IMPACT OF SUPPLEMENTAL PEPTIDES AND NEUTRAL DETERGENT FIBER ON MICROBIAL PROTEIN SYNTHESIS IN THE RUMEN OF CATTLE

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IN THE RUMEN OF CATTLE

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CHAPTER I

INTRODUCTION

The protein synthesizing capability of the rumen microbial population was highlighted by the research of Virtanen (1966), who demonstrated that the protein requirement of an average producing lactating cow could be met by the microbial mass with urea as the only dietary N source. Research in ruminant nutrition since these studies has corroborated the importance of the rumen microbial population both in carbohydrate digestion and the contribution of microbial protein to the ruminant's protein requirement.

The ruminant receives 40 to 80 % (Owens and Bergen, 1983) of its daily amino acid requirements from microbial protein flowing to the small intestine. It is important to quantify factors affecting microbial growth in the rumen and how these factors might be manipulated to improved microbial protein flow to the duodenum.

One of the many nutritional factors that has been suggested to influence rumen microbial growth is peptide supply. It is generally accepted that rumen degradable protein is beneficial to rumen microorganisms, and that, in mixed cultures *in vitro* and *in vivo*, fiber digestion, microbial growth and growth yield might be stimulated by peptides or amino acids (Hume, 1970; Maeng and Baldwin, 1976; Maeng et al., 1976; Cotta and Russell, 1982; Merry et al., 1990; McAllan, 1991). Based on culture studies cellulolytic bacteria use ammonia as their main source of nitrogen for growth, while non-cellulolytic

species use preformed peptides and amino acids (Russell et. al., 1992) though any benefit from preformed peptides or amino acids on *in vivo* yield of microbial protein has not been consistently demonstrated. The Cornell system for modelling rumen fermentation (Russell et. al., 1992) and the NRC 1996 publication on nutrient requirements for beef cattle used these assumption: that amino acids and peptides would stimulate protein yield in their prediction equations to estimate microbial growth. These assumptions although potentially valid *in vitro* may not prove valid *in vivo* because chemostat conditions of continuous input of nutrients differ from *in vivo* conditions where intake and supply of feed nutrients often is intermittent.

Not all *in vivo* studies found that preformed peptides/amino acids are beneficial to rumen microorganism. Cruz Soto et al. (1994) found that cellulytic rumen bacteria exhibited a variable response to amino acids and peptides: cellulose breakdown was unaffected, but growth rate on soluble sugars increased several fold when preformed amino acids were present. They proposed that, in general, rumen fermentation would be stimulated by preformed peptides and amino acids only when the rate of provision of energy permitted (Cruz Soto, et al., 1994). According to Chikunya et al. (1996) rumen microbial growth responds to preformed amino acids when fiber fermented rapidly. Based on these suggestions microbial growth response to peptides and amino acids is may be much more complicated than appreciated previously.

Second nutritional factor that influence microbial protein synthesis is effective neutral detergent fiber (eNDF) which is a stimulus for rumination, saliva flow, ruminal buffering, and health of the rumen wall. The NRC (1985) indicate that diets containing

less than 40 % forage (20 % NDF) have produce lower microbial yield, primarily due to depressed ruminal pH; mixed ruminal bacteria that were incubated in vitro produced less microbial protein at pH 5.7 than at pH 6.7 (Strobbel and Russell, 1986). Weakley and Owens (1983) in their studies involving steers fed corn diets observed that efficiency of microbial protein synthesis was quite variable and not related to ruminal pH over a range from 5.8 to 6.7. Russell et al. (1992) proposed that microbial yield is reduced 2.2 percent for every 1 percent decrease in forage eNDF below 20 % NDF. Additional factors not accounted for in the eNDF system that influence pH are total grain intake and its digestion rate, and form of grain.

Supplementation of the diet with amino acids/peptides failed to influence the efficiency of microbial protein synthesis in cattle fed grass-silage diets (Rooke and Armstrong, 1988; Rooke et al., 1989) and in sheep fed grass hay (Cruz Soto et al., 1994); they suggested that this was because fermentation was not limited by availability of peptides or amino acids when ammonia is available and the growth rate is limited by a slowly degradable energy source. Despite the fact that very little information is available on the effects of peptides and eNDF on microbial protein synthesis, the fact that these factors are included in models of ruminal fermentation indicate that it is important to verify or refute effects in cattle fed highly fermentable diets.

The objectives of the research trial reported in this dissertation were1) to determine the effects of dietary level and physical form of cottonseed hulls, a source of eNDF, on ruminal digestion and efficiency of microbial protein synthesis 2) to evaluate the effects of concentration of ruminal peptides on extent of ruminal digestion and

efficiency of microbial protein synthesis. 3) to evaluate the relationship and interactions between nitrogen and energy source on extent of ruminal digestion and efficiency of microbial protein synthesis.

CHAPTER I I

REVIEW OF LITERATURE

Microbial yield is the magnitude of the amount of substrate available for growth and the efficiency at which microbes use that substrate or microbial efficiency (MOEFF). The faster the growth rate of microorganisms, the greater the efficiency of microbial protein synthesis and, if the same amount of substrate is fermented, the greater the daily yield of microbial mass and protein.

This review will discuss the following factors influencing the efficiency of microbial protein synthesis: effects of peptides/amino acids, the level of feed intake, the feeding frequency, the concentrate to forage ratio, the carbohydrate source, the protein source and, finally the interaction of carbohydrate and protein.

Ruminant animals derive their protein from three sources: microbial protein synthesized in the rumen, undegradedable dietary protein and endogenous protein. Under most dietary conditions, microbial protein constitutes the major source of protein to the ruminant. The magnitude of this supply depends on the ability of rumen microbes to grow on fermentable carbohydrate, growth conditions, and the efficiency of microbial protein synthesis. Several nutritional factors have been found to influence microbial growth including source of nitrogen (N) and level of effective neutral detergent fiber (eNDF).

Formation of peptides in the rumen

Most protein in feed are hydrolyzed rapidly in the rumen, although the precise rate and extent of breakdown varies greatly between different proteins (Wallace, 1998). Early

work suggested that protein degradation in the rumen was proportional to solubility (Chalmers and Synge, 1954; Hendrickx, 1976). However, subsequent research detected other additional properties are also important (Kuafman and Lupping, 1982). For example, some soluble proteins are cleaved more slowly than insoluble proteins, depending on the degree of secondary and tertiary structure, and cleavage of disulfide bonds enhances the breakdown of albumin and similarly heavily cross-linked molecules (Mahadevan et al., 1980; Nugent et al., 1983; Wallace and Kopecny, 1983). Heating and formaldehyde treatments, affecting both solubility and cross-linking, have been used to protect proteins from degradation thereby provide bypass protein to the lower tract (Kaufman and Lupping, 1982). Peptides accumulate in the rumen fluid transiently after feeding, and thereafter peptide concentrations decline (Chen et al., 1987; Kim et al., 1998; Broderick and Wallace, 1988; Wallace and McKain, 1990: Williams and Cockburn, 1991). With hydrolysis of casein, peptides accumulate rapidly (Broderick and Wallace, 1988; Williams and Cockburn, 1991), but otherwise the extent to which peptides accumulate in the rumen contents does not appear to be related to either the rate or extent of degradation of the protein supplement (Williams and Cockburn, 1991).

The nature of diet has a major influence on the proteolytic activity of rumen contents. Fresh herbage promotes proteolytic activity up to nine times higher than that found with dry rations, the higher soluble-protein content of the herbage serving to the population of proteolytic bacteria (Nugent and Mangan, 1981; Hazlewood et al., 1983; Nugent et al., 1983). There has been speculation that endogenous plant proteases may play an important role in the breakdown of the fresh herbage protein in the rumen

(Theodorou, 1995), largely on the grounds that such enzymes exist and are predominantly responsible for protein breakdown in the silo. Cereal diets also yield higher proteolytic activities than do dry forage diets, probably because proteolytic rumen microorganisms tend to be amylolytic rather than cellulolytic (Siddons and Paradine, 1981).

Effects of peptides and amino acids on microbial growth

Ammonia is a central intermediate in the degradation and assimilation of dietary N in the rumen and is required by many species of bacteria (Hungate, 1966; Nolan, 1975). Most species of rumen bacteria can grow with ammonia as a sole source of N provided certain volatile fatty acids (VFA) are present as amino acids (AA) precursors (Bryant and Robinson, 1962; Bryant 1973). Indeed ruminants can survive for months or years with urea as the only N source in the diet (Virtanen, 1966). However some researchers have reported that ruminants have higher rates of production when dietary N is present in the form of protein (Stock et al., 1986; Rooke and Armstrong, 1989). Sometimes it is difficult to distinguish whether such a benefit stems from the increased supply of preformed N sources amino acids or peptides to rumen microorganisms or to the escape of some dietary protein to the abomasum. Nevertheless, it is generally accepted that ruminally degradedable protein is beneficial to rumen microorganisms, and that, in mixed cultures both in vitro and in vivo, fiber digestion, microbial growth and growth yield may be stimulated by protein or amino acid N (Hume, 1970; Maeng and Baldwin, 1976; Maeng et al., 1976; Cotta and Russell, 1982; Merry et al., 1990; McAllan, 1991).

There has been considerable controversy concerning the requirements for AA or peptides by rumen microbes for efficient growth. In pure culture, most species of bacteria grow better when pre-formed AA are present (Bryant and Robinson, 1962; Bryant, 1973: Cotta and Russell, 1982, Merry et al., 1990). These results supported suggestions that protein added to poor quality diets may increase microbial protein synthesis and protein flow from the rumen (Hume, 1970; Ben-Ghendalia et al., 1978). Argyle and Baldwin (1989) supported the suggestion that increased microbial growth efficiencies resulted when AA and peptides provided a proportion of the N in an incubation medium containing rumen microorganism. In addition, they found that any benefit of peptides over a complete mix of the same AA was small and concentration dependent, with a maximum of 10 mg peptides per liter giving improving growth yield by only 10% above the corresponding AA. Griswold et al. (1996) studied microbial metabolism in continuous culture and reported that replacing all the urea N in the diet with peptide N enhanced microbial growth, but when an equal amount of total N was provided, half from urea and half from peptides, microbial yield was not increased. According to Owens and Zinn (1988), AA in media can enhance bacteria growth rate in vitro but addition of AA to typical ruminant diets does not increase efficiency of microbial growth in the rumen.

Several *in vivo* studies have detected no benefit from supplemental amino acids on efficiency of growth or yield of rumen organisms (Leng and Nolan, 1984; Rooke and Armstrong, 1989; Cruz Soto et al., 1994). In studies with steers fed high roughage diets, no improvement in efficiency of microbial protein synthesis were observed by supplementing urea-based basal diets with either casein (Redman et al., 1980;

Sriskandarajah et al., 1982) or soybean meal (Kropp et al., 1977). In fact in these three studies, urea supplemented high roughage diets produced slightly greater microbial protein synthesis. In studies with higher concentrate diets fed to sheep (Mercer et al., 1980) and (Smith et al., 1978), again no improvement in efficiency of microbial protein synthesis was observed from substituting a variety of protein sources for urea. The results of studies by Maeng et al. (1989) may explain some of the contradictory results. These researchers indicated that rumen microbes growing on different carbohydrate substrates have requirements for different N-substrates; cellulolytic organisms may not require AA to the same extent as organisms growing on starch or sugar as the major substrate. For microbes utilizing sugars or starches, they suggested that the requirement for preformed AA/peptides was high; for cellulolytic organisms, they suggested that this was not true. Cruz Soto et al. (1994) studying the effect of peptides, amino acids and urea on microbial growth in rumen of sheep fed grass hay concluded that fermentation by rumen bacteria is not limited by the availability of peptides or amino acids when ammonia was available and the growth rate was limited by a slowly degradedable energy source. In an experiment by Chikunya et al. (1996) in which peptides were supplied with diets containing either rapidly or slowly fermented energy, results indicated that the benefit of peptides would only be evident if the energy source supported a growth rate which enabled the organism to respond.

Supplementation of sheep fed a poor quality forage with branched chain VFA increased the flow of microbial-N to the duodenum (Kay and Phillipson, 1964). Such stimulation of microbial growth with branched VFA has also been shown to increase feed

intake in some experiments (Helmsley and Moir, 1963). Certainly, cellulolytic microbes have a requirement for branch chained VFA that can be produced from branch chained AA but the requirement is probably very minimal. There is no solid evidence of increased fiber digestibility with degradeable protein supplementation (Hungate and Dyer, 1956). The consensus of evidence now suggests that cellulolytic organisms when growing in the rumen are rarely if ever deficient in AA, peptides or branched chain VFA in the rumen (Maeng et al., 1989). This is not to say that these organisms may still need the peptides, AA and branched chain fatty acids in "catalytic" amounts, but just that these compounds are rarely if ever at such low concentrations that they reduce microbial activity or growth.

Level of feed intake.

When level of feed intake was increased, flow of microbial N from the rumen has increased in numerous experiments (Greife et al., 1985; Robinson et al., 1985; Merchen et al., 1986). In other studies (Chamberlin et al., 1976; Tamminga et al., 1979; Firkins. 1984), however, no correlation was found between the feed intake, and the microbial yield. This might be due to the narrow feed intake levels used (between 1% and 2.5% of the body weight) in the above-cited studies carried out in sheep and in beef cattle, respectively. According to the results obtained by Tamminga (1981) and Robinson (1983), in dairy cows a clear-cut close correlation could be established between feed intake and the microbial yield. Tamminga's experiments included a total of 43 dairy cows fed a ration containing 47% hay. The lowest microbial yield was measured at a dry matter

intake of 10 kg (1.8% of the body weight). Intakes either above and below 10 kg resulted in an increased yield.

Van Soest (1982) suggested that at higher feed intakes, more particle flow from the rumen and thus more bacteria adhering to feed particles will pass into the duodenum. As a result, the higher flow of dietary organic matter will increase output and decrease microbial recycling. The turnover rate of the solid and liquid phase of the rumen content is positively correlated with feed in intake (Evans, 1981). The outflow rate of the ruminal liquid increases in parallel with increase feed intake. The higher microbial yield found in such cases probably a consequence of the enhanced "wash-out" of microorganisms from the rumen (Leng et. Al., 1984). Attempts to increase microbial yield by raising the outflow rate of rumen fluid above a certain basal level (4-6%/h) have failed. Indeed, in many cases, increasing the rumen fluid dilution rate caused the opposite effect, i.e. a reduction in the microbial yield (Chamberlin and Thomas, 1980; Hadjipanayiotou et al., 1982; and Goetsch and Owens, 1985), perhaps due to a reduction in the amount of substrate fermented.

Feeding frequency.

Only a few researchers have studied the relationship between microbial flow and the feeding frequency, even though this appears to be the most one logical way of influencing the microbial yield. An increased yield would not be surprising, as it would seem logical to expect that by increasing the feeding frequency diurnal fluctuations in the quantity of metabolites derived from microbial fermentation might be avoided and, through a more balanced rumen fermentation, microbial yield might be increased. The

results obtained by Tamminga (1981) support this hypothesis. As compared to twice daily feeding, six feedings per day resulted in an increased microbial N flow and enhanced ruminal true digestibility of organic matter (TDOM). In contrast, other investigators (Beever et al., 1972; Brandt et al., 1981; Robinson and Sniffen, 1985) either did not find any increase in microbial flow as a result of more frequent feeding, or – as opposed to Tamminga's findings – they observed a higher bacterial yield (Robinson, 1983) and protozoal yield (John and Ulyatt, 1984) with less frequent feeding. Sniffen and Robinson (1987) attribute this higher microbial yield from less frequent meals to an increased ruminal outflow over a short period following rapid feed intake. This higher flow rate will increases the outflow of bacteria adhering to feed particles and thus decrease bacterial recycling. This later in turn should leads to enhanced microbial growth. The higher protozoa output also might be expected because after feeding an increased amount soluble substrate, the number of protozoa in the liquid phase of the rumen content increases markedly; an increased ruminal outflow rate will enhance "wash-out" of protozoa.

Concentrate to forage ratio.

Several *in vivo* studies have indicated that the raising forage content of the ration results in a higher MOEFF. In sheep, the maximum MOEFF was measured when the forage constituted 70 % of the ration. Forage intakes below, but especially those above that level were found to lower MOEFF (Chamberlain and Thomas, 1979; Mathers and Miller, 1981). After increasing the forage level of the ration from 0 % to 25 % and from 10 % to 40 % in steers and dairy cows, respectively, a higher MOEFF was measured

(Cole et al., 1976; Oldham et al., 1979). The report issued by NRC (1985) also supports the observation that diets containing less than 40 % forage (20 % NDF) decreases microbial growth yield. From the results of Russell et al. (1992), the authors concluded that whenever NDF content was lower than 20 % of dry matter, microbial yield is reduced by 2.5 % for every 1 % decrease in NDF. The reduction in yield at higher concentrate levels was explained by an uncoupling of energy metabolism during fermentation. Namely, at higher forage levels the slow degradation of organic matter allows more energy to be trapped by microorganisms. In the studies cited above, the concentrate contained mainly barley and corn grains. In earlier work, no correlation was found between the concentrate to forage ratio and the microbial yield when the concentrate was composed of high-fiber by-products (Tamminga, 1981).

A positive correlation was established between the proportion of forage in the diet and the rumen liquid turnover rate (Evans, 1981). At the same time, at a forage level exceeding 70-75 %, microbial yield was reduced. Presumably, this drop is due to slower washout of microbial matter and greater bacterial recycling in the rumen that reduces yield of bacteria, and as well as diversion of a larger proportion of energy for maintenance. This is the only explanation that has been used to account for the observations of Chamberlain and Thomas, (1979) and Mathers and Miller (1981), who measured low microbial yield with an all-forage diet.

The effect of the concentrate to forage ratio may be markedly modified by the level of feed intake. Studying the relationship between these two variables, Merchen et al. (1986) found that at a low feed intake there was no difference in the efficiency of

microbial protein synthesis between rations containing 75 % or 25 % concentrate. In contrast, increasing the intake of the ration containing 75 % concentrate increased higher microbial N outflow and improve microbial efficiency. The complexity of the fermentation processes taking place in the rumen is shown by the fact that, as opposed to what has been described above, these authors explained the increase in duodenal flow of bacterial N and the enhanced efficiency of microbial protein synthesis to the larger amount of starch present in diets high in concentrates. This reasoning is supported by the *in vitro* study by Stern et al. (1978) who, stressing the importance of an exact knowledge of feed constituents, experimentally confirmed that the efficiency of microbial protein synthesis increased when the non structural carbohydrate (NSC) content of the ration was raised.

Carbohydrate source

Like animal cells, the cells of plants contain nitrogenous compounds, carbohydrate, lipids and minerals. The cell wall of plants contains molecules, that are not be found in the animal cells.

The carbohydrates of plants can be assigned to two main groups: nonstructural carbohydrates including e.g., sugars and starch, and structural cell-wall constituent carbohydrates such as pectin, cellulose and hemicellulose.

The ruminal utilization of carbohydrates is decisively influenced by the ease of solubilization. Correspondingly, sugar, starch and pectin are the carbohydrates most rapidly fermented. When feeding a diet of 77:23 % forage to concentrate, the quantity of starch and simple sugars in the rumen decreased to a minimal level by 8 h post feeding

(Leedle et al., 1986). With diets of higher concentrate ratio (68 %), the degradation of carbohydrates took place differently. Soluble starch again was fermented rapidly but the soluble sugar level first increased and decreased only 4h after feed the meal. After it initial decrease, the soluble pectin content of the rumen remained constant for 24 h; this can be explained by the concurrent pectin solubilization and galacturonic acid utilization.

Of the structural carbohydrates, hemicellulose was fermented more rapidly than cellulose, as indicated by the relatively constant cellulose content until 12 h post feeding (Leedle et al., 1986). In contrast, Glenn and Canale (1990) in their studies reported that ruminal digestion of hemicellulose and xylose was less extensive than for cellulose and glucose. The slower rate of hemicellulose hydrolysis is supported by the observation that xylan, the major constituent of hemicellulose, produced the lowest microbial yield, whereas cellobiose produced the highest microbial yield (Strobiel and Russell, 1986).

The degradation rate of carbohydrates may be modified by the pH value of the rumen fluid. At a constant pH value (6.7) the efficiency of microbial dry matter production (Y_{ATP}) during the degradation of starch, sugars, cellobiose, xylan and pectin was similar (Strobel and Russell, 1986). Lower pH markedly decreased the digestion of cellulose, hemicellulose and pectin while it only slightly affected that of starch (Marounek and Bartos, 1983; Hoover, 1986; Shriver et al., 1986; Ben Ghendalia et al., 1989). According to some studies (Russell and Dombrowski, 1980; Finlayson, 1986), the value of Y_{ATP} markedly decreases when pH decreases from 6.5 to 5.5. As compared to the level measured at pH 6.7, Strobel and Russell (1986) observed a 50 % decrease in the efficiency of microbial protein synthesis at pH 5.7. However, in other studies lowering

pH did not affect or in some cases increased the microbial efficiency, measured as Y_{DOM} (digestible OM) (Hoover et al., 1984; Shriver et al., 1986).

The rate of carbohydrate degradation also is influenced by the chemical structure of the carbohydrate. The degradation rate of plant cell wall is lower when the structural carbohydrates contain large quantities of lignin, acetal and phenol ester (Hespell, 1988). Despite their simpler chemical structure, nonstructural carbohydrates differ in their ruminal degradation, and this influence the total microbial protein yield per day. The starch present in certain plants (oats, tapioca, barley) is degraded 2 to 2.8 times as fast as in corn (Cone et al., 1989). Certain treatments of the feed (e.g. extrusion) may double the digestion of starch. The efficiency of microbial protein synthesis (g N/kg ADOM) was higher for dairy cows fed barley grains than for those fed corn (Oldham et al., 1979). This effect was even more pronounced when poor quality hay (containing 6.4 % crude protein) was fed as basal feed. Microbial yield was markedly higher for ruminants fed pelleted ryegrass hay when it was supplemented with barley than when it was supplemented with corn (Voight et al., 1977). On the other hand, no difference was found between barley and corn supplementation when the hay was fed in a chopped form. Pelleting facilitates the access of bacteria to fibre and, thus improves the ruminal degradation of hay. The barley starch added to the hay provided a rapidly degraded source of energy to support microbial growth. In contrast to the above study, sorghum grain, barley and corn did not change the efficiency of microbial protein synthesis of animals fed a ration containing 20 % forage (Spicer et al., 1986).

Protein source.

Rumen bacteria can utilize N-containing substances of different origin for protein synthesis. In most cases, ammonia acts as the principal intermediate in nitrogen metabolism. Depending on the carbohydrate source from which they obtain the energy necessary for their growth, bacteria markedly differ in terms of N utilization. Thus, bacteria fermenting structural carbohydrates utilize exclusively ammonia (Bryant, 1973), whereas those capable of degrading nonstructural polysaccharides (NSC) are proposed to use either ammonia or amino acids and peptides for protein synthesis (Russell et al., 1992). According to the results of *in vitro* studies, 66% of the microbial protein was derived from peptides or amino acids, and 34 % came from ammonia (Russell et al., 1983). This ratio was not affected by the growth rate of bacteria but was markedly influenced by the available energy. Namely, in the absence of easily fermentable carbohydrates the bacteria were unable to incorporate peptides which would have been degraded to ammonia.

Bacteria utilize products of proteolysis with dissimilar efficiencies. Presumably, large peptides are taken up more rapidly and are used more efficiently for microbial growth than amino acids and small peptides (Prins et al., 1979; Chen et al., 1987; Broderick et al., 1988; Wallace et al., 1990). As compared to peptides of a molecular weight less than 1500, peptides with a molecular weight exceeding 10,000 increase microbial growth (Thomsen, 1985).

Microbial growth may be influenced by sources of N. Compared with ammonia, amino acids improved microbial efficiency, measured as weight microbial N/weight

carbohydrate digested (Maeng and Baldwin, 1976). Higher microbial growth yield was obtained when amino acids and peptides were used in combination than when their effect was measured separately (Argyle and Baldwin, 1989). Still, an intact protein with high degradability (e.g. casein, soybean) proved to be the most efficient substrate; digestion of starch was doubled and the degradation of cellulose also was increased considerably (Hugue and Thomsen, 1984; Thomsen, 1985). Despite the observations cited above, ammonia represents the principal nitrogen source for microbial protein synthesis and therefore, its importance must not be underestimated. At a low ammonia level the growth rate of obligate ammonia-utilizing bacteria (e.g. cellulolytics) is slow, this results in a decreased carbohydrate degradation and reduced feed intake (Tamminga, 1980). Protein sources resistant to ruminal degradation (fish meal, blood meal) resulted in a decreased acetate to propionate ratio and produced a lower ruminal ammonia concentration (Febel et al., 1993; Kellems et al., 1993).

Opinions vary as to the optimum ammonia concentration necessary for maximum microbial growth; the reported values range between 2.9 to 13.5 mmol/L (5 to 23 mg/100 ml). According to Satter and Slatter (1974), microbial protein synthesis reached a plateau when ammonia was only 2.9 mmol/l (5mg/100 ml). Mehrez et al. (1977) observed a plateau at a much higher ammonia concentration (11.2 – 13.5 mmol/l or 19-23mg/100 ml). Hespell and Bryant (1979) suggested that a higher ammonia level is required for maintaining maximum digestion and fermentation rates when the ration contains a large amount of rapidly degraded carbohydrate.

The ammonia concentration of the ruminal fluid markedly influences the nitrogen assimilation, too (Leng and Nolan, 1984). At a high ammonia level glutamate dehydrogenase is active whereas at low ammonia levels glutamate synthetase and glutamine synthetase are responsible for ammonia fixation. Glutamine synthetase requires ATP for its activity; thus, in the absence of sufficient ammonia, microbial yield will be reduced by 14%.

Several studies have confirmed that urea supplementation of low N diet will increase MOEFF. This increase was evident only up to a certain urea level above which a reduction was noted (Kang- Meznarich and Broderick, 1981). Other authors have suggested that the microbial yield cannot be increased further by incremental urea supplementation (Hume et al., 1970; Leibholz, 1980). Parallel to increasing the dietary amount of a very rapidly degraded source of nitrogen, microbial yield will first increase and then drastically decrease in response to urea supplementation (Veira et al., 1980; Lu et al., 1982; Veira and Ivan, 1982). The efficiency of protein synthesis was found to decrease when supply of urea-N or rapidly degradedable N exceeding 25 g N/kg of dry matter intake.

The interaction of carbohydrate and protein.

During proteolysis ammonia production rate often exceeds the utilization rate for ammonia. Absorbed from the rumen, ammonia no longer remains fully available for the microbes: some of it is transformed into urea and excreted in the urine. By the use of N^{15}, Nolan (1975) found that as much as 25 % of the protein N may be lost for the bacteria

and, eventually, the host through absorption and excretion. Carbohydrates have little effect on the rate of protein degradation by extracellular proteinases. However, the further fate of amino acids arising during proteolysis is markedly affected by the quantity of fermentable carbohydrate available. If sufficient ATP is present, amino acids entering the microbes can be incorporated into microbial protein. Bacteria utilizing non-structural carbohydrate consume peptides at a rate of .07g peptide/g bacteria/h. This N is used for microbial protein synthesis or ammonia production (Russell and Martin, 1984; Hino and Russell, 1985). If energy (ATP) supply is not sufficient to drive protein synthesis, amino acids will be fermented as an energy source and ammonia will accumulate. Thus, the diversion of peptides to microbial protein or ammonia is theoretically could be regulated by the availability of carbohydrate.

The ruminal degradation of carbohydrate thus may be one of the major factors influencing the microbial protein yield. Carbohydrate degradation is positively influenced by the raising nonstructural carbohydrate content of total dietary carbohydrate and the increasing quantity of degradable intake protein (DIP); however, NSC is more effective (Hoover and Stokes, 1991). The efficiency of microbial protein synthesis increased when the NSC and DIP level of diets was raised, but at dissimilar rate. Based on *in vitro* studies, the efficiency of microbial protein synthesis increased, irrespective of DIP, when the NSC content was raised from 25 % to 37 %. However, this was the plateau, with, no further improvement by raising the dietary NSC content from 37 % to 54 %, which indicates that optimum NSC must be around 37 % of the dry matter (Stokes et al., 1991*b*). However, this assertion is at variance with the results reported by Chamberlin

and Thomas (1979), Oldham et al. (1979), and Mathers and Miller (1981), all suggest that the efficiency of microbial protein synthesis will decrease when the concentrate level of the ration exceeds 30 %. In their experiments the DIP remained constant when the concentrate intake and thus the NSC content was increased, which meant a wider NSC:DIP ratio. If the ratio of protein to carbohydrate is inadequate, microbial growth may be limited by energetic uncoupling. In their experiment cited above, Stokes et al. (1991*b*) found that a decrease of the DIP, i.e., an increase in the NSC:DIP ratio impaired the efficiency of microbial protein synthesis with all three levels of NSC used. Varga et al. (1988) also stressed the importance of having an accurate knowledge of the NSC:DIP ratio. At NSC:DIP ratios higher than 6:1 they observed a decrease in microbial growth as well as in the degradation of fiber and protein. When the NSC:DIP ratio was decreased to 3.4:1, the efficiency of microbial protein synthesis increased.

Stokes et al. (1991*a*) conducted another trial with dairy cows equipped with rumen and duodenal cannulas. Their experiment provided further valuable data on relationship between the NSC:DIP ratio and the efficiency of microbial protein synthesis. They fed diet containing 38, 31 or 24 5 NSC and 13.1, 11.8 or 9 % DIP. Parallel to the decrease of NSC and DIP, the ruminal degradation of organic matter, carbohydrate and protein decreased and the content of microbial N in the duodenum become lower. At the same time, the results of *in vivo* experiments (Stokes et al., 1991*a*; Febel et al., 1994) indicated that, the efficiency of microbial protein synthesis was not impaired if the NSC and DIP were decreased proportionately (low NSC:DIP ratio).

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CHAPTER III

EFFECTS OF LEVEL AND PHYSICAL FORM OF COTTONSEED HULLS ON MICROBIAL PROTEIN SYNTHESIS IN THE RUMEN OF STEERS FED HIGH CONCENTRATE DIETS

ABSTRACT

Six steers fitted with ruminal and duodenal cannulas were used in a replicated 3X3 Latin square. Ruminal digestion and efficiency of microbial protein synthesis were measured. Three corn based diets were formulated to contain the following percentages of cottonseed hulls (CSH) and effective neutral detergent fiber (eNDF): 1) 25% CSH and 14.1 % eNDF (CSH25) 2) 18% CSH (ground) and 6.1 % eNDF (CSH18g) and 3) 18% CSH and 10.4 % (CSH18). Mean ruminal pH values increased as eNDF content of the diet was incrased. Ruminal NH_3-N, peptide-N, and amino acid-N were similar among treatments averaging 96.4, 1.56, and 2.56 mg/L Efficiency of microbial protein synthesis in the rumen was unaffected by concentration of eNDF, and averaged 13.67 g of microbial N/kg of organic matter (OM) digested for all treatments. Increasing eNDF increased true ruminal OM and starch digestion in the rumen. Grinding CSH reduced ruminal starch digestion and total and entodiniomorph populations in the rumen. Reducing the eNDF content of the diet depressed pH less than expected and did not reduce efficiency of microbial growth to the degree proposed by NRC (1996).

(Key words: Effective neutral detergent fiber, Peptide-N, Microbial synthesis, Ruminal digestion, Protozoa.)

Introduction

The current beef NRC (1996) employs a model that uses effective neutral detergent fiber (eNDF) concentrations of dietary ingredients to predict ruminal pH that in turn is used to estimate fiber digestion and efficiency of microbial protein synthesis. Prediction equation predicts that microbial growth, and fiber digestion rate both decline linearly with pH as pH falls below 6.2. This equation is currently a component of the Cornell Net Carbohydrate and Protein model. Effective NDF is defined as the proportion of NDF that remains on a 1.18 mm screen after dry seiving and presumably represents that fraction of the diet that will stimulate rumination

Fine grinding and pelleting will reduce the eNDF level in forage diet and thereby would be expected to decrease the quantity of microbial N as well as the proportion of microbial N that reaches the duodenum. In most cases, net microbial production is unaffected or slightly decreased when ground and pelleted forages are fed although it has been increased in some studies (Rode et al., 1985) due to grinding.

The rumen sub-model for predicting the effect of eNDF on microbial growth efficiency in the beef NRC (1996) is based on continuous culture data. Extrapolating from chemostatic condition to the rumen condition can be erroneous because other than eNDF can influence ruminal pH, e.g., grain form, grain intake, starch digestion rate. The objective of this experiment was to determine the effect of form and dietary level of cottonseed hulls on ruminal digestion and efficiency of microbial protein synthesis in the rumen of steers fed high corn diets.

Materials and methods

Animals and treatments

The six steers (2 Hereford, 2 Angus and 2 Limousin) averaging 363 kg in weight were used in the experiment. Each was equipped with rumen fistula and duodenal re-entrant cannula in the duodenum proximal to the bile duct. The steers were randomly allotted to individual 3 X 5 meter stalls and had continuous free access to water. The steers were assigned to three dietary treatments in two identical 3 X 3 Latin squares. experiment. Dietary treatments included supplemented ground corn containing either a) ground corn + 18% cottonseed hulls plus supplements (CSH18) b) ground corn + 18% ground cottonseed hulls plus supplements (CSH18g) or c) ground corn + 25% cottonseed hulls plus supplements (CSH25). To ground cottonseed hulls (CSH) to include in the CSH18g diet, CSH were ground through 2 mm screen. Ingredients and chemical composition were analyzed and computed of each diet are shown in Table 1.

Diets were fed twice daily at 0800 and 1600 in equal portions. Feed dry matter was provided at a rate of 1.8 % of body weight daily. Chromium oxide (Cr) was used as a nonabsorbable marker for measurement of digesta flow. Chromium oxide (0.2 % of the total diet) was mixed with the supplements for all diets.

Sampling procedures

Before the start of the experiment, the steers were given the diet U for a period of three weeks for diet adaptation. Each experimental period lasted 21 d long, with 16 d for adjustment and 5 d for sampling. On day 17 through 19, approximately 250 ml of duodenal digesta and 200g of wet feces were collected at 2 and 8 h after feeding. On the d 20, approximately 1000 ml of strained rumen fluid were collected at 2 and 8 h after

feeding and frozen for later isolation of bacteria. On d 21 approximately 250 ml of rumen fluid were withdrawn at 1, 2, 4 and 8 h after feeding. A 0.5ml sub-sample of the rumen fluid from each sampling time was added to a formaldehyde/phosphate buffer/methyl green solution according to procedure developed by Department of Animal Sciences and Industry, Kansas State University and stored for later protozoa counting. The remaining samples were frozen and used later for ammonia and peptides analyses. All rumen fluid collected was strained through 4 layers of cheesecloth and the pH was measured immediately. Before freezing all rumen samples were acidified with 1ml of 20% v/v sulfuric acid per 50 ml strained fluid to stop microbial activity.

Feed samples were obtained prior to each sampling day and composited within each diet and period. All samples were ground in Wiley Mill fitted with a 2mm screen and stored for analysis.

Laboratory analyses and calculations

Feed, duodenal and fecal samples were analyzed for dry matter (DM), organic matter (OM), ash (AOAC, 1984), starch (Herrera-Saldana and Huber, 1989) and chromium (Cr) (Fenton and Fenton, 1979). The N content of feed, duodenal digesta, bacterial composites, and feces were analyzed by macro-Kjeldahl analysis (AOAC, 1984). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) in feed, duodenal and fecal samples were analyzed using procedures of Goering and Van Soest, 1970. Rumen NH_3-N was analyzed colorimetrically using a spectrophotometer (UV-VIS Spectrophotometer, Gilford-Respond Series, 1987) following the procedures of Broderick and Kang (1980). Protozoal numbers in the rumen fluid were counted using Sedgewick Rafter chamber. Bacteria were isolated from the ruminal fluid using the procedures of

Weakley and Owens (1983). Dried duodenal and bacterial samples were analyzed for nucleic acid-N by the procedure of Zinn and Owens (1986). To improve recovery of RNA pellets after precipitation with silver chloride, the RNA pellets were washed with a solution (100 ml) that consisted of 5ml solution containing 12.5 % $HCLO_4$ in .0285*M* $NH_4H_2PO_4$ + 5 ml of .4 *M* $AgNO_3$ + 90 ml of .2 *M* $NH_4H_2PO_4$. Rumen samples for peptide-N analysis were prepared using the procedures of Chen et al., (1987). Prehydrolyzed and hydrolyzed rumen fluid samples were analyzed colorimetrically at 570 nm using a spectrophotometer (UV-VIS Spectrophotometer, Gilford-Respond Series, 1987). The concentrations of ninhydrin reactive material in hydrolyzed and unhydrolyzed were measured and leucine was used as a standard (Moore and Stein, 1954; Moore, 1968). The concentration of peptide-associated α-amino N was calculated as the difference between the α-aminoN content of hydrolyzed and the unhydrolyzed samples. The concentrations of α-amino N in the hydrolyzed and unhydrolyzed were corrected for NH_3-N in samples and ninhydrin (by subtracting NH_3-N concentrations in the samples and ninhydrin from α-amino N concentrations in the hydrolysed and unhyrolysed samples).

Flows of DM at the duodenum were calculated by dividing daily Cr intake (grams) by Cr concentration (g/kg) in duodenal digesta. Nutrient flows were calculated by multiplying DM flow by the concentration of the given nutrient in duodenal DM. Bacterial N flow (g/d) at the duodenum was calculated by multiplying daily N flow at the duodenum by the proportion of bacterial N in the duodenal N. This proportion was

calculated by dividing the bacterial N:purine ratio of ruminal bacteria isolated from each steer in each period.

Daily amounts of non-microbial DM and N flowing past the duodenal cannula were calculated by subtracting the microbial contributions from the total. Bacterial DM was determined by oven drying the freeze-dried bacteria samples (ground) at 60 °C for 24 h Daily duodenal organic matter (OM) flow, corrected for microbial contributions, was calculated from the corrected duodenal DM flow X duodenal OM percentage.

Statistical analysis

Variables measured were analyzed as replicated 3X3 Latin square with animal (6), period (2) and dietary treatment (2) as factors (SAS Institute, Inc. 1988). Differences between treatments were determined using a multiple comparison test (PDIFF options of SAS Institute Inc. 1988). Statistical significance was considered to exist where $P<0.05$, whereas a trend was considered to exist if $0.05 \leq P \leq 0.10$. Simple correlations also were calculated across all observations. Contrast were utilized to make specific treatments comparison i.e., 1) between CSH18 and CSH18g 2) between CSH18 and CSH25. All values presented in tables are all least square means.

Results and Discussion

Feed DM intake for CSH25, CSH18g and CSH18 averaged 6.76, 6.51 and 6.51 kg/d. Mean ruminal pH, and concentrations of NH_3-N, peptide-N, amino acid-N and MOEFF are shown in Table 2. Ruminal pH was lower ($P<0.01$) for steers fed CSH18g diet than CSH18 diet but was higher ($P<0.06$) than for steers fed the CSH25 than CSH 18

diet. The lower level of fiber in diet CSH18 as compared to diet CSH25 appeared to lower rumen pH. Lower ruminal pH for steers fed ground CSH than non-ground CSH was due to the smaller particle size of ground CSH which presumably decreased salivary input during chewing and rumination. This reduction in pH would be expected to reduce activity of fiber degrading bacteria. Effects of sampling time on pH among treatments are shown in Figure 1. Cattle fed all diets had a lower pH at 1-2 h after feeding presumably due to rapid fermentation of starch to VFA, but after 4-6 h after feeding, the ruminal pH had rebounded. No differences were noted for rumen NH_3-N among diets; the mean NH_3-N concentration for all treatments was 9.64 mg/dL. This concentration is well above the value (5mg/dL) suggested by Satter and Slater (1974) for supporting maximal microbial growth. Reduction in percentage of eNDF (25.3%) (Table 1) in CSH18g diet did not affect the levels of rumen NH_3-N but tended to lower ruminal pH when compared to other diets with higher percentage of eNDF. The ruminal pH values for steers fed CSH18g was still above 6.0 probably because the percent of eNDF used in this experiment was slightly higher than the 20 % minimum value suggested by NRC Beef, (1996). Forms and level of CSH did not affect concentrations of peptide-N or amino acid-N in the rumen since all treatments received corn and urea as N source. The correlation coefficient between rumen peptide-N concentration and MOEFF is low and positve ($r = 0.27$; $P<0.48$). Ludden and Cecava (1995) in their studies using steers fed a high corn diet (81 % cracked corn) supplemented with urea (1.5 %) reported that the rumen peptide concentration was 14.6 mM that is ten fold than measured in this experiment. William and Cockburn (1991) reported a rumen peptide concentration of 2.4 mg/L for steers fed barley straw plus tapioca supplemented with casein.

Ruminal amino acid-N and peptide-N concentrations among treatments were similar averaging 2.56 and 1.56 mg/L respectively. Effect of sampling time on ruminal concentrations of peptide-N (Figure 2) and amino acid-N (Figure 3) showed that both of these N-compounds presumably had increased slightly after feeding because they were higher at 1 hr than later,: but decreased thereafter. This agrees with others (Chen et al., 1987; Broderick and Wallace, 1988; Williams and Cockburn, 1991) who reported that peptides accumulate in rumen fluid transiently after feeding, and thereafter their concentrations decline. Concentrations of peptide-N and amino acid-N were only 1 to 2% of the concentration of NH_3-N with all treatments; thus their contribution to the N supply of ruminal microbes in this study should be low. Efficiency of microbial protein synthesis (MOEFF) tended to be highest (14.5 g) for steers supplemented with CSH25 and to be lowest for steers fed with CSH18g (13.03 g) but the differences among treatments were not significant. Non- ground CSH diet, the diet that had higher percentage of eNDF tended to give a slightly higher MOEFF than the ground CSH diet that had less eNDF. Firkins et al., (1986) also detected no significant difference in MOEFF for steers fed ground versus non-ground hay. However Rode et al. (1985) with lactating cows fed ground and non-ground hay observed that MOEFF was higher in cow receiving ground hay; MOEFF in their study was directly related to ruminal solids turnover rate but inversely related to liquid dilution rate.

Table 3 shows the quantities of microbial N and non-ammonia non-microbial nitrogen (NANMN) entering duodenum. Although the amounts of N entering duodenum were not significantly different, steers fed CSH25 and CSH18 had values 5.6 % higher than steers fed diet CSH18g. Steers fed CSH18g had lower ($P<0.15$) N entering

duodenum than steers fed CSH18 suggesting that less N was utilized for microbial growth in the rumen. Supplementation with different physical forms and levels of CSH had very limited influence on microbial N and NANMN flow to duodenum. Average quantities of microbial N entering duodenum were 60.7, 50.4 and 56.7 g N/d for diets CSH25, CSH18 and CSH18g, respectively with microbial N flow for the non-ground CSH diet averaging 14.5% higher ($p<0.21$) than for the ground CSH diet. Feeding more CSH (25 vs 18%) also did not significantly ($P<0.47$) influence microbial N entering duodenum. Grams of NANMN flow to duodenum were a slightly bit higher for steers fed CSH18 than for steers fed CSH18g (54.1 vs. 53.6 g/d). This trend was similar to other reports on NANMN flow to duodenum in steers fed ground versus non-ground hay (Rode et al., 1985; Firkins et al., 1986). Adjusted ruminal N digestion and apparent total tract N digestibilities were similar between diets averaging 57.4 % and 75.2 % respectively indicating that level and form of CSH did not influence ruminal and post-ruminal N digestion.

Level of eNDF in the diet (25.3% for CSH18g vs 43.3% for CSH18) did not appear to affect N digestion and MOEFF in this experiment. Even though mean rumen pH in steers fed CSH18g was lower (6.11) than rumen pH of steers fed CSH18 (6.20) and CSH25 (6.31), MOEFF for CSH18g was similar to the other treatments. The in vitro data of Russell et al. (1992) and Pitt et al. (1996) indicated that when rumen pH fell below 6.2, microbial protein production decreased linearly with pH; this conflicts with our in vivo results. Ludden and Cecava (1995) studied the effect of feeding high corn diet (81 % cracked corn) supplemented with urea (1.5 %) reported that MOEFF was high (21 g N/kg true OM digested) even though ruminal pH (6.13) was low. A summary of two studies

(Weakly and Owens, 1983) involving steers fed corn diets demonstrated that efficiency of microbial synthesis was quite variable and not related to ruminal pH across a pH range from 5.8 to 6.7.

Organic matter intake (Table 4) was higher for steers fed CSH 25 than for steers fed either CSH18g or CSH18. Organic matter flows to duodenum were similar among diets averaging 2903 g/d. Apparent and true ruminal OM digestibilities percentages were higher ($P<0.02$, $P<0.13$) in the rumen of steers fed CSH18 than of steers fed CSH18g, but values were similar to those for steers fed CSH25. These results are similar to data from Lessard and Fisher (1980) who fed dairy cows long and ground alfalfa hay and showed that apparent and true ruminal OM digestibility was lower when ground forage was fed. Non-ground CSH were probably retained in the rumen longer for digestion than ground CSH although the greater surface area per g DM of ground CSH should have allowed more rapid colonization by ruminal microbes. Beever et al. (1981) also observed less OM digested in the rumen of sheep fed a pelleted than a ground grass. The correlation coefficient between ruminal organic matter digestibility and MOEFF in this experiment was low and not significant ($r = 0.14$, $P= 0.72$). Feeding a higher percentage of cottonseed hulls in the diet (25 vs 18%) increased ($P < .05$) fecal output and tended to decrease ($P < .08$) diet digestibility. Replacing corn by cottonseed hulls at a rate of 7% of diet dry matter increased adjusted duodenal OM flow by only 1.1% and fecal output by only .4%. If the ground corn had a digestibility of 88% (TDN from NRC, 1996), ruminal and total tract digestibility of added CSH were 82 and 93%, respectively. These values greatly exceed the NRC (1996) estimate of 42% TDN for cottonseed hulls. Similar values contrasting CSH25 with CSH18g revealed that CSH had true ruminal and total tract

digestibilities of 43 and 81%, respectively. These values suggest that added CSH had a positive associative effect on OM digestion with these diets. Post ruminal digestion of ground CSH compensated for two-thirds of the depression in ruminal digestion noted from grinding the cottonseed hulls.

Starch intakes were similar for all diets (Table 4). There was no significant differences ($P<0.12$) in starch duodenal flow attributable to grinding CSH or adding more CSH to the diet. However, ruminal starch digestibility tended to increase ($P < .08$) with addition of CSH to the diet and to be decreased ($P < .02$) by grinding of CSH. This may reflect increased chewing or rumination of grain with higher amounts or larger particle size of CSH. Total tract digestion values were similar among treatments averaging 95.2 %. The correlation coefficient between ruminal starch digestion and MOEFF was $r = 0.27$ ($P< 0.48$) but the correlation of ruminal starch digestion and dietary NDF and eNDF was considerably greater ($r = .86$; $r = .99$).

Intakes, duodenal flows, and fecal output of NDF and ADF (Table 5) all were higher with the CSH25 diet than the CSH18 diet because of higher DMI and the higher percentage of cottonseed hulls in the diet. Yet, ruminal and total tract ADF and NDF digestion percentages were not altered by level of cottonseed hulls in the diet. Grinding CSH tended to reduce ruminal and total tract digestibilities of NDF and ADF. Rode et al., (1985) studying the effect of hay particle size in cows and reported that digestibility of ADF was lower with ground than with non-ground hay.

Total numbers of protozoa (Table 6) were significantly lower ($P<0.003$) in the rumen of steers fed CSH18g than in the rumen of steers fed CSH18 largely due to an increase ($P < .01$) in the number of entodiniomorphs. Due again to a higher ($P < .09$)

population of entodiniomorphs, there was no different in protozoa numbers between treatment CSH18 and CSH25. Reducing particle size of CSH may increase particulate passage rate from the rumen. This would reduce particulate retention time in the rumen; long particulate retention time is considered essential for protozoal replication and survival in the rumen.. Reducing protozoa numbers were associated with a decreased feed particle size in a study reported by Christiansen et al. (1964). Small feed particle size also will promote an acidic rumen environment through reducing salvation; greater acidity is unfavorable for protozoal survival in the rumen. Feeding slightly higher percentage of fiber did not appear to stimulate protozoa growth in steers fed CSH25 diet because total protozoa numbers were slightly lower than with the CSH18 diet. The holotrich protozoa population in steers fed CSH18g was less than 10% that of steers fed CSH 18, but this difference was not statistically significant ($P<0.15$). Effects of sampling time on total protozoa numbers are shown in Figure 4.The number of protozoa increased after 2h after feeding and then remain stable thereafter with all treatments. This agrees with others (Abe et al., 1981; Bragg et al., 1986; Dehorty and Mattos, 1978; Leng et al., 1981) who reported that rumen fluid protozoa were more numerous immediately after feeding and thereafter their population stabilized.

Implications

Increasing the amount of cottonseed hulls in a corn based diet from 18 to25% by replacing ground corn grain increased ruminal pH and ruminal starch digestion but tended to decrease ruminal protozoa concentration and total tract organic matter digestibility. Grinding the cottonseed hulls fed at 18% of the diet, to decrease effective NDF concentration, tended to decrease ruminal pH and ruminal digestion of organic matter,

starch, and NDF and protozoal concentration in ruminal fluid. Increasing the effective NDF content of the diet from 6 to 10 and 14% of diet dry matter increased ruminal pH and ruminal and total tract starch digestibility but had little effect on NDF or ADF digestion or efficiency of microbial growth in the rumen.

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Table 1. Composition of diets in experiment 1.

	Diets		
	CSH25[2]	CSH18g[1]	CSH18[2]
	% of dry matter		
Ground corn	67.4	74.4	74.4
Molasses	3.59	3.59	3.59
Chromic oxide	0.2	0.2	0.2
Supplement			
Cottonseed hulls	25.0	18.0	18.0
Urea	1.4	1.4	1.4
Dicalcium phosphate	1.4	1.4	1.4
Limestone, 38%	0.7	0.7	0.7
Trace mineralized salt	0.3	0.3	0.3
Vitamin A	0.01	0.01	0.01
Crude protein, %	11.7	12.1	12.1
Starch, %	50.3	52.3	52.3
NDF, %	29.5	24.1	24.1
eNDF (% of NDF)[3]	47.9	25.3	43.3
eNDF (% of diet D.M)			
ADF, %	18.3	13.9	13.9

[1]CSH ground through 2-mm screen

[2]CSH non-ground

[3]Calculated from data of Fox et al. 1990

Table 2. Mean rumen pH, concentrations of nitrogen-containing compounds and microbial synthesis in the rumen of steers fed a ground corn based diet supplemented with different forms and levels of cottonseed hulls.

	Diets				Contrast	
Item	CSH18[2]	CSH18g[1]	CSH25[2]	SEM	CSH18g[1] vs. CSH18[2]	CSH18[2] vs. CSH25[2]
pH	6.20[a]	6.11[b]	6.31[a]	0.02	0.01	0.06
pH predicted from eNDF:						
Pitt et al., 1996	6.00	5.69	5.85			
NRC, 1996	6.02	5.68	5.86			
NH_3-N, mg/dL	9.53	10.00	9.40	0.41	0.41	0.86
Amino acid-N,mg/L[3]	2.43	2.36	2.39	0.01	0.16	0.37
Peptide-N,mg/L[4]	1.59	1.53	1.57	0.02	0.37	0.20
Microbial efficiency[5]	13.50	13.03	14.47	0.37	0.47	0.13
Predicted MOEFF, % of maximum on eNDF:						
Russell et al.1992	87	69	79			
NRC, 1996	85	65	76			
Predicted MOEFF, % of maximum on pH:						
Pitt et al.1996	91	85	87			

[ab] Means in a same row with different superscripts differ significantly ($p<.05$)

[1] CSH ground through 2-mm screen

[2] CSH non-ground

[3] prehydrolysed fluid

[4] hydrolysed fluid

[5] g microbial N per kg OM fermented

Table 3. Nitrogen (N) digestion in steers fed a ground corn based diet supplemented with different forms and levels of cottonseed hulls.

Item	Diets			SEM	Contrast	
	CSH18[2]	CSH18g[1]	CSH25[2]		CSH18g[1] vs. CSH18[2]	CSH18[2] vs. CSH25[2]
Nitrogen intake, g/d	125.5	125.5	126.6	_	_	_
Entering duodenum, g/d	117.6	112.0	119.7	1.8	0.08	0.15
Microbial N, g/d	56.7	50.4	60.7	3.1	0.27	0.14
NANMN, g/d[3]	54.1	53.6	52.9	4.4	0.52	0.72
Ruminal digestion, %						
Unadjusted	6.3[a]	10.8[b]	5.6[a]	0.1	0.01	0.01
Adjusted[4]	56.9	57.4	57.8	0.4	0.49	0.52
Fecal N, g/d	31.0	32.1	30.8	1.4	0.57	0.63
ATT digestion, %[5]	75.5	74.3	75.7	1.1	0.48	0.55

[ab] Means in a same row with different superscripts differ significantly ($p<.05$)

[1] CSH ground through 2-mm screen

[2] CSH non-ground

[3] Non-ammonia non-microbial nitrogen

[4] Adjusted for microbial and ammonia nitrogen

[5] Apparent Total Tract Digestibility

Table 4. Organic matter (OM) and starch digestion by steers fed a ground corn based diet supplemented with different forms and levels of cottonseed hulls.

Item	Diets				Contrast	
	CSH18[2]	CSH18g[1]	CSH25[2]	SEM	CSH18g[1] vs. CSH18[2]	CSH18[2] vs. CSH25[2]
OM intake, g/d	6209.0	6209.0	6433.0	_	_	_
OM flow at duodenum, g/d	2852.4	2969.3	2889.0	41.9	0.19	0.60
Ruminal digestion,						
Apparent, g/d	3356.6[a]	3239.7[ab]	3544.0[a]	36.6	0.08	0.10
% of intake	54.4[a]	51.1[b]	55.3[a]	0.4	0.02	0.33
True, g/d	3956.3[a]	3734.3[ab]	4143.8[a]	65.4	0.09	0.11
% of intake[3]	63.1	60.7	64.2	0.8	0.08	0.13
Fecal OM, g/d	1291.5[a]	1390.8[b]	1361.5[b]	19.0	0.14	0.05
ATTOMD, %[4]	79.2	77.6	78.8	0.3	0.47	0.13
Starch intake, g/d	3411.0	3411.2	3406.0	_		
Flow at duodenum, g/d	1510.8[ab]	1577.0[b]	1446.7[a]	17.9	0.12	0.13
Ruminal digestion,						
Apparent, g/d	1900.2[a]	1834.2[b]	1959.3[a]	9.8	0.01	0.02
% of intake	55.7[a]	53.8[b]	57.5[a]	0.5	0.04	0.11
Fecal starch, g/d	143.0	186.1	144.4	16.4	0.21	0.21
ATTSD, %[5]	95.3	94.5	95.8	0.3	0.28	0.41

[ab] Means in a same row with different superscripts differ significantly ($p<.05$)

[1] CSH ground through 2-mm screen

[2] CSH non-ground

[3] Adjusted for microbial OM

[4] Apparent Total Tract OM digestibility

[5] Apparent Total Tract Starch digestibility

Table 5. NDF and ADF digestion in steers fed a ground corn-based diet supplemented with different forms and level of cottonseed hulls.

Item	Diets			SEM	Contrast	
	CSH18[2]	CSH18g[1]	CSH25[2]		CSH18g[1] vs. CSH18[2]	CSH18[2] vs. CSH25[2]
NDF intake, g/d	1569.0	1569.0	1993.0	_	_	_
Entering duodenum, g/d	790.0[a]	827.3[a]	981.6[b]	10.0	0.12	0.01
Ruminal digestion, g/d	779.0[a]	741.7[a]	1011.4[b]	9.6	0.11	0.002
As % of intake	49.6[ab]	47.3[b]	50.7[a]	0.5	0.04	0.09
Fecal NDF, g/d	752.6[a]	787.2[a]	945.7[b]	10.5	0.14	0.01
ATTNDFD, %[3]	52.0	49.8	52.5	0.6	0.08	0.12
Expected digestion, % of maximum based on pH[4]	95.8	88.0	92.5			
ADF intake, g/d	905.0	905.0	1237.0	_	_	_
Entering duodenum, g/d	489.6[a]	499.3[a]	663.8[b]	11.2	0.57	0.01
Ruminal digestion, g/d	415.4[a]	407.7[a]	573.2[b]	11.1	0.55	0.01
As % of intake	45.9	45.0	46.3	1.08	0.51	0.42
Fecal NDF, g/d	466.6[a]	478.7[a]	645.1[b]	11.2	0.40	0.01
ATTNDFD, %[5]	48.4	47.1	47.8	1.1	0.29	0.35

[ab] Means in a same row with different superscripts differ significantly ($p<.05$)

[1] CSH ground through 2-mm screen

[2] CSH non-ground

[3] Apparent Total Tract NDF Digestibility

[4] Pitt et al. 1996

[5] Apparent Total Tract ADF Digestibility

Table 6. Mean ruminal protozoa numbers in steers fed a ground corn based supplemented with different forms and levels of cottonseed hulls.

Item	Diets			SEM	Contrast	
	CSH18[2]	CSH18g[1]	CSH25[2]		CSH18g[1] vs. CSH18[2]	CSH18[2] vs. CSH25[2]
Entodiniomorph	55.88[a]	49.62[b]	53.78[a]	0.48	0.03	0.60
Holotrich	0.92	0.08	1.32	0.26	0.08	0.15
Total	55.70[b]	49.60[a]	55.10[a]	0.29	0.003	0.58

[ab] Means in a same row with different superscripts differ significantly ($p<.05$)

[1] CSH ground through 2-mm screen

[2] CSH non-ground

Figure 1. Effect of sampling time on rumen pH in steers fed corn based diet supplemented with different forms and levels of cottonseed hulls.

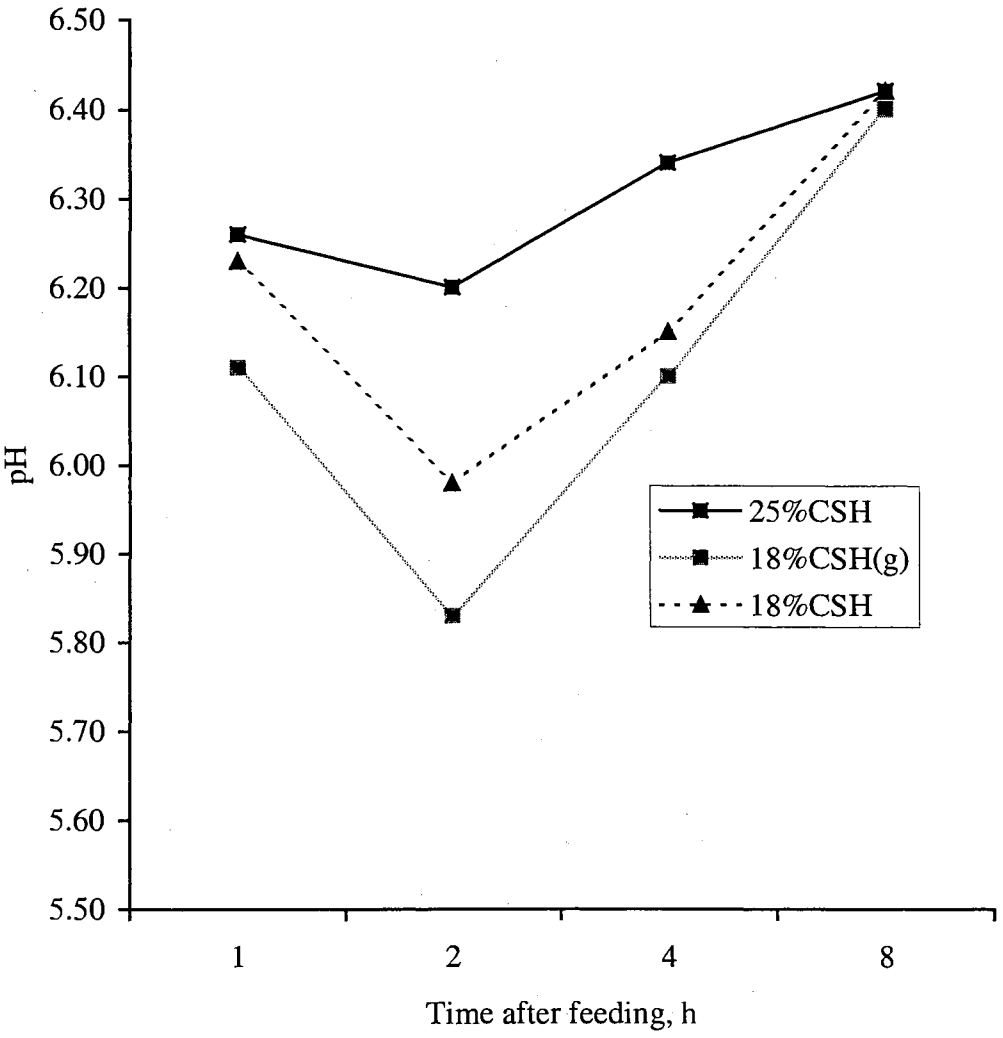


Figure 2. Effect of sampling time on concentrations of peptide-N in steers fed corn based diet supplemented with different forms and levels of cottonseed hulls.

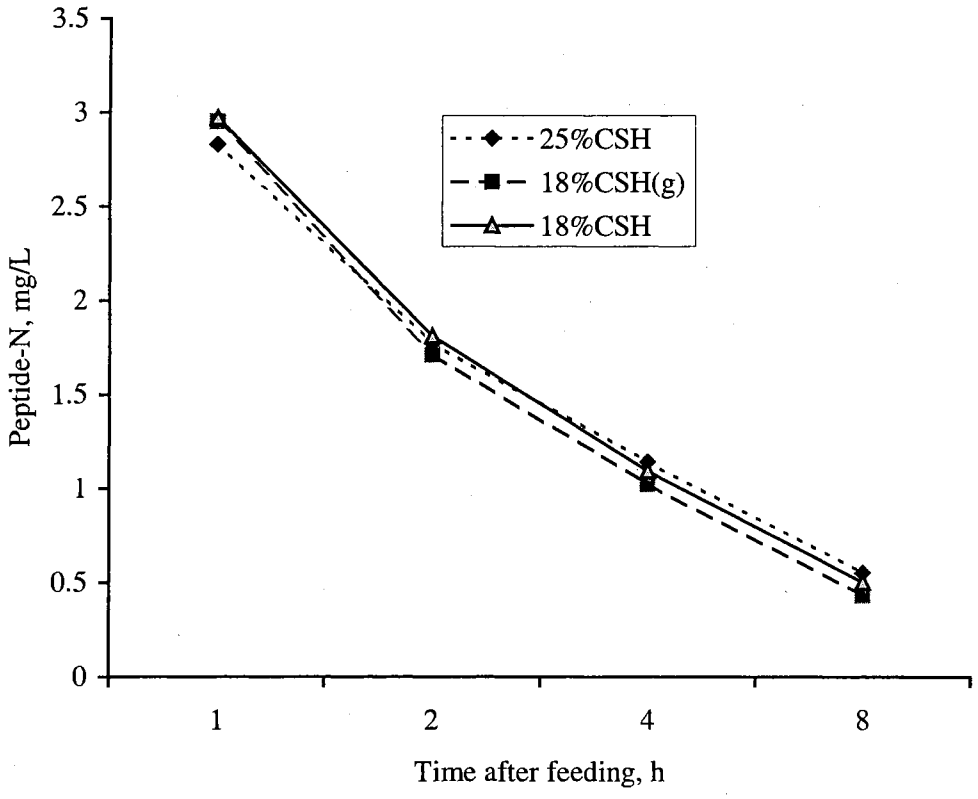


Figure 3. Effect of sampling time on concentrations of amino acids-N in steers fed corn based diet supplemented with different forms and levels of cottonseed hulls.

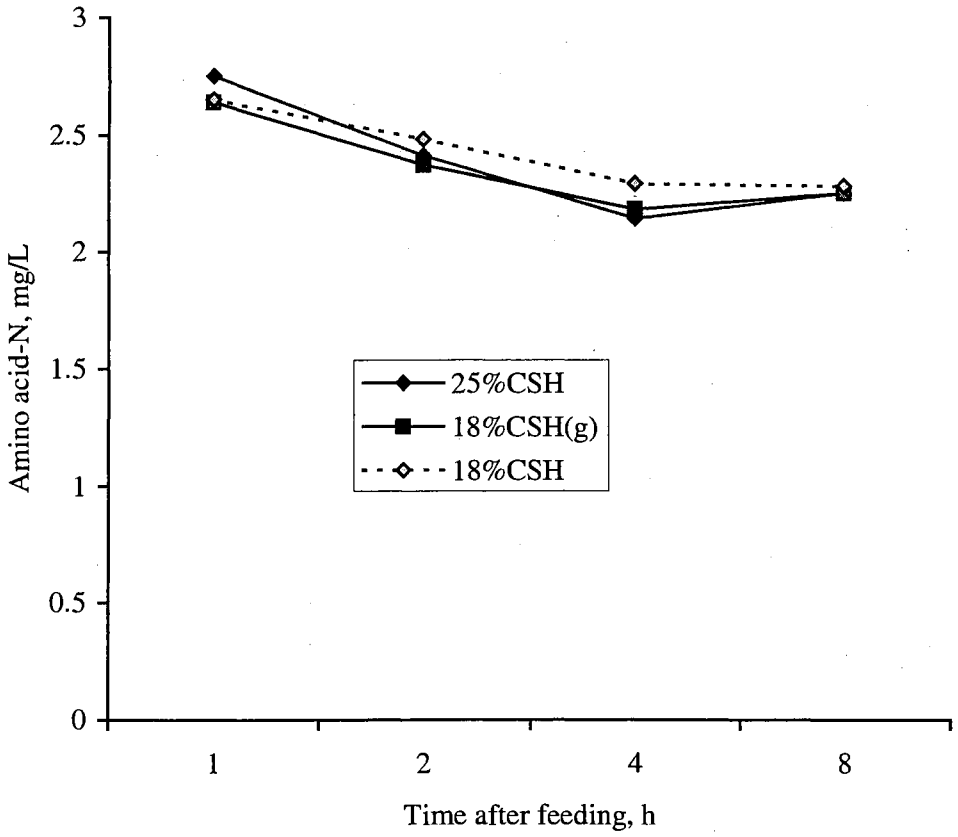
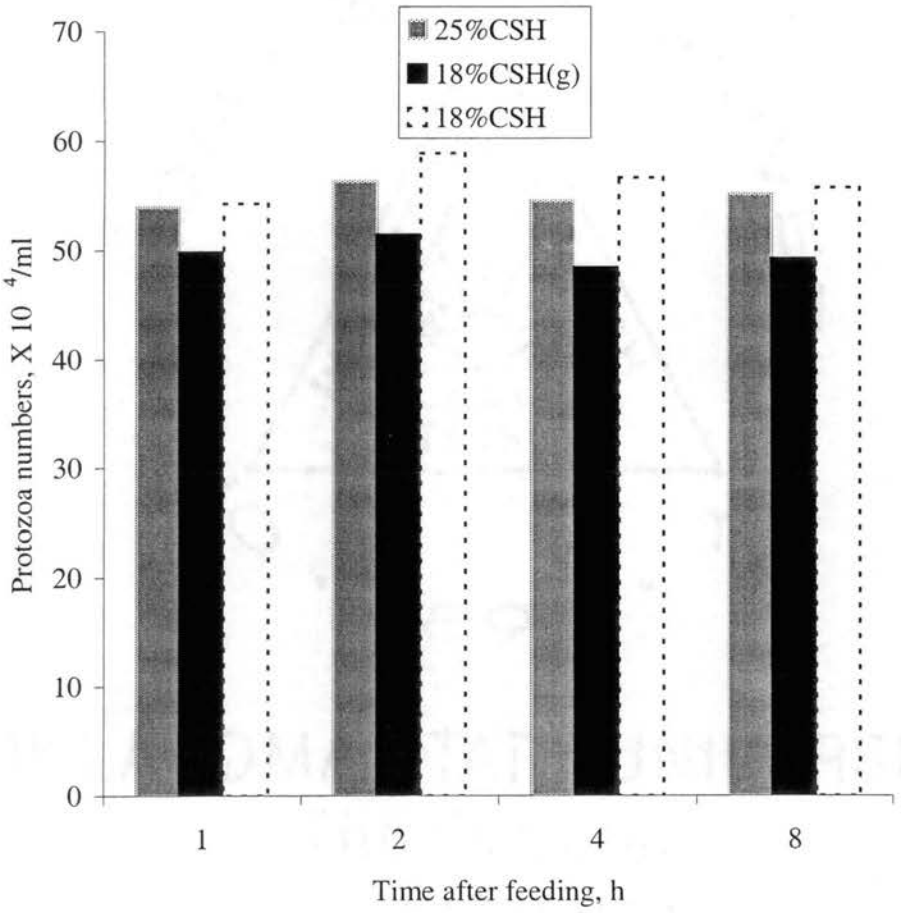


Figure 4. Effect of sampling time on ruminal protozoa numbers in steers fed corn based diet supplemented with different forms and levels of cottonseed hulls.



CHAPTER IV

INFLUENCE OF NITROGEN/PEPTIDE SOURCES ON MICROBIAL PROTEIN SYNTHESIS IN THE RUMEN OF STEERS FED HIGH CONCENTRATE DIETS

ABSTRACT

Six steers fitted with rumen and duodenal cannulas were used in a replicated 3X3 Latin square to determine the effects of peptides on ruminal digestion and efficiency of microbial protein synthesis. Steers were fed corn-based diets supplemented with three different source of nitrogen. The three treatment diets consisted of 1) corn + casein (CC) 2) corn + soybean meal (CSBM) and 3) corn + urea (CU). Mean ruminal NH_3-N concentrations for CC, CSBM and CU diets were 7.13, 5.93 and 9.27 mg/dL respectively. Steers supplemented with SBM had higher ruminal peptide-N concentration ($P<0.02$) and amino acid-N concentration ($P<0.03$) than steers supplemented with casein. Mean ruminal concentrations of peptide-N for CC, CSBM and CU diets were 56.67, 75.31 and 1.66 mg/L respectively. Efficiency of microbial protein synthesis was not altered by ruminal peptide-N concentrations and averaged 13.89 g of microbial N/kg of organic matter (OM) digested for all treatments. True ruminal digestion of OM averaged 66.5, 67.0 and 64.2 % for diets CC, CSBM and CU respectively, resulting in duodenal microbial flows of 65.0, 67.2 59.0 g/d. Ruminal starch, ADF and ADF digestion were greater in steers supplemented with SBM than in steers supplemented with casein or urea. Concentrations of Total protozoa numbers in ruminal samples were similar among treatments though the holotrich population tended to be greater with higher ruminal

treatments though the holotrich population tended to be greater with higher ruminal peptide concentrations. Efficiency of microbial protein synthesis was not increased by feeding diets that increased the concentration of peptides in the rumen. .

(Key words: Peptide-N, Microbial protein, Ruminal digestion , Protozoa.)

Introduction

Ammonia, a central intermediate in the degradation and assimilation of dietary N in the rumen, is required by many species of bacteria (Hungate, 1966; Nolan, 1975). However, Most species of rumen bacteria will grow with ammonia as their sole source of N provided that certain volatile fatty acids (VFA) are present as amino acids (AA) precursors (Bryant and Robinson, 1962; Bryant 1973). Indeed ruminants can survive for a prolonged period with urea as N; the sole source in the diet (Virtanen, 1966). When grow in vitro in mixed cultures, microbial growth often is greater when additional dietary N is provided in the form of peptides or amino acids (Maeng and Baldwin, 1976; Maeng et al., 1976; Cotta and Russell, 1982; Merry et al., 1990; McAllan, 1991). With in vitro studies, it is difficult to distinguish whether the benefit from an increased supply of preformed amino acids in the diet is due to effects on the rumen microorganisms or to increased escape of some dietary protein to the abomasum (McAllan, 1991).

Because peptides and amino acids stimulate the growth of rumen microorganisms grown in vitro, and they are often considered essential for optimal growth rates of many bacteria species growing on rapidly fermented substrates; in a rich medium, bacteria may prefer preformed amino acids as a source of N (or as source of carbon, too) for using

amino acid N for synthesis of more than 90 % of their amino acids. Cellulolytic species are an exception in this respect, but still about half of their cell N in vitro is derived from N obtained from preformed amino acids. However, the extent to which bacteria use ammonia vs. peptides and amino acids for protein synthesis also depends on the concentrations of each; consequently, preformed amino acids and peptides probably are may be used to a lesser extent in vivo than these in vitro experiments would suggest.

Cruz Soto et al. (1994) presented evidence that suggests that bacterial stimulation by peptides and amino acids does not always occur. However, Peptides and amino acids might be expected to benefit even cellulolytic rumen bacteria, when such bacteria are grown on cellobiose, instead of cellulose. Subsequent experiments (Chikunya et al., 1996), in which peptides were supplied with diets containing either rapidly or slowly degraded fiber, suggested that the benefit of peptides would be evident if only when the energy source supported a very rapid growth rate. Results from these studies indicate that the in vivo situation is much more complicated than the simplistic assumptions about the effect of peptides and amino acids on microbial growth efficiency in that form part of the Cornell Net Carbohydrate and Protein Model.

The objective of this experiment was to determine the effect of peptides on ruminal digestion and microbial protein synthesis in the rumen steers fed corn-based diets.

Materials and methods

Animals and treatments

The same six steers (402 kg) used in experiment 1 were used to measure the influence of different N sources on efficiency of microbial protein synthesis in the

rumen.in this experiment .The steers were randomly allotted to individual 3 X 5 meter stalls and had free access to water. The steers were assigned to three dietary treatments in a replicated 3 X 3 Latin square experiment. Treatment diets consisted of supplemented ground corn diet containing 18% cottonseed hulls; N was added from either a) casein (C) b) soybean meal (SBM) or c) urea (U). Ingredients and chemical composition of each diet are shown in Table 1. Diets were fed twice daily at 0800 and 1600 in equal portions. Feed dry matter was provided at a rate of 1.8 % of body weight daily.

Chromium sesquioxide (Cr2O3.1.5H2O) was used as a nonabsorbable marker for measurement of digesta flow. Chromium sesquioxide (0.2 % of the total diet) was mixed with the supplement for each diet.

Sampling procedures

Before the start of the experiment, all steers were fed diet U for a period of three weeks for diet adaptation. Each experimental period lasted 21 d, with 16 d for adjustment and 5 d for sampling. On day 17 through 19, approximately 250 ml of duodenal digesta and 200g of wet feces were collected at 2 and 8 h after feeding. On d 20, approximately 1000 ml of strained rumen fluid were collected at 2 and 8 h after feeding and frozen for later isolation of bacteria. On d 21 approximately 250 ml of rumen fluid were withdrawn at 1, 2, 4 and 8 h after feeding. A .5ml sub-sample of the rumen fluid from each sampling time was added to a formaldehyde/phosphate buffer/methyl green solution according to a procedure developed by Department of Animal Sciences and Industry, Kansas State University and stored for later protozoa counting. The remainder of each sample was frozen and used later for ammonia and peptides analyses. All rumen fluid collected was strained through 4 layers of cheesecloth and the pH was measured immediately. Before

freezing, each rumen sample was acidified with 1ml of 20% v/v sulfuric acid per 50 ml strained fluid to stop microbial activity.

Feed samples were obtained prior to each sampling day and composited within each diet and period. All samples were ground through a Wiley Mill fitted with a 2mm screen and stored for analysis.

Laboratory analyses and calculations

Feed, duodenal and fecal samples were analyzed for dry matter (DM), organic matter (OM), ash (AOAC, 1984), starch (Herrera-Saldana and Huber, 1989) and chromium (Cr) (Fenton and Fenton, 1979). The N content of feed, duodenal digesta, bacterial composites, and feces was analyzed by macro-Kjeldahl analysis (AOAC, 1984). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) in feed, duodenal and fecal samples were analyzed using procedures of Goering and Van Soest, (1970). Rumen NH_3-N was analyzed colorimetrically using a spectrophotometer (UV-VIS Spectrophotometer, Gilford-Respond Series, 1987) following the procedures of Broderick and Kang (1980). Protozoal numbers in the rumen fluid were counted using a Sedgewick Rafter chamber. Bacteria were isolated from the ruminal fluid using the procedures of Weakley and Owens (1983). Dried duodenal and bacterial samples were analyzed for nucleic acid-N by the procedure of Zinn and Owens (1986). To improve recovery of RNA pellets after precipitation with silver chloride, the RNA pellets were wash with a solution (100 ml) that consisted of 5ml solution containing 12.5 % $HCLO_4$ in .0285M $NH_4H_2PO_4$ + 5 ml of .4 M $AgNO_3$ + 90 ml of .2 M $NH_4H_2PO_4$. Rumen samples for peptide-N analysis were prepared using the procedures of Chen et al., (1987). Prehydrolyzed and hydrolyzed rumen fluid samples were analyzed colorimetrically at 570 nm using a spectrophotometer

(UV-VIS Spectrophotometer, Gilford-Respond Series, 1987). The concentrations of ninhydrin reactive material in hydrolyzed and unhydrolyzed were measured and leucine was used as a standard (Moore and Stein, 1954; Moore, 1968). The concentration of peptide-associated α-amino N was calculated as the difference between the α-aminoN content of each hydrolyzed and its corresponding unhydrolyzed samples. The concentrations of α-amino N in the hydrolyzed and unhydrolyzed were corrected for NH_3-N in samples and ninhydrin (by subtracting NH_3-N concentrations in the samples and ninhydrin from α-amino N concentrations in the hydrolysed and unhyrolysed samples).

Flows of DM at the duodenum were calculated by dividing daily Cr intake (grams) by Cr concentration (g/kg) in duodenal digesta. Nutrient flows were calculated by multiplying DM flow by the concentration of the given nutrient in duodenal DM. Bacterial N flow (g/d) at the duodenum was calculated by multiplying daily N flow at the duodenum by the proportion of bacterial N in the duodenal N. This proportion was calculated by dividing the bacterial N:purine ratio of duodenal digesta by the ratio found for ruminal bacteria isolated from each steer in each period.

Daily amounts of non-microbial DM and N flowing past the duodenal cannula were calculated by subtracting the microbial contributions from the total. Bacterial DM was determined by oven drying the freeze-dried bacteria samples (ground) at 60 °C for 24 h. As a percentage of DM, these samples ranged from 7.9 to 9.1% N. Daily duodenal organic matter (OM) flow, corrected for microbial contributions, was calculated from the corrected duodenal DM flow X duodenal OM percentage.

Statistical analysis

Variables measured were analyzed as replicated 3X3 Latin square with animal (6), period (2) and dietary treatment (2) as factors (SAS Institute, Inc. 1988). Differences between treatments were determined using a multiple comparison test (PDIFF options of SAS Institute Inc. 1988). Statistical significance was considered to exist where $P<0.05$, whereas a trend was considered to exist if $0.05 \leq P \leq 0.10$. Simple correlations also were calculated across all observations. Orthogonal contrasts were utilized to make specific treatments comparison, i.e., 1) between urea and intact protein sources and 2) between the two protein sources. All values presented in tables are all least squares means.

Results and Discussion

Source of N supplementation of the diet casein, SBM or urea had no significant effect on rumen pH. The mean pH values for C, SBM and U diets were 6.26, 6.27 and 6.24 respectively (Table 2). Effect of sampling time on rumen pH for all treatments are shown in Figure 1. Rumen pH declined from 1 h to 3 h after feeding but then increased until 8 h after feeding with all diets. Ruminal NH_3-N concentrations were significantly lower ($P<0.02$) for SBM and C than for U but no significant difference ($P<0.12$) was detected between SBM and C. Mean ruminal NH_3-N concentrations for C, SBM and U were 7.13, 5.93 and 9.27 mg/dL, respectively (Table 2). Concentrations of NH_3-N at 8 h were below the 5 mg/dL concentration that was recommended by Satter and Slater (1974) for optimum microbial synthesis in vitro. Kang-Merznarich and Broderick (1981) suggested that ruminal NH_3-N concentrations between 3.3 and 8.5 mg/dL were required

for maximal microbial growth when diets contain 74 % corn grain. Supplementing SBM as a protein source resulted in the lowest NH_3-N concentrations; but despite the lower NH3-N concentration, SBM resulted in the numerically highest microbial N flows to the duodenum, and a higher microbial efficiency when compared with urea and casein. According to Cotta and Russell (1982), Chen et al., (1987), Williams and Cockburn (1991) when ruminal ammonia concentrations are low, ruminal microbes may preferentially utilize soluble amino acids and peptides as N source, so these relatively low ruminal ammonia concentrations should enhance any potential benefits from non-ammonia sources of N.

Mean ruminal peptide-N and amino acid-N concentrations are shown in Table 2. Mean concentrations of peptide-N and amino acid-N concentrations were significantly higher ($P<0.004$, $P<0.003$) in ruminal samples from steers supplemented with SBM than in samples from steers receiving either casein or urea. Concentrations of peptide-N in the rumen averaged 45 and 34 times greater ($P < .04$) for steers fed SBM or casein than for steers fed urea. However, temporal patterns differed with N source. For steers supplemented with SBM, the ruminal peptide-N concentration peaked at 2 h after feeding (Figure 2) but for steers fed casein, peptide-N concentrations peaked at or before 1 h after feeding. Amino acid-N concentrations peaked at 1 hour after feeding for both SBM and casein treatments. Concentrations of peptide-N and amino acid-N both declined rapidly after peaking. This trend is similar to that reported by other workers (Chen et al., 1987; Wallace and McKain, 1990; Kim et al., 1998; William and Cockburn, 1991). However, According to William and Cockburn (1991) accumulation of peptide in rumen contents

does not appear to be related to either the rate or the extent of degradation of the protein supplement.

Although supplementing diets with SBM or casein was sufficient to markedly increase ruminal peptide-N and amino acid-N concentrations, it failed to increase ($P>0.39$) efficiency of microbial growth. The correlation coefficient between mean peptide-N in ruminal fluid and MOEFF was not significant ($r = 0.39$, $P= 0.71$). In vitro data, from Maeng and Baldwin (1976), Argyle and Baldwin (1989), Fujimaki et al., (1992), Kaur et al., (1992), Russell et al., (1983) and Grisswold et al., (1995) all have demonstrated that preformed amino acids stimulate microbial growth. In vivo data, from Hume (1970), McAllan and Smith (1984) and Rooke et al., (1987) also suggested that bacterial protein synthesis was stimulated by peptides and amino acids. In contrast, Cruz Soto et al., (1994) infused urea, amino acids and peptides into the rumen of sheep fed hay diet and reported that microbial protein flow to duodenum was unaffected by concentrations of either peptides or amino acids in the rumen. They concluded that fermentation by rumen bacteria is not limited by availability of peptides or amino acids when ammonia is available in sufficient quantities and bacterial growth rate is limited by a slowly degradable energy source. Even though this statement might suggest that rumen microbial growth should responds only when a concentrate energy source is provided, results from our experiment found no benefit in MOEFF even when concentrate was provided in the diet. With concentrate diets, the microbial population of the rumen increases so that the supply of readily available soluble energy in the rumen still remains limited.

Intake of N, duodenal N flow, ruminal N digestion, NANMN and apparent total tract N digestibility was not significantly different among diets (Table 3). Supplementation with SBM and casein tended to increase microbial N and NANMN flow but the difference between them was not significant ($P<0.08$). Mean microbial N entering duodenum averaged 65.0, 67.2 and 59.0 g/d for treatments CC, CSBM and CU respectively. Steers receiving SBM had 7.1 % more microbial N flow to duodenum than steers receiving urea. Fecal N excretion was greater for steers receiving urea or casein than for steers receiving SBM ($P<0.02$, $P<0.04$) but total tract N digestibilities were similar among treatments which suggest that the fecal N in the feces was not being derived from the diets.

Means for OM intake, OM flow to duodenum, OM digestibility, starch intake and digestibility are shown Table 4. Organic matter and starch intakes were slightly higher for steers supplemented with SBM than with casein or urea. True ruminal OM digestion was significantly greater ($P<0.01$) for steers fed either SBM or casein than for steers fed urea, but there was no difference ($P<0.23$) between steers fed the two different sources of supplemental intact protein. True OM digestibility for diets C, SBM and U averaged 66.5, 67.0 and 64.2 % respectively. The increases in true ruminal OM digestibility that occurred when SBM and casein were supplemented suggest that the diet contained enough N for maximal ruminal digestion, but they might be interpreted to indicate that the additional amino acids or peptide-N may have increased microbial digestion activity in the rumen. Apparent total tract OM digestibility was not affected by source of dietary N.

Duodenal starch flow was lower ($P<0.08$) for steers fed the C or SBM diet than for steers fed the urea diet (Table 4). Ruminal starch digestion also tended to be higher ($P<0.1$) with the SBM diet than with the C diet. Fecal starch and apparent total tract starch digestibility values were similar for all diets, with means of between 143.7 g/d and 95.9 %. It is not possible to determine from this data what proportion of the dietary starch that disappeared past the duodenum, about 40% of dietary starch, was digested in the small intestine and what portion was fermented in the large intestine.

Table 5 shows NDF and ADF intake, ruminal digestion and apparent total tract digestion. As percentage of total tract NDF and ADF digestion, 94 to 96 % of NDF and 91 to 93% of ADF was fermented in the rumen; these values are quite similar to those reported by other researchers. Intakes of NDF and ADF were higher with the SBM supplemented diet than casein or urea due to replacement of some grain by SBM. Ruminal NDF and ADF flow to duodenum were significantly higher ($P<0.02$) for the SBM diet than for the C diet. Ruminal NDF and ADF digestion also were significantly higher with the SBM diet than either casein ($P<0.003$) or urea ($P<0.01$) diets. The correlation coefficient between peptide and ruminal NDF digestion was significant ($P<0.02$, $r = 0.75$) but might be due to the increased proportion of dietary NDF being derived from SBM in the diet that produced the highest mean ruminal peptide-N concentration. Apparent total tract NDF digestion also was higher for steers fed the SBM diet than for steers fed the casein ($P<0.01$) or urea ($P<0.002$) diets. The ADF duodenal flow and ruminal digestion were significantly higher ($P<0.005$, $P<0.004$) from SBM diet than the casein diet and higher ($P<0.002$) with the SBM or casein than the urea diet. The correlation between peptide-N and ruminal ADF digestion was significant ($P<0.02$, $r =$

0.79). Whether these alterations in digestion of the fiber components are due to addition of NDF from the added SBM or to stimulation of fiber digestion by SBM is not certain. Even though the percentage digestibilities often were significantly greater with the SBM diet, total duodenal flow and fecal output of both ADF and NDF remained numerically greater with the SBM diet. This could be interpreted to suggest that the increases in fiber digestibility on are due to alterations in dietary fiber source, and not due to increased digestibility of dietary fiber derived from other ingredients.

Although the concentration of total protozoa in ruminal samples from steers fed various diets was similar (Table 6), holotrich protozoa numbers were significantly higher in ruminal samples from steers fed the SBM diet than steers fed either the casein ($P<0.05$) or the urea diet ($P<0.007$). Steers supplemented with the urea diet had the lowest concentration of holotrich protozoa. This might be interpreted to suggest that the holotrich population might be enhanced by increasing the ruminal peptide concentration. Total protozoa numbers varied with time of sampling (Figure 4) with the population peak occurring 2 h after feeding and a decline thereafter with all diets. The correlations between MOEFF and the entodiniomorph, holotrich and total concentration of protozoa in the was low and insignificant ($P<0.36$, $r = .34$; $P<0.43$, $r = .25$; $P<40$, $r = .28$) though all were positive. If the protozoa engulf and digest ruminal bacteria, one would expect protozoal presence to decrease MOEFF as suggested by Leng et al 1981.

Implications

Concentrate diets supplemented with different nitrogen (N) sources (casein, soybean meal, urea) produced markedly different concentrations of peptides in ruminal

samples. Diets producing higher ruminal peptide and amino acid concentrations tended to increase ruminal digestion of starch and organic matter and to increase duodenal flow of bacterial N slightly. But efficiency of microbial protein synthesis in the rumen, calculated as grams of bacterial N per kg of organic matter fermented in the rumen, was not increased when ruminal peptide and amino acid concentrations were elevated. These results suggest that ruminal peptide concentrations do not alter efficiency of microbial growth in vivo as was proposed by NRC (1996) based on in vitro experiments.

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Table 1. Composition of diets in experiment 2.

	Diets		
	Casein	SBM	Urea
	% of dry matter		
Ground corn	71.2	66.4	74.4
Cottonseed hulls	18.0	18.0	18.0
Molasses	3.59	3.59	3.59
Supplement			
Urea	–	–	1.4
SBM	–	9.4	–
Casein	4.6	–	–
Dicalcium phosphate	1.4	1.4	1.4
Limestone, 38%	0.7	0.7	0.7
Trace mineralized salt	0.3	0.3	0.3
Vitamin A	0.01	0.01	0.0
Chromic oxide	0.2	0.2	0.2
Crude protein, %	11.7	11.5	12.1
Starch, %	50.3	52.3	52.4

Table 2. Mean ruminal pH, concentrations of nitrogen-containing compounds and microbial protein synthesis in rumen of steers fed a ground corn-based diet diet supplemented with isonitrogenous amounts of either urea, casein or SBM.

	Diets				Contrast	
Item	Casein	SBM	Urea	SEM	Urea vs. Others	SBM vs. Casein
pH	6.26	6.27	6.24	0.03	0.41	0.64
NH_3-N, mg/dL	7.13[a]	5.93[a]	9.27[b]	0.33	0.02	0.12
Amino acid-N,mg/L[1]	4.13[a]	5.06[b]	2.40[c]	0.11	0.004	0.03
Peptide-N,mg/L[2]	56.67[a]	75.31[b]	1.66[c]	2.64	0.003	0.04
Microbial efficiency[3]	13.73	14.27	13.67	0.25	0.39	0.27

[abc]Means in a same row with different superscripts differ significantly ($p<.05$)

[1] prehydrolysed fluid

[2] hydrolysed fluid

[3] g microbial N per kg OM fermented

Table 3. Nitrogen(N) digestion in steers fed a ground corn-based diet supplemented with isonitrogenous amounts of either urea, casein or SBM.

Item	Diets			SEM	Contrast	
	Casein	SBM	Urea		Urea vs. Others	SBM vs. Casein
Nitrogen intake, g/d	136.3	136.2	136.1	_	_	_
Entering duodenum, g/d	128.7	133.40	124.6	1.3	0.06	0.013
Microbial N, g/d	65.0	67.2	59	1.8	0.08	0.47
NANMN, g/d[1]	56.7	60.0	57.6	2.7	0.86	0.49
Ruminal digestion, %						
Unadjusted	5.6[a]	2.1[b]	8.4[c]	0.4	0.01	0.01
Adjusted[2]	58.4	55.9	57.7	1.4	0.79	0.33
Fecal N, g/d	33.1[a]	32.0[b]	33.7[a]	0.1	0.02	0.04
ATT digestion, %[3]	75.7	78.8	75.2	1.5	0.37	0.27

[abc] Means in a same row with different superscripts differ significantly ($p<.05$)

[1] Non-ammonia non-microbial nitrogen

[2] Adjusted for microbial and ammonia nitrogen

[3] Apparent Total Tract Digestibility

Table 4. Organic matter (OM) and starch digestion by steers fed a ground corn-based supplemented with isonitrogenous amounts of either urea, casein or SBM

Item	Diets			SEM	Contrast	
	Casein	SBM	Urea		Urea vs. Others	SBM vs. Casein
OM intake, g/d	6909.0	7045.0	6726.0	_	_	_
OM flow at duodenum, g/d	2938.1	2973.0	3015.4	39.3	0.34	0.59
Ruminal digestion,						
Apparent, g/d	3970.9[a]	4072.0[a]	3710.6[a]	39.4	0.02	0.21
% of intake	57.5	57.8	55.20	0.6	0.07	0.74
True, g/d	4595.0[a]	4723.2[b]	4320.4[c]	16.4	0.03	0.03
% of intake[1]	66.5[a]	67.0[a]	64.2[b]	0.2	0.01	0.23
Fecal OM, g/d	1437.6[a]	1380.2[b]	1392.5[b]	14.4	0.04	0.01
ATTOMD, %[2]	79.2	80.4	79.3	0.8	0.78	0.43
Starch intake, g/d	3729.0	3729	3744.0	_	_	_
Flow at duodenum, g/d	1593.2[a]	1540.6[a]	1628.3[b]	14.4	0.08	0.12
Ruminal digestion,						
Apparent, g/d	2135.8	2188.4	2115.7	12.7	0.23	0.08
% of intake	57.3[a]	58.7[b]	56.5[a]	0.4	0.23	0.14
Fecal starch, g/d	142.1	127.8	162.1	9.8	0.85	0.12
ATTSD, %[3]	96.2	96.6	95.7	0.3	0.61	0.88

[abc]Means in a same row with different superscripts differ significantly ($p<.05$)

[1] Adjusted for microbial OM

[2] Apparent Total Tract OM digestibility

[3] Apparent Total Tract Starch digestibility

Table 5. NDF and ADF digestion in steers fed a ground corn basa-diet supplemented with isonitrogenous amounts of either urea, casein or SBM

Item	Diets			SEM	Contrast	
	Casein	SBM	Urea		Urea vs. Others	SBM vs. Casein
NDF intake, g/d	1689.0	1829.2	1698.7	–	–	–
Entering duodenum, g/d	832.3[a]	871.1[b]	853.0[ab]	3.9	0.02	0.02
Ruminal digestion, g/d	857.7[a]	958.1[b]	845.7[b]	3.9	0.01	0.003
As % of intake	50.8[a]	52.4[b]	49.8[b]	0.2	0.34	0.04
Fecal NDF, g/d	780.2[a]	822.2[b]	817.1[ab]	1.5	0.02	0.03
ATTNDFD, %[1]	53.8[a]	55.1[b]	51.9[c]	0.1	0.10	0.01
ADF intake, g/d	993.0	1074.0	980.0	–	–	–
Entering duodenum, g/d	527.5[a]	564.1[b]	526.3[a]	1.84	0.02	0.005
Ruminal digestion, g/d	465.5[a]	509.9[b]	453.7[b]	1.84	0.02	0.004
As % of intake	46.9	47.5	46.3	0.18	0.90	0.18
Fecal NDF, g/d	485.6	512.8	491.4	8.14	0.23	0.13
ATTNDFD, %[2]	51.1	52.3	49.9	0.80	0.89	0.47

[abc] Means in a same row with different superscripts differ significantly ($p<.05$)

[1] Apparent Total Tract NDF Digestibility

[2] Apparent Total Tract ADF Digestibility

Table 6. Mean protozoa numbers in the rumen of steers fed a ground-corn based diet Supplemented with isonitrogenous amounts of either urea, casein or SBM

	Diets				Contrast	
Item	Casein	SBM	Urea	SEM	Urea vs. Others	SBM vs. Casein
		X 10^4 /ml				
Entodiniomorphs	54.97	54.88	54.23	0.33	0.23	0.86
Holotrichs	1.58[a]	2.00[b]	0.83[c]	0.07	0.007	0.05
Total	56.55	56.88	55.06	0.34	0.72	0.86

[abc] Means in a same row with different superscripts differ significantly ($p<.05$)

Figure 1. Changes of pH in the rumen of steers fed corn based diet supplemented with isonitrogeneous amounts of casein, SBM and urea.

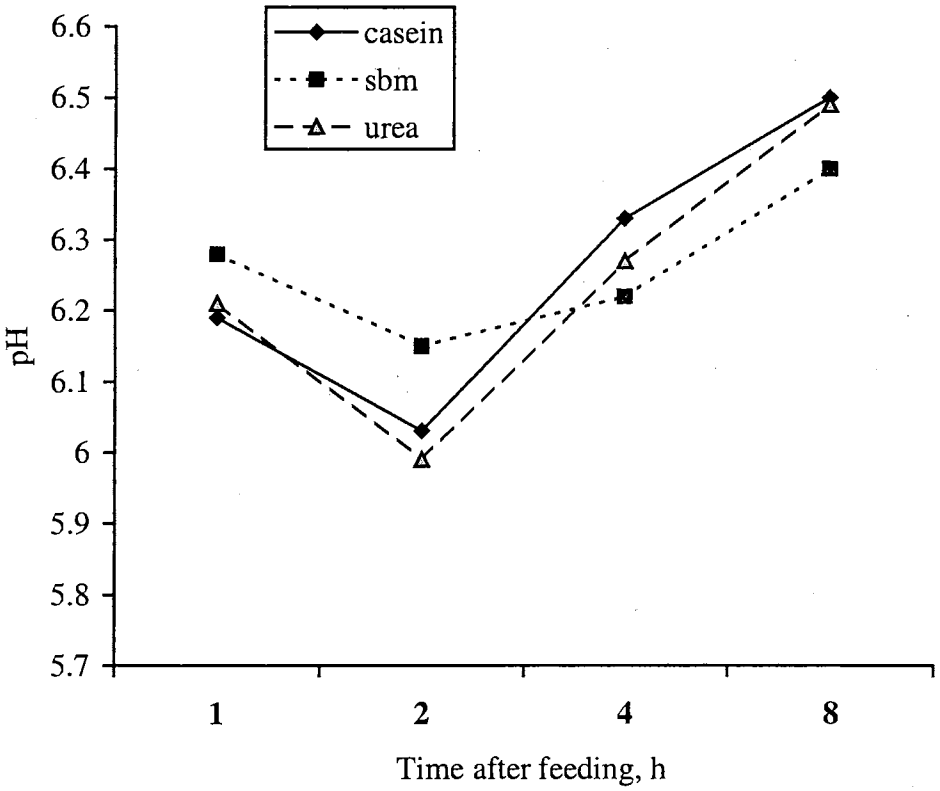


Figure 2. Changes in ruminal peptide-N concentrations in steers fed corn basal diet supplemented with isonitrogeneous amount of casein, SBM and urea.

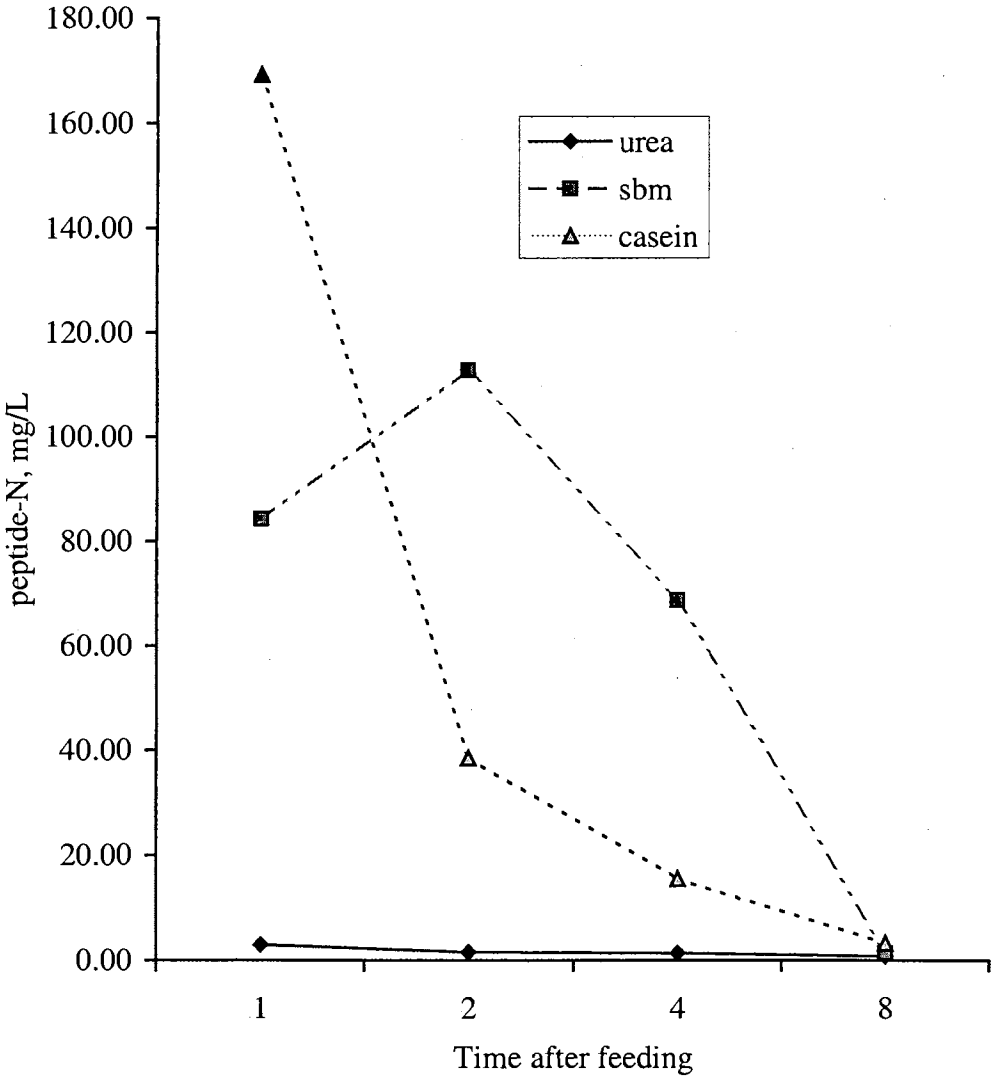


Figure 3. Effect of sampling time on concentrations of ruminal amino acid-N in steers fed corn based diet supplemented with isonitrogeneous amounts of casein, SBM and urea.

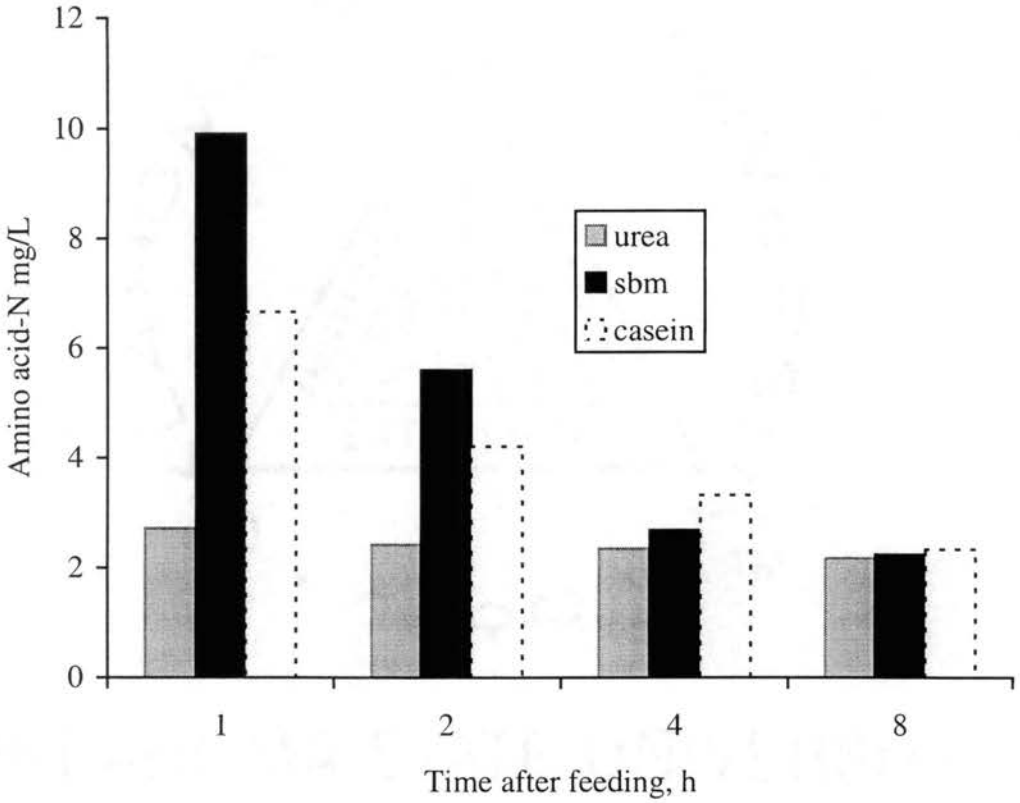
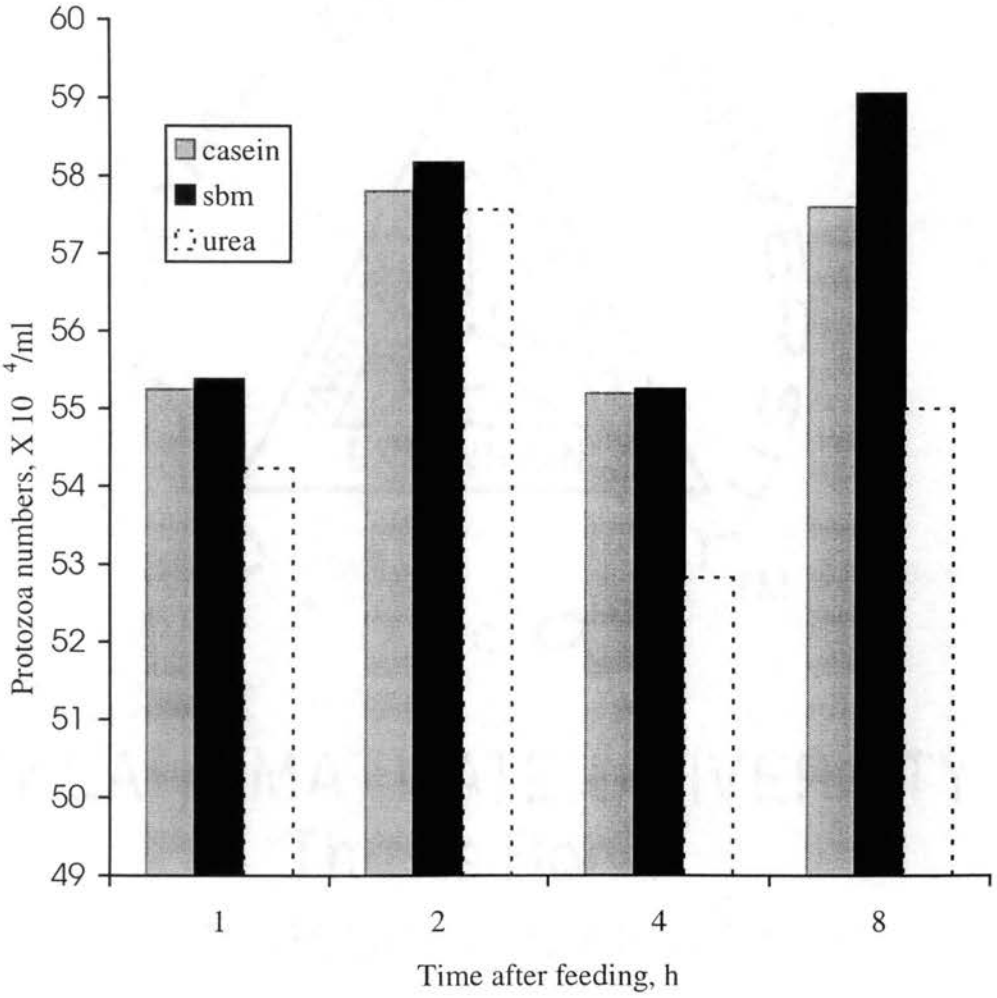


Figure 4. Effect of sampling time on total protozoa numbers in steers fed corn based diet supplemented with isonitrogeneous amounts of casein, SBM and urea.



CHAPTER V

EFFECT OF ENERGY AND NITROGEN SOURCE ON EFFICIENCY OF MICROBIAL PROTEIN SYNTESIS IN THE RUMEN OF STEERS FED CONCENTRATE OR ROUGHAGE DIETS

ABSTRACT

An experiment with ruminally and duodenally cannulated steers was undertaken to determine how the dietary energy source affected the ability of rumen fermentation to respond to peptides compared to non-protein nitrogen (N) in the diet. Four steers received diets of corn (C) or hay grass (H) supplemented with either urea (U) or casein in a 4X4 Latin square with 2X2 factorial arrangement. Measurements of rumen fermentation, microbial protein synthesis and protozoa numbers were made the third week of each 21-day period. Ruminal pH was significantly higher for steers fed hay than corn diet. Concentrations of ruminal NH_3-N were higher for steers supplemented with urea than for steers supplemented with casein. Steers receiving corn or hay diets supplemented with casein had higher ruminal amino acid-N and peptide-N concentrations than those supplemented with urea. Averaged rumen peptide-N concentrations were 56.50, 1.99, 56.24 and 4.21 mg/L for treatment CC, CU, HC and HU respectively. The source of N/peptides had no influence on efficiency of microbial protein synthesis (MOEFF) in the rumen. Nevertheless, steers receiving hay had 33% greater ($P < .0001$) MOEFF than steers receiving corn (18.61 vs. 14.03 g of microbial N/kg of OM digested). Total N flow

to the duodenum was similar among treatments but non-ammonia non-microbial N flow to duodenum was higher in corn based diet than hay based diet. Despite having a lower ($P < .0003$) ruminal pH (6.21 vs 6.52), steers fed the corn-based diet had greater ruminal organic matter, starch, ADF and NDF digestibilities than steers fed the hay-based diets, presumably reflecting a difference in the source of fiber with the two diets. Ruminal concentrations of total protozoa were higher for corn-fed steers than hay-fed steers but holotrichs were more prominent in hay-fed steers. Failure of peptides to increase efficiency of microbial growth in steers as noted by others with in vitro studies may reflect greater potential for nutrient crossfeeding, microbial adaptation to nutrient supply, and (or) energy limited growth and the resulting slower microbial growth rate under ruminal conditions in vivo.

Key words: Nitrogen source, Energy source, Peptide-N, Microbial efficiency, Ruminal digestion.)

Introduction

Ammonia is often the main nitrogen precursor for rumen microbial protein synthesis under practical dietary conditions (Nolan, 1975; Aharoni et al., 1991), and it is essential for the growth of several species of rumen bacteria (Blackburn, 1965; Allison, 1970; Bryant, 1973). Indeed, Virtanen (1966) established beyond doubt ammonia can meet the entire nitrogen requirement of the rumen microbial population as a whole. It is equally clear that in many studies pre-formed amino acids and peptides increased the rate or efficiency of microbial growth compared to ammonia alone (Hume, 1970; Amos and

Evans, 1976; Maeng and Baldwin, 1976; Maeng et al., 1976; Ben-Ghendalia et al., 1978; Cotta and Russell, 1982; Rooke and Armstrong, 1989; Merry et al., 1990; McAllan, 1991). Growth of pure cultures of rumen bacteria is stimulated by peptides and amino acids (Bryant, 1973; Chen et al., 1987; Cruz Soto et al., 1994).

Not all studies have found that pre-formed amino acids and peptides were beneficial, however (Cruz Soto et al., 1994), and such a variation in response was recognized in the Cornell model of protein and energy requirements for ruminants (Russell, et al., 1992). In this model, estimated requirements for pre-formed amino acids were recommended based on pure culture studies which show that bacteria which use starch and sugars require peptides and amino acids for optimal growth, whereas those which ferment cellulose do not. Cruz Soto et al. (1994) found that cellulolytic rumen bacteria actually had a variable response to amino acids and peptides: cellulose breakdown was unaffected, but their growth rate on soluble sugars increased severalfold when pre-formed amino acids were present. They proposed that in general, rumen fermentation would be stimulated by pre-formed amino acids and peptides only when the rate of provision of energy permitted (Cruz Soto et al., 1994). The objective of our experiment was to determine the effect of energy and nitrogen source on efficiency of microbial protein synthesis in the rumen of steers fed concentrate or roughage diets.

Materials and Methods

Animals and treatments

Four steers (1 Hereford, 2 Angus and 1 Limousine) averaging 447 kg in weight

which were used in the experiment 2, were used in experiment 3. Each was equipped with rumen fistula and duodenal re-entrant cannula in the duodenum proximal to the bile duct. The steers were randomly allotted to individual 3 X 5 meter stalls and had free access to water. The steers were assigned to four treatments in a 4 X 4 Latin square design with 2 X 2 factorial treatment arrangement. Treatments included 1) ground corn based diet + casein (CC) 2) ground corn based diet + urea (CU) 3) hay based diet + casein (HC) and 4) hay based diet + urea (HU). Ingredients and chemical composition of each diet are shown in Table 1.

Diets were fed twice daily at 0800 and 1600 in equal portions. Feed dry matter was provided at a rate of 1.8 % of body weight daily. Chromium oxide (Cr) was used as a nonabsorbable marker for measurement of digesta flow. Chromium oxide (0.2 % of the total diet) was mixed with the supplements for all diets.

Sampling procedures

Before the start of the experiment, the steers were fed the diet CU for a period of three weeks for diet adaptation. Each experimental period lasted 21 d long, with 16 d for adjustment and 5 d for sampling. On day 17 through 19, approximately 250 ml of duodenal digesta and 200g of wet feces were collected at 2 and 8 h after feeding. On d 20, approximately 1000 ml of strained rumen fluid were collected at 2 and 8 h after feeding and frozen for later isolation of bacteria. On d 21 approximately 250 ml of rumen fluid were withdrawn at 1, 2, 4 and 8 h after feeding. A .5ml sub-sample of the rumen fluid from each sampling time was added to a formaldehyde/phosphate buffer/methyl green solution according to procedure developed by Department of Animal Sciences and

Industry, Kansas State University and stored for later protozoa counting. The remaining samples were frozen and used later for ammonia and peptides analyses. All rumen fluid collected was strained through 4 layers of cheesecloth and the pH was measured immediately. Before freezing, all rumen samples were acidified with 1ml of 20% v/v sulfuric acid per 50 ml strained fluid to stop microbial activity.

Feed samples were obtained prior to each sampling day and composited within each diet and period. All samples were ground in Wiley Mill fitted with a 2mm screen and stored for analysis.

Laboratory analyses and calculations

Feed, duodenal and fecal samples were analyzed for dry matter (DM), organic matter (OM), ash (AOAC, 1984), starch (Herrera-Saldana and Huber, 1989) and chromium (Cr) (Fenton and Fenton, 1979). The N content of feed, duodenal digesta, bacterial composites, and feces were analyzed by macro-Kjeldahl analysis (AOAC, 1984). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) in feed, duodenal and fecal samples were analyzed using procedures of Goering and Van Soest, (1970). Rumen NH_3-N was analyzed colorimetrically using a spectrophotometer (UV-VIS Spectrophotometer, Gilford-Respond Series, 1987) following the procedures of Broderick and Kang (1980). Protozoal numbers in the rumen fluid were counted using a Sedgewick Rafter chamber. Bacteria were isolated from the ruminal fluid using the procedures of Weakley and Owens (1983). Dried duodenal and bacterial samples were analyzed for nucleic acid-N by the procedure of Zinn and Owens (1986). To improve recovery of RNA pellets after precipitation with silver chloride, the RNA pellets were washed with a

solution (100 ml) that consisted of 5ml solution containing 12.5 % $HCLO_4$ in .0285M $NH_4H_2PO_4$ + 5 ml of .4 M $AgNO_3$ + 90 ml of .2 M $NH_4H_2PO_4$. Rumen samples for peptide-N analysis were prepared using the procedures of Chen et al., (1987). Prehydrolyzed and hydrolyzed rumen fluid samples were analyzed colorimetrically at 570 nm using a spectrophotometer (UV-VIS Spectrophotometer, Gilford-Respond Series, 1987). The concentrations of ninhydrin reactive material in hydrolyzed and unhydrolyzed were measured and leucine was used as a standard (Moore and Stein, 1954; Moore, 1968). The concentration of peptide-associated α-amino N was calculated as the difference between the α-amino N content of hydrolyzed and the unhydrolyzed samples. The concentrations of α-amino N in the hydrolyzed and unhydrolyzed were corrected for NH_3-N in samples and ninhydrin (by subtracting NH_3-N concentrations in the samples and ninhydrin from α-amino N concentrations in the hydrolysed and unhydrolysed samples).

Flows of DM at the duodenum were calculated by dividing daily Cr intake (grams) by Cr concentration (g/kg) in duodenal digesta. Nutrient flows were calculated by multiplying DM flow by the concentration of the given nutrient in duodenal DM. Bacterial N flow (g/d) at the duodenum was calculated by multiplying daily N flow at the duodenum by the proportion of bacterial N in the duodenal N. This proportion was calculated by dividing the bacterial N:purine ratio of ruminal bacteria isolated from each steer in each period.

Daily amounts of non-microbial DM and N flowing past the duodenal cannula were calculated by subtracting themicrobial contributions from the total. Bacterial DM

was determined by oven drying the freeze-dried bacteria samples (ground) at 60 °C for 24 h. Daily duodenal organic matter (OM) flow, corrected for microbial contributions, was calculated from the corrected duodenal DM flow X duodenal OM percentage.

Statistical analysis

Variables measured were analyzed as 4X4 Latin square with animal (4), period (4) and dietary treatment (4) as factors (SAS Institute, Inc. 1988). Differences between treatments were determined using a multiple comparison test (PDIFF options of SAS Institute, Inc. 1988). Statistical significance was considered to exist where $P<0.05$, whereas a trend was considered to exist if $0.05 \leq P \leq 0.10$. Simple correlations also were calculated across all observations. Contrasts were utilized to make specific treatments comparison, i.e., 1) between corn and hay 2) between casein and urea and 3) the protein-energy interaction.

Results and Discussion

Mean ruminal pH, peptide-N, amino acid-N and NH_3-N concentrations are as shown in Table 2. Ruminal pH was significantly higher ($P<0.0001$) for steers fed hay than corn diet because hay diet contains a large amount of fiber that stimulates rumination and increases saliva flow to the rumen. Concentrations of rumen NH_3-N were significantly higher ($P<0.03$) for steers supplemented with urea than for steers supplemented with casein. Mean concentration of ruminal NH_3-N for treatments CC, CU, HC and HU were 7.55, 8.55 7.40 and 8.68 mg/dL respectively. These values were higher than the concentration adequate (5mg/dL) for supporting microbial crude protein

synthesis (Satter and Slyter, 1974) and also within the values (3.3 to 8.5 mg/dL) suggested by Kang-Merznarich and Broderick (1981) for maximal microbial growth when diets contained 74 % corn grain. Ammonia level was maximal at 2 h (Figure 2) in steers supplemented with casein and remained slightly lower at 4 and 8 h. By 2 h after feeding urea, ruminal NH_3-N was very high then rapidly declined thereafter indicating less N was available to rumen microorganisms 2 h post feeding. The more rapid accumulation of NH_3-N with urea than with casein reflects slight resistance of casein to ruminal degradation (Wallace et al., 1987).

Ruminal amino acid-N concentration was higher ($P<0.0001$) for steers supplemented with casein when fed either corn or hay diets. The values for all for amino acid-N in all treatments were low confirming the results of Annison (1956), Wright and Hungate (19670), and Harmeyer (1971). Amino acid-N peaked rapidly after 1 h of feeding in steers supplemented with casein before declining rapidly thereafter (Figure 1) suggesting that amino acid-N always remained available to microorganism as N source but the level was always low 1h post-feeding.

Mean rumen peptide-N concentrations were 56.50, 1.99, 56.24 and 4.21 mg/L for treatment CC, CU, HC and HU respectively. Steers receiving hays supplemented with urea had slightly higher ($P<0.61$) peptide-N concentration than steers receiving corn supplemented with urea because hay in general has higher ruminally degraded protein than corn. However, the ruminal peptide concentrations in steers fed either corn or hay and supplemented with casein were higher ($P<0.0001$) than for steers supplemented with urea. At 1, 2, 4 and 8 h after feeding, the concentrations of peptide-N remained

consistentlly higher in the group of steers receiving diets supplemented with casein (Table 3). The ruminal peptide-N concentration peaked at 1 h after feeding for steers supplemented with casein when the concentrations were 167.0 mg/L in steers fed CC diet and only 145.1 mg/L in steers fed HC diet, the difference was significant between these diets ($P<0.02$). However there was no difference ($P<0.12$) in mean peptide-N concentration between CC and HC treatment. Peptide-N concentration in the rumen of steers fed casein supplement were close to those (169 ± 12.6 mg/L) reported by Williams and Cockburn (1991) for steers 1 h after being fed diets containing casein. The peptide-N concentrations at 2 h after feeding ranged from 2.6 to 60.9 mg/L in all treatments. These values are somewhat lower than those (149-225 mg/L) reported by Chen et al. (1987) for cows 2 h after being fed corn silage/barley diets supplemented with SBM, SBM and extruded SBM, fish meal once daily. Kim et al. (1998) studying effects of popped and raw soybean in the rumen of calves reported that peptide-N concentrations at 2 h after feeding were 99.31 and 113.89 mg/L respectively. In studies with sheep given hay/concentrate diets supplemented with casein, Broderick and Wallace (1988) found peptide-N concentrations that were only about 30-50 % of those reported by Chen et al. (1987). Values were only 10% as high when casein was replaced by either urea or albumin. Different methods for determining peptides also may affect their estimated concentrations in the rumen. Nevertheless, feeding casein in this trial accomplished our objective of increaseing the rumen concentrations of peptide-N in steers receiving either corn or hay and thereby gave us an opportunity to evaluate the impact of an increased ruminal supply of peptides on ruminal digestion and microbial yield and efficiency.

. Efficiency of microbial protein synthesis (MOEFF) expressed as grams of N per kg of OM truly digested in the rumen (Table 1) were 14.2, 13.9, 18.8 and 18.4. Those steers receiving hay supplemented with either casein or urea had higher ($P<0.0003$) MOEFF than steers receiving corn supplemented either casein or urea. Source of N did not significantly ($P<0.62$) affect MOEFF and there was no energy-protein interaction among treatments. Lower MOEFF with the corn based diet than hay based diet may have been due to the fact that diets which contain more than 70% ground corn may caused rapid rate of nonstructural carbohydrate degradation in the rumen, resulting in uncoupled fermentation (Polan, 1988). Uncoupled fermentation is presumed to occur when energy is released much faster than it can be captured and utilized by the ruminal bacteria (Clark et al., 1992). For the hay based diet, one would expect greater saliva flow, higher ruminal pH, an improved cation exchange capacity, improved hydration, and an improved rumial mat formation, leading to decrease liquid retention times that should lead to greater microbial growth as microbial generation times would be reduced (Sniffen et al., 1987). The effect of readily fermentable carbohydrate supplementation on the efficiency of microbial protein synthesis may depend on the level of supplementation. Efficiency tends to be increased when readily fermentable carbohydrate is supplemented at less than 30% of the total diet (Feng et al., 1993), but decreased when the supplementation level is greater than 70% (Mathers and Miller, 1981). The correlation between peptide-N concentrations and MOEFF was low ($r = 0.07$, $P = 0.8$). Although peptide-N concentration was greatest in steers supplemented with casein, it did not appear to stimulate microbial growth in this experiment. Cruz Soto et al. (1994) also presented evidence that suggested

that stimulation by peptides and amino acids would not always occur. In vitro data, from Maeng and Baldwin (1976), Argyle and Baldwin (1989), Fujimaki et al. (1992), Kaur et al. (1992) and Russell et al. (1983), all have indicated that microbial growth will be increased when preformed amino acids are supplied; further, Argyle & Baldwin (1989) noted that, under certain combinations of substrate concentrations, peptides had a stimulatory effect greater than that seen with free amino acids. Chikunya et al. (1996) supplied peptides with diets containing either rapidly or slowly degraded fiber; their results suggested that the benefit of peptides would be evident only when the energy source supported a growth rate which enabled the organism to respond. In our trial, steers receiving hay appeared to have an energy source that supported greater microbial growth efficiency regardless whether there was high rumen peptide-N concentrations or not. Although the rumen NH_3-N and peptide-N concentrations were high in corn fed steers, the lower bacterial yield and efficiency with this diet indicated that factors other than ruminally available energy, ammonia and peptides limited microbial growth and efficiency.

Total N flow (Table 4) to the duodenum was affected by source of energy. Nitrogen flow to the duodenum was greater ($P<0.0001$) for steers fed corn than steers fed the hay diet. Compared with urea, casein supplementation also increased ($P<0.02$) N flow to the duodenum. Source of energy significantly ($P< 0.04$) affected microbial N flow to the duodenum; hay diet fed steers had higher flow than steers fed corn diet but the effect of N source was not significant ($P<0.12$) suggesting that nitrogen source or peptides had no effect on microbial N flow. Microbial N accounted for 49.9, 47.0, 61.3 and 61.6 % of

total N flow to the duodenum for treatments CC, CU, HC and HU respectively. NANMN flow to the duodenum was greater (P<0.0002) for steers fed corn diets than those steers fed hay diets but the effect of N was small (P<0.51). Ruminal N digestibility (corrected for microbial N flow) were 58.9, 59.3, 73.9 and 76.3 for diets CC, CU, HC and HU (Table 4). Steers fed the hay diet had higher (P<0.0001) ruminal N digestibility than corn fed steers. Source of N had no effect on ruminal N digestibility (P<0.17) suggesting that ruminal peptides did not play an important role in runinal N digestibility. Apparent total tract N digestibility (ATTND) was unaffected by N source but was influenced by energy source (P<0.0001) and being higher in steers receiving corn diets than those receiving hay diets. Post-ruminal supply of digestible N was about 50% greater with the corn than the hay diet (106 vs 70 g/d). Protein-energy interactions for all these measurements were insignificant.

Mean ruminal OM and starch digestion are presented in Table 5. Intake of OM was higher for the CC diet than other diets. Duodenal OM flow was higher (P<0.0001) for steers fed the hay diet than steers fed the corn diet and N source did not influenced (P < .78) duodenal OM flow. Apparent and true OM digested in the rumen both were higher in steers receiving the corn than those receiving the hay diets. True rumen OM digestibilities were significantly higher (P<0.0001) in steers fed corn than those fed the hay diet and there was also an effect (P<0.03) of N source for true ruminal OM digestion attributable primarily to greater ruminal OM digestion with the corn-based diet. The in rumen OM digestibility was due to the greater content of non-structural carbohydrate in the corn diet as compared to hay diet which was made up largely of structural

carbohydrate. Peptides did not influence digestibilities of rumen OM; the correlation between peptide-N and rumen digestibility was low but positive ($r = 0.24$, $P < 0.38$). Total tract OM digestibilities (TTOMD) for treatments CC, CU, HC and HU were 78.4, 77.7, 59.8 and 61.1 % respectively (Table 5). Total tract OM digestibilities were higher for steers receiving the corn than for steers receiving the hay diet regardless whether they were supplemented with either urea or casein.

Starch intake and flow to duodenum were higher for steers receiving corn diet than those fed hay diet (Table 5). Apparent ruminal and starch digestibilities, duodenal starch flow, fecal starch, and apparent total tract starch digestibilities were similar for steers fed corn supplemented with either casein or urea. Duodenal starch flow was much lower for steers receiving the hay diet than for steers fed the corn diet. Higher peptide-N concentrations in the rumen of steers fed the CC diet (56.5 mg/L) than in the rumen of steers fed the CU diet (1.99 mg/L) did not appear to improve rumen starch digestibility. Feeding the rapidly fermented corn-based diets, like those fed in this experiment, despite greater ruminal digestion, did not increase microbial N flow to the duodenum because MOEFF was decreased. Ludden and Cecava (1995) feeding steers with corn based diets supplemented with urea, SBM, soyplus and corn gluten/blood meal reported higher MOEFF (19.4 g/kg OM truly digested) than we observed in this trial.

Intakes of fiber fractions (ADF and NDF) were much lower ($P < .001$) with the corn diet than hay diet. Ruminal digestibilities of NDF and ADF for treatments CC, CU, HC and HU were 50.5, 48.1, 43.0, 42.9 and 47.7, 46.4, 39.2, 39.1 respectively (Table 6). Ruminal ADF and NDF digestion were influenced by Energy source ($P < .0001$) but not

by N source. This observation is in conflict with the results of McCarthy et al., (1989) and Cameron et al., (1991) who noted a depression in NDF digestion as ruminal availability of starch was increased in the diets of dairy cows. Apparent total tract NDF and ADF digestibilities were significantly higher ($P<0.0001$) for steers fed corn diet than hay diet. Higher starch intake in corn diets which lowered the pH did not reduce extent of ruminal NDF or ADF digestion in this study. However, in our study, the source of dietary NDF and ADF differed with diet, with most of the fiber being derived from grain with the corn diet and from forage with the hay diet and fiber source can readily alter fiber digestibility.

Basal diet affected the ruminal concentrations of the major protozoa species numbers (Table 7 and Figure 3). The concentration of entodiniomorph was significantly higher ($P<0.001$) in the rumen of steers fed corn-based diet than in the rumen of steers fed the hay diet. An effect of N sources also was detected ($P<0.02$) as well as a protein-energy interaction in entodiniomorph population The population density of rumen protozoa in ruminants fed forage plus concentrate is generally higher than that in ruminants fed forage only (Hinkson et al., 1976; Nakamura and Kanegasaki, 1969). Dennis et al. (1983) fed Holstein heifers diets that varied in the ratio of roughage to concentrate: 70:31, 50:50, and 30:70 and found that the numbers of protozoa increased as proportion of concentrate increased in the diet. Meyer et al. (1986) reported that protozoa numbers increased with each increase in the increment of ground corn given to sheep. This undoubtedly is a reflection of dietary quality and the ready availability of highly digestible carbohydrate in corn based diets, which will increase entodiniomorph numbers.

In contrast feeding 100% concentrate diets rich in fermentable carbohydrate tended to decrease the protozoal population in sheep. Holotrich protozoa numbers were significantly higher ($P<0.0001$) for hay-fed steers than for corn-fed steers with N source also playing highly significant role ($P<0.007$) although the total protozoal population was not significantly altered by N source ($P < .13$). The lower total protozoa numbers in steers fed hay diet might be involved with the increased microbial flow was NOT greater with the hay diet, only MOEFF was. Smith et al. (1978) working with steers and Sutton et al. (1983) working with sheep also found an increased MOEFF when the number of protozoa was reduced. Diurnal variations of protozoa numbers in the rumen are as shown in Figure 4. The Protozoa population tended to increase after feeding and then become constant thereafter as noted in two previous experiments.

Implications

Efficiency of converting digested organic matter to microbial protein in the rumen of steers was not increased by an increased supply of peptides either in hay or corn diets. Rumen microorganisms in corn-based diets may not require peptides as N source to synthesize their protein when an adequate supply of NH_3-N is available. Consequently, the degradable protein content in the concentrate should be considered before supplementation with highly degradable protein is exercised. No benefit in terms of ruminal or total tract digestion of organic matter or starch was noted when a dietary source of peptides (casein) replaced dietary urea. Nevertheless, microbial efficiency was 32 % greater with a hay-based than a corn-based diet.

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Table 1. Composition of diets in experiment 3.

	Diets			
	Corn + Casein	Corn + Urea	Hay + Casein	Hay + Urea
	% of dry matter			
Ground corn	74.79	77.99	_	_
Cottonseed hulls	11.0	11.0	_	_
Brome hay	_	_	25.0	25.0
Prairie hay	_	_	60.79	64
Supplement				
Soybean hulls	7.0	7.0	7.0	7.0
Urea	_	1.4	_	1.40
Casein	4.6	_	4.6	_
Dicalcium phosphate	1.4	1.4	1.4	1.4
Limestone, 38%	0.7	0.7	0.7	0.7
Trace mineralized salt	0.3	0.3	0.3	0.3
Vitamin A	0.01	0.01	0.01	0.01
Chromic oxide	0.2	0.2	0.2	0.2
Crude protein, %	12.3	12.7	12.2	12.4
Starch, %	54.2	56.5	2.9	2.9

Table 2. Rumen pH, ammonia, peptide-N, amino-N and microbial protein synthesis in steers fed diets of grass hay or corn supplemented with either urea or casein

	Diets					Contrast		
Item	Corn + Casein	Corn + Urea	Hay + Casein	Hay + Urea	SEM	Corn vs. Hay	Urea vs. Casein	E[4] X P
pH	6.18[a]	6.24[a]	6.58[b]	6.47[b]	0.04	0.0003	0.53	0.10
NH_3-N, mg/dL	7.55[a]	8.55[b]	7.40[a]	8.68[b]	0.23	0.96	0.003	0.57
Amino acid-N, mg/L[1]	3.89[a]	2.32[b]	3.95[a]	2.36[b]	0.13	0.73	0.0001	0.92
Peptide-N,mg/L[2]	56.50[a]	1.99[b]	56.24[a]	4.21[b]	1.81	0.61	0.0001	0.52
Microbial efficiency[3]	14.18[a]	13.88[a]	18.78[b]	18.78[b]	0.65	0.0003	0.62	0.97

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

[1] prehydrolysed fluid

[2] hydrolysed fluid

[3] g microbial N per kg OM fermented

[4] Energy- protein interaction

Table 3. Effect of sampling time on concentration of petipde-N in the rumen of steers receiving diets of grass or corn supplemented with either urea or casein.

	Diets					Contrast		
Time of sampling[1]	Corn + Casein	Corn + Urea	Hay + Casein	Hay + Urea	SEM	Corn vs. Hay	Urea vs. Casein	E[2] X P
	peptide-N, mg/L							
1 hour	167.03[a]	3.05[b]	145.08[c]	6.88[b]	5.00	0.12	0.0001	0.04
2 hour	50.02[a]	2.58[b]	60.86[c]	5.75[b]	3.02	0.02	0.0001	0.10
4 hour	15.36[a]	1.61[b]	14.24[a]	2.57[b]	1.12	0.94	0.0001	0.39
8 hour	3.60[a]	0.69[b]	6.00[c]	1.65[b]	0.23	0.003	0.0001	0.02

[abc] Means in a same row with different superscripts differ significantly ($p<.05$)

[1] Hour after feeding

[2] Energy- protein interaction

Table 4. Nitrogen digestion in steers receiving diets of grass hay or corn supplemented with either urea or casein.

	Diets					Contrast		
Item	Corn + Casein	Corn + Urea	Hay + Casein	Hay + Urea	SEM	Corn vs. Hay	Urea vs. Casein	E[5] X P
Nitrogen intake, g/d	162.8	163.1	162.6	162.7	_	_	_	_
E. duodenum, g/d[1]	149.6[a]	144.3[a]	136.1[b]	131.6[b]	2.2	0.0001	0.02	0.81
Microbial N, g/d	74.5[a]	67.8[a]	83.4[b]	81.1[ab]	2.4	0.004	0.12	0.40
NANMN, g/d[2]	66.9[a]	66.4[a]	42.4[b]	38.5[b]	2.7	0.0002	0.51	0.61
Ruminal digestion, %								
Unadjusted	8.1[a]	11.5[b]	16.3[c]	19.1[d]	_	0.0001	0.001	0.58
Adjusted[3]	58.9[a]	59.3[a]	73.9[b]	76.3[b]	_	0.0001	0.17	0.29
Fecal N, g/d	39.5[a]	41.6[a]	63.2[b]	63.8[b]	2.2	0.0001	0.57	0.74
ATTND, %[4]	75.8[a]	74.5[a]	61.1[b]	60.8[b]	1.4	0.0001	0.58	0.74

[abcd] Means in a same row with different superscripts differ significantly ($p<.05$)

[1] Entering duodenum

[2] Non-ammonia non-microbial nitrogen

[3] Adjusted for microbial and ammonia nitrogen

[4] Apparent Total Tract Nitrogen Digestibility

[5] Energy- protein interaction

Table 5. Organic matter and starch digestion in steers receiving diets of grass hay or corn supplemented with either casein or urea.

	Diets					Contrast		
Item	Corn + Casein	Corn + Urea	Hay + Casein	Hay + Urea	SEM	Corn vs. Hay	Urea vs. Casein	E[4] X P
OM intake, g/d	7857.0	7676.0	7686.0	7559.0	11.5	0.0001	0.0001	0.06
Flow at duodenum, g/d	3440.8[a]	3537.8[a]	4273.3[b]	4221.8[b]	78.9	0.0001	0.78	0.38
Ruminal digestion,								
Apparent, g/d	4416.3[a]	4138.3[b]	3412.8[c]	3338.5[c]	79.0	0.0001	0.78	0.24
% of intake	56.2[a]	53.9[b]	44.4[c]	44.2[c]	1.0	0.0001	0.07	0.35
True, g/d	5099.0[a]	4796.8[b]	4183.0[c]	4094.8[c]	68.7	0.0001	0.26	0.17
% of intake[1]	64.9[a]	62.5[a]	54.4[b]	54.2[b]	0.9	0.0001	0.03	0.27
Fecal OM, g/d	1698.0[a]	1716.5[a]	3091.8[b]	2937.8[c]	41.8	0.0001	0.16	0.08
ATTOMD, %[2]	78.4[a]	77.7[a]	59.8[b]	61.1[b]	0.5	0.0001	0.19	0.27
Starch intake, g/d	4255.0	4334.0	225.0	221.0	0.13	0.0001	0.0001	0.0001
Flow at duodenum, g/d	1807.0[a]	1879.0[b]	12.8[c]	13.5[c]	21.7	0.0001	0.15	0.15
Ruminal digestion,								
Apparent, g/d	2448.0[a]	2455.0[a]	215.8[b]	210.8[b]	22.0	0.0001	0.97	0.79
% of intake	57.5[a]	56.7[a]	95.9[b]	95.3[b]	0.7	0.0001	0.36	0.89
Fecal starch, g/d	193.3[a]	217.0[a]	3.0[b]	3.0[b]	7.8	0.0001	0.18	0.18
ATTSD, %[3]	95.4[a]	95.6[a]	98.7[b]	98.7[b]	0.3	0.0001	0.79	0.64

[abc] Means in a same row with different superscripts differ significantly ($p<.05$)

[1] Adjusted for microbial OM

[2] Apparent Total Tract OM digestibility

[3] Apparent Total Tract Starch digestibility

[4] Energy- protein interaction

Table 6. NDF and ADF digestion in steers receiving diets of grass hay or corn diets supplemented with either urea or casein.

Item	Diets					Contrast		
	Corn + Casein	Corn + Urea	Hay + Casein	Hay + Urea	SEM	Corn vs. Hay	Urea vs. Casein	E X P[5]
NDF intake, g/d	1885.0	1901.0	4752.0	4832.0	_	_	_	_
E. duodenum, g/d[1]	932.5[a]	986.3[a]	2707.8[b]	2756.8[b]	18.3	0.0001	0.03	0.90
R. digestion, g/d[2]	952.5[a]	914.7[a]	2044.2[b]	2075.3[b]	14.4	0.0001	0.38	0.09
As % of intake	50.5a	48.1[a]	43.0[b]	42.9[b]	0.8	0.0001	0.13	0.25
Fecal NDF, g/d	885.0[a]	917.8[a]	2629.5[b]	2687.0[b]	18.0	0.0001	0.05	0.62
ATTNDFD, %[3]	53.5[a]	51.3[a]	44.6[b]	44.4[b]	0.5	0.0001	0.06	0.09
ADF intake, g/d	1179.0	1159.0	3169.0	3223.0	_	_	_	_
E. duodenum, g/d[1]	616.5[a]	621.3[a]	1902.8[b]	1962.3[b]	18.8	0.0001	0.14	0.19
R. digestion, g/d[2]	562.5[a]	537.7[a]	1266.2[b]	1260.7[b]	19.7	0.0001	0.77	0.38
As % of intake	47.7a	46.4[a]	39.2[b]	39.1[b]	1.0	0.0002	0.50	0.56
Fecal NDF, g/d	557.3[a]	579.0[a]	1828.8[b]	1895.0[b]	21.2	0.0001	0.08	0.33
ATTNDFD, %[4]	52.7[a]	50.1[a]	42.2[b]	41.2[b]	1.0	0.0001	0.12	0.45

[ab] Means in a same row with different superscripts differ significantly ($p<.05$)

[1]E. duodenum, g/d

[2]Ruminal digestion

[3] Apparent Total Tract NDF Digestibility

[4]Apparent Total Tract ADF Digestibility

[5] Energy- protein interaction

Table 7. Least square mean of ruminal protozoa numbers in rumen fluid from steers receiving diets of grass hay or corn supplemented with either urea or casein.

	Diets					Contrast		
Item	Corn + Casein	Corn + Urea	Hay + Casein	Hay + Urea	SEM	Corn vs. Hay	Urea vs. Casein	E[1] X P
Entodiniomorph	50.18[a]	54.02[b]	11.63[c]	12.21[c]	0.67	0.0001	0.02	0.05
Holotrich	0.96[a]	0.55[b]	2.36[c]	1.45[d]	0.13	0.0001	0.007	0.57
Total	51.19[a]	54.57[b]	14.52[c]	14.48[c]	1.44	0.0001	0.13	0.12

[abcd] Means in a same row with different superscripts differ significantly ($p<.05$)

[1] Energy- protein interaction

Figure 1. Effect of time on concentrations of amino–N in the rumen of steers fed corn or hay based supplemented with isonitrogeneous amount of either urea or casein.

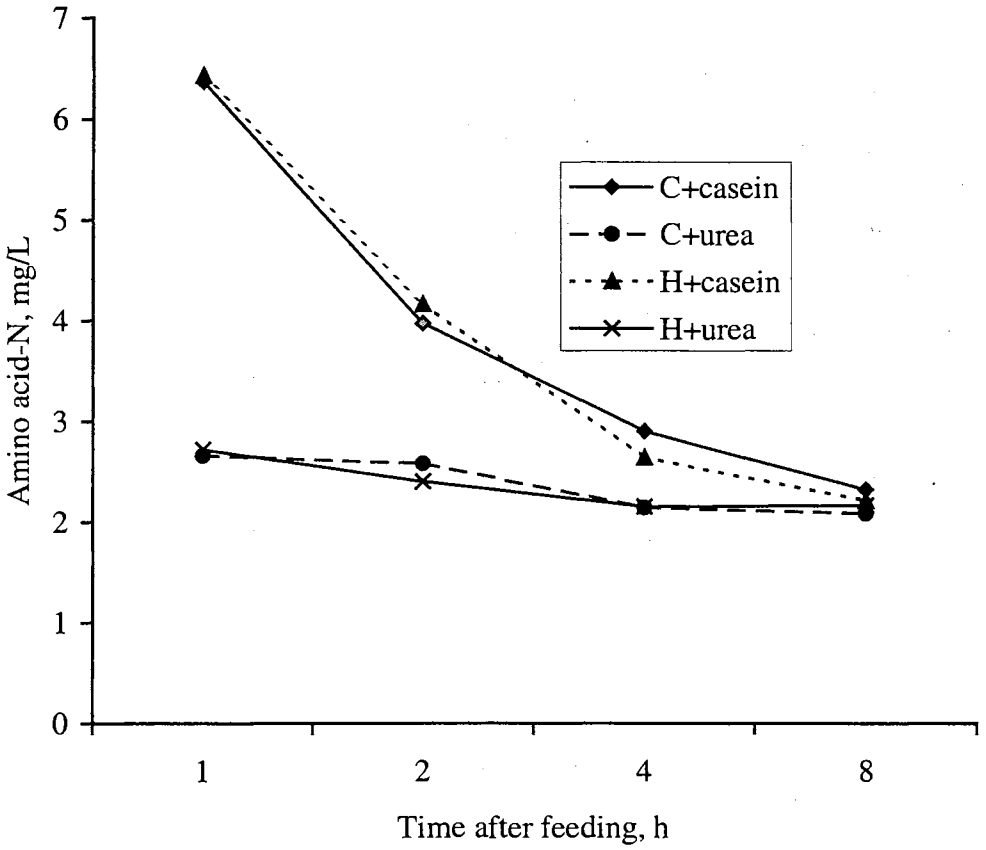


Figure 2. Effect of time on ruminal NH3-N concentrations in the rumen of steers fed corn or hay based diet supplemented with isonitrogeneous amount of either urea or casein.

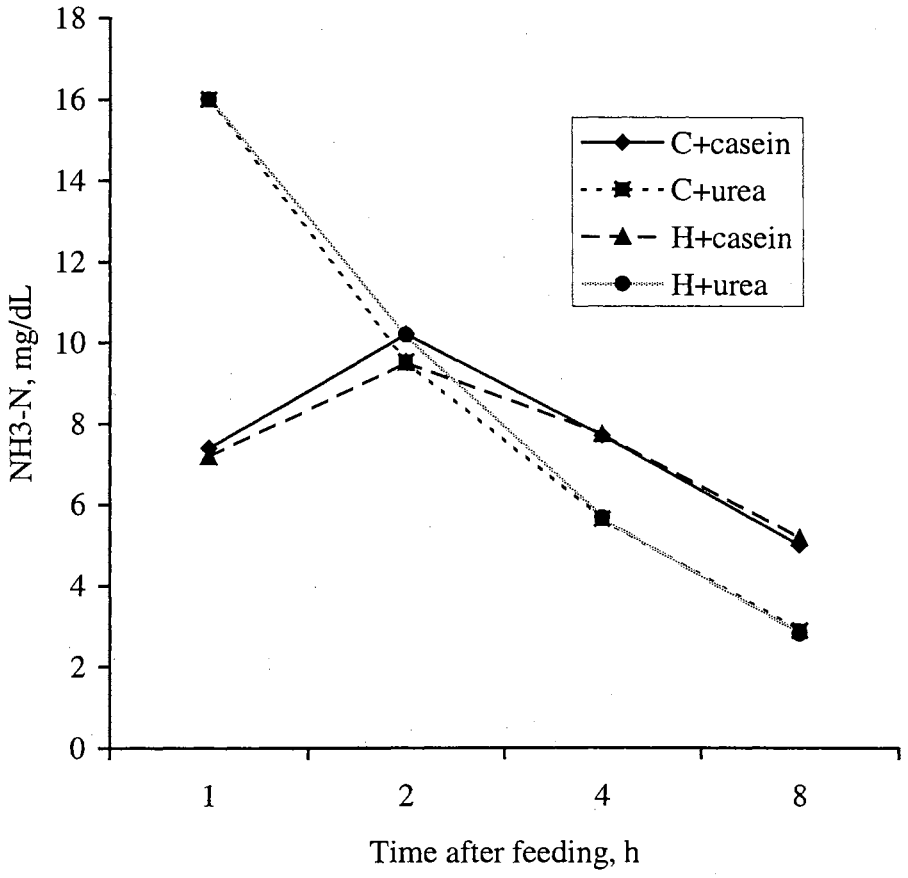


Figure 3. Mean population of entodiniomorph and holotrich protozoa in the rumen of steers fed corn or hay based diet supplemented with isonitrogeneous amount either urea or casein.

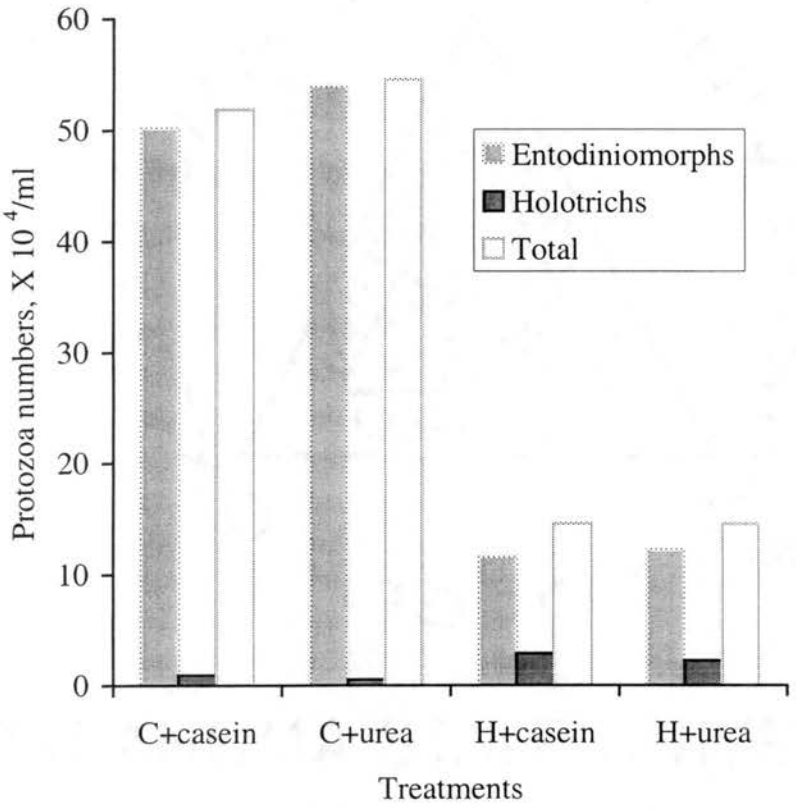
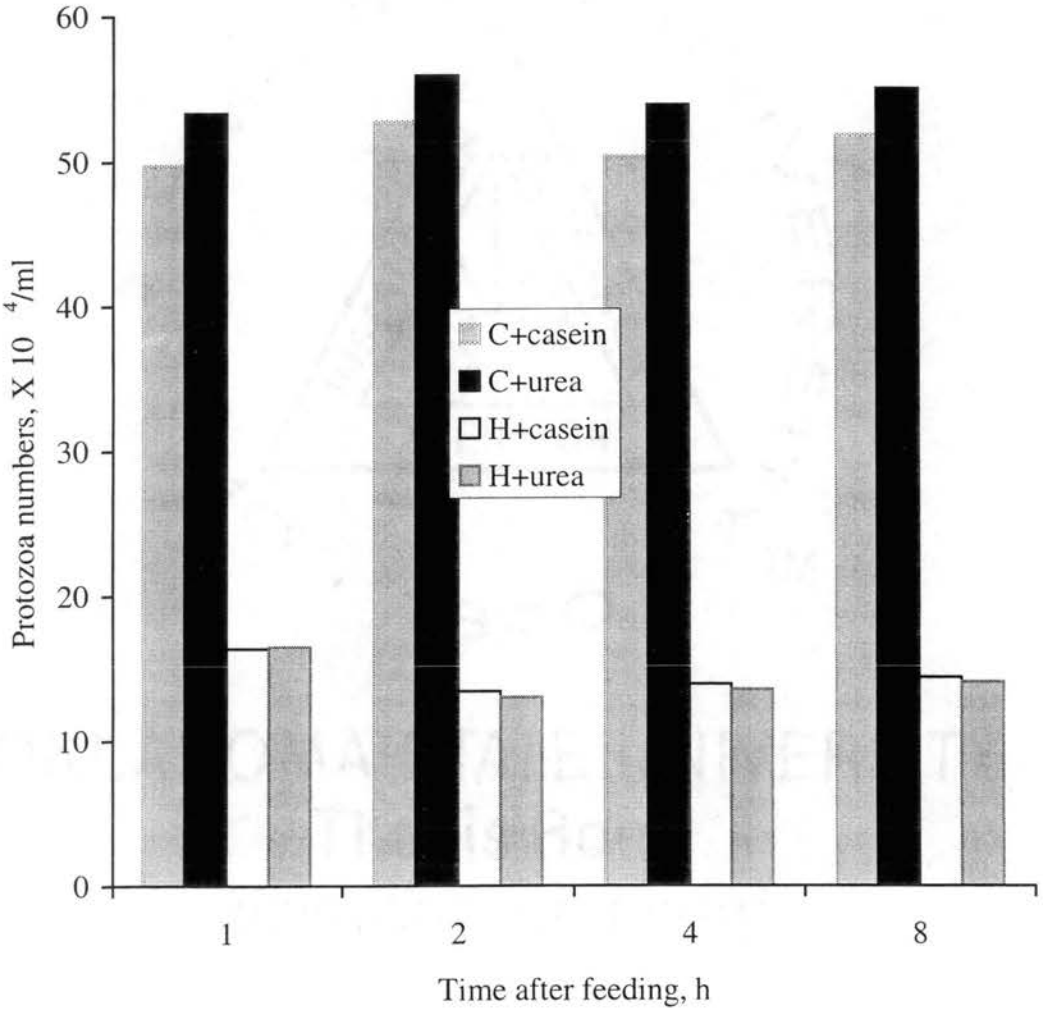


Figure 4. Effect of sampling time on total protozoa numbers in the rumen of steers fed corn or hay based diet supplemented with isonitrogeneous amount of either urea or casein.



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