THE EFFECTS OF A NONIONIC SURFACTANT 'TWEEN 80' ON THE PERFORMANCE OF LACTATING DAIRY COWS FED A TOTAL MIXED RATION

by

JEFFREY DAVID BERKSHIRE

B.Sc., Nova Scotia Agricultural College, 1996

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

THE FACULTY OF AGRICULTURAL SCIENCES

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

May 1999

© Jeffrey David Berkshire, 1999

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Agricultural Sciences

The University of British Columbia
Vancouver, Canada

Date Nov 1, 1999

DE-6 (2/88)

ABSTRACT

The effects of a nonionic surfactant, Tween 80 (T-80), on the performance and nutrient digestibility of dairy cows fed a total mixed ration (TMR) were evaluated. Fifteen, multiparous Holstein cows in early lactation were randomly assigned to treatment groups in a replicated 3 x 3 Latin Square design involving three, 28 d experimental periods. Treatment groups were balanced in terms of days in milk and estimated 305 d milk production. Cows had *ad libitum* access to one of the following diets: 1) TMR consisting of 45% dairy concentrate, 33% grass silage and 22% corn silage (Control); 2) TMR treated with 0.1% T-80; and 3) TMR treated with 0.2% T-80; all on a DM basis. Individual feed intake and milk yield were recorded daily. Milk, blood, and rumen fluid samples were taken during the third week of each period. Total collection of urine and feces from twelve cows (four from each group) occurred during the last five days of each experimental period.

Average daily intake and milk yield was not influenced by level of T-80 in the TMR ($P>0.05$). Differences in milk protein percentage ($P<0.05$) with 0.2% T-80 having lower milk protein than the Control was compensated by higher milk yield when expressed as daily protein yield (kg d^{-1}). No differences were observed for fat, lactose or somatic cell count ($P>0.05$). Blood urea nitrogen was approaching significance ($P=0.08$) and implicative of 0.2% T-80 having positive effects on N-utilization over the Control. Cows consuming 0.2% T-80 had higher rumen levels of acetate (mol 100 mol^{-1}) ($P<0.05$) than cows consuming 0.1% T-80, however, neither treatment was different ($P>0.05$) from the Control. Observed differences in acetate molar percentages between treatments did not contribute to differences in molar percentages of volatile fatty acids within treatments.

The acetate to propionate ratio between T-80 treatments was approaching significance (P=0.08) with 0.2% T-80 having increased acetate production and 0.1% T-80 increased propionate production, however, neither treatment was different (P>0.05) from the Control. No differences (P>0.05) were observed in apparent digestibility estimates of dry matter, crude protein, acid-detergent (ADF) or neutral-detergent fiber (NDF). A P-value of 0.10 was suggestive of apparent NDF digestibility being lower in 0.2% T-80 treated cows, and when compared to ADF, indicative of decreased hemicellulose degradation. No treatment difference (P>0.05) was observed for efficiency of milk yield.

Further research is required before definite conclusions concerning the experimental treatment levels of T-80 are made. Future work to: 1) investigate pre-feeding aerobic nutrient losses from the TMR as a result of T-80 application; 2) confirm the effective level of T-80 in prepared diets; and 3) complete a detailed examination of the effects of T-80 on the rumen microbial population balance and acclimatization rates were suggested.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

AA	amino acid
ADF	acid-detergent fiber
ADIN	acid-detergent insoluble nitrogen
ANIN%CP	acid-detergent insoluble nitrogen expressed as a percentage of crude protein
ATP	adenosine triphosphate
BUN	blood urea nitrogen
BW	body weight
CMC	carboxymethylcellulose
CP	crude protein
d	day
DIM	days in milk
DIP	degradable intake protein
DM	dry matter
DMI	dry matter intake
FCM	fat corrected milk
h	hour
IVTD	*in vitro* true digestibility
NEL	net energy for lactation
min	minute
mM	millimolar
MY	milk yield
N	nitrogen

NDF	neutral-detergent fiber
NPN	non-protein nitrogen
NSC	nonstructural carbohydrate
RDP	rumen degradable protein
RUP	rumen undegradable protein
SC	structural carbohydrate
SCC	somatic cell count
SE	standard error
T-80	Tween 80, polyoxyethylene monooleate, polysorbate 80
TMR	total mixed ration
UIP	undegradable intake protein
v	volume
VFA	volatile fatty acid
w or wt	weight

ACKNOWLEDGEMENTS

I would like to thank Dr. Jim Shelford of the Faculty of Agricultural Sciences at UBC for his support and guidance throughout this academic pursuit. Your academic expertise and good will were a continuous source of motivation and inspiration. Thank you for believing in me. Thanks are also extended to Dr. John Baah and Nelson Dinn for the abundance of academic assistance provided and to Mary Riddols for her technical help in running the experiment. I am grateful to all the friends I have made at the UBC Dairy Education and Research Center in Agassiz, BC as well as the lab staff in the Faculty of Agricultural Sciences at UBC. The friendship and input from my colleagues at UBC has been appreciated.

Many individuals have supported me and contributed to my personal development leading to the completion of this project. Thanks are extended to Christia Roberts and Dr. Alan Fredeen for their professional advice and personal interest in my academic success. Deepest appreciation and admiration is extended to special friends including Rene Siguenza, Jane Peebles, Martin Payne, Lana Norman, Carter Kagume, Kevin Gillard, Robert Sampson, Donnie MacAualy and the members of Pride UBC and Victory for the roles you have played in my life.

Above all I am thankful to my parents, David and Mavis Berkshire and my sisters Lisa, Nancy and Julie for the completion of this project was made possible through their love, acceptance and support.

1. GENERAL INTRODUCTION

Difficulties encountered in ration formulation and increasing genetic potential for milk production place the lactating dairy cow in a situation of nutritional stress. A lactating cow in nutritional stress has difficulty meeting milk production potential and may suffer from decreased reproductive performance. To meet production and maintenance requirements, dairy cows need adequate protein and energy supplementation. Dietary energy is often limiting in the rumen environment, decreasing efficiency of nutrient utilization and subsequent microbial production and volatile fatty acid (VFA) synthesis. In the absence of adequate energy from carbohydrate sources, ruminally degradable protein (RDP) is deaminated to supply energy rather than being used for microbial protein synthesis (Nocek and Russell, 1988). The nonionic surfactant, Tween 80 (T-80), may help improve energy availability to ruminal microbial digestion by increasing fermentation of structural carbohydrates (SC). T-80's positive impact on the rate and extent of degradation of the primary SC cellulose in anaerobic bioreactors is documented (Helle et al., 1993; Castanon and Wilke, 1981). Preliminary applications in ruminant animals have shown positive responses (Baah, 1998; Shelford and Baah, 1998; Shelford and Baah, 1997, Shelford et al., 1996, Kamande, 1994), however, further research is required to evaluate T-80's function in the complicated metabolic processes of the dairy cow.

Rumen microorganisms metabolize dietary components to produce microbial protein and VFAs. To achieve maximal microbial metabolism, proteolysis of RDP and supply of energy from carbohydrates must be synchronized (Tamminga, 1992). However, rumen microbes are highly proteolytic (Orskov, 1992) and subsequently energy is often limiting. Grain concentrates are energy rich but due to economic and

physiological limitations, forages must be fed as well. Forages are necessary for normal rumen function and provide a more cost-efficient means of feeding dairy cattle. Although an abundant energy source, the contribution of SCs as found in forages is limited by slow rates of microbial fermentation. A means of more rapidly and completely enhancing energy release from forages in the rumen would be beneficial to increasing digestive efficiency in the dairy cow.

The energy supply of forages is limited by the degree to which cellulose and hemicellulose are combined with lignin. Increased lignification reduces microbial fermentation and release of potential energy. An abundance and diversity of rumen microorganisms is necessary to supply enzymes to degrade the multitude of complex fibrous dietary components (Van Soest, 1994). Microbial enzyme production, release and action of enzymes can be improved upon. Characteristics of the substrate itself influence nutrient supply. For example, the surface area of fiber particles available for degradation is related to enzymatic attack and fermentation rate. Fiber surfaces become available for attack through lesions caused by mechanical processing, mastication by the cow and some ruminal microbial (i.e., fungal penetration) degradation.

The nonionic surfactant T-80 has been used in anaerobic bioreactors to increase the rate and extent of cellulose degradation (Helle et al., 1993; Castanon and Wilke, 1981). When utilized in the ruminant, T-80 facilitated microbial degradation of both dietary protein (Shelford et al., 1996; Kamande, 1994) and carbohydrates (Shelford et al., 1996). Enhanced proteolysis is undesirable in the already highly proteolytic rumen environment. Kamande (1994) suggested that the positive effects of T-80 on cellulose degradation could be maximized and the negative effects on protein degradation

minimized at lower concentrations of T-80 in the diet, and this has been verified in several ruminant studies (Baah et al., 1998; Shelford & Baah, 1998; Shelford & Baah, 1997; Shelford et al., 1996). It is hypothesized that the positive milk yield (MY) response is related to improvements in fibrous carbohydrate fermentation leading to increased energy availability for microbial protein synthesis and MY.

2. REVIEW OF LITERATURE

2.1 Carbohydrates

Carbohydrates are the largest component in dairy cow diets and contribute 60 to 70% of the net energy used for milk production (Mertens, 1992). Rumen microorganisms use energy from carbohydrates to convert amino acids (AA) and ammonia from dietary protein to microbial protein, releasing VFAs as a byproduct of this fermentative process. VFAs absorbed by the cow are metabolized to provide energy for maintenance and synthesis of milk and tissues. The chemical composition, physical characteristics, and digestion properties of carbohydrates affect intake, digestion, and utilization of the total diet and availability to the udder of nutrients for milk synthesis (Van Soest, 1994). Carbohydrates can be classified into two major categories based on their: 1) function in plants; or 2) nutritional characteristics.

2.1.1 Function in Plants

2.1.1.1 Non-Structural Carbohydrates

Non-structural carbohydrates (NSCs) are those contributing to the storage of energy in plants, primarily in the form of starch and simple sugars (Mertens, 1992; Chesson and Forsberg, 1988). These carbohydrates are found abundantly in the grain fraction of forages, some leaves and stems and are readily fermentable upon entering into the rumen (Mertens, 1992). NSCs are the major carbohydrates found in phloem and mesophyll cells in grass and legume leaves, and the phloem and immature parenchyma of grass stems (Akin, 1989). Chesson and Forsberg (1988) reported that readily digestible parenchyma cell walls are heavily colonized by rumen bacteria and rapidly digested. The proportions of forage tissues vary due to plant species, stage of

growth and management factors (Akin, 1980). NSCs high degree of rumen solubility contribute to an abundance of readily available energy to be used by rumen microbes. As a supplement to the lactating dairy cows diet, concentrate NSCs are often more expensive than forages and thus they are only added to the diet as needed.

2.1.1.2 Structural Carbohydrates

SCs are those derived from the support mechanisms or cell walls of the plant or forage species (Van Soest, 1994). Pectin, hemicellulose, cellulose and lignin are the major components of these support mechanisms (Mertens, 1992). Minson (1990) provides an overview of forage components and their relative degree of digestibility. Anatomically, SCs are found in the bundle sheath, epidermal cells, vascular bundles, sclerenchyma and cuticle, in decreasing order of microbial digestibility, respectively (Akin, 1978). Unlike the NSCs that are all considered potentially digestible, only a fraction of the SCs are considered potentially digestible. In fact, only the bundle sheath, epidermal cells and part of the vascular bundles are classified as potentially digestible with the remaining fraction of the vascular bundles, sclerenchyma and silica/cuticle considered indigestible, even by rumen fermentative processes (Minson, 1990).

SCs are unique in that the energy and nutrients they contain are exclusively available to microorganisms through microbial fermentation processes (Cheng et al., 1990), and not susceptible to the digestive enzymes of monogastric species. However, the ruminant has the ability to utilize the byproducts of microbial fermentation in the rumen to help meet its energy and protein requirements. The fermentation of SCs by

ruminant microorganisms results in the transformation of energy to a form which can be utilized in the production of microbial protein and the by-product VFAs.

2.1.2 Nutritional Characteristics

Classification of carbohydrates based on structural function in plants allows the nutritionist to relate nutrient availability to plant structure. However, in terms of nutritional characteristics, classification into fiber or nonfiber fractions is a better expression of nutrient availability. Van Soest (1965) indicates how all plants consist of variously modified cells with two main components, the cell contents and the cell wall constituents. This de-emphasizes the importance placed upon chemical purity or relationship to plant characteristics, and focuses importance on nutritional availability.

2.1.2.1 Non-Fibrous Carbohydrates

Non-fibrous carbohydrates represent the rapidly digested fractions that include pectin's, starches and sugars (Mertens, 1992) and is most comparable to the aforementioned NSCs with the exception of the addition of pectin. This is the fraction referred to as the cell contents and includes organic acids, soluble carbohydrates, crude protein (CP), fats and soluble ash (Van Soest, 1965). The non-fiber carbohydrate fraction of the plant can be regarded as completely digestible, since in herbivores the cell solubles are either completely removed by enzymatic digestion or by fermentation (Prins and Kreulen, 1990).

2.1.2.2 Fibrous Carbohydrates

The fibrous carbohydrate fraction of the diet is similar to the SCs, representing components of feed that are indigestible or slowly digested, including hemicellulose, cellulose, lignin, cutin and silica while excluding pectin (Mertens, 1992; Van Soest, 1965). Pectin is excluded due to its high ruminal solubility. Fiber is noted for occupying space in the gut thus limiting potential intake, and also for its requirement for extensive mastication to assist in microbial fermentation. According to Prins and Kreulen (1990), the chief determinant of the indigestible fraction is the degree of lignification, which typically ranges from 5 to 25% of the cell wall material depending upon species and plant maturity.

2.1.3 Laboratory Fiber Analysis

2.1.3.1 Neutral-Detergent Fiber

There are two main fiber fractions evaluated in forage analysis based on nutritional characteristics. Neutral- detergent fiber (NDF) is the fiber fraction of the diet containing cellulose, hemicellulose and lignin and is indicative of the fibrous fraction available to microbial digestion (plus the unavailable lignin fraction). The residue also contains minor cell wall components, including some protein and bound N, minerals, and cuticle (Van Soest, 1994). Digestible NDF is unique because it is the only digestible component in feed that cannot be digested by the cows enzymes, but must be digested by microbial enzymes (Mertens, 1992). In the ruminant, NDF is negatively correlated with intake, however, a minimum quantity of NDF is required by the ruminant in order to stimulate normal rumen function (Mertens, 1992, 1980, 1982, 1985a, 1985b; Varga and Hoover, 1983). Thus, diets of high producing dairy cows should seek to

attain NDF content in the lower ranges specified in NRC requirements (NRC, 1989) of 25 to 28% NDF on a dry matter (DM) basis. NDF is negatively correlated with digestibility, however, not to the extent of acid-detergent fiber (ADF). According to Van Soest (1994), intake is more relevant to animal production than is digestibility and therefore NDF is often considered in ration preparation and balancing.

2.1.3.2 Acid-Detergent Fiber

The second fiber fraction is ADF which recovers only cellulose and lignin, however, does not recover hemicellulose as does NDF (Van Soest, 1994). ADF is used as a quick method for estimating the fiber in feeds, it is suggested to be a better indicator of apparent digestibility than NDF (Mertens, 1985a) and like NDF it is negatively correlated with intake but not as representative (Mertens, 1985a; Van Soest, 1994). Like NDF, diets of high producing dairy cows should seek to attain ADF content in the lower ranges specified in NRC requirements (NRC, 1989) of 19 to 21% ADF (DM basis) as not to limit intake of high energy and protein dietary inputs thus restricting potential milk yield and quality.

2.1.4 Cellulose, Hemicellulose, Pectin and Lignin as Components of Carbohydrates

The four major dietary components of the plant cell wall are cellulose, hemicellulose, pectin and lignin (Mertens, 1992; Van Soest, 1965). Silica and cutin are components of the plant cell wall but not considered as actual carbohydrates (Van Soest, 1994). Physiologically these fractions are unique and vary in their degree of

utilization by ruminant microbes. Akin et al. (1974) reported that various plant tissues differ in their rate and extent of degradability by rumen bacteria.

Cellulose is the most abundant carbohydrate and amounts to 20 to 40% of the DM of all higher plants (Van Soest, 1994). It is the major component of monocot and dicot cell wall types (Chesson and Forsberg, 1988). All structural celluloses are combined to some degree with lignin, hemicellulose, cutin and minerals, granting them both potentially digestible and indigestible fractions (Prins and Kreulen, 1990; Dinsdale et al., 1978; Dehority and Johnson, 1961). The nutritional availability of cellulose ranges from 0 to 100% depending on fermentation time and its integration with lignin (Van Soest, 1994). In terms of solubility, cellulose is considered hydrophobic or insoluble in water (Lamed and Bayer, 1988).

Hemicellulose is a mixture of polysaccharides that varies greatly from one plant species to the next and makes up from 37 to 48% of plant cell walls (Chesson and Forsberg, 1988). It consists of heterogeneous polymers of a number of five- and six-carbon sugars (pentoses and hexoses, respectively), together with some uronic acids (Prins and Kreulen, 1990). Many of the cellulolytic strains of bacteria hydrolyze xylans, but as with most starch and cellulose hydrolysers, they will ferment only the disaccharide, xylobiose, and not the monosaccharide, xylose, from the polysaccharide (Hobson, 1976). Hemicellulose is generally less resistant to hydrolysis than cellulose (Chesson and Forsberg, 1988) and abundant production of xylanases by rumen bacteria contribute to their increased degradability (Chesson and Forsberg, 1988). Wherever the fermentation of cellulose is referred to, hemicelluloses are implicated as well, since the latter occur in intimate association with cellulose in the plant cell wall (Van Soest, 1994; Woolcock, 1991; Prins and Kreulen, 1990). Van Soest (1994)

indicates how ruminants digest about equal amounts of both cellulose and hemicellulose, but a substantial portion of hemicellulose escapes the rumen to be fermented in the lower tract. This could be due to the inability of xylan (of hemicellulose) to be digested until arabinosyl side chains are removed or perhaps the removal of a cellulosic protective layer (Francis et al., 1978).

Pectin occurs in the middle lamella and other cell wall layers (Van Soest, 1994). Although its presence in the cell wall classifies it as a SC, it is completely available to fermentation and considered as a soluble carbohydrate in terms of its nutritive characteristics (Van Soest, 1994). Van Soest (1994) indicated that rumen microorganisms usually fermented pectin faster than starch.

Lignin is the most indigestible of the SCs and is often associated with cellulose and hemicellulose, potentially reducing their susceptibility to microbial fermentation to almost zero (Van Soest, 1994). Sclerenchyma and xylem were the tissues most consistently indicative of lignin as reported by Akin (1989). Lignin is thought to arise from enzymatic polymerization of the primary precursors of sinapyl, coniferyl, and p-coumaryl alcohols (Akin, 1980). Lignin is considered to be one of the main factors limiting digestibility in ruminant diets (Van Soest, 1994). Structural characteristics that limit forage breakdown are primarily highly lignified support tissues like sclerenchyma and xylem (Akin, 1989). In most cases lignin was considered to be more closely associated with hemicellulose than cellulose (Van Soest, 1994).

Silica and cutin are not carbohydrates but their association within the cell wall contributes to variation in digestibility estimates that may naively be attributed to degree of lignification (Van Soest, 1994). Silica is a mineral found in the soil that becomes incorporated into the plant, and its natural abundance and impact on digestibility

warrant its presence in this review. Although associated with the cell wall, very little silica in plants is actually present in the structural components of the cell wall (McManus et al., 1977) but rather as soluble silica in the vicinity of the cell wall. It is the presence of soluble silica that depresses organic matter digestibility (Smith and Nelson, 1975). Van Soest (1994) reported that *in vitro* studies have provided direct evidence that silica reduces cell wall digestibility, however, the mode of action by which this operates is unresolved. Shimojo and Goto (1989) hypothesized that the depressing effect of soluble silica appears to be associated with a direct effect on the activity of the enzymes involved in forage digestion and is not due to any protection of the cell wall.

More structurally interrelated to lignin than silica, cutin is the major nonphenolic fraction of crude lignin, which is separated from crude lignin as the fraction resistant to oxidation (Meara, 1955). The proportions of cutin and lignin vary in different plant species and among tissues of the same plant. The cuticular surface of leaves offers a barrier to digestion and is the main indigestible fraction of leafy nonlignified vegetable tissues (Van Soest, 1994).

2.1.4.1 The Significance of Cellulose

Cellulose is a SC and of variable nutritional availability depending on the degree to which it is combined with other compounds such as lignin or hemicellulose (Van Soest, 1994). Cellulose is an abundant, renewable resource. It is formed in plants *via* photosynthetic processes, trapping the energy of the sun and combining it with organic compounds full of potential energy. Human beings lack the digestive enzymes necessary to release the potential energy and nutrients of cellulose. Humans also lack the capacity to host fiber degrading microorganisms. However, ruminant animals have

the unique ability to utilize cellulosic compounds. This is achieved through microbial fermentation in the rumen, coupled with the buffering capacity, rumination, natural turning and end product removal of the ruminal environment. The capacity for ruminants to digest cellulose grants them the ability to utilize a renewable energy source otherwise unavailable to humans and convert it into meat and dairy products.

2.1.4.2 Cellulose Structure

In its most simplistic molecular structure, cellulose is a repeating sequence of cellobiose units. Cellobiose [4-O-(β-D-glucopyranosyl)-D-glucopyranose] is a simple disaccharide often referred to as D-glucose (Lamed and Bayer, 1988) or β-1,4 linked glucose (Chesson and Forsberg, 1988). The cellulose molecule is a linear polymer consisting of up to 10^4 D-glucose molecules (Lamed and Bayer, 1988). To achieve this, repeating cellobiose units form linear chains called fibrils. Fibrils are insoluble, hydrophobic and more resistant to chemical and enzymatic attack than the glucan chains from which they were formed (Krassig, 1985). Fibril chains lie parallel to each other, and are extensively cross-linked by hydrogen bonding (Helle et al., 1993; Chesson and Forsberg, 1988) granting the cellulose its enzymatic resistance (Lamed and Bayer, 1988).

Cellulose is classified according to the complexity within and between fibrils (Chesson and Forsberg, 1988) into two main forms. That which is bound more tightly and with a higher degree of structural complexity is termed "crystalline" cellulose and accounts for over 75% of all cellulose (Lamed and Bayer, 1988; Cowling and Kirk, 1976) formed. Cellulose that is bound less tightly and with less complicated structure is called "amorphous" cellulose. The complexity of the order of the cellulose molecule

contributes to its digestibility and enzymatic resistance (Dinsdale et al., 1978; Chesson, 1981), with amorphous cellulose more readily fermented by ruminal microbes (Bertran and Dale, 1985) followed by more extensive fermentation to degrade crystalline forms (Huang, 1975; Ladisch et al., 1983; Ljungdahl, 1989). It was concluded that the two structural features considered most important in the enzymatic hydrolysis of cellulose are surface area and degree of crystallinity (Walker and Wilson, 1991), however, Helle et al. (1993) indicated crystallinity is a less important variable than previously perceived to be.

2.1.4.3 Enzymes of Cellulose Hydrolysis

The enzymatic hydrolysis of cellulose is a heterogeneous reaction system involving water-soluble, particulate, or cell-bound enzymes attacking a water-insoluble substrate (Lamed and Bayer, 1988) and is modeled after the fungal cellulase system (Wood, 1985; Mendels and Reese, 1964). Unique to cellulose hydrolysis is the synergism existing between endoglucanase and cellobiohydrolase bringing about the solubilization of crystalline cellulose (Wood & McCrae, 1979). Many sources often refer to the enzyme "cellulase" or the "cellulase complex" when referring to cellulose degradation (Abrha and Gashe, 1992; Oguntimein, 1991; Forsberg et al., 1981; Lamed and Bayer, 1988; Groleau and Forsberg, 1981), however, this enzyme does not exist as a single entity. In fact, the enzymatic hydrolysis of crystalline cellulose requires the simultaneous action of three main enzymes (Bernier and Stutzenberger, 1989; Chesson and Forsberg, 1988; Lamed and Bayer, 1988; Mullings, 1985), coupled with end-product removal as to prevent inhibition of the enzymes involved. Each of the enzymes noted below come in slightly different forms. Enzyme variation in molecular

weight arises from differences in the conditions of the bacterial cultivation from which they were derived, and also from simplification of the complex terminology. Despite minor differences in molecular weights and names, all enzymes of the cellulase system function to achieve the same outcome. For example, Beldman et al. (1988) identified six distinct endoglucanases, three distinct cellobiohydrolases and β-glucosidase from a commercial preparation of *T. viride* cellulase. Lamed and Bayer (1988) reviewed the contributing factors to the overall ease in which a particular enzyme system acts on cellulose.

Chesson and Forsberg (1988) provided an overview of enzymatic cellulose hydrolysis. Cellulose degradation begins when the enzyme endoglucanase (endo-1,4-β-glucanase, endo-1,4-β-D-glucan 4-glucanohydrolase, endocellulase) initiates the reaction by attacking the readily fermentable, amorphous cellulose from the more complex SC structures (White et al., 1993; Lamed and Bayer, 1988; Castanon and Wilke, 1980). This process called "amorphogenesis" involves the breaking of the hydrogen bonds that hold the cellulose fibrils together (Coughlan, 1991). Endoglucanase is inefficient at attacking crystalline cellulose, however, its attack on amorphous cellulose achieves a reduction in cellulose chain length and exposes free chain ends (Walker and Wilson, 1991; Henrissat et al., 1985; Lamed and Bayer, 1988). Amorphous cellulose is found in two main forms, either "phosphoric acid swollen cellulose" or "carboxymethylcellulose" (CMC), both of which are derivatives of cellulose and soluble in water (Minato and Suto, 1978). If endoglucanase acts upon CMC, the products formed are cello-oligosaccharides or "oligomers" (Ladisch et al., 1983; Wood, 1985) having varying degrees of solubility. Depending on the extent of degradation and their resulting solubility, cello-oligosaccharides either proceed to be acted upon by

cellobiohydrolase or β-glucosidase, for the water-soluble and insoluble, respectively. According to Coughlan and Ljungdahl (1988), endoglucanase breaks the cellulose into insoluble eight-sugar pieces that are then degraded to cellobiose.

This initial reaction exposes free chain ends of the more structured crystalline cellulose to the attack of the second major cellulosic enzyme, cellobiohydrolase (also called 1,4-β-D-glucan cellobiohydrolase, exoglucanase, endocellulase) (Walker and Wilson, 1991; Chesson and Forsberg, 1988). Cellobiohydrolase takes over the reaction, acting upon numerous available substrates including the cellulose altered by endoglucanase, "acid swollen" cellulose, the water-soluble cello-oligosaccharides and finally, Avicel (a highly crystalline cellulose with a low degree of polymerization) (Walker and Wilson, 1991). Cellobiohydrolase does not attack CMC. The enzymatic degradation by cellobiohydrolase of the above compounds results in cellobiose units being cleaved from the non-reducing end of the cellulose chain.

The third and final enzyme of cellulose hydrolysis is β-glucosidase (also called cellobiase). Acting on cellobiose and the soluble cello-oligosaccharides, β-glucosidase degrades these substances to glucose. β-glucosidase prevents end-product inhibition of the various forms of cellulose by the degradation of cellobiose, a potent inhibitor of many cellulolytic systems (Lamed and Bayer, 1988; Wood, 1985). Glucose, the end product of cellulose hydrolysis, is then available for microbial utilization in the assimilation and maintenance of new and existing microorganisms.

2.1.5 Nutritional Contribution of Structural Carbohydrates (Fiber) to the Cow

The major dietary contribution of the SCs is energy. Microbial enzymes act upon the complex fibrous structures converting their energy into organic products including VFAs, lactic acid and ethanol (Van Soest, 1994). As a result of this oxidation more microbial cells are produced. To obtain these final end products, the SCs are first hydrolyzed to glucose by microbial enzymes (i.e. the cellulolytic process described in 1.3.3). This energy is made available for assimilation into microbial cells. Energy from VFAs is available to the host ruminant once absorbed from the rumen and is then available for aerobic oxidation. The contribution of fiber towards promoting rumen function should not be overlooked (Van Soest, 1994).

2.1.5.1 Contribution to Volatile Fatty Acid Production

VFAs are the end products of anaerobic microbial fermentation (Latham et al., 1977) and provide the ruminant with a major source of metabolizable energy. VFAs are growth factors for many cellulolytic organisms, and other species use them for long-chain fatty acid synthesis, and sometimes, for AA synthesis (Van Soest, 1994). The main VFAs produced include acetic, propionic, butyric, isobutyric, valeric and isovaleric acids (Van Soest, 1994). Acetic, propionic and butyric are the three most abundant and influential on ruminant production. In general, acetate and butyrate are the major energy sources for oxidation, and propionate is reserved for gluconeogenesis in the liver (Van Soest, 1994). Under optimal conditions for ruminal fermentation, increased microbial metabolic rates contributed to increasing total VFA production.

2.1.5.1.1 Diet and VFA Production

The ratio of VFAs produced is dependent upon the carbohydrate sources available for microbial fermentation. Rapidly fermenting, NSCs such as starch promote the production of propionic acid (Mertens, 1992; Bachman, 1992) where as slowly degrading, fibrous or SCs stimulate acetic and butyric acid production (Mertens, 1992). Thus, inadequate roughage relative to fermentable carbohydrates within a diet result in a decrease in microbial production of the milk fat precursors, acetic and butyric acids (Bachman, 1992). When rates of starch degradation in the rumen are too fast, pH decreases rapidly and acidosis and milk fat depressions occur. If too slow, reductions in microbial digestion and protein synthesis occur with resultant decreases in MY and protein content (Bachman, 1992). Most VFAs absorbed by cows are metabolized to provide energy for maintenance and synthesis of milk and tissues (Mertens, 1992).

The proportions of the VFAs can be highly influenced by diet and microbial status within the rumen (Van Soest, 1994). They are not constant and are influenced by microbial fermentation balance, the escape of nutrients from the rumen, and endogenous production (particularly acetate) by body tissues (Mertens, 1992). Mertens (1992) summarizes the relationship of the two major VFAs, propionic and acetic acid, to milk components. Simply put, propionic acid from rapidly fermenting NSCs provides the precursors for glucose synthesis that in turn are used by the mammary gland to produce lactose. Lactose production is positively correlated with milk yield. The fermentation of compounds in SCs or fibrous carbohydrates leads to higher levels of acetate and butyrate production. Increased levels of acetate are indicative of higher fat percentages in milk and increased efficiency of fiber utilization. Acetic acid from the

slowly fermenting carbohydrates is needed for approximately 50% of milk fat that is produced.

2.1.5.1.2 The Acetate to Propionate Ratio

Van Soest (1994) discussed the contribution of VFAs in regard to the relative efficiency of adenosine triphosphate (ATP) production. Although this has yet to be determined in mixed microbial populations, studies using individual microorganisms grown in pure culture provided good estimates. The synthesis of one mole of acetate is thought to produce two ATPs, whereas one mole of propionate synthesized *via* the succinate route or butyrate formation each produces three ATPs per mole, respectively (Van Soest, 1994). This suggests how an increased ratio of propionate, and to a lesser extent butyrate (by virtue of its relative proportion to propionate and acetate) contribute to increased efficiency of milk production in the ruminant. Propionate's function as a precursor to gluconeogenesis in the liver followed by lactose production in the mammary gland (Mertens, 1992) also contributed to increased efficiency of milk production in the ruminant.

Propionate's contribution to milk production and the resulting efficiency of production do not acknowledge the physiological necessity and significance of acetate production. Physiologically, forages are necessary to promote optimal rumen function and their fermentation yields both acetic and butyric acids. The moles of individual VFAs fermented from one mole of glucose by a mixed rumen population were provided by Isaacson et al. (1975). At a dilution (turnover) rate of 0.02 h^{-1}, 1.18, 0.16 and 0.23 moles of acetate, propionate and butyrate, respectively, were formed from one mole of glucose. Combining this data with the ATP production estimates of Van Soest (1994),

yields of 2.36, 0.48 and 0.69 moles of ATP per mole of glucose metabolized for acetate, propionate and butyrate, respectively. Baldwin (1970) described how an increase in the A : P ratio from 1 : 1 to 3 : 1 demonstrated an increase in digestive efficiency as less methane was produced relative to glucose input. Methane is an energy loss in the fermentative process (Van Soest, 1994). Thus, a ration optimally balanced for concentrate and forage components could attain greater efficiency of digestion and milk production by maximizing acetate production from the SC fraction of the ration. Decreased A : P ratio's were not necessarily indicative of increased efficiency of milk yield if the forage to concentrate ratio was such that normal rumen function was jeopardized. Bachman (1992) discussed the relationship between dietary factors and ruminal responses of pH and volatile fatty acid concentrations.

Due to the relative proportions of acetic and propionic acids in the rumen and their significance to milk composition and energy balance, they are often expressed as a ratio of each other as a means of simplifying this relationship. Acetate production is positively correlated with fiber digestion (SCs) and increased levels of fat in the milk, where as propionate production is positively correlated with soluble carbohydrate digestion (i.e. starch) and increases in milk protein, lactose and total yield (Van Soest, 1994; Mertens, 1992; Bachman, 1992). With increases in ATP formation, propionate has been related to increased efficiency of milk production (Van Soest, 1994). According to Mertens (1992), if acetic acid production is low during ruminal fermentation relative to propionic acid, availability of milk fat precursors and energy to the udder is reduced and milk fat production is decreased. If too little propionic acid is produced, milk lactose synthesis and energy from glucose are reduced, and milk production is inhibited. Bachman (1992) exemplified, stating barley-based concentrates relative to

corn-based concentrates tend to reduce fiber digestibility with a resultant decrease in acetate to propionate ratio. Thus, higher ruminal degradability of barley promotes propionic acid production and thereby reduction in milk fat percentage (Bachman, 1992), particularly when propionic acid exceeds 25 molar percent (Bachman, 1992). Low acetate to propionate ratios may modify metabolism of lactating cows and result in a shift from milk production to body fattening.

2.1.5.2 Contribution of Structural Carbohydrates to Microbial Crude Protein

The net metabolizable protein received by the animal is the sum of the true digestible microbial protein and the feed protein that escapes the rumen (Van Soest, 1994). Microbial yield or production determines the microbial protein available to the animal. Microorganisms are able to reproduce by utilizing nitrogen (N) (primarily in the form of ammonia) and energy sources derived from carbohydrates for their maintenance and reproduction needs. Crucial to the success of this reproduction is for N and energy sources to be available simultaneously. Compared to the SCs, the fermentable NSCs tend to be highly adept at meeting the microbial energy demands for the readily available N sources in the diets of most high-producing dairy cattle. The SCs are also energy rich, but their energy is not as available and thus their contribution does not parallel that of the NSCs at least in terms of rate of energy production.

The energy contribution of SCs should not be underestimated. High quality forages containing SCs with a minimal degree of lignification are energy rich and have high rates of microbial fermentation. Quality forages are only limited by the rate of microbial enzymatic reaction, microbial enzyme availability and available fermentation time before passage rate out of the rumen. A means of optimizing SC utilization would

serve to make SCs a more significant contribution to total energy supply in the ruminant fermentation process. Greater yield of ATP from acetate per mole of glucose in relation to propionate and butyrate emphasize the energy contribution of SCs and the potential for increased microbial protein production with improved SC degradation.

2.2 Rumen Microbes

It is well known that rumen microbes facilitate the anaerobic degradation or fermentation of the fibrous carbohydrates (Akin, 1989) including cellulose, hemicellulose and other substances resistant to digestion by enzymes secreted by the host animal (Minson, 1990; Groleau and Forsberg, 1981; Akin, 1989). They provide the enzymes necessary to degrade the respective fractions, and in turn reproduce themselves creating microbial protein that can be digested as it passes to the lower digestive tract. There are three main types of rumen microbes contributing to the fermentation process, these are bacteria, protozoa and fungi (Minson, 1990; Akin, 1989; Chesson and Forsberg, 1988; Bauchop, 1981; Groleau and Forsberg, 1981; Clarke, 1977; Hungate, 1966). They colonize plant materials that enter the rumen, with the exception of intact, outer plant surfaces that reportedly are not colonized by any microbe (Bauchop, 1980).

2.2.1 Microbial Contribution to Fiber Fermentation

2.2.1.1 Bacteria

In comparison to ruminant protozoa and fungi, bacteria make up the most prominent and active fiber-digesting microbial group in the rumen under most conditions (Windham and Akin, 1984). Analysis of the factors affecting adhesion of bacteria to

cellulose revealed that temperature and ionic strength were important, whereas pH had less effect (Gong and Forsberg, 1989). Although less critical than temperature or ionic strength in adhesion, pH is influential on microbial metabolic activity. To achieve optimal cellulolytic digestion, a pH of 6.7 (± 0.5 pH units) is required and deviations on either side of this are inhibitory (Van Soest, 1994). In particular, pH below 6.2 inhibits the rate of digestion and increases lag and the cellulolytic, hemicellulolytic, and pectinolytic organisms are all inhibited under these circumstances (Grant and Mertens, 1992). Mertens concurred (1992) indicating how poor digestion of fiber occurs because fiber-digesting bacteria do not grow and compete for nutrients effectively when pH is low. Van Soest (1994) acknowledged that not all inhibition of cellulose digestion in animals on high-grain diets is the result of low pH, however, and substrate composition resulting in microbial competition is also of importance.

Bacteria became attached to cell walls in the initial step of the degradation process (Akin, 1986; Cheng et al., 1983/84), however, the degree of this attachment was positively correlated with the thickness of the plant cell walls in question (Akin, 1978; Akin et al., 1974). Morris and Cole (1987) referred to the significance of this attachment due to the number of cellulolytic enzymes that must act in combination. The three major cellulolytic bacteria species are *Ruminococcus albus, Ruminococcus flavefaciens and Fibrobacter succinogenes* (Van der Linden et al., 1984; Cheng et al., 1983/4, Latham et al., 1977; Hungate 1966) which are commonly found on damaged cell surfaces (Akin, 1986; Latham et al., 1978a). All combined, they accounted for over 70% of all fiber digesting bacteria (Akin, 1980). Akin and Rigsby (1985) reported that *R. flavefaciens* degraded more forage than did other cultures, followed by *Butyrivibrio fibrisolvens* (a hemicellulolytic species) and then *R. albus*.

2.2.1.1.1 *Ruminococcus albus*

R. albus is one of the most important cellulolytic rumen bacteria, and has been shown to adhere to cellulose fibers (Patterson et al., 1975; Minato and Suto, 1978) and contribute greater than 20% of the total endoglucanase in the rumen (Leatherwood, 1965). Morris and Cole (1987) confirmed *R. albus'* contribution to CMCase production. *R. albus* appeared to be loosely associated with cell walls (Forsberg et al., 1981; Cheng et al., 1983/4; Akin, 1978), primarily degrading the more easily digested tissues. Morris (1988) investigated factors influencing the adhesion of *R. albus* to cellulose and found that pH changes between 5.5 and 8.0 had little effect but below 5.0 there was a significant decrease in adhesion. Optimal adherence was noted at approximately pH 5.5. Patterson et al. (1975) showed that extracellular fibrils from cells of pure culture of *R. albus* were involved with attachment of the bacteria to cellulose. Morris (1988) studied the characteristics of the adhesion of *R. albus* to cellulose. It was suggested that the lack of inhibition of potassium thiocyanate on *R. albus*, which is known to disrupt hydrophobic bonds, confirms that hydrophobic interactions are not involved in attachment (Morris and Cole, 1987).

2.2.1.1.2 *Ruminococcus flavefaciens*

Ruminococcus flavefaciens contributes both endo- and exoglucanase to cellulose hydrolysis. Akin (1978) found that *R. flavefaciens* degraded more forage than did other rumen microbe cultures. This was attributed to a capsule which held the enzymes and substrate in close proximity (Akin and Rigsby, 1985). *R. flavefaciens* appeared to be loosely associated with cell walls (Forsberg et al., 1981; Cheng et al., 1983/4), attacking the more rapidly degrading cellulosic tissues without attachment.

2.2.1.1.3 *Fibrobacter succinogenes*

Fibrobacter succinogenes, formerly known as *Bacteroides succinogenes*, is noted for its high contribution of endoglucanase and β-glucosidase to cellulose hydrolysis (Groleau & Forsberg, 1981) and xylanase for hemicellulose degradation (Forsberg et al, 1981). Latham et al. (1977) observed *F. succinogenes* in mixed culture with *R. flavefaciens*, and noted that *F. succinogenes* predominated on the cut edges and on the uncut surfaces of the mesophyll cell walls, and rapid digestion of epidermal, mesophyll and phloem cell walls was noted in both bacterial species. *F. succinogenes* exhibits a tight attachment, frequently conforming to the surface of the material being digested (Cheng et al., 1983/4; Forsberg et al., 1981). Observations *of F. succinogenes* led Gong and Forsberg (1989) to deduce that adhesion to crystalline cellulose is specific and that it may involve surface proteins. *F. succinogenes* was reported to be an efficient degrader of the more resistant cellulose (Dinsdale et al., 1978) They also suggested that growth of *F. succinogenes* on different forms of cellulose required different degrees of adhesion affinity. Using transmission electron microscopy, Latham et al. (1977) observed extremely fine fibers originating at the cell surface of *F. succinogenes* that both attached it to the substrate and adjacent organisms. It was concluded that the ability of *F. succinogenes* to attach to, and potentially migrate over, undamaged surfaces must contribute significantly to its superior cellulolytic activity. Dehority and Scott (1967) reported that *F. succinogenes* digested significantly more cellulose from intact forages than other cellulolytic rumen bacteria.

2.2.1.2 Protozoa

The contribution of ruminant protozoa remains controversial (Akin, 1993). It is well known that rumen protozoa ingest plant tissues (Amos and Akin, 1978; Bauchop, 1979a) however they are not essential to fiber degradation (Hobson, 1976; Hungate, 1988) and have been shown to have negative effects on ruminant duodenal protein supply (Jouany and Ushida, 1990). Their large size relative to the bacteria contribute to them playing a large part in total rumen microbial mass (Akin, 1989; Olubobokun et al., 1983), and estimates of as much as 50% of rumen microbial mass have been suggested (Woolcock, 1991). It is well known that they colonize and degrade cellulose (Jouany and Ushida, 1990; Akin and Amos, 1979), although to a lesser extent than bacteria (Bryant, 1973). Like rumen bacteria, rumen protozoa first attack plant fragments and then degrade them to varying degrees. It has been reported by Yoder et al (1976) that ruminant protozoa contribute up to seven percent of total cellulolytic degradation. As noted, their role in digestion is controversial. They have been considered to be like "miniature ruminants", harboring cellulolytic bacteria within their cell walls, however, the benefits of this cellulose degradation for the host ruminant are minimal (Hobson, 1976). Also, the slow rate of degradation (positively correlated with their larger size) suggests that they provide few substrates to the host ruminant. Jouany and Ushida (1990) acknowledged the positive effects of active utilization of lactic acid by protozoa and how this helps prevents pH fluctuations in the rumen of faunated animals receiving carbohydrate rich diets (i.e., high starch). They concluded that protozoa (*Entodiniomorphid sp.*) have positive action on cellulose and hemicellulose degradation in the rumen.

2.2.1.3 Fungi

Aerobic fungi provide the basis upon which the model for enzymatic hydrolysis has been modeled (Wood, 1985; Mendels and Reese, 1964) although this is not a perfect representation of the *anaerobic* bacterial cellulolytic enzyme system (Ljungdahl, 1989). Similar to ruminal bacteria and protozoa, ruminal fungi are significant fiber degraders of unlignified plant tissues. (Akin, 1989; Akin et al., 1983; Bauchop, 1979; Bauchop and Mountfort, 1981; Orpin et al., 1979). They are slower acting, but more complete in their cellulosic degradation than either the bacteria or protozoa of the rumen environment and can be of importance under high fiber dietary conditions (Fonty et al., 1990; Bauchop, 1979; Orpin and Letcher, 1979). Akin and Rigsby (1987) suggested that ruminant fungi, in the absence of bacteria, were potentially able to degrade quantitatively almost as much fiber as the whole population, however, the tissues degraded (e.g. lignocellulose in sclerenchyma) were different from that degraded by bacteria. Given adequate time, fungi can attack the most resilient and lignified fibers of the SCs, cleaving their bonds and making them readily available to potential digestion by bacteria and protozoa (McAllister, 1994; Akin, 1989). Fonty et al. (1990) indicated that the main fermentation products of rumen fungi described to date included hydrogen, lactate, formate, ethanol and acetate as major products and sometimes succinate as a minor product. Akin (1989) indicated that fungi are better able to colonize the lignocellulosic tissues and can partially degrade the most resistant xylem tissues. This contributed to reducing fiber retention time in the rumen and potentially increasing ruminant intake (Fonty et al., 1990; Akin et al, 1983). Fungi do not digest lignin, but appear to have little difficulty breaking its complex bonds.

2.2.2 Microbial Attachment

There are practical implications of microbial attachment in the rumen. It is the first step in the digestion of insoluble nutrients (McAllister et al., 1994). The constant movement of the rumen contents dictates that microorganisms must attach or be adjacent to their dietary substrates in order to present their enzymes to the substrate and reap the nutrients of their degradations (McAllister, 1994). Attachment to newly ingested substrate also enables microbes to be retained in the rumen as degraded substrates are passed to the abomasum and lower gastro intestinal tract. Retention in the rumen ensures microorganisms continued ability to live and reproduce.

An interface is created when two immiscible bulk phases come into contact with each other, i.e., the forage substrate and/or rumen microbes and the rumen fluid. Fletcher et al. (1980) indicated that both the solid and the liquid and not just the solid surface charge determined the properties of the interface. When a solid was immersed in an aqueous environment, it usually acquired a surface charge, either by adsorption of ions or by the ionization of surface groups (Denyer et al., 1993). The surface charge, therefore, depends to a great extent on the composition of the aqueous phase. Once a surface became charged, it attracted oppositely charged ions from the surrounding aqueous phase (Fletcher et al., 1980).

Most plant cell membranes and walls have a net negative surface charge (Rosenberg and Doyle, 1990) which can be related to the presence of surface ionized carboxyl groups (Mozes and Rouxhet, 1990). It has been observed that bacteria as well as forage substrates have an overall negative charge, and for attachment to take place, the resulting electrostatic repulsion barrier must be overcome by attractive forces (Fletcher, 1996; Denyer et al., 1993). Fletcher (1996) noted that repulsive forces

decrease with an increase in ionic strength of the medium. Thus, as bacteria approach the surface of the substrate a repulsive interaction comes into play, resulting from the overlapping ionic atmospheres around the two surfaces (Denyer et al., 1993).

There is an increasing realization that hydrophobic interactions play a role in many, if not most, microbial adhesion phenomena (Rosenberg and Doyle, 1990). Hydrophobic interactions are brought about because water molecules situated around a non-polar substrate are more highly ordered than the bulk water. Thus, the establishment of a hydrophobic interaction displaces the water and thus increases the disorder of the system (Denyer et al., 1993). Rosenberg and Doyle (1990) indicated that hydrophobicity is considered to be an important determinant of interfacial processes such as cell adhesion. Displacement of adsorbed water to allow close approach of the two surfaces is energetically unfavorable. However, if either surface has nonpolar groups or patches, these can assist the exclusion of water by the aforementioned hydrophobic interactions (Pell and Schofield, 1993; Busscher and Weerkamp, 1987) facilitating the short-range reactions (Pell and Schofield, 1993). Fletcher (1996) suggested that two basic attachment processes occurred with hydrophobic and hydrophilic surfaces. Adsorption to hydrophobic surfaces was rapid, and binding thought to be stronger than on hydrophilic surfaces. In contrast, adhesion to hydrophilic surfaces was more variable and was suggested to follow the model of "reversible" and "irreversible" adhesion (Marshall et al., 1971). In this model, a bacterium that encounters the surface is first weakly held at the surface at a distance of several nanometers which potentially progresses into irreversible adhesion.

In order for enzymatic degradation to occur, the microbes must associate with the substrate (i.e., cellulose) in order to present their enzymes to the substrate and

absorb the products of the hydrolysis (ZoBell, 1943). The closeness of this association varies with different microbes and substrate types. For example, adhesion to crystalline cellulose is specific and involves surface proteins (McAllister et al., 1994; Gong and Forsberg, 1989). McAllister et al. (1994) commented that the focus of most microbial adhesion studies was on a capsular carbohydrate coat or glycocalyx and its associated binding proteins, a structure crucial in the attachment of the principal cellulolytic bacteria. Disruption of the glycocalyx prevented bacterial attachment (McAllister et al., 1994). Using a transmission electron microscope, Akin (1980) observed digestion of rapidly degraded orchard grass blades revealed that mesophyll, parenchyma bundle sheath, and parts of the epidermal cell wall apparently were degraded without direct attachment of bacteria although bacteria were near the cell walls undergoing digestion.

Microbes adhere to fiber by two main mechanisms, specific binding and non-specific ionic interactions (Whitehouse et al, 1993; Forsberg, 1986). Specific binding includes cell surface-associated enzymes, capsules or external fibrous material, and adhesions (molecules on the microbial cell surface that bind to receptors on the plant material) (Chesson and Forsberg, 1988). Non-specific interactions include physiochemical forces (i.e., Vander Waals, hydrophobic, electrostatic and ionic interactions), conformational adjustments to substrate surfaces (Czerkawski and Cheng, 1988), or by the consortia formed from cellulolytic fermentation (Costerton et al., 1985). Outside the range of the Vander Waals forces, bacteria are carried toward a surface by gravity, diffusion, taxis, motility or convection (Pell and Schofield, 1993). There is evidence that extracellular polysaccharides, proteins, and lipopolysaccharide can play a role in attachment, possibly at different stages in the attachment process. For example, protein (i.e., outer membrane proteins, flagella, enzymes) may be

required in early stages of adhesion whereas the aforementioned extracellular polysaccharides are synthesized by attached bacteria (Allison and Sutherland, 1987) and strengthens their binding to surfaces (Costerton et al., 1985) and facilitates intercellular binding (Fletcher, 1996). Bayer and Lamed (1986) described how upon binding, the polysaccharide cellulose binding factors underwent a dramatic conformational change forming passageways between the cell and the substrate.

2.2.2.1 The Microbial Consortia

The concept of the microbial "consortia" acknowledges the complex structure of cellulose and acknowledges the inability of the enzymes of any individual microbe species to fully degrade this complex molecule (McAllister et al., 1994; Cheng et al., 1990; Lamed and Bayer, 1988; Morris and Cole, 1987; Forsberg et al., 1981). Instead, the "consortia" concept states that many microbes partake in the fermentation of cellulose in the rumen (Akin and Amos, 1975), and that these microbes have a nutritional interdependence on each other (Miura et al, 1982) arising from their close relationships. Cheng et al. (1990) and Miura et al. (1982) reported that nutritional interdependence among rumen bacteria affects rate of cellulose digestion. Williams and Strachan (1984) defined consortia in regards to bacteria engulfing cellulose in complex layers. Primary degrading bacteria tightly colonize cellulose, followed by the secondary consortium of organisms that do not directly degrade cellulose but "drive" the digestive process by removing substrates (i.e. cellobiose) contributing to end-product inhibition (McAllister et al., 1994; Cheng et al., 1981). Forsberg et al. (1981) described how bacteria resembling *F. succinogenes* provided a source of sugars for microbes lacking polymer-degrading activity, thereby, contributing to a stable heterogeneous

microbial population. Evidence to verify the "consortia" concept was provided by Cheng et al (1981) in which this phenomenon of bacterial species combining for complex digestive functions was observed.

2.2.2.2 The Cellulosome Concept

Work by Bayer et al. (1983) led to the discovery of the "cellulosome" , which is important in the mechanism of both binding and cellulose hydrolysis. Lamed and Bayer (1988) verified the presence of the cellulosome using the anaerobic bacteria *Clostridium thermocellum*. Gong and Forsberg (1989) described the cellulosome as a proteinaceous cellulose-binding factor important in microbial binding and hydrolysis of cellulose and agreed concept integrates microbial adhesion and enzymatic breakdown. Stack and Hungate (1984; 1985) and Wood et al. (1982) identified a large cellulase complex on the surface of *R. albus*. This was proposed to compare to the cellulosome of *Clostridium thermocellum* and was expected to contain the enzyme and adhesive properties.

2.3 Surfactants

The term surfactant, or surface-active agent, refers to any substance that when mixed with water will congregate at interfaces rather than in the bulk of the solution (Stutz et al., 1973; Stauffer, 1990). This is in contrast to most solutes, which concentrate in the bulk of the solution away from the surfaces (Cross, 1987). Thus, the most well known property of surfactants is their interfacial activity or ability to align at the interface when placed in solution due to their bi-polar nature or their "head" and "tail" regions. Their bi-polarity reflects their tendency to assume the most energetically

stable orientation (Kamande, 1994) and facilitates a substantial decrease in interfacial tension (Helle et al., 1993; Cross, 1987).

The hydrophobic or lipophilic tail region is usually a long-chain fatty acid from a food grade fat or oil (Stauffer, 1990). Subsequently, surfactants are classified according to the charge of their hydrophilic head which can be either positive, negative or neutral (cationic, anionic or nonionic, respectively) and also the chemical nature of their tail (Kamande, 1994; Stauffer, 1990).

Surfactants have the effect of increasing the substrate's "wettability", making it readily available to microbial enzymes and their subsequent digestion. It is suggested that this is due to the favorable orientation of the surfactant molecules at the solid-liquid interface (Desai and Madamwar, 1994; Madamwar et al., 1991). To indicate that substrate wettability was increased indicates that the interactions between the solid and liquid molecules surrounding it are equal to or stronger than those between the liquid molecules themselves (Fletcher et al., 1980). Helle et al. (1993) supported the hypothesis that surfactant addition resulted in improved substrate wettability, suggesting that the adsorption of the surfactant was due to hydrophobic interactions between the tail of the surfactant and the hydrophobic cellulose.

In addition to improving wettability of substrate, surfactants have been suggested to increase the permeability of the microbial plasma membrane *via* exogenously supplied fatty acids that become combined in the plasma membrane and alter its properties (Asther & Corrieu, 1987). Surfactants are reported to improve enzyme production and enhance enzyme activity (Madamwar et al., 1991; Elworth et al., 1968). Abrha and Gashe (1992) suggested that surfactant alteration of the plasma membrane facilitated increased enzyme secretion of the microbes they associated with. Ooshima

et al. (1986) reported that nonionic, amphoteric, and cationic surfactants enhanced the saccharification of cellulose.

2.3.1 Nonionic Surfactants

Nonionic surfactants are those having a hydrophilic head with a neutral charge, i.e. it is uncharged (Cullum, 1994). The proposed surfactant in this experiment, T-80, is a nonionic surfactant. Stauffer (1990) indicated that nonionic surfactants are relatively insensitive to pH and ionic strength in an aqueous environment in contrast to the ionic (anionic or cationic) types. The mechanism of this is based on the large hydrophilic portion, i.e., as is found in the polyoxyethylene chain of the Tweens. The hydrophobic tail is anchored at the corresponding nonpolar substrate while the large head is strongly hydrated and generates a layer of "bound" water surrounding the substrate making it insensitive to ionic changes (Stauffer, 1990). Stauffer's (1990) observations regarding nonionic surfactants insensitivity to pH and ionic strength may help explain T-80's effectiveness in the chemically fluctuating rumen environment.

An abundance of cellulosic waste produced by the pulp and paper industry prompted research focusing on improving the enzymatic degradation of cellulosic wastes. Addressing similar issues in regards to fiber digestion in the anaerobic rumen, these scientists incorporated nonionic surfactants in anaerobic industrial bioreactors. As reported by Castanon and Wilke (1981) and Helle et al, (1993), nonionic surfactants were found to increase the rate and extent of cellulose saccharification by almost 40%. Ooshima et al. (1986) referred to nonionic surfactants as having exceptionally great effects on anaerobic digestion, especially with longer reaction time and higher concentrations within the ranges tested. This was attributed to the nonionic surfactants

ability to enhance the synergistic action of exoglucanase and endoglucanase on the surface of cellulose by restricting endoglucanase attachment and holding it in the liquid phase (Ooshima et al., 1986). The nonionic surfactant Tween 20 was hypothesized to vary the enzyme adsorption balance, creating more favorable situations for the unique synergistic action of endoglucanase and cellobiohydrolase in the initial steps of cellulose hydrolysis (Ooshima et al, 1986). The activation of cell and bacterial membrane associated enzymes by nonionic surfactants is widely documented (Rothfield and Finkelstein, 1968; Triggle, 1970; Machtiger and Fox, 1973). It was noted that nonionic surfactants act as accelerators of cellulosic saccharification without any significant denaturation of hydrolytic enzyme (Helle et al., 1993), and that they had a greater impact on more crystalline cellulose (Ooshima et al, 1986).

2.3.1.1 Tween 80

Polyoxyethylene monooleate or polysorbate 80, better known as "Tween 80" (Sigma Chemical Co., St. Louis, MO, USA) is a nonionic surfactant. Artz (1990) described how polyoxyethylene sorbitan esters are synthesized by the addition, *via* polymerization, of ethylene oxide to sorbitan fatty acid esters. Sisley and Wood (1952) provided detailed information on T-80's composition, surfactant class, form, properties and application.

> *Composition:* Condensation product of ethylene oxide and sorbitan monooleate. *Class:* III/B = Nonionic agents = Natural Products = saponines; products obtained by condensation of fatty substances and their derivatives with ethylene oxide *Form:* Semiviscous, amber-colored, oily liquid; viscosity 600 to 800 cp at 25 °C; density 1.05 to 1.1.

Properties: soluble in hard water, dilute acids and alkalis, and most of the organic solvents; insoluble in oils; can be used in combination with Span 80; the Tween is soluble in the aqueous phase, the Span in the oil phase. *Application:* Emulsifying agent (Sisley and Wood, 1952).

T-80 is considered safe for human or animal consumption and already has wide applications in the baking industry (Griffin and Lynch, 1972). In terms of chemical composition, T-80 contains many esterified fatty acids, of which the most abundant was oleic acid (99.0 x 104%, w / v) (Asther and Corrieu, 1987). T-80 has a tendency for spontaneous dispersion in aqueous mediums due to its hydrophilic and hydrophobic portions (Stauffer, 1990; Asther and Corrieu, 1987).

T-80 has shown numerous positive effects in a wide range of applications. It was found to be non-damaging to cellulolytic enzymes (Ooshima et al., 1986; Castanon and Wilke, 1981), and was found to increase fungal cellulolytic enzymes (Pardo, 1996; Long and Knapp, 1991; Asther and Corrieu, 1987; Reese and Maguire, 1969). When added to the culture media, T-80 was the best compound for enhancing the production of all enzymes in the fungi *Nectria catalinensis* of the cellulase complex. It caused an increase of 70% of endoglucanase (at 1.7 mM T-80) and 72% of exoglucanase (at 0.85 mM T-80) production, respectively, and the cellobiase yield was approximately fourfold higher that the Control (Pardo, 1996). Increases in enzyme yields were attributed to improvements in cell permeability (Pardo, 1996; Reese and Maguire, 1969). Variations in enzyme yields in microbes were found to vary for different organism species and between different enzymes of the same species (Machtiger and Fox, 1973; Triggle, 1970; Reese and Maguire, 1969). Improvements in anaerobic cellulose digestion were noted (Akin, 1980) and 0.1% T-80 (v / v) in the anaerobic dilution solution was used to

increase viable bacterial numbers in rumen contents (Dehority and Grubb, 1980). Akin (1980) observed increases in bacterial counts by as much at 70% when 0.1% T-80 (v / w) was added to the incubation media. White et al. (1988) found a 10% increase in the activity of exoglucanase from *R. flavefaciens* with 0.1% (v / v) T-80. Castanon and Wilke (1981) reported that 0.1% T-80 (v / v) added to the reaction solution in anaerobic reactors increased the rate and extent of cellulose saccharification by 33%. Other studies reported the ability of T-80 to increase cellulase stability and prevent its inactivation (Helle et al., 1993; Kim et al., 1981; Reese, 1980).

The use of T-80 is not without its drawbacks. Minato and Suto (1978) and Dehority and Grubb (1980) observed lysis of fiber degrading bacterial cells in the presence of T-80. Levels above 0.1% (v / v) T-80 were found to be inhibitory of ruminal bacterial growth (Dehority and Grubb, 1980). Under conditions of cooling and agitation, 70 to 80% of *Fibrobacter succinogenes* cells were lysed during the treatment having greater than 0.001% (v / v) T-80 whereas about ten percent of *Ruminococcus albus* and *R. flavefaciens* cells were lysed by treatment greater than 0.01% (v / v) T-80 (Minato and Suto, 1978). In rumen studies White et al. (1988) observed similar lysis phenomenon. Gong and Forsberg (1989) used 0.5% (w / v) T-80 in the culture medium and observed that adhesion of *F. succinogenes* to crystalline cellulose was unaffected. Akin (1980) observed increases in viable bacterial counts by as much as 70% in the presence of 0.1% (v / v) T-80 in the culture media.

Elution of microorganisms from substrates in the presence of 1g L^{-1} T-80 was observed by Legay-Carmier and Bauchart (1988), however the rotation rates utilized in this experiment were in excess of that occurring in natural rumen turnover (Kamande, 1994). Whitehouse et al. (1993) used 0.1% (v / v) T-80 to remove rumen

microorganisms from digesta solids, however, best results were obtained with both chemical and physical (i.e., shaking) methods combined. Kopecny and Wallace (1982) simply used gentle physical methods such as shaking to extract rumen bacterial enzymes. Akin (1980) incubated rumen bacteria in the presence of 0.1% (v / v) T-80 to remove them from forages, however, for the first 55 min of this incubation increased viable amounts of bacteria were observed of up to 59%. Removal of bacteria did not occur until more than 60 min after incubation. Gong and Forsberg (1989) observed that *F. succinogenes* cells bound to cellulose were not released by addition of T-80 at 0.5% (w / v). Thoman et al. (1989) was unsatisfied with 9.1 mM T-80 used as a phase transfer catalyst due to its separation from product after reaction. Minato and Suto (1978) used 0.001-0.5% (v / v) T-80 to elute bacteria from cellulose under conditions of cooling and agitation but considered it undesirable due to lysis occurring in some of the cells. Reese and Maguire (1969) suggested that cellulase production of the fungi *Trichoderma viride* could either be stimulated or inhibited in the presence of T-80 depending on the conditions of growth. In their studies, cellulase production of *T. viride* was inhibited by 0.1% (v / v) T-80, yet when grown under more favorable conditions having 0.3% (v / v) T-80, cellulase production was increased eightfold by the surfactant.

2.3.1.1.1 Suggested Mode of Action of T-80

The mode of action of T-80 remains unclear. Like other surfactants, T-80 improves wettability (Desai and Madamwar, 1994; Whitehouse et al., 1993; Merry and McAllan, 1983; Dehority and Grubb, 1980) and the potential for a substrate to be attacked by microbial enzymes. It is suggested that it aligns at the interface and mediates the substrate-enzyme (microbial) attachment (Madamwar et al., 1991). Helle

et al. (1993) suggested the charge of the surfactant was not an important consideration in increasing the total yield of reducing sugars from the hydrolysis of cellulose, as cationic, anionic and the neutrally charged T-80 all enhanced cellulose hydrolysis. The structure of the head group of the surfactant also appeared to have little significance.

It was suggested that the surfactant is protecting the cellulolytic enzymes from the postulated non-productive binding in which the enzymes become permanently attached to the substrate after hydrolysis (Helle et al., 1993; Walker and Wilson, 1991; Castanon and Wilke, 1981; Marshall et. al. 1971). Mozes and Rouxhet (1990) described T-80 as inhibitory of microbial adhesion due to its interference with the hydrophobic interactions existing between bacteria and substrate. Castanon and Wilke (1981) hypothesized that this difficulty of adsorption of enzyme prevents the immobilization of enzyme on the surface of substrate, resulting in the enhancement of saccharification of cellulose. The nature of this protective effect instilled by T-80 remains unclear. According to Castanon and Wilke (1981), the activity of endoglucanase could be held much longer by existing in a liquid phase.

The initiation of bacterial membrane associated enzymes by surfactants has been reported (Sami et al., 1988; Machtiger and Fox, 1973; Triggle, 1970). For example, Sami et al. (1988) reported that when 0.1% (v / v) T-80 was added to the culture medium of the cellulolytic bacteria *Cellulomonas flavigena*, more than a twofold increase in CMCase was noted in the culture supernatants. According to Baah et al. (1996), T-80 increases enzyme stabilization or possibly enzyme activity.

Numerous authors suggested T-80 appears to effect cell membrane permeability, promoting both entrance and exit of compounds from the cell (Sami et al., 1988; Reese and Maguire, 1969). In a later study, Reese (1972) suggested T-80

encouraged the release or solubilization of exoenzymes as a result of the altered membrane permeability. Increases in membrane permeability were observed in the presence of the oleic acid rich T-80. Oleic acid is a fatty acid that contributes to membrane permeability (Asther and Corrieu, 1987; Jager and Kirk, 1985; Greenway and Dyke, 1979), thus increasing potential enzymes yields. Oleic acids were proven to have significant effects on the membrane permeability of microorganisms (Greenway and Dyke, 1979). Jager and Kirk (1985) challenged the hypothesis of the T-80 contribution of fatty acids increasing membrane permeability as similar phenomenon were observed using surfactants unable to contribute fatty acids to the cell membrane. Sukan et al. (1989) reported the presence of low concentrations of fatty acids have both inhibited and stimulated microbial growth depending on culture conditions.

2.3.1.1.2 Tween 80 and Experimental Treatment Levels

Interpretation of results from previous experiments in terms of level of T-80 supplementation and their application is complicated by the variability of treatment levels expressed in previous T-80 studies. For example, Ooshima et al. (1986) studied enzymatic saccharification of cellulose using T-20 levels at, "0 to 1 wt %" using ten milliliter screw-capped test tubes and under the shaking of 70 oscillations per min. Pardo (1996) evaluated the effect of surfactant on the cellulase production of the fungi *Nectria catalinensis* and noted maximal yield of endoglucanase with 1.7 mM T-80, where as exoglucanase and cellobiase were optimized at 0.85 mM T-80.

Abrha and Gashe (1992) used T-80 at 0.1% (w / v) and increased production of cellulase in the fungal species *Cladosporium*. Ligninase production was increased in the fungal species *Phanerochaete chrysosporium* in the presence of 0.04% (w / v) T-80

(Asther and Corrieu 1987) and Jager and Kirk (1985) observed optimal ligninase activity in the same species at 0.05 to 0.1% (v / v) T-80 in culture. Reese and Maguire (1969) reported T-80 was added at 0.05 – 0.2% (v / v) (usually 0.1%) to fungal cultures in shaken flasks on a simple salts medium. (Dehority and Grubb, 1980) increased total colony counts of rumen contents when 0.1% (v / v) T-80 was added to anaerobic dilution solution, whereas (Sami et al., 1988) reported improvements in production of bacterial cellulases of *Cellulomonas flavigena* cultured in the presence of 0.1% (v / v) T-80, 0.2% yeast extract, and 0.5% Avicel (cellulose). In an evaluation of ligninase production and degradation by the fungi *Phanerochaete chrysosporium* in agitated submerged cultures. Jager and Kirk (1985) observed ligninase activity dropped off at the lowest concentrations of 0.01% (v / v) T-80, but persisted at the higher concentrations. The best results were obtained with 0.05 to 0.1% (v / v) T-80. Oguntimein (1991) using T-80 at a concentration of 0.1% (v / v) increased all enzyme activities of the fungal species *Neurospora sitophila* in fermentation's carried out in Erlenmeyer flasks, however, the optimum T-80 concentration for CMCase activity was 0.1% (v / v) while the total cellulase and β-glucosidase activities increased as the concentration was increased from 0.05 to 0.2% (v / v). Sukan et al. (1989) evaluated cellulase growth and production by the fungal species *Trichoderma reesei* and *S. pulverulentum* using 0.1% (v / v) T-80 in shaker flasks. There was no effect on cellulase growth and production rates, however, a trend for increased enzyme activity was observed (Sukan et al., 1989). Nystrom (1976) reported that T-80 is routinely incorporated into growth media at concentrations of 0.1% (v / v) for the production of cellulases. Abrha and Gashe (1992) increased cellulase production of a *Cladosporium* species by 1.5- to 4.5-fold compared with the Control using T-80 at 0.1% (v / v).

Experimental work in ruminant studies have reported dietary T-80 treatment levels of 1.2% (Kamande, 1994), 0.2% (Shelford and Baah, 1997) and 0.1% (Baah, 1998), all on a DM basis. Shelford and Baah (1998) indicated that diets were formulated to give each cow 100 or 150 g d^{-1} of a supplement containing T-80.

An optimal means of expressing the level of T-80 under experiment conditions to accommodate all biological systems has yet to be decided upon. There were two predominant means on which T-80 levels were expressed. First is based on the total volume of the reaction vessel rather than the quantity of substrate within that vessel. The majority of previous research has implemented this method using concentration units of w / v and v / v. The second means is based on quantity of substrate and was reported on a DM basis of the substrate utilized. Both means of expression have limitations. The addition of T-80 on a DM basis does not account for dilution of the product in the aqueous, fermentative environment. Kamande (1994) agreed, reporting the consumption of water would have a diluting effect on the T-80 concentration in the digesta. However, addition of T-80 based on reaction volume does not account for varying amounts of substrate within the reaction solution.

Estimations can be made to superimpose from one experiment to the next, but error is high and experimental parameters necessary for conversion are not always provided. The volume of the rumen of a live cow can be estimated by emptying the contents through a rumen fistula or by the dilution technique with a liquid marker (Van Soest, 1994). Using data from the current experiment as an example, cows having a DMI of 26.4 kg d^{-1} of 0.1%T-80 would consume 26.4 g T-80 d^{-1}. In an estimated 300 L rumen and on a v / v basis, that would be equivalent to approximately 0.009% T-80 v / v assuming that T-80 has the same passage rate through the rumen as the ration. An all-

inclusive means of T-80 expression would incorporate both total reaction vessel volume and quantity of substrate on a DM basis.

2.4 Ruminant Nutrition

Feeding of the ruminant animal, and in particular that of lactating dairy cows, is not a simple matter. The dietary composition of what the cow consumes is different from what is metabolized due to microbial fermentation in the rumen. As previously mentioned, the majority of carbohydrates consumed by the cow are indigestible in the absence of the enzymatic action of the ruminal microorganisms. Microbes metabolize fibrous carbohydrates to VFAs that can be absorbed and aerobically oxidized by the cow as a source of energy. Rumen microorganisms not only alter the composition of carbohydrates, but also of dietary protein. Proteolysis occurs, releasing ammonia and AAs that are combined with energy from carbohydrates into microbial CP. Inhibition of protein and AA degradation to ammonia is one of the main aims of improving N use by the ruminant (Jouany, 1994). Microbial protein becomes available to the cow as it passes from the rumen into the lower GI track, but is in a very different form from what it originally started.

Thus, the goal of the dairy nutritionist is to focus on achieving optimal microbial production. The modern dairy cow in early-lactation cannot meet her metabolic demands by rumen microbial fermentation alone, and must have supplementation above and beyond that required by the microorganisms to maintain a state of positive energy balance and maximal production of quality milk.

2.4.1 The Importance of Protein and Energy Synchronization

Rumen microbes are highly proteolytic (Orskov, 1992) resulting in imbalances in fermentation rate between protein and carbohydrates resulting in inefficient N-utilization. To achieve optimal N-utilization and subsequent microbial CP production, fermentation rates of protein and carbohydrates must be synchronized (Tamminga, 1992). Blood urea nitrogen (BUN) levels are indicative of N status in the dairy cow with elevated levels indicating excessive dietary N and the resulting inefficiency of utilization. BUN concentrations were shown to decrease when more optimal levels of ruminally fermentable carbohydrate were supplied to enhance incorporation of RDP into microbial protein (Roseler et al. 1993). Synthesis of microbial protein is improved by varying the source and degradability of energy incorporated into the diet (Petit and Tremblay, 1995). Aldrich et al. (1993) reported that microbial N synthesis is highest when high ruminally available NSC is combined with high ruminally available protein and lowest when high ruminally available NSC is combined with low ruminally available protein.

2.4.2 Tween 80 and Carbohydrates

The potential contribution of SCs from forages as an energy source and the abundance of cellulose within this fraction have been discussed. Similarly, the benefits of T-80 on cellulose hydrolysis were reported in several studies, and mechanisms of its action were proposed but not verified. Petit and Tremblay (1995) discussed how efficiency of microbial protein synthesis is low when cows were fed diets based on grass silage alone and subsequently did not meet the protein requirements of high-producing cows. Most protein in grass silage is in the form of non-protein nitrogen (NPN) that has slower fermentation rates than true protein resulting in decreased

microbial cell production. Microbial cell production is limited by energy availability or the synchronization of energy and protein sources (Tamminga, 1992). T-80 may be able to help fill this gap, facilitating natural enzymatic hydrolysis and attaining more efficient energy release from SCs.

2.4.3 Tween 80 and Dietary Protein

Two strategies are used to increase the amount of protein reaching the small intestine: increased microbial protein synthesis or supplementation of a source of RUP (Petit and Tremblay, 1995). Dairy rations balanced solely on CP are usually formulated to contain excess protein to ensure the need of RUP is met as not to limit milk production in high-producing dairy cows. This conventional feeding method often leads to inefficiency of N-utilization in the rumen as the abundant RDP is quickly degraded to AAs. In the lack of available energy, AAs are deaminated to supply energy rather than being used for microbial protein synthesis or lost as urea in the urine or milk (Kamande, 1994).

T-80 does not exclusively effect cellulolytic organisms, and *in vitro* work by Kamande (1994) and Shelford et al. (1996) demonstrated T–80 increased both cellulose and protein degradation. Kopecny and Wallace (1982) investigated the locations and properties of ruminant proteolytic microbes. The rumen microbes are highly proteolytic (Orskov, 1992) compared to their fiber degrading microbial counterparts, limiting the use of forage as a energy source due to imbalances created by SCs slow rate of fermentation (Kamande, 1994). In feeding situations where high-energy diets are required to meet maintenance and production needs, synchronization of carbohydrate and protein fermentation are desired to optimize microbial production

(Tamminga, 1992). In the ruminal environment, enhancing degradation of DIP is countered productive. Kamande (1994) suggested the positive impacts of T-80 on cellulose degradation could be realized and the negative impacts on protein degradation minimized at lower levels of T-80 supplementation in the ration.

2.4.4 Previous Ruminant Work

Application of concepts of T-80 cellulose hydrolysis is a relatively new concept in ruminant nutrition. Kamande (1994) achieved increases of almost 40% in DM intake and almost ten percent in apparent digestibility parameters in four fistulated sheep when T-80 was added to a 100% grass hay diet at 1.2% (DM basis). Work by Shelford et al. (1996) reported improvements of 3.22 kg d^{-1} in milk yield with T-80 supplementation in a preliminary trial, and in a follow-up lactation trial both treatments containing T-80 produced more than the Control animals having no T-80. Cows in their second or greater lactation fed 0.2% T-80 (DM basis) realized 1.31 kg d^{-1} more milk than Control animals (Shelford and Baah, 1997). Similarly, cows fed 100 g d^{-1} of supplement containing T-80 realized 1.12 kg d^{-1} over Control animals, however, cows consuming 150g d^{-1} of supplement containing T-80 were not different from Controls in terms of production (Shelford and Baah, 1998). Finally, in a study using beef cattle, Baah (1998) fed a TMR having 0.1% T-80 (DM basis) and observed significantly higher ($P<0.05$) total weight gain (kg) than steers fed a Control diet, however, no differences were observed in feeding efficiency.

2.4.5 Selection of Experimental Treatment Levels

Positive impacts of T-80 supplementation in ruminants have been observed from treatment levels as high as 0.5% (DM basis) (Kamande, 1994) to as low as 0.1% (DM basis) (Baah et al., 1996). In the dairy cow, improvements in milk production of 1.12kg d^{-1} for 100 g d^{-1} T-80 (Shelford and Baah, 1998) and 1.31 kg d^{-1} for 0.2% (DM basis) T-80 (Shelford and Baah, 1997) were reported. In light of the potential for decreased rate of ruminal microbial proteolysis compared to increases in the rate of cellulose degradation (Kamande, 1994) as well as increased milk production with lower levels of T-80 supplementation, the treatment levels of 0.2% T-80 (DM basis) and 0.1% T-80 (DM basis) were selected to examine an even greater reduction in the level of T-80 supplementation.

2.5 Summary

Microbial degradation of dietary protein and carbohydrates must be synchronized in order to achieve optimal production of microbial protein and the metabolic by-product VFAs (Tamminga, 1992). These products are metabolized by the cow to provide protein and energy, respectively, for milk production and growth. Forages in the cow's diet provide necessary fiber for normal rumen function, as well as an economical source of protein and energy over that of the more costly concentrate dietary components. Carbohydrates from forages are energy rich but are limited in their contribution to microbial protein production by their slow rate of ruminal fermentation relative to the faster protein degradation. The surfactant T-80 was shown to have positive implications on microbial enzymatic action. The majority of work has looked at T-80s positive impact on cellulose enzymatic degradation, however, Kamande (1994)

observed undesirable increases in ruminal protein enzymatic degradation in the presence of T-80. However, it appeared that T-80 had a decreased effect on ruminal protein degradation at lower concentrations in the diet while still maintaining the positive effects on cellulose degradation. Finding an optimal level of T-80 supplementation to reduce negative protein degradation while maintaining the beneficial effects of improved carbohydrate fermentation could lead to increasing production and efficiency of N utilization.

2.6 Objectives

The objective of this study was to compare a Control diet to those treated with T-80 at either 0.2% or 0.1% (DM basis) to determine the optimal level of supplementation as indicated by milk production, milk composition and apparent digestibility parameters.

2.7 References

Abrha, B. and Gashe, B. 1992. Cellulase production and activity in a species of *Cladosporium*. World Journal of Microbiology, 8:164-166.

Akin, D. 1978. Microscopic evaluation of forage digestion by rumen microorganisms - a review. Journal of Animal Science, 48(3):701-709.

Akin, D. 1980. Evaluation by electron microscopy and anaerobic culture of types of rumen bacteria associated with digestion of forage cell walls. Applied and Environmental microbiology, 39(1):242-252.

Akin, D. 1986. Chemical and biological structure in plants as related to microbial degradation of forage cell walls. Pp. 139-157 in: Milligan, L.P., Grovum, W. L., and Dobson, A. (Eds.) Control of Digestion and Metabolism in Ruminants. Prentice-Hall, Englewood Cliffs, New Jersey.

Akin, D. 1989. Histological and physical factors affecting digestibility of forages. Agronomy Journal, 81:17-25.

Akin, D. 1993. Perspectives of cell wall biodegradation – session synopsis. Pp. 73-82 in: Jung, H.G., Buxton, D.R., Hatfield, R.D. and Ralph, J. (Eds.) Forage Cell Wall Structure and Digestibility. American Society of Agronomy, Inc., Madison, WI.

Akin, D. and Amos, H.E. 1975. Rumen bacterial degradation of forage cell walls investigated by electron microscopy. Applied Microbiology, 29:692-701.

Akin, D. and Amos, H. 1979. Mode of attack on Orchard grass leaf blades by rumen protozoa. Applied and Environmental Microbiology, 37(2):332-338.

Akin, D. and Rigsby, L. 1985. Degradation of Bermuda and Orchard grass by species of ruminal bacteria. Applied and Environmental Microbiology, 50(4):825-830.

Akin, D. and Rigsby, L. 1987. Mixed fungal populations and lignocellulosic tissue degradation in the bovine rumen. Applied and Environmental Microbiology, 53:1987-1995.

Akin, D., Burdick, D. and Michaels, G. 1974. Rumen bacterial interrelationships with plant tissue during degradation revealed by transmission electron microscopy. Applied Microbiology, 27(6):1149-1156.

Akin, D., Gordon, G. and Hogan, J. 1983. Rumen bacterial and fungal degradation of *Digitaria pentzii* grown with or without sulfur. Applied and Environmental Microbiology, 46(30):738-748.

Aldrich, J.M., Muller, L.D., Varga, G.A. and Griel, L.C. 1993. Nonstructural carbohydrate and protein effects on rumen fermentation, nutrient flow, and performance of dairy cows. Journal of Dairy Science, 76:1091.

Allison, D.G. and Sutherland, I.W. 1987. The role of exopolysaccharides in adhesion of freshwater bacteria. Journal of General Microbiology, 133:1319-1327.

Amos, H.E. and Akin, D.E. 1978. Rumen protozoal degradation of structurally intact forage tissues. Applied Environmental Microbiology, 36:513-522.

Artz, W.E. 1990. Emulsifiers. Pp. 347-393 in: Branen, A.L, Davidson, P.M. and Salminen, S. (Eds.). Food Additives, University of Illinois, Urbana, Illinois.

Asther, M. and Corrieu, G. 1987. Effect of Tween 80 and oleic acid on ligninase production by *Phanerochaete chrysosporium* INA-12. Enzyme Microb. Technol., 9:245-249.

Baah, J. 1998. Effect of enzyme enhancing agent and fibrolytic enzyme on performance of feedlot cattle. The University of British Columbia, Vancouver, BC, Personal Communication.

Baah, J. 1996. Personal communication. Department of Animal Science. The University of British Columbia, Vancouver, BC.

Bachman, K.C. 1992. Managing Milk Composition. Pp. 336-346 in: Van Horn, H.H. and Wilcox, C.J. (Eds.) Large Dairy Herd Management. American Dairy Science Association, Champaign, IL.

Baldwin, R.L. 1970. Energy efficiency in anaerobes. American Journal of Clinical Nutrition, 23:1508-1518.

Bauchop, T. 1981. The anaerobic fungi in rumen fiber digestion. Agric. Environ., 6:339-48.

Bauchop, T. 1979. Rumen anaerobic fungi of cattle and sheep. Applied Environmental Microbiology, 38:148-158.

Bauchop, T. 1979a. The rumen ciliate Epidinium in primary degradation of plant tissues. Applied Environmental Microbiology, 37:1217-1223.

Bauchop, T. 1980. Scanning electron microscopy in the study of microbial digestion of plant fragments in the gut. Pp. 305-326 in: Ellwood, D.C., Hedger, J.N., Latham, J.N., Lynch, J.M. and Slater, J.H. (Eds.) Contemporary Microbial Ecology, Academic Press, London.

Bauchop, T. and Mountfort, D.O. 1981. Cellulose fermentation by a rumen anaerobic fungus in both the absence and the presence of rumen methanogenes. Applied Environmental Microbiology, 42:1103-1110.

Bayer, E.A., Kenig, R. And Lamed, R. 1983. Adherence of *Clostridium thermocellum* to cellulose. Journal of Bacteriology, 156:818-27.

Bayer, E.A. and Lamed, R. 1986. Ultrastructure of the cell surface cellulosome of *Clostridium thermocellum* and its interaction with cellulose. Journal of Bacteriology, 167:828-836.

Beldman, G., Voragen, A.G., Rombouts, F.M. and Pilnik, W. 1988. Synergism in cellulose hydrolysis by endoglucanases and exoglucanases purified from *Trichoderma viride*. Biotechnol. Bioeng., 31:173-178.

Bernier, R. And Stutzenberger, R. 1989. Beta-glucosidase biosynthesis in *Thermomonospora curvata*. Journal of Applied Microbiology and Biotechnology, 5:15-25.

Bertran, M.S. and Dale, B.E. 1985. Enzymatic hydrolysis and recrystallization behaviour of initially amorphous cellulose. Biotechnol. Bioeng., 27:177-81.

Bryant, M.P. 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. Fed. Proc. Fed. Am. Soc. Exp. Biol., 32:1809-1813.

Busscher, H.J., and Weerkamp, A.H. 1987. Specific and non-specific interactions in bacterial adhesion to solid substrata. FEMS Microbiol. Rev., 46:165-173.

Castanon, M. and Wilke, C. 1980. Adsorption and recovery of cellulases during hydrolysis of newspaper. Biotechnology and Bioengineering, 22:1037-1053.

Castanon, M. and Wilke, C. 1981. Effects of the surfactant Tween 80 on enzymatic hydrolysis of newspaper. Biotechnology and Bioengineering, 23:1365-1372.

Cheng, K.J., Fay, J., Coleman, R., Milligan, L. and Costerton, J. 1981. Formation of bacterial microcolonies on feed particles in the rumen. Applied and Environmental Microbiology, 41(1):298-305.

Cheng, K.J., McAllister, T. A., Kudo, H. and Costerton, J.W. 1990. The importance of adhesion in the microbial digestion of plant materials. Pp. 129-135 in: Hoshino, S., Onodera, R., Minato, H. and Itabashi, H. (Eds.) The Rumen Ecosystem: The Microbial Metabolism and Its Regulation. Japan Scientific Societies Press, Tokyo.

Cheng, K.J., Stewart, C.S., Dinsdale, D. and Costerton, J.W. 1983/84. Electron microscopy of bacteria involved in the digestion of plant cell walls. Animal Feed Science Technology, 10:93-120.

Chesson, A. 1981. Effects of sodium hydroxide on cereal straws in relation to the enhanced degradation of structural polysaccharides by rumen microorganisms. J. Sci. Food Agric., 32:745-58.

Chesson, A. and Forsberg, C.W. 1988. Polysaccharide degradation by rumen microorganisms. Pp. 251-284 in: Hobson, P.N. (Ed.) The Rumen Microbial Ecosystem. Elsevier Applied Sciences, London.

Clarke, R.T. 1977. Protozoa in the rumen ecosystem. Pp. 251-275 in: R.T. Clarke and T. Bauchop (Eds.) Microbial ecology of the gut. Academic Press, Inc., New York.

Costerton, J.W., Marrie, T.J., and Cheng, K.J. 1985. Phenomena of bacterial adhesion. Pp. 3 in: Savage, D.C. and Fletcher, M. (Eds.) Bacterial Adhesion: Mechanism and Physiological Significance. Plenum Press, New York.

Coughlan, M.P. 1991. Mechanisms of cellulose degradation by fungi and bacteria. Animal Feed Science Technology, 32:77-100.

Coughlan, M.P. and Ljungdahl, L.G. 1988. Comparative biochemistry of fungal and bacterial cellulolytic enzyme systems. Pp. 11-30 in: Aubert, J.P., Beguin, P., and Millet, J. (Eds.) Biochemistry and Genetics of Cellulose Degradation. Academic Press, New York.

Cowling, E.B. and Kirk, T.K. 1976. Properties of cellulose and lignocellulosic materials as substrates for enzymatic conversion processes. Biotechnology and Bioengineering Symposium, 6:95-103.

Cross, J. 1987. Introduction to nonionic surfactants. Pp. 3-28 in: Cross, J. (Ed.) Nonionic Surfactants: Chemical Analysis. Marcel Dekker, Inc., New York.

Cullum, D. C. 1994. Introduction to Surfactant Analysis. Blackie Academic & Professional, New York.

Czerkawski, J.W. and Cheng, K.J. 1988. Compartmentation in the rumen. Pp. 251 in: Hobson, P.N. (Ed.) The Rumen Microbial Ecosystem. Elsevier Applied Sciences, London.

Dehority, B.A. and Johnson, R.R. 1961. Effect of particle size upon the *in vitro* cellulose digestibility of forages by rumen bacteria. Journal of Dairy Science, 44:2242-2249.

Dehority, B. and Grubb, J. 1980. Effect of short-term chilling of rumen contents on viable bacterial numbers. Applied and Environmental Microbiology, 39(2):376-381.

Dehority, B.A. and Scott, H.W. 1967. Extent of cellulose and hemicellulose digestion in various forages by pure cultures of rumen bacteria. Journal of Dairy Science, 50:1136-1141.

Denyer, S.P., Hanlon, G.W. and Davies, M.C. 1993. Mechanisms of microbial adherence. Pp. 13-27 in: Denyer, S.P., Gorman, S.P. and Sussman, M. (Eds.) Microbial Biofilms: Formation and Control. Blackwell Scientific Publications, Boston.

Desai, M. and Madamwar, D. 1994. Surfactants in anaerobic digestion of cheese whey, poultry waste, and cattle dung for improved biomethanation. Transactions of the ASAE, 37(3):959-962.

Dinsdale, D., Morris, E. and Bacon, J. 1978. Electron microscopy of the microbial populations present and their modes of attack on various cellulosic substrates undergoing digestion in the sheep rumen. Applied and Environmental Microbiology, 36(1):160-168.

Elworth, P.H., Florence, A.T. and Macfarlane, C.B. 1968. Solubilization by Surface Active Agents and Its Application in Chemistry and the Biological Sciences. London, England: Chapman and Hall.

Fletcher, M. 1996. Bacterial Attachment in aquatic environments: A diversity of surfaces and adhesion strategies. Pp. 1- 24 in: Fletcher, M. (Ed.) Bacterial Adhesion Molecular and Ecological Diversity. A John Wiley & Sons, Inc. Toronto.

Fletcher, M., Latham, M.J., Lynch, J.M. and Rutter, P.R. 1980. The characteristics of interfaces and their role in microbial attachment. Pp. 67-78 in: Berkeley, R.C., Lunch, J.M., Melling, J., Rutter, P.R. and Vincent, B. (Eds.) Microbial Adhesion to Surfaces. John Wiley & Sons Canada Ltd., Rexdale, ON.

Fonty, G., Joblin, K.N., and Brownlee, A. 1990. Contribution of anaerobic fungi to rumen functions. Pp 93-100 in: Hoshino, S., Onodera, R., Minato, H. and Itabashi, H. (Eds.) The Rumen Ecosystem: The Microbial Metabolism and Its Regulation. Japan Scientific Societies Press, Tokyo.

Forsberg, C.W. 1986. Mechanism of bacteria attachment to dietary fibre in the rumen. Pp. 193-195 in: Taylor, T.G. and Jenkins, N.K (Eds.) Proceedings of 13th International Congress of Nutrition, Libbey, London.

Forsberg, C., Beveridge, T. and Hellstrom, A. 1981. Cellulase and xylanase release from *Bacteroides succinogenes* and its importance in the rumen environment. Applied and Environmental Microbiology, 42(5):886-896.

Francis, G.L., Gawthorne, J.M., and Storer, G.B. 1978. Factors affecting the activity of cellulases isolated from the rumen digesta of sheep. Applied Environmental Microbiology, 36:643-649.

Gong, J. and Forsberg, C. 1989. Factors affecting adhesion of *Fibrobacter succinogenes* subsp. *succinogenes S85* and adherence-defective mutants to cellulose. Applied and Environmental Microbiology, 55(12):3039-3044.

Grant, R.J. and Mertens, D.R. 1992. Influence of buffer pH and raw corn starch addition on *in vitro* fiber digestion kinetics. Journal of Dairy Science, 75:2762-2768.

Greenway, D.L. and Dyke, K.G. 1979. Mechanism of the inhibitory action of linoleic acid on the growth of *Staphylococcus aurous*. Journal of General Microbiology, 115:233-45.

Griffin, W.C. and Lynch, M.J. 1972. Surface active agents. Pp. 397 in: Furia, T.E. (Ed.) Handbook of Food Additives, 2nd Ed., CRC Press, New York.

Groleau, D. and Forsberg, C. 1981. Cellulolytic activity of the rumen bacterium *Bacteroides succinogenes*. Canadian Journal of Microbiology, 27:517-530.

Helle, S.S., Duff, S.J. and Cooper, D.G. 1993. Effect of surfactants on cellulose hydrolysis. Biotechnology and Bioengineering, 46:611-617.

Henrissat, B., Driguez, H., Viet, C. and Schulein, M. 1985. Synergism of cellulases from *Trichoderma reesei* in the degradation of cellulose. Bio/Technol., 3:722-726.

Hobson, P.N. 1976. The Microflora of the rumen. Meadowfield Press Ltd. Bushey, England.

Huang, A.A. 1975. Kinetic studies on insoluble cellulose-cellulase system. Biotechnol. Bioeng., 17:1421-33.

Hungate, R.E. 1966. The Rumen and Its Microbes. Academic Press, New York.

Hungate, R.E. 1988. The ruminant and the rumen. Pp. 1-20 in: Hobson, P.N. (Ed.) The Rumen Microbial Ecosystem. Elsevier Applied Sciences, London.

Isaacson, H.R., Hinds, F.C., Bryant, M.P., and Owens, F.N. 1975. Efficiency of energy utilization by mixed bacteria in continuous culture. Journal of Dairy Science, 58:1645-1659.

Jager, A. and Kirk, T. 1985. Production of ligninases and degradation of lignin in agitated submerged cultures of *Phanerochaete chrysosporium*. Applied and Environmental Microbiology, 50(5):1274-1278.

Jouany, J.P. 1994. Methods of manipulating the microbial metabolism in the rumen. Ann. Zootech., 43:49.

Jouany, J.P. and Ushida, K. 1990. Protozoa and fibre digestion in the rumen. Pp. 139-150 in Hoshino, S., Onodera, R., Minato, H. and Itabashi, H. (Eds.) The Rumen Ecosystem: The Microbial Metabolism and Its Regulation. Japan Scientific Societies Press, Tokyo.

Kamande, G.M. 1994. Manipulation of rumen and silage fermentation, Ph.D. Thesis, Department of Animal Science, The University of British Columbia, Canada.

Kim, M.H., Lee, S.B. and Ryu, D.Y. 1981. Surface deactivation of cellulase and its prevention. Enzyme Microb. Technol., 4:99-103.

Kopecny, J. and Wallace, R.J. 1982. Cellular location and some properties of proteolytic enzymes of rumen bacteria. Applied Environmental Microbiology, 43:1026.

Krassig, H. 1985. Structure of cellulose and its relation to properties of cellulose fibers. Pp. 3-25 in: Kennedy, J.F., Phillips, G.O., Wedlock, D.J. and Williams, P.A. (Eds.) Cellulose and its Derivatives, Ellis Horwood, Chichester.

Ladisch, M.R., Lin, K.W., Voloch, M. and Tsao, G.T. 1983. Process considerations in the enzymatic hydrolysis of biomass. Enzyme Microb. Technol., 5:8-160.

Lamed, R. and Bayer, E. 1988. The cellulosome of *Clostridium thermocellum.* Advances in Applied Microbiology, 33:1-46.

Latham, M., Brooker, B., Pettipher, G. and Harris, P. 1977. Adhesion of *Bacteroides succinogenes* in pure culture and in the presence of *Ruminococcus flavefaciens* to cell walls in leaves of perennial ryegrass (*Lolium perenne*). Applied and Environmental Microbiology, 35(6):1166-1173.

Latham, M.J., Brooker, B.E., Pettipher, B.L. and Harris, P.J. 1978. *Ruminococcus flavefaciens* cell coat and adhesion to cotton cellulose and to cell walls in leaves of perennial reyegrass (*Lolium perenne*). Applied Environmental Microbiology, 35:156-65. Applied Environmental Microbiology

Leatherwood, J.M. 1965. Cellulase from *Ruminococcus albus* and mixed rumen microorganisma. Applied Microbiology, 13:771-775.

Legay-Carmier, F. and Bauchart, D. 1988. Distribution of bacteria in the rumen contents of dairy cows given a diet supplemented with Soya-bean oil. British Journal of Nutrition, 61:725-740.

Long, K. and Knapp, J. 1991. The effect of Junlon PW110 and Tween 80 on the production of cellulolytic enzymes by *Coprinus cinereus*. Mycol. Res. 95(9):1077-1081.

Ljungdahl, L.G. 1989. Mechanisms of cellulose hydrolysis by enzymes from anaerobic and aerobic bacteria. Pp. 5-16 in Coughlan, M.P. (Ed.) Enzyme Systems for Lignocellulose Degradation. Elsevier Applied Science, New York.

Machtiger, N.A. and Fox, C.F. 1973. Biochemistry of bacterial membranes. Annual Rev. Biochem., 42:575.

Madamwar, D., Patel, A. and Patel, V. 1991. Effects of various surfactants on anaerobic digestion of water hyacinth-cattle dung. Bioresource Technology, 37:157-160.

Marshall, K.C., Stout, R., and Mitchell, R. 1971. Mechanisms in the initial events in the sorption of marine bacteria to surfaces. Journal of General Microbiology, 68:337-348.

McAllister, T.A., Bae, H.D., Jones, G.A. and Cheng, K.J. 1994. Microbial attachment and feed digestion in the rumen. Journal of Animal Science, 72:3004-3018.

McManus, W.R., Robinson, V.N. and Grout, L.L. 1977. The physical distribution of mineral material on forage plant cell walls. Aust. J. Agric. Sci., 28(4):651-662.

Meara, M.L. 1955. Fats and other lipids. Pp. 317-402 in: Paech, K. and Tracey, M. V. (Eds.) Moderne Methoden der Pflanzenanalyse, vol. 2., Springer Verlag, Berlin.

Mendels, M. and Reese, T. 1964. Fungal cellulases and the microbial decomposition of cellulosic fabric. Dev. Ind. Microbiol., 5:5-20.

Merry, R.J. and McAllan, A.B. 1983. A comparison of the chemical composition of mixed bacteria harvested from the liquid and solid fractions of rumen digesta. British Journal of Nutrition, 50:701.

Mertens, D. 1980. Fiber content and nutrient density in dairy rations. Proc. Distillers Feed Conf., 35:35.

Mertens, D. 1982. Using Neutral detergent fiber to formulate dairy rations. Pp. 116-126 in: Proc. GA. Nutr. Conf. For the Feed Industry. University of Georgia, Athens.

Mertens, D. 1985a. Effect of fiber on feed quality for dairy cows. Pp. 209-224 in: Proc. 46th Minn. Nutr. Conf., University of Minnesota, St. Paul.

Mertens, D. 1985b. Factors influencing feed intake in lactating cows; Form theory to application using neutral detergent fiber. Pp. 1-18 in: Proc. 46th Ga. Nutr. Conf., University of Georgia, Athens.

Mertens, D. 1992. Nonstructural and Structural Carbohydrates. Pp. 219-235 in: Van Horn, H.H. and Wilcox, C.J. (Eds.) Large Dairy Herd Management, American Dairy Science Association, Champaign, IL.

Minato, H. and Suto, T. 1978. Technique for fractionation of bacteria in rumen microbial ecosystem. II. Attachment of bacteria isolated from bovine rumen to cellulose powder in vitro and elution of bacteria attached therefrom. Journal of General and Applied Microbiology, 24:1-16.

Minson, D. J. 1990. Forage in Ruminant Nutrition. Academic Press, Inc., Toronto.

Miura, H., Horiguchi, M, Ogimoto, K and Matsumoto, T. 1982. Nutritional interdependence among rumen bacteria during cellulose digestion *in vitro*. Applied and Environmental Microbiology, 45(2):726-729.

Morris, E. 1988. Characteristics of the adhesion of *Ruminococcus albus* to cellulose. FEMS Microbiology Letters, 51:113-118.

Morris, E. and Cole, O. 1987. Relationship between cellulolytic activity and adhesion to cellulose in *Ruminococcus albus*. Journal of General Microbiology, 133:1023-1032.

Mozes, N., Marchal, F., Hermesse, M.P., van Haecht, J.L., Reuliaux, L., Leonard, A.J. and Rouxhet, P.G. 1987. Immobilization of microorganisms by adhesion: interplay of electrostatic and nonelectrostatic interactions. Biotechnol. Bioeng., 30:439-450.

Mozes, N. and Rouxhet, P.G. 1990. Microbial hydrophobicity and fermentation technology. Pp. 75-105 in: Doyle, R. and Rosenberg, M. (Eds.) Microbial Cell Surface Hydrophobicity. American Society for Microbiology, Washington, DC.

Mullings, R. 1985. Measurement of saccharification by cellulases. Enzyme and Microbial Technology, 7:586-91.

National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev ed. National Academy of Sciences, Washington, DC.

Nocek, J.E. and Russell, J.B. 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. Journal of Dairy Science, 71:2070-2107.

Nystrom, J.M. and Allen, A.L. 1976. Pilot scale investigations and economics of cellulase production. Biotechnology and Bioengineering Symposium, 6:55-74.

Oguntimein, G. 1991. Production of cellulolytic enzymes by *Neurospora sitophila* grown on cellulosic materials. Bioresource Technology, 39:277-283.

Olubobokun, J., Craig, W. and Nipper, W. 1983. Characteristics of protozoal and bacterial fractions from microorganisms associated with ruminal fluid or particles. Journal of Animal Science, 66:2701-2710.

Ooshima, H., Sakata, M. and Harano, Y. 1986. Enhancement of Enzymatic Hydrolysis of Cellulose by Surfactant. Biotechnology and Bioengineering, 28:1727-1734.

Orpin, C.G. and Letcher, A.J. 1979. Utilization of cellulose, starch, xylan, and other hemicelluloses for growth by the rumen phycomycete *Neocallimastix frontalis*. Current Microbiology, 3:121-124.

Orskov, E.R. 1992. Protein Nutrition in Ruminants. 2nd ed. Academic Press, London.

Pardo, A. 1996. Effect of surfactants on cellulase production by *Nectria catalinensis*. Current Microbiology, 33:275-278.

Patterson, H., Irvin, R., Costerton, J.W. and Cheng, K.J. 1975. Ultrastructure and adhesion properties of *Ruminococcus albus*. Journal of Bacteriology, 122:278-287.

Pell, A.N. and Schofield, P. 1993. Microbial adhesion and degradation of plant cell walls. Pp. 397-423 in: Jung, H.G., Buxton, D.R., Hatfield, R.D. and Ralph, J. (Eds.) Forage Cell Wall Structure and Digestibility. American Society of Agronomy, Inc., Madison, WI.

Petit, H. and Tremblay, G. 1995. Ruminal fermentation and digestion in lactating cows fed grass silage with protein and energy supplements. Journal of Dairy Science, 78(2):342-352.

Prins, R.A. and Kreulen, D.A. 1990. Comparative aspects of plant cell wall digestion in mammals. Pp. 109-120 in Hoshino, S., Onodera, R., Minato, H. and Itabashi, H. (Eds.) The Rumen Ecosystem: The Microbial Metabolism and Its Regulation. Japan Scientific Societies Press, Tokyo.

Reese, E. 1980. Inactivation of cellulase by shaking and its prevention by surfactants. Journal of Applied Biochemistry, 2:36.

Reese, E. and Maguire, A. 1969. Surfactants as stimulants of enzyme production by microorganisms. Applied Microbiology, 17(2):242-245

Reese, E.T. 1972. Enzyme production from insoluble substrates. Biotechnology and Bioengineering Symposium, 3:43-62.

Roseler, D.K., Fergusen, J.D., Sniffen, C.J. and Herrema, J. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. Journal of Dairy Science, 76:525-534.

Rosenberg, M. and Doyle, R.J. 1990. Microbial cell surface hydrophobicity: History, measurement and significance. Pp. 1-38 in Doyle, R. and Rosenberg, M. (Eds.) Microbial Cell Surface Hydrophobicity. American Society for Microbiology, Washington, DC.

Rothfield, L. and Finkelstein, A. 1968. Membrane biochemistry. Annual Rev. Biochem., 37:463.

Sami, A., Akhtar, M., Malik, N. and Naz, B. 1988. Production of free and substrate-bound cellulases of *Cellulomonas flavigena*. Enzyme Microb. Technol., 10:626-631.

Shelford, J. A. and Baah, J. 1997. Effect of enzyme enhancing agent on milk production. The University of British Columbia, Vancouver, BC, personal communication.

Shelford, J. A. and Baah, J. 1998. Evaluation of a bioactive agent to enhance milk production. The University of British Columbia, Vancouver, BC, personal communication.

Shelford, J.A., Cheng, K.J. and Kamande, G.M. 1996. Enzyme Enhancers: The key to unlocking the energy from feed. Advances in Dairy Technology, Proceedings of the 1996 Western Canadian Dairy Seminar, 8:269-275.

Shimojo, M., and Goto, I. 1989. Effect of sodium silicate on forage digestion with rumen fluid of goats or cellulase using culture solutions adjusted for pH. Animal Feed Science Technology, 24:173-177.

Sisley, J. P. and Wood, P. J. 1952. Encyclopedia of Surface-Active Agents. Chemical Publishing Co., Inc., New York.

Smith, G.S. and Nelson, A.B. 1975. Effects of sodium silicate added to rumen cultures on forage digestion, with interactions of glucose, urea and minerals. Journal of Animal Science, 41:891-898.

Stack, R.J. and Hungate, R.E. 1984. Effect of 3-phenylpropanoic acid on capsule and cellulases of *Ruminococcus albus* 8. Applied and Environmental Microbiology, 48:218-223.

Stack, R.J. and Hungate, R.E. 1985. Biochemical properties of the cellulase complex of *Ruminococcus albus*. Abstracts of the Annual Meeting of the American Society for Microbiology, 85, K129.

Stauffer, C.E. 1990. Functional additives for bakery foods. Van Nostrand, Reinhold, New York.

Stutz, R.L., Del Vecchio, A.J., Tenney, R.J. 1973. The role of emulsifiers and dough conditioners in foods. Food Development, 62:52-56.

Sukan, S., Guray, A., and Suakn, F. 1989. Effects of Natural Oils and surfactants on cellulase production and activity. J. Chem. Tech. Biotechnol., 46:179-187.

Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. Journal of Dairy Science, 75:345-357.

Thoman, C., Habeeb, T., Huhn, M., Korpusik, M. and Slish, D. 1989. Use of polysorbate 80 (Tween 80) as a phase-transfer catalyst. Journal of Organic Chemistry, 54:4476-4478.

Triggle, D.J. 1970. Some aspects of the role of lipids in lipid-protein interactions and cell membrane structure and function. Recent Progress in Surface Sci., 3:273.

Van der Linden, Y., Van Gylswyk, N.O. and Schwartz, H.M. 1984. Influence of supplementation of corn stover with corn grain on the fibrolytic bacteria in the rumen of sheep and their relation to the intake and digestion of fiber. Journal of Animal Science, 59:772-83.

Van Soest, P. J. 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. Journal of Animal Science, 24:834-843.

Van Soest, P.J. 1994. Nutritional Ecology of the Ruminant, 2nd ed. Cornell University Press, Ithaca, NY.

Varga and Hoover, 1983. Rate and extent of neutral detergent fiber degradation of feedstuffs *in situ*. Journal of Dairy Science, 66:2109.

Walker, L.P. and Wilson, D.B. 1991. Enzymatic Hydrolysis of Cellulose. Reprinted from Bioresource Technology, 36(1), Elsevier Science Publishers Ltd., New York.

White, B.A., Rassumssen, M.A. and Gardner, R.M. 1988. 1988. Methylcellulose inhibition of exo-β-1,4-glucanase A from *Ruminococcus flavefaciens* FD-1. Applied Environmental Microbiology, 54:1634-1636.

White, B.A., Mackie, R.I., and Doerner, C. 1993. Enzymatic hydrolysis of forage cell walls. Pp. 455-484 in Jung, H.G., Buxton, D.R., Hatfield, R.D. and Ralph, J. (Eds.) Forage Cell Wall Structure and Digestibility. American Society of Agronomy, Inc., Madison, WI.

Whitehouse, N., Olson, V., Schwab, C. Chesbro, W., Cunningham, K. and Lykos, T. 1993. Improved Techniques for dissociating particle-associated mixed ruminal microorganisms from ruminal digesta solids. Journal of Animal Science, 72:1335-1343.

Williams, A.G. and Strachan, N.S. 1984. The distribution of polysaccharide-degrading enzymes in the bovine rumen digesta ecosystem. Current Microbiology, 10:215-220.

Windham, W.R. and Akin, D.E. 1984. Rumen fungi and forage fiber degradation. Applied Environmental Microbiology 48:473-76.

Wood, T.M. 1985. Properties of cellulolytic enzyme systems. Biochem. Soc. Trans., 13:407-10.

Wood, T.M. and McCrae, S.I. 1979. Synergism between enzymes involved in the solubilization of native cellulose. Pp. 181-210 in: Brown, R.D. and Jurasek, L. (Eds.) adv. Chem. Ser. 181, American Chemical Society, Washington.

Wood, T.M., Wilson, C.A. and Stewart, C.S. 1982. Preparation of the cellulase from the cellulolytic anaerobic rumen bacterium *Ruminococcus albus* and its release from the bacterial cell wall. Biochemical Journal, 205:129-137.

Woolcock, J.B. 1991. World Animal Science: Microbiology of Animals and Animal Products. Elsevier, New York.

Yoder, R.D., Trenkle, A. and Burroughs, W. 1976. Influence of rumen protozoa and bacteria upon cellulose digestion *in vitro*. Journal of Animal Science, 25:609-612.

ZoBell, C.E. 1943. The effect of solid surfaces upon bacterial activity. Journal of Bacteriology, 46:39-56.

3. THE EFFECTS OF A NONIONIC SURFACTANT 'TWEEN 80' ON THE PERFORMANCE OF LACTATING DAIRY COWS FED A TOTAL MIXED RATION

3.1 Introduction

One of the ultimate goals of the dairy nutritionist is to help the cow reach her potential for MY and composition using a cost-effective diet while minimizing the negative implications of this diet on physiological process and waste production. It is well known that ruminants maintain a symbiotic relationship with the microorganisms in their rumen. In this relationship, microorganisms ferment ruminally available dietary components into microbial protein and VFAs that in turn are absorbed and metabolized by the cow as a source of protein and energy, respectively. In effect, feeding the dairy cow involves optimizing ruminal microbial production and supplying the additional nutrients needed to maximize milk. The nonionic surfactant, T-80, has been shown to facilitate the degradation of cellulose in anaerobic bioreactors (Helle et al., 1993; Castanon and Wilke, 1981). When this information was applied to the ruminant, positive production and digestibility responses were observed (Shelford and Baah, 1998; Baah, 1998; Shelford and Baah, 1997, Shelford et al., 1996; Kamande, 1994). Further research is needed on the mode of action and dietary treatment levels to optimize the positive impacts of T-80 supplementation in the ration of the lactating dairy cow.

To achieve optimal microbial production, synchronization of N and energy sources and their rates of fermentation must occur (Tamminga, 1992). Rumen organisms are highly proteolytic (Orskov, 1992) and thus energy is often limiting of microbial growth, resulting in inefficient N utilization and reduced microbial and VFA

production. To complement high rumen degradability of protein, diets rich in NSCs (sugars and starch) are required to match protein fermentation rates, however the use of such carbohydrates is limited by various factors. Some of these limitations include negative implications on rumen pH, low fiber content inadequate to stimulate normal rumen function and high feed costs over those of carbohydrate sources unavailable for human consumption (i.e., forages). Forages contain both structural and NSCs. The NSCs or cell solubles are rapidly degraded similar to the sugars and starch of cereal grains, leaving behind a wealth of potentially available SCs in the cell wall. The energy from the SCs is not as readily available, requiring microbial fermentation for its release. In comparison to carbohydrates supplied in cereal grains or concentrate rations, forages have more desirable fiber levels to stimulate normal rumen function and are more economical to feed.

Cellulose is the most abundant SC in forages and amounts to 20 to 40% of the DM of all higher plants (Van Soest, 1994). Rumen microorganisms provide the enzymes necessary to degrade cellulose provided adequate fermentation time in a hospitable rumen environment. Thus, cellulose's energy availability and contribution to microbial synthesis as a carbohydrate source is limited by its slower rate of fermentation with respect to the rapidly degraded dietary protein and soluble carbohydrates. A means of enhancing ruminal cellulose fermentation would be desirable.

The nonionic surfactant polyoxyethylene monooleate or 'Tween 80' has been shown to have positive effects on enzymatic cellulose degradation in numerous *in vitro* studies using various microorganisms. Work by Castanon and Wilke (1981) and Helle et al. (1993) has shown T-80 to increase the rate and extent of cellulose hydrolysis in

anaerobic bioreactors. When this information was applied to the ruminant, positive production responses were observed (Shelford et al., 1996; Shelford and Baah, 1998; Shelford and Baah, 1997; Baah, 1998; Kamande, 1994).

The mode of action of T-80 remains unclear. Its chemical characteristics as noted in its use in the baking and confectionery industry for tolerance of ionic and pH fluctuations (Stauffer, 1990) are promising for its use in the variable rumen environment. In addition, T-80's positive implications on improving substrate wettability, microbial membrane permeability, enzyme production and total microbial count were noted. T-80's ability to decrease non-productive enzymatic binding to substrate has been suggested, as well as its ability to positively influence the adsorption balance of cellulolytic enzymes. However, the use of T-80 is not without its potential drawbacks as have been observed under some experimental conditions. Negative implications of T-80 include lysis of some fiber degrading microorganisms and their elution from substrate to which they were previously attached. Kamande (1994) and Shelford et al. (1996) reported that T-80 increased rumen proteolysis, however Kamande (1994) suggested that proteolysis could be minimized while maintaining beneficial effects on cellulose fermentation at lower T-80 treatment levels.

In previous ruminant studies, T-80 has displayed positive responses at dietary concentrations of 0.5% (Kamande, 1994) and 0.2% (DM basis) (Shelford and Baah, 1997). Thus, the objective of this study was to compare a Control diet to those treated with T-80 at either 0.2% or 0.1% (DM basis) to determine the optimal level of supplementation as indicated by MY, milk composition and apparent digestibility parameters.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Design

Fifteen, multiparous Holstein cows in early lactation ranging from three to eight years of age (mean = 4.7 years) were blocked based on estimated 305 d production and days in milk (DIM) and randomly assigned to three different treatments. Experimental cows were housed in a separate area, adjacent to the main milking herd in a free-stall barn located at the UBC Dairy Research and Education Center in Agassiz, BC. Cows were fed using Calan feeding doors (American Calan, Inc., Northwood, NH) as a means of monitoring individual feed intakes and had *ad lib* access to water and resting areas. One week was allotted to train the cows to the doors prior to the beginning of the experiment.

The statistical design implemented was a 3 X 3 Latin Square consisting of three, four-week periods. Each period consisted of two weeks for diet adjustment followed by two weeks of data collection. During the last five days of each period a total tract digestibility study was performed on 12 of 15 cows (four blocks). One block was omitted from the total tract digestibility study due to limitations in availability of metabolism stalls. Cows were fed their respective diets in order to facilitate total collections in which only six cows could be collected from at any given time. For example, at the end of the fourth week of the study the six cows that had just completed total collections plus the three cows (from the block omitted from total collections) were changed to their new TMR for the second period. In the fifth week, the six remaining cows were subject to total collections before they were changed to their new TMR for their second period that began in the sixth week of the study.

3.2.2 Ration Composition

A representative sample of a commercial dairy concentrate [barley based, 18% CP, as fed basis], corn silage and grass silage was taken for pre-trial ration formulations. Samples were dried in a forced air oven at 60 °C until a constant weight was achieved, and then ground through a 1 mm screen. Prior to chemical analysis, residual DM determinations were made on duplicate, 1 g ground samples placed in aluminum trays in a forced-air oven at 100 °C until a constant weight was achieved (24-h). Samples were then analyzed for CP and NDF. CP was determined using the Leco FP-428 N analyzer (Leco Corp., St. Joseph, MI., USA) and NDF using the modified method of Van Soest et al. (1991) called the filter bag technique (ANKOM Co., Fairport, NY; Komarek et al., 1994) using the Ankom fiber analyzer (#F200, ANKOM Co., Fairport, NY). In feeds (i.e., grain concentrates) known to contain high quantities of starch, the NDF procedure included the use of a heat stable alpha-amylase (#FAA, Ankom Co., Fairport, NY) as recommended by Van Soest et al. (1991). Corn and grass silages were balanced to achieve a 16% CP content from the forage fraction of the diet resulting in a 33:66 corn silage to grass silage ratio. The resulting silage mixture was combined with the 18% CP (as fed basis) commercial dairy concentrate to achieve a 55:45 forage to concentrate ratio (DM basis).

3.2.2.1 Dietary Treatments and Ration Preparation

Three different treatments were applied to the TMR: Control where the TMR was fed as is, and 0.1% and 0.2% T-80) on a DM basis. Rations were prepared once daily at 1100 h by combining individual feed components in a Tycrop mixing wagon equipped

with an electronic scale. Freshly prepared ration was stored in uncovered, plastic storage containers until designated feeding times.

To prepare the ration, individual feed components were added to the mixing wagon to adequately meet the daily requirements of the three treatment groups. Requirements were based on an estimated goal of feeding the cows 110% of *ad lib* intake. The ration in its entirety was mixed for five min and the control fraction (approximate one third of the total, or ~220 kg) was removed first. A pre-calculated volume of T-80 was diluted in two liters of hot water in a four liter watering can. This T-80 dilution solution was added to the mixing wagon by slowly pouring over the turning contents in the wagon. Sufficient T-80 was added to the ration to bring the contents to the 0.1% T-80 level based on DM determinations of feed components (See Table 3.1). The ration was mixed for three min post T-80 application and prior to removal from the mixing wagon. The remaining TMR (now at 0.1% T-80) had additional T-80 dilution solution added to bring the final level to 0.2% T-80, was mixed for three min and removed from the mixing wagon. For example, after removal of the 0.1% T-80 ration, 220 kg of 0.1% T-80 TMR remained in the mixing wagon. To bring this to 0.2% T-80, an additional 116 ml of T-80 diluted in two liters of hot water was added, mixed and removed as the 0.2% T-80 ration.

Table 3.1 TMR ration formulation ratios with corresponding Tween 80 application levels on an as fed basis.

Total TMR*, kg, as fed	Concentrate, kg, as fed	Corn Silage, kg, as fed	Grass Silage, kg, as fed	0.1% Tween 80**, ml	0.2% Tween 80**, ml
180	47.6	67.4	65.1	95	189
200	52.8	74.9	72.3	105	210
220	58.1	82.4	79.5	116	231
240	63.4	89.8	86.7	126	252
260	68.7	97.3	94.0	137	273

* Based on TMR ratio of 9.9 : 4.84 : 7.26 (kg, DM basis) or 11.06 : 15.67 : 15.13 (kg, as fed) of concentrate : corn silage : grass silage.
** Volume (ml) of Tween 80 required to bring total TMR mass (kg, as fed) to the respective percentage concentration on a *DM basis*

Given that approximately 230 kg of TMR was prepared daily for each treatment group (about 690 kg total), the contribution of the T-80 dilution solution to overall change in DM content was considered negligible.

Cows were fed 25 kg of TMR each morning at 0800 h with the remainder of the daily ration being fed at 1600 h. Weighbacks (WB) totaled approximately ten percent of feed offered and were determined each morning prior to feeding. Feed intakes were calculated daily, averaged by week, and corrected for DM content to calculate daily dry matter intake (DMI).

3.2.2.2 Ration Sampling

The TMR was sampled three times per week, composited weekly, and frozen at –4 °C. WB samples were taken twice weekly for each cow, composited by week for

each ration and frozen at –4 °C. DM content was determined on the samples by drying them in a forced air oven at 60 °C until a constant weight was achieved (72 h). Samples were ground through a 1mm screen and stored until further chemical analysis of CP, NDF and ADF. Similar to NDF, ADF was determined using the modified method of Van Soest et al. (1991) called the filter bag technique (ANKOM Co., Fairport, NY; Komarek et al., 1994) using the Ankom fiber analyzer (#F200, ANKOM Co., Fairport, NY).

3.2.3 Milk

Milk yield was recorded twice daily at 0600 h and 1800 h, totaled and weekly averages determined for each cow. Only week three MY data of each period was reported to accommodate the two week ration adjustment at the beginning of each period and due to total collections in week four of each period. Milk samples were collected for four consecutive milkings on d 19 and d 20 of each period and were analyzed for fat and protein by infrared spectroscopy and for somatic cell count (SCC) using a Fossimatic cell counter (BC DHIS Lab, Chilliwack, BC). Total yields of the individual components were calculated using the average of the four MY values from the respective two-day collection period. Fat, protein and lactose milk compositional data were reported as percentages in milk and calculated yield (kg d^{-1}). Somatic cell count (SCC) was measured as number of cells (x 10^3) $ml^{-1}$of milk sample. Four percent fat corrected milk (4% FCM) yield was calculated using the following equation (NRC, 1989):

4% FCM (kg d^{-1}) = (0.4 + (0.15 *x* % fat)) *x* kg milk d^{-1}

3.2.4 Rumen Fluid and Blood

Rumen fluid samples (200 ml) were collected by vacuum stomach tube from each cow on d 19 of each period between 1000 h and 1130 h. Immediately post collection, pH was determined and two, ten milliliter samples were centrifuged. Duplicate samples were acidified to pH 2.0 by combining four milliliters of centrifuged rumen liquor with 1 ml 25% H_3PO_4 solution. Samples were frozen at –4 °C until VFA analysis. VFA composition was determined by gas chromatography using a Shimadzu gas chromatograph equipped with a capillary column (30 x 0.25 mm I.D. Stabilwax-DA). Injection port temperature was set at 170 °C. The column temperature was set at 120 °C to 180 °C at 10 °C min^{-1}, with an initial time of one min and a final time of two min. The internal standard used was isocaproic acid (0.70 g in 200 ml water). VFA data were reported as molar proportions (mol $100mol^{-1}$ or percentage of total VFA) and total production (mg ml^{-1}).

Blood samples (20 ml) were taken in duplicate via jugular venipuncture into Vacutainers containing heparin on d 19 of each period between 1000 h and 1130 h. Vacutainers were gently swirled immediately post collection to distribute the heparin and placed on ice until further processing. To prepare samples, blood was centrifuged and plasma was separated and frozen for later analysis of BUN by colorimetric procedures using Kodak Ektachem DT 60 analyzer with Disc Two Module (Clinical Products Division, Eastman Kodak Co., Rochester, NY).

3.2.5 Total Collections

Cows were confined to metabolism stalls for the total collection of urine and feces from d 23 to d 28 or each period. Feces and urine were collected as described

by Dinn (1996). Urine was collected to prevent the contamination of feces. Fecal output was expressed in kg DM d^{-1}, while urine output was given as L d^{-1}. Fecal samples were dried in a forced-air oven at 60 °C until a constant weight was achieved to determine DM. Dried samples were ground through 1 mm screen, composited for each cow-treatment-period and stored. Samples were analyzed for NDF, ADF and N by the same methods as the ration samples, excluding the use of alpha-amylase.

Apparent Digestibility (AD_x) was calculated for individual cows from total collections TMR intake and fecal output data,:

$\mathbf{AD_x}$ = [(*X* fraction in TMR – *X* fraction in Feces) / *X* fraction in TMR] x 100%, where *X* = ADF, NDF, CP and DM

Individual water intakes were recorded daily for cows in the metabolism stalls. Cows were weighed at the beginning of the trial and again on d 20, 21 and 22 at 1100 h of each period (prior to total collections) to monitor changes in body weight (BW) throughout the experiment.

3.2.6 Statistical Analysis

The data were analyzed using least squares analysis of variance (ANOVA) following the general linear model (GLM) procedure of the Statistical Analysis System (1990) and the program in Appendix 1. Differences between treatments were considered significant at $P<0.05$. The statistical model is given below:

$$Y_{ijkl} = \mu + B_k + T_j + P_l + C_{i(k)} + e_{ijl(k)}$$

Where:

Y_{ijkl} = response variable

μ = overall mean

B_k = effect of block, k = 1,..., 5

T_j = effect of treatment, j = 1,..., 3

P_l = effect of period, l = 1,..., 3

$C_{i(k)}$ = effect of cow within block, i = 1,..., 3

$E_{ijl(k)}$ = experimental error

3.2.7 Efficiency Calculations

Efficiency of MY ($\mathbf{E_{MY}}$) was calculated for individual cows by the following formula for each observation of the respective variables in the third week of each period. Units were reported as kilograms of MY per kilogram of DMI. :

$$\mathbf{E_{MY}} = [\text{MY (weekly mean, kg)} / \text{DMI (weekly mean, kg)}] \times 100\%$$

3.2.8 Post Trial Analysis

3.2.8.1 True Digestibility

Lack of treatment differences in apparent digestibility estimates prompted a post trial analysis of *in vitro* true digestibility (IVTD). Duplicate samples of TMR from each treatment and week were weighed out into filter bags (#F57, Ankom Co., Fairport, NY) and heat-sealed. The prepared filter bags (#F57, Ankom Co., Fairport, NY) were subject to the first step of the Tilley and Terry (1963) rumen *in vitro* digestion procedure. An *in vitro* apparatus (IV100, Daisy II, Ankom Co., Fairport, NY) facilitated this digestion, maintaining a constant temperature of 39.5 °C and rotation rate of one rpm to simulate rumen conditions. Samples were removed after 8 h incubation, washed in distilled water and dried in a forced-air oven at 60 °C until constant weight was achieved. NDF was determined on samples post *in vitro* digestion to obtain IVTD by difference from the original sample weight (Goering and Van Soest, 1970).

IVTD = [weight of dry sample post *in vitro* and NDF analysis, g / (weight of original sample, g x residual DM of respective sample, %)] x 100%

3.2.8.2 Additional T-80 and IVTD

Samples of TMR from each of the respective experimental treatments from week four (week four was arbitrarily chosen from the 13 week experimental study) had a calculated volume of T-80 added to achieve 0.2% T-80 (DM basis), regardless of prior treatment level (i.e., Control, 0,1% T-80 and 0.2% T-80 were all increased by 0.2% T-80). To achieve this, a five percent T-80 solution (v / v) was prepared by combining five milliliters of T-80 in 95 ml of distilled water. Approximately five grams of sample (DM

basis) was placed in a beaker and the corresponding volume of five percent T-80 solution (v / v) was added to the dry sample to increase its T-80 concentration by 0.2% and stirred. To ensure that equal volumes of liquid were added to each sample, additional distilled water was added to each sample to bring the total volume of liquid added to one milliliter. Each sample was stirred again and subjected to the same procedure for IVTD described in 3.2.8.1 with the exception that samples were subject to digestion times of 8 h and 24 h.

3.2.8.3 Acid-Detergent Insoluble Nitrogen

Analysis for acid detergent insoluble nitrogen (ADIN) was conducted on individual silage samples that were collected in the latter half of the trial once it became evident that the quality was not improving. Factors indicative of poor silage quality included visible signs of mold and spoilage, increases in silage temperature as determined by tactile manipulation and finally, an "off" odor compared to silage considered to be good quality. The samples analyzed included eight grass silage samples collected from November 21, 1997 to January 15, 1998, two corn silage samples collected within the same time frame and the respective TMR samples were analyzed for ADF. The N content of the ADF was determined using the Leco-428 N analyzer (Leco Corp., St. Joseph, MI) to yield ADIN.

ADIN as a percentage of total N was calculated for each of the respective TMR samples and corresponding grass and corn silage samples (See Appendix 2 for sample calculations). The TMR was analyzed to provide a basis on which to evaluate the contribution of ADIN from the silage.

3.3 RESULTS

3.3.1 Ration Composition

3.3.1.1 Pre-Trial Ration Component Analysis

The results of the pre-trial ration analysis are given in Table 3.2. All TMR components (i.e. dairy concentrate and silages) were analyzed for on a DM basis for NDF and CP. NDF of the pre-trial concentrate sample was 39.3%, unusually high for a concentrate, however, analysis of concentrate samples collected later in the trial revealed a more acceptable NDF in the range of 25%. NDF content of individual feed components was not used in ration balancing and thus did not affect the final ration composition.

3.3.1.2 Composition of TMR

TMR ration composition data is given in Table 3.3. Reported values were on a DM basis. There were no differences ($P>0.05$) observed for CP, ADF or NDF for the three treatment levels as expected, confirming the TMR was equal in protein and fiber quality for each dietary treatment.

3.3.2 Dry Matter Intake and Body Weight

The influence of diet on DMI and BW is given in Table 3.4 for the three treatment levels. There were no differences ($P>0.05$) in DMI with all groups maintaining intake levels in excess of 26 kg DM d^{-1}. Similarly, there were no differences observed in BW change as a result of treatment or DMI as a percentage of BW.

3.3.3 Milk Parameters

The influence of diet on MY and component yield is given in Table 3.5 for the three treatment levels. There were no differences ($P>0.05$) observed between treatment levels for MY (kg d^{-1} cow^{-1}). All treatment levels maintained production over the 12 week experimental duration at 37 kg of 4% FCM milk cow^{-1} d^{-1}.

Protein percentage was significantly greater ($P<0.05$) in Control over 0.2% T-80 cows, although the same as 0.1% T-80. T-80 treatment groups were not significantly different from each other for protein percentage. Differences observed in protein percent were compensated for when expressed as yield (kg d^{-1}) as differences were no longer significant ($P>0.05$). No differences ($P>0.05$) were observed in the fat, lactose or SCC data.

3.3.4 Blood Urea Nitrogen

The influence of diet on BUN is given in Table 3.6. No differences ($P>0.05$) were observed for the three treatment groups, however, BUN in cows on the 0.2% T-80 treatment tended to be lower ($P=0.8$). There was a significant period effect ($P<0.05$) having BUN levels of 15.81, 17.50 and 20.16 mg dl^{-1} for periods one, two and three, respectively (data not shown).

3.3.5 Ruminal Fluid

The influence of diet on ruminal fluid pH and VFAs are given in Table 3.7. There was no difference ($P>0.05$) observed for pH. The VFAs measured included acetic, propionic, butyric, caproic and the branched-chain isovaleric and isobutyric. The A : P ratio was calculated and reported as well. There were significant differences in acetic

acid production (P<0.05) when expressed as a molar proportion of total VFA, however no other differences were noted in VFA compositional data, nor when acetic and propionic acids were expressed as a function of each other in the A : P ratio (P=0.8). At the given level of significance for the A : P ratio, 0.2% (DM basis) had a higher A : P ratio than 0.1% (DM basis) T-80 although neither treatment was different from the Control.

3.3.6 Digestibility Parameters

3.3.6.1 Total Collections

The influence of diet on water intake and fecal and urinary output are given in Table 3.8. There were no differences (P>0.05) observed for each of the three total collections parameters measured, nor of the fecal DM percentages.

3.3.6.2 Apparent Digestibility Estimates

Table 3.9 depicts the influence of diet on apparent nutrient digestibility during week four of each experimental period for DM, ADF, NDF and CP. There were no differences (P>0.05) observed for each of the respective categories, however, apparent NDF digestibility in cows on 0.2% T-80 tended to be lower than either the Control or 0.1% (DM basis) treatments (P=0.10).

3.3.7 Efficiency of Milk Yield

Calculated values for the efficiency of milk yield are given in Table 3.10. No differences (P>0.05) were observed for the calculated values of efficiency.

3.3.8 Post Trial Analysis

3.3.8.1 True Digestibility

For verification of the DM digestibility estimates observed, an *in vitro* study was performed on TMR samples from each week (1 through 13) and treatment with the subsequent estimates of true digestibility given in table 3.11. Results revealed no significant differences ($P>0.05$) in true digestibility. Numerical differences paralleled those of apparent DM digestibility with the Control ration displaying higher estimates than the treatments.

3.3.8.2 True Digestibility: Additional T-80

As only one TMR sample of each treatment from week four was subject to the $IVTD_{additional\ T\text{-}80}$ analysis, statistical analysis was not performed. Observed data are reported in Table 3.12. Numerical comparisons were made between 8 h and 24 h digestion times and for control and T-80 supplemented treatments for each of the three main experimental treatments of Control, 0.1% (DM basis) T-80 and 0.2% (DM basis) T-80.

3.3.8.3 Acid Detergent Insoluble Nitrogen

Results of a post-trial ADIN determination are given in table 3.13. ADIN%CP levels in TMR samples ranged from 6.29% to 8.08%, for grass silage ADIN%CP ranged from 4.80 to 6.82 and for two individual corn silage samples tested for verification, ADIN%CP levels were 3.90 and 4.09. There were not enough samples to conduct a statistical analysis.

3.4 DISCUSSION

3.4.1 Ration Composition

3.4.1.1 Pre-Trial Ration Component Analysis

Values for NDF and CP for corn and grass silage were within the ranges reported by NRC (1989) and Mertens (1992). For comparison with forages of local farms, data were compared to work by Shelford et al. (1994) in which samples were collected in BC on individual farms and analyzed for their nutritional variation. The grass and corn silage used on trial were both approaching the maximum values reported for DM% within the ranges specified (Shelford et al., 1994). In particular, grass silage was much drier at 48.0% DM than the mean of the reported grass silage samples at 27.4% DM. Likewise, the experimental corn silage was 30.9 %DM, approaching the maximum value reported by Shelford et al. (1994) for corn silage at 32.0% DM and several percentage units above the reported mean of 27.4 %DM. High silage DM was reflected in the apparent silage quality in which there was a high degree of spoilage, particularly visible in the grass silage.

The overestimate of the reported pre-trial NDF value for the dairy concentrate was attributed to the accidental omission of heat stable alpha-amylase (#FAA, Ankom Co., Fairport, NY) as recommended by Van Soest et al. (1991) for feeds containing high quantities of starch. Analysis of concentrate samples collected for the trial revealed NDF in the range of 25%, more representative of that reported by NRC (1989) although still somewhat high. NDF content of individual feed components was not used in ration balancing and thus did not affect the final ration composition. Had it been taken into account, fiber content of overall TMR would have been closer to NRC (1989) recommendations.

Questionable Silage Quality

The high DM silage was more susceptible to aerobic conditions favoring molding, heating and subsequent potential losses of the heat stimulated Maillard reaction. If silage of slightly lower DM content was utilized perhaps spoilage level and losses would have been reduced. A great effort was made to manually remove the most visibly damaged of this material (i.e., growth of mold) but some contamination was unavoidable.

According to Van Soest (1994), there are many advantages to using well prepared, high DM silage (greater than 30% DM) including increased nutritive value and palatability resulting in greater intakes. There are also the disadvantages of potential increased molding and heating, as oxygen removal becomes more difficult in drier silage, leading to losses of carbohydrates, proteins and a subsequent reduction in digestibility. Van Soest (1994) recommended that the most stable hay crop silages are from 40 to 50% DM. In the current study, grass silage ranged from 36.5 to 62.1 %DM and corn silage from 30.9 to 36.2 %DM (data not reported; silage was prepared in August 1997) indicating the variability in DM observed.

Silage management recommendations suggest that up to 15 cm should be removed from the silage 'face' each day, especially in warm weather, to minimize contact of silage with oxygen and to ensure quality of forage fed (Chr. Hansen BioSystems, 1991). As only 15 cows were being fed from the horizontal silo bag, a relatively small amount of silage was required each day. Progression through the silage was slow and the time the face of the silage was exposed to oxygen was high relative to the quantity utilized. The removal of 15 cm of silage from the entire face was not always practical and thus the quality of the forage fed was jeopardized.

Contamination was not exclusively due to aerobic conditions arising at the silage-air interface. Spoilage was occurring deep within the bag as was discovered in aggressive attempts to move beyond the spoilage at the silage-air interface. Heating and molding of both grass and corn silage was evident early in the trial, but cooler temperatures in the second (beginning November 16, 1997) and third periods (ending January 16, 1998) helped minimize apparent spoilage in the corn however the grass spoilage continued throughout the trial.

Van Soest (1994) acknowledged the effects of heat on forage protein, noting that heat damages protein. Excessive heating can bind proteins to carbohydrate fractions and make both unavailable for digestion by the animal (Chr Hansen Biosystems, 1991). More specifically, this is called the Maillard reaction and involves the condensation of amino groups with carbonyls and dehydroreductones derived from carbohydrate followed by polymerization into an indigestible, lignin-like matrix (Van Soest, 1994). Animals fed a mildly heated feed may show increased protein utilization despite the slight reduction in protein digestibility. This is possible due to the benefits of decreasing rumen protein solubility through denaturation from RDP to RUP, subsequently overriding the losses of excessively available RDP.

It is unquestionable that the silage, and in particular grass silage, used on this trial was experiencing negative, aerobic fermentation. This issue was not addressed early in the trial as it was presumed that this spoilage was due to factors arising from opening a new silo bag – that the material in the end of the bag was subject to air contamination due to the inability of it to be adequately packed at the bag end. Silage quality factors were not addressed until post-trial and are referred to at a later point in the discussion (Post-Trial Analysis: Acid-Detergent Insoluble Nitrogen).

3.4.1.2 Composition of TMR

Crude Protein

Observed CP values of almost 19% were adequate to meet production needs. Dairy rations that are not balanced precisely for RDP and ruminally undegradable protein (RUP) are usually formulated with extra protein to ensure that the quantity of RUP is met as it helps to fulfill protein requirements beyond that produced by microbial metabolism. According to NRC (1989), dairy cows in early lactation (weeks zero to three) require approximately 19% CP, which later falls to 16 to 17% CP as production stabilizes in early lactation (weeks 4 to 15), and continues downward to about 12% CP in late lactation as dry-off approaches. The mean stage of lactation of all cows on trial was 45 DIM (6.4 weeks) at the beginning of the first 28 d period. This confirmed that cows began the trial in early lactation and would finish early in their mid-lactation trimester at an average of 129 DIM (18.4 weeks) assuming an estimated 305 d production cycle.

Neutral-Detergent Fiber

NRC (1989) recommendations for daily intake include a minimum NDF of 25 to 28% on a DM basis, with higher producing cows on the lower end of this scale. NDF was negatively correlated with intake (Mertens 1992, 1980, 1982, 1985a&b, Varga and Hoover, 1983) and digestibility (Van Soest, 1994). The observed values for the TMR of about 40% NDF were higher than NRC (1989) recommendations but did not appear to limit intake. NRC (1989) suggested that 75% of NDF be provided as forage. Despite marginal week to week variations in ration composition, calculations (not shown) suggest that the experimental ration had approximately 70% of the NDF originating

from forage. Although approaching NRC suggestions, NDF of the TMR could have been reduced if closer attention was given to NDF of the concentrate and the TMR adjusted accordingly.

Acid-Detergent Fiber

NRC (1989) recommends minimum ADF values for ruminant diets, and for the dairy cow in early lactation they range from 19 to 21% on a DM basis. The lower values within this range are more applicable to higher producing cows so as not to limit intake of high energy and protein dietary inputs (NRC, 1989). The observed values of about 24 % ADF met the minimum requirement and did not seem to impair intake levels, paralleling NDF data and intake observations.

3.4.2 Dry Matter Intake and Body Weight

3.4.2.1 Dry Matter Intake

NRC recommendations (1989) suggested DMI of cows in early lactation might be up to 18% less than the predicted values for mid- to late-lactation because of lowered appetite during that period. According to formulas provided by NRC (1989) and 4% FCM and BW data from the current study, cows in mid-to late-lactation should consume approximately 3.57% of BW as DM to meet nutrient demands of milk production and normal weight gain. Cows in the current study were in early-lactation and thus their intake levels were expected to be lower than the calculated intake as a percentage of BW value. However, this was not the case and intake levels and DMI as a percentage of BW were excellent. Although not significantly different from each other, animals on Control, 0.1% T-80 and 0.2% T-80 diets consumed 3.88%, 3.92% and 3.97% of BW,

respectively. Obviously, the TMR and fiber levels observed were not limiting intake. Thus, observed values in DMI as a percentage of BW accounted for experimental cows (in early lactation) consuming from 2.2 to 2.7 kg DM d^{-1} more than NRC (1989) suggestions for cows in mid- to late-lactation and as much as 2.6 to 3.2 kg DM d^{-1} more than cows in early-lactation.

Intake: Previous results

Despite excellent intake levels observed in the current study, there were no differences in intake among treatment groups. Similarly, in a previous study by Shelford et al. (1996), cows were fed a diet based on grass and corn silage, grass hay, a partial mixed ration (barley, Canola, bypass mix, mineral and limestone) and a concentrate. The treatments included the Control where the ration was fed as prepared and two levels of T-80 supplementation ("High" and "Low", exact T-80 concentrations not provided) added to the ration. There were no differences in intake observed by Shelford et al. (1996) for both the high and low levels of T-80 supplementation compared to the Control. No differences in intake were observed in the current study ($P>0.05$) despite the fact that experimental cows were considered to be very uniform in terms of their stage of lactation.

In a trial conducted by Shelford and Baah (1997), cows were fed a TMR based on grass silage, corn silage, grass hay, barley and Canola meal. The treatments applied were TMR-0 (no additive), TMR-1 (45gT-80 d^{-1}, equivalent to 0.2% on a DM basis) and TMR-2 (45 g T-80 + 10 g xylanase enzyme d^{-1}). There were no differences in intake reported by Shelford and Baah (1997) when T-80 was incorporated at the 0.2%(DM basis) level with or without enzyme treatment.

In a lactation trial (Shelford and Baah, 1998) conducted during the summer of 1998 on the same farm as that of the current experiment (UBC Dairy Education and Research Center, Agassiz, BC), cows were fed a TMR based on grass silage, corn silage, grass hay and a dairy concentrate. The treatments were the Control where the ration was fed as is (treatment 1), 100g d^{-1} (treatment 2) or 150g d^{-1} (treatment 3) of a supplement containing T-80. No differences were reported in intake levels for the first ten weeks of the trial when a supplement containing T-80 was included at 100 or 150 g d^{-1}. During the last two weeks of the trial ambient temperatures exceeded 40 °C resulting in considerable heat stress. Cows on all treatments displayed reduced intake, however, cows on treatment 2 and 3 displayed improvements in intake of up to 15.9% and 17.7%, respectively, over that of the Control treatment. Although an experimental response in group feed intakes was not observed, there was an increase in production efficiency noted early in the study with treated cows performing better than Control. The current study reported intakes in period 1 of 25.3, 26.9 and 26.5 kg DM d^{-1} for Control, 0.1% T-80 and 0.2% T-80, respectively (data not shown), however, neither treatment ($P=0.69$) or period ($P=0.21$) statistical differences were observed.

The influence of T-80 on intake in non-dairy ruminants was reported. For example, Baah (1998) fed a TMR of rolled barley, corn silage and Canola meal to beef steers. Four treatment levels were evaluated having either: 1) no additives (Control); 2) 0.1% (DM basis) T-80; 3) one containing a fibrolytic enzyme; and finally, 4) fibrolytic enzyme and 0.1% (DM basis) T-80. Exact intake levels were not discussed, however, no differences in feeding efficiency were observed indicating that steers with greater weight gains ($P<0.05$) also ate more. In work by Kamande (1994) utilizing four ewes fitted with rumen fistulae, T-80 was added at 0.5% (DM basis) to a 100% grass hay

ration and increased DMI as a percentage of BW by almost 40%. Due to similarities in their digestive processes, the studies of Baah (1998) and Kamande (1994) are of interest when comparing intakes of the dairy cows in the current study.

When applicable, the Control treatments of Dinn (1996) and Wright (1998) were referred to provide a comparison for the current study to two previous studies on the same farm. Despite differences in dietary composition, the work of Dinn (1996), Wright (1998) and that of the current study all had similar goals of high MY and efficient feed utilization. In reference to intake, Dinn (1996) observed intake levels of 3.56 and 3.67% of BW in two consecutive studies and Wright (1998) reported an intake of 3.8% of BW. In the current study, intake as a percentage of BW of the Control exceeded that of Dinn (1996) and was comparable to Wright (1998).

Intake Rationale

Intake is limited by the bulky nature of the fibrous fraction of the diet, the time required for mastication and microbial degradation to reduce this fiber to a point where it can be passed from the rumen to the lower digestive tract. According to Prins and Kreulen (1990), the chief determinant of the indigestible fraction is the degree of lignification, which typically ranges from 5 to 25% of the cell wall material depending upon species and plant maturity. The results of this experiment in terms of the lack of significant differences observed in DMI are not entirely unexpected. As mentioned, the majority of ruminant studies using T-80 have not reported dramatic increases in intake levels but rather efficiencies in ration utilization (Shelford and Baah, 1998; Shelford and Baah, 1997).

Intake of Neutral-Detergent Fiber

In relating intake levels to fiber composition of the TMR, Mertens (1992) proposed values for maximum NDF as percentage of BW for cows at various stages of lactation. According to this theory, cows in early lactation should consume no more than 1.1% of BW as NDF, increasing to 1.3% by mid-lactation as production demands decrease (Mertens, 1992). This was based on the principal of ensuring adequate concentrates in the ration as not to limit MY. In light of the fact that average intake of NDF as percent of BW for the duration of the trial was 1.58%, this theory suggested that cows on trial were consuming a diet of excess fiber and thus deficient in energy. Thus, experimental cows may not have reached their greatest potential MY.

Application of T-80 to TMR in Relation to Feeding Time

In the current study the ration was sampled for laboratory analysis immediately after preparation at 1100h, however the feed was fed at 1600h and the following morning at 0800h, or 5 and 21 h later, respectively. Signs of aerobic fermentation in the silages were observed as moldy patches and manually felt as heat when the TMR was prepared. Heating was also evident in the prepared TMR at the respective feeding times. Under anaerobic conditions, *in vitro* work reported by Shelford et al. (1996) observed an increase in proteolytic activities of 80% above controls, and increases in the initial rate of cellulose degradation by 75% when T-80 was included. It is speculated that the addition of T-80 to the TMR would result in increases in aerobic fermentation pre-feeding based on the observations by Shelford et al. (1996).

Carry-Over Effects

The impact of carry-over effects can minimize the impact of a particular treatment in statistical designs where animals are subject to more than one of the experimental treatments in succession. This occurs when the properties of the treatment remain active in the experimental animal for longer than the adaptation period. For example, in cows fed live yeast culture, Woodward (1999) experienced positive impacts of yeast supplementation on MY well beyond the removal of the yeast treatment from the diet. This was attributed to the positive changes the yeast had on the rumen microbial populations and the cow's ability to maintain these more favorable populations once yeast supplement was removed. If cows were subjected to more than one treatment level of yeast, the compounding effect of the existing microbial populations would make statistical analysis and interpretation impossible.

In the current study, the Latin Square design dictated that all animals receive all treatments for three periods of equal duration. Question arose as to the potential for carry-over effects contributing to the lack of differences observed in intake. The effect of T-80 supplementation on the rumen microbial population balance and acclimatization rates has not been determined and previous ruminant studies reported varied results in terms of evidence of carry-over effects. For example, a preliminary lactation trial by Shelford et al. (1996) demonstrated an immediate production response of 3.22 kg d^{-1} during a short, 25 d study. The production response dropped off as quickly as it began when the animals were removed from treatment, suggesting a lack of carry-over effects. Work by Kamande (1994) successfully used a 2 x 2 Latin Square design to show the positive impact of T-80 on sheep. The remainder of the studies using T-80 and ruminant animals (Baah, 1998; Shelford and Baah, 1998; Shelford and Baah 1997;

Shelford et al., 1996) have not subjected animals to more than one experimental treatment during the experiment.

Measures to Avoid Cross-Contamination of Treatments With T-80

Rations were prepared daily, with the Control and treatments removed in succession of increasing level of T-80. The mixing wagon was used after experimental preparation of TMR'S to prepare rations for other cows on the farm. It was assumed that this would serve to 'clean' the mixing wagon of traces of T-80 remaining post experimental ration preparation. As mentioned earlier, a means of determining the exact concentration of T-80 in a ration has yet to be determined and so the possibility of contamination cannot be completely ruled out. If contamination were occurring, the Control animals would have received trace amounts in their rations, significantly less than either of the experimental treatments. Precautions were taken in all other feeding methods to ensure there was no cross-contamination.

3.4.2.2 Body Weight

Difficulties in achieving adequate intake in early-lactation to meet MY and maintenance needs often place cows in a negative energy balance resulting in weight loss (NRC, 1989). This phenomenon was not observed in this study. All cows were in early lactation however maintained BW despite high production levels. In fact, average gains of 0.37, 0.12 and 0.31 kg d^{-1} over the three experimental periods were reported for Control, 0.1% T-80 and 0.2% T-80, respectively.

In a trial conducted in Oyster River, weight gains of cows on TMR-1 (0.2% T-80) were the same as TMR-0 (no additives) cows, and TMR-2 (0.2% T-80 plus xylanase

enzyme) was higher than that of TMR-0. Weight gain in treated dairy cattle was reported in a lactation trial (Shelford and Baah, 1998) where treatment 1 cows (Control) lost about 4.5 kg over the 90 d experimental period, however, cows on treatment 2 and 3 (100 and 150 g d^{-1} supplement with T-80) gained 10 kg and 7 kg, respectively. Baah et al. (1998) fed a TMR to steers having T-80 and experienced significantly higher ($P<0.05$) total weight gain (kg) than steers fed a control diet or one containing a fibrolytic enzyme either on its own or in combination with T-80. Shelford et al. (1996) reported all animals on trial lost weight; however, the inclusion of T-80 significantly decreased the amount of BW loss.

The above studies report positive influences of T-80 supplementation on weight gain. In comparison to the Control treatments in previous work on the same farm, Dinn (1996) observed daily weight gains of 0.198 kg d^{-1} and 0.339 kg d^{-1} in two consecutive studies and Wright (1998) observed a daily weight gain of 0.158 kg d^{-1}. A daily weight gains of 0.37, 0.12 and 0.31 kg d^{-1} for Control, 0.1% T-80 and 0.2% T-80 diets were observed in the current study. There were no significant differences observed in BW or BW change for all treatment groups in the current reported study, however, numerical differences indicate that Control animals gained 31 kg, followed by 0.2% T-80 cows at 26 kg and 0.1% T-80 cows at 10 kg. Although not significant, these differences show a tendency for T-80 treated cows on this trial to show reduced weight gain compared to those on the Control diet.

It is common for lactating cows to lose weight during the high-production of early lactation. This is due to inadequate energy supply to meet MY and maintenance needs (Staples et al., 1992). Weight gains of all experimental animals in the current study were indicative of a positive energy balance. The TMR fed was high in CP (~19%),

although not balanced for RDP and RUP, and given the forage to concentrate ratio of 55:45, potentially limiting in energy. In the absence of adequate energy from carbohydrate sources, excess AAs are used for energy in an inefficient metabolic process (Nocek and Russell, 1988).

3.4.3 Milk Parameters

3.4.3.1 Milk Yield

The results of this experiment were compared to previous work in lactating dairy cows regarding the influence of T-80 supplementation on MY. Shelford et al. (1996) reported positive impacts of T-80 supplementation in two separate studies. In the first study, cows were fed alfalfa hay supplemented with dairy concentrate and T-80. Significant responses to T-80 were observed by day seven, and a production response of up to 3.22 kg d^{-1} was noted in the 22 d study. There was a dramatic decrease in production when the cows were taken off treatment in the preliminary trial. In the full lactation trial, both the 'high' and 'low' treatments supplemented with T-80, or level 1 and level 2, respectively, reported higher yield than the Control. Milk yield of level 1 supplemented cows was greater than MY of level 2 cows. When the animals were classified according to DIM (either above or below 28 DIM at the beginning of the trial), positive responses were noted in both groups, however, responses were more dramatic in animals over 28 DIM. Thus, a lag in response to T-80 on MY was noted and it was concluded that cows greater than 28 DIM at the start of the 12 week trail displayed considerable advantages to the inclusion of the agent at the lower level. Similar delays to response of additive in early lactation were found in work on enzyme inclusion in lactating cow diets (Shelford et al., 1996). Cows in the current study averaged 45 DIM

(± 18 DIM) on day one of period one indicating that by the time data collection began in week three, all would have exceeded the minimum 28 DIM age requirement discussed in Shelford et al. (1996).

The positive impacts of T-80 on MY were observed by Shelford and Baah (1997). Yield of cows with 0.2% T-80 was increased by 0.76kg d^{-1} above the Control, and 0.2% T-80 plus xylanase enzyme yielded 0.96kg d^{-1} above the Control. A dramatic response was observed in heifers on 0.2% T-80 plus enzyme over the Control (2.6 kg d^{-1}). Cows in their second or greater lactation on 0.2% T-80 produced 1.31kg d^{-1} more than Control cows. All cows in the current study were selected to be in their second or greater lactation.

Shelford and Baah (1998) observed that cows consuming 100 g d^{-1} supplement containing T-80 produced 1.12 kg d^{-1} more than Control cows for the first ten weeks of the study. There were no differences between 150 g d^{-1} supplement containing T-80 and Control cows. During the last two weeks of the trial, cows experienced unexpected heat stress where ambient temperatures exceeded 40 °C. In this two week period, Control animals realized reductions in production from 2.6 to 8.3% greater than those on treatment. A positive response to lower T-80 level was noted in first calf heifers that calved at least 21 d prior to the start of the trial and also in mature cows. It was noted (Shelford and Baah, 1998) that the MY response to treatment was greater early in the trial. This was not observed in the current study, although numerical differences in period 1 indicate Control, 0.1% T-80, 0.2% T-80 were 40.2, 41.8 and 39.4 kg d^{-1}, respectively, with P=0.40 for the treatment by period interaction. In addition, pre-trial MY was statistically compared to yield in the first period, however, the variability noted in fresh cows was not conducive to achieving statistical significance.

Shelford (1996) used a 3 x 3 Latin Square design and observed a decrease in MY when T-80 was included in the ration at 0.25% (DM basis). There was no difference in MY of Control cows and those having 0.12% (DM basis) T-80 of total dietary intake.

In comparison to the Control treatments in previous work on the same farm, Wright (1998) reported 4% FCM MY of 33.8 kg d^{-1} and Dinn (1996) reported 4% FCM MY of 33.7 and 32.8 kg d^{-1} in two reported trials. In the current study, yields of 4% FCM of 37.1, 37.0 and 37.0 kg d^{-1} for Control, 0.1% T-80 and 0.2% T-80 were observed which are considerably higher and therefore would provide a greater opportunity to identify treatment differences.

Milk Yield Rationale

There were no treatment differences ($P>0.05$) in the current study. All cows produced exceptionally well at 37 kg d^{-1} of 4% FCM providing an excellent opportunity for treatment differences to be realized. High MY indicated that the current experimental diet was of excellent quality and sufficient to facilitate high MY. Positive responses in MY as a result of T-80 supplementation were noted in previous studies (Shelford and Baah, 1998; Shelford and Baah, 1997; Shelford et al., 1996). One of the main contributing factors to optimal MY is abundant supply of the VFA milk precursor propionic acid. Propionic acid is produced from rapid fermentation of NSCs in the rumen. Glucose is derived from propionic acid in the liver and later synthesized by the mammary gland into lactose which is positively correlated with MY (Bachman, 1992). Apparently in this experiment, T-80 did not accelerate propionate production or net glucose formation resulting in increased MY.

3.4.3.2 Milk Composition

Conflicting results were observed in previous studies in terms of milk compositional data. Shelford et al. (1996) observed depression in milk fat and slight improvements in protein percentage in response to T-80 supplementation in a preliminary lactation trial. In a full lactation trial, milk fat percentage was significantly lower in level 1 treated cows than Control. Differences observed in milk protein percentage between Control and level 1 were approaching significance and milk protein percentage in level 2 was significantly lower. In work by Shelford and Baah (1997), milk fat percentage tended to increase with time from the beginning of trial to the end in all cows, while no differences were observed in milk protein percentage levels and SCC. Shelford and Baah (1997) demonstrated that milk fat percentage tended to decrease with increasing levels of a supplement containing T-80 in the TMR (0, 100 and 150 g d^{-1} T-80 supplement yielded 3.5, 3.35 and 3.32% fat, respectively). Milk protein percentage was the same in Control and 100 g d^{-1} T-80 supplement although lower in 150 g d^{-1} T-80 supplement (3.12, 3.12, 3.05 % protein, respectively).

In comparison to the Control treatments in previous work on the same farm, Dinn (1996) observed milk fat of 3.91 and 3.63% (yield not provided) and milk protein of 3.02 and 3.20% (yield not provided) in two consecutive studies. Dinn (1996) did not report lactose percentage or yield. Wright (1998) observed milk fat of 3.82% (1.33 kg d^{-1} fat), milk protein of 3.25% (1.14 kg d^{-1} protein) and milk lactose of 4.41% (1.56 kg d^{-1} lactose). For the Control treatment in the current study, milk fat of 3.42% (1.38 kd d^{-1} fat), milk protein of 3.21% (1.30 kg d^{-1} protein) and milk lactose of 4.57% (1.85 kg d^{-1} lactose) were observed demonstrating exceptional milk component characteristics.

Milk Protein

Milk protein is a reflection of adequate quantity and quality of protein sources reaching the lower GI track where it can be absorbed for maintenance and metabolic purposes, such as milk protein production (Bachman, 1992). This is achieved through a desirable combination of RDP, RUP and ruminally available energy, and the subsequent production of microbial protein and VFAs in adequate supply to exceed maintenance requirements and supply excess for MY (Bachman, 1992).

Previous studies using lactating dairy cows reported decreases in milk protein percentage at the higher levels of T-80 supplementation (Shelford, 1996; Shelford et al.,1996; Shelford and Baah, 1998). The same effect was observed ($P<0.05$) in the current study indicating a trend for T-80 supplementation to be negatively related to protein percentage in milk. In Shelford and Baah (1998) no differences were observed in milk protein percentage between the lower levels (Control and 100 g d^{-1} T-80 supplement), while in the current study there were no differences between Control and 0.1% (DM basis) T-80, but there were differences between Control and 0.2% (DM basis) T-80.

In comparison to the Control treatments in previous work on the same farm, cows in the current trial had similar to superior milk protein percentage at 3.21% protein for Control to that reported by Dinn (1996) and Wright (1998). When expressed as protein yield (1.30 kg d^{-1}), results from the current study were superior to that produced by either Dinn (1996) or Wright (1998). According to data from the BC Milk Marketing board (Abbottsford, BC), the average protein content in milk for the UBC dairy herd was 3.308% in September 1997 and 3.395% in January 1998. These comparisons suggest that the diet was fully adequate to meet protein production needs.

Milk Fat

Percentage milk fat was lowest in Shelford et al. (1996) level 1 treatment while in Shelford and Baah (1997) paralleled protein data for tending to decrease with increasing levels of T-80 in the TMR. In the current study, no differences were observed in fat percentage. In comparison to the Control treatments in previous work on the same farm, Control cows in the current trial had a slightly lower milk fat percentage at 3.42% than observed by either Dinn (1996) or Wright (1998). Lower milk fat percentage in the current study was compensated for when expressed as fat yield (kg d^{-1}) as total fat produced was in excess of either Wright (1998) or Dinn (1996). According to data from the BC Milk Marketing board (Abbottsford, BC), the average fat content in milk for the UBC dairy herd was 3.408% in September 1997 and 3.445% in January, 1998. Similar phenomenon to that observed between the current study and that of Wright (1998) and Dinn (1996) for decreased fat percentage compensated for by exceptional yield was expected in comparing experimental results to herd milk fat yield (kg d^{-1}).

Milk Lactose

Propionic acid and gluconeogenic AAs provide the precursors for glucose *via* gluconeogenesis. This is the tie-in between energy and protein. In turn, glucose is needed by the mammary cells to synthesize the milk sugar known as lactose (Mertens, 1992). The role of lactose in determining milk volume is due to its solubility in water. As lactose is synthesized by mammary cells it creates an osmotic imbalance within cells which is corrected by drawing water into the cells (Bachman, 1992). Lactose production was not reported in previous lactation studies using T-80 supplementation.

Cows in the current trial had lactose percentages of 4.57, 4.54 and 4.59 (1.85, 1.83 and 1.87 kg d^{-1}) for Control, 0.1% T-80 and 0.2% T-80, respectively, paralleling observations in the current study in total MY.

Somatic Cell Count

Somatic cell count (SCC) was not reported in previous lactation studies using T-80 supplementation. Cows in the current trial had SCCs of 158, 397 and 59 ($x10^3$ ml^{-1}) for Control, 0.1% T-80 and 0.2% T-80, respectively, similar to that observed in the Control treatment of Wright (1998) at 223 cells $x10^3$ ml^{-1}.

3.4.4 Blood Urea Nitrogen

In an attempt to justify the observed BUN values, NRC (1989) was used to calculate total CP utilized by experimental cows. The experimental data used for this calculation included live weight, 4% FCM, MY, intake, live weight change and stage of lactation (i.e., early-lactation). Requirements were calculated for each of the treatment groups to yield 16.3%, 15.9% and 16.2% CP for Control, 0.1% T-80 and 0.2% T-80, indicating the ability of the TMR to meet CP needs.

Kaufmann and Lupping (1982) summarized the relationship between observed urea levels, dietary N intake and milk protein percentage levels. BUN is a reflection of N metabolism in the dairy cow (Roseler et al., 1993). It is well known that when diets of optimal energy and protein requirements (both RDP and RUP balanced) and fermentation rates are utilized, N use and efficiency in the formation of microbial CP is optimized (Tamminga, 1992). There appears to be an optimal range of BUN, above which the diet is excessive in CP or unbalanced in protein sources. Below this

hypothesized range, the diet is considered deficient in N (Roseler et al., 1993; Baker et al., 1995). Journet et al. (1983) concluded that BUN concentrations greater than eight to ten mg dl^{-1} were required to maximize organic matter digestion in the rumen of the lactating cow. Excess N supplied to the rumen or to postruminal tissues increased the concentration of urea in plasma (Baker et al, 1995). Christensen et al. (1993) discussed how the concentration of urea in plasma was reduced by decreasing the CP in the diet or by decreasing the ruminal degradability of the CP fed to cows. Findings were similar when the ruminal degradability of the dietary CP was decreased by heat treatment (Wright, 1998). Diets balanced for RDP and RUP tend to be more efficient at N-utilization than those balanced by more traditional CP% means (Baker et al., 1995; Dinn, 1996).

This is the fist trial in which BUN was observed in lactating dairy cows supplemented with T-80. As stated above, there were no significant differences observed between treatment groups of the current study. Numerically, BUN levels decreased with increasing level of T-80 supplementation (18.52, 18.21 and 17.57 mg dl^{-1} for Control, 0.1%T-80 and 0.2% T-80, respectively). BUN values of the current study were significantly different ($P<0.05$) between periods, with observed values of 15.81, 17.5 and 21.16 mg dl^{-1} for periods one, two and three, respectively. These levels indicate that N was not limiting in the TMR and exceeded the minimum BUN requirements of eight to ten mg dl^{-1} specified by Journet et al. (1983) to maximize organic matter digestion.

The Control treatments of two previous studies on the same farm were examined to provide a basis upon which to evaluate the TMR CP percentage and BUN (mg dl^{-1}) in the current study. Wright (1998) observed BUN of 18.6 mg dl^{-1} corresponding to TMR

CP% of 17.3. In two separate studies by Dinn (1996), BUN of 14.0 mg dl^{-1} (TMR CP= 15.3%) and 15.9 mg dl^{-1} (TMR CP = 18.3%) were observed. Dinn's (1996) observations demonstrate the differences in efficiency of N-utilization of different ration formulations. BUN and corresponding CP levels observed in the current study of 18.52 mg dl^{-1} (TMR CP= 18.9%) did not display the N efficiency of utilization of Dinn (1996) or Wright (1998).

Roseler et al. (1993) reported that plasma urea nitrogen on a balanced NRC diet was 14.8 mg dl^{-1} and increased with imbalances of degradable intake protein (DIP), undegradable intake protein (UIP) or both. Baker et al. (1995) demonstrated that a diet balanced for CP intake with imbalances in RDP and RUP produced plasma urea nitrogen concentrations that were lower than a similar diet with excess CP, but higher than those produced on isonitrogeneous diets balance for RDP and RUP. A BUN of 16.0mg dl^{-1} when lactating cow rations were balanced for CP, DIP and UIP with high quality protein sources was observed (Roseler et al, 1993). Garcia-Bojalil et al. (1992) reported that lactating cows fed 20% CP diets containing either 72.5 or 56.5% RDP had BUN of 21.9 and 17.3 mg dl^{-1}. Finally, using a 16% CP ration, Wohlt, et al. (1991) observed BUN levels comparable to those reported in the current study.

Observed BUN levels were considered appropriate for the similarly high level of protein in the diet. When compared to previous studies on the same farm in which diets were formulated for improved N utilization, BUN data show better levels (i.e., lower BUN) than the controls, however, lack the efficiencies of the superior diets. BUN increased from 15.81 to 20.16 mg dl^{-1} over the duration of the experimental study. Assuming a relatively consistent %CP in the TMR, this reflected greater protein

requirements and utilization in early lactation and provided indication of excessive dietary protein supply in the later stages of the trial.

3.4.5 Ruminal Fluid: pH and VFA

3.4.5.1 pH

Shelford et al. (1996) indicated total VFA production was increased in the presence of T-80 in an *in vitro* system. Giesecke (1970) reported increased production rate of VFAs induces higher ruminal VFA concentrations. It is known that the accumulation of acids (most of which are VFAs) in the rumen contribute to its acidic condition and thus can be considered indicative of a decline in rumen pH of T-80 treated animals. The magnitude of the hypothesized decrease in pH as a result of T-80 supplementation cannot be determined from the above information.

The pH values observed in this study were relatively low considering the ideal ruminal fluid pH of 6.7 $\pm$0.5 (Van Soest, 1994) and approaching the suggested minimal pH of 6.2, below which fiber digesting microorganisms are inhibited (Grant and Mertens, 1992). There was some question as to the legitimacy of the observed pH due to the crude methods of pH determination employed. In addition, difficulties in determining rumen pH by samples collected *via* rumen stomach tube were noted due to the error associated with contamination of sample by saliva. Saliva contamination tends to increase observed pH values due to its alkalinity and buffering capacity. Rumen samples were collected at 1100 h, roughly three hours after feeding time. Van Soest (1994) indicated how fermentation peaks about four hours after feeding on a hay diet, but occurs sooner if the diet contains much concentrate. This suggested that rumen

fluid samples were collected at peak fermentation time that potentially contributed to the lower pH values observed.

3.4.5.2 Volatile Fatty Acids

Rumen microorganisms produce VFAs and gases as a result of their metabolic processes. Gill (1997) reviewed the gas production technique and discussed the positive relationship between rumen microbial gas production and digestibility where increased gas production is associated with increased digestibility. However, Gill (1997) stated how this relationship does not always hold true and was also dependant upon the type of feedstuff (Priva et al., 1988). Wolin (1960) reported estimates of gas production during the production of VFAs and indicated how acetate and butyrate participate directly in gas production whereas propionate does not. As gas losses are associated with energy losses (Van Soest, 1994), propionate production was associated with increased energy efficiency relative to acetate and butyrate.

Shelford et al. (1996) observed differences in the ratio of *in vitro* VFA concentration and an increase in total VFA concentration in the presence of T-80. Acetate production was not changed, however, the proportion of propionate was increased significantly. Propionate is associated with increased MY and efficiency of production. Lower milk fat percentage was expected with a higher level of propionate production. Shelford et al. (1996) observed an improvement in gas produced by rumen organisms and concluded the amount of gas produced by a given amount of feedstuff was reduced with the addition of T-80.

Work of the current study did not correspond with the observations of Shelford et al. (1996) in regards to *in vitro* VFA compositional data. In the current study, total VFAs

were unaffected by the treatment levels. The molar proportions of VFA were changed, but different from that observed by Shelford et al. (1996). The molar proportion of acetate was significantly higher in the 0.2% T-80 over the 0.1% T-80, however the Control was neither lower than the 0.2% T-80 or higher than the 0.1% T-80. According to Van Soest (1994), the drop in molar proportions of acetate is usually caused by the dilutor effect of a large increase in propionate. With the marginal increases in acetate production observed it is not unrealistic that there was only a numerical increase in molar proportions of propionate (P=0.15) in 0.1% T-80 treated cows.

In two separate studies, Dinn (1996) observed an A:P ratio of 2.59 in the first experiment, followed by an A:P ratio of 1.96 in the second experiment having a superior balanced diet for N-utilization. In contrast, Wright (1998) observed an A:P ratio of 2.73. A decrease in the A:P ratio (i.e., from 2.44 to 2.16) results from a decrease in acetic acid and/or increase in propionic acid production with respect to each other. Increased propionic acid production is related to increased MY and efficiency of production. Although decreased A : P ratio's are indicative of increased efficiency of production, they are unique to the ration fed and are not necessarily comparable between different rations. Bachman (1992) discussed the relationship between dietary factors and VFA concentration indicating how diets either excessive in forages or concentrates alter the rumen pH and A : P ratio to varying extremes, both of which are detrimental to MY.

T-80 was shown to increase the rate and extent of cellulose hydrolysis in anaerobic bioreactors (Helle et al., 1993; Castanon and Wilke, 1981) and work with microorganisms has suggested improvements in enzyme-substrate interactions and enzyme efficiency (Pardo, 1996; Long and Knapp, 1991; Asther and Corrieu, 1987). Similar improvements were expected in the rumen, with T-80 facilitating dietary

fermentation and subsequent production of microbial CP and VFAs. Changes in relative proportions of VFAs between control and treated animals were only expected if T-80 altered the rate of protein versus carbohydrate fermentation. Increases in carbohydrate (with respect to protein) fermentation would be realized in increased acetic and butyric acid production without subsequent compromise of total propionic acid production, MY or protein percentage. It was suggested how acetic acid yielding fermentation may provide more ATP per unit digested for the synthesis of microbial cells (Van Soest, 1994; Isaacson, 1975).

3.4.6 Digestibility Parameters

Apparent Digestibility

Supplementation with T-80 had no effect on apparent DM digestibility in the current study. According to Cheeke (1999), maximum DMI occurred at 65 to 70% apparent DM digestibility, and below this value, rumen fill limits consumption where as in higher values chemostatic regulation of feed intake occurred. The apparent DM digestibility values of the current study were within Cheeke's (1999) specified range and complimented the excellent intake level observed. Differences in apparent DM digestibility as compared to the Control treatments in previous work on the same farm by Dinn (1996) and Wright (1998) suggested the slightly lower levels observed in the current study were due to differences in intake levels, where cows on the current study had the highest DM intakes of the reported trials. Lower estimates of apparent DM digestibility were attributed to the significant concentrate fraction of the diet (i.e., 45% concentrate on DM basis of total TMR). In addition, the concentrate was barley-based which could have further contributed to an overall reduction in digestibility estimates.

There was no effect of supplementation with T-80 on apparent CP digestibility at either of the experimental treatment levels above the Control ration in the current experiment. In addition to carbohydrate degradation, experimental work has acknowledged the stimulatory effects of T-80 on protein degradation in the rumen (Kamande, 1994; Shelford et al., 1996). Observed estimates of apparent CP digestibility were similar to that of Dinn (1996) and Wright (1998). Considering both of these studies placed high emphasis on balancing RDP and RUP, it was also supportive of the quality of ration fed in the current study in the absence of balancing for protein degradability. BUN levels in the current study indicated adequate dietary protein and estimates of apparent CP digestibility verify protein utilization as comparable to previous studies.

There was no effect ($P>0.05$) of T-80 supplementation on apparent NDF or ADF digestibility in the current experiment. Apparent fiber (NDF and ADF) digestibility was lower than expected in light of positive responses to T-80 supplementation in previous studies (Kamande, 1994; Castanon and Wilke, 1981, Helle et al., 1993). High intake as a percentage of BW, high rate of passage (Van Soest, 1990; Mertens, 1992) and a TMR having a significant barley-based concentrate (Bachman, 1992) are all factors that could have contributed to the overall apparent fiber digestibility estimates observed. However, this does not explain the lack of response observed to T-80 supplementation at the given treatment levels of 0.2%(DM basis) and 0.1% (DM Basis) in the current study. For cows with intakes above maintenance levels, the average depression in an ordinary dairy diet is about four percent per unit of intake (Van Soest, 1994). Observed estimates of NDF and ADF apparent digestibility were both less than that observed by Dinn (1996) and greater than that of Wright (1998).

Numerical differences in apparent fiber digestibility indicated that apparent ADF digestibility of 0.2% T-80 cows (51.3%) was 3.5% higher than the Control (47.8%), and 3.3% higher than 0.1% T-80 (48.0%). The opposite effect was observed in NDF apparent digestibility (P=0.10) with 0.2% T-80 displaying reduced apparent digestibility of NDF compared to both the 0.1% T-80 and control treatments. Numerically, apparent digestibility estimates for NDF of 0.8% (55.6%) and 4.1% (52.4%) in 0.1% T-80 and 0.2% T-80 were observed, respectively, under that of the Control (56.5%). These differences were numerical but not significant, making speculation difficult. Nevertheless, the main difference between ADF and NDF fractions is the presence of hemicellulose in NDF, with both fiber fractions having cellulose and lignin. As it is well known that lignin is indigestible, differences in effectiveness of T-80 in degradation of cellulose versus hemicellulose were questioned. Apparently T80 had a negative influence on microbial hemicellulosic degradation.

The Contribution of Hemicellulose

The impact of T-80 solely on hemicellulose digestion has not been reported. However, hemicellulose's intimate association with cellulose in the plant cell wall (Van Soest, 1994; Woolcock, 1991; Prins and Kreulen, 1990) and ease of microbial degradation in comparison to cellulose (Hobson, 1976) suggest T-80 would benefit its fermentation on the same premise. Hemicellulose is often excluded from discussions of cellulose due to its less complicated hydrolysis and assumption that what pertains to cellulose will, when necessary, apply to the more complex of the hemicellulosic structures (Van Soest, 1994). According to Chesson and Forsberg (1988), hemicellulose-degrading xylanases are more widely distributed than are cellulases

among rumen bacteria. Magnified positive effects of T-80 were expected in the hemicellulose containing fiber fraction (NDF) in the observations of apparent fiber digestibility as opposed to the ADF fraction, however, numerical differences indicate the opposite of this was observed.

Was TMR "*as-sampled*" the Same as "*as-fed*"?

These results raise question as to the quality or availability of the nutrients in the TMR. Samples collected for laboratory analysis were frozen immediately after the ration was prepared at 1100 h, however, the TMR was not fed to cows until 1600 h and 0800h the next day or 5 h and 21 h post preparation, respectively. There was active aerobic fermentation occurring in the silages used to prepare the rations. This was manually felt as heat when the silage (more apparent in the grass silage) was removed from the silo bags on a daily basis to prepare the TMR and observed as moldy patches. Further aerobic fermentation post TMR preparation and prior to feeding was observed, particularly in TMR that was not fed until 21 h post preparation and in warmer weather in the first half of the study. Differences in aerobic fermentation between treatments were not evaluated, however, T-80 was thought to contribute to increased aerobic fermentation rates in treated rations on the same premise as reported in previous anaerobic studies (Shelford et al., 1996, Helle et al., 1993; Castanon and Wilke, 1981).

Aerobic fermentation has its greatest impact on the soluble carbohydrates and protein of the ration (Van Soest, 1994). It was suggested that the rations containing T-80 could have experienced magnified levels of aerobic fermentation and nutrient loss. Fermentation occurring pre-feeding may have resulted in the cows consuming a TMR different from that sampled at mixing time and may have contributed to the lack of

production responses. Thus, it is hypothesized that when the rations were consumed the positive effects of T-80 were masked by pre-feeding aerobic losses. Further work is needed to verify this speculation.

3.4.7 Efficiency of Production

Estimates of efficiency are derived from the expression of MY (kg) DMI^{-1} (kg). Intake of quality ration and ability to efficiently utilize the nutrients are prerequisites for optimal efficiency of MY. Propionate production was positively related to efficiency of production (Shelford et al., 1996; Van Soest, 1994) due to its positive impact on MY and energy availability. In the current study there was no difference observed in propionate production, MY or intake between the three experimental treatments supporting the similar lack of difference ($P>0.05$) in efficiency of MY.

In vitro studies using T-80 by Shelford et al. (1996) concluded that the proportion of propionate was increased significantly. Propionate is associated with increased efficiency of MY. VFA analysis was not determined in Shelford's (1996) second lactation trial. In Shelford and Baah (1997), TMR-1 (0.2% T-80) had the highest feed efficiency over both TMR-0 (Control) and TMR-2 (T-80 plus enzyme) rations. Finally, Kamande (1994) observed both intake and apparent digestibility estimates increased together by almost 40% and 10% respectively. Therefore, Kamande (1994) concluded that T-80 improved efficiency of the digestive enzymes, particularly in the rumen. Efficiency in the current study was expressed as a function of daily milk yield (kg d^{-1}) over daily DMI (kg d^{-1}) resulting in a unitless expression of efficiency. Values of 1.55, 1.53 and 1.52 milk (kg d^{-1}) DMI^{-1} (kg d^{-1}) for Control, 0.1% T-80 and 0.2% T-80 were observed in the current experiment.

Results from previous *in vitro* and *in situ* lactation trials demonstrate the ability for T-80 treated cows to display increased efficiency of production over control animals. There was no effect of T-80 supplementation on efficiency of MY in the current study. In the absence of any significant differences in efficiency estimates in the current study ($P>0.05$), it was suggested that T-80 facilitated the aerobic fermentation of the TMR pre-feeding. The aerobic fermentation was thought to be facilitated to such an extent by T-80 that net nutritive losses endured pre-feeding equaled net fermentative gains in the rumen resulting in no observable differences in the production response. Unfortunately this idea cannot be tested for the current experiment as TMR samples were only collected immediately after ration preparation, however, examining the impact of T-80 on facilitating aerobic fermentation in silages and TMR would be worthy of future research efforts.

3.4.8 Post Trial

3.4.8.1 True Digestibility

For verification of the DM digestibility estimates observed, an *in vitro* study of true digestibility was performed on each of the 39 TMR samples (Control, 0.1% T-80 and 0.2% T-80 sampled each week for the 13 week experimental period) with an eight hour incubation time. Results indicated no significant differences ($P>0.05$) in true digestibility as a result of T-80 supplementation when the rations were originally prepared at either 0.1% (DM basis) or 0.2% (DM basis). Similarly, there were no differences observed in apparent DM digestibility estimates.

3.4.8.2 Additional T-80 Added to Samples

A TMR sample from each of the three experimental treatment levels from week four of the current study was evaluated in a *in vitro* analysis of true digestibility in which additional T-80 was added to each of the respective TMR samples and incubated for either 8 or 24 h. In both the 8 and 24 h digestion times, true digestibility for the samples displayed increases in digestibility estimates with 0.2% (DM basis) T-80 > 0.1% (DM basis) T-80 > Control verifying the presence and positive effects of the original T-80 supplementation at the time of diet preparation. Similarly, with the addition of T-80 to individual TMR samples at the time of *in vitro* analysis, the Control samples displayed the greatest increases in digestibility estimates. These results demonstrate the potential ability of T-80 to favorably influence *in vitro* true digestibility estimates and also suggest the TMR samples, as prepared, were consistent in their T-80 concentration and that the 0.2% T-80 (DM basis) was more favorable than either 0.1% T-80 (DM basis) or the Control. Further analysis was suggested to verify these observations statistically.

3.4.8.3 Acid-Detergent Insoluble Nitrogen

Speculated Aerobic Spoilage in Silage

The quality of both the corn and grass silage used on this trial was questionable. Pre-trial analysis revealed both were in the upper limits of acceptable DM content, however, excellent protein levels warranted their continued use. Upon opening the silage bags, evidence of aerobic fermentation was apparent. Both corn and grass silages were warm and even hot to touch, had an 'off' odor and a multitude of moldy patches. This spoilage was attributed to air contamination at the end of the silo bag. Fluctuations in visible signs of aerobic fermentation varied from day to day. Aggressive

attempts to move past the silage-air interface were unsuccessful and perhaps misleading as the spoilage relentlessly continued. When rations were prepared the most evidently spoiled fractions were manually removed, however, some contamination was inevitable. Regardless of fluctuations observed in silage quality, the trial proceeded using the same forage source as to avoid the analytical complications of a complete ration change.

It is well known that heating of silages brought about by aerobic fermentation can lead to decreased N availability as a result of the Maillard reaction (Wright, 1998; Van Soest, 1994), increasing silage ADF (Rotz and Muck, 1994; Pennells, 1984; Weiss and Conrad, 1986) and ADIN (Rotz and Muck, 1994; Pennells, 1984; Van Soest and Mason, 1991; Goering and Van Soest, [JDSVol 56]; Yu and Thomas, 1976; Weiss and Conrad, 1986). Nitrogen contained in ADIN is not distinguishable in normal N analysis as the sample must first be subjected to the ADF procedure and then analyzed for N content of the residue for this determination. There was conflict in the literature as to the nutritive availability of ADIN as a N source. Traditionally it was thought to be entirely unavailable, however, Waters et al. (1992) suggest that ADIN may not be entirely indigestible as 'naturally' present ADIN is considered undegradable. However, ADIN produced as a result of the Maillard reaction is thought to be partially available. Weiss and Conrad (1986) reported that the digestibility of ADIN is highly variable but averages about 30%. Increased levels of ADIN attributed to aerobic fermentation of silage's and the Maillard reaction were linked to decreased levels of N apparent digestibility (Van Soest and Mason, 91; Van Soest, 94; Goering and Van Soest, Vol 56..; Yu and Thomas, 1976; Weiss and Conrad, 1986).

ADIN Analysis Results

ADIN in experimental grass silage ranged from 4.80% to 6.82% and in two corn silage samples were 3.90% and 4.09% of total N. Van Soest (1965) and Yu and Thomas (1976) suggested normal unheated forages contain from seven to nine percent of their total N in the ADF fraction, so forages with ADIN%CP levels greater than nine percent were considered heat damaged (Yu and Thomas, 1976). Weiss and Conrad (1986) related ADIN levels in silages to heating temperatures achieved, where ADIN in 35°C silages equals 9.5% of total N compared to 60°C silage in which 22.9% of total N is held in the ADIN fraction.

The results of the current study confirmed the quality of the TMR and ruled out the possibility of heat damage in the corn and grass silages prior to ration preparation. This seemed logical as levels of CP apparent digestibility and BUN levels did not correspond with a diet severely lacking or unbalanced in protein. Conclusions could not be made regarding changes in ADIN%CP post ration preparation and pre-feeding resulting from enhanced aerobic fermentation in the presence of T-80.

3.5 EXPERIMENTAL CONCLUSION

Results of this study indicated that addition of T-80 at 0.1% or 0.2% (DM basis) to the TMR of high-producing dairy cattle had no effect ($P>0.05$) on DMI, MY, milk component yield, digestibility estimates or efficiency of production when compared to the Control treatment. Excellent DMI and MY data and laboratory analysis confirmed the TMR as prepared was fully acceptable to meet production requirements.

High TMR fiber levels did not impair intake, but may have limited potential energy intake resulting in decreased microbial production and subsequent reductions in overall milk protein and total yield. All animals ate exceptionally well and gained weight in early lactation despite the tendency of negative energy balance during this time. The potential for treatment carry over effects resulting from the 3 x 3 Latin Square design was discussed as previous ruminant studies that reported significant production responses did not expose cows to more than one experimental treatment.

There were no differences ($P>0.05$) in BW gain, however, numerical differences indicated T-80 treated cows did not gain as much weight as Control animals with 0.2% (DM basis) T-80 gaining more weight than 0.1% (DM basis) T-80 treated cows. Apparently T-80 did not enhance NSC fermentation and subsequent propionate production to an extent to be realized in increased MY in treatment rations. Differences were observed in milk protein percentage ($P<0.05$) with 0.2% (DM basis) T-80 lower than the Control, however, differences were not significant ($P>0.05$) when expressed as daily protein yield (kg d^{-1}). Milk protein is reflective of dietary and microbial protein supply. Considering dietary protein supply was consistent among treatments, it appears that 0.2% (DM basis) T-80 had inhibitory effects on microbial protein supply. According to Kaufmann (1982), the low milk protein levels (<3.2%) observed, coupled

with higher BUN levels was indicative of a diet limiting in energy. The forage to concentrate ratio of 55:45 compared to a more common 50:50 ratio certainly contributed to the above suggestion regarding energy deficiency in this ration. Lack of differences in BUN levels indicated no effect of treatment on N utilization, however, differences in BUN between periods paralleled NRC (1989) recommendations for decreasing CP requirement as lactation progressed. At $P<0.10$, decreased BUN levels in 0.2% (DM basis) below that of Control were suggestive of increased N utilization. N utilization as evident by decreased BUN in 0.2% (DM basis) T-80 treated animals was thought to result from pre-feeding TMR aerobic microbial protein degradation.

Cows consuming TMR having 0.2% (DM basis) T-80 produced more acetate (mol 100 mol^{-1}) ($P<0.05$) than cows consuming 0.1% (DM basis) T-80 indicating greater fiber fermentation, however, neither treatment was significantly different from the Control ration. Observed differences in molar percentages of acetate between treatments were not large enough to contribute to differences in molar percentages within treatments. Similarly, the slight increase in acetate production in 0.2% (DM basis) T-80 observed did not contribute to increased fat production as expected. At $P<0.10$, the A : P ratio of 0.2% (DM basis) was greater than 0.1% (DM basis) T-80 treated cows, although neither was different from the Control.

Apparent digestibility estimates were comparable, and in certain cases either higher or lower than previous studies by Wright (1998) and Dinn (1996). The contribution of barley to decreased fiber (NDF and ADF) digestibility was noted and later dismissed upon comparisons to the similar rations of Wright (1998) and Dinn (1996). At $P<0.10$, apparent NDF digestibility was lower in 0.2% (DM basis) T-80 treated cows suggesting reduced hemicellulose fermentation and contradicting acetic

acid and A:P ratio VFA hypotheses of increased fiber fermentation. In the current experiment 0.2% (DM basis) T-80 did not enhance ($P<0.10$) ruminal microbial hemicellulose fermentation. *In vitro* true digestibility analysis post-trial contradicted apparent digestibility estimates. When additional T-80 was added to TMR samples collected in week four of the study, numerical differences in an *IVTD* suggested digestibility was greater in 0.2% (DM basis) T-80, followed by 0.1% (DM basis) T-80 and Control, respectively, opposite to that observed in the apparent digestibility estimates. It appears that T-80 may have evoked a positive *in vitro* digestibility response in the current experimental diets after several months' storage. Post trial analysis for ADIN indicated that corn and grass silages were not heat damaged prior to TMR preparation, but could not confirm heat damage that may have occurred post TMR preparation and prior to feeding.

Further studies using transmission electron microscopy would be beneficial to identify shifts in microbial populations. The resulting alterations in enzyme activity from T-80 supplementation would serve to verify treatment levels and provide more information towards Kamande's (1994) concept that ruminal cellulose degradation was enhanced and protein degradation stabilized at lower concentrations of T-80.

It was speculated that T-80 facilitated the aerobic fermentation of the TMR pre-feeding, accelerating the nutritive loss of soluble energy and proteins. This suggestion was supported by a positive numerical response in a post trial *IVTD* analysis. Further work is needed to evaluate the effect of T-80 on aerobic degradation in prepared rations. Changes in TMR quality post-preparation and pre-feeding could not be verified

as samples were not collected. Further research is required before recommendations concerning the experimental T-80 treatment levels are made.

3.6 REFERENCES

Asther, M. and Corrieu, G. 1987. Effect of Tween 80 and oleic acid on ligninase production by *Phanerochaete chrysosporium* INA-12. Enzyme Microb. Technol., 9:245-249.

Baah, 1998. Effect of enzyme enhancing agent and fibrolytic enzyme on performance of feedlot cattle. The University of British Columbia, Vancouver, BC, personal communication.

Bachman, K.C. 1992. Managing milk composition. Pp. 336-346 in: Van Horn, H.H. and Wilcox, C.J. (Eds.) Large Dairy Herd Management, American Dairy Science Association, Champaign, IL.

Baker, L.D., Ferguson, J.D. and Chalupa, W. 1995. Responses in urea and true protein of milk to different protein feeding schemes for dairy cows. Journal of Dairy Science, 78: 2424-2434.

British Columbia Milk Marketing Board. 1997. M.C.P. Test Statement, UBC Dairy Research and Education Centre. Abbottsford, BC.

Castanon, M. and Wilke, C. 1981. Effects of the surfactant Tween 80 on enzymatic hydrolysis of newspaper. Biotechnology and Bioengineering, 23:1365-1372.

Cheeke, P.R. 1999. Applied Animal Nutrition, Feeds and Feeding, 2nd ed. Prentice Hall, NJ.

Chesson, A. and Forsberg, C.W. 1988. Polysaccharide degradation by rumen microorganisms. Pp. 251-284 in: Hobson, P.N. (Ed.) The Rumen Microbial Ecosystem. Elsevier Applied Sciences, London.

Chr. Hansen, 1991. Insure high-quality silage with good management and a silage inoculant. Chr. Hansen BioSystems, Milwaukee, WI.

Christensen, R.A., Lyncy, G.L., Clark, J.H., and Yu, Y. 1993. Influences and degradability of protein on production of milk and milk components by lactating Holstein cows. Journal of Dairy Science, 76:3490-3496.

Dinn, N.E. 1996. Manipulating ration formulations to reduce nitrogen excretion from lactating cows, while maintaining milk production. M.Sc. Thesis, Department of Animal Science, The University of British Columbia, Canada.

Garcia-Bojalil, C.M., Staples, C.R., Thatcher, J.D., and Risco, C. 1992. Effect of dietary protein degradability and calcium salts of long chain fatty acids (CaLCFA) on reproductive performance of lactating Holstein cows. Journal of Dairy Science, 75(Suppl. 1):203.

Giesecke, D. 1970. Comparative microbiology of the alimentary tract. Pp.306-318 in: Phillipson, A.T. (Ed.) Physiology of Digestion and Metabolism in the Ruminant.. Oriel Press, Newcastle upon Tyne, England.

Gill, C.S. 1997. The effect of enzyme and mild hydrothermal treatment on the nutritive value of barley grain and canola meal for dairy cattle. M.Sc. Thesis, Department of Animal Science, The University of British Columbia, Canada.

Goering, H.K., and Van Soest, P.J. 1973. Relative susceptibility of forages to heat damage as affected by moisture, temperature and pH. Journal of Dairy Science, 56:137-143.

Goering, H.K., and Van Soest, P.J. 1970. Forage Fiber Analyses. (Apparatus Reagents, Procedures, and some Applications). Agric. Handbook No. 379., ARS-USDA, Washington, DC.

Goering, H.K., Gordon, C.H., Hemken, R.W., Waldo, D.R., Van Soest, P.J., and Smith, L.W. Analytical estimates of nitrogen digestibility in heat damaged forages. Journal of Dairy Science, 55(9):1275-1280.

Grant, R.J. and Mertens, D.R. 1992. Influence of buffer pH and raw corn starch addition on *in vitro* fiber digestion kinetics. Journal of Dairy Science, 75:2762-2768.

Helle, S.S., Duff, S.J. and Cooper, D.G. 1993. Effect of surfactants on cellulose hydrolysis. Biotechnology and Bioengineering, 46:611-617.

Hobson, P.N. 1976. The Microflora of the rumen. Meadowfield Press Ltd. Bushey, England.

Isaacson, H.R., Hinds, F.C., Bryant, M.P., and Owens, F.N. 1975. Efficiency of energy utilization by mixed bacteria in continuous culture. Journal of Dairy Science, 58:1645-1659.

Journet, M., Champredon, C., Pion, R., and Verite, R. 1983. Physiological basis of the protein nutrition of high producing cows. Critical analysis of the allowances. Pp. 433 in 4th Int. Symp. Protein Metab. Nutr. Colloques de l'INRA no. 16, vol. 1. Versailles, France: INRA Publications.

Kamande, G.M. 1994. Manipulation of rumen and silage fermentation, Ph.D. Thesis, Department of Animal Science, The University of British Columbia, Canada.

Kaufmann, V.W., and Lupping, W. 1982. Protected proteins and protected amino acids for ruminants. Pp. 36-75 in: Miller, E.L., Pike, I.H., and Van Es, A.J.H. (Eds.) Protein Contribution of Feedstuffs for Ruminants: Application to Feed Formulation, Studies in the Agricultural and Food Sciences, Washington, D.C.: Butterworth Scientific.

Komarek, A.R., Robertson, J.B., and Van Soest, P.J. 1994. A comparison of methods for determining ADF using the Filter Bag Technique versus conventional filtration. Journal of Dairy Science (Suppl. 1), 77:114. (Abstract)

Long, K. and Knapp, J. 1991. The effect of Junlon PW110 and Tween 80 on the production of cellulolytic enzymes by *Coprinus cinereus*. Mycol. Res. 95(9):1077-1081.

Mertens, D. 1982. Using Neutral detergent fiber to formulate dairy rations. Pp. 116-126 in Proc. GA. Nutr. Conf. For the Feed Industry. Athens: University of Georgia.

Mertens, D. 1980. Fiber content and nutrient density in dairy rations. Proc. Distillers Feed Conf. 35:35.

Mertens, D. 1985a. Effect of fiber on feed quality for dairy cows. Pp. 209-224 in Proc. 46th Minn. Nutr. Conf. St. Parul: University of Minnesota.

Mertens, D. 1985b. Factors influencing feed intake in lactating cows; Form theory to application using neutral detergent fiber. Pp. 1-18 in Proc. 46th Ga. Nutr. Conf. Athens: University of Georgia.

Mertens, D. 1992. Nonstructural and Structural Carbohydrates. Pp. 219-235 in: Van Horn, H.H. and Wilcox, C.J. (Eds.) Large Dairy Herd Management, American Dairy Science Association, Champaign, IL.

National Research Council. 1989. Nutrient requirements of dairy cattle. 6th rev ed. National Academy of Sciences, Washington, DC.

Nocek, J.E., and Russell, J.B. 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. Journal of Dairy Science, 71:2070-2107.

Orskov, E.R. 1992. Protein nutrition in ruminants. 2nd ed. Academic Press, London.

Pardo, A. 1996. Effect of surfactants on cellulase production by *Nectria catalinensis*. Current Microbiology, 33:275-278.

Pennells, G.C.L. 1984. The effect of heat damage on the digestibility and nitrogen utilization of grass silages by sheep. M.Sc. Thesis, Department of Animal Science, The University of British Columbia, Canada.

Prins, R.A. and Kreulen, D.A. 1990. Comparative aspects of plant cell wall digestion in mammals. Pp. 109-120 in: Hoshino, S., Onodera, R., Minato, H. and Itabashi, H. (Eds.) The Rumen Ecosystem: The Microbial Metabolism and Its Regulation. Japan Scientific Societies Press, Tokyo.

Priva, G., Santi, E., Belladonna, S. and Curto, O. 1988. Kinetics of in vitro fermentability of forages. Reprod. Nut. Develop. 28 (suppl. 1):101-102.

Roseler, D.K., Fergusen, J.D., Sniffen, C.J. and Herrema, J. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. Journal of Dairy Science, 76:525-534.

Rotz, C.A. and Muck, R.E. 1994. Changes in forage quality during harvest and storage. Pp. 828-868 in: Fahey, G.C., Collins, M., Mertens, D.R. and Moser, L.E. (Eds.) Forage Quality, Evaluation, and Utilization. American Society of Agronomy, Madison, WI.

Shelford, J.A. and Baah, J. 1997. Effect of enzyme enhancing agent on milk production. Personal communication. The University of British Columbia, Vancouver, BC.

Shelford, J.A. and Baah, J. 1998. Evaluation of a bioactive agent to enhance milk production. Personal communication. The University of British Columbia, Vancouver, BC.

Shelford, J.A., Cheng, K.J. and Kamande, G.M. 1996. Enzyme Enhancers: The key to unlocking the energy from feed. Advances in Dairy Technology, Proceedings of the 1996 Western Canadian Dairy Seminar, 8:269-275.

Shelford, J.A., von Keyserlingk, M.A.G. and Swift, M.-L. 1994. Forage Protein: Quality is more important than Quantity. Advances in Dairy Technology, Proceedings of the 1994 Western Canadian Dairy Seminar, 6:77-84.

Staples, C.R., Thatcher, W.W., Garcia-Bojalil, C.M., and Lucy, M.C. 1992. Nutritional influences on reproductive function. Pp. 382-392 in: Van Horn, H.H. and Wilcox, C.J. (Eds.) Large Dairy Herd Management, American Dairy Science Association, Champaign, IL.

Statistical Analysis System (SAS), 1990. SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC.

Stauffer, C.E. 1990. Functional additives for bakery foods. Van Nostrand, Reinhold, New York.

Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. Journal of Dairy Science, 75:345-357.

Tilley, J.M.A. and Terry, R.A. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Brit. J. Grassl. Soc., 18:104-111.

Van Soest, P. J. 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. Journal of Animal Science, 24:834-843.

Van Soest, P.J. 1994. Nutritional Ecology of the Ruminant, 2nd ed. Cornell University Press, Ithaca, NY.

Van Soest, P.J. 1990. Fiber utilization. In: Proceedings of the 26th annual nutrition conference For Feed Manufacturers. Guelph, ON.

Van Soest, P.J., and Mason, V.C. 1991. The influence of the Maillard reaction upon the nutritive value of fibrous feeds. Animal Feed Science and Technology, 32:45-53.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fiber, NDF and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74:3583-3597.

Varga and Hoover, 1983. Rate and extent of neutral detergent fiber degradation of feedstuffs in situ. Journal of Dairy Science, 66:2109.

Waters, C.J., Kitcherside, M.A. and Webster, A.J.F. 1992. Problems associated with estimating the digestibility of undegraded dietary nitrogen from acid-detergent insoluble nitrogen. Animal Feed Science and Technology, 39:279-291.

Weiss, W.P. and Conrad, H.R. 1986. Digestibility of nitrogen in heat-damaged alfalfa. Journal of Dairy Science, 69:2658-2670.

Wohlt, J.E., Chmiel, S.L., Zajak, P.K., Backer, L., Blethen, D.B., and Evans, J.L. 1991. Dry matter intake, milk yield and composition, and nitrogen use in Holstein cows fed soybean, fish, or corn gluten meals. Journal of Dairy Science, 74:1609-1622.

Wolin, M.J. 1960. A theoretical rumen fermentation balance. Journal of Dairy Science, 43:1452-1459.

Woodward, N.L. 1999. Effect of live yeast culture supplementation on dry matter intake and milk production on transition cows. M.Sc. Thesis, Department of Animal Science, The University of British Columbia, Canada.

Woolcock, J.B. 1991. World Animal Science: Microbiology of Animals and Animal Products. Elsevier, New York.

Wright, C.F. 1998. The evaluation of heat and lignosulfonate treated canola meal as sources of rumen undegradable protein for lactating cows. M.Sc. Thesis, Department of Animal Science, The University of British Columbia, Canada.

Yu, Y. and Thomas, J.W. 1976. Estimation of the extent of heat damage in alfalfa haylage by laboratory measurement. Journal of Animal Science, 42:766-774.

Table 3.2 Pre-trial Ration Component Analysis

nutrient[1]	dairy concentrate	corn silage	grass silage
		(%, DM basis)	
DM	89.5	30.9	48.0
NDF	39.3	49.1	51.4
CP	22.8	8.3	19.4

[1]DM, NDF, and CP stand for dry matter, neutral detergent fibre and crude protein, respectively.

Table 3.3 Composition of total mixed rations

		diet			
nutrient[1]	n	Control	0.1% T-80	0.2% T-80	S.E.
			(%, DM basis)		
CP	36	18.9	18.9	18.7	± 0.18
ADF	36	23.7	24.6	24.2	± 0.39
NDF	36	40.1	40.7	39.9	± 0.64

[1]CP, ADF and NDF stand for crude protein, acid detergent fiber and neutral detergent fiber, respectively.

Table 3.4 The influence of diet on dry matter intake and body weight.

			diet		
		n	Control	0.1% T-80	0.2% T-80	S.E.
[1]DMI						
	kg d^{-1}	43	26.2	26.4	26.9	± 0.57
	% of BW	43	3.88	3.92	3.97	± 0.08
[2]BW						
	kg	44	677	674	677	± 2.94
	gain, kg d^{-1}	42	0.37	0.12	0.31	± 0.18

[1]DMI and [2]BW stand for dry matter intake and body weight, respectively.

Table 3.5 The influence of diet on milk yield and composition

		diet			
	n	Control	0.1% T-80	0.2% T-80	S.E.
[1]Milk, kg d^{-1}	44	40.6	40.4	40.6	± 0.47
4% [2]FCM, kg d^{-1}	44	37.1	37.0	37.0	± 0.42
Milk Composition, %					
Protein	42	3.21[a]	3.16[ab]	3.13[b]	± 0.018
Fat	42	3.42	3.43	3.40	± 0.13
Lactose	42	4.57	4.54	4.59	± 0.016
Component Yield, kg d^{-1}					
Protein	42	1.30	1.27	1.27	± 0.014
Fat	42	1.38	1.39	1.39	± 0.057
Lactose	42	1.85	1.83	1.87	± 0.025
Milk [3]SCC, x 10^3 ml^{-1}	42	158	397	59	± 138

[1]MY, [2]FCM and [3]SCC stand for milk yield, fat corrected milk and somatic cell count, respectively.

[a,b] Means with different letters differ significantly ($P<0.05$)

Table 3.6 The influence of diet on blood urea nitrogen (BUN)

		diet			
	n	Control	0.1% T-80	0.2% T-80	S.E.
BUN, mg dl^{-1} (P=0.0751)	41	18.52[a]	18.21[ab]	17.57[b]	± 0.27

[a,b] Means with different letters differ significantly (P=0.08)

Table 3.7 The influence of diet on rumen fluid pH and volatile fatty acid concentration (mg ml^{-1}) and molar proportions (% of total VFA)

		diet			
	n	Control	0.1% T-80	0.2% T-80	S.E.
pH	42	6.37	6.50	6.33	± 0.12
VFA, mol $100mol^{-1}$					
Acetate (A)	42	62.86^{ab}	62.34^{b}	63.70^{a}	± 0.35
Propionate (P)	42	20.89	21.42	20.11	±0.44
Isobutyrate	42	0.74	0.77	0.72	± 0.03
Butyrate	42	12.56	12.68	12.77	± 0.31
Isovalerate	42	0.98	1.00	0.96	± 0.04
Valerate	42	1.49	1.43	1.35	± 0.08
Caproate	42	0.47	0.36	0.38	± 0.09
Total VFA, mg ml^{-1}	42	4.70	4.46	4.85	± 0.24
[1]A:P ratio (mol $100mol^{-1}$, P=0.08)	42	3.07^{ab}	2.97^{b}	3.23^{a}	± 0.06

[1]A: P stands for acetate to propionate ratio

[a,b] Means with different letters differ significantly

Table 3.8 The influence of diet on water intake and fecal and urine output

		diet			
	n	Control	0.1% T-80	0.2% T-80	S.E.
Water intake, l d^{-1}	36	69.5	70.6	68.5	± 2.25
Urine output, l d^{-1}	36	22.0	22.5	22.6	± 0.98
Feces output, kg d^{-1}	36	50.5	50.5	51.0	± 1.04
Feces output, kg DM d^{-1}	36	7.90	7.84	7.85	± 0.17
Feces dry matter, %	36	15.69	15.61	15.49	± 0.17

Table 3.9 The influence of diet on apparent nutrient digestibility (AND) during week four of each experimental period

		diet			
AND	n	Control	0.1% T-80	0.2% T-80	S.E.
		(%, DM basis)			
DM	36	69.05	67.71	68.25	± 0.69
CP	36	67.42	69.27	68.50	± 1.05
NDF	36	56.47[a]	55.62[a]	52.40[b]	± 1.31
ADF	36	47.77	47.98	51.31	± 1.41

* DM, CP, NDF and ADF stand for dry matter, crude protein, neutral detergent fiber and acid detergent fiber, respectively.
[a,b] Means with different letters differ significantly ($P<0.10$)

Table 3.10 The influence of diet on efficiency of milk yield.

		diet			
	n	Control	0.1% T-80	0.2% T-80	S.E.
$^1E_{MY}$	43	1.55	1.53	1.52	± 0.035

$^1E_{MY}$ is efficiency of milk yield [yield (kg d^{-1}) / intake (kg d^{-1})]

Table 3.11 Post Trial Analysis: *In vitro* true digestibility (8 h)

		diet			
	n	Control	0.1% T-80	0.2% T-80	S.E.
			(%, DM basis)		
IVTD	39	64.36	63.34	63.30	± 0.81

Table 3.12 Post Trial Analysis: *In vitro* true digestibility$_{Additional\ T\text{-}80}$ of TMR samples collected in week 4

		diet		
time (h)	treatment	Control	0.1% T-80	0.2% T-80
			(IVTD$_{additional\ T\text{-}80}$, %, DM basis)	
8	Control	65.67	67.65	69.12
8	0.2% (DM basis) T-80	68.46	66.49	68.22
24	Control	80.82	80.82	83.07
24	0.2% (DM basis) T-80	82.47	81.80	83.74

Table 3.13 Post Trial Analysis: Acid detergent insoluble nitgrogen (ADIN) in the TMR and grass silage expresses as a percentage of dietary crude protein

Date	TMR	Grass Silage (ADIN %CP)	Corn Silage
Nov 21	8.02	5.52	3.90
Nov 28	7.24	6.82	N/A.
Dec 12	8.08	5.05	N/A.
Dec19	6.29	4.80	4.09
Dec 26	6.75	5.72	N/A
Jan 5	7.41	5.45	N/A
Jan 9	6.44	5.55	N/A
Jan 15	7.34	6.45	N/A

4. APPENDICES

4.1 Statistical Analysis System Program

```
data;
title 'T-80 Lactation Trial';
options ps=60 ls=75;
infile 'c:\ [name of respective file]';
input cow treatment block period [response variables];
proc glm;
        class cow treatment block period;
        model [response variables] = treatment block per cow(block)/ ss3 nouni;
        random block period cow(block) / test;
        means period treatment;
        lsmeans treat / stderr pdiff;
proc print;
run;
```

Post statistical analysis it was observed that 'block' was not significant for any of the response variables measured, and thus the more simplified GLM procedure was used instead.

```
data;
title 'T-80 Lactation Trial';
options ps=60 ls=75;
infile 'c:\ [name of respective file]';
input cow treatment period [response variables];
proc glm;
        class cow treatment period;
        model [response variables] =  cow treatment period treatment*period/ ss3;
        lsmeans treat / stderr pdiff;
proc print;
run;
```

4.2 ADIN Calculations

Calculations for ADIN%N_x , where ***x*** = TMR, grass silage or corn silage. Sample numbers taken from the TMR sample collected on November 21, 1998.

1) Determine weight of ADF fraction of 1 g of sample

    24% ADF (TMR-Nov21) x 1 g = 0.24 g or 240 mg

2) ADIN is the N content of the ADF residue. Calculate N content of sample ADF residue.

0.994 % N x 240 mg = 2.386 mg

3) Calculate N content of original 1 g forage sample
   2.983 % N x 1000 mg = 29.83 mg

4) Calculate ADIN as a percentage of total N, ADIN %N
   (2.386 mg / 29.83 mg) x 100% = 8.0%

Therefore, 8% of the nitrogen in the TMR sampled on November 21, 1998 was present in the ADF fraction.