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Effect of roughage to concentrate ratio on ruminal fermentation and protein degradability in dairy cows

by

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DECLARATION

I declare that this dissertation for the degree MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria, has not been submitted by me for a degree at any other University.

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SUMMARY

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Published research suggests that it might be beneficial to increase the amount of rumen undegradable protein (RUP) that passes out of the rumen, through manipulation of rumen fermentation to establish a lower rumen pH. To test this hypothesis, a study was conducted in which three ruminally cannulated Holstein cows, 722 kg ±25.6 kg fed three different diets (treatments) were used in a Latin square design experiment to determine effects of increasing levels of dietary concentrate on some rumen parameters and ruminal crude protein (CP) degradability. The *in situ* method was used to determine the ruminal protein degradability of sunflower oilcake, cottonseed oilcake and roasted soya. The three treatments differed in roughage:concentrate ratio, being 60:40 (Treatment UP 60), 45:55 (Treatment UP 45) and 30:70 (Treatment UP 30). Intake of dry matter (DM) (kg/day) did not differ between treatments. The mean rumen pH in cows receiving the three experimental diets differed and was 6.00, 6.27 and 6.44 for treatments UP 30, UP 45 and UP 60 respectively. The time (hours) below pH 5.8, which is considered to be the pH where fibre degradation is substantially negatively affected, was approximately 2.5 hours, but only on treatment UP 30. Mean rumen ammonia nitrogen (N) and total volatile fatty acid (VFA) concentrations did not differ among cows receiving different treatments but, cows fed treatment UP 30 had a lower ruminal acetic acid:propionic acid (A:P) ratio compared to the other treatments. There were no differences in ruminal CP degradation within the three feedstuffs when incubated in cows fed diets with different roughage:concentrate ratios. Results suggest that roughage:concentrate ratios ranging from 60:40 to 30:70, which

resulted in mean pH values ranging from 6.4 to 6.0, did not affect ruminal CP degradation of sunflower oilcake, cottonseed oilcake and roasted soya.

LIST OF ABBREVIATIONS

AA	Amino acid
A:P	Acetic acid to propionic acid
ADIN	Acid detergent insoluble nitrogen
AFMA	Animal Feed Manfacturers Association
ADF	Acid detergent fibre
AMTS	Agricultural Modeling and Training Systems
ARC	Agricultural Research Council
CF	Crude fibre
CNCPS	Cornell Net Carbohydrate and Protein System
CPM	Cornell Penn Miner Dairy Model
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
IVOMD	*In vitro* organic matter digestibility
MCP	Microbial crude protein
ME	Metabolizable energy
MIU	Million international units
MJ	Mega joules
N	Nitrogen
NAN	Non ammonia nitrogen
NDF	Neutral detergent fibre
NFC	Non fibre carbohydrate
NPN	Non protein nitrogen
NRC	National Research Council
NSC	Non-structural carbohydrate
OM	Organic matter
RDP	Rumen degradable crude protein
RUP	Rumen undegradable crude protein
SC	Structural carbohydrate
SEM	Standard error of the mean
TMR	Total mixed ration
VFA	Volatile fatty acid

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CHAPTER 1.

INTRODUCTION AND MOTIVATION

The protein requirements of high yielding dairy cows or and rapidly growing feedlot animals are met by a combination of microbial crude protein (MCP), produced by micro-organisms in the rumen, and rumen undegradable protein (RUP) which escapes proteolytic activity in the rumen. Since there is a limited capacity of rumen micro-organisms to produce MCP, the proportional contribution of RUP to fulfilling the requirements of high yielding ruminants becomes more important. In general, the RUP fraction is relatively more expensive because rumen micro-organisms can utilise the products (i.e., NH_3, peptides, amino acid's (AA)) of relatively poor quality CP sources to synthesize better quality MCP. Apart from ruminal CP degradability, factors such as the AA profile and digestibility of RUP should be taken into account when estimating the CP requirements of ruminants (National Research Council (NRC), 2001). Nutritional models such as Cornell Penn Miner Dairy (CPM-Dairy, 1998), Cornell Net Carbohydrate and Protein System (CNCPS) (Fox *et al*., 1995) and Agricultural Modeling and Training Systems (AMTS, 2006) are becoming increasingly important tools to enable nutritionist's to predict the nutritional requirements of high yielding ruminants. In order to more accurately simulate the duodenal flow of CP and AA's, better descriptions of the effects of dietary composition, more accurate prediction of MCP production and CP degradability, and factors affecting it, as well as a description of interactions among dietary factors needs to be developed (Bateman *et al.*, 2001).

It is important to understand factors affecting CP degradability, since both the requirement for degradable CP, and amount of CP actually degraded, depend on diet composition as well as conditions in the rumen. Results in the published literature indicate that CP degradability is sometimes lower with high concentrate diets and situations where low rumen pH prevails. However, there are still many questions to be answered, such as: is this due to a change in microbial population that degrades ruminal CP or due to a structural change in the protein source, such as solubility of CP or accessibility of CP to the microbes and the proteolytic enzymes? Or is it due to pH *per se* or to other factors, such as the nutrients available to the microbes? O is it due to reduced proteolysis *per se* or is it due to reduced cellulose degradation at low ruminal pH, which then reduces the degradation of CP that is associated with or protected by this cellulose? Many of these aspects will be dealt with in this literature review.

The emphasis of this study, however, was on how level of concentrate, and the resulting lower pH, would affect rumen CP degradability I three feeds. Lower rumen degradability would mean less MCP synthesis but also more RUP would be available for digestion in the small

intestine. Protein sources high in RDP can then be used to replace some of the more expensive RUP, thereby rectifying the shortfall in MCP. This would be of economic importance since CP sources that are highly ruminally degradable are usually less expensive than those that are high in rumen undegradable CP.

Published research results report more than a 20% reduction in CP degradability or proteolytic activity with a lower rumen pH (Siddons & Paradine, 1981; Strobel & Russel., 1986; Assoumani *et al.,* 1992). Endres & Stern (1993) observed a reduction in CP and NDF digestion when pH decreased from 6.3 to 5.9. Assuming the lower pH causes CP degradability to be 10% lower than previously thought, a diet containing 15% lucerne hay, 15% *Eragrostis curvula* hay, 15% wheat straw, 40% maize meal, 0.5% urea, 4% sunflower oilcake, 6% heat treated soya, 3% cottonseed oilcake and minerals, would have a degradability of approximately 60% (based on average CP values for these feedstuffs and degradability values published by Erasmus *et al.* (1988; 1990a; 1990b) at an outflow rate of 0.08/h). Assuming a 10% decrease in degradability of CP in all ingredients except urea, which is always 100% degradable, degradability would be 54%. To bring it back up to the desired level, all the cottonseed oilcake and half the heat-treated soya would need to be replaced with sunflower oilcake. Assuming prices of R2200/ tonne for sunflower oilcake, R2900/ tonne for cottonseed oilcake and R3400/ tonne for heat-treated soya, this could reduce the price of the total diet by almost R60 per tonne. According to AFMA (Animal Feed Manufacturers Association) the National Dairy Feed Production in South Africa in 2005/2006 was 1 482 683 tonne. Assuming this much feed is still produced, and assuming a typical ration is similar to the one in the above calculation, the South African dairy feed industry could save about R 88 million a year by replacing expensive CP sources with less expensive ones. Similarly heat-treated soya could be partially replaced by raw soya.

The aim of this experiment was to determine effects of roughage:concentrate ratio and the resulting effects of a variable pH on the ruminal CP degradability of three protein sources. When there is a more accurate estimate of the quantitative effect of ruminal pH on CP degradability, this could be incorporated into ration formulation models making these models more accurate at estimating CP degradability. This would require more experimentation to obtain values for all the CP sources, as the effect of ruminal pH on the degradability of individual feedstuffs may differ (Loerch *et al.*, 1983; Cronjé, 1992). Alternatively results could be used to create guidelines whereby the ration should be formulated for a higher RDP, and lower RUP, when a lower ruminal pH is expected. This would be of economic advantage to the feed industry as there is a need to use more cost effective CP sources while maintaining sufficient RUP in the diet.

CHAPTER 2.
LITERATURE REVIEW

2.1 INTRODUCTION

It is important to understand the concept of dietary CP degradability since, the extent and rate of its degradation determines N available for rumen micro-organisms and undegradable CP available for digestion in the small intestine (Ørskov, 1992). There are many factors affecting degradability of CP in the rumen, including roughage:concentrate ratio in the basal diet and the resulting ruminal pH.

Since rumen pH needed to be modified and controlled in this study, the literature study commences with a review of factors affecting rumen pH, and how it can be modified. Thereafter general aspects of CP degradability, factors affecting CP degradability and methods of estimating CP degradability were discussed. Lastly effects of basal diet and rumen pH on CP degradability are discussed in detail.

2.2 GENERAL FACTORS AFFECTING RUMINAL pH

Ruminal pH is affected by the fibre concentration of the diet (i.e., neutral detergent fibre (NDF)) and the balance between production of fermentation acids and secretion of salivary buffers (Krause *et al.*, 2002). The pH of the rumen can vary from more than 7.0 on a roughage diet to less than 5.0 on a high grain diet (Russell & Dombrowski, 1980; Erfle *et al.*, 1982; Erdman, 1988) even though the rumen is well buffered by bicarbonates, phosphates and protein (Russell & Dombrowski, 1980). When feeding a dairy cow a total mixed ration (TMR) twice a day, the ruminal pH may range from 5.5 to 6.5 and, on fresh high quality pasture, the pH may range from 5.6 to 6.8 within a 24 hour period (de Veth & Kolver, 2001).

Decreasing the diet crude fibre (CF), NDF or acid detergent fibre (ADF) level generally decreases rumen pH (Erdman, 1988; Kolver & de Veth, 2002), while increasing the amount of concentrate or non-structural carbohydrates (NSC) in the ration generally decreases ruminal pH (Mould & Ørskov, 1983; Ørskov 1992; Ørskov, 1994; Kolver & de Veth, 2002). Readily fermentable carbohydrates decreased rumen pH (Strobel & Russell, 1986) due to rapid microbial fermentation, and less rumination and salivation (Mould & Ørskov, 1983). Reduction in the ruminal pH due to rapid fermentation of a grain-based diet with high starch content occurs to a lesser extent if cows are adapted to the high grain diet (Clayton *et al.*, 1999), as this is associated with alterations in the microbial species in the rumen (West *et al.*, 1987).

Rumen pH is largely a function of the VFA concentration (Erdman, 1988; Stokes *et al.*, 1991), and pH will drop if there is a reduced rate of VFA absorption (Owens *et al.*, 1998). In a

diet with high NSC and RDP, VFA concentrations are high and ruminal pH is low (Zhao *et al.*, 1993). Russell (1998) found that cows fed 90% concentrate had lower ruminal pH values (6.22 vs. 6.86), higher VFA concentrations, and lower acetate:propionate ratios than cows fed forage only, and concluded that as much as 25% of the decrease in acetate:propionate ratio could be explained by pH alone.

Feed intake and salivary secretion affect pH in the rumen (Maekawa *et al.*, 2002b). At a higher level of feed or DM intake, the pH of the rumen is lower (Robinson *et al.*, 1986; Madsen & Hvelplund, 1988; Zhao *et al.*, 1993). Haaland *et al.* (1982) found pH at maintenance to be 6.57 and at two times maintenance to be 6.35, and Madsen (1986) found that doubling the feeding level decreased rumen pH from 6.59 to 6.47.

Multiparous cows tend to have a lower pH in the rumen than primiparous cows, because higher feed intake leads to more fermentation acids produced in the rumen, which is not compensated by increased salivary secretions associated with increased chewing (Maekawa *et al.*, 2002b).

Ruminal pH declines following a meal, the drop depending partly on the initial pH (Maekawa *et al.*, 2002a; Nocek *et al.*, 2002). Ruminal pH generally continues to decline 4-6 hours after feeding with the lowest rumen pH generally occuring 4 - 6 hours after feeding (Lindberg, 1981a; Madsen & Hvelplund, 1988). The duration of low pH generally increases with increasing grain levels in the diet (Nocek *et al.*, 2002) although Maekawa *et al.* (2002a) found no effect of roughage:concentrate ratio on ruminal pH. This could have been because increased chewing time, and hence saliva addition during mastication, in cows consuming diets with more forage was offset by less saliva secretion (Maekawa *et al.*, 2002a).

Infrequent feeding is conducive to large fluctuations in the rumen environment, including pH (Vanzant *et al.*, 1998). Variation in pH is smaller when feed intake is more frequent, which can be achieved by feeding restricted amounts of feeds several times a day (Madsen & Hvelplund, 1988; Ørskov, 1994; Le Liboux & Peyraud, 1999). As meal frequency decreases the fluctuation in ruminal production of VFA is more pronounced and correlated to a larger fluctuation in ruminal pH. The minimum meal frequency needed to maintain a steady rumen pH increases as dietary effective NDF decreases and *vice versa* (Pitt & Pell, 1997). Pitt and Pell (1997) predicted, with the use of metabolic models, that DM intake and body weight, as well as use of dietary buffers at levels less than 1% of DM, have relatively small effects on the magnitude of ruminal pH fluctuations.

Feeds with rapid ruminal rates of starch degradation may result in low ruminal pH (Batajoo & Shaver, 1998), and so diets with higher levels of rapidly fermented carbohydrates tend to

exaggerate diurnal fluctuations in pH (Robinson *et al.*, 1986). Fulton *et al.* (1979) observed that steers fed a wheat grain based diet had a lower voluntary intake and rumen pH, and experienced wider fluctuations in rumen pH than steers fed a maize grain based diet. Martín-Orou *et al.* (2000) fed diets with differing ratios of maize and barley grains in the concentrate portion of the diet. Ruminal pH was slightly lower (6.29 vs. 6.46) for cattle offered a higher proportion of barley than those with a higher proportion of maize, because barley grain is more fermentable in the rumen. However, ruminal pH never decreased below 5.50, and there was no difference in effective rumen degradability between the two diets. Increasing dietary CP increases pH of the rumen (Haaland *et al.*, 1982).

Physical disintegration and heat processing of cereals increases their rate of fermentation and thereby reduces rumen pH (Lindberg, 1985). The amount of barley grain needed to depress rumen pH depends on the degree of processing (Mould *et al.*, 1983). Krause *et al.* (2002) found that decreasing forage particle size, or increasing the level of rumen fermentable carbohydrate, decreased ruminal pH and minimum diurnal ruminal pH, and increased the time below pH 5.80. Le Liboux & Peyraud (1999) found that if lucerne was ground and pelleted, as opposed to chopped, mean diurnal pH was lower and the range of pH and time when it was low, were higher.

Method of sampling also has an effect on rumen pH as rumen fluid samples by stomach tube tend to have a higher pH than those taken via a rumen fistula (Erdman, 1988). Site of sampling and time post feeding probably have more extensive effects on rumen pH than molar proportions of VFA (Erdman, 1988).

There are many factors affecting ruminal pH and, although some metabolic models have attempted to predict pH fluctuations, limited information is available on the effects of these fluctuations on microbial fermentation and nutrient digestion (Calsamiglia *et al.*, 2002). These factors need to be managed to obtain maximum benefits of ruminal CP degradation.

2.3 RUMINAL PROTEIN DEGRADABILITY

2.3.1 Introduction

Ruminal CP degradability of a feedstuff is an important characteristic to determining the CP value of a feed (Madsen & Hvelplund, 1987; Michalet-Doreau & Ould-Bah, 1992). Dietary CP requirements are best expressed in terms of RDP and RUP, as duodenal amino acid (AA) needs of ruminants are met mainly by a combination of MCP synthesized by rumen microbes and RUP that escapes degradation in the rumen (Kirkpatrick & Kennelly, 1987; Stern *et al.*, 1994; Halgerson *et al.*, 1995). The AA makeup of RUP is important because the cow has a metabolical requirement for individual AA's (Schwab *et al.,* 2007). Passage of protein to the small intestine

was previously assumed to originate entirely from ruminally synthesized MCP and RUP (NRC, 2001), although endogenous CP however contributes to total CP passage to the duodenum and should be considered in models that predict passage of protein to the small intestine (NRC, 2001). The Dairy NRC (2001) takes endogenous CP into account by predicting passage of endogenous N to the duodenum from dry matter intake (DMI) (NRC, 2001).

Degradability of dietary CP describes the amount of RDP that is available as a source of N for rumen microbes as well as indicating the amount of RUP potentially entering the small intestine (Miller & Ørskov, 1986; Madsen & Hvelplund, 1987). The CP in feed serves either as an N source for microbial growth in the rumen through feed CP that is degraded in the rumen, or as a source of AA for the ruminant body through AA that reach the small intestine undegraded (Hvelplund & Madsen, 1990). When formulating ruminant diets, there needs to be an adequate supply of RDP to meet microbial growth requirements to optimize rumen fermentation, and an adequate supply of RUP to make up any deficit which could exist between intestinal supply of rumen synthesized MCP and tissue requirements for intestinally absorbed AA (Lindberg, 1981b; Siddons *et al.*, 1985; Hvelplund & Madsen, 1990). Hence diet formulation is dependant on reliable estimates of the extent to which the CP of feedstuffs is degraded in the rumen (Siddons *et al.*, 1985; Broderick *et al.*, 1988; Tamminga *et al.*, 1991). High producing ruminants need to be supplemented with individual AA in certain instances (Schwab *et al*., 2007)

Proteins are degraded in the rumen by bacterial proteases, peptidases and deaminases to create peptides, AA and NH_3 that are major sources of N for rumen bacteria and influence microbial growth rates in the rumen (Ørskov, 1992; Stern *et al.*, 1994). Most species of rumen bacteria have some proteolytic activity (Wallace & Cotta, 1988) and few, if any, major strains of rumen bacteria are obligate proteolytics (Ørskov, 1992). The combined action of many microbial species is involved in proteolysis in the rumen (Russell & Hespell, 1981). Strains of *Butyrivibrio, Bacteroides, Streptococcus, Selenomonas, Clostridium, Lachnospira*, *Borrelia, Eubacterium* and *Succinivibrio* are examples of proteolytic bacteria, although they also have cellulolytic, xylanolytic, pectinolytic, amylolytic and saccarolytic activities (Lindberg, 1985; Wallace, 1988). The three main proteolytic species are *Bacteroides amylophilus*, *Butyrivibrio fibrisolvens* and *Bacteroides ruminicola* (Wallace & Cotta, 1988). Rumen protozoa are also proteolytic (Lindberg, 1985; Wallace & Cotta, 1988), and Russell & Hespell (1981) concluded that shift in microbial species numbers could influence the rate of proteolysis in the rumen.

It is important to supply enough RDP to meet the requirements of rumen bacteria and to maximise MCP synthesis in the rumen because MCP has a very good amino acid makeup (Stern *et al.*, 1994; Klopfenstein *et al.*, 2001). Dietary CP is the most important source of N for rumen

microbes, which makes CP degradability important (Ørskov, 1992). In diets that are lacking RDP, such as diets with a lot of cereal grain, microbial fermentation could be limited, which could negatively affect ruminal fibre digestion (Martín-Orou *et al.*, 2000). Models recognize that N requirements of the different species of bacteria differ in the CNCPS, micro-organisms are categorized into those that ferment structural carbohydrate (SC), such as cellulose and hemicellulose, and those that ferment NSC such as starch, pectins and sugars (Russell *et al.,* 1992). Structural carbohydrate fermenting bacteria utilize ammonia as their primary N source, while NSC fermenters utilize ammonia and amino acids as N sources for MCP synthesis.

Rates of proteolysis and deamination are independent of rates of MCP synthesis (Loerch *et al*., 1983). Rapid microbial degradation of CP leads to high ruminal NH_3 levels (Crawford *et al.*, 1978). If the rate of NH_3 production from the fermentation of AA exceeds rate of NH_3 utilization for MCP synthesis, it accumulates in the rumen, is absorbed across the gut wall and ultimately is excreted in urine to reduce N retention (Russell *et al.*, 1983; Siddons & Paradine, 1983). Increased absorption of NH_3 through the rumen wall decreases efficiency of N utilization (Crawford *et al.*, 1978). Because degradability of CP influences N losses as NH_3, and the quantity and quality of AA available to ruminants (Halgerson *et al.*, 1995), changes in degradability of N could alter required concentration of dietary CP (Ørskov, 1992).

If there is an N deficiency in the rumen, due to a deficiency of dietary RDP, digestibility and intake could be depressed (Miller & Ørskov, 1986). However, feeding excess CP as RDP decreases performance (Garcia-Bojalil *et al.*, 1998) and negatively affects fertility (Canfield *et al*., 1990; Klopfenstein *et al.*, 2001). There is little advantage to feeding formulated levels of RDP or RUP above proposed levels (Sloan *et al.*, 1988).

Feed proteins with low degradability are especially valuable in ruminants with high CP requirements (Broderick *et al.*, 1988). Lactating and growing cattle with high metabolizable protein requirements respond to supplementation with RUP, even when RDP is adequate (Klopfenstein *et al.*, 2001). As milk yield increases, a substantial amount of additional dietary CP from CP supplements needs to leave the rumen undegraded to meet CP requirements of the cow (Stern *et al.*, 1994). For example, the RUP requirement of a cow is increased by 98% when milk production increases from 35 to 55 kg/day (NRC, 2001).

Several studies have indicated increased milk yield with an increase in dietary RUP (McCormick *et al.*, 1999) whereas other studies have reported no response (Chiou *et al.*, 1997; Chiou *et al.*, 1999). When applying the concept of RUP in diet formulation, several factors need to be considered, such as the mean milk yield and lactation period, as well as digestion and adsorption of the diet, energy content of the diet and AA profile of the RUP that also influences

performance (Chiou *et al.*, 1997). The dairy cow has requirements for individual AA rather than metabolizble protein *per se* (Schwab *et al.,* 2007). Garthwaite *et al*., (1998), summarized 12 feeding studies in which addition of metabolizable lysine and methionine to create a more favourable ratio of 3:1 when compared to the control, were investigated. As noted by Schwab *et al*., 2007, in seven of the studies there was an increase in the average milk yield, milk protein and milk protein percentage. Research results have indicated that methionine is often the first limiting AA for milk production and lysine is often the second limiting AA (Schwab *et al.,* 2007). The first limiting AA varies depending on type of diet and histidine has been identified as first limiting when grass silage, barley grain and oat grain diets were fed (Huhtanen *et al*., 2002).

Ruminal degradability of CP is neither a positive or negative characteristic of feed as in some situations, a high and in others a low CP degradation is needed for optimum production (Madsen & Hvelplund, 1985; Madsen & Hvelplund, 1987). While an optimum ratio of RUP:RDP is not fixed (Erasmus *et al.*, 1988), when the correct balance of RUP and RDP are fed for a particular situation, dietary N is used efficiently for production (Kirkpatrick & Kennelly, 1987). With a wide variety of feed CP sources available, it should be possible to provide diets with the optimal RUP:RDP balance (Nocek *et al.*, 1979). However accurate measures of CP degradability are needed for successful application of this concept (Stern *et al.*, 1994; Alexandrov, 1998; Klopfenstein *et al.*, 2001). By measuring the amount of CP degraded in the rumen it is possible to estimate the amount of N available for rumen microbes and amount of CP made available for digestion in the small intestine (Zhao *et al.*, 1993). Estimates of ruminal CP degradability, and the amount of CP escaping the rumen are variable (Batajoo & Shaver, 1998; Holtshausen & Cruywagen, 2000). Rumen degradability of a CP depends on feedstuff, conditions in the rumen of the animal consuming the feed and experimental procedure employed to determine the value. Many dietary and ruminal factors need to be considered when value of a feed CP source is assessed (Zinn & Owens, 1983).

Feed CP can be divided into an undegradable fraction, a potentially (slowly) degradable fraction and a rapidly degradable fraction (NRC, 2001). Degradability of a feed CP is determined by the fraction that is undegradable, while degradation or disappearance of CP is determined by the relationship of rate of degradation and rate of passage out of the rumen (Van Straalen & Tamminga, 1990; Broderick *et al.*, 1991), with the latter mainly influenced by DMI. The NRC applies a discount factor to decrease energy value and ruminal CP degradability at high intakes (NRC, 2001). For example, when DMI increases from 25 to 30 kg/day, the metabolizable energy (ME) value of the feed is discounted by 4% and the diet CP degradability is decreased by about 3% (NRC, 2001).

Factors affecting the amount of CP degraded in the rumen include the amount of CP ingested, solubility of CP in rumen fluid and the time the CP is retained in the rumen (Netemeyer *et al.*, 1980). Differences in degradability can be caused by differences in the rumen environment and by differences in resistance to proteolytic enzymes (Ørskov, 1992). Degradability of CP varies among feeds, within feeds and due to chemical or physical treatments of the feed (Lindberg, 1985; Madsen & Hvelplund, 1987; Ørskov, 1992). It is important to obtain estimates of CP degradability on local feeds (Cronjé, 1983).

There is no single degradability value for a CP source that applies to all feeding conditions (Miller & Ørskov, 1986; Kirkpatrick & Kennelly, 1987), and even determined values only apply under specific conditions (Owens & Zinn, 1988). It is better to obtain relative RDP and RUP values that rank CP sources and diets relative to one another under specific feeding conditions (Siddons & Paradine, 1983; Kirkpatrick & Kennelly, 1987). The rate, as opposed to the extent, of CP degradability needs to be known in order to determine how much will be degraded at specific feeding conditions (Miller & Ørskov, 1986). Sources of CP can be classified into groups with high, medium and low degradability (Owens & Zinn, 1988), although in the latest NRC (2001), CP degradability is not a constant value, but can vary due to differences in DMI, thereby emphasizing the dynamic nature of the NRC (2001) model.

Degradation of CP in a mixed diet can be manipulated by selecting ingredients with high or low degradabilities (Tamminga, 1979; Van Straalen & Tamminga, 1990). When diets are often balanced for RDP and RUP, average or book values for these fractions of individual feeds are used (Stallings *et al.*, 1991; Aldrich *et al.*, 1996). The degradability of the diet can be predicted using CP degradability values of individual ingredients (Stallings *et al.*, 1991; Aldrich *et al.*, 1996).

2.3.2 Techniques to estimate crude protein degradability

Methods of evaluating feed CPs include *in vivo* (i.e., sampling of digestive contents throughout digestion), *in situ* (i.e., incubation of feeds in bags suspended in the rumen) and *in vitro* (i.e., laboratory) techniques (Janicki & Stallings, 1988; Van Straalen & Tamminga, 1990; Broderick *et al.*, 1991).

Estimates of CP degradability are sensitive to conditions of measurement (Mohamed & Smith, 1977). Even when measured under standardized conditions, the CP degradation in roughages and concentrates is variable. There are substantial differences between laboratories in effective CP degradation of feedstuffs (Van Straalen & Tamminga, 1990; Cottrill, 1993).

However the sequence of degradation of the feedstuff is usually similar (Van Straalen & Tamminga, 1990).

The *in situ* procedure, as proposed by Ørskov & McDonald (1979), is a widely used method to predict rumen CP degradability (Negi *et al.*, 1988; Beckers *et al.*, 1995; Kohn & Allen, 1995), and this procedure was also accepted by the NRC (2001) as the preferred procedure.

A three-step *in vitro* procedure was developed to estimate intestinal digestion of CP in ruminants (Calsamiglia & Stern, 1995). This procedure provides an alternative to using ruminally cannulated cows to determine CP degradation. Calsamiglia & Stern, (1995) stated that, compared with *in vivo* determination of intestinal digestion of CP, the three step procedure resulted in a substantial reduction in cost and labour, provided evidence of reliability and could be routinely used for screening intestinal digestion of CPs in ruminants.

2.3.2.1 In vivo

The *in vivo* measurement of feed N rumen degradability requires animals equipped with simple or re-entrant cannulas in the abomasum or duodenum, as well as reliable markers to calculate flow of digesta and of MCP (Lindberg, 1985). Errors with the *in vivo* technique are due to difficulties with markers, or differentiation between proteins of microbial and dietary origin (Hvelplund & Madsen, 1990; Broderick *et al.*, 1991; Kohn & Allen, 1995), or the digesta samples might not be representative (Van Straalen & Tamminga, 1990).

While *in vivo* determinations are good for reference data and even though it is still the technique against which other methods are validated, other techniques that are less time consuming and labour intensive are used for more extensive evaluations of CP degradability (Lindberg, 1985; Chiou *et al.*, 1995; Kohn & Allen, 1995).

2.3.2.2 In situ

The ruminal *in situ* bag technique is the most closely related to the environment in which CP degradation takes place *in vivo* (Madsen & Hvelplund, 1987; Nocek, 1988; Michalet-Doreau & Ould-Bah, 1992). It can give rapid and reasonable estimates for a wide variety of feedstuffs, although there are factors that can influence results (Chiou *et al.*, 1995). There is a close relation between *in vivo* degradation and *in situ* degradability (Madsen & Hvelplund, 1985; Lindberg, 1987), especially when an outflow rate of 0.08 h^{-1} is used (Madsen & Hvelplund, 1985). The NRC (2001) describes a standardized procedure used to describe *in situ* ruminal CP degradation. It is however difficult to obtain absolute CP degradation values using the *in situ* technique

makeing it realistic to determine relative values that rank CP sources (Kirkpatrick & Kennelly, 1987; Erasmus *et al.*, 1990b).

In situ CP disappearance is influenced by properties of the sample and experimental technique (Vik-Mo & Lindberg, 1985). Routine *in situ* procedures should be standardized in terms of fineness of grinding, pore size of bag material and washing procedure (Lindberg, 1987; Cottrill, 1993; Batajoo & Shaver, 1998) in order to minimize differences in results and make it easier to compare values obtained in different studies as there is considerable inter- and intra-laboratory variation with *in situ* measures of ruminal digestion (Vanzant *et al.*, 1998). *In situ* measurement of CP disappearance is also affected by factors such as size of the nylon bag, sample weight to bag surface area ratio, diet, washing technique, microbial contamination of bag residue, bag introduction sequence into the rumen, bag location in the rumen, animal species and time variation, pre-ruminal incubation and preparation (pre-soaking and milling) of sample prior to incubation (Nocek, 1988; Van Straalen & Tamminga, 1990; Vanzant *et al.*, 1998).

With the *in situ* technique, the assumption is that CP washed out of the bags (i.e., CP leaving the bag after 0 hour incubation), has been degraded and is available to the rumen micro-organisms (Broderick *et al.*, 1991; Michalet-Doreau & Ould-Bah, 1992; Kohn & Allen, 1995), which may not always be the case (Cottrill, 1993). Some soluble CP may be washed out of the bag without being degraded (Tamminga, 1979) and Michalet-Doreau & Ould-Bah (1992) stated that CP disappearance correctly simulates rumen CP degradation, except for rapidly degraded feeds.

The *in situ* procedure measures the rate of disappearance of CP in the rumen and this is combined with an estimated fractional outflow rate of the rumen contents to predict the proportion of dietary CP that will escape degradation in the rumen (Freer & Dove, 1984; Cottrill, 1993; Klopfenstein *et al.*, 2001). Thus, to be able to predict CP degradability, an estimate of rumen retention time, or outflow rate of the feedstuff from the rumen, is required (Siddons *et al.*, 1985; Cottrill, 1993). In most cases, it is assumed and so CP loss at a specific incubation time, or CP loss relative to DM, an index of CP degradability (Siddons *et al.*, 1985). Degradability of CP is however not static and in new metabolic models it is calculated by the model. Factors that affect ruminal CP degradability include ruminal retention time, microbial proteolytic activity and ruminal pH (NRC, 2001). The RDP and RUP values for a feedstuff are calculated using different equations when using the (NRC, 2001) model.

In situ results vary between animal species and also between days in the same animal and between bags for the same day and animal, because of variations in ruminal environment during

the course of the day and in different parts of the rumen at any given time of the day (Michalet-Doreau & Ould-Bah, 1992).

2.3.2.3 In vitro

It would be more rapid and cost effective to estimate degradability using *in vitro* techniques than the *in situ* technique and these techniques would be useful when fistulated ruminants are not available (Janicki & Stallings, 1988; Ørskov, 1992; Klopfenstein *et al.*, 2001). These techniques include solubility in water, salt, alkali or buffer solutions; neutral detergent insoluble CP; incubation with proteolytic enzymes; incubation with rumen fluid; continuous culture systems; measuring the accumulation of end products such as NH_3, the inhibitor method; gel electrophoresis; and near infrared reflectance spectroscopy (Miller & Ørskov, 1986; Ørskov, 1992; Klopfenstein *et al.*, 2001). There are high correlations between *in situ* degradabilities and some *in vitro* measurements (Lindberg, 1985; Janicki & Stallings, 1988; Michalet-Doreau & Ould-Bah, 1992), and different feedstuffs have higher correlations with different techniques (Janicki & Stallings, 1988).

In vitro solubility of feed CP could be an effective way of estimating relative CP degradability within a class of feed although, among feedstufs there is a poor agreement between buffer solubility and effective rumen degradability of CP (Erasmus *et al.*, 1988; Owens & Zinn, 1988; Hvelplund & Madsen, 1990) and buffer solubility is not a reliable method for estimating CP degradability of feeds (Hvelplund & Madsen, 1990).

While small amounts of soluble CP may leave the rumen undegraded, most will be degraded (Crawford *et al.*, 1978). Hence CP solubility is a reliable way of estimating rapidly degradable, or 'a' fraction CP, but not total CP degradability (Crawford *et al.*, 1978; Cottrill, 1993). Determination of CP solubility would only be adequate for CP sources where degradable CP consists mainly of the 'a' fraction such as silages (Ørskov, 1992). The correlation between CP solubility and CP disappearance from nylon bags decreases as exposure time of bags in the rumen increases (Stern & Satter, 1984).

Continuous culture systems provide a way of evaluating effects of nutrients on metabolism of microbes maintained under controlled pH, turnover rate and nutrient intake (Michalet-Doreau & Ould-Bah, 1992; Stern *et al.*, 1997). A limitation of *in vitro* techniques is that the results do not represent actual degradation *in vivo* (Tamminga, 1979).

2.3.3 Factors affecting crude protein degradability

There are inherent differences in CP degradability among feedstuffs due to source of ingredients, processing method and particle size (Batajoo & Shaver, 1998). Extent of CP degradation in the rumen is affected by composition and activity of microbes (i.e., proteolytic bacteria and protozoa), chemical and physical properties of CP and its residence time in the rumen (Van Nevel & Demeyer, 1988).

Feeding management, such as changing the ratio, sequence, level and frequency of feeding concentrate and roughage, can also affect CP degradation (Van Straalen & Tamminga, 1990). The diet being fed, physical nature of the diet, level of feed intake, ruminal retention time and rate of passage of digesta, method and frequency of feeding, experimental animals used, rumen environment (such as ruminal pH) and microbial proteolytic activity also effect ruminal CP degradability (Stern *et al.*, 1994; Holtshausen & Cruywagen, 2000; NRC, 2001).

Degradability can be changed by changing the growing conditions of the forage or by processing feedstuffs physically or chemically (Tamminga, 1979; Van Straalen & Tamminga, 1990).

Sources of CP degradability variation can be analytical error (Holtshausen & Cruywagen, 2000), as different laboratories use different analytical procedures (Batajoo & Shaver, 1998), different experimental techniques (Kirkpatrick & Kennely, 1987) and part of *in vivo* variation is due to inappropriate techniques (Tamminga, 1979).

Different factors affecting ruminal CP degradability have been well described in the literature (NRC, 2001). The purpose here is not to extensively review the topic, but rather summarize the most important factors affecting degradability.

2.3.3.1 Characteristics of dietary crude protein

The extent of degradation of dietary CP and *in situ* CP degradability values are affected by characteristics of the feed, such as solubility of the CP and structural differences caused by, for example, disulphide bridges between proteins and cross linkages of protein to carbohydrates (Kirkpatrick & Kennely, 1987; Wallace & Cotta, 1988; Zhao *et al.*, 1993). Protein structure affects degradability of CP in the rumen by influencing the accessibility to proteolytic enzymes (Leng & Nolan, 1984; Stern *et al.*, 1994), because of this some feeds are naturally resistant to ruminal microbial degradation (Stern *et al.*, 1994).

Solubility of CP is one of the factors influencing rumen proteolysis (Krishnamoorthy *et al.*, 1982; Lindberg, 1985; Stern *et al.*, 1994) and varies among feed and type of solvent (Russell & Hespell, 1981; Krishnamoorthy *et al.*, 1982). Insoluble CP is solubilized before it can be

hydrolyzed (Owens & Zinn, 1988). In contrast, soluble CP's are rapidly degraded because they are rapidly attached to bacterial cell walls (Owens & Zinn, 1988; Ørskov, 1992). Less soluble CP become attached to bacteria and are degraded at various rates (Ørskov, 1992). Although related, CP solubility and CP degradation are not synonymous (Nocek *et al.*, 1979; Cottrill, 1993). Solubility is more useful as an index of rate of proteolysis than extent of proteolysis (Owens & Zinn, 1988). Solubility of CP is not itself a reliable indication of its susceptibility to hydrolysis by rumen bacterial proteases as other factors, such as structural characteristics of the proteins, also influence CP fermentation in the rumen (Siddons & Paradine, 1981; Wallace & Cotta, 1988; Ørskov, 1992). For practical purposes, it is often assumed that CP solubility in a buffer solution is the same as immediate degradability of CP in the rumen (Leng & Nolan, 1984).

Solubility of CP is affected by ruminal pH as well as treatments of both forages and concentrates (Tamminga, 1979). As proteins are made up of amino acids, whose side chains can contain acidic or basic groups, at the iso-electric point of the protein it is uncharged and displays minimum water solubility. At high pH, the protein carries a negative charge and, at low pH, a positive charge. These ionic forms of protein are water soluble (Shakhashiri, 1983). Protein solubility is largely determined by distribution of hydrophobic and hydrophilic AA on the surface of the protein molecule (Russell & Hespell, 1981; Owens & Zinn, 1988). More soluble proteins (i.e., ones with more hydrophilic residues near the surface) have, in some cases, been degraded at a faster rate by rumen bacteria (Russell & Hespell, 1981). Proteins that are composed mainly of albumins and globulins have a higher solubility and are degraded more rapidly than those composed mainly of prolamins and glutelins (Erasmus *et al.*, 1990a; Assoumani *et al.*, 1992). Heat treatment, which unfolds the tertiary structure and exposes the hydrophobic AA, decreases solubility and causes a reduction in fermentation.

Some soluble CP is resistant to degradation (Wallace & Cotta, 1988; Cottrill, 1993) and *vice versa* (Wallace, 1988). Soluble proteins such as serum albumin, ovalbumin, chloroplast protein extract and soluble proteins from soybean meal and rapeseed meal have variable resistance to degradation (Mahadevan *et al.*, 1980; Leng & Nolan, 1984). The major soluble protein in raw soybean is because of high quantities of soluble albumins and globulins (Cronje, 1983). Although casein and ovalbumin are both soluble, they differ in proteolytic rate, the former being rapidly degraded while the latter has a slow rate of proteolysis, possibly due to structural differences (Russell & Hespell, 1981; Lindberg, 1985).

The presence of cross-linking disulphide bonds is one of the properties that makes a protein more resistant to proteolytic enzymes (Erasmus *et al.*, 1990a; Ørskov, 1992). Proteins such as albumins and immunoglobulins, with extensive disulphide bonding, or proteins with

cross-linkages caused by chemical treatment, are degraded more slowly than less ordered proteins (Broderick *et al.*, 1991). Disruption of disulphide bonds increases rate of degradation (Wallace & Cotta, 1988; Broderick *et al.*, 1991). Non-protein polymers, such as polysaccharides, may limit access of proteolytic organisms to substrate (Wallace & Cotta, 1988). Protein degradation can also be influenced by the carbohydrate moieties bound to glycoproteins such as γ-globulins (Broderick *et al.*, 1991).

2.3.3.2 Feedstuff characteristics

Most of the variation in *in situ* is due to the origin of the feed (Madsen & Hvelplund, 1987). Chemical, physical and structural properties of feeds are important factors affecting rumen degradation (Russell & Hespell, 1981). Obviously each feedstuff would have its own rumen degradability of CP, for example, there are large differences between grasses and legumes in rate of CP degradation as CP in legumes degrades at a faster rate (Van Straalen & Tamminga, 1990) and CP is generally more degradable in barley vs maize grain (Stern *et al.*, 1994; Martín-Orou *et al.*, 2000) mainly due to different properties of the protein matrix that affect access of rumen bacterial enzymes to the starch granules (Martín-Orou *et al.*, 2000).

Protein supplements of animal origin are generally degraded rapidly, but incompletely and hence have a low degradability over a range of rumen retention times. Plant proteins are generally degraded more slowly, but potentially completely making rumen escape dependend on ruminal proteolytic activity and particle outflow rate that result from other components of the diet (Wallace, 1988).

Degradation of CP in the rumen is negatively correlated to the level of fibre in the feedstuff (Lindberg, 1985; Nocek, 1985). Part of the nitrogenous compounds in many feedstuffs, including roughages and oilseed cakes, are protected from degradation by a fibrous structure that needs to be fermented by rumen micro-organisms before the CP is available for degradation (Lindberg, 1985).

In cottonseed meal, the presence of gossypol induced cross-linkages seems to provide a hypothesis for its atypical rate and extent of degradation (Cronje, 1983). According to Cronje (1983) this also provides evidence to validate the meaning of the factors derived from the equation (i.e., $p = a + b\ (1 - e^{-ct})$), that shows a small soluble 'a' fraction with a major slowly degradable 'b' fraction, which is in accord with the interpretation of the determinants of tertiary protein structure from whence it is logical that induced cross-linkage would mainly affect CP solubility.

***2.3.3.3* Variation within feedstuffs**

There can be substantial variation in CP degradability within feeds (Madsen & Hvelplund, 1985), as well as roughage samples from different regions. Hence, use of average CP values for roughages in formulation equations could lead to inaccuracy, since they might not reflect the roughages being used in that region (von Keyserlingk *et al.*, 1996). Rumen degradable CP content in grasses range from 41 - 60% and in legumes from 69 - 79% (Ibrahim *et al.*, 1995). Dynamic models such as NRC and CPM, use various factors to estimate the potential CP degradability of a feedstuff. Therefore no tabular values are provided, and CP degradability is estimated by the model for each specific set of conditions.

Large variation in ruminal degradation can also occur among and within different rendering by-products such as meat and bone meal, feather meal and blood meal (Howie *et al.*, 1996). Preparation methods alter ruminal degradation of fishmeal CP (Yoon *et al.*, 1996). Yoon *et al.* (1996) evaluated fishmeal samples from five processing plants and found ruminal CP degradation ranged from 29 - 57%.

Degradability of CP differs among cultivars of the same species. For example Mustafa *et al.* (2002) reported that different cultivars of pea silage have different CP degradabilities.

Degradability of CP in the rumen increases with increasing application of N fertilizen (Van Straalen & Tamminga, 1990; van Vuuren *et al.,* 1991), as N fertilization increases the CP content, and size of rapidly degradable CP (i.e., non protein nitrogen (NPN)) fraction, and the rate at which the slowly degradable CP fraction is degraded in the rumen, and decreases the undegradable CP fraction (Van Straalen & Tamminga, 1990; van Vuuren *et al.,* 1991).

As forages mature, CP degradability generally decreases (Hoffman *et al.*, 1993; Antoniewicz *et al.*, 1995; Rinne *et al.*, 1997) because the proportion of soluble CP decreases thereby resulting in a decreased rate of CP degradation and a higher proportional escape from the rumen. However, the amount of unfermented plant protein reaching the small intestine per kg DM ingested may not differ much, since CP content decreases with increasing maturity (van Vuuren *et al.,* 1991).

Some legumes contain high levels of tannins, which are polyphenolic compounds that bind with proteins to protect them from degradation in the rumen, while they can be digested in the small intestine where the pH is lower (Broderick *et al.*, 1991). Protein bownd to tannins is less extensively degraded in the rumen (Erasmus *et al.*, 1990a). The level of tannin in plants is influenced by the genetic strain, growing conditions and season (Broderick *et al.*, 1991). Bird resistant sorghum is an example of a feed high in tannins (Erasmus *et al.*, 1990a).

2.3.3.4 Forage preservation

Degradability of CP in the rumen is affected by the method used to preserve the forage (Verbič *et al.*, 1999), and is more extensive in ensiled forages than in hays (Petit & Tremblay, 1992; Verbič *et al.*, 1999). Wilting prior to ensilage reduces CP degradability in most forages (Van Straalen & Tamminga, 1990; Hristov & Sandev, 1998; Verbič *et al.*, 1999). Feed additives can also affect CP degradability, as demonstrated by Hristov (1994) and Hristov & Sandev (1998) where lucerne ensiled with sodium metabisulphite had higher ruminal CP degradability than the control silage. The CP degradability of grass is also affected by harvesting systems (Petit & Tremblay, 1992), and CP degradability generally decreases with a higher DM concentration in the preserved forage (Verbič *et al.*, 1999).

2.3.3.5 Processing or treatment of feedstuffs

Physical processing and treatment with chemical agents can influence CP degradation. This topic has been extensively reviewed (Cronjé, 1983; Erasmus *et al.*, 1988; NRC, 2001) and will only be summarized in this section.

Degradability of CP is decreased by treatment of feed with aldehydes, such as formaldehyde and glutaraldehyde (Miller & Ørskov, 1986; Van Straalen & Tamminga, 1990; Ørskov, 1992), and strong acids such as formic acid and sulphuric acid (Nagel & Broderick, 1992; Hristov & Sandev, 1998; Verbič *et al.*, 1999). Alcohol, such as ethanol, changes protein structure, making it more hydrophobic and less rumen degradable (Van Straalen & Tamminga, 1990; Broderick *et al.*, 1991), whereas alkali also decreases CP degradability (Wallace, 1988; Broderick *et al.*, 1991).

Heat treatment decreases ruminal CP degradability (Ørskov, 1992; Pereira *et al.*, 1998; Goelema *et al.*, 1999) by reducing CP solubility, by blocking reactive sites for microbial proteolytic enzymes (Broderick & Craig, 1980) and by denaturation and Maillard reactions. The effect on ruminal CP degradation depends on the temperature reached, processing time and moisture content during processing (Ørskov, 1992; Dakowski *et al.*, 1996; Goelema *et al.*, 1999). Heat treatment has been shown to increase both the undegradable and indigestible CP fractions (Van Straalen & Tamminga, 1990; Broderick *et al.*, 1991; Ørskov, 1992). A number of processing techniques, such as pelleting, extrusion, expander treatment, pressure toasting and roasting of feedstuffs, either require or generate heat which can reduce CP degradability in the rumen (Zaman *et al.*, 1995; Goelema *et al.*, 1999; Prestløkken, 1999). The correct amount of heat needs to be applied in order not to compromise intestinal digestibility (Broderick *et al.*, 1991; Pereira *et al.*, 1998).

Hydrolytic lignin (Zahedifar *et al.*, 2002), animal proteins such as feather meal, blood meal and meat meals (Ørskov, 1992; Matsumoto *et al.*, 1995) and lipid substances such as cottonseed oil, fish oil, beef tallow and soap-stock (Rossi *et al.*, 1999; Manterola *et al.*, 2001) can be used to protect CP from degradation in the rumen. Tannins can be added directly to feedstuffs to protect CP from degradation (Broderick *et al.*, 1991; Weimer, 1998) through binding of proteases (Weimer, 1998) and causing cross-linkages between proteins and carbohydrates (Ørskov, 1992).

Storage conditions have been shown to affect ruminal CP degradability. Mehrez *et al.* (1980) found that the most important factor influencing CP degradability of fishmeal was the length of time it was stored prior to processing. Freezing lucerne for 30 days at -18^{o}C prior to processing increased the extent of initial CP disappearance *in situ* (Hristov, 1998).

2.3.3.6 Animal variation

Ruminal CP degradability is also affected by the species of animal consuming the CP generally (cattle vs. sheep) (Lindberg, 1985; Šebek & Everts, 1999), as different species have different capacities to degrade feeds. Cows have a lower *in situ* rumen CP degradation rate than sheep for concentrates and higher rumen undegradable CP residue for hays (Šebek & Everts, 1999). Terramoccia *et al.* (2000) found that CP degradability is higher in buffalo than in cattle and sheep due to different rumen micro flora, longer retention time in the rumen and more intense ruminal contractions in buffalo. There are also differences among sheep breeds (Šebek & Everts, 1999). Reference CP values for feeds that are based on values for cows should not be used for sheep (Šebek & Everts, 1999) and *vice versa*. Some of these differences may be due to differences in diet composition and amount of nutrients recycled in the rumen (Šebek & Everts, 1999). If sheep and cattle were fed the same diet, microbial populations would be expected to be similar. However, there are differences because of different outflow rates (Ørskov *et al.*, 1983). In the study by Ørskov *et al.* (1983), no consistent difference in rate of CP degradation between sheep and cattle occurred, suggesting that degradation rates of CP obtained with sheep can be applied to cattle fed similar diets. However it cannot be assumed that this will apply for all diets. Nandra *et al.* (2000) found similar *in situ* CP degradabilities in sheep and cattle for lucerne, ryegrass, kikuyu, and soybean meal, although there were species differences for wheat grain. Degradability of CP seems to be similar in sheep and cattle when they are fed at maintenance levels (Nandra *et al.,* 2000). Siddons & Paradine (1983) stated that at a maintenance feeding level, soluble CP's are degraded at similar rates in sheep and cattle, whereas insoluble feed CP's tend to have higher CP degradability in sheep. Casein degrading activity of rumen liquor did not differ between sheep and steers, whereas rumen *in situ* degradation of feed DM was higher in

sheep. The ranking of feed, however, remained similar between species (Siddons & Paradine, 1983). In general it would seem that, whenever possible, use of the same species for which values are intended is advisable.

Dry matter and CP degradability in calves differs from that of mature cows up to the age of 10 - 12 weeks after which the calves ability to degrade feed CP approaches that of mature cows (Holtshausen & Cruywagen, 2000). This is to be expected due to the young calf's rumen still being in development.

Rate of disappearance of CP supplements from nylon bags suspended in the rumen differs among animals, and even within the same animal on different days (Lindberg, 1985; Broderick *et al.*, 1991). Animals have different proteolytic microbial populations, even if they receive the same or similar diets (Broderick *et al.*, 1991).

2.3.3.7 Rumen retention time and frequency of feeding

Level of DM intake, residence time in the rumen and fractional outflow rate has an effect on degradability, and extent of CP degradation in the rumen (Miller & Ørskov, 1986; NRC, 2001). It is important to correct for outflow rates from the rumen to determine effective CP degradability, as outflow rates may effect degradation of CP supplements in the rumen (Eliman & Ørskov, 1984). Erasmus (1985) reported fractional outflow rates to vary from 0.02/h to 0.06/h for cows with DMI's between 12.3 and 19.1 kg/day and suggested high-producing cows exhibit fractional outflow rates of 0.07/h to 0.10/h. It is suggested that, for practical purposes, degradability values of CP should be given in feed tables for three fractional outflow rates of small particles, 0.02/h for cattle given a low intake of mixed diets or completely milled diets; 0.05/h for calves and low-yielding dairy cows (fed energy at < 2 x maintenance = yield of < 15 kg/d for a Friesian) and 0.08/h for high-yielding dairy cows (fed energy at > 2 x maintenance = yield of > 15 kg/d for a Friesian) fed mixed diets (ARC, 1984). A slower outflow rate is expected to increase the maintenance cost of the microbes because there is more microbial recycling in the rumen (Verbič, 2002). These factors are taken into account in NRC (2001). Degradability of CP is lower at a higher level of DM intake with its higher rumen outflow rate (Erasmus *et al.*, 1988; Van Straalen & Tamminga, 1990; Zhao *et al.*, 1993). At any given degradation rate, the extent of CP degradation decreases as rumen passage rate increases (Broderick *et al.*, 1991). Using the NRC (2001) model, ruminal CP degradability of a diet decreased from 64% to 59% when DMI increased from 20 kg/day to 35 kg/day. With more extensive fluid turnover in the rumen, more soluble carbohydrates and proteins are expected to escape rumen degradation (West *et al.*, 1987).

More frequent feeding stabilizes rumen fermentation by reducing diurnal changes that the micro-organisms undergo (Lindberg, 1985; Van Straalen & Tamminga, 1990). Thus at a high level of DM intake, feeding concentrate-based diets more frequently increases CP degradation, while there is no affect when the diet consists mainly of roughages. The effect of increased frequency of feeding and level of feed intake on CP degradation seems to be restricted to concentrate based diets and is mainly evident at high levels of DM intake (Van Straalen & Tamminga, 1990).

2.3.3.8 Particle size

Degradability of CP could be related to particle size (Netemeyer *et al.*, 1980; Ehle *et al.*, 1982). Freer and Dove (1984) estimated CP degradation of coarse, medium and fine lupin seed meal to be 0.71, 0.79 and at least 0.90, respectively. However, Ehle *et al.* (1982) found that rates of CP degradation were not affected by particle size within feed samples and Netemeyer *et al.* (1980) found that neither rumen NH_3 concentrations nor digestibility of ration components were changed by particle size. Ruminal MCP synthesis and microbial efficiency are improved with increasing forage particle size (Yang *et al.*, 2002a).

With conflicting results, such as those described above, it is important to assure that feedstuffs are ground to the same particle size when a comparison between CP degradabilities are being determined, again emphasizing the importance of using a standardized technique.

2.3.3.9 Supplementation

Degradation of CP is decreased with supplementation of ionophores such as monensin (Broderick *et al.*, 1991; Ørskov, 1992; McGuffy *et al.*, 2001), ardacin, an antibacterial glycopeptide with ruminal activity, (Moloney *et al.*, 1996), defaunation agents and sodium bentonite and zinc salts (Broderick *et al.*, 1991). However, agents inhibiting deaminative activity of the rumen micro-organisms can reduce microbial degradation of dietary CP while haveing a negative effect on MCP synthesis (Tamminga, 1979).

Microbial feed additives, such as probiotics, can stabilize rumen pH to enhance the population of cellulolytic bacteria, thereby increasing MCP synthesis (Broderick *et al.*, 1991). Erasmus *et al.*, (1992) found that inclusion of a yeast culture in diets of lactating dairy cattle improved the flow of microbial CP to the duodenum, and the pattern of AA in duodenal digesta CP was also altered.

2.4 EFFECT OF BASAL DIET AND RUMEN ENVIRONMENT ON CRUDE PROTEIN DEGRADABILITY

Since there are numerous dietary factors that affect *in situ* degradation of CP, it would be ideal to perform incubations in conditions as close as possible to the environments of the rumens of the animals consuming the diet of interest (Kirkpatrick & Kennely, 1987; Assoumani *et al*., 1992; Vanzant *et al*., 1998). The protein concentrate should be evaluated using the experimental diet in which it will be included (Cronjé, 1992; Ørskov, 1992), since the rate and extent of CP degradation in nylon bags depends on the basal diet and roughage level fed to the animal being used for the incubations (Madsen & Hvelplund, 1985; Lees & Miller, 1988; Cronjé, 1992). The diet being fed, especially the form of carbohydrate, is one of the most important sources of variation in results when applying *in situ* techniques (Šebek & Everts, 1999). Most effects are when high amounts of starch are fed (Hvelplund & Madsen, 1990), although changes in roughage source can also affect *in situ* degradation results (Lindberg, 1985), thereby explaining why the nature of the diet has a major influence on proteolytic activity of rumen contents and the type of proteolytic enzymes found therein (Wallace & Cotta, 1988).

2.4.1 Forage to concentrate ratio

Differences have been shown in degradation of protein supplements in sheep fed concentrate or hay diets (Ørskov, 1992). Results from many studies have shown that rate of *in situ* CP disappearance is generally lower for animals fed a diet with a lower forage:concentrate ratio (Siddons & Paradine, 1981; Ørskov, 1992; Vanzant *et al.*, 1998). Weakly *et al.* (1983) found that disappearance of CP from soybean meal suspended in dacron bags was lowest when the animal was fed a high grain diet. An increase in maize grain level of the diet decreases the numbers amount of cellulotic species at the expense of amylolytic species (Cronje, 1992). Diets higher in NSC such as starch normally cause a decrease in microbial growth efficiency due to a decrease in ruminal pH and a slower ruminal passage rate (Mabjeesh *et al*., 1997). The diet that is fed affects rumen CP degradation and estimates of RUP. Vik-Mo & Lindberg (1985) found reduced protein degradation when feeding grass silage and barley grain instead of grass silage, barley and soybean meal. Lees & Miller (1988) found CP disappearance from nylon bags to be more pronounced when sheep were offered dried grass cubes, than a starch concentrate plus hay (in a ratio of 40:60). Inclusion of barley grain in the diet has decreased rate of CP degradation (Aldrich *et al.*, 1996), in agreement with Ganev *et al.* (1979) where degradability of CP from soybean meal, groundnut meal and sunflower meal was lower in nylon bags incubated in sheep receiving whole barley grain based diets as opposed to dried grass diets.

Differences in CP degradability are substantial for protein supplements of vegetable origin, such as soybean meal and groundnut meal, but only minor for protein supplements of animal origin (Ørskov, 1992). It is possible that the more viscous consistency of rumen liquor when cereals are fed could hinder digesta movement in and out of the bags and hence reduce rate of CP degradation of material in the bags (Siddons & Paradine, 1981; Vik-Mo & Lindberg, 1985). When the diet contains a high amount of concentrate, the pores of the nylon bags are more likely to clog due to particulate matter, and grains of starch or bacterial slime cause a reduction in solubilisation and degradation (Michalet-Doreau & Ould-Bah, 1992). In diets containing high levels of concentrates, efficiency of MCP synthesis in the rumen is lower than in well-balanced roughage-based diets (Verbič, 2002). Although amylolytic bacteria tend to be more proteolytic than cellulolytic bacteria (Siddons and Paradine, 1981; Wallace *et al*., 1997), CP degradation in studies of Cardozo *et al*. (2000) and Cardozo *et al.* (2002) was consistently lower when high-concentrate diets provided substrate to microbes, regardless of the rumen pH.

These results indicate that CP degradation is affected by pH and type of diet, which may dictate the predominant type of microbial population in the rumen. Devant *et al* (2001) incubated soybean meal and heat-processed soybean meal in the rumen of dairy cattle fed a 60:40 roughage:concentrate diet, or in the rumen of beef cattle fed a 10:90 roughage:concentrate diet. Using the *in situ* technique, results indicated that CP degradation was lower with the beef-type diet, despite that pH was 6.0 in both groups. This illustrates that the reduction of CP degradation is not only due to pH, but is also related to the type of substrate being fermented and the predominant microbial population induced by a diet. Assoumani *et al*. (1992) demonstrated that starch interferes with CP degradation in the rumen, they also noted that the addition of amylase increased ruminal CP degradation of cereal grains between 6 and 20 percentage units. Debroas & Blanchart (1993) found that proteolytic bacteria degraded NDF- bound CP, but only after microbial depolymerization of cellulose had begun.

2.4.2 Conflicting results

Tamminga (1981) varied the ratio of long roughage to pelleted concentrates from 0.29:0.71 to 0.81:0.19 and found little effect on ruminal degradation of dietary CP or efficiency of MCP synthesis. Siddons & Paradine (1981) reported that starch in the diet increased degradation of soybean meal protein in the rumen. Russell *et al.* (1981) found that higher levels of soluble carbohydrate stimulated microbial proteolytic activity (Aldrich *et al.*, 1996). In the study by Lindberg (1981b) low roughage diets (i.e., roughage:concentrate 30:70 vs. 70:30) generally had a faster ruminal CP disappearance.

Santra & Karim (2002), however, reported conflicting results compared to most other studies on the effect of roughage:concentrate ratio on ruminal CP degradability. They fed defaunated and faunated lambs diets with roughage:concentrate ratios of 50:50, 65:35 and 80:20 and found that, irrespective of the presence or absence of rumen protozoa, digestibility of DM, organic matter (OM), CP and energy increased with increasing proportion of concentrate while digestibility of cell wall constituents decreased. With a decreasing proportion of concentrate in the diet, body weight and feed conversion efficiency decreased.

Siddons & Paradine (1983) found that when barley grain was included in the roughage diet, the casein degrading activity was higher whereas *in situ* degradability of feedstuff (i.e., soybean meal, cotton seed meal, groundnut meal, meat and bone meal and dried grass) was reduced.

Conflicting results in studies conducted to compare different basal diets could be due to differences in rumen microbial population size and species composition, which is influenced by factors such as rumen pH, NH_3 concentration, type of energy substrate in the diet and the balance between rumen NH_3 concentration and fermentable carbohydrate supply (Cronjé, 1992).

2.4.3 Microbial population

The basal diet is the main factor determining numbers and species of microbes in the rumen and, therefore, rate and extent of CP degradation (Siddons & Paradine, 1981; Nocek, 1988). Differences in CP degradability in the rumen with different diets may, at least in part, be due to diet induced changes in microbial population (Siddons & Paradine, 1981; Lindberg, 1985; Kirkpatrick & Kennelly, 1987) since either types or number of micro-organisms in the rumen, or both, vary with diet (Siddons & Paradine, 1981). Any dietary factor that has an influence on the ruminal microbial population, such as starch content of the diet, could affect rate and/or extent of CP digestion in the *in situ* bags (Vanzant *et al.*, 1998).

Proteolytic activity is associated with the range of micro-organisms (Siddons & Paradine, 1981). Cereal diets have higher proteolytic activity than dry roughage diets, probably because amylolytic bacteria tend to be more proteolytic than cellulolytic bacteria (Wallace & Cotta, 1988; Calsamiglia *et al.*, 2002). Level of proteolytic activity in the rumen is also likely a function of microbial numbers, which are often higher with higher cereal diets (Siddons & Paradine, 1981). This could explain why Siddons & Paradine (1981) found that levels of proteolytic and deaminase activity in the rumen were higher when a predominantly cereal diet was fed, compared to a predominantly roughage diet, resulting in a higher degradability of casein, since soluble proteins such as casein are readily accessible to microbial proteases and their rate of degradation is determined by the level of proteolytic activity in the rumen. However, with most feed, level of

proteolytic activity in the rumen is not as important in determining rate of degradation of CP, since only a small proportion of CP is soluble in the rumen. This solubility decreases at lower pH, which could partly explain why CP degradability is lower with high concentrate diets. The accessibility, and therefore degradability, of insoluble CP to microbial proteases probably depends on rate of digestion of non-protein OM as there is often a similar disappearance of CP and DM from nylon bags (Siddons & Paradine, 1981).

All major proteolytic bacteria are found in the rumens of animals fed both low and high grain diets, while cellulolytic bacterial numbers decrease in those fed high grain diets (Loerch *et al.*, 1983). Addition of starch or sugars has resulted in decreased fibre digestibility, which is often accompanied by a drop in CP degradation (Lindberg, 1985). Reduction in cellulose digestion could explain decreased degradability of plant CP supplements with cereal diets (Siddons & Paradine, 1981).

Since the rumen of an animal contains a large number of microbial species, all having interactive effects on CP degradability, this is an important, but complex, aspect in investigating CP degradability in the rumen.

2.4.4 Differences between feeds

Some protein sources are more sensitive to changes in the rumen environment than others, resulting in the effect of roughage:concentrate ratio on CP degradability differing among feeds (Loerch *et al.*, 1983; Cronjé, 1992). Lindberg (1981a) stated that roughage seems to be more susceptible to changes in the diet than concentrate components, but Lees & Miller (1988) found that all feeds studied (i.e., soya, rapeseed, barley grain, hay), were similarly influenced by the basal diet. According to Madsen & Hvelplund (1985) the difference in CP degradability when concentrate diets were fed was considerable for vegetable proteins, but negligible for fishmeal.

Cronjé (1992) tested effects on *in situ* degradability in sheep of three basal diets with roughage:concentrate (*Eragrostis curvula* hay: maize grain) ratios of 75:25, 50:50, and 25:75. Increasing dietary maize content depressed CP degradation rate of *E. curvula* hay by 48%, but did not affect CP degradation rates of lucerne hay, maize grain, sunflower oilcake and cottonseed oilcake. Ruminal DM degradation rate of *E. curvula* was decreased by 58% and that of lucerne hay by 44%, while DM degradation rates of maize grain, sunflower oilcake and cottonseed oilcake were not affected (Cronjé, 1992). Since there was a similar response for both CP and ruminal DM degradation of *E. curvula* hay, it is likely that N components are associated with carbohydrate fractions. A decrease in CP degradation with a high maize grain diet could be due to a decrease in the number of cellulolytic species at the expense of amylolytics. These changes

in composition of rumen microbes could be due to a fall in pH from 6.2 for the high roughage diets to 5.8 for the low roughage diets. The different degradibility responses of CP and DM fractions of lucerne hay may indicate that CP degradation of lucerne is not limited by its association with fibrous components of the plant, as might be the case for *E. curvula* hay (Cronjé, 1992). This supports results of Šebek & Everts (1999), who reported that, for roughages, an increase in concentrate proportion in the diet generally resulted in a decreased degradation rate of cell walls and CP, but when CP degradation was not limited by its association with fibrous components, as for high quality roughages, there was no influence of roughage:concentrate ratio on CP degradation. In the study by Ganev *et al.* (1979), feeding sheep whole barley grain based diets or dried grass did not affect rate of CP disappearance of fishmeal, while it did for vegetable CP sources, probably because of cellulose in vegetable protein sources protecting CP from exposure to degradation. Thus when cellulose digestion is reduced, as in high concentrate diets, so is CP disappearance.

Different studies have been completed, as reported above, showing conflicting results and there are still many questions that need to be answered regarding this topic.

2.4.5 Implications

Since many dietary factors affect *in situ* and *in vitro* ruminal degradation of feeds, it would be ideal to do incubations in conditions as close as possible to the environments of the rumens of animals consuming the diet of interest (Assoumani *et al.*, 1992; Vanzant *et al.*, 1998). Therefore the test feed should be evaluated using the basal diet in which it will be included (Cronjé, 1992; Ørskov, 1992). In practical feed evaluation work, where a large number of samples need to be handled, it is not always possible to estimate rumen CP degradability of a feed in the rumen of an animal fed a diet similar to the diet where it will be used. Thus a basal diet should be formulated containing feeds that should allow a variety of micro-organisms to be established and maintained (Lindberg, 1985). Small amounts of a wide variety of CP supplements should be fed to cows used for the incubation to minimize effects of CP source on degradability (Madsen & Hvelplund, 1985). There was no specific recommendations made relative to the basal diet to be fed by the NRC (2001).

2.5 THE EFFECT OF RUMINAL pH ON CRUDE PROTEIN DEGRADABILITY

The pH of the rumen, brought about primarily by the diet being consumed, is one of the factors of the rumen environment influencing CP solubility and degradability (Assoumani *et al.,* 1992; Stern *et al.,* 1994; NRC, 2001). Alterations in pH have been shown to affect fermentation

patterns in different ways when comparing results among experiments (Van Nevel & Demeyer, 1988). Protein metabolism is the result of metabolic activity of ruminal micro-organisms, and protein structure is a key factor in determining its susceptibility to microbial proteases and its degradability. Approximately 70 - 80% of ruminal micro-organisms attach to undigested feed particles in the rumen and 30 - 50% of those have proteolytic activity (Prins *et al*., 1983). A large number of microbial species form a consortium that attaches to a feed particle, acting symbiotically to degrade and ferment a variety of nutrients, including protein. The synergistic action of different proteases is necessary for complete protein degradation because there are a number of different bond types within a single protein (Wallace *et al*., 1997). The rate and extent of CP degradation depends on the proteolytic activity of the ruminal micro-flora and the susceptibility and the accessibility of the peptide bonds. Peptides and AA resulting from extra-cellular rumen proteolytic activity are transported inside microbial cells. Peptides can be further degraded by peptidases into AA which can be incorporated into MCP or further deaminated to VFA's and ammonia (Tamminga, 1979).

According to Kopecny & Wallace (1982), the optimal pH of rumen proteolytic enzymes ranges from 5.5 to 7.0. Protein degradation is lower at the lower end of this range and so, with high concentrate diets, CP degradation is normally reduced (Ganev *et al*., 1979, Lindberg, 1981a). The reduction in CP degradability has been attributed to lower ruminal pH, which causes changes in CP solubility (Loerch *et al*., 1983) and reduces the fibrolytic activity of rumen micro flora (Mould & Ørskov, 1983). It has been concluded that cellulose protects proteins from degradation at least to some extent and it must be degraded to allow proteolysis (Ganev *et al*., 1979, Lindberg, 1981a, Devant *et al*., 2001).

Under most feeding situations, pH in the rumen is in a range where extensive degradation of dietary CP can occur. Many plant proteins are trapped in a fibre matrix that needs to be degraded before proteases can gain access for degradation. Therefore, it appears that CP degradation in the rumen requires the presence of several proteolytic and non-proteolytic enzymes, and that the combination of several microbial and enzymatic activities is required for maximum CP degradation. This was illustrated in a study by Endres & Stern (1993), who observed a reduction in CP and NDF digestion when ruminal pH decreased from 6.3 to 5.9. In this case, proteolytic bacteria counts were not affected by pH, but cellulolytic bacteria counts were reduced about 50%. It is likely that with a high-concentrate ration, even if pH is high, starch-degrading bacteria predominate, and fibre digestion is limited by the reduced number of cellulolytic bacteria, thereby reducing degradation of CP (Bach *et al*., 2005). Therefore, effects of pH and/or the substrate being fermented may affect the predominant microbial population and

modify CP degradation caused by interactions among nutrients (Endres & Stern, 1993). It was hypothesized that reduction in cellulotic bacteria as a consequence of low pH leads to a reduction in fibre degradation, reducing access of proteolytic bacteria to proteins, indirectly diminishing CP degradation (Bach *et al*., 2005).

Bartle *et al*. (1986) found that CP degradation responded to pH in a quadratic manner with degradation being lower at pH 5.5 and pH 7, but highest at pH 6.0 – 6.5. The period of time that the ruminal pH is below optimal may be more critical for digestion than the relationship between mean diurnal pH and optimal pH (de Veth & Kolver, 2001). Microbial activity is inhibited even for short periods (i.e., four hours) of a pH lower than 5.4 (de Veth & Kolver, 2001), because such a low pH alters the microbial populations in the rumen and the microbes are only able to recover when pH is back to optimum (de Veth & Kolver, 2001). Cerrato-Sánchez *et al*. (2007), found that the effect of time at suboptimal pH on rumen fermentation appeared to take place as soon as the pH started to become suboptimal (pH 5.5), and concluded that the largest effects occurred within the first 12 hours of suboptimal pH and, in most cases, longer periods had only small additional effects.

2.5.1 Decline in ruminal pH and protein degradability with high concentrate diets

When cows were fed high moisture maize grain or increasing concentrations of maize, rumen pH was reduced and *in situ* degradability declined and there was a linear decrease in ruminal CP disappearance as dietary maize level increased (Loerch *et al.,* 1983). Madsen & Hvelplund (1985) found no decrease in ruminal degradability when maize had been treated with sodium hydroxide suggesting that the CP degradability may be partly related to a lower rumen pH. Loerch *et al.* (1983) stated that decreased degradation of soybean meal in the rumen of animals fed high concentrate diets appeared to be mainly due to the associated decrease in rumen pH.

Rumen CP degradation is generally faster when roughage rich diets are fed compared to diets rich in concentrates, probably due to a higher rumen pH that stimulates microbial activity, especially fiber digesting microbes (Van Straalen & Tamminga, 1990). Elizalde *et al.* (1992), however found that effective CP degradability in wheat straw was higher in cows grazing winter oats than in cows that were also supplemented with maize silage, even though rumen pH of the latter was higher.

2.5.2 Reduced degradability of fibre-associated proteins with low pH

Acids that are produced during fermentation of grains keep the rumen pH slightly below 7.0 or at 7.0 (NRC, 2001). How far the pH drops below neutral depends on how much acid is produced, the rate at which it is absorbed from the rumen and the amount of salivary secretions which is released to neutralize the acids (NRC, 2001). When ruminants are fed a high forage diet the bacterial population shifts away from lactate producers (NRC, 2001), and Zhao *et al.* (1993) reported that ruminal forage CP degradability was higher when incubated in the rumen of goats fed lucerne hay cubes than in goats fed lucerne hay cubes and a concentrate mixture. Decrease in degradability of forage CP was associated with a reduction in pH of rumen fluid. Degradability of CP was positively correlated with rumen pH measured prior to feeding and three hours after feeding. Degradability of CP was correlated with disappearance of DM, which possibly decreased due to less cellulolytic bacteria in the rumen when the pH declined. The optimum ruminal pH range for maximum cellulose digestion is between 6.4 and 6.8 (Erdman, 1988), and fibre digestion rates (cellulolysis) decrease when ruminal pH declines below about 6.0 - 6.2 (Ørskov, 1994; Mabjeesh *et al.*, 1997; Pitt & Pell, 1997) as cellulolytic bacteria are sensitive to rumen pH and their growth is inhibited. The lower numbers of cellulolytic bacteria would reduce rupture of plant cell walls and slow release of CP for microbial degradation, reducing access of bacteria and enzymes to the CP, which would decrease degradability of forage CP (Zhao *et al.*, 1993; Calsamiglia *et al.*, 2002). If fibre is a barrier to proteolytic attack or solubilization, a higher pH can increase exposure and extent of proteolysis (Owens & Zinn, 1988). The small amount of cellulose and hemicellulose in protein supplements of vegetable origin exerts a protective action against degradation (Ørskov, 1992). A major part of CP in plant based feeds is protected from degradation by a fibrous structure, as indicated by the high correlation between CP and cell wall degradation (Lindberg, 1981a; Vik-Mo & Lindberg, 1985). The rate at which protein supplements are degraded can differ according to whether the rumen environment can support a high or low rate of cellulose digestion, especially for protein supplements of vegetable origin as opposed to supplements of animal origin (Vik-Mo & Lindberg, 1985; Ørskov, 1992). If this is the case, the effect of rumen pH is not directly related to differences in proteolytic activity but is related to differences in cellulolysis (Ørskov, 1992).

A low rumen pH value may inhibit MCP yield by inhibiting degradation of fibre (Verbič, 2002). However, Kolver & de Veth (2002) found that in cows fed fresh pasture a low ruminal pH was associated with higher MCP flow from the rumen. They found that the performance of cows fed high quality pasture was not limited when mean ruminal pH decreased to 5.8, suggesting that rumen pH has to decline below 5.8 before MCP synthesis and fibre digestion is compromized.

Many of the rumen pH studies on pastures have been conducted with limited numbers of rumen cannulated cows, but negative effects have been observed at pH values below just 6.0 (2008, Dr. R. Meeske, Pers. Comm., Department of Agriculture, Western Cape, Outeniqua Experimental Farm, PO Box 249, George 6503, South Africa).

2.5.3 Diet effect or rumen pH?

As offering a diet high in NSC decreases rumen pH, the effect of ruminal pH and type of diet are often confounded (Calsamiglia *et al.*, 2002). Altering pH of the rumen by changing the ratio of grain to roughage does not differentiate between effect of type of diet and pH *per se* on rumen microbial fermentation (Erfle *et al.*, 1982). When studying effects of pH on microbial fermentation, the buffering capacity of feeds, saliva flow and ruminal outflow rate could also be confounded (Calsamiglia *et al.*, 2002). Whether it is pH as such that causes shifts in bacterial population was questioned and the nature and concentration of nutrients were suggested to play a more important role (Therion *et al.*, 1982; Lindberg, 1985; Vik-Mo & Lindberg, 1985).

Changes in fermentation pattern after feeding a high concentrate diet could be due to a combination of substrate and pH (Calsamiglia *et al.*, 2002). Mould & Ørskov (1983) reported that micro-flora associated with an all-hay diet are inhibited, and ultimately destroyed, when rumen fluid pH is maintained below 6.1 but, when rumen pH of sheep consuming barley grain was increased, there was a minor effect on micro-flora and feed CP degradation, indicating that both type of substrate in the rumen and rumen pH are important. Strobel & Russell (1986) found that, in general, ruminal pH has a more important effect on fermentation patterns than the type of carbohydrate fed.

In vitro simulation systems (i.e., continuous culture fermentation) allow studies of the true effect of ruminal pH under conditions where diet type, feed intake, dilution rates and saliva input are controlled (Stern *et al.,* 1997; Calsamiglia *et al.*, 2002). Shriver *et al.* (1986) controlled pH in a continuous culture system at levels of 5.8, 6.2, 6.6 and 7.0, by using NaOH and HCl and found that digestibility of OM, NDF and CP were depressed at pH 5.8, increased considerably at pH 6.2 and increase slightly at pH 7.0.

Calsamiglia *et al.* (2002) used dual-flow continuous culture fermenters to study effects of pH and pH fluctuations on microbial fermentation and nutrient flow, are reported that constant low ruminal pH of 5.7 decreased CP degradation and increased non NH_3-N and dietary CP flow compared to a constant high ruminal pH of 6.4. Different pH treatments had no effect on bacterial CP flow or efficiency of MCP synthesis but the flow of essential AA was higher for low ruminal pH treatments.

Yang *et al.* (2002b) used a dual effluent flow continuous culture system to investigate effect of pH level (5.5, 6.0 or 6.5) on fermentation of a diet consisting of 50% roughage and 50% barley grain-based concentrate, and found that degradabilities of DM, OM and fibre were affected by the fermenter pH, but not degradability of CP. Addition of buffers to the diet has, in some cases, increased CP degradation rate, which was positively correlated with rumen pH (Van Nevel & Demeyer, 1988).

2.5.4 Protein solubility or microbial population?

The decline in extent of ruminal degradation of CP as ruminal pH decreases could be due to changes in the access of microbes to it, or changes in the microbial population (Owens & Zinn, 1988). The effect of pH on ruminal CP degradability is probably mediated through a decrease in rumen CP solubility or a shift to less proteolytic bacteria in the rumen (Loerch *et al.*, 1983).

Solvent pH is one of the factors influencing solubility of CP (Nocek, 1988; Owens & Zinn, 1988), as most proteins are more soluble at a pH near or above 7.0 (Owens & Zinn, 1988). Solubility of proteins is an important factor influencing CP degradability as soluble CP are generally rapidly degraded (Siddons & Paradine, 1981; Loerch *et al.*, 1983).

Proportion of the total CP soluble in the rumen is likely to be lower with cereal diets than with roughage diets due to lower rumen pH (Siddons & Paradine, 1981). Wohlt *et al.* (1973) found that as pH increased from 5.5 - 7.5 there was an increase in mean solubility of N of purified casein and isolated soy protein from 27 - 57%, but no difference in solubility between pH 6.5 and 7.5.

Rumen pH is one of the factors affecting rate of degradation of soluble CP (Leng & Nolan, 1984). In a study by Loerch *et al.* (1983), solubility was more important than *in situ* shifts in microbial population on extent of rumen degradation of soybean meal, blood meal and maize gluten meal CP. However with dehydrated lucerne, reduced CP degradation was more likely due to a shift towards less cellulolytic bacteria and hence less microbial degradation of fibre bound CP.

Ruminal pH is one of the factors influencing microbial population dynamics (Cronjé, 1992) and can be a major factor in determining competition among bacteria (Russell *et al.*, 1979; Grant & Mertens, 1992). The bacterial fraction of rumen micro-organisms has a broad pH optimum of between 6 and 7 (Wallace, 1988). While different bacterial species grow in different pH ranges (Russell *et al.*, 1979), individual species of bacteria vary in their sensitivity to low pH (Strobel & Russel, 1986; Grant & Mertens, 1992). Cellulolytic bacteria are sensitive to acid pH whereas amylolytic species are more acid tolerant (Strobel & Russel, 1986; Grant & Mertens, 1992).

Dietary induced changes in rumen micro-flora are accompanied by changes in rumen fermentation pattern. When a high cereal, as opposed to high roughage, diet is fed, soluble sugars and starch are rapidly fermented, reducing rumen pH and causing a shift to a more amylolytic population at the expense of cellulolytics and protozoa (Vik-Mo & Lindberg, 1985; Nocek, 1988).

Growth rates and yields of rumen bacteria are depressed as pH is reduced (Strobel & Russel, 1986; Stokes *et al.*, 1991), and if pH drops below 6, there is a reduction in proteolytic activity and a low count of proteolytic bacteria (Lindberg, 1985). Usually protozoa numbers also decline as pH declines, which could be partially involved in reduction in the extent of ruminal proteolysis at a low rumen pH (Owens & Zinn, 1988).

2.5.5 Proteolysis and deamination

Proteolysis and deamination are affected by ruminal pH (Tamminga, 1979; Madsen, 1986; Stern *et al.,* 1997), and protein conformation depends on the ruminal pH to which the protein is exposed (Stern *et al.,* 1997). Optimum pH for most ruminal proteolytic enzymes appears to be between 6 and 8, or about 6.5 or 7.5 (Owens & Zinn, 1988; Wallace, 1988) and, according to Reis & Reid (1959) optimum ruminal pH for production and activity of bacterial deaminases are about 7.0. Wallace & Cotta (1988) stated that proteolytic activity has a broad pH optimum at around pH 5.5 - 7.0 in rumen fluid. Lower ruminal pH inhibits protease and especially deaminase reactions (Erfle *et al.*, 1982; Van Nevel & Demeyer, 1988; Verbič *et al.*, 1999). At a ruminal pH of 5.0, protease activity measured in bacteria from the artificial rumen was 22% and the deaminase activity 10% of that at pH 7.0 (Erfle *et al.*, 1982). It is probably loss of proteolytic bacteria that caused the protease activity to be much lower at pH 5.0 than 7.0, as there were less proteolytic organisms at pH 5.5 than pH 6.0 or 7.0 (Erfle *et al.*, 1982).

Although a reduction in CP degradation at low pH has been reported (Assoumani *et al.,* 1992; Calsamiglia *et al.*, 2002), the proteolytic enzymes produced by rumen microbes are generally active over a wide pH range (Calsamiglia *et al.*, 2002) and, under most nutritional circumstances, the rumen pH will allow extensive breakdown of dietary CP (Tamminga, 1979). Proteolytic bacteria can withstand low ruminal pH (de Veth & Kolver, 2001) since acid tolerant bacteria, such as amylolytic bacteria, have proteolytic activity.

2.5.6 Microbial protein synthesis

Apart from affecting CP degradation, rumen pH could also affect MCP synthesis. Efficiency of MCP synthesis and yield of MCP are affected by rumen pH and outflow rate of

solid particles and liquid from the rumen (Mabjeesh *et al.*, 1997; Verbič *et al.*, 1999). Lower microbial efficiency when diets contain high NSC and RDP is due to a decrease in ruminal pH and lower ruminal passage rate (Mabjeesh *et al*, 1997). Increased microbial yield occurs with faster rumen dilution rate (Stern & Hoover, 1979; West *et al.*, 1987), because faster outflow rates reduce maintenance costs of microbes because they spend less time in the rumen (Verbič, 2002). Low ruminal liquid and solid turnover rates could have increased microbial recycling in the rumen and caused a large portion of energy and CP to be used for maintenance rather than growth of microbes (Feng *et al.*, 1993).

Efficiency of MCP production depends on the maintenance requirements of microbes and amount of N that microbes recycle in the rumen (Leng & Nolan, 1984). Efficiency is optimal, and MCP passage higher, when degradable CP in the rumen is synchronized with availability of fermentable energy (Stern *et al.*, 1994; Mabjeesh *et al.*, 1997; Verbič, 2002). Microbial CP synthesis depends on the availability of carbohydrates and N in the rumen (NRC, 2001). Studies by Herrera-Saldana *et al*. (1990) indicated that MCP passage to the duodenum was highest when starch and CP degradability were synchronized for fast rates of digestion, where the feeds used included barley grain and cottonseed oilcake. Flows of MCP to the small intestine were slower when the primary fermentable carbohydrate, and the CP source, were asynchronized (barley grain and brewer's dried grain) or synchronized for slow degradability (milo grain and brewer's dried grain) (NRC, 2001).

A higher and more stable ruminal pH facilitates more efficient MCP synthesis (Clayton *et al.*, 1999), which can be related to a higher rumen pH and better conditions for cellulolysis (Verbič, 2002). At lower pH, more energy is diverted from growth to non-growth processes in micro-organisms (Strobel & Russell, 1986; Verbič, 2002).

Changes in ruminal pH can explain at least part of the variability in MCP yield (Verbič, 2002). A decrease in rumen pH generally decreases MCP yield (Russell & Dombrowski, 1980; Krause *et al.*, 2002; Verbič, 2002). Strobel & Russell (1986) reported that bacterial CP synthesis was reduced when bacteria were incubated at an initial pH of 6.0 compared to 6.7. Hoover *et al.* (1984) found that in a continuous culture, CP degradation, as well as microbial growth and efficiency were higher at pH 6.5 vs 5.5. Therion *et al.* (1982) found that ruminal pH optima for growth of their microbial species was between 6.1 and 6.6, with the upper limit being between 7.3 and 7.8 and the lower limit varying between 4.4 and 5.4, depending on species. Efficiency of MCP synthesis is only affected when the pH decreases below 5.5 (Calsamiglia *et al.*, 2002).

Feng *et al.* (1993) found that microbial efficiency, and flow of non-ammonia N (NAN) and microbial CP to the duodenum were decreased by diets with 39% NSC as opposed to 29%. This

low microbial yield appeared to be due to a low turnover rate of ruminal contents, rather than from depressed ruminal pH (Feng *et al.*, 1993).

2.5.7 Differences between protein sources

The pH-mediated effect on potential degradative activity of rumen contents differs among feeds (Cronjé, 1992). Some CP sources are more sensitive to changes in the rumen environment than are others (Loerch *et al.*, 1983; Aldrich *et al.*, 1996). Ruminal pH influences solubility of plant CP's more than animal CP's (Owens & Zinn, 1988), and effects of lower rumen pH are more pronounced for degradable (i.e., soybean meal, dehydrated lucerne) than undegradable (i.e., blood meal, meat and bone meal, maize gluten meal) CP supplements (Loerch *et al.*, 1983; Aldrich *et al.*, 1996). Increasing solvent pH from 5 - 7 resulted in a 3.6- to 23.3-fold increase in N solubility for soybean meal and casein respectively while blood meal, meat and bone meal, pelleted dehydrated lucerne and maize gluten meal were not affected (Loerch *et al.*, 1983). The solvent pH x protein source interaction also differs among CP sources for example effects of ruminal pH for groundnut and soybean meals was more pronounced between pH 5 and 6.5, while for cottonseed meal the major influence occurred between 7.5 and 9 (Loerch *et al.*, 1983).

2.5.8 Extent and duration of pH drop

Even small declines in ruminal pH, typical of those seen in dairy cattle, can be detrimental to rumen microbial synthesis (Strobel & Russel, 1986). The period of time that ruminal pH is below optimal may be more critical for digestion than the relationship between mean diurnal pH and optimal pH (de Veth & Kolver, 2001). de Veth & Kolver (2001) found a negative linear relationship between time at sub-optimal pH and microbial CP flow and a large diurnal variation in ruminal pH may reduce microbial growth to a larger extent than when mean ruminal pH is less variable. Variation is less when feeding is more frequent, probably by avoiding drops in pH below 6 which negatively affect digestion (Madsen & Hvelplund, 1988).

Microbial activity is inhibited even when exposed to short periods (i.e., four hours) of sub optimal pH (5.4) (de Veth & Kolver, 2001), which affect the microbial population, with microbes being able to recover in intervals when ruminal pH is optimal. Therefore the depression in microbial growth is not as large when reductions in rumen pH are cyclic and of short duration (Stokes *et al.*, 1991). In the study by Calsamiglia *et al.* (2002), the effect of a short time period of decrease in pH was small. It seems clear that pH can drop below 6 for up to four hours without substantially affecting microbial fermentation.

2.5.9 Implications

Effects of ruminal pH on microbial fermentation are generally considered in current feeding systems for dairy cattle (Calsamiglia *et al.*, 2002). However metabolic models such as the CPM does take ruminal pH effects into account (CPM-Dairy, 1998). From the above discussion it is clear that low ruminal pH could either reduce CP degradability or MCP synthesis, or both. A possible benefit from decreased rumen proteolysis would be an increase in dietary CP escaping rumen degradation (i.e., increased RUP supply (Erfle *et al.*, 1982), which is needed for high producing ruminants. Reduced CP degradation due to low rumen pH would be a way to increase the RUP fraction of CP. If this effect is significant, and is not taken into account with diet formulation, there is a possibility that RUP might be overfed. The resulting lower RDP would mean less CP available for MCP synthesis, suggesting that a highly degradable CP source would need to be included in the diet. This would be of economic significance, since CP sources that are highly degradable are more cost effective than those that are high in RUP, which are usually more expensive. Thus short periods of low pH could be beneficial in terms of CP nutrition, by increasing flow of NAN and essential AA to the small intestine. However, low pH for a long period of time would impare rumen fermentation, reduce fibre digestion and reduce saliva production resulting in accumulation of VFA which could lead to digestive disturbances such as acidosis (Calsamiglia *et al.*, 2002).

2.6 BOTTOM LINE

High concentrate diets and low ruminal pH have been reported to cause reduced CP degradability whilst in others it has not. The latter could have been due to more energy being available for rumen microbes and, probably, more amylolytic bacteria that have a higher proteolytic activity being present in the rumen. Lower CP degradability in most cases was probably due to cellulolysis being inhibited, which would reduce degradability of CP that is partially protected by fibre. Results from these studies suggest that effects of diet and rumen pH on CP degradability depend on the CP source. If CP is readily accessible, reducing proteolytic activity in the rumen can reduce degradability, whereas if the CP is protected by cellulose, degradability can be reduced by inhibiting cellulose degradation.

If all or most factors affecting CP degradability could be quantified, these could be incorporated into a model to predict CP degradability in the rumen. Some of the factors that affect ruminal CP degradability, such as rate of passage, are taken into account in current diet formulation models, while others, such as rumen pH, are only induced to a limited extent.

In some situations a high and in others a lower, CP degradation is necessary for optimal animal production (Madsen & Hvelplund *et al.*, 1985; Erasmus *et al.*, 1990b). The ratio of RDP to UDP needs to be balanced for each situation to meet the requirements of the animal, but the many factors affecting CP degradability complicate the process of attaining this balance.

The objective of this study, therefore, was to investigate effects of different roughage:concentrate ratios in the diet has on ruminal pH and fermentation. Effects that these changes caused in ruminal fermentation and rumen pH had on CP degradability of sunflower oilcake, cottonseed oilcake and roasted soya was investigated.

CHAPTER 3.

MATERIALS AND METHODS

3.1 ANIMALS AND EXPERIMENTAL DESIGN

Three rumen cannulated Holstein cows from the University of Pretoria's Hatfield Experimental Farm were used. The cows were late in lactation with a body weight of 722 ± 25.6 Kg at the onset of the trial. The cannulas (10 cm diameter) were obtained from Ankom Technology Co. (Macedon, NY).

The experimental design was a 3 x 3 Latin Square (Table 1) in which each cow received a diet differing in roughage:concentrate ratio during each of the three periods. The three roughage:concentrate diets, namely UP 30 (30% roughage:70% concentrate), UP 45 (45% roughage:55% concentrate) and UP 60 (60% roughage:40% concentrate) were intended to create low, medium and high ruminal pH values.

Each experimental period consisted of 10 days for adaption followed by six days for *in situ* incubation and ruminal sampling. Each six-day period was devided into a 72 hour *in situ* incubation and ruminal sampling period followed by a repeated 72 hour *in situ* incubation period only. Three protein concentrates differing in expected ruminal CP degradability, namely sunflower oilcake, cottonseed oilcake and roasted soya beans were incubated in each animal during each period.

Table 1 Experimental design: Latin Square

	Cow number		
Period	30	117	121
1	UP 30[1]	UP 60	UP 45
2	UP 45[1]	UP 30	UP 60
3	UP 60[1]	UP 45	UP 30

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45 % roughage: 55% concentrate) and UP 60(60% roughage:40% concentrate)

3.2 EXPERIMENTAL DIETS, COWS AND MANAGEMENT

The experimental diets (treatments) were formulated to create different rumen environments resulting in different pH values. The ingredient and chemical composition of the experimental diets is shown in Table 2. Care was taken to formulate diets that differed in roughage:concentrate ratio, but would still be realistic diets to feed under practical conditions. The cows were fed *ad libitum* twice a day and DMI's were determined on a daily basis. Feed bunks were cleared of orts and fresh feed was provided every day at 6:00 h. Treatment UP 30 was expected to cause the lowest pH value resulting in more amylolytic species to be present in the rumen than cellulolytic species, when compared with treatment UP 45. Treatment UP 60 was expected to cause the highest pH value resulting in more cellulolyic species compared to amylolytic species to be present in the rumen.

Cows were housed separately and individual DMI were monitored. Cows were milked three times per day; the first milking occurring at 5:00 h, the second at 12:00 h and the third at 19:00 h. Before each experimental phase cows were adapted to the new diet for 10 days, but were fed *ad libitum* during the adaptation and experimental phases so that the least disturbance to flow rate and rumen retention time was caused. Clean water was available *ad libitum*. When samples were taken, or *in situ* bags added or removed, the cows were herded into a crush pen. The cows received no additional treatments during the measurement period. Feed samples were collected daily at feeding; a sample from the bottom, middle and top part of the bag was pooled to give a representative sample of that bag. All samples were then pooled at the end of each period to give a representative sample for that period.

3.3 PROTEIN CONCENTRATES USED FOR *IN SITU* EVALUATION

Three protein concentrates that differed in potential CP degradability were selected, with roasted soya representing a low RDP concentrate, cottonseed oilcake a medium and sunflower oilcake a highly RDP concentrate.

3.4 THE *IN SITU* TECHNIQUE

For the *in situ* incubations, 10 X 20 cm polyester monofilament bags with a pore size of 53µm (±10) were used (Ankom Technology Co., Macedon, NY). The bags were numbered with a permanent marker, oven dried at 65˚C for 30 minutes and weighed after being cooled to room temperature in a desiccator to determine empty mass (Osuji *et al.*, 1993). Before incubation, the protein concentrates were ground through a 2 mm screen (Erasmus *et al.*, 1990a; Osuji *et al.*, 1993), and these samples were then dried in an oven at 103˚C for 24 hours to determine the

Table 2 Ingredient and chemical composition (%) of the three experimental diets (treatments) as estimated using the CPM Dairy Model (CPM-Dairy, 1998)

Item	Treatments[1]		
	UP 30	UP 45	UP 60
Ingredient (As fed basis)			
Lucerne hay	14.82	22.31	29.81
Eragrostis curvula hay	14.82	22.31	29.81
Maize grain (Med ground)	42.08	33.12	24.12
Molasses	6.43	5.05	3.66
Cottonseed Meal	6.49	5.12	3.7
Soybean meal (Roasted)	4.27	3.36	2.45
Sunflower Meal	8.56	6.74	4.91
Energy Booster [2]	0.80	0.63	0.57
Calcium Carbonate	0.40	0.31	0.23
Trace Mineral Premix[3]	0.88	0.69	0.50
Salt	0.44	0.34	0.25
Chemical composition (DM basis)			
DM	88.91	89.14	89.38
Roughage (%DM)	30.01	45.06	60.03
ME (MJ/kg)	11.43	10.49	9.55
CP (%DM)	16.18	15.44	14.68
NDF (%DM)	26.41	32.56	38.66
NFC (%DM)	46.70	41.40	36.07
Fat (%DM)	5.29	4.76	4.35
Ca (%DM)	0.74	0.81	0.88
P (%DM)	0.44	0.41	0.39

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

[2] Rumen Protected Fat, Milk Specialities Company, Dundee, IL

[3] Contains per kg of premix: 4.25 million international units (MIU) Vitamin A, 0.75 MIU Vitamin D_3, 7.5g Vitamin E, 1500mg Cobalt, 2g Potassium, 200mg Selenium, 50g Manganese, 15g Copper, 40g Zinc, 200g Magnesium and 25g Iron

DM content (procedure 934.01 AOAC, 2000). Five grams of air-dried ground feedstuff (weighed precisely to three decimal places) were placed in each bag (Cronjé, 1992). Bags were tightly tied using nylon string (Osuji *et al.*, 1993).

Each of the three protein concentrates was incubated in the rumen for 2, 4, 8, 16, 24, 48 and 72 hours, in each of the cows for each period. There was thus one bag, for each of the three protein concentrates in the rumen, at each of the seven incubation periods. Bags were added sequentially and removed at the same time (Erasmus *et al*., 1988), in the complete exchange method (Paine, *et al.*, 1981). This sequence was repeated twice within each period and the two sequences of disappearance were treated as replications for the statistical analysis. There were a total of 216 samples per repetition and, since the 0 time incubation sample did not need to be incubated, there was a maximum of 21 bags in the rumen of any cow.

Bags were tied to a stainless steel disc prior to suspension, and placed in the rumen at the following times: 12:00 h on day 10 of each period (for 72 h incubation), 12:00 h on day 11 (48 h), 12:00 h on day 12 (24 h), 20:00 h on day 12 (16 h), 4:00 h on day 12 (8 h), 8:00 h on day 12 (4 h) and 10:00 h on day 12 (2 h). All the bags were removed at 12:00 h on day 13. The experimental phase was then repeated on days 13 - 16, yielding two bags per protein concentrate per cow per experimental period for each incubation time. There were therefore six bags per protein concentrate for all incubation times.

All the bags, including the 0 h time bags, were washed under running tap water until the fluid squeezed from the bags were clear, and then dried for 48 h at 65˚C. The contents of the bags were weighed after being cooled in a desiccator (Osuji *et al.*, 1993). Dry matter was determined by drying samples in an oven at 103˚C for 24 hours (procedure 934.01 AOAC, 2000). These results were used to determine CP disappearance for each bag. The degradability was calculated as the difference between the CP initially present in the bag and that present after the incubation, stated as a proportion of the initial CP (McDonald *et al*., 2002).). The model proposed by Ørskov & McDonald (1979), was used to determine the CP degradability. This technique is subject to several inherent sources of error (i.e., sample size, bag size, porosity of the bag material and treatment of the bags following the removal from the rumen (McDonald *et al*., 1981), which must be controlled if reproducible results are to be obtained. This method makes the basic assumption that loss of CP from the bag, reflecting solubility in the rumen fluid, is synonymous with degradability (McDonald *et al*., 1981). It is known that small amounts of feed CP that are solubilized leave the rumen without being degraded, casting doubt on the veracity of the values obtained using the technique. It is also known that acid-detergent insoluble N (ADIN), known to be largely undegradable, may disappear during incubation. Another complicating

factor is the presence of rumen bacteria in the bags, which contribute to its N content (McDonald *et al*., 1981). Even though the technique has shortcomeings, it is the most practical method to determine CP degradability, if a standard procedure is followed to reduce variation.

3.5 PARAMETERS MEASURED

During the experimental phase from days 11 - 13, samples of rumen fluid were collected for pH determination, NH_3-N analysis and VFA analysis for each cow at the following times: 12:00 h, 18:00 h, 24:00 h, 6:00 h and 8:00 h on day 10, 14:00 h, 24:00 h, 2:00 h, 4:00 h and 9:00 h on day 11, 16:00 h, 22:00 h, 4:00 h and 10:00 h on day 12. This sequence resulted in obtaining a sample every two hours for the 24 hour day. The same procedure was repeated for only rumen pH during days 13 - 16 of each period. For rumen parameters, only rumen pH sampling was duplicated, but CP disappearance was estimated by calculating the amount of CP that disappeared at different time intervals, as described in section 3.4.

Ruminal pH was determined by collecting equal proportions of ruminal fluid from different locations in the rumen. This rumen fluid was then pooled and the pH measured immediately using a portable pH meter (Mini Lab ISFET, Model IQ 120 pH meter, IQ Scientific, Carlsbad, CA, USA). The sampling technique, as well as the site of sampling, has effects on average rumen pH (Erdman, 1988), which is a complication when comparing pH values among studies. In our study, the same amount of rumen fluid was drawn from the top, middle and bottom layers of the rumen. The fluid was then filtered through a double layer of cheesecloth and the pH measured immediately after withdrawal from the rumen so that minimum exposure to oxygen occurred as exposure to oxygen can cause an increase in ruminal pH. Indwelling continuous ruminal pH measurement devices normally results in values that are about 0.05 units lower than manual measurements of pH taken from the same location within the rumen (Penner *et al*., 2006).

After the rumen fluid was pooled and the pH measured, 60 ml of fluid was collected and filtered through four layers of cheesecloth (De Bruin, 1995). From each sample 5 ml of rumen filtrate was preserved with 1 ml of 25% H_3PO_4 for VFA analysis and stored frozen at -20˚C (Beauchemin *et al.*, 2003), 30 ml of the fluid was preserved with 5 ml of 50% H_2SO_4 for the NH_3-N analysis and stored frozen at -20˚C till further analysis could be done (De Bruin, 1995). For VFA analysis and NH_3-N analysis samples were analyzed for 3 cows X 3 periods X 14 samples per period for a toatal of 126 analyses.

When samples were removed from the rumen they were dried and weighed to create a total of 450 DM analyses (i.e., 18, 0 hour incubations (3 protein concentrates X 3 periods X 1 repeat)

plus 432 after incubations (i.e., 3 animals X 3 periods X 3 protein concentrates X 8 incubation times X 1 repeat).

The content of the incubated protein concentrates was analyzed for all three periods using Leco analysis (i.e., 432 analyses for each phase (procedure 968.06 AOAC, 2000)). Values were used in CP disappearance as well as CP degradation calculations.

During all three periods, a sample of feed was collected from each of the three diets for analysis as described in section 3.6. For each of the protein concentrates, a sample was collected at the start of the study and analyzed as described in section 3.6.

3.6 SAMPLE ANALYSIS

Laboratory analyses were done at Nutrilab, Department of Animal and Wildlife Sciences, University of Pretoria. The protein concentrate samples and the samples from the three experimental diets were analyzed in duplicate for DM (procedure 934.01 AOAC, 2000), ash (procedure 942.05 AOAC, 2000), ether extract (EE)(procedure 920.39 AOAC, 2000); CP (Leco analysis (procedure 968.06 AOAC, 2000)), soluble CP (Tilley & Terry, 1963; procedure 968.06 AOAC, 2000), NPN (Faichney & White, 1983), starch (MacRae & Armstrong, 1968; Faichney & White, 1983; AOAC, 1990), acid detergent lignin (ADL) (Goering & Van Soest, 1988), NDF (Robertson & Van Soest, 1981), ADF (Goering & Van Soest, 1988), gross energy (GE)(MC – 1000 Modular Calorimeter), Ca (Giron, 1973), P (procedure 965.17 AOAC, 2000) and *in vitro* organic matter digestibility (IVOMD) (Tilley & Terry, 1963). The metabolizable energy value for treatments and protein concentrates was determined by using the equation ME = 0.82 x (GExIVOMD) (Robinson *et al*., 2004).

Volatile fatty acids of rumen fluid were analyzed using gas chromatography (Webb, 1994), and rumen ammonia nitrogen (NH_3-N) in rumen fluid was analyzed using the procedure of Broderick & Kang, (1980).

3.7 STATISTICAL ANALYSIS

For all the repeated variables (i.e., pH, VFA, rumen ammonia and N disappearance), an analysis of variance with GLM model (Statistical Analysis Systems, 2006) was used to determine the significance of differences between the treatments, periods and cows for the balanced data in a Latin square design. When time was included, the GLM Repeated Measures Analysis of variance was used (Statistical Analysis Systems, 2006). Means and standard error of the mean (SEM) were calculated. Significance of differences ($P < 0.05$) and tendencies ($P \leq 0.10$) between means was determined using Fischer's test (Samuels, 1989).

Linear or quadratic regression of effects of pH on N disappearance as a dependant variable and rumen ammonia on N disappearance was analyzed with the GLM model (Statistical Analysis Systems, 2006). The analyses were done for all the incubated time periods, but only those that indicated a correlation are discussed. The incubation periods not discussed showed no relationship between pH and N disappearance of rumen ammonia and N disappearance.

The CP degradation data were used utilizing the Ørskov model (Ørskov & McDonald, 1979), an analysis of variance with the GLM model (Statistical Analysis Systems, 2001) was used to determine the significance between different treatments, protein concentrates, periods and cows for the balanced data in a Latin square design.

The percentage CP disappearance for each incubation was calculated from the proportion remaining after rumen incubation (Ørskov & McDonald, 1979). The extent of degradation was fitted to the equation of Ørskov and McDonald (1979): $p = a + b (1 - e^{-ct})$ where p = proportion degraded at time t, a = an intercept representing soluble CP, b = the insoluble potentially degradable CP fraction, and thus a + b would represent the maximum extent of degradation or the asymptote of the equation (Erasmus *et al.*, 1990a). Non-linear parameters a, b and c were estimated by an iterative least-square procedure (Erasmus *et al.*, 1990a). This equation has the advantage of providing factors (a,b,c) which have biological relevance (Ørskov & McDonald, 1979). The degradation rate of the b fraction would be described by c, the fractional rate constant / h. Intercept 'a' is thus the CP which is water-soluble and which is considered to be immediately degradable, and 'b' represents the CP that is degraded more slowly. By using the fractional outflow rate, *k*, the effective protein degradation (p) was calculated (Ørskov & McDonald, 1979).

$$p = a + [\, bc/(c+k)]$$

As indicated in the literature, fractional outflow rates vary from 0.02/h for animals at maintenance to 0.08/h for high producing dairy cows (Erasmus, 1993). In this study 0.05 and 0.08 were used for *k* in calculating effective degradability (Ørskov & McDonald, 1979).

CHAPTER 4.

RESULTS AND DISCUSSION

4.1 EXPERIMENTAL DIETS

4.1.1 Composition of experimental diets

The feed samples of the three different treatments were analyzed and the chemical and ingredient composition are in Table 3.

Table 3 The chemical composition of the three experimental diets differing in roughage:concentrate ratio

	Treatments[1]		
Composition (%DM)	UP 30	UP 45	UP 60
Dry matter	91.65	92.16	92.55
Crude protein	15.90	15.29	14.38
Non protein nitrogen	0.12	0.15	0.20
Soluble protein (As % of CP)	39.17	42.59	42.95
Metabolizable energy, MJ/kg[2]	12.12	11.31	11.15
OM	92.77	92.10	91.47
Ether extract	6.70	4.34	3.07
In vitro OM digestibility	78.24	75.89	75.75
Neutral detergent fibre	33.36	37.08	42.63
Acid detergent fibre	20.10	24.35	25.88
Acid detergent lignin	4.96	5.78	5.19
Non fibre carbohydrates[3]	37.52	36.49	32.88
Starch	25.36	19.53	17.21
Calcium	0.76	0.71	0.75
Phosphorus	0.35	0.30	0.28

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

[2] Metabolizable enery (ME) = 0.82x(GExIVOMD) (Robinson *et al*., 2004)

[3] NFC=100-(%NDF+%CP+%Fat+%Ash) (NRC, 2001)

The DM levels were comparable among the treatments. The CP value for treatment UP 30 was numerically higher than that of treatment UP 45 and UP 60. Variations like these are likely to occur where TMR's are analyzed for nutritional parameters. The NPN levels were comparable across the three treatments with treatment UP 60 haveing the highest and treatment UP 30 the lowest value. The estimated ME value was highest for treatment UP 30, lowest for treatment UP 60, with treatment UP 45 having an intermediate value due to the higher maize grain level in treatment UP 30. The OM content, IVOMD, Ca and P levels were similar among treatments. Treatment UP 30 had the highest starch percentage with treatment UP 45 intermediate and treatment UP 60 resulting in the lowest value, probably due to the higher maize grain level in treatment UP 30. Neutral detergent fibre levels were lowest for treatment UP 30, with treatment

UP 60 being the highest, due to the high hay and lusern levels in treatment UP 60. These chemically determined values are not the same as were predicted by the CPM model (Table 2), possibly because of matrix differences in the different raw materials databases. Nutrient levels within a feed can vary and, in this case, the South African feeds might have had different nutrient values than those in CPM Dairy. Sampling errors can be another cause of variation, and laboratories use different analytical procedures (Batajoo & Shaver, 1998) and different experimental techniques (Kirkpatrick & Kennely, 1987) could also have caused some variation.

The diet fed, especially the form of carbohydrate and roughage source are important sources of variation when estimating ruminal CP degradation using an *in situ* technique (Šebek & Everts, 1999; Lindberg, 1985). Diets higher in NSC, such as starch, normally cause a decrease in microbial efficiency due to a decrease in ruminal pH and a slower ruminal passage rate (Batajoo & Shaver, 1998; Mabjeesh *et al*., 1997). Krause *et al*. (2002) found that increasing the level of rumen fermentable carbohydrate decreased the ruminal pH and the minimum diurnal ruminal pH. The nature of the diet has a major influence on proteolytic enzymes and proteolytic activity of the rumen fluid (Wallace & Cotta, 1988). In this study the same feeds were used in all treatments and only the in levels in the experimental diets were altered to keep the variation in CP degradation caused by different feeds to a minimum.

4.1.2 Composition of protein concentrates used for *in situ* evaluation

Three protein concentrates that differed in expected CP degradability were selected. Roasted soya represents a low RDP concentrate, cottonseed oilcake a medium and sunflower oilcake a high RDP concentrate. Differences in the chemical composition of the protein concentrates are in Table 4. Cottonseed oilcake had a CP value of 43.88% compared to the 44.9% (DM basis) reported in the NRC 2001. The 37.27% CP value for roasted soya is lower than the 43% CP (DM basis) reported in the NRC (2001). The 40.27% CP value for sunflower oilcake is comparable to the 36.0% CP reported by Ewing (1997). Sunflower oilcake had the highest level of soluble CP followed by cottonseed oilcake and then roasted soya. Due to the higher fat value of roasted soya, it had a much higher ME content than the other two protein concentrates. Cottonseed oilcake had the highest NDF value followed by sunflower oilcake and then roasted soya. The NRC (2001) reported an NDF value of 30.8% for cottonseed meal, 40.3% for sunflower oilcake and 22.1% for roasted soya. Sunflower oilcake had the highest ADF levels. The NRC (2001) reported an ADF value of 19.9% for cottonseed meal, 30.0% for sunflower meal and 14.7% for roasted soya.

Table 4 Chemical composition of the protein concentrates ruminally incubated

Composition (%DM)	Protein concentrates		
	Sunflower oilcake	Cottonseed oilcake	Roasted soya
Dry matter	90.78	90.55	93.59
Crude protein	40.27	43.88	37.27
Non protein nitrogen	0.06	0.34	0.23
Soluble protein (As % of CP)	70.43	26.53	17.50
Gross energy, MJ/kg DM	20.71	20.91	22.76
Metabolizable energy, MJ/kg DM[1]	11.74	11.97	15.68
OM	94.65	92.90	93.42
Ether extract	3.77	5.84	16.53
In vitro OM digestibility	69.14	69.82	84.02
Neutral detergent fibre	34.75	35.49	33.34
Acid detergent fibre	27.17	16.89	18.83
Acid detergent lignin	11.16	8.28	11.42
Non fibre carbohydrates[2]	15.35	6.93	7.13
Starch	0.19	0.44	2.19
Calcium	0.27	0.22	0.28
Phosphorus	1.01	1.32	0.58

[1] ME = 0.82x(GExIVOMD) (Robinson *et al*., 2004)

[2] NFC=100-(%NDF+%CP+%Fat+%Ash) (NRC, 2001)

Ewing (1997) reported a starch value of 3% for cottonseed meal, 1.5% for sunflower meal and 3.7% for roasted soya. Even though there are differences the overall values obtained in this study, they are comparable to previously published values.

4.1.3 Experimental diets and its effect on DMI and rumen pH

There were no differences ($P > 0.05$) in DMI of the different treatments (Table 5). Intake of feed has a major effect on ruminal pH which generally declines after feeding. The decrease in pH will be dependent on the initial pH (Maekawa *et al*., 2002a; Nocek *et al*., 2002), with the lowest pH generally occuring four to six hours after feeding (Lindberg, 1981a). Infrequent feed intake causes major fluctuations in pH. Maekawa *et al*. (2002b) found that feed intake and salivary secretion affect the pH in the rumen. Higher levels of feed or DM intake cause a lower rumen pH (Madsen and Hvelplund, 1988; Zhao *et al*., 1993).

Table 5 Effect of roughage:concentrate ratio on DMI (Dry Matter Intake)

Treatment[1]	Intake (kg/day)[2]
UP 30	22.41
UP 45	21.84
UP 60	22.55

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

[2] Standard error of the mean (SEM)=0.68

In a study conducted by Haaland *et al*. (1982), ruminal pH was 6.57 at maintenance and 6.35 at a feeding level of two times maintenance. Madsen (1986) found that doubling the feeding level altered rumen pH from 6.59 to 6.47. A higher feed intake leads to more fermentation acids produced in the rumen which is not compensated by the increased salivary secretion associated with increased chewing time (Maekawa *et al*., 2002b). There were no treatment differences in DMI in this study suggesting that any differences in pH among treatments would not be as a result of differences in the level of intake.

There were differences in the average pH values obtained from feeding the three treatments ($P < 0.05$) (Table 6). Ruminal pH can vary from more than 7.0 to less than 5.0 depending on the type of diet fed to an animal as well as on the rate and frequency of feeding (Erfle *et al.*, 1982). Treament UP 30 caused the pH to drop below pH 5.80 for approximately two and a half hours and below pH 6.00 for approximately nine hours in the day (Figure 1). Generally a rumen pH of 5.80 is considered as a critical threshold for fibre degradation (Rotger *et al*., 2005). The time of ruminal pH below the critical threshold for fibre degradation of 5.80 increases with increasing grain level in the diet (Nocek *et al*., 2002). With cows consuming treatment UP 45 and treatment UP 60 the pH never dropped below a pH of 6.00. In a study by Rotger *et al*., (2005) four Holstein heifers were fed one of two TMR's, being a 12:88 and a 30:70 roughage:concentrate ratio on an *ad libitum* basis, that were iso-energetic (11.5 MJ of ME/kg DM) and iso-nitrogenous (151 g/kg of CP). The average rumen pH was 6.0 for both diets and the time pH below 5.8 was the same for both treatments (i.e., 10.4 ± 1.6h).

In our study, feed was provided *ad libitum* to reduce fluctuations in ruminal pH normally associated with intake of feed to simulate feeding of a TMR diet under practical conditions. The ruminal pH values during this study ranged from 5.73 to 6.59 indicating that values were compatable to the physiological boundaries of the cow. When ruminal pH is lower than 5.50 the

Table 6 The effect of roughage:concentrate level on rumen pH at different time intervals

	Treatment[1]			
Time	UP 30	UP 45	UP 60	SEM
2:00	$5.87^{a}{}_{d}$	$6.15^{a}{}_{e}$	$6.49^{b}{}_{de}$	0.10
4:00	$6.03^{a}{}_{d}$	$6.30^{ab}{}_{e}$	$6.59^{b}{}_{d}$	0.11
6:00	$6.07^{a}{}_{d}$	$6.37^{ab}{}_{e}$	$6.43^{b}{}_{de}$	0.10
8:00	6.09_{d}	6.21_{de}	6.37_{e}	0.11
10:00	6.01^{a}	6.28^{b}	6.55^{c}	0.08
12:00	$6.12^{a}{}_{d}$	$6.41^{ab}{}_{e}$	$6.48^{b}{}_{de}$	0.12
14:00	6.02^{a}	6.23^{ab}	6.38^{b}	0.10
16:00	6.16^{a}	6.25^{a}	6.50^{b}	0.06
18:00	6.12^{a}	6.35^{ab}	6.47^{b}	0.10
20:00	5.73^{a}	6.06^{b}	6.26^{b}	0.09
22:00	5.82^{a}	6.24^{b}	6.24^{b}	0.10
24:00	5.87^{a}	6.10^{ab}	6.36^{b}	0.11
Averages	6.00^{a}	6.27^{b}	6.44^{c}	0.05

^{abc} Means in the same row with different superscripts differ ($P < 0.05$)

$_{de}$ Means in the same row with different subscripts tend to be different ($P \leq 0.1$)

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

risk of sub-clinical and clinical acidosis increases significantly. Strobel & Russell (1986) found that bacterial protein synthesis was reduced when bacteria were incubated at an initial pH of 6.00 compared to a pH of 6.70.

The purpose of this study was to investigate whether there would be any benefit from feeding a diet that causes a lower pH, but is still practical under normal feeding conditions. If cows were under a constant threat of acidosis, the value from increasing the RUP level from a lower pH would be irrelevant and unrealistic. The experimental diets therefore succeeded in creating ruminal environments that differed ($P < 0.05$) in pH, but were still within the pH boundaries experienced with diets normally fed in practice.

Treatment UP 30 resulted in a lower average ruminal pH value than treatments UP 45 and UP 60 ($P < 0.05$) (Table 6). This was expected due to the reduced NDF from 38.66% (DM basis) in treatment UP 60 to 26.41% (DM basis) in treatment UP 30, and that NFC increased from 36.07% (DM basis) in treatment UP 60 to 46.70% (DM basis) in treatment UP 30 (Table 2). Rumen pH of cows receiving treatment UP 30 varied from 5.73 to 6.16, treatment UP 45 varied from 6.06 to 6.41 and treatment UP 60 varied from 6.24 to 6.59. The lowest pH for an individual

animal was 5.41 for cow 117 on treatment UP 30 (Table 7). The pH value of cows receiving treatment UP 45 and treatment UP 60 increased and decreased over approximately the same time intervals (Figure 1). Cows fed these diets had a decrease in pH from 6:00 till 9:00 h, then again decreasing from 12:30 till 15:00 h, and also a decrease in pH occurring from 19:00 to 21:00h. Cows fed treatment UP 30 followed the same pattern except for not haveing a decrease from 8:00 h and not 6:00 h, as was the case with cows fed treatments UP 45 and UP 60. The pH values of the cows were at their lowest from about 19:00 h to 02:00 h, possibly due to a reduced bicarbonate flow from saliva and an accumulation of fermentation acids (Krause *et al*., 2002). In general, the fluctuation in rumen pH compared well with other studies for example de Veth & Kolver (2001) indicated that pH could vary from 5.50 to 6.50 within 24 hours in a dairy cow fed a total mixed diet twice daily.

Table 7 The mean, minimum and maximum ruminal pH values of cows receiving diets with differing roughage:concentrate ratio's

	Treatments[1]		
	UP 30	UP 45	UP 60
Cow 30			
Minimum	5.51	5.93	6.06
Maximum	6.41	7.03	6.88
Average	6.01	6.38	6.40
Cow 117			
Minimum	5.41	6.00	5.91
Maximum	6.70	6.93	6.91
Average	6.03	6.34	6.47
Cow 121			
Minimum	5.54	5.71	5.70
Maximum	6.43	6.61	6.82
Average	5.97	6.07	6.45

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

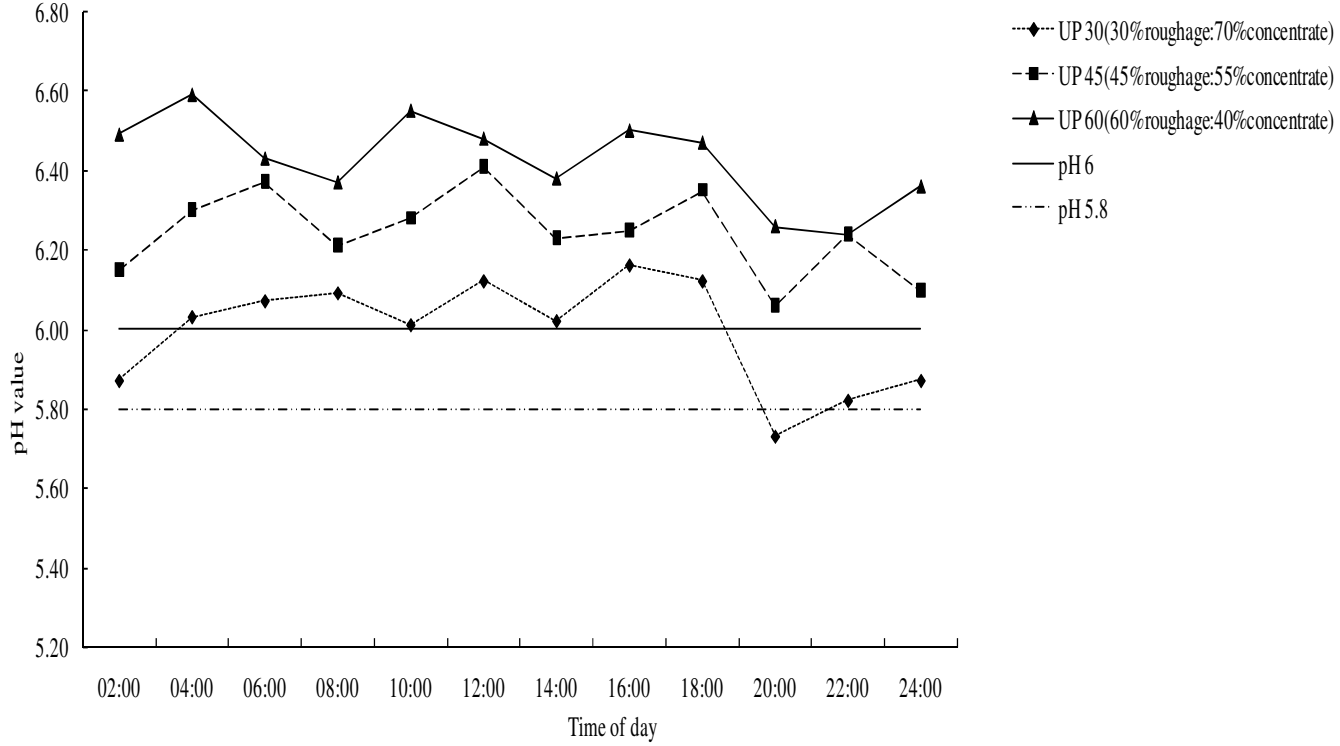

Figure 1 Effect of increasing roughage:concentrate level on variation in pH over time

4.2 EFFECT OF ROUGHAGE:CONCENTRATE RATIO ON RUMINAL PROTEIN DEGRADATION

4.2.1 Crude protein degradation of different protein concentrates

Average CP degradability for the different protein concentrates at the different fractional outflow rates is in Table 8. The effective CP degradability values obtained in this study are summarized in Table 9, and compared to values obtained from previously published research studies.

Table 8 The effect of the type of protein concentrate on factors derived from the exponential equation $p = a+b(1-e^{-ct})$ and effective degradability at an outflow rate of kr = 0.02, kr = 0.05 and kr = 0.08 respectively

	Protein concentrate			
	Sunflower oilcake	Cotton seed oilcake	Roasted soya	SEM
a	28.02[a]	18.07[b]	23.83[c]	1.09
b	68.96[a]	78.87[b]	79.18[b]	1.24
c	0.57[a]	0.07[b]	0.05[b]	0.02
ED (kr=0.02)	94.49[a]	78.07[b]	79.97[b]	0.48
ED (kr=0.05)	91.10[a]	62.46[b]	63.18[b]	0.72
ED (kr=0.08)	88.06[a]	53.37[b]	54.20[b]	0.75

[abc] Means in the same row with different superscripts differ (P < 0.05)

Table 9 Protein degradability of *in situ* incubated protein concentrates calculated at different fractional outflow rates compared to literature values

	0.02/h			0.05/h			0.08/h		
Protein concentrate	Present study	Ørskov *et al.*, 1981	Erasmus 1993	Present study	Ørskov *et al.*, 1981	Erasmus 1993	Present study	Ørskov *et al.*, 1981	Erasmus 1993
Sunflower oilcake	94.49	96.00	90.60	91.10	91.00	85.40	88.06	87.00	81.00
Cottonseed oilcake	78.07	80.00	67.40	62.46	69.00	51.90	53.37	62.00	44.20
Roasted soya	79.97	80.00	79.90	63.18	62.00	55.60	54.20	50.00	45.30

The comparisons between effective CP degradability were at the same assumed rumen outflow rates. The fractional outflow rate of undegraded CP from the rumen (kr) must be considered in calculating effective degradation (P). Both outflow rates of 0.05/h and 0.08/h were used in calculating effective degradability (Ørskov & McDonald, 1979), although fractional outflow rates vary from 0.02/h for animals at maintenance to 0.08/h for high producing dairy cows (Erasmus, 1993). The sum of fractions 'a' soluble CP and 'b' potentially degradable CP should not exceed 100%. However in this study the values for roasted soya did slighly exceed 100%. Others (Cronje, 1983; Kirkpatrick & Kennelly, 1987; Erasmus, 1993) have also reported a+b values exceeding 100%, possibly because the model fits a curve to the data points, which are concentrated during the periods of high CP degradation (i.e., 0-16 hours) with very few data points at the later stages (i.e., 16 -72 hours) thereby causing overestimation during these latter stages (2007, P.H. Robinson, Pers. Comm., University of California, Davis, CA, USA).

Sunflower oilcake had an effective CP degradability of 91.10% at an outflow rate of kr=0.05. This is a high degradability value for a protein concentrate, but compares well to the 85.40% reported by Erasmus (1993) and the 91.0% reported by (Ørskov *et al.,* 1981) (Table 9). Cottonseed oilcake had an effective degradability value of 62.46% at an outflow rate of kr=0.05, slightly lower the 69.00% reported by (Ørskov *et al.,* 1981) and higher than the 51.90 reported by Erasmus (1993). This could have been caused by a difference in processing between the batches (Cronje, 1983). Crude protein degradability values throughout this study were comparatively higher than values reported by Erasmus (1993) (Table 9). Roasted soya had an effective degradability value of 63.18% at an outflow rate of kr =0.05, similar to the 62.00% reported by (Ørskov *et al.,* 1981) but is higher that the 55.60% reported by Erasmus (1993). This lower degradability reported by Erasmus (1993) could possibly be due to more intense heat treatment (Cronje, 1983), but values are still comparable. These values illustrate that sunflower oilcake is a highly degradable CP concentrate, while values of cottonseed oilcake and roasted soya indicate a medium to low degrability for these two concentrates. The value for roasted soya was expected to be closer to that reported by Erasmus (1993), which indicates a lower degradable CP concentrate.

Values for the model parameters in our study were compared to previous studies (Table 10) (Ørskov & McDonald (1979). Although the values differ among studies, a similar pattern for soluble CP and slower degradable fractions of CP are evident in all sources of data.

Table 10 Comparison of model parameters among studies

Model parameter	Present study	Erasmus 1993	Cronje 1983
(Sunflower oilcake)			
a	28.02	35.6	39.3
b	68.96	64.1	53.4
c	0.57	0.29	0.22
(Cottonseed oilcake)			
a	18.07	21.7	23.9
b	78.87	69.6	89.0
c	0.07	0.04	0.04
(Roasted soya)			
a	23.83	20.2	-
b	79.18	80.0	-
c	0.05	0.03	-

The 'a' values in Table 10 indicate that sunflower oilcake had the highest fraction of soluble CP, with roasted soya intermediate and cottonseed oilcake having the lowest fraction. The 'c' value is the degradation rate of the b fraction of different protein concentrates. Sunflower oilcake had a degradation rate of 0.57. Erasmus (1993) reported 'c' values as high as 0.21 for lucerne hay. While Cronje (1983) reported a value of 0.22 for sunflower oilcake they calculated a flow rate of 0.039 /h making values not directly comparable. Cronje (1983) reported a degradation rate of 0.04 for cottonseed oilcake. The heat-treated roasted soya resulted in the slowest rate of CP degradation, 0.05, with sunflower oilcake having the highest rate of degradation as expected. All oilcakes had been processed which likely induced protein modification, with one of the most important protein reactions being the Maillard reaction (Cronje, 1983). Heat treatment of soyabeans generally decreases the rapidly soluble CP fraction, rate of degradation and estimated extent of CP degradation, but increases the slowly degradable fraction (Erasmus, 1993).

4.2.2 The effect of feeding diets with diffent roughage:concentrate ratios on ruminal crude protein degradation

The effect of dietary treatments on effective CP degradability of sunflower oilcake and the factors derived from the exponential equation $p = a+b(1-e^{-ct})$ are in Table 11.

Table 11 The effect of roughage:concentrate level on factors derived from the exponential equation $p = a+b(1-e^{-ct})$ for sunflower oilcake at different outflow rates

	Sunflower oilcake			
	UP 30[1]	UP 45[1]	UP 60[1]	SEM
a	24.73[a]	26.34[ab]	33.00[b]	1.89
b	72.68[a]	70.39[a]	63.81[b]	2.15
c	0.58	0.59	0.54	0.40
ED (kr=0.05)	91.27	91.02	91.00	1.24
ED (kr=0.08)	88.11	88.03	88.03	1.30

[ab] Means in the same row with different superscripts differ ($P < 0.05$)

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

There were differences in the 'a' values for sunflower oilcake between treatment UP 30 and treatment UP 60. There were differences in the 'b' value of sunflower oilcake between treatments UP 30, UP 45 and UP 60 ($P < 0.05$). There were no differences in degradation rate between the treatments. The average CP degradability was 85.4% (±2.1) for sunflower oilcake in a study reported by Erasmus (1993), here degradability for sunflower oilcake was 91% (±1.24) at an outflow rate of 0.05/h and 88% (±1.30) at an outflow rate of 0.08/h. Differences in roughage:concentrate ratio and the resulting differences in pH (i.e., 6.00 – 6.44) did not affect ruminal CP degradability of sunflower oilcake. In a study by Rotger *et al*., (2005), roughage:concentrate ratio did not affect the rumen CP degradability of any of the protein supplements, which included alfalfa hay, solvent-extracted soybean meal, solvent-extracted sunflower meal, peas, lupin seeds, broadbean, horsebean and vetch. Woods *et al*., (2002) found no differences in the *in situ* degradation of protein concentrates among diets differing in the roughage:concentrate ratio.

The effect of dietary treatments on effective CP degradability of cottonseed oilcake and the factors derived from the exponential equation $p = a+b(1-e^{-ct})$ are in Table 12. There were no differences in the soluble fraction of protein 'a', the potentially degradable fraction 'b' or the degradation rate 'c' between the different treatments. Degradability of CP tended ($P \leq 0.10$) to be influenced by dietary treatment and indirectly rumen pH (Table 12), but the small difference in ruminal degradability it is unlikely to be biologically significant when formulating diets.

Table 12 The effect of roughage:concentrate level on factors derived from the exponential equation $p = a+b(1-e^{-ct})$ for cottonseed oilcake at different outflow rates

	Cottonseed oilcake			
	UP 30[1]	UP 45[1]	UP 60[1]	SEM
a	19.86	17.86	16.47	1.89
b	77.75	78.80	80.10	2.15
c	0.06	0.07	0.07	0.04
ED (kr=0.05)	51.85_{c}	53.56_{cd}	54.70_{d}	1.30
ED (kr=0.08)	60.71_{c}	62.70_{cd}	63.98_{d}	1.24

$_{cd}$ Means in the same row with different subscripts tend to be different ($P \leq 0.1$)

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

Degradability of CP was 51.85% at an outflow rate of 0.05/h in cows consuming treatment UP 30, vs. 54.70% in cows consuming treatment UP 60, and CP degradability was 60.71% at an outflow rate of 0.08/h in cows consuming treatment UP 30, vs. 63.98% in cows consuming treatment UP 60. Thus the different roughage:concentrate ratios in the treatments did thus not cause a difference in the effective CP degradability of cottonseed oilcake. This contrasts to data reported by Loerch *et al.*, (1983), in which CP disappearance decreased linearly when cows were fed increasing concentrations of maize grain. Degradation of CP in studies from Cardozo *et al.,* (2002) was consistently lower when high concentrate diets were fed. Protein degradability values of cottonseed meal at a passage rate of 0.08/h, varied from 42% up to 73% with a mean value of 56% (Madsen & Hvelplund, 1985). Erasmus (1993) reported the degradability of cottonseed oilcake at 51.9% (±1.9%). The effective CP degradability values reported by these researchers were similar to what was obtained in my study.

The effect of dietary treatments on effective CP degradability of roasted soya and the factors derived from the exponential equation $p = a+b(1-e^{-ct})$ are in Table 13. There were no differences in the soluble fraction 'a', the potentially degradable fraction 'b' or the degradation rate 'c' of roasted soya between the different treatments (Table 13). The effective CP degradability value of roasted soya, in cows fed treatment 30, had the tendency ($P \leq 0.1$) to be higher than in cows fed treatment UP 45. Treatment UP 30 had a degradability of 64.95% at an outflow rate of 0.05/h, vs. 62.44% for treatment UP 60. Treatment UP 30 had a degradability of 56.19% at an outflow rate of 0.08/h, vs. 53.31% for treatment UP 60.

Table 13 The effect of roughage:concentrate level on factors derived from the exponential equation p = a+b(1-$e^{-ct)}$ for roasted soya at different outflow rates

	Roasted soya			
	UP 30	UP 45	UP 60	SEM
a	24.54	23.74	23.23	1.89
b	78.37	79.34	79.82	2.15
c	0.06	0.05	0.05	0.04
ED (kr=0.05)	64.95_{c}	62.16_{d}	62.44_{cd}	1.24
ED (kr=0.08)	56.19_{c}	53.10_{d}	53.31_{d}	1.30

$_{cd}$ Means in the same row with different subscripts tend to be different (P ≤ 0.1)

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

Thus the different roughage:concentrate ratios in the treatments did thus not cause a significant difference in the effective CP degradability of roasted soya. This contrasts to results by Devant *et al.,* (2001), which indicated that CP degradation was lower in beef cattle fed a diet with a roughage:concentrate ratio of 10:90 when compared to dairy cattle fed a 60:40 roughage:concentrate ratio. Erasmus (1993) reported the protein degradability of roasted soya to be 55.6% at an outflow rate of 0.05/h.

4.2.3 Ruminal pH and its effect on crude protein disappearance

This discussion deals mainly with the 2-16 hour incubation period during which the highest extent of CP disappearance occurred. During the early incubation periods (i.e., 0-2 hours) the extent of CP degradation was not high enough to detect a relationship between rumen pH and *in situ* CP disappearance. During the long incubation periods (24 -72 hours), most of the CP had disappeared from the *in situ* bags and no relationship could be observed between rumen pH and *in situ* CP disappearance. In Figure 2 the CP disappearance of sunflower oilcake, cottonseed oilcake and roasted soya, are expressed as a percentage of the potentially degradable fraction, at different pH levels for a four hour rumen incubation period.

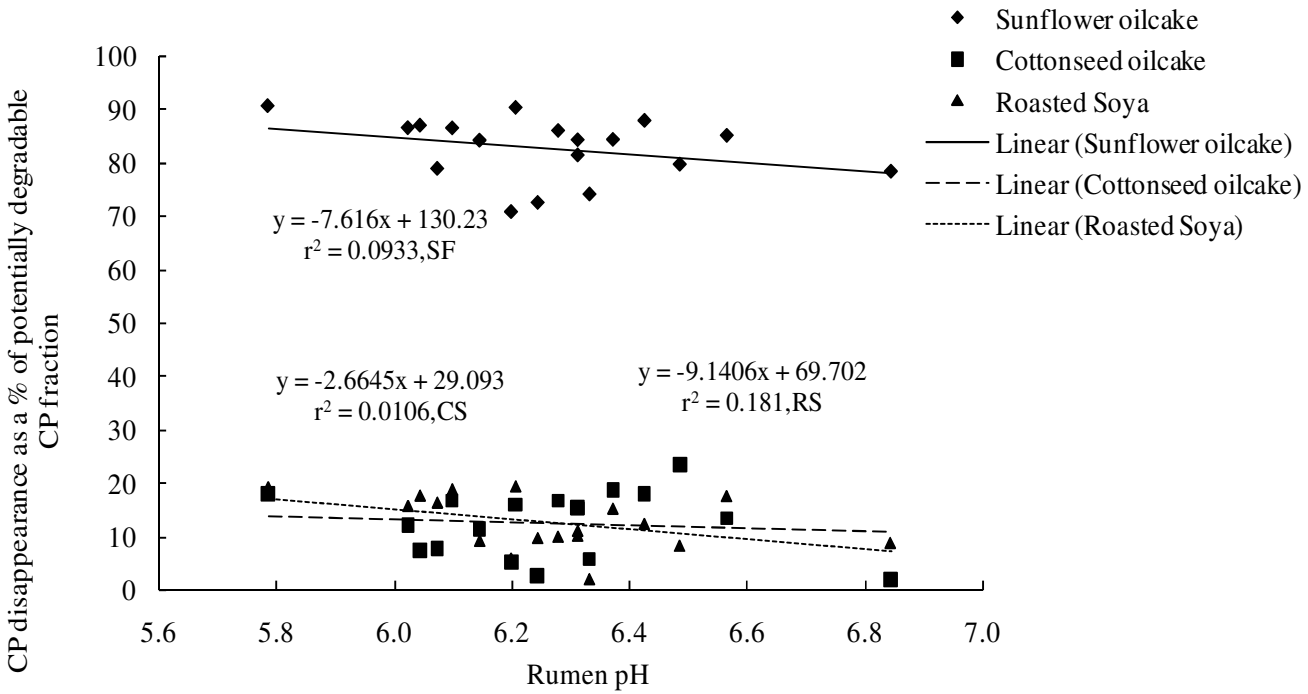

Figure 2 Crude protein (CP) disappearance of three protein sources expressed as a percentage of the potentially degradable CP fraction at different pH levels (4 hour incubation).

After four hours of incubation time there was a poor correlation between pH value and CP disappearance for all three protein concentrates. For sunflower oilcake, about 85% of the potentially degradable fraction had already disappeared after four hours of incubation. There was clearly no correlation between the ruminal pH and CP disappearance of sunflower oilcake in the rumen after four hours of incubation. It could be that the initial high rate of CP degradation of the 'b' fraction could have contributed to this, and that the degradability of sunflower oilcake was so high that pH did not influence degradability. These results are in agreement with Wallace & Cotta (1988), who stated that proteolytic activitity has a broad pH optimum of about pH 5.50-7.00 in rumen fluid. Even though a reduction in CP degradation at low pH has been reported (Assoumani *et al.,* 1992). The proteolytic enzymes in the rumen are generally active over a wide pH range (Calsamiglia *et al.,* 2002). Under most nutritional circumstances, rumen pH will allow extensive degradation of dietary CP (Tamminga, 1979).

After four hours of incubation, the CP disappearance of cottonseed oilcake and roasted soya was about 15% of the potentially degradable CP fraction. There was no correlation between ruminal pH and the CP disappearance of cottonseed oilcake. This agrees with data from Calsamiglia *et al*. (2002), which indicated that pH can drop below 6 for four hours without affecting microbial fermentation.

There was a negative correlation between the CP disappearance of roasted soya and ruminal pH, as the CP disappearance increased slightly as the pH decreased ($r^2 = 0.18$). This is in contrast to what was expected, but the correlation was weak and not significant.

In Figure 3 the CP disappearance of sunflower oilcake, cottonseed oilcake and roasted soya, are expressed as a percentage of the potentially degradable CP fraction at different pH levels for an eight hour rumen incubation period. At eight hour of incubation in the rumen, most of the N from sunflower oilcake had disappeared. The r^2 values in Figure 3 illustrates that there was no correlation between ruminal pH and N disappearance of sunflower oilcake.

Between 26% and 51% of the potentially degradable CP from cottonseed oilcake had disappeared by eight hours of incubation. There was a tendency to a positive correlation between the pH in the rumen and CP disappearance of cottonseed oilcake ($P \leq 0.1$). However the correlation was not significant and therefore not relevant under practical feeding conditions. The relationship between pH and N disappearance was best, albeit poorly, described by a linear equation ($y = -45.736 + 13.367x$; $r^2 = 0.18$).

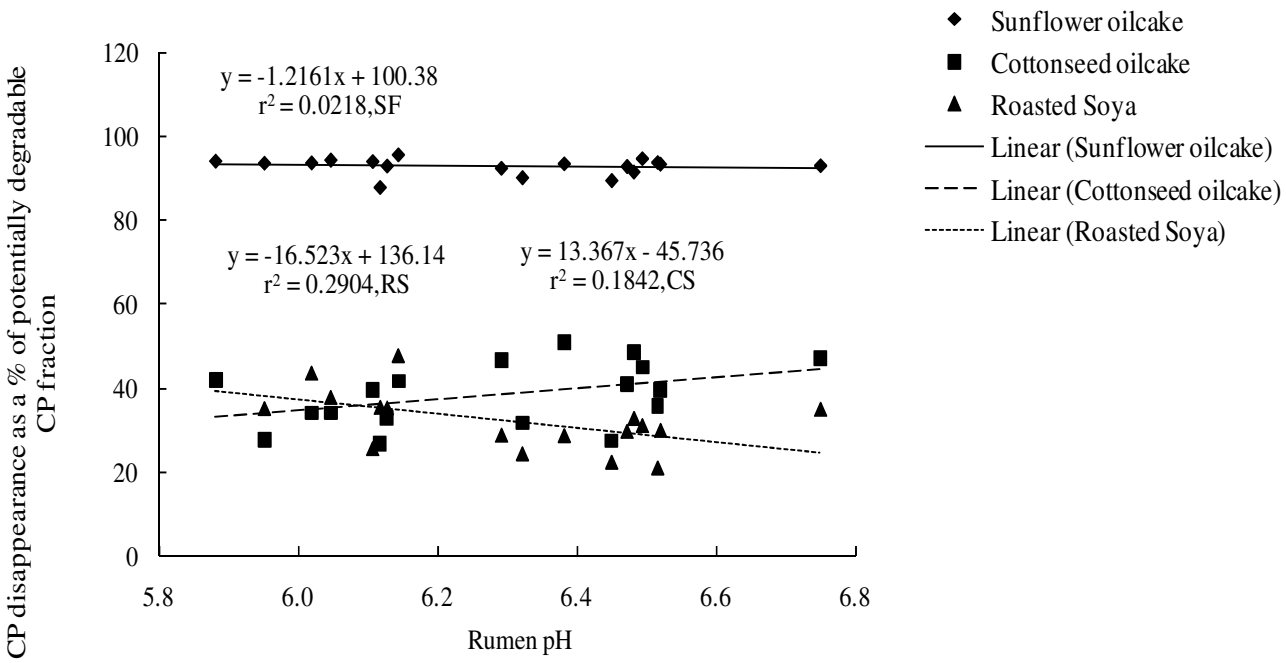

Figure 3 Crude protein (CP) disappearance of three protein sources expressed as a percentage of the potentially degradable CP fraction at different pH levels (8hour incubation).

This is in contrast to data of Endres & Stern (1993), who observed a reduction in CP degradation when ruminal pH decreased from 6.30 to 5.90. Even though these researchers observed an effect at relatively high pH levels it is possible that the pH value was not low enough to cause a decrease in CP degradation. Cerrato-Sánchez *et al.* (2007) reported that the negative effect on rumen fermentation started as soon as pH decreased to 5.50. In my study, the pH level did not decrease to 5.50 for any treatment however, fibre digestion rates decrease when ruminal pH declines below 6.00-6.20 (Ørskov, 1994), which reduces access of bacteria and enzymes to protein thus decreasing CP degradability (Calsamiglia *et al*., 2002). The same results were not obtained in my study, possibly because of the way CP is bound to fibre in these protein concentrates. Similar to what was observed in my study, Yang *et al.* (2002b) reported no effect on degradability of CP at pH levels of 5.50, 6.00 or 6.50 in a dual effluent flow continuous culture system.

Between 20% and 48% of the potentially degradable fraction of CP from roasted soya had disappeared by eight hours incubation, the opposite situation from cottonseed oilcake occurred in roasted soya. As pH increased, the CP disappearance from roasted soya decreased linearly, with the relationship best described by the equation: $y = 136.14 – 16.523x$; $r^2 = 0.29$; P=0.02. The relationship could also be described quadratically by the equation $y = 433.53 + 6.57x^2 – 105.32x$; $r^2 = 0.42$; P = 0.02. There was thus a negative correlation ($P < 0.05$) between the pH in the rumen and the CP disappearance of roasted soya.

In Figure 4, the CP disappearance of sunflower oilcake, cottonseed oilcake and roasted soya, are expressed as a percentage of the potentially degradable fraction, at different pH levels for a 16 hour rumen incubation period. At 16 hours of incubation in the rumen, the entire potentially degradable CP fraction from sunflower oilcake had disappeared (Figure 4). There was thus no effect from pH on N disappearance.

Between 38% and 69% of the potentially degradable CP fraction from cottonseed oilcake had disappeared. The pH in the rumen did effect CP disappearance with it increasing as the pH value increased (P=0.04), creating a positive correlation ($P < 0.05$) between pH in the rumen and CP disappearance of cottonseed oilcake. The relationship was best described by the linear equation: $y = -57.85 + 19.153x$; $r^2 = 0.27$. Rumen pH could affect CP disappearance by altering microbial activity and by changing protein structures. Rumen pH is normally between 5.50 and 7.00, and proteins with an iso-electric point in this range would have altered solubility and possibly altered CP degradability (Bach *et al*., 2005). The iso-electric points of cottonseed oilcake vs. roasted soya might be one explanation why these two protein sources

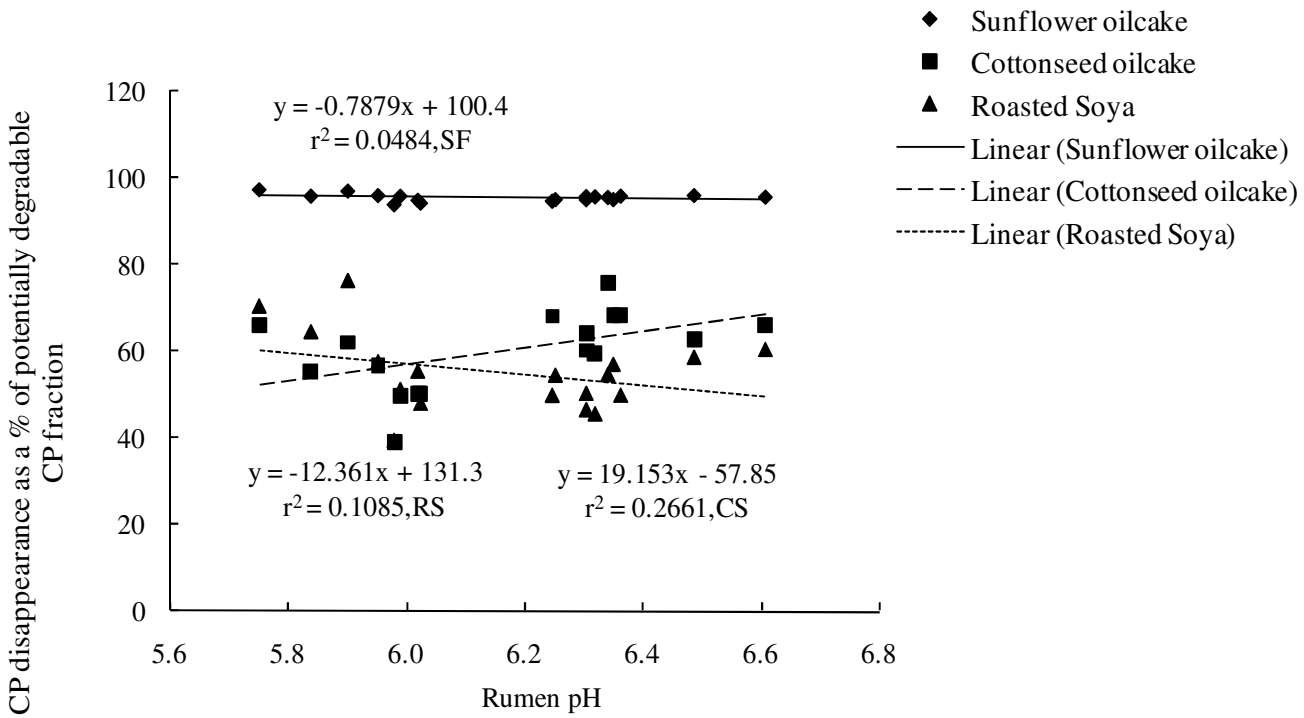

Figure 4 Crude protein (CP) disappearance of three protein sources expressed as a percentage of the potentially degradable CP fraction at different pH levels (16 hour incubation).

reacted so differently to a lower pH in the rumen. Fibre may also limit microbial access to plant protein and reduced fibre digestion at a lower pH might be involved as well (Ganev *et al*., 1979). This could possibly explain why there was lower CP degradation for cottonseed oilcake at this incubation period. Proteolysis and deamination are affected by pH, but experimental results are conflicting. Evidence suggests that the optimum pH for both proteolysis and deamination is between 6.00 and 7.00 (Tamminga, 1979).

Between 39% and 76% of CP from roasted soya had disappeared by 16 hours of incubation. The pH in the rumen did not effect CP disappearance in roasted soya ($r^2 = 0.11$; $P > 0.05$). The relationship was better described by the quadratic equation: $y = 3930.70+100.15x^2-1246.97x$; $r^2 = 0.49$; $P = 0.006$. This indicates a correlation between rumen pH and the CP disappearance of roasted soya. Bartle *et al*. (1986) found that soybean meal CP disappearance responded to pH in a quadratic manner, with disappearance being highest at pH 6.00 - 6.50 and lower at pH 5.50 and pH 7.00. The pH values in my study were not at these extremes, which could explain why we did not obtain similar results.

4.2.4 The effect of feeding diets differing in roughage:concentrate ratio on ruminal crude protein disappearance of different protein concentrates

The effect of roughage:concentrate ratio on the *in situ* CP disappearance of sunflower oilcake is in Table 14 and Figure 5. Treatment did not affect percentage CP disappearance of sunflower oilcake at the different incubation times (Table 14). Results reported by Freer & Dove (1984), indicated that disappearance of CP from untreated sunflower meal increased from 40% (±3.2%) at two hours of incubation to 95% (±2.1%) at 24 hours of incubation. In my study CP disappearance was about 74% after the first two hours of incubation (Figure 5). Two hour N disappearance values reported by Freer & Dove (1984) were numerically lower than found here, but CP disappearance at 24 hours were similar. In a study conducted by Cronje (1983), 81.5% of CP disappearance occurred within the first three hours of incubation, similar to CP disappearance values recorded in my study.

Sunflower oilcake was highly rumen degradable with about 75% of CP having disappeared after only two hours and, after four hours, almost 90% had disappeared. There was no difference in CP disappearance among treatments at any incubation time. After four hours of *in situ* incubation, sunflower oilcake had 89.33% disappearance in cows receiving treatment UP 30, 87.11% on treatment UP 45 and 87.07% on treatment UP 60.

Table 14 Effect of roughage:concentrate ratio on *in situ* crude protein (CP) disappearance of sunflower oilcake

	CP disappearance %						
Treatment[1]	2h	4h	8h	16h	24h	48h	72h
UP 30	73.68	89.33	95.10	96.91	97.69	97.49	98.03
UP 45	75.63	87.11	94.97	96.93	97.30	97.13	97.48
UP 60	74.41	87.07	94.79	96.86	96.61	97.41	97.28

[ab] Means in the same column with different superscripts differ ($P < 0.05$)

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

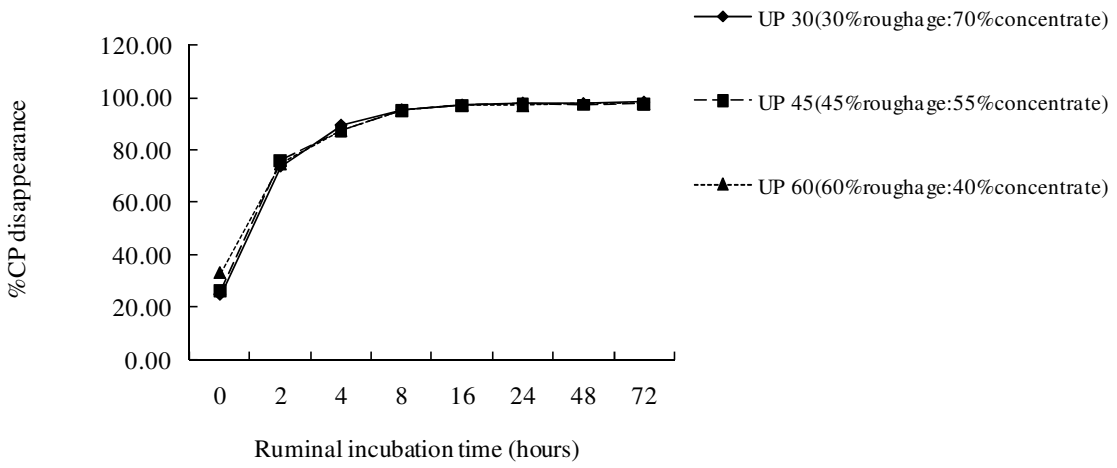

Figure 5 The effect of roughage:concentrate ratio on the *in situ* crude protein (CP) disappearance of sunflower oilcake over time

These differences are minor and will not likely have a significant impact under practical feeding conditions. Ganev *et al.* (1979) and Lindberg (1981b) demonstrated clear effects of experimental diet (or indirectly pH) on sunflower oilcake as well as on cottonseed oilcake. Cronje (1983) found no dietary effects and concluded that the contradiction might have been because of differences in processing methods between the different studies. Similar to Cronje (1983), the sunflower oilcake used in my study was highly degradable with about 75% of CP having disappeared by two hours, an extent of disappearance only reached after 15 hours in Ganev *et al.* (1979). The probability of the expression of dietary effects is greatly reduced as the initial insoluble potentially degradable CP fraction of a protein concentrate declines (Cronje, 1983). It is possible that a more significant extent of CP degradation at an earlier incubation period could have caused dietary effects to be negligible.

The effect of roughage:concentrate ratio on *in situ* CP disappearance of cottonseed oilcake is in Table 15 and Figure 6. There were no differences in CP disappearance of cottonseed oilcake among the treatments at the two hour incubation. Treatment UP 30 resulted in the lowest CP disappearance value of 26.49%, which is only 2.42% lower than that caused by treatment UP 45 and 0.43% lower than that caused by treatment UP 60. At an incubation time of four hours, CP disappearance was similar among treatments but from four hours incubation to 72 hours incubation, cottonseed incubated in cows consuming treatment UP 30 had the lowest numerical CP disappearance (Figure 6). During these incubation times, treatment UP 60 had numerically the highest CP disappearance with treatment UP 45 having an intermediate value. Differences in the *in situ* CP disappearance of cottonseed oilcake occurred at only 16 and 48 hours. At 16 hours of incubation, there were differences ($P < 0.05$) in CP disappearance of cottonseed oilcake among treatments (Table 15) and (Figure 6). At 48 hours of incubation, there were differences ($P < 0.05$) in CP disappearance of cottonseed oilcake between treatment UP 30 and the other two treatments. The most substantial difference between treatments occurred at 16 hours with a 10.81% difference between treatment UP 30 and treatment UP 60. At 8 hours there was a 3.14% difference and at 24 hours incubation time there was a 2.53% difference in CP disappearance. In general, roughage:concentrate ratio did not affect CP disappearance and these small numeric differences are biologically unimportant. Cronje (1983) explains that a decrease in *in vitro* degradability of cottonseed oilcake might be concurrent with an increase of gossypol bound amino groups upon heat treatment.

Table 15 Effect of roughage:concentrate ratio on *in situ* crude protein (CP) disappearance of cottonseed oilcake

	CP disappearance %						
Treatment[1]	2h	4h	8h	16h	24h	48h	72h
UP 30	26.49	34.36	51.49	65.70[a]	78.61	91.82[a]	95.39
UP 45	28.91	33.38	53.32	69.42[a]	78.47	93.83[b]	95.82
UP 60	26.92	33.22	53.45	75.76[b]	80.07	94.11[b]	95.95

[ab] Means in the same column with different superscripts differ ($P < 0.05$)

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

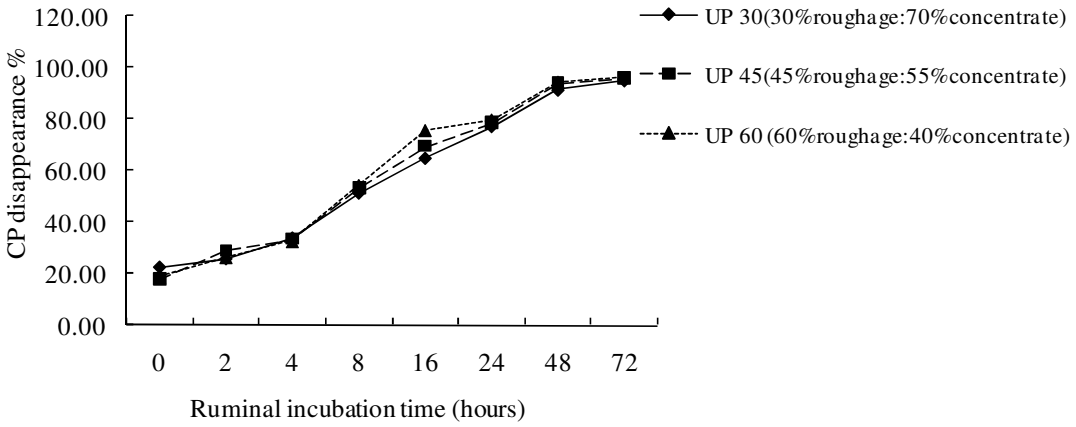

Figure 6 The effect of roughage:concentrate ratio on the *in situ* crude protein (CP) disappearance of cottonseed oilcake over time

Broderick (1977) concluded that gossypol may act as a naturally occurring cross-linking agent if both carbonyls react with lysine ϵ-amino groups of different peptide chains. Broderick & Craig (1980) reported rumen degradable values of 55.6 to 71.1% for different batches of solvent extracted cottonseed meals and rumen degradable CP values of 34.8 – 40.0% for screw press cottonseed meals. In South Africa, all oilseeds are processed by the same procedure (i.e., solvent extraction) and therefore the effect of processing on oilcakes was not further investigated. In this study, all the protein concentrates were obtained from the same company.

The effect of roughage:concentrate ratio on the *in situ* CP disappearance of roasted soya is in Table 16 and Figure 7. There was a difference in CP disappearance of roasted soya between treatment UP 30 and the other two treatments at the eight hour incubation period ($P < 0.05$). Disappearance of CP on treatments UP 30 and treatment UP 45 tended to differ at 16 hours ($P \leq 0.1$), but differences were minor and will probably have no practical importance. Treatment UP 30 caused a numerically higher rate of CP disappearance compared to treatment UP 45 and treatment UP 60 (Figure 7). Disappearance of CP on treatment UP 45 and treatment UP 60 followed similar patterns, with treatment UP 60 causing numerically a lower disappearance than treatment UP 45 at two and eight hours respectively. The difference in CP disappearance was most prominent from two hour of incubation to 24 hours, after which the difference became smaller due to a higher proportion of the CP already having disappeared. The distinctly different responses of cottonseed oilcake and roasted soya on different treatments might be because of morphological, or other inherent, differences among the CP concentrates. Differential digestion of various cell wall types leads to differential digestion of various cell wall components (Morris & Bacon, 1977). Cottonseed oilcake was higher in fibre than roasted soya (Table 4). These findings would substantiate previous findings that pH mediated effects on CP disappearance is due to variations in cellulotic bacteria (Siddons & Paradine, 1981). There is thus a difference in CP disappearance of different CP concentrates at different pH values. Ruminal pH, however, seems to have a more pronounced effect on the specific protein concentrate than on total dietary CP degradation. This could be due to the different populations of micro-organisms that flourish at different pH levels (Loerch *et al.*, 1983). Ruminal pH could possibly play a larger role in the digestion of various cell wall fractions, reduced CP degradation at a lower pH due to a smaller population of cellulolytic bacteria that exists (Calsamiglia *et al.,* 2002). The decrease in CP degradability could be due to the proteins being linked to the cell wall fraction and thus dependent fibre degradation, which will be dependent on the protein concentrate and the way in which the protein is bound (Calsamiglia *et al.,* 2002).

Table 16 Effect of roughage:concentrate ratio on *in situ* crude protein (CP) disappearance of roasted soya

	CP disappearance %						
Treatment[1]	2h	4h	8h	16h	24h	48h	72h
UP 30	33.30	37.41	54.70[a]	70.02$_{c}$	79.92	96.19	98.77
UP 45	31.02	34.51	49.52[b]	64.41$_{d}$	78.46	96.29	98.80
UP 60	30.16	35.76	47.16[b]	66.82$_{cd}$	78.78	96.96	98.52

[ab] Means in the same column with different superscripts differ ($P < 0.05$)

$_{cd}$ Means in the same column with different subscripts tend to be different ($P \leq 0.1$)

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

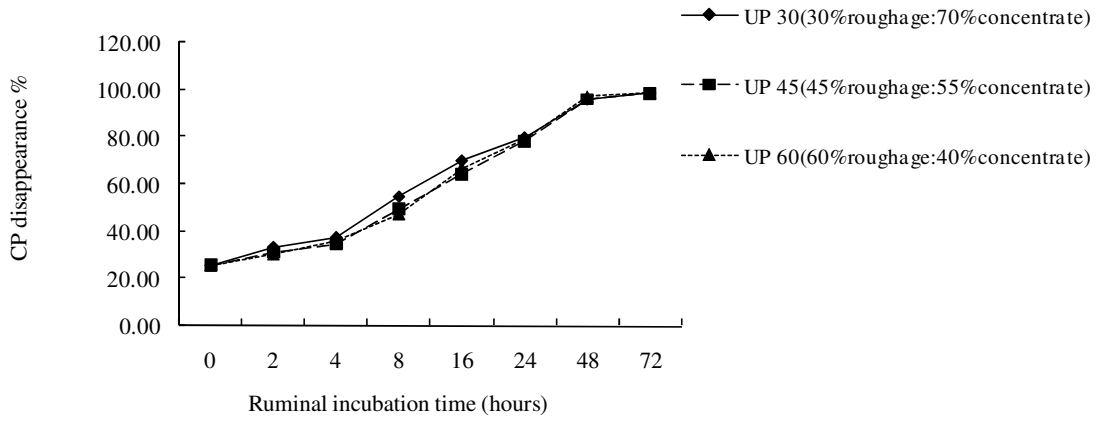

Figure 7 The effect of roughage:concentrate ratio on the *in situ* crude protein (CP) disappearance of roasted soya over time

It does seem that a major determinant would be the degree of association of CP with fibrous components within a feed.

The way in which the different protein concentrates responded to varying levels of roughage:concentrate in the diet are in Figures 8, 9 and 10. The protein concentrates did not follow the same pattern of CP disappearance on the different treatments. From Figure 8 it can be seen that on treatment UP 30, which resulted in the lowest pH values, CP disappearance of roasted soya was numerically higher than that of cottonseed oilcake. On treatment UP 45, CP disappearance of cottonseed oilcake was numerically higher than that of roasted soya from 8 to 24 hours of incubation (Figure 9). The same pattern occurred on treatment UP 60, with the difference between cottonseed oilcake and roasted soya being slightly bigger (Figure 10). The most prominent difference between the protein concentrates on treatment UP 45 is 5% and on treatment UP 60 it is 8.8%. In general, however, CP disappearance patterns appeared to be the same, and I conclude that within the pH ranges of 6.00 to 6.40, ruminal pH did not have an important effect on CP disappearance or CP degradation in these protein concentrates.

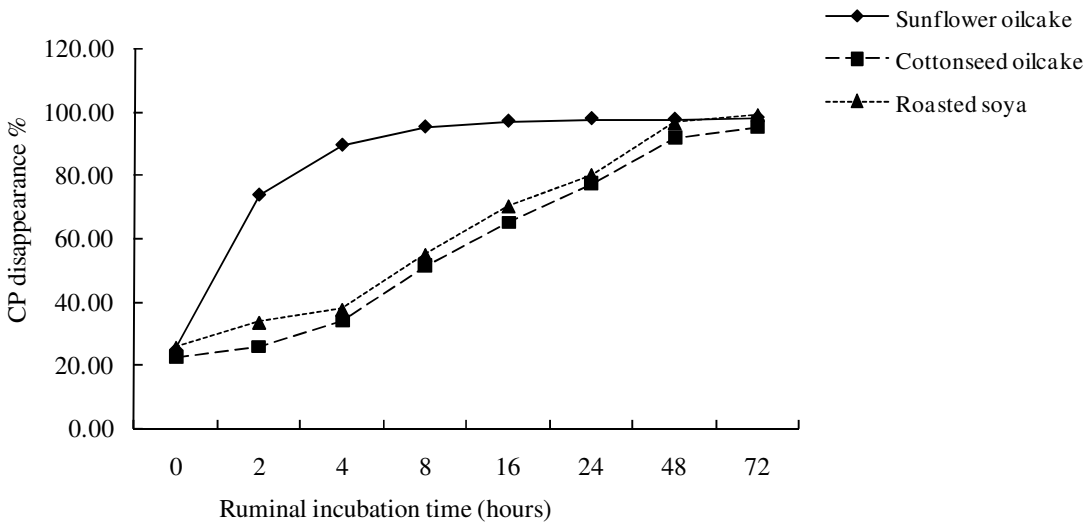

Figure 8 Comparison of crude protein (CP) disappearance of different protein concentrates in animals fed a ration containing 30% roughage:70% concentrate

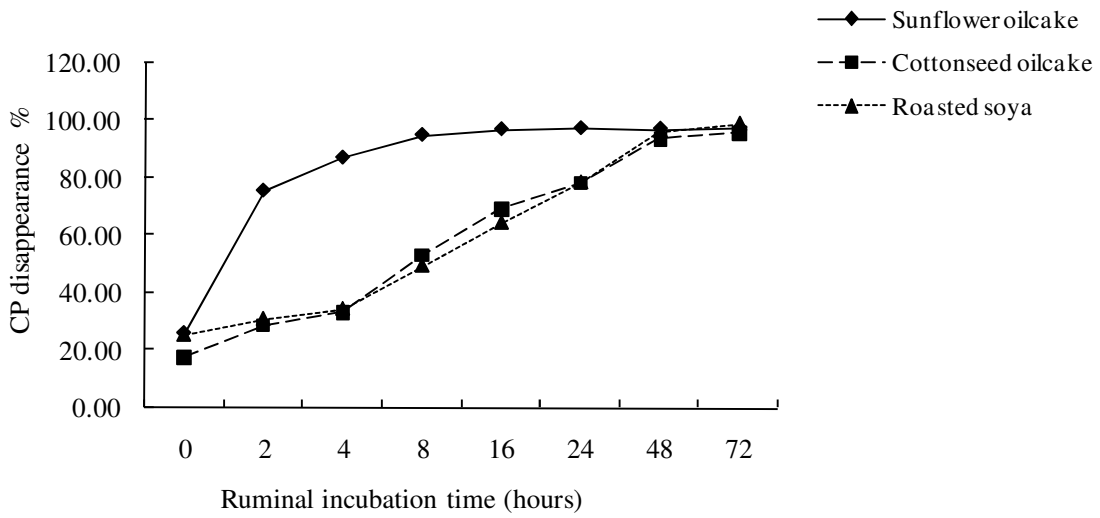

Figure 9 Comparison of crude protein (CP) disappearance of different protein concentrates in animals fed a ration containing 45% roughage:55% concentrate

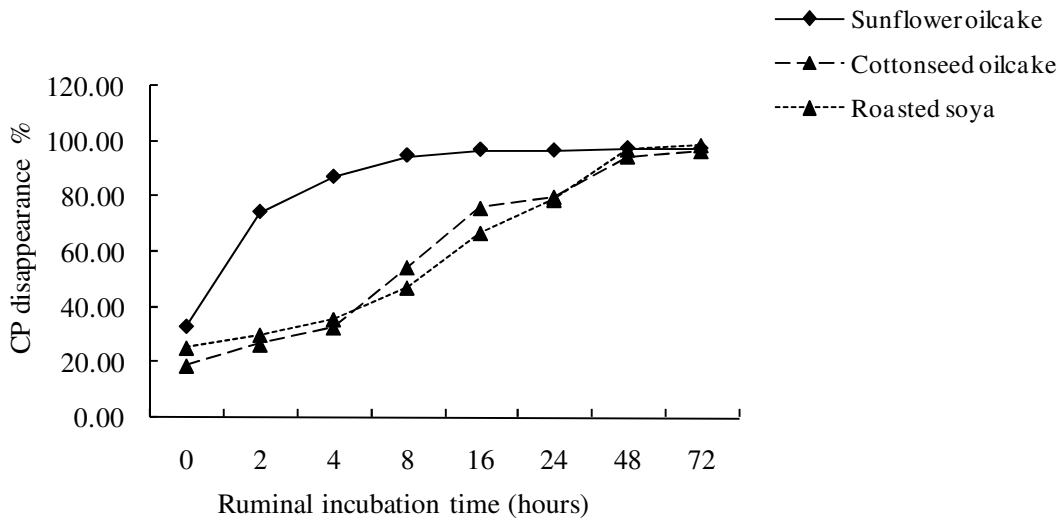

Figure 10 Comparison of crude protein (CP) disappearance of different protein concentrates in animals fed a ration containing 60% roughage:40% concentrate

4.3 EFFECT OF ROUGHAGE:CONCENTRATE RATIO ON VOLATILE FATTY ACIDS

The molar proportions and total VFA's produced in cows fed rations differing in roughage:concentrate ratio is in Table 17. In this study, total VFA production was 128.0 mM for cows on treatment UP 30, 123.06 mM for cows on treatment UP 45 and 121.09 mM for cows on treatment UP 60 (Table 17). The total VFA concentration varied considerably over a 24 hour period (Figure 11).

Table 17 Molar proportions and total volatile fatty acids produced in cows fed rations differing in roughage:concentrate ratio

	Treatment[1]			
	UP 30	UP 45	UP 60	SEM
Total VFA production (mM)	128.10	123.06	121.09	4.39
Acetic acid (mM)	82.45	86.08	84.91	3.36
Propionic acid (mM)	29.78[a]	20.97[b]	21.28[b]	0.85
Butyric acid (mM)	13.16	13.48	12.43	0.79
Iso butyric acid (mM)	0.94	0.94	0.87	0.05
Valeric acid (mM)	1.68	1.59	1.60	0.08
A:P Ratio	2.77[a]	4.13[b]	3.98[b]	0.14
Acetic acid (%)	58.10[a]	63.52[b]	63.61[b]	0.84
Propionic acid (%)	25.65[a]	18.95[b]	19.63[b]	0.27
Butyric acid (%)	13.30	14.53	13.75	0.61
Iso butyric acid (%)	0.98	1.02	0.98	0.08
Valeric acid (%)	1.97	1.99	2.03	0.11

[ab] Means in the same row with different superscripts differ ($P < 0.05$)

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

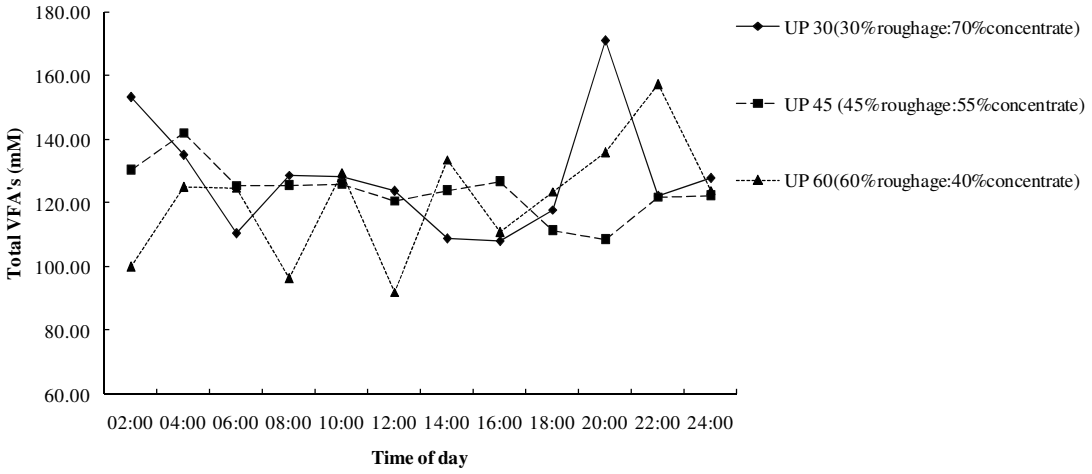

Figure 11 The effect of roughage:concentrate ratio on the total volatile fatty acid levels in the rumen over the day

The findings in my study is consistent with McDonald *et al.* (1995), who reported that the total concentration of VFA's vary widely according to the animal's diet and the time elapsed since the previous meal, although normally it is in the range of 70-150 mmol/litre. VFA concentrations are in general higher with a diet high in NSC and RDP, and this is associated with a low ruminal pH (Zhao *et al*., 1993). In my study, the total concentration of VFA's was 128.10 mM for treatment UP 30, 123.06 mM for treatment UP 45 and 121.09 mM for treatment UP 60 which this is in agreement with Zhao *et al.,* (1993). This could explain, in part, the average pH values of 6.00 on treatment UP 30, 6.27 for treatment UP 45 and 6.44 for treatment UP 60 (Table 17), as rumen pH is inversly related to VFA concentration (Erdman, 1988; Stokes *et al*., 1991), if there is a reduced rate of VFA absorption it will cause ruminal pH to drop (Owens *et al*., 1998). This is constant with data from my study this trial in which cows fed treatment UP 30 which produced the lowest pH had the highest concentration of VFA's; treatment UP 60, which had the lowest concentration in VFA's, had the highest average pH value. Difference in the concentration of acetic acid and propionic acid on different treatments was significant ($P < 0.05$). Russell (1998) found that cows fed 90% concentrate diets had lower ruminal pH values, higher VFA concentrations, and lower A:P ratios than cows fed roughage only, and concluded that as much as 25% of the decrease in

A:P ratio could be explained by ruminal pH alone. The same results were observed in my study with treatment UP 30, which had the highest dietary NSC concentration, and the lowest A:P ratio. As meal frequency decreases, there is a more pronounced fluctuation in ruminal production of VFA (Pitt & Pell, 1997).

In evaluation of VFA concentrations, the ratio of acetic: propionic acid (A:P ratio) reflects rumen fermentation. When rumen fermentational conditions are optimal, the A:P ratio should be greater than 2.2:1 (McDonald *et al*., 1995). In my study, cows on treatment UP 30 a ratio of 2.77:1, on treatment UP 45 a ratio of 4.1:1 and on treatment UP 60 a ratio of 3.98:1 ($P < 0.05$) (Table 17). The A:P ratio for treatment UP 30 differed from that of treatment UP 45 and treatment UP 60 ($P < 0.05$), but the A:P ratio of treatment UP 30 was just within the conditions of optimal fermentation. A higher level of fermentable carbohydrate in the diet could have led to higher levels of propionic acid, and thus reduced fibre digestion and possibly acidosis. High levels of acetic acid can indicate a high fibre, low fermentable carbohydrate diet (McDonald *et al*., 1995). The lower A:P ratio caused by treatment UP 30 was most probably due to the higher level of concentrate in that diet (Table 2).

Cerrato-Sánchez *et al.,* (2007) investigated effects of time at a sub-optimal pH (pH 5.5) on rumen fermentation in a dual-flow continuous culture system. These researchers found that total VFA concentrations had a cubic response ($VFA = 112.7 – 2.09x + 0.17x^2 -0.0054x^3$-; $r^2 = 0.82$) to suboptimal pH (5.5). The proportion of acetate decreased linearly ($acetate = 58.7-0.61x$; $r^2 = 0.79$) and the proportion of propionate increased ($propionate = 17.6 + 2.09 x -0.044x^2$; $r^2 = 0.85$) with increasing time at suboptimal pH. In my study, molar proportions for treatment UP 30 were acetic 0.58, propionic 0.26, butyric 0.13 and others 0.02. Molar proportions for treatment UP 45 were acetic 0.64, propionic 0.19, butyric 0.15 and others 0.02. Molar proportions for treatment UP 60 were acetic 0.64, propionic 0.20, butyric 0.14 and others 0.02. The typical VFA proportions in cattle fed 40% pelleted hay and 60% concentrates are total VFA (m moles/liter) 140, molar proportions acetic 0.5, propionic 0.3, butyric 0.11 and others 0.09. In cattle fed 40% long hay and 60% concentrates it changed to:total VFA (m moles/liter) 96, molar proportions acetic 0.61, propionic 0.18, butyric 0.13 and others 0.08 (McDonald *et al*., 1995). The ratios in my study were similar to those reported by McDonald *et al.* (1995). The ratios changed depending on the roughage:concentrate ratio, as was expected and in agreement with published data (McDonald *et al.,* 1995). The proportion of acetic acid on treatment UP 30 was lower than on the other two treatments, treatment UP 30 had the highest ($P < 0.05$) level of NFC (Figure 12). Ratios for cows fed treatment UP 45 and treatment UP 60 were similar. Treatment UP 45 caused a higher level of butyric acid than treatment UP 60. The effect of roughage:concentrate ratio on

the molar level of propionic acid in the rumen at different times of the day are in Figure 13, and the effect on the molar percentage of propionic acid in the rumen are in Figure 14.

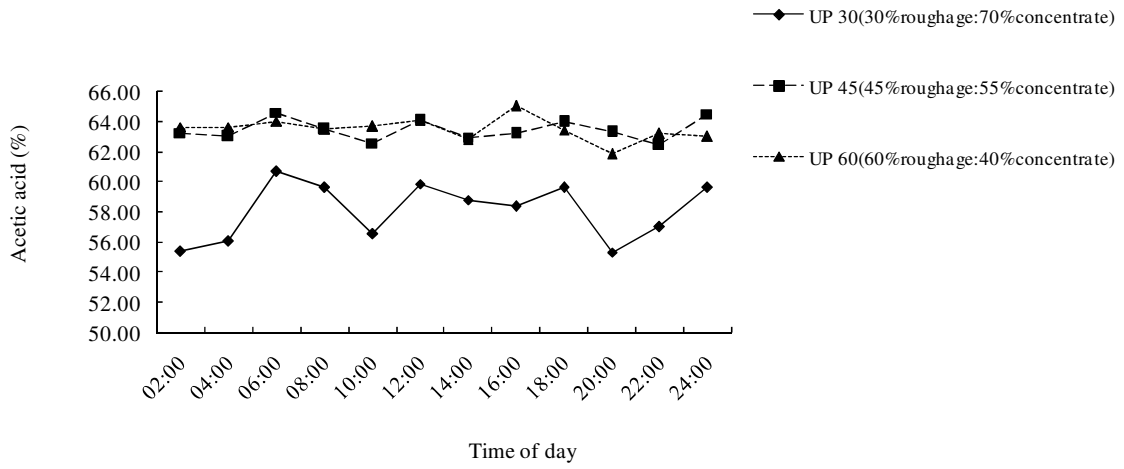

Figure 12 The effect of roughage:concentrate ratio on the molar percentage of acetic acid in the rumen at different times of the day

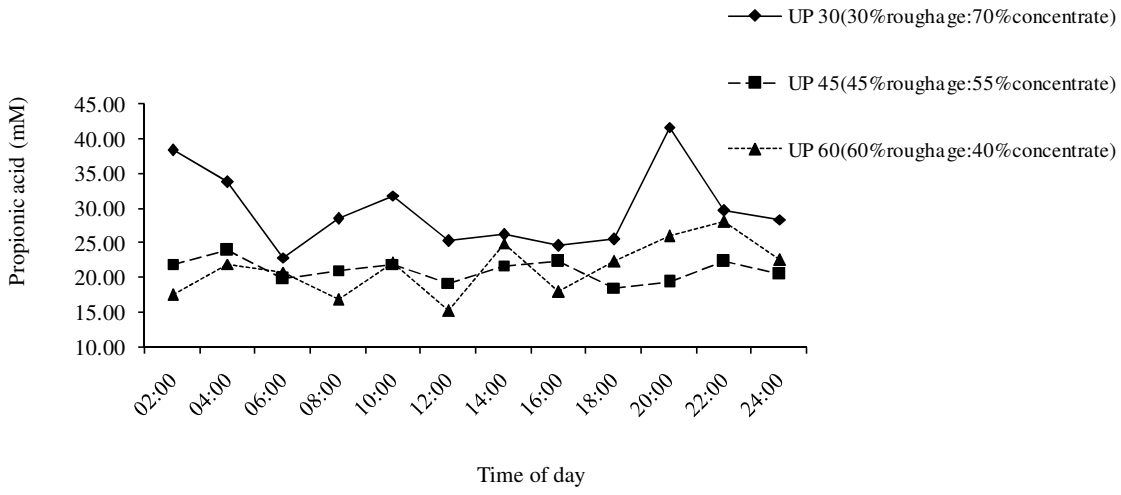

Figure 13 The effect of roughage:concentrate ratio on the molar level of propionic acid in the rumen at different times of the day

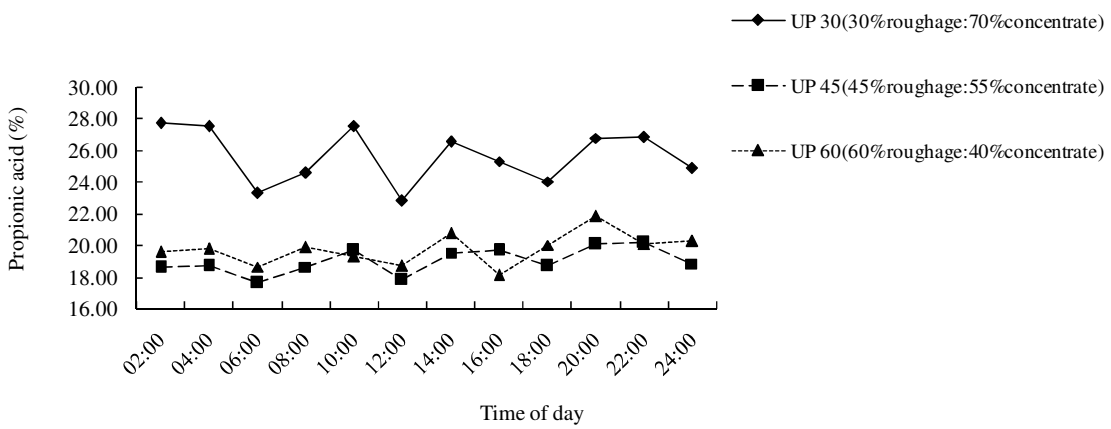

Figure 14 The effect of roughage:concentrate ratio on the molar percentage of propionic acid in the rumen at different times of the day

As the maize grain level increased (Table 2), the proportions of propionic and butyric acid increased numerically at the expense of acetic acid. There was no increase in propionic acid level when treatment UP 60 was compared to treatment UP 45. There was, however, a numerical increase in butyric acid levels when these two treatments were compared (Figure 15).

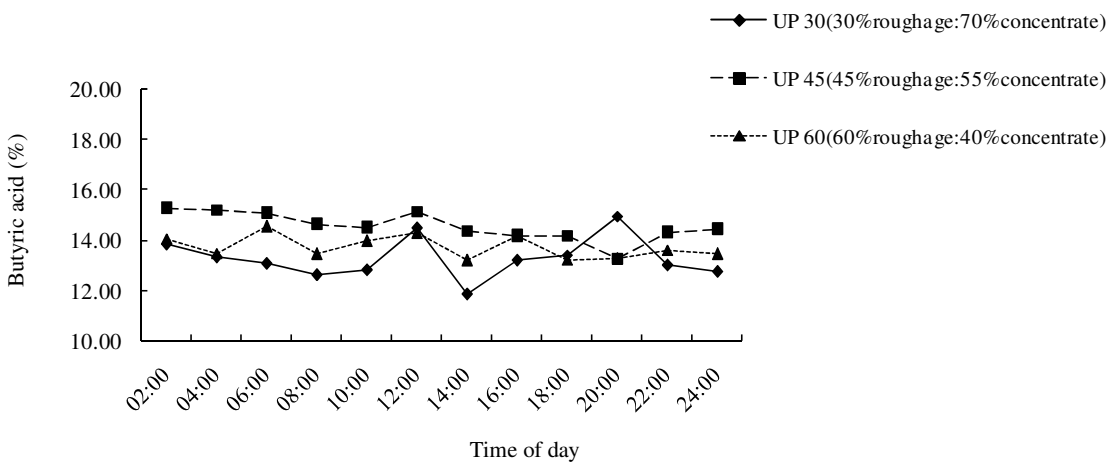

Figure 15 The effect of roughage:concentrate ratio on the molar percentage of butyric acid in the rumen at different times of the day

In an *in vitro* study, Erfle *et al.* (1982) reported that the ratios of acetic, butyric, isobutyric and isovaleric acids decreased, whereas those of propionic, valeric and caproic increased when ruminal pH decreased from 7 to 5. In some studies, feeding a protein source that is low in ruminal CP degradability has decreased total VFA concentrations in rumen fluid (Zerbini *et al*., 1988; McCarthy *et al*., 1989; Klusmeyer *et al*., 1990). Veen *et al.* (1988) suggested that total VFA concentrations might be reduced, whereas the A:P ratio would be higher, when less extensively degraded CP are in the diet. This effect could be attributed to a slower, more gradual fermentation of dietary CP (Erasmus, 1993).

In my study, the same protein sources were used in all three treatments, with the only difference being the level of protein in the treatments. Treatment UP 30 was formulated to have a CP level of 16.18%, treatment UP 45, 15.44% and treatment UP 60 a level of 14.68% CP (DM basis). In all three treatments, the major protein sources were cottonseed oilcake, roasted soya and sunflower oilcake and, in all treatments, these sources were included at a ratio of 1.5:1:2 to eliminate variation of feeding less extensively degraded proteins on VFA levels.

4.4 EFFECT OF ROUGHAGE:CONCENTRATE RATIO ON RUMEN AMMONIA

4.4.1 Treatment effects on rumen ammonia levels

Effects of rations with different roughage:concentrate ratios on rumen NH_3-N concentrations at various time intervals are in Table 18.

Table 18 The effect of roughage:concentrate ratio on rumen ammonia levels at various time intervals

	Treatment[1]			SEM
	UP 30	UP 45	UP 60	
Time	mg NH_3-N/100ml	mg NH_3-N/100ml	mg NH_3-N/100ml	
2:00	21.4	11.5	11.2	2.95
4:00	23.1	17.8	22.4	3.04
6:00	22.1	8.0	14.5	4.08
8:00	23.6	15.2	19.3	5.78
10:00	19.2	12.6	17.4	3.36
12:00	$18.2^{a}{}_{c}$	$9.4^{b}{}_{cd}$	$10.9^{ab}{}_{d}$	1.35
14:00	16.4	12.0	18.4	3.40
16:00	16.3	12.9	12.0	3.53
18:00	17.5	13.4	19.9	6.43
20:00	23.8	14.1	19.2	4.45
22:00	17.2	14.5	22.1	2.06
24:00	21.8	12.4	17.2	3.91
Average	19.7	13.3	16.9	2.64

[ab] Means in the same row with different superscripts differ ($P < 0.05$)

[cd] Means in the same row with different subscripts tend to be different ($P \leq 0.1$)

[1] Experimental diets: UP 30 (30% roughage:70% concentrate), UP 45(45% roughage:55% concentrate) and UP 60(60% roughage:40% concentrate)

Cows fed treatment UP 30 had an average rumen NH_3-N value of 19.7(±2.64) mg /100ml rumen fluid, cows consuming treatment UP 45 had an average rumen NH_3-N value of 13.3(±2.64) mg /100ml rumen fluid and cows consuming treatment UP 60 had an average rumen NH_3-N value of 16.9 (±2.64) mg /100ml rumen fluid (Table 18). These values are high compared to 10.3(±2.26) mg NH_3-N /100ml reported by Rotger *et al*., (2005) on 30:70 roughage:concentrate and 12:88 roughage:concentrate ratio containing 15.1% CP (DM basis).

Cows fed treatment UP 30 had a numerically higher level of ammonia in the rumen compared to treatment UP 45 and treatment UP 60. Only at 12:00 h was this difference

significant ($P < 0.05$). Rumen ammonia values obtained from cows fed treatment UP 60 were variable, ranging from 11.2 mg NH_3-N /100ml to 22.1 mg NH_3-N /100ml. Rumen ammonia values in cows fed treatments UP 30 and UP 45 followed the same trend throughout the day (Figure 16).

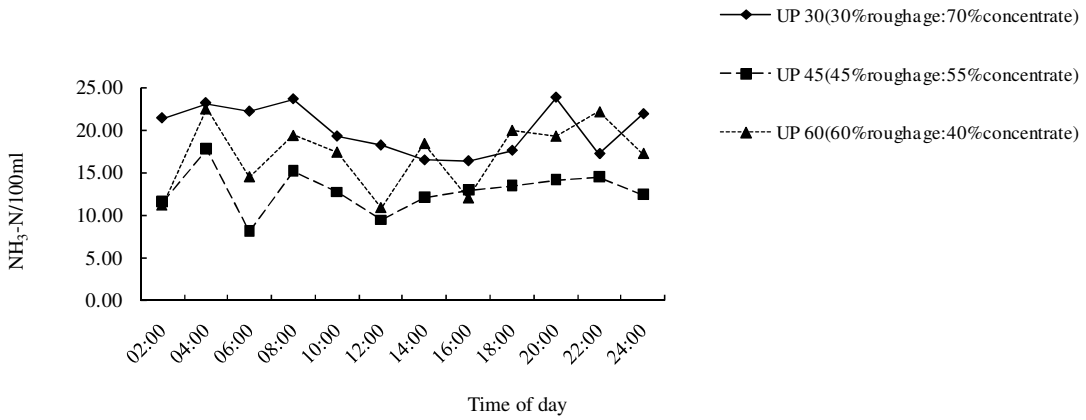

Figure 16 Variability in the rumen ammonia concentrations in cows fed rations differing in roughage:concentrate ratio

The numerically higher rumen ammonia values could indicate a higher rate of CP degradation (Crawford *et al*., 1978). Rumen ammonia values for treatment UP 30 could indicate that the rate of degradation of CP in the rumen was higher on this treatment than on UP 45, but the differences were not significant. The higher CP level for treatment UP 30 could also have contributed to a higher rumen ammonia level. When the rate of rumen ammonia production from the fermentation of AA exceeds the rate of rumen ammonia utilization from MCP synthesis, it will accumulate in the rumen (Russell *et al*., 1983; Siddons & Paradine, 1983). Broderick *et al.* (1981) obtained a curvilinear response in ruminal AA concentration to increasing rumen ammonia concentrations (maximal at 22.4 mg NH_3-N /100ml). Reis & Reid (1959) found deaminative activity to be more dependent on levels of fermentable carbohydrate inclusion in the diet through a possible effect of pH on enzymes responsible for deamination.

Results reported by Siddons & Paradine (1981) showed that *in situ* CP degradation closely followed that of DM degradation. The optimum level of rumen ammonia for *in situ* OM degradation was reported to be 19.3 mg NH_3-N /100ml (Mehrez *et al.*, 1977). If the rate of ruminal protein degradation is dependent on DM degradation, then there will be a level of rumen ammonia N at which CP degradation is highest. Wallace (1979) showed that as levels of rumen

ammonia increased from 8.4 – 18.3 mg NH_3-N /100ml, CP degradation and microbial population size increased. In studies by Cronje (1983), a change in AA metabolism believed to be due to improved hydrolytic activity at high rumen ammonia levels accompanied these changes in microbial population. This theory is based on the premise that the bacteria responsible for degradation at these levels require a higher rumen ammonia concentration for growth and assimilate ammonia by the alanine pathway (Cronjé, 1983). From this evidence, it is clear that rumen ammonia levels are not just an indicator of rate of CP degradation that has taken place, it also plays an important role in rate of CP degradation that will take place. Results reported by Cronjé (1983), where three diets with varying levels of concentrate were fed, found no effect on crude protein or DM disappearance by applying different levels of urea to the feed. This is in strong contrast with the dramatic responses obtained by Mehrez *et al.* (1977) and Wallace (1979) when feeding diets based on barley grain. This could possibly be explained by morphological and other inherent differences between maize and barley grains.

Ammonia exists as free NH_3 at a high pH level but as ammonia ions (NH_4+) at a low pH (Erasmus, 1985). Bacterials cell membranes are permeable to lipid soluble NH_3, but is impermeable to (NH_4+) ions. Thus more ammonia is absorbed at a high pH (Bartley & Deyoe, 1981), which may be the reason why there was a higher level of ammonia in the rumen on treatment UP 30 than on treatments UP 45 and UP 60. It is important that ruminal pH values are taken into account when proteolytic activity and protein degradability is considered as a measure of rumen ammonia alone will give no indication of these parameters (Erfle *et al.,* 1982). Results of Kertz *et al*. (1983) supports the hypothesis that a low rumen pH traps rumen ammonia and inhibits its absorption to the blood. According to Erfle *et al.* (1982), measurement of rumen ammonia alone would not give any indication of the change in rumen proteolysis and CP degradability, the effect of ruminal pH has to be considered. In a study by Cerrato-Sánchez *et al.,* (2007) in a dual flow continuous culture system, ammonia N concentration and flow decreased linearly as the time at suboptimal pH increased, and Calsamiglia *et al*., (2002) reported a lower ammonia N concentration when incubations were conducted at suboptimal pH for 12 hours vs. a constant pH of 6.40. The reduction in ammonia N concentration at low pH might be a result of a reduction in dietary CP degradation.

Under some conditions, a low rumen ammonia value equates to more MCP synthesis and a high rumen ammonia value equates to lower MCP synthesis (Erasmus, 1985). This is the case when there is substantial highly degradable CP available with significant amounts of highly fermentable energy sources. Rumen ammonia values vary depending on feed intake pattern, increases after feeding, and the level of increase will depend on the amount and form of CP as

well as energy available to bacteria (Erasmus, 1985). My average rumen ammonia level of 16.9 mg NH_3-N/100ml compares to the level of 17.5 mg NH_3-N/100ml found by Proctor-Howell *et al*. (1983) to be the optimal concentration for ruminal CP degradation.

The above literature suggests that in my study the lower pH with treatment UP 30 did not inhibit degradation of the easily degradable CP sources, but that the lower pH could have inhibited absorption of ammonia from the rumen to some extent thereby causing the numerically higher levels of rumen ammonia on treatment UP 30.

4.4.2 Rumen ammonia levels and its effect on crude protein disappearance

Disappearance of CP calculated as a percentage of the potentially degradable CP fraction was calculated for the two hour incubation period and this was correlated with the rumen ammonia N value for that specific period determined (Figure 17). The lowest rumen ammonia value for the two hour incubation period was 4.8 mg NH_3-N/100ml with 25.0 mg NH_3-N/100ml being the highest. This gives a wide variation for a period of only two hours. If a highly degradable CP source is fed with a low amount of soluble carbohydrates, a sharp increase in rumen ammonia concentration and low MCP synthesis might be expected (Erasmus, 1985). If the same amount of degradable CP is fed, but combined with a high amount of soluble carbohydrates, there would still be an increase in rumen ammonia but not to the same extent because more MCP will be produced. In Grummer *et al*. (1984), Holstein steers were fed twice daily a diet of urea supplemented maize grain and maize silage. Ruminal NH_3-N concentrations ranged from 3.0 mg NH_3-N/100ml at six hours post feeding to 46.0 mg NH_3-N/100ml at one hour post feeding (Grummer *et al*., 1984), clearly illustrating how rumen NH_3-N levels can vary over a short period of time, consistent with the findings in my study.

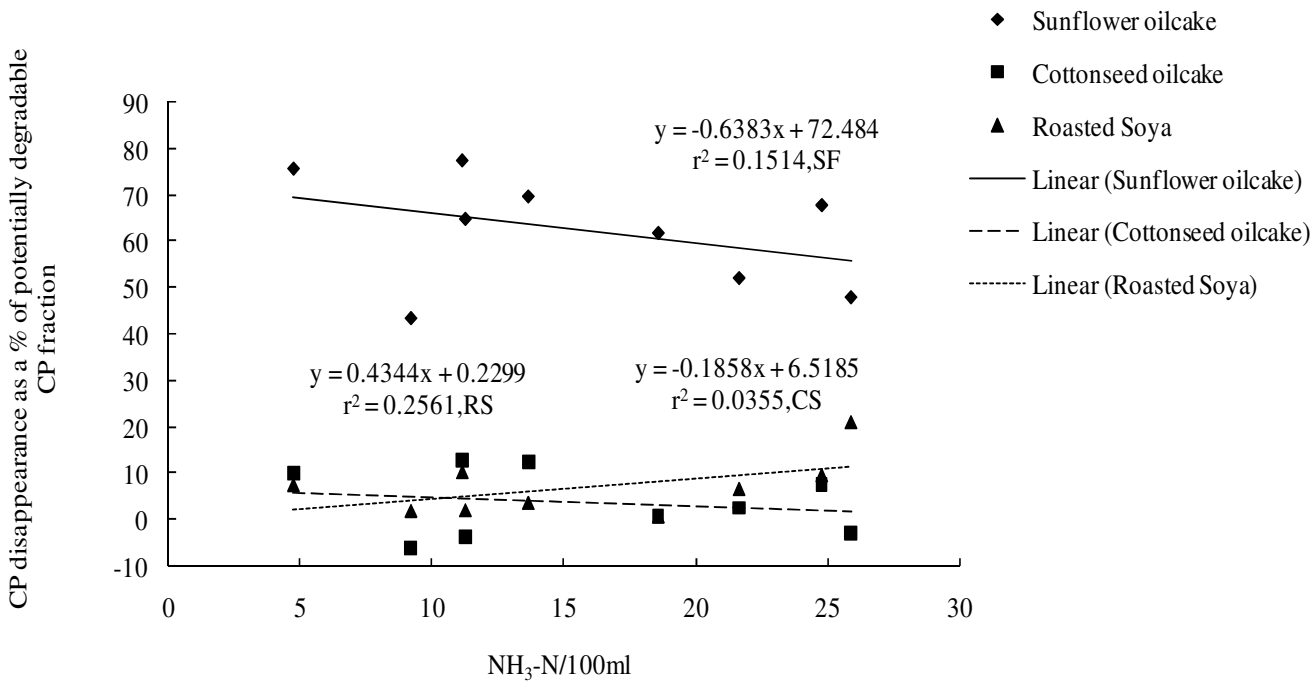

Figure 17 Crude protein (CP) disappearance expressed as a percentage of the potentially degradable CP fraction at different rumen ammonia levels (2 hour incubation).

For sunflower oilcake, CP disappearance was high at two hours of incubation. As the rumen ammonia values increased, CP disappearance values decreased, $r^2 = 0.15$. Because this is a small data set, the r^2 value is influenced by outliers. If one outlier is removed from the data set, the r^2 value changes to 0.58. The level of significance with the outlier in the data set was P=0.3, suggesting no correlation between rumen ammonia level and CP disappearance of sunflower oilcake. When the outlier is removed, the correlation was significant (P = 0.03), although the r^2 was only 0.58, suggesting that there might be a negative correlation between rumen NH_3-N and CP disappearance of sunflower oilcake. The rumen ammonia requirement for maximum MCP synthesis is a highly controversial topic (Clark & Davis, 1983). Karsli & Russell (2002) reported that 5.0 mg NH_3-N /100ml maximized MCP synthesis *in vitro*, microbial growth was limited at concentrations closer to 2.0 mg NH_3-N /100ml. More than 5.0 mg NH_3-N /100ml may be needed for maximal *in vivo* microbial growth because *in vitro* conditions are normally static while *in vivo* conditions are dynamic (Karsli & Russell, 2002). According to Satter & Roffler (1975) an ammonia value of higher than 5.0 mg NH_3-N/100ml had no effect on MCP production. Satter & Slyter (1974) concluded that MCP synthesis is maximum when the rumen ammonia value is 2-5 mg NH_3-N /100ml. Erasmus (1985) state that in a study where the usage of urea as an N supplement was tested fermenters were continuously infused *in vitro* with soluble carbohydrates and urea, there the rumen ammonia concentration would have been suppressed because there was always energy available for microbes to hydrolyze the urea. A cow's normal feeding pattern differs, as she does not continuously consume energy and urea. Mehrez *et al.* (1977) indicated fermentation was maximum when rumen ammonia concentration was below 23.5 mg NH_3-N /100ml. In my study, the average rumen ammonia level of 16.9 mg NH_3-N/100ml compares with the level of 17.5 mg NH_3-N/100ml found by Proctor-Howell *et al.* (1983) to be the optimal concentration for ruminal CP disappearance.

For cottonseed oilcake and roasted soya, CP disappearance was minimal at this short incubation time, and no correlation occured.

The lowest rumen ammonia value for the eight hour incubation period was 6.2 mg NH_3-N/100ml with 26.3 mg NH_3-N/100ml being the highest (Figure 18). After an eight hour incubation time, CP disappearance of sunflower oilcake had a poor correlation with the rumen ammonia because a high percentage of the potentially degradable CP fraction had already disappeared. For cottonseed oilcake, the relationship was

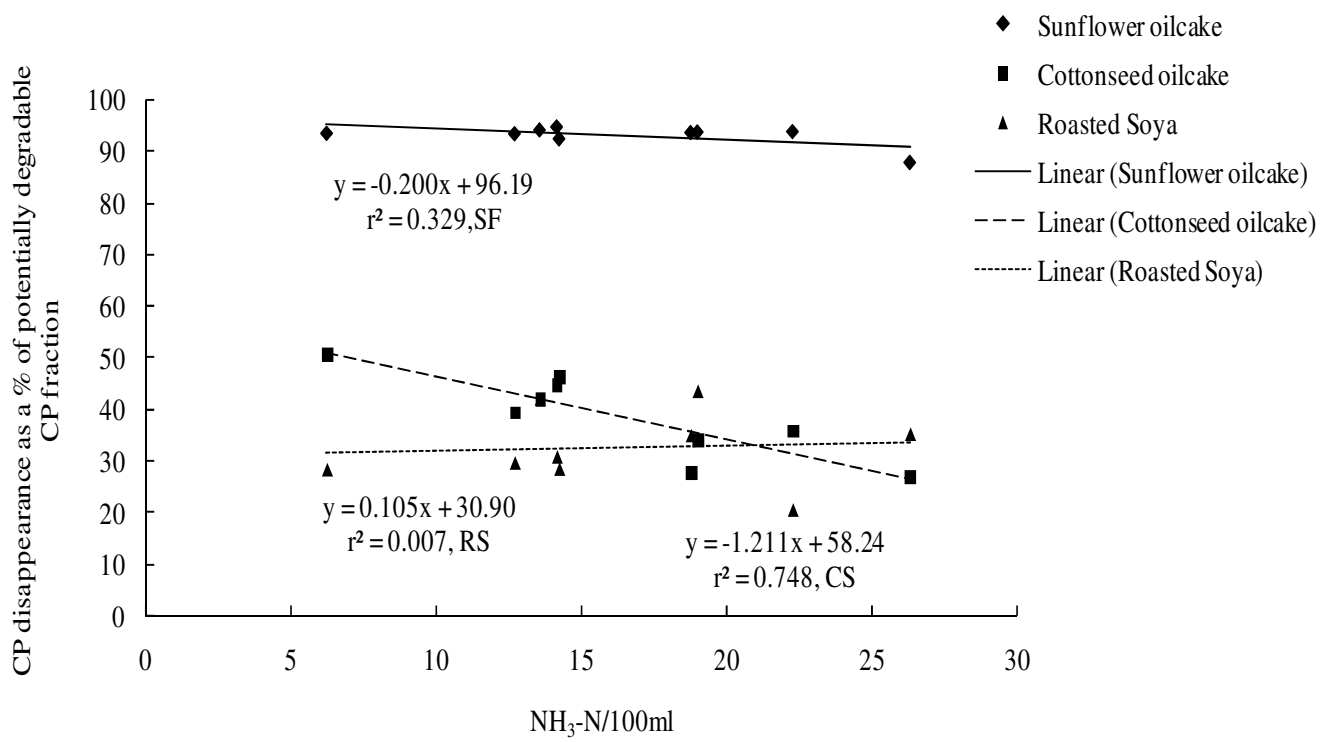

Figure 18 Crude protein (CP) disappearance expressed as a percentage of the potentially degradable CP fraction at different rumen ammonia levels (8 hour incubation).

best described by a linear equation: $y = 58.249-1.2113x$; $r^2 = 0.75$; $P = 0.0026$, which suggests that there was a negative correlation between the level of rumen ammonia and the N disappearance of cottonseed oilcake. Under certain instances, a low rumen ammonia value equates to more MCP synthesis, and a high rumen ammonia value equates to lower MCP synthesis (Erasmus, 1985). This relationship is only true when there is a continuous source of energy available with a highly degradable source of CP. According to Clark & Davis (1983) the level of rumen ammonia that will cause maximal MCP synthesis will not be constant under all feeding conditions. Interactions that occur are not known and using rumen ammonia as an indicator for effectiveness of ammonia utilization will thus not be possible (Ørskov, 1982). More research is needed in this regard, but even if our understanding is better, usage of rumen ammonia will still be limited because rumen ammonia levels varies considerably with feed intake patterns (Erasmus, 1985). There was no correlation between CP disappearance of roasted soya and an increased rumen ammonia level. This is in agreement with Grummer *et al*. (1984) in which *in situ* rates of CP and DM disappearance from soybean protein supplements with 10.2 or 50.1% soluble N was not affected by an increase of rumen NH_3-N from 4.8 mg NH_3-N/100ml to 17.3 mg NH_3-N/100ml.

The lowest rumen ammonia value for the 16 hour incubation period was 5.9 mg NH_3-N/100ml with 26.6 mg NH_3-N/100ml being the highest (Figure 19). Nearly all CP from sunflower oilcake had disappeared from the bags by this time, and so there was no correlation between CP disappearance of sunflower oilcake and rumen ammonia levels. There was still a positive correlation between CP disappearance of cottonseed oilcake and rumen ammonia with the linear equation: $y = 81,82-1.2443x$; $r^2 = 0.53$: $P = 0.04$ best describing the relationship. The quadratic equation describing the relationship is: $y = 61.25-0.09x^2 + 1.69x$; $r^2 = 0.73$; $P = 0.04$. There was no correlation between rumen ammonia and CP disappearance for roasted soya.

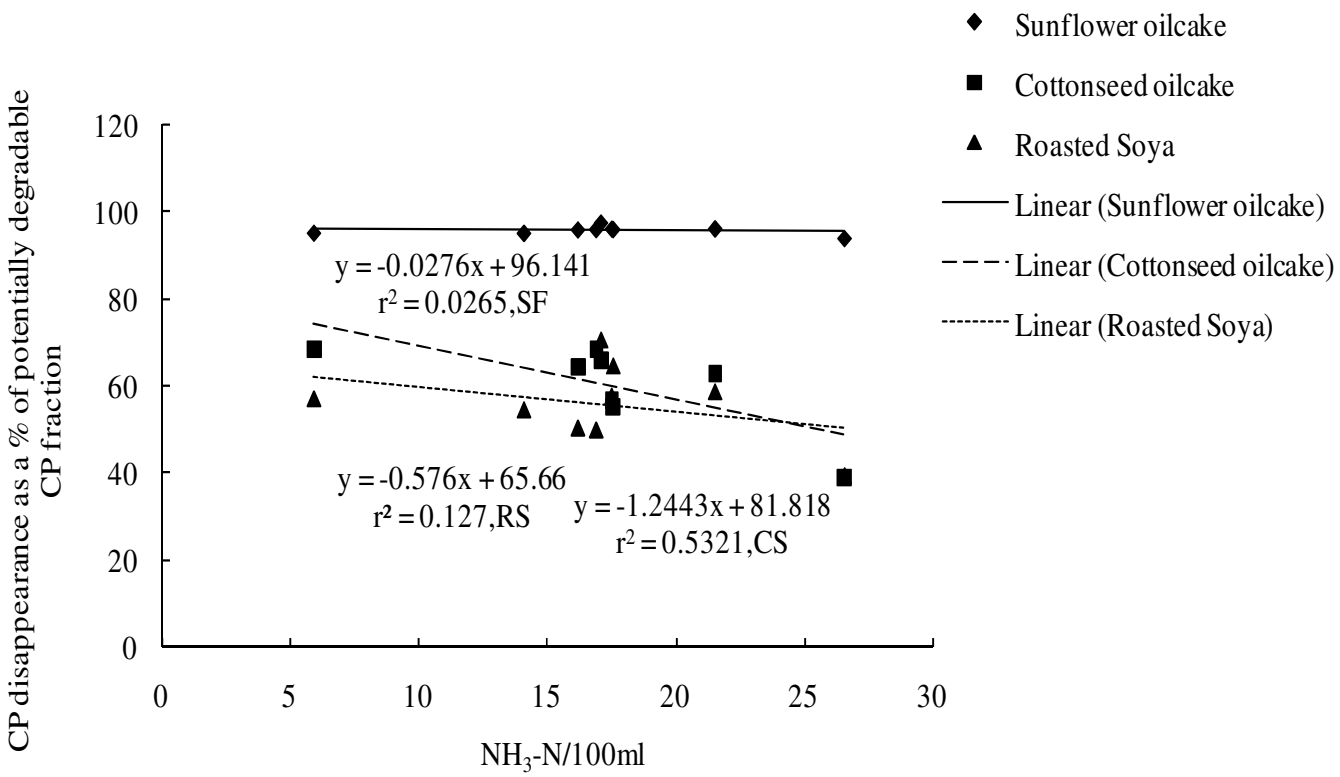

Figure 19 Crude protein (CP) disappearance expressed as a percentage of the potentially degradable CP fraction at different rumen ammonia levels (16 hour incubation).

The lowest rumen ammonia value for the 24 hour incubation period was 5.9 mg NH_3-N/100ml, with 25.3 mg NH_3-N/100ml being the highest (Figure 20). As nearly all CP from sunflower oilcake had left the bags by this time, there was no correlation between CP disappearance of sunflower oilcake and rumen ammonia levels. The relationship between CP disappearance of cotton seed oilcake and the rumen ammonia value was best desribed by the equation: $y = 91.253-1.09x$; $r^2 = 0.47$; $P = 0.04$. The quadratic equation that describes the relationship is: $y = 77.19-0.06x^2+0.94x$; $r^2 = 0.57$; $P = 0.07$. There was no correlation between rumen ammonia value and CP disappearance for roasted soya.

There is limited information about effects of ammonia concentrations on proteolysis or deamination in the rume. The main pathway of ammonia fixation by rumen bacteria may differ according to the prevailing concentration of ammonia (Erfle *et al*., 1982). Perhaps catabolic processes in rumen bacteria are influenced by ammonia concentrations, and it might alter the rate of protein hydrolysis through end product inhibition. However, Nikolic & Filipovic (1981) were not able to demonstrate an effect of ammonia concentration on the degradation rate of maize CP.

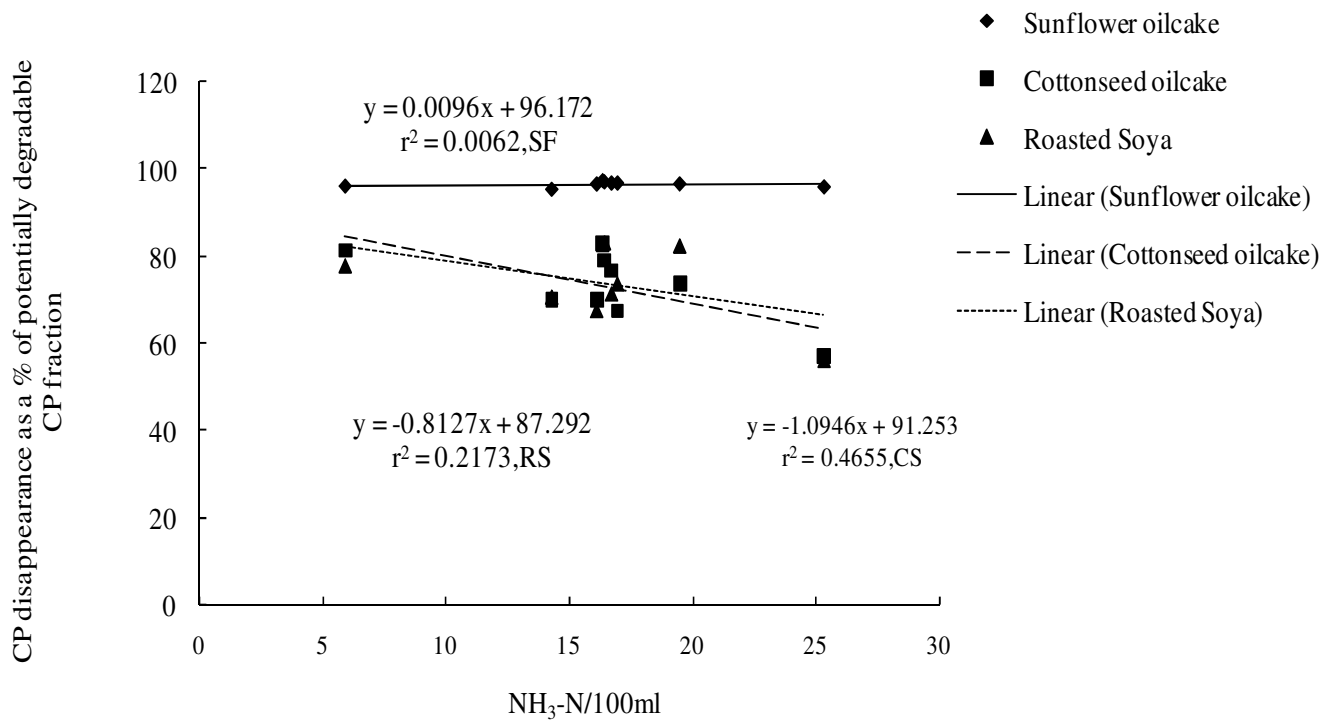

Figure 20 Crude protein (CP) disappearance expressed as a percentage of the potentially degradable CP fraction at different rumen ammonia levels (24 hour incubation).

CHAPTER 5.
CONCLUSION

This study was conducted to investigate effects of roughage:concentrate ratio on CP degradability of three protein concentrates differing in expected CP degradability. The hypothesis was that the resulting low pH brought about by the low roughage diet would decrease ruminal CP degradability, thereby affecting a potential saving in more costly high RUP sources. If a reponse to this hypothesis was obtained, then these effects could be incorporated in metabolic nutritional models to account for effects of rumen pH on CP degradability.

The most logical approach to investigate effects of pH on ruminal CP disappearance is to concentrate on the period where maximum rate and extent of CP disappearance occurs, which in this study, was the period from 2 – 16 hours of incubation. The way in which results were expressed (i.e., CP disappearance as a percentage of the potentially degradable fraction and its possible correlations with rumen parameters (i.e., pH, NH_3-N, VFA's)) were the most sensitive way of interpreting and expressing results. That r^2 values with regard to CP disappearance and rumen pH were in general poor, and mostly non significant, suggests that rumen pH had little effect on effective ruminal CP degradability. The different responses in CP disappearance between cottonseed oilcake and roasted soya due to different treatments might be because of morphological differences between the protein concentrates. High rumen ammonia N values caused a decrease in CP disappearance at some time intervals, but there was no effect over the whole incubation period.

The experimental diets were formulated to cover the range of diets that could be considered as practical under South African conditions. The roughage:concentrate ratio varied from 30% roughage: 70% concentrate to 60% roughage: 40% concentrate, and NDF varied from 33.36% to 42.63% (DM basis) and NFC from 32.88% to 37.58% (DM basis). Overall when practical South African formulation strategies were followed it failed to induce a pH low enough to influence ruminal CP degradation. Other means, such as heat or chemical treatment, might be more feasible.

CHAPTER 6.

CRITICAL EVALUATION

The pH value that was determined in this study was probably not low enough to have a significant effect on CP degradation of the different protein concentrates. The main source of concentrate used was coarsely ground maize grain, which does not have the highest fermentation rate in the rumen. If a follow up study is considered, different sources or combinations of grains that have a more rapid rate of fermentation, such as wheat, should be used. This would likely create the lower pH necessary to investigate effects of pH on CP disappearance. The maize grain in the diet should also be steam flaked to increase its fermentation rate in the rumen. The maize could also be milled more finely to increase the fermentation rate in the rumen.

By pooling rumen fluid from the different layers in the rumen there is a disturbance to the normal rumen environment: use permanently inserted pH meters with data loggers woul be more effective in investigating effects of rumen pH on CP degradability.

The degradability of the roasted soya CP was higher than expected, possibly due to under processing. In a follow up study, it would be recommended to have the protein concentrates tested for the level of processing they were exposed to, before they are used.

The cows used in this study were late in lactation. It is recommended that in a follow up study high producing cows be used as they have higher DM intake and, having a higher load of total VFA's, a potentially lower rumen pH.

Beef cattle in feedlot situation have a much lower ruminal pH than dairy cows; it would be recommended to rather use beef cattle when testing the effect of a low ruminal pH on protein degradability in the rumen.

The concentration of lactic acid in the rumen was not analyzed in this study, and it is recommended that the effect of lactic acid be investigated in the future.

From this study, it seems that rumen ammonia might play an important role in CP degradation of protein concentrates. In a follow up study it is suggested to give more attention to this topic. More rumen ammonia analyses will be needed because the ammonia data set in this trial was too small.

The use of dietary manipulation to induce a low rumen pH, and thereby increase the flow of RUP to the small intestine, seems to be unfeasible in practice. Diets that would result in such low pH values would be impractical and would most likely lead to metabolic disturbances such as acidosis.

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