EFFECT OF TREATING RAPE SEED MEAL WITH FORMALDEHYDE ON GROWTH PERFORMANCE IN YOUNG CALVES AND FLOW OF NUTRIENTS THROUGH G.I. TRACT OF HOLSTEIN STEERS

A THESIS

Presented to

the Faculty of Graduate Studies and Research

University of Manitoba

In partial fulfillment

of the requirements for the degree

DOCTOR OF PHILOSOPHY

by

HARI RAM SHARMA

Department of Animal Science

March, 1973

THE UNIVERSITY OF MANITOBA LIBRARIES

ABSTRACT

Ten (Holstein and Holstein X Brown Swiss) calves of both sexes and 55-63 days of age were fed two test rations containing either 26% untreated or 26% formaldehyde (FA) treated (5.6 g FA/100 g protein) rape seed meal (RSM) during a 14-week growth trial. No significant differences ($P > 0.05$) were found in dry matter (DM) consumption, daily gain, or feed efficiency between the two groups of animals. Treatment of RSM with formaldehyde reduced ($P < 0.01$) the levels of ruminal ammonia, blood plasma urea, concentration of total VFA's ($P < 0.05$) in rumen fluid and apparent digestibility ($P < 0.05$) of DM and crude protein (CP). Nitrogen (N) balance trials indicated no significant differences ($P > 0.05$) in DM and N consumption and N retention between the treated and untreated RSM fed calves. Calves fed the treated RSM had reduced ($P < 0.01$) urinary N and increased fecal N excretion.

Twenty-four Holstein calves of both sexes and eight weeks of age were randomly allotted to three rations containing either 14% soybean meal (SBM), 20% RSM or 20% FA-treated (0.7 g FA/100 g protein) RSM. Calves were fed the test rations ad libitum for 14 weeks. Feed consumption, DM intake, live weight gain and feed efficiency did not differ significantly ($P > 0.05$) among the three groups. A significant reduction in ruminal ammonia levels was observed

in calves receiving the treated RSM compared with those fed the RSM ration. Urea N levels in blood plasma and VFA's (mmoles/100 ml and molar percent) in ruminal fluid did not differ significantly ($P > 0.05$). Formaldehyde treatment of RSM tended to lower the DM, CP, acid detergent fibre and energy digestion coefficients.

Four fistulated Holstein (rumen, abomasal and ileal) steers with an average weight of 189 Kg were used in a change over design, to study the effect of treating RSM or casein protein with FA(0.7 g FA/100 g protein) on DM and CP digestibility, N retention and flow of various fractions of N through the G.I. tract. Animals were fed at 10 minute intervals using an automatic feeder device. FA-treatment of RSM or casein did not show any significant ($P > 0.05$) effect on apparent digestibility of DM, CP and N retention in steers fed treated diets as compared with untreated diets. There was a trend for lower rumen NH_3-N and blood urea N levels for steers receiving the treated diets compared with the untreated diets. FA-treatment of casein lowered ($P < 0.05$) the molar percentages of acetic and isobutyric acids in the rumen fluid of steers fed the FA-casein diet compared with those receiving the casein diet.

FA-treatment of casein but not RSM influenced the flow of total N and non-ammonia N (NAN) through the rumen. Flow of total N and NAN through abomasal digesta of steers

fed treated RSM and casein diets was significantly ($P<0.01$) higher than those receiving the untreated diets. Bacterial N flowing through the abomasum was significantly ($P<0.05$) lower for steers on casein diets compared with those fed RSM diets. Apparently a higher amount of bacterial protein per 100 g of digested dry matter flowed in the abomasum of steers fed RSM diets compared with those fed casein diets. The total N flowing through the terminal ileum of steers fed both RSM diets was significantly ($P<0.01$) more than those fed the casein diets. However, FA-treatment did not affect the flow of NAN through the terminal ileum of steers fed the different diets.

The percentages of total amino acids were significantly higher in rumen digesta ($P<0.01$) and abomasal digesta dry matter for steers fed the FA-casein diet compared with those receiving the casein diet or RSM diets ($P<0.05$). FA-treatment of casein protein significantly ($P<0.01$) increased the flow of amino acids, as a percent of amino acids consumed, through the rumen and abomasum of steers, but had no effect ($P>0.01$) when RSM was treated with FA. The flow of ileal amino acids as a percent of ruminal amino acids were significantly less ($P<0.05$) for steers fed FA-casein compared with those fed the other three diets, indicating that the apparent absorption of amino acids was higher in the small intestines of steers fed the treated casein diet compared

with the other three diets. The level of cystine was increased in the rumen digesta of steers fed casein diets and decreased for the steers fed RSM diets. Glutamic acid level in the rumen digesta was decreased for all the steers as compared to dietary level. However, glutamic acid was significantly ($P < 0.01$) higher for steers fed the FA-casein diet than the other steers. Except leucine and DAP, no significant differences were observed in the amino acid composition of bacteria isolated from the rumen of steers fed the various diets. There were noticeable increases in the level of glutamic acid (all diets) and decreases in the levels of lysine (RSM diet) and DAP (all diets) in the abomasal digesta of steers when compared with the ruminal digesta.

ACKNOWLEDGEMENT

The author is greatly indebted to Dr. J. R. Ingalls, Associate Professor of Dairy Cattle Nutrition, for his assistance, advice, guidance in planning, close supervision, execution of this project and in the preparation of this manuscript.

Sincere thanks are due to Dr. T. J. Devlin, Associate Professor Beef Cattle Nutrition and Dr. R. R. Marquardt, Associate Professor of Biochemistry for their critical reading and assistance in preparation of this manuscript.

Thanks are extended to Dr. G. D. Phillips for the Surgery of my experimental animals and to Dr. E. W. Stringam, Head of the Department Animal Science, for providing the opportunity for study and research assistantship in the department.

The author also wishes to acknowledge to Dr. B. E. McDonald, Associate Professor of Food and Nutrition for reading this manuscript, and to Mr. J. A. McKirdy, Research Associate, for suggestion and aid with technical analysis. Acknowledgement is due also to Mr. Gordon Hamilton, Mrs. Y. Lewanski and Peter A. Mills for their help during this investigation. Finally, I express my sincere appreciation to my wife Chandra Sharma for help, encouragement and patience during the present work.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

TABLES IN APPENDICES

Page

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Alpha
AA	Amino acids
Ad lib	Ad libitum
B	Beta
CP	Crude protein
CO_2	Carbon dioxide
CWC	Cell wall constituents
DAP	Diaminopimelic acid
DES	Diethylstilbestrol
DM	Dry matter
DNA	Deoxyribonucleic acid
ε	Epsilon
FA	Formaldehyde
FA-casein	Formaldehyde treated casein
FA-RSM	Formaldehyde treated rape seed meal
g	Gram
GA	Glutaraldehyde
Kg	Kilogram
LSM	Linseed meal
Ug	Microgram
MHA	Methionine hydroxy analogue
mg	Milligram
ml	Milliliter
M	Moles/liter
N	Nitrogen

NAN	Non-ammonia Nitrogen
NH_3	Ammonia
OM	Organic matter
%	Percent
RSM	Rape seed meal
RNA	Ribonucleic acid
SBM	Soy bean meal
SFM	Sunflower meal
VFA's	Volatile fatty acids

INTRODUCTION

Previous studies in ruminant nutrition have indicated that when the amount or solubility of protein fed is high, the extent of protein degradation probably exceeds protein synthesis by rumen microorganisms. Thus, substantial amounts of dietary protein may be lost due to excessive production of ammonia in the rumen and consequently excreted in the urine as urea. Proteins introduced directly into the abomasum or duodenum have been effective in increasing nitrogen retention, body weight gain and wool production in sheep. In order to achieve similar results with dietary protein it is necessary to protect the protein (an expensive moiety) from microbial degradation in the rumen, thus allowing more protein to be digested in the lower gut. It should then be possible to force the rumen microbiota to use the dietary non-protein nitrogen (urea) as a source of N for the synthesis of microbial protein and spare the dietary protein for digestion and absorption in the small intestines.

Protection of protein could be brought about by heat treatment, vegetable tannins or with formaldehyde (FA). However, the nutritional value of both heated and tannin treated proteins may be impaired. Therefore, FA treatment of protein for reducing its solubility in rumen fluid and increasing its resistance to microbial degradation in the

rumen was used in these studies.

Australian workers reported a response by sheep to FA treated casein supplementation in the diet and to abomasal infusions of casein and amino acids. Little information is available regarding the fate of nitrogenous compounds flowing after the rumen of ruminants fed FA-treated proteins, however, some workers have reported the effect of FA-treatment of protein on apparent digestibility and N retention in sheep and calves. Research is required to explore the fate of nitrogenous components which pass beyond the rumen of ruminants fed FA-treated proteins. The solubility of protein in rape seed meal (RSM) is somewhat higher than that of soy bean meal (SBM). Thus treatment of RSM with FA could be of some value in increasing N retention, especially when urea is used to supply part of the N requirement.

Rape seed meal has become readily available as a protein supplement, but the quantity of RSM used in ruminant ration has been restricted to a lower level. A limited amount of research has been conducted with ruminants on digestibility, acceptability, feed efficiency and comparative value of RSM as compared with other commonly used high protein meals. Studies were undertaken to obtain further information regarding the nutritive and comparative values of SBM with respect to RSM or FA RSM for young dairy calves.

REVIEW OF LITERATURE

Degradation of Protein and Other Nitrogenous Compounds

Under practical condition of feeding, nitrogen (N) entering the rumen is made up chiefly of protein together with varying amounts of non-protein nitrogenous substances (peptides, amino acids, amides, purines and other simple bases). Ruminants do not secrete proteolytic enzymes in the saliva or from the reticulo-rumen wall (Koningsberger et al., 1946). Therefore, protein digestion in the rumen can only be brought about by the proteolytic enzymes produced by the rumen microorganisms.

The proteolytic activity of the rumen content was first recognized by Syme (1938) and was confirmed by Pearson and Smith (1943). They suggested that the value of protein to ruminants depended upon the extent to which it is degraded in the rumen. The importance of ammonia as an end product of protein digestion was clearly defined by McDonald (1948, 1952). His studies showed that there was an increased production of NH_3 in the rumen of sheep after ingestion of certain proteins. In subsequent years other workers also observed similar results when pure proteins were incubated with rumen liquor or washed suspensions of rumen bacteria (El-Shazly, 1952 a, b; Annison, 1956; Warner, 1956; Moore and King, 1958; Blackburn and Hobson,

1960, 1962; Abou Akkada and Blackburn, 1963). Proteolytic activity has also been observed in ruminal protozoa by several workers (Warner, 1956; Blackburn and Hobson, 1960, a; Williams et al. 1961; Abou Akkada and Howard, 1962).

Protein solubility is suspected as one of the factors which influences the rate of hydrolysis of protein within the rumen. Ammonia production in the rumen is related to the solubility of protein (Chalmers et al., 1954; Annison et al., 1954; Chalmers and Synge, 1954; Chalmers and Marshall, 1964; and Mangan, 1972). The extent of ammonia production from different proteins depends upon a wide variety of factors: (1) surface of the protein available to microbial attack, (2) physical consistency of the protein, (3) effects due to the protein encased within cell walls, (4) chemical nature of a protein which affects its susceptibility to enzymic attack (Chalmers and Synge, 1954; Blackburn and Hobson, 1960, b).

Annison (1956) reported that casein, arachin (ground nut protein) and soy protein were rapidly degraded by the washed rumen microbes, whereas bovine albumen, wheat gluten and zein were degraded slowly. Lewis (1962) divided the proteins roughly into three groups in terms of the rate of ammonia release in the rumen: casein, gelatin, ground nut protein $>$ soy protein, wheat gluten $>$ bovine albumen and zein. Recently, Mangan (1972) showed that the half-life of

casein and ovalbumen in the rumen of steers was in the range of 20 and 180 minutes, respectively. Although both are water soluble proteins, their rates of digestion in the rumen are quite different. The disparity in the rate of digestion of these proteins in the rumen is dependent upon their tertiary structures and the availability of the terminal amino or carboxyl groups (Porter, 1950) for microbial enzymic digestion.

The proteolytic activity of the rumen content is not dependent upon the composition of the diets, as most of the proteolytic bacteria are found in significant numbers in animals on a wide variety of diets (Warner, 1956; Blackburn and Hobson, 1960, a). El-Shazly (1952, b) and Lewis (1955) indicated that the activity of rumen bacterial deaminases is influenced by the type of protein consumed by ruminants. The majority of bacteria contain both exo- and endopeptidases and produce hydrolysate with a high content of amino acids and small peptides (Abou Akkada and Blackburn, 1963). In vitro studies with rumen protozoa have shown that ciliate protozoa can engulf stained particles of casein and after digestion, incorporate them into protein, (Blackburn and Hobson, 1960, a; Abou Akkada and Howard, 1962), the principal products of digestion being peptides and amino acids, (Abou Akkada and Howard, 1962).

In the process of digestion in the rumen, the

proteins are converted to amino acids before their degradation to ammonia by the microbes. The concentration of the free amino acids, however, is usually low which Annison (1956) assumed to be due to their rapid uptake or degradation by the microorganisms. El-Shazly, (1952, a) observed that volatile fatty acids (VFA's) were produced when ammonia was being liberated during the fermentation of protein. Any increment in the ammonia concentration was correlated with increased concentration of C_4 and C_5 acids, particularly of the branched chain isomers, which could be attributed to the break down of amino acids. He further demonstrated (1952, b) the degradation of amino acids by a Stickland type of reaction in which some amino acids are oxidized and others are reduced. The products of the reaction are ammonia, Co_2 and three isomeric forms of the C_2 - C_5 VFA's.

The metabolism of the amino acids has been studied mainly in washed suspension of mixed rumen microorganisms and pure cultures. Incubation of individual amino acids with washed suspension and acetone dried rumen bacteria indicated that most of the amino acids are degraded during <u>in vitro</u> fermentation. The principal degradation products from aspartic acid, glutamic acid, serine, cystine, lysine, histidine, L-threonine, arginine, citrulline, ornithine, proline and delta-amino valeric acid were hydrogen, CO_2, NH_3, VFA's and keto acids (Sirotnak, 1953, 1954; Lewis, 1955;

Lewis and Emery, 1960; Van den Hende et al., 1963, a, b; Portugal and Sutherland, 1966). The other end products obtained in the fermentation of various amino acids by the washed suspensions of rumen bacteria and strained rumen liquor were delta-amino valeric acid, indole and skatole, putrescine, cadaverine, phenylpyruvic acid and phenyl-acetic acid (Lewis and Emery, 1962 b; Van den Hende et al. 1963, a, b; Van den Hende et al., 1964).

Warner (1964) indicated that asparagine, glutamine, nicotinamide and formamide were rapidly broken down by the sheep rumen micro-organisms with the production of ammonia but acetamide and propionamide were attacked slowly. He further suggested that asparaginase was associated with the bacteria and glutaminase to a large extent with the protozoa. The amino acids formed by deamination of amides were further deaminated.

Smith and McAllan (1970) reported that RNA and DNA added to the calf rumen were completely degraded after one hour in the rumen and also destroyed fairly rapidly on in vitro incubation with rumen fluid. Xanthine, guanine-HCl and uric acid were degraded completely at a slow rate by washed cell suspensions of bovine bacteria (Jurtshuk et al., 1958). The end products formed were CO_2, NH_3 and acetic acid. Hypoxanthine was degraded incompletely, whereas adenine was neither decarboxylated nor deaminated. Thymine and uracil

were broken down into B-amino isobutyric acid and B-alanine respectively, (Van der Horst, 1965).

Urea entering the rumen is very rapidly hydrolysed to ammonia. In fact it is difficult to detect urea in rumen contents at any time after administration (Chalmers, Hughes and Jaffrey, 1968). Carbon dioxide and ammonia are formed when urea is incubated with a suspension of rumen micro-organisms (Sirotnak et al., 1953). Rumen urease activity is entirely associated with the microbial fraction of the rumen contents, and bacteria rather than protozoa appear to be mainly responsible for urease production (Gibbons and McCarthy, 1957; Abou Akkada and Howard, 1962; Jones et al., 1964). Other workers suggested that the urease activity of the ruminal mucosa also originates from the rumen contents (Abdel Rehman and Decker, 1966). Houpt (1969) presented the hypothesis that the bacterial urease enzyme penetrates the cornified layers of the rumen epithelium and hydrolyse a portion of the urea diffusing from the blood vessel into the rumen wall. Any unchanged urea continues to diffuse into the rumen interior where it is rapidly hydrolysed by microbial urease.

Conversion of Dietary Nitrogen into Microbial Nitrogen

The extent to which dietary nitrogen is converted to microbial protein in the rumen is dependent upon: (a) the

rate at which the dietary protein is degraded to amino acids and ammonia, (b) the rates of absorption of amino acids and ammonia through the rumen wall, (c) the rates of passage of digesta out of the rumen, (d) the availability of carbohydrates as a source of energy and (e) the synthetic power of the microorganisms in the rumen. Since Syme (1938) demonstrated proteolytic activity in the rumen contents, it has become evident that proteins and other nitrogous compounds undergo extensive transformation in the rumen before passing down to the lower gut to become available to the host. Various workers have confirmed that ammonia is the major end product of protein degradation in the rumen, (McDonald, 1948, 1952; El-Shazly, 1952, a, b; Chalmers et al., 1954; Annison, 1956; Warner, 1956; Blackburn and Hobson, 1960). The importance of ammonia in rumen N metabolism was suggested by McDonald (1948, 1952). Part of the ammonia produced may be resynthesized to microbial protein. Some of the ammonia passes directly through the rumen epithelium and is converted into urea in the liver and can then be recycled via saliva (Somers, 1961) or through simple diffusion into the rumen from blood (Houpt, 1959) and hydrolyzed into NH_3 again. The ammonia thus evolved can be assimilated by rumen bacteria for the synthesis of microbial protein (Pearson and Smith, 1943). Phillipson et al. (1959) indicated that ammonia from other sources (urea and ammonium

salts) could also be utilized for the growth of bacteria. Many rumen bacteria are capable of using ammonia as a sole source of nitrogen (Bryant and Robinson, 1963) and some are known to have an obligatory requirement for peptides or amino acids per se. (Hungate, 1966). Portugal and Sutherland (1966) reported that glutamic acid and aspartic acid, under physiological conditions, are degraded to CO_2, VFA's and NH_3 by rumen microorganism. Direct incorporation of these amino acids into bacterial protein was very small. Therefore, ammonia and VFA's were utilized by the microbes for de-novo synthesis of the microbial protein rather than the dietary amino acids. Tracer studies with ^{15}N (Boggs, 1959) have shown incorporation of ammonia into amino acids by rumen bacteria in sheep on a purified ration. Pilgrim, Gray and Weller (1970) measured the synthesis of microbial protein from $(^{15}NH_4)_2SO_4$ in the rumen of sheep and the proportion of dietary N converted into microbial nitrogen. They reported that 76-78% of the bacterial N was derived from $(^{15}NH_4)_2SO_4$ when the animals were fed a low nitrogen diet. The values for protozoal nitrogen were variable (64-43%). On high N diets (lucerne hay), the corresponding values were lower for bacterial N (62-64%) and protozoal N (41 and 35%). Mathison and Milligan (1971) also observed similar results when $^{15}N(NH_4Cl)$ was infused into the rumen of sheep fed either a barley diet or a hay diet with a continuous feeder.

The mixed bacterial population appear to digest starch more efficiently in the presence of ammonia than in the presence of amino acids (Acord et al., 1966, 1968). On the other hand ciliate protozoa have an absolute requirement for amino acids essential to higher animals and preformed purine and pyrimidine bases (Kidder, 1967). Coleman (1967 a, b and 1968) showed that rumen ciliate Entodinium caudatum incorporate amino acids into its protein without interconversion and incorporate nucleotides derived from bacterial nucleic acids. Wallis and Coleman (1967) also indicated that washed I. intestinalis and I. prostoma incorporated free exogenous amino acids and amino acids arising from E. coli cells. Amino acids, arising partly from the diet and partly from the proteolysis of proteins in the rumen are either assimilated by rumen microbes (Annison, 1956) or absorbed through the rumen wall (Cook et al., 1961; Leibholz, 1965, 1969). Wright and Hungate (1967) reported that the concentration of individual free amino acids in rumen fluid rarely exceed 0.3 mM which agrees with the findings of Annison (1956).

Procedures for the direct determination of the quantity of microbial and food protein which pass from the rumen into the abomasum and the extent of conversion of food protein to microbial protein are dependent on methods which can differentiate between microbial and food proteins in the

digesta. Working with purified proteins, McDonald (1954) made use of the properties of Zein, (its solubility in 80% ethanol and the absence of lysine in the zein protein) and observed that 40% of the zein N was converted into microbial protein in the rumen of sheep. Ely et al. (1967) confirmed the low conversion of zein N and indicated that the replacement of cellulose with an equal weight of starch in the diet containing zein did not affect the synthesis of microbial protein. The conversion of casein into microbial protein was assayed on the basis of its phosphorus content by McDonald and Hall (1957). They reported that 90% of the dietary casein passed into the lower gut as microbial protein. Weller et al. (1958) recommended the use of 1-6 diaminopimelic (DAP) as a marker for calculating the bacterial N, because of its absence in plant and protozoal protein. Using DAP, they showed that 63-82% of the total N of the rumen was of microbial N. El-Shazly and Hungate (1966) arrived at the same conclusion that the bacterial N in the rumen of cows fed hay or hay and concentrate ration varied from 69-80% of the total N present in the rumen digesta. Hutton et al. (1971) used the N:DAP ratio for estimating the bacterial N and indicated that the contribution of bacterial N to the total N leaving the abomasum of a lactating cow was found to be 50%. Ibrahim and Ingalls (1972) showed that the rumen microorganism contributed 54-92% of the total amino

acids in the rumen digesta of dry Holstein cows and 24-31% of which was in the form of bacterial protein. Sharma et al. (1969) using ∝-amino N as a measure of protein synthesis in the rumen of sheep fed a semipurified diet containing urea, reported that 72% of the total N passing through the duodenum was microbial protein N.

Several workers have studied the formation of microbial protein in the rumen of cows and sheep by using the rate of incorporation of ^{35}S added in an inorganic form (Conrad et al., 1967; Robert and Millers, 1969). Methionine synthesis ranged from 33 to 46 mg per Kg body weight of cow and increased with the addition of starch in alfalfa diets (Conrad et al., 1967b). They further reported that methionine synthesis increased at a rate of 1.5 g per Kg of feed consumed which represents 63 g microbial protein synthesized per Kg of feed consumed.

Gray et al. (1958) reported that 11 g of microbial protein was synthesized for every 100 g of fermentable carbohydrates. Bloomfield et al. (1964) reported that the assimilation of urea N by bacteria required 55 g carbohydrate for each g of nitrogen fixed in an in vitro fermentation system. Hungate (1966) suggested that the synthesis of microbial protoplasm from carbohydrates under anaerobiosis is in the order of 10% which is significantly less than under aerobic condition. Hogan and Weston (1967) reported

that the quantity of microbial protein synthesized did not exceed 44-49 g per day, which is equivalent to 15-16 g per 100 g organic matter digested in the rumen. Conrad and Hibbs (1968) calculated the conversion of organic matter into microbial cells and concluded that 10.2% of the fermentable organic matter was converted into microbial protein equal to 15.8% of dry cell material. These values are quite similar to the figures calculated by Hungate (1966). Walker and Nadar (1968) used $Na_2{}^{35}S$ to label the sulphide pool of rumen contents which allowed the estimation of microbial protein synthesis in untreated rumen ingesta from animals under normal feeding conditions. They indicated that the microbial protein was synthesized at the rate of 94 ug per g of rumen content per hour. Hume (1970) suggested that the maximum cell yield may exceed 20 g per 100 g organic matter digested in the rumen. Lindsay and Hogan (1972) estimated the growth of rumen bacteria in defaunated sheep and indicated that about 32 g of bacterial organic matter and 23 g of bacterial crude protein were synthesized in the rumen for each 100 g of plant organic matter digested. Recently use of nucleic acids as a measure of microbial protein was recommended by Telmer-Kucharski and Gausseres, (1965), Ellis and Pfander (1965), Smith and McAllan (1970).

Nutritional Value of Nitrogenous Compound in the Digesta Flowing to the Lower Gut

Protein nutrition of all species is dependent on the adequacy of the nitrogen containing compounds which are absorbed to meet the need for tissue protein synthesis. Monogastric animals are dependent on the quality and quantity of nitrogenous substances consumed. Ruminants are less dependent on the quality of dietary nitrogenous compounds because of microbial fermentation and synthesis of proteinaceous material. Smith and Baker (1944) reported that the synthesized material obtained from urea N in in vitro fermentation was very much similar to a typical feedingstuff (linseed meal) in its chemical composition.

Various experimental approaches have been used to assess the amino acids that are available to ruminant animals by feeding either semipurified diets containing urea or natural feeds and examining the amino acid composition of the rumen digesta and the rumen microbes (Loosli et al., 1949; Duncan et al., 1953; Schelling et al., 1967; Ibrahim and Ingalls, 1972). They indicated that all the essential amino acids are synthesized by the rumen microorganisms from urea N in semi-purified diets. However, the quantity of some of the essential amino acids were less in the rumen contents of the animals fed on purified diets compared to natural diets. Virtanen (1966) reported that the only difference in

the amino acid composition of the rumen protein was as an increase in the percentage of diaminopimelic acid in the ingesta of cows fed a purified diet with non-protein nitrogen as compared with cows fed natural diets. Johnson et al. (1949) analysed rumen bacteria of sheep for sulfur containing amino acids and reported that ruminal bacterial protein is rich in both cystine and methionine. Reed et al. (1949) found similar results for the rumen bacteria isolated from the rumen of sheep fed green or dry feeds. They indicated that rumen bacterial protein was mildly deficient in methionine. Holmes et al. (1953) showed that bacterial protein was inferior to whole egg protein as a source of leucine, threonine and phenylalanine and markedly inferior as a source of methionine and isoleucine. Schelling et al. (1967) indicated that the relative amount of lysine and threonine were somewhat higher in microbial protein, whereas histidine and the sum of the sulfur containing amino acids were lower as compared to whole egg protein. The essential amino acids of microbial protein accounted for less than 50% of the total amino acids rather than 60% as in whole egg protein.

The amino acid composition of bacterial preparations were similar to those reported for pasture leaf proteins (Weller, 1957; Purser and Buechler, 1966 and Bergen et al., 1968b). However, the amino acid composition of bacteria and protozoa were quite different (Meyer et al., 1967). Lysine

content is higher in the protozoa than in bacteria (Purser and Buechler, 1966; Weller, 1957; and Bergen et al., 1968, b). The constancy in amino acid composition of rumen bacteria does not infer that all the rumen bacteria are of similar protein quality, since Bergen et al. (1967) demonstrated that 14 strains of rumen bacteria with similar amino acid composition were of widely differing protein quality. This discrepancy could be due to the differences in total digestibility and the pattern in which amino acids are released from the bacterial protein in the lower gut. The findings of McNaught et al. (1954) were confirmed by Bergen et al. (1968, a) who also concluded that the first limiting amino acids in bacterial and protozoal proteins were cystine and histidine respectively.

The true digestibility of sheep rumen bacteria was 60% when fed to rats as compared to 100% for casein. The biological values, which were approximately the same, were slightly less than 80%. McNaught et al. (1954) reported similar biological values for dried rumen bacteria and protozoa but the true digestibility of the bacterial protein was lower than the protozoal protein.

Dry preparations of mixed ruminal bacteria and protozoa were evaluated as a potential source of protein and B-vitamins for monogastric animals (Abdo et al., 1964). Freeze dried microorganisms gave a protein efficiency ratio

of 3.6 with a true digestibility of 74 to 80%. The biological values determined by Bergen et al. (1968, a) for rumen bacteria and protozoa were 85 and 82%, respectively, and the true digestibility of the rumen protozoa was higher than that of the rumen bacteria. These results agree well with the results reported earlier.

Blaxter (1964) reported that ruminants in general show a greater relative loss of N in the feces than do simple stomach animals. This is particularly apparent with low nitrogen intake. He further indicated that this high excretion of N in feces was caused by trapping of N in the digesta as a result of microbial activity in the rumen. Mason (1969) indicated that 57-81% of the nondietary fecal N was associated with bacterial material. Furthermore, Mason (1971) reported that grinding and pelleting a grass ration for steers resulted in an increase in the excretion of nondietary fecal N and bacterial plus endogenous debris N, 28 and 30% respectively, compared with the chopped material. It was concluded that these responses reflect the dominating influence of N of microbial residues from the rumen and hind gut on the excretion of bacterial plus endogenous debris and nondietary fecal N. The bacterial mucopeptides constituents (2-6-diaminopimelic and muramic acids) synthesized in the rumen of sheep are not digested in the abomasum and small intestine but are extensively degraded by bacteria in the

caecum and colon (Mason and White, 1971 and Mason and Milne, 1971).

Due to the microbial activity an appreciable portion of the dietary N is converted into nucleic acids in association with microbial protein. Smith et al. (1968) reported as high as 250 mg/100 ml, in the rumen fluid of calves or 10-16% of the total nonammonia N is in the form of nucleic acids. Ellis and Pfander (1965) indicated 10% or more of the microbial nitrogenous compounds formed is accounted for as nucleic acids. Smith et al. (1968) found that the nucleic acids entering the duodenum of ruminating calves (comprising 8-13% of the total N on many diets) were digested and absorbed to an extent of about 80% in the small intestine. In experiments in which pure RNA or DNA was fed to rats and preruminant calves, only about 25-30% of nucleic acid nitrogen appeared in the urine as allantoin (Smith et al., 1969). Digestibility of abomasally infused RNA-N was estimated to be 82% and urinary recovery of purine N from infused RNA was estimated to be 110% with low N intake and 92% with high N intake (Condon and Hatfield, 1971). This shows that the ruminally produced nucleic acid nitrogen is not used directly for nucleic acid synthesis by the host.

Abomasal Administration of Protein and Amino Acids in Ruminants

When dietary protein content is high, the extent of

protein degradation exceeds protein synthesis by rumen microorganisms. Thus there is a substantial loss of dietary protein to the host. However, when proteins are administered directly into the abomasum or duodenum, N retention and wool growth are markedly improved (Chalmers et al., 1954; Reis and Schinckel, 1963, and 1964). Eagen and Moir (1965) confirmed that post ruminal administration of casein improved its utilization over oral or ruminal administration. Little and Mitchell (1967) observed that abomasal administration of casein or soybean protein produced a greater N retention in lambs than when these proteins were fed orally. Schelling and Hatfield (1968) reported that infusion of certain mixtures of essential amino acids, particularly those containing lysine, improved N utilization by sheep. Similar results were obtained with lysine infusion into the abomasum of steers (Devlin and Woods, 1965). Abomasal infusion of sulfur containing amino acids and MHA resulted in considerable increased wool growth in sheep (Reis and Schinckel, 1963, 1964 and Reis, 1970).

Formaldehyde treated casein in the diet and casein per abomasum caused large increases in the concentration of the branched chain amino acids in plasma. In contrast, untreated casein in the diet did not increase the concentration of these amino acids in plasma and had little effect on the proportions of most essential amino acids (Reis and Tunks,

1970). Substantial increases have been shown in the concentration of most essential amino acids in sheep plasma and a decrease in glycine during abomasal supplementation with casein (Hogan et al., 1968). Schelling (1970) reported that infusion of DL-methionine or casein per abomasum in growing lambs resulted in a greater N retention on low protein diets as compared with the high protein diets. He further indicated that methionine was limiting the N retention in lambs fed high quality diets at two different protein levels. Hudson et al. (1970) suggested that supplementation of either regular or heated soybean meal into the abomasum did not significantly affect the protein digestibility or nitrogen retention in sheep. Reis and Downes (1971) observed the greatest increases in wool growth during the first four days of 100 g casein infusion into the abomasum of sheep and very little further change was noticed after 8 days. Bird and Moir (1972) obtained a response in wool growth and body weight gain when methionine (2 g/day) was continuously infused either ruminally or abomasally in sheep over a 6 week period, the gains were greater ($P < 0.05$) with abomasal infusion than with ruminal infusion. Abomasal infusion of methionine also improved the N and sulfur balances when compared with ruminal infusion. Steinakar et al. (1972) reported a higher N retention in steers when methionine was infused via the abomasum compared with dietary methionine or no

dietary methionine supplementation but with dietary sulfur supplementation. Scott et al. (1972) indicated that the administration of methionine into the abomasum of growing wethers showed a trend for increased nitrogen balance and a significant increase in plasma methionine and a decrease in plasma threonine.

Reis and Schinkel (1964) reported that infusion of S-amino acids (methionine or cystine) with casein gave an additional wool growth response, suggesting that the first limiting factor in the nutritional value of casein for wool growth is S-amino acid content. McDonald (1968) suggested that casein protein has approximately the same content of S-amino acids as the protein of pasture plants and rumen microbes. Therefore, it may be concluded that the amount of these amino acids absorbed in the intestines is a primary factor in influencing the rate of wool growth in grazing sheep.

Protection of Protein from Degradation in the Rumen

The preceding discussion has shown that the nitrogen of several different proteins was utilized more efficiently for tissue growth and wool production when added directly into the abomasum or duodenum compared with being given orally or added to the rumen (Chalmers et al., 1954; Blaxter and Martin, 1962; Reis and Schinkel, 1963; Reis, 1970; Little

and Mitchell, 1967; Hogan et al., 1968; Schelling and Hatfield, 1968; Reis and Downes, 1971). Feeding of encapsulated methionine to beef cattle resulted in a marked increase in N retention (Sibbald et al., 1968). Ingalls et al. (1970b) reported that there was small increase in feed intake and weight gain when encapsulated methionine was added to urea and soy diets. However, methionine supplementation had no significant effect ($P > 0.05$) on feed intake, weight gain or feed efficiency of a basal, basal plus urea or basal plus soy diets. These findings suggest that performance of ruminant animals can be improved if the feed proteins or S-amino acids were protected from degradation in the rumen and allowed to pass unchanged to the abomasum for digestion and absorption in the small intestine.

Protection of the protein from rumen degradation might be achieved in many ways. Orskov and Benzie (1969) showed that degradation of protein in the rumen could be prevented by stimulating closure of the oesophageal groove which facilitates the liquid diet to flow directly into the abomasum for enzymic digestion and by pass rumen fermentation. This could only be possible in young ruminants. The other promising methods are to modify the protein by increasing their resistance to proteolysis. The modification of protein can be brought about by two methods: (1) physical treatments and (2) chemical treatments. The physical

treatments involve dry heating or toasting and steaming oil-meals under pressure. Heat-treated casein (Chalmers et al., 1954) in diets for sheep and heat-treated ground nut meal (Chalmers et al., 1964) in diets for goats have been reported to give better overall nitrogen utilization than untreated proteins. Lower ammonia levels in the rumen with the heated proteins suggested that this was due to decreased degradation in the rumen. Whitelaw et al. (1961) reported that heating of ground nut meal improved growth rate and nitrogen retention in calves. Loosli et al. (1961) observed an increase in milk production in dairy cows fed heated soy bean. Tagari et al. (1962) reported improvements in nitrogen retention in rams given heat-treated soy bean meal compared with untreated soy bean meal fed rams.

Glimp et al. (1967) reported that heating soy bean meal (S B M) at 149°C for 4 hours improved the gain in lambs receiving rations with 12% crude protein and resulted in gains comparable to lambs receiving rations with 17% crude protein. Hudson et al. (1970) indicated that heating soy bean meal significantly ($P < 0.05$) increased the concentration of dry matter and non protein nitrogen in the abomasal digesta of sheep. Autoclaving the cotton seed and SBM resulted in decreased protein solubility and increased feed efficiencies and N retention in sheep (Sherrod and Tillman, 1962; Sherrod and Tillman, 1964, and Danke et al., 1966).

Dysli et al. (1967) observed a significant linear increase in average daily gain as the heating time for the soy beans increased from 0 to 30 minutes. The efficiency of nitrogen utilization obtained with sheep was maximum with 30 minutes heating compared with 45 or 60 minutes heating of full fat soy beans.

Several experiments have shown that heat treatment of protein, usually in the presence of carbohydrates will decrease their solubility and rumen degradation. Severe heating will also cause protein denaturation and decreased digestibility and availability of the amino acids.

Chemical modification of dietary protein can be brought about by treating with vegetable tannins (Leroy et al., 1965; Zelter and Leroy, 1966). Later Delort-Laval and Zelter (1968) reported that tannin treated peanut and linseed meals resulted in slightly increased efficiency of N utilization by experimental animals. Feed efficiency was increased when tannin treated milk powder was given to growing goats. Driedger and Hatfield (1970) found that N retention as a percent of intake was higher for lambs infused with tannin-treated SBM per rumen or abomasum compared to the control group. Pelleting the tannin treated SBM resulted in superior N retention over the treated unpelleted meal in lambs (Driedger and Hatfield, 1972). Tagari et al. (1965) suggested a possible disadvantage of the use of tannins for

protecting protein, because certain tannins interfere with the cellulolytic activity of the rumen microorganisms.

Ferguson et al. (1967) showed that treatment of casein with formaldehyde (FA) reduced the solubility of the protein and its susceptibility to microbial degradation. Formaldehyde treated casein appeared to be well utilized by sheep and resulted in a marked improvement in wool growth, weight gain (Ferguson et al., 1967), and N retention (Reis and Tunks, 1969). The response to FA treated casein diminished with increasing energy intake (Hughes and Williams, 1970, 1971b). Peter et al. (1971) reported that lambs receiving FA-treated SBM without methionine hydroxy analog(MHA) gained significantly faster and more efficiently ($P < 0.05$) than lambs which received FA-treated SBM plus MHA. Lambs fed glyoxal treated SBM without MHA gained significantly ($P < 0.08$) faster than lambs receiving glyoxal treated SBM plus MHA. Similar results were obtained by Wright (1971). Langlands (1971, b) did not observe any significant difference between diets containing untreated or FA-treated cotton seed meal in either wool production or live weight change. Wool production was up by 51% when the diet of grazing sheep was supplemented with 40 or 80 g of FA-treated casein in the rumen (Langlands, 1971, a). Nimrick et al. (1972) showed that treatment of fish meal with glyoxal significantly ($P < 0.05$) decreased nitrogen digestibility, urinary N

excretion, plasma urea N and increased fecal N excretion and N retention. Faichney and Davies (1972) observed little improvement in weight gain in calves receiving a FA treated peanut meal ration. Formaldehyde treatment was associated with a reduction in N digestibility and plasma urea levels. MacRae et al. (1972) reported that when FA-treated casein was given, the flow of most of the amino acids to the duodenum was increased over that observed with dried grass by an amount similar to that supplied in the supplement. However, for amino acids other than histidine, alanine and glycine the additional flow of amino acids to the duodenum with untreated casein was much less than the extra dietary amino acids supplied by the treated casein supplement. MacRae (1970) reported that there was an increase in the duodenal methionine over the dietary intake when the sheep were fed dried grass plus 60 g of treated casein. When untreated casein was given the level was much lower than the treated casein but comparatively higher than the dried grass diet. This indicated that there was a considerable degradation of methionine within the rumen with the untreated casein, whereas formaldehyde treatment of casein protected the methionine from rumen degradation. Ileal and fecal levels of methionine for all three diets were similar, showing that the absorption of methionine within the small intestine was the greatest with the treated casein and lowest with the dried grass diet.

Influence of Feeding Frequency, Grinding, Pelleting and Purified Diets on Rumen Fermentation and Protozoal Populations

The even supply of substrate and supposedly even production of metabolites that may occur under frequent feeding system may (a) give rise to an alimentary environment better suited to growth and metabolism of microorganisms, and (b) it may enable the animal to utilize the metabolites more efficiently than under the single feeding system where there may be a more rapid release of metabolites than compatible with an optimal rate of utilization (Gordon and Tribe, 1952). Disappearance of dry matter from the rumen depends upon the liberation of fermentation gases, absorption of metabolites and the passage of digesta to the omasum. Boyne et al. (1956) suggested that the quantity of dry matter in the rumen at various times after feeding varies according to the time between feeding and the level of feedings.

Animals fed twice daily showed significantly lower volatile fatty acids (VFA's) production than those animals fed 8 times daily (Knox and Ward, 1960). The relative proportions of propionic acid was higher and acetic acid was lower in the rumen of frequently fed heifers. Putnam et al. (1961) observed somewhat greater VFA concentrations in heifers fed ten times as compared with twice daily. However, comparisons within the pairs were inconsistant and the differences did not reach the level of significance.

Satter and Baumgardt (1962) reported that ruminal ammonia and VFA levels as well as pH values fluctuated significantly less when the animals were frequently fed. The absence of change in the average pH values due to feeding frequency is consistent with the findings of Rakes et al. (1961) but differ from another study by Rakes et al. (1957), where pH was lowered by more frequent feeding. Moir and Somers (1957) observed the lowest dry matter digestibility, N retention, ruminal pH and protozoal population in sheep fed once daily as compared with twice or four times a day feeding. In all cases diurnal fluctuation about the mean appeared to be less when the animals were frequently fed than compared to once or twice daily feedings. Frequent feeding did not effect the digestibility of dry matter, energy and N but influenced the N retention (Satter and Baumgardt, 1962). Ibrahim et al. (1969) suggested that with the continuous feeding system the rate of fermentation in the rumen of dairy cows virtually became constant as fluctuations in ruminal pH, ammonia and nyctohemeral variation in concentration of ciliate protozoa were insignificant. This facilitated the sampling for studies of the effect of diet on the concentration and types of protozoa. The total protozoa numbers were significantly higher ($P < 0.01$) for cows fed natural diets than for cows fed semipurified diets. In addition, inclusion of DES in the diets resulted in a significant increase in total number

of protozoa and aided in the establishment and retention of different ciliate protozoa. Chalupa et al. (1965) found no difference in the protozoa numbers when the heifers were fed forage twice or four times daily. Feeding pelleted forage significantly reduced the protozoa number after 7 days. Holotrichs were absent and remained so for the entire duration of the experiment. Changes in the molar proportions of VFA's were noticed on pelleted diets, which might be due in part to the variation in protozoa population. Christiansen et al. (1964) reported that lambs consuming some unground hay harboured relatively large protozoal numbers while the feeding of pelleted or finely ground mixed rations resulted in small numbers of rumen protozoa. Inclusion of DES not only prevented the disappearance of rumen protozoa from lambs fed a high concentrate pelleted ration but it also increased protozoal numbers. These increases were accompanied by improved live weight gain and feed conversion.

Oltjen et al. (1965) showed that the resting salivary flow and buffering capacity of saliva were significantly less for steers consuming purified and pelleted rations. Oltjen et al. (1962) reported that rumination time was markedly reduced in sheep fed the purified diets. Bailey and Balch (1961) indicated that secretion of saliva during rumination was two and one half times greater than during rest. Saliva of steers fed either purified diets or pelleted rations had

less ($P > 0.05$) buffering ability than saliva from steers on conventional rations. Reduced salivary flow could also affect the rumen pH. This might explain the lowered ruminal pH observed in steers fed on pelleted rations. Oltjen et al. (1965) indicated that pelleted rations reduced saliva flow when compared with conventional rations. Reduced salivation could result in lowered secretion of mucin which some investigators believe has antifoaming properties. This could be a causative factor in many observed cases of bloat. Reduction in salivary secretion also results in a reduced flow of electrolytes to the rumen. This may be associated with a low buffering capacity of saliva. Therefore, ruminal pH does not rise to an optimum level in animals fed pelleted diets as compared with those receiving non pelleted diets.

An important effect of pelleting is to increase the concentration of dry matter in the rumen. This leads to greater fermentation rate per unit volume and often greater gains in the animals. Feeds can be ground to varying degrees before pelleting. Grinding increases the consumption partly by increasing the turn over due to more rapid escape of finely comminuted material from the rumen (Hungate, 1966). Christiansen (1963) reported that grinding and pelleting the feed may eliminate the protozoa from the rumen. Purser and Moir (1959) indicated that ciliate protozoal population is profoundly influenced by pH conditions within the rumen.

The extent of the depression in pH and the period during which low pH conditions prevail, appear to be a major factor in controlling the concentration of the ciliate protozoa in the rumen.

The pH-VFA relationship for different diets may be considerably modified by variation in salivary secretion and in the accumulation of ammonia-N in the rumen after feeding (Briggs et al., 1957). Lactic acid only accumulated in the rumen on diets containing high levels of soluble carbohydrates or starch. Agrawala et al. (1953) reported that in five out of seven calves receiving purified diet containing 87% - starch, glucose and cellophane, the pH fell below 5.0.

Certain drugs such as antibiotics also influence rumen metabolism and the microbial population. Rumen protozoa increased following tylosin or aureomycin supplementation, while no difference was observed in the total viable bacterial counts (Purser et al., 1965). Klopfenstein et al. (1964) indicated that significant improvements were noticed in apparent digestibilities of DM, N, protozoal concentration and N balance by the addition of antibiotics.

Considerable interest has been shown during the past few years in the contribution of rumen ciliate protozoa to the metabolism and growth of ruminants. Ciliate free calves (Pounden and Hibbs, 1950; Eadie, 1962) and lambs (Abou Akkada and El-Shazly, 1964) had rough hair coats and were

pot bellied as compared with the faunated animals. The cause of this difference is not known. Though ciliate free calves have been noted to have poorer coats than faunated animals, there was no obvious difference in wool growth in lambs (Eadie and Gill, 1971). No significant difference was found in weight gain, food intake or general performance between animals with and without ciliates even though calves were subjected to a stress of low energy diets (Eadie, 1962). However significant differences in growth rates were noticed in faunated lambs (Abou Akkada and El-Shazly, 1964; Christiansen et al., 1965), water buffalo calves (Bohrami et al., 1967) and early weaned zebu calves (El-Sayed Osman, et al., 1970) compared to the defaunated animals.

Increased ammonia production in the faunated animals has been observed as compared with the unfaunated animals (Abou Akkada and El-Shazly, 1964; Christiansen et al., 1965; Klopfenstein et al., 1966; Chalmers et al., 1968; El-Sayed Osman et al., 1970 and Eadie and Gill, 1971). Even on low N diets, rumen ammonia almost doubled by faunation, while N retention and percent absorbed N retained were increased (Klopfenstein, 1966). Ammonia is also excreted by the ciliate protozoa as an end product of protein metabolism (Williams et al., 1961; Abou Akkada and Howard, 1962; Warner, 1965). Ciliate protozoa appear to enhance nutrient utilization when N intake is limited relative to energy availability.

From the literature it is clear that, although a high concentration of total VFA's are associated with the presence of ciliates in animals on roughage and concentrate rations, the changes in the proportions of VFA's are not consistant (Christeansen et al., 1965; Luther et al., 1966; Klopfenstein et al., 1966; Eadie and Gill, 1971). In contrast Eadie et al. (1970) found higher concentrations of total VFA's in the absence of or with low ciliate population in steers given restricted amounts of barley diet and found a high proportion of butyrate to be associated with very large ciliate populations in these animals. The restriction of feed intake in the absence of ciliate protozoa appeared to lead to an increase in rumen pH relative to that with ad lib. intake and suggests that this initial change in pH is a prerequisite to the eventual establishment of ciliates. However, Whitelaw et al. (1971) indicated that the rumen pH values during the restricted ciliate free periods were similar to those commonly observed in steers on ad lib. intake of barley diet found by Eadie et al. (1970). Despite this low pH, ciliates were established with ease after a single inoculation. This discrepancy between the two set of observations is not clear.

The influence of defaunation on the plasma amino acid concentration was demonstrated by Purser et al. (1966) Klopfenstein et al. (1966) and Virtanen, (1966). Defaunated

lambs showed higher concentration of plasma amino acids and a greater decrease in concentration following starch or glucose infusion than the faunated lambs (Purser et al., 1966). Lysine was one of the amino acid found to be limiting in the defaunated lambs and plasma amino acids were lower in the faunated lambs (Klopfenstein et al., 1966).

Host specificity for certain types of ciliates has been demonstrated by Naga et al. (1969) with cross inoculation among buffaloes, cows and sheep. Certain protozoa from sheep rumen inoculum failed to survive in young or adult cows and buffaloes thus suggesting the host specificity for particular species of protozoa.

Rape Seed Meal as a Protein Supplement in Ruminant Rations

Rape seed meal (RSM) is a relatively new protein supplement for ruminant animals in Canada, although it has been used extensively in other parts of the world for many years. RSM has become available as an ingredient for livestock and poultry feeds, however, the quantity used in ruminant rations is low. Bell et al. (1967) indicated that when rape seed is extracted by the solvent or prepress solvent process, RSM is a satisfactory protein supplement for dairy cows. Seale (1952) fed a ration of hay and grain mixture to dairy cows, which contained either 20% RSM or 20% linseed meal and noticed that the cows receiving the 20% RSM

grain ration produced 0.2 Kg more milk per day as compared to ration containing linseed meal (LSM). There was no difference in palatability between the ration or in taste or odor of the milk produced. Asplund (1961) fed RSM at 0, 10 and 20% of the dry matter of the ration to milking cows. The cows that received 10% RSM produced as much milk as those that received 10% LSM, but those that received 20% RSM declined in milk production almost twice as fast as the control. In a further experiment a concentrate mixture containing 10% of either RSM or linseed oil meal was fed to cows on pasture at 1 Kg per 6 Kg or 1 Kg per 12 Kg milk produced during a 13 week trial which resulted in cows consuming 3.6 Kg RSM per day. The substitution of RSM for linseed meal had no effect on milk yield.

No depression in feed intake or milk production was noticed in Holstein Friesian cows when fed a ration containing 10% RSM in a grain mixture (Bell, unpublished data). Bell (1969) further indicated that dairy cattle found RSM rations less palatable than the control rations but this was overcome by use of molasses and by gradually introducing RSM into the ration. Glucosinolates in RSM are apparently inactivated or altered when the meal is fed to ruminants as these have not been reported in the milk of lactating cows (Asplund and McElroy, 1961; Virtanen, 1963). Ingalls et al. (1968) reported that the replacement of 10% SBM in the dairy

rations with 12-13% RSM resulted in a decrease in ad libitum grain consumption. These levels of RSM had no significant effects on milk composition or production when used in place of soybean meal.

Jarl (1951) fed cows 2.5 Kg per day of an oil cake mixture containing 25 and 50-60% RSM. The cows that were fed 0 and 25% RSM in the mixture produced 0.5 Kg more milk daily than those cows that received the 50 to 60% RSM ration. Palatability of RSM was not a problem as soon as the cows become accustomed to it. He suggested that Swedish RSM was a good high protein concentrate for dairy cows and could be fed at a daily amount of at least 2 Kg per cow and it should always be fed dry. Ingalls and Seale (1971) indicated no significant difference in milk production during the first 60 days after calving among heifers that had received rations containing 0, 6.8 and 13.7% RSM from birth to calving. However, milk yield was about 10% less for heifers that had received the 13% RSM treatments compared with the other treatments.

Lactation studies were conducted at Fredericton, New Brunswick on 24 milking cows fed on grass hay and grain mixtures containing either 15% SBM, 22.5% RSM or 1.75% urea in a 12 week trial. Milk production of cows fed urea declined more rapidly then those fed RSM and production of RSM fed cows declined more rapidly than those fed SBM Ingalls and Waldern, 1972). From the studies at Aggasiz,

Ingalls and Waldern (1972) reported that the daily milk production, rate of decline in milk production and percentage of butter fat were lower for those cows fed a grain mixture containing a 24% RSM compared with those receiving a 27% SBM diet.

Chanet (1970) suggested a limit of 20% RSM must be set for the total concentrate feed with regard to dairy cattle and especially high producers, otherwise the animals will refuse to eat it. On the other hand, the percentage of RSM must be allowed to reach 40% in the case of a complementary nitrogen feed made from grains. He further indicated that toasting of meal enhanced the palatability and feed utilization without affecting the digestibility.

Seale (1952) studied the effect of feeding 8 to 10% of linseed meal, sunflower meal, mustard seed meal and RSM in the grain mixture to fattening steers. No difference was observed in efficiency feed utilization among the groups receiving sunflower seed meal, RSM or mustard seed meal. Clark and Bezeau, as cited by Whiting (1965), indicated that 6% RSM in place of LSM in the ration of young Holstein calves had no effect on feed intake or growth rate. Ingalls and Seale (1971) reported no significant ($P > 0.05$) effect on feed intake, weight gain or feed efficiency of heifers up to breeding weights by replacing SBM with 0, 6.8 or 13.7% RSM in calf starter rations. Studies were carried out at

the University of Guelph to examine the rate of growth of calves receiving calf starters containing four levels of RSM (0, 8, 16 and 24% of the grain mixture). RSM as a % of total ration was 0, 6.2, 12 and 17.6. The data suggested that up to 16% RSM in the grain mixture had no effect on animal performance. The 24% level of RSM had little effect on weight gain but appeared to result in higher hay intake and lower daily starter intake. It is of interest to note that the daily hay intake appeared to increase at a more rapid rate than the decrease found in starter consumption as the level of RSM increased (Ingalls, Waldern and Stone, 1970). Bezeau et al. (1960) reported that the digestibility of dry matter and protein was higher in a ration which contained 20% linseed meal than in a ration which contained 20% RSM (64 vs 61% for DM and 73 vs 66% for CP). The two rations contained the same percent of protein. Jarl (1951) indicated the digestibility coefficient of RSM for organic matter and crude protein were 76 and 83% respectively. Wood and Stone (1970) observed no significant differences in the digestibility of dry matter and crude protein for the calves receiving the basal, rape-basal and soy-basal diets during the growth trial. The growth rates of 0.55 and 0.75 Kg per day were supported by intakes of 1.66 and 1.92 X maintenance for rape-basal and soy-basal diets respectively. Stakes et al. (1972) found no significant differences ($P > .05$) for protein

digestibility of SFM, RSM and SBM diets. Sunflower meal had the lowest digestion coefficient for dry matter and energy. In each instance, it was significantly less ($P < 0.05$) digestible than SBM but not significantly different than RSM. Dry matter intake was significantly ($P < 0.01$) reduced by RSM compared with SFM or SBM from birth to eight weeks of age when RSM made up 26% of the ration. No significant differences in average daily gain or feed efficiency were observed from birth to 14 weeks of age.

PART ONE

Nutritive and Comparative Values of Rape Seed Meal (Treated or Untreated with Formaldehyde) and Soy Bean Meal for Young Dairy Calves

MATERIALS AND METHODS

EXPERIMENT I

The influence of treating RSM with 2 vol (W/V) of a 1% FA solution (5.6 g FA/100 g protein) was investigated using ten young Holstein & Holstein X Brown Swiss calves of 55-63 days of age. The growth trial was conducted for 14 weeks. Rumen fluid and jugular blood samples were collected at the start and after 1, 2, 4, 6 and 8 weeks of the growth trial for the measurement of NH_3 release in the rumen and urea level in blood plasma (Appendix no. 1). A digestibility and N balance trial was conducted by taking two male calves from each group and using a switch back design. The animals were given a 14 days adjustment period and total collections for urine and feces were made for seven days (Appendix no. 1).

In vitro studies were carried out by treating various oil meals RSM, SBM and linseed meal (LSM) with four solutions of FA and two of glutaraldehyde (GA). In addition the oil meals were also treated with heat at 180° C for 20 minutes. The solubility of protein was determined in 0.02 N NaOH and the NH_3 release was measured in an artificial rumen from the commercial and treated meals (for details see Appendix no. 1).

EXPERIMENT II

Solvent extracted RSM was treated with FA solution (0.7 g FA/100 g protein) by spraying it on the meal in a mixer. After treating with FA the RSM was stored in burlap bags lined with plastic. Three to four days later the FA-RSM was mixed with other ration ingredients. This method of application was much more practical compared to that used in the first experiment.

Twenty four Holstein dairy calves of both sexes were weaned from milk at 35 days of age and placed on the experimental diets at the age of seven to nine weeks. Calves were randomly assigned to the three test diets (Appendix no. 2) for 14 weeks. Calves were housed in individual pens with feed and water available free choice.

Rumen samples for ammonia and volatile fatty acids (VFA's) and blood samples for Urea N were collected and stored as indicated in "Nutritive value of formaldehyde treated rape seed meal in dairy calves", (Appendix no. 1). Biweekly body weights and daily feed consumptions were recorded during the growth trial.

Chromium oxide (0.3%) was mixed in the test rations as a marker for determining the digestion coefficients for dry matter, nitrogen, acid detergent fibre (ADF) and energy. Six calves from each group were used for digestibility studies (Appendix no. 2).

RESULTS

EXPERIMENT I For results see Appendix No. 1.

EXPERIMENT II For results see Appendix No. 2.

DISCUSSIONS

Protein Solubility

The solubility of RSM protein in 0.02 N NaOH was decreased from 64.7 to 5.4% by treating with 1% FA (2 V/W). FA treatments (1, 2, 3 and 4%) reduced the solubility of protein to zero as measured after incubating for one hour in rumen fluid. The present findings are in agreement with those of Peter et al. (1971) in which the protein solubility of SBM was depressed ($P < 0.01$) by treatment with FA, GA or glyoxal. Ferguson et al. (1967) reduced the solubility of casein protein from 83 to 8% by treating with 4% FA solution (10 V/W or 40 g FA/100 g protein). Hughes and Williams (1971, a) indicated that FA treatment of casein (2.1 g FA/100 g protein) markedly lowered the solubility of casein protein from 90 (untreated) to 4.9% (treated) in buffered rumen liquor after incubating for 48 hours in an artificial rumen. While working with ground nut meal they could lower the protein solubility in the rumen liquor from 75.8 to 30.8% after treating with FA solution (Hughes and Williams,

1971, b). Faichney and Davies (1972) reported a 15 fold reduction of protein solubility of peanut meal in a 1.0 M-NaCl solution after FA treatment. However, very little depression in solubility of protein was found by Langlands (1971) with cotton seed meal, indicating that mechanically extracted cotton seed meal protein was less soluble in a 1.0 M-NaCl solution as compared to peanut meal.

Barry (1972) reported that FA treatment of casein (3.5 g FA/100 g protein) reduced the solubility of protein from 94.6 to 3.4%, however, the acid pepsin digestibility of the treated casein was depressed as compared with the untreated casein. Zelter et al. (1970) indicated that FA did not reduce the susceptibility of treated peanut meal (1.31 g FA/100 g protein) to pepsin digestion in the in vitro rumen system.

Treating RSM, SBM and LSM with heat or various levels of FA and GA solution significantly ($P < 0.01$) decreased deamination in the artificial rumen as measured by ammonia production after a 24 hour fermentation period (Appendix no. 1). Zelter et al. (1970) obtained total inhibition of the bacterial deamination of peanut protein after treating with 0.6% formal and 1.5-1.8% glyoxal or GA. Hughes and Williams (1971) reported a significant reduction in ammonia production from treated meals after 48 hours incubation with rumen liquor. Faichney and Davies indicated

that FA (4.37 g FA/100 g protein) treatment of peanut meal completely prevented ammonia release after a 12 hours *in vitro* fermentation with rumen fluid. Peter *et al.* (1971) and Langlands (1971) obtained similar results by incubating treated SBM or cotton seed meal *in vitro* with rumen liquor.

Animal Performance

Treatment of RSM protein with FA (5.6 g FA/100 g protein) did not influence the feed intake, weight gain, and feed efficiency in young calves receiving FA-RSM ration compared with those fed untreated RSM during the growth trial (Appendix 1). In the second experiment FA treatment of RSM (0.7 g FA/100 g protein) did not improve feed consumption, live weight gain, or feed conversion over those calves receiving untreated RSM or SBM diets during the 14 week growth trial (Appendix 2). Other workers also reported that treatment of SBM protein (Mowat and Deelstra, 1970, Satter *et al.*, 1970), peanut meal protein (Hughes and Williams, 1971; Faichney and Davies, 1972) and cotton seed meal protein (Langlands, 1971, b) did not improve the animal performances over the untreated meals in sheep and dairy calves. However, Peter *et al.* (1971) obtained a substantial increase in body weight gain in sheep or growing lambs fed treated SBM (0.6 g FA or 1.5 g glyoxal/100 g of SBM). Increased performances as measured by live weight gain or wool

growth were observed in sheep or lambs when the FA-treated casein was given as a protein supplement in the abomasum or in the diet compared with those given the untreated casein (Ferguson et al., 1967; Reis and Tunks, 1969; Wright, 1971; Hughes and Williams, 1971; Langlands, 1971, a; Barry, 1972). The disparities observed in calves and sheep or lambs after feeding treated meal diets or treated casein diets for growth performance and wool growth could be either due to the species difference relative to amino acid requirements, the proteins difference relative to amino acid composition or the method of treating the casein or vegetable protein, or both.

Stake et al. (1972) compared RSM, sunflower meal (SFM) and SBM as protein supplements for dairy starter rations and indicated that no significant differences in average daily gain or feed efficiency were observed from birth to 14 weeks of age among the three treatments. Ingalls and Seale (1971) reported no significant ($P > 0.05$) effect on feed intake, weight gain or feed efficiency of heifers upto breeding age by replacing SBM with 0, 6.8 or 13.7% RSM in the calf starter rations. However, inclusion of 30% RSM in the calf starter grower ration resulted in reduced feed consumption and weight gain compared with a ration containing 20% RSM (Ingalls and Waldren, 1972). The results of the present studies also indicated that inclusion of RSM at 20% of the ration for young calves (8 to 22 weeks of age) did

not affect the dry matter intake, daily gain or feed efficiency when compared with a SBM ration.

Ruminal Ammonia and Blood Urea

Ruminal ammonia levels were significantly depressed ($P < 0.01$) in the calves receiving FA-treated RSM compared with the control calves (Experiment I, Appendix 1, Table 5). In Experiment II rumen ammonia levels of FA-RSM calves were also found to be significantly ($P < 0.05$) lower than those calves receiving the RSM ration but did not differ significantly from those fed the SBM ration (Appendix 2, Table 3). Satter et al. (1970) observed lower ruminal ammonia level in cows receiving the FA-treated SBM compared with those receiving the control ration. Barry (1972) reported that FA treatment of casein reduced the concentration of ammonia in rumen fluid of young sheep.

Blood plasma urea N was significantly ($P < 0.01$) lower for calves fed FA-treated RSM compared with those receiving untreated RSM (Appendix 1, Table 5). Similar results were obtained by Satter et al. (1970) in cows fed FA-treated SBM. Peter et al. (1971) reported that glyoxal treated SBM lowered plasma urea N levels ($P < 0.05$) as compared with those lambs receiving the water treated SBM. Faichney (1971) indicated that the plasma urea levels in the lambs given the FA-treated casein declined steadily until, at nine hours

after feeding, they were only 60% ($P < 0.01$) of the levels in the lambs given untreated casein. Faichney and Weston (1971) also reported a significant decrease ($P < 0.01$) in the level of urea N in the plasma of lambs given the treated casein diet. Blood plasma urea levels observed in experiment II tended to be lower in the FA-RSM calves than those receiving the SBM or RSM ration, however, the difference was not significant ($P > 0.05$), (Appendix 2, Table 3). Faichney and Davies (1972) observed no significant ($P > 0.05$) decrease in the plasma urea of calves fed FA-treated peanut meal at lower protein levels (13%) but at a higher protein level (20%) the reduction was significant ($P < 0.05$). Nimrick et al. (1972) reported a significant decrease ($P < 0.05$) in plasma urea N levels in lambs fed glyoxal treated fish meal and SBM compared to the untreated groups. Barry (1972) observed that plasma urea N was lowered by FA treatment of casein ($P < 0.01$) but increased with increasing level of casein intake ($P < 0.01$). In experiment II, the level of FA used for treating RSM (0.7 g FA/100 g protein) was comparatively lower than that used in experiment I (5.6 g FA/100 g protein) and the other workers (Table 1). The depression in the ruminal NH_3 and blood plasma urea N in experiment I and II are associated with the formaldehyde treatment and are in agreement with Satter et al. (1970); Peter et al. (1971); Faichney (1971); Faichney and Weston (1971) and Barry (1972).

These data indicate that FA treatment partially protected the RSM protein from degradation in the rumen and/or reduced the rate of ammonia production thus resulting in more intact protein passing to the lower G.I. tract. The levels of FA applied for treating the different proteins which significantly lowered the blood plasma urea N were found to be 1.09 g FA/100 g peanut meal protein, 3.16 g - 3.51 g FA/100 g casein protein and 5.62 g FA/100 g RSM protein (Faichney and Davies, 1972; Faichney, 1971; Faichney and Weston, 1971; Barry, 1972 and Appendix 1, Table 5). Ruminal ammonia release from casein and RSM proteins was effectively reduced, when these proteins were treated at the rate of 3.16 to 3.5 g, and 0.7 to 5.6 g FA per 100 g of proteins, respectively (Faichney, 1971; Faichney and Weston, 1971; Barry, 1972; and Appendices 1, 2). Furthermore, the studies carried out in the _in vitro_ rumen by several workers indicated that lower levels of FA were sufficiently effective in decreasing the production of ammonia from the treated proteins (Ferguson _et al._, 1967; Zelter _et al._, 1971; Hughes and Williams, 1971; Langlands, 1971; Peter _et al._, 1971; Appendix 1 and 2; Faichney and Davies, 1972; and Barry, 1972).

Ruminal VFA's

Total volatile fatty acids (VFA's) in rumen fluid were significantly higher for calves receiving control RSM

compared with those on FA-treated RSM in Experiment I. However, no significant difference was noted in the concentration (m moles/100 ml) of total VFA's for the calves fed SBM, RSM and FA-RSM diets in experiment II. The level of FA treatment was much lower in experiment II. Except for propionic acid no significant differences ($P > 0.05$) were observed in the concentration or molar percent of VFA's (acetic, butyric, isobutyric, valeric and isovaleric acid) between the control and FA-treated RSM groups in experiment I. In experiment II also, no significant differences ($P > 0.05$) were observed in the concentration or molar percentages of VFA's in the rumen fluid of calves fed SBM, RSM and FA-RSM rations. Faichney and Weston (1971) indicated that FA treatment (3.16 g FA/100 g protein) of casein caused a significant reduction in the concentrations of VFA's in the rumen fluid of lambs.

Apparent Digestibility and N Balance

In experiment I, there was a significant decrease in apparent digestibilities of dry matter ($P < 0.05$) and crude protein ($P < 0.01$) in the FA-treated RSM (5.62 g FA/100 g of protein) group compared with the untreated RSM (control) group. However, in experiment II no significant ($P > 0.05$) differences were found in the digestion coefficients of dry matter, nitrogen, ADF and gross energy of the SBM, RSM and FA-RSM (0.7 g FA/100 g protein) diets. However, the

digestibility of dry matter, N, ADF and energy tended to be higher for the RSM diet compared to FA-RSM diet.

The discrepancy between experiments I, II in the digestibility of nutrients could be due to the difference in the level of FA-applied for treatment of RSM. Slightly lower digestibility of FA-treated casein (40 g FA/100 g C.P.) was reported by Reis and Tunk (1969) in sheep when it was included in the diet as compared with the untreated casein. Hemsley et al. (1970) indicated that digestion of cell wall constituents (C.W.C.) and organic matter (O.M.) were little affected by the FA-treatment of dried forage (4 g FA/100 g C.P.). Faichney (1971) reported a significant decrease in apparent digestibility of OM ($P < 0.05$) and N ($P < 0.01$) in one experiment when FA-treated casein (3.16 g FA/100 g protein) was fed in the ration, but no difference in a later experiment. Faichney and Weston (1971) reported that inclusion of FA-treated casein (3.16 g FA/100 g protein) in the diet of lambs had no effect on OM digestion in the whole gastro-intestinal tract, however, significantly ($P < 0.01$) less OM was digested in the stomach of lambs fed treated casein compared with those fed untreated casein ration. The digestibility of N was found to be significantly ($P < 0.001$) lower in lambs fed treated casein compared with untreated casein. Faichney and Davies (1972) found no significant difference in the digestibility of dry matter and OM, however,

treatment of peanut meal was associated with a reduction in the digestibility which was significant at lower protein (13%) level but not at the higher protein (20%) level. Hughes and Williams (1971) reported that nitrogen digestibility of FA-treated ground nut meal was significantly lower in sheep at 15% protein level but not at the 11% protein. Langlands (1971, b) also observed depression in OM and N digestibility in sheep by treating cotton seed meal with FA. In the present studies FA-treatment of RSM (5.6 g FA/100 g protein) significantly depressed the apparent digestibilities of dry matter ($P < 0.05$) and crude protein ($P < 0.01$), but not when 0.7 g FA/100 g protein was used for treating the RSM. Langlands (1971, b) indicated that breakdown of protein *in vitro* was insignificant when more than 0.68% FA (W/W) was added in the treatment of cotton seed meal. Nicholson *et al.* (1972) reported that FA treatment of RSM (2.53 g FA/100 g protein) did not affect the digestibility of dry matter or crude protein in sheep. The level of FA used in experiment II and III was very low (0.7 g FA/100 g CP) as compared to other workers, however, even at this low level there was a trend to lower ration digestibility in young dairy calves, but little affect in young steers (Experiment III).

Excretion of N in the feces and urine was significantly different ($P < 0.01$) in the untreated and FA-treated RSM groups. Larger amounts of N were observed in the feces

of FA-RSM fed calves than the untreated calves. The opposite trend was observed in the excretion of urinary N (Appendix 1, Table 6). These results are in agreement with those of Reis and Tunks (1969) obtained when sheep were fed FA-treated casein. Similar observations were made by Faichney (1971) with lambs fed FA-treated casein. The FA-treated RSM apparently became less susceptible to enzymic digestion in the intestine, which resulted in more N excretion in the feces of calves. Zelter et al. (1970) indicated that FA did not reduce the susceptibility of treated peanut meal (0.6 g FA/100 g) to pepsin digestion in the in vitro system. Hughes and Williams observed a marked depression in the digestibility of FA-treated casein (2.1 g FA /100 g protein) and ground nut meal (4.37 g FA/100 g protein) in rumen fluid + pepsin digestion in vitro. FA treatment appears to make the protein less susceptible to enzymic digestion in the lower gut. A positive N balance was observed for the treated and untreated RSM fed calves in experiment I but the difference was not significant ($P > 0.05$). Reis and Tunks (1969) obtained a significantly higher N retention in sheep with FA-treated casein compared to the untreated casein. Faichney (1971) found that N retained in the body tended to be higher at all times in the lambs given the diet containing FA-treated casein. The difference in N retention could be due to the higher digestibility of the

casein protein as compared with the RSM protein and other vegetable proteins. RSM is fairly high in fibre content (cell wall constituents) and the protein encased in the cell walls is not digested in the rumen and in the G.I. tract which results in lower protein digestibility.

The studies from experiment I indicate that different levels of FA and GA treatments of meals affect the microbial degradation of RSM protein *in vitro*. Data of ammonia release in the rumen of calves in experiment I, II and III showed a partial protection of RSM protein from bacterial deamination. However, treating RSM with FA(5.6 g FA/100 g protein) reduced the rate of deamination in the rumen and reduced enzymic digestion in the lower gut in experiment I. The level of FA used in experiment II and III (0.7 g FA/100 g protein) was comparatively lower than the FA level applied in experiment I, however, even at this low level digestibility tended to be reduced. The higher levels of FA and GA treatments of RSM resulted in only slightly greater inhibition of the bacterial degradation in the artificial rumen. These observations are in agreement with those reported by Peter *et al.* (1971) for SBM and Langlands (1971, b) for cotton seed meal. This suggested that the lower levels of aldehydes used in the present experiments and reported by other workers were enough to cause a marked depression of the microbial degradation of the treated meals or casein *in vitro* and also produced partial protection *in vivo*.

TABLE 1

LEVELS OF FORMALDEHYDE USED FOR TREATING THE DIFFERENT TYPES OF PROTEINS *

Type of Protein	Aldehyde used	Level of FA /100g material used (g)	Level of FA /100g protein used (g)	Reference
1. Casein 100%	FA	40.0	40.00	Ferguson et al., 1967
2. Casein 100%	FA	40.0	40.00	Reis and Tunks, 1969
3. Peanut meal 45.8% (C.P.)	FA	0.6	1.31	Zelter et al., 1970
4. Forages (25%)	FA	1.0	4.00	Hemsley et al., 1970
5. Casein (95%)	FA	2.0	2.10	Hughes and Williams, 1970
6. Casein (95.6%)	FA	3.0	3.10	Langlands, 1971
7. Cotton seed meal (41.88%)	FA	1.0	2.39	Langlands, 1971
8. Ground nut meal (45.8%)	FA	2.0	4.37	Hughes and Williams, 1971
9. Casein (95%)	FA	3.0	3.16	Faichney, 1971
10. Casein (95%)	FA	3.0	3.16	Faichney and Weston, 1971
11. Soy bean meal (50%)	FA	0.6	1.20	Peter et al., 1971
12. Casein (100%)	FA	40.00	40.00	Wright, 1971
13. Soy bean meal (44%)	FA	0.74	1.68	Mowat and Deelstra, 1972
14. Peanut meal (45.8%)	FA	0.5	1.09	Faichney and Davies, 1972

continued.....

TABLE 1 (continued)

Type of Protein	Aldehyde used	Level of FA /100g material used (g)	Level of FA /100g protein used (g)	Reference
15. a) Rape seed meal (35.6%)	FA	2.0	5.62	Sharma et al., 1972
b) RSM (35.6%)	FA	0.25	0.70	Sharma and Ingalls, 1972 (unpublished)
c) Casein (87%)	FA	0.61	0.70	unpublished, 1972
16. a) Soy bean meal (50%)	FA	0.5	1.00	Nimrick et al., 1972
b) Fish meal (66%)	FA	0.6	1.00	Nimrick et al., 1972
17. Casein (85%)	FA	3.0	3.50	Barry, 1972
18. a) Rape seed meal	FA	18.5	51.97	Nicholson et al., 1972
b) Rape seed meal	FA	9.25	25.98	Nicholson et al., 1972
c) Rape seed meal	FA	0.93	2.59	Nicholson et al., 1972
d) Rape seed meal	FA	0.62	1.74	Nicholson et al., 1972

PART TWO

Effect of Formaldehyde Treatment of Rape Seed Meal and Casein Protein on Digestibility and N Retention by Young Holstein Steers

MATERIALS AND METHODS

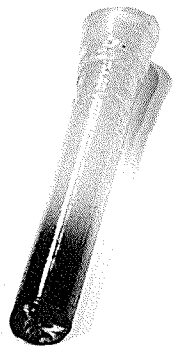
EXPERIMENT III

Four young Holstein steers were fistulated in the pyloric portion of the abomasum and in the ileum, six inches before the ileo-caecal valve, with soft plastic T cannulae (Figure 1, 2). Animals were given about one month for complete recovery from the operation. The animals were then used in the feeding experiment of a planned 4 X 4 latin square design. The rations were randomly alloted to the four animals for four periods. Each experimental period consisted of a 14-days preliminary period for adjustment, seven days of balance trial and seven days of digesta collections (Table 2). A 12-day rest period was given between each experimental period.

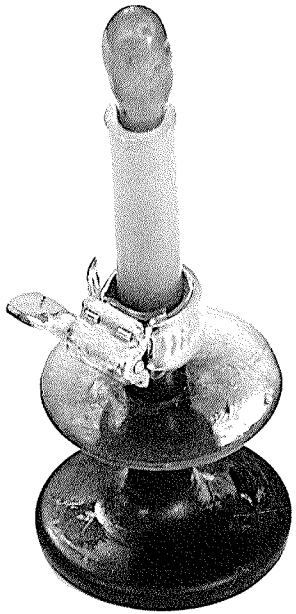
Feeding Regime

The steers were fed by a continuous belt feeder (Ibrahim *et al.*, 1969). The pelleted feeds were spread evenly on the two-ply rubber conveyer belts of the automatic feeders, which move towards the centre of the unit, dropping feed into the respective feed box of each steer. The feeds were placed on the belt once in the morning at 9 AM for each steer and the feeders were so set that a small quantity of the pellets dropped from the conveyors into the respective

Figure 1. Cannulae used in experimental steers with their discs, clamps and plugs. (A) Rumen cannula, (B) abomasal cannula and (C) ileal cannula.



A



B

C

Figure 2. Position of the abomasal and ileal cannulae in the Holstein steer used for the collection of digesta.

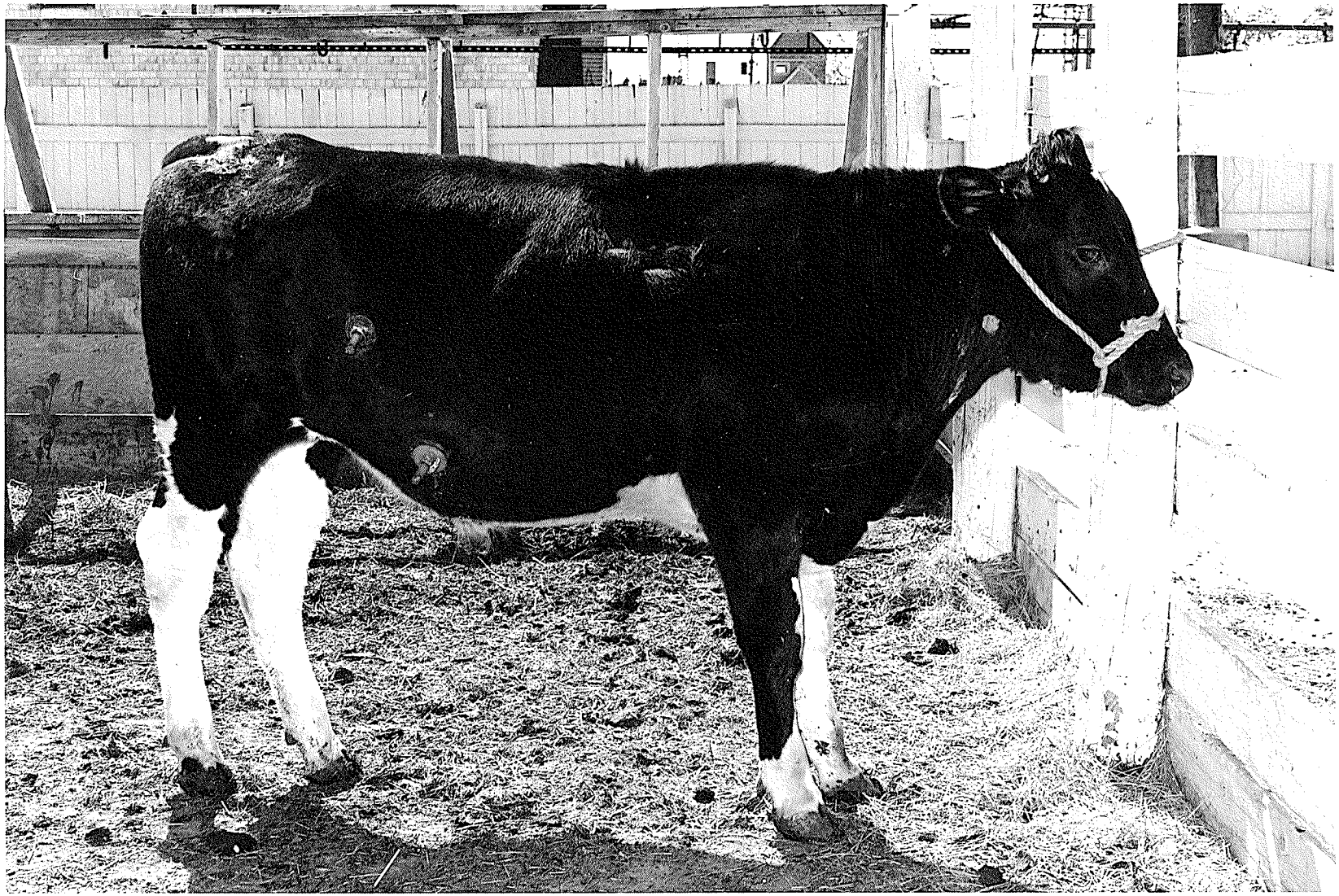


TABLE 2

PLAN OF EXPERIMENT AND COLLECTION OF SAMPLES

Preliminary period	Digestibility and N balance trials (Total collection of urine and feces	Collection of abomasal, ileal and fecal grab samples	Collection of rumen fluid for pH, NH_3 and VFA's	Collection of rumen contents	Rest Period
14 days	15 to 22 days	I 22nd to 24th day II 26th to 28th day	25th day	29-30th day	30-42 days

	Abomasal, ileal and fecal samples			Rumen Fluid	Abomasal, ileal and fecal samples			Whole rumen contents
	Digesta Collection Days							
Time	22	23	24	25	26	27	28	29 + 30
9 AM								++
10 AM				+			+	
11 AM				+		+		
12 AM				+	+			
1 PM			+	+				
2 PM		+		∓				
3 PM	+			+				
4 PM							+	
5 PM				+		+		
6 PM					+			
7 PM			+					
8 PM		+						
9 PM	+							

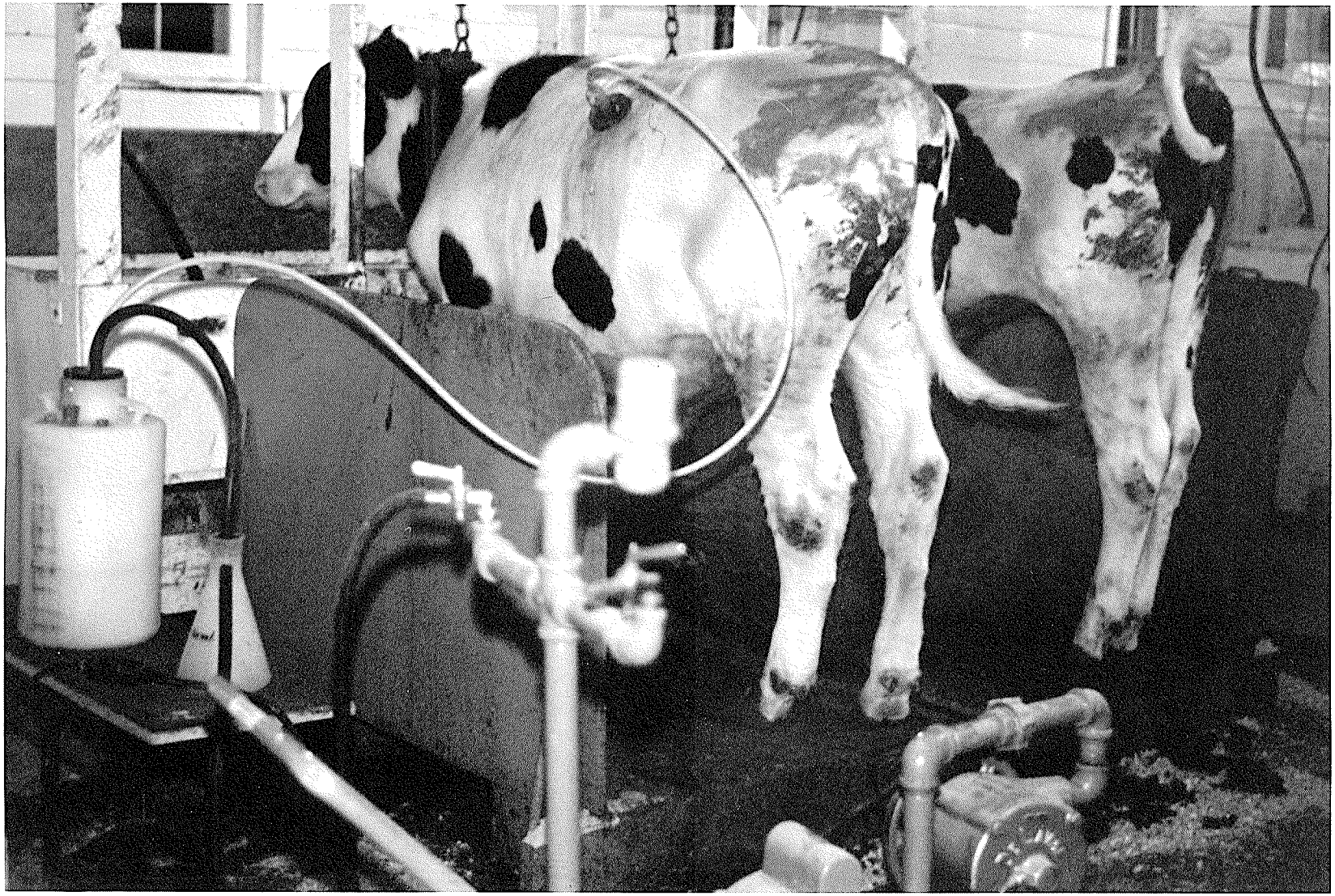
\+ = Indicates the samples collected at the particular time from each steer.

∓ = Indicates collection of jugular blood sample from each steer at 2 PM.

mangers for 20 seconds after every 10 minute interval (Figure 4). The animals were housed in stalls under continuous artificial light and temperature (60°F) and had free access to water at all times (Figure 4). The steers quickly adapted to the continuous feeding regimen and ate frequently. Early morning and late evening check indicated that little if any feed accumulated during any time period. The feed was adjusted to the maximum intake by each steer during the preliminary period and the same amount of the feed was given during the balance trial and the digesta collection period. Near steady state conditions were established in the rumen and gastro-intestinal (G.I.) tract using this feeding system (Ibrahim et al., 1969).

Feed samples were taken daily from each ration during the experimental period, placed in plastic bags and composite samples were stored for chemical analysis. The experimental treatments consisted of feeding the semipurified pelleted diets containing corn starch, cellulose powder, urea, rape seed meal (RSM), formaldehyde treated rape seed meal (FA-RSM), casein, FA-treated casein (FA-casein), minerals, vitamins, chromium oxide and polyethyleneglycol (PEG), (Table 3). RSM and casein protein were treated with formaldehyde as follows: Three liters of 3.6% FA solution was sprayed onto 43 Kgs of commercial RSM in a mixer and was mixed for 20-30 minutes. After thorough mixing the meal was

Figure 3. Collection of rumen digesta sample from the experimental animal during the present studies (Experiment III).



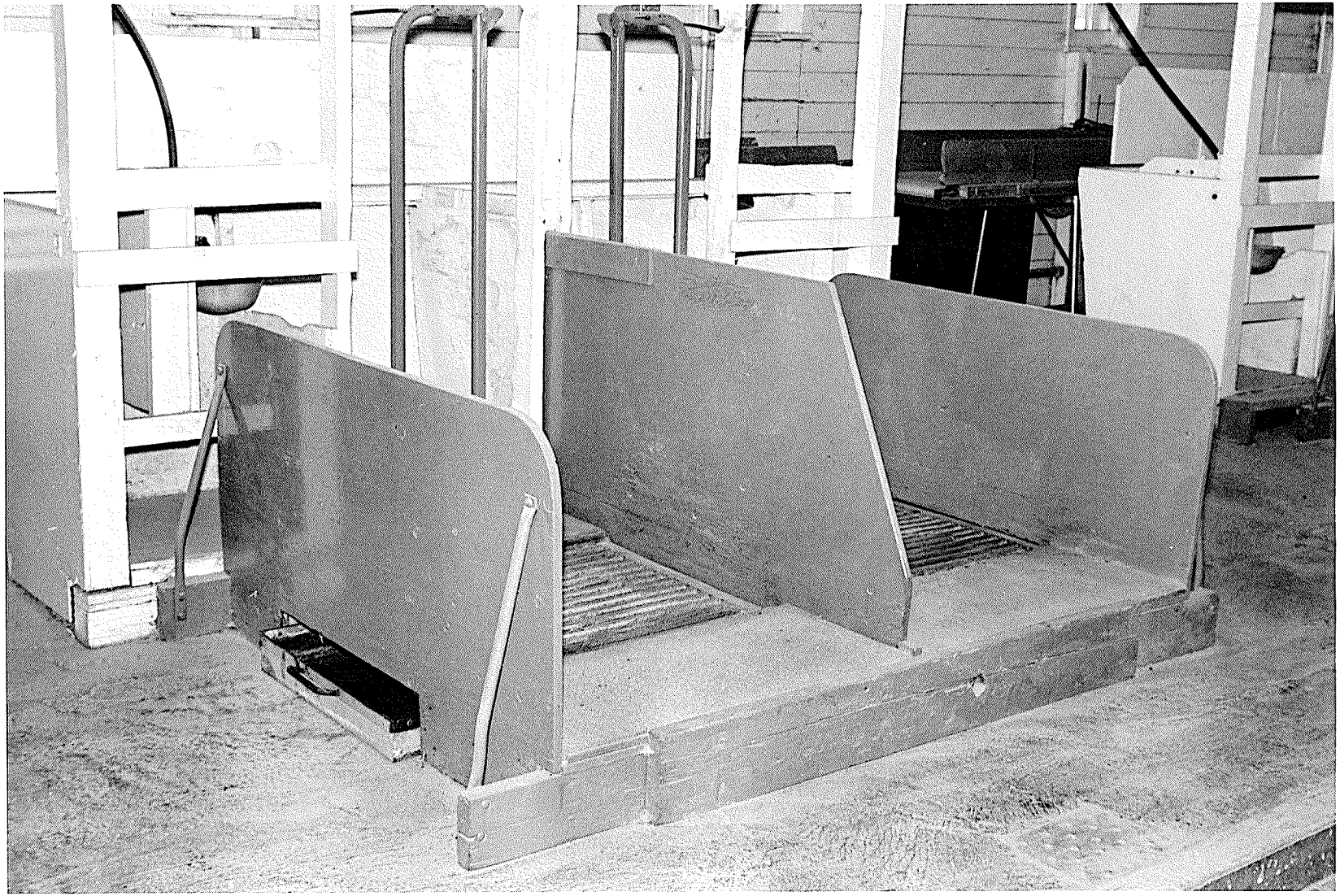
kept in burlap bags lined with plastic. Similarly 17 Kgs of casein was treated with 1.5 liters of 6.9% FA solution and after thorough mixing it was stored in a burlap bag lined with plastic. Experimental rations were mixed 3-4 days later. This technique provided 0.7 g FA per 100 g protein. All the feeds were pelleted with a 3/16th inch die and stored in burlap bags for feeding the steers.

Management of the Experimental Animals

During the preliminary and treatment comparison periods the animals were kept in stanchion stalls with a central slatted floor area for the collection of urine in metal trays (Figure 4).

Two of the steers developed swollen knees due to some physical problems in the stalls during the second period and had to be dropped from the experiment. The experiment was continued with the remaining two steers. Within one month soft plastic T type cannulae were fixed in the rumen, abomasum and ileum of two new steers of the same breed and similar initial weights. The procedure was the same as described above. After the recovery period the new steers were also placed on the test diets for four different periods.

Figure 4. Stalls and the automatic feeders used for nitrogen balance trials and digesta collection periods in Experiment III.



Digestibility and N Balance Trials

The stalls were thoroughly cleaned before the collection of feces and urine. In order to separate the feces and urine, a plastic (006 mils) tube of about one meter long and 55 cms wide was fixed on the rump of each steer. The tail was kept free by passing it through a small hole made in the tube. The lower end of the plastic tube was tied tightly with a string and thus served as the fecal bag for each steer during the balance trials. The bags were emptied several times during the day and the feces were stored in separate plastic bags for sampling. Urine was collected in the metal trays placed underneath the slatted floor area of each stall (Figure 4). Toluene was added in the trays to reduce loss of N from the urine. Total collection of feces and urine was over a seven day period. One-tenth of the feces and 200 ml of the acidified urine were stored at -20°C. At the end of each period, after thawing, the feces and urine were composited separately for each animal and sub sampled for analysis. Fecal samples were dried in a forced air oven at 70°C. Total N in the feces and urine samples were determined according to the Kjeldahl method (AOAC. 1965). Chromium oxide in the feed and feces was estimated by atomic absorption spectrophotometery (Williams et al., 1962).

Collection of Digesta and Fecal Samples

Digesta and fecal grab samples were collected during two three-day periods following the balance trials (22, 23, 24th day and 26, 27 and 28th day, Table 2). On the 25th day six rumen samples (10 AM, 11 AM, 12 AM, 1 PM, 3 PM and 5 PM) were collected from each steer for the estimation of ruminal pH, VFA's and NH_3 levels (Table 2).

A 50 ml abomasal digesta sample was collected directly into a measuring cylinder after opening the cannula. After mixing 25-30 ml of the digesta was transferred to prelabelled plastic bags and immediately frozen in liquid nitrogen. The remaining portion was pooled in another container for PEG, NH_3 and dry matter analysis.

For ileal digesta no fixed volume was taken from the ileal cannula because the flow of the digesta was quite variable. Therefore, digesta that flowed through the cannula during a 10 to 15 minute period was considered a representative sample. This sample was collected in plastic bags and frozen as given above.

Fecal grab samples were taken directly from the rectum of each animal at the time of collection of digesta and frozen in the same manner as given above for digesta samples. During the day (9 AM to 9 PM) 12 abomasal, 10 to 12 ileal and 12 fecal samples were collected from each steer (Table 2).

TABLE 3

COMPOSITION OF THE EXPERIMENTAL DIETS (EXPT. III)

Ingredients	Treatments			
	RSM	FA-RSM	CASEIN	FA-CASEIN
Rape seed meal	19.0	19.0	--	--
Casein	--	--	7.5	7.5
Corn starch	38.0	38.0	41.5	41.5
Cellulose powder†	20.0	20.0	20.0	20.0
Ground barley straw	8.0	8.0	16.0	16.0
Sun flower oil	2.0	2.0	2.0	2.0
Cane molasses	5.0	5.0	5.0	5.0
Urea (281 % C P)	1.9	1.9	1.9	1.9
Trace mineralized salt	3.0	3.0	3.0	3.0
Calcium Phosphate	1.0	1.0	1.0	1.0
Choline Chloride	0.2	0.2	0.2	0.2
Sulphur	0.2	0.2	0.2	0.2
Vitamin premix‡ (A, D, and E)	0.9	0.9	0.9	0.9
Cr_2O_3	0.3	0.3	0.3	0.3
P E G	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0
Crude protein (%)§	14.7	15.1	14.8	14.1
Acid detergent fibre§ (%)	23.6	23.7	24.0	23.9

† Alpha-floc -BNB -20 0-11-26-A, Brown Company, New Hampshire, USA.

‡ Vitamin A 500,000 in IU, Vitamin D_3 50,000 IU and Vitamin E 50,000 IU per 500 lb of feed mixed.

§ Determined by chemical analysis.

On the 25th day of each experimental period six rumen samples (four at hourly and two at 2-hourly) were drawn with the help of a plastic syringe attached to a hard plastic tubing. The rumen fluid was aspirated into a 50 ml syringe. After straining the fluid through one layer of cheese cloth, pH was measured immediately using a glass electrode-pH meter. A few drops of 1% mercuric chloride solution were added to each sample for preservation. Jugular blood samples were collected at 2 PM from each steer in centrifuge tubes containing a thin layer of potassium oxalate. Plasma was separated immediately and kept at -20°C for plasma urea N analysis.

Rest Period

After each experimental period the animals were transferred to pens and about 5-6 Kg of alfalfa pellets and a small amount of mineral mixture were offered to each steer during a 12-day rest period, in order to remove any carry over effect of the previous feed.

Determination of Ruminal VFA's and Ammonia

Volatile fatty acids of the rumen fluid were determined by the method of Erwin et al. (1961). One ml of 25% of metaphosphoric acid containing acrylic acid (65 mM) as an internal standard was mixed with 5 mls of strained rumen

fluid and centrifuged at 1500 XG for ten minutes. The supernatent was analyzed for VFA's by gas liquid chromatagraph (Burrell) with a flame ionization detector. The stainless steel column (180 X 0.318 cm outer diameter) was filled with 20% neopentylglycol succinate + 2% H_3PO_4 on 100/120 mesh Gas Chrom R, and conditioned 17 hours at 200°C. Helium (30 psig) was used as a carrier gas with a flow rate of 40 ml/minute plus hydrogen (26 psig) with flow rate of 30 ml/minute and air (20 psig) with a flow rate of 330 ml /minute. The gas liquid chromatograph was attached to a Honeywell recorder with a one millivolt range with a chart speed of 0.5 inches per minute.

Ammonia N in rumen fluid and plasma urea N were determined according to Conway (1957).

Separation of Protozoa and Bacteria from Rumen Fluid

After the end of each period 3 to 4 liters of rumen digesta was removed from the rumen with the help of a vacuum pump (Figure 4). Rumen digesta was transferred from a plastic bottle to a warm thermos flask. After mixing a small sample was taken and frozen for further analysis. Dry matter content of the digesta was determined in a forced air oven at 70°C. Rumen fluid was squeezed through four layers of cheese cloth to remove the feed particles. Protozoa and bacteria were separated according to Ibrahim et al. (1970).

Protozoa and bacterial fractions were also frozen for lyophylization.

Lyophylization and Sub Sampling

Rumen digesta, protozoa, bacteria, abomasal digesta, ileal digesta and fecal grab samples were freeze dried and stored at -20°C for analyses. All the abomasal digesta samples were separately macerated in a pestle and mortar. Sub sampling was done separately for the first and second three-days of each period by taking an equal weight of digesta from the sample bags. In this way 32 composite samples were obtained for the abomasal digesta. Ileal digesta samples were also pooled in the same manner. Fecal samples were finely ground through a 1 mm screen in a small grinding mill and pooled as given for abomasal and ileal digesta samples. Rumen contents, abomasal and ileal digesta were also ground through a 1 mm screen and then stored in plastic bags at -20°C till further analyses.

Chemical Analysis of the Digesta

Dry matter in rumen, abomasal, ileal and fecal samples was determined in the vacuum oven at 98°C for three hours. Nitrogen content was estimated by microkjeldahl A.O.A.C. procedures (1971). Chromium oxide in the digesta and fecal grab samples was determined as given earlier.

PEG in the rumen fluid and abomasal digesta was estimated according to Stig Hyden (1960). From feed, dry digesta and dry fecal grab sample, PEG was extracted by taking 0.5 g of these samples, separately in screw cap centrifuge tubes with 10 ml of distilled water. Then the tubes were heated in boiling water for 10 minutes and centrifuged for 15 minutes at 15000 g. PEG was analyzed as given for fluid samples. Due to poor recovery of PEG in feed and other digesta samples, it was not used for the calculation of the flow of digesta in the alimentary tract.

Amino Acid Analyses

Amino acids in the feed, rumen contents, abomasal digesta, ileal digesta and fecal grab samples were determined by an automatic amino acid analyzer (Beckman model no 116/119 with a sample injector).

Dried samples (300-400 mg) of the ground rations, rumen contents, abomasal digesta, ileal digesta, feces and 200 mg of rumen bacteria were hydrolysed under reduced pressure in 10 ml of 3 N HCl in a sealed flask at 121°C for 15 hours (Bragg et al., 1966). The hydrolysate was evaporated to dryness at 40°C under reduced pressure in a Buchii Rotavapor 'R'. The residue was dissolved in pH 2.2 (0.20N) sodium citrate buffer, filtered through a medium porocity sintered glass crucible and adjusted to a final volume of

100 ml. An aliquot of 0.25 ml of HCl acid hydrolysate was injected with a self injector on the ion exchange column (Beckman type AA15 and PA35 resins). Amino acids were eluted from the ion exchange column with sodium citrate buffers, pH 3.25 (.2N Na^+), 4.25 (0.2N Na^+) and 5.10 (0.3N Na^+) at 0, 86, and 180 minutes, respectively. In order to locate the peak time of DAP, 0.25 ml of a standard solution containing 0.4 u moles of α - ε - diaminopimelic acid (Grade A, Calbiochem, Los Angeles, California) was placed on the column with the amino acid caliberation mixture (type 1, Beckman, Palo Alta, California). Cystine recovery was variable and low in preliminary trials. In order to get better recovery of cystine and separation of methionine from diaminopimelic acid in the digesta, rumen bacteria and fecal samples, the samples were oxidized by the modified method of Hirs (1967) as follows: One hundred mg of the sample was transferred to an oxidation tube (2X20 cm), cooled in an ice bath to which was added 4 ml of freshly prepared performic acid. Performic acid was prepared by mixing 30% H_2O_2 and 88% formic acid (1:9) at room temperature and allowed to stand for one hour, cooled in an ice bath and used immediately. Oxidation was allowed to proceed for 20 hours in an ice bath. Excess of performic acid was neutralized by adding 0.6 ml of 48% HBr acid. The mixture was evaporated to dryness at 40°C under vacuum in a rotary evaporator. The dried residue was hydrolysed with

3 ml of 3 NHCl and filtered through a medium porosity sintered glass crucible in order to remove the humin. The filterate and washings were transferred to a clean hydrolysis flask and evaporated to dryness under vacuum. The amino acids were dissolved in 5 to 10 ml of pH 2.2 sample diluting buffer and quantitatively transferred into a 25 ml volumetric flask and made upto the volume. After thorough mixing the sample was stored in a plastic bottle at $4^{o}C$ for amino acid analyses. Cysteic acid, methionine sulphone and DAP were eluted with peak times of 19, 50 and 134 minute, respectively. The total time for each run was set at 145 minutes.

Calculation of Passage of Digesta Dry Matter, N and other Nutrients

The amount of dry matter passing through various segments of the gut was calculated with the help of chromic oxide as a marker in the following manner:

$$\text{Flow of dry matter through rumen (Kg)} = \frac{\text{Concentration of } Cr_2O_3\text{/g diet DM X g DM intake/day}}{\text{Concentration of } Cr_2O_3\text{/g rumen digesta DM X 1000}}$$

$$\text{Flow of dry matter through the abomasum/day (Kg)} = \frac{\text{Concentration of } Cr_2O_3\text{/g diet DM X g DM intake/day}}{\text{Concentration of } Cr_2O_3\text{/g abomasal digesta DM X 1000}}$$

$$\text{Flow of dry matter through the ileum /day (Kg)} = \frac{\text{Concentration of } Cr_2O_3\text{/gm diet DM X g DM intake/day}}{\text{Concentration of } Cr_2O_3\text{/g ileal digesta DM X 1000}}$$

$$\text{Excretion of DM in feces per day (Kg)} = \frac{\text{Concentration of } Cr_2O_3\text{/gm diet DM X g DM intake/day}}{\text{Concentration of } Cr_2O_3\text{/g fecal DM X 1000}}$$

Calculation of nitrogen and other nutrient flow was made by multiplying the concentration of N or other nutrient per g of dry digesta or feces times the amount of dry matter flow per day through the particular segment of the gastro-intestinal tract.

Example.

$$\text{Amount of N flowing through abomasum/day (g)} = \text{Concentration of N/g digesta DM X g DM per day}$$

Calculation of Bacterial Nitrogen

Bacterial N in the abomasal digesta was calculated according to Hutton et al. (1971)

Bacterial N(g/day) = R X DAP (g/day)

where R is the N:DAP ratio determined for bacteria isolated from the rumen of fistulated Holstein steers.

Determination of N:DAP ratio of rumen bacteria.

N = gm of nitrogen per 100 gm dry bacteria

DAP = gm of 1-6 diaminopimelic acid per 100 gm dry bacteria

$$\text{N:DAP or R} = \frac{\%\text{ of bacterial N}}{\%\text{ of diaminopimelic acid}}$$

Ruminal protozoal N was not determined because protozoa were isolated from only two of the steers in some periods. Also the samples were found to be contaminated with DAP indicating the presence of bacteria.

Statistical Methods

The data collected for all measurements except data given in Table 11 were analyzed statistically as a change over design (Snedecor, 1956) and not as a Latin Square design, because this design was no longer applicable in the present experiment. The treatment means were subjected to the Duncan's multiple range test (1955). No statistical analysis was made on the amino acid composition of the experimental diets (Table 11). Orthogonal comparisons were made on some of the data and are indicated in footnotes below each table.

RESULTS

Digestibility and N Balance Trials

Dry matter (DM) digestibility of RSM, FA-RSM, casein and FA-casein diets appeared to be similar and there was no significant difference ($P > 0.05$) among the four treatments (Table 4). Apparent digestibility of DM determined by grab sampling technique was about two and three units lower for RSM and FA-RSM rations respectively, whereas the differences for the casein and FA-casein rations were 0.6 and 1.0 units, respectively. Apparent digestibility of crude protein tended to be higher in both the casein diets as compared with the RSM diets, however, only FA-RSM was significantly ($P < 0.10$) lower when compared with the other diets. Apparent digestibility of crude protein determined by grab sampling technique was significantly ($P < 0.05$) higher for both the casein diets as compared to the RSM diets. Apparent digestibility values determined by fecal grab samples technique for crude protein were also lower than the values found by total collections method. However, the difference between the two methods were small. No significant differences ($P > 0.05$) were found in the consumption and excretion of DM during the seven-day digestion trials among the four treatments.

No significant ($P > 0.05$) differences were observed

TABLE 4

APPARENT DIGESTIBILITIES OF DRY MATTER, CRUDE PROTEIN AND N RETENTION IN HOLSTEIN STEERS FED THE EXPERIMENTAL DIETS DURING THE 7-DAY TRIALS

Parameters	Treatments				SE ±
	RSM	FA-RSM	CASEIN	FA-CASEIN	
DM intake (Kg)	44.8	39.7	38.2	42.5	2.6
DM excreted (Kg)	14.8	13.3	13.4	14.6	0.7
Apparent DM digestibility (%)					
a) Total collection	66.9	66.4	65.1	65.6	0.9
b) Grab samples	65.0	63.2	64.5	64.6	0.8
Apparent C.P. digestibility (%)					
a) Total collection†	73.7^{ab}	73.4^{b}	77.8^{a}	77.6^{a}	1.2
b) Grab samples	71.3^{b}	71.2^{b}	75.9^{a}	76.2^{a}	1.4
N intake (g)	1054.7	958.8	904.2	959.3	52.6
N excreted in feces(g)	278.4^{a}	256.2^{ab}	200.9^{c}	213.6^{bc}	15.7
N excreted in urine(g)	552.0	495.5	509.3	514.9	43.9
Total N excreted (g)	830.5	751.7	710.2	728.5	49.3
N retained (g)	224.2	207.1	194.1	230.8	11.9
N as a % of N intake					
Urine	52.3	51.8	56.2	53.2	2.4
Feces	26.3	26.6	22.3	22.4	1.2
N retained	21.4	21.7	21.6	24.4	2.1
% of absorbed N retained	29.0	28.0	27.4	31.5	2.3

abc values not sharing the common superscripts are significantly different ($P < 0.05$).

† Level of significance at $P < 0.10$.

for N consumption, urinary N excretion, total N excretion and N retention for steers receiving the four experimental diets (Table 4). FA-treatment of RSM or casein protein did not influence the total N excretion and retention. However, significantly ($P < 0.05$) less fecal N was observed for the steers receiving the casein diet compared with those fed the RSM and FA-RSM diets. FA-treatment did not show any effect on the excretion of N in feces of steers fed FA-RSM or FA-casein diets (Table 4). When the excretion of N in the urine and feces was expressed as a percentage of N intake, no significant ($P > 0.05$) differences were found among the treatments. The excretion of fecal N as a percentage of N intake was about four units higher for RSM and FA-RSM fed steers compared with those receiving casein diets. N retention as a percentage of intake was slightly more for the steers fed FA-casein diet but the difference among the treatments did not reach the level of significance ($P > 0.05$).

Ruminal pH, Ammonia, VFA's and Plasma Urea

Rumen pH was slightly lower in the rumen of steers receiving casein and FA-casein diets as compared with the RSM and FA-RSM diets. FA-treatment of RSM or casein did not influence rumen pH (Table 5). Rumen ammonia level was highest in steers fed RSM as compared with the other treatments. The difference among the treatments, however, was not

significant ($P > 0.05$).

No significant ($P > 0.05$) differences were observed in the concentrations of VFA's (mmoles/100 ml) in the rumen fluid of steers receiving the experimental diets (Table 5). A trend of lower concentration of total VFA's was found for steers fed RSM diet compared to those fed the FA-RSM, casein and FA-casein diets. Molar percentages of propionic and butyric acids in the rumen fluid of steers fed the experimental diets were not significantly ($P > 0.05$) different. The molar percentages of acetic acid for steers receiving casein diet were comparatively higher than those fed FA-casein, RSM and FA-RSM diets. Orthogonal comparison showed a significant difference ($P < 0.05$) in the molar percentages of acetic acid for steers fed casein and FA-casein diets and not for the steers receiving RSM diets. RSM fed steers did not show any difference in the isobutyric acid level but steers on casein diet had significantly ($P < 0.05$) higher molar percentage of isobutyric acid when compared with the RSM, FA-RSM and FA-casein fed steers. Butyric acid molar percentages tended to be higher for steers fed FA-casein and FA-RSM diets compared with those receiving the RSM or casein diets, but failed to reach the level of significance ($P > 0.05$). Isovaleric acid tended to be higher for steers on untreated casein diet than those on the other treatments. Valeric acid molar percentages were higher for FA-RSM and FA-casein

TABLE 5

PARAMETERS MEASURED IN THE RUMEN AND BLOOD PLASMA OF HOLSTEIN STEERS FED EXPERIMENTAL DIETS (EXPT. III)

Parameters	Treatments				SE ±
	RSM	FA-RSM	CASEIN	FA-CASEIN	
Rumen pH†	5.8	5.7	5.5	5.5	
Rumen NH_3-N mg/100 ml	25.2	20.5	21.7	17.4	3.4
Total VFA's m moles/ 100 ml	11.8	13.5	13.2	13.6	1.3
Rumen VFA's molar percentages (%)					
Acetic acid‡	55.1^{b}	50.1^{b}	61.8^{a}	48.2^{c}	4.2
Propionic acid	27.5	29.5	22.0	24.5	4.6
Isobutyric acid‡	0.4^{c}	0.5^{c}	1.1^{a}	0.8^{b}	0.2
Butyric acid	9.7	15.1	10.5	19.8	4.1
Isovaleric acid	0.8	0.9	1.7	0.9	0.4
Valeric acid	2.4	4.2	3.0	5.9	2.0
Plasma urea N(mg/100 ml)	10.4	10.1	10.4	8.4	0.6

† Pooled data were not analysed statistically.

‡ Orthogonal comparison between protein sources were significant ($P < 0.05$).

diets compared with RSM and casein diets. However, there was a significant interaction between treatments and animals, which was used for calculating the level of significance.

There was a slight nonsignificant ($P \leq 0.05$) depression of plasma urea N levels for steers fed FA-casein diet compared with the other three treatments (Table 5).

Flow of Dry Matter and N through the Rumen, Abomasum and Ileum

No significant difference ($P > 0.05$) in the daily DM and N consumption was observed for the steers fed the four experimental diets (Table 6). The steers receiving FA-RSM and casein diets, however, tended to consume less dry matter which would tend to reduce the amount of DM passing through the rumen (Table 6). No significant differences ($P > 0.05$) were found for dry matter flow as a percent of DM intake among the experimental treatments.

Nitrogen content (%) of rumen digesta of steers receiving the FA-casein diet was higher ($P < 0.05$) than that of casein fed steers. The highest amount total N and non-ammonia nitrogen (NAN) were observed in the rumen digesta of steers fed FA-casein diet when compared with those fed RSM and FA-RSM and casein diets. However, no significant ($P \geq 0.05$) difference was recorded among the four treatments. NAN tended to be higher for steers receiving FA-casein

TABLE 6

DATA ON SOME OF THE PARAMETERS MEASURED IN THE RUMEN DIGESTA OF HOLSTEIN STEERS FED EXPERIMENTAL DIETS IN 24 HOURS (EXPT. III)

Parameters	Treatments				SE ±
	RSM	FA-RSM	CASEIN	FA-CASEIN	
DM intake (Kg)	6.4	5.6	5.6	6.1	1.1
Total N intake (g)	146.8	135.5	133.3	139.5	6.0
Flow of rumen contents					
DM %	12.4	12.2	12.7	13.6	0.6
DM (Kg)	3.8	3.3	3.5	4.1	0.4
DM as % of intake	57.2	57.7	62.8	66.1	5.3
Nitrogen (%)	3.3^{a*}	3.5^{a}	2.8^{b}	3.4^{a}	0.1
Total N (g)	121.4	111.9	97.4	139.9	13.0
Ammonia N(g)	6.4	5.3	5.9	5.6	1.1
NAN (g)	115.0	106.7	91.5	134.3	12.9
NAN as % of N intake	78.2	78.5	68.6	96.6	9.0
Bacterial N (g)	59.1	84.2	70.0	65.8	9.9
Bacterial N as % of N intake	40.0	61.1	52.3	47.0	7.6
Bacterial N as % of rumen N	51.9	74.4	69.7	46.6	8.1

* Values not sharing the common superscripts in the same row are significantly different ab($P < 0.05$).

DM - Dry matter
NAN - Non-ammonia nitrogen
SE - Standard error of the treatment means.

compared with those fed the RSM diets. The bacterial N as a percentage of total rumen N tended to be higher for FA-RSM fed steers compared with the other three treatments, however, the differences among the treatments were not significant ($P > 0.05$).

No significant difference was observed in abomasal digesta dry matter (%) for steers fed the four different rations during the experimental period (Table 7). The amounts of DM passed through the abomasum as a percent of DM intake were comparatively higher for RSM diets than those receiving the casein diets, but the differences did not reach the level of significance ($P > 0.05$). The passage of N through the abomasum as a percent of N consumed was increased by FA treatment of casein ($P < 0.01$) and tended to increase by FA treatment of RSM. Nitrogen flow as a percent of N intake was significantly ($P < 0.05$) higher for the steers receiving RSM, FA-RSM and FA-casein compared with those steers fed the casein diet. A similar trend was observed for NAN as a percent of N intake for the steers fed on various experimental diets (Table 7).

The total amount of bacterial N passing through the abomasum of steers receiving RSM diets was significantly ($P < 0.05$) higher than those fed on casein diets (Table 7). Bacterial N as a percent of total abomasal N tended to be higher for the steers receiving the RSM diets compared with

TABLE 7

PASSAGE OF DRY MATTER AND VARIOUS FRACTIONS OF N THROUGH THE ABOMASUM OF FISTULATED YOUNG HOLSTEIN STEERS FED THE EXPERIMENTAL DIETS IN 24 HOURS (EXPT. III)

Parameters	Treatments				SE ±
	RSM	FA-RSM	CASEIN	FA-CASEIN	
DM intake (Kg)	6.3	5.7	5.6	6.1	0.4
Total N intake (g)	148.4	136.5	132.5	138.0	6.9
Non-urea N intake (g)	96.1	89.6	86.0	87.1	--
Abomasal digesta					
DM (%)	7.2	7.8	7.5	7.7	0.2
DM passed (Kg)	3.9	3.7	3.2	3.5	0.2
DM passed as % of intake	60.7	65.5	57.2	58.1	2.0
Nitrogen (%)	3.6	3.7	3.4	4.0	0.1
Total N flowed (g)	137.3	134.7	108.8	139.9	7.8
Total N flowed as % of intake	91.9^{ABa}*	98.3^{A}	82.2^{Bb}	101.7^{A}	3.0
NAN (g)	131.7	128.9	103.8	135.0	7.8
NAN as % of N intake	88.1	94.1^{A}	78.5^{B}	98.1^{A}	3.2
Bacterial N (g)†	77.1^{a}	74.5^{a}	54.2^{b}	48.2^{b}	10.9
NAN-Bacterial N (g)	54.5	54.3	49.6	86.8	9.6
Bacterial N as % of N intake	50.7	53.4	40.9	34.6	6.5
Bacterial N as % of abomasal N	53.9	54.4	49.1	34.1	6.7

* Values not sharing the common superscripts in the same row are significantly different AB($P < 0.01$) and ab($P < 0.05$).

† Orthogonal comparison indicated differences between protein sources were significant ($P < 0.05$).

those fed casein diets. The bacterial N as a percent of abomasal N was appreciably lower for FA-casein fed steers compared with other treatments but was not significantly different ($P \geq 0.05$).

The percentage of dry matter in the ileal digesta and the amount of total dry matter flow through the ileum of steers fed RSM, FA-RSM, casein and FA-casein diets did not differ significantly ($P > 0.05$) among the treatments (Table 8). Dry matter as a percent of intake tended to be higher for FA-RSM fed steers, however, no significant differences ($P > 0.05$) were observed among the treatments. Nitrogen as a percent of DM tended to be lower in the ileal contents of steers fed FA-casein compared to other diets. The amount of total N flowing through the ileum to the large intestine tended to be higher for steers getting the RSM diets compared to those fed casein diets (Table 8). Orthogonal comparison between the two sources of proteins showed a significant difference between the RSM and casein diets. A similar trend was observed for NAN passing through the ileum. There was no difference between the two rape seed meal diets or between the two casein diets. NAN as a percent of N intake was slightly more for FA-RSM steers than for the other three treatments. When NAN was expressed as a percent of abomasal N the FA-casein treatment tended to be lower than the other treatments.

TABLE 8

DAILY FLOW OF NUTRIENTS THROUGH THE ILEUM OF FISTULATED YOUNG HOLSTEIN STEERS FED THE EXPERIMENTAL DIETS (EXPT. III)

Parameters	Treatments				
	RSM	FA-RSM	CASEIN	FA-CASEIN	SE+
DM (Kg)	2.7	2.6	2.4	2.7	.2
% of DM intake	43.0	46.3	42.1	44.0	1.5
DM (%)	9.4	10.4	9.4	9.1	.4
Nitrogen (%)	1.9	2.0	1.8	1.6	.1
Total N (g)‡	51.0^{a}	50.7^{a}	42.5^{b}	42.2^{b}	3.1
Non-ammonia N (NAN)(g)‡	49.4^{a*}	49.8^{a}	41.7^{b}	41.4^{b}	3.1
NAN as % of N intake	33.2	36.5	31.4	30.1	2.5
NAN as % of abomasal N	36.3	37.2	38.2	30.7	3.8

‡ Orthogonal comparisons between the protein sources.

* Values not sharing the common superscripts in the same row are significantly different ab($P < 0.05$).

Amino Acids

Total amino acid content as a percent DM in the rumen digesta for the steers fed on the untreated casein diets was significantly ($P < 0.01$) lower than the FA-casein fed steers (Table 9). Total amino acids in the rumen content of steers receiving FA-casein and FA-RSM diets were not significantly ($P > 0.05$) different but differed significantly from steers fed the casein diet. There was no significant difference between the two RSM diets for ruminal total amino acid content. Total amino acids in the bacterial fraction of rumen digesta of steers fed on the four experimental diets did not show any significant differences among treatments.

Abomasal digesta total amino acid concentration for steers on the casein diet was significantly ($P < 0.01$) lower than for steers fed FA-casein diet. The percentage of DM as amino acids in the abomasal contents of steers on the FA-casein diet was significantly ($P < 0.05$) higher than those receiving the RSM diets (Table 9). Amino acid content of ileal digesta was slightly more for steers on both the rape seed meal diets as compared with those on casein diets. However no significant ($P > 0.05$) difference was observed among the treatments. Fecal total amino acids concentration for steers on RSM diet was comparatively higher than for steers fed FA-RSM, casein and FA-casein diets, but the difference among the treatments was not significant ($P > 0.05$).

TABLE 9

TOTAL AMINO ACIDS (% DM) FOUND IN VARIOUS SEGMENTS OF THE GUT FOR FISTULATED HOLSTEIN STEERS FED EXPERIMENTAL DIETS (EXPT. III)

Parameters		Treatments				
		RSM	FA-RSM	CASEIN	FA-CASEIN	SE ±
Ration amino acid	(%)	7.7	7.8	8.9	7.9	
Rumen contents	(%)	15.5^{ABb*}	16.4^{ABab}	13.0^{Bc}	18.3^{Aa}	0.8
Bacteria†	(%)	37.0	32.2	39.1	34.6	2.6
Abomasum	(%)	17.1^{ABb}	17.3^{ABb}	15.6^{B}	19.9^{Aa}	0.6
Ileum	(%)	8.2	8.0	7.4	7.3	0.6
Feces	(%)	8.5	7.4	6.9	6.9	0.4

* Values not sharing the common superscripts in the same row are significantly different AB($P < 0.01$) and abc ($P < 0.05$).

† Bacteria isolated from rumen contents.

The Amount of Total Amino Acids Synthesized or Degraded in the Gastro-intestinal Tract of Holstein Steers

The amount of total amino acids consumed by steers fed FA-RSM was slightly lower than those receiving the RSM, casein and FA-casein diets, however, the difference was not significant ($P > 0.05$) among the treatments (Table 10). The amount of total amino acids in the rumen digesta of steers fed FA-casein diet was significantly ($P < 0.05$) higher than the steers receiving casein and FA-RSM diets (Table 10), but did not differ from RSM steers. Orthogonal comparison indicated a significant increase of amino acids when casein was treated with FA (Table 10).

The flow of total amino acids through the abomasal digesta for steers fed on casein diet was significantly lower ($P < 0.05$) than for steers fed the FA-casein or RSM diets. Flow of amino acids through the abomasum as a percent of amino acid consumed was lower for steers fed the casein diet ($P < 0.01$) compared to those fed RSM, FA-RSM and FA-casein diets. However, no significant difference was found among FA-casein, RSM and FA-RSM rations. Passage of total amino acids through the terminal ileum for steers receiving RSM diets was significantly ($P < 0.05$) higher than those steers fed on casein diets. Flow of ileal amino acids as a percent of intake was significantly ($P < 0.01$) higher for the steers receiving the FA-RSM diet as compared with those

TABLE 10

AMOUNTS OF TOTAL AMINO ACIDS SYNTHESIZED OR DEGRADED PER DAY IN THE GASTROINTESTINAL TRACT OF FISTULATED HOLSTEIN STEERS FED EXPERIMENTAL DIETS (EXPT. III)

Parameters	Treatments				SE ±
	RSM	FA-RSM	CASEIN	FA-CASEIN	
AA consumed/day (g)	493.0	434.7	500.4	489.9	39.9
AA flow from rumen (g)	579.8^{ab*}	522.1^{b}	449.3^{b}	751.1^{a}	59.2
Ruminal AA as a % of intake	120.6	119.1	90.9^{B}	154.5^{A}	14.7
AA consumed/day (g)**	495.8	436.6	499.4	477.0	33.0
AA flow in the abomasal digesta (g)	659.6	599.0	500.5	704.4	42.6
Abomasal AA as a % of intake	132.1^{A}	137.1^{A}	101.8^{B}	147.2^{A}	5.4
AA flow in ileal digesta (g)	$223.0^{a\dagger}$	206.8^{a}	173.7^{b}	195.5^{b}	13.6
Ileal AA flow as a % of intake	45.0^{a}	47.7^{A}	34.6^{Bb}	41.0	2.5
Ileal AA flow as a % of ruminal AA	43.3^{a}	40.8^{a}	38.5^{a}	26.0^{b}	3.1
Ileal AA flow as a % of abomasal AA	34.2	34.7	35.1	27.8	3.4
AA excreted out in feces (g)	187.4^{Aa}	153.9^{ABb}	137.4^{B}	145.8^{B}	8.2
AA excreted in feces as % of ileal AA	84.9	75.1	83.9	75.8	6.4

† Orthogonal comparison between RSM and casein proteins ($P < 0.10$).

* Values not sharing the common superscripts in the same row are significantly different AB($P < 0.01$) and ab($P < 0.05$).

**Mean values calculated on the basis of daily DM consumed during the two digesta collection periods in each experimental period.

receiving the untreated casein diet. When the amounts of total amino acids passing through the ileum were expressed as a percent of ruminal amino acids, significantly ($P < 0.05$) less amino acids flowed through the ileal digesta of FA-casein fed steers as compared with the other treatments. Steers on the RSM diet excreted more ($P < 0.01$) amino acids in their feces than steers receiving the casein diets. Fecal excretion of amino acids from FA-RSM steers was significantly ($P < 0.05$) lower than from RSM steers.

Total amino acids content in the casein diet was slightly higher as compared with RSM, FA-RSM and casein diets (Table 11). Threonine, glutamic acid, proline, glycine, alanine, cystine, histidine and arginine levels in rumen digesta of steers fed the experimental diets (Table 12) were significantly different ($P < 0.05$) while the remaining amino acids levels were not different ($P > 0.05$) among the treatments. Treatment of RSM with FA resulted in an increased ($P < 0.05$) rumen level of histidine. Treatment of casein with FA resulted in increased ($P < 0.05$) glutamic acid and proline with decreased threonine and glycine, alanine and arginine levels in rumen contents.

No significant ($P > 0.05$) difference was observed in the individual amino acid concentration of ruminal bacteria for the steers fed on various diets except leucine and diaminopimelic acid (DAP), (Table 13). FA treatment of RSM

TABLE 11

AMINO ACID COMPOSITION OF THE EXPERIMENTAL DIETS (EXPT. III)

Amino Acids*	Treatments			
	RSM	FA-RSM	CASEIN	FA-CASEIN
Aspartic acid	9.3	9.3	8.3	7.8
Threonine	4.7	4.7	4.1	3.9
Serine	4.9	5.1	5.5	5.7
Glutamic acid	20.8	20.7	23.1	22.4
Proline	6.7	7.5	9.3	8.0
Glycine	5.7	5.6	2.7	2.5
Alanine	5.9	6.2	4.4	4.2
Cystine	2.2	2.2	0.7	0.6
Valine	5.4	5.2	6.0	6.0
Methionine	2.2	2.0	2.3	2.6
Isoleucine	4.1	4.2	4.6	4.8
Leucine	7.4	7.6	8.9	9.1
Tyrosine	2.7	2.7	4.8	3.7
Phenylalanine	4.4	4.4	5.0	4.9
Lysine	5.1	4.9	6.2	6.8
Histidine	2.5	2.1	1.9	2.9
Arginine	6.1	5.8	3.3	4.2
Total (% DM)	7.7	7.9	8.9	7.9

* gm amino acid per 100 g of total amino acids.

TABLE 12

AMINO ACID COMPOSITION OF THE RUMEN CONTENTS FROM THE YOUNG HOLSTEIN STEERS FED THE EXPERIMENTAL DIETS. (EXPT. III)

Amino Acids*	Treatments				SE ±
	RSM	FA-RSM	CASEIN	FA-CASEIN	
Aspartic acid	11.9	10.9	11.4	10.6	0.3
Threonine	5.2^{A**}	5.0^{AB}	5.2^{A}	4.7^{B}	0.1
Serine	4.9	4.8	4.8	5.0	0.2
Glutamic acid	14.8^{B}	14.4^{B}	14.9^{B}	17.5^{A}	0.5
Proline	3.8^{b}	4.6^{b}	4.0^{b}	6.4^{a}	0.5
Glycine	5.8^{A}	5.6^{A}	5.4^{A}	4.1^{B}	0.2
Alanine	6.8^{ABb}	6.9^{ABb}	7.8^{Aa}	6.0^{Bc}	0.3
Cystine	1.7^{ABa}	1.9^{A}	1.3^{BCb}	1.1^{C}	0.1
Valine	5.6	5.7	5.9	5.8	0.1
Methionine	2.4	2.7	2.6	2.7	0.1
Isoleucine	5.1	5.0	4.9	4.7	0.2
Leucine	7.8	7.8	7.7	8.3	0.2
Tyrosine	3.5	3.5	3.6	3.8	0.1
Phenylalanine	4.6	4.5	4.6	4.8	0.2
Lysine	8.7	8.3	8.5	7.8	0.3
Histidine	2.1^{b}	2.4^{a}	2.0^{b}	2.2^{ab}	0.1
Arginine	5.1^{ab}	5.6^{a}	4.8^{b}	4.1^{c}	0.2
DAP	0.5	0.6	0.8	0.5	0.1
Total (% DM)	15.5	16.4	13.0	18.3	0.8

* gm amino acid per 100 gm of total amino acids.

** Values not sharing the common superscripts in the same row are significantly different ABC($P < 0.01$) and abc($P < 0.05$).

TABLE 13

AMINO ACID COMPOSITION OF RUMEN BACTERIA OF YOUNG HOLSTEIN STEERS FED THE EXPERIMENTAL DIETS. (EXPT. III)

Amino Acids*	Treatments				SE ±
	RSM	FA-RSM	CASEIN	FA-CASEIN	
Aspartic acid	12.0	11.7	12.4	11.9	0.3
Threonine	5.5	5.4	5.6	5.3	0.1
Serine	4.5	4.4	4.4	4.5	0.1
Glutamic acid	12.2	12.2	12.4	12.3	0.3
Proline	3.2	2.9	3.3	2.9	0.2
Glycine	5.7	5.2	5.6	5.6	0.2
Alanine	7.7	7.8	7.9	7.7	0.3
Cystine	1.2	1.2	1.2	1.1	0.3
Valine	6.0	6.0	5.3	5.9	0.3
Methionine	2.4	2.5	2.5	2.5	0.1
Isoleucine	5.2	5.3	5.3	5.0	0.1
Leucine	7.4^{ABb} **	7.6^{Aa}	7.3^{B}	7.0^{c}	0.1
Tyrosine	5.3	5.2	5.2	5.0	0.1
Phenylalanine	4.6	4.8	4.5	4.4	0.1
Lysine	9.8	9.7	9.6	9.4	0.5
Histidine	2.0	2.0	1.9	2.6	0.2
Arginine	4.8	5.2	4.9	5.9	0.3
DAP	0.86^{b}	0.83^{b}	1.06^{a}	1.10^{a}	0.06
Total (% DM)	37.0	32.2	39.1	34.6	2.6

* g. amino acid per 100 g of total amino acids.

** Values not sharing the common superscripts in the same row are significantly different AB($P < 0.01$) and abc($P < 0.05$).

resulted in an increased ($P < 0.05$) level of leucine while treatment of casein resulted in a decreased ($P < 0.01$) level of leucine in rumen bacteria. The concentration of DAP in rumen bacteria for steers receiving casein diets were higher ($P < 0.05$) than those fed RSM diets.

No significant differences ($P > 0.05$) were found in the concentration of serine, valine, methionine, isoleucine, phenylalanine, histidine, arginine and DAP in the abomasal digesta of steers fed the experimental diets (Table 14). FA treatment of RSM resulted in increased ($P < 0.05$) proline and cystine in the abomasal digesta. FA treatment of casein resulted in increased ($P < 0.05$) glutamic acid, proline, leucine, and decreased ($P < 0.05$) aspartic acid, threonine, glycine, alanine, cystine and lysine in abomasal digesta. RSM as the protein supplement in place of casein resulted in decreased ($P < 0.05$) alanine and lysine with an increase level of cystine in abomasal digesta. FA treated casein as a protein supplement in place of FA-RSM resulted in increased ($P < 0.05$) levels of glutamic acid, threonine, glycine, alanine, and cystine in abomasal digesta.

No significant differences were found in the amino acid concentration of the ileal digesta for steers receiving the four experimental diets except for glutamic acid, alanine and leucine (Table 15). Glutamic acid content in the ileal digesta of steers fed FA-casein diet was significantly

TABLE 14

AMINO ACID COMPOSITION OF THE ABOMASAL DIGESTA OF YOUNG HOLSTEIN STEERS FED THE EXPERIMENTAL DIETS. (EXPT. III)

Amino Acids *	Treatments				SE ±
	RSM	FA-RSM	CASEIN	FA-CASEIN	
Aspartic acid	11.7^{A**}	11.2^{A}	11.6^{A}	10.4^{B}	0.2
Threonine	5.4^{a}	5.2^{a}	5.2^{a}	4.9^{b}	0.1
Serine	5.1	5.0	5.0	5.1	0.1
Glutamic acid	15.4^{B}	16.3^{ABb}	15.2^{B}	17.9^{Aa}	0.4
Proline	3.9^{C}	4.6^{B}	3.8^{C}	5.9^{A}	0.2
Glycine	5.7^{A}	5.7^{A}	5.4^{A}	4.3^{B}	0.1
Alanine	7.1^{ABb}	6.8^{B}	7.5^{Aa}	5.9^{C}	0.1
Cystine	1.5^{Ba}	1.7^{A}	1.4^{Bb}	1.0^{C}	0.04
Valine	5.7	5.7	5.7	5.7	0.1
Methionine	2.1	2.2	2.4	2.3	0.1
Isoleucine	4.9	4.8	4.8	4.7	0.1
Leucine	7.8^{b}	7.8^{b}	7.6^{b}	8.1^{a}	0.1
Tyrosine	3.7^{ab}	3.6^{b}	3.7^{ab}	3.9^{a}	0.1
Phenylalanine	4.6	4.5	4.5	4.5	0.1
Lysine	7.8^{Bbc}	7.1^{Bc}	9.0^{Aa}	8.2^{ABb}	0.3
Histidine	2.1	2.1	2.1	2.3	0.1
Arginine	5.2	5.2	4.9	4.5	0.3
DAP	0.47	0.47	0.57	0.40	0.06
Total (%DM)	17.1	17.3	15.6	19.9	0.6

* g amino acid per 100 g of total amino acids.

** Values not sharing the common superscripts in the same row are significantly different ABC($P < 0.01$) and abc($P < 0.05$).

TABLE 15

AMINO ACID COMPOSITION OF ILEAL DIGESTA OF YOUNG HOLSTEIN STEERS FED THE EXPERIMENTAL DIETS (EXPT. III)

Amino Acids*	Treatments				
	RSM	FA-RSM	CASEIN	FA-CASEIN	SE±
Aspartic acid	9.6	9.3	10.0	10.4	0.4
Threonine	5.8	5.5	5.1	5.6	0.2
Serine	6.0	5.8	6.2	6.0	0.2
Glutamic acid	14.2^{B**}	14.7^{B}	14.0^{B}	17.3^{A}	0.4
Proline	5.6	6.9	4.5	5.8	1.2
Glycine	5.7	5.7	5.5	5.4	0.2
Alanine	8.0^{b}	7.7^{b}	9.3^{a}	7.9^{b}	0.3
Cystine	2.9	3.1	2.8	2.8	0.1
Valine	5.7	5.5	5.4	5.2	0.1
Methionine	1.7	1.8	2.4	1.7	0.2
Isoleucine	4.1	4.3	4.2	4.0	0.1
Leucine	7.4^{a}	7.2^{a}	7.0^{ab}	6.4^{b}	0.2
Tyrosine	3.0	2.9	3.8	2.9	0.5
Phenylalanine	4.2	3.9	4.4	3.6	0.2
Lysine	7.6	7.3	7.4	7.4	0.2
Histidine	2.7	2.7	2.3	2.4	0.2
Arginine	4.4	4.1	3.9	3.8	0.2
DAP	1.55	1.54	1.79	1.52	0.12
Total (% DM)	8.2	8.1	7.4	7.3	0.6

* gm amino acid per 100 g of total amino acids.

** Values not sharing the common superscripts in the same row are significantly different ABC($P < 0.01$) and abc($P < 0.05$).

more ($P < 0.01$) than those steers receiving the RSM, FA-RSM and casein diets. Alanine was significantly ($P < 0.05$) different for steers on the casein diet as compared with the other three treatments. Leucine was found to be significantly ($P < 0.05$) lower in the ileal digesta of steers on FA-casein diet as compared with steers receiving the RSM diets, but not different from casein fed steers (Table 15).

The concentrations of amino acids in the fecal matter did not differ significantly ($P > 0.05$) among the four treatments except alanine, which was significantly ($P < 0.05$) higher for casein fed steers compared with those fed the other three diets (Table 16).

TABLE 16

AMINO ACID COMPOSITION OF FECES OF YOUNG HOLSTEIN STEERS FED THE EXPERIMENTAL DIETS (EXPT. III)

Amino Acids*	Treatments				SE ±
	RSM	FA-RSM	CASEIN	FA-CASEIN	
Aspartic acid	11.3	10.3	11.4	11.3	0.6
Threonine	5.6	5.3	5.4	5.3	0.2
Serine	5.2	5.1	5.2	5.0	0.1
Glutamic acid	13.1	13.5	13.3	13.6	0.4
Proline	3.6	3.8	3.1	3.0	0.2
Glycine	5.9	5.9	6.0	5.7	0.2
Alanine	8.3^{b}**	8.4^{b}	9.4^{a}	8.6^{b}	0.2
Cystine	2.1	2.5	2.1	2.2	0.3
Valine	5.9	5.9	6.1	5.6	0.2
Methionine	2.5	2.7	2.7	2.6	0.2
Isoleucine	5.1	4.9	5.2	4.9	0.1
Leucine	7.5	7.3	7.6	7.1	0.2
Tyrosine	3.3	3.0	3.3	3.0	0.1
Phenylalanine	4.5	4.1	4.9	4.2	0.2
Lysine	8.6	9.0	8.3	9.3	0.7
Histidine	2.5	2.8	1.9	3.6	0.4
Arginine	4.1	4.3	3.4	3.7	0.3
DAP	0.93	1.18	1.07	1.27	0.15
Total (% DM)	8.5	7.4	6.9	6.9	0.4

* g amino acid per 100 g of total amino acids.

** Values not sharing the common superscripts in the same row are significantly different ab($P < 0.05$).

DISCUSSIONS

Treating of RSM or casein with formaldehyde (0.7 g FA/100 g of protein) did not appear to influence the apparent digestibilities of dry matter (DM) and crude protein (CP) in experiment III. Apparent digestibility of CP was slightly higher in both the casein diets as compared with the RSM diets. This could be due to the difference in protein source. No significant differences in digestion coefficients of DM and CP were observed among the experimental diets, indicating that the FA-treated or untreated diets were equally digested by the young steers during the present studies. However, the digestibilities of DM ($P < 0.05$) and N ($P < 0.01$) were significantly decreased when FA was used at the rate of 5.6 g FA per 100 g protein (Appendix I, Table 6). In experiment II FA-treatment of RSM did not significantly ($P > 0.05$) influence the DM and N digestion coefficients for young calves. But even at such a low level (0.7 g FA/100 g protein) of formaldehyde there was a trend towards lower digestibility when compared with the untreated diets. Similar results were obtained by Reis and Tunks (1969) when FA-treated casein was included in the diet as compared to the untreated casein. Weston (1971) indicated that FA-treatment of casein caused some impairment of the digestion of treated casein. Faichney (1971) observed significantly lower ($P < 0.05$) organic matter digestibility in the first

balance trial but not in the later experiments when FA-treated casein was given in the ration of lambs. The apparent digestibility of N was lower in these lambs in all balance trials. Faichney and Weston (1971) found no effect of treating casein on organic matter digestion in the whole gastrointestinal tract, whereas N digestibility was significantly ($P < 0.001$) lower when treated casein was given to lambs. The digestibility of N posterior to the abomasum was higher when casein in the diet of lambs was treated. Nimrick et al., (1972) stated that glyoxal treatment of fish meal and SBM significantly ($P < 0.05$) lowered N digestibility in growing lambs. Faichney and Davies (1972) reported similar reduction in N digestion of the treated peanut meal fed calves, however, no differences were observed in the dry matter and organic matter digestibility between treated and untreated peanut meal diets. Nicholson et al. (1972) reported that dry matter and crude protein digestibility of RSM were reduced in sheep fed the FA-treated RSM (18.5 g FA/100 g meal). However, when lower levels (0.93 g and 0.62 g FA/100 g meal) of FA were used for treating RSM, no effect was observed on the digestibility of DM or CP. The results found in the present studies followed a similar trend.

Total N excretion was comparatively more for steers fed on RSM diets than the casein diets, however, no significant ($P > 0.05$) difference was noticed between the

experimental treatments. There was a significant difference ($P < 0.05$) in the excretion of fecal N for the steers fed on the untreated RSM and casein diets. When N excretion in feces and urine or N retention in the body were expressed as a percentage of intake, no significant differences ($P > 0.05$) were observed between the treated and untreated diets. The percentage of absorbed N retained also did not differ ($P > 0.05$) among the various treatments. The fecal N was about four units higher for steers fed RSM diets than for the casein fed steers. This suggested that treatment of RSM or casein protein with FA (0.7 g/100 g protein) did not appear to influence the excretion of N in the feces, urine and the N retention in Holstein steers.

Larger amounts of N were observed in the feces of FA-treated RSM than the untreated RSM fed calves (Expt. I) and an opposite trend was observed in the excretion of N in the urine of these calves. FA-treatment of RSM protein significantly influenced the excretion of N in the feces and urine of Holstein calves fed ration containing 26% FA-treated RSM (Appendix 1, Table 6). Similar results were obtained by Reis and Tunks (1969), Barry (1972), and Faichney (1971) with FA-treated casein in sheep and lambs. Nimrick *et al.* (1972) also obtained a similar trend in the excretion of urinary and fecal N for growing lambs fed glyoxal treated fish meal (3.75 g/100 g meal).

A positive N balance was observed in steers receiving the experimental diets and FA treatments of RSM or casein did not influence the N retention over the untreated RSM and casein diets (Expt. III). Similar results were obtained in experiment I (Appendix 1, Table 6) for Holstein calves fed untreated RSM and FA-treated RSM rations. Though slightly more N was deposited in the FA-RSM fed calves, the difference was not significant. Nimrick et al. (1972) showed a significant increase in N retention for lambs receiving glyoxal treated fish meal as compared with the untreated fish meal. An increase in N retention was observed in sheep and lambs when FA-treated casein was given in the ration of these animals (Reis and Tunks, 1969; Barry, 1972; Faichney, 1971). The possible explanation for the lack of response obtained in the present experiment could be due to the high level of protein (14-16%) in the rations of Holstein calves and young steers used in experiments I and III. The requirements of these animals seems to be fully met by the untreated protein and FA-treatment did not improve the performance in young Holstein calves and steers. Another explanation for discrepancy in animal response could be the level and method of treating the vegetable or animal proteins and differing amino acids requirements of lambs and calves.

The results of the present experiment indicated that

the rumen pH in the steers fed the semipurified diets containing RSM, FA-RSM, casein and FA-casein plus 30% urea N ranged from 5.46 to 5.78. Agrawala et al. (1953) recorded very low pH in the rumen of calves fed purified diets. The low pH could be partly due to the higher concentration of VFA's (Storry and Rook, 1966; Whitelaw et al.,1972). Briggs et al. (1957) indicated that the rumen pH is closely related to the VFA's levels and the pH rarely falls outside the range of 5.0-7.5 on diets which do not result in the accumulation of lactic acid in the rumen. Generally a low pH is associated with a reduction or complete absence of rumen protozoa. Giesecke et al. (1966) reported the complete disappearance of the protozoa from the rumen of sheep fed a semipurified diet. In the present studies, a limited number of protozoa were observed in two steers in different periods of the experiment and complete disappearance in the other two steers fed experimental diets. Inclusion of sodium bicarbonate did help in the establishment of the ciliate protozoa in the rumen of test steers.

The low pH (5.46-5.78) could be beneficial for the steers fed test diets by decreasing the absorption of NH_3 from rumen. Hogan (1961) indicated that absorption of VFA's across the rumen wall at pH 6.5 increased the transport of NH_3 but not at the lower pH (4.5). Therefore, the available NH_3 in the rumen could be utilized by the microbes for the

synthesis of protein.

In the present studies, the results showed no significant difference in the rumen NH_3 - N levels for the steers fed on experimental diets. Treatment of RSM or casein slightly depressed rumen NH_3 levels. A significant reduction in NH_3 production was observed in young calves self fed on treated rations compared with those on untreated RSM or SBM rations in experiment I and II.

Treating of proteins with different levels of aldehydes markedly reduced the ammonia release from the treated proteins in *in vitro* and *in vivo* studies (Ferguson *et al.*, 1967; Zelter *et al.*, 1970; Hemsley *et al.*, 1970; Peter *et al.*, 1971; Faichney and Weston, 1971; Barry, 1972; Faichney and Davies, 1972; Appendix no. 1 Table 3). Satter and Baumgardt (1962) observed significantly less fluctuations in ruminal NH_3, VFA's and pH values when the animals were frequently fed. Ibrahim *et al.*(1969) observed little fluctuation in the ruminal pH and NH_3 levels with frequent feeding. In the present studies slight fluctuations were observed in the ruminal NH_3 levels and not in the ruminal pH values within each period of sampling. Probably frequent feedings in the present experiment affected the NH_3 levels in the steers receiving the treated and untreated ration and the influence of formaldehyde on rumen NH_3 level was not clearly indicated. Less ammonia concentration was recorded

in the rumen liquor of frequent fed animals (Satter and Baumgardt, 1962; Hungate, 1966) compared with the once or twice feedings (Hungate, 1966, and Virtanen, 1969). However, in experiment I and II the NH_3 - N levels recorded from the rumen liquor of calves fed free choice ranged from 4.0 to 7.2 (unt-RSM), 1.0 to 6.0 (FA-RSM); 4.1 to 9.6 (SBM); 6.8 to 11.2 (RSM) and 4.7 to 8.1 (FA-RSM) mg N/100 ml, respectively (Appendix 1, Table 5; Appendix 2, Table 3). Whereas in steers fed semipurified diets, the levels of ammonia N were 25.2 (RSM), 20.5 (FA-RSM), 21.7 (casein) and 17.4 (FA-casein) mg/100 ml of rumen fluid. The higher level of rumen NH_3 in experiment III probably is due to differences in diet composition. The ingredients used in experiment I and II are somewhat similar to conventional diets while diets used in experiment III were more like a semipurified diet. The values reported here are similar to those observed by Ibrahim et al., (1969).

The results of the present experiment (III) did not show any significant difference in the concentration of VFA's in the rumen liquor of steers receiving the experimental diets (Table 5). Similar results were obtained in experiment II with young calves fed SBM, RSM and FA-RSM diets. However, significantly lower concentrations of VFA's were recorded in rumen liquor of calves receiving the FA-treated RSM (5.6 g FA/100 g protein), (Appendix 1, Table 5). Faichney and

Weston (1971) reported that FA-treatment (3.16 g FA/100 g protein) of casein caused a significant reduction in the concentration of VFA's in the wether lambs. Protein source did not appear to influence the total concentrations of VFA's in the steers fed RSM or casein diets. Acetic acid showed a significantly lower concentration in the rumen liquor of FA-casein compared with the untreated casein fed steers. A similar trend in the molar percentages of acetic acid was noticed in steers fed RSM diets but the difference was not significant (Table 5). This trend was not apparent in experiment I and II. FA-treatment did not influence the molar percentages of isobutyric acid in the rumen liquor of RSM fed steers but showed a significant affect in rumen liquor from steers receiving the casein diets. El-Shazly (1952, a) reported that valine, leucine and isoleucine were degraded by bacteria into branched chain VFA's (isobutyric, isovaleric and B-methylbutyric acids). In the present studies casein protein supplied more of these amino acids as compared with the RSM diets, which were catabolized in the rumen to VFA's, NH_3 and CO_2.

In the present experiment, FA treatment did not show any significant influence on the blood plasma urea N for the steers fed FA-RSM and FA-casein diets compared with those receiving the untreated diets (Table 5). There was a slight depression in the plasma urea N level in FA-casein fed

steers compared with those on the untreated casein diet. In experiment II similar results were obtained when FA-RSM was fed in the calf starter ration containing urea. Faichney and Davies (1972) observed no significant ($P>0.05$) decrease in the plasma urea of calves fed FA-treated peanut meal at lower protein levels (13%) but at a higher protein level (20%) the reduction was significant ($P<0.05$). In experiment I a significant ($P<0.01$) reduction in the plasma urea N was noticed in calves receiving FA-treated RSM compared to the untreated RSM rations containing 16% crude protein. Barry (1972) reported that plasma urea N was lowered by FA treatment of casein ($P<0.01$) in sheep but increased with increasing level of casein intake ($P<0.01$). Significant reductions in plasma urea N in lambs and sheep were recorded by feeding glyoxal treated SBM (Peter et al., 1971); fish meal (Nimrick et al., 1972) and FA-treated casein diets (Faichney, 1971; Faichney and Weston, 1971).

A significantly ($P<0.05$) lower N content in rumen digesta was recorded in the steers fed casein diets compared with those fed RSM, FA-RSM and FA-casein diets. This would suggest that a larger proportion of the untreated casein was deaminated and lost as NH_3 through the rumen wall, which is supported by the fact that the non-ammonia nitrogen (NAN X 6.25 or protein) as a percent of N intake in the rumen digesta of steers fed FA-casein was, appreciably higher than

the untreated casein fed steers and also from those on RSM diets. FA treatment did not show any significant influence on the N content of the rumen digesta when FA-RSM protein was fed to steers. The level of FA used for treating RSM and casein was the same on the basis of protein contents (0.7 g FA per 100 g protein), whereas it was different on the basis of dry materials (0.25 g FA/100 g RSM and 0.61 g FA/100 g casein). Casein being an isolated protein provoided more free groups for cross linkages with FA as compared with the RSM protein.

The bacterial N levels (bacterial protein), as a percent of rumen N, levels for RSM, FA-RSM, casein and FA-casein fed steers were 51.9, 74.4, 69.7 and 46.6%, respectively. However, the bacterial N levels, as a percent of N intake, were in the order of 40.0, 47.0, 52.3 and 61.1% for the RSM, FA-casein, casein and FA-RSM, respectively. The lower percentages of bacterial N for RSM and FA-casein fed steers could be either due to higher amount of N consumed per day or the higher amount of total N recorded in the rumen digesta of these steers. Weller et al. (1958) reported 61-80% of the N in the rumen was present as microbial N. Pilgrim et al. (1970) calculated the microbial N in the rumen of sheep and suggested that 73% and 58-59% of the dietary N in the low and high N diets were converted into microbial N. In the present studies the bacterial N found

in the rumen digesta of steers fed on various experimental diets as a percent of N intake varied from 40.0 to 61.1%.

The total amount of N flowing from the abomasum as a percent of intake for the FA-casein and FA-RSM steers were 101.7 and 98.3% whereas 82.2 and 91.9% were recorded for casein and RSM steers, respectively. A substantial gain in the total N from the mouth to the duodenum was observed when the dietary N was low in the ration of ruminant animals (Gray et al., 1958; Harris and Phillipson, 1962; Clark et al., 1966; Hogan and Weston, 1967; Topps et al., 1968; Sharma et al., 1969; Van't Klooster and Rogers, 1969), but less N passed through the abomasum (Hogan, 1965; Clark et al., 1966; Hogan and Weston, 1967) on high protein rations. Nicholson et al. (1972) reported that treatment of RSM protein increased the flow of total N and protein N through the duodenum of sheep. The results of the present experiment also showed that FA-treatment of RSM and casein protein affected the N flowing from the abomasum of the steers.

The non-ammonia-N in the abomasal digesta as a percent of N intake for the steers receiving the casein diet was significantly ($P < 0.01$) lower compared with those of FA casein and FA-RSM fed steers. About 98 and 94% of the dietary N flowed as NAN through the abomasal digesta of steers fed FA casein and FA-RSM diets and comparatively less (78.5 and 88%) in the untreated casein and RSM diets,

respectively. McRae (1970) indicated that protection of casein with FA allowed virtually all the extra N to reach the duodenum as NAN where only half of the untreated casein N emerged from the rumen to the site of enzymic digestion in the lower gut of the host animal. In the present studies the amount of dietary protein N (NAN - Bacterial N) flowing through the abomasum of steers fed RSM and FA-RSM diets were (54.5 and 54.3 g) similar but somewhat different (49.6 and 86.8 g) for casein and FA-casein fed steers, respectively. MacRae et al. (1972) reported that the daily amounts of NAN reaching the duodenum of the sheep receiving dried grass (21.4 g) and the treated casein (30.1 g) diets were similar to amounts of N consumed (20.6 g and 29.4 g respectively). However, the amount of NAN reaching the duodenum of sheep receiving untreated casein (26.3 g) was less than the N consumed (29.7 g). Quantities of NAN reaching the duodenum, relative to the dietary N intake were high in their studies compared with the reports on high N diets (Clarke et al., 1966; Hogan and Weston, 1967). Weston (1971) and Faichney and Weston (1971) observed significantly more NAN leaving the stomach when FA-treated casein was included in the sheep's ration. Similar results were obtained by Hemsley et al., (1970) when FA-treated clover rich forage was offered to sheep in equal proportions at 3 hourly intervals during the digestion trials. The reason for a high

duodenal flow of NAN could be the very high content of soluble carbohydrates in the present experiment and in the diets used by other workers (Hemsley et al., 1970; Faichney and Weston, 1971; MacRae et al., 1972). The results of the present experiment are in agreement with the studies reported earlier.

In the present studies, the bacterial N which flowed through the abomasum was more for steers receiving the RSM and FA-RSM rations than observed for those on casein and FA-casein diets. The bacterial N flowing through the duodenum of the steers in the present experiment was calculated on the basis of N:DAP ratio and the amount of DAP flowing through the abomasal digesta (Hutton et al., 1971). Weller et al. (1958) showed that the N:DAP ratio for mixed rumen bacterial samples from sheep on a fixed feeding regimen is reasonably constant and was confirmed by Hutton et al. (1971). About 51-53% of the dietary N in the abomasal digesta was accounted for as bacterial N for the steers fed RSM and FA-RSM diets, whereas lower percentages (35 and 41) were recorded for the steers fed FA-casein and casein diets. Hutton et al. (1971) reported that bacterial N as a percent of the total N leaving the abomasum of a lactating cow was 50%. Smith (1969) indicated that on most of their diets approximately 60 to 70% of non-ammonia N (NAN) was found as microbial N in calf's ruminal and duodenal samples. With

decorticated ground nut meal in the diet these values sometimes decreased by about one-third. Similar to Smith's (1969) calculations Coehlo da Silva et al. (1972) reported that on chopped and cobbed diets 44-46% of the total N entering the small intestine was of microbial origin, the value falling to 32% for the pelleted diet. On all three diets, 61-63% of the microbial N was contributed by the bacterial N. Ellis and Pfander (1965) reported that about 41 to 52% of the total ingesta N was contributed by the microbial N. Ely et al. (1967) stated that 26-30% of the N in the abomasal digesta of lambs was microbial protein.

The bacterial protein in the present experiment was calculated from the bacterial N flowing through the abomasal digesta and found to vary from 11.4 g to 21.8 g of bacterial protein per 100 g of the digested dry matter for steers fed the experimental diets. The amounts of bacterial protein in the abomasal digesta of steers fed RSM and FA-RSM were 20.4 and 21.8 g/100 g digested DM, respectively, whereas lower values (14.7 g and 11.4 g) were recorded for the steers receiving the casein and FA-casein diets, respectively. Gray et al. (1958) reported that 11 g of bacterial protein was synthesized per 100 g of fermentable carbohydrates. Hungate (1966) suggested that synthesis of microbial protoplasm from carbohydrates was in the order of 10%. Hogan and Weston (1967) showed that 15-16 g of microbial

protein was synthesized in the rumen per 100 g of the organic matter digested. Conrad and Hibbs, (1968) also observed 10.2% of the fermentable organic matter was converted into microbial protein equal to 15.8% of the dry cell material. Hume et al.(1970) observed 13.3 g protein per 100 g of dietary organic matter fermented. Hogan and Weston (1970); Lindsay and Hogan (1972) reported that 23 g of bacterial protein was synthesized /100 g OM digested. Ørskov et al. (1972) observed 15.6 g bacterial protein per 100 g of the dietary organic matter fermented in the rumen. The values for the bacterial protein found in the abomasal digesta of the steers fed the various experimental diets are somewhat higher than reported by other workers.

The passage of dry matter as a percent of DM intake through the terminal ileum was slightly affected by the FA-treatment of the RSM and casein and varied from 42 to 46.3% $\pm$ 1.5 for the various experimental diets. A significantly higher ($P < 0.05$) total N was flowing through the terminal ileum of steers fed both RSM diets compared with those fed on casein diets. NAN as a percent N intake flowing through the ileum was not affected by FA treatment of the protein. When the NAN values were expressed as a percent of abomasal N, very little difference was found among steers fed RSM, FA-RSM and casein diets with somewhat lower values for FA-casein fed steers.

Bacterial protein is reported to be less digestible (McNaught et al., 1954 and Bergen et al., 1968, a) than casein protein. This suggests that more of the abomasal N or protein was absorbed in the small intestines of FA-casein fed steers than those receiving the casein diet. A greater amount of NAN or protein was absorbed in the intestines of steers receiving the FA-casein diet compared with those receiving the casein diets which is in agreement with other workers using sheep (MacRae, 1970; Weston, 1971; Faichney and Weston, 1971; MacRae et al., 1972).

The percentage of rumen DM as amino acids was significantly ($P < 0.01$) higher for steers receiving the FA-casein diet compared with the untreated casein and RSM fed steers. Whereas, in the bacterial fractions, the concentration of total amino acids tended to be higher for steers fed the untreated diets compared to those receiving FA-casein and FA-RSM diets. The results of the present experiment for total amino acid content of the rumen digesta of steers were similar to the levels in the rumen digesta of cows receiving a semipurified diet (Ibrahim and Ingalls, 1972) but the amino acid content of the bacterial DM were higher than those observed by them for the bacterial fractions.

In the present experiment, the percentage of total amino acids in the abomasal digesta DM was significantly more ($P < 0.05$) for the steers receiving FA-casein diet than

the other diets. FA treatment of proteins showed a significant influence on the total amino acid concentrations in the abomasal digesta of steers on casein diets but not RSM diets. This response might be related to the different levels of FA used per 100 g of RSM or casein. The level of total amino acid content in the ileal digesta DM decreased markedly in all steers compared to the abomasal digesta. There was a slight reduction compared to ration in the total amino acid concentration of the fecal DM of steers receiving the FA-RSM, casein and FA-casein diets with a little increase for the untreated RSM steers.

MacRae (1970) reported that treatment of casein protein with FA allowed virtually all the treated casein N to reach the duodenum as NAN whereas only half of the untreated casein N emerged from the rumen to the lower gut for digestion and absorption. As a result, a much higher amount of treated casein (65%) as compared to untreated casein (30%) was digested and absorbed within the small intestine. MacRae et al. (1972) reported that the extra NAN increases observed in the duodenum of sheep over that in the dried grass diet was equivalent to the amount of N given as treated casein. Only 50-55% of the extra NAN increases were observed when untreated casein was used as a protein supplement. They further reported that the flow of most of the amino acids to the duodenum was increased over that observed

with the dried grass by an amount similar to that supplied by the FA-treated casein.

In the present studies, the amount of total amino acids passing from the rumen content of the steers receiving the FA-casein diet was significantly higher ($P<0.05$) than casein and FA-RSM fed steers but not higher than RSM fed steers. Comparatively, a higher percentage of the amino acids consumed were recorded in the rumen contents of steers fed the FA-casein, RSM and FA-RSM diets than those on untreated casein diet. The level of total amino acids as a percent of intake in the abomasal content of steers fed FA-casein diet was significantly more than untreated casein fed steers, however, not different from the RSM diets (Table 10). The quantities of total amino acids leaving the terminal ileum were significantly ($P<0.10$) lower in the ileal digesta of steers fed casein diets compared to those fed RSM diets. The flow of ileal amino acids as a percent of ruminal amino acids was significantly lower ($P<0.05$) for the steers receiving the FA-casein diet compared with those fed the casein, RSM and FA-RSM diets. The ileal amino acids as a percent of abomasal amino acids also followed similar trend but did not reach the level of significance. This suggested that FA-treatment of casein tended to increase digestion and absorption of protein in the small intestines of these steers. Fecal total amino acids expressed as a

percent of total ileal amino acids showed that 75.1 and 75.8% were excreted in the feces of steers fed the treated RSM and casein diets, respectively, where as 84.9 and 83.9% of the ileal amino acids were excreted in feces of RSM and casein fed steers.

In the present studies, the amino acid composition of rumen digesta of steers receiving the experimental diets showed significant differences for some of the amino acids (threonine, glutamic acid, proline, glycine, alanine, cystine, histidine and arginine). The other amino acids in the rumen digesta of steers did not differ significantly (Table 10). The differences observed for some of the amino acids could either be due to higher dietary consumption of amino acids by RSM and casein steers than those fed FA-RSM and FA-casein diet or to the degradative or synthetic capacity of the microbes in the rumen of these steers. Ibrahim and Ingalls (1972) observed no significant difference in the amino acid composition (except histidine) of rumen digesta of cows fed semipurified and conventional diets. Duncan et al.(1953) indicated a similar amino acid pattern for rumen contents of calves fed a purified diet containing urea and those fed natural diets. Some of the amino acids (aspartic acid, threonine, glycine, alanine, cystine (casein diets), methionine, isoleucine and lysine) showed an upward trend when compared with the amino acid composition of the experimental

diets. The other amino acids like serine, glutamic acid, histidine, cystine (RSM diets) and arginine showed the downward trend. The level of glutamic acid was significantly ($P < 0.01$) higher in the rumen contents of steers fed FA-casein diet than those on other diets, showing some protection of dietary protein due to FA-treatment. The level of cystine in the rumen digesta of steers fed casein diets was increased as compared with the dietary level, indicating bacterial synthesis of cystine in these steers. Arginine level in the rumen digesta was increased for steers fed casein diet and decreased for other steers receiving the RSM, FA-RSM and casein diets. The levels of cystine or histidine observed in the present studies were lower than the levels reported by Ibrahim and Ingalls (1972) but similar to Schelling et al. (1967). Valine, leucine, tyrosine and phenylalanine were increased compared to the dietary amino acids for steers receiving RSM diets and decreased in those receiving the casein diets. Bigwood (1964) indicated that the rumen processes led to a decrease in proline, arginine and glutamic acid and an increase in lysine, threonine and isoleucine. Similar results were observed in the present studies.

The results of the present studies, showed a uniform amino acids composition (except leucine and α-ε-diaminopimelic acid) of the rumen bacterial hydrolysate of the steers fed on semipurified diets containing RSM and casein

protein plus urea. Weller (1957) reported a uniform composition of the bacterial hydrolysates obtained from rumen contents of sheep fed on a wide variety of diets and suggested that the amino acid composition of the mixed bacterial proteins is almost constant. Similar results were observed by other workers (Purser and Buechler, 1966; Bergen et al., 1968, b; Ibrahim and Ingalls, 1972). A significantly lower ($P < 0.01$) level of leucine was found in the ruminal bacteria of steers fed FA-casein diet than those steers fed on casein, RSM and FA-RSM diets. However, bacteria separated from the rumen digesta of steers fed FA-RSM diet showed a higher ($P < 0.05$) leucine content compared with those maintained on RSM diet. The concentration of α- ε-diaminopimelic acid (DAP) in the bacterial fractions of steers fed RSM diets was significantly less ($P < 0.05$) compared with those receiving casein diets. The level of DAP in the ruminal bacteria was not affected by the FA-treatment of RSM and casein protein but was influenced by the source of protein in the diets.

In the present studies higher levels of lysine, valine, leucine, arginine, serine, alanine and lower levels of cystine, methionine, tyrosine, histidine and DAP were found in the rumen bacteria isolated from the steers fed experimental diets than those reported by Ibrahim and Ingalls (1972) for rumen bacteria of dairy cows fed semipurified and

natural diets. Bergen et al. (1968, a) indicated that the rumen bacterial protein appeared to be deficient or limiting in cystine. Constancy in amino acid compositions of the rumen bacteria has been reported by several workers, this does not infer that all rumen bacteria are of similar protein quality. Bergen et al. (1967) demonstrated that 14 strains of rumen bacteria with similar amino acid compositions were of widely differing protein quality. These variations may arise from differences in both digestibility and the pattern in which amino acids are released from bacterial proteins.

The amino acids composition of abomasal digesta showed some changes over the dietary amino acids. These modifications to a great extent could be due to the microbial activities in the rumen (Bigwood, 1964) and to some extent could be due to the mixing of digesta with the gastric secretions in the abomasum (Phillipson, 1964). In the present studies, there were noticeable increases in the concentrations of glutamic acid (all diets) and decreases in the levels of lysine (RSM diets) and DAP (all diets) in the abomasal digesta of steers when compared with ruminal amino acids. The other amino acids levels of abomasal digesta showed only slight change over the ruminal amino acids of these steers. Coehlo da Silva et al. (1972) reported that contribution of amino acids from gastric juice

represented 9-17% of each of the amino acids entering the small intestine of sheep on lucerne hay given as either chopped, cobbed or pelleted form. They further indicated that contributions for arginine, lysine and histidine were negligibles from the gastric juice. Clarke et al. (1966) reported that more of each amino acid passed to the duodenum of sheep when hay with or without maize was eaten, while less of each amino acid passed to the duodenum when the diet was hay with soy bean protein. The greatest losses were observed for glutamic acid and substantial losses for aspartic acid, proline, arginine and leucine, when the dietary hay and soy protein passed through rumen to the duodenum of sheep. The smallest losses were noticed with cystine and threonine. Little et al. (1968) reported that the amino acid patterns in the abomasal contents were similar with soy bean, casein and gelatin in Wether lambs. Neudorffer et al. (1971) observed marked increases in amounts of glycine, isoleucine, lysine, arginine and decreases in the amounts of glutamic acid, alanine and leucine in abomasal digesta of heifers fed poor quality grass hay and maize diet compared with dietary amino acid. Coehlo da Silva et al. (1972) showed a substantial loss of phenylalanine, proline and aspartic acid and increases of methionine, lysine, tryptophan and cystine in the abomasal digesta. MacRae et al. (1972) indicated that the amounts of isoleucine,

leucine, lysine, methionine, threonine, valine, aspartic acid and tyrosine reaching the duodenum of sheep receiving dried grass and FA-casein were almost as high as those consumed in the food. However, there was a net loss of all amino acids passing through the stomach in sheep given untreated casein. In the present studies formaldehyde treatment of casein protein did show some influence on the level of total amino acids (19.9% of DM) in the abomasal digesta of steers receiving the FA-casein diet compared with those fed casein diet (15.6% of DM). But did not affect the level of total amino acids for the steers fed the RSM and FA-RSM diets. Clarke et al. (1966) suggested that losses of proline before the small intestine is associated with its participation in a stickland-type of reaction with subsequent formation of δ - amino valeric acid (El-Shazly, 1952, b). Amino acids liable to be reduced include proline, glycine, arginine, tyrosine, cystine and methionine and those liable to be oxidized are alanine, leucine, isoleucine, valine, histidine, phenylalanine, tyrosine and serine. A decrease in the concentration of glutamic acid was observed in the rumen contents of steers fed the experimental diets. The reduction in glutamic acid concentration could be due to its incorporation into the transamination reaction for the synthesis of other amino acids. It could also be possible that part of the glutamic acid was converted into

glutamine after reacting with ruminal ammonia in the bacteria and rumen mucosa (Hoshino et al., 1966). A portion of glutamic acid is absorbed into circulation after it is converted to glutamine in the rumen mucosa. In a similar manner ammonia will be fixed in the rumen microorganisms. Glutamine which was absent in the current study may have been used by the bacteria for the synthesis of microbial amino acids, absorbed in the blood plasma through the rumen wall and then in the general circulation, or in the digesta flowing to the abomasum.

There is some discrepancy in the net loss or gain of certain amino acids during fermentation of dietary proteins which were passing through stomach to the duodenum of steers in the present studies from those of other workers. This could be due to the differences in the make up of diets and the species of animals used in their studies.

With the exception of serine, proline, glycine (FA-casein diet), alanine, cystine, histidine and DAP, the concentrations of individual amino acids at the terminal ileum were lower than those at the proximal duodenum. Similar results were obtained by Coehlo da Silva et al. (1972). The results of the present studies also agrees with those reported by Purser (1970) from an examination of the results of Clarke et al. (1966). Bergen et al. (1967) suggested that some of the differences in amino acid

disappearance may be related to the nature of protein supplied ie. bacterial protein. These differences may arise from differences in both digestibility and the pattern in which amino acids are released from bacterial protein. Clarke et al. (1966) reported that in the duodenal digesta of sheep, marked losses of arginine, an oxidant like proline, occurred with all diets. But amongst the reductant amino acids the losses were smaller and less consistant. Barker (1961) indicated that amino acids reacts in pairs, one partner being reduced while the other oxidized. The losses of amino acids which occurred in the duodenal digesta of steers were similar to those observed by Clarke et al. (1966).

The amino acids composition of the feces dry matter (except threonine, glutamic acid and arginine) showed a reverse picture of the ileal digesta. Some increase in the concentrations of aspartic acid, glycine, alanine, valine, methionine, isoleucine, leucine, phenylalanine and lysine were noticed in the feces of steers receiving the experimental diets. The lower level of DAP observed in the feces of steers when compared with the ileal digesta, indicated that DAP was degraded by the microorganisms of the large intestine. Similar observations were reported by White and Milne (1971). The amino acid increases in the feces over the ileal digesta could be due to the biosynthesis of these

amino acids in the caecum which was passed out in the feces. Treatment of RSM and casein protein did not show any appreciable influence on the concentrations of individual amino acids in feces of Holstein steers receiving the experimental diets. About 75 to 76% of the ileal digesta total amino acids were excreted in feces of steers fed formaldehyde treated diets, where as 84 to 85% of total amino acids of ileal contents were excreted in feces of those getting the untreated diets. This indicated that either the amino acids were absorbed in the large intestine or deaminated by the caecum and colon microorganisms. MacRae et al. (1972) reported that the disappearances of amino acids from the large intestine were small with dried grass, but larger with the untreated and largest with treated casein.

SUMMARY

Young dairy calves and young Holstein steers were used to study the effect of treating rape seed meal (RSM) with formaldehyde (FA) on the dry matter (DM) consumption, apparent digestibilities of DM and crude protein (CP), N retention and flow of nutrients through the gastro-intestinal (G.I.) tract. Experimental rations containing either 26% untreated or FA-treated (5.6 g FA/100 g protein) RSM were fed for 14 weeks to dairy calves. Weight gain, DM consumption and feed efficiency did not differ significantly ($P > 0.05$) for the two groups. Treatment of RSM with FA significantly ($P < 0.01$) depressed the ruminal ammonia and plasma urea levels in the experimental calves. Total volatile fatty acids (VFA) (mmoles/100 ml) concentration was significantly higher ($P < 0.05$) in ruminal fluid of calves fed RSM compared with those fed FA-treated RSM. Molar percentages of the VFA's except propionic acid were not significantly different ($P > 0.05$) between the two groups. In the digestibility and N balance trials, DM and N consumptions were higher for the FA-RSM group but did not differ significantly ($P > 0.05$). FA-treatment of RSM significantly decreased the apparent digestibilities of DM ($P < 0.05$) and CP ($P < 0.01$) for young dairy calves. Treatment of RSM with FA resulted in decreased ($P < 0.01$) urinary N and increased fecal N excretions in calves with no significant ($P > 0.05$) effect on N consumption

and retention.

In experiment II replacement of soy bean meal (SBM) protein with RSM or FA-treated (0.7 g FA/100 g protein) RSM did not significantly ($P > 0.05$) affect the DM consumption, weight gain and feed conversion for the young calves during the 14 week growth trial.

FA-treatment of RSM significantly ($P < 0.05$) depressed the ammonia release in the rumen of calves fed FA-RSM compared with those receiving RSM and SBM rations. Blood plasma urea levels tended to be lower in FA-RSM fed calves but did not differ significantly ($P \geqslant 0.05$) compared with those fed SBM and RSM rations. The concentrations and molar percentages of VFA's were not significantly different among the treatments and were not affected by replacing SBM with RSM or treatment of RSM with FA.

No significant differences ($P > 0.05$) were observed for digestion coefficients of DM, N, acid detergent fibre and energy among the calves fed SBM, RSM and FA-RSM rations.

In experiment III, four young Holstein steers with cannulae in the rumen, abomasum and ileum were used in a change over design to study the effect of treating RSM and casein protein with FA (0.7 g FA/100 g protein) on DM and CP digestibility, N excretion, N retention, blood urea, rumen metabolism and flow of nutrients through the various segments of G.I. tract. Feed intake by each steer was adjusted during

the preliminary period using an automatic feeder device and was offered at 10-minute intervals.

FA-treatment (0.7 g FA/100 g protein) of RSM or casein did not show any significant ($P > 0.05$) effect on apparent digestibility of DM as determined by total collection or by the fecal grab sample technique. FA-treatment of RSM or casein protein did not affect the total N excretion and retention. However, less ($P < 0.05$) fecal N was observed for steers fed casein diet compared with RSM and FA-RSM fed steers. Ruminal ammonia and blood plasma urea N levels tended to be lower for steers fed treated diets but did not differ significantly ($P > 0.05$) from those fed untreated diets. No significant differences were observed in the concentrations of VFA's (mmoles/100 ml) in the rumen fluid of steers fed the experimental diets. Molar percentages of acetic acid were higher ($P < 0.05$) for casein diets compared with RSM diets.

No significant differences ($P > 0.05$) were found in the flow of DM as a percent of DM intake through the rumen, abomasum and terminal ileum of steers fed the experimental diets.

The passage of total N through the abomasum as a percent of N consumed was increased by FA-treatment of casein ($P < 0.01$) and tended to be increased by FA treatment of RSM. The bacterial N as a percent of total ruminal N

and of total abomasal N tended to be higher for the RSM diets and lower for casein diets. FA-treatment of proteins did not show any significant ($P > 0.05$) affect on the flow of bacterial N from rumen or abomasum of the steers. Total N and non ammonia nitrogen (NAN) flowing through the terminal ileum were influenced by the source of protein but not by FA treatment of the two protein supplements.

FA-treatment of RSM and casein protein significantly influenced the concentrations (as % DM) of total amino acids of rumen ($P < 0.01$) and abomasal ($P < 0.05$) digesta but did not show any significant ($P > 0.05$) effect on the rumen bacterial total amino acid concentrations. The amount of total amino acids flowing, as a percent of amino acids consumed, through the abomasum of steers were significantly influenced ($P < 0.01$) by FA-treatment of the casein diets and not by the RSM diets. There was a significant ($P < 0.01$) increase in the amount of total amino acids flowing, as a percent of amino acid consumed, through the ileum of steers receiving FA-RSM diets compared with casein diet. Steers fed RSM diets excreted more ($P < 0.01$) of amino acids in their feces than the steers receiving the casein diets. FA treatment of RSM or casein did influence the total excretion of amino acids in the feces of steers fed treated diets.

Treatment of casein with FA, in contrast to FA treatment of RSM, significantly increased the amount of

total amino acids flowing in the rumen digesta of steers compared with the untreated casein. The amino acid composition of the rumen digesta indicated that FA-treatment of casein significantly increased ($P < 0.05$) the levels of glutamic acid and proline. Bacterial amino acid levels, except for leucine and DAP, were not significantly ($P > 0.05$) influenced by FA treatment or protein source.

FA treated casein as a protein supplement in place of FA-RSM resulted in increased ($P < 0.05$) glutamic acid, threonine, glycine, alanine and cystine levels in the abomasal digesta of steers. Treatment of casein with (0.7 g FA /100 g protein) significantly ($P < 0.01$) influenced the concentration of glutamic acid in the ileal digesta of steers receiving FA-casein diet compared with untreated casein fed steers. However, no effect of FA was observed on the other amino acids in the ileal digesta and also in the fecal matter of the steers fed the test diets.

CONCLUSIONS

The following conclusions could be drawn from the present studies.

1. Treating of RSM with FA (5.6 g FA/100 g protein) reduced the rate of deamination in the rumen and reduced enzymic digestion in the lower G.I. tract.
2. A lower level of FA treatment (0.7 g FA/100 g protein) tended to reduce ration digestibility.
3. Consumption of FA-treated RSM (0.7 g FA/100 g protein) did not appear to influence animal performance.
4. FA and GA treatment of meals reduced *in vitro* degradation of RSM, SBM and LSM.
5. Treatment of casein with FA (0.7 g FA/100 g protein) increased the N content in the rumen, abomasum and increased the flow of total N, NAN, total amino acids through the rumen and abomasum of Holstein steers.
6. Treatment of casein with FA (0.7 g FA/100 g protein) inhibited gradation in the rumen but had little affect on protein digestion in the lower G.I. tract.
7. FA-treatment of RSM (0.7 g FA/100 g protein) tended to increase the flow of various N fractions through the G.I. tract of Holstein steers compared with those fed RSM diet.
8. Treatment of RSM or casein increased the levels of certain amino acids in rumen digesta; histidine for FA-RSM, glutamic acid and proline for FA-casein fed steers, respectively.

LITERATURE CITED

Abdel Rehman, S. and Decker, P. 1966. Comparative study of the urease in the rumen wall and contents. Nature. 209:618-619.

Abdo, K. M., King, K. W. and Engel, R. W. 1964. Protein quality of rumen microorganisms. J. Anim. Sci., 23:734-736.

Abou Akkada, A. R. and Howard, B. H. 1962. Biochemistry of protozoa. The nitrogen metabolism of Entodinium. Biochem. J. 82:313-320.

Abou Akkada, A. R. and Blackburn, T. H. 1963. Some of the observations on the nitrogen metabolism of rumen proteolytic bacteria. J. Gen. Microbiol. 31:461-469.

Abou Akkada, A. R. and El-Shazly, K. 1964. Effect of absence of ciliate protozoa from the rumen on microbiol activity and growth of lambs. Appl. Microbiol. 12: 384-390.

Acord, C. R., Mitchell, Jr., G. E., Little, C. O. and Karr, M. R. 1966. N sources for starch digestion by rumen microorganisms. J. Dairy Sci. 49:1519-1522.

Acord, C. R., Mitchell, Jr., G. E. and Little, C. O. 1968. Combination of N sources by starch digestion by mixed rumen bacteria. J. Dairy Sci. 51:1249-1251.

Agrawala, I. P., Duncan, C. W., and Huffman, C. F. 1953. A quantitative study of rumen synthesis in the bovine on natural and purified rations. I. Protein, dry matter, and non-protein nitrogen. J. Nutrition. 49: 29-39.

Annison, E. E., Chalmers, M. I., Marshall, S. B. and Synge, R. L. M. 1954. Ruminal ammonia formation in relation to the protein requirement of sheep. III. Ruminal ammonia formation with various diets. J. Agric. Sci. 44:270-273.

Annison, E. E. 1956. Nitrogen metabolism in sheep. Protein digestion in the rumen. Biochem. J. 64:705-714.

A.O.A.C. 1965. Official methods of analysis. 10th ed. George Banta Publishing Co. Menasha, Wis.

A.O.A.C. 1971. Official methods of analysis. 11th ed. George Banta Publishing Co. Menasha, Wis.

Asplund, J. M. 1961. Rape seed oil meal for dairy cattle. Univ. Alberta. 40th Annual feeders' day report.

Asplund, J. M. and McElroy, L. W. 1961. Effect of high levels of rape seed oil meal (RSOM) in dairy cows ration on milk production. J. Dairy Sci. 44:2338 (Abstract).

Bailey, C. B. and Balch, C. C. 1961. Saliva secretion and its relation to feeding in cattle. 2. The composition and rate of secretion of mixed saliva in the cow during rest. Br. J. Nutr. 15:383-

Barker, H. A. 1961. The bacteria. Vol II. p. 195 (Ed. I. C. Gunsalus and R. Y. Stanier) New York and London: Academic Press.

Barry, T. N. 1972. The effect of feeding formaldehyde-treated casein to sheep on nitrogen retention and wool growth. N.Z. J. Agric. Res. 15:107-116.

Bell, J. M., Downey, R. K. and Wetter, L. R. 1967. Oil and meal from Canadian rape seed. CDA publi. no. 1183. p. 17.

Bell, J. M. 1969. Rape seed meal for livestock and poultry. Publ. no. 3. p. 15-16. Published by the rape seed association of Canada.

Bergen, W. G., Purser, D. B. and Cline, J. H. 1967. Enzymatic determination of the protein quality of individual rumen bacteria. J. Nutr. 92:357-364.

Bergen, W. G., Purser, D. B. and Cline, J. H. 1968, a. Determination of limiting amino acids of rumen-isolated microbial proteins fed to rats. J. Dairy Sci. 51:698-1700.

Bergen, W. G., Purser, D. B. and Cline, J. H. 1968 (b). Effect of ration on the nutritive quality of rumen microbial protein. J. Anim. Sci. 27:1497-1501.

Bezeau, L. M., Slen, S. B. and Whiting, F. 1960. The nutritional value of rape seed oil meal for lamb and wool production in mature range ewes. Can. J. Anim. Sci. 40:37-43.

Bigwood, E. J. 1964. In Symposium on The role of gastro-intestinal tract in protein metabolism, p. 155. (Ed. H. N. Munro) Oxford: Blackwell Sci. Publ. Oxford. England.

Bird, P. R. and Moir, R. J. 1972. Sulpher metabolism and excretion studies in ruminants. VIII. Methionine degradation and utilization in sheep when infused into the rumen or abomasum. Aust. J. Biol. Sci. 25:835-848.

Blackburn, T. H. and Hobson, P. N. 1960. Proteolysis in the sheep rumen by whole and fractionated rumen contents. J. gen. Microbiol. 22:272-281.

Blackburn, T. H. and Hobson, P. N. 1960, b. The degradation of protein in the rumen of the sheep and redistribution of the protein N after feeding. Br. J. Nutr. 14:445-456.

Blackburn, T. H. and Hobson, P. N. 1960. Breakdown of protein and proteolytic activity in the sheep rumen at different times after feeding. J. Gen. Microbiol. 22:290-294.

Blackburn, T. H. and Hobson, P. N. 1962. Further studies on the isolation of proteolytic bacteria from the sheep rumen. J. Gen. Microbiol. 29:69-81.

Blaxter, K. L. and Martin, A. K. 1962. The utilization of protein as a source of energy in fattening sheep. Br. J. Nutr. 16:397-407.

Blaxter, K. L. 1964. In the role of the Gastro-intestinal Tract in Protein Metabolism. p. 143. (Ed. H. N. Munro) Oxford: Blackwell.

Bloomfield, R. A., Wilson, R. P. and Thompson, G. B. 1964. Influence of energy levels on urea utilization. J. Anim. Sci. 23:868 (Abst).

Boggs, D. E. 1959. An in vivo N^{15} Tracer study of amino acid metabolism in the rumen of sheep on a purified diet. (Cornell University) Dissert. Abst. 20:55-56.

Borhami, B. E. A., El-Shazly, K., Abou Akkada, A. R. and Ahmad, I. A. 1967. Effect of early establishment of ciliate protozoa in the rumen on microbial activity and growth of early weaned buffalo calves. J. Dairy Sci. 50:1654-1660.

Boyne, A. W., Campbell, R. M., Davidson, J. and Cuthbertson, D. P. 1956. Changes in the composition of the digesta along the alimentary tract of sheep. Br. J. Nutr. 10:325-333.

Bragg, D. B., Ivy, C. A., Greene, D. E., Waldroup, P. W. and Stephenson, E. L. 1966. Comparison of amino acids in milo and corn. Poultry Sci. 45:1072-

Briggs, P. K., Hogan, J. P. and Reid, R. L. 1957. The effect of VFA's, lactic acid and ammonia on rumen pH in sheep. Aust. J. agric. Res. 8:674-

Bryant, M. P. and Robinson, I. M. 1963. Apparent incorporation of ammonia and amino acid carbon during growth of selected species of ruminal bacteria. J. Dairy Sci. 46:150-154.

Chalmers, M. I., Cuthberston, D. P. and Synge, R. L. M. 1954. Ruminal NH_3 formation in relation to the protein requirement of sheep. I. Duodenal administration and heat processing as influencing fate of casein supplement. J. Agr. Sci. 44:254-262.

Chalmers, M. I. and Synge, R. L. M. 1954. Ruminal ammonia formation in relation to the protein requirement of sheep. II. Comparison of casein and herring meal supplements. J. Agric. Sci. 44:263-269.

Chalmers, M. I. and Marshall, S. B. M. 1964. Ruminal ammonia formation in relation to the utilization of ground nut meal and herring meal as protein sources for milk production. J. Agric. Sci. 63:277-282.

Chalmers, M. I., Jaysinghe, J. B. and Marshall, S. B. M. 1964. The effect of heat treatment in the processing of ground nut meal on the value of the protein for ruminants with some additional experiments on copra. J. Agric. Sci. 63:283-288.

Chalmers, M. I., Hughes, A. D. and Jaffrey, A. E. 1968. Estimation of urea in rumen contents and the rate of urea degradation in the rumen of adult sheep. Proc. Nutr. Soc. 28:35A.

Chalupa, W., Kutches, A. J., Lavker, R. and O'Dell, G. D. 1965. Variations in protozoa populations of pellet fed heifers. J. Anim. Sci. 24:876 (Abstract).

Chanet, M. 1970. The utilization of rape seed meal in animal feeding. Proc. Int. Rape seed Conf. St. Adele, Quebec, Canada. p. 311-320. Published by Rape Seed Association of Canada.

Christiansen, W. C. 1963. Nutrient and other environmental influences upon rumen protozoal growth and metabolism. Dissertation Abst. 24:1315-1316.

Christiansen, W. C., Woods, W. and Burroughs, W. 1964. Ration characteristics influencing rumen protozoal populations. J. Anim. Sci. 23:984-988.

Christiansen, W.C., Kawashima, R., Burroughs, W. 1965. Influence of protozoa upon rumen acid production and live weight gains in lambs. J. Anim. Sci. 24:730-734.

Clarke, E. M. W., Ellinger, G. M. and Phillipson, 1966. The influence of diet on the nitrogenous components passing to the duodenum and through the lower ileum of sheep. Proc. R. Soc. B. 166:63-79.

Coelho da Silva, J. F., Seeley, R. C., Thomson, D. J., Beever, D. E. and Armstrong, D. G. 1972. The effect in sheep of physical form on the sites of digestion of a dried lucerne diet. Brit. J. Nutr. 28:43-61.

Coleman, G. S. 1967, a. The metabolism of free amino acids by washed suspensions of the rumen ciliate Entodinium Caudatum. J. Gen. Microbiol. 47:433-447.

Coleman, G. S. 1967, b. The metabolism of the amino acids of Escherichia Coli and other bacteria by the rumen ciliate Entodinium Candatum. J. Gen. Microbiol. 47: 449-464.

Coleman, G. S. 1968. The metabolism of bacterial nucleic acid and free components of nucleic acid by the rumen ciliate Entodinium Caudatum. J. Gen. Microbiol. 54: 83-96.

Condon, R. J. and Hatfield, E. E. 1971. Metabolism of exogenous nucleic acids by ovine. Fed. Proc. 30:403(Abst).

Conrad, H. R., Miles, R. C. and Butdorf, J. 1967, a. Estimation of methionine synthesis in intact cows after administering sulfide-^{35}S. J. Nutrition. 91:337-342.

Conrad, H. R., Hibbs, J. W. and Pratt, A. D. 1967. Effect of plane of nutrition and source of N on methionine synthesis in cows. J. Nutrition. 91:343-347.

Conrad, H. R. and Hibbs, J. W. 1968. Nitrogen utilization by the ruminant. Appreciation of its nutritive value. J. Dairy Sci. 51:276-285.

Conway, E. J. 1957. Microdiffusion analysis and volumetric errors. 4th ed. London Crosby, Lockwood and Sons, Ltd. University Press, Glasgow. p. 98-100, 162-165.

Cook, R. M., Brown, R. E. and Davis, C. L. 1961. Ruminal absorption of amino acids. J. Dairy Sci. 44:1203(Abst).

Danke, R. J., Sherrod, L. B., Nelson, E. C. and Tillman, A. D. 1966. Effects of autoclaving and steaming of cotton seed meal for different lengths of time on N solubility and retention in sheep. J. Anim. Sci. 25:181-184.

Delort-Laval, J. and Zelter, S. Z. 1968. Improving the nutritive value of proteins by tanning process. Proc. 2nd world conf. Anim. Prod. p. 457-458. College Park, Md.

Devlin, T. J. and Woods, W. 1965. Nitrogen metabolism as influenced by lysine administration posterior to the rumen. J. Anim. Sci. 24:878 (Abstract).

Downes, A. M. 1961. On the amino acids essential for the tissues of the sheep. Aust. J. Biol Sci. 14:254-259.

Driedger, A. and Hatfield, E. E. 1972. Influence of tannins on the nutritive value of soy bean meal for ruminants. J. Anim. Sci. 34:465-468.

Driedger, A. and Hatfield, E. E. 1970. Utilization of tannin treated SBM infused per rumen or abomasum. J. Anim. Sci. 31:1038 (Abst).

Duncan, C. W., Agrawala, I. P., Huffman, C. F. and Luecke, R. W. 1953. A quantitative study of rumen synthesis in the bovine on natural and purified rations. J. Nutrition 49:41-49.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics. 11:42.

Dysli, R. R., Amerman, C. B., Loggins, P. E., Moore, J. E. and Arrington, L. R. 1967. Effect of steam-heating upon the nutritive value of full-fat soy beans for sheep and rats. J. Anim. Sci. 26:618-623.

Eadie, J. M. 1962. The development of rumen microbial populations in lambs and calves under various conditions of management. J. Gen. Microbiol. 29:563-578.

Eadie, J. M., Hyldgaard-Jensen, J., Mann, S. O., Reid, R. S. and Whitelaw, F. G. 1970. Observations on the microbiology and biochemistry of the rumen in cattle given different quantities of a pelleted barley ration. Br. J. Nutr. 24:157-177.

Eadie, J. M. and Gill, J. C. 1971. The effect of the absence of rumen ciliate protozoa on growing lambs fed on a roughage-concentrate diet. Br. J. Nutr. 26:155-167.

Eagen, A. R. and Moir, R. J. 1965. Nutritional status and intake regulation in sheep. I. Effects of duodenally infused single dose of casein, urea and propionate upon voluntary intake of a low-protein roughage by sheep. Aust. J. Agric. Res. 16:437-449.

Ellis, W. C. and Pfander, W. H. 1965. Rumen microbial polynucleotide synthesis and its possible role in ruminant nitrogen utilization. Nature. 205:974-975.

Ely, D. G., Little, C. O., Woolfolk, P. G. and Mitchell, Jr. G. E. 1967. Estimation of the extent of conversion of dietary zein to microbial protein in the rumen of lambs. J. Nutrition. 91:314-318.

El-Sayed Osman, H., Abou Akkada, A. R. and Agabawe, K. A. 1970. Influence of rumen ciliate protozoa on conversion of food and growth rate in early-weaned zebu calves. Anim. Prod. 12:267-271.

El-Shazly, K. and Hungate, R. E. 1966. Method for measuring diaminopimelic acid in total rumen contents and its application to the estimation of bacterial growth. Appl. Microbiol. 14:27-30.

El-Shazly, K. 1952, a. Degradation of protein in the rumen of the sheep. 1. Some volatile fatty acids including branched chain isomers found in vivo. Biochem. J. 51:640-647.

El-Shazly, K. 1952, b. 2. The action of rumen microorganisms on amino acids. Biochem. J. 51:647-653.

Erwin, E. S., Marco, G. J. and Emery, E. M. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768-1771.

Faichney, G. J. 1971. The effect of formaldehyde-treated casein on the growth of ruminant lambs. Aust. J. Agric. Res. 22:453-460.

Faichney, G. J. and Davies, H. L. 1972. The effect of formaldehyde treatment of peanut meal in concentrate diets on the performance of calves. Aust. J. Agric. Res. 23:167-175.

Faichney, G. J. and Weston, R. H. 1971. Digestion by ruminant lambs of a diet containing formaldehyde-treated casein. Aust. J. Agric. Res. 22:461-468.

Ferguson, K. A., Hemsley, J. A. and Reis, P. J. 1967. Nutrition and wool growth. The effect of protecting dietary protein from microbial degradation in the rumen. Aust. J. Sci. 30:215-217.

Gibbons, R. J. and McCarthy, R. D. 1957. Obligately anaerobic urea-hydrolysing bacteria in the bovine rumen. J. Dairy Sci. 40:635 (Abstract).

Giesecke, D., Lawlore, M. J. and Karin Walser-Karst. 1966. Comparative studies on the digestive physiology of sheep fed on semi-purified or roughage-concentrate diets. 2. Microbiological investigations. Br. J. Nutr. 20:383-392.

Glimp, H. A., Karr, M. R., Little, C. O. and Woolfolk, P. G. 1967. Effect of reducing soy bean protein solubility by dry heat on the protein utilization of young lambs. J. Anim. Sci. 26:858-861.

Gordon, J. G. and Tribe, D. E. 1952. The importance to sheep of frequent feeding. Brit. J. Nutr. 6:89-93.

Gray, F. V., Pilgrim, A. F. and Weller, R. A. 1958. The digestion of food stuffs in the stomach of the sheep and the passage of digesta through its compartments. 2. Nitrogenous compounds. Brit. J. Nutr. 12:413-420.

Hatfield, E. E., Driedger, A. and Garrigus, U. S. 1968. Unpublished observations cited by E. E. Hatfield in selected topics related to the amino acid nutrition of the growing ruminant. Fed. Proc. 29:44-50.

Hemsley, J. A., Hogan, J. P. and Weston, R. H. 1970. Protection of forage protein from ruminal degradation. Proc. XI. Int. Granland Congr. p. 703-706.

Hirs, C. H. W. 1967. (6) Determination of cystine as cysteic acid. cited in Methods in Enzymology. Vol. XI p. 59 (ed. Hirs, C. H. W.), Academic Press, New York.

Hogan, J. P. 1961. The absorption of ammonia through the rumen of the sheep. Aust. J. Biol. Sci. 14:448-460.

Hogan, J. P. 1965. The digestion of food by the grazing sheep. III. The quantity of protein reaching the small intestine. Aust. J. Agric Res. 16:179-188.

Hogan, J. P. and Weston, R. H. 1967. The digestion of two diets differing protein content but with similar capacities to sustain wool growth. Aust. J. Agric. Res. 18:973-981.

Hogan, J. P., Weston, R. H. and Lindsay, J. R. 1968. Influence of protein digestion on plasma amino acid levels in sheep. Aust. J. Biol. Sci. 21:1263-1275.

Hogan, J. P. and Weston, R. H. 1970. "In Physiology of Digestion and Metabolism in the Ruminant", ed. A. T. Phillipson. p. 474, (Oriel Press: New Castle-Upon Tyne).

Holmes, P., Moir, R. J. and Underwood, E. J. 1953. Ruminal flora studies in sheep. V. Amino acid composition of rumen bacterial protein. Aust. J. Biol. Sci. 6:637-644.

Hoshino, S., Sarumaru, K. and Morimoto, K. 1966. Ammonia anabolism in ruminants. J. Dairy Sci. 49:1523-1528.

Houpt, T. R. 1959. Utilization of blood urea in ruminant. Amer. J. Physiol. 197:115-120.

Houpt, T. R. 1969. Transfer of urea and ammonia to the rumen, in physiology of digestion and metabolism in the ruminant. Ed. by A. T. Phillipson. Oriel Press, England.

Hudson, L. W., Glimp, H. A., Little, C. O. and Woolfolk, P. G. 1970. Ruminal and post ruminal nitrogen utilization by lambs fed heated soy bean meal. J. Anim. Sci. 30:609-613.

Hughes, J. G. and Williams, G. L. 1970. Utilization of formaldehyde treated casein by sheep. Anim. Prod. 12: 356.

Hughes, J. G. and Williams, G. L. 1971a. The utilization of formaldehyde-treated ground nut meal by sheep. Proc. 52nd Br. Soc. Anim. Prod. London (April 5th-7th).

Hughes, J. G. and Williams, G. L. 1971b. The effect of formaldehyde treatment of protein supplements upon their in vitro fermentation and utilization by sheep. Proc. Nutr. Soc. 30:41A.

Hume, I. D. 1970. Synthesis of microbial protein in the rumen, III. The effect of dietary protein. Aust. J. Agric. Res. 21:305-314.

Hungate, R. E. 1966. The rumen and its microbes. Academic Press, New York.

Hutton, K., Bailey, F. J. and Annison, E. F. 1971. Measurement of the bacterial nitrogen entering the duodenum of the ruminant using diaminopimelic acid as a marker. Br. J. Nutr. 25:165-173.

Hyden, S. 1955. A turbidometric method for the determination of higher polyethyleneglycol in biological materials. Annaler. 22:139-145.

Ibrahim, E. A., Ingalls, J. R. and Phillips, G. D. 1969. Effect of continuous feeding on the composition of rumen digesta. Can. J. Anim. Sci. 49:399-401.

Ibrahim, E. A., Ingalls, J. R. and Bragg, D. B. 1970. Separation and identification of amino acids present in rumen microorganisms. Can. J. Anim. Sci. 50:397-400.

Ibrahim, E. A. and Ingalls, J. R. 1972. Microbial protein biosynthesis in the rumen. J. Dairy Sci. 55:971-978.

Ingalls, J. R., Seale, M. E. and McKirdy, J. A. 1968. Effect of rape seed meal and urea on ad libitum consumption of grain rations by dairy cows. Can. J. Anim. Sci. 48:437-442.

Ingalls, J. R., Waldern, D. E. and Stone, J. B. 1970. Rape seed meal in ruminants rations - Dairy Cattle. Proc. Int. Rape Seed Conf. St. Adele, Quebec, Canada. p. 281-291. Published by Rape Seed Association of Canada.

Ingalls, J. R., Sharma, H. R., Devlin, T. J. and Phillips, G. D. 1970. Supplemental methionine for growing calves. J. Dairy Sci. 53:675 (Abst.).

Ingalls, J. R. and Seale, M. E. 1971. Effect of continuous feeding of rape seed meal on growth of dairy calves and subsequent first lactation yield. Can. J. Anim. Sci. 51:681-686.

Ingalls, J. R. and Waldern, D. E. 1972. Rape seed meal in rations for dairy cattle. Canadian rape seed meal in poultry and animal feeding. Publi. no. 16:24-27.

Jarl, F. 1951. Kungl. Lantbrukshogskolan och statens Lantbruksforsok statens Husdjursforsok Meddelande Nr. 45.

Johnson, R., Moir, R. J. and Underwood, E. J. 1949. Sulpher containing amino acids in the rumen bacteria of sheep. Nature. 163:101.

Jones, G. A., MacLeod, R. A. and Blackwood, A. C. 1964. Ureolytic rumen bacteria. 1. Characteristics of the microflora from a urea-fed sheep. Can. J. Microbiol. 10:371-378.

Jurtshuk, Jr., P., Doestsch, R. N. and Shaw, J. C. 1958. Anaerobic purine dissimilation by washed suspensions of bovine rumen bacteria. J. Dairy Sci. 41:190-202.

Kidder, G. W. 1967. In chemical Zoology (gen. eds. M. Florkin and B. T. Scheer) Vol. 1. Protozoa. p. 93 (ed. G. W. Kidder). New York: Academic Press.

Klopfenstein, T. J., Purser, D. B. and Tyznik, W. J. 1964. Influence of aureomycin on rumen metabolism. J. Anim. Sci. 23:490-495.

Klopfenstein, T. J., Purser, D. B. and Tyznik, W. J. 1966. Effects of defaunation on feed digestibility, rumen metabolism and blood metabolites. J. Anim. Sci. 25: 765-773.

Knox, K. L. and Ward, G. M. 1960. Rumen volatile fatty acid production as affected by feeding frequency. J. Dairy Sci. 43:875 (Abstract).

Koningsberger, V. J., Slijper, E. J. and Vonk, H. J. 1946. Tabulae biol. Amst. v 21. pt. 1. cited in Biological Handbook, Blood and other body fluids by P. L. Altman 1961. p. 420. Federation of American Society for Expt. Biol. Washington, D.C.

Langlands, J. P. 1971, a. The wool production of grazing sheep supplemented with casein and formaldehyde-treated casein. Aust. J. Expt. Agric. and Anim. Husbandry 11:9-13.

Langlands, J. P. 1971, b. The wool production of sheep supplemented with cotton seed meal and formaldehyde-treated cotton seed meal. Aust. J. Expt. Agric. and Anim. Husbandry 11:493-497.

Leibholz, Jane. 1965. The free amino acid occurring in the blood plasma and rumen liquor of the sheep. Aust. J. Agric. Res. 16:973-979.

Leibholz, J. 1969. Effect of diet on the concentration of free amino acids, ammonia and urea in the rumen liquor and blood plasma of the sheep. J. Anim. Sci. 29:628-633.

Lewis, D. 1955. Amino acid metabolism in the rumen of the sheep. Br. J. Nutr. 9:215-230.

Lewis, D. 1962 Interrelationships of individual proteins and carbohydrates during fermentation in the rumen of the sheep. II. The fermentation of starch in presence of protein and other substances containing N. J. Agric. Sci. 58:73-79.

Lewis, T. R. and Emery, R. S. 1962. Intermediate products in the catabolism of amino acids by rumen microorganisms. J. Dairy Science. 45:1363-1368.

Lewis, T. R. and Emery, R. S. 1960. Amino acid metabolism by rumen bacteria. J. Dairy Sci. 43:876. (Abstract).

Lewis, T. R. and Emery, R. S. 1962. Metabolism of amino acids in the bovine rumen. J. Dairy Sci. 45:1487-92.

Lewis, T. R. and Emery, R. S. 1962. Relative deamination rates of amino acids by rumen microorganisms. J. Dairy Sci. 45:765-768.

Leroy, R., Zelter, S. Z. and Francois, A. C. 1965. Protection of proteins in feeds against deamination by bacteria in the rumen. Abst. Rev. 35:444.

Lindsay, J. R. and Hogan, J. P. 1972. Digestion of two legumes and rumen bacterial growth in defaunated sheep. Aust. J. Agric. Res. 23:321-330.

Little, C. O. and Mitchell, G. E. 1967. Abomasal vs. oral administration of protein to wethers. J. Anim. Sci. 26:411-413

Little, C. O., Mitchell, Jr. G. E. and Potter, G. D. 1968. Nitrogen in the abomasum of wethers fed different protein sources. J. Anim. Sci. 27:1722-1726.

Loosli, J. K., Williams, H. H., Thomas, W. E., Ferris, F. H. and Maynard, L. A. 1949. Synthesis of amino acids in the rumen. Science. 110:144-145.

Loosli, J. K., Warner, R. G. and Hintz, H. F. 1961. Value of corn distillers dried grains, soy bean oil meal, heated soy beans, and soy bean oil meal plus starch for milk production. J. Dairy Sci. 44:1910-1914.

Luther, R., Trenkel, A. and Burrough, W. 1966. Influence of rumen protozoa on volatile acid production and ration digestibility in lambs. J.Anim.Sci. 1116-1122.

MacRae, J. C. 1970. Studies on the intestinal digestion of nitrogen by sheep fed formaldehyde treated casein diets. Proc. N.Z. Soc. Anim. Prod. 50:218-226.

MacRae, J. C., Ulyatt, M. J., Pearce, P. D. and Hendtlass, J. 1972. Quantitative intestinal digestion of nitrogen in sheep given formaldehyde-treated and untreated casein supplements. Brit. J. Nutr. 27:39-50.

Mangan, J. L. 1972. Quantitative studies on nitrogen metabolism in the bovine rumen. The rate of proteolysis of casein and ovalbumin and the release and metabolism of free amino acids. Br. J. Nutr. 27:261-283.

Mason, V. C. 1969. Some observation on the distribution and origin of N in sheep feces. J. Agric. Sci. 73:99-111.

Mason, V. C. 1971. Some preliminary observations on the nature of factors influencing the excretion of non-dietary fecal N by ruminant animals. J. Agric. Sci. 76:157-166.

Mason, V. C. and Milne, G. 1971. The digestion of bacterial mucopeptide constituents in the sheep. 2. The digestion of muramic acid. J. Agric. Sci. 77:99-101.

Mason, V. C. and White, F. 1971. The digestion of bacterial mucopeptide constituents in the sheep. 1. The metabolism of 2-6-diaminopimelic acid. J. Agric. Sci. 77:91-98.

Mathison, G. W. and Milligan, L. P. 1971. Nitrogen metabolism in sheep. Br. J. Nutr. 25:351-366.

McDonald, I. W. 1948. Absorption of ammonia from the rumen of sheep. Biochem. J. 42:584-587.

McDonald, I. W. 1952. The role of ammonia in the ruminal digestion of protein. Biochem. J. 51:86-90.

McDonald, I. W. 1954. The extent of conversion of food protein to microbial in the rumen of sheep. Biochem. J. 56:120-125.

McDonald, I. W. and Hall, R. J. 1957. The conversion of casein into microbial proteins in the rumen. Biochem. J. 67:400-405.

McDonald, I. W. 1968. Nutritional aspects of protein metabolism in ruminants. Aust. Vet. J. 44:145-150.

McNaught, M. L., Owen, C. E., Henry, K. M. and Kon, S. K. 1954. The utilization of non-protein nitrogen in the bovine rumen. 8. The nutritive value of the proteins of preparations of dried rumen bacteria, rumen protozoa and brewer's yeast for rats. Biochem. J. 56:151-156.

Meyer, R. M., Bartley, E. E. and Deyoe, C. W. 1967. Feed processing. 1. Ration effects on rumen microbial protein synthesis and amino acids composition. J. Dairy Sci. 50:1327-1332.

Moir, R. J. and Somer, M. 1957. Ruminal flora studies. VIII. The influence of rate and method of feeding a ration upon its digestibility upon ruminal functions and upon ruminal population. Aust. J. Agric. Res. 8:251-265.

Moore, W. E. C. and King, K. W. 1958. Determination of the intra ruminal distribution of soluble nitrogen. J. Dairy Sci. 41:1451-1455.

Mowat, D. N. and Deelstra, K. 1972. Encapsulated methionine supplement for growing-finishing lambs. J. Anim. Sci. 34:332-335.

Naga, M. A., Abou Akkada, A. R. and El-Shazly, K. 1969. The establishment of rumen ciliate protozoa in cow and water buffalo (Bos bubalus L.) calves under late and early weaning systems. J. Dairy Sci. 52:110-112.

Neudoerffer, T. S., Leadbeater, P. A., Horney, F. D. and Bayley, H. S. 1971. The influence of level of grain intake on protein digestion in the intestine of cattle. Br. J. Nutr. 25:343-350.

Nicholson, J. W. G., Gorrill, A. D. L. and Burgess, P. L. 1972. Utilization by sheep of RSM protein treated with various concentrations of formaldehyde. Can. J. Anim. Sci. 52:586. (Abst.).

Nimrick, K., Peter, A. P. and Hatfield, E. E. 1972. Aldehyde-treated fish and soy bean meals as dietary supplements for growing lambs. J. Anim. Sci. 34:488-490.

Oltjen, R. R., Sirny, R. J. and Tillman, A. D. 1962. Purified diet studies with sheep. J. Anim. Sci. 21: 277-283.

Oltjen, R. R., Putnam, P. A. and Davis, R. E. 1965. Salivary and metabolic studies with steers fed pelleted or unpelleted conventional and purified rations. J. Anim. Sci. 24:1126-1130.

Ørskov, E. R. and Benzie, D. 1969. Studies on the oesophageal groove reflex in sheep and on the potential use of the groove to prevent the fermentation of food in the rumen. Br. J. Nutr. 23:415-420.

Ørskov, E. R., Fraser, C. and McDonald, I. 1972. Digestion of concentrates in sheep. 4. The effects of urea on digestion, nitrogen retention and growth in young lambs. Br. J. Nutr. 27:491-501.

Pearson, R. M. and Smith, J. A. B. 1943. The utilization of urea in the bovine rumen. 3. The synthesis and breakdown of protein in rumen ingesta. Biochem. J. 37:153-164.

Peter, A. P., Hatfield, E. E., Owens, F. N. and Garrigus, U. S. 1971. Effects of aldehyde treatments of soy bean meal on in vitro ammonia release, solubility and lamb performance. J. Nutrition. 101:605-612.

Phillipson, A. T. 1964. The digestion and absorption of nitrogenous compounds in the ruminant. In Mammalian Protein Metabolism Vol 1. p. 71 (H. N. Munro and J. B. Allison, editors) London. Academic Press.

Phillipson, A. T., Dobson, M. J. and Blackburn, T. H. 1959. Assimilation of ammonia N by rumen bacteria. Nature 183:402-404.

Pilgrim, A. F., Gray, F. V. and Weller, R. A. 1970. Synthesis of microbial protein from ammonia in the sheep's rumen and the proportion of dietary nitrogen converted into microbial nitrogen. Br. J. Nutr. 24:589-598.

Porter, R. R. 1950. A chemical study of rabbit antiovalbumin. Biochem. J. 46:473-478.

Portugal, A. V. and Sutherland, T. M. 1966. Metabolism of glutamic and aspartic acids in whole rumen contents. Nature. 209:510-511.

Pounden, W. D. and Hibbs, J. W. 1950. The development of calves raised without protozoa and certain other characteristics rumen microorganisms. J. Dairy Sci. 33: 639-644.

Purser, D. B. and Moir, R. J. 1959. Ruminal flora studies in the sheep. IX. The effect of pH on the ciliate population of the rumen in vivo. Aust. J. Agric. Res. 10:555-564.

Purser, D. B., Klopfenstein, T. J. K. and Cline, J. H. 1965. Influence of Tylosin and aureomyein upon rumen metabolism and the microbial population. J. Anim. Sci. 24:1039-1044.

Purser, D. B. and Buechler, S. M. 1966. Amino acid composition of rumen microorganisms. J. Dairy Sci. 49:81-84.

Purser, D. B., Klopfenstein, T. J. and Cline, J. H. 1966. Dietary and defaunation effects upon plasma amino acid concentrations in sheep. J. Nutrition, 89:226-234.

Purser, D. B. 1970. Nitrogen metabolism in the rumen: Microorganisms as a source of protein for the ruminant animal. J. Anim. Sci. 30:988-1001.

Putnam, P. A., Gutierrez, J. and Davis, R. E. 1961. Effect of frequency of feeding upon rumen volatile acids, protozoal populations and weight gain in Angus heifer calves. J. Dairy Sci. 44:1364-1365.

Rakes, A. H., Hardison, W. A., Albert, J., Moore, W. E. C. and Graf, G. C. 1957. Effect of frequency of feeding on animal response, feed digestibility and rumen activity. J. Dairy Sci. 40:633 (Abst.).

Rakes, A. H., Lister, E. E. and Reid, J. T. 1961. Some effects of feeding frequency on the utilization of isocaloric diets by young and adult sheep. J. Nutr. 75:86-92.

Reed, F. M., Moir, R. J. and Underwood, E. J. 1949. Ruminal flora studies in the sheep. 1. Nutritive value of rumen bacterial protein. Aust. J. Sci. Res. Series B. 2:304-317.

Reis, P. J. and Schinckel, P. G. 1963. Some effects of sulphur containing amino acids on the growth and composition of wool. Aust. J. Biol. Sci. 16:218-230.

Reis, P. J. and Schinckel, P. G. 1964. The growth and composition of wool. II. The effect of casein, gelatin, and sulphur containing amino acids given per abomasum. Aust. J. Biol. Sci. 17:532-547.

Reis, P. J. and Tunks, D. A. 1969. Evaluation of formaldehyde-treated casein for wool growth and nitrogen retention. Aust. J. Agric. Res. 20:775-781.

Reis, P. J. and Tunks, D. A. 1970. Changes in plasma amino acid patterns in sheep associated with supplements of casein and formaldehyde-treated casein. Aust. J. Biol. Sci. 23:673-680.

Reis, P. J. 1970. The influence of abomasal supplements of amino acids and sulphur-containing compounds on wool growth rate. Aust. J. Biol. Sci. 23:441-446.

Reis, P. J. and Downes, A. M. 1971. The rate of response of wool growth to abomasal supplements of casein. J. Agric. Sci. 76:173-176.

Roberts, S. A. and Miller, E. L. 1969. An estimate of bacterial protein synthesis in sheep on a constant feeding regime. Proc. Nutr. Soc. 28:32A.

Satter, L. D. and Baumgardt, B. R. 1962. Changes in digestive physiology of the bovine associated with various feeding frequencies. J. Anim. Sci. 21:897-900.

Satter, L. D. Brook, G. P. and Schwale, C. G. 1970. Effect of formaldehyde treated soy bean oil meal on performance of growing lambs and lactating cows. J. Dairy Sci. 53668 (Abst.).

Schelling, G. T., Hinds, F. C. and Hatfield, E. E. 1967. Effect of dietary protein levels, amino acid supplementation and N source upon the plasma free amino acid concentrations in growing lambs. J. Nutrition. 92: 339-347.

Schelling, G. T. and Hatfield, E. E. 1968. Effect of abomasally infused nitrogen sources on nitrogen retention of growing lambs. J. Nutrition. 96:319-326.

Schelling, G. T. 1970. The effect of abomasally infused methionine and casein on N retention and plasma amino acid concentration in lambs. Fed. Proc. 20:759 (Abst.).

Scott, R. A., Little, C. O., Amos, H. E. and Mitchell, Jr. G. E. 1972. Abomasal nitrogen, plasma amino acids and nitrogen balance responses of weathers to fed or abomasally infused methionine. J. Anim. Sci. 35:446-449.

Seale, M. E. 1952. Comparison of protein supplements in steer feeding. Univ. of Manitoba. 2nd Ann. Livestock day report, p. 1-3.

Seale, M. E. 1952. Comparative value of rape seed meal and linseed oil meal for milk production. 2nd Ann. Livestock day report. p. 11-16.

Sharma, H. R., Van't Klooster, A. Th. and Frens, A. M. 1969. In vivo measurements of the synthesis of protein from urea in the rumen of sheep. Zeitschrift fur Tierphysiologie, Tierernahrung und Futtermittelkunde Band. 24: 371-383.

Sherrod, L. B. and Tillman, A. D. 1962. Effects of varying the processing temperatures upon the nutritive values for sheep of solvent extracted soy bean meal and cotton seed meals. J. Anim. Sci. 21:901-910.

Sherrod, L. B. and Tillman, A. D. 1964. Further studies on the effects of different processing temperatures on the utilization of solvent-extracted cotton seed protein by sheep. J. Anim. Sci. 23:510-516.

Sibbald, I. R., Loughheed, T. C. and Linton, J. H. 1968. A methionine supplement for ruminants. Proc. Second World Conf. on Anim. Prod. paper 113. College Park, Md.

Sirotnak, F. M., Doetsch, R. N., Brown, R. E., Shaw, J. C. 1953. Amino acid metabolism of bovine rumen bacteria. J. Dairy Sci. 36:1117-1123.

Smith, J. A. B. and Baker, F. 1944. The utilization of urea in the bovine rumen. 4. Isolation of the synthesized material and the correlation between protein synthesis and microbial activities. Biochem. J. 38: 496-505.

Smith, R. H. 1969. Reviews of the progress of dairy science. Section G. General. Nitrogen metabolism and the rumen. J. Dairy Sci. 36:313-331.

Smith, R. H. and McAllan, A. B. 1970. Nucleic acid metabolism in the ruminant. 2. Formation of microbial nucleic acids in the rumen in relation to the digestion of food nitrogen, and the fate of dietary nucleic acids. Br. J. Nutr. 24:545-556.

Smith, R. H., McAllan, A. B. and Hill, W. K. 1968. Nucleic acids in bovine nutrition. 2. Production of microbial nucleic acids in the rumen. Proc. Nutr. Soc. 27:48A.

Smith, R. H., McAllan, A. B. and Hill, W. B. 1969. Nucleic acids in bovine nutrition. 3. Fate of nucleic acids presented to the small intestine. Proc. Nutr. Soc. 28:28A.

Snedecor, G. W. 1956. Statistical methods. 5th ed. The Iowa State University Press, Ames. Iowa.

Somers, M. 1961. Factors influencing the secretion of N in sheep saliva. 1. The distribution of N in the mixed and parotid saliva of sheep. Aust. J. Exp. Biol. Med. Sci. 39:111-122.

Stake, P. E., Owens, M. J. and Schingoethe. 1972. Evaluation of three protein supplements for dairy calf starter meals. J. Dairy Sci. 55:704 (Abst).

Steinacker, G., Devlin, T. J. and Ingalls, J. R. 1970. Effect of methionine supplementation posterior to the rumen on nitrogen utilization and sulphur balance of steers on high roughage ration. Can. J. Anim. Sci. 50:319-324.

Storry, J. E. and Rook, J. A. F. 1966. The relationship in the cow between milk-fat secretion and ruminal volatile fatty acids. Br. J. Nutr. 20:217-223.

Sym, E. A. 1938. Hydrolase activity of the contents of the calcum in the horse and of the rumen in cattle. 1. Introduction: general methods and proteolytic action. Acta. Biol. Exp. 12:192-210.

Tagari, H., Ascarelli, I. and Bondi, A. 1962. The influence of heating on the nutritive value of soy bean meal for ruminants. Br. J. Nutr. 16:237-243.

Tagari, H., Henes, Y., Tamir, M. and Volcani, R. 1965. Effect of carob pod extract on cellulolysis, proteolysis, deamination, and protein biosynthesis in an artificial rumen. Appl. Microbiol. 13:437-442.

Telmer-Kucharski, A. and Gausseres, B. 1965. Quantitative evaluation of the microbial population of the digestive tract of ruminants from its content of nucleic acids. 2. Application to the study of digestion in the weaned calf by analysis of the contents of the duodenum. Ann. Biol. Anim. Biochem. Biophys. 5:207-221.

Topps, J. H., Kay, R. N. B., Goodall, E. D., Whitelaw, F. G. and Reid, R. S. 1968. Digestion of concentrate and of hay diets in the stomach and intestines of ruminants. Br. J. Nutr. 22:281-290.

Van den Hende, C., Oyaert, W. and Bouckaert, J. H. 1963, a. Fermentation of histidine by rumen bacteria. Res. Vet. Sci. 4:77-88.

Van den Hende, C., Oyaert, W. and Bouckaert, J. H. 1963, b. Fermentation of serine and threonine by rumen bacteria. Res. Vet. Sci. 4:89-95.

Van den Hende, C., Oyaert, W., Bouckaert, J. H. 1964. The metabolism of arginine, citrulline, ornithine, proline, delta-amino valeric acid and phenylalanine by rumen bacteria. Res. Vet. Sci. 5:491-496.

Van der Horst, C. J. G. 1965. Cited by R. H. Smith in Reviews of the progress of Dairy Science. Section G. General. Nitrogen metabolism and the rumen. J. Dairy Res. 1969. 36:313-331.

Van't Klooster, A. Th. and Rogers, P. A. M. 1969. The rate of flow of digesta and the net absorption of dry matter, organic matter, ash, nitrogen and water. Mededlingen Landbouwhogeschool Wageningen. Nederland 69-11, p. 3-19.

Virtanen, A. I. 1963. Investigations on the alleged goiterogenic properties of milk. Report of the Biochem. Institute, Helsinki, Finland.

Virtanen, A. I. 1966. Milk production of cows on protein free feed. Science. 153:1603.

Virtanen, A. I. 1969. On nitrogen metabolism in milking cows. Fed. Proc. 28:232-240.

Walker, D. J. and Nader, C. J. 1968. Method for measuring microbial growth in rumen content. Appl. Microbiol. 16:1124-1131.

Wallis, O. C. and Coleman, G. S. 1967. Incorporation of ^{14}C-labelled components of Escherichia coli and amino acids by Isotricha intestinalis and Intestinalis Prostoma from the sheep rumen. J. Gen. Microbiol. 49: 315-323.

Warner, A. C. I. 1956. Proteolysis by rumen microorganisms. J. Gen. Microbiol. 14:749-762.

Warner, A. C. I. 1964. The break down of asparagine, glutamine and other amides by microorganisms from the sheep rumen. Aust. J. Biol. Sci. 17:170-182.

Weller, R. A. 1957. The amino acid composition of microbial preparations from the rumen of sheep. Aust. J. Biol. Sci. 10:384-389.

Weller, R. A., Gray, F. V. and Pilgrim, A. F. 1958. The conversion of plant nitrogen to microbial nitrogen in the rumen of sheep. Br. J. Nutr. 12:421-429.

Weston, R. H. 1971. Factors limiting the intake of feed by sheep. V. Feed intake and the productive performance of the ruminant lamb in relation to the quantity of crude protein digested in the intestines. Aust. J. Agric. Res. 22:307-320.

Whitelaw, F. G., Preston, T. R. and Dawson, G. S. 1961. The nutrition of the early-weaned calf. II. A comparison of commercial ground nut meal, heat-treated ground nut meal and fish meal as the major protein source in the diet. Anim. Prod. 3:127-133.

Whitelaw, F. G., Eadie, J. M., Mann, S. O. and Reid, R. S. 1972. Some effects of rumen ciliate protozoa in cattle given restricted amounts of a barley diet. Br. J. Nutr. 27:425-437.

Williams, P. P., Davis, R. E., Doetsch, R. N. and Gutierrez, J. 1961. Physiological studies of the rumen protozoan Ophryoscolex caudatus Eberlein. Appl Microbiol. 9:405-409.

Wright, P. L. 1971. Body weight gain and wool growth response to formaldehyde treated casein and sulpher amino acids. J. Anim. Sci. 33:137-141.

Wood, A. S. and Stone, J. B. 1970. Digestibility, nitrogen retention and caloric value of rape seed and soy bean meals when fed at two dietary levels to calves. Can. J. Anim. Sci. 50:507-512.

Wright, D. E. and Hungate, R. E. 1967. Amino acid concentrations in rumen fluid. Appl. Microbiol. 15:148-151.

Zelter, S. Z., Leroy, F. and Tissier, J. P. 1970. Protection of proteins in the feed against bacterial deamination in the rumen. I. Studies in vitro: Behaviour in the rumen of some proteins tanned with tannin from the chestnut wood or certain aldehydes (formaldehydes, glutaraldehyde, glyoxal). Ann. Biol. Anim. Biochem. Biophysics 10:111-122.

APPENDICES

APPENDIX 1

NUTRITIVE VALUE OF FORMALDEHYDE-TREATED RAPESEED MEAL FOR DAIRY CALVES

H. R. SHARMA, J. R. INGALLS, and J. A. McKIRDY

Department of Animal Science, University of Manitoba, Winnipeg, Man. *Received Oct. 1, 1971, accepted Mar. 2, 1972.*

ABSTRACT

Treatment of oilseed meals with formaldehyde (FA) and glutaraldehyde (GA) significantly ($P < 0.01$) reduced the solubility of protein in 0.02 N NaOH and the production of NH_3 in the artificial rumen. Ten Holstein and Holstein × Brown Swiss calves of both sexes were fed two test rations containing either 26% untreated or formaldehyde-treated (5.6 g FA per 100 g protein) rapeseed meal (FA-treated RSM) for 14 weeks. Plasma urea nitrogen (N) and rumen NH_3 levels were significantly ($P < 0.01$) lower in the FA-treated RSM calves compared with the control calves during the first 8 weeks of the growth trial. No significant difference ($P > 0.05$) in dry matter consumption, daily gain, or feed efficiency was observed. Total volatile fatty acids (VFA) (mmoles/100 ml) concentration was significantly higher ($P < 0.05$) in rumen fluid from the control calves compared with those receiving treated RSM. Molar percentages of the VFA's were not significantly different ($P > 0.05$) between the two groups. In the N balance and digestibility trial, dry matter consumption did not differ significantly ($P > 0.05$); however, the FA-treated RSM resulted in decreased ($P < 0.05$) dry matter and crude protein digestibility. Calves receiving the treated RSM had reduced ($P < 0.01$) urinary N excretion; however, there was no significant effect ($P > 0.05$) on N consumption and retention.

RESUME

Le traitement de tourteaux à l'aldéhyde formique (FA) ou glutarique (GA) a abaissé de façon significative ($P < 0.01$) la solubilité de la protéine au NaOH à 0.02 N, ainsi que le dégagement de NH_3 en rumen artificiel. Dix veaux Holstein de race pure ou croisés avec la Brune des Alpes (Suisse brune), ont reçu pendant 14 semaines deux rations expérimentales contenant, à raison de 26%, du tourteau de colza respectivement traité (5.6 g FA par 100 g de protéine) ou non à l'aldéhyde formique. Le niveau de l'azote uréique du plasma et du NH_3 du rumen a été significativement ($P < 0.01$) moindre chez les veaux nourris au tourteau traité que chez les veaux témoin au cours des 8 premières semaines de l'essai de croissance. On n'a noté aucune différence significative ($P > 0.05$) quant à la consommation de matière sèche, le gain quotidien ou l'indice de conversion. La concentration totale en acides gras volatils (AGV) en mmoles/100 ml était significativement plus forte ($P < 0.05$) dans le liquide du rumen des veaux témoin que de celui des veaux recevant le tourteau traité. Il n'y a pas eu de différence significative ($P > 0.05$) entre les deux groupes quant aux pourcentages molaires d'AGV. Dans l'essai de bilan azoté, la consommation de matière sèche n'a pas différé significativement ($P > 0.05$) mais le tourteau traité a entraîné une baisse significative de la digestibilité de la matière sèche et de la protéine brute. Les veaux nourris au tourteau traité ont manifesté un moindre taux d'excrétion d'azote urinaire mais on n'a constaté aucun effet significatif sur la consommation et la rétention azotées.

INTRODUCTION

Previous studies in ruminant nutrition have indicated that proteins of high solubility are rapidly hydrolyzed in the rumen and may result in elevated ruminal ammonia levels that the microflora cannot utilize efficiently for protein synthesis, and thus a sizeable portion of the dietary N is lost through ruminal absorption. Chemical modification of dietary protein can be brought about by treating with vegetable tannins (Leroy et al. 1965) or formaldehyde (FA) (Ferguson et al. 1967). Both these treatments have been shown to decrease the solubility of protein and increase its resistance to bacterial degradation. Ferguson et al. (1967) and Reis and Tunks (1969) reported that FA-treated casein was well utilized by sheep and resulted in a marked improvement in wool growth. Peter et al. (1971) also obtained similar results with FA-treated soybean meal (SBM).

Rapeseed meal (RSM) has become readily available as a protein supplement, and data are required on the solubility of rapeseed protein, which appears to be higher than that of soybean meal. Information is required as to how the solubility of the protein might be decreased and thus result in increased nitrogen retention. Rumen microorganisms could thus be forced to obtain a higher percentage of their nitrogen from dietary nonprotein nitrogen (NPN). The objectives of the present investigation were to measure the solubility of FA- or glutaraldehyde (GA)-treated RSM protein in 0.02 N NaOH solution and rumen fluid and to determine the effect of FA-treated RSM on nitrogen retention and growth of dairy calves.

EXPERIMENTAL PROCEDURES

In vitro Studies

TREATMENT WITH ALDEHYDES. Four different solutions of formaldehyde (FA-1, 2, 3, and 4%) and two of glutaraldehyde (GA-1.25 and 2.5% concentrations) were prepared by diluting formalin and GA solutions with distilled water. One hundred g of each of the commercial RSM, linseed meal (LSM), and SBM were placed in six small containers with lids and mixed thoroughly with 200 ml of the various aldehyde solutions. The containers were closed and allowed to react for 1 hr at room temperature, then dried at 80 C in a forced-air oven. After drying, the meals were reground through a 1-mm screen and stored in plastic bags for analysis.

HEAT TREATMENT. One pound of RSM, LSM, and SBM were taken in 3 iron trays and heated at 180 C for 20 min. After cooling to room temperature, the meals were reground as indicated for the chemically treated meals. Untreated meals were also reground in a similar manner.

PROTEIN SOLUBILITY. One g each of the untreated, FA-treated (1–4% solution), GA-treated (1.25% solution), and heat-treated RSM were incubated according to Lyman et al. (1953) for 1 hr in 100 ml of 0.02 N NaOH solution at 37 C with frequent shakings. After incubation, the mixtures were centrifuged at 1500 $\times$ *g* for 10 min and the supernatant filtered to remove any floating particles. The filtrate was used for the determination of soluble protein by the Kjeldahl method. The above test was repeated four times on different dates.

Protein solubility in rumen fluid was determined by incubating 1 g of the untreated and FA-treated RSM in 100 ml of fresh rumen fluid for 1 hr. Procedures followed were then similar to those used above after incubation with NaOH. Rumen fluid for this test was obtained from a fistulated cow and strained through four layers of cheesecloth into a thermos flask. The fluid was bubbled with CO_2 for 1 min and kept in the incubator for 30 min to allow the particulate matter to rise. Nonparticulate rumen fluid was syphoned off into another beaker and used immediately for the protein solubility test.

Ammonium release was determined after a 24-hr in vitro fermentation. Two hundred mg of the untreated, heat-treated, or aldehydes-treated meals were incubated with 10 ml of the nonparticulate rumen fluid (obtained in the same manner as given above) for 24 hr at 37 C. The fermenting tubes were flushed with CO_2 before being put into the incubator. After the 24-hr fermentation, the volume was made up to 50 ml in the tubes by adding distilled water. Ammonia content was

measured according to Conway (1957). Observations were made on 4 different days.

In vivo Studies

In vitro ammonia release and solubility of protein indicated that a 1% FA solution was sufficiently effective in protecting the protein from bacterial degradation and decreasing the solubility of RSM protein. Therefore, one part of the commercial RSM was thoroughly mixed with 2 vol (w/v) of a 1% FA solution and allowed to react in a closed container for 1 hr. The mixture was dried at 80 C in a forced-air oven and reground through a 3-mm sieve in a Wiley mill and kept in burlap bags for mixing in the ration. Aliquots of the unground commercial RSM and reground FA-treated RSM were tested through standard sieves no. 10, 20, and 40 on a shaker for 10 min. Most of the material passed through the no. 10 sieve in both instances. About 14% of the untreated and 32% of the treated RSM were retained on sieve no. 20. Equal amounts of the meals passed through sieve no. 40.

GROWTH TRIAL. Reground FA-treated RSM and commercial RSM were used in feeding 10 dairy calves (Holstein and Holstein × Brown Swiss) of both sexes for 14 weeks. Calves were weaned from milk at 32 days of age and placed on experiment at 55–63 days of age. All calves were randomly distributed into two groups. Calves were housed in separate pens with feed and water freely available at all times. Animals were fed experimental rations (Table 1) at 10:30 AM. Rumen samples for ammonia and volatile fatty acids (VFA) were taken by stomach tube from each calf (3–4 hr after feeding) before putting the calves on test diets and at the end of 1, 2, 4, 6, and 8 weeks on test. Blood samples for urea N were also taken at the same time from the jugular vein in potassium oxalate tubes. Blood plasma and rumen fluid samples were stored at −20 C for further analyses.

Table 1. Composition of experimental rations

Item	Rations	
	RSM	FA-treated RSM
Ingredients (%)		
Rapeseed meal† (RSM)	26	–
Formaldehyde (FA) – treated RSM	–	26
Rolled barley	30	30
Corn starch	20	20
Barley straw	7.55	7.55
Ground hay	5.0	5.0
Corn oil	2.0	2.0
Cane molasses	8.0	8.0
Trace mineral salt	0.5	0.5
Calcium carbonate	0.72	0.72
Vitamin A and D_3‡	0.20	0.20
Aurofac–50	0.03	0.03
Total	100.0	100.0
Crude protein	16.4	16.5
Acid detergent fiber	13.2	13.7

†Commercial rapeseed meal was supplied by Co-op Vegetable Oils Ltd. Altona, Manitoba.
‡Vitamin A 2740 IU/kg, vitamin D_3 274 IU/kg of final ration.

One of the animals from the FA-treated group bloated several times and was dropped from the experiment after 10 weeks.

NITROGEN BALANCE STUDIES. At the end of the growth trial, four male calves, two from each treatment, were used for nitrogen balance trials. A switch-back design using two animals and three periods resulted in six observations for each treatment. Each period consisted of a 14-day adjustment period and a 7-day collection period of total feces and urine. One-tenth of the feces and 200 ml of the acidified urine were taken from each calf daily and stored at −20 C. At the end of each period the samples were composited and subsampled for analyses.

Dry matter contents of the feed and feces samples were determined by drying at 70 C in a forced-air oven. Nitrogen contents in feed, feces, and urine samples were determined according to the Kjeldahl method and plasma urea nitrogen was measured according to Conway (1957). Statistical analyses were carried out according to Snedecor (1956) and Duncan (1955).

RESULTS

The solubility of RSM protein (Table 2) in 0.02 N NaOH solution was significantly ($P < 0.01$) decreased by the various levels of FA, 1.25% GA, and heat-treated RSM compared with untreated RSM. Incubation for 1 hr of untreated and FA-treated RSM in strained rumen fluid also indicated a marked depression in the solubility of protein.

The influence of heating at 180 C and the various levels of FA and GA solutions on ammonia production from the treated meals (RSM, LSM, and SBM) were tested in the artificial rumen. The results of the in vitro ammonia release (Table 3) indicated that treating the meals with heat or with aldehydes significantly decreased deamination as indicated by ammonia levels after incubation. There were significant ($P < 0.01$) differences among the meals as well as among treatments applied to them.

When RSM data alone were subjected to analysis of variance, heated and aldehyde-treated RSM resulted in significantly ($P < 0.01$) less ammonia production compared with that of untreated RSM. Duncan's (1955) test indicated that heating at 180 C or treating the RSM with 1.25 or 2.5% GA solutions and 1 or 2% FA solutions caused a similar reduction in ammonia release into the in vitro

Table 2. Solubility of rapeseed meal (RSM) protein in 0.02 N NaOH solution and in strained rumen fluid

Treatment	Solubility of protein (%)	
	NaOH	Rumen fluid
Rapeseed meal (control)	64.7±1.4	30.3±0.5
1% FA†-treated‡ RSM	5.4±0.1	0.0
2% FA-treated RSM	5.4±0.2	0.0
3% FA-treated RSM	4.7±0.2	0.0
4% FA-treated RSM	4.5±0.2	0.0
1.25% GA§-treated RSM	18.6±0.1	–
Heated RSM (180 C for 20 min)	11.5±0.3	–

†FA = formaldehyde — prepared from formalin (37% formaldehyde), Ingram and Bell Ltd., Winnipeg, Manitoba.
‡2 ml of FA or GA solution per g meal.
§GA = glutaraldehyde — prepared from a 25% glutaraldehyde solution, J. T. Baker Chemical Co., USA.

Table 3. Ammonia levels† (mg N/100 ml) found after incubating 200 mg of meals in the in vitro rumen for 24 hr

	Treatments							
Meals‡	Control	Heated	1.25% GA	2.5% GA	1% FA	2% FA	3% FA	4% FA
RSM	88.3±2.0	40.5±1.6	45.6±1.4	38.5±1.4	37.5±2.9	37.0±2.5	31.6±2.6	29.7±1.5
LSM	59.7±1.9	31.2±0.1	35.8±2.0	31.9±0.1	33.2±1.6	–	–	–
SBM	71.2±1.6	31.1±0.4	32.7±2.4	30.5±2.0	33.0±3.0	–	–	–

†Initial ammonia N level in rumen fluid = 34.0 mg/100 ml.
‡RSM rapeseed meal, LSM linseed meal, SBM soybean meal.

system. The 3 and 4% FA solutions resulted in a further decrease in ammonia release, such that no increase in ammonia level was noticed after fermentation for 24 hr. A 1% FA solution decreased the ammonia release from 88.3 to 37.5 mg N/100 ml showing a substantial influence on the microbial deamination and protecting the RSM protein from bacterial attack in the artificial rumen; therefore, in the in vivo experiment a 1% FA solution (2 v/w) was used for treating the commercial RSM.

In vivo Growth Experiment

Untreated (commercial) and 1% FA-treated RSM were used in rations for young dairy calves. One of the animals in the FA-treated group bloated several times after about 8 weeks on test and was dropped from the experiment at 10 weeks. Total dry matter intake was slightly higher (Table 4) in the treated group than the control, but the difference between the two was not significant ($P > 0.05$).

Daily gains in the untreated and FA-treated RSM groups were not significantly ($P > 0.05$) different. Feed conversion was slightly lower for calves receiving the treated RSM. However, this difference was not significant ($P > 0.05$).

Ammonia levels in the rumen fluid of the calves were significantly ($P < 0.01$) less for those animals receiving FA-treated RSM compared with the control animals (Table 5). During the 8-week sampling period, blood urea ranged from 4.6 to 9.0 mg and 3.5 to 6.2 mg per 100 ml of plasma for calves receiving the untreated and FA-treated RSM, respectively. Blood plasma urea N was significantly ($P < 0.01$) lower for calves receiving FA-treated RSM compared with those

Table 4. Effects of treating rapeseed meal with 1% formaldehyde solution on the growth and performance of dairy calves

	Ration		
Growth trial (14 weeks)	Control	Formaldehyde-treated	SE
No. calves†	5	4	
Initial wt (kg)	79.5	88.8	
Daily gain (kg)	0.87 *a*	0.87 *a*	±0.25
Total DM intake (kg)	311.3 *a*	344.0 *a*	±16.3
Feed/gain	3.7 *a*	4.1 *a*	±0.3

a Figures with similar letters are not significantly different ($P > 0.05$).
†One of the animals in FA-treated group bloated several times and was therefore dropped from the experiment.

Table 5. Rumen ammonia nitrogen (mg/100 ml), blood plasma urea nitrogen (mg/100 ml), and rumen volatile fatty acids (VFA) (mmoles/100 ml and molar %) of calves receiving treated and control rapeseed meal

	Weeks on exp						
	0	1	2	4	6	8	SE
Blood urea N							
Control	–	6.5	6.6	9.0	4.6	8.2	
Treated	5.6†	3.6	3.5	4.8	3.8	6.2	±1.0
Rumen NH_3-N							
Control	4.6	4.0	6.5	7.2	5.6	6.0	
Treated	6.0	0.6	2.4	2.2	1.0	1.3	±1.4
Rumen VFA's (mmoles/100 ml)							
Total VFA's							
Control	15.33	13.39	14.16	9.44	11.35	12.81	
Treated	11.95	10.51	7.91	9.92	10.60	10.79	±1.73
Propionic acid							
Control	5.85	5.34	4.22	3.11	4.64	4.38	
Treated	4.82	3.44	2.30	4.64	3.44	3.21	±0.75
VFA's molar percentages							
Acetic acid							
Control	47.82	49.08	45.02	50.38	50.31	51.48	
Treated	46.15	49.78	49.29	49.55	53.95	51.01	±2.44
Propionic acid							
Control	37.46	39.95	36.74	35.01	40.00	34.55	
Treated	37.84	38.24	30.02	33.16	32.21	34.19	±3.17
Butyric acid							
Control	8.29	8.85	11.46	8.77	6.20	9.57	
Treated	9.81	6.52	13.58	11.33	9.73	9.43	±1.71

†Based on three calves.

receiving untreated RSM. This indicates that the FA-treated RSM protein was at least partly protected from the microbial attack and bypassed the rumen as intact protein.

Total VFA's in rumen fluid were significantly ($P < 0.05$) higher for calves receiving control RSM compared with those on FA-treated RSM. The concentration of propionic acid ranged from 3.11 to 5.85 and 2.30 to 4.82 mmoles per 100 ml of rumen fluid of the calves receiving the untreated and FA-treated RSM rations, respectively. Except for propionic acid, no significant differences ($P > 0.05$) were observed in the concentration or molar percent of VFA's (acetic, butyric, isobutyric, valeric, and isovaleric acid) between the control and FA-treated RSM groups. Rumen fluid concentration of the propionic acid was significantly higher for calves receiving the control ration compared with those receiving the FA-treated RSM.

Digestibility and Nitrogen Balance Trials

Digestibility of the dry matter in FA-treated RSM ration was significantly lower ($P < 0.05$) than that of the untreated RSM ration (Table 6). Apparent protein digestibility was 20.4% lower ($P < 0.01$) in the FA-treated RSM ration compared with the control ration. Dry matter and N consumption were slightly higher

Table 6. Influence of treating rapeseed meal with 1% formaldehyde solution on digestibilities of dry matter (DM), crude protein (CP), and nitrogen retention in young calves

Nitrogen balance trial (7 days)	Rations		
	Control	Formaldehyde-treated	SE
No. observations	6	6	
DM intake (kg)	36.9 *a*	39.4 *a*	±1.2
DM digestibility (%)	75.4 *a*	70.6 *b*	±1.3
N intake (g)	969.3 *a*	1043.5 *a*	±27.6
N in feces (g)	248.6 A	421.5 B	±18.8
N in urine (g)	408.4 A	257.9 B	±27.4
N retained (g)	312 *a*	364 *a*	±20
CP digestibility (%)	74.5 A	59.3 B	±2.1

a,*b* Figures with different letters are significantly different ($P < 0.05$).
A,B Figures with different letters are significantly different ($P < 0.01$).

in the treated group but did not differ significantly ($P > 0.05$) from the untreated RSM group. Excretion of N in the feces and urine was significantly different ($P < 0.01$) in the untreated and FA-treated RSM groups. Nitrogen excreted in the feces as a percent of N intake was 25.6 and 40.4 in the untreated and FA-treated RSM groups, respectively. Urinary N excretion was 42.1 and 24.7% of the N consumed in the untreated and treated RSM groups, respectively. The decrease in the urinary N ($P < 0.01$) and increase in fecal N ($P < 0.01$) with FA treatment resulted in no significant difference ($P > 0.05$) in the total N retained as a percent of N intake.

DISCUSSION

The solubility of RSM protein in NaOH was decreased from 64.7 to 5.4% by treating RSM with 1% FA (2 v/w). FA treatment (1, 2, 3, and 4%) reduced the solubility of protein to zero as measured after a 1-hr fermentation in rumen fluid. The present findings are in agreement with those of Peter et al. (1971) in which the protein solubility of SBM was depressed ($P < 0.01$) by treatment with FA, GA, and glyoxal. Ferguson et al. (1967) reduced the solubility of casein from 83 to 8% by treating the casein with 4% FA (10 v/w, or 40 g FA per 100 g casein).

Treating RSM, LSM, and SBM with heat or various levels of FA and GA solutions significantly ($P < 0.01$) decreased deamination in the artificial rumen as measured by ammonia levels after a 24-hr fermentation period. Zelter et al. (1970) obtained total inhibition of the bacterial deamination of peanut protein after treating with 0.6% formol and 1.5–1.8% glyoxal or GA. Peter et al. (1971) reported similar results. These studies indicate that the higher levels of FA and GA treatments resulted in only slightly greater inhibition of the bacterial degradation of treated RSM protein in the artificial rumen. This suggests that the lower levels of the aldehydes are enough to cause a maximum depression of the microbial degradation of the treated oil meals in in vitro studies.

No appreciable difference in weight gain was noticed in young calves fed on FA-treated or untreated RSM for a 14-week period. Feed intake, weight gain, and feed efficiency did not differ significantly ($P > 0.05$) for the two groups.

Mowat and Deelstra (1970) and Satter et al. (1970) reported that FA-treated SBM was less efficiently utilized for weight gain and feed efficiency, whereas Reis and Tunks (1969) and Peters et al. (1971) showed a substantial increase in body weight gain in sheep or growing lambs fed on rations containing either FA-treated casein (40 g FA/100 g) and treated SBM (.6 g FA or 1.5 g glyoxal/100 g of SBM). This discrepancy between the calves and lambs may be due to the species difference relative to amino acid requirements or method of treating the vegetable protein or casein, or both.

Ammonia levels in the rumen and plasma urea N were significantly depressed ($P < 0.01$) in the calves receiving FA-treated RSM compared with the control group. Similar results were obtained by Satter et al. (1970) in cows fed FA-treated SBM. Peter et al. (1971) reported that glyoxal-treated SBM lowered plasma urea N level ($P < 0.05$) as compared with lambs receiving the control (water-treated SBM). The data indicate that oil seed meal protein after treatment with aldehydes is protected from microbial degradation in the rumen of sheep and cattle.

Results of the present digestibility and N balance trials showed a significant decrease in apparent digestibilities of dry matter ($P < 0.05$) and crude protein ($P < 0.01$) in the FA-treated RSM (2 g FA/100 g RSM) group compared with the untreated RSM (control) group. Higher feed intake was noticed for the treated group during the growth studies and N balance trials, though the values were not significantly different ($P > 0.05$). Slightly lower digestibility of FA-treated casein was reported by Reis and Tunks (1969) in the sheep when it was included in the diet as compared with the untreated casein.

Larger amounts of N were observed in the feces of FA-treated RSM than the control group. An opposite trend was noticed in the excretion of urinary N. These results are in agreement with those Reis and Tunks (1969) obtained when sheep were fed FA-treated casein. The FA-treated RSM apparently became less susceptible to enzymatic digestion in the intestine, which resulted in more N excretion in the feces of the calves. However, Zelter et al. (1970) indicated that formaldehyde did not reduce the susceptibility of treated peanut meal (.6 g FA/100 g) to pepsin digestion in the in vitro system. In our experiment we obtained a positive N balance for the treated and untreated RSM group. The retention of N was slightly higher in the FA-treated RSM but the difference was not significant ($P > 0.05$). Reis and Tunks (1969) obtained a significantly higher N retention in sheep with the FA-treated casein than the untreated casein. This difference could be due to the higher digestibility of the casein as compared with the RSM protein.

The present studies indicate that different levels of formaldehyde and glutaraldehyde treatment of meal affects the microbial degradation of rapeseed meal protein.

Treating RSM with FA (5.6 g FA/100 g protein) reduced the rate of deamination in the rumen and reduced enzymatic digestion in the lower gastrointestinal tract. Further work is required to find a level of FA treatment that will slow deamination rate in the rumen but not reduce apparent protein digestion of RSM.

ACKNOWLEDGEMENTS

We thank Mrs. Y. Lewanski for technical assistance, the Manitoba Department of Agriculture and the National Research Council for financial assistance, and Co-op Vegetable Oils Ltd., Altona, Manitoba, for the supply of solvent extracted rapeseed meal.

LITERATURE CITED

CONWAY, E. D. 1957. Microdiffusion analysis and volumetric errors. 4th ed. London Crosby, Lockwood and Sons, Ltd., University Press, Glasgow. p. 98–100, 162–165.

DUNCAN, D. B. 1955. Multiple range and multiple F tests. Biometrics **11**: 42.

FERGUSON, K. A., HEMSLEY, J. A. and REIS, P. F. 1967. Nutrition and wool growth. The effect of protecting dietary protein from microbial degradation in the rumen. Aust. J. Sci. **30**: 215–217.

LEROY, F., ZELTER, S. Z. and FRANCOIS, A. C. 1965. Protection of proteins in feeds against deamination by bacteria in the rumen. Abstr. Rev. **35**: 444.

LYMAN, C. M., CHANGE, W. Y. and COUCH, J. R. 1953. Evaluation of protein quality in cotton seed meal by chick growth and by a chemical index method. J. Nutr. **49**: 679–690.

MOWAT, D. N. and DEELSTRA, K. 1970. Encapsulated methionine supplements for lambs. J. Anim. Sci. **31**: 1041 (Abstr.).

PETER, A. P., HATFIELD, E. E., OWENS, F. N. and GARRIGUS, U. S. 1971. The effect of aldehyde treatments of soybean meal on *in vitro* ammonia release, solubility and lamb performance. J. Nutr. **101**: 605–612.

REIS, P. J. and TUNKS, D. A. 1969. Evaluation of formaldehyde treated casein for wool growth and nitrogen retention. Aust. J. Agr. Res. **20**: 775–781.

SATTER, L. D., BROOK, G. P. and SCHWALE, C. G. 1970. Effect of formaldehyde treated soybean oil meal on performance of growing lambs and lactating cows. J. Dairy Sci. **53**: 668 (Abstr.).

SNEDECOR, G. W. 1956. Statistical methods. 5th ed. The Iowa State University Press, Ames, Iowa.

ZELTER, S. Z., LEROY, F. and TISSIER, J. P. 1970. Protection of proteins in the feed against bacterial deamination in the rumen. I. Studies *in vitro*: Behaviour in the rumen of some proteins tanned with tannin from the chestnut wood or certain aldehydes (formaldehydes, glutaraldehyde, glyoxal). Ann. Biol. Anim. Biochem. Biophys. **10**: 111–122.

COMPARATIVE VALUE OF SOYBEAN, RAPESEED AND FORMALDEHYDE TREATED RAPESEED MEALS IN UREA CONTAINING CALF RATIONS

H. R. Sharma and J. R. Ingalls
Department of Animal Science
University of Manitoba
Winnipeg, Manitoba,
Canada

A B S T R A C T

Twenty four Holstein calves were fed three experimental rations containing either 14% soybean meal, 20% rapeseed meal or 20% formaldehyde treated (0.7 g formaldehyde per 100 g protein) rapeseed meal from 8 to 22 weeks of age. No significant differences ($P>0.05$) were observed in feed consumption, dry matter intake, live weight gain and feed conversion among the three groups. There was a significant ($P<0.05$) reduction in rumen ammonia levels in calves receiving the treated rapeseed meal compared with rapeseed meal rations. Plasma urea levels and rumen fluid volatile fatty acids (m moles per 100 ml and molar percent) did not differ significantly ($P>0.05$) among treatments. There was no significant difference in the digestibilities of dry matter, nitrogen, acid detergent fibre and gross energy among treatments, although treating the rapeseed meal with formaldehyde tended to reduce dry matter, nitrogen, fibre and energy digestibility.

INTRODUCTION

The quantity of rapeseed meal (RSM) used in ruminant ration has been limited to 10%. A limited amount of research has been conducted with ruminants on digestibility, acceptability, feed efficiency and comparative value of RSM as compared with other commonly used high protein meals. Clark and Bezeau, as cited by Whiting (1965), indicated that 6% RSM in place of linseed meal in the diet of young Holstein calves had no effect on feed intake or growth rate. Ingalls and Seale (1971) reported no significant ($P>0.05$) effect on feed intake, weight gain or feed efficiency of heifers up to breeding weights by replacing soybean meal (SBM) with 0, 6.8 or 13.7% RSM in the calf starter rations. Ingalls et. al. (1968) indicated that rations containing 12% RSM or 6% RSM + 0.8% urea decreased grain consumption ($P<0.05$) in dairy cows when compared with SBM ration.

The protein moiety in calf starter rations of early weaned calves is expensive. Protection of this protein from microbial degradation could be brought about by formaldehyde treatment (Ferguson et. al., 1967), thus, allowing more protein to be digested in the lower gut. This should force the rumen microbiota to use the non-protein nitrogen (urea) that is present as a source of nitrogen for the synthesis of microbial protein and spare the vegetable protein for digestion and absorption in the lower gastrointestinal tract.

The present experiment was undertaken to study the

effect of substituting SBM protein by RSM or formaldehyde treated rapeseed meal (FA-RSM) in the ration of young dairy calves when urea supplied about 30% of the total crude protein on feed intake, live weight gain, feed conversion, digestibility and some of the rumen parameters.

MATERIALS AND METHODS

Previous studies from this laboratory indicated that formaldehyde when used at 5.6 g per 100 g protein caused a marked depression on the crude protein digestibility. In vitro studies suggested that 0.7 g formaldehyde per 100 g protein would reduce protein solubility. Therefore, this lower level of formaldehyde was used in order to obtain protection and may not affect the digestibility of dry matter or crude protein.

Formaldehyde treatment of RSM

Formaldehyde solution was prepared by diluting 676 ml of 37% formaldehyde solution in 4 litres of distilled water. This solution was sprayed onto 100 kg of RSM in a horizontal mixer and thoroughly mixed for 30 minutes. This provided 0.7 g of formaldehyde per 100 g of protein. After treating with formaldehyde the FA-RSM was stored in burlap bags lined with plastic. Three to four days later the FA-RSM was mixed with other ingredients.

Growth trial

The nutritive value of commercial RSM and FA-RSM was compared with SBM by feeding isonitrogenous rations to

Holstein dairy calves. About 60% of the crude protein of the ration was provided by SBM, RSM or FA-RSM and 30% by urea, (Table 1). Twenty-four Holstein dairy calves of both sexes were weaned from milk at 35 days of age and equal numbers of female (six) and male (two) calves were placed on each treatment at the age of 7 to 9 weeks. The experiment was started with a small number of calves which were randomly assigned to the three test diets (Table 1) for 14 weeks. The remaining calves were randomly alloted to the three groups as available from the University dairy herd. Calves were housed in individual pens with feed and water available free choice.

Rumen samples were taken by stomach tube for ammonia and volatile fatty acids (VFA'S) analysis and jugular blood samples for urea N analyses were collected and stored as previously indicated (Sharma et al., 1972). Bi-weekly body weights and daily feed consumption were recorded during the growth trial.

Digestibility trial

Chromium oxide (0.3%) was mixed in the test rations as a marker for determining apparent digestibility of dry matter, nitrogen, acid detergent fibre (ADF) and energy. Six calves from each group were used for the digestibility studies. After 10 weeks on the test diets, the pens were cleaned and fecal samples were collected at 10 a.m. and 5 p.m. from the floor for five days. During the collection

period fecal samples were stored at -20C for each animal in plastic bags. After thawing to room temperature fecal samples for each animal were mixed and subsamples were dried in a forced-air oven at 70C and then ground in a Wiley mill. The digestibility coefficients for dry matter, nitrogen, ADF and energy were determined by using the feed: feces ratio of Cr_2O_3.

Dry matter content in the feed samples was determined by drying at 70C in a forced-air oven. Nitrogen in the feed and feces was estimated according to the Kjeldahl method (AOAC, 1965). ADF was determined according to Goering and Van Soest (1970). Chromium oxide in feed and feces was measured by atomic absorption spectrophotometer according to Williams et al., (1962). Rumen fluid ammonia was estimated according to Conway (1957) and VFA'S according to Erwin et al., (1961) by gas liquid-chromatography. Plasma urea N was measured by an autoanalyser using a standard urea solution as reference (Marsh et al., 1965). Gross energy values of the feed and feces were determined by adiabatic oxygen bomb calorimeter. Data were analysed by analysis of variance using a completely randomized design according to Snedecor (1956) and mean differences were tested according to Duncan's multiple range test (1955).

RESULTS AND DISCUSSIONS

Calves receiving the SBM and FA-RSM gained slightly but not significantly ($P>0.05$) more than the commercial RSM group

(Table 2). Feed consumption was slightly but not significantly (P>0.05) lower in the SBM group compared to that of RSM and FA-RSM groups. Ingalls and Seale (1971) reported that 0, 6.8 and 13.7% RSM in the ration of dairy heifers did not significantly (P>0.05) affect feed consumption whereas Ingalls and Waldern (1972) reported that a starter-grower ration containing 30% RSM resulted in reduced daily feed intake and weight gain compared with a 20% RSM ration. Sharma et al. (1972), with a limited number of calves observed no significant difference (P>0.05) in the dry matter intake or weight gain when 26% of either RSM or FA-RSM were fed in the calf starters. Stake et al. (1972) stated that dry matter intake was significantly lower (P<0.01) for RSM vs sunflower meal (SFM) or SBM when fed to dairy calves from birth to 8 weeks of age when RSM made up 26% of the ration but they observed no significant difference in average daily gain or feed efficiency from birth to 14 weeks. In the present studies the feed conversion was not significantly (P>0.05) poorer for calves receiving the untreated RSM compared to those receiving SBM or FA-RSM (Table 2).

Hughes and Williams (1971) fed rations containing untreated and formaldehyde (FA) treated ground nut meal to lambs for 123 days. With 11, 13 and 15% protein rations, weight gains were not significantly (P>0.05) different between the treated and untreated ground nut meal rations. However, there appeared to be a weight gain depression for lambs

receiving the 15% protein ration containing FA-treated rather than untreated ground nut meal. Faichney and Davies (1972) observed a significantly ($P<0.05$) lower intake of dry matter of a FA-peanut meal ration compared with an untreated peanut meal ration. When a high protein (20%) ration was fed FA treatment of peanut meal had no effect on live weight gain or dry matter conversion. However, there were small improvements in live weight gain (2.5%) and dry matter conversion (4.5%) when FA-treated meal was fed in a lower protein (13%) ration.

The levels of urea N in blood plasma of calves after one week on the test diets was significantly ($P<0.05$) lower in the SBM group as compared with the RSM and FA-RSM fed calves. Later on the plasma urea levels tended to be lower in the FA-RSM calves than those receiving the SBM and RSM rations(Table 3), however, the differences were not significant ($P>0.05$). Nimrick et al. (1972) observed a significant decrease ($P<0.05$) in plasma urea N levels in lambs fed glyoxal treated fishmeal and SBM compared to the untreated groups. Sharma et al. (1972) reported a significant ($P<0.01$) decrease in the plasma urea N in calves receiving FA-RSM compared to the untreated RSM ration containing 16% crude protein. Faichney and Davies (1972) observed no significant ($P>0.05$) decrease in the plasma urea of calves fed FA-treated peanut meal at lower protein levels (13%) but at a higher protein level (20%)the reduction was significant ($P<0.05$). In the

present work the level of formaldehyde (0.7 g/100 g protein) used for treating the RSM was comparatively lower than that used by Sharma et al. (1972), (5.6 g formaldehyde/100 g protein) and Faichney and Davies (1972), (.5 g formaldehyde/ 100 g dry meal).

Ammonia levels in the rumen of FA-RSM calves were significantly ($P<0.05$) lower than for ~~SBM and~~ RSM fed calves (Table 3). Sharma et al. (1972) observed a significant ($P<0.01$) reduction in rumen ammonia level of calves fed FA-RSM compared with an untreated RSM diet. The present studies are in agreement with those of Sharma et al. (1972), in which FA-treatment partially protected the RSM from degradation in the rumen or reduced the rate of ammonia formation.

Although RSM is more soluble (Sharma et al., 1972) than SBM, the presence of 30% of the N as urea in a RSM ration appeared to result in animal performance similar to a SBM ration with 30% of the N as urea. These data would suggest that urea can be used to furnish part of the crude protein requirement in a RSM ration as well as in a SBM ration.

No significant ($P>0.05$) difference was noted in the concentration (m moles per 100 ml) of total volatile fatty acids and molar percentages of the VFA'S (acetic, propionic, butyric, isobutyric and valeric acids) in the rumen fluid of calves receiving SBM, RSM and FA-RSM diets (Table 3). Similar results were reported by Sharma et al. (1972) when RSM and FA-treated RSM were fed to young dairy calves.

There were no significant (P>0.05) differences in the digestion coefficients of dry matter, nitrogen, acid detergent fibre and gross energy between the SBM, RSM and FA-RSM diets (Table 4). The digestibilities of dry matter, nitrogen, ADF and energy tended to be higher for the RSM diet compared to FA-RSM diet. Stake et al. (1972) reported no significant difference (P>0.05) between protein digestibility of SFM, RSM and SBM diets. Also dry matter and energy digestibilities of RSM and SBM diets were not different (P>0.05). Wood and Stone (1970) observed no significant differences at maintenance levels of intake between basal, rape-basal and soy-basal diets or at levels of intake for growth. However, there was a tendency for the crude protein coefficient of digestibility of the rape-basal diet but not the soy-basal diet, to be lower at maintenance than at the growth level of intake. Faichney and Davies (1972) indicated that the digestibilities of dry matter and organic matter were similar for FA-treated and untreated diets containing 13% and 20% crude protein. FA-treatment was associated with a reduction of about 7 units in protein digestibility for both the 13% and 20% dietary protein levels. In the present work, FA tended to lower the digestibilities of dry matter, nitrogen, ADF and energy as compared with the RSM diet but these differences were not significant (P>0.05). In a previous trial higher levels of formaldehyde treatment (Sharma et al., 1972) reduced crude protein digestibility with little effect on nitrogen balance.

The present studies indicated that inclusion of RSM at 20% of the ration of calves (8 weeks to 22 weeks of age) did not affect the dry matter intake, daily gain or feed efficiency when compared with a SBM diet. Digestibility of dry matter, nitrogen, ADF and energy was similar for rations containing SBM and RSM. Inclusion of FA-RSM in calf ration did not appear to influence animal performance. The level of formaldehyde used was very low, however, even at this level there was a trend of lower ration digestibility. The present study suggested that urea can be used to furnish part of the crude protein requirement of a calf ration when RSM is used as a protein supplement in place of SBM.

ACKNOWLEDGEMENTS

The authors wish to thank the Manitoba Department of Agriculture and the National Research Council of Canada for financial assistance, Co-op Vegetable Oil Ltd., Altona, Manitoba for the supply of solvent extracted rapeseed and soybean meals. Acknowledgement is due also to Mr. G. Hamilton and Mrs. Y. Lewanski for technical assistance.

LITERATURE CITED

AOAC. 1965. Official methods of analysis. 10th ed. Assoc. offic. Agr. Chem. Washington, D. C.

CONWAY, E. J. 1957. Microdiffusion analysis and volumetric errors. 4th Ed. London Crosby, Lockwood and Sons, Ltd. University Press, Glasgow p. 98-100, 162-165.

ERWIN, E. S., MARCO, G. J. AND EMERY, E. M. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci., 44:1768-1771.

FAICHNEY, G. J. AND DAVIES, H. L. 1972. The effect of formaldehyde treatment of peanut meal in concentrate diets on the performance of calves. Aust. J. Agric. Res. 23:167-175.

FERGUSON, K. A., HEMSLEY, J. A. AND REIS, P. J. 1967. Nutrition and wool growth. The effect of protecting dietary protein from microbial degradation in the rumen. Aust. J. Sci. 30:215-217.

GOERING, H. K. AND VAN SOEST, P. J. 1970. Forage fibre analysis (apparatus reagents, procedures and some applications). Agriculture Handbook No. 379. p 8-9. U. S. D. A.

HUGHES, J. G. AND WILLIAMS, G. L. 1971. The Utilization of formaldehyde-treated ground nut meal by sheep. Proc. of the 52nd Meeting of the Brit. Soc. of Anim. Prod. London, (April 5-7th).

INGALLS, J. R. AND SEALE, M. E. 1971. Effect of continuous feeding of rapeseed meal on growth of dairy calves and subsequent first lactation yield. Can. J. Anim. Sci. 51:681-686.

INGALLS, J. R., SEALE, M. E. AND McKIRDY, J. A. 1968. Effect of rapeseed meal and urea on ad libitum consumption of grain rations by dairy cows. Can. J. Anim. Sci. 43:437-442.

INGALLS, J. R. AND WALDERN, D. E. 1972. Rapeseed meal in rations for dairy cattle, Canadian rapeseed meal in poultry and animal feeding. Publi. No. 16:24-27.

MARSH, W. H., FINGERHUT, B. AND MILLER, H. 1965. Modified procedure for determination of blood urea nitrogen. Technicon method No. AA11-1. Clinical Chem. 11:624-627.

NIMRICK, K., PETER, A. P. AND HATFIELD, E. E. 1972. Aldehyde-treated fish and soybean meals as dietary supplements for growing lambs. J. Anim. Sci. 34:488-490.

SHARMA, H. R., INGALLS, J. R. AND McKIRDY, J. A. 1972. Nutritive value of formaldehyde-treated rapeseed meal for dairy calves. Can. J. Anim. Sci. 52:363-371.

SNEDECOR, G. W. 1956. Statistical methods. 5th ed. The Iowa State University Press, Ames. IOWA.

STAKE, P. E., OWENS, M. J., AND SCHINGOETHE, D. J. 1972. Evaluation of three protein supplements for dairy calf starter meals. J. Dairy Sci. 55:704 (Abstract).

WHITING, F. 1965. Feeding value of rapeseed meal for ruminant animals. In Rapeseed meal for livestock and poultry Ed. J. P. Bowland, D. R. Clandinin and L. R. Wetter. CDA Publ. 1257, p. 61-67. Queen's Printer, Ottawa.

WILLIAMS, C. H., DAVID, D. J. AND IISMAA, O. 1962. The determination of chromic oxide in feces samples by atomic absorption spectrophotometery. J. Agri. Sci. 59:381-385.

WOOD, A. S. AND STONE, J. B. 1970. Digestibility, nitrogen retention and caloric value of rapeseed and soybean meals when fed at two dietary levels to calves. Can. J. Anim. Sci. 50:507-512.

TABLE 1. Ingredient and chemical composition of experimental rations

	SBM	RSM	FA-RSM
	(kg)	(kg)	(kg)
SBM*	14	--	--
RSM*	--	20	--
FARSM†	--	--	20
Corn starch	41	41	41
Ground barley	11	5	5
Ground alfalfa hay	5	5	5
Ground barley straw	15	15	15
Cane molasses	6	6	6
Tallow	2.4	2.4	2.4
Trace mineralized salt	3	3	3
Calcium Phosphate	1	1	1
Urea (281% CP)	1.4	1.4	1.4
Premix‡	0.2	0.2	0.2
Total (Kg)	100.0	100.0	100.0
Analysis of Rations (DM basis)			
Crude protein (%)	13.8	13.9	13.8
Acid detergent fibre (%)	12.0	14.0	14.1
Energy (k cals /kg.)	4021	4070	4048

* SBM and RSM were supplied by Co-op Vegetable Oils Ltd., Altona, Manitoba, Canada.

† Formaldehyde treated Rapeseed meal (.7g per 100g of protein)

‡ Vitamin A 220,500 IU, Vitamin D_3 22,050 I.U., Vitamin E 22,050 IU and Aurofac-50, 30.21g per 100 kg of feed.

Table 2. Effect of feeding SBM, RSM and FA-RSM on body weights, feed consumption, dry matter intake and feed efficiency of young dairy calves from about 8-22 weeks of age

Parameter	Rations			
	SBM	RSM	FA-RSM	S. E.†
No. of calves	8	8	8	
Av. gain in wt. (kg)	90.6	87.5	91.8	± 6.8
Av. daily gain (kg)	0.92	0.89	0.94	
Av. DM intake (kg)	307.9	332.9	325.3	±23.0
Av. daily DM intake (kg)	3.14	3.39	3.32	± .24
Av. DM intake per 100 kg body wt.	2.86	2.67	2.87	± .08
Dry matter intake/gain	3.42	3.83	3.60	± .16

† SE = standard error of the treatments mean.

Table 3. Effect of feeding SBM, RSM and FA-RSM on blood plasma urea nitrogen, rumen ammonia nitrogen and rumen VFA's in young dairy calves

	Weeks on test					
	0	1	2	4	8	Treatment mean
Blood Urea N (mg/100 ml)						
SBM	4.3	3.8[b]	5.3	5.4	5.2	4.80
RSM	4.4	7.4[a]	4.5	4.9	4.3	5.11
FA-RSM	4.4	6.0[ab]	3.4	3.3	4.0	4.22
						$S\bar{X}$† ± 0.32
Rumen NH_3-N (mg/100 ml)						
SBM	9.6	7.3	7.1	6.8	4.1	7.34[ab]
RSM	8.3	11.2	11.1	12.3	6.8	9.94[a]
FA-RSM	8.1	7.9	5.8	6.7	4.7	6.63[b]
						$S\bar{X}$ ± 0.93
Rumen VFA's (m moles/100 ml)						
SBM	12.1	8.9	9.6	10.5	12.6	11.3
RSM	12.3	8.7	9.2	10.3	11.2	10.3
FA-RSM	13.3	6.9	9.6	10.8	12.5	10.6
						$S\bar{X}$ ± 0.6
Rumen VFA's molar percentages (%)						
Acetic acid						
SBM	46.4	56.5	52.1	54.0	50.9	52.0
RSM	48.1	55.4	54.5	52.4	49.1	51.9
FA-RSM	45.4	53.2	51.9	52.9	50.5	50.8
						$S\bar{X}$ + 0.8
Propionic acid						
SBM	39.2	33.5	35.7	36.8	39.0	35.6
RSM	37.9	33.6	33.8	37.1	39.1	36.3
FA-RSM	39.1	36.4	38.7	38.9	39.6	38.6
						$S\bar{X}$ ± 1.1
Butyric acid						
SBM	8.0	9.4	9.1	7.8	7.2	8.3
RSM	9.0	7.4	7.3	7.6	8.6	8.0
FA-RSM	10.5	7.1	6.2	5.8	6.2	7.1
						$S\bar{X}$ ± 0.6

ab - Means not sharing the same superscript are significantly different ($P<0.05$)

† $S\bar{X}$ - Standard error of the treatment means

Table 4. Apparent digestibilities of dry matter, nitrogen, acid detergent fibre and energy of SBM, RSM and FA-RSM rations fed to the growing dairy calves

Ration	Dry matter	Nitrogen	Acid detergent fiber	Energy
	%	%	%	%
SBM	69.2	66.7	9.1	68.3
RSM	69.7	68.9	8.6	69.4
FA-RSM	66.3	63.3	3.4	66.3
$S\bar{X}$†	±1.4	±2.3	±2.9	±1.6

† $S\bar{X}$ - Standard error of the treatment means.

APPENDIX 3

TABLE 1

DATA ON SOME OF THE PARAMETERS MEASURED ON FISTULATED YOUNG HOLSTEIN STEERS FED EXPERIMENTAL DIETS (EXPT. III)

Animals	Treatments			
	RSM	FA-RSM	CASEIN	FA-CASEIN
	DM Intake per Day (Kg)			
1	7.1	5.3	5.5	5.5
2	6.6	5.4	5.6	7.7
3	5.5	5.0	5.9	5.5
4	6.4	6.8	5.5	5.9
	Rumen Digesta DM (Kg)			
1	1.9	2.1	2.9	3.8
2	5.8	3.4	4.2	5.1
3	3.3	3.0	3.6	3.6
4	4.2	4.7	3.4	4.0
	Rumen Digesta DM (%)			
1	8.9	9.9	10.0	11.7
2	12.0	9.1	13.4	12.1
3	15.4	16.1	14.4	15.5
4	13.5	13.8	13.1	15.0
	Nitrogen in Rumen Contents (%)			
1	3.8	4.0	3.6	3.7
2	3.3	3.5	2.4	3.4
3	3.1	3.5	2.4	3.3
4	2.9	3.1	2.9	3.3
	Daily flow of Non-Ammonia-N in Rumen Contents (g)			
1	69.6	79.8	100.6	138.3
2	189.8	111.1	95.3	161.6
3	91.3	99.2	75.9	112.7
4	114.5	136.7	94.2	124.2
	NAN as a % of N intake in Rumen Digesta			
1	44.0	58.8	74.4	110.5
2	118.2	82.8	71.7	95.7
3	72.8	82.3	58.2	92.6
4	77.7	90.1	70.0	87.7

continued.....

APPENDIX 3 - TABLE 1 (continued)

Animals	Treatments			
	RSM	FA-RSM	CASEIN	FA-CASEIN
	Total N Consumption in 7 Days (g)			
1	1121.0	960.4	959.9	843.3
2	1078.0	951.5	887.2	1191.2
3	1024.7	808.2	850.2	905.6
4	995.1	1115.2	919.6	896.8
	N retained as a % of N Intake			
1	15.8	21.0	19.3	26.0
2	21.2	17.2	17.5	20.0
3	22.5	25.2	27.6	25.5
4	26.3	23.2	21.9	26.2
	Percentage Recovery of Cr_2O_3 in Feces			
1	92.2	97.8	107.2	101.4
2	102.8	100.8	93.8	107.5
3	83.1	97.4	100.2	91.6
4	102.9	92.3	107.1	107.3
	1-6, Diaminopimelic acid % Bacterial AA			
1	0.9	0.8	1.1	1.2
2	0.6	0.8	0.9	1.0
3	1.0	1.0	1.2	1.2
4	1.0	0.6	1.0	1.0
	N:DAP Ratio of Rumen Bacteria			
1	23.3	25.3	17.3	17.4
2	34.1	25.6	22.1	18.9
3	19.8	17.1	14.9	14.3
4	18.7	34.4	20.9	18.3

APPENDIX 3

TABLE 2

DAILY FLOW OF NUTRIENTS THROUGH G.I. TRACT OF FISTULATED HOLSTEIN STEERS (EXPT. III)

Animals	Treatments			
	RSM	FA-RSM	CASEIN	FA-CASEIN
	Abomasal Digesta DM (Kg)			
1	4.7	4.3	3.1	3.1
	4.4	4.1	3.4	3.6
2	4.4	3.5	3.2	4.7
	4.2	3.8	3.3	4.1
3	3.0	2.8	3.4	3.1
	3.0	2.9	3.4	3.2
4	3.5	3.9	2.8	3.4
	3.5	4.2	3.0	3.2
	Abomasal Digesta DM %			
1	5.5	6.0	6.5	7.9
	5.8	6.6	5.8	5.9
2	7.0	8.5	8.2	6.8
	7.8	7.3	8.1	8.5
3	7.4	9.3	9.0	8.0
	8.0	8.8	7.8	7.9
4	7.9	8.1	7.8	8.7
	8.5	7.8	7.0	7.9
	Nitrogen in Abomasal Digesta (%)			
1	3.5	3.4	3.2	4.5
	3.5	3.6	3.4	3.9
2	3.8	3.5	3.8	3.7
	3.7	3.5	3.5	4.2
3	3.6	3.7	3.1	3.6
	3.4	3.9	3.0	4.0
4	3.5	3.8	3.6	3.9
	3.6	3.7	3.7	4.1

continued.....

APPENDIX 3 - TABLE 2 (continued)

Animals	Treatments			
	RSM	FA-RSM	CASEIN	FA-CASEIN
	NAN as a % of N Intake in Abomasal Digesta			
1	93.9	96.7	68.2	110.4
	92.2	102.5	78.5	106.7
2	102.2	89.8	97.1	96.0
	95.1	97.1	83.1	99.4
3	81.1	85.3	74.8	91.8
	78.8	88.4	72.4	100.4
4	80.2	92.1	74.4	90.8
	81.1	100.7	79.3	89.2
	Bacterial N as a % of Abomasal Digesta N			
1	58.1	35.8	56.1	35.7
	68.0	36.2	72.9	39.7
2	68.1	84.3	62.9	38.1
	75.5	57.6	58.8	30.3
3	36.4	35.4	32.2	20.7
	42.9	35.7	28.4	21.5
4	42.5	68.9	45.5	47.0
	39.9	80.9	35.8	39.6
	Bacterial Protein/100 g Abomasal Digesta DM			
1	25.4	12.6	14.5	12.9
	31.1	17.7	25.5	17.4
2	32.0	34.9	23.4	14.1
	30.1	29.2	18.2	9.2
3	10.1	11.2	8.3	6.6
	11.4	12.1	7.0	7.3
4	12.2	23.0	10.9	11.2
	11.1	33.7	9.9	12.1

APPENDIX 3

TABLE 3

TOTAL AMINO ACIDS (%DM) IN THE FEED AND VARIOUS SEGMENTS OF THE G.I. TRACT FOR FISTULATED HOLSTEIN STEERS (EXPT. III)

Animals	Treatments			
	RSM	FA-RSM	CASEIN	FA-CASEIN
	Total AA[1] in feed (% DM)			
1	8.14	7.01	10.18	7.35
2	7.22	8.04	8.37	8.33
3	7.78	8.18	9.02	8.00
4	7.81	7.77	8.11	8.07
	Total AA in rumen contents (% DM)			
1	17.91	19.07	16.03	17.99
2	16.39	17.19	11.17	18.36
3	14.10	13.84	11.03	18.16
4	13.50	14.30	13.76	18.77
	Total AA[1] in ruminal bacteria (% DM)			
1	35.29	24.46	27.92	22.56
2	24.43	30.34	41.21	37.78
3	43.67	36.48	41.79	35.96
4	44.52	37.58	45.29	42.16
	Total AA in abomasal digesta (% DM)			
1	16.68	16.25	13.93	16.85
	16.14	16.07	14.48	19.33
2	19.14	17.04	19.16	18.70
	18.14	16.31	15.87	22.50
3	16.55	17.48	14.13	18.03
	15.24	20.15	13.38	21.58
4	16.71	17.40	16.55	20.66
	18.02	17.64	17.60	21.34

1 AA - Amino acids (g) per 100 g dry matter.

continued.....

APPENDIX 3 - TABLE 3 (continued)

Animals	Treatments			
	RSM	FA-RSM	CASEIN	FA-CASEIN
	DAP[1] in abomasal digesta			
1	0.52	0.41	0.74	0.55
	0.64	0.37	0.97	0.46
2	0.40	0.68	0.56	0.40
	0.45	0.48	0.59	0.30
3	0.40	0.44	0.47	0.29
	0.49	0.40	0.43	0.28
4	0.47	0.44	0.47	0.49
	0.42	0.50	0.36	0.41
	NAN in ileal digesta as a % of N intake			
1	33.6	37.0	41.8	33.2
	30.4	37.0	48.0	37.9
2	34.1	39.9	37.7	34.8
	42.0	52.1	29.1	38.3
3	38.3	34.1	26.9	29.7
	30.0	29.6	23.0	30.5
4	30.8	31.1	21 0	24.3
	26.8	31.3	23.7	22.5
	DAP[1] in ileal digesta			
1	2.07	1.35	1.72	1.44
2	1.13	1.40	1.39	1.10
3	1.15	1.34	1.58	1.52
4	1.88	2.06	2.48	2.01
	DAP[1] in fecal contents			
1	1.30	1.42	1.47	1.20
2	1.07	1.37	1.17	1.07
3	0.65	0.79	0.75	1.77
4	0.69	1.12	0.89	1.05

1 Diaminopimelic acid (g) per 100 g total amino acids.

APPENDIX 3

TABLE 4

AMOUNTS OF TOTAL AMINO ACIDS CONSUMED AND FLOWED THROUGH ABOMASUM OF HOLSTEIN STEERS (EXPT. III)

		RSM	FA-RSM	CASEIN	FA-CASEIN
		Amount of total Amino acids consumed/day			
25 - 70	1	578.7	398.2	559.9	425.5
	2	534.7	383.5	559.9	405.0
27 - 70	1	507.3	436.0	457.3	526.7
	2	507.3	436.8	465.5	636.3
47 - 71	1	423.1	401.0	532.4	414.9
	2	423.9	401.0	532.4	442.1
48 - 71	1	495.8	526.0	443.7	488.2
	2	495.8	510.5	443.7	477.6
		Amount of total amino acids passed through abomasum/day			
25 - 70	1	790.7	501.4	433.7	519.7
	2	714.8	571.8	497.9	687.6
27 - 70	1	833.6	602.2	637.8	870.0
	2	754.6	618.0	517.3	923.1
47 - 71	1	498.2	494.2	473.9	550.6
	2	459.7	576.6	448.8	688.4
48 - 71	1	589.6	685.5	468.5	706.9
	2	635.6	741.9	525.9	689.1

APPENDIX 3

TABLE 5

CHANGE OVER DESIGN AND TREATMENT SEQUENCE (EXPT. III)

Treatments/Periods	I	II	III	IV	I	II	III	IV
RSM			27	25			48	47
FA-RSM		27	25		47			48
CASEIN	27	25			48	47		
FA-CASEIN	25			27		48	47	