THE EFFECT OF DIETARY CRUDE PROTEIN LEVEL, FORMALDEHYDE TREATED RAPESEED (CANOLA) MEAL AND DIETARY TYROSINE SUPPLEMENTATION ON THE PERFORMANCE OF LACTATING DAIRY COWS

A Thesis

Presented to
the Faculty of Graduate Studies
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In partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

by

ROBERT CAMERON RAE

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ABSTRACT

The effect of lowering the ruminal protein degradability of canola meal (CM) on milk production of Holsteins fed a CM/barley/corn silage based diet was studied. Preliminary experiments showed that CM protein degradability could be substantially reduced by treatment with 1.2g formaldehyde (FA)/100g crude protein (CP). Formaldehyde treated CM was fed to dairy cows in a lactation study. Forty Holsteins were assigned to one of four isocaloric diets 2 weeks postpartum in a 2 x 2 factorial arrangement. Factor I was dietary CP level, either 17.4% or 14.6% CP. Factor II was FA treatment of the CM, either treated or untreated. The experimental period lasted 12 weeks. Formaldehyde treatment had no effect on milk yield, milk composition, dry matter (DM) intake, or the apparent digestibilities of DM, nitrogen (N), ether extract or acid detergent fibre. Formaldehyde treatment reduced ($p < .05$) the concentration of ruminal ammonia-N (NH_3-N) and tended to increase ($p < .1$) the plasma concentration of total essential amino acids. High dietary CP level increased ($p < .05$) milk and lactose yields and increased ($p < .05$) the plasma concentrations of urea and total essential amino acids. Reasons suggested for the lack of response to FA treated CM are (a) amino acid supply to the mammary gland did not limit milk synthesis, or (b) FA treatment failed to increase the absorption of specific amino acids, particularly lysine and tyrosine, whose availability may have limited milk yield.

In the next part of the study, experiments were conducted to determine if the lack of response to FA treated CM was due to failure

to increase the uptake of tyrosine. Results from preliminary experiments with two duodennaly cannulated sheep suggested that orally administered tyrosine could partially escape degradation in the rumen and reach the small intestine.

Tyrosine was then fed to 6 lactating Holsteins in a switchback design with three experimental periods of 10 days duration. Tyrosine supplementation (50g tyrosine/cow/day) increased milk yield by 1.0kg/day (3.4%).

Next, tyrosine was fed to 12 lactating Holsteins to determine the effect of supplementing tyrosine to diets containing FA treated CM. The design was a switchback with three periods of 14 days duration. Formaldehyde treatment tended to increase ($p < .1$) the plasma concentration of total essential amino acids but did not affect milk yield or DM intake. Tyrosine supplementation of the FA treated diet increased ($p< .05$) milk yield by 1.6kg/day but tyrosine supplementation of the untreated diet did not affect milk yield. Possibly, the lack of response of milk yield to FA treated CM was partially due to failure to increase the absorption of tyrosine.

Tyrosine supplementation may stimulate milk production by increasing the supply of tyrosine to the mammary gland or by a catecholamine mediated response. The potential interactions between tyrosine, catecholamines, and growth hormone are discussed in relation to the protein requirements of lactating dairy cows.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADF-N	Acid detergent insoluble nitrogen
AEP	2-Aminoethylphosphonic acid
ATP	Adenosine triphosphate
BCAA	Branched chain amino acids
BMM	Burroughs mineral mixture
BVFA	Branched chain volatile fatty acids
C	Carbon
Ca	Calcium
CH_4	Methane
CM	Canola meal
cm	Centimetre
CO_2	Carbon dioxide
CP	Crude protein
CS	Canola seeds
DAP	Diaminopimelic acid
DE	Apparent digestible energy
DM	Dry matter
DNA	Deoxyribonucleic acid
EAA	Essential amino acids
EE	Ether extract
FA	Formaldehyde
FCM	Fat corrected milk
g	Grams

GE	Gross Energy
GH	Growth hormone
h	Hours
HCl	Hydrochloric acid
H_2O	Water
kg	Kilogram
l	Litre
L-DOPA	L-Di hydroxyphenylalanine
LNAA	Large neutral amino acids
ME	Metabolizable energy
μg	Micrograms
μmole	Micromoles
mg	Milligrams
min	Minutes
ml	Milliltres
mm	Millimetre
mmole	Millimoles
N	Nitrogen
NaCl	Sodium chloride
NAN	Non ammonia nitrogen
NaOH	Sodium hydroxide
NE_l	Net energy for lactation
NH_3	Ammonia
NH_3-N	Ammonia nitrogen
NNDM	Non nitrogenous dry matter

NPN	Non protein nitrogen
O	Oxygen
OM	Organic matter
P	Phosphorus
PCS	Partially extracted canola meal
RDP	Rumen degradable protein
REP	Rumen escape protein
RNA	Ribonucleic acid
rpm	revolutions per minute
RSM	Rapeseed meal
S	Sulphur
SBM	Soybean meal
VFA	Volatile fatty acids
vit	Vitamin
YATP	Yield of microbial dry matter in grams/mole adenosine triphosphate utilized.

INTRODUCTION

Over the past few years, rapeseed has become established as a major crop in Canada. This has been largely due to the development of "double low" cultivars which are low in erucic acid and glucosinolates, leading to increased use of rapeseed as a source of supplementary protein for livestock and poultry and edible oil for humans. To distinguish the new cultivars from older rapeseed varieties, the term "canola" has been adopted. The Canola Council of Canada (1980) states, "...the generic term 'canola' applies to the seed, meal, oil, hulls, or any other derivatives of the varieties with less than 3mg/g normally measured glucosinolates and with 5% or less erucic acid. Currently included are the Brassica napus varieties, Tower, Regent and Altex and the Brassica campestris variety, Candle." Several studies have been conducted to assess the nutritional value of canola meal (CM) as a protein supplement for lactating dairy cows (Sharma et al. 1977; Papas et al. 1978; Papas et al. 1979; Laarveld et al. 1981; Sanchez and Claypool (1982) and results show that CM compares favourably with soybean meal (SBM).

Like some other protein supplements, e.g. SBM, cottonseed meal and peanut meal, there is evidence that CM protein is readily degraded in the rumen (Burroughs et al. 1975). The high ruminal degradability of CM protein may limit the efficiency of protein utilization by lactating cattle because of failure to supply an adequate amount of rumen escape protein (REP) to meet the animals' protein requirements. Several systems proposed recently for assessing the protein

requirements of ruminant livestock incorporate the concept of REP and rumen degradable protein (RDP) (Burroughs et al. 1975; Satter and Roffler 1975; Kaufmann 1977; Verite et al. 1979; Agricultural Research Council 1980). Protein entering the abomasum from the forestomach is of microbial origin or undigested dietary protein. In the case of an animal with a high protein requirement, the yield of microbial protein is unlikely to be adequate so the animal must depend on undegraded dietary protein, i.e., REP, to meet the balance of its needs. This is particularly true of high producing dairy cows which have an extremely high requirement for protein. Thus, protein supplements, such as CM, which are extensively degraded in the rumen may not provide sufficient REP for optimal milk production.

A deficiency of REP is more likely to occur if CM is fed in combination with other dietary ingredients which are low in REP. In Western Canada, rations based on CM, barley and corn silage are commonly fed to dairy cattle and all three ingredients have low levels of REP. Therefore, there is a possibility that protein utilization of this type of diet is inefficient. The objective of the present study was to determine if milk production and nitrogen (N) utilization of cows fed CM, barley and corn silage could be improved by lowering the ruminal degradability of CM protein.

LITERATURE REVIEW

Introduction

The amino acid requirements of ruminants are met by absorption of amino acids from the small intestine (Agricultural Research Council 1980). These amino acids are supplied by microbial protein synthesized in the rumen, undegraded dietary protein and endogenous protein secretions. However, since digestion of endogenous proteins does not represent a net gain of amino acids, the former two sources determine the quantity and composition of amino acids available to the animal. Therefore, factors which affect microbial protein synthesis or the degradability of dietary protein in the rumen will influence the supply of amino acids to the intestine. Discussion of these factors as they relate to the protein requirements of lactating dairy cows forms the basis of the literature review. The potential role of tyrosine in the control of milk production is also discussed.

Microbial Protein Synthesis

Comparative contributions of bacteria and protozoa to microbial protein synthesis

Protein is synthesized in the rumen by protozoa and bacteria. Most available information concerns bacterial protein synthesis and relatively little is known about the contribution made by protozoa. Attempts have been made to quantitate protozoal protein and there is

little doubt that a substantial portion of microbial protein in the rumen is protozoal (Ibraham and Ingalls 1972). However, there are conflicting reports about the quantity of protozoal protein actually reaching the small intestine. Weller and Pilgrim (1974) estimated that the rate of passage of protozoa through the omasal canal was less than 25% the rate of water, implying that protozoa have a tendency to remain in the rumen. Other workers (Hagemeister 1975; Abou Akkada and El Shazly 1976) found large quantities of 2-aminoethyl phosphonic acid (AEP), a protozoal marker, in the duodenum and suggested that the flow of protozoa to the small intestine was substantial. However, although AEP is supposedly found only in protozoa, Ling and Buttery (1978) found considerable amounts of AEP in dietary and bacterial material so the validity of using AEP as a protozoal marker has been questioned.

Assessing the overall quantitative contribution of protozoal protein is further complicated by the method protozoa sequester nutrients. Although protozoa obtain N from sources such as particulate proteins, peptides, amino acids and ammonia (NH_3), the major source is bacterial N which is obtained by engulfment of whole bacteria (Russell and Hespell 1981). Therefore, protozoal protein is derived from recirculation of proteins which have already been synthesized by bacteria. This is not to say that protozoa are unimportant in the protein nutrition of ruminants, it merely points out the difficulty of assessing the relative contributions of protozoal and bacterial protein in meeting the requirements of the host.

General mechanism of bacterial protein synthesis

The mechanism of protein synthesis in the rumen varies between bacterial species (Buttery 1976). However, the major events have been summarized by Smith (1979). Bacterial amino acids are synthesized from NH_3 and α-keto acids. Ammonia entering the rumen is combined with glutamate and aspartate to form glutamine and asparagine. The amide groups on the latter two molecules are used to convert α-ketoglutarate to glutamate and for synthesis of alanine and aspartate from their respective keto acids. Glutamine, aspartate and alanine accumulate and their amino groups are transferred to suitable keto acids for the synthesis of other amino acids. These, along with preformed amino acids, are used for the synthesis of polypeptide chains. Many of the enzymes and mechanisms required for this general pathway have been identified (Smith 1979).

Factors affecting the yield of microbial protein

It has been known for some time that energy supply is a major factor limiting microbial protein synthesis (Walker 1965). Basically, adenosine triphosphate (ATP) must be generated from the digestion of organic matter (OM) in the rumen then utilized for the synthesis of protein and other cell constituents. Dietary carbohydrate provides the bulk of the energy required for microbial growth, but the contribution of dietary protein is also considered significant (McMeniman et al. 1976). Carbohydrates which are readily fermentable, such as starches and sugars, are more effective at promoting efficient microbial growth and protein synthesis, possibly because they yield more energy (Stern and Hoover 1979). In addition, the source of starch is important, e.g. the starch in corn does not appear to be utilized

in the rumen as efficiently as barley starch (Orskov et al. 1971).

Smith (1979) suggested that bacteria produce an average of 20 moles ATP per kg OM truly digested in the rumen, although the actual value will vary considerably depending on the nature of the diet. A large part of the ATP produced is used for protein synthesis (Hespell and Bryant 1979). From a survey of available data, Smith (1979) concluded that the average yield of microbial N is 23 g/kg OM truly digested in the rumen, or 30 gN/kg OM apparently digested. A similar survey by Stern and Hoover (1979) led the authors to report an average yield of 27 g microbial N/kg OM apparently digested in the rumen. A more recent review (Johnson and Bergen 1982) suggests an average yield of 19 g microbial N/kg OM truly digested or 25 gN/kg OM apparently digested.

The supply of digestible OM is not the only factor involved in the utilization of energy for microbial protein synthesis. Until recently, it was believed that the efficiency of utilization of ATP in the rumen was a biological constant, but this is no longer the case (Bergen 1980). For any given energy supply, the efficiency of ATP utilization by rumen microorganisms is extremely variable. Hespell and Bryant (1979) estimated that, in theory, mixed rumen bacteria could achieve a maximum YATP value of 26 g/mole, where YATP is the yield of microbial dry matter (DM) in g/mole ATP (Bauchop and Elsden 1960). However, from a review of studies where YATP values for various bacterial species were measured, Hespell and Bryant (1979) found the majority of measured values were in the range 10 to 12 g microbial DM/mole ATP. Thus, it appears that the actual efficiency of microbial growth, in terms of YATP, is considerably lower than the

theoretical maximum.

The practical implications of improving the efficiency of ATP utilization by rumen microorganisms have been pointed out by Smith (1979). If YATP can be increased for a given energy supply, the result would be an increased synthesis of microbial protein and a greater supply of microbial protein to the small intestine. Using calculations from the Agricultural Research Council (1980) protein system, Smith (1979) suggested that a high producing dairy cow could obtain virtually all the protein it requires from urea if a YATP value of 20 g/mole could be achieved. He further suggested that YATP could be increased in practice by manipulating ruminal dilution rate and nutrient supply.

There are many reports, as cited by Stern and Hoover (1979), showing that microbial growth increases as dilution rate in the rumen increases, e.g. Isaacson et al. (1975) observed an increase in YATP from 7.5 to 16.7 g/mole when dilution rate was increased from 2% to 12% per hour. At high dilution rates, the mean age of the microbial population is lowered and younger cells have a greater potential for rapid growth than mature cells (Van Soest 1982). Other factors which may account for the effect of dilution rate on microbial growth are reduced autolysis of bacteria, reduced phagocytosis of bacteria by protozoa and changes in the microbial population caused by changes in substrate availability (Kennedy et al. 1976).

Microbial protein synthesis will be efficient only if there is an adequate supply of all the nutrients required for microbial growth. All rumen bacteria need a supply of sulphur (S) for the synthesis of cysteine and methionine. Therefore, an inadequate S intake may limit

microbial protein synthesis (Goodrich et al. 1978). Hume and Bird (1970) reported that sheep consuming 0.6 gS/day produced 82 g microbial protein but when S intake was 2.0g/day, 94 g microbial protein was produced. A deficiency of S is more likely to limit microbial protein synthesis when urea is used in significant amounts because of a potential deficiency of methionine (Stern and Hoover 1979). Because S supply affects microbial N utilization, requirements for S are often expressed as a proportion of dietary N, e.g. the Agricultural Research Council (1980) suggests a dietary N:S ratio of 14. Although bacteria require S, not all species can utilize inorganic S so methionine supplementation may be more beneficial to the microbial population than supplementation with inorganic S (Goodrich et al. 1978).

Volatile fatty acids (VFA) are essential or stimulatory for growth in many rumen bacteria. Hume (1970) observed a 14% increase in microbial protein production when branched chain VFA (BVFA) were supplemented to sheep fed a semi-purified diet containing urea. Cline et al. (1966) reported an increase in cellulose and DM digestibility when BVFA were added to high cellulose diets containing urea. Similarly, van Gylswyk (1970) and Roche et al. (1973) found that BVFA supplementation led to increased growth of cellulolytic bacteria. Recently, supplementation of BVFA to lactating dairy cow rations increased milk production with no effect on feed intake (Papas et al. 1981; Cook et al. 1981; Sniffen et al. 1981). The BVFA are used by bacteria as precursors for the synthesis of branched chain amino acids (BCAA) and longer branched chain fatty acids (Hespell and Bryant 1979). Although BVFA are synthesized de novo by bacteria, it has been

suggested that substantial quantities of BVFA are derived from deamination of BCAA. Therefore, a deficiency may occur when ruminants are fed low protein diets (Umunna et al. 1975).

Amino acids and peptides can contribute substantially as sources of N for microbial protein synthesis. Reviewing available data, Hespell and Bryant (1979) suggested that 50 to 70% of microbial N can be derived from NH_3 with the remainder coming from amino acids and peptides. Maeng et al. (1976) reported that supplementation of BCAA to viable rumen fluid stimulated microbial growth when cellobiose, but not glucose, was the carbohydrate source. The authors suggested this was due to BCAA contributing to the requirements of cellulytic bacteria for BVFA. The same authors also found a large increase in microbial growth when urea was replaced by S containing amino acids and several other amino acid mixtures. Similar responses have been reported by Maeng and Baldwin (1976a, b). Using a different approach, Salter et al. (1979) supplied N^{15} -labelled urea to steers. When the animals were fed vegetable protein to ensure an adequate supply of preformed amino acids, bacteria utilized proline, arginine, tyrosine, phenylalanine and methionine directly from the medium to a greater extent than other amino acids. However, when most dietary N was in the form of urea, de novo synthesis of proline and arginine appeared to increase but synthesis of methionine, tyrosine, and phenylalanine did not. The authors concluded that methionine and phenylalanine may limit bacterial growth when low protein, high urea diets are fed. Tyrosine was not included because the authors presumed any requirement for tyrosine could be met by phenylalanine. Interestingly, Salter et al. (1979) did not find that availability of BCAA limited bacterial

growth. However, the authors pointed out that the deaminated and, therefore, unlabelled carbon skeletons of dietary BCAA may have been utilized for bacterial amino acid synthesis. Teather et al. (1980) reported that the population of rumen bacteria was 70% greater on diets containing SBM or a silage-urea mixture compared to diets containing urea as the sole N source. The bacterial species whose numbers increased were those which are able to utilize amino acids or peptides. Cotta and Russell (1982) reported that addition of amino acids or peptides to bacterial cultures increased the efficiency of energy utilization. Some bacteria use oligopeptides in preference to NH_3 (Pittman and Bryant 1964), possibly because less energy is required for transport across the cell wall (Hespell and Bryant 1979).

Thus, there is considerable evidence that the efficiency of microbial protein synthesis can be improved if oligopeptides, amino acids and BVFA are supplied in adequate quantities. Hespell and Bryant (1979) suggested this could be achieved by feeding dietary proteins which are slowly degraded in the rumen so that peptides and amino acids, including BCAA, would be released over a prolonged period. Ben-Ghedalia et al. (1978) found that the addition of corn gluten, which is slowly degraded in the rumen, to a purified diet containing urea as the sole N source increased the efficiency of microbial protein synthesis. However, when casein was added, there was no effect on microbial protein synthesis.

The remaining dietary factor of major importance to microbial protein synthesis is the provision of NH_3. Most bacterial species in the rumen use NH_3 as the principle source of N and for some, NH_3 is an essential nutrient (McMeniman et al. 1976). Various estimates have

been made regarding the contribution of NH_3 to total microbial protein synthesis. Maeng and Baldwin (1976a) suggested 60 to 80% of microbial N was derived from NH_3-N under normal feeding conditions. Stern and Hoover (1979) suggested the value could be as low as 40% and as high as 100% under extreme conditions, and Hespell and Bryant (1979) suggested a range of 50 to 70%.

A supply of NH_3-N is essential for microbial growth (Hespell and Bryant 1979), but there is still disagreement about the optimal level of NH_3 for maximum microbial activity in the rumen. The optimal level is difficult to define because the amount of NH_3 which can be utilized in the rumen depends on several factors, particularly the supply of energy (Satter and Roffler 1975) and the availability of carbon skeletons for amino acid synthesis (Huber and Kung 1981). Satter and Roffler (1975) describe the optimal level of ruminal NH_3 in terms of "NH_3 overflow." For a given situation, when production of NH_3 in the rumen exceeds utilization, NH_3 accumulates and there is an "NH_3 overflow." This excess NH_3 is removed from the rumen. The level at which "NH_3 overflow occurs has been referred to as the "breakpoint" by Smith (1979) and accumulation of NH_3 above the breakpoint is evidence that the supply of NH_3 has exceeded demand. Smith (1979) concludes that NH_3 in the rumen will be used with greatest efficiency under conditions where the uptake of NH_3 into bacterial cells is always just sufficient to support maximum growth, given a particular supply of energy and other nutrients.

Many attempts have been made to determine the ruminal NH_3 level which will support maximum microbial growth. Estimates have been obtained from *in vitro* and *in vivo* studies.

Allison (1970) reported that NH_3 became limiting for *in vitro* growth of *Bacteroides amylophilus* at concentrations below 6.4 mg/100 ml. Satter and Slyter (1974), using *in vitro* continuous culture fermenters, determined that microbial protein synthesis reached a maximum at a concentration of 5 mg NH_3 -N/100 ml. In a similar type of study, Nikolic et al. (1975) found that microbial protein synthesis *in vitro* was not significantly affected by NH_3 -N concentrations ranging from 1.6 to 16.7 mg/100 ml. However, the authors suggested that protein catabolism may be increased in the rumen when NH_3 -N concentration is low and recommended that rumen NH_3 -N levels be maintained at 5 to 6 mg/100 ml. This is similar to the value of 8 mg NH_3-N/100 ml suggested by Annison (1975). Edwards and Bartley (1979) added various levels of Starea (a urea-starch complex) to viable rumen fluid and found that maximum microbial protein synthesis occurred when the NH_3-N concentration of the medium was 76 mg/100 ml. This is considerably higher than other estimates obtained from *in vitro* studies. Schaefer et al. (1980) determined the NH_3 saturation constants of several predominant species of rumen bacteria and concluded that the major bacterial species could achieve at least 95% of their specific growth rates in a medium containing only 1.7 mg NH_3 -N levels because less energy would be required to assimilate the available NH_3.

Many estimates of the optimal ruminal NH_3 concentration have been obtained from *in vivo* studies. Hume et al. (1970) reported that synthesis of microbial protein was greatest at ruminal NH_3 -N levels of 8.8 mg/100 ml in sheep fed a purified diet. The flow of microbial protein from the rumen reached a maximum when ruminal NH_3-N

concentration was 13.3 mg/100 ml. Miller (1973) fed a similar type of diet to sheep and found that 29 mg/100 ml was the optimal concentration of ruminal NH_3-N for microbial protein synthesis. Roffler et al. (1975) infused urea into the rumina of steers and concluded that microbial assimilation of N was unaffected by ruminal NH_3-N levels in excess of 5 mg/100 ml. Allen and Miller (1976) reported that the flow of non NH_3-N (NAN) through the abomasum of sheep was greatest at ruminal NH_3-N levels of 15.8 mg/100 ml. Using the artificial fibre bag technique, Mehrez et al. (1977) found that maximum DM disappearance from bags suspended in the rumina of sheep occurred when ruminal NH_3-N concentration was 23.5 mg/100 ml. In a similar type of study, Ortega et al. (1979) did not observe any effect on DM disappearance when ruminal NH_3-N level was raised in increments from 6.3 to 27.5 mg/100 ml. Okorie et al. (1977) found that maximum microbial synthesis occurred with ruminal NH_3-N levels of 7 mg/100 ml when sheep were infused intraruminally with urea. Slyter et al. (1979) infused urea into the rumina of steers and observed greatest microbial protein synthesis when ruminal NH_3-N concentration reached 2.2 mg/100 ml. They also found that ruminal VFA concentration and N retention increased up to ruminal NH_3-N levels of 4.5 mg/100 ml but did not increase significantly beyond this level. The authors concluded that a concentration of 2 to 5 mg NH_3-N/100 ml is sufficient to allow maximum growth of rumen microorganisms. Veira et al. (1980) fed four diets of varying N content to weaned calves and found that microbial protein synthesis was not increased when ruminal NH_3-N concentration rose above 1.9 mg/100 ml. There was also no effect on

VFA production or the flow of bacterial protein through the abomasum. Kang-Meznarick and Broderick (1980), using an automated procedure for the simultaneous determination of NH_3 and amino acids in rumen liquor from steers fed various levels of urea, found that the level of amino acids produced in the rumen reached a maximum when ruminal NH_3-N concentration was 8.5 mg/100 ml. A similar study by these authors (Broderick et al. 1981) suggested the optimal NH_3-N level was 27 mg/100 ml.

Estimates of the NH_3-N breakpoint range from about 1 to 76 mg/100 ml although the majority of studies found that 1 to 10 mg NH_3-N/100 ml provided an adequate supply of NH_3 for efficient microbial protein synthesis and microbial growth. Estimates from in vivo studies tended to be higher than estimates obtained from in vitro experiments. It is not surprising that the breakpoint level of ruminal NH_3 has not been clearly elucidated because the optimal level of NH_3 will vary depending on the supply of other nutrients, particularly energy (Satter and Roffler 1975). Many different dietary regimes were used in the studies discussed above. Smith (1979) discussed the breakpoint in terms of NH_3 absorption through the rumen wall. At pH values below 7.0, NH_3 may remain in the rumen for several hours because it will be present in its ionized form and will, therefore, be unable to pass through the rumen wall at a rapid rate. Although there may be considerable uptake of NH_3 from the omasum, high NH_3 concentrations may remain in the rumen to provide a reserve of NH_3 for bacterial growth over a prolonged period. So, all the accumulated NH_3 is not necessarily wasted. Smith (1979) concluded that the high levels of ruminal NH_3-N reported by some authors to be necessary for maximum

microbial growth may have resulted from the beneficial effects of this reserve in sustaining microbial growth over a longer period of time. The ammonium ion acts as a buffer in the rumen so part of the effect of high ruminal NH_3-N concentrations may be due to the effect of NH_3 on rumen fluid pH.

Another likely reason for the variability in breakpoint estimates is the extensive presence of bacterial microcolonies in the rumen, which are intimately associated with small food particles such as starch granules (Cheng and Costerton 1980; Allison 1982). Individual cells in these microcolonies are likely to be exposed to local NH_3 concentrations which differ markedly from the NH_3 levels measured in samples of rumen fluid because energy supply within the colony is high and fluid flux is low (Allison 1982).

Finally, Mahadevan et al. (1982) reported that ruminal NH_3 concentrations fluctuate greatly throughout the day in a cyclic pattern and concluded that the practice of measuring and reporting ruminal NH_3 at one time point or as a mean of several time points does not adequately represent the complex pattern of NH_3 utilization in the rumen. The authors gave convincing examples of how a researcher could easily make an incorrect interpretation from analysis of rumen fluid NH_3 levels. This type of error may have contributed to the wide variability in estimates of NH_3 breakpoint concentration.

Degradation of Nitrogenous Compounds in the Rumen

General mechanism of protein and NPN degradation

Nitrogenous compounds entering the rumen are commonly referred to as protein or NPN. The NPN fraction is comprised largely of nucleic acids, amino acids, NH_3 and urea (Huber and Kung 1981).

It has been known for many years that dietary protein entering the rumen is subject to proteolysis (Syme 1938; Pearson and Smith 1943). Most of the available information concerns bacteria, but protozoa are also proteolytically active (Chalupa 1975; Tamminga 1979). Bryant and Burkey (1953) studied several bacterial strains and found that 20% had the capacity to liquify gelatin. Several authors, as cited by Chalupa (1975), have reported that species of Bacteroides, Butyrivibrio and Selenomas are more proteolytic than other species. Bacteroides amylophilus strains are reputed to be particularly active (Blackburn 1968; Mahadevan et al. 1980). Streptomyces griseus also appears to be proteolytically active (Poos et al. 1980; Sniffen 1982).

Results from several in vitro experiments in which casein degradation was studied (Annison 1956; Warner 1956; Blackburn and Hobson 1960) have suggested that the composition of the diet has little effect on proteolysis in vivo. However, recent findings by Russell et al. (1981) indicate that Streptococcus bovis can become the predominant proteolytic species within 3 h of adding a large dose of soluble carbohydrates to viable rumen fluid. This species appears to be extremely proteolytic and the authors suggest that proliferation of Streptococcus bovis after ingestion of a meal rich in readily

fermentable carbohydrate may be an important factor in the rapid degradation of protein observed 1 to 3 h after feeding. Therefore, the nature of the diet may influence proteolytic activity in the rumen.

The enzymes responsible for proteolysis by bacteria are attached to the outer bacterial cell membrane (Blackburn and Hobson 1960). Therefore, bacteria must make physical contact with the substrate for proteolysis to occur. Tamminga (1979) has reviewed the basic mechanisms of protein degradation in the rumen. Once bacteria make contact with a protein, the polypeptide chain is hydrolysed to form small peptides and amino acids. These are transported across the cell wall into the bacterial cell. Here, the peptides are further hydrolysed to amino acids by peptidases. Amino acids can be incorporated directly into microbial protein but most are catabolised. Deamination is probably the first step in the degradative pathway, followed by decarboxylation of the subsequent α-keto acids, although some amino acids may be decarboxylated prior to deamination leading to formation of the respective amides. There is also transamination of some amino acids in the bacterial cells. The end products of this degradative pathway, as outlined by Tamminga (1979), are VFA, NH_3, carbon dioxide (CO_2) and methane (CH_4). It is not clear whether proteolysis or amino acid catabolism is the rate limiting process. The increased levels of amino acids in the rumen observed after a meal imply that proteolysis is not rate limiting; however, Nugent and Mangan (1978) suggest this may not always be the case, particularly if the diet contains a high proportion of slowly degradable protein.

The contribution of protozoa to proteolysis is unclear. Species of Entodinium, Isotrichia, Eudiplodinium and Osphryoscolex are known

to be proteolytic (Chalupa 1975). The mechanism of digestion is different from that of bacteria. Protozoa engulf bacteria and small food particles so that digestion of protein by exo- and endopeptidases occurs within the protozoal cell (Coleman 1975).

Proteolytic activity of rumen microorganisms often exceeds their requirement for NH_3, as can be seen by the rapid rise in ruminal NH_3 concentration following ingestion of a high protein meal (Mahadevan et al. 1982). Orskov et al. (1976) reported that addition of urea did not spare protein from degradation in the rumen, although Nikolic et al. (1975) observed some sparing effect. The reasons why protein is degraded in excess to NH_3 requirement are not clear. Possibly, microorganisms continue to have a requirement for NAN compounds, such as amino acids and peptides, after the need for NH_3 has been met (Chalupa 1975). A more likely explanation is that excess dietary protein is degraded to supply energy (Tamminga 1979). However, the exact stoichiometric relationship between amino acid catabolism and ATP production is not known. An alternative explanation for excess degradation of protein is a possible lack of transport mechanisms to remove intact amino acids from the cell. In order to excrete amino acids from the cell, bacteria may first have to degrade them (Tamminga 1979).

Amino acids and peptides are considered NPN compounds (Huber and Kung 1981). As discussed above, they are produced in the rumen by proteolysis of larger proteins. However, peptides and amino acids are also present in the diet, especially in hays and silages (Sniffen 1982). Ammonia can also be a relatively important source of dietary N, particularly when ammoniated silages are fed (Huber and Kung 1981).

A substantial portion of dietary N consists of nucleic acids,

e.g. 5.2 to 9.5% of forage - N is comprised of nucleic acids (Smith 1975). Smith and McAllan (1970) added nucleic acids to the rumina of calves and found complete destruction within 1 h. Similar observations have been made by Razzaque and Topps (1972). In vitro degradation of nucleic acids has been reported by Smith and McAllan (1970) and McAllan and Smith (1973). The pathways of nucleic acid catabolism have been discussed by Smith (1975). Although nucleic acids are rapidly degraded in the rumen, several products of degradation, namely uracil, xanthine, hypoxanthine, and thymine, are partially resistant to further degradation. These conclusions were drawn from *in vitro* studies (Smith 1975). However, uracil, xanthine, hypoxanthine and thymine are rapidly removed from the rumen, *in vivo*, but it is not clear whether they are absorbed across the rumen wall or if further catabolism occurs *in vivo*. The quantitative importance of nucleic acid N to microbial protein synthesis is not known.

Urea enters the rumen in saliva or by direct passage through the rumen wall (Houpt 1970). Nolan and Leng (1972) suggested that the salivary route is quantitatively more important than direct passage. However, a recent study by Kennedy and Milligan (1980) indicated that the salivary route accounted for only 15 to 50% of urea transfer in sheep fed a forage diet. Urea is also commonly added to ruminant diets as a source of supplemental N (Huber 1975). Urea is rapidly degraded to NH_3 and CO_2 by the action of bacterial ureases (EC 3.5.1.5) (Pearson and Smith 1943). Hydrolysis of urea in the rumen usually exceeds the rate of NH_3 assimilation (Allison 1970) so attempts have been made to supply urea in a form which is degraded more slowly (Huber and Kung 1981).

Degradability of nitrogenous compounds in the rumen

Studies on the degradation of nitrogenous compounds in the rumen have centred on the rate and extent of N degradation rather than the actual mechanisms. This is because information on N degradability is required to implement the new protein systems (e.g. Agricultural Research Council 1980). The various techniques used to estimate N degradability will be discussed briefly since the schemes used to describe protein and NPN utilization in the rumen were developed from the use of these techniques.

In vivo methods have been reviewed by Stern and Satter (1982) and there are two basic techniques. The amount of undegraded protein can be estimated directly using duodenally fistulated animals. This is done by measuring total N intake and total dietary and microbial N passing to the duodenum. The amount of protein degraded is calculated by difference. This method requires that microbial protein be quantitated by using a marker such as diaminopimelic acid (DAP) or ribonucleic acid (RNA). The alternative in vivo technique described by Stern and Satter (1982) is to measure the increase in duodenal amino acids from a known increase in amino acid intake and then calculate the amount of ruminally degraded protein indirectly. Relatively few in vivo measurements have been made and most of these have been made using sheep. Results from these studies may not accurately be extrapolated to cattle because of differences in the digestive systems between sheep and cattle (Stern and Satter 1982).

Several in vitro techniques have been used to estimate N degradability in the rumen. Ammonia accumulation during incubation of

a protein source in viable rumen fluid has commonly been used as a measure of N degradability. However, serious errors occur because this method fails to account for assimilation of NH_3 by microorganisms (Broderick 1982). Broderick (1978) attempted to overcome the problem of N assimilation by rumen microbes by adding hydrazine to the incubation medium. Hydrazine inhibits NH_3 and amino acid assimilation by bacteria.

Feedstuffs have been incubated with proteases to estimate rumen degradability and several proteolytic enzymes were tested by Poos et al. (1980). These workers found that degradation of dietary protein by a fungal protease from Aspergillus oryzae was more closely correlated with in vivo digestibility than degradation by a protease from Streptomyces griseus, a proteolytic species of rumen bacteria. Mahadevan et al. (1980) used a bacterial protease prepared from Bacteroides amylophilus to determine the degradability of several soluble and insoluble proteins. This method has the advantage that Bacteroides amylophilus does not appear to contain deaminase enzymes so a crude preparation of the protease will not deaminate amino acids. This means that amino acids in the incubation medium can accumulate and be measured by the ninhydrin reaction (Mahadevan et al. 1980).

A serious problem when measuring protein degradation using rumen fluid inoculums is the accurate separation of microbial and dietary N. Mahadevan et al. (1979a) used a diazonium salt solution to treat various dietary proteins. Diazonium dyes bind reversibly to protein and are released following hydrolysis of the polypeptide chain. Therefore, undegraded dietary protein can be measured by precipitating

the protein which remains in the medium after incubation and quantitating the amount of dye. The dye will only be bound to undigested dietary protein.

Nitrogen solubility has received considerable attention as a method for estimating degradability in the rumen largely because of its simplicity. A variety of solvents have been used, e.g. sodium hydroxide (NaOH) solution (Lyman et al. 1953; Sharma et al. 1972), autoclaved rumen fluid (Little et al. 1963; Wohlt et al. 1973), cold water (Little et al. 1963), hot water (Waldo and Goering 1979), sodium chloride (NaCl) solution (Salobir et al. 1970), 0.1N hydrochloric acid (HCl) solution (Waldo and Goering 1979), a dilute solution of pepsin in 0.1N HCl (Beever et al. 1977), artificial saliva as described by McDougal (1949) (Crooker et al. 1978), a mineral solution described by Burroughs et al. (1950) (Henderickx and Martin 1963; Wohlt et al. 1973) and 70% ethanol (Sprague and Breniman 1969). There are pronounced differences among solvents (Waldo and Goering 1979; Krishnamoorthy et al. 1982) and no agreement has been reached on which, if any, solvent should be used on a routine basis.

The use of N solubility as a measure of degradability has fallen into disrepute because (a) many insoluble proteins are degradable in the rumen (Crooker et al. 1978; Waldo and Goering 1979) and (b) some soluble proteins are not readily degradable (Mangan 1972; Mohamed and Smith 1977; Mahadevan et al. 1980). Solubility is a good estimator of degradability for some feeds but not for others, and this makes interpretation of N solubility data extremely difficult (Broderick 1982). There is still some support for using N solubility, however. Mertens (1977) and Zinn et al. (1981) have proposed systems for

predicting protein degradability based partially on N solubility data. Zinn et al. (1981) found extremely high correlations between predicted and measured N degradability; however, the authors pointed out that the system is dependent on a number of important assumptions. Satter (1981) suggested that N solubility be used within a feedstuff to predict N degradability. Some feedstuffs, e.g. oil seed meals and byproducts of the distilling industry, are subject to heat during processing. The temperature during processing can vary from factory to factory so the protein degradability of a given feedstuff may vary depending on the factory of origin. Most of the soluble N of silages is comprised of NPN and is rapidly degraded in the rumen (Sniffen 1982), particularly if the plant is wilted prior to ensiling and active proteases have broken down plant protein to peptides and amino acids (Tamminga 1979). Therefore, solubility may be an appropriate estimator of degradability of silage N.

A method for estimating N degradability which has gained some acceptance is the artificial fibre bag technique. This procedure has been used for many years (Quin et al. 1938) and has become popular for estimating N degradation following a report on its use by Mehrez and Orskov (1977). The technique involves placing a known quantity of food in a small bag which is suspended in the rumen of a fistulated animal for a measured period of time. The quantity of N disappearing from the bag is taken as an estimate of protein degradability. Variations of the technique have been discussed by several authors (Van Hellen and Ellis 1977; Orskov et al. 1980; Kempton 1980;

Broderick 1982; Stern and Satter 1982).

Limitations of the technique were discussed by Orskov et al. (1980). Firstly, the food is not subject to mastication or rumination as is the case for ingested food. Secondly, the food is not free to leave the rumen once a desirable particle size has been reached. Pore size of the bag material determines when food particles can leave the bag. Thirdly, the technique equates N disappearance from the bag with N degradation but the two are not the same. Soluble proteins disappear rapidly from the bag and are assumed to be degraded; however, not all soluble proteins are rapidly degraded (Mangan 1972; Mohamed and Smith 1977; Mahadevan et al. 1980). In addition, material is allowed to pass from the bag when its particle size is sufficiently small to pass through the pores in the bag material, but this does not mean that the material has been degraded to simple chemical compounds (Broderick 1982).

Most studies using the bag technique have not taken rate of passage through the rumen into account. This is likely to be a major source of error since the extent of degradation depends on the length of time spent in the rumen (Tamminga 1979). Orskov and McDonald (1979) and Stern et al. (1980) have reported methods which correct for rate of passage. In spite of its limitations, the artificial fibre bag technique appears to be a useful tool for estimating N degradability in the rumen.

Although imperfect, the invivo, in vitro and in situ techniques discussed above have given nutritionists an overview of protein and NPN utilization in the rumen. Peptides, amino acids, urea and nucleic acids are rapidly degraded in the rumen (Pearson and

Smith 1943; Smith 1975; Chalupa 1976) but the degradability of protein varies greatly depending on dietary source (Sniffen 1982). Although there is evidence suggesting that some soluble proteins are not rapidly degraded, schemes for N utilization generally assume that most soluble protein is rapidly catabolized in the rumen. It is apparent that many insoluble proteins are degradable (Crooker et al. 1978; Waldo and Goering 1979) so solubility is obviously not the only factor determining degradability. The protein tertiary structure appears to be an important factor in determining degradability. Disulphide bonds can help maintain a tertiary structure which is resistant to attack by microbial proteases (Nugent and Mangan 1978; Mahadevan et al. 1979b, 1980). Proteins which are associated with the plant cell wall are likely to be degraded more slowly or not at all (Sniffen 1982).

Another important factor determing the extent of protein degradation is the rate of passage through the rumen (Bull et al. 1979). Therefore, factors which increase rate of passage, e.g. increased food intake (Thornton and Minson 1973), reduced food particle size (Hogan and Weston 1967), infusion of mineral salts into the rumen (Harrison et al. 1975) and reduced environmental temperature (Kennedy et al. 1976) will tend to decrease the degradability of protein in the rumen.

Nutritionists have attempted to place absolute values on the protein degradability of certain feedstuffs for convenience in formulating diets (Satter 1981; Sniffen 1982). However, it is widely recognized that the protein degradability of a particular feedstuff may vary considerably for reasons discussed in the preceding paragraph. Physical and chemical methods of manipulating protein

degradability are discussed in a subsequent section.

Protein Requirements of Lactating Cattle

Nitrogen requirements of the rumen microorganisms

Meeting the N requirements of rumen microbes has usually implied determining how much RDP to feed to meet the NH_3 requirements (Satter and Roffler 1975). However, peptides (Pittman and Bryant 1964) and amino acids (Maeng et al. 1976) also stimulate growth so future protein systems may have to take these factors into account.

The amount of NH_3 which can be utilized by rumen microorganisms depends on the supply of other nutrients, particularly the level and source of dietary energy (Satter and Roffler 1975). Estimates of the optimal ruminal NH_3 level were reviewed in a previous section and ranged from 1 to 76 mg NH_3-N/100 ml. Thus, it is not yet possible to state with any degree of certainty the amount of RDP required by the rumen microorganisms.

Burroughs et al. (1975) proposed a system for estimating the amount of urea which could be included in the diet. This system is based on the CP and TDN content of the diet. The Agricultural Research Council (1980) suggests that the amount of RDP fed to an animal should be estimated from the intake of metabolizable energy (ME). The accuracy of this system has still to be determined. Based on the results of an *in vitro* study, Satter and Roffler (1975) concluded that diets containing 12 to 13% CP will normally meet the requirements of the microbial population of cattle in early lactation,

but this estimate will vary depending on the energy content of the diet, the level of dietary NPN, the degradability of dietary protein, the efficiency of bacterial growth in the rumen, and the extent of N transfer into the rumen from saliva and blood (Satter 1981). The value of 12 to 13% dietary CP is supported by many in vivo studies in which the response of milk production and DM intake to various levels of dietary CP was measured. These studies are discussed in more detail in the next section. Ideally, the requirement for N should be expressed as an absolute value because the use of percents can be inaccurate unless feed intake is also known (Clark and Davis 1980).

When excess RDP is fed, rumen microorganisms are not able to utilize all the NH_3 produced in the rumen (Satter and Roffler 1975). Excess NH_3 is absorbed into the blood, converted to urea in the liver and excreted in the urine (Satter and Roffler 1975). Plasma NH_3 may also be detoxified by urinary excretion as ammonium salts or by transamination of glutamate to glutamine (Najarian and Harper 1956). Thus, excess RDP is essentially wasted. Toxicity may result from feeding excess RDP if NH_3 production in the rumen exceeds the capacity of the animal's detoxifying systems (Clark and Davis 1980). There are many reports, as cited by Clark and Davis (1980), suggesting that increased plasma NH_3 can interfere with intermediary carbohydrate metabolism and decrease the availability of glucose for milk synthesis. There are also reports that excess dietary protein can increase the incidence of reproductive disorders and downer cow syndrome (Gould 1969; Julien et al. 1977; Jordan and Swanson 1979).

A deficiency of RDP results in an insufficient supply of NH_3 (Satter and Roffler 1975) and, possibly, peptides (Pittman and Bryant

1964), amino acids (Maeng et al. 1976) and BVFA (Umunna et al. 1975) for optimal microbial growth. This results in a decreased capacity to digest OM, increased retention time of ingesta in the rumen and decreased feed intake (Huber and Kung 1981). Therefore, a deficiency of RDP results in a decreased intake of other nutrients. This is particularly important during early lactation since a nutrient deficiency at this time decreases peak milk production and may reduce milk production during the remainder of lactation (Clark and Davis 1980). Cows which are severely protein deficient will have poor body condition and may be predisposed to infections and metabolic disorders (National Research Council 1978).

Effect of dietary CP level on milk production

The effect of dietary CP level on milk production during early lactation has been studied extensively in recent years, and many of these studies have been reviewed by Clark and Davis (1980), Huber and Kung (1981) and Van Horn (1982). Results from these experiments are not always easy to interpret because a wide range of diets were used with varying amounts of RDP, NPN, energy and other nutrients.

Raising dietary CP from less than 13% to 13% or more has often resulted in increased milk production. Polan et al. (1976) compared five dietary CP levels between 9.4 and 16.2% with and without urea, and noted a declining rate of increase in milk yield as dietary CP was raised. They also found that urea was a less effective N source than SBM, particularly at the higher protein levels. Kwan et al. (1977) raised dietary CP level from 11.7 to 13.9% using SBM and urea and observed an increase in milk production. Wohlt and Clark (1978)

reported a response in milk yield when dietary CP was raised from 9.8 to 11.5 using either urea or SBM. Milk yield was increased further by raising dietary CP to 14% with SBM but raising CP level to 14% with supplemental urea had no further effect on milk yield. Roffler et al. (1978) reported a substantial increase in milk production when dietary CP was raised from 12.2 to 16.2%. Clay and Satter (1979) found a difference between dietary CP levels of 10.5 and 12.5%. Gordon and McMurray (1979), Laird et al. (1979) and Oldham et al. (1979) reported an increase in milk production following supplementation of protein to low protein diets. Cressman et al. (1980) observed an increase in milk yield when dietary CP was raised from 12 to 18%. Kung and Huber (1981) reported that increasing dietary CP from 11 to 14% caused an increase in milk yield. Lundquist et al. (1982) found a substantial increase in milk yield when dietary CP was raised from 12.5 to 15.5% and Holter et al. (1982) reported that raising dietary CP from 11.1 to 13.7% caused an increase in milk production. Norman et al. (1982) increased milk production by raising dietary CP from 11 to 15%.

In several studies, increasing dietary CP level to 13% or more did not improve milk production. Chandler et al. (1976) raised dietary CP from 12.5 to 15.5% with no effect on milk production. Van Horn et al. (1976) observed no significant effect on milk yield when dietary CP was raised from 11.5 to 14.5% although the difference was significant at $p < .1$. In the same study, there was no difference between dietary CP levels of 12 and 17%. Treacher et al. (1976) compared a low protein diet with a high protein diet and did not find any effect on milk yield. Clay and Satter (1979) reported that increasing dietary CP from 12.5 to 14.5% did not increase milk yield,

and Foldager and Huber (1979) found no difference between CP levels of 12.5 and 16.0%. Claypool et al. (1980) found no significant difference in milk yield when diets containing 12.7, 16.3 and 19.3% CP were compared although there was a non-significant difference of 3 kg milk/day between the 12.7 and 19.3% CP levels. Stokes et al. (1981) did not find a reponse in milk production when dietary CP level was raised from 11.5 to 14.2%. Crooker et al. (1982) found no difference between 12 and 14% dietary CP.

The majority of these studies suggest that raising dietary CP from below 13% to above 13% leads to an increase in milk production. This is in agreement with the suggestion that diets containing 12 to 13% CP are necessary to meet the N requirements of the rumen microbial population (Satter and Roffler 1975).

It appears that NPN can be utilized efficiently when dietary CP level is about 13% or less (Polan et al. 1976; Kwan et al. 1977; Wohlt and Clark 1978; Kung and Huber 1981). Increasing dietary CP above 13% using NPN seems to be of little value (Polan et al. 1976; Wohlt and Clark 1978; Murdock and Hodgson 1979; Foldager and Huber 1979; Kung and Huber 1981) although improvements in milk yield have been reported when urea was included in a diet containing 16.6% CP (Kwan et al. 1977). Urea may be utilized more efficiently when fed in a complete ration rather than once or twice daily in a concentrate (Huber and Kung 1981).

Supplementing plant protein to diets containing more than 13% CP has had variable results. Gardner and Park (1973) raised dietary CP from 13 to 15.5% and from 14 to 16% and observed a 10% increase in milk production in both instances. Sparrow et al. (1973) increased

milk yield by raising dietary CP level from 13.5 to 17.5%. Grieve et al. (1974) reported an increase in milk production when dietary CP was raised from 14 to 16% and there was a further increase on dietary CP from 16 to 18%. Van Horn et al. (1979) observed an increase in milk yield when cottonseed meal was used to raise dietary CP from 13.5 to 16.3%, and Edwards et al. (1980) found that 15% dietary CP was superior to 13% in terms of increased milk yield. Murdock et al. (1981) raised dietary CP from 13.5 to 15.7% with SBM and observed a large increase in milk yield. Barney et al. (1981) decreased dietary CP from 17 to 13% at three stages during early lactation and noted a linear decrease in milk production. Holter et al. (1982) increased milk yield in one experiment by raising dietary CP from 13.7 to 15.7%, however, in a similar experiment by the same authors there was no effect on milk production.

Results of Polan et al. (1976) and Van Horn et al. (1976) suggest a declining rate of increase in milk production in response to increases in dietary CP. Kwan et al. (1977) compared a 13.9% CP diet with urea, a 16.6% CP diet with urea and a 16.6% CP diet with plant protein only. There was no difference in production between the 13.9% CP diet and the 16.6% CP plant protein diet; however, milk yield was higher when the 16.6% CP urea diet was fed. Clay et al. (1978) found no difference between diets containing 13 and 16% dietary CP, and Murdock and Hodgson (1979) found no difference in milk yield when cows were fed 14.4% CP or 17.5% CP diets. Edwards et al. (1980) raised dietary CP from 15 to 17% with no effect on milk yield. Claypool et al. (1980) reported there was no difference between diets containing 16.3 and 19.3% CP, and Gordon (1980) found no difference between high

and medium protein diets. Folman et al. (1981) compared diets with 16 or 20% CP and did not find a difference in milk production. Murdock et al. (1981) found no increase in milk yield when dietary CP was raised from 15.7 to 18.4%. Kung and Huber (1981) reported that milk yield was not increased by raising dietary CP from 14 to 17%. Holter et al. (1982) increased dietary CP from 16 to 18% with no effect on milk yield, and Lundquist et al. (1982) found no difference between diets containing 15.5 and 18.0% CP.

Much of the variability in production response can be attributed to differences in productivity between cows from the various locations and to the wide range of diets used. The energy density of the diet is an important factor in the response of cows to increased dietary CP (Krohn and Andersen 1980; Macleod et al. 1982), particularly when NPN is included in the diet (Burroughs et al. 1975).

Some studies suggest that first lactation cows do not respond to increased dietary CP to the same extent as multiparous animals (Roffler et al. 1978; Cressmam et al. 1980). However, Macleod et al. (1982) reported that milk yields of 63 first lactation cows increased in a curvilinear manner with dietary CP levels of 12, 15, and 18% during early lactation.

In general, it appears that cows in early lactation will produce more milk when dietary CP is raised to 13 or 14%. However, increasing dietary CP above this level usually produces less of a response and often no response at all.

In most studies where milk yield has increased in response to dietary CP level, DM intake has also been increased. When DM intake was not increased, usually there was no effect on milk yield (Chandler

et al. 1976; Van Horn et al. 1976; Treacher et al. 1976; Clay et al. 1978; Foldager and Huber 1979; Claypool et al. 1980; Folman et al. 1981; Stokes et al. 1981). A large part of the response of lactating cows to increased dietary CP level may be mediated by an increased intake of energy (Van Horn and Zometa 1978). Wohlt et al. (1978) and Oldham et al. (1979) have reported an increase in OM digestibility in lactating cattle in response to increased dietary CP level.

Abomasal infusions of protein and amino acids

Until recently, it was believed that the quantity and quality of microbial protein reaching the small intestine was sufficient to meet the requirements even of high producing cows. However, results from experiments where proteins and/or amino acids were administered post-ruminally suggest this is not the case (Clark 1975). Studies conducted prior to 1975 which involved abomasal infusions of casein and amino acids, or intravenous infusions of amino acids have been thoroughly reviewed by Clark (1975). In general, post-ruminal infusions of casein increased milk production by 1 to 4 kg/day and milk protein by 10 to 15%. Cows producing more than 20 kg milk/day usually showed the greatest response. Infusions of amino acids intravenously or abomasally did not increase milk production to the same extent as casein infusion (Clark 1975).

In a subsequent study, Orskov et al. (1977) reported an increase in milk yield, milk fat concentration, and milk protein concentration in response to abomasal infusions of casein, but not glucose. Clark et al. (1977) also reported that abomasal infusion of casein, but not glucose, caused an increase in milk production and milk protein concentration. Using lactating goats, Ranawana and Kellaway (1977)

reported a linear increase in milk production in response to four levels of abomasally infused casein.

Clark (1975) has suggested that the response of lactating animals to abomasal infusion of casein may be mediated by an increased supply of glucose and/or amino acids to the mammary gland or could result from hormonal regulation by one or more amino acid. The results of Clark et al. (1977) and Orskov et al. (1977) suggest that increased glucose supply is not the predominant mechanism.

In reviewing many of the post-ruminal infusion studies, Clark (1975) was unable to conclude which amino acids were most limiting for milk production. Methionine and phenylalanine were cited frequently as limiting amino acids; however, lysine, threonine, tyrosine, histidine, glutamate, proline, valine, isoleucine, and leucine were also considered limiting by some authors. A subsequent report by Clark et al. (1977) suggested that the availability of methionine, phenylalanine and lysine limits milk synthesis. The difficulties of elucidating limiting amino acids for milk synthesis were discussed by Mepham (1982). He suggested three groups of amino acids could limit milk synthesis. Methionine, phenylalanine, tyrosine and tryptophan may be limiting because there is a stoichiometric relationship between uptake of these amino acids by the mammary gland and their output in milk. Lysine and threonine may also fall into this category. The other essential amino acids may limit milk synthesis because although their uptake by the mammary gland is generally in excess to their output in milk, they may be required for essential purposes other than providing residues for milk protein. Finally, the supply of non-essential amino acids may limit milk synthesis because intra-mammary synthesis may be inadequate. Mepham (1982) concluded that the availability

of methionine, phenylalanine and possibly lysine and threonine could limit milk synthesis. Clark (1975) suggested that addition of a single amino acid may give a small response in milk yield but a measurable response may not be detectable until the accumulative effect of several amino acids is added together, i.e., no single amino acid may clearly limit milk synthesis.

Rumen escape protein

Results of infusion studies suggest that the supply of microbial protein to the small intestine is inadequate to meet the requirements of high producing cattle (Clark 1975). Although abomasal infusion of casein effectively increases the level of protein reaching the duodenum, the technique can be used only for experimental purposes. If duodenal protein supply is to be increased on a practical basis, either the level of microbial protein must be increased or the amount of dietary protein escaping degradation in the rumen must be elevated. Increasing the supply of microbial protein is attractive because it could lead to increased use of inexpensive NPN (Smith 1979). However, the emphasis has been on increasing the amount of rumen escape protein (Chalupa 1975; Tamminga 1979). The principle is to feed proteins which are poorly degraded in the rumen but are efficiently digested in the duodenum. There should also be no reduction in microbial protein synthesis (Tamminga 1979). Degradable and undegradable proteins should be included in the diet, but a correct balance must be maintained (Clark and Davis 1980). If too much REP is included, there may be a deficiency of NH_3 for optimal microbial growth whereas an excess of RDP results in increased excretion of N in the urine (Clark and Davis 1980). Methods of manipulating the level of REP are discussed in a later section.

Digestion of nitrogenous compounds in the small intestine

Bacterial N entering the duodenum is comprised of 75 to 85% protein and 13 to 19% nucleic acids (Smith 1975). The limited information on the digestion of nucleic acids in the small intestine of ruminants has been reviewed by Smith (1979). It appears that nucleic acids are extensively degraded in the first quarter of the small intestine and 80 to 90% of the ribonucleic acid (RNA) and 75 to 85% of the deoxyribonucleic acid (DNA) are digested.

Estimates of total microbial N digestion in the small intestine of ruminants have been reviewed by Smith (1979) and most are in the range of 75 to 85%. The author suggests that the actual value may be lower because the estimates of microbial N digestibility included nucleic acid - N, which is highly digestible. Steinhour and Clark (1982) reported that the digestibility of bacterial and protozoal N fed to rats was 74 to 79% and 87 to 91%, respectively. The authors warned that estimates of the amount of N flowing to the small intestine which ignore the contribution of protozoal N may be seriously in error. Values for microbial protein digestibility currently being applied in various protein systems are 70% (Verite et al. 1979; Agricultural Research Council 1980), 80% (Satter and Roffler 1975) and 85% (Kaufmann 1977).

The digestibility of REP in the small intestine depends on the dietary source. For example, the intestinal digestibility of REP derived from raw soybeans appears to be less than that from SBM (Satter 1981). Similarly, the digestibility of REP from distillers dried grains is lower than that of wet brewers grains and corn gluten meal (Satter 1981). Chemical and physical processes, e.g.

formaldehyde or heat treatment, which protect protein from degradation in the rumen may also decrease intestinal digestibility if the protein is overprotected (Chalupa 1975).

Digestion of nitrogenous compounds in the small intestine appears to be fundamentally the same in ruminants and non-ruminants (Smith 1979). However, in the ruminant, a low pH extends further along the small intestine, and this may prolong the activity of abomasal pepsin but delay the onset of activity of pancreatic enzymes (Smith 1979). Pancreatic ribonuclease appears to be more abundant in ruminants than non-ruminants, reflecting the greater supply of nucleic acids (Barnard 1969).

The amino acid composition of microbial protein entering the small intestine appears to be fairly constant over a variety of dietary regimes (Bergen et al. 1968) although methionine may be reduced when animals are fed urea as the primary source of N (Salter et al. 1979). There are some differences between the amino acid compositions of bacterial and protozoal protein (Bergen et al. 1968) but the biological values of bacterial and protozoal protein appear to be the same (Purser 1970). A comparison of the amino acid composition of bacterial protein and milk protein suggests that bacterial protein supplies a reasonable balance of most essential amino acids. However, the supply of histidine, leucine and valine is disproportionately lower than the other essential amino acids (Kaufmann 1979). Compared to most dietary proteins, microbial protein is of good quality; it is high in lysine and is a good source of methionine (Satter 1981). The amino acid composition of REP depends on the nature of the diet. There is evidence that the amino acid profile of REP may differ from

that of the original dietary protein (Macgregor et al. 1978).

Methods of Manipulating REP

Selection of dietary ingredients

The amount of REP in the diet can be manipulated in a variety of ways. A simple method of increasing REP is to select feedstuffs which are naturally resistant to degradation in the rumen. Majdoub et al. (1978) observed a dramatic increase in milk yield by lowering N solubility of the diet, primarily by inclusion of corn meal. These researchers used N solubility as a measure of N degradability. Zook et al. (1981) also observed an increase in milk production when dietary N solubility was reduced, this time by replacing linseed meal with soybean meal. However, this response may not have been due to an increased supply of amino acids to the small intestine because linseed meal and SBM are both readily degradable in the rumen in spite of differences in N solubility (Sniffen 1982). Tamminga (1979) pointed out that when diets are formulated according to N solubility or degradability, other properties of the diet may change and this could affect animal performance. Grieve and Forster (1982) reported a large increase in milk yield when highly degradable SBM was replaced by less degradable corn gluten meal. Results obtained for growing lambs and cattle (Stock et al. 1981) suggest that inclusion of slowly degradable protein in the diet can increase the efficiency of N utilization.

Heat treatment

Heat treatment reduces the degradability of protein in the rumen (Chalmers et al. 1954). When heat is applied to a protein, carbonyl groups of sugars and, to a lesser extent, proteins bind with free amino groups (Bjarnason and Carpenter 1969; Bjarnason and Carpenter 1970). In this way, the protein becomes resistant to microbial attack. However, damage can occur if excess heat is applied, due to irreversible binding of amino acids with sugars or destruction of amino acids, particularly lysine (Chalupa 1975). Heat damage decreases the amino acid availability to the host (Chalupa 1975).

In studies with growing ruminants, heat treatment of protein supplements has often resulted in more efficient utilization of dietary N (Chalupa 1975). Although feeding heat treated protein supplements to dairy cows has sometimes resulted in increased milk production (Mielke and Schingoethe 1979; Netemeyer et al. 1982), often there has been no positive response (Ahrar and Schingoethe 1979; Mielke and Schingoethe 1981; Kung and Huber 1981; Block et al. 1981; Grummer and Clark 1982). The reasons for the apparent lack of response are not known. Grummer and Clark (1982) reported no decrease in apparent N digestibility and Block et al. (1981) reported an increase in plasma essential amino acids due to heat treatment, yet milk production was not increased in either experiment.

Increased digesta turnover rate in the rumen

Protein degradability in the rumen can be decreased by increasing the rate of passage through the rumen. Bull et al. (1979) reported marked reductions in degradability of cottonseed meal and corn gluten meal when digesta turnover rate in the rumen was increased from

9.1%/hour to 11.1%/hour, although digestibility of SBM was only slightly reduced. Factors which increase rate of passage through the rumen, e.g. reduced particle size (Hogan and Weston 1967), might increase the level of REP. However, reducing N degradability by increasing rate of passage may not be practical because cellulose digestion will also be decreased and less energy will be obtained from forages (Huber and Kung 1981).

Oesophageal groove reflex

Orskov and Benzie (1969) suggested that the oesophageal groove reflex could be exploited to provide protein postruminally. Recently, Huber et al. (1982) administered whole milk via the oesophageal groove to first lactation cows which had been trained to suckle. Milk yield was not significantly affected but milk protein yield was increased by 10%.

Whole blood

The application of whole blood to SBM reduces protein degradability in the rumen (Mir et al. 1982a). Feeding the treated SBM did not increase milk yield in dairy cattle (Mir et al. 1982b), however, N retention in calves was increased even though overall DM digestibility was decreased (Mir et al. 1982c).

Monensin

Monensin appears to have a protein sparing effect which has been

attributed to an increase in REP (Poos et al. 1979; Chalupa et al. 1980). Whetstone et al. (1981) reported that in vitro protein degradation decreased linearly as the amount of monesin added to the medium increased. It is possible that the growth response observed in cattle fed monensin may not be due entirely to the effect on VFA production in the rumen. The increased growth rate may be partly due to increased amounts of protein reaching the small intestine (Poos et al. 1979; Hanson and Klopfenstein 1979). Since monensin decreases the ratio of acetate to propionate in the rumen, there is an unwillingness to feed it to dairy cattle because low acetate/propionate ratios generally reduce milk fat concentration (Oldham and Sutton 1979).

Chemical treatment

A variety of chemical treatments have been used to reduce protein degradability in the rumen. These chemical agents form reversible cross-linkages with proteins which make the protein less susceptible to microbial degradation. The cross-linkages are broken in the abomasum due to the low pH. The protein can then be digested by the proteolytic enzymes of the host (Chalupa 1975). Treatment with phosphonitrilic halides, polymerized unsaturated carboxylic acids, halo-triazines, sulphonyl halides, acrolein acetals, hexamethylene-tetramine and acetylenic esters reduces protein degradability (Chalupa 1975).

Friedman et al. (1982) reported that treatment with dimethylurea effectively reduces the degradability of proteins in the rumen, but

experiments to measure animal performance have not been reported. Treating SBM with NaOH solution decreases protein degradability (Mir et al. 1982a). Feeding NaOH treated SBM has resulted in increased milk production in dairy cattle (Sidhu and Ashes 1977; Mir et al. 1982b).

Addition of tannins to protein supplements reduces protein degradability in the rumen and has been reported to increase the efficiency of protein utilization in lambs (Driedger and Hatfield 1972). However, steers fed tannin treated SBM did not show a positive response (Nishimuta et al. 1974).

Several aldehydes have been used to protect protein against ruminal degradation (Peter et al. 1971). Increased daily gains have been reported for lambs fed glyoxal treated SBM (Peter et al. 1971). Mangan et al. (1980) suggested that gluteraldehyde may be useful for reducing the protein degradability of fresh herbage. However, formaldehyde has received particular attention (Ferguson et al. 1967).

Formaldehyde treatment

Barry (1976) discussed the chemistry of FA-protein complexes. The initial reaction is the rapid formation of a methylol compound:

$$R\text{-}XH + HCHO \rightleftarrows R\text{-}X\text{-}CH_2OH.$$

The -XH can be a terminal amino group, the ε-amino group of lysine, the primary amide groups of asparagine and glutamine, 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 the imadazole group of histidine. Following the formation of the methylol compounds, condensation reacions occur slowly over a period of time and stable methylene cross-linkages are formed between protein chains:

$$R\text{-}X\text{-}CH_2OH + R^1\text{-}NH_2 \rightleftarrows R\text{-}X\text{-}CH_2\text{-}NH\text{-}R^1 + H_2O$$

$$R\text{-}XH + R^1\text{-}NH\text{-}CH_2OH \rightleftarrows R\text{-}X\text{-}CH_2\text{-}NH\text{-}R^1 + H_2O$$

These reactions of formaldehyde with amino acids are dependent on pH and temperature. At pH 7.0 and room temperature, the principle reactions are thought to involve the terminal amino groups, the primary amide groups of asparagine and glutamine, the ε-amino group of lysine and the guanidyl group of arginine (Barry 1976). Reports that tyrosine can be extensively modified (Reis and Tunks 1973; Sidhu and Ashes 1977) suggest that the phenol group of tyrosine is actively involved. The methylene linkages are hydrolysed in acid conditions such as those found in the abomasum, allowing the protein to be digested by the host's proteolytic enzymes (Barry 1976). In this way, the supply of amino acids to the small intestine can be increased.

Mills et al. (1972) studied the metabolism of ingested FA. In a series of experiments, sheep and goats were fed C^{14}-FA as an aldehyde-casein-safflower oil complex. Approximately 60 to 80% of the label was found in expired air. The lower level (60%) was found when the FA treated supplement had been stored for a longer period of time before feeding. Eleven to 27% of the label was found in faeces and the higher level (27%) in faeces corresponded to the lower level in the expired air, suggesting that FA may form irreversible links with protein over a period of time. The remainder of the label was found in urine (5%), carcass (5%), and the alimentary tract (5%). The C^{14} label in expired air was present as CH_4 and CO_2 in the approximate ratio $1CH_4 : 2CO_2$. The authors suggested that some CO_2 and most CH_4 originated from metabolism of FA in the rumen. The remainder of the labelled CO_2 was, presumably, derived from tissue metabolism. Most of the label in the digestive tract was found in gut contents, particularly in the rumen and caecum. Very little C^{14} found in tissues was present as FA. Sheep fed the FA treated diets for 18 months had the same level of FA in tissues, as determined by a colorimetric assay, as sheep fed an untreated control diet. Feeding the FA treated diets to lactating goats for 6 months did not increase the levels of FA in milk. The authors concluded that feeding FA treated proteins does not result in accumulation of FA in tissues or milk.

Formaldehyde has been used to treat casein (Fergusen et al. 1967), whey proteins (Rodriguez et al. 1975), SBM (Peter et al. 1971), peanut meal (Faichney 1972), meat meal (Rattray and Joyce 1970),

linseed meal (Rattray and Joyce 1970), RSM (Sharma et al. 1972), sunflower meal (Amos et al. 1974), dried forage (Hemsley et al. 1970), fresh forage (Mangan et al. 1980) and silage (Brown and Valentine 1972). The protein-FA matrix has also been exploited to protect dietary lipids from ruminal degradation (Scott et al. 1969).

It is clear that treating protein supplements with FA can increase the level of amino acids reaching the small intestine (Faichney and White 1979). However, there is no consensus regarding the optimal level of FA application. The optimal level of FA application is defined in the present discussion as the amount of FA which reduces protein digestibility in the rumen to a desired level without adversely affecting amino acid availability in the small intestine. This ideal level of FA application may be difficult to achieve in practice. A wide range of FA application rates have been used to protect proteins, e.g. Reis and Tunks (1969) treated casein with 40g FA/100g casein whereas Spears et al. (1980) treated SBM with 0.3g FA/100g CP. Broderick (1975) suggested that optimum levels for the application of FA are 0.8 to 1.2g FA/100g protein for casein, 2.0g FA/100g CP for oilseed meals and 3.0g FA/100g CP for silages. Barry (1976) suggested that the concentration of FA to be used should be based on the level of degradable true protein in the feedstuff, rather than the total CP content. He proposed that 3.0 to 5.2g FA/100g degradable true protein offers adequate protection.

There is evidence that the method of FA application can affect the degree of protection. Apparently, FA administered as a concentrated solution is more effective than the same amount of FA administered as a dilute solution (Peter et al. 1971; Wachira et al.

1974). The length of time after treatment also affects the degree of protection. Mills et al. (1972) reported the degree of protection of FA treated casein-safflower oil was greater 40 days after treatment than after 10 days. However, Caja et al. (1977) showed that the degree of protection of FA treated SBM and sunflower meal started to decrease 2 months after treatment.

With the exception of SBM, little comparative information is available concerning the optimal level of FA for protecting oilseed meals. There is evidence that SBM protein can be adequately protected with levels of FA lower than those suggested by Broderick (1975). Peter et al. (1971) reported that maximum depression of in vitro NH_3 production in viable rumen fluid was approached when SBM was treated with 1.3g FA/100g CP, although there was also a significant reduction in NH_3 production with 0.5 to 0.6g FA/100g CP. Feeding SBM treated with 1.3g FA/100g CP improved liveweight gain and feed efficiency in lambs (Peter et al. 1971). Schmidt et al. (1973) treated SBM with 0.4, 0.8, 1.2 and 1.6g FA/100g CP and fed the treated meals to rats. Treatment with 0.4g FA/100g CP did not adversely affect animal performance; however, the higher levels of FA reduced N retention and growth rate. All FA treatments markedly reduced in vitro NH_3 production. In a similar study, Spears et al. (1980) treated SBM with 0.3, 0.6 and 0.9g FA/100g CP. All three treatments reduced NH_3 production during in vitro incubation of the treated SBM in viable rumen fluid. When fed to chicks, growth rates and feed efficiencies were not affected by the 0.3g FA/100g CP treatment, but the higher levels of FA depressed chick performance. When growing steers were fed the treated diets, higher daily gains were reported for all three

levels of FA application. Spears et al. (1980) concluded that application of 0.3g FA/100g CP adequately protects SBM protein against ruminal degradation without decreasing protein digestibility in the small intestine. However, when SBM treated with 0.3g FA/100g CP was fed to lactating cattle, apparent N digestibility was reduced (Crooker et al. 1982). On the other hand, Kaufmann and Lupping (1979) observed an increase in milk yield when FA treated SBM (0.34g FA/100g CP) was fed to dairy cows.

Apparently, in the case of SBM, application of 0.3 to 0.4g FA/100g CP reduces protein degradability in the rumen without decreasing protein digestibility in the small intestine. However, the results of Crooker et al. (1982) suggest that intestinal digestibility may be reduced even at these low levels of FA application.

Several studies have been conducted using FA treated RSM. Nicholson et al. (1972) treated RSM with approximately 50g FA/100g CP and fed the treated material to sheep. The apparent digestibilities of DM and N were reduced, but the amount of protein reaching the duodenum was increased and N retention was doubled. Sharma et al. (1972) treated RSM with 5.4, 10.8, 16.2 and 21.4g FA/100g CP. All treatments markedly reduced the solubility of RSM-N in 0.01N NaOH solution. There was no effect on daily gain or feed efficiency when RSM treated with 5.4g FA/100g CP was feed to steers; however, FA treatment caused a decrease in apparent DM and N digestibility and a decrease in urinary N excretion. Sharma and Ingalls (1973) fed FA treated RSM (0.7g FA/100g CP) to Holstein calves. Although apparent DM and N digestibilities were not significantly affected, they tended to be lowered by FA treatment. Treatment with FA had no effect on

liveweight gain or feed efficiency. Sharma and Ingalls (1974) reported that treating RSM with 0.7g FA/100g CP did not affect apparent DM or N digestibilities or N retention. There was also no effect on the quantities of amino acids leaving the rumen. Sharma et al. (1974) found that feeding RSM treated with 0.7g FA/100g CP tended to reduce the digestibilities of DM and N in the small intestine of Holstein steers and did not affect the level of amino acids flowing through the abomasum. Sharma and Nicholson (1975) treated RSM with 1g FA/100g CP and fed the treated material to sheep. There was no effect on the digestibilities of DM or N but N retention tended to increase. In addition, treatment with FA increased the flow of DM, total N and true protein N to the abomasum. Kowalczyk et al. (1982) fed FA treated RSM (approximately 5.5g FA/100g CP) to young bulls. The apparent digestion of N and DM in the small intestine was reduced by FA treatment.

From the above experiments, it appears that treating RSM with 0.7g FA/100g CP does not significantly increase the flow of amino acids to the intestine but at higher levels of FA application, overall DM and N digestibilities are reduced. Kowalczyk et al. (1982) concluded, "...procedures still have to be defined for the treatment of rapeseed meal with formaldehyde so as to minimize the degradability of its protein in the rumen, yet prevent depression of its digestion and absorption in the stomach and small intestine."

Numerous studies have been conducted to determine the response of sheep and growing cattle to FA treated protein supplements (Chalupa 1975; Fergusen 1975; Barry 1976). Administration of FA treated casein has often resulted in improved wool growth in sheep and increased N

retention and liveweight gains in sheep and cattle. However, the responses to FA treated plant proteins have been very inconsistent (Chalupa 1975). The best response to FA treated protein has been observed when FA treated casein was fed to sheep. Often, wool growth was increased (Fergusen 1975). The response of wool growth to FA treatment is thought to be due to increased absorption of methionine, the limiting amino acid for wool growth (Fergusen 1975). The more variable response of liveweight gains of sheep and cattle to FA treated protein supplements may reflect the relatively less important role of methionine in muscle deposition compared to wool growth (Tamminga 1979).

Several studies have been conducted to determine the response of lactating dairy cows to FA treated feedstuffs. Valentine and Radcliffe (1975) fed silage treated with 0.6 or 1.2g FA/100g CP to lactating cattle and noted an increase in milk production. There was also an increased DM intake in response to FA treatment. Tayler et al. (1979) treated grass silage with a mixture of formic acid and FA. Feeding the treated silage to dairy cows had no effect on milk yield or DM intake. However, Thomas et al. (1981) reported that treatment of grass silage with a solution of FA/formic acid caused an increase in DM intake and tended to increase milk yield. It is possible that treating silages with FA results in an increased milk production by increasing the DM and energy intake of cows (Thomas et al. 1981).

The majority of studies with lactating dairy cows have involved FA treated protein supplements or concentrates, and most studies have been conducted in mid-lactation when milk production had passed its peak. Wilson et al. (1970) fed FA treated casein and found no effect

on milk yield. Satter et al. (1970) did not observe any improvement in milk yield when SBM was treated with FA. Clark et al. (1974) fed SBM treated with 1.8g FA/100g CP to lactating cows and reported no increase in milk yield. Wachira et al. (1974) conducted two experiments with FA treated protein supplements. In the first experiment, treating SBM with 1.6g FA/100g CP did not increase milk yield. In the second experiment, treatment of a concentrate mix with 0.5g FA/100g CP also failed to increase milk production. Kellaway et al. (1974) conducted two feeding trials with dairy cows. In one experiment, feeding FA treated casein (1g FA/100g casein) increased milk yield but in the other experiment, feeding FA treated casein had no effect on milk yield. Muller et al. (1975) treated whey protein with 1g FA/100g CP. When the treated protein was fed to lactating cattle, 4% fat corrected milk (FCM) yield increased by 3.2 kg/day. However, this increase was not statistically significant. Verite and Journet (1977) treated a mixture of RSM and SBM with 0.6g FA/100g CP. When the treated supplement was fed to dairy cows in mid-lactation, there was a significant increase in milk yield. Broderick and Lane (1978) fed FA treated casein (0.8g FA/100g casein) to cattle in early-mid lactation. Cows fed the treated casein did not produce more milk than cows fed untreated casein; however, cows fed treated casein produced more milk than those fed no casein at all. Stobbs et al. (1977) fed casein treated with 1g FA/100g casein to pasture fed Jerseys in mid-lactation and observed an increase in milk yield. Flores et al. (1979) conducted a similar experiment and also reported increased milk production in response to feeding FA treated casein. Rogers et al. (1980) fed FA treated casein (1g FA/100g CP) to

Jersey-Friesian crossbred cows in early lactation and observed an increase in milk yield. Kenna and Schwab (1981) fed FA treated casein (0.8g FA/100g casein) to cattle in early-mid lactation and noted an increase in milk yield compared to cows fed no casein.

These studies were conducted with cows which were in mid-lactation or, in the case of Rogers et al. (1980), were low producers. In three of the studies (Stobbs et al. 1977; Flores et al. 1979; Rogers et al. 1980), Jerseys or Jersey-Friesian crossbreeds were used and a positive response to FA treated casein was observed each time. However, it is not possible to tell from these three experiments if Jerseys are more responsive to protected protein than Holsteins. In general, supplementing FA treated casein to the diet increased milk production, but with the exception of the study by Verite and Journet (1977), treatment of plant proteins with FA has not caused an increase in milk production during mid-lactation.

Some studies have been conducted in early lactation with high producing Holsteins and Friesians when the demand for dietary protein is greatest. Verite and Journet (1977) conducted two trials during early lactation using a mixture of RSM/SBM treated with 0.6g FA/100g CP. In one trial there was no significant effect on milk production but in the other, FA treatment increased milk yield by 7%. Kaufmann and Lupping (1979) fed FA treated SBM (0.34g FA/100g CP) to lactating cows and reported an increase in milk yield. Folman et al. (1981) found no significant difference in milk yield between cows fed untreated SBM or SBM treated with 0.34g FA/100g CP. Hunter et al. (1981) compared a high protein diet containing untreated SBM to a low protein diet containing FA treated SBM and found that cows fed the FA

treated SBM produced less milk. However, the authors suggested that the level of REP in the FA treated diet may have been insufficient. Crooker et al. (1982) compared untreated and FA treated SBM (0.3g FA/100g CP) and did not observe any difference in milk production between cows fed the two supplements. A similar study by Lundquist et al. (1982) found that cows fed FA treated SBM (0.3g FA/100g CP) produced more milk than cows fed a low protein control diet. However, there was no significant difference in milk production between cows fed treated or untreated SBM.

Of the studies conducted in early lactation, only Verite and Journet (1977) and Kaufmann and Lupping (1979) reported a significant increase in milk production in response to FA treatment. Although Folman et al. (1981) and Lundquist et al. (1982) reported non-significant increases of 1.5 and 1.4kg milk/day, respectively.

The fact that cows generally produce more milk in response to supplemental FA-treatedcasein suggests that, in principle, FA-treated plant proteins should also elicit a response. Several reasons have been suggested for the lack of response in milk production observed by many researchers when FA treated plant proteins were fed to lactating cows.

(a) The experimental periods may have been too short in some experiments (Tamminga 1979). The experiments of Wachira et al. (1974) and Muller et al. (1975) had experimental periods of only 12 days duration and no significant effect on milk yield was observed. However, positive responses have been reported in experiments with 14-day periods (Stobbs et al. 1977; Flores et al 1979; Rogers et al. 1980). In addition, Folman et al. (1981) and Crooker et al. (1982)

did not observe an effect on milk yield when FA treated SBM was fed for 160 and 273 days, respectively. Therefore, the lack of response observed by some researchers was not due solely to the short experimental periods.

(b) The protected protein may have been naturally resistant to ruminal degradation (Wachira et al. 1974). In the experiments discussed above, however, the plant protein supplements were RSM and SBM, both of which are readily degraded in the rumen. Thus, it is unlikely this accounts for the lack of response.

(c) The supply of amino acids to the duodenum may already have been adequate under the conditions of the experiment (Clark et al. 1974; Wachira et al. 1974; Broderick and Lane 1979; Tamminga 1979). In these circumstances, factors other than amino acid availability would limit milk production.

(d) The diet may not have provided sufficient RDP for optimal microbial activity (Tamminga 1979). If a diet is low in CP or if a high proportion of the protein intake of the cow is FA treated, there may be an inadequate supply of RDP to meet the NH_3-N requirements of the microbial population (Tamminga 1979). Since there is still considerable doubt about the "optimal" level of rumen NH_3-N, as discussed in a previous section, this possibility is difficult to assess.

(e) Underprotection of the protein supplement will result in little or no additional protein reaching the small intestine (Wachira et al. 1974). However, the concentrations of FA used in the above experiments are considered adequate to protect the protein against ruminal degradation, e.g. the low level of 0.34g FA/100g CP used by

Folman et al. (1981) provided enough protection to significantly reduce rumen NH_3-N levels. In addition, Spears et al. (1980) reported that treatment of SBM with only 0.3g FA/100g CP significantly reduced protein degradability in the rumen. In most of the studies with dairy cattle, the concentration of FA was 0.3 to 1.8g FA/100g CP so it does not seem likely that underprotection would occur unless the material was not thoroughly mixed during treatment.

(f) Overprotection of the protein supplement will reduce the digestibility of protein in the small intestine, and this may compensate for any benefit derived from increasing the amount of REP (Clark et al. 1974; Wachira et al. 1974; Chalupa 1975; Tamminga 1979). Decreased digestibility of FA treated protein supplements has been reported in vitro (Wachira et al. 1974), and in vivo in sheep (Wachira et al. 1974), steers (Sharma et al. 1972) and lactating cattle (Clark et al. 1974). Several authors suggest that decreased digestibility of FA treated protein in the small intestine may be responsible for the lack of response in milk production in their experiments (Clark et al. 1974; Wachira et al. 1974; Crooker et al. 1982).

(g) The treated protein may have a poor amino acid balance and a low biological value for milk production (Clark et al. 1974; Wachira et al. 1974). The treated protein may have a poor amino acid profile to begin with. However, it has also been suggested that FA treatmant may decrease the availability of specific amino acids, particularly tyrosine (Sidhu and Ashes 1977), and this may account for the lack of response observed by many researchers. The availability of lysine and cysteine may also be reduced by FA treatment (Reis and Tunks 1973).

In principle, feeding FA treated plant proteins to lactating cows

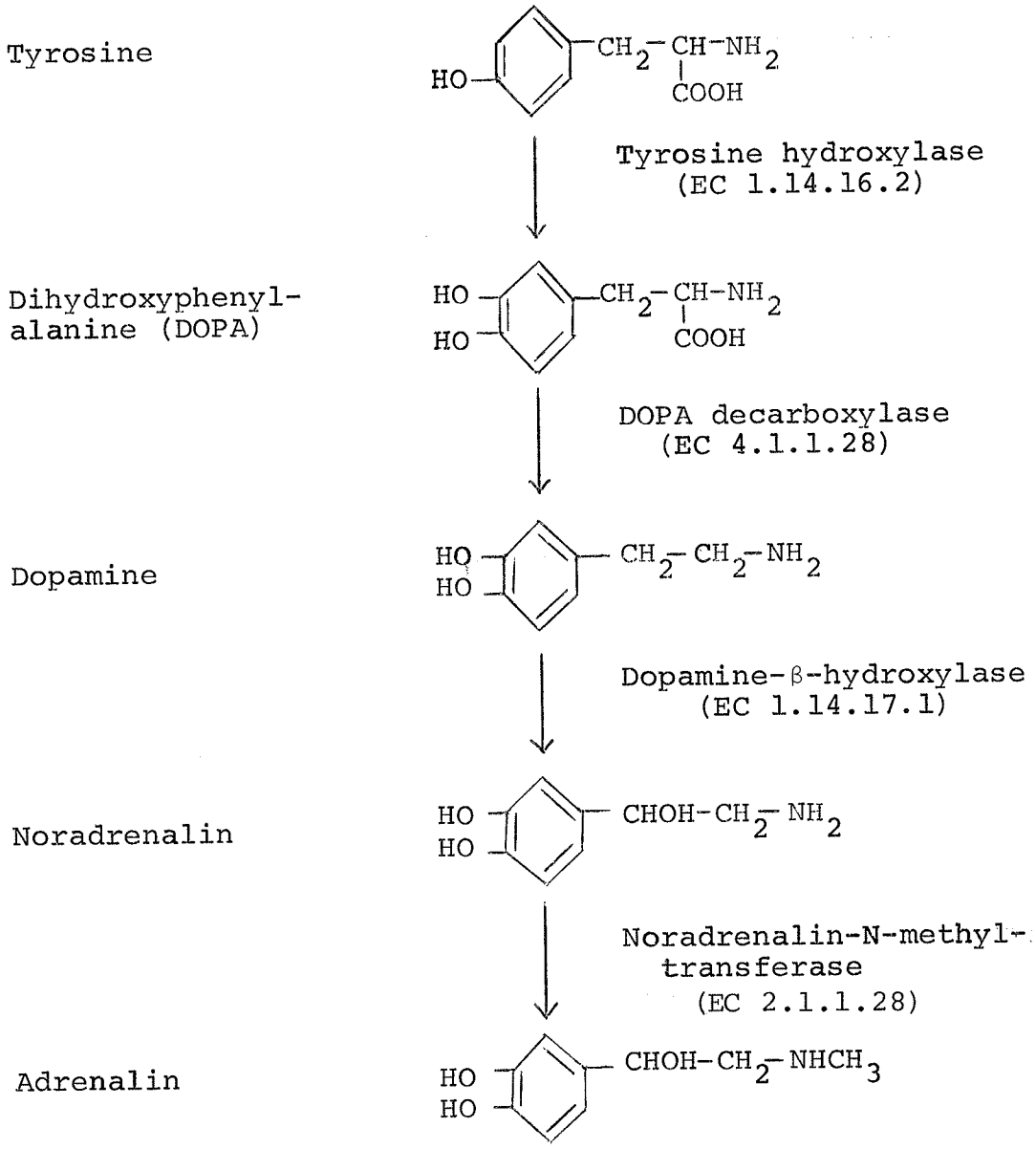
should increase the supply of amino acids to the small intestine and, ultimately, increase milk yield. In practice, however, responses to FA treated plant proteins have generally been poor, even during early lactation. Reasons for the poor response are not known.

Effect of Tyrosine on Brain Catecholamine Synthesis and Possible Implications for Milk Production

Sidhu and Ashes (1977) suggested that the lack of response when dairy cows are fed FA treated SBM may be due to destruction of SBM-tyrosine by FA. Mepham (1982) proposed that the supply of tyrosine, phenylalanine, methionine and tryptophan to the mammary gland may limit milk synthesis. Therefore, a tyrosine deficiency resulting from feeding FA treated SBM (Sidhu and Ashes 1977) may prevent an increase in milk production because of an inadequate supply of tyrosine to the mammary gland.

However, a tyrosine deficiency could, conceivably, affect the hormonal regulation of milk synthesis. Tyrosine appears to have an important role in neurohormonal regulation due to its role as precursor of dopamine, adrenalin and noradrenalin (Wurtman 1982). The latter three compounds are collectively known as the catecholamines. The catecholamines are synthesized in sympathetic neurons and in chromaffin cells of the adrenal medulla (Bentley 1980). The pathway of catecholamine synthesis was first described by Blaschko (1939) and is outlined in Figure 1. Tyrosine hydroxylase (EC 1.14.16.2) has been considered the rate limiting enzyme for catecholamine synthesis

Figure 1. Biosynthesis of dopamine, noradrenalin and adrenalin from tyrosine [from Bentley (1980)]



(Bentley 1980). However, it appears that tyrosine hydroxylase in the brain is not saturated at physiological levels of tyrosine and the conversion of tyrosine to L-dihydroxyphenylalanine (DOPA) is regulated in part by substrate availability. That is, catecholamine synthesis in brain neurons is dependent on the concentration of brain tyrosine.

Tyrosine crosses the blood-brain barrier by means of a carrier system. This is the same carrier system used to transport the other large, neutral amino acids (LNAA), namely phenylalanine, tryptophan, leucine, isoleucine and valine (Wurtman 1982). These amino acids compete with one another for sites on the carrier molecules. Therefore, if the plasma concentration of tyrosine increases in relation to the other LNAA, more tyrosine will be taken up by the brain. This causes an increase in brain catecholamine synthesis (Wurtman 1982). Anderson (1979) has speculated that the ratio of plasma tyrosine: phenylalanine may be particularly important in determining the rate of tyrosine uptake by the brain because of the close structural relationship between these two molecules. One could speculate that the ratio of plasma tyrosine: phenylalanine is unimportant because phenylalanine is the precursor of tyrosine so it should not matter which amino acid is taken up by the brain. Gibson et al. (1982) reported that intraperitoneal injection of phenylalanine caused an increase in brain tyrosine levels but there was a decrease in the levels of dopamine and noradrenalin. Phenylalanine is an inhibitor of tyrosine hydroxylase and this may explain the decrease in noradrenalin and dopamine concentrations (Gibson et al. 1982). Therefore, the ratio of plasma tyrosine: phenylalanine appears to be important in determining the rate of catecholamine synthesis in the

brain.

Administration of tyrosine to rodents has produced several physiological responses which can be linked with catecholamine action. Gibson et al. (1982) injected tyrosine intraperitoneally in mice and noted an increase in behavioural activity which was similar to the response of mice to administration of amphetamine. The authors observed an increase in brain tyrosine concentration but did not detect an increase in the concentrations of dopamine and nonadrenalin in the brain. Adrenalin was not measured. The authors speculated that an increased turnover of catecholamines, i.e., an increased synthesis and degradation, may have prevented a detectable rise in brain catecholamine levels (Gibson et al. 1982).

Wurtman (1982) reported that administration of tyrosine to hypotensive rats increased blood pressure whereas tyrosine decreased blood pressure in hypertensive rats. This apparent contradiction was explained as follows. Tyrosine administration increased the synthesis of catecholamines in sympathetic neurons of the brain. However, it is only those cells which are actually firing which can respond to the additional catecholamines. That is, the additional catecholamines, particularly noradrenalin, within the cell do not stimulate the cell to fire but the additional noradrenalin provides an extra supply of neurotransmitter to sympathetic cells which are already firing. This allows the rate of firing to increase so that the signal is amplified. Sympathetic cells which are not firing will not respond to an increased supply of noradrenalin because they have no need for the neurotransmitter. In the case of the hypertensive rats, the cells which are normally firing are the noradrenalin-releasing neurons in

the brain stem. Noradrenalin released from these cells suppresses the activity of neurons in the brain stem. The result is a decreased activity of sympathetic neurons in the peripheral nervous system and chromaffin cells of the adrenal medulla. Ultimately, the release of adrenalin and noradrenalin from the peripheral nervous system and adrenal medulla is decreased, vasodilation occurs and blood pressure is reduced. In this case, a small, local increase of noradrenalin in the brain appears to have lowered the release of adrenalin and noradrenalin in the body as a whole. Wurtman (1982) explained the response of hypotensive rats to tyrosine administration in a similar manner. In this situation, the noradrenalin-releasing cells of the brain stem are not firing because the rat is trying to increase blood pressure. Therefore, these cells do not respond to increased levels of noradrenalin. However, the sympathetic neurons in the peripheral nervous system and adrenal medulla are active as the rat attempts to stimulate the release of adrenalin and noradrenalin. The increased level of noradrenalin in the brain, caused by tyrosine administration, accelerates the firing of sympathetic neurons in the peripheral nervous system and adrenal medulla so that adrenalin and noradrenalin increase is stimulated, vasoconstriction occurs and blood pressure is increased. Therefore, it appears that tyrosine is involved in regulating blood flow in rats.

A third response of rodents to administration of tyrosine is an increased energy intake. Anderson (1979) reported that the energy intake of rats was increased in response to an increase in the plasma level of tyrosine in relation to the other LNAA, especially phenylalanine. Increasing the plasma ratio of tyrosine:

phenylalanine from approximately 1.0 to 1.6 caused energy intake to increase by an average of 30% in four experiments. The author (Anderson 1979) suggested that the response was mediated by catecholamines, possibly by the action of noradrenalin and/or adrenalin in the ventromedial and lateral regions of the hypothalamus.

Finally, there is convincing evidence that catecholamines, particularly noradrenalin, influence GH secretion in a variety of species (Martin 1980; Merimee 1979). In many species, GH is released episodically, i.e. in surges. Episodic release of GH is particularly prominent in the rat so this animal has been the subject of particular attention (Martin 1980). The episodic release of GH is dependent on stimuli from the ventromedial nucleus of the hypothalamus (Martin et al. 1974) and these stimuli appear to be due to the action of catecholamines. Administration of α-methyl-tyrosine, an inhibitor of tyrosine hydroxylase, depletes noradrenalin and dopamine in the hypothalamus and totally abolishes episodic GH secretion in the rat (Eden et al. 1979). It is thought that the action of catecholamines on episodic GH release is primarily due to α-adrenergic stimulation by noradrenalin or other α-adrenergic neurotransmitters. Therefore, it is conceivable that tyrosine induced synthesis of noradrenalin in the hypothalamus could stimulate the release of GH.

If tyrosine availability regulates brain catecholamine synthesis in cattle, there are several ways in which an increased plasma tyrosine concentration could potentially affect milk production in cattle. Firstly, animal behaviour may be altered to the point where milk production is affected. Secondly, DM intake may be increased. Thirdly, blood flow to the mammary gland may be affected. Fourthly,

GH release may be stimulated with a resultant increase in milk production (Machlin 1973).

PART I

The Nutritive Value of Formaldehyde Treated Canola Meal for Holstein Cows During Early Lactation

INTRODUCTION

Canola meal, barley, and corn silage contain a relatively low proportion of REP (Fisher 1981). Therefore, rations based on these ingredients may not provide sufficient quantities of protein to the small intestine to support high levels of milk production. The object of Part I of the study was to determine the effect of decreasing the ruminal degradability of canola-N on the performance of Holstein cows during early lactation. Experiment 1 was conducted to determine a suitable procedure for reducing N solubility and degradability of canola-N. Three possibilities were investigated. These were (a) treatment of CM with FA, (b) treatment of CM with heat, and (c) selection of canola products that may be less degradable than CM, namely unextracted canola seed (CS) and partially extracted canola seed (PCS). Results from experiment 1 were used as a basis for formulating diets of differing N degradabilities for a feeding trial with Holstein cows during early lactation (experiment 2).

MATERIALS AND METHODS

Experiment 1. The Effect of Heat and Formaldehyde Treatments on Nitrogen Solubility and Degradability of Canola Products.

Canola products

Samples of Tower CS, Tower PCS, and Tower CM were obtained from the production line at CSP Foods Ltd., Altona, Manitoba. All samples

were taken from the same consignment of Tower CS being processed at the time of sampling. The whole CS were broken by repeatedly grinding through a 2mm screen in a Wiley hammer mill until whole seeds could no longer be detected. Henceforth, the abbreviation CS refers to ground seeds. The nutrient compositions of the canola products are shown in table 1.

Experimental procedure for determining N solubility of feed samples in two solvents

The procedure was modified from the method of Wohlt et al. (1973). The two solvents were (a) 0.15N NaCl solution adjusted to pH 6.5 with 85% orthophosphoric acid, and (b) Burroughs mineral mixture (BMM) (Burroughs et al. 1950) diluted to 10% with distilled water. Feed samples containing 30mg N were added to 100ml solvent in 125ml Erlenmeyer flasks. The flasks were stoppered, placed in a Controlled Environment Incubator Shaker (New Brunswick Scientific Company, Edison, New Jersey) and incubated at 39C with constant shaking at 380 rpm for a specified period of time. After the incubation, the flask contents were filtered through Whatman #4 filter paper held in a Buchner funnel. The N content of 90ml aliquots of filtrate was determined by the macro-Kjeldahl procedure. The soluble N content was expressed as a percentage of total feed N.

Effect of incubation time on N solubility of CM and SBM in NaCl solution

Samples of CM and commercial SBM were ground through a 1mm screen in a Wiley hammer mill. Nitrogen solubility of the ground material

Table 1. Nutrient composition of crushed canola seeds (CS), partially extracted canola meal (PCS) and fully extracted canola meal (CM) (experiment 1)

Nutrient composition by analysis (% dry matter)	Canola Products		
	CS	PCS	CM
Crude protein	23.3	34.7	40.6
Ether extract	35.7	17.5	2.6
Ash	4.2	6.1	6.9

was determined by incubating in NaCl solution for 1h, 2h, or 3h. The completely randomised design was arranged as a 2x3 factorial with two feedstuffs, three incubation times, and two replicates per treatment combination. Pairwise comparisons were made using Duncan's multiple range test (Duncan 1955).

Effect of particle size and incubation time on N solubility of CM and SBM in NaCl solution

Samples of CM and SBM were ground through 0.5mm and 1mm screens in a Wiley hammer mill. Nitrogen solubility of the ground material was determined by incubating in NaCl solution for 1h or 2h in the case of CM and 1h, 2h, 3h, or 4h in the case of SBM. Separate statistical analyses were made for CM and SBM. The CM data were analysed in a completely randomised design arranged as a 2x2 factorial with two particle sizes, two incubation times, and two replicates per treatment combination. The SBM data were analyzed in a 2x4 factorial arrangement with two particle sizes, four incubation times, and two replicates per treatment combination. Pairwise comparisons were made using Duncan's multiple range test (Duncan 1955).

Effect of various FA treatments on N solubility of CM in NaCl solution

The procedure for treating CM with FA was modified from the method of Sharma et al. (1972). Solutions containing 0, 0.08, 0.4, 0.8, 1.6 and 3.2% FA were prepared from formalin (37% FA) and distilled water. Aliquots containing 50ml of these solutions were added to 100g samples of CM in glass jars and mixed thoroughly. The rates of FA application were 0, 0.1, 0.5, 1.0 and 2.0g FA/100g CP.

The glass containers were sealed and the CM was left to react with FA overnight at room temperature. The treated material was then removed from the jars, dried at 60C in a forced air oven and ground through a 1mm screen in a Wiley hammer mill. Nitrogen solubility of the treated material was determined by incubating in NaCl solution for 2h. A completely randomised design was used with two replicates per treatment. Pairwise comparisons were made using Duncan's multiple range test (Duncan 1955).

Effect of various heat treatments on N solubility of CM in NaCl solution

Samples of approximately 500g CM were thinly spread on metal trays and autoclaved at 121C for 0, 5, 10, 20, 30, or 60 min. The treated material was dried at 60C in a forced air oven and ground through a 1mm screen in a Wiley hammer mill. Nitrogen solubility of the treated material was determined by incubating in NaCl solution for 2h. A completely randomised design was used with two replicates per treatment. Pairwise comparisons were made using Duncan's multiple range test (Duncan 1955).

Effect of FA and heat treatments on N solubility of canola products in 10% BMM or NaCl solution

The canola products were CS, PCS, and CM. Treatment with FA was achieved by the method described previously. Fifty ml aliquots of 0.95, 0.85, or 0.55% FA solutions were added to 100g samples of CM, PCS, and CS, respectively. This corresponded to an application rate of 1.2g FA/100g CP. Heat treatment was achieved by autoclaving the CM

at 121C for 5 min, as described previously. Treated and untreated CM and PCS were ground through a 1mm screen in a Wiley hammer mill. The treated and untreated CS was not ground through the 1mm screen since this caused the fat to separate and resulted in congelation. Nitrogen solubility was determined by incubating the materials in NaCl solution or 10% BMM for 2h. The completely randomised design was arranged as a 2x3x3 factorial, with two solvents, three treatments, three canola products, and three replicates per treatment combination. Pairwise comparisons were made using Duncan's multiple range test (Duncan 1955). The amino acid content of untreated CM, heat treated CM, FA treated CM and insoluble (in NaCl solution) CM was determined.

Experimental procedure for determining DM and N disappearance of feed samples from nylon bags

A woven nylon fabric was purchased from Arnotts Ltd., Ayr, Scotland. The material had approximately 760 pores/cm^2 and the average width of the pores was 36μm, as determined under a microscope. Bags measuring 15cm x 1.5cm when laid flat were prepared from the material. The bags were placed in a forced air oven at 60C for a 24h period immediately prior to use. Concentrate and hay samples were air dried and corn silage was dried in a forced air oven at 60C. Samples were ground through a 1mm screen in a Wiley hammer mill and 1g aliquots were placed in the nylon bags. The bags were folded and sealed with nylon thread. The final dimensions of the sealed bags were 10cm x 1.5cm. The bags were labelled, attached to a length of baling twine and suspended in the rumen of a fistulated steer for a measured period of time. On removal from the rumen, the bags were

rinsed thoroughly with cold water to remove any particulate material attached to the bag surface. The bags were then dried to constant weight at 60C in a forced air oven. Dry matter disappearance was determined and expressed as a percentage of total feed DM. The residue was removed from the bags and N content was measured by the macro-Kjeldahl procedure. Nitrogen disappearance was expressed as a percentage of total feed N.

Effect of incubation time on DM and N disappearance of CM from nylon bags

Samples of CM were placed in nylon bags and suspended in the rumen of a fistulated steer, as described above. Bags were removed after 1, 2, 4, 18, or 24h. The disappearance of DM and N from the bags was determined. A completely randomised design was used with two replicates per incubation time. Pairwise comparisons were made using Duncan's multiple range test (Duncan 1955).

Effect of FA treatment on DM, N and non N-DM (NNDM) disappearance of CM from nylon bags

Solutions containing 0, 0.12, 0.55, 0.95, and 2.4% FA were used to treat CM, as described previously. This corresponded to application rates of 0, 0.15, 0.7, 1.2, and 3.0g FA/100g CP, respectively. Samples of treated CM were placed in nylon bags and suspended in the rumen of a fistulated steer for 24h. The disappearance of DM and N was determined and NNDM disappearance was measured by difference. A completely randomised design was used with three replicates per treatment. Pairwise comparisons were made using Duncan's multiple

range test (Duncan 1955).

Chemical analyses

Feed DM's were determined by drying at 60C to constant weight in a forced air oven. Ash, ether extract (EE) and CP were measured according to AOAC (1975) procedures. Dried feed samples were hydrolysed in 6N HCl at 121C for 16h. The hydrolysates were evaporated to dryness, redissolved in citrate buffer and filtered through Whatman #4D filter paper. The amino acid composition of the filtrate was determined using a Beckman model 119C amino acid analyser (Beckman Instruments, Palo Alto, California).

Experiment 2. The Effect of Dietary Crude Protein Level and Formaldehyde Treated Canola Meal on Milk Production and Nitrogen Utilization of Holstein Cows During Early Lactation

Treatment of CM with FA

Tower CM was protected with FA by slowly adding 18 l of a 13.3% FA solution to 500kg CM in a feed mixer. The material was mixed thoroughly for at least 5 min. This corresponded to an application rate of 1.2g FA/100g CP. The treated material was stored for at least 7 days in burlap bags lined with plastic before inclusion in the experimental diets.

Formulation of experimental diets

The nutrient and ingredient compositions of the diets are shown in table 2. For the purpose of formulating the experimental diets,

Table 2. Ingredient and nutrient composition of the diets used to determine the response of lactating cows to dietary crude protein (CP) level and formaldehyde (FA) treated canola meal (experiment 2)

	Dietary Treatment			
	FH	FL	UH	UL
Ingredients (%DM)				
Corn silage	43	43	43	43
Brome hay	7	7	7	7
Barley	21.2	32.0	21.2	32.0
FA Canola meal[a]	23.5	13.0	--	--
Canola meal	--	--	23.5	13.0
Tallow	2.7	2.0	2.7	2.0
Urea	0.5	0.5	0.5	0.5
Limestone	0.9	0.9	0.9	0.9
Dicalcium phosphate	0.5	0.7	0.5	0.7
Salt (cobalt-iodized)	0.4	0.4	0.4	0.4
Premix[b]	0.4	0.4	0.4	0.4
Nutrient composition by analysis (%DM)				
Crude protein	17.4	14.6	17.3	14.7
Ether extract	4.5	3.5	4.4	3.7
ADF[c]	21.2	19.8	20.9	19.6
ADF-N[d]	1.5	1.4	1.2	1.2
Calcium	0.8	0.8	0.8	0.8
Phosphorus	0.6	0.6	0.6	0.6
DE (M cal/kg)[e]	2.64	2.63	2.69	2.61
Rumen escape protein[f] (g/kg DM)	99	73	74	60
Rumen degradable protein[f] (g/kg DM)	75	73	99	87

[a]Canola meal treated with 1.2g FA/100g CP.

[b]Provided per kg of diet: 3500 IU vit A, 1750 IU vit D, 10 IU vit E, 44 mg ZnO, 45 mg $MnO_2.H_2O$, 0.3 mg $CaCl_2$, 1 mg KI, 1.6 mg MgO.

[c]Acid detergent fibre.

[d]Acid detergent insoluble nitrogen.

[e]Apparent digestible energy, based on digestibility trial.

[f]Estimated from results of nylon bag study (table 13).

the assumption was made that treatment of CM with 1.2g FA/100g CP reduced N degradability by 40 percentage points (see table 11). Actual N degradabilities of 55% and 15% were assigned to untreated and FA treated CM, respectively. For the other dietary ingredients, estimates of N degradability were taken from the literature (Burroughs et al. 1975). Values of 70% were used for each of barley, corn silage and brome hay. Urea-N was assumed to be 100% degradable. It was anticipated that animals would consume 10kg DM/day and that concentrate, corn silage, and brome hay would be consumed in the ratio 50: 45: 5 (DM basis). Diets FL and UH were formulated to provide approximately 1300g REP/day. Diet FH was identical to UH except FA treated CM replaced untreated CM. Diet UL was identical to FL except untreated CM replaced FA treated CM.

Nitrogen solubility of the experimental diets in NaCl solution

The concentrate and hay were air dried and corn silage was dried at 60C in a forced air oven. Nitrogen solubility of ground samples was determined by incubating in NaCl solution for 2h. Since the corn silage and hay were common to all treatments in the lactation trial, data from these ingredients were not included in the statistical analysis. The concentrate data were analysed in a completely randomised design with three replicates per treatment. There was one missing observation for concentrate UH. Pairwise comparisons were made using Duncan's multiple range test (Duncan 1955).

Disappearance of the experimental diets from nylon bags

Samples of the ingredients used in the solvent extraction study

were placed in nylon bags and suspended in the rumen of a fistulated steer for 24h. The disappearance of DM and N was measured. The statistical design was similar to that used in the solvent extraction study, but there were two replicates per treatment and no missing observations.

Lactation trial

Production study: Forty Holstein cows from the university herd at Glenlea Research Station were assigned to one of four isocaloric diets approximately 2 weeks post-partum in a 2x2 factorial arrangement. There were eight multiparous cows and two first lactation heifers per diet. Factor I was dietary CP level, either 17.4% or 14.6% CP on a DM basis, denoted H and L, respectively. Factor II was FA treatment of the CM, either treated (F) or untreated (U). The four treatment combinations were FH, FL, UH, and UL (table 2). Concentrate, corn silage, and brome hay were fed ad libitum for 12 weeks in the ratio 50: 43: 7 (DM basis). Concentrate and silage were offered as a complete mix once per day, long hay was added separately. Refusals were weighed every morning before feeding. Animals were weighed on two consecutive days at the start of the experiment and at the end. Feed intake and milk yields were recorded daily, and milk samples were taken for three 24h periods per week for fat, protein, and lactose analysis. Additional milk samples were taken from each cow once per month, composited and stored for iodide analysis. Feed samples were taken weekly and composited monthly for laboratory analysis. Samples of tail vein blood and rumen liquor were taken by venipuncture and stomach tube, respectively, from each

cow once per month immediately before feeding. An additional sample of rumen liquor was taken from each of 24 cows (6 per treatment combination) 3h after feeding. Blood plasma and centrifuged rumen liquor were frozen and stored for future analysis.

Digestion study: Twenty-four animals (6 per treatment combination) were put on a 5-day total collection trial 6 to 11 weeks after assignment to the experimental diets. Total urine output was collected under toluene in jugs via urinary catheter. Total feces output was collected on plastic sheets. Measured aliquots of urine and feces were taken twice daily and frozen for future analysis to determine N balance and the apparent digestibilities of DM, N, EE and acid detergent fibre (ADF).

Chemical analyses: The DM, EE and CP of feed and feces samples were determined by the procedures described in the materials and methods section of experiment 1. Gross energy (GE), calcium (Ca) and phosphorus (P) were measured according to AOAC (1975) procedures. Acid detergent fibre and acid detergent insoluble N (ADF-N) were determined by the method of Goering and Van Soest (1970). Amino acids in feed sample hydrolysates and deproteinated blood plasma were determined using a Beckman model 119C amino acid analyser. Milk fat, protein and lactose were measured by infra-red spectroscopy (Milkoscan model 203, Foss Electric, Cornwall, Ontario), courtesy of Manitoba Department of Agriculture. Milk iodide was determined using a specific iodide electrode (Lacroix and Wong 1980). Urinary N was measured by the macro-Kjeldahl procedure. Plasma urea, glucose and total protein were measured using a Technicon autoanalyser II (Technicon Industrial Systems, New York). Rumen liquor NH_3-N was

determined by specific NH_3 electrode (Model 95-10, Orion Research, Cambridge, Massachussetts) and VFA by gas liquid chromatography (Erwin et al. 1961).

Post-experimental observations: Following the 12-week experimental period of the production study, cows were returned to the general herd management program. Milk yields and composition (fat, protein and lactose) were measured weekly until the end of lactation. Subsequent reproductive performance was assessed by determining the number of services per conception.

Statistical analyses: Data from the production and digestion studies were analysed by factorial analysis of variance (Snedecor and Cochran 1980). Unless otherwise indicated, interactions were not significant. One multiparous cow assigned to diet FL suffered severe mastitis so data from this animal were not used in the analysis of data from the production study. The missing value was calculated by the procedure suggested by Snedecor and Cochran (1980). There were no missing values from the digestion study. Factorial analysis of variance was also made on milk yield and composition data collected during the post-experimental period and again there was one cow missing from diet FL. Chi-squared analysis was made to determine if dietary treatment affected the number of cows requiring more than one service per conception (Parker 1973).

Experiment 3. The Effect of Formaldehyde Treated Canola Meal on Littering Rate and Litter Size of Mice

Laboratory mice were obtained from the Department of Animal Science mouse colony which is a closed population of randomly mated

mice. Seventy-eight mature females were randomly assigned to one of two experimental diets for 6 weeks. Diet MF contained FA treated CM whereas diet MU contained untreated CM (table 3). The rations were fed in pellet form. The females were confined three to a cage and had unlimited access to food and water. Food intake was measured for three consecutive days during the second week of the experiment. After 6 weeks, the experimental diets were replaced by a control diet (Rodent Laboratory Chow #5001, Ralston Purina Company, St. Louis, Missouri) and a male mouse was placed in each cage. The male remained in the cage for 12 days. Statistical analyses were made according to Parker (1973). The number of litters produced by mice on the two treatments were compared by chi-squared analysis. Litter sizes were compared using Student's t-test.

RESULTS

Experiment 1

Effect of incubation time on N solubilities of CM and SBM in NaCl solution

Nitrogen solubility of CM was significantly ($p < .01$) higher than SBM (table 4). Incubation time had no effect on N solubility of CM ($p < .05$), but N solubility of SBM was greater after the 3h incubation compared to the 1h incubation ($p < .01$).

Table 3. Ingredient composition of the diets used to determine the effect of formaldehyde (FA) treated canola meal on the reproductive performance of mice (experiment 3)

Ingredients (g/100g)	Treatment	
	MF	MU
Canola meal	--	25.0
FA-canola meal[a]	25.0	--
Rolled barley	70.6	70.6
Tallow	1.4	1.4
Dicalcium phosphate	1.0	1.0
Limestone	1.0	1.0
Vitamin premix[b]	0.5	0.5
Mineral premix[c]	0.5	0.5

[a]Canola meal treated with 1.2g FA/100g CP.

[b]Provided per kg diet: 7500 IU vit A, 250 IU vit D, 10 IU vit E, 1.5mg vit K, 425mg choline chloride, 15mg niacin, 2mg riboflavin, 0.5mg pyridoxine HCl, 0.3mg folic acid, 0.05mg biotin, 2.5Mg vit B12, 4.25mg pantothenic acid, 1.5g methionine.

[c]Provided per kg diet: 0.17g MnO, 0.015g ZnO, 0.03g $FeSO_4.7H_2O$, 0.026g $CuSO_4.5H_2O$, 4.76g NaCl (iodized).

Table 4. Effect of incubation time on nitrogen solubility of canola meal and soybean meal in NaCl solution

Incubation time	Nitrogen solubility (%)	
	CM	SBM
1h	$^{a}38.4$	$^{b}12.2$
2h	$^{a}37.8$	$^{bc}14.5$
3h	$^{a}36.9$	$^{c}16.5$
SE	0.7	

a, b, c Means with unlike superscripts are significantly different ($p < .01$).

Effect of particle size and incubation time on N solubilities of CM and SBM in NaCl solution

Neither particle size nor incubation time affected ($p > .05$) N solubility of CM in NaCl solution (table 5). The N solubility of SBM was higher ($p < .01$) when the material was ground through the 0.5mm screen. Incubation time did not significantly ($p > .05$) affect the N solubility of SBM, but there was a trend towards higher N solubility at the longer incubation times, particularly for the more finely ground material.

Effect of various FA treatments on N solubility of CM in NaCl solution

Treating CM with 0.1g FA/100g CP significantly ($p < .01$) reduced N solubility (table 6) in NaCl solution compared to the control (0g FA/100g CP). Nitrogen solubility was further reduced ($p < .01$) by treatment with 0.5g FA/100g CP, but there was no further effect on N solubility at higher levels of FA application ($p > .05$).

Effect of various heat treatments on N solubility of CM in NaCl solution

Autoclaving CM at 121C for 5 min significantly ($p < .05$) reduced N solubility (table 7) in NaCl solution compared to the control (0min). However, autoclaving for longer periods did not further reduce N solubility.

Effect of FA and heat treatments on N solubility of canola products in BMM or NaCl solution

Factorial analysis of variance revealed that N solubility of the canola products was significantly ($p < .01$) higher in NaCl solution than 10% BMM (table 8). Treating CM with heat or FA significantly ($p <$

Table 5. Effect of particle size and incubation time on nitrogen solubility of canola meal and soybean meal in NaCl solution

	[a]Nitrogen Solubility (%)			
	Canola meal[b]		Soybean meal[c]	
Incubation time	0.5mm	1.0mm	0.5mm	1.0mm
1h	35.6	36.9	15.1	11.9
2h	36.3	36.4	15.5	13.2
3h	--	--	16.1	13.4
4h	--	--	16.5	15.0
SE	0.6		1.0	

[a]Separate statistical analyses were made for CM and SBM data.

[b]Neither incubation time nor particle size affected N solubility ($p > .05$)

[c]Incubation time had no significant ($p > .05$) effect on N solubility but particle size significantly affected N solubility ($p < .05$).

Table 6. Effect of various levels of formaldehyde application on the nitrogen solubility of canola meal in NaCl solution

Level of formaldehyde application (g formaldehyde/100g crude protein)	Nitrogen solubility (%)
0	^{a}37.4
0.1	^{b}29.4
0.5	^{c} 3.5
1.0	^{c} 2.7
2.0	^{c} 3.7
4.0	^{c} 1.9
SE	1.0

$^{a, b, c}$Means with unlike superscripts are significantly different ($p < .01$).

Table 7. Effect of autoclaving at 121C on nitrogen solubility of canola meal in NaCl solution

Time in autoclave at 121C (min)	Nitrogen solubility (%)
0	[a]35.7
5	[b] 6.9
10	[b] 5.6
20	[b] 5.2
30	[b] 5.3
60	[b] 6.1
SE	0.9

[a, b]Means with unlike superscripts are significantly different ($p < .01$).

Table 8. Effect of formaldehyde (FA)[a] and heat[b] treatments on nitrogen solubility of fully extracted canola meal (CM), partially extracted canola meal (PCS) and crushed canola seeds (CS) in 10% Burroughs Mineral Mixture (BMM) and NaCl solution

Solvent	Treatment	Canola Product		
		CM	PCS	CS
10% BMM	Untreated	36.8	42.1	29.2
	Heat	2.5	2.4	2.0
	FA	1.1	0.7	0.9
NaCl	Untreated	44.0	53.2	39.8
	Heat	4.5	2.5	1.0
	FA	1.6	1.4	-0.2
SE = 1.4				

[a] 1.2g FA/100g crude protein.

[b] Autoclaved for 5 min at 121C.

[c] Significant difference between solvents ($p < .01$).

[d] Nitrogen solubility was significantly ($p < .01$) reduced by heat and FA treatments.

[e] Comparing means of untreated canola products, nitrogen solubility of PCS was highest, CM was next and CS had the lowest level of soluble N.

.01) reduced N solubility to almost zero. Differences in N solubility between canola products were significant ($p < .01$). Pairwise comparisons of the untreated canola products showed that PCS-N was much more soluble than CM-N ($p < .05$) and CM-N was more soluble than CS-N ($p < .01$).

Amino acid composition of FA treated, heat treated and untreated CM, and insoluble CM

Autoclaving CM at 121C for 5 min did not appear to affect the amino acid composition (table 9). Treatment of CM with 1.2g FA/100g CP reduced the tyrosine content by 57.7% and lysine by 18.9%. There was also a 17.4% increase in phenylalanine which is probably related to the decreased tyrosine content. There were marked differences between the amino acid compositions of untreated CM and the CM residue remaining after solvent extraction in NaCl solution. In particular, the insoluble residue contained a much higher proportion of methionine than CM.

Dry matter and N disappearance of CM from nylon bags over time

The disappearance of DM and N from nylon bags was significantly ($p < .01$) higher after 18h in the rumen compared to 1h (table 10). However, there was no significant ($p > .05$) difference in DM or N disappearance between the 18h and 24h incubations.

Effect of FA treatment on DM, N and NNDM disappearance of CM from nylon bags

The disappearance of DM and N was not affected ($p > .05$) by treatment with 0.15g FA/100g CP (table 11). However, both were significantly ($p < .05$) reduced by treatment with 0.7g FA/100g CP.

Table 9. Amino acid composition of untreated, formaldehyde (FA) treated and heat treated canola meal, and insoluble canola meal.

Amino acid[a]	Canola Meal			
	Untreated	Heat[b]	FA[c]	Insoluble residue[d]
Lysine	6.1	5.9	4.4	6.5
Histidine	3.0	2.9	2.9	2.9
Arginine	6.9	6.7	7.0	7.4
Aspartic acid	8.3	8.4	8.6	8.7
Threonine	4.5	4.7	4.8	5.5
Serine	4.2	4.3	4.4	4.7
Glutamic acid	19.2	19.2	19.5	14.7
Proline	6.5	6.6	6.7	6.3
Glycine	5.6	5.6	5.8	5.5
Alanine	4.9	4.9	5.1	5.3
Cystine	2.6	2.5	2.5	2.9
Valine	6.0	6.0	6.4	6.4
Methionine	2.3	2.2	2.2	3.3
Isoleucine	4.5	4.5	4.7	4.1
Leucine	7.9	7.9	8.3	7.9
Tyrosine	2.9	3.1	1.2	3.5
Phenylalanine	4.5	4.8	5.3	4.4
Total (% DM)	40.5	41.2	41.3	35.4

[a] g amino acid per 100g total amino acids.

[b] 5 min at 121°C.

[c] 1.2g FA/100g crude protein.

[d] Residue remaining after incubation of CM in NaCl solution for 2h.

Table 10. Dry matter (DM) and nitrogen (N) disappearance of canola meal from nylon bags over time

Time in rumen	DM disappearance (%)	N disappearance (%)
1h	$^{a}15.6$	$^{a}18.2$
2h	$^{b}26.7$	$^{ab}25.4$
4h	$^{b}29.3$	$^{b}31.8$
18h	$^{c}55.3$	$^{c}57.2$
24h	$^{c}58.4$	$^{c}55.1$
SE	2.2	2.1

a, b, c
Means within columns having unlike superscripts are significantly different ($p < .05$).

Table 11. Effect of formaldehyde (FA) treatment on dry matter, nitrogen and non-nitrogen dry matter (NNDM) disappearance of canola meal from nylon bags after 24h.

Level of FA (g FA/100g CP)	Percent disappearance		
	Dry Matter	Nitrogen	NNDM
0	$^{a}56.4$	$^{a}54.5$	$^{a}58.2$
0.15	$^{a}50.8$	$^{a}51.3$	$^{b}50.5$
0.7	$^{b}39.7$	$^{b}26.5$	$^{bc}48.5$
1.2	$^{c}32.1$	$^{c}15.8$	$^{c}43.0$
3.0	$^{d}21.3$	$^{d}8.4$	$^{d}29.9$
SE	2.1	1.9	2.1

a, b, c, d Means within columns having unlike superscripts are significantly different ($p < .05$).

Treating CM with 1.2g FA/100g CP caused a further reduction ($p < .05$) in the disappearance of DM and N, and treatment with 3.0g FA/100g CP reduced ($p < .05$) the disappearance still further. The disappearance of NNDM was reduced by treatment with 0.15g FA/100g CP, but there was no difference ($p > .05$) between the 0.15 and 0.7g FA/100g CP rates of application. Treatment of CM with 1.2g FA/100g CP lowered ($p < .05$) NNDM disappearance compared to treatment with 0.15 or 0.7g FA/100g CP. There was a further decrease ($p < .05$) in NNDM disappearance following treatment with 3.0g FA/100g CP.

Experiment 2

Nitrogen solubility of the experimental diets in NaCl solution

There were small but significant ($p < .05$) differences in N solubility between concentrates FH and FL, and between UH and UL (table 12). The concentrates which contained FA treated CM had N solubilities which were substantially lower ($p < .01$) than concentrates containing untreated CM.

Disappearance of the experimental diets from nylon bags

Dry matter disappearance of concentrate FH was significantly ($p < .01$) lower than DM disappearance of the other concentrates (table 13). The DM disappearance of concentrate FL was less ($p < .05$) than that of UH and UL but UH and UL did not differ significantly ($p > .05$). Nitrogen disappearance of concentrate FH was significantly ($p < .01$) lower than N disappearance of the other concentrates. Concentrate FL had a lower ($p < .01$) N disappearance than UH and UL but there was no

Table 12. Nitrogen solubility of the lactation diets in NaCl solution (experiment 2)

Ingredients	Nitrogen solubility (%)
Concentrates	
FH	$^{aA}16.7$
FL	$^{bA}19.4$
UH	$^{cB}30.3$
UL	$^{dB}32.7$
SE	0.8
Corn silage	40.9
Brome hay	28.6

a, b, c, d Means having unlike superscripts are significantly different ($p<.05$).

A,B Means having unlike superscripts are significantly different ($p<.01$).

Table 13. Dry matter, nitrogen and non-nitrogen dry matter (NNDM) disappearance of the lactation diets from nylon bags after 24h (experiment 2).

Ingredients	Percent disappearance		
	Dry Matter	Nitrogen	NNDM
Concentrates			
FH	$^{aA}_{49.0}$	$^{aA}_{40.4}$	$^{aA}_{51.3}$
FL	$^{bB}_{55.8}$	$^{bB}_{50.4}$	$^{abAB}_{56.5}$
UH	$^{cB}_{59.1}$	$^{cC}_{59.1}$	$^{bAB}_{58.0}$
UL	$^{cB}_{60.9}$	$^{cC}_{63.8}$	$^{bB}_{59.3}$
SE	0.8	1.3	1.5
Corn silage	51.1	50.0	51.2
Brome hay	42.7	46.2	42.2

$^{a, b, c}$Means within colums having unlike superscripts are significantly different ($p < .05$).

$^{A, B, C}$Means within columns having unlike superscripts are significantly different ($p < .01$).

significant ($p < .05$) difference between UH and UL. There was no difference ($p > .05$) in NNDM disappearance between concentrates FH and FL or UH and UL. However, NNDM disapperance of FH was lower ($p < .05$) than NNDM disappearance of UH or UL.

Lactation trial

Production study: There was no significant ($p > .05$) difference in liveweight changes during the experiment. The average liveweight at the start of the experiment was 585kg and the average weight loss was 9.8kg. Cows assigned to the high protein diets tended to have higher DM intakes (figure 2 and table 14), but the difference was significant only at $p < .1$. Formaldehyde treatment had no effect on DM intake. The CP intake of cows on the high protein diets was equal to the amount recommended by NRC (National Research Council 1978) for animals at this level of production whereas the low protein diets provided 80% of the suggested level. Based on the results from the nylon bag study (table 13), crude estimates of the REP content of the diets were made (table 2). It is unlikely that these estimates provide a precise measure of the absolute REP content. However, they probably reflect the relative amounts of REP in the diets. It appears that diets FL and UH contained approximately equal amounts of REP. Diet FH contained relatively more REP whereas diet UL contained relatively less.

Animals assigned to the high protein diets had higher ($p < .05$) milk yields and 4% fat corrected milk (FCM) yields (figure 2 and table 14). Figure 2 illustrates that most of the difference was due to higher milk production during weeks 4 to 12 of the experiment. On the low protein diets, milk production peaked at about week 4 of lactation

Figure 2. Response of milk yield and dry matter intake to two levels of dietary crude protein and formaldehyde treated canola meal (experiment 2)

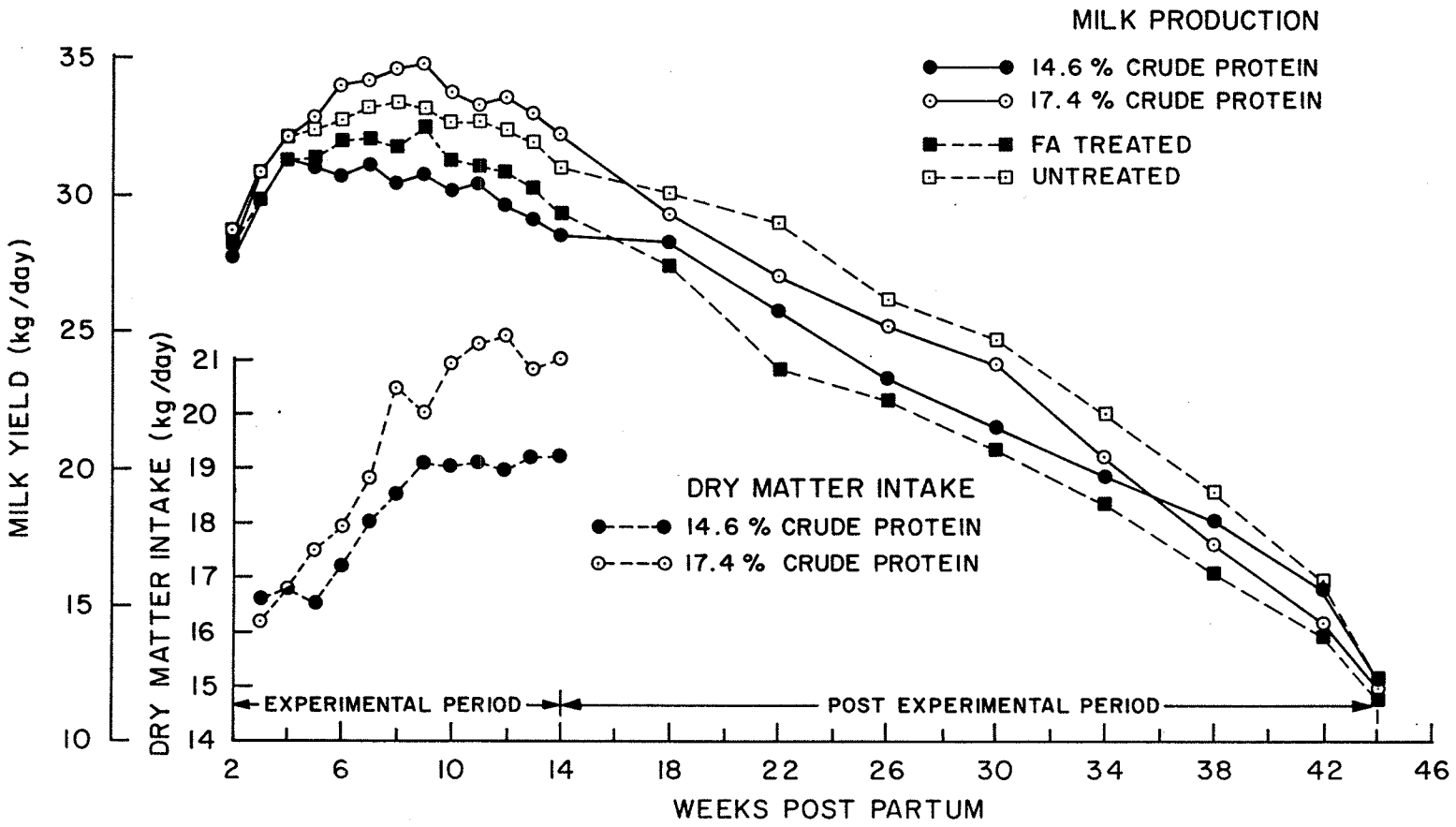

MILK PRODUCTION
14.6 % CRUDE PROTEIN
17.4 % CRUDE PROTEIN
FA TREATED
UNTREATED
DRY MATTER INTAKE
14.6 % CRUDE PROTEIN
17.4 % CRUDE PROTEIN
MILK YIELD (kg/day)
DRY MATTER INTAKE (kg/day)
EXPERIMENTAL PERIOD
POST EXPERIMENTAL PERIOD
WEEKS POST PARTUM
35
30
25
20
15
10
21
20
19
18
17
16
15
14
2
6
10
14
18
22
26
30
34
38
42
46

Table 14. Dry matter (DM) intake, milk yield and milk composition of cows in response to two levels of dietary crude protein (CP) and formaldehyde (FA) treated canola meal (experiment 2)

	CP level (%DM)		FA treatment		SE
	17.4	14.6	[a]F	[b]U	
DM intake (kg/day)[c]	19.5	18.3	19.0	18.7	0.7
CP intake (kg/day)[d]	3.4	2.7	3.1	3.0	0.1
Milk yield (kg/day)[d]	33.2	30.4	31.2	32.4	1.4
4% FCM (kg/day)[de]	33.3	30.0	31.4	31.9	1.6
% milk fat	4.03	3.95	4.06	3.93	0.17
Fat yield (kg/day)[c]	1.34	1.19	1.26	1.27	0.08
% milk protein	2.92	2.99	2.94	2.97	0.05
Protein yield (kg/day)[c]	0.97	0.90	0.91	0.96	0.04
% lactose	4.87	4.86	4.85	4.88	0.05
Lactose yield (kg/day)[d]	1.62	1.47	1.51	1.58	0.07
Milk iodide (g/l)	331	328	330	328	53

[a]Formaldehyde treated diets.

[b]Untreated diets.

[c]Difference between protein levels ($p < .01$).

[d]Difference between protein levels ($p < .05$).

[e]4% fat corrected milk.

whereas yields on the high protein diets continued to increase and did not peak until 8 weeks post-partum. The difference was maintained throughout the remainder of the experimental period. Formaldehyde treatment had no effect on milk yield or 4% FCM yield. There was no difference in the concentrations of milk fat, protein, or lactose among treatments (table 14). The average concentration of milk fat was 3.99% which is high for Holsteins at this level of production. Lactose yields were higher ($p < .05$) on the high protein diets, reflecting the increased milk yields. Although fat and protein yields tended to be higher, the differences were significant only at $p < .1$. Formaldehyde treatment had no effect on fat, protein, or lactose yields. Neither dietary CP nor FA treatment affected the concentration of milk iodide (table 14).

Dietary treatment had no effect on the NH_3-N concentration in rumen liquor sampled immediately before feeding (table 15). However, when samples were taken 3h after feeding, animals assigned to the FA treated diets had significantly ($p < .05$) lower rumen fluid NH_3-N levels (table 15). Dietary CP level had no effect on rumen NH_3-N levels, but this was not altogether surprising because of the different levels of RDP in the diets (table 2). Although protein level x FA treatment interaction was not significant, rumen liquor NH_3-N levels 3h after feeding tended to be higher on diet UH and UL. Means for individual diets were 9.4, 8.5, 16.2, and 13.9mg/100ml for diets FH, FL, UH and UL, respectively. Total VFA concentration and the molar ratios of VFA in rumen liquor did not vary among treatments (table 15).

Plasma urea was higher ($p < .05$) on the high protein diets but was

Table 15. Response of rumen liquor profile to two levels of dietary crude protein (CP) and formaldehyde (FA) treated canola meal (experiment 2).

Metabolite		CP level (%DM)		FA treatment		SE
(hours post feeding)		14.6	17.4	[a]F	[b]U	
NH_3-N (mg/100ml)	0h	6.3	6.1	5.8	6.5	1.1
	3h[c]	12.8	11.3	9.0	15.1	2.1
VFA (molar %)[d]						
Acetate	0h	68.7	66.7	67.6	67.8	1.3
	3h	60.5	61.6	60.0	62.1	1.4
Propionate	0h	17.9	19.4	18.8	18.5	1.0
	3h	20.9	20.0	20.8	20.2	1.0
Butyrate	0h	11.3	11.3	11.3	11.3	0.7
	3h	16.2	15.7	16.8	15.1	1.1
Acetate:Propionate	0h	3.8	3.5	3.6	3.7	0.2
	3h	2.9	3.1	2.9	3.1	0.2
Total VFA (mmoles/l)	0h	54.5	64.8	58.3	61.0	6.8
	3h	81.6	87.6	87.7	81.5	8.2

[a]Formaldehyde treated diets.

[b]Untreated diets.

[c]Decreased by FA treatment ($p < .05$).

[d]Volatile fatty acids.

not affected by FA treatment (table 16). There was no diference in the levels of total plasma protein among treatments. Treatment main effects on plasma glucose were not significant (table 16); however, protein level X FA treatment interaction was significant ($p < .05$). This was due to higher ($p < .05$) plasma glucose levels on diet FL compared to FH. The actual values were 69.8 and 52.5mg glucose/100ml plasma for FL and FH, respectively. Of the non-essential plasma amino acids, glycine and serine were reduced ($p < .05$) by FA treatment and alanine tended to be reduced ($p < .1$). Plasma citrulline concentration was increased ($p < .05$) by the high protein diets. Dietary CP level had no effect on the levels of non-essential plasma amino acids. The essential amino acids tyrosine, valine, isoleucine and leucine were increased ($p < .05$) by the high protein diets, and plasma histidine tended to increase ($p < .1$). Because of the high demand for tyrosine and phenylalanine for milk production (Mepham 1982), tyrosine will be considered an essential amino acid in this discussion. Formaldehyde treatment increased ($p < .05$) plasma valine, leucine and histidine, and tended to increase ($p < .1$) plasma isoleucine. In addition, FA treatment decreased ($p < .05$) plasma threonine. Total plasma essential amino acids were increased ($p < .05$) by high dietary CP and tended to be increased ($p < .1$) by FA treatment. The trend towards increased total plasma essential amino acids caused by FA treatment was almost entirely due to increased valine, leucine, isoleucine, and histidine

Digestion study: Neither FA treatment nor dietary CP level affected apparent DM digestibility (table 17). Formaldehyde treatment had no effect on apparent N digestibility; but, as expected, apparent

Table 16. Response of plasma profile to two levels of dietary crude protein and formaldehyde (FA) treated canola meal (experiment 2).

Plasma metabolite	Protein level (% DM)		FA treatment		SE
	17.4	14.6	Treated	Untreated	
Urea (mg/100ml)[a]	15.7	11.3	14.2	12.9	1.1
Glucose (mg/100ml)[b]	58.1	63.2	60.7	60.5	3.8
Total protein (mg/100ml)	10.6	10.0	10.4	10.2	0.8
Amino acids (μ moles/l)					
Non-essential					
Aspartic acid	27	30	27	29	2
Asparagine	37	33	33	36	5
Glutamic acid	122	133	127	128	18
Glutamine	444	421	458	411	53
Proline	108	100	112	97	20
Glycine[c]	560	576	500	629	63
Alanine[d]	313	265	239	337	42
Citrulline[a]	114	88	107	97	13
Ornithine	66	62	66	63	10
Serine[c]	116	118	105	129	10

Table 16 continued. Response of plasma profile to dietary crude protein level and formaldehyde (FA) treated canola meal (experiment 2).

Plasma metabolite	Protein level (% DM)		FA treatment		SE
	17.4	14.5	Treated	Untreated	
Amino acids (μmoles/l)					
Essential					
Tyrosine[a]	55	47	50	52	5
Threonine[c]	157	151	139	168	16
Valine[ac]	456	340	448	360	40
Isoleucine[ad]	175	139	169	147	15
Leucine[ac]	231	176	228	183	20
Methionine	25	23	24	24	2
Phenylalanine	53	48	51	50	5
Tryptophan	10	10	10	10	1
Lysine	123	110	116	118	16
Histidine[ce]	61	50	63	50	7
Arginine	114	106	108	112	16
Total EAA[adf]	1462	1202	1408	1276	64

[a]Protein level significant ($p < .05$).

[b]Interaction significant ($p < .05$).

[c]FA-treatment significant ($p < .05$).

[d]FA- treatment significant ($p < .1$).

[e]Protein level significant ($p < .1$).

[f]Essential amino acids.

Table 17. Effect of dietary protein level and formaldehyde (FA) treated canola meal on the apparent digestibilities of dry matter, gross energy, ether extract, acid detergent fibre (ADF) and nitrogen, and the partition of dietary nitrogen between feces, urine and milk (experiment 2).

	Protein level (% DM)		FA treatment		SE
	17.4	14.6	Treated	Untreated	
Dry matter					
Intake (kg/day)	19.5	19.3	19.3	19.5	1.2
Fecal excretion (kg/day)	7.2	7.1	7.1	7.2	0.4
Apparent digestibility (%)	62.4	62.9	62.9	62.5	2.4
Gross energy					
Intake (M cal/day)	85.3	82.4	83.3	84.3	5.2
Fecal excretion (M cal/day)	31.2	30.3	30.6	30.9	1.7
Apparent digestibility (%)	63.1	62.7	63.0	62.9	2.4
Ether extract					
Intake (g/day)	872	754	818	808	71
Fecal excretion (g/day)	136	126	131	131	15
Apparent digestibility	84.0	82.5	83.3	83.2	2.3
ADF					
Intake (g/day)	414	389	396	407	23
Fecal excretion (g/day)	259	231	241	249	13
Apparent digestibility	37.0	40.1	38.6	38.5	3.2

Table 17 continued. Effect of dietary protein level and formaldehyde treated canola meal on the apparent digestibilities of dry matter, gross energy, ether extract, acid detergent fibre (ADF) and nitrogen, and the partition of dietary nitrogen between feces, urine and milk (experiment 2)

	Protein level (% DM)		FA treatment		SE
	17.4	14.6	Treated	Untreated	
Nitrogen					
Intake (g/day)[a]	550	436	485	504	45
Excretion (g/day)					
Feces	201	184	198	186	11
Urine[a]	202	116	153	164	15
Milk	150	147	146	151	9
Nitrogen partition (% of intake)					
Feces[b]	37.2	44.0	41.9	39.3	3.3
Urine[a]	37.2	26.6	31.6	32.2	2.7
Milk[a]	27.6	35.1	30.8	31.9	2.5
Retained	-2.0	-5.6	-4.3	-3.4	6.4
Apparent Digestibility (%)	62.8	56.0	58.1	60.7	3.3

[a]Difference between protein levels ($p < .01$).

[b]Difference between protein levels ($p < .05$).

N digestibility was increased ($p < .05$) by the high protein diets. Formaldehyde treatment did not affect the partitioning of dietary N between feces, urine, and milk. However, animals assigned to the high protein diets apparently excreted a lower ($p < .05$) proportion of dietary N in feces and in milk, and a higher ($p < .05$) proportion in urine. Dietary treatment did not affect ($p > .05$) the apparent digestibilities of EE or ADF.

Post-experimental observations: During the post-experimental period, i.e. weeks 15 to 42 of lactation, animals which had been assigned to the FA treated diets tended to produce less milk ($p < .1$) (figure 2 and table 18). Dietary CP level during the experimental period had no effect on subsequent milk production. There was no difference in milk composition among treatments during the post-experimental period (table 18). There was no significant ($p > .1$) difference in the number of services per conception among dietary treatments although the number of animals requiring more than one service tended to be increased by FA treatment and, to a lesser extent, by low dietary CP (table 18). The comparison of one versus more than one service was chosen because (a) the cows were still consuming the experimental diets at first service, and (b) and overall mean for number of services cannot reasonably be calculated because an unusually large number of animals were subsequently culled from the herd because of their inability to conceive after five or more services. The average number of days open for the cows was also not calculated because of the large number of animals culled. Of the animals which were culled, five were on diet FL, one was on FH and one was on UL. The trend towards poorer fertility of animals on diet FL

Table 18. Milk yield and composition during the post-experimental period (days 99 through 305 of lactation) and number of cows requiring 1 or >1 service per conception (experiment 2).

	Protein level (% DM)		FA treatment		SE
	17.4	14.6	F	U	
Milk yield (kg)[a]	4591	4303	4145	4741	166
% milk fat	3.79	3.80	3.86	3.72	0.15
% milk protein	3.17	3.21	3.18	3.19	0.06
% lactose	4.72	4.65	4.67	4.69	0.06
Number of cows requiring 1 or >1 service[bc]					
1	8	5	4	9	
>1	11	13	15	9	

[a]Decreased by FA treatment ($p < .1$).

[b]Not significant ($p > .1$) by chi-squared analysis.

[c]Includes seven animals culled because of inability to conceive after five or more services.

may have been due to FA treatment, low dietary CP, or may have been an anomaly. Several cows contracted bladder infections as a result of catheterization. In some cases, the bladder infections were so severe that several cows were removed from the herd.

Experiment 3

The diets which were fed to the mice in experiment 3 were similar to those fed to the cows in experiment 2. There was no difference in food intake between diets MF and MU. The overall mean ± SE was 5.45± 0.22g/mouse/day. Based on an application rate of 1.2g FA/100g canola-CP, mice on diet MF consumed up to 0.66mgFA/day. One mouse assigned to diet MU died during the experiment, so data from 38 mice on diet MU and 39 mice on diet MF were analysed. There was no significant difference ($p > .1$) in littering rate or average litter size between the two treatments (table 19).

Table 19. Effect of formaldehyde treated canola meal on littering rate and litter size in laboratory mice (experiment 3)

	Treatment[a]		
	MF	MU	
No. of mice that littered[b]	29	32	
No. of mice that did not litter	10	6	
Total no. of mice	39	38	
			SE
No. of offspring per litter[b]	9.38	9.77	0.48

[a]See table 3 for composition of diets.

[b]Differences between treatments were not significant ($p > .1$).

DISCUSSION

Determination of a Suitable Method for Reducing the Ruminal Degradability of Canola-N

The inaccuracies of using N solubility as a measure of N degradability were discussed in the literature review. The solvent extraction technique was used in the present study to provide preliminary information for selecting products for the nylon bag study. It was presumed that physical or chemical treatments which did not reduce N solubility would not reduce N degradability. On the other hand, if a treatment reduced N solubility, possibly N degradability was reduced also. The most promising treatments were selected for further study using the nylon bag technique.

The solubility of CM-N was greater than SBM-N (table 4). This has been reported elsewhere (Wohlt et al. 1973). Canola meal and SBM are both readily degraded in the rumen (Fisher 1981), so although SBM-N solubility is relatively low, ruminal degradability is high. Incubation time did not affect the N solubility of CM. However, the 2h incubation period was chosen instead of the 1h incubation for subsequent solvent extractions as a precautionary measure. Because there was no difference in CM-N solubility between material ground through the 0.5mm or 1mm screens (table 5), the 1mm screen was chosen for subsequent solvent extractions. Although incubation time and particle size did not affect N solubility of CM, SBM-N solubility increased with longer incubation times and the smaller particle size

(tables 4 and 5).

The experiments to determine the effect of various rates of FA application on CM-N solubility showed that mild treatment with FA (0.1g FA/100g CP) substantially lowered the solubility of CM-N. Treatment with higher levels of FA reduced N solubility to approximately zero (table 6). Sharma et al. (1972) reported that the solubility of RSM-N in fresh rumen fluid was reduced to zero following treatment with 5.4g FA/100g CP. In the present experiment, treatment with 0.5g FA/100g CP reduced CM-N solubility to the same extent as higher rates of FA application. However, the level of 1.2g FA/100g CP was chosen for subsequent solvent extractions with CM to ensure a high degree of protection.

Autoclaving CM at 121C for 5min reduced N solubility by approximately 80% but autoclaving for longer periods did not further reduce N solubility (table 7). For the subsequent solvent extraction with heat treated CM, the material was autoclaved for 5min. Although the CM was kept at 121C for the times listed in table 7, there was an additional length of time while the autoclave chamber reached 121C and then cooled down again. Sharma et al. (1972) reported that N solubility of RSM in 0.02N NaOH solution was reduced by 82% when RSM was heated in an oven at 180C for 20 minutes.

Based on results from studies discussed above, heat (5 min at 121C) and FA (1.2g FA/100g CP) were used to treat the other canola products. Because of the known variation between solvents (Crooker et al. 1978), N solubility of treated and untreated canola products was measured in two solvents. The difference in N solubilities observed between 10% BMM and 0.15N NaCl solution (table 8) has been reported elsewhere

(Crooker at al. 1978; Krishnamoorthy et al. 1982). The reduction in N solubility following treatment with heat or FA was similar to the responses observed in the previous solvent extractions (tables 6 and 7). Nitrogen solubility of CM was expected to be lower than the other canola products because heat is applied during processing at the factory. The lower N solubility of CS compared to CM may have been due to the much larger particle size of the CS material. For reasons discussed earlier, CS could not be ground through the 1mm screen.

Except for the likely higher energy contents, neither CS nor PCS had any discernible advantage over CM as a protein supplement for lactating cattle. Given the much larger particle size of CS compared to CM, the 5.9 percentage point difference in N solubilities was not considered biologically important. Therefore, CM was selected as the canola product for further study. Canola meal has a higher CP content than the other two products (table 1) and is widely used as a commercial protein supplement. In addition, potential problems (Palmquist and Jenkins 1980) which may have arisen from the high fat content of CS and PCS (table 1) were avoided by using CM. Formaldehyde treatment was selected to reduce the degradability of CM-N because FA can be conveniently applied on a large scale under carefully controlled conditions which are easily repeatable. In addition, a positive response in milk production to FA treatment of a RSM/SBM mixture had been reported (Verite et al. 1977). A potential drawback to using FA was the apparent decrease in CM tyrosine and lysine following treatment (table 9).

The first nylon bag study was conducted to find the incubation time at which CM-DM and CM-N disappearance plateaued. The plateau

appeared to occur between 18h and 24h (table 10) which is within the range of 12h to 36h suggested by Orskov et al. (1980) for measuring DM and N disappearance of concentrates. Therefore, subsequent incubation periods lasted 24h.

The second nylon bag study (table 11) was conducted to determine the effectiveness of various levels of FA application at reducing N disappearance of CM and to assess the effect on the non-N portion of CM. Although treatment of CM with 0.1g FA/100g CP reduced N solubility in NaCl solution (table 6), treatment of CM with 0.15g FA/100g CP did not reduce the disappearance of CM-N from nylon bags (table 11) which suggests that the protection achieved by treating CM with 0.1 to 0.15g FA/100g CP is insufficient to prevent protein degradation by microbial proteases. Surprisingly, NNDM disappearance was reduced by the low rate of FA application for reasons which are not clear. Treating CM with 0.7, 1.2, or 3.0g FA/100g CP reduced N disappearance by 51%, 71%, and 85%, respectively, compared to the control (0 g FA/100g CP) (table 11). The corresponding values for the reduction in NNDM disappearance were 17%, 16%, and 49%. Therefore, FA treatment not only reduced the ruminal degradability of protein, the degradability of non-nitrogenous material was also reduced, though to a lesser extent. Treatment of CM with 0.7g FA/100g CP substantially decreased N disappearance from nylon bags in the present study. However, in a previous study (Sharma and Ingalls 1974), treating RSM with this level of FA had no effect on the flow of amino acids through the abomasum of steers. As a precautionary measure, the application rate of 1.2g FA/100g CP was selected for the lactation trial (experiment 2) to ensure an adequate level of protection, knowing that

this higher level increased the risk of lowering protein digestibility in the small intestine (Chalupa 1975).

In summary, after a series of solvent extraction and nylon bag studies, treatment with 1.2g FA/100g CP was chosen as the method for reducing the ruminal degradability of CM-N.

Ruminal Degradability of the Diets Used in Experiment 2

The criteria for formulating diets FH, FL, UH and UL was discussed in the materials and methods section. Treatment with FA substantially reduced N solubility of the diets (table 12). However, measurements of N solubility do not give a true measure of N degradability. Treatment of CM with FA reduced the N disappearance of the diets from the nylon bags (table 13). This observation is a more reliable indicator that FA treatment reduced the N degradability of the diets. However, the N disappearance values in table 13 may not be equal to the actual N degradabilities of the diets for reasons discussed in the literature review. In the present study, the values have been used to provide an estimate of the relative amounts of REP of one diet compared to another. Based on the CP intakes of the cows and N disappearance of the diets from nylon bags, the relative amounts of REP consumed daily by animals on diets FH, FL, UH and UL were 2000g, 1320g, 1390g, and 1100g, respectively. Similarly, RDP consumption was estimated to be 1480g, 1330g, 1890g and 1610g for diets FH, FL, UH and UL, respectively. Although these values cannot be taken as a measure of the actual amounts of REP and RDP consumed by the cows, they can be used to compare the relative quantities of the

two N fractions which were consumed. Diets FL and UH apparently provided approximately equal amounts of REP, as planned. Diet FH provided considerably more REP whereas UL provided less than the other two diets. The decrease in NNDM disappearance of concentrate FH (table 13) is unfortunate because there is a possibility that in vivo carbohydrate digestibility was lowered.

Effect of FA Treated CM on the Performance of Lactating Cows

Potential goitrogenic effect of CM on lactating cows

The goitrogenic effect of RSM has received considerable attention (Papas et al. 1979; Laarveld et al. 1981). In experiment 2, there was no indication that the high concentration of low glucosinolate CM in the diets had any detrimental effects on milk production. Several other authors have reported that feeding CM to dairy cows does not adversely affect milk production (Sharma et al. 1977; Papas et al. 1979; Laarveld et al. 1981; Sanchez et al. 1982). If first lactation heifers are removed from the analysis, multiparous cows on the high protein diets produced an average of 34.8kg milk/day while consuming 5kg CM/day. Low milk iodide levels associated with feeding RSM or CM may indicate impaired iodine utilization by the cow (Papas et al. 1979). In the present study, milk iodide levels (table 14) were in the range reported by Lacroix and Wong (1980) for raw milk produced in various locations in the Washington, D.C., area. In addition, dietary treatment had no effect on milk iodide concentration (table 14) even though the high protein diets contained approximately twice as much CM as the low protein diets. The data suggest that the inclusion of high

concentrations of CM in the diets did not impair animal performance.

Effect of FA treated CM on various production and metabolic parameters

Although milk composition was not affected by dietary treatment (table 14), milk fat concentration was higher than expected. The high milk fat content can be partially explained by the adequate levels of forage in the diets and the favourable ADF content (table 2), reflected in the relatively high molar ratios of acetate:propionate in rumen liquor (Oldham and Sutton 1979). The inclusion of tallow in the diets may also have contributed to the high level of milk fat (Palmquist and Jenkins 1980).

In theory, treatment of CM with FA should have caused an increase in milk production, but in practice no increase was observed (table 14). Many authors, as cited in the literature review, have reported that feeding FA treated protein supplements to dairy cows did not increase milk production. Reasons for the lack of response are not clear. In the present experiment, the lack of response cannot be explained by a short experimental period (Tamminga 1979) because the animals had 12 weeks in which to show a response. Natural resistance of the untreated protein to ruminal degradation (Wachira et al. 1974) is also unlikely to be a factor because CM, like SBM, is readily degradable in the rumen (Fisher 1981).

Another possible reason for the lack of response to FA treatment is underprotection of the protein supplement (Wachira et al. 1974). Animals are not likely to respond to FA treated diets if the level of FA application is too low to protect the protein against ruminal degradation. Spears et al. (1980) reported a substantial decrease in

the ruminal degradability of SBM-protein when SBM was treated with 0.3g FA/100g CP. In most of the studies discussed in the literature review in which FA treated protein supplements were fed to lactating cattle, the concentration of FA was in the range of 0.3 to 1.8g FA/100g CP so underprotection was probably not a factor. In the present study, CM was treated with 1.2g FA/100g CP. This level of application substantially reduced the disappearance of dietary N from nylon bags (table 13). In addition, rumen liquor NH_3-N concentration 3h after feeding was reduced by FA treatment (table 15), suggesting that protein digestion in the rumen was reduced. Therefore, it is unlikely that the CM was underprotected.

Tamminga (1979) pointed out that treatment of dietary ingredients to reduce protein degradability in the rumen should not result in a decrease in microbial activity. Possibly, animals assigned to the FA treated diets did not consume enough RDP to maintain an adequate level of rumen NH_3-N for microbial growth. Ammonia levels in the rumen should be at a minimum just before feeding (Mahadevan et al. 1982), so the NH_3-N concentration of rumen liquor sampled immediately prior to feeding (table 15) will be considered the basal level. Formaldehyde treatment did not affect the basal level of rumen liquor NH_3-N. In addition, the rumen NH_3-N levels were above 5mg/100ml which is the level considered adequate by Satter and Roffler (1975) and similar to the level considered adequate by many other authors for microbial growth, as described in the literature review. Formaldehyde treatment lowered the NH_3-N concentration of rumen liquor samples taken 3h after feeding. This is hardly surprising since the purpose of FA treatment was to reduce the degradation of protein and prevent excess

accumulation of NH_3-N in the rumen. The "ideal" concentration of rumen liquor NH_3-N to support microbial growth has not been clearly established, as was discussed in the literature review, so it is not possible to state whether the rumen liquor NH_3-N levels of animals assigned to the FA treated diets were adequate or not. Indirect evidence suggests that rumen NH_3-N did not limit microbial activity. The typical response to a decrease in microbial activity is a reduction in the digestibilities of DM and ADF, and a decreased feed intake (Clark and Davis 1980; Huber and Kung 1981), none of which occurred in the present study (tables 14 and 17). Ruminal VFA concentration has also been used as an indirect measure of the response of microbial activity to various levels of rumen liquor NH_3-N (Slyter et al. 1979). Folman et al. (1981) reported a decrease in ruminal VFA concentration when lactating dairy cows were fed FA treated SBM and suggested this effect might be due to the reduction in ruminal NH_3-N. In the present experiment, the total concentration of VFA in rumen liquor sampled immediately before feeding or 3h after feeding was not affected by FA treatment (table 15). This observation suggests that the concentration of NH_3-N in the rumen did not limit microbial activity. The indirect evidence discussed above indicates that animals assigned to the FA treated diets consumed enough RDP to maintain adequate levels of ruminal NH_3-N to support microbial growth.

In addition to providing NH_3-N to the microbial population, dietary RDP also provides peptides and amino acids, both of which stimulate microbial growth (Pittman and Bryant 1964; Maeng et al. 1976). In addition, BCAA are precursors of BVFA and BVFA are also stimulatory for microbial growth (Umunna et al. 1975). Therefore, one

could speculate that FA treatment caused a deficiency of peptides, amino acids, and/or BVFA for optimal microbial activity. However, for reasons discussed in the previous paragraph, there was no apparent effect of FA treatment on microbial activity so the data suggest that a deficiency of RDP was not a factor in the present study.

A deficiency of RDP is not the only factor which could potentially lower microbial activity. Treatment of CM with FA lowered DM and NNDM disappearance from nylon bags (table 13) so it is possible that energy availability in the rumen was lowered. The presence of unbound FA in the rumen could also adversely affect the rumen microbial population, although Dinius et al. (1974) reported that casein treated with 5g FA/100g casein did not affect rumen microorganisms during an in vitro incubation.

Although FA treatment could potentially affect microbial activity in a variety of ways, there was no evidence that the lack of response to FA treated diets in the present experiment was due to impaired microbial activity. The indirect evidence available suggests that microbial activity was not affected by FA treatment.

A commonly cited reason for the lack of production responses to FA treatment is overprotection of the protein supplement leading to a decrease in protein digestibility in the small intestine (Chalupa 1975; Tamminga 1979). From studies with lactating cows, Clark et al. (1974), Wachira et al. (1974) and Crooker et al. (1982) suggested that overprotection was a primary reason why the animals did not produce more milk in response to FA treated SBM. In the present experiment, FA treatment did not significantly affect the apparent digestibilities of DM or N (table 17), although there was a small,

non-significant decrease in apparent N digestibility. Apparently, the in vivo digestibilities of DM and N were not affected by FA treatment (table 17), even though DM and N disappearance from nylon bags was reduced by FA treatment (table 13). Reductions in apparent N digestibility have been reported when RSM was treated with 5 to 6g FA/100g CP (Sharma et al. 1972; Kowalczyk et al. 1982) but not with 0.7g FA/100g CP (Sharma and Ingalls 1974) or 1g FA/100g CP (Sharma and Nicholson 1975). There are several reports, as discussed in the literature review, suggesting that FA treatment of SBM reduces the overall digestibility of the protein. Crooker et al. (1982) reported a decrease in apparent N digestibility when lactating cows were fed SBM treated with only 0.3g FA/100g CP. Although there was no significant difference in apparent N digestibilities between the untreated and FA treated diets in the present experiment, the possibility that FA treatment reduced the digestibility of protein in the small intestine cannot be ruled out entirely. The coefficient of variation for apparent N digestibility was fairly high (13.6%) so it is possible that the variation between animals was too high to allow a difference in apparent N digestibilities to be detected. Nevertheless, the results indicate that FA treatment of CM did not affect apparent N digestibility. The results in table 17 and those of Sharma and Ingalls (1974) and Sharma and Nicholson (1975) suggest that treatment of RSM/CM with 0.7 to 1.2g FA/100g CP has little effect on overall N digestibility. This is in contrast to SBM whose N digestibility appeared to be reduced by treatment with 0.3g FA/100g CP (Crooker et al. 1982) and 0.6g FA/100g CP (Spears et al. 1980). Possibly, RSM or CM protein is less sensitive to FA treatment than SBM protein.

The apparent increase in total plasma essential amino acids in response to FA treatment (table 16) strongly suggests that protecting the protein increased the amount of amino acids being absorbed from the gut. This observation is a further indication that decreased protein digestibility was not a major factor in the lack of response to FA treated CM. The response of plasma amino acids to FA treatment was similar to the response obtained by increasing the level of dietary CP, with the notable exceptions of tyrosine and threonine, and was similar to the response reported when casein is abomasally infused into lactating cattle (Clark 1975; Clark et al. 1977) and lactating goats (Ranawana and Kellaway 1977).

Data from experiment 2 suggest that the lack of response to FA treated CM was not caused by underprotection, overprotection, or by the adverse effect of FA treatment on rumen microbial activity. Amino acid uptake from the gut appears to have been increased by raising the level of dietary CP and by treating CM with FA. However, milk yield was increased by raising the level of dietary CP but not by FA treatment (table 14). Discussion will now focus on possible reasons why milk yield was increased by raising the level of dietary CP.

At least part of the increased milk production in response to dietary CP can be accounted for by an increase in DM intake and, therefore, an increased energy intake (figure 2 and table 14). Animals assigned to the high protein diets consumed an additional 3.20 Mcal DE/day while producing an extra 3.3kg 4% FCM. According to NRC (National Research Council 1978) recommendations, these animals would require 4.75 McalDE/day to produce this amount of milk. Therefore, almost 70% of the increased milk yield could be accounted

for by the increased energy intake. In the experiments discussed in the literature review, when milk yield was increased by raising dietary CP from about 13% to a greater level, generally there was an increase in DM intake. There are reports that OM digestibility in the rumina of cattle is increased by raising dietary CP level (Wohlt et al. 1978; Oldham et al. 1979) which implies that rumen microbial activity is increased. Therefore, the response to increased dietary CP level observed by many authors could result from an increased supply of RDP.

However, this is not the only explanation for increased feed intake in response to increased dietary CP level. Journet and Remond (1981) reported that the intake of corn silage was increased when cows were fed FA treated SBM and the response could not be explained by an increased supply of RDP. The authors suggested that an increased feed intake during early lactation may not necessarily be due to increased microbial activity in the rumen. Instead, the higher consumption may be partly due to an increased absorption of amino acids from the small intestine resulting in greater milk production and a greater intake capacity of the cow, where "intake capacity" is defined by Journet and Remond (1981) as "the quantity of food that an animal fed ad libitum can ingest." Presumably, the increased milk production would result from an elevated supply of amino acids to the mammary gland or, possibly, in response to the effects of one or more amino acid elsewhere in the body (Clark 1975).

Thus, there are two possible mechanisms by which an increased supply of dietary protein could increase feed intake, namely (a) an increased microbial activity in the rumen, or (b) an increase in the

intake capacity of the cow resulting from an increased uptake of amino acids into the blood (Verite and Journet 1981). In the present study, the increased DM intake in response to the high protein diets (table 14) does not appear to have resulted from an increase in rumen microbial activity. Dietary CP level had no significant ($p > .1$) effect on total VFA concentration in rumen liquor (table 15) although total VFA concentration tended to be increased by the high protein diets. In addition, dietary CP level did not affect apparent ADF digestibility (table 17) so fibre digestion in the rumen does not appear to have been affected. Estimates of RDP intake provide a further indication that DM intake was not increased by an increase in rumen microbial activity. Animals assigned to diet UL consumed an estimated 1610g RDP/day compared to 1480g RDP/day for animals on diet FH. Yet, cows on UL tended to eat less and produced less milk (table 14).

The explanation of Journet and Remond (1981) seems to be more applicable to the present study. Figure 2 illustrates that milk yields of animals assigned to the high and low protein diets started to diverge approximately 5 weeks post-partum, i.e., week 3 of the experimental period. However, DM intakes did not diverge until 8 weeks post-partum, i.e. week 6 of the experimental period. Using the logic expounded by Journet and Remond (1981), a possible interpretation of these observations is as follows. Animals on the high protein diets absorbed a greater quantity of amino acids. The increased absorption of one or more of these amino acids stimulated an increase in milk production which became apparent after week 3 of the experiment (figure 2). Thus, animals on the high protein diets would

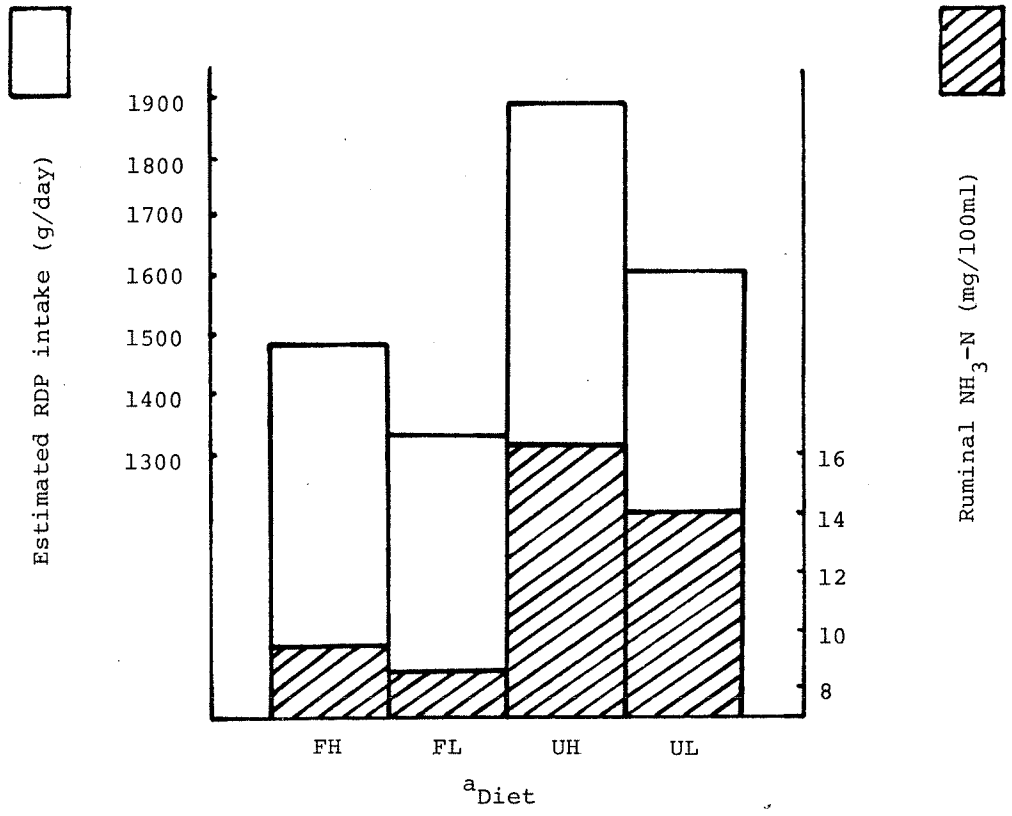
be excreting nutrients at a greater rate than animals on the low protein diets. This may have increased the intake capacity of the cows so that by week 6 of the experiment, animals on the high protein diets had higher DM intakes (figure 2).

In summary, the response of DM intake to the high protein diets could conceivably be due to an increase in rumen microbial activity. However, the experimental results suggest that the response was caused by the effect of an increased uptake of amino acids from the gut.

Results from the digestion study (table 17) indicate that the additional CP of the high protein diets was not utilized efficiently. The increased apparent N digestibility caused by increasing dietary CP level was expected because the proportion of endogenous N in feces decreases as dietary N increases (National Research Council 1978). On the high protein diets, a greater proportion of dietary N was apparently excreted in the urine. This observation is consistent with the elevated concentration of plasma urea (table 16). The elevated plasma urea levels seem to contradict the observation that dietary CP level had no significant effect on ruminal NH_3-N levels (table 15). However, bear in mind that one of the two high protein diets was FA treated and did not contain a high level of RDP. The relationship between ruminal NH_3-N concentration 3h after feeding and the estimated RDP intakes of the indivual diets is illustrated in figure 3. Treatment with FA reduced ruminal NH_3-N levels, but there was no significant difference between treatments FH and FL or between UH and UL. However, ruminal NH_3-N concentration was highly correlated with estimated RDP intake ($r = 0.95$).

Part of the increased plasma urea caused by the high protein

Figure 3. Effect of dietary crude protein level and formaldehyde treated canola meal on the estimated intake of rumen degradable protein (RDP) and ammonia-nitrogen (NH_3-N) concentration of rumen liquor sampled 3h after feeding (experiment 2)



[a]See table 2 for composition of the experimental diets.

diets (table 16) may have been due to catabolism of excess amino acids. So, although raising the level of dietary CP increased the quantity of amino acids being absorbed into the blood and resulted in higher milk production, the absorbed amino acids appear to have been utilized less efficiently. Increased catabolism of amino acids may also explain why FA treatment lowered rumen liquor NH_3-N levels but did not affect plasma urea concentration or the proportion of dietary N apparently excreted in urine. On the FA treated diets, a relatively greater proportion of plasma urea-N may have been derived from catabolism of excess amino acids whereas the contribution of NH_3 absorbed from the rumen may have been quantitatively more important on the low protein diets.

The decrease in plasma levels of glycine, alanine, and serine caused by FA treatment (table 16) may have been due to lower levels of rumen liquor NH_3-N. In the presence of suitable carbon sources, rumen epithelium can convert NH_3 to glycine and alanine in great enough quantities to contribute to their respective plasma pools (Boila and Milligan 1980). Serine is also released but at lower rates. The drop in plasma threonine caused by FA treatment may have been due to a decreased uptake of threonine from the gut or, possibly, activation of serine-threonine dehydratase (EC 4.2.1.16) in liver (Clark 1975). The latter explanation may also account, in part, for the drop in plasma serine levels. Citrulline is an intermediate in the urea cycle. The increased plasma citrulline concentration caused by the high protein diets may have been due to increased activity of the urea cycle. Journet and Remond (1981) reported increased plasma levels of urea cycle amino acids (citrulline + ornithine +

arginine) when lactating cows were fed high protein diets.

The response of plasma levels of essential amino acids to the FA treated diets was similar to that of increasing dietary CP level (table 16). Levels of plasma valine, leucine, isoleucine, and histidine were increased by FA treatment and high dietary CP levels. However, plasma tyrosine was increased only by the high protein diets and plasma threonine was decreased by FA treatment. Levels of the other amino acids were not affected. The similarity in responses suggests that FA treatment did, in fact, increase the uptake of amino acids from the gut.

The significant difference in plasma glucose levels between treatment combinations FH and FL may be an indication that animals on diet FL required less glucose to support the lower level of milk production (Rook 1976). However, there was no corresponding difference in plasma glucose between UH and UL, even though milk yields on these diets were similar to those on FH and FL, respectively.

The preceding discussion can be summarized as follows. Increasing the level of dietary CP or treating the CM with FA appears to have increased the absorption of several amino acids from the gut. Although milk yield was increased by raising the level of dietary CP, FA treatment had no effect on milk production. The lack of response to FA treatment does not appear to have been due to underprotection, overprotection, or a deficiency of RDP. The results suggest that the response of cows to the increased level of dietary CP was mediated by an increased uptake of amino acids from the gut rather than an increase in rumen microbial activity.

The next step is to determine why milk production was not

increased by treating CM with FA. There are two possibilities. Either (a) milk production was not limited by amino acid supply, or (b) FA treatment did not increase absorption of those amino acids which were limiting milk production. The latter explanation does not preclude the suggestion that the response of lactating cows to an increased uptake of amino acids may be partially due to hormonal regulation by specific amino acids (Clark 1975; Ranawana and Kellaway 1977; Oldham et al. 1978).

The suggestion that milk production was not limited by amino acid supply may be correct. A possibility which has not yet been discussed is that the response of cattle to the high protein diets was not due to a higher protein intake. Although the diets were formulated to be isocaloric on the basis of NE content (National Research Council 1978) and the digestion study found no significant difference between the measured DE content of the diets, the actual metabolizable energy (ME) or NEl contents may have been different. This explanation does not seem likely but still remains a possibility. Further evidence that milk production may not be limited by amino acid supply has come from studies using exogenously administered growth hormone (GH). Injection of GH in lactating cattle causes substantial increases in milk production (Machlin 1973; Bines et al. 1980; Peel et al. 1981). Because GH administration appears to increase milk production by partitioning available nutrients towards increased milk synthesis, Fronk et al. (1981) suggested that nutrient availability may not be the limiting factor for milk production under most feeding conditions. Therefore, the amino acid requirements of the cows in experiment 2 may have been met by the untreated diets. The apparent increased uptake

of amino acids caused by FA treatment may have been redundant.

The suggestion that FA treatment did not increase the uptake of those amino acids which were limiting milk synthesis may also be correct. An increased milk production in response to an increased absorption of one or more amino acids could result from an elevated supply of glucogenic amino acids for glucose synthesis, an elevated supply of the amino acid(s) to the mammary gland or from the effects of the amino acid(s) elsewhere in the body (Clark 1975). The observations made in experiment 2 do not provide information about the contribution of glucogenic amino acids to glucose synthesis. However, results from studies where casein and glucose were infused into lactating cows post-ruminally (Clark et al. 1977; Orskov et al. 1977) suggest that the increased milk yield due to casein infusion is not mediated by an increased uptake of glucogenic amino acids.

If the supply of essential amino acids to the mammary gland limited milk synthesis in the present experiment, then, using the approach of Clark (1975), potential candidates as rate limiting amino acids are methionine, phenylalanine, lysine, tryptophan and arginine. There was no change in the plasma levels of these amino acids in response to increased protein level (table 16) in spite of the apparent increase in the plasma level of total essential amino acids, which suggests they were utilized for the additional milk synthesis. Threonine is not included in the list because plasma threonine decreased in response to FA treatment with no effect on milk production. Therefore, FA treatment may not have increased milk production because of failure to increase absorption of one or more of methionine, phenylalanine, lysine, tryptophan or arginine. As

discussed in the literature review, all of these amino acids, with the possible exception of arginine, have been cited at one time or another as being limiting for milk synthesis. Methionine, phenylalanine and lysine were considered limiting by numerous authors as cited by Clark (1975) and Mepham (1982).

Interestingly, the lysine content of CM was reduced by 29% following treatment with FA (table 9). Losses of lysine have been reported elsewhere when protein sources were treated with FA (Reis and Tunks 1973; Faichney 1974; Faichney and White 1979). Lysine reacts readily with FA to form ε -N-methyl lysine (NML) (Reis and Tunks 1973). ε -N-Methyl lysine has been found in the plasma of sheep fed FA treated casein (Carrico et al. 1970; Reis and Tunks 1973). In the present experiment, the decreased lysine content of CM may have resulted from the formation of NML, although canola meal - NML was not measured. Wachira et al. (1974) conducted a growth experiment with rats fed FA treated casein to determine if lysine availability limited animal performance. The results of these authors suggested that lysine availability did not limit the growth rate of rats fed FA treated casein. However, the loss of canola meal-lysine in the present experiment may have prevented a response to the FA treated CM.

An alternative approach is to consider the effect of amino acids on hormonal regulation, particularly the stimulation of GH release. Oldham et al. (1978) reported a 50% increase in plasma GH when lactating goats were infused abomasally with casein. Barry (1980) observed a 74% increase in plasma GH when lactating ewes were abomasally infused with casein. Bines and Hart (1982) found an increase in plasma GH in first lactation cows fed casein or SBM

treated with FA. The amino acid(s) responsible for the increased GH release have not been identified. An obvious candidate is arginine since intravenous injection of arginine is known to stimulate GH release in sheep (Hertelendy et al. 1970). However, Oldham et al. (1978) did not observe an effect on plasma GH when lactating goats were infused abomasally with arginine even though plasma GH levels of the same goats were increased by casein infusion. In experiment 2, failure to increase absorption of the amino acid(s) responsible for increasing plasma GH may account for the lack of response to FA treatment. For an amino acid to influence hormone release, one would expect a change in its plasma level. In the present study, plasma tyrosine was the only essential amino acid to increase in response to increased dietary CP level but not to FA treatment. Possibly, the lack of response to FA treatment may have been due to failure to increase the absorption of tyrosine.

The possibility that tyrosine is involved in the lack of response of lactating cows to FA treated protein supplements has previously been suggested by Australian workers. Sidhu and Ashes (1977) reported a total loss of SBM-tyrosine following treatment with 3g FA/100g CP but only minor losses of tyrosine with NaOH treatment. When the NaOH treated and FA treated SBM were fed to lactating cattle, animals assigned to the NaOH treated diets produced more milk. The authors suggested that the lack of response to FA treated protein supplements may be due to destruction of tyrosine and partial unavailability of other amino acids. Mir et al. (1982b) also reported that cattle fed NaOH treated SBM had higher milk yields than animals fed untreated SBM. Loss of casein tyrosine due to FA treatment has been reported

(Reis and Tunks 1973; Faichney 1974; Sharma and Ingalls 1974) but no effect was observed on the tyrosine content of RSM (Sharma and Ingalls 1974), peanut meal (Faichney 1974) or a concentrate mix treated with FA (Faichney and White 1979).

Tyrosine is the precursor of the catecholamines and plasma tyrosine concentration appears to have an important role in regulating brain catecholamine synthesis in rats (Wurtman 1982). Catecholamines are known to stimulate GH release from the pituitary; therefore, GH release may be stimulated by increased levels of plasma tyrosine.

Another possible role of tyrosine is in appetite control and this also appears to be related to the function of tyrosine as precursor of the catecholamines (Anderson 1979). In monogastric animals, increasing the ratio of tyrosine: phenylalanine in plasma appears to stimulate feed intake, possibly by a catecholamine mediated mechanism (Anderson 1979). However, there is no evidence that this mechanism occurs in ruminants (Baile and Della-Fera 1981).

The possible involvement of tyrosine in the response of lactating animals to an increased supply of protein to the small intestine was investigated in Part II of the study.

Post-experimental observations

The tendency for animals which had been fed the FA treated diets to produce less milk during the post-experimental period (table 18) is in contrast to the report by Folman et al. (1981) that lactating cows tended to produce more milk throughout lactation when fed FA treated SBM. Similarly, animals assigned to the FA treated diets tended to be less fertile, on the basis of number of services for conception (table

18), whereas fertility in Folman's experiment tended to improve. Because of differences in the rate of FA application and the amount of protected protein fed, animals used by Folman's group were consuming up to 2.5g bound FA/day compared with an average of up to 18g/day in the present study. This may account for the contrasting responses. The effect of FA treatment on reproductive performance during the present experiment was not significant ($p > .1$) and may have been an anomaly. Experiment 3 was conducted to investigate further the possible detrimental effect of FA treated CM on fertility.

Effect of FA Treated CM on the Reproductive Performance of Mice

There were 32 litters produced by mice on the untreated diets compared to 29 litters on the FA treated diets (table 19). Three of the mice which did not litter when fed the FA treated diets were confined in the same cage. Possibly, the male in this cage was infertile, and not the three females. If the three females had littered, there would have been 32 litters produced by mice on the FA treated diets. The average litter size tended to be lower on the FA treated diets (table 19) but the difference was not significant ($p > .1$). The results suggest that FA treatment did not impair the reproductive performance of mice.

Based on an application rate of 1.2g FA/100g canola-CP, the mice were consuming up to 0.2mg bound FA/g body weight/day compared to an average of 0.03mg/day for the cows in experiment 2. This line of evidence suggests that the apparent effect of FA treatment on fertility in dairy cattle was an anomaly.

SUMMARY OF PART I

Results from experiments 1, 2, and 3 suggest that treatment of CM with 1.2g FA/100g CP substantially reduces N degradability in the rumen and increases the amount of protein reaching the small intestine. However, feeding the treated CM to cows in early lactation did not increase milk production. The lack of response to FA treatment does not appear to have been caused by underprotection, overprotection, or by a deficiency of RDP. The most likely explanations for the lack of response are (a) amino acid supply did not limit milk production in this study, (b) FA treatment did not increase the absorption of amino acids whose supply to the mammary gland may have limited milk synthesis, particularly lysine, phenylalanine, methionine, tryptophan, and arginine, or (c) FA treatment did not increase the absorption of amino acids, especially tyrosine, which may stimulate milk production indirectly via hormonal regulation. The effect of supplemental tyrosine on milk production was investigated in Part II of the study.

PART II

The Effect of Orally Administered Tyrosine on Plasma and Duodenal Fluid Tyrosine Levels of Sheep, Dry Matter Intake and Milk Production in Holstein Cows, and Feed Intake in Rats and Chicks

INTRODUCTION

The objectives of Part II of the study were firstly, to find a suitable method of increasing tyrosine absorption into the blood and secondly, to determine the effect of increased tyrosine absorption on milk production in dairy cows. It was originally intended that a solution containing tyrosine be infused into the jugular vein of lactating cows. However, during preliminary studies to determine a suitable medium for tyrosine infusion, tyrosine was found to be only sparingly soluble in distilled water or NaCl solution. The solubility of tyrosine in water is 0.45g/l at 25C and 1.05g/l at 50C (Windholz 1976). By interpolation, tyrosine solubility in water was estimated to be 0.72g/l at 39C. A similar calculation for phenylalanine gave a value of 37.0g/l at 39C, i.e., there is a 50 fold difference between the solubilities of tyrosine and phenylalanine in water. However, tyrosine was found to be readily soluble at pH2. This raised the possibility that relatively insoluble tyrosine administered orally could partially escape ruminal degradation and reach the abomasum. The low pH in the abomasum would solubilize any free tyrosine and, presumably, tyrosine would be absorbed into the blood from the small intestine.

Experiment 4 was conducted to determine if, in fact, orally administered tyrosine could partially escape ruminal metabolism, reach the small intestine and be absorbed into the blood. In experiments 5 and 6, tyrosine was fed to dairy cows to determine the effect on milk production. Anderson (1979) reported that the level of dietary

tyrosine can affect feed intake in rats. Experiments 7A, 7B and 7C were conducted to determine the effect of supplemental tyrosine on feed intake in rats and chicks.

MATERIALS AND METHODS

Experiment 4. The Effect of Orally Administered Tyrosine on Plasma and Duodenal Fluid Tyrosine Levels in Sheep.

Disappearance of free tyrosine from nylon bags

A preliminary experiment was conducted to determine the disappearance of tyrosine from nylon bags. Samples of 0.5g tyrosine were placed in nylon bags and suspended in the rumen of a fistulated steer for 24h or 48h. Tyrosine disappearance was measured by the procedure described in experiment 1 for measuring DM disappearance.

Effect of supplemental dietary tyrosine on plasma and duodenal fluid tyrosine levels in cannulated sheep

The experiment was conducted using one sheep and repeated using another. Both sheep were fitted with duodenal cannulae and were kept in metabolism crates. Sheep #1 was fed 1kg alfalfa pellets per day for 7 days (treatment C). The sheep was fed at 9.00 a.m. and 4.30 p.m. every day. For the next 7 days, 6g tyrosine/day was added to the alfalfa pellets as a top dressing (treatment T). This amount of tyrosine is approximately equal to the amount of tyrosine contained in 1.5kg alfalfa pellets (Hubbell 1979). Duodenal fluid and blood samples were taken at approximately 2.00 p.m. daily during the final 5

days of the two periods. Duodenal fluid was collected by opening the cannula and allowing duodenal fluid to flow into a wide mouthed plastic vial. Jugular blood was sampled by venipuncture. When the experiment was repeated with sheep #2, the experimental procedure was identical except (a) the alfalfa pellets were fed continuously by means of a slowly moving conveyor belt which provided 1kg alfalfa pellets/24h, and (b) blood was taken via an indwelling jugular catheter.

Tyrosine assays

Plasma tyrosine was measured using a diagnostic kit (diagnostic kit No. 70-F, Sigma Chemical Company, St. Louis, Missouri) by the procedure described in Sigma Technical Bulletin No. 70-F. The tyrosine content of duodenal fluid was determined in a similar manner to plasma tyrosine but with several modifications. Ten ml duodenal fluid was transferred to a test tube. Six drops of a 37% HCl solution were added by Pasteur pipette to reduce the duodenal fluid pH to approximately 1.0. This step was to ensure complete solubilization of free tyrosine. The duodenal fluid was then centrifuged at 3500 rpm for 1h. The tyrosine content of the supernatant was determined by the procedure described in Sigma Technical Bulletin No. 70-F.

Statistical analyses

Data for the 24h and 48h incubations of the nylon bag study were presented as means with 95% confidence intervals (Parker 1973). For the feeding trial, data from each sheep were analysed separately. Means for the 5 day control and treatment periods were compared using

Student's t-test (Parker 1973).

Experiment 5. Effect of Feeding Supplemental Dietary Tyrosine on Plasma Tyrosine Levels and Milk Production in Holstein Cows During Early-Mid Lactation

Feeding and sampling

Six multiparous Holsteins in early-mid lactation (average 93 days post-partum within a range of 33 to 148 days) were assigned to one of two treatment sequences in a switchback design. The ingredient and nutrient compositions of diet CU (no supplemental tyrosine) and diet TU (50g supplemental tyrosine/day) are shown in table 20. There were three experimental periods of 10 days duration. Three cows were assigned to treatment sequence CU/TU/CU and three to sequence TU/CU/TU. Diet TU was identical to diet CU except 50g/day tyrosine was added to the concentrate as a top dressing. Concentrate and good alfalfa hay were fed ad libitum twice per day in the ratio 59: 41 (DM basis). Refusals were weighed every morning before feeding. Feed intake and milk yields were recorded daily during the final 7 days of the 10 day experimental periods. Composite samples of milk from the morning and afternoon milkings were taken three times during the final 7 days of each experimental period for fat, protein, and lactose analysis. Feed samples were taken once per week and composited biweekly for nutrient analysis. Samples of tail vein blood were taken by venipuncture from each cow 1h after morning feeding on day 9 of each experimental period. Blood plasma was frozen and stored for tyrosine analysis.

Table 20. Nutrient and ingredient composition of the diets used to determine the response of lactating cows to tyrosine supplementation (experiment 5)

	Diet CU
Ingredient composition (% DM)	
Alfalfa hay	41.0
Canola meal	8.2
Rolled barley	46.9
Tallow	1.3
Limestone	0.8
Dicalcium phosphate	0.8
Salt (cobalt-iodized)	0.6
Premix[b]	0.4
Nutrient composition by analysis (% DM)	
Crude protein	18.7
Ether extract	3.2
ADF[c]	15.6
ADF-N[d]	0.5
Gross energy (Mcal/kg)	4.42

[a]Diet TU was identical to diet CU but 50g L-tyrosine/cow/day was added as a top dressing.

[b]Provided per kg of diet:- 3500 IU vit A, 1750 IU vit D, 10IU vit E, 44 mg ZnO, 45mg $MnO_2.H_2O$, 0.3mg $CoCl_2$, 1mg KI, 1.6mg MgO.

[c]Acid detergent fibre.

[d]Acid detergent insoluble nitrogen.

Chemical analyses

Procedures for the analyses of feed and milk samples were described in the materials and methods sections of experiments 1 and 2. Plasma tyrosine was measured by the procedure described in Sigma Technical Bulletin No. 70-F.

Statistical analyses

Data were analysed according to Lucas (1956) for switchback designs with two treatments and three experimental periods.

Experiment 6. Response of Lactating Holstein Cows to Tyrosine Supplementation of Diets Containing Formaldehyde Treated Canola Meal

Feeding and sampling

Twelve multiparous cows in early lactation (average 58 days post-partum within a range of 39 to 89 days) were assigned to one of 12 treatment sequences in a Lucas design (Lucas 1956) as described in table 21. Diets UC and FC were identical except UC contained untreated CM whereas FC contained CM treated with 1.2g FA/100g CP (table 22). The method of FA application was described in the materials and methods section of experiment 2. Diets UT and FT were identical to diets UC and FC, respectively, except tyrosine was supplemented at the rate of 50g/cow/day. Treatment periods lasted 14 days and during this time concentrate, corn silage and brome-alfalfa hay were fed to each cow ad libitum in the ratio 52: 43: 5 (DM

Table 21. Plan of treatment sequences used in experiment 6.

Cow #	Days post-partum	[a]Treatment sequence
1	87	UT: UC: UT
2	87	UC: UT: UC
3	67	UC: FC: UC
4	67	FC: UC: FC
5	62	FC: FT: FC
6[b]	-	FT: FC: FT
7	48	FT: UT: FT
8	44	UT: FT: UT
9	54	UT: FC: UT
10	39	FC: FT: UC
11	53	UC: FT: UC
12	41	FT: UT: FT

[a]See table 22 for the nutrient and ingredient composition of diets UT and UC.

[b]Missing value calculated for this animal.

Table 22. Nutrient and ingredient composition of the diets used to determine the response of lactating cows to tyrosine supplementation (experiment 6)

	Treatment			
	UT	UC	FC	FT
Ingredient composition (% DM)				
Corn silage	42	42	42	42
Alfalfa hay	5	5	5	5
Barley	29.8	29.8	29.8	29.8
Canola meal	17.0	17.0	--	--
FA-Canola meal[a]	--	--	17.0	17.0
Tallow	2.3	2.3	2.3	2.3
Limestone	1.2	1.2	1.2	1.2
Dicalcium phosphate	1.1	1.1	1.1	1.1
Urea	0.6	0.6	0.6	0.6
Salt (cobalt-iodized)	0.6	0.6	0.6	0.6
Premix[b]	0.4	0.4	0.4	0.4
Tyrosine (g/cow/day)[c]	50	--	--	50
Nutrient composition by analysis (% DM)				
Crude protein	18.2	18.2	17.4	17.4
Ether extract	4.8	4.8	4.8	4.8
ADF[d]	18.9	18.9	18.6	18.6
ADF-N[e]	2.0	2.0	2.1	2.1
Calcium	0.9	0.9	0.9	0.9
Phosphorus	0.7	0.7	0.7	0.7
Gross energy (M cal/kg)	4.45	4.45	4.44	4.44

[a]Canola meal treated with 1.2g formaldehyde/100g crude protein.

[b]Provided per kg diet: 3500 IU vit A, 1750 IU vit D, 10 IU vit E, 44 mg ZnO, 45mg $MnO_2.H_2O$, 0.3mg $CaCl_2$, 1mg KI, 1.6mg MgO.

[c]L-Tyrosine (Sigma Chemical Company, St. Louis, Missouri).

[d]Acid detergent fibre.

[e]Acid detergent insoluble

basis). Concentrate and silage were fed as a total mix. Hay was added separately. Measurements were taken during the final 7 days of each treatment period. For diets UT and FT, 50g tyrosine was added as a top dressing to the concentrate-silage mixture. Feed refusals were weighed every morning before feeding. Feed intake and milk yields were recorded daily during the final 7 days of each treatment period. Composite samples of milk from morning and afternoon milkings were taken three times during the final 7 days of each period for fat, protein and lactose analysis. Feed samples were taken once per week and composited biweekly for nutrient analysis. Samples of tail vein blood were taken by venipuncture from each cow 1h after morning feeding on day 13 of each period. Blood plasma was frozen and stored for amino acid analysis.

Chemical analyses

Procedures for the analyses of feed and milk samples were described in the materials and methods sections of experiments 1 and 2. The amino acid composition of deproteinated blood plasma was determined using a Beckman model 119C amino acid analyser.

Statistical analyses

The animal assigned to treatment sequence FT/FC/FT contracted mastitis during the experiment, so data from this cow were not included in the analysis of variance. A missing value was calculated by the procedure suggested by Lucas (1956). Analysis of the data was made according to Lucas (1956) for switchback designs with four treatments and three experimental periods. Orthogonal comparisons

were made according to Snedecor and Cochran (1980).

Experiment 7A. Effect of Dietary Tyrosine Supplementation on Feed Intake and Weight Gain in Weanling Rats

Sixty male, Sprague-Dawley rats were obtained from Central Animal Care Services, University of Manitoba. The rats were supposedly weanlings aged approximately 21 to 30 days; however, the large variation in liveweights (25 to 90g) suggested that many of the rats were older than 30 days. Upon their arrival, the rats were weighed, placed in cages (1 rat per cage) and assigned to the control diet, RC (table 23). The rats were fed this diet for a 6 day adjustment period after which they were reweighed. Ten rats which did not appear to be performing as well as the others were removed at this time. Because of the variability in liveweights between rats, the animals were paired according to liveweight. One rat from each pair was assigned to diet RC, the other to RT (table 23). The experimental diets were fed ad libitum in pellet form for 10 days, after which time the rats were reweighed. Feed intake during the 10-day experimental period was measured, average daily gain was determined, and the ratio of feed intake: liveweight gain was calculated.

The data were analysed by the general linear model procedures of SAS (SAS Institutes Inc., 1979) using liveweight gain in the pre-experimental period as a covariate. Analysis of covariance was made because, by chance, rats which were subsequently assigned to diet FT tended to have higher liveweight gains during the pre-experimental period. During the experiment, two rats became ill so analysis of

Table 23. Ingredient composition of the diets used to determine the effect of dietary tyrosine supplementation on feed intake of rats and chicks (experiments 7A, 7B, and 7C).

Ingredient (g/100g)	Experiments 7A and 7B	Experiment 7C
	[a]RC	[e]FC1
FA-Canola meal[b]	12.5	17.0
Barley	35.3	34.0
Corn	50.0	45.0
Tallow	0.7	--
Dicalcium phosphate	0.5	1.0
Limestone	0.5	0.8
Lysine HCl	--	0.4
Methionine	--	0.2
Vitamin premix	[c]0.25	[f]1.0
Mineral premix	[d]0.25	[g]0.5

[a]Diet RT was identical to RC but was supplemented with 0.25g L-tyrosine per 100g diet.

[b]Canola meal treated with 1.2g formaldehyde/100g crude protein.

[c]Provided per kg diet: 3750 IU vit A, 125 IU vit D, 5 IU vit E, 0.75 mg vit K, 212mg choline chloride, 7.5mg niacin, 1mg riboflavin, 0.25mg pyridoxine HCl, 0.15mg folic acid, 25mg biotin, 1.25mg vit B12, 2.1mg pantothenic acid, 1.5g methionine.

[d]Provided per kg diet: 85mg MnO, 7.5mg ZnO, 15mg $FeSO_4.7H_2O$, 13mg $CuSO_4.5H_2O$, 2.4g NaCl (Iodized).

[e]Diets FC2, FC3 and FC4 were identical to FC1 but were supplemented with 0.2, 0.4 and 0.6g tyrosine/100g diet, respectively.

[f]Provided per kg diet: 8250 IU vit A, 180 IU vit D, 1.1 IU vit E, 2.3mg vit B12, 10mg menadione, 1g Pen-Strep, 250mg santoquin, 500mg DL-methionine, 17mg niacin, 180mg choline, 6mg riboflavin, 11mg pantothonic acid.

[g]Provided per kg diet: 170mg MnO, 14mg ZnO, 31mg $FeSO_4.7H_2O$, 25mg $CuSO_4.5H_2O$, 10g NaCl (iodized).

covariance was performed on 23 pairs of animals.

Experiment 7B. Effect of Dietary Tyrosine Supplementation on Feed Intake and Weight Gain in Weanling Rats

Experiment 7B was conducted to check the results of experiment 7A because the initial liveweights of rats used in experiment 7A were extremely variable causing possible flaws in the experimental design. Sixty male, Sprague-Dawley rats were obtained from Central Animal Care Services, University of Manitoba. This time, however, the liveweights were fairly uniform. The experimental procedures were identical to those of experiment 7A with the following exceptions. (1) The rats were assigned to diets RT and RC (table 23) such that the mean liveweights of animals on the two treatments were equal. (2) The adjustment (pre-experimental) period lasted 4 days. (3) The experimental period lasted 14 days. (4) Statistical analyses were made using Student's t-test as described by Parker (1973). Data were obtained from 23 rats per treatment.

Experiment 7C. Effect of Supplemental Dietary Tyrosine on Feed Intake and Weight Gain in Chicks.

Experiment 7C was conducted to further test the influence of supplementary tyrosine on feed intake. One day old White Leghorn cockerels were obtained from a commercial hatchery. Upon their arrival, the chicks were confined in a thermostatically controlled battery and fed a standard chick starter diet (21% CP) for a 4 day

adjustment period. The chicks were then divided into 24 groups of five such that the total liveweight of each group was approximately equal. The birds were starved for 4h prior to weighing. Each group of five chicks was considered a single experimental unit. The groups were randomly assigned to one of four experimental diets (table 23) and housed in thermostatically controlled batteries equipped with wire floors and continuous lighting. There was one group of chicks per cage unit. The birds had unlimited access to food and water. The ingredient composition of the diets is shown in table 23. All diets were identical except 0, 2, 4, and 6g tyrosine/kg diet were added to diets FC1, FC2, FC3, and FC4, respectively. Food intake and liveweight gain of each group during the 7 day experimental period were recorded. One way analysis of variance was performed according to Snedecor and Cochran (1980).

RESULTS

Experiment 4

Tyrosine disappearance from nylon bags

The amount of tyrosine which disappeared from nylon bags was relatively low (table 24) suggesting a slow rate of degradation in the rumen. This reflects the low solubility of tyrosine in water (0.72g/l).

Table 24. Disappearance of free tyrosine from nylon bags (experiment 4)

Time in rumen	Number of samples	Percent disappearance	95% confidence limits
24h	12	37.7	5.8
48h	6	73.1	8.3

Plasma and duodenal fluid tyrosine levels of sheep fed supplemental tyrosine

When tyrosine was fed to sheep #1, the level of tyrosine in duodenal fluid increased ($p < .086$) by 25% (table 25). In sheep #2, the increase ($p < .062$) was 50%. Because of the method by which duodenal fluid tyrosine was measured, the 25% and 50% increases do not take into account the tyrosine content of microbial protein or undigested food protein. The increases represent the rise in soluble tyrosine in duodenal fluid. Generally, the level of duodenal fluid tyrosine appeared to be much higher in sheep #2 than sheep #1, though the differences were not compared statistically. The coefficients of variation for duodenal fluid tyrosine concentration were high in both cases (17% and 29% for sheep #1 and sheep #2, respectively), reflecting a high variability between individual samples. In spite of the apparent increase in duodenal fluid tyrosine concentration, plasma tyrosine levels were not affected by dietary supplementation with tyrosine (table 25).

Experiment 5

Milk production, DM intake and plasma tyrosine levels of cows fed supplemental tyrosine

Supplementing the diet with tyrosine did not appear to affect the plasma tyrosine concentration of lactating dairy cows, although there was a non-significant increase of 10% (table 26). Dietary treatment had no effect on DM intake. However, supplementing the diet with tyrosine caused an increase ($p < .051$) in milk production of approximately 1kg milk/day. There was no effect on 4% FCM yield, milk composition, or milk fat yield but the yields of milk protein and

Table 25. Tyrosine concentration of blood plasma and duodenal fluid from sheep fed supplemental tyrosine (experiment 4)

	Sheep #1				Sheep #2			
	[a]Treatment				[a]Treatment			
	C	T	SE	[b]P	C	T	SE	[b]P
Plasma tyrosine (mg/100ml)	1.3	1.3	0.1	[c]NS	1.4	1.3	0.1	[c]NS
Duodenal fluid tyrosine (mg/100ml)	9.2	11.5	0.7	0.086	24.9	37.5	4.1	.062

[a]Treatment C: 1kg alfalfa pellets/day. Treatment T: 1kg alfalfa pellets plus 6g tyrosine/day.

[b]Probability that the difference between the treatments means is equal to zero.

[c]Difference between treatment means was not significant ($p > .1$).

Table 26. Plasma tyrosine levels, feed intake, milk yield and milk composition of cows fed supplemental dietary tyrosine (experiment 5)

	Treatment		SE	[b]P
	[a]CU	TU		
Plasma tyrosine (μmole/l)	82	91	6	[c]ns
Dry matter intake (kg/day)	22.4	22.9	.7	ns
Milk yield (kg/day)	29.3	30.3	.3	.051
4% FCM (kg/day)[d]	25.1	26.3	1.0	ns
% milk fat	3.03	3.11	.17	ns
Fat yield (g/day)	891	943	57	ns
% milk protein	3.16	3.15	.05	ns
Protein yield (g/day)	923	950	10	.054
% lactose	4.86	4.86	.02	ns
Lactose yield (g/day)	1424	1469	17	.059

[a]See table 20 for nutrient and ingredient composition of experimental diets.

[b]Probability that the difference between treatment means is equal to zero.

[c]Difference between treatment means was not significant (p> .1).

[d]4% fat corrected milk.

lactose were increased by tyrosine supplementation ($p < .054$ and $p < .059$, respectively), reflecting the increased milk yield.

Experiment 6

Amino acid composition of FA treated CM used in experiments 6, 7A, 7B, and 7C

Treating CM with 1.2g FA/100g CP reduced the lysine content by 18.5% (table 27). There was only a slight decrease in the tyrosine content (7.7%). The phenylalanine content appeared to increase by 12.9% and methionine appeared to increase by 36.2%.

Milk composition, DM intake and plasma amino acid composition of cows fed FA treated CM and supplemental tyrosine

Dry matter intake was not affected by dietary treatment (table 28). In addition, there was no effect on 4% FCM yield, milk composition, or the yields of milk fat and milk protein (table 28). There were significant differences in milk yields ($p < .05$) and lactose yields ($p < .01$) between diets FC and FT. However, differences between diets UT and UC were not significant. Comparing the two FA treated diets with the two untreated diets (comparison U vs F in table 28) showed that FA treatment had no effect on milk production.

The concentration of several plasma amino acids were affected by dietary treatment (table 29). Formaldehyde treatment increased plasma valine ($p < .01$), isoleucine ($p < .05$) and histidine ($p < .05$), and tended to increase ($p < .1$) the concentrations of leucine and total essential amino acids. The response of plasma amino acids to FA

Table 27. Amino acid composition of untreated and formaldehyde (FA) treated canola meal (experiments 6, 7A, 7B, and 7C).

Amino acid[a]	Canola Meal	
	Untreated	FA treated
Lysine	6.10	4.97
Histidine	2.96	2.93
Arginine	6.86	7.35
Aspartic acid	8.27	8.40
Threonine	4.54	4.74
Serine	4.22	4.12
Glutamic acid	19.21	18.51
Proline	6.54	6.52
Glycine	5.60	5.80
Alanine	4.91	5.01
Cystine	2.62	2.73
Valine	6.05	6.00
Methionine	2.32	3.16
Isoleucine	4.54	4.28
Leucine	7.93	7.77
Tyrosine	2.86	2.64
Phenylalanine	4.49	5.07

[a] g/100g amino acid.

Table 28. Effect of dietary tyrosine supplementation on dry matter (DM) intake, milk yield and milk composition of cows fed diets containing formaldehyde treated canola meal (experiment 6).

	[a]Treatment				SE	Orthogonal Comparisons		
	UT	UC	FT	FC		U vs F	UT vs UC	FT vs FC
DM intake (kg/day)	20.9	20.5	21.0	20.5	0.6	[b]NS	NS	NS
Milk yield (kg/day)	32.9	32.4	33.1	31.5	0.5	NS	NS	c
4% FCM (kg/day)[e]	29.5	29.7	28.8	29.6	1.6	NS	NS	NS
% milk fat	3.31	3.41	3.19	3.57	0.29	NS	NS	NS
Fat yield (g/day)	1090	1118	1041	1132	106	NS	NS	NS
% milk protein	2.95	3.00	3.02	3.03	0.08	NS	NS	NS
Protein yield (g/day)	968	972	1002	946	36	NS	NS	NS
% lactose	4.74	4.66	4.75	4.60	0.10	NS	NS	NS
Lactose yield (g/day)	1560	1518	1583	1451	35	NS	NS	d

[a]See table 22 for nutrient and ingredient composition of experimental diets.

[b]No difference between treatment means ($p > .05$).

[c]Difference between treatment means ($p < .05$).

[d]Difference between treatment means ($p < .01$).

[e]4% fat corrected milk.

Table 29. Effect of dietary tyrosine supplementation on plasma amino acid concentrations of cows fed diets containing formaldehyde treated canola meal (experiment 6)

	[a]Treatment				SE	Orthogonal comparisons		
	UT	UC	FT	FC		U vs F	UT vs UC	FT vs FC
Plasma amino acids (μmoles/l)								
Nonessential								
Aspartic acid	22	20	22	20	2	[b]NS	NS	NS
Asparagine	45	42	49	36	8	NS	NS	NS
Glutamic acid	139	156	148	110	40	NS	NS	NS
Glutamine	680	572	590	438	175	NS	NS	NS
Proline	134	113	147	149	21	NS	NS	NS
Glycine	415	383	392	412	45	NS	NS	NS
Alanine	292	292	312	276	43	NS	NS	NS
Citrulline	110	94	123	103	15	NS	NS	NS
Ornithine	89	80	86	91	7	NS	NS	NS
Serine	105	83	99	78	6	NS	c	c
Essential								
Tyrosine	59	52	68	51	11	NS	NS	NS
Phenylalanine	45	41	55	44	5	NS	NS	NS
Threonine	144	117	159	117	21	NS	NS	e
Valine	269	268	366	329	27	c	NS	NS
Isoleucine	133	130	179	141	18	d	NS	e
Leucine	162	160	217	181	23	e	NS	NS
Methionine	32	30	34	28	3	NS	NS	e
Tryptophan	7	7	6	9	1	NS	NS	NS
Lysine	116	110	131	101	18	NS	NS	NS
Histidine	61	52	79	66	8	d	NS	NS
Arginine	109	87	118	98	11	NS	e	NS

Table 29 continued. Effect of dietary tyrosine supplementation on plasma amino acid concentrations of cows fed diets containing formaldehyde treated canola meal (experiment 6)

	[a]Treatment				SE	Orthogonal Comparisons		
	UT	UC	FT	FC		U vs F	UT vs UC	FT vs FC
Plasma amino acids (μmoles/l)								
Essential								
EAA[f]	1137	1054	1412	1165	129	e	NS	NS
Urea cycle AA[g]	299	255	335	292	30	NS	NS	NS
Phenylalanine: tyrosine	0.77	0.79	0.81	0.83	0.09	NS	NS	NS
LNAA[h]: tyrosine	11.3	12.4	12.6	14.2	1.3	NS	NS	NS

[a]See table 22 for composition of experimental diets.

[b]No difference between treatment means ($p > .1$).

[c, d, e]Difference between treatment means (c: $p < .01$; d: $p < .05$; e: $p < .1$).

[f]Total essential amino acids.

[g]Ornithine + citrulline + arginine.

[h]Tryptophan + phenylalanine + valine + leucine + isoleucine.

treatment was virtually identical to the response observed in experiment 2 (table 16). Compared to treatment UC, treatment UT caused an increase ($p < .01$) in plasma serine and tended to increase ($p < .1$) plasma arginine. Treatment FT increased plasma serine concentration ($p < .01$) and tended to increase ($p < .1$) the concentration of plasma threonine, isoleucine and methionine, compared to treatment FC. Although plasma tyrosine tended to be higher on the two high tyrosine diets, i.e., diets UT and FT, the increases were not significant ($p > .1$).

Experiment 7A

Unadjusted treatment means

Analysis of variance suggested that supplemental dietary tyrosine increased ($p < .01$) feed intake by 7.9% (table 30). There was also an increase ($p < .05$) in average daily gain of 8.4%. The ratio of feed intake: liveweight gain was not affected by dietary treatment. The initial growth rate, i.e., growth rate during the pre-experimental adjustment period, of rats which were subsequently assigned to diet RT tended to be higher than the growth rate of rats assigned to diet RC. The difference was significant only at $p < 0.14$. However, as a precautionary measure, the statistical analysis was repeated using initial growth rate as a covariate.

Adjusted treatment means

When the data were analysed by analysis of covariance using initial growth rate as a covariate (table 30), the differences in

average daily gain and feed: gain ratios between treatments were found to be non-significant ($p > .1$). However, feed intake still tended to be increased ($p < .1$) by supplemental dietary tyrosine. The results do not conclusively show whether or not tyrosine supplementation of the diet affected feed intake.

Experiment 7B

Supplemental dietary tyrosine had no effect ($p > .1$) on feed intake, average daily gain, or feed: gain ratio (table 31).

Experiment 7C

Supplemental dietary tyrosine had no effect ($p > .1$) on feed intake, average daily gain, or feed: gain ratio in chicks (table 32).

Table 30. Effect of supplemental dietary tyrosine on feed intake, liveweight gain and feed: gain ratio of rats in experiment 7A

	Unadjusted Treatment Means		SE	
	[a]RC	RT		
Feed intake (g/day)	16.38	17.68	0.29	c
Average daily gain (g/day)	4.87	5.28	0.16	d
Feed: gain ratio	3.38	3.37	0.06	[e]NS
[b]Initial growth rate (g/day)	5.10	5.46	0.17	NS
	[f]Adjusted Treatment Means		SE	
	[a]RC	RT		
Feed intake (g/day)	16.64	17.41	0.30	d
Average daily gain (g/day)	4.97	5.19	0.16	NS
Feed: gain ratio	3.39	3.36	0.06	NS

[a]See table 23 for ingredient composition of experimental diets.

[b]Growth rate during the 6 day adjustment period.

[c]Difference between treatment means ($p < .01$).

[d]Difference between treatment means ($p < .1$).

[e]No difference between treatment means ($p > .1$).

[f]Adjusted by covariance for initial growth rate.

Table 31. Effect of supplemental dietary tyrosine on feed intake, liveweight gain and feed: gain ratio of rats in experiment 7B

	Treatment Means		SE	
	[a]RC	RT		
Feed intake (g/day)	15.48	15.15	0.43	[b]NS
Average daily gain (g/day)	3.89	3.79	0.16	NS
Feed: gain ratio	4.09	4.10	0.12	NS

[a]See table 23 for ingredient composition of experimental diets.

[b]No difference between treatment means ($p > .1$).

Table 32. Effect of supplemental dietary tyrosine in feed intake, liveweight gain and feed: gain ratio of chicks in experiment 7C

	Treatment Means				SE	
	[a]FC1	FC2	FC3	FC4		
Feed intake (g/day)	10.57	11.12	10.56	11.20	0.28	[b]NS
Average daily gain (g/day)	2.73	2.93	2.81	3.20	0.18	NS
Feed: gain ratio	3.89	4.00	3.82	3.46	0.19	NS

[a]See table 23 for ingredient composition of the experimental diets.

[b]No differences among treatments ($p > .1$).

DISCUSSION

Response of Plasma Tyrosine Concentration, DM Intake and Milk Production to Oral Administration of Tyrosine

Oral administration of tyrosine as a method of increasing tyrosine absorption

The slow rate of tyrosine disappearance from nylon bags (table 24) is an indication that the rate of degradation of tyrosine in the rumen was low. However, this is not proof that orally administered tyrosine would not be totally degraded in the rumen. When fed in a practical situation, tyrosine would be able to mix fully with the rumen contents so that the concentration of tyrosine would be diluted considerably. In the nylon bag study, tyrosine was not free to permeate throughout the rumen but remained concentrated in one location (i.e., in the bags). Thus, the *in situ* disappearance of tyrosine from nylon bags may underestimate the actual degradability of tyrosine *in vivo*.

There is no evidence that tyrosine in solution is slowly degraded in the rumen. Chalupa (1976) added tyrosine and some non-essential amino acids to viable rumen fluid in vitro. The concentration of tyrosine in the rumen fluid was 1.6mM. The apparent degradation of tyrosine after 6h was 73% compared to 82% for serine, 90% for alanine, and 97 to 98% for the other amino acids in the medium. When tyrosine and several essential and non-essential amino acids were added to viable rumen fluid, apparent degradation of tyrosine after 6h was only

19%. This compared with 29% for ornithine, 43% for methionine, 49% for valine and 69 to 100% for the other amino acids. The author (Chalupa 1976) suggested that the extremely low apparent degradability of tyrosine was due to the contribution of tyrosine derived from catabolism of phenylalanine. Even so, the results suggest that when there is a ready supply of tyrosine and phenylalanine in the rumen, tyrosine is not rapidly removed from the medium. Scheifinger et al. (1976) studied amino acid degradation by pure cultures of rumen bacteria. Of the seven bacterial species studied, three did not degrade tyrosine at all, one species degraded 7% of the added tyrosine, and the remaining three species degraded approximately 40% of the supplementary tyrosine after 24h incubations. Of the other 15 amino acids studied, only methionine degradation was lower.

Thus, tyrosine administered orally could conceivably escape ruminal metabolism in two ways. (a) Some tyrosine could escape degradation by passing through the rumen without going into solution. (b) Tyrosine in solution may partially escape metabolism because tyrosine appears to be degraded relatively slowly in the rumen (Chalupa 1976).

The daily administration of 6g tyrosine/day to sheep in experiment 4 was roughly equivalent to the estimated tyrosine content of 1.5kg alfalfa pellets (Hubbell 1979), i.e., the tyrosine supplement increased tyrosine intake by approximately 150%. A large dose was administered to increase the likelihood that a measurable amount of tyrosine would escape metabolism in the rumen. Supplemental dietary tyrosine increased duodenal fluid tyrosine in sheep #1 by 25% and sheep #2 by 50% (table 25), though the increases were significant only

at $p < .086$ and $p < .062$, respectively. The results indicate that some orally administered tyrosine did, in fact, escape metabolism in the rumen and reach the small intestine.

However, there was no indication that plasma tyrosine concentration was increased (table 25). Possibly, (a) there was no increased absorption of tyrosine, or (b) the absorbed tyrosine was rapidly cleared from the blood. When Clark et al. (1977) infused casein post-ruminally into lactating cattle, the concentrations of all the essential amino acids in plasma were increased with the exceptions of threonine, phenylalanine, and tyrosine. Similarly, Vik-Mo et al. (1974) did not observe an increase in plasma tyrosine concentration following abomasal infusion of casein into lactating cows. Ranawana and Kellaway (1977) reported no increase in plasma tyrosine concentration when goats were abomasally infused with casein. Thus, there is evidence that plasma tyrosine concentration is not particularly responsive to an increased supply of tyrosine to the small intestine. It is not clear if the lack of response in these studies was due to a high rate of clearance of tyrosine from the blood or failure of the tyrosine to be absorbed. Other authors have reported an increase in plasma tyrosine concentration in cattle following abomasal infusion of casein (Derrig et al. 1974; Spires et al. 1975; Rogers et al. 1979). Apparently, plasma tyrosine concentration can, in fact, respond to an increased supply of tyrosine to the small intestine. The conflicting results discussed above suggest that plasma tyrosine concentration is not necessarily indicative of an increased supply of tyrosine to the small intestine.

A primary object of experiment 5 was to study the effect

of feeding free tyrosine on the plasma tyrosine concentration of lactating cattle. In experiment 5, the supplemental tyrosine was estimated to increase tyrosine intake by about 50%. This is approximately equal to the amount of tyrosine contained in 3.5kg SBM (Hubbell 1979). Once again, tyrosine supplementation did not appear to increase plasma tyrosine concentration (table 26) though there was a non-significant increase of about 10%. However, there were small but significant increases in milk yield ($p < .051$), milk protein yield ($p < .054$) and lactose yield ($p < .059$) in response to tyrosine supplementation. Possibly, the response was mediated by the effect of an increased supply of tyrosine on microbial activity in the rumen, as discussed in a later section. A more likely explanation is that the cows responded to an increased uptake of tyrosine into the blood. This will also be discussed in more detail later. Thus, there is reason to believe that dietary supplementation with tyrosine did result in an increased absorption of tyrosine.

The response of plasma tyrosine to oral supplementation of tyrosine in experiment 6 was similar to the response observed in experiment 5. Plasma tyrosine levels did not increase significantly ($p > .1$) in response to orally administered tyrosine though there was a non-significant difference of 23% between the supplemented and unsupplemented diets (table 29). Several parameters were affected by tyrosine supplementation; namely, milk yield, lactose yield (table 28), and the plasma concentrations of several amino acids (table 29). Possibly, the response of these parameters was due to an increased uptake of tyrosine.

Results from experiments 4, 5, and 6 do not provide conclusive

evidence that oral administration of a large dose of tyrosine increased the absorption of tyrosine into the blood. However, indirect evidence, i.e., increased duodenal fluid tyrosine levels in sheep and increased milk production in dairy cows, suggests that tyrosine supplementation did increase the uptake of tyrosine by the animals.

Effect of formaldehyde treatment on the tyrosine content of canola meal

Treating CM with FA apparently reduced the tyrosine content by 57% in Part I of the study (table 9) but only by 8% in Part II (table 27). Reasons for the discrepancy are not clear. The apparent loss of tyrosine, particularly in Part I, may have been an artefact. Gruber and Mellon (1968) reported that the presence of FA during acid hydrolysis of protein samples interferes with subsequent quantitation of tyrosine during amino acid analysis. However, this observation does not rule out the possibility that some irreversible modification of tyrosine occurs during storage of FA treated protein supplements. Barry (1976) reported that the phenol group of tyrosine is methylolated by FA. The question then is whether the methylolation reaction is freely reversible. If the reaction of tyrosine with FA during storage of FA treated protein supplements is the same as that reported by Gruber and Mellon (1968) for tyrosine and FA during acid hydrolysis, then the reaction would appear not to be completely reversible. Therefore, tyrosine availability may be decreased by FA treatment. However, there is some evidence suggesting that the apparent loss of tyrosine during amino acid analysis is wholly an artefact caused by the presence of FA during acid hydrolysis of the

treated protein. Mortensen (1981) measured the tyrosine content of grass juice preserved with FA and noted a large decrease in tyrosine. The author suggested that the loss of tyrosine occurred during hydrolysis of the juice with HCl and not during preservation of the juice, based on preliminary experiments. However, no account was given of the nature of the preliminary experiments. In the present study, a firm conclusion cannot be reached as to whether the apparent loss of CM-tyrosine observed in experiment 2 (table 9) and to a lesser extent in experiment 6 (table 27) was due to irreversible binding of FA during storage of the treated CM, or an artefact caused by the presence of FA in the acid hydrolysis medium prior to amino acid analysis. In experiment 2, plasma tyrosine was the only essential amino acid whose concentration was increased by feeding the high protein diets but not by FA treatment (table 16) suggesting that tyrosine availability was increased by high dietary CP level but not by FA treatment.

There are several reports, as discussed in the literature review, of an apparent loss of tyrosine following treatment of protein supplements with FA. The loss of tyrosine in these studies may or may not have been an artefact. Other authors did not observe an effect of FA treatment on the tyrosine content of protein supplements. Perhaps, tyrosine was truly not affected by FA in these studies. Alternatively, the modified tyrosine product, possibly a methylol form of tyrosine (Barry 1976), may not have clearly separated from tyrosine during amino acid analysis. The chromatogram peak of the modified tyrosine molecule appears to be closely associated with the phenylalanine and tyrosine peaks. The phenylalanine and tyrosine

peaks are normally very close together so the modified tyrosine molecule could easily be hidden by either of the two former molecules. Incomplete separation of phenylalanine from the modified tyrosine molecule may explain why the phenylalanine content of CM was apparently increased by FA treatment in experiments 2 and 6 (tables 9 and 27).

In conclusion, there is some doubt as to whether treatment of CM with FA increased the absorption of tyrosine by cows in experiments 2 and 6. Feeding the protected protein may not necessarily cause a decrease in tyrosine absorption. However, if tyrosine availability limited milk production in experiments 2 and 6, failure to increase tyrosine absorption could account for the lack of response to FA treatment.

Effect of dietary tyrosine supplementation on the performance of lactating cows

The aim of part II of the study was to determine if the lack of response to FA treatment in experiment 2 could be due to failure to increase the absorption of tyrosine. Results from experiment 5 are interesting because they showed that milk production can be increased by supplementing the diet with a large dose of tyrosine (equal to 50% of the basal tyrosine intake). In this experiment, the basal diet contained 18.7% CP (table 20) which was somewhat higher than planned. The animals consumed 4.2kg CP/day on diet CU and 4.3kg CP/day on diet TU which is about 50% greater than the amount suggested by the National Research Council (1978) for cows at this level of production. Even so, the animals produced more milk in response to tyrosine

supplementation (table 26). The dietary ingredients used in experiment 5 contain a high proportion of RDP compared to REP (Fisher 1981). Therefore, a high proportion of tyrosine in the basal diet was probably degraded in the rumen.

In experiment 6, the response of cows to FA treated CM (comparison U vs. F in table 28) was similar to the response observed in experiment 2 (table 14), i.e., FA treatment did not improve milk production. Tyrosine supplementation of the FA treated diet (comparison FT vs. FC in table 28) significantly increased milk yield ($p < .05$) and lactose yield ($p < .01$). This observation is consistent with the hypothesis that the lack of response to FA treated CM in experiment 2 was due to failure to increase the absorption of tyrosine.

Supplementing tyrosine to the untreated diet (comparison UT and UC in table 28) did not affect milk production. A possible explanation is that diet UC provided an adequate amount of tyrosine to the small intestine whereas the amount supplied by diet FC was inadequate. In this event, diet FC would benefit from tyrosine to a greater extent than diet UC. In terms of milk production, tyrosine supplementation of the untreated diet produced a non-significant ($p > .1$) increase in milk yield of 0.5kg/day whereas tyrosine supplementation of the FA treated diet resulted in a significant ($p < .05$) increase of 1.6kg/day. However, a major criticism of this explanation is the high protein degradability of the ingredients of diet UC (Fisher 1981). Based on the response of milk production in experiment 5 (table 26), one would expect diet UC to benefit from tyrosine supplementation just as diet CU benefitted from tyrosine supplementation in experiment 5. Reasons for this inconsistency are not clear.

In experiment 6, dietary treatment affected the plasma concentrations of several amino acids (table 29). The response of plasma essential amino acids to FA treated CM was similar to the response observed in experiment 2 (table 16). Formaldehyde treatment increased the plasma concentrations of valine ($p < .01$), isoleucine ($p < .05$), leucine ($p < .1$) and histidine ($p < .05$). In addition, the concentration of total essential amino acids in plasma tended to increase ($p < .1$). This suggests that FA treatment increased the uptake of amino acids into the blood and is consistent with the results of experiment 2. Formaldehyde treatment did not affect the plasma concentrations of any non-essential amino acids in experiment 6. This is in contrast to the response in experiment 2 when the concentrations of plasma glycine, serine, and alanine were decreased by FA treatment. Since the diets in experiments 2 and 6 were very similar, there is no obvious reason for the differing responses of the plasma non-essential amino acids between the two experiments.

In experiment 6, supplemental dietary tyrosine increased ($p < .01$) the plasma concentration of serine and tended to increase ($p < .1$) threonine, isoleucine, methionine, tryptophan and arginine (table 29). No explanation can be offered regarding the cause or significance of this response, but a change in plasma amino acid profile suggests that amino acid metabolism was altered in some way.

In summary, results from experiments 5 and 6 suggest that milk yield can be increased by supplementing a large dose of tyrosine to the diet. In experiment 6, only the FA treated diet benefitted from tyrosine supplementation and this observation supports the contention that the lack of response to FA treated CM observed in experiment 2 may have been due to failure to increase the absorption of tyrosine.

Possible mechanisms of tyrosine stimulation of milk production

There are several possible mechanisms by which tyrosine supplementation could increase milk production. These are (a) stimulation of rumen microbial activity resulting in increased OM digestion in the rumen, (b) an increased supply of tyrosine to the mammary gland for milk synthesis, and (c) the effect of tyrosine on hormonal regulation. These will be discussed in turn.

(a) Stimulation of microbial activity. Although tyrosine was administered orally with the object of increasing the supply of tyrosine to the small intestine, there is no doubt that a substantial portion of supplemental tyrosine was degraded in the rumen. Since rumen bacteria appear to have a requirement for some preformed amino acids, as discussed in the literature review, the supplemental dietary tyrosine may have stimulated microbial activity causing an increase in OM digestion. There is only scant evidence linking tyrosine availability to microbial growth. Maeng and Baldwin (1976a, b) reported an increase in rumen bacterial activity in response to infusion of a solution containing a mixture of 18 amino acids, including tyrosine. However, the response could have been mediated by any combination of the amino acids. Maeng et al. (1976) found an increase in bacterial activity following addition of tyrosine and seven non-essential amino acids to the incubation medium. Once again, though, the response need not have been due to tyrosine. Based on results from a study in which N^{15}-labelled amino acids were infused into the rumina of steers, Salter et al. (1979) concluded that the rumen bacterial population have a minimum requirement for tyrosine, phenylalanine and methionine, though the authors presumed that the

requirement for tyrosine could be met from phenylalanine. From the above observations, the increased milk production observed in experiments 5 and 6 may conceivably have been due to stimulation of rumen microbial activity by tyrosine. However, there is at least one major criticism of this explanation. In experiment 5, the basal diet (i.e., diet CU) contained 18.7% CP, most of which was estimated to be highly degradable (Fisher 1981). Therefore, the supply of tyrosine to the microbial population would already be substantial so supplementary tyrosine would not be expected to stimulate microbial growth. An alternative explanation is that the tyrosine supplement provided an additional amount of N for microbial growth. This explanation is even more unlikely. Fifty g of tyrosine contains 7.73g N. The basal diet in experiment 5 provided 670g N/cow/day, therefore, tyrosine supplementation increased the N intake by only 1.2%. In addition, the basal diet contained a high level of RDP, so the level of ruminal NH_3 -N is not likely to have limited microbial growth. In conclusion, there is little reason to suppose that the response of milk production in experiments 5 and 6 was mediated by the effect of tyrosine on bacterial activity in the rumen.

(b) Milk yield may have been increased due to an elevated supply of tyrosine to the mammary gland. Tyrosine is considered a non-essential amino acid for growth in rats (Rose 1938) because it can be synthesized from phenylalanine in the presence of phenylalanine hydroxylase (EC 1.14.16.1). However, the enzyme does not appear to be present in significant amounts in mammary tissue (Davis and Mepham 1976). In addition, Verbeke et al. (1972) reported that C^{14}-labelled phenylalanine activity was not transferred to tyrosine during a goat

mammary perfusion study at physiological concentrations of phenylalanine. Mepham (1982) reported that plasma tyrosine was the sole precursor of tyrosine in milk protein. Given the high demand for tyrosine and phenylalanine for milk synthesis (Mepham 1982) and the apparent inability of the mammary gland to convert phenylalanine to tyrosine, an adequate supply of exogenous tyrosine is probably essential for maximum milk synthesis. In reviewing many studies which attempted to identify amino acids whose availabilities limited milk synthesis, Mepham (1982) concluded that the stoichiometric transfer of tyrosine, phenylalanine, methionine and tryptophan from blood to milk makes these amino acids prime candidates for limiting milk synthesis. These amino acids were designated group I amino acids by Mepham (1982). Group I may also include lysine and threonine. In an earlier review, Swan (1979) suggested that tyrosine, phenylalanine, methionine and lysine were group I amino acids. In several studies discussed by Clark (1975) in which amino acids were administered abomasally or intravenously to lactating animals, the response of milk yield was not as great as the response observed when casein was infused abomasally. However, in all but one of these studies, tyrosine was not included in the amino acid infusate. Tyrosine may have been omitted because it has been classified as non-essential for growth in rats.
Alternatively, tyrosine may not have been included because of its low solubility. Linzell and Mepham (1974) intravenously infused a mixture of amino acids in lactating goats. Tyrosine was included in the amino acid infusate but only at half the concentration of the other amino acids because of its low solubility. The milk yield of one goat was increased, but the amino acid infusion had no effect on the other two

goats. Although tyrosine was part of the amino acid infusate, the nature of the response suggested that the availability of methionine, threonine, and tryptophan limited milk synthesis.

In experiment 2 of the present study, primary candidates as rate limiting amino acids, i.e., amino acids whose availability to the mammary gland limited milk synthesis, were phenylalanine, methionine, lysine, threonine, and arginine. The basis for this conclusion was discussed in Part I. The supply of tyrosine to the mammary gland did not appear to limit milk synthesis. Since the diets in experiment 6 were very similar to those in experiment 2 (tables 2 and 22), there is no reason to suppose that tyrosine supply to the mammary gland limited milk synthesis in experiment 6. In conclusion, there is evidence that tyrosine availability to the mammary gland can limit milk synthesis (Mepham 1982). However, results from experiment 2 suggest that tyrosine supply to the mammary gland did not limit milk production in the present study. Therefore, the response of milk production to tyrosine supplementation in experiments 5 and 6 does not appear to have been mediated by an increased supply of tyrosine to the mammary gland.

(c) Effect of tyrosine on hormonal regulation. As discussed in the literature review, administration of tyrosine may affect milk production by the action of catecholamines. For this to occur, tyrosine transport across the blood-brain barrier would have to increase (Wurtman 1982). Apparently, a prerequisite for an increased uptake of tyrosine by brain neurons is an increase in plasma tyrosine in relation to the other LNAA. The plasma ratios of phenylalanine: tyrosine and LNAA: tyrosine were not significantly affected by

dietary treatment in experiment 6. However, given the high coefficients of variation for these parameters (17.0 and 16.6% for LNAA: tyrosine and phenylalanine: tyrosine, respectively), the results cannot be regarded as conclusive evidence that tyrosine uptake by the brain was not increased.

Since administration of tyrosine increased the behavioural activity of mice (Gibson et al. 1982), probably by a catecholamine mediated action, a similar behavioural response may have occurred in cattle in experiments 5 and 6. No objective test of animal activity was made in these two experiments. However, even if there were subtle changes in the behavioural activity of the cows, this is unlikely to have been responsible for the increased milk production.

Increased noradrenalin activity in the brain, due to an increased uptake of tyrosine, can stimulate or suppress a general release of adrenalin and noradrenalin from the peripheral nervous system and adrenal medulla resulting in a change in blood flow (Wurtman 1982). Milk production is dependent on the rate of blood flow through the mammary gland (Mepham 1982). Therefore, tyrosine administration in experiments 5 and 6 may conceivably have increased the flow of blood through the mammary gland resulting in an increase in milk production.

An alternative possibility is that the response to tyrosine administration in experiments 5 and 6 was mediated by an increased feed intake. Anderson (1979) reported that feed intake of rats was increased by supplemental dietary tyrosine. Tyrosine supplementation did not appear to affect DM intake in experiments 5 and 6 (tables 26 and 28). However, an increased DM intake of about 0.5 to 1.0kg/day could provide enough energy to increase milk production by 1 to

2kg/day (National Research Council 1978). An increase in DM intake of only 0.5 to 1.0 kg/day, i.e., an increase of about 2.5 to 5.0%, may be difficult to detect using small numbers of cows. Since tyrosine supplementation is known to influence food intake in rats (Anderson 1979), diets which had a similar ingredient content to the diets in experiment 6 were formulated and fed to rats in experiments 7A and 7B. The major difference between the diets was the replacement of corn silage with corn grain (tables 22 and 23). Results from experiment 7A (adjusted means in table 30) suggest that food intake tended to increase with tyrosine supplementation. However, because of the large variability in initial weights of the rats, there is some doubt as to the validity of these results. When the experiment was repeated with other rats (experiment 7B), tyrosine supplementation had no effect on food intake (table 31). This observation is in contrast to the report by Anderson (1979) that tyrosine supplementation increased the energy intake of rats by an average of 30% in four experiments. The environmental temperature was lower during experiment 7B than 7A and this probably explains why the average daily gains were lower in experiment 7B (tables 30 and 31). Possibly, the lower environmental temperature prevented a response of food intake to tyrosine supplementation. However, there appeared to be little difference in food intake between experiments 7A and 7B so food intake does not appear to have been much affected by the change in environmental temperature. The results of experiment 7B suggest that the response of food intake to tyrosine supplementation observed in experiment 7A was an anomaly and that tyrosine supplementation did not affect food intake.

To study further the effect of dietary tyrosine supplementation, tyrosine was fed to growing chicks (experiment 7C). The diets were very similar to those used in experiments 7A and 7B (table 23). Tyrosine supplementation did not affect food intake or weight gain in chicks (table 32). In summary, although there is evidence that tyrosine supplementation increases feed intake in rats (Anderson 1979), in the present study tyrosine supplementation did not appear to influence feed intake of cows or chicks and produced a questionable response in rats.

The potential influence of tyrosine on GH release was discussed in the literature review. Several amino acids are known to stimulate GH release in humans (Knopf et al. 1965) and sheep (Hertelendy et al. 1970; Davis 1972) when administered intravenously in pharmacological doses. In reviewing the literature, no reports were found regarding the effect of intravenous tyrosine administration on GH release. However, there is circumstantial evidence which strongly suggests that plasma tyrosine concentration could affect GH release. Catecholamines, particularly noradrenalin and dopamine, in the brain are known to stimulate the release of GH from the pituitary (Martin 1980). Intravenous injection of L-DOPA, an intermediate in the synthesis of noradrenalin and dopamine from tyrosine (figure 1), stimulates GH release in humans (Lal et al. 1975). This response is thought to be due to an increased synthesis of noradrenalin and/or dopamine. Since the enzyme which catalyses the conversion of tyrosine to L-DOPA (tyrosine hydroxylase, figure 1) in brain neurons is not fully saturated (Wurtman 1982), an increased supply of tyrosine to neurons in the brain will stimulate the synthesis of L-DOPA (Wurtman

1982), and, possibly, GH release.

Growth hormone appears to be a potent stimulator of milk production. Machlin (1973) reported an 18% increase in milk yield following injection of GH in dairy cows. Bines et al. (1980) and Peel et al. (1981) also observed dramatic increases in milk yield with no effect on feed intake in response to GH administration. The mechanism of GH action appears to involve a repartitioning of available nutrients towards milk production. Fronk et al. (1981) injected GH into dairy cows which were abomasally infused with a solution of casein plus glucose. Abomasal infusion of casein plus glucose increased milk yield by 3.5%. Injection of GH in addition to abomasal infusion of casein plus glucose increased milk yield by 16.8%. Injection of GH alone increased milk yield by 15.2%. Thus, nutrient availability did not appear to limit milk yield. However, GH somehow partitioned nutrients towards milk synthesis.

Possibly, the response to tyrosine administration in experiments 5 and 6 of the present study was mediated by stimulation of GH secretion. Dry matter intake did not appear to be affected by dietary treatment and the small increases in milk production would be consistent with a small increase in GH release (tables 26 and 28). Unfortunately, plasma GH concentrations were not measured in the experiments. Further studies are required to determine the response of plasma GH concentration to administration of tyrosine.

SUMMARY OF PART II

Oral administration of tyrosine appears to have increased the amount of tyrosine reaching the small intestine of sheep but did not affect plasma tyrosine concentration. In experiment 5, dairy cows which were fed an alfalfa hay/barley/CM diet produced 3.4% more milk in response to oral administration of tyrosine. Plasma tyrosine concentration was not significantly ($p > .1$) affected. In experiment 6, tyrosine supplementation of a corn silage/barley/FA treated CM diet increased milk yield by 5.1%. This response is consistent with the contention that FA treatment of CM did not elicit an increased production because of failure to increase the absorption of tyrosine. Tyrosine supplementation of the corn silage/barley/untreated CM diet had no effect on milk yield or plasma tyrosine concentration. This is in contrast to the increased milk production observed in experiment 5 when tyrosine was supplemented to the diet. Reasons for the inconsistency are not clear. The response to tyrosine supplementation in experiments 5 and 6 may have been due to stimulation of microbial activity in the rumen or to an increased supply of tyrosine to the mammary gland. However, these explanations are not considered likely. A more attractive explanation is that the increased milk production was mediated by a tyrosine induced elevation of catecholamine synthesis in the brain. Three mechanisms were postulated by which an increase in catecholamine activity in the brain could stimulate milk production, namely (a) an increased flow of blood to the mammary

gland, (b) an increase in feed intake, and (c) stimulation of GH release. The latter explanation is preferred because catecholamines have been shown to stimulate GH release (Martin 1980) and GH is known to be a potent stimulator of milk production (Bines et al. 1980; Peel et al. 1981).

GENERAL DISCUSSION

There is no obvious reason why dairy cows do not respond to FA treated plant proteins. Of the 10 studies discussed in the literature review where FA treated plant proteins were fed to dairy cows, only Verite and Journet (1977) and Kaufmann and Lupping (1979) reported an increase in milk production. In the present study, FA treatment had no effect on milk production in two separate experiments. Therefore, FA treatment of plant proteins has not increased milk production in 10 of 12 experiments. There is little doubt that dairy cattle can respond to an increased supply of protein to the small intestine. Abomasal infusions of casein have consistently increased milk production (Clark 1975; Clark et al. 1977; Orskov et al. 1977). In addition, there have been eight experiments using FA treated casein and milk yield was increased in all but two of these studies. Presumably, feeding FA treated casein increased the absorption of the amino acid(s) which limited milk production whereas feeding protected plant protein did not.

The most commonly cited reason for a lack of response to FA treated plant protein is a decrease in protein digestibility in the small intestine (Clark et al. 1974; Wachira et al. 1974; Tamminga 1979; Crooker et al. 1982). Crooker et al. (1982) reported that FA treatment of SBM reduced apparent N digestibility from 65.4 to 62.4% and the authors suggested that the small decrease in protein digestibility prevented an increase in milk yield. However, a small decrease in overall protein digestibility does not necessarily mean

that amino acid absorption was not increased. If the diet contains a large amount of RDP, apparent N digestibility will probably be high. But, much of the digested N will be converted to NH_3, absorbed into the blood and excreted as urea. Therefore, amino acid absorption may be low even though apparent N digestibility is high. When feeding protected protein, a small decrease in overall protein digestibility may be acceptable if amino acid absorption is increased. The frequent lack of response to FA treated plant proteins cannot adequately be explained by a decrease in protein digestibility unless the level of FA application was excessively high.

The suggestion by Sidhu and Ashes (1977) that the poor response of dairy cows to FA treated plant proteins is due to a loss of tyrosine is promising. Results from experiment 6 of the present study suggest that the value of FA treated CM is enhanced by supplemental dietary tyrosine. Possibly, the lack of response to FA treated CM was partly due to failure to increase the absorption of tyrosine.

Given the consistent lack of production responses, the continued use of FA treatment as a method of increasing the supply of protein to the intestine is questionable. The potential risks of handling and feeding FA do not appear to be justified. Reducing protein degradability by other chemical treatments, e.g. NaOH treatment, may be more beneficial. Sidhu and Ashes (1977) and Mir et al. (1982b) fed NaOH treated SBM to dairy cows and noted an increase in milk production.

The response of milk yield to oral administration of tyrosine is itself an interesting observation. Possibly, milk yield was increased by the effect of tyrosine on rumen microbial activity, an increased

supply of tyrosine to the mammary gland, a behavioural response to tyrosine, an increased flow of blood to the mammary gland, or an increased feed intake. However, the most attractive explanation is that tyrosine administration increased milk yield by stimulating the release of GH.

Abomasal infusion of casein has increased plasma GH concentration in lactating goats (Oldham et al. 1978) and lactating ewes (Barry 1980). Therefore, the response of lactating animals to abomasal infusion of casein may be due to stimulation of GH release rather than an increased supply of amino acids to the mammary gland. Fronk et al. (1981) showed that abomasal infusion of casein plus glucose did not enhance the response of lactating cows to GH injection. This implies that the supply of nutrients to the mammary gland did not limit milk synthesis. Rather, milk production was limited by the inability of the animals to utilize available nutrients. These observations led Fronk et al. (1981) to question the concept of limiting nutrients.

There is another line of indirect evidence suggesting that tyrosine may be the amino acid responsible for the increased plasma GH concentration following abomasal infusion of casein. As discussed previously, abomasal infusion of casein consistently increased milk production in dairy cows (Clark 1975; Clark et al 1977; Orskov et al. 1977). However, abomasal or intravenous infusions of amino acids generally have not stimulated milk yield to the same extent as casein infusion. Clark (1975) reviewed several studies which investigated the effect of post-ruminal administration of various combinations of amino acids. With the exception of one study, post-ruminal administration of several combinations of essential amino acids did

not appear to stimulate milk production. This was in spite of the fact that the basal diets contained only 10.8 to 12.0% CP and were, presumably, protein deficient (National Research Council 1978). The study reported by Schwab et al. (1976) was particularly extensive. In a series of experiments, abomasal infusions of casein appeared to stimulate a higher level of milk production than abomasal infusions of various combinations of amino acids. These observations imply that casein infusion increased the uptake of an amino acid which was not present in the amino acid infusates. With the exception of tyrosine, all the essential amino acids were present in one or more of the amino acid infusates. Therefore, at least part of the increased milk production following abomasal infusion with casein may be due to an increased uptake of tyrosine.

This argument can be taken a stage further. As discussed in the literature review, raising dietary CP level from less than 13% to more than 13% usually causes an increase in milk yield. The increased milk yield is usually accompanied by an increase in DM intake. A reasonable explanation of these results is that the higher level of dietary CP caused an increase in rumen microbial activity. When dietary CP level has been increased from 13% or more to a greater level, results have been more variable. Of the 21 studies discussed in the literature review, milk yield was increased in 10. If, as suggested by Satter and Roffler (1975), the N requirements of the rumen microorganisms are normally met by diets containing 12 to 13% CP, then the response to higher levels of dietary CP is probably not due to an increase in rumen microbial activity. Current concepts of ruminant protein physiology suggest that the response is due to an

increased supply of amino acids to the small intestine leading to an increased uptake of amino acids into the blood (Satter 1981). The increased supply of amino acids to the small intestine is derived from undigested dietary protein and the increased milk yield has been assumed to be due to an increased supply of amino acids to the mammary gland (Kaufmann 1977; Satter 1982). However, results from studies discussed in the previous two paragraphs, particularly those of Fronk et al. (1981), suggest the response of lactating animals to an increased supply of amino acids may not necessarily be due to an increased supply of so called "limiting" amino acids to the mammary gland. Instead, the response may be mediated by stimulation of GH release by one or more amino acids. Therefore, the increased milk production reported by several authors when dietary CP was raised from 13% or more to a greater level may have been due to the effect of a specific amino acid on GH release rather than an increased supply of amino acids to the mammary gland.

The amino acid(s) responsible for the increased plasma GH concentration following abomasal infusion with casein (Oldham et al. 1978; Barry 1980) have not been identified. However, a list of potential candidates can be compiled from the results of studies in which plasma GH response to intravenous infusions of amino acids was measured. Intravenous infusions of arginine, histidine, lysine, methionine, and phenylalanine consistently increased plasma GH levels in humans but the response to leucine, valine, isoleucine and threonine was inconsistent or insignificant (Knopf et al. 1965). Hertelendy (1970) reported an increase in plasma GH concentration in response to intravenous infusion of arginine in sheep. Davis (1972)

infused arginine and leucine in sheep and observed an increase in plasma GH concentration but infusion of phenylalanine had no effect. The physiological significance of these studies is not clear because of the pharmacological doses of amino acids. However, if the results are taken at face value, they suggest that one or more of arginine, histidine, lysine, phenylalanine, leucine, or methionine could be responsible for the casein induced increase in plasma GH concentration. The effect of tyrosine and tryptophan infusions on GH release do not appear to have been studied so these amino acids will also be added to the list of potential candidates. Oldham et al. (1978) found that the plasma GH levels of lactating goats were increased by abomasal casein infusion but not by abomasal infusions of arginine, methionine, or phenylalanine. Therefore, these three amino acids will be removed from the list of potential candidates. In experiments 2 and 6 of the present study, FA treatment appeared to increase the plasma concentrations of histidine and leucine (tables 16 and 29) but milk yield was not affected. Therefore, histidine and leucine will also be removed from the list. The remaining amino acids which can be considered potential mediators of the stimulation of GH release are tyrosine, lysine, and tryptophan. Of these three amino acids, there is a considerable amount of circumstantial evidence suggesting that tyrosine could stimulate GH release by a catecholamine mediated mechanism, as discussed previously.

The potential exists to stimulate GH release by feeding protected protein. In their review, Bines and Hart (1982) reported an experiment in which plasma GH concentration of first lactation heifers was increased by feeding FA treated SBM and FA treated casein. Milk

production was not affected, possibly because the increase in plasma GH concentration was not great enough to stimulate a detectable increase in milk yield. Actual levels of plasma GH were not reported so the relative responses of plasma GH to the two protein supplements cannot be compared. If the response of plasma GH concentration to the FA treated protein supplements was mediated by an increased uptake of tyrosine, then the response of GH to FA treated SBM appears to contradict the suggestion that treatment of CM with FA in the present study did not increase milk yield because of failure to increase the absorption of tyrosine. However, the effect of FA treatment on the tyrosine content of protein supplements has been variable, as discussed previously, and there is some doubt as to how much, if any, tyrosine is actually made unavailable by FA treatment. It remains to be seen if other forms of protected protein, e.g. NaOH treated SBM (Sidhu and Ashes 1977; Mir et al. 1982b), can stimulate an increase in plasma GH concentration.

There is one final observation which deserves comment. Majdoub et al. (1978) reported that milk yield of dairy cows fed high protein diets (15.3% CP) was increased by 4.9 kg/day (21%) when cottonseed meal was replaced by corn gluten meal on an equal protein basis. The corresponding increase of cows on the low protein diets (12.6% CP) was 3.1 kg/day (14%). Grieve and Forster (1982) reported that milk yield of cows fed diets containing 14% CP was increased by 3.4 kg/day (11%) when SBM was replaced by corn gluten meal. The estimated ruminal protein degradabilities of corn gluten meal, SBM and cottonseed meal are 45%, 70%, and 70%, respectively (Satter 1981). Possibly, the response of milk yield to corn gluten meal was mediated by an

increased supply of "limiting" amino acids to the mammary gland. However, there is an alternative explanation. Corn gluten meal contains approximately 50% more tyrosine than SBM or cottonseed meal (Hubbell 1979), on a protein basis. Because of differences in the ruminal protein degradabilities between corn gluten meal, cottonseed meal and SBM, the former protein supplement could supply about three times more tyrosine to the small intestine than the latter two supplements for a given protein intake. Possibly, the response to corn gluten meal was mediated by an increased uptake of tyrosine.

In conclusion, the following hypothesis is proposed. Under most feeding systems, when cows are fed diets containing 13 to 14% CP, the amino acid supply to the mammary gland does not limit milk synthesis. When additional protein is provided, an increase in milk production is due largely to stimulation of GH release by a specific amino acid, possibly tyrosine, rather than an increased supply of "limiting" amino acids to the mammary gland. The additional protein may be provided by increasing the level of dietary CP, replacing a highly degradable protein supplement with one which is less degradable, or by abomasal casein infusion. There is little direct evidence to support this proposal at present but the concept may provide a basis for future study.

CONCLUSIONS

1. Cows fed high protein diets (17.4% CP) produced more milk ($p < .05$) than cows fed low protein diets (14.6% CP).
2. Feeding FA treated CM to lactating cows lowered ruminal NH_3-N levels ($p < .05$), tended to increase ($p < .1$) the plasma concentrations of total essential amino acids, and had no effect on the apparent digestibilities of DM, N, or ADF.
3. Cows fed FA treated CM did not produce more milk than cows fed untreated CM. The lack of response did not appear to be due to over- or underprotection of the CM, or to a deficiency of RDP for optimal microbial activity. The lack of response to FA treatment may have been due to failure to increase the uptake of a specific amino acid, possibly tyrosine or lysine.
4. The persistent lack of response to FA treated plant proteins in the present study and in other studies suggests there is little justification for the inclusion of FA treated plant proteins in commercial dairy rations.
5. Tyrosine administered orally appeared to partially escape ruminal metabolism in sheep, though there is little doubt that oral administration of free tyrosine is an extremely inefficient method of increasing the supply of tyrosine to the duodenum.
6. Feeding supplemental tyrosine to dairy cows caused an increase in milk production, particularly when the diet contained FA treated CM.

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APPENDIX

Table 1A. Effect of formaldehyde (FA)[a] and heat[b] treatments on nitrogen solubility of fully extracted canola meal (CM), partially extracted canola meal (PCS) and crushed canola seeds (CS) in 10% Burroughs Mineral Mixture (BMM) and NaCl solution (individual data)

		Canola Product		
Solvent	Treatment	CM	PCS	CS
10% BMM	Untreated	37.2	39.5	30.7
		34.6	42.5	23.7
		38.7	44.6	33.1
	Heat	2.5	6.1	0.1
		0.9	0.1	2.8
		4.1	1.2	3.1
	FA	-0.8	-2.9	1.7
		2.4	3.7	0.7
		1.7	1.3	0.4
NaCl	Untreated	47.1	54.7	38.3
		41.1	54.7	39.5
		43.8	50.2	41.6
	Heat	4.9	2.3	2.1
		7.2	1.1	0.1
		1.3	4.2	0.9
	FA	1.3	1.9	0.9
		2.7	0.7	-3.1
		0.9	1.5	1.7

[a] 1.2gFA/100g crude protein

[b] Autoclaved for 5 min at 121C

Table 2A. Nitrogen solubility of the lactation diets in NaCl solution (experiment 2,individual data)

Ingredients	Nitrogen solubility(%)		
Concentrates			
FH	16.8	18.0	15.3
FL	20.4	18.3	19.6
UH	29.4	-	31.2
UL	31.6	34.2	32.3
Corn silage	40.9	34.2	32.3
Brome hay	28.9	29.6	27.3

Table 3A. Dry matter, nitrogen and non-nitrogen dry matter (NNDM) disappearance of the lactation diets from nylon bags (experiment 2, individual data)

	Percent disappearance					
Ingredients	Dry matter		Nitrogen		NNDM	
Concentrates						
FH	47.8	50.2	39.1	41.7	50.1	52.5
FL	53.7	57.9	48.9	51.9	54.2	58.7
UH	59.5	58.6	60.2	58.0	58.1	57.8
UL	62.4	59.4	64.9	62.7	60.9	57.6
Corn silage	48.8	53.4	52.1	47.9	48.6	53.8
Brome hay	41.9	43.5	45.1	47.3	41.4	42.9

Table 4A. Response of various parameters to two levels of dietary crude protein and formaldehyde treated canola meal (experiment 2, individual data)

Treatment	Cow#	Liveweight gain (kg/day)	DM Intake (kg/day)	CP Intake (kg/day)	Milk yield (kg/day)
	1	-0.44	22.8	3.96	39.1
	2	-1.12	20.1	3.50	38.5
	3	0.49	24.7	4.29	33.6
	4	-0.50	17.8	3.10	32.6
	5	-0.33	22.9	3.98	35.7
FH	6	0.35	19.7	3.43	32.2
	7	-0.23	19.2	3.34	30.8
	[a]8	0.07	14.1	2.45	22.5
	[a]9	-0.17	17.5	3.04	29.3
	10	0.29	21.6	3.76	35.0
	11	0.19	18.9	2.76	28.4
	[a]12	0.00	18.5	2.71	21.0
	13	-0.05	16.8	2.45	27.1
	14	-0.01	16.1	2.35	29.3
	15	-0.79	19.5	2.84	37.0
FL	[b]16	-	-	-	-
	17	0.25	18.1	2.65	30.5
	18	-0.08	18.7	2.73	28.5
	19	-0.23	18.9	2.76	29.6
	[a]20	-0.04	16.2	2.37	30.2
	21	-0.42	20.9	3.61	42.6
	22	-0.10	20.9	3.62	32.5
	23	-0.46	17.8	3.09	31.1
	24	-0.05	20.6	3.57	36.0
	25	0.02	20.4	3.53	36.1
UH	[a]26	0.67	16.7	2.89	27.5
	27	0.24	18.4	3.19	30.8
	28	-0.17	17.9	3.10	34.4
	29	0.02	18.6	3.22	35.8
	[a]30	-0.51	17.1	2.96	27.8
	[a]31	0.11	18.1	2.66	24.7
	32	-0.06	19.8	2.90	30.4
	33	-0.74	19.1	2.80	26.4
	34	-0.50	17.3	2.53	34.6
	35	0.33	20.5	3.01	32.0
UL	36	0.05	18.3	2.69	35.0
	37	-0.38	16.1	2.36	31.2
	[a]38	-0.19	16.7	2.45	28.6
	39	-0.35	20.8	3.05	37.3
	40	-0.67	17.9	2.63	32.9

[a]First lactation cows [b]Missing data

Table 4A continued. Response of various parameters to two levels of dietary crude protein and formaldehyde treated canola meal (experiment 2, individual data)

Treatment	Cow#	4% FCM yield (kg/day)	% milk fat	Fat yield (kg/day)	% milk protein (kg/day)
	1	40.1	4.17	1.63	3.02
	2	37.8	3.87	1.49	2.85
	3	34.0	4.06	1.37	2.86
	4	35.5	4.63	1.51	2.86
	5	41.0	4.99	1.78	2.91
FH	6	32.9	4.18	1.35	3.05
	7	25.3	2.82	0.87	2.83
	[a]8	20.9	3.54	0.79	2.81
	[a]9	30.6	4.37	1.28	3.10
	10	33.1	3.57	1.25	2.65
	11	27.4	3.87	1.10	2.86
	[a]12	22.0	4.30	0.90	3.26
	13	30.3	4.77	1.29	2.97
	14	32.1	4.45	1.30	2.95
	15	39.0	4.39	1.62	3.01
FL	[b]16	-	-	-	-
	17	32.4	4.39	1.34	3.01
	18	26.7	3.56	1.01	3.05
	19	28.9	3.83	1.13	2.92
	[a]20	27.4	3.44	1.04	2.92
	21	41.6	3.84	1.64	2.94
	22	33.6	4.22	1.37	3.01
	23	31.0	4.00	1.25	2.85
	24	39.6	4.65	1.67	3.05
	25	35.5	3.91	1.41	2.90
UH	[a]26	29.6	4.79	1.31	3.10
	27	28.4	3.48	1.07	2.98
	28	34.1	3.88	1.33	2.84
	29	33.3	3.56	1.28	2.82
	[a]30	28.0	4.04	1.12	2.97
	[a]31	23.7	3.75	0.92	3.12
	32	28.1	3.49	1.06	3.31
	33	27.9	4.41	1.16	3.21
	34	35.2	4.17	1.44	2.66
	35	34.5	4.49	1.44	3.07
UL	36	34.1	3.83	1.34	2.76
	37	23.9	2.55	0.80	2.89
	[a]38	26.4	3.52	1.01	3.06
	39	34.4	3.50	1.31	2.88
	40	34.8	4.40	1.45	2.95

[a]First lactation cows [b]Missing data

Table 4A continued. Response of various parameters to two levels of dietary crude protein and formaldehyde treated canola meal (experiment 2, individual data)

Treatment	Cow#	Protein yield (kg/day)	% lactose	Lactose yield (kg/day)	Milk iodide (µg/l)
FH	1	1.18	5.08	1.98	471
	2	1.10	4.92	1.90	305
	3	0.96	4.81	1.62	263
	4	0.93	4.74	1.55	462
	5	1.04	4.76	1.70	224
	6	0.98	4.89	1.57	290
	7	0.87	4.77	1.47	243
	[a]8	0.63	5.09	1.14	230
	[a]9	0.91	4.91	1.44	331
	10	0.93	4.85	1.70	257
FL	11	0.81	4.85	1.38	316
	[a]12	0.69	4.86	1.02	437
	13	0.81	4.91	1.33	324
	14	0.86	4.77	1.40	178
	15	1.11	4.71	1.74	405
	[b]16	-	-	-	-
	17	0.92	4.77	1.46	538
	18	0.87	4.87	1.39	325
	19	0.86	4.86	1.44	377
	[a]20	0.88	4.68	1.41	281
UH	21	1.25	4.84	2.06	1050
	22	0.98	5.02	1.63	316
	23	0.89	4.72	1.47	211
	24	1.10	4.82	1.73	290
	25	1.05	4.85	1.75	342
	[a]26	0.85	4.74	1.30	229
	27	0.92	5.08	1.56	394
	28	0.98	4.73	1.63	240
	29	1.01	4.79	1.72	258
	[a]30	0.83	5.00	1.37	208
UL	[a]31	0.77	4.91	1.21	244
	32	1.01	4.91	1.49	248
	33	0.85	5.23	1.38	240
	34	0.92	4.45	1.54	251
	35	0.98	4.77	1.53	679
	36	0.97	4.85	1.70	225
	37	0.90	4.75	1.47	230
	[a]38	0.88	5.23	1.50	376
	39	1.07	4.94	1.84	231
	40	0.97	5.00	1.64	300

[a]First lactation cows

[b]Missing data

Table 5A. Effect of dietary protein level and formaldehyde treated canola meal on ammonia-nitrogen (NH_3-N) and volatile fatty acid concentrations of rumen liquor sampled immediately before feeding (experiment 2, individual data)

Treatment	Cow#	NH_3-N (mg/100ml)	Acetate (mM)	Propionate (mM)	Butyrate (mM)
FH	1	8.9	45.6	13.0	7.4
	2	4.5	60.1	15.4	11.2
	3	2.4	62.4	19.0	11.6
	4	10.0	27.8	6.1	3.7
	5	6.7	13.0	3.4	2.2
	6	6.3	16.4	4.9	3.0
	7	4.8	43.8	10.4	5.3
	[a]8	3.7	41.3	10.3	6.1
	[a]9	4.2	42.2	14.3	9.6
	10	5.6	41.6	11.6	6.6
FL	11	2.7	41.5	10.5	5.9
	[a]12	4.5	37.3	9.8	5.9
	13	6.3	55.2	13.9	9.2
	14	8.1	31.2	6.6	3.8
	15	2.6	31.6	6.7	4.3
	[b]16	-	-	-	-
	17	4.8	29.2	6.4	4.1
	18	10.9	52.3	15.1	11.5
	19	11.0	49.9	16.1	10.3
	[a]20	2.0	35.9	8.4	5.6
UH	21	13.7	54.6	16.4	14.6
	22	7.2	34.1	7.0	5.9
	23	7.9	41.4	11.8	6.5
	24	3.2	42.8	13.4	6.2
	25	4.5	43.9	12.2	5.0
	[a]26	4.2	17.3	5.8	2.2
	27	2.5	28.8	8.6	4.4
	28	10.4	19.6	5.8	3.8
	[b]29	8.5	-	-	-
	[a]30	6.2	25.9	6.6	4.8
UL	[a]31	15.3	55.0	15.1	12.2
	32	7.7	44.2	14.3	8.0
	33	4.1	64.6	17.4	11.6
	34	2.8	22.2	6.6	4.2
	35	6.3	51.1	12.6	6.6
	36	3.1	55.3	13.6	13.3
	37	4.5	37.8	19.6	5.9
	[a]38	4.0	49.7	10.7	8.6
	39	4.7	46.6	15.5	5.5
	40	9.2	42.5	11.4	6.0

[a]First lactation cows [b]Missing data

Table 6A. Effect of dietary protein level and formaldehyde treated canola meal on ammonia-nitrogen (NH_3-N) and volatile fatty acid concentrations of rumen liquor sampled 3h after feeding (experiment 2, individual data)

Treatment	Cow#	NH_3-N (mg/100ml)	Acetate (mM)	Propionate (mM)	Butyrate (mM)
FH	6	6.4	46.6	17.8	12.2
	7	14.0	53.8	17.4	13.7
	[a]8	3.4	46.1	15.0	17.5
	[a]9	10.8	42.5	14.5	11.4
	10	13.9	52.8	17.0	11.8
	41	7.8	37.2	16.9	10.2
FL	14	7.6	52.8	18.4	14.9
	[a]15	5.8	40.8	15.8	10.1
	17	14.1	66.3	18.1	16.4
	18	15.2	60.8	20.3	15.1
	20	5.7	60.0	20.5	16.4
	[a]42	2.6	80.3	26.4	19.3
UH	25	15.9	49.4	15.7	14.3
	[a]26	11.6	51.7	18.6	11.8
	27	24.9	55.0	18.7	17.5
	28	23.0	66.7	24.4	22.0
	29	13.7	50.6	17.0	15.1
	[a]30	5.5	31.3	9.4	8.2
UL	35	11.9	46.3	12.2	9.7
	36	13.2	48.3	15.2	12.0
	[a]38	14.8	44.8	16.4	9.5
	39	9.7	58.2	21.0	17.8
	40	17.2	43.4	13.9	7.4
	[a]43	16.8	50.3	13.6	12.1

[a]First lactation cows

Table 7A. Response of plasma profile to two levels of dietary crude protein and formaldehyde treated canola meal (experiment 2, individual data)

Treatment	Cow#	Urea (mg/100ml)	Glucose (mg/100ml)	Total protein (mg/100ml)
FH	1	20.2	56.5	9.1
	2	18.9	66.5	15.3
	3	13.5	47.0	10.0
	4	18.0	54.5	10.1
	5	13.9	45.0	9.8
	6	16.5	49.0	10.4
	7	18.2	48.5	8.4
	[a]8	18.5	77.0	13.9
	[a]9	12.1	44.5	7.3
	10	9.0	36.5	5.3
FL	11	11.3	69.5	11.1
	[a]12	16.5	90.5	12.8
	13	13.7	54.5	10.1
	14	9.9	76.0	10.4
	15	15.5	82.5	13.2
	[b]16	-	-	-
	17	8.0	60.0	7.2
	18	12.7	70.0	8.6
	19	13.3	57.5	11.7
	[a]20	10.5	68.0	11.9
UH	21	15.5	65.0	10.4
	22	11.5	51.0	10.0
	23	15.2	60.5	11.7
	24	14.5	75.0	11.7
	25	16.1	84.5	12.6
	[a]26	18.2	63.5	10.2
	27	15.2	58.5	12.1
	28	16.6	52.0	11.3
	29	16.6	65.0	11.1
	[a]30	15.0	61.5	10.4
UL	[a]31	6.5	41.0	3.9
	32	14.1	66.5	13.4
	33	8.8	47.5	9.6
	34	10.8	61.0	11.1
	35	11.1	60.5	10.8
	36	15.8	82.0	11.8
	37	19.5	74.5	12.0
	[a]38	6.3	46.0	6.0
	39	5.2	44.5	7.4
	40	5.2	49.5	6.4

[a]First lactation cows

[b]Missing data

Table 7A continued. Response of plasma profile to two levels of dietary crude protein and formaldehyde treated canola meal (experiment 2, individual data)

Treatment	Cow#	Plasma amino acids (μmoles/l)				
		Methionine	Phenylalanine	Tryptophan	Histidine	Arginine
FH	1	29	62	13	63	118
	2	19	59	6	65	113
	3	26	56	6	79	76
	4	26	58	10	60	125
	5	24	48	2	70	78
	6	18	39	12	60	83
	7	30	68	19	72	159
	[a]8	25	53	10	61	97
	[a]9	18	46	11	50	113
	10	24	45	9	66	110
FL	11	14	38	6	54	81
	[a]12	24	47	4	61	110
	13	34	67	11	95	186
	14	19	32	8	48	79
	15	23	47	11	52	85
	[b]16	-	-	-	-	-
	17	22	44	9	50	86
	[b]18	-	-	-	-	-
	19	36	64	12	74	148
	[a]20	27	45	12	44	100
UH	21	31	89	14	100	191
	22	23	43	8	52	101
	23	19	36	12	51	97
	24	23	53	13	41	109
	25	28	48	4	48	104
	[a]26	21	47	9	35	80
	27	22	48	6	53	94
	28	32	58	9	65	178
	29	28	54	15	65	142
	[a]30	22	46	5	62	107
UL	[a]31	15	36	6	43	91
	32	30	59	17	56	149
	33	21	59	13	72	153
	34	18	52	10	38	82
	35	20	38	13	45	91
	36	19	32	13	15	79
	37	21	48	4	37	87
	[a]38	22	45	11	31	76
	39	31	62	19	62	138
	40	31	44	6	30	91

[a]First lactation cows

[b]Missing data

Table 7A continued. Response of plasma profile to two levels of dietary crude protein and formaldehyde treated canola meal (experiment 2, individual data)

Treatment	Cow#	Plasma amino acids (μmoles/l)				
		Aspartate	Asparagine	Glutamate	Glutamine	Proline
	1	23	36	159	310	135
	2	24	33	162	476	123
	3	22	48	90	475	135
	4	27	36	101	528	135
	5	23	32	105	378	84
FH	6	26	25	126	475	109
	7	30	38	159	505	177
	[a]8	26	21	115	612	83
	[a]9	24	20	110	473	74
	10	27	37	126	388	150
	11	21	23	107	395	90
	[a]12	22	27	129	368	97
	13	39	69	174	406	200
	14	31	26	121	451	52
	15	26	30	98	437	78
FL	[b]16	-	-	-	-	-
	[b]17	29	30	122	330	75
	[b]18	-	-	-	-	-
	19	33	38	183	660	124
	[a]20	29	39	97	560	95
	21	17	45	106	303	68
	22	26	30	105	323	87
	23	23	31	113	324	72
	24	28	45	112	471	86
	25	35	39	91	547	65
UH	[a]26	28	33	120	603	90
	27	29	39	115	453	93
	28	29	58	169	382	174
	29	35	43	156	337	90
	[a]30	31	42	101	513	131
	[a]31	26	23	129	277	69
	32	41	61	157	732	197
	33	33	43	137	514	198
	34	24	21	92	542	59
	35	29	30	112	269	61
UL	36	27	30	80	346	71
	37	23	25	99	294	62
	[a]38	26	30	113	385	50
	39	45	30	271	353	131
	40	32	25	172	249	78

[a]First lactation cows

[b]Missing data

Table 7A continued. Response of plasma profile to two levels of dietary crude protein and formaldehyde treated canola meal (experiment 2, individual data)

Treatment	Cow#	Plasma amino acids (μmoles/l)				
		Glycine	Alanine	Citrulline	Ornithine	Serine
FH	1	384	330	117	63	108
	2	568	242	99	33	85
	3	345	306	163	74	108
	4	505	281	190	80	122
	5	503	231	111	47	92
	6	441	235	95	63	77
	7	584	384	131	97	124
	[a]8	460	264	93	72	106
	[a]9	700	215	79	52	86
	10	461	361	105	78	112
FL	11	329	273	59	42	67
	[a]12	404	344	98	69	94
	13	813	444	119	114	80
	14	491	282	79	52	89
	15	420	343	67	53	98
	[b]16	-	-	-	-	-
	17	553	209	90	42	91
	[b]18	-	-	-	-	-
	19	567	385	134	109	134
	[a]20	467	237	92	53	110
UH	21	575	534	129	105	128
	22	461	273	163	71	105
	23	571	317	71	43	127
	24	696	276	97	55	121
	25	555	261	117	65	139
	[a]26	592	289	93	42	128
	27	520	301	105	67	133
	28	1011	464	129	104	154
	29	732	408	113	58	161
	[a]30	531	290	82	67	107
UL	[a]31	437	258	55	36	91
	32	649	521	131	98	155
	33	761	519	69	71	131
	34	531	237	80	38	92
	35	647	313	84	59	141
	36	477	239	61	48	130
	37	591	257	113	56	117
	[a]38	687	265	57	45	121
	39	853	427	97	70	156
	40	701	292	95	60	132

[a]First lactation cows [b]Missing data

Table 8A. Intake and excretion of dry matter (DM), nitrogen (N), acid detergent fibre (ADF), gross energy (GE) and ether extract (EE) of cows in response to two levels of dietary crude protein and formaldehyde treated canola meal (digestion study, experiment 2, individual data)

		Intake				
Treatment	Cow#	DM (kg/day)	N (kg/day)	ADF (kg/day)	EE (kg/day)	GE (Mcal/day)
FH	6	23.42	0.61	4.81	1.03	100.22
	7	20.64	0.61	4.29	0.98	92.02
	[a]8	15.31	0.37	3.06	0.76	67.16
	[a]9	17.26	0.47	3.69	0.72	75.00
	10	21.02	0.50	5.17	0.77	90.31
	41	21.23	0.58	4.52	0.89	92.32
FL	14	19.23	0.47	3.90	0.98	80.90
	[a]15	21.01	0.52	3.60	0.74	90.70
	17	21.23	0.53	3.90	0.99	91.68
	18	18.45	0.47	4.09	0.67	79.32
	20	18.14	0.42	3.45	0.78	78.20
	[a]42	14.61	0.28	3.03	0.51	61.96
UH	25	24.40	0.70	4.83	0.97	104.74
	[a]26	17.90	0.57	3.82	0.86	79.09
	27	19.34	0.62	4.16	0.93	86.28
	28	19.02	0.60	4.14	0.93	83.88
	29	20.62	0.65	3.89	1.12	91.00
	[a]30	14.20	0.36	3.27	0.50	61.28
UL	35	23.65	0.58	4.26	0.95	99.93
	36	22.50	0.61	4.01	0.92	96.60
	[a]38	15.04	0.32	3.36	0.58	63.87
	39	20.36	0.37	4.74	0.67	86.78
	40	19.24	0.36	4.54	0.62	81.87
	[a]43	18.12	0.32	3.75	0.64	76.76

[a]First lactation cows

Table 8A continued. Intake and excretion of dry matter (DM), nitrogen (N), acid detergent fibre (ADF), gross energy (GE) and ether extract (EE) of cows in response to two levels of dietary crude protein and formaldehyde treated canola meal (digestion study, experiment 2, individual data)

Treatment	Cow#	Fecal excretion				
		DM (kg/day)	N (kg/day)	ADF (kg/day)	EE (kg/day)	GE (Mcal/day)
FH	6	7.99	0.22	2.68	0.13	34.29
	7	6.31	0.20	2.39	0.09	27.23
	[a]8	5.35	0.15	1.94	0.09	23.40
	[a]9	7.84	0.23	2.73	0.14	33.89
	10	8.49	0.22	2.80	0.21	38.35
	41	8.91	0.24	3.19	0.15	36.41
FL	14	6.72	0.17	2.13	0.13	27.90
	[a]15	6.96	0.19	2.30	0.12	30.30
	17	6.49	0.19	2.08	0.14	28.47
	18	7.81	0.23	2.48	0.14	33.57
	20	5.53	0.16	1.92	0.09	23.77
	[a]42	6.89	0.17	2.27	0.14	29.48
UH	25	8.52	0.22	3.19	0.10	35.45
	[a]26	6.54	0.18	2.37	0.13	28.19
	27	6.70	0.20	2.31	0.13	28.92
	28	7.43	0.21	2.75	0.11	32.05
	29	6.48	0.17	2.34	0.24	28.57
	[a]30	6.40	0.16	2.37	0.11	27.52
UL	35	8.15	0.20	2.62	0.14	34.56
	36	7.06	0.21	2.46	0.10	30.73
	[a]38	7.02	0.17	2.36	0.13	30.21
	39	8.77	0.19	2.84	0.14	37.61
	40	6.88	0.17	2.04	0.13	29.11
	[a]43	6.45	0.15	2.15	0.11	27.57

[a]First lactation cows

Table 8A continued. Intakeand excretion of dry matter (DM), nitrogen (N), acid detergent fibre (ADF), gross energy (GE) and ether extract (EE) of cows in response to two levels of dietary crude protein and formaldehyde treated canola meal (digestion study, experiment 2, individual data)

Treatment	Cow#	Urinary N excretion(kg/day)	Milk N excretion(kg/day)
FH	6	0.20	0.18
	7	0.22	0.15
	[a]8	0.19	0.10
	[a]9	0.19	0.16
	10	0.14	0.14
	41	0.19	0.15
FL	14	0.13	0.15
	[a]15	0.11	0.17
	17	0.11	0.15
	18	0.17	0.15
	20	0.14	0.14
	[a]42	0.05	0.12
UH	25	0.27	0.20
	[a]26	0.22	0.13
	27	0.24	0.15
	28	0.21	0.17
	29	0.19	0.15
	[a]30	0.16	0.14
UL	35	0.16	0.16
	36	0.16	0.15
	[a]38	0.12	0.15
	39	0.08	0.17
	40	0.07	0.14
	[a]43	0.09	0.12

[a]First lactation cows

Table 9A. Milk yield and milk composition of cows during the post-experimental period (days 99 through 305 of lactation) of experiment 2 (individual data)

Treatment	Cow#	Milk yield (kg)	% fat	% protein	% lactose
FH	1	4353	4.33	3.47	4.68
	2	5313	3.48	3.05	4.86
	3	2987	3.31	2.95	4.46
	4	3310	4.24	3.17	4.57
	5	5010	4.10	3.44	4.36
	6	5562	4.31	3.38	4.89
	7	2846	3.07	2.97	4.73
	[a]8	4796	4.25	3.09	5.05
	[a]9	4361	3.65	3.20	4.88
	10	4293	3.62	2.79	4.69
FL	11	3962	3.54	3.05	4.83
	[a]12	2273	4.49	3.59	4.52
	13	3110	5.34	3.51	4.75
	14	5032	3.51	3.10	4.72
	15	6214	3.62	3.18	4.48
	[b]16	-	-	-	-
	17	3260	3.97	3.25	4.56
	18	3101	3.45	3.25	4.39
	19	3248	3.75	3.03	4.62
	[a]20	5092	3.38	3.04	4.70
UH	21	4816	3.40	3.27	4.62
	22	3890	4.27	3.33	4.94
	23	4388	3.60	3.12	4.64
	24	6508	4.10	3.26	4.77
	25	4030	3.21	3.16	4.32
	[a]26	3802	4.54	3.52	4.51
	27	5884	3.57	3.06	4.96
	28	6171	3.06	3.17	4.73
	29	3402	3.66	2.94	4.59
	[a]30	5454	3.86	3.11	4.99
UL	[a]31	3744	3.97	3.46	4.70
	32	4683	3.84	3.42	4.79
	33	3619	4.27	3.49	4.36
	34	4642	3.39	2.99	4.31
	35	6918	3.98	3.22	4.78
	[b]36	5529	-	-	-
	37	2065	3.33	3.17	5.05
	[a]38	5478	3.16	3.07	4.63
	39	5429	3.99	3.06	4.70
	40	4349	3.48	2.94	4.68

[a]First lactation cows

[b]Missing data

Table 10A. Effect of formaldehyde treated canola meal on littering rate and litter size in laboratory mice (experiment 3, individual data)

Treatment	Mouse#	Litter Size	Mouse#	Litter Size
MU	1A	13	7C	none
	1B	5	8A	10
	1C	11	8B	13
	2A	8	8C	6
	2B	eaten	9A	12
	2C	11	9B	5
	3A	11	9C	14
	3B	none	10A	12
	3C	none	10B	11
	4A	10	10C	11
	4B	8	11A	female died
	4C	6	11B	11
	5A	13	11C	7
	5B	7	12A	8
	5C	12	12B	6
	6A	13	12C	12
	6B	none	13A	13
	6C	none	13B	12
	7A	9	13C	3
	7B	none		
MF	14A	7	20C	4
	14B	11	21A	7
	14C	8	21B	6
	15A	14	21C	13
	15B	13	22A	10
	15C	13	22B	none
	16A	10	22C	12
	16B	11	23A	none
	16C	11	23B	none
	17A	5	23C	none
	17B	6	24A	9
	17C	none	24B	12
	18A	8	24C	none
	18B	9	25A	7
	18C	10	25B	6
	19A	11	25C	none
	19B	9	26A	9
	19C	none	26B	none
	20A	10	26C	none
	20B	11		

Table 11A. Tyrosine concentration of plasma and duodenal fluid from sheep fed supplemental tyrosine (experiment 4, individual data)

	Duodenal fluid tyrosine (mg/100ml)		Plasma tyrosine (mg/100ml)	
	Treatment		Treatment	
	C	T	C	T
Sheep #1	9.1	9.8	1.4	1.5
	9.6	10.7	0.9	1.4
	9.3	10.3	1.6	1.3
	8.4	[a] -	1.4	1.3
	9.8	15.3	1.4	1.2
Sheep #2	18.4	34.5	1.5	1.2
	21.6	26.1	1.6	1.3
	33.8	51.1	1.3	1.4
	25.0	47.6	1.4	1.3
	25.8	28.4	1.4	1.3

[a]Missing value

Table 12A. Effect of dietary tyrosine supplementation on dry matter (DM) intake, milk yield, milk composition and plasma tyrosine concentration of dairy cows (experiment 5, individual data)

Cow	Treatment	DM Intake (kg/day)	Milk Yield (kg/day)	4% FCM Yield (kg/day)	%fat	% protein
Vision	CU	24.04	26.7	21.4	2.67	3.13
	TU	26.79	27.4	24.5	3.30	3.13
	CU	24.97	26.1	22.3	3.02	3.14
Loena	CU	21.10	31.7	25.4	2.67	3.16
	TU	24.06	31.9	27.6	3.09	3.06
	CU	24.07	33.1	28.1	2.99	3.17
Queenie	CU	19.71	35.0	30.8	3.19	3.09
	TU	20.63	33.8	30.9	3.43	3.14
	CU	20.69	30.9	27.3	3.22	3.25
Kathy	TU	22.30	31.0	25.9	2.90	3.11
	CU	23.63	30.8	25.9	2.94	3.23
	TU	26.10	34.1	29.9	3.18	3.12
Peggy	TU	18.43	30.8	24.2	2.56	3.05
	CU	22.33	28.5	26.7	3.58	3.00
	TU	21.02	27.1	22.8	2.95	3.34
Rosa	TU	21.37	27.4	23.5	3.04	3.11
	CU	21.05	25.0	20.5	2.79	3.26
	TU	23.10	26.4	22.8	3.08	3.35

Table 12A continued. Effect of dietary tyrosine supplementation on dry matter (DM) intake, milk yield, milk composition and plasma tyrosine concentration of dairy cows (experiment 5, individual data)

Cow	Treatment	% lactose	Fat yield (g/day)	Protein yield (g/day)	Lactose yield (g/day)	Plasma tyrosine (μmoles/l)
Vivian	CU	4.82	713	836	1287	94
	TU	4.82	904	858	1321	77
	CU	4.86	788	820	1268	110
Leona	CU	4.54	846	1002	1439	66
	TU	4.63	986	976	1477	66
	CU	4.60	990	990	1523	94
Queenie	CU	5.14	1117	1082	1799	83
	TU	5.16	1159	1061	1744	110
	CU	5.08	995	1004	1570	122
Kathy	TU	4.60	899	964	1426	94
	CU	4.72	906	995	1454	61
	TU	4.68	1084	1064	1596	94
Peggy	TU	4.76	788	939	1466	77
	CU	4.79	1020	858	1365	72
	TU	4.72	799	905	1279	99
Rosa	CU	5.16	833	852	1414	110
	TU	5.13	698	815	1283	77
	CU	5.18	813	884	1368	105

Table 13A. Response of dry matter (DM) intake, milk yield and milk composition to tyrosine supplementation of diets containing formaldehyde treated canola meal (experiment 6, individual data)

Cow	Treatment	DM Intake (kg/day)	Milk Yield (kg/day)	4% FCM Yield (kg/day)	% Fat	Fat Yield (g/day)
Queenie	UT	17.2	30.4	27.8	3.41	1037
	UC	18.6	31.2	29.6	3.65	1139
	UT	19.4	32.3	30.6	3.64	1176
Peggy	UC	20.1	24.4	21.8	3.27	798
	UT	21.1	23.5	21.1	3.32	780
	UC	20.4	23.4	20.3	3.13	732
Beatrice	UC	20.9	35.8	31.1	3.14	1124
	FC	21.8	35.0	37.9	4.53	1586
	UC	20.1	33.5	36.4	4.57	1531
Vivian	FC	20.7	26.9	23.8	3.22	866
	UC	23.3	26.1	22.1	2.99	780
	FC	22.2	21.6	19.1	3.22	696
Emmy	FC	21.1	33.1	26.0	2.57	851
	FT	23.2	34.1	31.5	3.49	1190
	FC	22.0	34.5	30.2	3.16	1090
Joan	FT	20.3	38.7	33.9	3.17	1227
	UT	21.3	36.1	32.6	3.34	1206
	FT	20.2	35.1	29.0	2.84	997
Bonny	UT	15.8	26.4	25.0	3.64	961
	FT	15.6	23.4	21.8	3.56	833
	UT	16.0	20.6	18.1	3.21	661
Royalle	UT	21.4	35.3	28.7	2.76	974
	FC	20.8	32.9	30.6	3.54	1165
	UT	21.4	34.2	29.4	3.07	1050
Janice	FC	21.0	35.9	35.1	3.84	1379
	UT	21.4	38.5	35.4	3.46	1332
	FC	24.2	36.4	32.1	3.21	1168
Nora	UC	21.1	39.3	41.8	4.43	1741
	FT	22.8	40.6	32.1	2.61	1060
	UC	22.9	39.9	33.7	2.96	1181
Lady	FT	22.6	36.4	33.6	3.48	1267
	UC	21.4	36.9	34.0	3.48	1284
	FT	21.8	39.6	34.7	3.18	1259

Missing value calculated for cow on sequence FT/FC/FT

Table 13A continued. Response of dry matter (DM) intake, milk yield and milk composition to tyrosine supplementation of diets containing formaldehyde treated canola meal (experiment 6, individual data)

Cow	Treatment	% Protein	Protein Yield (g/day)	% Lactose	Lactose Yield (g/day)
Queenie	UT	2.90	882	4.81	1462
	UC	2.97	927	4.83	1507
	UT	3.29	1063	4.89	1579
Peggy	UC	3.10	756	4.34	1059
	UT	3.09	726	4.53	1065
	UC	3.30	772	4.17	976
Beatrice	UC	2.82	1010	4.64	1661
	FC	2.80	980	4.43	1551
	UC	3.14	1052	4.48	1501
Vivian	FC	3.08	829	4.49	1208
	UC	3.08	804	4.39	1146
	FC	3.43	741	4.33	935
Emmy	FC	2.83	937	5.11	1691
	FT	2.76	941	5.10	1739
	FC	3.07	1059	5.07	1749
Joan	FT	3.28	1269	4.96	1920
	UT	2.79	1007	4.64	1675
	FT	3.02	1060	4.88	1713
Bonny	UT	2.82	744	5.01	1323
	FT	2.74	641	4.86	1137
	UT	2.77	571	4.92	1014
Royalle	UT	2.85	1006	4.78	1687
	FC	3.01	990	4.52	1487
	UT	2.99	1023	4.80	1642
Janice	FC	3.31	1188	4.44	1594
	UT	3.15	1213	4.70	1810
	FC	2.99	1088	4.88	1776
Nora	UC	2.88	1132	4.81	1890
	FT	3.04	1234	4.81	1953
	UC	3.05	1217	4.82	1923
Lady	FT	2.78	1012	4.46	1623
	UC	2.91	1074	4.43	1635
	FT	2.87	1137	4.53	1794

Missing value calculated for cow on sequence FT/FC/FT

Table 14A. Response of plasma amino acid concentrations to tyrosine supplementation of diets containing formaldehyde treated canola meal (experiment 6, individual data)

		Plasma amino acid concentration (μmoles/l)				
Cow	Treatment	Aspartate	Asparagine	Glutamate	Glutamine	Proline
Queenie	UT	17	53	164	534	128
	UC	16	35	97	840	64
	UT	17	14	105	453	91
Peggy	UC	23	51	173	617	157
	UT	25	83	112	1448	156
	UC	27	95	124	785	148
Beatrice	UC	21	35	142	216	110
	FC	20	42	110	254	160
	UC	14	23	86	316	83
Vivian	FC	21	30	146	446	130
	UC	23	70	285	1047	163
	FC	21	46	90	740	139
Emmy	FC	20	40	114	449	203
	FT	16	44	104	955	105
	FC	17	32	91	597	139
Joan	FT	23	53	107	578	157
	UT	19	48	111	722	137
	FT	22	31	273	160	226
Bonny	UT	10	27	122	664	130
	FT	14	44	91	474	130
	UT	26	27	213	334	140
Royalle	UT	17	53	82	782	115
	FC	17	23	167	299	109
	UT	21	38	121	455	98
Janice	FC	17	42	89	606	120
	UT	23	35	207	375	119
	FC	23	37	180	566	135
Nora	UC	34	23	194	390	125
	FT	34	55	203	447	163
	UC	32	65	204	421	146
Lady	FT	23	39	133	501	157
	UC	20	39	111	605	99
	FT	26	53	135	725	183

Missing value calculated for cow on sequence FT/FC/FT

Table 14A continued. Response of plasma amino acid concentrations to tyrosine supplementation of diets containing formaldehyde treated canola meal (experiment 6, individual data)

Cow	Treatment	Plasma amino acid concentrations(μmoles/l)				
		Tyrosine	Phenylalanine	Threonine	Methionine	Isoleucine
Queenie	UT	76	55	142	38	138
	UC	41	37	101	28	108
	UT	94	64	151	35	192
Peggy	UC	63	52	167	34	166
	UT	94	61	223	48	179
	UC	114	80	218	48	254
Beatrice	UC	54	55	118	31	137
	FC	57	60	139	30	144
	UC	38	39	80	21	92
Vivian	FC	68	46	124	28	157
	UC	101	57	183	42	164
	FC	74	53	138	33	160
Emmy	FC	50	43	136	27	169
	FT	42	41	137	24	144
	FC	31	39	106	20	137
Joan	FT	52	41	215	30	177
	UT	39	27	149	28	92
	FT	62	49	85	32	170
Bonny	UT	27	33	100	24	100
	FT	65	58	136	38	190
	UT	49	52	81	33	156
Royalle	UT	46	38	124	32	138
	FC	42	36	30	24	134
	UT	48	39	95	27	146
Janice	FC	43	32	138	25	123
	UT	53	41	111	32	134
	FC	54	37	135	32	136
Nora	UC	44	46	66	27	126
	FT	50	54	152	36	189
	UC	58	53	170	36	154
Lady	FT	57	31	143	26	98
	UC	44	24	128	28	83
	FT	60	44	191	30	127

Missing value calculated for cow on sequence FT/FC/FT

Table 14A continued. Response of plasma amino acid concentrations to tyrosine supplementation of diets containing formaldehyde treated canola meal (experiment 6, individual data)

		Plasma amino acid concentration (μmoles/l)				
Cow	Treatment	Glycine	Alanine	Citrulline	Ornithine	Serine
Queenie	UT	253	338	76	92	110
	UC	343	225	89	63	68
	UT	291	310	113	96	77
Peggy	UC	411	306	82	98	98
	UT	497	340	129	118	132
	UC	452	604	82	144	127
Beatrice	UC	500	307	140	97	122
	FC	521	262	161	108	114
	UC	337	204	91	74	82
Vivian	FC	327	276	71	88	90
	UC	277	352	138	102	115
	FC	241	217	117	102	93
Emmy	FC	396	242	57	73	89
	FT	373	224	79	65	105
	FC	299	206	85	65	80
Joan	FT	509	332	123	95	82
	UT	421	284	104	48	87
	FT	495	397	138	127	77
Bonny	UT	385	237	78	69	64
	FT	212	236	113	76	72
	UT	371	311	105	95	90
Royalle	UT	408	233	139	61	87
	FC	452	238	113	84	51
	UT	419	197	137	73	81
Janice	FC	411	178	157	67	81
	UT	500	292	113	76	102
	FC	437	260	90	70	92
Nora	UC	507	343	71	111	70
	FT	557	437	154	118	111
	UC	576	398	127	113	114
Lady	FT	289	298	85	56	84
	UC	337	253	94	54	77
	FT	413	325	101	61	93

Missing value calculated for cow on sequence FT/FC/FT

Table 14A continued. Response of plasma amino acid concentration to tyrosine supplementation of diets containing formaldehyde treated canola meal (experiment 6, individual data)

		Plasma amino acid concentration (μmoles/l)					
Cow	Treatment	Leucine	Lysine	Valine	Tryptophan	Arginine	Histidine
Queenie	UT	171	125	268	8	113	48
	UC	115	66	242	11	82	36
	UT	212	135	380	15	132	56
Peggy	UC	208	138	332	4	129	75
	UT	204	177	355	3	187	105
	UC	279	244	496	15	167	121
Beatrice	UC	186	139	315	11	111	72
	FC	199	193	366	12	129	94
	UC	127	64	219	5	75	48
Vivian	FC	148	139	388	4	83	70
	UC	229	193	368	3	93	89
	FC	231	140	431	3	111	81
Emmy	FC	227	132	379	5	84	61
	FT	183	111	345	2	93	69
	FC	186	90	338	6	78	66
Joan	FT	208	119	369	7	126	73
	UT	117	75	192	9	64	43
	FT	241	134	368	8	70	74
Bonny	UT	112	66	219	7	84	52
	FT	224	126	295	10	96	67
	UT	185	135	209	9	89	67
Royalle	UT	146	95	161	8	77	43
	FC	174	91	296	8	88	55
	UT	177	108	303	4	99	56
Janice	FC	148	88	267	4	87	57
	UT	163	121	251	7	98	47
	FC	155	101	265	4	84	50
Nora	UC	150	97	288	8	101	57
	FT	200	110	390	12	149	85
	UC	175	117	350	12	153	66
Lady	FT	115	80	224	7	76	52
	UC	100	75	174	13	59	30
	FT	169	87	303	10	84	70

Missing value calculated for cow on sequence FT/FC/FT

Table 15A. Effect of supplemental dietary tyrosine on feed intake, liveweight gain and feed:gain ratio of rats (experiment 7A,individual data)

		Preexperimental period		Experimental period		
Pair#	Treatment	Final weight(g)	Liveweight gain(g/6 days)	Liveweight gain(g/10days)	Feed intake (g/10days)	Feed: gain ratio
1	RC	106.74	28.36	78.16	242.02	3.10
1	RT	115.79	24.90	57.85	225.70	3.90
2	RC	102.73	24.27	40.73	162.11	3.98
2	RT	103.97	29.92	77.38	241.99	3.13
3	RC	101.23	20.36	65.13	197.73	3.04
3	RT	99.35	29.67	58.05	200.05	3.45
4	RC	95.34	16.16	47.87	168.29	3.52
4	RT	99.08	21.41	51.62	185.97	3.60
5	RC	95.20	28.13	50.74	191.06	3.77
5	RT	95.94	28.36	59.45	212.30	3.57
6	RC	93.77	21.27	39.83	148.36	3.72
6	RT	92.81	24.70	54.49	186.77	3.43
7	RC	92.62	24.70	63.87	207.47	3.25
7	RT	91.86	21.09	52.92	189.64	3.58
8	RC	91.55	18.44	54.46	175.91	3.23
8	RT	86.29	23.33	53.36	177.42	3.32
9	RC	89.31	22.12	54.93	166.19	3.03
9	RT	84.11	17.02	40.88	154.35	3.78
10	RC	88.62	22.12	47.86	159.57	3.33
10	RT	81.87	26.67	68.36	205.18	3.00
11	RC	83.89	21.27	63.92	188.76	2.95
11	RT	81.72	27.16	70.87	211.92	2.99
12	RC	80.65	23.03	57.48	187.36	3.26
12	RT	80.04	16.51	53.48	165.54	3.10

Table 15A continued. Effect of supplemental dietary tyrosine on feed intake, liveweight gain and feed:gain ratio of rats (experiment 7A, individual data)

		Preexperimental period		Experimental period		
Pair#	Treatment	Final weight(g)	Liveweight gain(g/6 days)	Liveweight gain(g/10days)	Feed intake (g/10days)	Feed: gain ratio
13	RC	79.87	20.23	46.94	163.19	3.48
13	RT	77.97	21.49	52.28	170.08	3.25
14	RC	76.82	20.56	50.79	166.60	3.28
14	RT	75.19	21.72	58.01	181.12	3.12
15	RC	67.66	15.20	46.64	153.27	3.29
15	RT	69.71	19.13	46.07	155.25	3.37
16	RC	64.01	25.73	53.21	182.31	3.43
16	RT	66.97	22.84	58.56	191.96	3.28
17	RC	62.27	18.58	58.59	176.13	3.01
17	RT	66.31	24.57	57.54	193.40	3.36
18	RC	61.91	16.44	27.87	111.60	4.01
18	RT	64.08	20.89	45.58	152.60	3.35
19	RC	54.05	19.85	39.10	129.32	3.31
19	RT	63.31	15.73	40.37	141.85	3.51
20	RC	51.38	17.48	41.65	139.09	3.34
20	RT	63.03	23.91	61.38	189.97	3.09
21	RC	49.39	14.14	36.91	129.63	3.51
21	RT	45.85	16.04	39.47	129.66	3.29
22	RC	48.41	17.06	36.68	123.77	3.37
22	RT	42.11	12.91	30.63	107.97	3.52
23	RC	37.76	13.44	27.15	97.70	3.60
23	RT	37.70	12.04	26.05	92.77	3.56

Table 16A. Effect of supplemental dietary tyrosine on feed intake, liveweight gain and feed :gain ratio of rats (experiment 7B, individual data)

Rat#	Treatment	Initial liveweight(g)	Liveweight gain(g/14days)	Feed intake (g/14days)	Feed:gain ratio
1	RC	65.06	62.93	223.07	3.54
2	"	73.18	53.68	218.56	4.07
3	"	59.65	56.45	192.72	3.41
4	"	67.68	48.54	201.68	4.15
5	"	59.64	42.75	181.35	4.24
6	"	69.81	34.61	219.47	6.34
7	"	54.55	56.10	192.88	3.44
8	"	62.20	77.87	257.51	3.31
9	"	78.77	35.58	213.96	6.01
10	"	68.85	63.67	236.84	3.72
11	"	81.22	43.20	186.83	4.32
12	"	59.38	46.92	197.00	4.20
13	"	74.19	51.49	202.86	3.94
14	"	84.16	74.55	286.37	3.84
15	"	86.64	57.28	233.19	4.07
16	"	74.95	66.34	239.45	3.61
17	"	68.82	55.97	221.01	3.95
18	"	77.00	64.77	237.49	3.67
19	"	72.91	45.40	189.33	4.17
20	"	73.58	41.98	185.33	4.43
21	"	74.18	53.59	215.47	4.02
22	"	63.73	52.66	215.10	4.08
23	"	70.62	66.99	238.14	3.55
24	RT	76.13	42.85	164.14	3.83
25	"	62.85	60.65	222.27	3.66
26	"	66.22	54.01	217.07	4.02
27	"	59.37	40.62	166.80	4.11
28	"	72.00	29.91	154.42	5.16
29	"	68.11	41.98	180.28	4.29
30	"	55.38	46.29	189.92	4.10
31	"	89.73	80.61	294.24	3.65
32	"	63.59	52.95	196.72	3.72
33	"	66.11	63.70	225.85	3.55
34	"	79.93	63.31	231.72	3.66
35	"	74.24	60.72	243.00	4.00
36	"	71.69	69.21	230.27	3.33
37	"	78.83	47.24	243.82	5.16
38	"	73.15	72.39	222.90	3.08
39	"	82.19	58.95	230.86	3.92
40	"	66.81	43.11	218.59	5.07
41	"	61.29	44.34	183.20	4.13
42	"	66.53	56.86	235.03	4.13
43	"	75.22	41.35	215.54	5.21
44	"	71.40	52.18	203.67	3.90
45	"	60.06	50.41	211.84	4.20
46	"	60.17	45.16	195.40	4.33

Table 17A. Effect of supplemental dietary tyrosine on feed intake, liveweight gain and feed:gain ratio in chicks (experiment 7C, individual data)

Treatment	Group#	Initial liveweight (g/chick)	Liveweight gain (g/chick/7days)	Feed intake (g/chick/7days)	Feed: gain ratio
FC1	1	44.4	19.4	69.5	3.58
	2	44.0	19.8	76.7	3.87
	3	44.0	16.4	69.5	4.24
	4	44.0	20.6	77.9	3.78
	5	44.6	21.8	81.9	3.76
	6	44.8	16.8	68.7	4.09
FC2	7	44.6	20.8	76.2	3.66
	8	43.4	23.8	82.0	3.45
	9	44.0	24.5	84.5	3.45
	10	44.2	24.4	80.4	3.30
	11	43.6	15.8	71.4	4.52
	12	44.4	13.6	72.7	5.35
FC3	13	44.0	20.4	75.8	3.72
	14	43.0	23.8	80.2	3.37
	15	44.0	15.8	66.0	4.18
	16	44.0	16.4	70.3	4.29
	17	44.4	20.8	76.3	3.67
	18	44.0	20.4	75.1	3.68
FC4	19	43.8	22.6	76.2	3.37
	20	45.0	22.2	77.7	3.50
	21	44.0	23.6	81.1	3.44
	22	44.2	21.0	76.0	3.62
	23	43.4	24.4	79.6	3.26
	24	43.8	20.4	72.5	3.55

Table 18A. Glucosinolate content of the canola meal used in experiments 6, 7A, 7B and 7C

Glucosinolate	Concentration (μmoles/g oil free meal)
Butenyl	3.99
Pentonyl	0.42
Hydroxypentenyl	0.19
Hydroxybutenyl	10.32
Indole	0.31
Hydroxyindole	2.41
Total	17.64