EVALUATION OF RUMINAL ESCAPE POTENTIAL OF CRAB MEAL AND OTHER PROTEIN SUPPLEMENTS AND INFLUENCE OF STEAM EXPLOSION ON RUMINAL DEGRADABILITY OF CRAB MEAL

by

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(ABSTRACT)

Four experiments were conducted, three to study the value of crab meal and other protein supplements, and the other to explore the potential of steam explosion technique to improve the nutritive value of crab meal. In Expt. 1, 48 Angus x Hereford and Angus x Simmental steers (avg. BW, 223 kg) were used in a 126-d growth study. Diets were formulated to contain 10.5% CP and 63% TDN, DM basis. In each diet, one third of the N was supplied by the protein supplement. Steers were randomly allotted to the following six supplements: 1) soybean meal (SBM); 2) supplement based on industrial byproducts of both plant and animal origin (IPA); 3) experimental supplement based on byproducts of animal origin (ESA); 4) hydrolyzed supplement No. 3 (HESA); 5) commercial supplement (Pro-Lak®) based on animal protein (CS) and 6) crab meal (CM). There were no significant positive responses in performance and feed efficiency for any of the protein supplemented groups compared to SBM. There was a trend for a positive response in gain to feed for steers fed CS and IPA. Lower weight gain and gain:feed were recorded for the steers fed HESA supplement. Steers fed CM diet had numerically higher growth and gain:feed than those fed SBM.

In Expt. 2, two metabolism trials were conducted, each with 24 wether lambs (avg. BW, 25 kg). In addition to the six diets that were used for the growth
trial, two other diets were used, a negative control (NC) with no supplemental N, and a diet supplemented with urea (U). The supplements supplied one third of the total dietary N. There were no differences in DM and OM digestibilities among the lambs fed the different protein supplements. Lower (P < .05) apparent absorption of N was recorded for the lambs fed the HESA and NC diets. There were no differences in ruminal fluid pH among the sheep fed different protein supplements. Sheep fed CM tended to have higher total VFA compared to other supplements. Highest (P < .05) ruminal NH₃ N and blood urea N were observed in lambs fed the U diet.

In Expt. 3, the ruminal degradability of DM and CP of crab meal and other protein supplements were estimated in situ, in a ruminally cannulated steer. The highest DM degradability was for SBM. The ruminal escape of protein was lowest (P < .05) for SBM (23.2%) and the highest (P < .05) for the ESA supplement (79.8%). The respective values for IPA, HESA, CS and CM were, 60.6, 67.3, 69.8, and 48.4%. The IVDMD of feather meal and blood meal combinations (ESA and HESA) were lowest (P < .05). The IVDMD of crab meal was 67%.

In Expt. 4, the potential of steam explosion technique to enhance the nutritive value of crab meal was explored. Crab meal was steam exploded in a batch steam explosion reactor at two levels of severities. Steam explosion decreased (P < .05) N content of crab meal by 20%, and did not improve DM degradability nor increase escape of CP. A 60% increase in chitin degradability, from 21.5 to 34.2% was observed for steam exploded CM. Steam explosion improved IVDMD of CM from 65.9% to 75.2%.

These studies illustrated that substituting other protein supplements used in this study for SBM did not elicit a significant positive response in steer performance. Crab meal is comparable to SBM or other commercial products as a

Abstract
protein supplement for steers. Steam explosion does not seem to be a promising processing method for improving utilization of crab waste for ruminants.
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To my wife, Amritha
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Chapter I
Introduction

Most of the protein and non-protein N (NPN) ingested by ruminants is converted to microbial protein in the rumen, which is utilized by the animal. Some protein sources which escape ruminal degradation are utilized more efficiently than microbial protein. Thus, if these high-quality proteins escape the rumen, the protein will be utilized more efficiently. Only a portion of the N ingested by growing ruminants and lactating dairy cows is used for microbial action in the rumen. In order to maximize the performance of high producing animals, much of the dietary protein from protein supplements should escape rumen degradation and be available for absorption in the small intestine.

Studies have been conducted in cattle to assess the escape protein potential of various protein supplements of waste materials such as feather meal, blood meal, and distillers dried grains. Little information is available on the escape protein potential of some commercial protein supplements based on plant and animal protein, and crab processing waste, a co-product obtained from the sea food industry. Studies have shown that crab processing waste is a good source of protein for ruminants which could be fed as dehydrated form (crab meal) or by ensiling with roughage.
Although crab meal is a good source of protein, it has been shown that the digestibility of protein in the crab waste-straw silage was lower than that of fish-waste straw silage (Samuels et al., 1991). The lower digestibility of crab processing waste diets may be due to chitin, which is a component of exoskeleton of crustaceans. Chitin, a poly-N-acetylglucosamine, an analogue of cellulose, is less digestible in ruminants than cellulose. Therefore, a method which would cleave the chitin-protein matrix would be beneficial for the better utilization of the waste.

Steam explosion is a technique employed in industries for fractionation of lignin, hemicellulose, and cellulose. As chitin is structurally similar to cellulose, perhaps when this material is subjected to steam explosion, there would be a structural change in the chitin-protein linkage. This technique has been employed in the field of ruminant nutrition. Steam exploded wheat straw was found to be better utilized by steers, compared to non exploded straw (Sciaraffia and Marzetti, 1988). However, no information is available on the effect of steam explosion on the degradability of chitin-protein complex of shell fish wastes. Therefore, the overall objective of this program was to evaluate the ruminal escape potential of crab processing waste and other protein supplements and also to investigate the effect of steam explosion on the nutritive value of crab waste in ruminants.
Chapter II

Review of Literature

Evaluation of Protein Supplements

*Protein Degradation in the Rumen.* Dietary protein entering the rumen is extensively degraded by both bacteria and protozoa (Russell et al., 1991). This degradation involves two steps. Initially, the protein chain is broken by hydrolysis of peptide bonds (proteolysis), resulting in peptides and amino acids (AA). The terms degradation and fermentation are often used interchangeably, but there are differences. Protein degradation is a multistep process involving solubilization, extracellular hydrolysis, transport, and the formation of endproducts (e.g., NH₃, VFA, CO₂, CH₄). The term fermentation refers only to the last few steps, which is a more absolute criterion for complete degradation.

The quality and quantity of ruminal fermentation products are dependent on the types and activities of microorganisms in the rumen. The ruminal microbial ecosystem is very diverse (Hungate, 1966). Russell and Hespell (1981) indicated that the ruminal microbial ecology is complicated by the fact that there are numerous interrelationships among the various ruminal microbes. The complexity
of the ruminal ecosystem has led many nutritionists to conclude that the ruminal ecosystem is so complex that it cannot be understood or described in quantitative terms.

The proteolytic enzymes appear to be associated primarily with the bacterial cell wall with a small amount of cell-free activity probably resulting from cell lysis (Allison, 1970). The protease is present on the outer cell surface and hydrolyzes protein extracellularly (Blackburn and Hullah, 1974). Proteolytic enzymes are associated with many ruminal bacteria, and proteolytic activity of rumen microorganisms is not greatly altered by the diet. Diet can have an effect on protein degradation in the rumen, perhaps indirectly through altering pH and bacterial numbers or types (Allison, 1970).

Protease activity appears to be “trypsin-like” in nature (NRC, 1985). The liberated amino acids after proteolysis may leave the reticulo-rumen, may be utilized for microbial growth, or will be degraded to NH₃ and fatty acids. Amino acids are rapidly degraded in the rumen, and therefore only limited amounts of AA will be available for absorption. Chalupa (1976) reported that the half life of eight essential AA incubated with strained rumen fluid was 2 h or less. Some strains of rumen bacteria require NH₃, even if AA are present in their growth medium. Bacteroides ruminicola can however, utilize peptides from the growth medium, suggesting a lack of transport system for the individual AA across the cell wall (Pittman et al., 1967).

Volatile fatty acids produced by the ruminal fermentation form an important energy source for the ruminant. Acetate and butyrate are well utilized for the growth of the animal, while, propionate is used for gluconeogenesis. Slyter (1976) reported that lactate accumulation leads to ruminal acidosis, lowered fiber digestion, lowered feed intake and in extreme cases death occurs.
Ammonia Production and Microbial Growth. Most ruminal bacteria are able to use NH₃ as a N source for microbial protein synthesis. Normally ruminal protein fermentation produces more NH₃ than the microorganisms can utilize. A portion of the NH₃ will be utilized by microbes, while the other portion may be absorbed through the ruminal wall and carried to the liver where it is converted into urea. A part of the urea thus produced will be recycled back into the rumen through saliva or directly into the rumen through the rumen wall. The remaining portion will be excreted through the urine. Since protein is the most expensive ingredient in most ruminant diets there has been considerable interest in reducing ruminal protein fermentation. Nolan (1975) reported that in most cases ≥ 25% of the protein can be lost as NH₃.

Tamminga (1979) reported that if the protein goes into solution quickly, the rate of enzymatic degradation is often increased. Many of the proteins found in forages and soybeans are very soluble and are degraded rapidly by ruminal bacteria. Heat treatment that can denature the protein can decrease solubility and the rate of protein degradation. Byproduct feeds such as brewers dried grains (BDG), corn gluten meal (CGM), blood meal (BM), and fish meal (FM) contain protein of low solubility.

Carbohydrate has little effect on the rate of protein degradation by extracellular proteinases, but it can greatly affect the end product of AA metabolism (Russell et al., 1983). Russell and Martin (1984) reported that microorganisms that utilize non structural carbohydrate take up peptides at the rate of .07 g of peptide per gram of microorganism per hour, and this N is used for microbial protein synthesis or NH₃ production. The diversion of peptides to microbial protein synthesis or NH₃ production is regulated by the availability of carbohydrate. When there is availability of carbohydrate, 66% of microbial protein
comes from peptides and 34% comes from NH₃ (Russell et al., 1983). All the peptide N will be converted to NH₃ in the absence of carbohydrate.

The capacity of various ruminal bacteria to ferment protein hydrolyzate and produce ammonia was studied by Bladen et al. (1961). They concluded that *Bacteroides ruminicola* was the most important amino acid fermenting bacterium in the rumen of cattle. Russell et al. (1983) reported that when mixed ruminal bacteria were incubated with an excess of casein and with amounts of mixed carbohydrates that were inadequate to support maximum growth rate, there was a linear decline in NH₃ production as the growth rate increased. Studies with ¹⁴N indicated that 34% of the NH₃ was not influenced by carbohydrate availability. Excessive NH₃ production and absorption increases N excretion and the energy cost of urea synthesis.

Microbial colonization of the digestive system of newborn ruminants follows a typical ecological succession in that bacteria proliferate in the fluid phase immediately after birth and colonize the digestive tract (Fonty et al., 1987). These fluid-phase bacteria facilitate the subsequent sequential colonization of the fluid by fungi and protozoa (at 8 to 10 and 12 to 20 d, respectively) which results in the formation of complex multispecies that develop in the fluid, on feed particles, and on tissue surfaces, and result in the complex digestive activities of mature ruminants (Fonty et al., 1988; Stewart et al., 1988).

Cheng and Costerton (1980) reported that the types of microbial populations that develop at various locations of the ruminant digestive tract are complex, and they depend on the chemical microenvironments provided by nutrients. Also, once established, they are very stable and will change only when the nutrients are changed.
Ruminal microorganisms derive most of their energy from the fermentation of carbohydrates, and the ruminal bacteria may be categorized in a general way according to the type of carbohydrate that they ferment (Russell et al., 1992). Microorganisms that ferment cellulose and hemicellulose (SC) grow slowly and utilize NH₃ as N for microbial protein synthesis. Those that ferment starch, pectin, and sugars (NSC) grow more rapidly than those that ferment SC and utilize either NH₃ or AA as a N source. Nocek and Russell (1988) reported that ruminal microbial growth yields are a constant function of DMI or OM digestion. The yield of microbial N per kilogram of TDN was calculated to be 26.12 g (NRC, 1989).

Ammonia N can serve as principal N source for microbial protein synthesis. The concentration of NH₃ N ranged from 5 (Roffler and Satter, 1975) to >20 mg/dL (Mehrez et al., 1977) of ruminal fluid. In the case of rapidly degraded feedstuffs a higher level of NH₃ N concentration may be necessary for maximum digestion and fermentation rates (Hespell and Bryant, 1979). Some microbes require N in the form of NH₃, and the feeding of slowly degraded proteins may create an NH₃ deficiency in the rumen (Klopfenstein et al., 1976), but this can be avoided by urea supplementation to meet microbial NH₃ needs (Klopfenstein, 1976). Higher ruminal NH₃ levels (37 mg/dL) were observed at 12 h in lambs fed a urea control diet.

Lambs fed BM with urea had lower NH₃ N (18.2 mg/dL), compared to natural protein, indicating a lower degradability in the rumen (Stock et al., 1981). Thomas and Beeson (1976) reported that ruminal NH₃ N of steers fed soybean meal (SBM), feather meal (FTM) and hair meal were 14.1, 5.9 and 6.9 mg/dL. Santos et al. (1984) conducted a trial in lactating dairy cows to estimate the protein degradability of SBM, CGM, wet brewers grain and distillers dried grains. It was
observed that ruminal NH$_3$ N concentrations were 10.1, 5.6, 6.9 and 6.4 mg/dL, respectively.

Clark et al. (1992) reported that with ruminal NH$_3$ N > 5 mg/dL, passage of microbial N to small intestine is correlated more highly with organic matter truly digested in the rumen than with the concentration of NH$_3$ in ruminal fluid. Further, the increased proportions of AA passage to small intestine arise from increased intact protein escaping the rumen. They also reported that with typical dairy diets, bacterial CP synthesis peaks at 12 to 13 % dietary CP. 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 contained 74% corn grain. Feeding CGM and BM (combination of 50:50 on N basis) resulted in NH$_3$ N concentrations below 3.3 mg/dL, numerically lower microbial N flows to the duodenum, and lower microbial efficiency compared with urea, SBM and SoyPLUS$^\circledR$ a high ruminal escape SBM (Ludden and Cecava, 1995).

Schloesser et al. (1993) reported that ruminal NH$_3$ N concentration decreased linearly as BM replaced SBM; however, feeding BM did not affect the total N, microbial N, or non NH$_3$ N (NAN) flows to the small intestine. Titgemeyer et al. (1989) reported that replacement of corn starch in diets with protein sources resulted in decreases in efficiency of microbial protein synthesis. Ruminal NH$_3$ N had the greatest increase when SBM was fed. Blood meal supplementation resulted in only nonsignificant increases in ruminal NH$_3$ N. Urea supplementation resulted in the highest ruminal NH$_3$ concentration, whereas BM supplementation resulted in the lowest ruminal NH$_3$ concentration (Lardy et al., 1993).

In a study conducted in four steers with ruminal, duodenal, and ileal cannulas, Cecava et al. (1991), found out that total N flows to the small intestine
were 13.1% greater for a combination of CGM and BM than for SBM because of increased passage of nonmicrobial N. Feeding SBM vs a combination of CGM and BM increased efficiency of microbial CP synthesis (27.3 vs 23.3 g of N/kg of OM truly fermented) and microbial N flow to the small intestine (127.5 vs 112.5 g/d). Microbial growth yields have often been expressed as microbial N or CP per kilogram or 100 g of digested organic matter (DOM).

Hsu et al. (1991a) conducted a study in five ruminally, duodenally, and ileally cannulated sheep to investigate the applicability of using a combination of defaunation with various combinations of N supplements (SBM, CGM, BM, urea, and casein). They concluded that defaunation did not limit the supply of free AA and peptides for ruminal bacterial growth or activity. Defaunation did not have a consistent effect on ruminal microbial deaminase activity (Hsu et al., 1991b), and did not decrease the total free AA concentrations in ruminal fluid, but it altered the AA profile. They also reported that there were no increases in total microbial CP or OM concentrations in ruminal contents even though the defaunation increased the ruminal bacterial numbers.

Rooke et al. (1986) reported that maximum ruminal bacterial protein synthesis was with SBM in place of barley in the basal diet. They also reported that ruminal microbial protein synthesis would be maximally efficient when N concentration was 27 g N/kg DM intake (~16.8% CP) and thereafter the synthesis would drop. Microbial protein synthesis was at the peak when the feed contained 50% ruminal degradable N (Cummins et al., 1983). The microbial protein synthesis exceeded the protein degradation which might be due to the utilization of recycled N. *In Vitro* studies conducted by Strobel and Russell (1986) showed that there was a significant decline in efficiency of microbial protein synthesis at pH values < 6.0.
Factors Affecting Microbial Growth. Efficiency of microbial growth is dependent on the availability of specific substrate for fermentation in the rumen; pattern, composition and rate of substrate availability; and environmental factors (NRC, 1985). Also, microbial flow is dependent on rumen volume/passage and particle size relationships. The factors affecting microbial efficiency are numerous and complex. Forage intake and rumen dilution rate have been shown to have a significant impact on microbial flow (Van Soest, 1982).

Interaction of carbohydrate and protein in the diet has a profound effect on microbial protein synthesis. Harrera-Saldana et al. (1990) determined the influence of synchronization of protein and starch degradation in the rumen of lactating cows using barley or milo as the carbohydrate source and BDG or cottonseed meal (CSM) as the source of protein. Crude protein and starch digestion in the rumen were greater for diets containing barley than milo but were not affected by protein source. Efficiency of microbial protein synthesis in the rumen and microbial CP flow to the small intestine were greatest when a combination of barley and CSM were fed to cows. This indicates that synchronization of rapid fermentation stimulated ruminal microbial protein production.

Stokes et al. (1991) studied the effects of different levels of nonstructural carbohydrate (NSC) and degradable intake protein (DIP) on ruminal microbial protein production in lactating cows. Diets formulated to contain 31 or 39 % NSC and 11.8 or 13.7% DIP, as percentages of DM, produced greater microbial protein synthesis than a diet containing 25% NSC and 9% DIP. They concluded that NSC greater than 24% and DIP greater than 9% will increase the microbial protein flow from the rumen. Maeng et al. (1993) and Taniguchi et al. (1993) also reported a trend that increased levels of rumen available energy and N in diets improved the efficiency of microbial synthesis in the rumen.
Aldrich et al. (1993) formulated diets for cows in early lactation for high and low ruminal availabilities of NSC and CP. A diet containing 36% NSC, of which 80% was digested in the rumen resulted in greatest passage of bacterial N to the small intestine. Decreasing the proportion of NSC to protein digested in the rumen reduced passage of bacterial N. Henning et al. (1993) provided the same daily allowance of energy or protein intraruminally to sheep at 12-hourly pulse-doses or continuous infusion. Better microbial growth was recorded for the continuous infusion, compared to the pulse-infusion. This study indicated that continuous availability of energy is more important to microbial growth than the pattern of energy and N release.

A positive correlation occurs between increased dilution rate and increased microbial growth (Cole et al., 1976). Rumen dilution rate is defined as proportion of total rumen volume leaving the rumen per hour. Cole et al. (1976) observed that there was an increase in dilution rate (3 to 5%/h) along with an increase in microbial protein synthesis (7.5 to 11.8 g/100 g OMD) when steers were switched from an all concentrate diet to one containing 14% roughage.

Dietary S affects microbial protein synthesis. Sulfur is required by the rumen microorganism for the synthesis of methionine and cysteine. Hume and Bird (1970) reported that when sheep were fed a diet supplying .6 g of S per day (N:S = 34.3), 82 g of microbial protein were produced daily in the rumen. Raising S intake to 2 g/d (N:S = 10.9) increased protein synthesis to 94 g/d, but no improvement was noticed when the ratio was narrowed further to 6.4 (3.4 g/d). They reported that the optimum N:S ratio is 10:1 for the microbial growth.

Protein Fractions and Degradation. Dietary protein entering the forestomach is usually extensively degraded by both bacteria and protozoa (Tamminga, 1979). This degradation involves two steps. Firstly, the protein chain
is broken by hydrolysis of peptide bonds (proteolysis) which results in peptides and AA and secondly the deamination of AA. Proteolysis has been reported to be rate limiting (Nugent and Mangan, 1978; Russell et al., 1983). Proteolysis and deamination were both found to be affected by pH. Conflicting results have been reported. Lewis and Emery (1962) reported that the optimum pH for both proteolysis and deamination was between 6 and 7. Hussein and Jordan (1991) reported that at low pH the degradability of N of SBM will be much lower.

Feed protein is partitioned into three fractions: Fraction A (NPN), Fraction B (true protein), and Fraction C (unavailable N) (NRC, 1985). Methods have been developed for assessing the rumen degradability of protein using rate of degradation and rate of passage (Orskov and McDonald, 1979; Mathers and Miller, 1981). Passage rate may be influenced by animal (Poppi et al., 1980), and intake level (Colucci et al., 1982). With high feed intake ruminal degradability decreases (NRC, 1985). Escape protein values of forages differed between years and among months within year (Karges et al., 1992). The extent of degradation can be described as \( A + k_{db} \times B/(k_{db} + k_{pb}) \), where \( A \) is soluble protein, \( B \) is degradable protein, and \( k_{db} \) and \( k_{pb} \) are rate constants for degradation rate and passage rate of degradable protein, respectively. The fraction of total protein that is passed, \( P = k_{pb} \times B/(k_{db} + k_{pb}) + C \), where \( B \), \( k_{db} \) and \( k_{pb} \) are the same as above; \( C \) is unavailable N.

*Methods of Estimation of Ruminal Degradability and Escape Protein.* There are several *in vitro* and *in vivo* techniques that have been used to estimate the degradability of dietary N in the rumen. Degradability experiments with animals are expensive, time consuming and laborious. Therefore many attempts have been made to predict the protein degradability of feeds from relatively simple methods.
In vitro methods based on N solubility (Verite et al. 1979) have been used for the estimation of ruminal protein degradability. Krishnamoorthy et al. (1983) proposed a model using commercial proteases. Mahadevan et al. (1987) and Kohn and Allen (1995) used proteases isolated from mixed ruminal microbes to estimate protein degradability. Mahadevan et al. (1987) reported that accumulation of NH$_3$ and AA in ruminal in vitro incubations may be misleading indicators of protein degradation because protein degradation and microbial protein synthesis occur simultaneously. Broderick (1987) could overcome this handicap by using inhibitors of NH$_3$ and AA anabolism to obtain quantitative recovery of the products of protein degradation. Aufrere et al. (1991) compared the enzymatic technique and a method using solubility in a buffer. The prediction was that the enzymatic was very precise and much better than the solubility method.

An in vitro procedure was developed by Hristov and Broderick (1995) to estimate rate and extent of ruminal protein degradation using $^{15}$NH$_3$ to quantify uptake of protein degradation products for microbial protein synthesis. They reported that microbial protein synthesis was a linear function of extent of degradation.

Chalupa et al. (1991) presented a model-generated protein degradation system which analyzes protein fractions using borate-phosphate buffer, neutral detergent, and acid detergent solutions which separate fractions of protein into A, B$_1$, B$_2$, B$_3$, and C. Ruminal degradabilities of fractions (%/h) were A, instantaneous; B$_1$: 200 to 300; B$_2$: 5 to 15; B$_3$: .1 to 1.5; and C: 0%. Ruminal degradation (K$_d$) of protein is based on literature values where feeds were incubated either in situ or in vitro with proteolytic enzymes. They generated the dietary degradability estimates of protein from the above model. They found that 58% of N of alfalfa haylage was in fraction A, large percentages of protein of high
moisture corn, SBM and BM were in fraction B₂, and 38% of BM protein was fraction B₃. The ruminal degradation of the B₂ fraction is variable depending on rates of passage or turnover rates.

Three enzymes, Pronase E, papain and bromelain were compared for the efficiency of in vitro rumen protein degradation (Tomiankova and Kopecny, 1995). The highest correlation coefficient for protein degradation estimated by the in sacco method compared with the enzymatic method was obtained for the bromelain. Correlation coefficients of linear regression equations for rumen protein degradability estimated by the in sacco method and bromelain were 0.839 for concentrates, .730 for fresh forage, .741 for hays and .876 for silages. They suggested that feeds with high starch content had to be treated with α-amylase to obtain the correlation coefficient of .839.

An in vitro method applying Michaelis-Menton saturation kinetics was developed as an alternative approach for estimating protein degradation rates in the rumen (Broderick and Clayton, 1992). Non-linear regression (NLR) analysis of the integrated Michaelis-Menten equation yielded fractional degradation rates, \( k_d \), from direct estimates of the maximum velocity: Michaelis constant ratio (\( k_d = \frac{V_{\text{max}}}{K_m} \)). They suggested that degradation rates estimated using the NLR method were more rapid than those obtained with a limited substrate approach. The NLR rates were found to be more consistent with the in vivo estimates of rumen protein escape.

The in situ or in sacco method measures the disappearance of feed components from bag containing the test diet after incubation, for variable periods in the rumen of an animal fitted with a rumen cannula. This technique has been used for several years and is the basis of prediction of digestion in several feeding systems (Waldo and Glenn, 1984). Poos Floyd et al. (1985) reported that among
different methods tested for measuring protein quality, the *in situ* method has the highest correlation with *in vivo* degradability at 12 and 24 h. The major criticism of this technique is low repeatability as suggested by the diversity of values obtained by different researchers for similar feed samples (Nocek, 1988). Further, various factors that may affect the results are, bag porosity, particle size, sample size, contamination by rumen bacteria and location of the bag in the rumen.

Lindberg and Knutsson (1981) observed increased loss of DM as bag porosity increased from 10 to 36 μm. A similar trend was reported by Weakly et al. (1983) and Uden and Van Soest (1984). Too small a bag porosity (< 35μm) may cause clogging of pores, bring about a reduction in the solubilization and degradation of the sample and even accumulation of gas in the bag (Nocek and Hall, 1984).

There is controversy as to the degree of particle breakdown associated with microbial digestion (Nocek and Kohn, 1988). Weakley et al. (1983) showed that DM and N degradation were less with coarse (2000 μm) than with finer particle size (520). Ehle et al. (1982) reported that rate of N digestion of many feed ingredients are not affected by particle size. Grinding alfalfa and timothy hay increased digestibility compared to field chopped material (Nocek and Kohn, 1988).

Sample size to bag surface ratio also provides a measure of the appropriate sample size. Uden et al. (1974) showed that cell wall digestibility decreased from 54 to 34% when sample size was increased from 6.5 to 50 mg/cm². Mehrez and Orskov (1977) noticed an increase in DM disappearance from 37.5 to 85% when sample size decreased from 55 to 15 mg/cm². Uden and Van Soest (1984) recommended 6 to 7 mg/cm². Playne et al. (1978) has reported DM degradation of forages varies little if the sample size to surface area ratio remains constant.
Potential contamination of test feed with ruminal microflora has been reported (Kennedy et al., 1984). Concentrate ingredients generally contain little microbial contamination (Mathers and Aitchison, 1981). Extensive DM contamination was found on dacron bags that were incubated for prolonged periods of time in the rumen of steers fed alfalfa hay (Van Milgen et al., 1992). Use of X-ray analysis showed the presence of hydroxylapatite and synthetic Ca-Mg phosphate (whitelokite). Van Milgen et al. (1992) reported that DM contaminant appears as a smooth coating on the dacron fiber, suggesting that contamination was a gradual process. It was found that DM contamination was less than .04 g per bag (average bag weight was 1.2 g) during 10 d of incubation.

The effect of bag location on DM digestibility in cattle involved a reduction of 6 to 10% units from the free to cannula end (Hawley, 1981). The decrease in DMD in cattle as the bags were located closer to the cannula can be attributed to the stratification of digesta resulting in a greater digestive capacity in the ventral than in the dorsal part of the rumen.

The ideal method of estimation of ruminal degradation of feeds is by using a duodenally-cannulated animal by which bacterial and protozoal CP and undegraded intake protein can be separated (NRC, 1985). In this method direct measurement of dietary protein intake and total protein flow at duodenum with indirect measurement of bacterial crude protein (BCP) are measured. Digesta flow is determined by use of an indigestible digesta marker (Zinn et al., 1980). The measurement of digesta flow to the duodenum may be subject to error (NRC, 1985). The amount of undegradable intake protein (UIP) is calculated by the difference. UIP = duodenal protein - BCP. This calculation does not take into account endogenous protein.
Procedures for estimating BCP are by utilizing microbial markers such as nucleic acids, diaminopimelic acid (DAPA), or by aminoethylenephosphonic acid (AEP). Clark (1977) developed a method using one of the radioisotopes, $^{35}\text{S}$, $^{32}\text{P}$, or $^{15}\text{N}$. This BCP estimate using microbial marker is also subjected to error (NRC, 1985). Since endogenous protein is difficult to estimate, usually this is ignored, which may lead to an overestimate of UIP.

Stern and Satter (1982) developed another procedure for the estimation of UIP, which will take care of the endogenous protein. In this method flow of protein to the duodenum is increased by increasing the intake protein in small increments. The disadvantage of this method is that it can be used only when the feed contains high protein.

**Factors Affecting Ruminal Degradation of Protein.** Several factors affect the amount of protein in feed that escapes ruminal degradation (NRC, 1985). Two important factors that determine the ruminal degradation, 1) physical and chemical characteristics of the protein in feed and 2) methods by which they are processed. In general, the soluble N fraction of a feed is degraded more rapidly in the rumen than the insoluble fraction. In the case of SBM a higher proportion of the total N is in the soluble fraction than for BM, CGM, dried distillers grains (DDG), BDG, MBM, and dried distillers grains with solubles.

Sniffen (1974) reported that cereal grains and protein supplements contain four types of protein, albumins, globulins, prolamines, and glutelins. Albumins and globulins are low molecular weight proteins and because of this property they are soluble in ruminal fluid. Prolamines and glutelins are higher molecular weight proteins which render them insoluble in rumen liquor. Nugent and Mangan (1978) reported that proteins that have low solubility in ruminal fluid and that possess
extensive cross linkages like disulfide bonds are less accessible by proteolytic enzymes produced by the microorganisms.

Corn contains no albumin and only 5 to 6% globulins (Sniffen, 1974). The remainder of the protein consists of prolamines and glutelins. Similarly, albumin content in barley is only 3 to 4% and globulins, 10 to 20 %, the major portion of the protein being prolamins and glutelins. Hence, corn and barley contain predominantly prolamins and glutelins which are insoluble and undegradable in the rumen. Distillers grains, BDG, and CGM are mainly produced from corn and barley. Since the soluble proteins are removed in the process of fermentation, mainly undegradable protein fractions will be left in these products.

Losses of lysine and arginine are found to be greater than losses of other AA in the rumen (Craig and Broderick, 1984; Crooker et al. 1986). Craig and Broderick (1984) suggested that microbial enzymes that cleave peptide bonds at lysine residues may be important for regulating protein degradation in the rumen. Cereal grain byproducts such as distillers grains with solubles, DDG, CGM, and BDG contain lower concentrations of lysine and arginine than SBM or byproducts of animal origin. Protein of animal byproducts such as MM, MBM, and FM are generally resistant to ruminal degradation due to the collagen (connective tissue) content in the products which renders them inaccessible to microorganism because of the cross linking (Nugent and Mangan, 1978).

During processing feeds undergo physical changes because of cooking or drying. In the processing of MM and MBM, considerable loss of rapidly degradable protein fractions occurs or they are converted into a insoluble form during cooking grinding and pressing (Bohme, 1982). Bohme (1982) also reported that decreased surface area for microbial attack and random relinkage of bonds on cooling renders these animal byproducts more resistant to ruminal degradation.
Heat applied during drying cereal byproduct feeds also coagulates and denatures the protein which render the protein less soluble and less degradable in the rumen. Heat processing renders the protein undegradable in the rumen. Treatment with chemicals such as formaldehyde to lower the rumen protein degradability of protein supplements have been done (Crooker et al., 1986).

Other factors that affect the rumen degradability of protein are DM intake and associative effect of other feeds. Zinn and Owens (1983) increased the DM intake of steers fed a high-concentrate diet containing corn, dehydrated alfalfa pellets, cottonseed hulls, SBM, and molasses from 1.2 to 2.1% of BW. The protein that escaped ruminal degradation was increased from 44 to 71%. Tamminga et al. (1979) fed lactating dairy cows a mixed diet consisting of long meadow hay and ground, pelleted concentrates that contained a mixture of proteins. They reported that as the DM intake of the feeds was increased from 8.2 to 12.9 kg·cow⁻¹·day⁻¹, the fraction of ruminal nondegradable protein in the diet increased from 29 to 45%.

Owens (1986) observed that as feed intake was increased from 1 to 2% of BW, the quantity of protein in the feed supplemented with SBM that escape ruminal degradation increased from 16.9 to 27.2%. The explanation for this increase in ruminal escape was that the decreased retention time of feed in the rumen resulted in lowered microbial growth which resulted in a lowered degradation of protein. The effect of DM intake on ruminal protein escape was lower when the DM intake was increased from medium to high, compared to lower to medium intake of DM.

Ratio of forage to concentrate in the diet alters the ruminal degradation due to an altered ruminal fermentation (Owens, 1986). He reported that increasing the concentrates from 30 to 60% of the diet increased the ruminal escape of protein.
from 22 to 29%; which is attributed to lower ruminal pH, causing reduction in protein solubility. Another reason for increased ruminal escape might be due to a lowered fiber content in the feed which lowers the microbial mass and hence a lowered microbial degradation of protein.

Dry matter intake is much greater for high-producing cows than for low producing cows, steers, and sheep. The concentrate portion of the diet will be high for dairy cows in early lactation compared to low-producing cows, steers, and sheep. Protein degradability values determined by using low-producing cows will be overestimating the degradability of protein in high producing dairy cows.

Nocek and Russell (1988) indicated that the rate of feedstuff degradation in the rumen will have a profound effect on fermentation end product and animal performance, if, 1) the rate of protein degradation exceeds the rate of carbohydrate fermentation, large quantities of N can be lost as NH₃; 2) the rate of carbohydrate fermentation exceeds the protein degradation rate, microbial protein production can decrease; 3) feedstuffs are degraded slowly, rumen fill will decrease intake and 4) the degradation rate is low, some of the feed may escape ruminal fermentation and pass directly to lower gut.

The fate of the feed is ultimately determined by the relative rates of fermentation and passage (Waldo et al., 1972). Russell et al. (1992) indicated that fermentation rate is an inherent property of the feed and is described by first order kinetics (substrate-limited, enzyme excess) and specific rate constants (hours⁻¹). Passage rates (hours⁻¹) are regulated by feed intake, processing (chopping, grinding, and other means of particle size reduction), and the type of feed that is consumed (e.g., forage vs cereal grain).

Protein Supplements. High resistance to ruminal degradation is seen in by-product protein sources such as BDG, DDG, CGM, FM, BM, and MBM (NRC,
The feeding of optimum undegradable protein in diets seems to be important. Too high a protein supply in the form of highly degradable protein such as SBM may result in poor utilization of the extra protein.

The demand for undegradable protein is less in low-producing animals and more in high-producing animals. According to Waldo and Glenn (1984) undegradability of the feed must be specified in addition to a total protein requirement. As high milk production requires more total protein and available degradable intake protein (DIP), more undegradable protein sources increase the efficiency of protein use.

Many animal byproduct feeds have a high level of undegradable intake protein (UIP), which could be utilized to increase the total UIP concentration in dairy heifer diets as well as high proportion of essential AA that may be required by ruminants (Amos, 1986). Amos (1986) also reported that ADG has been enhanced when UIP percentage in the ration was increased from 36 to 56% while total DM intake did not change. A linear relationship between UIP level and weight gain has been reported in beef cattle (Newbold et al., 1987). Undegradable proteins, when fed as the major N source may sometimes reduce the microbial growth (Polan, 1988).

Corn gluten meal is the residue remaining after removal of the larger part of the starch and germ and separation of the bran (Church, 1991). The nature of protein resembles that of the parent grain in that lysine and tryptophan are usually the most limiting amino acids, but S containing AA are found in higher concentrations than in the grains. Much work has been conducted in feeding of CGM with finishing steers and with lactating cows, considering its higher ruminal escape nature of protein compared to SBM.
Seymour et al. (1992) observed a higher duodenal flow of total N, total protein N, microbial N, and duodenal recovery of ingested N for the SBM and CGM diet, compared to a SBM diet. Higher levels of ruminal NH₃-N, plasma urea, ruminal butyrate and valerate were recorded for the SBM diet. Stock et al. (1981) reported that SBM is degraded to a greater extent than more slowly degraded proteins such as CGM.

Romagnolo et al. (1994) conducted electromagnetic and densitometric analysis on the CGM residues after ruminal incubation for 72 h. The prolamin fraction, zein for corn and CGM were more resistant to ruminal degradation than albumins, globulins, and glutelins. The total degradability of protein fractions of CGM was 18.6%. Zorrilla-Rios et al. (1991) conducted a trial in 80 steers to determine protein-sparing value of the N added to wheat straw during the ammoniation process and to determine the effects of supplementing ammoniated straw diets with energy and ruminal escape protein. Addition of CGM did not alter the straw consumption but ADG was increased by .35 kg. Ruminal and blood N concentration indicated that the N from ammoniated straw was contributing to the ruminal N pool and that CGM was compensating for microbial protein deficiencies postruminally.

Over 1% of the corn crop in the United States is used for manufacturing beer and distilled liquors, and most of the residue, minus part of the starch, is returned as animal feed (Church, 1991). In the manufacture of liquors various cereal grains such as, corn, rye, and malted barley in different proportions are cooked, cooled and fermented. Following fermentation and distillation, solids are recovered and dried. Various feeds produced are, dried distillers solubles, distillers dried grains, and distillers dried grains with solubles. Distillers dried grain account
for the largest volume of distillery byproducts. Distillers dried grains have a protein content of 27 to 29%, which is relatively deficient in lysine and phenylalanine. Phosphorus, S, and Se levels are relatively high.

Protein sources from the beer industry include BDG dried grains, dried spent hops, malt sprouts, and brewers yeast (Church, 1991). Brewers dried grains are the dried residue of the barley malt and other grains that have been used to provide maltose and dextrins for fermenting. Protein content varies from 26 to 29%. Lysine is most limiting, while tryptophan is relatively high in all brewery byproducts. Dried grains have proteins that are resistant to degradation by rumen microorganisms.

Ruminal digestion (predicted from in situ degradation) of BDG, brewers wet grains and SBM was 42, 73 and 83%, respectively (Armentano et al., 1986). This indicates that BDG deliver more protein and DM to the duodenum than isonitrogenous amounts of SBM. Release of N from BDG is slower and more sustained than that from SBM. The sustained release may have greater impact when protein supplement is fed less frequently.

Santos et al. (1984) conducted a trial in lactating dairy cows to estimate the protein degradability of SBM, CGM, wet brewers grain and DDG. It was observed that ruminal NH3 N concentrations (mg/dL) were 10.1, 5.6, 6.9 and 6.4 respectively. Protein degradation in the rumen was higher for the SBM diet (70%) than for CGM (45%), wet brewers grain (WBG) (52%) and DDG (46%) diets. Apparent absorption of AA from the small intestine was 70, 77, 71 and 66% of the AA entering the duodenum for the SBM, CGM, WBG and DDG diets, respectively. Actual amounts absorbed (g/d) were lowest for the SBM diet. It was
concluded that diets containing CGM, WBG and DDG will supply more AA to the intestine than a diet containing SBM.

Firkins et al. (1983) reported that non-NH₃ non-bacterial N reaching the duodenum was lowest for steers fed a urea-supplemented diet (20.9 g/d) whereas the values were higher for wet distillers grain (64.1 g/d) and dried distillers grain (74.7 g/d) when compared to wet corn gluten feed (41.3 g/d) and dry corn gluten feed (32.7 g/d). They reported that percentage protein apparently escaping rumen fermentation was greater for steers fed wet distillers grain (47%) or dried distillers grain (54%) than for those fed wet corn gluten feed (26%) or dry corn gluten feed (14%).

In a study conducted in Holstein cows, Cozzi and Polan (1994) supplemented the diet with SBM alone or with CGM or BDG. Ruminal isovalerate was found to be highest in the diet supplemented with CGM because of its high leucine content. Dried brewers grain gave good production response due to a more favorably balanced AA profile in the ruminally undegraded protein.

Hydrolyzed poultry feathers is the product resulting from the treatment under pressure of clean, undecomposed feathers from slaughtered poultry free of additives, and/or accelerators (Boehme, 1982). Not less than 75% of its CP content must be digestible by the pepsin digestible method. The American rendering industry in 1978 produced 318,000 t of FTM (Boehme, 1982). In the process of preparation of hydrolyzed FTM, the feathers are cooked under steam pressure for 30 to 45 min and the resulting slurry is cooked for an additional hour to drive off excess moisture. The material is then transferred to a steam tube or hot air dryer where the material is dried to 6 to 8% moisture (Ellingson, 1993).
Feather meal is limiting in methionine, lysine, histidine, and tryptophan, but it has a greater amount of several other AA, particularly cystine (Baker et al., 1981). Cystine disulfide bonds within the keratin contribute to the insolubility and indigestibility of the protein. Therefore, these bonds have to be cleaved before feather protein can be digested by animals (Moran et al., 1966). Extensive studies have been conducted with FTM as a source of protein in poultry diets. Feather meal has been used as a protein supplement for ruminants too. Jordan and Croom (1957) reported that FTM could replace SBM as a supplement to a corn and hay diet without reducing lamb performance.

Karges et al. (1992) fed steers on summer pasture with increasing levels of ruminally degradable (.15, .27 and .37 kg/d) and escape protein (.07, .14 and .21 kg/d) replacing a cornstarch and molasses (energy control) supplement. Treated SBM and FTM were the sources of escape protein. Corn steep liquor supplied the degradable protein. They observed that low level of degradable protein produced numerically the greatest ADG (1.96 kg), compared to the high level (.96 kg). A linear increase in ADG was also observed in steers fed escape protein in addition to ruminally degradable protein (1.02 vs 1.12 kg). An in vitro study indicated that more microbial protein was synthesized from the energy supplement than from the degradable protein. As level of corn starch mixture decreased in the supplement and level of steep liquor increased, gains by steers fed degradable protein treatments tended to decline.

Thomas and Beeson (1977) reported that N digestibility was reduced from 68% to 59% when FTM replaced SBM in the diet. Digestibility of protein from SBM, FTM and hair meal were 90, 62.8 and 49.7%, respectively. Dry matter and gross energy digestibility tended to be higher on the SBM supplemented diet.
However, steers fed the FTM protein supplement retained a higher percentage of absorbed N than those fed SBM. Church et al. (1982) reported that protein digestibility was reduced by 5.7 and 8.5 percentage units in two digestion studies when FTM replaced 70% of the SBM protein in the diet. The replacement of SBM by FTM may have reduced the amount of protein degraded in the rumen and ruminal NH₃ concentration may have been reduced below the optimal level for maximal ruminal digestion.

Ruminal NH₃ concentration of animals fed SBM, FTM and hair meal were 14.1, 5.9 and 6.9 mg/dL, respectively (Thomas and Beeson, 1977). The ruminal NH₃ concentration of animals fed with the SBM supplement diet were higher at 1, 2, 4 and 6 h. If the ruminal NH₃ values appear to be too low DM and energy digestibility may have been limited by the lack of N (Klopfenstein et al., 1976). Satter and Roffler (1975) reported that ruminal NH₃ N levels greater than 6 mg/dL ruminal fluid may have had no beneficial effect on N balance.

When growing calves were fed a basal diet of corn cob:sorghum silage (61:39) and were individually supplemented with DDG, heat damaged DDG, feather meal (FTM), or urea, the plasma concentration of methionine responded linearly and quadratically to increased escape protein (Gibb et al., 1992). There was a linear and quadratic decrease in plasma concentration of lysine with an increase in supply of DDG. They reported that plasma AA responses may identify AA that become limiting with increasing escape protein. Of the essential AA, threonine, valine and isoleucine were more resistant to ruminal degradation; methionine, cysteine, histidine and arginine were more extensively degraded than the total AA supply (Titgemeyer et al., 1989).
Blood meal, conventional cooker dried is produced from clean fresh animal blood, exclusive of all extraneous material such as hair, stomach belchings, and urine except in such traces as might occur unavoidable in good manufacturing processes (Boehme, 1982). The protein content of BM is about 85 % and is a good source of AA except isoleucine (Church, 1991). Blood meal is low in minerals except iron. By virtue of its low rumen degradability of protein, the material is a potential protein supplement for high producing dairy cows.

Titgemeyer et al. (1989) reported that replacement of corn starch in diets with protein sources resulted in decreases in efficiency of microbial protein synthesis. Ruminal NH₃ N had the greatest increase when SBM was fed. Blood meal supplementation resulted in only a nonsignificant increase in ruminal NH₃ N. Soybean meal had the lowest proportion of N escaping ruminal degradation (21%). Corn gluten meal-N (86%) and BM-N (92%) escaped ruminal degradation to the greatest extent, and FM-N was intermediate (68%). They also reported that absorbable non bacterial N from the small intestine followed the same trend, 13, 69, 68 and 50%, respectively for SBM-N, corn gluten-N, BM-N, and FM-N, respectively.

Lambs fed BM with urea had lowest ruminal NH₃ N (18.2 mg/dL), indicating a lower digestibility in the rumen (Stock et al., 1981). Lardy et al. (1993) reported that urea supplementation resulted in the highest ruminal NH₃ concentration, whereas BM supplementation resulted in the lowest ruminal NH₃ concentration. Blood meal resulted in greater dietary protein flow to the duodenum than the other treatments. They also reported that the ruminal escape value of rapeseed meal was greater than that of SBM but less than BM.

Schloesser et al. (1993) did not find any advantage of feeding BM over SBM in sheep fed grass hay diets. They reported that ruminal NH₃ N
concentration decreased linearly as BM replaced SBM; however, feeding BM did not affect the total N, microbial N, or non NH₃ N flows to the small intestine. They also reported that the different dietary treatments (SBM, 2/3 SBM:1/3 BM, 1/3 SBM:2/3BM; or BM) had no influence on ewe BW or body condition score changes. Protein source did not influence serum protein, urea N, creatinine, glucose, or nonesterified fatty acid concentrations.

Rusche et al. (1993) reported that when cows were fed a diet that contained high CP (150% NRC recommendations) which contained gluten meal/BM (high escape) as the principal supplemental protein source, there was an increase in ADG as well as a decrease in plasma glucose and urea N. They also reported that calves had an increased ADG from 0 to 97 d. Calves did not have access to cows' diet and they assume that the increased milk production might be one of the reason for having an increased ADG. In contrast, Wiley et al. (1991) reported no effect on milk production or gain in calves from supplying additional CP from a high-escape source.

In a study conducted in Holstein calves, Swartz et al. (1991) observed that calves fed the highest undegradable protein (BM) diet had the least amount of CP intake, 553 g/d compared to 589 g/d for the lowest undegradable protein. Feed efficiency (kg feed/kg gain) was increased with increasing rumen undegradable protein, 3.99, 3.77 and 3.39, respectively, for low, medium and high undegradable protein intake.

Petersen et al. (1982) reported that escape protein from BM did not improve winter weight gains in cows or steers. Cattle performed similarly when they were fed 40% CP supplements formulated with BM-urea and SBM-urea although BM-urea contained three times the quantity of escape protein as the SBM-urea.
supplement. The escape protein values were calculated to be 16.5 and 54.2%, respectively for SBM-urea diet and BM.

Meat meal is defined as the dry rendered product from mammal tissues, exclusive of hair, hoof, horn, hide trimmings, manure and stomach contents, except in such amounts as may occur unavoidably in good factory practice (Boehme, 1982). It should not contain added BM, and not more than 14% pepsin indigestible residue, and not more than 11% of the CP in the product shall be pepsin indigestible. In the case of MBM the only difference from MM is that it may contain more than 4.4% P. Usually the products are marketed to contain 45, 50, 53 to 55 % CP (as fed). The protein quality is generally considered to be lower than that of FM because of lower isoleucine (Church, 1991).

Anderson et al. (1988), in a study conducted in eight ruminally-cannulated heifers on wheat pasture, recorded an increase of 39% DM intake (expressed as percentage of BW) by the supplementation of MM. There was no effect on N balance by supplementation of MM. The ruminal escape of MM was estimated to be 56 % (Vogel et al., 1988). Loerch et al. (1983) reported that percentage of N escape of MBM was 49.3 compared to 28.7 that for SBM.

Fishmeal is defined as the clean, dried, ground tissue of undecomposed whole fish or fish cuttings, with or without extraction of part of oil (Church, 1991). It must not contain more than 10% moisture and, if it contains more than 3 % salt, the amount of salt must be specified and in no case should salt content exceed 7%. Fish meals are excellent sources of proteins and essential AA especially lysine which is deficient in cereal grains. They are a good source of B-vitamins and most of the minerals. Therefore, FM is an excellent protein supplement for swine and poultry feeds. Fish meal is being used in ruminant feeding also by virtue of its slowly degraded protein fraction (NRC, 1985).
Fish meal is processed by cooking, pressing, drying and grinding of fish (Barlow and Windsor, 1983). Fish is cooked to coagulate proteins, free water and fat from the tissues, and to sterilize the material. Cooked fish is then pressed to remove the water and fat. The pressed material is then dried to about 90% DM. Barlow and Windsor (1983) reported that CP content of FM varied from 60.4 to 72% and is higher in long chain (20 carbon atoms or more) polyunsaturated fatty acids than vegetable fat. They also reported that FM is rich in essential AA specially S containing amino acids. Although there are differences in CP content among FM of different origin, AA profiles are similar.

Ruminal CP degradation of FM ranges from 30 to 70% (Orskov et al., 1971). The ruminal CP degradation of FM depends on several factors such as, storage period of the raw fish before processing, dryer types, formaldehyde addition, heating duration and proportion of fish solubles added to the meal (Kaufmann and Løpping, 1982).

Growing lambs fed FM diets had higher ADG than those fed SBM diets (Orskov et al., 1970; Adam et al., 1982). Beerman et al. (1986) reported a 16% increase in ADG when FM replaced approximately one-third of SBM protein in corn-based diets. In contrast, Hussein and Jordan (1991) did not observe any difference in ADG when growing wether lambs were fed FM or SBM. Oldham and Smith (1982) also reported greater ADG for young growing heifers (weighing less than 200 kg) fed diets supplemented with FM than those fed diets supplemented with urea or SBM. In contrast, Steen (1989) did not find any difference in ADG or feed efficiency in finishing steers (weighing 450 kg) when FM was replaced with SBM or sunflower meal.

Donaldson et al. (1991) reported that when steers on pasture were fed high ruminal escape protein (FM and dried brewers yeast), low ruminal escape protein,
or corn, the escape protein flows were 1137, 1027 and 844 g/d, respectively. Similarly, abomasal non NH₃ N (NAN) tended to be greater for steers receiving high ruminal escape protein. Non NH₃ N flows were 1044, 955 and 777 g/d for steers receiving high ruminal escape protein, low ruminal escape protein and corn, respectively. The results show that escape protein can increase post ruminal protein flow and also showed that there were no adverse effect on fiber digestion in steers grazing annual rye pasture.

Feeding diets supplemented with either SBM or FM to cows in early lactation resulted in similar milk yields (Zerbini et al., 1988; McCarthy et al., 1989). Their studies using ruminally and intestinally cannulated dairy cows indicated that replacing SBM with FM in the diet did not affect NAN flow to the small intestine. This showed that the increased duodenal flow of dietary N as a result of FM supplementation was balanced by a depression in ruminal microbial protein synthesis. Orskov et al. (1981) fed high-producing dairy cows in early lactation with high- or low-concentrate diets supplemented with groundnut meal or FM. Milk yield was not affected when FM supplemented high-concentrate diets, but was increased by 1.5 kg/d with the low-concentrate diets. The explanation given for this effect was due to the increased dietary AA flow to the duodenum and (or) improved ruminal fiber fermentation through a continuous release of nitrogenous substrates from FM to the ruminal microbes.

Milk protein yield increased, 61, 95, and 130 g/d with SBM, high soluble FM, and low soluble FM versus no supplement in dairy cows (Broderick, 1991). They also reported that ruminal escape protein (estimated in vitro) was 27% (SBM), 43% (high soluble FM), and 63% (low soluble FM). The results indicated that greater rumen escape of FM protein, relative to SBM, increased efficiency of protein utilization in lactating cows fed alfalfa silage. Fish meal supplementation
increased dietary N intake and significantly increased the flow of total N, NAN and AA at the duodenum (Dawson et al., 1988). Shqueir et al. (1984) reported that the feeding of liquified fish (LF) resulted in increased rumen fluid-N (NH₃ N) (16.2 mg/dL) compared with cottonseed meal (CSM) (9.6 mg/dL) and feather meal (FM) (10.6 mg/dL).

**Combinations of Different Protein Supplements.** In a study conducted in four lactating Holstein cows fitted with duodenal cannula, Erasmus (1994) fed different protein supplements, BM, CGM, a combination of BM and CGM and sunflower meal. They observed that there were no differences in DM intake or in milk yield. Ruminal NH₃ N concentration was higher for the sunflower diet, while molar percentage of propionate decreased with the BM diet. Bacterial N flows were 46.0, 45.3, 46.4, and 65.8% of NAN for the respective diets. The essential AA profiles of the duodenal digesta and duodenal flow of individual AA closely reflected the AA composition of the respective protein supplements, indicating that the composition of rumen undegradable protein profoundly affected the composition of protein entering the intestine when supplemental protein provided 35% of total CP intake.

Grummer et al. (1994) reported that Holstein cows fed a combination of MBM plus BM had a higher milk yield (45.5 kg/d) over those received raw soybeans (43.2 kg/d). Increasing the ratio of undegradable to degradable dietary protein also increased yields of milk protein and fat, but, DM intake was not influenced by treatment. A growth trial conducted by Blasi et al. (1991) with grazing calves using combinations of BM and FTM showed a complementary effect in calves fed 87.5:12.5 FTM:BM on a CP percentage basis. Calves fed the control diet that contained urea gained .38 kg/d, whereas, maximal gain due to protein supplementation was .71 kg/d. They attributed the increase in gain above
the control to the ruminal escape protein supplied by FTM and BM. Goedeken et al. (1990) reported that the escape protein value for FTM (69.1%) was less than the values for BM (82.8%) and CGM (80.4%) but greater than that for SBM (26.6%). They also reported that protein efficiency was similar for BM:CGM, BM:FM and BM:FM:CGM combinations.

Sindt et al. (1992) reported that calves supplemented with a combination of BM/FTM/urea were 6% more efficient during the first 41 d than calves supplemented with urea alone. At 76 d and by the end of the trial (164 d) calves fed diets supplemented with natural protein and urea were 7 and 6% more efficient, respectively, than calves fed diets supplemented with urea alone (Sindt et al., 1993). Calves fed dry-rolled corn or high-moisture corn basal diets supplemented with BM25/FTH25/urea50 were 10 and 8% more efficient, respectively, than calves supplemented with urea alone.

Crab Processing Waste

Crab processing waste (CPW) is a good source of protein for ruminants. It can be utilized for feeding of animals either as dehydrated meal (Patton et al., 1975; Velez et al., 1991) or by ensiling with roughage (Samuels et al., 1991; 1992). Low digestibility for the crab waste straw silage (55%) compared to fish waste straw silage (83.6%) was observed by Samuels et al. (1991). There was a lower rate of ruminal degradation and/or deamination of the acetic acid treated CPW silage. It is expected that CPW could be a potential source of escape protein supplying AA to the lower intestinal tract. Apparent digestibility of DM, OM, CP, energy, NDF, cellulose, and hemicellulose of silage containing CPW decreased
linearly with increased levels of CPW (Ayangbile, 1989). The decreased digestibility of CPW was due to the very low digestibility of chitin (15 to 29%).

Samuels et al. (1991) observed that urinary N excretion was greater in sheep fed fish waste silage diets than by those fed CPW silage diets. As a percentage of absorbed, N retention was greater (27.9%) by sheep fed crab waste-straw silage (60:40) when compared to 9.7% of retention for those fed diets containing fish waste-straw silage (70:30). Ayangbile (1989) reported that N retention expressed as percentage of intake or absorbed, was similar among the sheep fed diets with different levels of crab waste straw silage.

Ayangbile (1989) reported that N intake increased linearly (P < .05) with level of CPW. Total N excretion increased linearly with level of crab waste straw silage. Nitrogen retention, expressed as g/d, increased linearly with level of crab waste-straw silage. This is in agreement with the study of Abazinge et al. (1994). Lubitz et al. (1943) stated that the quality of N from shell fish waste is superior to that from fish waste.

Ruminal NH₃ N was lower for sheep that were fed the acetic acid treated crab waste silage, compared to those on molasses treated crab waste silages (Abazinge et al., 1994). Patton and Chandler (1975) found that solubility of DM and CP of crab meal in the rumen ranged from 25 to 42% and 37.5 to 50.2%, respectively.

The chitin-N forms about 20% of total N content of CPW (Lovell et al., 1968). Chitin, which is a component of exoskeleton of crustaceans is a (1-4)-β-linked homopolymer of N-acetyl-D-glucosamine, an analogue of cellulose (Figure 1), which is less digestible than cellulose in ruminants (Velez et al., 1991). The deacetylated chitin is chitosan. The lower digestibility of crab processing waste diets may be due to chitin. Chitin has been shown to be resistant to degradation by
microorganisms in the gastrointestinal tract of ruminants (Morgavi et al., 1994). The CP digestion coefficients for the crustacean waste meal diets were 8 to 18% lower than that for an isonitrogenous diet with SBM as the protein source.

Steam Explosion

The steam explosion process has been studied widely as it is an effective method to increase the accessibility of cell wall components (Chornet and Overend, 1985; Kokta, 1988). The process as it is now practiced is best characterized as a "flash hydrolysis". The steam explosion method is a thermo-mechano-chemical process in which the combined presence of heat (as steam), mechanical forces (shear effects) and chemistry (as hydrolysis) results in massive disruption of the lignocellulosic structure. The process combining thermal,
mechanical and chemical effects can be traced back to the early patents of Mason (1929).

In the steam explosion technique the material is introduced into the reactor and heated under steam pressure of 20 to 50 bars (unit of pressure, bar = one million dynes / cm²) and 200 to 260°C for a few minutes (Toussaint et al., 1991). During this process the hemicelluloses are hydrolyzed and become water soluble, the cellulose is slightly depolymerized, the lignin melts and is depolymerized. The reaction is immediately stopped by opening the reactor to depressurize the material which can be expelled through a bar nozzle. Materials such as poplar wood (Toussaint et al., 1991), wheat straw (Sciaraffia and Marzetti, 1988; Beltrame et al., 1992) and other lignocellulosic materials (Greethlein and Converse, 1991) have been studied.

Augustin et al. (1991) reported that steam explosion technique could alter profoundly the microstructure of cellulose. A gel type compound was formed. This shows that physical action of this method induces a structural change to the cellulose. Kamide and Okajima (1987) have shown that when an aqueous suspension of cellulose was treated for a very short time at high temperature (~250°C) and the pressure suddenly released by opening a discharge valve, the cellulose undergoes structural changes, which were not identified as H-bonding modifications.

The changes were probably induced by structural H-bond or shifts due to enhanced water penetration between adjacent cellulose molecules. This effect was due to the sudden decompression. They also noticed that direct exposure to 250°C without decompression did not lead to structural changes.
The explosion treatment process results in a profound change in the enzyme accessibility, compared to the untreated wood (Maschessault and St-Pierre, 1980). They also claimed that the morphological modification involving disruption into microfibrillar elements of the cell wall as well as the fiber fracture would be responsible for this phenomenon. Steam explosion process leads to a major increase in the enzyme accessible surface. This factor is responsible for enhanced enzymatic degradation of steam exploded material.

Sciarraffia and Mazetti (1988) conducted an in vivo digestion trial with steam exploded wheat straw with sheep for 4 wk. The straw was steam exploded at 210°C for 2 min. Due to high moisture content, the steam exploded material was conserved as alkaline silage (adding urea). The digestibility of steam exploded straw was up to 58% compared to 45% to that for untreated straw. It was also showed that the steam explosion did not negatively affect the blood parameters considered. There was an increase in the nutritional value and palatability of straw. It was found from the in vivo studies, that steam exploded wheat straw could be a promising feed for ruminants because of its better palatability.

Chitin-protein matrix (Complex) of crab cuticle shows a supra molecular organization geometrically comparable to a "Twisted Plywood" appearance (Bouligard, 1972). Fully grown crab cuticle is composed of four distinct superimposed layers, the outer epicuticle with a layer of lipoprotein coating and the three layers of procuticle whose general structure is stratified. The organic fibril forming the procuticle are composed of chitin crystallites and protein (Giraud-Guille and Bouligard, 1986). They also reported that a chitin-protein microfibril structure consists of a crystalline chitin core surrounded by a sheath of ordered proteins. Chitin is considered as forming clear rods embedded in a dark
protein matrix, but there is high variation, depending upon the chitin content (Hackman, 1984)

Covalent links between chitin and protein occur via aspartic acid (Hackman, 1984). Brine and Austin (1981) showed that predominant AA in the residual chitin after vigorous alkaline hydrolysis were aspartic acid, serine and glycine which may be involved in the chitin-protein linkage.

A part of N in the shell fish waste is present in the chitin which may not be available to the animal. Lovell (1968) reported that crayfish contained 40% CP. The extracted chitin contained 19.8% of the total N of the waste. Only 32.2% formed the corrected CP. This value is similar to the value of 32.7% CP obtained for crab meal (Lubitz et al., 1943). The mean digestion coefficients of CP for crayfish was lower (70.2), compared to soy protein (85.7) when the diet containing 8% of either of the above ingredient was fed to rats. Ayangbile (1985) reported that apparent digestibility of DM, OM, CP, Energy, NDF, ADF, cellulose and hemicellulose in silage containing crab waste decreased linearly (P < .01) with increased level of crab waste straw silage.

From the foregoing literature it is seen that as the chitin content in the feed increases there is a linear decrease in the overall digestibility. If chitin complex could be cleaved the nutrients would be available to the animal. Therefore, the proposed research was to explore the possibilities of any structural changes in chitin by steam explosion techniques so that the material would be better utilized by ruminants.
Objectives

The overall objective of the research was to evaluate the ruminal escape potential of crab meal and other protein supplements and to study the effect of steam explosion on physico-chemical characteristics of crab processing waste and rumen degradability.

Specific objectives:

I. To determine the relative value of different protein supplements included in high-roughage diets on:
   - Performance of cattle
   - N utilization by lambs
   - Blood urea and ruminal ammonia
   - Ruminal degradability of DM and protein

II. To investigate the effect of steam explosion on:
   - Ruminal degradability of DM and chitin
   - Ruminal escape potential of crab meal
Chapter III. Journal Paper 1. Effects of Feeding Crab Processing Waste and Other Protein Supplements on Growth and Ruminal Characteristics of Steers Fed High-Roughage Diets

Abstract: A growth trial was conducted with 48 Angus x Hereford and Angus x Simmental steers (avg. BW, 223 kg) for 126-d to study the relative value of crab meal and other protein supplements included in high roughage diets on growth and feed efficiency. Steers were blocked by weight and breeding into eight blocks, and randomly allotted within blocks to six supplements (eight steers per treatment). Diets were formulated to contain 10.5% CP and 63% TDN, DM basis. Ingredients were orchard grass hay, cottonseed hulls, corn grain, and protein supplements. The six supplements were, 1) soybean meal (SBM), control; 2) supplement based on industrial byproducts of both plant and animal origin (IPA); 3) experimental supplement based on byproducts of animal origin (ESA); 4) hydrolyzed supplement No 3. (HESA); 5) commercial supplement based on animal protein (CS), Pro-Lak®; and 6) crab meal (CM). In each diet, one third of the N was supplied by the protein supplement. Steers were individually full fed during a 16 h period daily. Average daily gains were 1.16, 1.13, 1.23, .98, 1.26 and 1.19 kg,
respectively, for the cattle fed the six supplements. Gain/feed values were .138, .148, .141, .118, .147, and .146, respectively. There were no significant positive responses (P > .05) for any of the protein supplemented groups over those fed SBM. However, there was a trend (P < .1) for a positive response in gain/feed for steers fed CS and IPA, compared to SBM fed steers. Lower (P < .05) weight gain and feed efficiency were recorded for the cattle fed the HESA supplement. Steers fed ESA and CM had intermediate gain/feed values. Steers fed the CM diet had a numerically higher growth and feed efficiency than those fed the SBM diet. The blood urea N and ruminal pH at 56 d and 126 d of the trial showed no significant differences (P > .05) among the treatments. At 126 d ruminal NH$_3$N was higher (P < .05) for the steers fed SBM than for those fed the IPA and HESA supplements. Blood urea was numerically highest for the steers fed the ESA and lowest for those fed the HESA. There were no differences (P > .05) in the total VFA, propionate, isobutyrate, butyrate, or valerate concentrations. Numerically, highest ruminal acetate concentration (72.4 mol/100 mol) was for the steers fed CM. Substituting other protein supplements used in this study for SBM did not elicit a significant positive response in weight gain or gain/feed in growing steers on a high-roughage diet. Crab meal was comparable to SBM or other products as a protein supplement for steers.

Key Words: Protein Supplements, Steers, Feed efficiency, VFA

Introduction

It has been shown that quantity of microbial protein synthesized in the rumen is inadequate for rapidly growing steers (Chalupa, 1975) or high producing dairy cows (Santos et al., 1984; Keery et al., 1992). There may not be any
any advantage in increasing the protein content which is highly degradable in the 
diet for high producing animals and fast growing steers, unless the diet contains 
sufficient energy for maximum microbial growth. Diets supplemented with protein 
Sources that will degrade slowly in the rumen has improved N and amino acid 
(AA) flows to the small intestine. (Santos et al., 1984; Stern et al., 1985; 
Titgemeyer et al., 1989; Coomer et al., 1993; Ludden and Cecava, 1995).

Several byproduct feeds of plant, animal and marine origin have been 
shown to be good protein supplements for high-producing animals due to 
resistance to ruminal degradation. Most of these byproducts are deficient in one or 
more essential AA. Corn gluten meal (CGM) and brewers dried grains (BDG) are 
deficient in lysine, while fish meal (FM) is rich in lysine. Microbial protein has 
been found to be most limiting in lysine, methionine, and threonine for growth in 
steers (Richardson and Hatfield, 1978). Therefore, these deficiencies have to be 
met by supplementing the diet with protein sources which can provide these AA. 
Studies have been conducted to elicit the complimentary responses by feeding 
combinations of the rumen undegradable protein sources such as blood meal (BM) 
and CGM (Stock et al., 1981), or BM and feather meal (FTM) (Goedeken et al., 
1990; Blasi et al., 1991).

Another protein source, crab processing waste (CPW), a byproduct 
obtained from seafood industry, has been shown to be a good protein supplement 
for ruminants. Crab processing waste can be fed either as a dehydrated meal 
(Patton et al., 1975; Velez et al., 1991) or by ensiling with roughage (Samuels et 
degradation and/or deamination for the acetic acid treated crab waste silage. This 
property reflects its potential as a source of escape protein.
The objective of this study was to evaluate crab processing waste and other protein supplements on growth, feed efficiency, and ruminal and blood parameters in steers.

Experimental Procedures

Forty eight Angus x Hereford and Angus x Simmental steers (avg. BW 233 kg) were used in a growth study. Steers were blocked by weight and breeding into eight blocks and were randomly allotted within blocks to six diets containing the following protein supplements: 1) soybean meal (SBM), control; 2) supplement based on industrial byproducts of both plant and animal origin (IPA); 3) experimental supplement based on byproducts of animal origin (ESA); 4) hydrolyzed supplement No 3. (HESA); 5) commercial supplement based on animal protein (CS), Pro-Lak®, and 6) crab meal (CM).

The hydrolyzed supplement No 3. (HESA) was processed in a high intensity mixer and hydrolyzed under basic conditions at atmospheric pressure for approximately 10 min. Temperature of the product reached 110°C (Meacham, 1995). Protein supplements, IPA, ESA, and HESA were obtained from Harmony Products Inc. Chesapeake, Virginia. The commercial supplement (CS) is manufactured and marketed as Pro-Lak® by H. J. Baker and Bro. Inc. N.Y, NY. Crab meal was obtained from Graham and Rollins, Hampton, Virginia. The other ingredients were orchard grass hay, cottonseed hulls, corn grain and mineral and vitamin supplements. The mixed protein supplements, IPA, ESA, and HESA were formulated as shown in Table 1. The chemical composition of protein supplements and other ingredients are presented in Table 2. The CP content of protein supplements used in this study was 24.0, 68.1, 64.3, 76.6, 38.4, and 49.3%,
### Table 1. Composition of mixed protein supplements\(^a,b\)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground corn grain</td>
<td>17.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wheat middlings</td>
<td>31.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>8.75</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Distillers dried grains</td>
<td>5.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Feather meal</td>
<td>-</td>
<td>73.20</td>
<td>73.20</td>
</tr>
<tr>
<td>Blood meal</td>
<td>8.75</td>
<td>9.15</td>
<td>9.15</td>
</tr>
<tr>
<td>Megalac(^d)</td>
<td>20.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fat(^e)</td>
<td>-</td>
<td>9.15</td>
<td>9.15</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Monoammonium phosphate</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Yea-sacc(^f)</td>
<td>.50</td>
<td>.50</td>
<td>.50</td>
</tr>
<tr>
<td>Niacin(^g)</td>
<td>.30</td>
<td>.30</td>
<td>.30</td>
</tr>
<tr>
<td>Dairy flavor(^h)</td>
<td>.15</td>
<td>.15</td>
<td>.15</td>
</tr>
</tbody>
</table>

\(^a\) As fed basis
\(^b\) Harmony Products Inc. Chesapeake, VA.
\(^c\) IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.
\(^d\) Ca salts of palm oil fatty acids, Church and Dwight Co., Inc., Princeton, NJ.
\(^e\) Mixture of animal and plant fats
\(^f\) Alltech Inc.,
\(^g\) 2767 mg/kg
\(^h\) Feed flavor, Inc.
Table 2. Chemical composition of ingredients used for the formulation of the diets for the steers\(^a\)

<table>
<thead>
<tr>
<th>Feed(^b)</th>
<th>DM</th>
<th>OM</th>
<th>CP</th>
<th>NDF(^c)</th>
<th>ADF</th>
<th>Lignin</th>
<th>Cellulose</th>
<th>Ash</th>
<th>Ca</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay</td>
<td>89.0</td>
<td>93.9</td>
<td>9.3</td>
<td>68.5</td>
<td>45.3</td>
<td>9.1</td>
<td>35.4</td>
<td>6.1</td>
<td>0.38</td>
<td>0.25</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>86.9</td>
<td>97.2</td>
<td>5.2</td>
<td>93.0</td>
<td>66.8</td>
<td>12.4</td>
<td>55.4</td>
<td>2.8</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>Corn</td>
<td>90.3</td>
<td>98.6</td>
<td>9.2</td>
<td>-</td>
<td>3.2</td>
<td>1.0</td>
<td>2.5</td>
<td>1.4</td>
<td>0.02</td>
<td>0.25</td>
</tr>
<tr>
<td>IPA</td>
<td>90.2</td>
<td>87.0</td>
<td>24.0</td>
<td>-</td>
<td>5.1</td>
<td>1.5</td>
<td>3.4</td>
<td>13.0</td>
<td>1.80</td>
<td>0.96</td>
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<tr>
<td>ESA</td>
<td>93.0</td>
<td>88.9</td>
<td>68.1</td>
<td>-</td>
<td>20.3</td>
<td>-</td>
<td>-</td>
<td>11.1</td>
<td>1.28</td>
<td>0.83</td>
</tr>
<tr>
<td>HESA</td>
<td>92.3</td>
<td>76.5</td>
<td>64.3</td>
<td>-</td>
<td>9.7</td>
<td>-</td>
<td>-</td>
<td>23.5</td>
<td>1.43</td>
<td>1.17</td>
</tr>
<tr>
<td>CS</td>
<td>92.0</td>
<td>88.0</td>
<td>76.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.0</td>
<td>2.68</td>
<td>1.56</td>
</tr>
<tr>
<td>CM</td>
<td>93.3</td>
<td>54.6</td>
<td>38.4</td>
<td>-</td>
<td>15.7</td>
<td>-</td>
<td>-</td>
<td>45.4</td>
<td>11.68</td>
<td>1.47</td>
</tr>
<tr>
<td>SBM</td>
<td>88.6</td>
<td>92.6</td>
<td>49.3</td>
<td>-</td>
<td>12.8</td>
<td>2.0</td>
<td>9.1</td>
<td>7.4</td>
<td>0.36</td>
<td>0.56</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>97.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>23.99</td>
</tr>
<tr>
<td>Limestone</td>
<td>100.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32.28</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)DM basis except DM

\(^b\)IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal; SBM = soybean meal, control.

\(^c\)Proper filtration could not be done in the case of concentrate feedstuffs.
respectively for IPA, ESA, HESA, CS, CM, and SBM. Crab meal contained an average of 15.7% chitin, DM basis. Since ADF is a measure of chitin in CM (Ayangbile, 1989), chitin content in CM is the value obtained for ADF content. It has been reported that chitin contains 6.9% N (Black and Schwartz, 1950). Thus nonchitin CP of CM was calculated to be 31.7%.

Diets were formulated to contain 10.5% crude protein (CP) and 63% TDN, DM basis. In each diet one third of the N was supplied by the protein supplement. Diets were formulated to meet or exceed NRC (1989) requirements. Diet formulation was adjusted periodically based on the chemical composition of ingredients and the mixed diets. At every mixing of diets, samples of hay, protein supplements, feed ingredients, and diets were collected. These samples were ground through a 1 mm mesh in a mill (Thomas-Wiley, Laboratory Mill, Model 4, Arthur H. Thomas Co. Philadelphia, PA.). Samples were analyzed for DM and CP by AOAC (1990) procedures. Calcium was estimated by atomic absorption spectrophotometer (Perkin Elmer 5100, Norwalk, CT) and P by the colorimetric method of Fiske and Subbarow (1925) after wet ashing (Sandel, 1959). Diet samples were also analyzed for NDF (Van Soest and Wine, 1967), ADF (Van Soest, 1963), lignin, cellulose, and hemicellulose (Van Soest and Wine, 1968) and ash (AOAC, 1990). The ingredient and chemical composition of diets are presented in Table 3.

All steers were dewormed with Ivomec®(1 ml/50 kg body weight, s.c., MSD, Division of Merck & Co., Inc., Rahway, New Jersey) before the trial began. Initial and final weights were averages of two consecutive daily weights taken 16 h after removed from water. Steers were individually fullfed during a 16 h period daily. The amount of feed was adjusted as needed to minimize refusals. Animals were weighed every 14 d. Ruminal fluid and blood samples were collected at 56 d
Table 3. Ingredient and chemical composition of diets fed to steers

<table>
<thead>
<tr>
<th>Item</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
</tr>
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<td><strong>Ingredient composition</strong></td>
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<tr>
<td>Hay</td>
<td>31.86</td>
<td>48.96</td>
<td>22.26</td>
<td>25.41</td>
<td>25.48</td>
<td>29.39</td>
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<tr>
<td>Cottonseed husls</td>
<td>28.76</td>
<td>14.19</td>
<td>32.67</td>
<td>31.82</td>
<td>33.39</td>
<td>26.33</td>
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<tr>
<td>SBM</td>
<td>7.11</td>
<td>-</td>
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<td>-</td>
<td>5.04</td>
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<td>-</td>
<td>-</td>
<td>5.43</td>
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<td>-</td>
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<td>CS</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>4.57</td>
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<tr>
<td>CM</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>9.31</td>
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<tr>
<td>Corn grain</td>
<td>30.53</td>
<td>21.63</td>
<td>37.50</td>
<td>34.92</td>
<td>35.09</td>
<td>34.23</td>
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<tr>
<td>Trace mineralized salt</td>
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<td>.50</td>
<td>.50</td>
<td>.50</td>
<td>.50</td>
<td>.50</td>
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<tr>
<td>Dicalcium phosphate</td>
<td>.81</td>
<td>.30</td>
<td>.75</td>
<td>.46</td>
<td>.43</td>
<td>.23</td>
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<tr>
<td>Lime stone</td>
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<td>.18</td>
<td>.52</td>
<td>.69</td>
<td>.54</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin mineral premix</td>
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<td>.80</td>
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<td><strong>Chemical composition</strong></td>
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<tr>
<td>Dry matter c</td>
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<td>89.51</td>
<td>89.20</td>
<td>89.11</td>
<td>89.08</td>
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<tr>
<td>Organic matter</td>
<td>94.19</td>
<td>92.82</td>
<td>93.73</td>
<td>93.60</td>
<td>94.53</td>
<td>91.86</td>
</tr>
<tr>
<td>Crude protein</td>
<td>10.45</td>
<td>10.47</td>
<td>10.78</td>
<td>10.69</td>
<td>10.79</td>
<td>10.63</td>
</tr>
<tr>
<td>RUP, %CP d</td>
<td>38.90</td>
<td>53.00</td>
<td>55.20</td>
<td>52.00</td>
<td>52.70</td>
<td>47.20</td>
</tr>
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<td>NDF</td>
<td>70.53</td>
<td>67.03</td>
<td>67.50</td>
<td>62.00</td>
<td>68.80</td>
<td>68.10</td>
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<td>ADF</td>
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<td>33.01</td>
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<td>33.86</td>
<td>35.09</td>
<td>32.22</td>
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<tr>
<td>Lignin</td>
<td>6.89</td>
<td>5.50</td>
<td>4.38</td>
<td>6.27</td>
<td>2.37</td>
<td>4.16</td>
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<tr>
<td>Cellulose</td>
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<td>25.82</td>
<td>23.28</td>
<td>28.45</td>
<td>31.21</td>
<td>27.36</td>
</tr>
<tr>
<td>Ash</td>
<td>5.81</td>
<td>7.18</td>
<td>6.27</td>
<td>6.40</td>
<td>5.47</td>
<td>8.14</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.52</td>
<td>0.55</td>
<td>0.53</td>
<td>0.55</td>
<td>0.54</td>
<td>1.13</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.35</td>
<td>0.32</td>
<td>0.38</td>
<td>0.36</td>
<td>0.35</td>
<td>0.36</td>
</tr>
</tbody>
</table>

*SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal. All diets were supplemented with 2200 IU Vitamin A / kg.

*DM basis

*Average of 18 samples

*Contains 98% NaCl, .35% Zn, .34% Fe, .2% Mn, .033% Cu, .007% I, .005% CO

*RUP = rumen undegraded CP, based on measured ruminal escape N of protein supplements and reported values for other ingredients.
and at the end of the trial (126 d). Ruminal liquor was collected from each steer using a stomach tube with the help of a vacuum pump, filtered through eight layers of cheese cloth and pH of the fluid was measured immediately after the collection (Accumet®, Mini pH Meter, Model 640 A, Fisher Scientific Company). Samples for the estimation of VFA and NH₃ N were taken in 15 mL tubes containing 1mL of 25% metaphosphoric acid and 1 drop of concentrated H₂SO₄, respectively. Samples were centrifuged at 1,800 x g for 15 min. Volatile fatty acids were determined by gas chromatography (Varian Vista 6000 gas chromatograph). A glass column packed with 10% SP-1200/10% H₃PO₄ liquid phase on 80/100 chromosorb WAW packing (Supelco Inc.) was used to separate acetic, propionic, butyric, valeric, isobutyric, and isovaleric acids. The detector temperature was 175°C, the column temperature as 125°C, and inlet temperature was 180°C. The VFA concentrations were determined by integration, using a VFA standard containing acetic (51.66 μmol/mL), propionic (30.63 μmol/mL), butyric (10.4 μmol/mL), valeric (5.18 μmol/mL), isobutyric (4.96 μmol/mL), and isovaleric (4.95 μmol/mL) acids. Ruminal NH₃ N was determined by the method described by Beecher and Whitten (1970).

Blood was drawn by jugular venipuncture from all steers in vacutainer tubes and was placed on ice until centrifuged at 1,800 x g for 15 min. Blood urea nitrogen (BUN) in serum was estimated in an Autoanalyzer, Centrifichem® System 500, using BUN (Rate) reagent. Sigma Diagnostic, St. Louis, MO.

Statistical Analysis

All data are presented as least squares means. Data were analyzed using the GLM procedure of SAS (1989) as a completely random arrangement of treatments. All parameters were subjected to the design shown in model. The model included block and diet.
Model. Randomized block design

\[ Y_{ij} = \mu + b_i + t_j + e_{ij} \]

Where,

- \( Y_{ij} \) = observation of steer in the \( i^{th} \) block given \( j^{th} \) diet
- \( \mu \) = unknown constant
- \( b_i \) = effect of \( i^{th} \) block, where \( i = 1, 2, \ldots, 8 \)
- \( t_j \) = effect of \( j^{th} \) diet, where \( j = 1, 2, \ldots, 6 \)
- \( e_{ij} \) = \( Y_{ij} - (\mu + b_i + t_j) \) is the experimental error of observation of steer randomly assigned to diet \( j \) in block \( i \)

Tukey’s Studentized Range (HSD) Test was used for comparing the treatments for different variables. Significance of \( (P < .05) \) was used throughout unless otherwise mentioned.

**Results**

Data on ADG, DMI, and feed efficiency are presented in Table 4. The DMI averaged 2.8% BW. Daily gains averaged 1.16 kg/d. Numerically, the highest weight gain was recorded for steers fed CS (1.26 kg/d) and the lowest were for the steers fed HESA supplement (.98 kg/d) which was lower \( (P < .05) \) than those fed other protein supplements except IPA (Figure 2). Differences in rate of gain were similar throughout the trial. There was a trend for a higher \( (P = .09) \) rate of gain for steers fed CS compared to those fed IPA supplement (1.13 kg/d). Lowest DMI were for the steers fed IPA supplement (2.61% BW) which was lower \( (P < .05) \) than for those steers fed SBM, ESA, and CS, expressed as kilogram per day and lower \( (P < .05) \) than for ESA and HESA, expressed as percent of BW. Expressed as percent of BW, there was a trend for intake to be higher \( (P < .10) \) for steers fed
Table 4. Performance of steers fed different protein supplements

<table>
<thead>
<tr>
<th>Item</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight, kg</td>
<td>225</td>
<td>221</td>
<td>225</td>
<td>221</td>
<td>221</td>
<td>227</td>
<td>-</td>
</tr>
<tr>
<td>Final weight (126-d), kg</td>
<td>371</td>
<td>364</td>
<td>380</td>
<td>343</td>
<td>379</td>
<td>377</td>
<td>-</td>
</tr>
<tr>
<td>Daily gain, kg/d</td>
<td>1.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.13&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.05</td>
</tr>
<tr>
<td>DM intake, kg/d</td>
<td>8.36&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.09&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8.45&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.09&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>.20</td>
</tr>
<tr>
<td>DM intake, % of body weight</td>
<td>2.82&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.81&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.69&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>.05</td>
</tr>
<tr>
<td>Feed efficiency, gain:feed (kg/kg)</td>
<td>.138&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.148&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.141&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.118&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.147&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.146&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.01</td>
</tr>
</tbody>
</table>

<sup>a</sup>SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.

<sup>b,c</sup>Means with the different superscript letters differ (P < .05)
Figure 2. Average cumulative daily gain of steers fed different protein supplements

SBM = soybean meal; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal. At 84 d HESA differs from all others (P < .05), IPA differs from CS (P < .05); 98 and 112 d = HESA differs from all others (P < .05); and 126 d = HESA differs from ESA, CS, CM, and SBM (P < .05)
SBM (2.82% BW), CS (2.81% BW) and CM (2.69%) than those fed the IPA supplement. The steers fed CM supplement tended to have a higher (P < .10) DMI (8.09 kg/d) compared to those fed the IPA supplement (7.59 kg/d). Differences in DMI were present after 56 d and followed a similar pattern until the end of the experiment, but differences were larger at 126 d (Figure 3).

Gain:feed was lower (P < .05) for steers fed HESA than for those fed the other supplement. There was a trend for a higher (P < .10) gain:feed for steers fed supplements IPA, CM and CS than those fed the SBM supplemented diet. Steers fed CM supplement had numerically higher gain:feed (.146) than those fed SBM, ESA, and HESA supplements, also. Throughout the trial period of 126 d, the steers fed HESA supplement recorded the lowest (P < .05) gain:feed and the other trends remained constant (Figure 4). In general, gain:feed of the steers tended to decline as experiment advanced because of linear increase in DMI with almost a constant ADG.

Data on average ruminal pH, ruminal NH₃ N, and blood urea N (BUN) at 56 d and 126 d of the experiment are presented in Table 5. Ruminal pH averaged 7.0 and 7.3 at 56 d and 126 d, respectively. No significant differences (P > .05) were observed in pH, at either 56 or 126 d among the steers fed different protein supplements. Steers fed SBM had numerically highest ruminal NH₃ N (10.05 mg/dL) and lowest values were for the steers fed ESA and HESA supplements (4.00 mg/dL, for each) at 56 d. However, values were not significantly different (P > .05), perhaps due to individual variation within the treatment (SEM=1.54). At 126 d, highest ruminal NH₃ N were also for the steers fed the SBM (21.03 mg/dL) which was higher (P < .05) than those fed the IPA and HESA supplements (8.86 and 9.39 mg/dL, respectively). Steers fed ESA, CS, and CM had intermediate NH₃ N concentrations (12.29, 19.28, and 19.16 mg/dL, respectively).
Figure 3. Average cumulative dry matter intake of steers fed different protein supplements
SBM = soybean meal; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No. 3; CS = commercial supplement based on animal protein; and CM = crab meal. At 84 d = IPA differs from ESA (P < .05); 98, 112, and 126 d = IPA differs from ESA, CS, and SBM (P < .05)
Figure 4. Average cumulative feed efficiency (gain:feed) of steers fed different protein supplements
SBM = soybean meal; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal. At 28, 42, 70, 98, 112, and 126 d = HESA differs from all others (P < .05); 56 d = ESA differs from CS (P < .05)
Table 5. Ruminal pH and blood urea of steers fed different protein supplements

<table>
<thead>
<tr>
<th>Item</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
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</thead>
<tbody>
<tr>
<td>56 d</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ruminal pH&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>7.1</td>
<td>7.1</td>
<td>7.0</td>
<td>7.1</td>
<td>6.9</td>
<td>.09</td>
</tr>
<tr>
<td>Ruminal NH3-N&lt;sup&gt;b&lt;/sup&gt;, mg/dL</td>
<td>10.05</td>
<td>5.19</td>
<td>4.00</td>
<td>4.00</td>
<td>7.65</td>
<td>8.40</td>
<td>1.54</td>
</tr>
<tr>
<td>Blood urea N&lt;sup&gt;b&lt;/sup&gt;, mg/dL</td>
<td>3.88</td>
<td>3.92</td>
<td>3.26</td>
<td>2.94</td>
<td>4.00</td>
<td>5.04</td>
<td>.62</td>
</tr>
<tr>
<td>126 d</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruminal pH&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.4</td>
<td>7.2</td>
<td>7.1</td>
<td>7.5</td>
<td>7.2</td>
<td>7.3</td>
<td>.14</td>
</tr>
<tr>
<td>Ruminal NH3-N, mg/dL</td>
<td>21.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.86&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.29&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>9.39&lt;sup&gt;d&lt;/sup&gt;</td>
<td>19.28&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>19.16&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>2.58</td>
</tr>
<tr>
<td>Blood urea N&lt;sup&gt;b&lt;/sup&gt;, mg/dL</td>
<td>3.67</td>
<td>3.88</td>
<td>5.34</td>
<td>3.52</td>
<td>4.71</td>
<td>4.22</td>
<td>.55</td>
</tr>
</tbody>
</table>

<sup>a</sup>SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.

<sup>b</sup>Treatments do not differ (P > .05)

<sup>c</sup><sup>d</sup>Means with the different superscript letters differ (P < .05)
Blood urea N averaged 3.84 and 4.22 mg/dL at 56 and 126 d, respectively. The steers fed the HESA supplement had numerically lowest BUN, 2.94 and 3.52 mg/dL, respectively at 56 d and 126 d, among the steers fed various supplements. However, there were no significant differences (P > .05) in BUN.

Data on the ruminal VFA of steers fed various supplements at 56 and 126 d are presented in Table 6 and 7, respectively. Total ruminal VFA averaged 46.23 and 43.06 µmol/mL, respectively at 56 and 126 d, which were not significantly different. At 56 d, lowest (P < .05) acetate concentrations (61.72 mol/100 mol) were for the steers fed CS supplement, compared to those fed other supplements. Highest propionate concentrations (22.48 mol/100 mol) were observed for the steers fed CS supplement which were higher (P < .05) than those for the steers fed other supplements except that for HESA supplement. Isobutyrate concentration was lowest for the steers fed IPA and CM supplements. Values were lower (P < .05) than for steers fed the ESA supplement. Butyrate concentrations were lower (P < .05) for the steers fed iPA supplement (10.10 mol/100 mol) compared to those fed ESA and CM supplements (13.64 and 13.37 mol/100 mol, respectively). Isovalerate was highest (P < .05) in steers fed ESA supplement compared to those fed other supplements except for CS. Molar concentration of valeric acid was shown to be numerically highest in steers fed CS supplement and lowest in those fed IPA and HESA supplements. However, these values were not significantly different (P > .05). Higher (P < .05) acetate to propionate ratio was for the steers fed ESA, IPA, and CS supplements (4.36, 4.1, 4.06, respectively) compared to those fed CS (2.87).

At 126 d, highest acetate levels were for the steers fed CM supplement (72.39 mol/100 mol) which were higher (P < .05) than those fed ESA and CS supplements (67.91 and 68.26 mol/100 mol, respectively). Propionic acid,
Table 6. Ruminal volatile fatty acids of steers fed the different protein supplements (56 d)

<table>
<thead>
<tr>
<th>Item</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total VFA&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>µmol/mL mol/100 mol</td>
<td>48.23</td>
<td>45.10</td>
<td>42.46</td>
<td>47.41</td>
<td>41.74</td>
<td>52.44</td>
<td>2.82</td>
</tr>
<tr>
<td>Acetate</td>
<td>66.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>69.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>61.72&lt;sup&gt;d&lt;/sup&gt;</td>
<td>66.90&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup>SBM = soybean meal, control, IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.

<sup>b</sup>Treatments did not differ (P > .05)

<sup>cd</sup>Means with different superscript letters differ (P < .05)
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<sup>a</sup>SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.

<sup>b</sup>Treatments did not differ (P > .05)

<sup>cd</sup>Means with different superscript letters differ (P < .05)
isobutyric acid, butyric acid, and valeric acid and the acetate to propionate ratio were not significantly different (P > .05) among the steers fed the different supplements. Steers fed SBM and IPA supplements recorded lower (P < .05) isovaleric acid (1.24 and 1.13 mol/100 mol, respectively) compared to those fed ESA, HESA and CS supplements (1.73, 1.77 and 1.86 mol/100 mol, respectively).

Discussion

The lower DMI of IPA fed steers might be due to the low density of the diet as a result of low level of cottonseed hulls and high levels of hay compared to that of other diets. It was observed that as the level of cottonseed hulls increased there was an increase (numerical) in DMI expressed as both kilograms per day as well as percentage of BW. Nonsignificant lower DMI were observed for the steers fed CM supplement. These results tend to agree with the previous findings of Velez et al. (1991) who reported that DMI of Holstein heifers fed crab waste meal were significantly lower from those fed SBM supplemented diet. In the present study, lack of large and consistent differences in DMI of steers fed different protein supplements is in agreement with the previous reports of Harvey and Spears (1989), Sun et al. (1990), Johnson et al. (1994), and Mansfield et al. (1994), while Donaldson et al. (1991) found that escape protein supplementation (combination of fish meal and distillers dried grains) increased DMI in steers.

Substituting rumen undegradable protein sources for SBM did not elicit a profound effect in growth or gain:feed in growing steers. These results agree with the results of Coomer et al. (1993) who did not record any differences in ADG, feed efficiency or CP efficiency (defined as the daily gain observed above the urea control per unit of natural protein supplemented) when calves were fed different diets supplemented with SBM, heated SBM (HSBM), CGM, or combination of
HSBM and CGM. Adam et al. (1988) and Derouen et al. (1988) also reported that feeds supplemented with undegradable intake protein (UIP) did not affect the growth of beef heifers and Holstein heifers. However, an increased ADG and feed efficiency has been reported in Holstein heifers fed increased UIP supplied by CGM and dehydrated alfalfa meal (Amos, 1985). Ewes gained more weight when they were fed a combination of BM and SBM, compared to those fed either SBM or urea (Hoaglund et al., 1992).

Results of the present study agree in part to Ludden et al. (1995) who fed three groups of steers, in a 175 d trial, with SBM; SoyPLUS®, a high rumen escape soybean product; and a 50:50 CGM and BM at different levels of supplemented true protein in the diet (20, 30, or 40% of total dietary protein by altering the proportions of true protein and urea in the supplement). They observed no differences in ADG, DMI or efficiency.

The inability to detect responses to feeding ruminal escape protein sources such as IPA, ESA, HESA, and CS compared to that of SBM in the present study may be related to a relatively high escape N content for SBM. Ludden et al (1995) reported that the estimate of the ruminal escape N content of SBM was more than twice as great as the average estimate of 28% reported by NRC (1989). Perhaps, the relatively high escape of N of SBM may be related to the ruminal environment of the diet (70% corn grain) in that study. In another study (Chapter V), the escape N of SBM estimated by in situ technique was only 23% when bags were incubated in the rumen of a cannulated steer fed 50:50 alfalfa grass hay diet. The diet formulated for the steers in the present study contained on an average 30% ground corn which would have affected the degradability of SBM.

Loerch et al. (1983) observed that the decrease in ruminal pH associated with feeding diets based on cereal grains may reduce the protein degradability of
SBM. The escape N content of SBM was increased to 54.2% when bags were incubated in the rumen of steers fed 80% high moisture corn-based diets. When Ludden et al. (1995) incubated SBM in the rumen of steers fed high corn diet with a ruminal pH of 6.2, escape protein was found to be 66.0%. In the metabolism study conducted, the average ruminal pH of the SBM fed sheep was 6.39 (Chapter IV). Hussein and Jordan (1991) reported that at low pH the escape of N in SBM will be much higher and it is close to the escape value of fish meal. However, in the present study no differences of ruminal pH were observed for SBM fed steers compared to those fed other protein supplements. Further, average ruminal pH of steers fed different protein supplements was higher (7.0 and 7.3, respectively at 56 and 126 d) than expected. Veen (1986) suggested that in general the pH will be higher immediately before morning feed than afterwards. One possible explanation given by Veen (1986) is that before feeding there is no easily fermentable substrate remaining in the rumen. Another explanation may be the fact that the concentration of NH₃ rises before the morning feed through the endogenous metabolism of bacteria and/or through lysis of bacteria (Wohlt et al. 1976). In addition, the concentration of NH₃ may increase again many hours after feeding, as a result of increased salivary secretion (Wohlt et al., 1976). In the present study for most of the steers there was a lapse of at least 12 h between the time the steers were fed and sampling of ruminal fluid which may have caused the ruminal pH to be higher.

At a low ruminal pH, ruminal degradability of protein decreases due to reduction of electrostatic repulsion between neighboring protein molecules due to the lack of net charge (Clark, 1975). As a result, the reduction in ruminal pH observed when concentrate-based diets are fed, could increase the escape N content of certain proteins by reducing solubility of these proteins in the rumen.
Thus, low ruminal pH would enhance the escape nature of protein in the feedstuffs. The incorporation of corn in the present study probably lowered the degradability of SBM.

Loerch and Berger (1981) recorded, numerically, higher ADG and DMI when SBM was supplemented to a 75% corn based diet, compared to BM and MBM supplements. Sindi et al. (1993a) did not observe any differences in ADG or feed efficiency when urea was replaced with a combination of BM and FTM in finishing calves although there was a small response in the beginning. Similar results were obtained by Swartz et al. (1991) when Holstein calves were fed different levels of undegradable intake protein (UIP). They did not observe any difference in ADG in calves fed different levels of UIP (low, 30%; medium, 34%; high, 38% of the feed) but, significant differences in feed efficiency for the calves fed different levels of UIP were observed due to differences in DMI.

Ludden and Cecava (1995) could not explain the reason for not having a significant response to escape protein supplementation. They suggested that the reason might be due to increased availability for microbial protein synthesis by feeding concentrates. Another reason for not getting a response may be due to the escape nature of corn grain protein, zein, which is 58 to 73% undegraded when feed contain proportionately high levels of corn in the diet (Zinn and Owens, 1983). In the present study, the levels of corn in the diet (~30%) would have contributed considerable escape protein. Further, ruminal NH₃ concentrations were much higher at 126 d than the minimum concentration of ruminal NH₃ N of 5 mg/dL required for the optimum microbial protein synthesis, as suggested by Satter and Slyter (1974). This suggests that optimum microbial protein synthesis occurred in the steers fed the different supplements.
In the current study, steers fed protein supplement based on plant protein and BM had a lower DMI compared to those fed SBM, but the daily gain of the steers were similar. Feed efficiency of the steers fed IPA was numerically higher than for those fed SBM, however, the differences were not significant. Similar trends were observed in ESA, CS, and CM supplemented steers. This trend for a response, perhaps due to the complementary effect of protein sources.

Goedeken et al (1990a) reported that when steers were fed a diet containing 72% ensiled corn cobs and 20% alfalfa (DM basis), supplemented with BM, FTM, BM with CGM, BM with FTH and a combination of BM, FTH and CGM, they gained faster than steers supplemented with urea. They suggested that BM may have supplied needed lysine while CGM may have provided needed sulfur amino acids. They also reported that protein efficiency for BM and CGM was improved, compared to BM or FTM alone, but it was similar to BM and FTM and combination of BM, FTM, and CGM. Stock et al. (1981) reported a 22% improvement in protein efficiency when BM and CGM were fed, compared to BM alone.

In another study, Goedeken et al (1990b) fed steers diets containing 50% ground corn cobs and 40% corn silage supplemented with protein supplements such as SBM, FTM, BM and a combination of FTH and BM, compared to urea control. They suggested that the most efficiently used protein sources were BM and FTM and BM combinations compared to SBM. Also, a complementary effect has been observed when BM and FTM were fed together as the protein efficiency for the mixture was improved compared to the calculated average of BM and FTM treatments. In the current study the steers fed ESA, which is a combination of BM and FTM recorded numerically higher weight gain and gain:feed compared to
those fed SBM. This trend for a response perhaps was due to the complementary
effect of FTM and BM to provide a better balance of AA.

Microbial protein which is limiting in methionine and lysine (Nimrick et al.,
1970; Fendonon and Bergen, 1975; Richardson and Hatfield, 1978) would have
been complemented with high levels of lysine (BM) and sulfur AA (CGM and
FTM) from the protein sources. Blasi et al. (1991) reported that steers fed 100%
BM had maximum protein efficiency compared with those fed urea, FTH, and
combination of BM and FTM. They also reported that no differences were
observed in protein efficiency among the supplements containing the various
proportions of FTH:BM. Protein value of FTH for growing ruminants can be
dramatically improved with small additions of blood to FTH during the processing,
so that deficiency of AA profile of microbial protein can be complemented (Blasi
et al., 1991).

A linear increase in body weight was observed when wether lambs were fed
FTM, compared to SBM in a 56 d experiment (Thomas et al., 1994). The authors
suggested that, the growth response obtained from a linear increase in dietary
protein supplied by FTM was due to an increased availability of protein and not
due to sulfur containing AA though there was a linear increase in the S
concentration in the diet as the FTM level increased in the diet.

Gibb et al. (1992) reported that as the level of FTM increased in the diet,
level of histidine also decreased, because of its low histidine content. In our study,
although AA acids were not estimated, ESA which is a combination of FTM and
BM is a rich source of S AA produced similar weight gain and feed efficiency as
other protein supplements except for HESA.

Complementary effects of feeding combinations of FTM and BM with urea
supplementation have been reported by Sindt et al. (1993a). They reported that,
although not significant, calves finished after grazing corn stalks and supplemented with SBM were 7% more efficient than calves supplemented with urea alone. They also suggested that supplementing SBM/FTM/urea or BM/FTM/urea improved feed efficiency compared with supplementing FTM/urea alone. These data show that supplementing the diet with combinations of protein sources are more beneficial than feeding single source. In another study Sindt et al (1993b) reported that calves supplemented with urea-BM/FTM were more efficient during the first 41 d of the trial than were calves supplemented with urea alone (basal diet contained either dry rolled corn or dry rolled grain sorghum diets). However, ADG and feed efficiency were not affected from 42 d to slaughter (188 d) by protein sources such as urea, urea + 60:40 combination of BM and FTM. The data strongly suggest that calves in the early stage, i.e., from 0 to 41 d were deficient in metabolizable protein (MP) in the urea fed calves, but were adequate in MP from d 42 to slaughter (188 d). The data also show that supplementing urea-BM/FTM improved feed efficiency when the diet was limiting in MP and not when the MP was adequate.

Although the above study cannot be compared in toto with the present study due to urea incorporation in their diets, the results of the present study agree with this trend. The SBM supplemented diet perhaps provided adequate MP for the optimum feed efficiency for the calves, so the other protein supplements did not elicit a pronounced response when compared to SBM. The findings agree with the suggestion of Chalupa (1975) who reported that the potential of rumen escape protein is shown highest in young, growing ruminants, where microbial protein synthesis may not be sufficient for the optimum requirement for rapid growth. Earlier, Orskov et al. (1971) observed increased ADG and gain:feed when post weaning lambs were fed FM substituted for SBM. Oldham and Smith (1982)
suggested that growing cattle weighing more than 200 kg fed a diet with adequate energy intake can meet their protein requirements from the MP synthesized in the rumen. They postulated that the protein sources that are rapidly degraded in the rumen will be sufficient to meet the N requirement through MP synthesis.

The reason for the lower (P < .05) ADG and gain:feed for steers fed HESA compared to other protein supplements is not known. One reason might be the low degradability of the protein which would have created a deficiency of NH$_3$ N and would have resulted in low microbial growth. In the present study ruminal NH$_3$ N concentration was only 4.0 mg/dL which is lower than the minimum concentration of 5 mg/dL for the optimum microbial growth as suggested by Satter and Slyter (1974). However, concentration of NH$_3$ N was higher than this at 126 d. Further, the digestibility of the material in the lower tract would be low compared to the other supplements. Metabolism studies conducted in sheep revealed that sheep fed HESA supplement had the lowest DM, OM, and cellulose digestibility among the sheep fed different protein supplements (Chapter IV). Disulfide bonds in cystine of FTM cause poor digestibility (Moran et al., 1966). Correct balance of time and temperature during hydrolysis of feathers is necessary for optimum protein digestibility (Morris and Balloun, 1973; Latshaw, 1990). Cystine is converted to lanthionine during processing (Robbins et al., 1980). As processing increases, more lanthionine is produced which limit the bioavailability of S AA (Robbins et al., 1980) and possibly other essential AA as well (Papadopoulos et al., 1986). Goedken et al. (1990b) indicated that protein quality of FTM and BM combination will be reduced if the BM is added before hydrolysis of FTM due to reduced ruminal protein escape and total tract protein digestibility. In the present study, perhaps HESA supplement was processed for too long a period of time.
Numerically, higher feed efficiency was obtained for steers fed the CM supplemented diet compared to those fed SBM supplemented diet. Higher feed efficiency has been reported by Ayangbile (1989) when cattle were fed 30% crab waste straw silage compared to a control diet. Similar results were obtained by Patton et al. (1975) who observed no reduction in weight gain, but improved feed efficiency for steers fed CM. In contrast, Brundage (1986) reported a reduction in daily gain in steers fed 7.5 to 22.5% tanner crab meal. Lower weight gains and poor feed efficiency has been reported in minks fed 10 and 20% crustacean meal (Watkins et al., 1982). Results of the present study contradicts the findings of Velez et al. (1991) who reported that DMI and feed conversion efficiency of crab waste meal fed Holstein heifers were significantly lower than those fed SBM supplemented diet. They reported that overall performance of Holstein heifers on a crab waste meal diet was inferior to those that received a SBM supplemented diet (Velez et al., 1991). They suggested that crab waste meal was inferior to SBM when supplemented in corn silage based diets. The inferiority of the crab waste meal, according to the authors, might be due to less ruminal microbial degradation leading to low microbial protein synthesis. Another possibility might be due to possible mineral imbalance. In the present study we did not observe any negative response compared to those fed SBM.

Preston and Bartle (1990) reported that maximum gain and efficiency were obtained when steer calves were fed a basal diet comprised of cottonseed hulls (25%), corn silage (40%) and steam flaked sorghum grain (23.4%) supplemented with protein having 60% ruminal escape protein (41% of total CP). This conclusion was further strengthened by the results of Clark et al. (1992) who suggested that diets should contain at least 35% of the dietary protein from the protein supplement to elicit a positive response. In the present study proportion of
the diet was only 33.3% which was marginal to elicit any response as suggested by Clark et al. (1992).

Ruminal pH was higher than expected in all the treatments. Similar results were obtained when different UIP sources, SBM, heated SBM, CGM and combination of heated SBM and CGM were fed to steers (Coomer et al., 1993), and dairy cows (Seymour et al., 1992; Robinson and McQueen, 1994). Increased rumen pH when cows were fed rumen undegradable feeds have been reported with different protein sources (Zerbini et al., 1988; Broderick et al., 1993; Christenson et al., 1993). Rodriguez (1994) reported a higher pH when Holstein cows were fed 41% RDP diets compared to those fed 29% RDP diets.

The high ruminal NH₃ N in steers fed the SBM diet shows that SBM is more degradable than other protein supplements. Differences in protein solubility in rumen resulted in a significant increase (P < .01) in ruminal NH₃ N from 5.57 to 23.53 mg/dL (Sniffen, 1974). He reported that an increase of 2.7 times in solubility of intake protein would result in 4.2 times the production of ruminal NH₃.

Several workers have reported that ruminal NH₃ N decreased linearly as FTM replaced SBM (Thomas and Beeson, 1977; Daugherty and Church, 1982; Ellingson, 1993; Thomas et al., 1994). The authors suggested that ruminal NH₃ N concentrations in wethers fed FTM supplements were probably related to a lower ruminal degradation of FTM. Feeding low ruminal degradable protein sources in dairy cows resulted in low levels of ruminal NH₃ N (McCarthy et al., 1989; Klusmeyer, 1990; Stokes et al., 1991).

Titgemeyer et al. (1989) reported decreased ruminal NH₃ N concentration in steers fed CGM compared with those fed SBM. Coomer et al. (1993) observed that ruminal NH₃ N peaked at 2 h after feeding and were 16.9, 16.2, 11.2, and 13.4
mg/dL for SBM, heated SBM, CGM, and combination of heated SBM and CGM. They observed a reduction (P < .01) in ruminal NH₃-N in steers fed CGM, compared to those fed SBM. Koeln and Paterson (1986) also reported a lower ruminal NH₃-N from in vitro fermentation of heated SBM and CGM than from SBM. This low availability of NH₃-N would have directed microbes to use other sources of N such as peptides and AA (Chen et al., 1987).

In our study, BUN did not show any significant differences among the steers fed different protein supplements. This is in agreement with the findings of Swartz et al. (1991) who reported that when calves were fed three levels of ULP, 33, 37, and 46% up to 12 wk, and 30, 34, and 38% from 12 to 28 wk, there were no differences in BUN. They suggested that plasma urea N did not change with diet and suggested that when total CP in the diet was the same, protein utilization in the body is not likely changed by varying degradable protein in the diet. Although the sources of protein were different in the present study, total protein concentration in the diet was same in all the diets. Steers fed the SBM supplemented diet showed higher ruminal NH₃-N concentrations even though there were no differences in BUN among the steers fed different protein supplements. These results do not agree with the reports of Preston et al. (1965), who found a high correlation between ruminal NH₃-N and blood urea. Jordan et al. (1983) has reported that increased plasma N concentrations were observed when increased levels of CP were fed. They suggested that high levels of plasma urea were expressed because of high amounts of NH₃ formed from high levels of CP with high ruminal degradability. Corbett and Edey (1977) observed lower plasma urea N in ewes fed formaldehyde-treated casein, compared with ewes fed isonitrogenous diets supplemented with untreated casein.
Feeding different protein supplements did not alter the total VFA at the middle and end of the experiment. These results are in agreement with those obtained by Wandersee et al., (1990) when cows were fed UIP sources such as FTM. In the present study, individual VFA were not affected except for acetate and valerate when steers were fed different protein supplements. Higher concentration of acetate in CM diet fed animals may be due to the relatively higher amount of acetate formed from the chitin degradation in the rumen. Acetate has been reported to be the major end product of chitin fermentation (Pel et al., 1990).

Molar concentration of isobutyrate was not affected, while isovalerate was higher for steers fed CS, HESA, and ESA supplemented diets, compared to those fed SBM. Hussein et al. (1991b) reported that higher concentration of isobutyrate and isovalerate were detected for diets containing SBM than for those containing slowly degraded protein sources such as FM. Hussein et al. (1991a) suggested that the higher levels of these branched VFA were produced as a result of higher degradation of SBM. isobutyrate and isovalerate are produced from the deamination of the branched-chain AA, valine and leucine (Harwood and Canale-Paula, 1981). The reason for lower molar concentration of isovalerate for the steers fed SBM is not known. Ratio of acetate to propionate did not differ for steers fed different protein supplements in the present study. Rodriguez (1994) also did not observe any difference in acetate:propionate ratio when cows were fed 41% and 29% rumen undegradable protein (RUP) diets, while, valerate concentration was found to be lower (P < .05) in cows fed a higher RUP (41%). Reduced concentration of butyrate and valerate with high RUP diets were also observed by Seymour et al. (1992). The authors suggested that this low concentration of butyrate and valerate may indicate a decreased ruminal
fermentation of AA. In the present study no differences were observed for butyrate and valerate among steers fed various protein supplement diets.

Implications

Substituting SBM with other commercial protein supplements did not elicit a significant difference in weight gain or gain:feed in growing steers on a high roughage diet. However, there was a trend for positive response in gain:feed for the protein supplements, IPA, and CS. Crab meal appears to be similar to SBM and other protein supplements for growing steers. Feeding HESA (hydrolyzed feather meal and blood meal) had a negative response. It can be concluded that protein supplements such as IPA, CS, ESA, and CM may be beneficial in replacing SBM in the diets for growing steers.

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Chapter IV. Journal Paper 2. Effects of Feeding Crab Meal and Escape Protein Supplements on Nitrogen Balance, Digestibility and Calcium and Phosphorus Availability in Sheep

Abstract: Two metabolism trials were conducted with 24 wether lambs to investigate the effects of feeding crab meal and other protein supplements on N utilization, digestibility and Ca and P balance in sheep. The lambs (avg. BW, 25 kg) were randomly allotted to eight diets in each of two trials. The supplements were: 1) none, negative control (NC); 2) soybean meal (SBM), control; 3) supplement based on industrial byproducts of both plant and animal origin (IPA); 4) experimental supplement based on byproducts of animal origin (ESA); 5) hydrolyzed supplement No 4. (HESA); 6) commercial supplement based on animal protein (CS), Pro-Lak®, 7) crab meal (CM); and 8) urea (U). The supplements supplied 33% of the total dietary N (CP, 9.8%; DM basis). Lambs fed NC diets had lower (P < .05) DM and OM digestibility. There were no differences in DM and OM digestibilities among the different protein supplement groups. Lower (P <
.05) apparent absorption of N was recorded for the lambs fed the HESA and NC diets. Numerically, higher Ca absorption was observed for lambs fed IPA, ESA, and CS compared to those received SBM. Sheep fed CM had lower Ca absorption compared to SBM. Highest (P < .05) P absorption was observed for lambs fed CS and CM and lowest for U and NC diets. Sheep fed CM had higher (P < .05) VFA concentration (65.7 μmol/mL), compared to those fed ESA, CS, and NC diets (47.3, 49.8, and 49.5 μmol/mL, respectively). There were no differences (P > .05) in propionate, butyrate and valerate concentrations among animals fed the different protein supplements. There were no differences in ruminal fluid pH for lambs fed the different diets. Highest (P < .05) ruminal NH₃-N (29.6 mg/dL) was observed in lambs fed the U diet, while those fed the NC diet had the lowest (P < .05) average value (7.66 mg/dL). Lambs fed the U diet had the highest blood urea N (10.67 mg/dL) and those fed the NC diet had the lowest (P < .05) average value (3.06 mg/dL). The other protein supplements, CS, ESA, IPA, and CM may be advantageous in supplementing the diet for the growing steers, considering the numerically higher retention of N compared to SBM.

**Key Words:** Escape Protein, Sheep, Protein Supplement, Digestibility, N Balance

**Introduction**

Feeding combinations of different escape protein supplements such as blood meal (BM) and feather meal (FTM) (Blasi et al., 1991; Sindt et al., 1993) and BM and corn gluten meal (CGM) to growing calves (Ludden and Cecava,
1995) have been shown to be more efficient than feeding these protein supplements separately. These protein supplements of high rumen escape potential are deficient in one or more amino acids (AA). Corn gluten meal and FTM are high in S AA especially methionine while, BM is high in lysine. These AA have been found to be limiting in microbial protein (William and Smith, 1974). Various combinations of different protein supplements are available commercially, but their rumen escape potential and their effect on digestibility are not known.

Several studies conducted in this laboratory (Ayangbile, 1989; Samuels et al., 1991; 1992; Abazinge et al., 1993; 1994) and elsewhere (Lubitz et al., 1943; Patton et al., 1975; Velez et al., 1991) have shown that crab processing waste could be processed and fed as protein supplement either in the form of a silage in combination with roughage or as dehydrated meal for ruminants. Lubitz et al. (1943) reported that quality of crab meal protein is higher than that of fish meal (FM) protein. Later, Patton et al. (1975) reported that there were no significant reductions in digestibility of DM, N, and Ca when cattle were fed 10 or 20% CM. Higher DM and OM digestibilities were observed when sheep were fed silage containing 60% crab waste than those fed silage containing 40% crab waste (Samuels et al., 1992). Higher N retention has been reported when sheep were fed crab waste-straw silage compared to wheat straw silage (Samuels et al., 1992; Abazinge et al., 1994). Ayangbile (1989) reported that DM, OM, CP, energy, NDF, ADF, cellulose and hemicellulose decreased linearly (P < .01) with increased levels of crab waste-straw silage. Nitrogen retention increased linearly (P < .05) with increased levels of crab waste-straw silage.

An experiment was conducted to determine digestibility, N balance, and Ca and P metabolism in lambs fed crab meal and different protein supplements.
Experimental Procedures

Two metabolism trials were conducted each with 24 wether lambs (avg. BW, 25 kg). In each trial, the lambs were blocked into three blocks of eight according to BW, and were randomly allotted within blocks to eight experimental diets containing the following supplements: 1) none, negative control (NC); 2) soybean meal (SBM), control; 3) supplement based on industrial byproducts of both plant and animal origin (IPA); 4) experimental supplement based on byproducts of animal origin (ESA); 5) hydrolyzed supplement No 4. (HESA); 6) commercial supplement based on animal protein (CS), Pro-Lak®; 7) crab meal (CM); and 8) urea (U). In randomizing the lambs for the second trial the lambs were not allowed to receive the same supplement as in the first trial. The ingredient and chemical composition of different experimental diets are presented in Tables 8 and 9, respectively. Diets were isonitrogenous (9.8%, CP) and isocaloric (58%, calculated TDN), DM basis, except for the negative control diet in which the CP was 6.5%.

Lambs were kept in metabolism stalls similar to those described by Briggs and Gallup (1949) designed for separate collection of feces and urine. All animals were treated with Ivomec® (1 ml/50 kg BW, s.c.; MSD, Division of Merck and Co., Inc. Rahway, New Jersey) for internal parasites and were given 500,000 I.U. of vitamin A and 75,000 I.U of vitamin D, i.m. before starting the first trial.

Metabolism trials consisted of 7 d adaptation, 2 d transition, 10 d preliminary, and 10 d collection periods. Each lamb was fed 700 g feed daily in equal portions at 12 h intervals at 0800 h and 2000 h. Proportionate quantities of hay, cottonseed hulls, and concentrate (combination of other ingredients of the respective diets) were weighed separately for each lamb at each feeding. Water
Table 8. Ingredient composition of experimental diets fed to sheep

<table>
<thead>
<tr>
<th>Item</th>
<th>None</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
<th>U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay</td>
<td>45.9</td>
<td>45.9</td>
<td>45.9</td>
<td>45.9</td>
<td>45.9</td>
<td>45.9</td>
<td>45.9</td>
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</tr>
<tr>
<td>Cottonseed hulls</td>
<td>33.0</td>
<td>31.0</td>
<td>28.2</td>
<td>29.0</td>
<td>30.2</td>
<td>31.7</td>
<td>27.7</td>
<td>30.5</td>
</tr>
<tr>
<td>Glucose&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.3</td>
<td>3.9</td>
<td>-</td>
<td>7.8</td>
<td>6.3</td>
<td>6.5</td>
<td>6.5</td>
<td>10.4</td>
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<td>7.5</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>IPA</td>
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<td>-</td>
<td>15.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>ESA</td>
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<td>-</td>
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<td>-</td>
<td>5.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CS</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>4.5</td>
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<tr>
<td>CM</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>9.4</td>
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<tr>
<td>Urea</td>
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<td>Salt</td>
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<td>.5</td>
<td>.5</td>
<td>.5</td>
<td>.5</td>
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<tr>
<td>Dicalcium phosphate</td>
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<td>.6</td>
<td>-</td>
<td>.5</td>
<td>.3</td>
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<td>.8</td>
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<tr>
<td>Limestone</td>
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<td>.6</td>
<td>.3</td>
<td>.6</td>
<td>.6</td>
<td>.5</td>
<td>-</td>
<td>.6</td>
</tr>
<tr>
<td>Vit. - min. premix&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>.8</td>
<td>.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

<sup>a</sup>DM basis

<sup>b</sup>SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3; CS = commercial supplement based on animal protein; and CM = crab meal.

<sup>c</sup>Cerelose, Corn Products, Summit-Argo, IL.

<sup>d</sup>Custom additive premix, Formulated by Wilson Enterprises, Disputana, VA.
Table 9. Chemical composition of experimental diets fed to sheep\textsuperscript{a}

<table>
<thead>
<tr>
<th>Component</th>
<th>None</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
<th>U</th>
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</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>90.1</td>
<td>89.9</td>
<td>90.1</td>
<td>90.1</td>
<td>90.1</td>
<td>90.0</td>
<td>90.2</td>
<td>89.8</td>
</tr>
<tr>
<td>Organic matter</td>
<td>93.9</td>
<td>93.5</td>
<td>93.2</td>
<td>93.4</td>
<td>92.4</td>
<td>93.9</td>
<td>91.3</td>
<td>94.0</td>
</tr>
<tr>
<td>Crude protein</td>
<td>6.5</td>
<td>9.8</td>
<td>9.7</td>
<td>9.9</td>
<td>10.0</td>
<td>9.9</td>
<td>9.9</td>
<td>10.1</td>
</tr>
<tr>
<td>RUP, %CP\textsuperscript{c}</td>
<td>28.0</td>
<td>36.6</td>
<td>50.1</td>
<td>55.0</td>
<td>51.7</td>
<td>52.7</td>
<td>45.4</td>
<td>28.0</td>
</tr>
<tr>
<td>ADF</td>
<td>40.8</td>
<td>40.6</td>
<td>38.3</td>
<td>39.3</td>
<td>39.3</td>
<td>40.1</td>
<td>39.3</td>
<td>39.1</td>
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<tr>
<td>Cellulose</td>
<td>33.7</td>
<td>33.5</td>
<td>31.9</td>
<td>32.0</td>
<td>32.4</td>
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<td>32.4</td>
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<tr>
<td>Calcium</td>
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<td>.63</td>
<td>.69</td>
<td>.68</td>
<td>.66</td>
<td>.66</td>
<td>1.34</td>
<td>.64</td>
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<tr>
<td>Phosphorus</td>
<td>.34</td>
<td>.31</td>
<td>.29</td>
<td>.33</td>
<td>.34</td>
<td>.33</td>
<td>.31</td>
<td>.32</td>
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</tbody>
</table>

\textsuperscript{a}DM basis, except the values for DM.
\textsuperscript{b}SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 2.; CS = commercial supplement based on animal protein; and CM = crab meal.
\textsuperscript{c}RUP = Rumen undegraded CP; based on measured ruminal escape N content of protein supplements and reported values for other ingredients.
was provided throughout the trials except during the two 2 h feeding periods. Samples of feed (hay, cottonseed hulls, and concentrate) were collected beginning 2 d prior to start of the trial until 2 d prior to the end of the trial. At the end of each trial, the feed samples (10 d) were composited, subsampled and ground in a Wiley mill (Thomas Wiley, Laboratory Mill Model 4, Arthur H. Thomas Co. Philadelphia, PA.) for chemical analysis.

Feces were collected daily in metal pans and dried in a forced draft oven maintained at a maximum of 60°C. The daily collections of feces of each lamb were composited in plastic buckets with loose fitting lids for equilibrating with atmospheric moisture. At the end of the trial, dried feces were subsampled and ground in a Wiley mill through a 1 mm mesh screen. Urine was collected in 4 L plastic jars containing 15 mL of 1:1 (w/w) concentrated H₂SO₄ and H₂O plus approximately 500 mL of H₂O. Urine was collected once daily, diluted to a fixed weight (5000 g) with H₂O and a 2% (100 mL) aliquot was taken from each lamb and placed in a bottle and refrigerated. At the end of the trial urine samples were subsampled and kept frozen for further analysis of N and Ca. At the end of the trial ruminal fluid samples were collected 2 h post feeding using a stomach tube and vacuum pump. Ruminal fluid was strained through eight layers of cheese cloth and pH was immediately measured using a portable pH meter (Accumet® Mini pH Meter, Model 640A, Fisher Scientific Company). Samples (5 mL each) for VFA and NH₃ N determination were collected in 15 ml tubes containing 1 ml of 25% metaphosphoric acid or one drop of sulfuric acid, respectively. Blood was drawn by jugular venipuncture from all wethers 6 h after feeding and was centrifuged at 1800 x g for 15 min. and serum was separated. Urea N in serum was determined in an Autoanalyzer, Centrifichem® System 500, using BUN (Rate) reagent, Sigma Diagnostic, St. Louis, MO.
Feed components and feces were analyzed for DM and ash (AOAC, 1990), Ca (atomic absorption Spectrophotometer, Perkin Elmer 5100 PC, Norwalk, CT), P (colorimetric method of Fiske and Subbarow, 1925), NDF (Van Soest and Wine, 1967), ADF (Van Soest, 1963), Cellulose (Van Soest and Wine, 1968). Feed, fecal and urinary N were determined by kjeldahl method (AOAC, 1990). Ruminal NH₃ N was determined by the method described by Beecher and Whitten (1970). Volatile fatty acid analyses were performed by gas chromatography (Varian Vista 6000 gas Chromatograph, column packed with 10% SP-1200 /10% H₃PO₄ on 80/100 chromosorb WAW). The detector, column, and inlet temperatures were 175, 125, and 180°C, respectively. Sample VFA concentrations were determined by integration, using a VFA standard containing acetic (51.66 µmol/mL), propionic (30.63 µmol/mL), butyric (10.4 µmol/mL), Valeric (5.18 µmol/mL), isobutyric (4.96 µmol/mL), and isovaleric (4.95 µmol/mL) acids.

Statistical Analysis

All data are presented as least squares means. Data were analyzed using the GLM procedure of SAS (1989). All parameters were subjected to the design shown in model. The model included trial, block, and diet.

Model. Randomized block design

\[ Y_{ijk} = \mu + b_i + d_j + t_k + e_{ijk} \]

Where,

\[ Y_{ijk} = \text{observation of steer in the } i^{th} \text{ block given } j^{th} \text{ diet} \]
\[ \mu = \text{unknown constant} \]
\[ b_i = \text{effect of } i^{th} \text{ block, where } i = 1, 2, \ldots, 6. \]
\[ d_j = \text{effect of } j^{th} \text{ diet, where } j = 1,2,\ldots,8 \]
\[ t_k = \text{effect of } k^{th} \text{ trial, where } k = 1 \text{ and } 2 \]
\[ e_{ijk} = Y_{ijk} - (\mu + b_i + d_j + t_k) \text{ is the experimental error of the} \]
observation of the sheep randomly assigned to diet j in block i in trial k.

Tukey's Studentized Range (HSD) Test was used for comparing the treatments for different variables.

**Results and Discussion**

*Apparent Digestibility.* Data on apparent digestibility of DM, OM, ADF, cellulose, and CP and Ca and P availability are presented in Table 10. Apparent digestibility of DM ranged from 57.0 to 62.5% across diets. The lowest numerical value for DM digestibility was for the lambs fed the diet with no supplemental N which was lower (\( P < .05 \)) than values for diets supplemented with SBM, IPA, ESA and CS. The lower digestibility of DM for steers fed the diet with no supplemental N might be due to lower microbial activity in the rumen. Christensen et al. (1993) did not observe any differences in DM digestibility when protein with low ruminal degradability (55%) was fed as the sole supplemental protein, compared to a high ruminally degradable protein (70%). Results of the present study agree with their findings. Blasi et al. (1991) reported that DM digestibility of the BM diet was higher than that of the SBM and urea diets. In the same study, the DM digestibility of the diet containing FTM hydrolyzed for 18 min was greater than that of the diet containing FTM hydrolyzed for 10 min and slightly higher than that of the diet containing FTM for 12 and 15 min. Stock et al. (1981) reported that lambs fed a urea control diet had lower (\( P < .05 \)) DM digestibilities.
<table>
<thead>
<tr>
<th>Item</th>
<th>None</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
<th>U</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>57.02e</td>
<td>61.46de</td>
<td>62.52e</td>
<td>61.76de</td>
<td>60.09cde</td>
<td>61.06de</td>
<td>59.68de</td>
<td>58.85cd</td>
<td>.68</td>
</tr>
<tr>
<td>OM</td>
<td>57.79e</td>
<td>62.17def</td>
<td>62.98f</td>
<td>62.51ef</td>
<td>60.56de</td>
<td>61.88def</td>
<td>61.42def</td>
<td>60.03d</td>
<td>.67</td>
</tr>
<tr>
<td>ADF</td>
<td>40.47c</td>
<td>45.66ef</td>
<td>46.03f</td>
<td>44.95def</td>
<td>43.87ddef</td>
<td>43.46cdef</td>
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<td>1.00</td>
</tr>
<tr>
<td>Cellulose</td>
<td>52.87c</td>
<td>57.61f</td>
<td>57.38f</td>
<td>56.74ef</td>
<td>53.96cde</td>
<td>55.91def</td>
<td>55.55cdef</td>
<td>53.83cd</td>
<td>.91</td>
</tr>
<tr>
<td>CP</td>
<td>28.19c</td>
<td>50.96c</td>
<td>51.58e</td>
<td>48.95e</td>
<td>41.15d</td>
<td>48.37e</td>
<td>48.26e</td>
<td>52.34e</td>
<td>.94</td>
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<tr>
<td>Ca</td>
<td>1.65c</td>
<td>8.51cde</td>
<td>11.99c</td>
<td>11.80c</td>
<td>6.76cde</td>
<td>9.18de</td>
<td>5.03cde</td>
<td>2.49cde</td>
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<tr>
<td>P</td>
<td>5.26c</td>
<td>16.83d</td>
<td>17.12d</td>
<td>15.61cd</td>
<td>15.55cd</td>
<td>27.88e</td>
<td>25.03de</td>
<td>5.49c</td>
<td>3.46</td>
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</tbody>
</table>

Each value represents the mean of six sheep.

SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.

c, d, e, f Numbers in the same row with different superscript letters differ (P<.05)
than lambs fed SBM-urea, BM-urea, or CGM-SBM-urea supplemented diets. In contrast, Goedsken et al. (1990) detected no differences ($P > .20$) in DM digestibilities when lambs were fed urea, SBM, BM, FTM or CGM supplemented diets.

Apparent digestibility of OM was in the range of 57.8 to 63.0%. The pattern of OM digestion was similar to that of DM digestion. Digestibility of OM was lower at ($P < .05$) for the negative control diet than for the other diets. The value of the IPA supplemented diet was higher ($P < .05$) than the diets supplemented with HESA and U. Values for the diets supplemented with SBM, ESA, CS, and CM were intermediate. Keery et al. (1993) found that whole tract digestibilities of OM were not influenced by supplemental protein sources (SBM, heated SBM, menhaden FM). Ludden and Cecava (1995) did not observe any difference ($P > .10$) in total tract OM digestibility for diets supplemented with BM compared to SBM, urea and SoyPLUS®- a high ruminal escape SBM. The authors reported that animals had an acceptability problem with BM which caused a slower rate of consumption of CGM-BM combination. They suggested that slower rate of intake of CGM-BM diet would have reduced passage rate and as a result increased OM digestibility.

Trends for ADF and cellulose digestibilities were similar as the OM digestibility. Apparent digestibility of ADF ranged between 40.5 and 46.0%, and that of cellulose was from 52.9 to 57.6%. When cows were fed a diet supplemented with CGM, Klusmeyer et al. (1990) did not find any decrease in digestibilities of OM, starch, NDF, and ADF compared to those fed a SBM supplemented diet. Hussein et al. (1991) suggested that replacing high ruminally degradable protein sources in the diets may improve ruminal fiber digestion. Contradictory to the above findings, McAllan and Griffith (1987) reported that
ruminal fiber digestion was inversely related to ruminal protein degradation when diets containing casein, SBM, or FM were fed to steers. Veen (1986) suggested that feeding protein low in degradability will produce gradual release of NH₃ N, peptides, and branched chain VFA. Therefore, cellulytic bacteria can utilize steadily these essential nutrients for a longer period of post feeding time.

The CM supplemented diet was comparable with other protein supplemented diets with regard to DM and OM digestibilities (59.7% and 61.4% respectively). These results are not in agreement with the results obtained by Velez et al. (1991). They reported that DM digestibility of a diet supplemented with CM was lower (P < .05) than for a diet supplemented with SBM. In the present study sheep fed the CM diet had a lower, numerically, ADF digestibility (42.6%) compared to those fed SBM supplemented diets (45.7%). However, there was no significant difference. This is in agreement with the results obtained by Velez et al (1991). In the present study, numerically lower ADF digestibility might be due to the low digestibility of chitin which forms 15.7% of CM, DM basis. The fiber content of crustacean waste meals is a reliable estimate of the amount of chitin present (Lovell et al., 1968; Ayangbile, 1989). A lower (P < .01) apparent digestibility of DM, OM, CP, energy, NDF, ADF, cellulose and hemicellulose with increase in the level of crab waste-straw silage for sheep has been reported (Ayangbile, 1989).

Nitrogen intake was lower for the sheep fed no supplemental N and intake were similar for the lambs fed the supplements (Table 11). The highest (P < .05) fecal N excretion was for the steers fed HESA supplement (5.82 g/d) which shows that N that passed to the lower gut was not digested as efficiently that of other supplements. Lowest fecal N excretion was for the steers fed no supplemental N (which was a reflection of low N intake) and IPA supplement. Sheep fed ESA, CS,
Table 11. Nitrogen balance by sheep fed experimental diets*  

<table>
<thead>
<tr>
<th>Item</th>
<th>None</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
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<th>SE</th>
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<tbody>
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<td>Excretion, g/d</td>
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<td></td>
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<tr>
<td>Fecal</td>
<td>4.67</td>
<td>4.80</td>
<td>4.66</td>
<td>5.08</td>
<td>5.82</td>
<td>5.10</td>
<td>5.08</td>
<td>4.80</td>
<td>.09</td>
</tr>
<tr>
<td>Urinary</td>
<td>2.05</td>
<td>4.28</td>
<td>3.90</td>
<td>3.61</td>
<td>3.71</td>
<td>3.57</td>
<td>3.76</td>
<td>4.90</td>
<td>.13</td>
</tr>
<tr>
<td>Total</td>
<td>6.72</td>
<td>9.08</td>
<td>8.56</td>
<td>8.69</td>
<td>9.53</td>
<td>8.67</td>
<td>8.86</td>
<td>9.70</td>
<td>.16</td>
</tr>
<tr>
<td>Apparent absorption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/d</td>
<td>1.84</td>
<td>4.99</td>
<td>4.99</td>
<td>4.88</td>
<td>4.07</td>
<td>4.78</td>
<td>4.74</td>
<td>5.28</td>
<td>.09</td>
</tr>
<tr>
<td>% of intake</td>
<td>28.19</td>
<td>50.96</td>
<td>51.58</td>
<td>48.95</td>
<td>41.15</td>
<td>48.37</td>
<td>48.26</td>
<td>52.34</td>
<td>.94</td>
</tr>
<tr>
<td>Retention</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/d</td>
<td>-0.21</td>
<td>.71</td>
<td>1.09</td>
<td>1.27</td>
<td>.36</td>
<td>1.21</td>
<td>.95</td>
<td>.38</td>
<td>.16</td>
</tr>
<tr>
<td>% of intake</td>
<td>-3.35</td>
<td>7.26</td>
<td>11.15</td>
<td>12.73</td>
<td>3.64</td>
<td>12.20</td>
<td>9.64</td>
<td>3.75</td>
<td>1.75</td>
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<tr>
<td>% absorbed</td>
<td>-20.40</td>
<td>14.21</td>
<td>20.02</td>
<td>25.56</td>
<td>8.35</td>
<td>25.06</td>
<td>18.66</td>
<td>6.23</td>
<td>5.58</td>
</tr>
</tbody>
</table>

*Each value represents the mean of six sheep

SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.

Numbers in the same row with different superscript letters differ (P<.05)
and CM had higher (P < .05) fecal N excretion compared to those fed IPA and no supplemental N. This shows that N of IPA, which is a combination of plant protein and BM is more digestible than that of FTM-BM combination (ESA) and the other animal protein based supplement. Sheep fed SBM and U supplement had intermediate values between those fed ESA, CS and CM supplement and IPA and no supplemental N.

Apparent absorption of N ranged between 28.2 to 52.3%. Expressed as g/d or as % of intake apparent absorption was lowest (P < .05) for the sheep fed no supplemental N. There were no differences in apparent absorption of N for the sheep fed different protein supplement except for those fed HESA supplement, which were lower (P < .05) than others. Feeding HESA supplements depressed the N absorption by 19.2% when compared with that of SBM fed animals. Cecava and Hancock (1994) found that when steers were fed diets supplemented with combinations of SBM and FM, N digestion was decreased (P < .05), compared to that for a urea-supplemented diet. Blasi et al. (1991) observed a lower (P < .05) protein digestion for hydrolyzed FTM diets than for BM, SBM, and urea diets. Apparent protein digestibilities of FTM hydrolyzed for 10 and 18 min were higher (P < .05) than those of FTM hydrolyzed for 12 and 15 min. The authors suggested that hydrolysis time is not a criterion in determining the quantity of CP digested and the quality of the raw materials are the primary factors. They suggested that quality factors include deterioration with time before hydrolysis and contamination with heads and feet.

Numerically, lower absorption was observed for the sheep fed ESA supplement which is a combination of FTM and BM compared with those fed IPA and SBM supplements. This tends to agree with the findings of Thomas and Beeson (1977) who reported that N digestibility was reduced from 68% to 59%.
when FTM replaced SBM in the diet. Church et al. (1982) reported that protein digestibility was reduced by 5.7% and 8.5% units in two digestion studies where FTM replaced 70% of the SBM protein in the diet. In their studies FTM substitution for SBM would have produced lower ruminal NH\textsubscript{3} N which could have caused lower microbial protein activity and thus caused a low digestion. Lardy et al. (1993) reported that supplementation with BM resulted in lower ruminal and total tract CP digestibilities than SBM and rapeseed meal, while U supplementation resulted in highest total tract CP digestibility. In contrast to the above findings, Goedeken et al. (1990) observed no differences in total tract N digestibility when lambs were fed urea, SBM, BM, FTM or CGM supplemented diets.

Numerically, a lower N digestibility of CM supplement (48.3%) was observed, compared to those fed SBM (51.0%), however, the values were not significantly different. These results tend to agree with those of Velez et al. (1991) who reported a lower (P < .05) CP digestibility of 51.1% for CM compared with that of 55.7% for an iso-nitrogenous diet supplemented with SBM. Patton et al. (1975) determined N digestibility of 67.8% for CM. However, the CP digestibility coefficient of CM observed in the present study does not agree with the higher value that was obtained for Patton et al. (1975). Lovell et al. (1968) reported that N content of chitin is indigestible and has no value for nonruminant animals. The N content of chitin is 6.9% and accounts for 19 to 20% of the total N in crustacean meals (Lovell et al., 1968). Velez et al. (1991) reported that the wide variation in the response may be because of the variability in the commercial CM obtained from different sources.

Highest (P < .05, 4.90 g/d) urinary N excretion was for the steers fed U and the lowest (P < .05, 2.05 g/d) was for those fed no supplemental N. Wethers fed
the ESA supplemented diet had a lower ($P < .05$) urinary N excretion (3.61 g/d) than those fed SBM supplemented diet (4.28 g/d). Stock et al. (1981) reported that urinary N excretion did not differ ($P > .05$) among lambs fed diets supplemented with various protein sources; however, N excretion was higher in those fed urea and SBM-urea diets. Cecava and Hancock (1994) observed higher urinary excretion of N in steers fed a urea diet compared to a SBM-FTM combination. In the present study, total N excretion was highest in steers fed U supplement, which was higher ($P < .05$) than for steers fed IPA, ESA, CS, and CM. This is probably a reflection of the lower efficiency of N utilization of urea. Sheep fed no supplemental N recorded the lowest ($P < .05$) total N excretion, due to low N intake.

Nitrogen retention (% of intake) ranged from -3.35 to 12.73%. A negative N balance was observed for those fed no supplemental N (-3.4%) which was lower ($P < .05$) compared to all other experimental groups, except U and HESA. This indicated that considerable amount of N was mobilized from body tissues. Higher ($P < .05$) N retentions (% of intake) were observed in ESA and CS (12.7% and 12.2%, respectively) compared to those fed U, NC, and HESA diets. Sheep fed IPA, CM, and SBM had intermediate values (11.15, 9.64, 7.26%, respectively). Retention of N was numerically higher for wethers fed CM compared to those fed SBM supplemented diet. These results agree with those reported by Abazinge et al. (1994). In the present study, sheep fed urea had lower ($P < .05$) N retention (3.8%) compared to ESA and CS fed sheep (12.7 and 12.2%, respectively). This shows that when urea was supplied as the sole source of supplemental CP with a moderate source of metabolizable energy, urea was used with low efficiency. In sheep fed the urea supplemented diet, urea would have been rapidly converted into NH$_3$ in the rumen and would have been converted into urea in the liver, causing
increased urinary N loss and decreased efficiency of N utilization compared with other protein supplements of true protein nature. Sheep fed ESA, CS, IPA, and CM had retained a numerically higher percentage of absorbed N (25.56, 25.06, 20.02 and 18.66 mg/dL) than steers fed SBM (14.21 mg/dL). Thomas and Beeson (1977) observed a similar trend in N retention as percentage of absorbed for animals fed SBM and keratin protein supplements (FTM and hair meal). Stoke et al. (1981) reported that N retention was greater (P < .05) for lambs fed meat meal(MM)-urea, CGM-urea, CGM-MM-urea, CGM-SBM-urea, and CGM-BM-urea compared to those fed urea diet. They suggested that some complementary effects might exist between the protein in CGM and that in SBM and BM.

Although N digestibility was numerically lower for ESA (which is a combination of FTM and BM) compared to SBM, there was numerically a higher N retention for ESA (12.7%) over SBM (7.3%). Feather meal N is less digestible than SBM N, but FTM would have supplied AA of better profile than SBM (Cecava and Hancock, 1994).

Calcium intake was higher for the lambs fed CM supplement, and intake were similar for the lambs fed the other supplements (Table 12). Fecal excretion was higher (P < .05) for lambs fed CM, a reflection of higher intake. Among sheep fed protein supplemented diets, Ca absorption was similar, but expressed as percent of intake CM fed sheep had a numerically lower Ca absorption. Urinary Ca excretion was low for the lambs fed all supplements with no difference among the different supplements. Absorption and retention of Ca were lower for sheep fed diets supplemented with U or no supplemental N, but differences were usually not significant. Absorption of Ca is dependent on its solubility at the point of contact with the absorbing membranes irrespective of the forms in which Ca is ingested (Maynard et al., 1979). Furthermore, animals absorb Ca from their gut
Table 12. Calcium balance by sheep fed different protein supplements

<table>
<thead>
<tr>
<th>Item</th>
<th>BM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
<th>U</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake, g/d</td>
<td>3.86</td>
<td>3.96</td>
<td>4.35</td>
<td>4.22</td>
<td>4.07</td>
<td>4.11</td>
<td>4.42</td>
<td>3.90</td>
</tr>
<tr>
<td>Excretion, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.83</td>
<td>3.86</td>
<td>3.84</td>
<td>3.72</td>
<td>3.79</td>
<td>3.77</td>
<td>3.83</td>
<td>3.77</td>
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<tr>
<td>Apparent absorption</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retention</td>
<td>1.55</td>
<td>.51</td>
<td>.34</td>
<td>.28</td>
<td>.28</td>
<td>.28</td>
<td>.38</td>
<td>.43</td>
</tr>
<tr>
<td>% of intake</td>
<td></td>
<td>51</td>
<td>34</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>% of intake</td>
<td>.06</td>
<td>.03</td>
<td>.02</td>
<td>.02</td>
<td>.02</td>
<td>.02</td>
<td>.02</td>
<td>.02</td>
</tr>
<tr>
<td>% of absorbed</td>
<td>50.00</td>
<td>76.58</td>
<td>91.42</td>
<td>86.84</td>
<td>78.07</td>
<td>89.83</td>
<td>69.94</td>
<td>40.00</td>
</tr>
</tbody>
</table>

Each value represents the mean of six sheep. BM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No. 3; CS = commercial supplement. Numbers in the same row with different superscript letters differ (P < .05).
according to their need and they can alter the efficiency of absorption depending upon the need (McDowell, 1992). Thus, lower absorption of Ca, expressed as percent of intake for the CM supplemented lambs is a reflection of intake exceeding the requirement. Excessive dietary Ca fed to dairy calves affected concentration of Zn, Fe, Cu, and Mn in some body tissues (Alfaro et al., 1988). In the present study Ca content of the CM diet was almost double (1.34%) than the average value for the other experimental diets (.66%).

The lowest P excretion were in sheep fed CS and CM which were lower (P < .05) than those fed HESA, U, and no supplemental N (Table 13). Highest P excretion was for sheep fed no supplemental N which was higher (P < .05) than for those fed SBM, IPA, CS, and CM supplements. The highest absorption of P were for the sheep fed CS and CM (27.9 and 25.0%, respectively) and the lowest was for those fed U and no supplemental N (5.5 and 5.3%, respectively). The values for sheep fed diets supplemented with SBM, IPA, ESA, and HESA were intermediate. The CM fed sheep had numerically a higher P absorption (25%) over those fed SBM (16.8%).

Volatile Fatty Acids. Total VFA ranged from 47.3 to 65.7 μmol/mL. Sheep fed CM had a higher (P < .05) total VFA (65.7 μmol/mL) compared to those fed NC, ESA and CS (49.5, 47.3 and 49.8 μmol/mL, respectively) (Table 14). Weathers fed SBM, IPA, and HESA had intermediate values. Acetate proportions were higher (P < .05) in wethers fed IPA (69.3 mol/100 mol) compared to those fed U and NC diets (59.9 and 60.7 mol/100 mol, respectively). However, there were no differences (P > .05) among the sheep fed diets supplemented with different protein sources. In contrast, Cheery et al. (1993) found that concentration of acetate was higher (P < .10) in steers fed the SBM diet (96.8 mM/L) than those steers fed the heated SBM, menhaden FM, and combination of protein sources.
### Table 13. Phosphorus absorption by sheep fed experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>None</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
<th>U</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake, g/d</td>
<td>2.05</td>
<td>1.95</td>
<td>1.81</td>
<td>2.05</td>
<td>2.12</td>
<td>1.99</td>
<td>1.95</td>
<td>1.96</td>
<td></td>
</tr>
<tr>
<td>Excretion, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal</td>
<td>1.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.62&lt;sup&gt;def&lt;/sup&gt;</td>
<td>1.50&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>1.74&lt;sup&gt;cdef&lt;/sup&gt;</td>
<td>1.79&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>1.44&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.45&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.85&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.07</td>
</tr>
<tr>
<td>Apparent absorption/retention&lt;sup&gt;g&lt;/sup&gt;, g/d</td>
<td>.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.33&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>.31&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>.32&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>.33&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>.56&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.50&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.07</td>
</tr>
<tr>
<td>% of intake</td>
<td>5.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.83&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>17.12&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>15.61&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>15.55&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>27.88&lt;sup&gt;d&lt;/sup&gt;</td>
<td>25.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.46</td>
</tr>
</tbody>
</table>

<sup>a</sup>Each value represents the mean of six sheep

<sup>b</sup>SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.

<sup>c</sup>d<sup>e</sup>f<sup>i</sup>Numbers in the same row with different superscript letters differ (P < .05)

<sup>g</sup>Phosphorus in urine were not in a detectable range, hence the value of absorption may be considered as retention could not be detected.
Table 14. Ruminal volatile fatty acids of sheep fed the different protein supplement diets

<table>
<thead>
<tr>
<th>Item</th>
<th>None</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
<th>U</th>
<th>SE</th>
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</thead>
<tbody>
<tr>
<td>Total VFA, µmol/ml</td>
<td>49.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54.65&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>58.02&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>47.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>51.57&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>49.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>65.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>55.19&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.42</td>
</tr>
<tr>
<td>mol/100 mol</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>60.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>65.84&lt;sup&gt;de&lt;/sup&gt;</td>
<td>69.30&lt;sup&gt;e&lt;/sup&gt;</td>
<td>63.41&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>67.03&lt;sup&gt;de&lt;/sup&gt;</td>
<td>64.04&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>65.98&lt;sup&gt;de&lt;/sup&gt;</td>
<td>59.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.13</td>
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<tr>
<td>Propionate</td>
<td>29.32&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>21.31&lt;sup&gt;e&lt;/sup&gt;</td>
<td>19.75&lt;sup&gt;e&lt;/sup&gt;</td>
<td>25.71&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>23.43&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>26.15&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>22.39&lt;sup&gt;de&lt;/sup&gt;</td>
<td>30.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.60</td>
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<tr>
<td>Isobutyrate</td>
<td>.40&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>.61&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>.65&lt;sup&gt;f&lt;/sup&gt;</td>
<td>.47&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>.52&lt;sup&gt;def&lt;/sup&gt;</td>
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<td>.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.04</td>
</tr>
<tr>
<td>Butyrate</td>
<td>8.57</td>
<td>10.56</td>
<td>8.68</td>
<td>8.93</td>
<td>8.15</td>
<td>7.81</td>
<td>9.66</td>
<td>8.58</td>
<td>.86</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>.49&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>.94&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.89&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.79&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>.76&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>.84&lt;sup&gt;de&lt;/sup&gt;</td>
<td>.69&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.08</td>
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<tr>
<td>Valerate</td>
<td>.60</td>
<td>.78</td>
<td>.77</td>
<td>.73</td>
<td>.71</td>
<td>.69</td>
<td>.73</td>
<td>.65</td>
<td>.04</td>
</tr>
<tr>
<td>Acetate/Propionate</td>
<td>2.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.14&lt;sup&gt;de&lt;/sup&gt;</td>
<td>3.56&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.49&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>2.94&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>2.52&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.02&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>2.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.21</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each value represents the mean of six sheep

<sup>b</sup> SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3; CS = commercial supplement based on animal protein; CM = crab meal.

<sup>c,de,f</sup> Numbers in the same row with different superscript letters differ (P<.05)
supplemented diets (83.5, 80.8, and 78.9 mM/L, respectively). Ayangbile (1989) reported that total VFA and acetate tended to be higher for sheep fed 50% crab waste-silage compared to those fed basal diet without crab waste.

Wethers fed the U diet had higher (P < .05) propionate proportions (30.1 mol/100 mol) compared to those fed CM, IPA and SBM supplements. Molar concentration of different volatile fatty acids for the sheep fed CM supplement were not significantly different from those fed SBM supplement. Ayangbile (1989) observed lower propionate, butyrate, and isovalerate concentrations for the sheep fed 50% crab waste-silage diet compared with those fed a basal diet without crab waste. Khorasani et al. (1994) reported a lower (P < .06) ruminal concentration of propionate, isobutyrate and valerate when cows were fed slowly degradable protein sources (FM, CGM, and MM) substituted for rapidly degradable protein sources (canola meal and SBM). Keery et al (1993) found that rumen concentrations of isobutyric acid and isovaleric acid were lower (P < .10) in steers received heated SBM and combination of protein sources diets than in steers received menhaden FM. Similar results have been obtained by Pena et al. (1986). When cows were fed diets supplemented with heat-treated cottonseed (roasted or extruded), concentration of isobutyrate and isovalerate were lower than in cows fed diets supplemented with raw cotton seed. In the present study generally, numerically lower proportions of branched chain VFA such as valerate, isovalerate, and isobutyrate were observed among the groups fed different protein supplemented diets compared to those fed SBM. For the optimum growth of cellulolytic bacteria branched chain fatty acids are needed. The branched chain fatty acids are formed from the fermentation of branched chain AA (Russell and Sniffen, 1984). Leng (1973) suggested that the high level of isovaleric acid may be an indication of ruminal proteolytic activity. The SBM supplemented diet
produced numerically higher isovalerate compared to other protein supplemented group, which agrees with the above statement. In the present study, there were no differences (P > .05) in the ratio of acetate to propionate among the sheep fed diets supplemented with different protein sources. However, lower (P < .05) ratios were observed in those sheep fed U and NC diets compared with those fed SBM diet.

Contradictory results were obtained by Ludden and Cecava (1995) who reported that feeding SoyPLUS®, high ruminal escape SBM increased total VFA and branched chain and isoacids. It has been shown that branched chain VFA stimulates microbial growth *in vitro* (Argyle and Baldwin, 1989). Khorasani et al. (1994) observed higher (P = .06) total branched chain fatty acids when cows were fed diets supplemented with slowly degradable protein sources, compared to those fed diets supplemented with highly degradable protein sources.

*Other Ruminai and Blood Parameters:* The ruminal pH of sheep fed different experimental diets averaged 6.38 which was lower than observed for steers in the growth study fed different experimental diets (Chapter III). In this study ruminal pH ranged from 6.24 (HESA) to 6.52 (U) (Table 15). There were no differences in ruminal pH for sheep fed different experimental diets. These results agree with the results of several studies conducted with protein supplements. Cozzi and Polan (1994) observed no differences in ruminal pH when cows were fed diets supplemented with SBM, CGM, or brewers dried grain. Titgemeyer et al. (1989) also reported that ruminal pH was not altered due to feeding escape protein supplements such as CGM or FM over SBM. Similar results of not having any change in ruminal pH by supplementing diets with different protein supplements have been reported (Seymour, et al., 1992; Schloesser et al., 1993; Sindic et al., 1993; Khorasani et al., 1994; Ludden and
Table 15. Blood urea nitrogen, and rumen pH and ammonia nitrogen of sheep fed experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>None</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
<th>U</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruminal NH$_3$-N, mg/dL</td>
<td>7.66</td>
<td>17.58</td>
<td>14.17</td>
<td>12.75</td>
<td>14.45</td>
<td>15.36</td>
<td>16.13</td>
<td>29.59</td>
<td>1.52</td>
</tr>
<tr>
<td>Blood urea-N, mg/dL</td>
<td>3.06</td>
<td>7.14</td>
<td>6.50</td>
<td>5.61</td>
<td>5.18</td>
<td>6.25</td>
<td>6.46</td>
<td>10.67</td>
<td>.75</td>
</tr>
</tbody>
</table>

*Each value represents the mean of six sheep

*SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.

c,d,eNumbers in the same row with different superscripts differ (P < .05)
Cecava, 1995). Ellingson (1993) observed low ruminal pH in heifers fed diets supplemented with SBM, CGM or FM. Optimum pH for high cellulolytic activity has been proposed to be 6.8 (Terry et al., 1969). Increased ruminal pH values have been reported when cows were fed rumen undegradable feeds with different protein sources (Zerbini et al., 1988). A higher pH has been reported for sheep fed crab waste-straw, compared to a diet that fed without crab waste (Ayangbile, 1989).

Ruminal NH$_3$ N of sheep fed different diets ranged from 7.66 to 29.59 mg/dL. Highest (P < .05) NH$_3$ N values were observed for sheep fed the U supplement (29.59 mg/dL) and sheep fed no supplemental N, numerically, had the lowest NH$_3$ N (7.66 mg/dL). Ruminal NH$_3$ N for sheep fed SBM, CS, and CM were higher (P < .05) than those not fed a N supplement. This is in agreement with the results reported by Khorasani et al. (1994). Ruminal NH$_3$ N observed in all the diet treatments were higher than the minimum concentration (5 mg/dL) reported to be essential for optimum ruminal microbial growth (Satter and Slyter, 1974). It has been shown that to achieve maximum microbial activity much higher NH$_3$ concentrations are required than for the maximum microbial protein production may be required (Mehrez et al 1977). Satter and Roffler (1975) reported that under normal feeding conditions this minimum level of 5 mg/dL rumen fluid will be achieved with a dietary CP between 11 and 14% in the diet DM. 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. From these studies it can be implied that ruminal NH$_3$ N produced by the various diets supplemented with different protein sources in the present study appears to be sufficient for maximum microbial activity.
Ludden and Cecava (1995) reported that free α-AA N decreased (P < .10) as the ruminal degradability of the preformed protein source decreased. They also observed that concentration of peptide-associated α amino N and the relative size of the peptides decreased (P < .10) when steers were fed SBM supplemented diet. The authors suggested that the peptides formed as a result of SBM degradation are immediately utilized by ruminal microbial colony which boost microbial growth.

Stock et al. (1981) reported that ruminal NH₃ levels were equal at 2 h post feeding for sheep fed urea, SBM-urea, MM-urea, BM-urea, CGM-urea, CGM-SBM-urea, CGM-BM-urea, and CGM-MM-urea. The reason for not having a difference in ruminal NH₃ N concentration would be because of urea incorporation in all the diets (40% of N). Later, at 4 and 6 h post feeding, lambs fed urea control diet had higher (P < .05) concentration of rumen NH₃ N than those fed the other seven diets. In that experiment urea masked any difference in in vivo rate of degradation of protein supplements. They also reported that ruminal NH₃ N levels in lambs on the urea treatment exceeded (P < .05) the average of the means of the lambs fed, SBM-urea, BM-urea and MM-urea. Lambs fed BM had the lowest (P < .05) ruminal NH₃ N compared to those fed urea, SBM-urea, and MM-urea. Lambs fed SBM had intermediate values at 3 h of post feeding.

Feeding more resistant protein will result in lower NH₃ N levels (McCarthy et al. 1989) in ruminal fluid and even NH₃ N may fall below the minimum level required for maximum microbial protein synthesis (Wohlt et al., 1976). Titgemeyer et al. (1989) reported that ruminal NH₃ N was 12.5 mg/dL when steers were fed a basal diet without any protein supplements. Addition of SBM caused the greatest (P < .05) increase in ruminal NH₃ N concentration and BM addition resulted in only a nonsignificant (P > .05) increase. They also reported that an increase in ruminal NH₃ N concentration was similar between lambs fed CGM and
FM. Soybean supplementation resulted in greater NH₃ production in the rumen than did a diet containing CGM (Klumeyer et al., 1990), CGM and BM (Cecava et al., 1991; Ludden and Cecava, 1995), CGM or brewers dried grain (Cozzi and Polan, 1994), CGM, wet brewers grain, or distillers dried grain (Santos et al., 1984), heated SBM or combination of protein sources (Keery et al., 1993) and FTM or hair meal (Thomas and Beeson, 1977). A higher ruminal NH₃ N has been observed in sheep fed crab waste-straw silage over those fed a diet without crab waste (Abazinge et al., 1994). Ludden et al. 1995 reported that feeding a CGM-BM combination resulted in lower microbial N flows to the duodenum and lower microbial efficiency, compared to urea, SBM and SoyPLUS®. Schloesser et al. (1993) observed a linear decrease in ruminal NH₃ N as they increased the level of BM replacing SBM in the diet for ewes. They also reported that ruminal NH₃ N concentration and other growth factors were in sufficient concentrations to meet the requirements for microbial protein synthesis when wethers were fed a diet supplemented with BM replacing SBM.

Lardy et al. (1993) observed highest (P < .01) mean ruminal NH₃ N concentration when they supplemented the diet with urea and lowest for those supplemented with BM. These results support the data obtained in the present study for the ruminal NH₃ N release in the animals fed diets supplemented with urea and other protein sources.

Blood urea N of sheep fed different experimental diets ranged from 3.06 to 10.67 mg/dL. Lowest BUN were for the sheep fed no supplemental N, which was lower (P < .05) than the value for those fed U supplement (10.7 mg/dL). The values for the other supplements were intermediate. The same trend as that was observed in ruminal NH₃ N was observed in BUN (Figure 5). As the level of ruminal NH₃ N increased there was a proportional increase in BUN. These results
Figure 5. Ruminal ammonia nitrogen and blood urea concentrations of sheep fed diets with no supplemental N (NC); U = urea; SBM = soybean meal; IPA = supplement based on industrial by-products of both plant and animal origin; ESA = experimental supplement based on by-products of animal origin; HESA = hydrolyzed supplement No 4.; CS = commercial supplement based on animal protein; and CM = crab meal. Within parameter, bars lacking a common letter differ.
are in agreement with the results of Thomas et al. (1984). Thomas and Beeson (1977) reported that plasma urea N concentration was higher (P < .05) for animals fed SBM compared to those fed FTM and hair meal. Christensen et al. (1993) found that concentration of urea in plasma was reduced by decreasing the ruminal degradability of the dietary CP. Effect of protein source contribute mainly to the level of BUN. Greater concentration of BUN would be expected when protein of high ruminal degradability was fed. In the present study BUN followed the same trend. Corbett and Edey (1977) reported a low BUN when ewes were fed formaldehyde-treated casein compound compared to those fed a isonitrogenous untreated casein.

Higher BUN obtained in this study when U was fed is in agreement with Cecava and Hancock (1994) who observed higher (P < .05) plasma urea-N when they fed urea or SBM-FTM combination in the diet for steers compared to those fed a diet based on corn silage and corn supplemented with urea. Higgingbothams et al (1989) found an increase of 2.6 mg/dL in BUN in cows fed isonitrogenous and isocaloric diets by increasing DIP from 58 to 65% of dietary CP. They suggested that ruminal and post ruminal excess of N are eliminated from the body in the same process of hepatic urea synthesis; therefore, excess protein, whether DIP or UIP, will elevate BUN.

Rusche et al. (1993) observed increased plasma urea N concentration with increased dietary protein which they explained that it may be largely by increased absorption of ruminal NH₃ N, resulting in greater quantities of NH₃ being converted into urea in the liver. In the present study a correlation of r = .79 has been recorded for ruminal NH₃ N and BUN (Figure 6). The level of BUN is relatively proportional to the concentration of ruminal NH₃ (Preston et al., 1965). Contrary to these reports, Kwak (1990) reported that concentrations of BUN did
Figure 6. Correlation between blood urea nitrogen and ruminal ammonia nitrogen of sheep fed diets supplemented with different protein sources. nn N (NC); U = urea; BM = soybean meal; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 4.; CS = commercial supplement based on animal protein; and CM = crab meal.
not vary consistently with ruminal NH₃ N levels. Thornton and Wilson (1972) suggested that urinary N was linearly related to plasma urea concentration (r = .97). The present study also agrees to these results (r = .62) (Figure 7), but BUN was more correlated to ruminal NH₃ N than urinary N. Kwak (1990) suggested that BUN concentrations reflected urinary N excretions instead of ruminal NH₃ N levels.

Implications

Protein digestibilities of diets supplemented with experimental supplement based on feather meal, blood meal; supplement based on animal protein, Prolak®; supplement based on plant protein and blood meal; and crab meal are comparable with that of soybean meal. The hydrolyzed experimental supplement was found to be lower to all other protein supplements tested. There seems to be no advantage in hydrolyzing feather meal-blood meal combination in improving the nutritive value of the material for ruminants might be because of varying processing conditions. The retention of N of the above protein supplements and crab meal have numerically higher retention of N compared to soybean meal and may be advantageous in feeding of growing steers.
Figure 7. Correlation between blood urea nitrogen and urinary nitrogen of sheep fed diets supplemented with different protein sources. Ruminal ammonia nitrogen and blood urea concentrations of sheep fed diets with no supplemental N (NC); U = urea; SBM = soybean meal; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 4.; CS = commercial supplement based on animal protein; and CM = crab meal.
Literature Cited


Chapter V. Journal Paper 3. *In Situ* Dry Matter and Crude Protein Degradability and *In Vitro* Dry Matter Digestibility of Crab Processing Waste and Other Protein Supplements.

**Abstract:** An *in situ* dacron bag experiment, consisting of two trials, was conducted with a ruminally cannulated steer to determine the ruminal degradability of DM and CP of crab meal and other protein supplements used in the feeding trial. Protein supplements were, 1) soybean meal (SBM); 2) supplement based on industrial byproducts of both plant and animal origin (IPA); 3) experimental supplement based on byproducts of animal origin (ESA); 4) hydrolyzed supplement No 3. (HESA); 5) commercial supplement based on animal protein (CS), Pro-Lak®; and 6) crab meal (CM). *In vitro* DM digestibility (IVDMD) of the above protein supplements was estimated also with ruminal fluid from the ruminally cannulated steer. The highest *in situ* ruminal DM degradability was for SBM (70.9%) which was higher (P < .001) than for other supplements, viz., IPA (38.8%), ESA (17.0%), HESA (26.8%), CS (24.5%), and CM (29.1%). Dry matter degradability of ESA was lower (P < .01) than IPA, while values for HESA, CS, and CM were intermediate. The *in situ* ruminal escape of protein was lowest for SBM (23.2%) which was lower (P < .01) than CM (48.4%). The highest value was for the ESA supplement (79.8%) which was higher (P < .05) than IPA.
Values for the HESA and CS (67.3 and 69.8%, respectively) were intermediate. The rate of degradation of degradable B (kDB) of N was highest (P < .0001, 12%/h) for SBM compared to IPA (4%/h), ESA (2.3%/h), HESA (2.4%/h), CS (3.4%/h), and CM (5.2%/h), however, the values for the latter supplements were similar (P > .05). The IVDMD were 89.0, 78.8, 40.5, 50.9, 63.4, 67.0, respectively, for SBM, IPA, ESA, HESA, CS, and CM. Values were different (P < .0001) among different supplements except between CS and CM (P > .05). A protein supplement based on feather meal and blood meal was found to have the highest ruminal escape protein. These data shows that CM is a digestible moderate escape protein source for ruminants.

**Introduction**

Advantages of feeding escape protein for enhancing performance of ruminants have been reported (Stock et al., 1981; Harvey and Spears, 1989; Titgemeyer et al., 1989; Blasi et al., 1991; Sindic et al., 1993; Ludden et al., 1995). It has been shown that amino acid (AA) requirements at the tissue level of the ruminant animal increases as production level increases (Hussein et al., 1991). Microbial protein has been found to be most limiting in lysine, methionine, and threonine for growth in steers (Richardson and Hatfield, 1978). Several byproduct feeds of plant, corn gluten meal (CGM) and brewers dried grain (BDG); and of animals, blood meal (BM), feather meal (FTM), meat meal (MM), meat and bone meal (MBM); and marine, fish meal (FM) have been used as protein supplements for high producing cows due to their high ruminal escape potential and AA profile (Waltz et al., 1989; Hussein et al., 1991; Christensen et al., 1993; Cozzi and Polan, 1994). Complimentary responses by feeding combinations of these

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byproducts such as BM and CGM (Stock et al., 1981), or BM and FTM (Goedeken et al., 1990b; Blasi et al., 1991) have been studied. Several commercial escape protein supplements of different combinations of these byproducts of different proportions are now available, but their ruminal degradability and escape potential are not known.

Another product of interest as a protein supplement for ruminants is crab processing waste which is a byproduct of sea food industry. Several studies have shown that crab waste meal can be fed to ruminants as a protein source. Patton and Chandler (1975) observed rumen degradation of crab waste meal to be 35.9% for DM and 50.2% for CP. Patton et al. (1975) observed no differences in average daily gain, feed intake, feed conversion, N retention, or digestibility of DM, crude fiber or N when Holstein heifers were fed 10 or 20% crab waste meal compared to those fed a diet with no crab waste meal. The apparent digestibility of DM and CP of silage containing crab processing waste has been shown to decrease linearly with increased levels of CPW silage in the diet (Ayangbile, 1989). Velez et al. (1991) observed lower rumen degradability of DM and CP of crab processing waste compared with those values obtained by Patton and Chandler (1975).

To measure the rumen DM and CP degradability, in situ (nylon bag, in sacco) technique has been the common method employed since it was first developed by Quin et al. (1938). Previously, this nylon bag technique was used primarily for forage evaluation. This method has been employed to evaluate DM degradation (Mehrez and Orskov, 1977; Nocek et al., 1979; Lindberg, 1989) and for the rate of protein degradation (Mehrez et al., 1977; Nocek et al., 1979; Weakly et al., 1983).

The objectives of this study were to determine the ruminal disappearance of DM and CP of crab meal and other protein supplements by measuring in situ
degradability and to determine the *in vitro* dry matter digestibility of the supplements.

**Experimental Procedures**

*In Situ Experiment.* A ruminally fistulated steer (550 kg, BW) was used to determine *in situ* DM, CP degradabilities of the protein supplements and crab meal. The steer was fed equal portions of alfalfa and orchard grass hay beginning 15 d before the start of the first *in situ* trial. Protein supplements tested were, 1) soybean meal (SBM); 2) supplement based on industrial byproducts of both plant and animal origin (IPA); 3) experimental supplement based on byproducts of animal origin (ESA); 4) hydrolyzed supplement No 3. (HESA); 5) commercial supplement based on animal protein (CS), Pro-Lak®; and 6) crab meal (CM). The supplements were ground through 1 mm mesh in a Wiley mill (Arthur Thomas Company, Philadelphia, PA).

Polyester bags of 10 x 23 cm size with 59 ± 2 μm porosity were used. Calculated sample size to bag surface area was 15 mg/cm² (Nocek, 1985). Six grams of samples were placed in each polyester bag. Bags were closed with 10 cm Bar-Lok® Cable ties (Dennison Manufacturing Co., Framingham, MA). Duplicate bags were tied with another Bar-Lok® Cable tie of 10 cm length. Duplicate bags of the supplements were tied to the metal chain attached to the polyester thread with bigger, 20 cm Bar-Lok® Cable ties. Bags for the 72 h incubation were tied to a thick polyester string (to which subsequent groups of bags were attached) of approximately 75 cm in length which was attached to a metal chain 20 cm in length. The incubation periods were 0, 2, 6, 12, 24, 48, and 72 h. The bags were introduced in the rumen in the reverse order. Prior to incubation, bags were soaked...
in lukewarm water (~38°C) for 15 min (Rodriguez, 1994) and then introduced into the rumen through the rumen cannula. Eighty four bags were suspended in the rumen. The bags for the 0 h incubation were not introduced in the rumen, instead, bags were soaked in lukewarm water for 15 min. After 72 h all bags were retrieved, washed in running tap water and duplicates were separated. Bags were washed thoroughly until rinse water was clear. Bags were dried at 60°C in a forced draft oven for 48h. Bags with dried residues left in the bags were weighed and DM disappearance was estimated. Residues left in the bags were used for the analysis of N by Kjeldahl method (AOAC, 1990).

Ruminal degradation of DM and CP were classified into three main fractions as proposed by Armentano et al. (1986). For CP, the fractions are A, B, and C which are as follows,

\[
A = \text{NPN or true protein that is solubilized rapidly in warm water}
\]

and is calculated by subtracting the percent remaining at 0 time from 100.

\[
B = \text{Protein that is degraded at a rate similar to the rate of passage and is calculated by subtracting sum of A and C from 100.}
\]

\[
C = \text{Protein that is undegraded in the rumen, which is the percent remaining at 48 h.}
\]

For DM, the definition was the same as above. Degradability of DM or CP depends on rate of passage of digesta \(k_{pb}\) which is expressed as percentage per hour (%)h and rate of degradation \(k_{db}\). The rate of passage in this study was assumed to be 5%/h. The residue at 48 h incubation was considered as C fraction and there was no change for this fraction. The B fraction was calculated as the difference between 0 h and 48 h sample weight. Then, the residues (expressed as
percentage of the material inserted) at each incubation time minus C fraction were converted into natural log, and subjected to linear regression. The degradation constant \((k_{db})\) is represented by the slope of this line. The *In situ* ruminal degradability of DM and escape of CP of protein supplements were estimated applying the model as prescribed by Orskov and McDonald (1979) considering the ruminal passage rate \((k_{pb})\).

\[
D = A + (B \times k_{db})/(k_{db} + k_{pb})
\]

where,

\(D\) = Protein degradability, (%)

\(A\) = Fraction A, readily degradable, (%)

\(B\) = Fraction B, slowly degradable, (%)

\(k_{db}\) = Degradation rate constant of degradable B

\(k_{pb}\) = Rate of passage (assumed to be 5%/h)

The escape of protein was also estimated by applying the model proposed by Orskov and McDonald (1979).

\[
\text{Escape} = k_{pb} \times B / (k_{db} + k_{pb}) + C
\]

Where,

\(B, k_{db}, \text{ and } k_{pb}\) are stated as in above model

\(C\) = Fraction that is undegraded

*In Vitro Experiment.* The *in vitro* procedure according to Tilley and Terry (1963) was followed for the estimation of DM digestibility of the protein supplements. The experiment was replicated. The protein supplements were prepared as for the *in situ* method. The ruminally cannulated steer was fed a diet containing equal portions of alfalfa and grass hay. The steer was fed this diet for a minimum of 15 d prior to the collection of rumen liquor. Ruminal fluid was
collected through the cannula and filtered through eight layers of cheese cloth into a prewarmed thermos flask. Fluid was then transported to laboratory where it was added to the buffer solution, which has been prepared prior to the collection of rumen liquor.

The buffer solution (McDougall, 1948) was prepared as follows.

\[
\begin{align*}
\text{NaHCO}_3 & \quad 9.80 \text{ g} \\
\text{Na}_2\text{HPO}_4\cdot12\text{H}_2\text{O} & \quad 9.38 \text{ g} \\
\text{NaCl} & \quad .47 \text{ g} \\
\text{KCl} & \quad .57 \text{ g} \\
\text{MgCl}_2 & \quad .06 \text{ g}
\end{align*}
\]

The above salts were thoroughly dissolved in about 500 mL of distilled water and adjusted to a volume of 1 L. pH of buffer at this time was around 8.2 to 8.4. Just prior to use, .04 g of CaCl$_2$ and enough (NH$_4$)$_2$SO$_4$ to make the solution .02 M (2.64 g/L) were added to the buffer and thoroughly dissolved. Temperature of the buffer was adjusted at 38 to 39°C and CO$_2$ was bubbled through the buffer to bring down the pH to 6.8 to 7.0. Rumen liquor was added to the buffer in the ratio 1:4. Thirty five mL of rumen liquor:buffer mixture were placed in the tubes containing protein supplements and flushed with CO$_2$ to create anaerobic condition. Tubes were closed with Bunsen valves, then incubated in a water bath for 48 h at 39°C, centrifuged at 1800 x g for 15 min and decanted. The tubes were again incubated in water bath for 48 h at 39°C after adding acid pepsin (2 g pepsin plus 83.3 mL of HCl per L of solution) loosely fitted with Bunsen stoppers. At the end of incubation, tubes were centrifuged at 1800 x g for 15 min, decanted, and dried at 60°C for 24 h. Percentage \textit{in vitro} DM digestibility (IVDMD) was calculated using the formula:

\[
\text{% IVDMD} = \frac{\text{wt of sample} - (\text{wt of residual} - \text{blank})}{\text{wt of sample}} \times 100
\]
**Statistical Analysis:** All data are presented as least squares means. Data were analyzed by analysis of variance using the GLM procedure of SAS (1989). Tukey's Studentized Range (HSD) Test was used for comparing the treatment for different variables. Significance of (P < .05) was used throughout unless otherwise stated.

**Results**

**In Situ Experiment:** Dry matter degradability of protein supplements at different incubation times is presented in Figure 8. Over 90% DM of SBM was degraded in first 12 h, whereas the values were only 40 to 45% for ESA, HESA, and CM. High degradability (60%) was observed for HESA at 0, and 2 h, then the degradability plateaued. More than 90% of DM of SBM was degraded in the first 12 h, whereas only about 40% of DM was degraded in the case of ESA, CS, and CM. About 60% of DM of HESA was degraded in the first 2 h and thereafter degradability was plateaued and only 10% was further degraded in another 46 h. Similar but slightly higher degradability was seen for IPA where almost 65% DM was degraded in first 6 h and in the next 42 h only 20% was degraded.

The data on fractions, degradation rate constant of B fraction, and degradability of DM of protein supplements are presented in Table 16. The soluble A fraction ranged from 8.8 to 33.9%. Highest soluble A fraction was for the HESA supplement (33.9%) which was higher (P < .05) than that for IPA (19.7%), CM (12.2%), CS (10.7%), and ESA (8.8%), while SBM had an intermediate value (25.0%). Degradable B fraction was highest in SBM (74.3%) which was higher (P < .05) than ESA (43.9), HESA (33.3%), and CS (47.7%). Lowest (P < .0001) undegradable C fraction of DM was observed in SBM (.7%). The supplements
Figure 8. In situ dry matter degradability of protein supplements. SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.
Table 16. Degradability of dry matter of protein supplements in situ

<table>
<thead>
<tr>
<th>Item</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter fractions, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble A ^</td>
<td>25.0 ^d</td>
<td>19.7 ^d</td>
<td>8.8 ^d</td>
<td>33.9 ^c</td>
<td>10.7 ^d</td>
<td>12.2 ^d</td>
<td>4.35</td>
</tr>
<tr>
<td>Degradable B ^</td>
<td>74.3 ^c</td>
<td>65.3 ^c</td>
<td>43.9 ^de</td>
<td>35.9 ^c</td>
<td>47.6 ^d</td>
<td>46.0 ^de</td>
<td>3.13</td>
</tr>
<tr>
<td>Undegradable C ^ ***</td>
<td>.7 ^c</td>
<td>15.0 ^d</td>
<td>47.3 ^c</td>
<td>30.2 ^f</td>
<td>41.7 ^e</td>
<td>41.8 ^e</td>
<td>1.57</td>
</tr>
<tr>
<td>k_{db}, %/h ^ ***</td>
<td>12.7 ^c</td>
<td>4.2 ^d</td>
<td>2.4 ^d</td>
<td>2.8 ^d</td>
<td>3.9 ^d</td>
<td>5.0 ^d</td>
<td>0.71</td>
</tr>
<tr>
<td>Degradability, % ^ b, ^ ***</td>
<td>70.9 ^c</td>
<td>38.8 ^d</td>
<td>17.0 ^c</td>
<td>26.8 ^de</td>
<td>24.5 ^de</td>
<td>29.1 ^de</td>
<td>3.31</td>
</tr>
</tbody>
</table>

^Least-squares mean
^SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No. 3.; CS = commercial supplement based on animal protein; and CM = crab meal.

*P < .05
**P < .01
***P < .001
^c,d,e,f Numbers in the same row with different superscript letters differ
^Degradation rate constant of degradable B fraction
^Degradation = A + B x k_{db}/(k_{db} + k_{pb})

where,

A = fraction A, readily degradable (%)
B = fraction B, degradable (%)
k_{db} = rate of degradation
k_{pb} = rate of passage(5%/h)
ESA (47.3%), CM (41.8%), and CS (41.7%) were higher (P < .001) than HESA (30.2%) which was higher (P < .001) than IPA (15.0%).

Degradation rate constants (k_{db}) of the B fraction of DM of feedstuffs ranged from 2.4 to 12.7 %/h. The k_{db} of SBM was the highest (P < .01; 12.7%/h) compared to other supplements where k_{db} ranged between 2.4 and 5%/h. Among these protein supplements, numerically, higher k_{db} was for CM (5%/h) and the lowest was for ESA (2.4%/h), however, differences were not significant (P > .05). Dry matter degradability was highest (P < .001, 70.9%) for SBM among the different protein supplements tested. The lowest value was for the ESA (17.0%) which was lower (P > .01) than IPA (38.8%). Values for HESA, CS and CM (26.8, 24.5, and 29.1%, respectively) were intermediate.

Similar trends were observed in the CP fractions of SBM, but the trend varied among other protein supplements (Figure 9). Crab meal, which had a lower DM degradability, showed a higher degradability of protein. Highest disappearance was for SBM.

Lowest soluble A fraction was for the ESA (6.2%) which was lower (P < .01) than that of IPA (21.3%), HESA (23.2%) and CM (20.7%) (Table 17). Values for SBM and CS were intermediate. Degradable B fraction was highest (P < .0001) in SBM (87.1%). Degradable B fraction of CM (59.8%) was higher (P < .0001) than for ESA and HESA. The value for CS was intermediate.

Undegradable C fraction was negligible (.2%) for SBM while ESA had the highest (53.2%), which was higher (P < .01) than for the other supplements. Undegradable C fraction of CM (19.5%) and IPA (21.7%) were lower (P < .01) than for HESA and CS. Degradation rate constant (k_{db}) of the degradable B fraction of the CP of protein supplements showed a similar trend as that of rate degradation of DM. Rate of degradation was highest (P < .01) for SBM (14%/h)
Figure 9. In situ crude protein degradability of protein supplements. SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3; CS = commercial supplement based on animal protein; and CM = crab meal.
Table 17. Degradability of crude protein of protein supplements *in situ*

<table>
<thead>
<tr>
<th>Item</th>
<th>SBM</th>
<th>IPA</th>
<th>ESA</th>
<th>HESA</th>
<th>CS</th>
<th>CM</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein Fractions, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble A **</td>
<td>12.7d</td>
<td>21.3d</td>
<td>6.2e</td>
<td>23.2d</td>
<td>12.4de</td>
<td>20.7d</td>
<td>2.59</td>
</tr>
<tr>
<td>Degradable B *</td>
<td>87.1d</td>
<td>57.0ef</td>
<td>40.6g</td>
<td>37.5g</td>
<td>49.4f</td>
<td>59.8g</td>
<td>2.02</td>
</tr>
<tr>
<td>Undegradable C **</td>
<td>2d</td>
<td>21.7e</td>
<td>53.2g</td>
<td>39.3f</td>
<td>38.2f</td>
<td>19.5e</td>
<td>1.99</td>
</tr>
<tr>
<td>[k_{db}, %/h^{b**}]</td>
<td>14.0d</td>
<td>4.0ef</td>
<td>2.3f</td>
<td>2.4f</td>
<td>3.4ef</td>
<td>5.2g</td>
<td>0.63</td>
</tr>
<tr>
<td>Escape, % CP**</td>
<td>23.2d</td>
<td>60.6e</td>
<td>79.8f</td>
<td>67.3ef</td>
<td>69.8ef</td>
<td>48.4g</td>
<td>3.14</td>
</tr>
</tbody>
</table>

*Least-squares mean

**SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.*

**DM basis

*Numbers in the same row with different superscript letters differ

**P < .05

**P < .01

Degradation rate of degradable B fraction

\[\text{Escape} = k_{pb} \times B / (k_{db} + k_{pb}) + C\]

where,

\[B = \text{fraction B, degradable (}\%\)]

\[C = \text{fraction C, undegradable (}\%\)]

\[k_{db} = \text{rate of degradation}\]

\[k_{pb} = \text{rate of passage (5}\%/h)\]
compared to other protein supplement, where the rate of degradation ranged from 2.3 to 5.2%/h. Rate of degradation of CM (5.2%/h) was highest (P < .01) than for ESA and HESA (2.3 and 2.4%/h). Values for IPA and CS were intermediate (4 and 3.4%/h).

The ruminal escape of CP was highest (79.8%) for ESA which was higher (P < .05) than IPA (60.6%), SBM (23.2%) and CM (48.4%). The escape of CP of CM was lower (P < .05) than IPA, whereas the values for HESA, and CS (67.3% and 69.8%, respectively) were intermediate. Lowest (P < .001) escape value was observed for SBM (23.2%).

In Vitro Dry Matter Digestibility (IVDMD). In vitro DM digestibility of different protein supplements ranged from 40.5 to 89% (Figure 10). Highest (P < .0001) DM digestibility was for SBM (89%) and the lowest (P < .0001) value was for ESA (40.5%). Respective values for IPA, CM, CS and HESA were 78.8, 67, 63.4, and 50.9%, which were different (P < .0001) except between CS and CM (P > .05).

Discussion

Degradation pattern of CP of SBM agrees with the findings of Kwak (1990) who reported that the rate of degradation of degradable B fraction of SBM was 10.3%/h, while Zerbini and Polan (1985) reported a still closer value of 12.2%/h compared to the value obtained in this study (14%/h). Blake and Stem (1988) reported a low k_{db} of 6%/h. The present study reveals that the degradable fraction B of CP of all the protein supplements was rapidly degraded during the first 2 h, then slower up to 12 h and from 12 to 48 h degradation was still lower. The 2 h N disappearance was assumed to contain soluble NPN plus a rapidly degradable
Figure 10. *In vitro* dry matter digestibility of protein supplements. SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.
protein fraction (Nocek et al., 1979). These rates will be referred to as $2 \ h \ N \ k_{db}$. In the present study, $N$ disappearance up to 2 h was rapid, especially for protein supplements of byproduct nature compared to that of SBM. The 2 to 12 or 2 to 24 h $N \ k_{db}$ represents a slowly degradable protein fraction which is assumed to be degraded linearly. A two sloped degradation characteristic has been demonstrated in wide variety of feeds (Mathers et al., 1977; Crawford et al., 1978). Nocek et al. (1979) reported that 2 h $k_{db}$ for $N$ show a wide range of degradation rates in feed ingredients ranging from 3.5%/h to 27.9%/h. Therefore, to eliminate this discrepancy, Nocek et al. (1979) excluded 2 h $k_{db}$ from the calculation of correlations. They also reported that $N$ of cottonseed meal and dehydrated alfalfa disappeared rapidly within the first 2 h, while other processed protein supplements such as SBM, distillers grain, and fish meal had lower 2 h $k_{db}$.

Dry matter disappearance was higher starting from 6 to 12 h period, with significant difference ($P < .01$) at 12, 24, 48 and 72 h ruminal incubation between the SBM and all other supplements. The same trend was observed for CP also, but the disappearance was rapid i.e., higher disappearance of SBM $N$ compared to other supplements was shown starting 2 to 6 h period with significant difference ($P < .01$) at 6 h and all other incubation periods thereafter. This indicates that SBM was more susceptible to microbial degradation in the rumen. At 0 h, numerically higher disappearance of CP was observed for IPA, HESA, and CM (23.8, 23.2, and 20.7%, respectively) than SBM (12.7%). This indicates the presence of highly soluble $N$ fraction in these supplements. Commercial supplement (CS) had similar CP disappearance at 0 h compared to that of SBM. The ESA had the lowest (6.7%) disappearance of CP at 0 h, showing a relative lower content of soluble $N$ fraction than all other protein supplements.
The soluble N fraction of a feed is degraded more rapidly in the rumen than the insoluble fraction (Clark et al., 1987). Clark et al. (1987) reported that more total N in the SBM is in the soluble fraction compared to those in BM, CGM or meat and bone meal. Sniffen (1974) reported that cereal grains and protein supplements contain four types of proteins, albumin, globulin, prolamin, and glutelin. They indicated that albumins and globulins are low molecular weight proteins which are soluble in ruminal fluid, whereas prolamins and glutelins are high molecular proteins that contain disulfide bonds which render them less soluble in ruminal fluid. Nugent and Mangan (1978) reported that protein that have a low solubility in ruminal fluid and that contain extensive cross linking such as disulfide bonds are less accessible to proteolytic enzymes and are relatively resistant to degradation. They also reported that albumin and globulin have better AA profile than glutelin and prolamin but they are degraded in the rumen. Sniffen (1974) reported that the largest portion of protein of corn and barley are prolamin and glutelin. Soybean meal contains largely globulin, hence the meal is highly degraded in the rumen.

Nitrogen disappearance of SBM in first 2 h in this study agree with results of Nocek et al. (1979). Nitrogen solubility has been considered as an indication of N degradability on the assumption that if N compounds are highly soluble, then these also will be highly degradable (Sniffen, 1974; Wohlt et al., 1976). Mertens (1977) proposed that in vivo all of the soluble N is degraded, and 40 to 50% of the insoluble N is degraded in the rumen. Some workers have disagreed to the above statements and reported that protein solubility is a poor indication of protein's resistance or susceptibility to degradation in the rumen (Mahadevan et al., 1980; Stern and Satter, 1984; Nicholson and Johnson, 1991). However, Nocek et al. (1979) reported that neither N solubility nor 2 h N disappearance are reliable
indicators of total N degradation of a feedstuff during ruminal residence. Solubility of protein is not a guarantee nor a prerequisite for degradability (Chalupa, 1982). Leng and Nolan (1984) suggested that soluble proteins such as serum albumin, ovalbumin, chloroplast protein extract, and soluble protein from SBM and rapeseed meal are degraded to varying degrees. It has been shown that the structure of protein is an important determinant of degradability (Chalupa, 1982; Leng and Nolan, 1984). Proteins which have no terminal amino or carboxyl groups (i.e., ovalbumin) and those with excessive cross linking (i.e., Ribonuclease A) appear to be less accessible to proteolytic enzymes.

The same trend as seen in CP degradation was shown in DM also. Nocek et al. (1979) reported that there was a positive correlation ($r^2 = .81$) between the $2\text{ h}$ and 2 to $48\text{ h}$ $k_{db}$ of DM of various feed ingredients. Therefore, they suggested that more reliable prediction of degradation of DM from DM solubility than from N solubility.

Ruminal degradability of CP of SBM obtained in this study agrees well with previous reports for the ruminal N degradability of SBM. Literature values for SBM N escaping ruminal degradation have ranged between 15 and 43% (Zinn et al., 1981; Zinn and Owens, 1983; Loerch et al., 1983a; Kirkpatrick and Kennelly, 1986; Kwak, 1990; Zerbini and Polan, 1990; Rodriguez, 1994). The escape value determined (23.2%) for the SBM N in the present study agrees with previously reported values. Sindt et al (1993) reported ruminal N escape of 52.3% for SBM where $k_{pb}$ was estimated to be 6.3%/h while, for BM and FTM values were 4.7%/h and 5.2%/h, respectively. The high escape of N of SBM in their study might be due to increased rate of passage which reflected on a lower $k_{db}$ of 5.7%/h compared to 14%/h in the present study. Orskov et al. (1983) reported that the rate of outflow of protein can range from approximately 1%/h with a low level of
feeding of ground diets to approximately 10%/h with very high levels of feeding of mixed diets. Normally, \( k_{ph} \) of 5%/h was considered optimum at maintenance level of feeding (Miller, 1982). They also reported that degradability cannot be expressed as a single value and will vary considerably with outflow rate. Their estimate of escape N in SBM (52 or 43%, depending on passage rate estimate) was higher than the value obtained in the current study (23.2%) where the steer was fed 50:50 alfalfa grass hay diet. Their low ruminal degradation values for the SBM N might be due to a low ruminal pH. Loerch et al. (1983a) reported that 12 h in situ escape N of SBM increased from 33 to 55% as level of high-moisture corn in the diet increased from 20 to 80%. Ruminal pH decreased with increased high-moisture corn level in the diet. Loerch et al. (1983a) suggested that this low ruminal degradation might be due to a low pH when cattle were fed high grain diets. They also suggested that this low pH might have reduced the number of proteolytic bacterial species. However, it is not likely that the ruminal pH of the steers used in the present study was low since the diet did not include concentrates.

A high level of escape protein for SBM (66.0%) has been reported by Ludden et al. (1995). Escape of protein of a combination of CGM and BM was 90.8%. Rate of degradation of SBM and combination of plant protein and BM was also lower than observed in the present study. One possibility for this high escape of SBM in their experiment would be due to the diet which contained 70% corn grain whereas, in the present study the host animal was fed a complete roughage diet (50:50, alfalfa - grass hay). A similar result as observed by Ludden et al. (1995) has been reported earlier by Barrio et al. (1986) who found that the rumen escape value for DM and N in SBM were lower when a diet with 80% as opposed to 40% concentrate was fed to cattle. Loerch et al. (1983b) had reported earlier
that the decrease in ruminal pH by feeding diets based on cereal grains may reduce the protein degradability of SBM. Ward et al (1986) reported that the amount of dietary protein that escaped ruminal degradation in lactating dairy cows fed concentrate, alfalfa hay and sorghum silage was about 2.5 times greater (46 vs 18%) when concentrates supplied 65% rather than 55% of the dietary DM. Owens (1986) indicated that increasing concentrate from 30 to 60% of the diet increased protein that escaped ruminal degradation from 22 to 29%, which may be attributed to lower ruminal pH, which reduces solubility, thus reducing the accessibility of protein for degradation (Loerch et al. 1983a) or due to a reduction in the fiber content of the diet, which inhibits microbial degradation of protein (Weakely et al., 1983).

Sindt et al. (1993) reported that estimated escape N determined by combining passage and digestion rates were highest for BM and FTM and lowest for corn silage while, SBM had an intermediate value. Reported values for N escape for BM were 85, 82, 81.4, 92, and 93.2 %, respectively, by Stock et al. (1981), Loerch et al. (1983b), Palmquist et al. (1983), Titgemeyer et al (1989), and Rodriguez (1994), while those for FTM were 65, 85.1 and 91.7%, respectively, by Titgemeyer et al. (1989), Palmquist et al. (1993) and Sindt et al. (1993). Titgemeyer et al. (1989) reported that BM contained the largest amount of N (22%) that was not available for either ruminal degradation or small intestinal absorption, whereas SBM contained the least nonavailable N (6.5%) followed by FTM (13%). The escape N for urea-BM/FTM combination was 59.2% (Sindt et al. 1993) which was lower than the value obtained for combination of FTM and BM in the present study, the reason for their low value might be due to the incorporation of urea.
The present study indicated that the CP of ESA which is a combination of BM and FTM was only about 25% as degradable as the protein in SBM, whereas for the HESA and CS, degradability was 43 and 39%, respectively, as degradable as the SBM protein. In the present study the rumen escape of CP of ESA and HESA supplement were 79.8 and 67.3%, respectively. Although both products are combinations of BM and FTM, the later was hydrolyzed with little differences in composition between the two. The escape of N of HESA was similar to that of CS (69.8%), but the nutritive value of HESA was significantly lower than CS (Chapter III and IV). This suggests that the hydrolyzed product of FTM and BM combination might be poorly digested postruminally nor had a poor AA profile. In fact CP digestibility was very low (41%) for the HESA supplemented diet (Chapter IV). These results agree with findings of King et al. (1990) who reported that BM N was poorly digested post ruminally, especially when BM was batch dried at high temp which may decrease available protein more than would spray or roller drying.

Garret et al. (1987) calculated undegradable intake protein (UIP) of BM and SBM as 58 and 30%, respectively, which indicated that various sources of BM have a lower UIP value than that reported (82%) by NRC (1989). Titgemeyer et al. (1989) reported UIP of 92% for BM. Howie et al. (1994) suggested that these inconsistencies may be due to variation among samples of BM processed by different methods or estimation of ruminal CP degradation.

Goedeken (1990b) reported that escape protein of FTM (73.2%) was greater than that of SBM (24.6%) but less than that of BM (90.0%). A combination of BM and FTM had an escape of 82% which was comparable with the values (80%) obtained in the present study. They also reported that weighted average of ruminal escape of FTM and BM is similar to the protein escape value.
for the FTM + BM (79 vs 82%). Hydrolyzing blood possibly increased ruminal fluid solubility and reduced ruminal escape (Goedeken et al., 1990b). They indicated that adding raw blood to feathers before hydrolyzing did not increase the escape above that of FTM without blood, whereas adding blood after hydrolyzing feather increased ruminal escape compared with FTH. The same workers (Goedeken et al., 1990a), in another experiment, estimated the in situ N undegradability of FTM and BM to be 69 and 83%, respectively. Waltz et al. (1989) reported that grain mixes containing FTM and BM + FTM had lower rates of N disappearances and protein degradation compared with the grain mix containing SBM. They also reported that rate of N disappearance and extent of protein degradation were lower in the grain mix containing the BM than in the grain mix containing SBM but was higher than in the grain mix containing FTM. Method of processing of BM had an effect on degradability of BM (Harvey and Spears, 1989). Waibel et al. (1977) reported that the conventional method of processing BM by vat drying is less expensive than the ring dried but lysine has been shown to be lower in vat dried BM.

Hvelplund et al (1993) reported that rumen undegraded protein (RUP) should be partitioned into intestinally available and unavailable fractions. The NRC assumes all the escape proteins are equally digested in the small intestine. The NRC models consider only RDP and RUP, ignoring the nature of protein, i.e., bound or unavailable that is present in RUP. Since bound protein may not be digested in the small intestine, the nutritional value and digestibility of RUP may be variable. This shortfall has been recognized by the Cornell Net Carbohydrate and Protein System for evaluating cattle diets. This system contains a sub model whereby protein is partitioned into six fractions. Fraction C represents proteins that are insoluble in acid detergent and considered to be indigestible in the
intestine (Fox et al., 1990). Although intestinal measurements have not been studied in this experiment, it is assumed that intestinal digestion of CP of HESA may be poor when the results of this experiment is related to the balance experiments and growth trials conducted (Chapter III and IV).

Wallace (1988) noted that proteins of animal origin are generally broken down rapidly but incompletely, whereas plant proteins are degraded more slowly, but potentially degraded almost completely. Results of the present study do not agree entirely with the above statement because CP disappearance of ESA and CS was lower than SBM, which might be due to collagenous nature of protein of FTM and other proteins of animal origin which are resistant to ruminal degradation. However, in the present study N disappearance from CM, CS, ESA, and HESA tended to be higher compared to plant protein supplement such as SBM and a combination of plant protein and BM for the 1st 2 h of incubation.

Clark et al. (1987) reported that protein of animal byproducts are less degraded compared to cereal by products. The low degradability of meat or meat and bone meal which contains relatively large amount of collagen, and is low in lysine (Happich, 1975). Therefore, these products will have a shortage of trypsin-sensitive peptide bonds. Accessibility of certain AA residues within the protein molecule may influence both rate and extent of protein degradation in the rumen (Craig and Broderick, 1984). Losses of lysine and arginine have been shown to be greater than average loss of other AA when proteins were degraded by ruminal microbes. (Craig and Broderick, 1984; Crooker et al, 1986). It has been shown that microbial enzymes cleave the peptide bonds at lysine residues, similar to the mammalian trypsin enzyme (Craig and Broderick, 1984). Obviously, a feedstuff with low lysine content (cereal grain by product) will have a slow degradation in the rumen. By product feedstuffs of animal origin such as FTM and hair meal are
extensively cross linked with disulfide bonds, which are not very susceptible to proteolytic enzymes (Clark et al., 1987). These cross linkages render these products resistance to ruminal degradation (Nugent and Mangan, 1978). The low ruminal degradation of the ESA and HESA supplements are explained by the above reason.

Processing of byproduct feeds undergo cooking or drying which denatures and coagulates the protein, making it insoluble (Boehme, 1982). Similarly, cooling causes a random relinkage of the protein molecule (Bendall, 1964). He also reported that decreased surface area for microbial attack and the formation of chemical bonds upon cooling render the protein in these byproduct feeds more resistant to ruminal degradation. Heat applied during drying of cereal byproduct feeds also denatures and coagulates protein, making it less degradable in the rumen.

In the present study ruminal DM degradability was lower (29.1%) for CM compared to that for CP (51.6%). Relatively higher degradability of CP of CM was observed compared to other protein supplements. However, the escape protein was found to be over twice as that of SBM. A similar trend was observed by Velez et al. (1991) but a lower DM and CP degradability of 18.3 and 34.9%, respectively, were reported for crab waste meal. They also reported a relatively lower degradability of DM and CP of SBM (55.9 and 63.6%, respectively). A lower rate of degradation of DM and CP of crab waste meal (.57%/h and .79%/h, respectively) have been reported (Velez et al., 1991), compared to higher values of 5%/h and 5.2%/h, respectively, for the present study. The differences observed in these two studies might be explained by the differences in the ruminal environment due to the diet variation or due to differences in the procedures. Degradability observed in this study for CM agrees with the findings of Patton and Chandler (1975) who reported the DM disappearance at 12, 24, 36, 48, and 60 h
for crab waste meal as 26.7, 33.2, 36.2, 41.2, and 38.3%, respectively. The low degradability of DM relative to CP might be due to high levels of ash (45%) which may be poorly soluble in rumen.

As expected, almost 90% of the DM of SBM was digested by *in vitro* estimation of DM digestibility (IVDMD). Feather meal and BM combination (ESA) had the lowest DM digestibility whereas the hydrolyzed product had a higher DM digestibility but this difference was not revealed in *in vivo* estimation (Chapter IV), perhaps because of the nutrient dilution. These results agree with the findings of King et al. (1990) who reported that BM N was poorly digested post ruminally, especially when BM was batch dried at high temperature. Relatively higher level of DM digestibility and ruminal escape of N for the CM, shows that it will be advantageous to use CM as a protein supplement for ruminants.

**Implications**

Crab meal contains over twice the ruminal escape protein of SBM. This seafood waste is a digestible moderate escape protein source that may be useful in diets for ruminants. Combination of feather meal and blood meal (ESA) had the highest escape protein among the supplements tested which shows its potential use as an escape protein supplement. Although hydrolyzed product of feather meal and blood meal combination had almost thrice the ruminal escape protein of SBM, its nutritive value is significantly lower than all the other protein supplements tested. This implies that caution should be taken to evaluate the feedstuffs based on escape value alone and it is suggested that other criteria such as amino acid profile and whole tract digestibility may be considered in evaluating the feedstuffs as a protein supplement for ruminants.
Literature Cited


Chapter VI. Journal Paper 4. Effect of Steam Explosion at Two Levels of Severity on Dry Matter and Chitin Digestibilities and Escape of Protein as Measured by In Situ and In Vitro Methods

Abstract: The potential was explored of the steam explosion technique to enhance the nutritive value of crab meal for ruminants. Crab meal and laboratory grade chitin (isolated chitin) were steam exploded in a 25 L, stainless steel, batch steam explosion reactor at reactor temperature 193°C, pressure 12.7 kg/cm², (low severity); and reactor temperature 226°C, pressure 25.5 kg/cm², (high severity); for 3 min and 30 sec. Steam explosions for each ingredient for each level of severity were replicated. Steam explosion decreased N content of crab meal by 20% in both severities. A ruminally-cannulated steer (550 kg, BW) was fed a diet containing 40 parts alfalfa hay, 40 parts grass hay, 10% ground corn, and 10% crab meal starting 15 d prior to the experiment. The materials tested for in vitro and in vivo experiments were, 1) crab meal, 2) steam exploded crab meal at low severity, 3) steam exploded crab meal at high severity, 4) isolated chitin, 5) steam exploded isolated chitin at low severity, and 6) steam exploded isolated chitin at high severity. An IVDMD experiment was replicated. Steam explosion at low and
high levels of severities improved (P < .01) IVDMD (71.0 and 75.2%, respectively) compared to unexploded (65.9%), while IVDMD of isolated chitin was reduced by 40% by steam explosion. Steam explosion did not show any effect on ruminal DM degradability. The escape protein of crab meal was estimated to be 37.4%. Steam explosion did not improve the escape value of crab meal protein (41.2% and 34.7%, respectively for crab meal steam exploded at low and high severities). Chitin content in the crab meal was 14.7%. A 60% increase in chitin degradability was observed for crab meal exploded at high severity. Chitin degradability in isolated chitin exploded at high severity was more than twice that of crab meal, but steam explosion lowered chitin degradability (P < .05). Steam explosion does not seem to be a promising processing method for better utilization of crab waste for ruminants.

**Introduction**

Substantial quantities of wastes are produced as byproduct from seafood processing plants around the world. It has been estimated that global annual production of dry shell wastes from crab, lobster, shrimp, krill, and clam/oyster, is 1.46 million t, DM basis (Knorr, 1991). The waste produced by seafood producing plants is highly perishable and has become a menace to the processor both in developed and developing countries due to the environmental problems. Shell fish wastes have been found to be suitable for feeding ruminants as dry meal (Patton et al., 1975; Velez et al, 1991) and as silage with wheat straw (Samuel et al., 1991). Although crab meal (CM) is a good source of protein, it has been shown that the digestibility of protein by crab waste-straw silage was lower than that of fish waste straw silage (Samuels et al., 1991). Further, Velez et al. (1991) reported that
CP digestibilities for the crustacean waste meal diets were 8 to 18% lower than for isoamitrogenous diets with SBM. Digestion trials conducted with sheep showed that animals fed 50% crab waste-straw silage and 50% basal diet had a lower digestibility (37%) of chitin than for sheep receiving 100% crab-waste straw silage (58%) (Ayangbile, 1989). Apparent digestibility of DM, OM, CP, energy, NDF, ADF, cellulose and hemicellulose in silage containing crab waste decreased linearly with increased level of crab waste straw silage.

The lower digestibility of crab processing waste diets may be due to chitin which is (1-4)-β-linked homopolymer of N-acetyl-D-glucosamine, an analogue of cellulose. Chitin has been shown to be resistant to degradation by microorganisms in the gastrointestinal tract of ruminants (Morgavi et al., 1994). This lower digestibility of chitin and protein is perhaps due to lower accessibility of microbial enzymes. Chitin and protein are covalently linked via aspartic acid (Brine and Austine, 1981; Hackman, 1984). If chitin-protein complex could be cleaved, perhaps more nutrients would be available due to more accessibility to enzymes. Therefore, the proposed research was to explore the possibilities of any structural changes in the components of crab waste by steam explosion technique.

Steam explosion is a technique employed in industries for fractionation of lignin, hemicellulose, and cellulose. In this method biomass is treated with high pressure steam followed by explosive release of pressure (Foody, 1982). Steam explosion of biomass normally produces a fibrous material that can be fractionated into water-soluble hemicelluloses, alkali-soluble lignin, and an insoluble cellulose-rich residue in proportions of 25, 25, and 50%, respectively (Jain and Glasser, 1992). Steam explosion technique could alter profoundly the microstructure of cellulose and it has been shown that physical action of this method induced a structural change to the cellulose. Kamide and Okajima (1987) have shown that
these changes were not due to H-bonding modification, but probably induced by enhanced water penetration between adjacent cellulose molecules which was due to sudden decompression. The changes are due to the explosive depressurization process which combines the chemical action of autohydrolysis with mechanical action (Grethlein and Converse, 1991). Steam explosion process leads to a major increase in the enzyme accessibility because of the morphological modification involving disruption into microfibrillar elements of the cell wall as well as the fiber fractions (Machessault and St-Pierre, 1980).

Steam explosion technique has been employed in the field of ruminant nutrition. Steam exploded wheat straw was found to be better utilized by steers compared to unexploded straw (Sciaraffia and Marzetti, 1988). Although no report is available on steam explosion of crustacean meal, it is assumed that there would be some structural changes for the chitin-protein complex and may improve digestion by ruminants. Therefore, the objectives of this experiment were 1) to study the effects of steam explosion at two levels of severity on ruminal degradation of DM, CP, and chitina of CM by in situ experiment and DM digestibility by in vitro method, and 2) to estimate the ruminal escape potential of CP of CM.

Experimental Procedures

Crab meal was obtained from Graham and Rollins, Hampton, Virginia and isolated chitin (practical grade) from crab shells was obtained from Sigma Chemicals Co, St Louis, MO. Steam explosion of CM and isolated chitin was performed in a 25 L, stainless steel, batch steam explosion reactor, located in the Pilot/Demonstration Laboratory of Thomas M. Brooks Forest Products Center.
located at Virginia Polytechnic Institute and State University, Blacksburg. Steam explosion of two severities were tested. 1) Low severity, reactor temp 193°C, pressure 12.7 kg/cm² 2) High severity, reactor temp 226°C, pressure 25.5kg/cm². Both treatments were for 3 min and 30 sec. For each level of severity (low and high) duplicate explosions were carried out with 1 kg of each material. Steam exploded material was collected in plastic buckets of 18 L capacity and transported to the laboratory. Steam exploded material contained moderately high water content due to the condensation of steam and washings. Samples of approximately 1 kg (to get approximately 200 g of DM for in situ and in vitro experiments) were collected from each exploded material and were frozen in ice trays. The frozen materials were freeze dried and kept for analysis for Kjeldahl N by AOAC (1990) and chitin (procedure for ADF estimation; Ayangbile, 1989).

In Vitro Experiment. The IVDMD were performed according to Tilley and Terry (1963) on the following materials: 1) crab meal, 2) steam exploded CM at low severity, 3) steam exploded CM at high severity, 4) isolated chitin, 5) steam exploded isolated chitin at low severity, and 6) steam exploded isolated chitin at high severity. The experiment was replicated. A ruminally-cannulated steer (550 kg, BW) was fed a diet containing 40 parts alfalfa hay, 40 parts grass hay, 10 parts ground corn and 10 parts crab meal, starting 15 d prior to the collection of rumen liquor. Rumen fluid was collected through rumen fistula and filtered through eight layers of cheese cloth into a pre-warmed thermos which was then taken to laboratory. Ruminal fluid was added to the buffer solution in the ratio 1:4 which had been adjusted to 39°C. Carbon dioxide was passed through the rumen liquor:buffer mixture until pH of 7 was reached.

Ruminal liquor-buffer solution (35 mL) was added to the tubes containing material and flushed with CO₂ on top of the fluid to create anaerobiosis. Tubes
were closed with stoppers fitted with Bunsen valves which to prevent accumulation of gas in the tubes. Tubes were incubated for 48 h at 39°C. At 48 h tubes were centrifuged at 2500 × g for 15 min and supernatant was discarded. Tubes were again incubated for 48 h at 39°C with 35 mL of acid pepsin. At the end of incubation the tubes were centrifuged and decanted and dried for 24 h. The difference in weight loss after incubation was expressed as percentage disappearance.

In Situ Experiment. Effect of steam explosion on CM and isolated chitin on ruminal degradation of DM, CP and chitin were estimated. The following were tested: 1) crab meal, 2) steam exploded CM at low severity, 3) steam exploded CM at high severity, 4) isolated chitin, 5) steam exploded isolated chitin at low severity, and 6) steam exploded isolated chitin at high severity. A ruminally-cannulated steer (550 kg, BW) fed as described above was used for the in situ experiment.

Six gram of samples were placed in polyester bags of 10 × 23 cm size with 59 ± 2 μm in porosity. Calculated sample size to bag surface area was 15 mg/cm² (Nocek, 1988). Samples were placed in duplicate bags and were closed with 10 cm Bar-Lok® cable ties (Dennison Manufacturing Co., Framingham, MA). Duplicate bags were tied together with another Bar-Lok® tie. Duplicate bags of six different materials were attached to a metal chain of ~20 cm in length which was attached to one end of the polyester thread of 60 cm in length. The bags for the 72 h incubation were tied to the metal chain which was attached to a thicker polyester thread of 75 cm which acted as the main string to which groups of bags for the subsequent incubations were attached by 20 cm Bar-Lok® tie.

The incubation periods were 0, 2, 6, 12, 24, 48, 72 h. The bags were introduced in the rumen in reverse order. Prior to incubation, bags were soaked in
lukewarm water for 15 min (Rodriguez, 1994) and then introduced into the rumen through the rumen cannula. A total of 84 bags were introduced into the rumen. The bags for the 0 h were not introduced in the rumen but were soaked in warm water for 15 min. At 72 h all bags were retrieved, and washed in running tap water. Bags were separated and washed thoroughly until the wash water was clear. The bags were then dried in a forced draft oven for 48 h. Bags were weighed and loss in weight was calculated as percentage DM degradability. Samples were kept for the analysis for Kjeldahl N (AOAC, 1990) and for chitin estimation. The procedure for the ADF estimation was used for the estimation of chitin (Ayangbile, 1989).

Degradability of DM and CP were calculated by using the model of Orskov and McDonald (1979),

\[ D = A + (B \times k_{db})/(k_{db} + k_{pb}), \]

where,

\[ D = \text{degradability} \, (\%), \quad A = \text{fraction} \, A, \text{readily degradable} \, (\%); \quad B = \text{fraction} \, B, \text{degradable} \, (\%); \quad k_{db} = \text{degradation rate constant of degradable} \, B; \text{and} \quad k_{pb} = \text{rate of passage constant (assumed to 5%/h)} \]

The model proposed by Orskov and McDonald (1979) for the estimation of escape of protein is as follows: \[ \text{Escape} = k_{pb} \times B/(k_{db} + k_{pb}) + C, \] where, B, k_{db}, and k_{pb} are stated as in above model; C = fraction that is undegraded (at 48 h).

Fraction A was calculated by subtracting the percent remaining at 0 h (after soaking in warm water) from 100. Fraction B is calculated by subtracting the sum of A and C from 100. Fraction C is the percent residue left undegraded at 48 h of incubation. Degradability depends on rate of passage of digesta (k_{pb}), and is estimated by subjecting the natural log of the residues (expressed as percentage of
the material inserted) at each incubation time minus C fraction to linear regression. The degradation constant is represented by the slope of this line.

Statistical Analysis: All data are presented as least squares means. Data were analyzed by analysis of variance using the GLM procedure of SAS (1989). Tukey's Studentized Range (HSD) Test was used for comparing the treatment for different variables. Effects of treatment were compared separately for sources (crab meal and isolated chitin). Significance of \( P < .05 \) was used throughout unless otherwise mentioned.

Results and Discussion

The CP averaged 41.0 and 42.2%, respectively for CM and isolated chitin, while chitin content averaged 14.4 and 93.0, respectively, DM basis (Table 18). The result shows that isolated chitin (laboratory grade chitin) had 93% chitin and 42.2% CP, which means that the source of this N is the acetylated amino group of chitin molecule. From the above values the percentage of N of chitin tested was calculated to be 7.26%. The CP of CM (41.0) was decreased (\( P < .0001 \)) to 32.6% and 31.6%, respectively for CM exploded at low and high severities. This loss of N perhaps was due to deamination of protein to NH\(_3\) at high temperature. This loss of N could not be measured. The temperature of the reactor at low severity may be sufficient for this deamination. This might be the reason for similar values for both severities. A small decrease in CP was observed for steam explosion of isolated chitin. Nitrogen in chitin is present in the chitin molecule as acetylated amino group (-\( \text{NHCOC}_\text{H}_3 \)) (Mathur and Navrang, 1990). Chitin content in CM was lowered (\( P < .05 \)) from 14.4 to 13.5% by steam explosion at low severity, but this difference was not observed on high severity steam explosion. Although there was
Table 18. Effect of steam explosion on crude protein and chitin of crab meal and isolated chitin

<table>
<thead>
<tr>
<th>Item</th>
<th>Crude Protein</th>
<th>Chitin ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crab meal</td>
<td>41.0 ²</td>
<td>14.4</td>
</tr>
<tr>
<td>Steam exploded crab meal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low ¹</td>
<td>32.6 ³</td>
<td>13.5</td>
</tr>
<tr>
<td>High ³</td>
<td>31.6 ³</td>
<td>14.4</td>
</tr>
<tr>
<td>SE</td>
<td>.50</td>
<td>.20</td>
</tr>
<tr>
<td>Isolated Chitin</td>
<td>42.2 ²</td>
<td>93.0</td>
</tr>
<tr>
<td>Steam exploded isolated chitin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low ¹</td>
<td>39.2 ³</td>
<td>92.9</td>
</tr>
<tr>
<td>High ³</td>
<td>40.7 ⁴</td>
<td>90.5</td>
</tr>
<tr>
<td>SE</td>
<td>0.42</td>
<td>0.84</td>
</tr>
</tbody>
</table>

³Least squares means, avg. of four values
²Did not differ (P > .05)
¹DM basis
⁴Means with in a column lacking a common superscript letter (P < .05) (crab meal and isolated chitin were compared separately)

¹Reactor temperature 193°C, pressure 12.7 kg/cm² for 3 min 30 sec
²Reactor temperature 226°C, pressure 25.5 kg/cm² for 3 min 30 sec
a decrease in chitin on steam explosion for isolated chitin, differences were not significant (P > .05). The same trend was observed for CP of isolated chitin, which shows that little deamination would have taken place at high severity explosion.

*In Vitro Experiment.* An increase in percent *in vitro* DM digestibility was observed when CM was steam exploded at low and high severity (Figure 11). About 10% increase (P < .0001) in DM digestibility (75.2%) was observed for CM steam exploded at high severity compared to unexploded CM (65.9%). Crab meal exploded at low severity had an intermediate value (71%) which was different (P < .05) from unexploded and exploded at high severity. Isolated chitin behaved differently on steam explosion. Steam explosion had a negative effect on DM digestibility of isolated chitin. Over 40% reduction (P < .0001) in DM digestibility was observed for isolated chitin exploded at high severity. Isolated chitin treated with low severity of explosion had a decrease (P < .001) of 16%, compared unexploded. Dry matter digestibility of unexploded isolated chitin appears to be higher, compared to CM.

This implies that chitin is well digested in the digestive tract of ruminants. Perhaps steam explosion caused structural changes in the chitin which would have made the molecule inaccessible for the microbial enzymes. In the case of CM, the increase in the digestibility may not be due to increase in the digestibility of chitin or protein, but perhaps due to the solubility of the minerals, especially CaCO₃ in the rumen liquor:buffer and acid pepsin. Steam explosion would have caused extensive destructive changes in the chitin-protein structure which is ‘reinforced’ with calcium especially CaCO₃ and to a lesser extent CaHPO₄. It has been reported that exoskeleton of crustacean contains several chitinous layers, generally impregnated with mineral salts, such as carbonates and phosphates of calcium (Mathur and Navrang, 1990; Madhavan, 1992). Johnson and Pennisten (1982)
Figure 11. Effect of steam explosion on *in vitro* dry matter digestibility of crab meal and isolated chitin. Low = steam exploded at low severity, High = steam exploded at high severity.
reported that crustacean shell contains 30 to 50% mineral matter, DM basis, mainly CaCO₃ and 8 to 10% calcium phosphate (of the mineral matter). The total ash determined for CM in the present study (Chapter III) was 45% which agrees with previous reports.

*In Situ Experiment.* Ruminal DM degradability of unexploded and exploded CM and isolated chitin at two severities at different ruminal incubations are presented in Figure 12. Ruminal DM degradability of CM, both exploded and unexploded, were higher until 36 h of incubation, compared to isolated chitin except for isolated chitin exploded at high severity which was lower than the other products. Ruminal degradability of DM of steam exploded CM was higher (P < .05) at 0 h incubation and this trend continued until 12 h incubation and after 24 h there were no differences. Low degradability of DM (~10%) was observed for unexploded and exploded isolated chitin until after 12 h incubation. Exploded isolated chitin both at low and high severities showed higher degradability (P < .05) than unexploded until 12 h incubation. After 12 h the trend changed for isolated chitin, where degradability increased, but there was a decrease (P < .005) in DM degradability of steam exploded chitin. At 72 h incubation DM degradability of CM and isolated chitin were similar.

Steam explosion of CM resulted in higher (P < .05) soluble fraction A of DM for both severities (Table 19). The higher soluble fraction A may be due to the physical action of steam explosion which would have broken the mineral bindings. Soluble A fraction was negligible for exploded and unexploded isolated chitins (Table 20). Surprisingly, a high level of degradable B fraction of DM was observed (75.6%) for isolated chitin which was higher than for CM (47.8%). Crab meal showed a decrease (P < .01) in B fraction of DM both in low and high severity explosion, while isolated chitin showed a significant decrease (P < .05).
Figure 12. Effect of steam explosion on degradability of dry matter of crab meal and isolated chitin. CM = unexploded crab meal, CML = steam exploded crab meal at low severity, CMH = steam exploded crab meal at high severity, CH = unexploded isolated chitin, CHL = steam exploded isolated chitin at low severity, CHH = steam exploded isolated chitin at high severity.
Table 19. Effect of steam explosion on fractionation and degradability of dry matter of crab meal by \textit{in situ} experiment$^a$

<table>
<thead>
<tr>
<th>Item</th>
<th>Unexploded</th>
<th>Exploded$^b$ Low</th>
<th>Exploded$^b$ High</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter fractions, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble A$^*$</td>
<td>17.2$^c$</td>
<td>21.9$^d$</td>
<td>24.6$^c$</td>
<td>.48</td>
</tr>
<tr>
<td>Degradable B$^*$</td>
<td>47.8$^c$</td>
<td>41.1$^d$</td>
<td>36.4$^c$</td>
<td>.75</td>
</tr>
<tr>
<td>Undegradable C</td>
<td>35.0$^c$</td>
<td>37.0$^d$</td>
<td>39.0$^c$</td>
<td>.36</td>
</tr>
<tr>
<td>kdb$^{**}$</td>
<td>5.9$^c$</td>
<td>4.9$^c$</td>
<td>5.5$^c$</td>
<td>.63</td>
</tr>
</tbody>
</table>

Degradability, %$^{***}$

<table>
<thead>
<tr>
<th>Exploded$^b$ Low</th>
<th>Exploded$^b$ High</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.6$^c$</td>
<td>42.2$^c$</td>
<td>43.5$^c$</td>
</tr>
</tbody>
</table>

$^a$Least squares mean

$^b$Steam exploded in a 25 L stainless steel, batch steam explosion reactor at reactor temperature 193$^\circ$C, pressure 12.7 kg/cm$^2$ for 3 min 30 sec (low severity); reactor temperature 226$^\circ$C, pressure 25.5 kg/cm$^2$ for 3 min 30 sec (high severity)

$^{c,d}$Means with in a row lacking a common superscript letter differ ($P < .05$)

$^*P < .01$

$^{**}$Estimated by regression analysis

$^{***}$Degradability = A + (k_{db} \times B)/(k_{db} + .05)

Where,

A = fraction A, readily degradable (%)
B = fraction B, degradable (%)
kdb = rate of degradation
.05 = rate of passage (5%/h)
Table 20. Effect of steam explosion on fractionation and degradability of dry matter of isolated chitin by *in situ* experiment

<table>
<thead>
<tr>
<th>Item</th>
<th>Unexploded</th>
<th>Exploded&lt;sup&gt;b&lt;/sup&gt;</th>
<th></th>
<th></th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Low</td>
<td>High</td>
<td>SE</td>
</tr>
<tr>
<td>Dry matter fractions, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble A</td>
<td>2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.26</td>
</tr>
<tr>
<td>Degradable B</td>
<td>75.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63.6&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>54.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.98</td>
</tr>
<tr>
<td>Undegradable C</td>
<td>22.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.2&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>43.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.48</td>
</tr>
<tr>
<td>kdb&lt;sup&gt;*&lt;/sup&gt;</td>
<td>9.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.05</td>
</tr>
<tr>
<td>Degradability, %**</td>
<td>51.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>44.1&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>31.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.72</td>
</tr>
</tbody>
</table>

<sup>*</sup>Least squares mean  
<sup>b</sup>Steam exploded in a 25 L stainless steel, batch steam explosion reactor at reactor temperature 193°C, pressure 12.7 kg/cm² for 3 min 30 sec (low severity); reactor temperature 226°C, pressure 25.5 kg/cm² for 3 min 30 sec (high severity)  
<sup>c,d</sup>Means with in a row lacking a common superscript letter differ (P < .05)  
<sup>*</sup>Estimated by regression analysis  
<sup>**</sup>Degradability = A + (k<sub>db</sub> x B)/(k<sub>db</sub> + .05)  
Where,  
A = fraction A, readily degradable (%)  
B = fraction B, degradable (%)  
kdb = rate of degradation  
.05 = rate of passage (5%/h)
only in high severity explosion. There was a decrease of more than 10 percentage units for degradable fraction B of CM exploded at high severity compared to unexploded. Undegradable C fraction of DM of CM was increased (P < .05) due to steam explosion at both severities. Fraction C of DM of isolated chitin was increased (P < .01) by almost 100% by high severity steam explosion compared to unexploded. Calculated rate of degradation (k_{db}) of DM based on regression calculation was found to be numerically higher for isolated chitin compared to CM. There was a trend for a decrease in k_{db} for the exploded meal isolated chitin compared to unexploded. A similar trend was observed for CM, but these differences were not significant (P < .05).

Steam explosion did not show any impact on DM degradability of CM while DM degradability was reduced (P < .005) in isolated chitin due to low and high severity explosion. Steam exploded isolated chitin at high severity had significantly lower (P < .05) DM digestibility compared to steam exploded at low and high severities. The results shows that steam explosion has caused some structural changes in the protein and chitin molecules which would have made the microbial enzyme inaccessible for the hydrolytic action. Marchessault (1988) suggested that a mechanical destructuring of biomass is a key feature of steam explosion. In the case of carbohydrate polymer the principal chemical transformation is hydrolysis producing soluble carbohydrate (Marchessault, 1988). Grethlein et al. (1984) reported that purpose of any pretreatment (e.g. steam explosion) is to increase the surface area available to the enzyme to act upon. In the case of cellulose, enzyme cellulase must be able to contact the glycosidic bonds for cleaving. Cellulose is very porous and has about 600 to 800 m² of surface area per g of substrate, but approximately 98% of this area is in pores that are available for smaller molecules (Grethlein and Converse, 1991). There is a
major increase in accessibility to enzymes due to increase of the internal porosity (Marchessault, 1988). If this is the case, it has to be assumed from the behavior of DM of isolated chitin when it was steam exploded at two severities that some molecular rearrangement which would have lead to a negative response by lowering the surface area. This would have restricted the accessibility of enzyme.

Digestibility of DM was high for in vitro compared to in situ. This higher DM digestibility for the in vitro technique, perhaps was due to simply the solubility of minerals in low acidic environment in abomasum and small intestine which would have overestimated the DM digestibility, because in the in vitro method the DM digestibility of the material is estimated only on the basis of loss in weight of the incubated material. This loss in weight does not give an estimate of the level of absorption. In the present case, the assumed soluble ash mainly comprised of Ca is poorly absorbed.

Ruminal CP degradability of steam exploded CM and isolated chitin at different incubation times are given in Figure 13. Although CM and isolated chitin contain similar level of CP, their pattern of degradability is different. Crab meal protein both unexploded and exploded had 50 to 60% degradability by 2 h of incubation, whereas isolated chitin had only ~10% at 2 to 12 h incubation. After 12 h degradability of CP in isolated chitin increased, but decreased (P < .005) with steam explosion. Although steam exploded crab meal showed higher (P < .05) degradability at 2, 6, 12 and 24 h incubation, there were no differences at 48 and 72 h.

Soluble A and degradable B fractions of N of CM did not change when steam exploded at low severity, but had an increase (P < .001) of 7.2% for soluble A and a decrease (P < .01) (7.3%), for fraction B on high severity explosion compared to unexploded (Table 21). The degradable B fraction of CM
Figure 13. Effect of steam explosion on degradability of crude protein of crab meal and isolated chitin. CM = unexploded crab meal, CML = steam exploded crab meal at low severity, CMH = steam exploded crab meal at high severity, CH = unexploded isolated chitin, CHL = steam exploded isolated chitin at low severity, CHH = steam exploded isolated chitin at high severity.
Table 21. Effect of steam explosion on fractionation and degradability of crude protein of crab meal by *in situ* experiment*\(^a\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Unexploded</th>
<th>Exploded</th>
<th>Low</th>
<th>High</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter fractions, %*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble A</td>
<td>33.9(^c)</td>
<td>33.1(^c)</td>
<td>41.1(^d)</td>
<td>.96</td>
<td></td>
</tr>
<tr>
<td>Degradable B</td>
<td>52.1(^c)</td>
<td>51.4(^c)</td>
<td>44.8(^d)</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>Undegradable C</td>
<td>14.0(^c)</td>
<td>15.5(^c)</td>
<td>14.1(^c)</td>
<td>.44</td>
<td></td>
</tr>
<tr>
<td>kdb**</td>
<td>6.2(^c)</td>
<td>5.1(^c)</td>
<td>6.1(^c)</td>
<td>.71</td>
<td></td>
</tr>
<tr>
<td>Escape, % CP***</td>
<td>37.4(^d)</td>
<td>41.2(^c)</td>
<td>34.7(^d)</td>
<td>1.47</td>
<td></td>
</tr>
</tbody>
</table>

*Least squares mean

*Steam exploded in a 25 L stainless steel, batch steam explosion reactor at reactor temperature 193°C, pressure 12.7 kg/cm² for 3 min 30 sec (low severity); reactor temperature 226°C, pressure 25.5 kg/cm² for 3 min 30 sec (high severity)

\(^c\)(d)Means with in a row lacking a common superscript letter differ (\(P < .05\))

*DM basis

**Estimated by regression analysis

***Escape = k\(_{db}\) x B/(k\(_{db}\) + k\(_{pb}\)) + C

Where,

B = fraction B, degradable (%)
C = fraction C, undegradable (%)
k\(_{db}\) = rate of degradation
k\(_{pb}\) = rate of passage (5%/h)
was decreased only by the high severity (\(P < .1\)). There was no difference in the undegradable C fraction of N between unexploded and exploded CM. Rate of degradation also showed no difference (\(P < .05\)).

Steam explosion at high severity reduced (\(P < .05\)) the soluble A fraction of N of isolated chitin (Table 22). A decrease in degradable B fraction of N resulted for isolated chitin exploded at low and high severity explosions, compared to unexploded. Undegradable C fraction of N of isolated chitin was increased at low and high severity steam explosions with a significant increase (\(P < .05\)) at high severity compared to unexploded. There was a decrease (\(P < .0001\)) for \(k_{ab}\) for the isolated chitin exploded at high severity compared to unexploded isolated chitin.

The escape protein of CM was estimated to be 37.4%. This value was lower than the value observed for CM (48.4%) in another in situ study conducted (Chapter V). The higher in situ ruminal degradability of CM N observed in the present study might be due to better adaptation to the CM diet because of ruminal microbial adaptation. Steam explosion at both levels of severity did not exert (\(P > .05\)) any effect on ruminal escape value of CP of CM, whereas there was an increase (\(P < .05\)) in escape of N for explosion at high severity. The difference in behavior of N of CM and isolated chitin shows that the main structural changes occurs for acetylated amino group (-NHCOCH\(_3\)). Focher et al. (1988) reported that X-ray diffraction of flash treated chitin shows large modification both in supramolecular structure and in morphology, while NMR analysis did not indicate any change in primary structure except in the acetyl group content.

In the present study an attempt has been made to fractionate chitin into three fractions as for DM and CP, soluble fraction A, degradable fraction B and undegradable fraction C. Chitin content of CM and isolated chitin tested in this study contained 14.4 and 93.0%, respectively. Chitin content observed in CM
Table 22. Effect of steam explosion on fractionation and degradability of crude protein of isolated chitin by *in situ* experiment*

<table>
<thead>
<tr>
<th>Item</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Low</td>
<td>High</td>
<td>SE</td>
</tr>
</tbody>
</table>
| Crude protein fractions, %
  Soluble A     | 7.0c       | 8.7c     | 3.8d     | 2.56     |
| Degradable B  | 74.9c      | 63.3d    | 59.4d    | 4.40     |
| Undegradable C| 18.1c      | 28.0cd   | 36.8d    | 2.87     |
| kdb**         | 9.3c       | 7.0cd    | 4.8d     | .96      |
| Escape, % CP***| 44.7c     | 55.0cd   | 68.0d    | 3.57     |

*Least squares mean

**Steam exploded in a 25 L stainless steel, batch steam explosion reactor at reactor temperature 193°C, pressure 12.7 kg/cm² for 3 min 30 sec (low severity); reactor temperature 226°C, pressure 25.5 kg/cm² for 3 min 30 sec (high severity)

^cd Means with in a row lacking a common superscript letter differ (P < .05)

*DM basis

**Estimated by regression analysis

***Escape = k_{pb} x B/(k_{db} + k_{pb}) + C

Where,
- B = fraction B, degradable (%)
- C = fraction C, undegradable (%)
- k_{db} = rate of degradation
- k_{pb} = rate of passage (5%/h)
tested in this study falls in the reported range of 13 to 15% for the crab processing waste (Ashford et al., 1977; Ayangbile, 1989).

The degradability pattern of chitin in isolated chitin was similar to the degradability of DM, obviously due to 93% chitin of isolated chitin DM. Ruminal degradability of chitin of both unexploded and exploded CM and isolated chitin is presented in Figure 14. For CM, degradability of chitin was lower compared to that of DM or CP. Steam exploded CM at high severity showed an increase of degradation at 0 h incubation compared to low severity exploded and unexploded CM. This trend continued throughout the incubation period until 72 h, where differences were not significant (P < .05). Chitin degradability of isolated chitin was higher for low severity at 0, 2, 6, and 12 h incubations. The other differences were similar to those for DM degradability.

Soluble A fraction of chitin of CM (Table 23) was negligible (0.6%) while that for isolated chitin was 9.5% (Table 24). Steam explosion of CM at high severity showed an increase (P < .001) in soluble A fraction of chitin (10.5%), while CM exploded at low severity did not show any change (P > 05). Degradable B fraction of chitin was 75% for isolated chitin which was higher than CM (47.6%). There was a tendency for a small decrease in degradable B fraction of chitin of CM on steam explosion. A decrease (P < .05) was observed for the B fraction of chitin on steam explosion, but differences between severities were not significant. Effect of steam explosion on undegradable fraction C of CM followed the same trend as that for degradable B fraction. Undegradable C fraction of chitin in isolated chitin was increased by low and high (25.6 and 34.8%, respectively) severity steam explosion compared to unexploded isolated chitin (15%). Rate of degradation of chitin fraction of isolated chitin was found to be more than twice as that of CM (9.7 and 4.0%, respectively). The effect of steam explosion at high
Figure 14. Effect of steam explosion on degradability of chitin of crab meal and isolated chitin. CM = unexploded crab meal, CML = steam exploded crab meal at low severity, CMH = steam exploded crab meal at high severity, CH = unexploded isolated chitin, CHL = steam exploded isolated chitin at low severity, CHH = steam exploded isolated chitin at high severity
Table 23. Effect of steam explosion on fractionation and degradability of chitin of crab meal by *in situ* experiment

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<td>Chitin fractions, %</td>
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<td>Soluble A</td>
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<td>44.6&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup>Least squares mean
<sup>b</sup>Steam exploded in a 25 L stainless steel, batch steam explosion reactor at reactor temperature 193°C, pressure 12.7 kg/cm² for 3 min 30 sec (low severity); reactor temperature 226°C, pressure 25.5 kg/cm² for 3 min 30 sec (high severity)
<sup>c</sup>Means with in a row lacking a common superscript letter differ (P < .05)

*DM basis

**Estimated by regression analysis

***Degradability = A + (k<sub>b</sub> x B)/(k<sub>b</sub> + .05)

Where,

A = fraction A, readily degradable (%)
B = fraction B, degradable (%)
kd = rate of degradation
.05 = rate of passage (5%/h)
Table 24. Effect of steam explosion on fractionation and degradability of chitin of isolated chitin by in situ experiment

<table>
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<td>Chitin fractions, %</td>
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<td>Soluble A</td>
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<tr>
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<td>7.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.91</td>
<td></td>
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<td>Degradability, %&lt;sup&gt;***&lt;/sup&gt;</td>
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<sup>a</sup>Least squares mean
<sup>b</sup>Steam exploded in a 25 L stainless steel, batch steam explosion reactor at reactor temperature 193°C, pressure 12.7 kg/cm<sup>2</sup> for 3 min 30 sec (low severity); reactor temperature 226°C, pressure 25.5 kg/cm<sup>2</sup> for 3 min 30 sec (high severity)
<sup>c,d</sup>Means with in a row lacking a common superscript letter differ (P < .05)
<sup>*</sup>DM basis
<sup>**</sup>Estimated by regression analysis
<sup>***</sup>Degradability = A + (k<sub>b</sub> x B)/(k<sub>b</sub> + .05)

Where,
- A = fraction A, readily degradable (%)
- B = fraction B, degradable (%)
- kdb = rate of degradation
- .05 = rate of passage (5%/h)
severity showed different trends for $k_{db}$ of chitin of CM and isolated chitin, the former increased while the latter decreased.

Chitin degradability was only 21.5% for the unexploded CM. Steam explosion of CM at high severity had a degradability of 34.2%, which was higher ($P < .05$) than unexploded and exploded at low severity. An opposite trend was observed for isolated chitin when steam exploded. Chitin degradability was decreased in steam exploded isolated chitin compared to unexploded (57.7%). There was a decrease ($P < .05$) in chitin degradability when isolated chitin was exploded at low and high severity (46.9 and 36.8%, respectively).

An increase of 60% chitin digestibility was observed for CM when steam exploded, whereas, degradability of chitin of isolated chitin was lowered due to steam explosion. The reason for this diverse result is not clear. Perhaps the higher degradability of chitin at high severity of steam explosion were due to morphological modification involving disruption of the structure. These changes would have resulted in improved enzyme accessibility. Marchessault and St-Pierrie (1980) reported that the steam explosion treatment process results in a profound change in enzyme accessibility, compared to the untreated. They suggested that morphological modification involving disruption into microfibrillar elements of the cell wall as well as the fiber fracture is responsible for this phenomenon. The increased enzymatic degradation of cellulose is due to increase in the enzyme accessible surface due to explosion process. Behavior of chitin in isolated chitin to steam explosion may partly explained by the chemical and processing changes. It has been reported that by simply manipulating the chemical and physical processing a single species source, yielded chitin products of various molecular weight ranges having significantly different properties (Bough et al., 1978).
Implications

Isolated chitin is well digested (70.9%) in the digestive tract of ruminants which shows that comparatively poor digestion of chitin in crab meal may not be due to poor digestibility of chitin but due to high mineralization of the chitin-protein complex. Although there was an increase of 10% DM digestibility due to steam explosion, the higher digestibility might be due to higher solubility of Ca. Furthermore, steam explosion did not improve the escape value of CM protein. Also there was a loss of over 20% N on steam explosion. The marginal increase in digestibility of CM may not be justifiable for the loss of N and the extra cost involved in the process. Therefore, steam explosion does not seem to be a promising processing method for the better utilization of crab waste for ruminants. Further studies in intestinal digestion and absorption of nutrients of steam exploded CM may bring more light to this field.

Literature Cited


Chapter VI
General Discussion

For rapidly growing animals or high producing dairy cows, the requirement for metabolizable protein must be met by a combination of microbial protein synthesized in the rumen and by dietary protein that passes to the small intestine. In order to increase the quantity of protein that escape ruminal degradation, it is necessary to use treatments such as physical (heating) or chemical (formaldehyde) or select feedstuffs which are resistant to microbial degradation, such as corn gluten meal (CGM), feather meal (FTM), blood meal (BM) and meat and bone meal. Utilization of these byproducts as a source of escape protein will obviously reduce the cost of livestock production (Thomas and Beeson, 1977). These industrial byproducts of both plant and animal origin are limiting in one or other essential amino acids (AA). Therefore judicial selection of byproducts is needed to mitigate the deficiencies of individual byproducts as well as microbial protein. Several studies have been conducted to elicit the complimentary responses by feeding combinations of the rumen undegradable protein sources such as CGM, BM and FTM (Stock et al., 1981; Goedeken et al., 1990; Blasi et al., 1991).

Another byproduct of importance is the shell fish waste, a byproduct of the seafood industry. It has been estimated that approximate global annual production of crustacean waste (DM basis) is 1.46 Million t and that of US is .1 Million t (Knorr, 1991). Studies conducted in this laboratory (Ayangbile, 1989; Samuels et
al., 1991; 1992; Abazinge et al., 1993; 1994) and elsewhere (Patton et al., 1975; Velez et al., 1991) have shown that crab processing waste is a good protein supplement for ruminants but lower digestibility has been reported. Lower digestibility may be attributed to the chitin, an analogue of cellulose, which occurs as a chitin-protein complex with mineral binding which restrict the accessibility of enzyme. An attempt was made to explore the feasibility of steam explosion, a technique of much importance in industries for the fractionation of lignin, hemicellulose and cellulose.

This study shows that substituting rumen undegradable protein sources for soybean meal (SBM) did not elicit a profound effect on growth or gain:feed in growing steers although there was a trend (P < .05) for a positive response for the supplement based on plant protein and BM and commercial supplement based on animal protein. Similar results of not having a positive response have been reported by Adam et al. (1988); Derouen et al. (1988); Coomer et al. (1993), and Ludden et al. (1995). Feeding low degradable protein to ruminants has often failed to increase the amount and quality of protein available in the small intestine and improve animal performance because of inadequate protection of protein from ruminal degradation (Calsamiglia et al., 1992), decreased microbial protein synthesis (Windschitl and Stern, 1988), decreased intestinal digestion (Waltz et al., 1989) and /or AA limitations (Rogers et al., 1989).

In the present study, one of the reasons for not getting a response, perhaps related to a relatively high escape protein content of SBM. Ludden et al. (1995) reported that the estimate of ruminal escape of N of SBM was almost twice as that reported by NRC (1989). Loerch et al. (1983) has reported that a decrease in ruminal pH associated with feeding diets based on cereal grains may reduce the protein degradability of SBM. In the study of Ludden et al. (1995), the high level
of corn grain (70%) in the diet would have lowered the pH. In the present study pH averaged 7.0, although the level of corn grain in the diet averaged 32%. Veen (1986) suggested that, in general the pH will be higher immediately before feeding than afterwards, perhaps due to nonavailability of fermentable substrate remaining in the rumen. Another reason for high pH is due to endogenous metabolism of bacteria and/or high lysis of bacteria (Wohlt et al., 1976). The time lag between the sampling of ruminal fluid and the time the steers were fed would have caused the ruminal pH to be higher in the present study. The ruminal pH of sheep in the metabolism study was generally lower, 6.4, than the ruminal pH for SBM, where the level of corn in the diet was only 10%. There are similar reports of high escape of SBM in low ruminal pH (Loerch et al., 1983; Hussein and Jordan, 1991).

Ludden et al. (1995) reported 66.0% escape protein for SBM at 6.2 pH. This shows that the escape protein observed in the in situ study, perhaps would be a lower than the actual escape in the growth study. The escape of protein of SBM and other protein supplements was estimated by dacron bags incubated in rumen of a steer fed equal proportions of alfalfa and grass hay diet. The probable reason for the lower escape protein (23.2%) observed in this study compared to the value (66.1%) reported by Ludden et al. (1995) may be due to the difference in the ruminal environment attributed to the differences in the diet.

Another reason for not getting a positive response may be due to escape nature of corn grain protein, zein, which is 58 to 73% undegradable when the diet contains proportionately high levels of corn (Zinn and Owens, 1983). In the present study, an average of 32% corn in the diet would have contributed to a considerable portion of escape protein.
Providing readily available N for the optimum microbial protein synthesis is an important criterion in the feeding of rumen escape supplements. All the steers fed different protein supplemented diets had higher levels of ruminal NH₃ N than the minimum (5 mg/dL) suggested for the optimum microbial protein synthesis (Satter and Slyter, 1974). It has been shown that to achieve maximum microbial activity much higher NH₃ concentration than required for the maximum microbial protein production, may be required (Mehrez et al., 1977). Furthermore, there are reports that some microbes require N in the form of NH₃ and the feeding of slowly degraded protein may create an NH₃ deficiency in the rumen (Klopfenstein et al., 1976). Therefore, perhaps the performance would have improved if escape protein supplements were supplemented with soluble N such as urea in the diets.

Steers fed SBM diet would have provided sufficient metabolizable protein for the optimum feed efficiency for the steers in the present study. The potential of escape protein supplements has been shown to be highest in young, growing ruminants, where microbial protein synthesis may not be sufficient for the optimum requirements for rapid growth (Chalupa, 1975). Oldham and Smith (1982) suggested that growing cattle weighing more than 200 kg fed diets with adequate energy intake can meet their protein requirements from the microbial protein synthesized in the rumen. They concluded that the protein sources that are rapidly degraded in the rumen will be sufficient to meet the N requirement through microbial protein synthesis.

Combinations of CGM, BM, and FTM have been found to be more efficient for growth in steers (Stock et al., 1981; Goedeken, 1990a; Sindt et al., 1993a; 1993b) than when fed alone. This complementary effect is due to the lysine content in BM and S AA of CGM. The microbial protein is limiting in methionine and lysine (Nimrick et al., 1970; Richardson and Hatfield, 1978). A trend for a
positive response in gain:feed for IPA, CS, and ESA may be attributed to the feed additives included as a premix in the supplement and in the case of IPA and ESA, there may also have been a complementary effect.

The reason for the poor response in steers fed HESA supplemented diet compared to other supplements is not known. One reason is perhaps due to low degradability or low availability of NH₃ for microbial growth. In the metabolism studies conducted in sheep, there was a numerical decrease in digestibility of OM and cellulose and a significant decrease (P < .05) in digestibility of CP, for those fed the HESA supplemented diet.

The IVDMD of HESA was lower (P < .0001) than for SBM, IPA, CS, and CM. The overall performance of steers fed HESA supplemented diet in the growth studies and the various parameters of blood and ruminal fluid of sheep in the metabolism studies show that lower performance may be due to low digestibility or low AA profile. Furthermore, incorrect balance of time and temperature during hydrolysis of FTM will affect the protein digestibility (Morris and Balloun, 1973). During processing, cystine is converted to lanthionine (Robbins et al., 1980) which will limit the bioavailability of S AA (Robbins et al., 1980) and possibly other essential AA as well (Papadopoulos et al., 1986). Goedeken et al (1990b) suggested that protein quality of FTM and BM combination will be reduced if the BM is added before hydrolysis of FTM due to reduced escape and total tract protein digestibility. In the present study perhaps the HESA supplement was processed for too long a period of time which would have caused poor digestibility of nutrients, especially N.

Conflicting results have been reported for the nutritive value of crab meal (CM). Improved feed efficiency has been observed in cattle when CM was included in the diet (Patton et al., 1975; Ayangbile, 1989) whereas negative
responses have been reported (Brundage, 1986; Velez et al., 1991). In the present study, CM was found to be comparable with SBM in weight gain and gain:feed. Results in the metabolism study also showed that there were no differences (P > .05) in digestibilities of nutrients compared to that of SBM. Phosphorus availability was found to be numerically higher than SBM. The escape protein of CM was 48.4% when incubated in the rumen of a cannulated steer fed equal portion of alfalfa and grass hay diet, but, the escape protein of CM was found to be only 37.4% when incubated after adapting the steer to a CM diet. Bunting et al. (1988) indicated that ruminal adaptation is required to elicit maximum degradation of crab-craw fish waste CP.

*In vitro* dry matter digestibility of CM was improved from 65.9 to 75.2% on steam explosion whereas isolated chitin behaved differently on steam explosion. Unexploded isolated chitin had 71% digestibility which was reduced on steam explosion. This shows that chitin is well digested in the digestive tract of ruminants. A higher IVDMD of steam exploded CM may not be due to increase in the digestibility of chitin or CP, but may be due to the solubility of the minerals especially calcium carbonate in the rumen liquor buffer and acid pepsin. Steam explosion would have caused extensive changes in protein structure in chitin with heavy mineralization with calcium carbonate.

*In situ* studies showed that there was no effect on the escape of steam exploded CM protein. There was a loss of about 20% N on steam explosion. There was an increase of 60% chitin digestibility i.e., from 21.5 to 34.2%, whereas chitin content of isolated chitin was decreased on steam explosion. The reason for this diverse result is not clear, perhaps due to higher degradability of chitin of CM might be due to morphological modification including disruption of the structures. Marchessault and St-Pierrie (1980) reported that the steam explosion treatment
results in a profound change in enzyme accessibility compared to the unexploded material. Decreased chitin degradability of isolated chitin may be due to the result of the chemical and processing effect of chitin preparation. Bough et al (1978) indicated that chemical and physical manipulation may change chitin products with different properties. An increase of 10% IVDMD of CM may not be justifiable for the loss of N and the cost involved in the processing.

In conclusion, substituting SBM with other protein supplements used in this study did not elicit a significant positive response in weight gain orgain:feed in growing steers on a high-roughage diet. However, there was a trend for a positive response for IPA and CS supplements. Crab meal was comparable to SBM or other commercial products as a protein supplement for steers. Steam explosion technique does not seem to be a promising processing method for the better utilization of crab waste for ruminants. Further studies on intestinal digestion and absorption of nutrients of steam exploded CM in ruminants may be of interest.
Literature cited


**Literature Cited**

190


Literature Cited


Literature Cited


Literature Cited
Appendix
Appendix Table 1. Dry matter disappearance of protein supplements *in situ*

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*SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.*
Appendix Table 2. Crude protein disappearance of protein supplements in situ

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<td>53.5</td>
<td>39.3</td>
<td>56.0</td>
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<td>60.1</td>
<td>38.0</td>
<td>53.3</td>
<td>42.0</td>
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<td>46.4</td>
<td>60.7</td>
<td>61.8</td>
<td>80.5</td>
</tr>
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<td>72</td>
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<td>88.1</td>
<td>58.2</td>
<td>67.3</td>
<td>68.9</td>
<td>84.4</td>
</tr>
</tbody>
</table>

*SBM = soybean meal, control; IPA = supplement based on industrial byproducts of both plant and animal origin; ESA = experimental supplement based on byproducts of animal origin; HESA = hydrolyzed supplement No 3.; CS = commercial supplement based on animal protein; and CM = crab meal.

*DM basis
Appendix Table 3. Effect of steam explosion on degradation of dry matter of crab meal and isolated chitin by *In situ* a

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Crab Meal</th>
<th>Steam Exploded</th>
<th>Chitin</th>
<th>Steam Exploded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>0</td>
<td>17.2</td>
<td>21.8</td>
<td>24.6</td>
<td>2.4</td>
</tr>
<tr>
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<td>34.0</td>
<td>38.7</td>
<td>41.9</td>
<td>4.8</td>
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<td>37.9</td>
<td>40.8</td>
<td>45.0</td>
<td>4.9</td>
</tr>
<tr>
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<td>42.8</td>
<td>44.5</td>
<td>46.5</td>
<td>5.6</td>
</tr>
<tr>
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<td>56.7</td>
<td>55.3</td>
<td>55.7</td>
<td>46.3</td>
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<tr>
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<td>65.1</td>
<td>63.0</td>
<td>61.2</td>
<td>77.9</td>
</tr>
<tr>
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<td>68.1</td>
<td>66.3</td>
<td>63.3</td>
<td>72.1</td>
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</tbody>
</table>

*a*Least squares mean, avg. of four values
Appendix Table 4. Effect of steam explosion on degradation of crude protein of crab meal and isolated chitin *in situ*.

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Crab Meal</th>
<th>Steam Exploded</th>
<th>Chitin</th>
<th>Steam Exploded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low</td>
<td>High</td>
<td>%</td>
</tr>
<tr>
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<td>34.0</td>
<td>33.1</td>
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<td>7.0</td>
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<td>58.4</td>
<td>61.3</td>
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<td>85.9</td>
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<td>88.7</td>
<td>87.0</td>
<td>88.1</td>
<td>80.1</td>
</tr>
</tbody>
</table>

*a* Least squares mean, avg. of four values.
Appendix Table 5. Effect of steam explosion on degradation of chitin of crab meal and isolated chitin by *in situ* \(^a\)

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Crab Meal</th>
<th>Steam Exploded</th>
<th>Chitin</th>
<th>Steam Exploded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low</td>
<td>High</td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
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<td>26.5</td>
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<tr>
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<td>21.8</td>
<td>19.7</td>
<td>29.8</td>
<td>8.7</td>
</tr>
<tr>
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<td>35.7</td>
<td>34.6</td>
<td>44.7</td>
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<td>45.5</td>
<td>53.7</td>
<td>85.3</td>
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<tr>
<td>72</td>
<td>54.9</td>
<td>51.1</td>
<td>55.4</td>
<td>81.9</td>
</tr>
</tbody>
</table>

\(^a\)Least squares mean, avg. of four values
Vita

T. V. Viswanathan was born in Trichur, Kerala, India on February 5, 1949. He received his bachelors degree in Veterinary Science in 1971 from Kerala Veterinary College, Kerala, India. Subsequently, he worked as Veterinary Officer in a sister organization of Anand Milk Union Limited (AMUL), Gujarat, and as Veterinary Surgeon in Indo-Swiss Project, Maduppetty, India. He received his M.V.Sc in Animal Nutrition in 1977. He joined Veterinary Faculty, Kerala Agricultural University, Mannuthy, Kerala, India in 1977. He entered Virginia Tech in August, 1992.