PROTEIN NUTRITION OF DAIRY COWS FED HIGH FAT DIETS

by

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(Under the Direction of MARK A. FROETSCHEL)

ABSTRACT

Two experiments were conducted to determine the value of specific protein supplements in lactating dairy cows fed high fat diets. Experiment 1, six feedstuffs (wheat silage, corn, soybean meal, soybean hulls, whole cotton seed, and poultry protein meal) and a protein blend (fish meal and dry distillers grains) were evaluated for ruminal dry matter and crude protein degradation kinetics. Duplicate nylon bags were incubated for 0, 2, 4, 8, 16, 24, 48, and 72 h in two Holstein steers fitted with ruminal cannulae, fed at 3.5 X maintenance. Degradation constants for most of these feeds, except wheat silage, compared closely to values listed in the dairy NRC (4 X maintenance). An NRC value for pet food grade poultry protein meal is not available. Our results indicate that its CP k_d is 2.45 %/h and its RUP is 58.49 %. In situ intestinal protein digestibility was obtained for six ruminally incubated feed ingredients (wheat silage, corn, soybean meal, whole cotton seed, and poultry protein meal) and a protein blend (fish meal and dry distillers grains). Quadruplicate nylon bags were introduced into the duodenum of two Hereford steers fitted with permanent cannulae. Six nylon bags were introduced per day with an interval of 15 min between bags. Digestibility values were higher for soybean meal. Poultry protein meal digestibility was lower than soybean meal but higher than the other feeds. Experiment 2, twenty-four high producing Holstein lactating dairy cows were used in a completely randomized design to determine the utilization of pet food grade poultry protein meal as a protein supplement in rations that were relatively high in both fat and fiber. Cows in early lactation ranging between 35 and 126 DIM, were distributed into six groups based on their level of milk production. The basal diet contained approximately 55% wheat silage and 6.5% fat. Treatments varied due to protein supplementation and were described as: 1) positive control-soybean meal (SBM), plus a ruminally undegraded protein (RUP) blend of fish meal (FM), blood meal (BM), and dry distillers grains (DDG); 2) negative control-SBM; 3) PPM-50%, 50% pet food grade poultry protein meal (PPM) substituted for the RUP blend in treatment 1; and 4) PPM-100%, 100% substitution PPM for RUP blend in treatment 1. Cattle were fed behind Calan gates, a common diet for two weeks and treatment diets for 12 wks afterwards. Intake (DMI) was lowest with the positive control diet and fat corrected milk production was higher in the 100% PPM diet. Fat corrected milk, milk fat, fiber digestibility and body weight loss were all lower in cattle fed the negative control. The most interesting response of practical value is the increase in DE

concentrations of diets supplemented with RUP sources. This corresponded with an increase in diet NE content. Effect that is complemented with higher fiber and OM digestibilities that appears to be the main responsible for the increased DE. Back fat change detected from ultrasonography was greater in cattle fed the negative control diet. Insulin and blood urea nitrogen (BUN) were influenced by treatment especially during the first 4 weeks. These results indicate that PPM is an economic alternative for more expensive animal based sources of RUP.

INDEX WORDS: Protein degradation, Protein digestibility, Protein supplement, High fat diets, Poultry protein meal.

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DEDICATION

To my parents, my daughter and son.

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CHAPTER 1

INTRODUCTION

As the milk yield per cow has increased, it is increasingly more difficult to meet nutrient requirements of the lactating dairy cow. This problem centers on meeting both the caloric and fiber requirements when these nutrients are inversely concentrated in most feeds. Feeding a dairy herd to maximize production necessitates that the energy and protein requirements are met within certain dry matter intake (DMI) limit. Protein requirement is met by a combination of microbial protein synthesis and ruminally undegraded protein (RUP). Ruminally microbial protein synthesis is constrained by limits on readily fermentable carbohydrate intake and supplies a decreasing proportion of the required metabolizable protein for higher producing dairy cows. Thus, diets for high producing dairy cattle (>30 kg/d) should contain significant amounts of ruminally undegraded protein (RUP) in order to meet protein needs (Santos, 1998). This is especially critical in cows that have compromised intake as in early lactation and those under heat stress.

After calving, dairy cows experience negative energy balance because energy intake cannot meet the requirements for maintenance plus milk yield. Although it is less recognized early lactating cows are also in a negative state of protein balance. Hence, high producing dairy cows in early lactation not only require more energy dense diets, but also a diet that contains high amounts of good quality protein. Microbial protein is

relatively good quality protein but it cannot be produced to supply enough metabolizable protein to support high levels of production. Therefore, high producing cows benefit when provided additional ruminally undegraded protein (Schingoethe, D.J. 1996). The intake of fermentable organic matter is the dietary parameter that most limits rumen microbial protein synthesis. The requirement for RUP is increased even more when intake of fermentable carbohydrates is decreased. During periods of heat stress, feed intake decreases and maintenance requirements increase. This challenges producers to alleviate heat stress by cooling the environment of the cow and providing proper nutrition.

The best approach to provide better nutrition to the heat stressed cow is to increase energy concentration of the diet. Energy intake is dependent on both level of feed intake and energy density of the ration. Energy density of the diet can be increased by incorporation of additional concentrate, supplemental fat, or both (Grum, D.E. et al 1996). The first option is often practiced, but there are upper limits to the amount of grain that can be fed. Moreover, high concentrate diets can lead to acidosis, milk fat depression, and decreased feed intake. Until recently, using fat in dairy rations as an energy supplement has been a challenge because of its detrimental effects on rumen microorganisms and fiber digestion. Since the development of ruminant inert fat sources, levels greater than 5 percent have been fed successfully to high producing cows (Holter et al, 1991). However, supplemental fat has not consistently improved performance of heat stressed dairy cattle. Addition of ruminally inert fat to dairy rations increases the energy density of the diet without reducing fiber levels below that required for optimal rumen fermentation (Coppock 1991, Grummer 1992). In addition, fat is an energy dense

feed ingredient associated with reduced metabolic heat production per unit of energy fed relative to other nutrients (Baldwin 1980).

The rationale for studying RUP in cows fed high fat diets is based on two reasons. First, when fat is added to a dairy ration, it usually replaces fermentable carbohydrates, and this may compromise ruminal microbial protein synthesis by lowering the substrate for microbial fermentation. Secondly, fat supplementation may result in lowered protein intake and decreased protein uptake by the mammary gland. The effectiveness of RUP supplementation was initially demonstrated with research (Orskov 1977) that showed abomasal infusions of casein increased milk and milk protein yields. It is recognized that supplying a protein supplement balanced in RUP and ruminally degraded protein (RDP) can maximize the amount of dietary and microbial protein reaching the duodenum, and may increase milk and milk protein yields. The benefit of supplementing RUP should be even greater with high fat diets.

The objective of this research was to evaluate the response to different levels and sources of RUP, in dairy cows fed relatively high fat, and high neutral detergent fiber (NDF) diets.

CHAPTER 2

LITERATURE REVIEW

Dietary Protein

Dietary protein generally measured as crude protein (CP), is the nitrogen (N) content of the diet X 6.25. The CP content includes both true protein and nonprotein N (NPN). True protein consists of amino acids linked by peptides bonds, whereas NPN consists of amino acids, and other N containing molecules such as ammonia and urea. Ruminant nutritionists have categorized protein into two fractions depending on whether it is degraded or not degraded in the rumen. Ruminally degraded protein (RDP) and ruminally undegraded protein (RUP) are the two components of dietary CP that have separate and distinct functions. RDP provides a mixture of peptides, free amino acids, and ammonia as N containing substrates for microbial growth and synthesis of microbial protein. RUP is the second most important source of absorbable amino acids to the animal (Dairy NRC, 2001).

Runinal Protein Metabolism

The pool of potentially degradable proteins includes those from feed protein plus the endogenous protein in saliva, sloughed epithelial cells, and the remains of lysed ruminal microorganisms (Dairy NRC, 2001). Non protein nitrogen (NPN) from the feed and urea recycled into the rumen either via saliva or the rumen wall also contributes to the pool of ammonia in the rumen (Wattiaux, 1998). The ruminal microbial population

uses peptides, amino acids, and ammonia (only bacteria) as N substrates for growth. The contribution of ammonia versus preformed amino acids to microbial protein synthesis is highly variable; however, the minimum contribution to microbial protein from ammonia is 26 percent when higher concentrations of intact proteins are present in the rumen, with a potential maximum of 100 percent when N from NPN is the only source (Dairy NRC, 2001).

In regard to feedstuffs, the extent of protein ruminal degradation is variable even within a given protein source. Of the total intake protein, 20 to 100 percent is degraded in the rumen for different feeds; while a portion resists ruminal fermentation and passes intact to the small intestine (Owens and Zinn, 1993).

Historically, proteins have been classified relative to their solubility in a variety of solvents. In general, intake proteins are partitioned into pools that are soluble or insoluble in the rumen fluid, and into pools that are degradable or undegradable by ruminal microorganisms. In general, proteins that are more soluble in the ruminal liquid phase are more rapidly and completely degradable than those that are insoluble (Chalupa, 1984).

Mechanisms of ruminal degradation

Many strains and species of bacteria, protozoa, and anaerobic fungi participate in ruminal protein degradation by elaborating a variety of proteases, peptidases, and deaminases. The liberated peptides, amino acids, and ammonia are nutrients for the growth of rumen microorganisms (Dairy NRC, 2001). Ammonia is the terminal breakdown product of the ruminal protein degradation process and the main source of N substrate for ruminal bacterial protein synthesis. The breakdown of protein to ammonia

consists of several steps. The first is the association between microorganism and substrate. The next step is proteolysis, the proteolytic cleavage of the protein to peptides, followed by peptide breakdown to amino acids, followed by deamination of the amino acids. Proteolysis is an ability shared by all of the main categories of ruminal microorganisms, but bacteria are the most important in the breakdown of soluble protein and probably in protein in general (Wallace, 1994).

Most bacterial proteases are associated with the cell surface, only about 10 percent of the total proteolytic activity occurs externally. An initial step in bacterial protein degradation is adsorption of soluble proteins to bacteria. Although a portion of protein is solubilized in the aqueous medium of ruminal digesta, extracellular proteolysis gives rise to oligopeptides which are degraded further to small peptides and free amino acids. Following bacterial uptake of small peptides and free amino acids, the following events occur: (1) cleavage of peptides to free amino acids, (2) utilization of free amino acids for protein synthesis, (3) catabolism of free amino acids to ammonia and carbon skeletons, (4) utilization of ammonia for resynthesis of amino acids, and (5) diffusion of ammonia out of the cell (Dairy NRC, 2001).

Protozoa also are significant participants in ruminal protein degradation. These microorganisms ingest particulate matter (bacteria, fungi, and small feed particles), of which bacteria are their principal source of ingested protein. As a result of this feeding behavior, protozoa are more active in degrading insoluble feed proteins. Ingested proteins are degraded within the protozoal cell to yield a mixture of peptides and free amino acids. Then the amino acids are incorporated into protozoal protein. However, it is important to remark that protozoa are not able to synthesize amino acids from

ammonia (Dairy NRC, 2001). It is also recognized that protozoa are associated with the ruminal epithelial tissue and enter the digesta at times of feeding. This process in known as sequestration and is involved in their ability to recycle N (Froetschel et al, 1990). Finally, as a result of significant secretory processes, significant autolysis, and death, protozoa release large amounts of peptides and amino acids as well as peptidases into ruminal fluid (Dairy NRC, 2001).

Not much is known about the involvement of fungi in ruminal protein catabolism, these microbes are most recognized for their involvement in the fibrolytic process. In fact, anaerobic fungi are considered to have negligible effects on ruminal protein digestion (Dairy NRC, 2001).

Metabolism of protein in the intestines

Protein available for intestinal absorption and ultimately used for cattle production is supplied by microbial protein, dietary protein escaping ruminal degradation, and endogenous secretions into the digestive tract (O'Connor et al, 1993). Microbial protein is composed by the ruminal bacteria, protozoa and fungi that pass to the small intestine. Bacteria provide most of the microbial protein leaving the rumen; protozoa do not contribute in proportion to the their contribution to the total microbial biomass in the rumen, because they are more extensively recycled in the rumen than bacteria (Dairy NRC, 2001). Ruminal bacteria have approximately 62.5 percent CP, of which it is assumed that 25 percent is cell wall N, 15 percent nucleic acid N, and 60 percent true protein (O'Connor et al, 1993).

Addition of endogenous protein

Endogenous protein also contributes to N passage to the duodenum and should be considered as part of the protein pool that passes to the small intestine; under normal circumstances most of it is reabsorbed before the terminal ileum (Hogan, 1975). Sources of endogenous protein that could contribute to duodenal protein include: (1) mucoproteins in saliva, (2) epithelial cells from the respiratory tract, (3) cellular debris from the sloughing and abrasion of the epithelial tissue of the mouth, esophagus, and reticulo-rumen, (4) cellular debris from the sloughing and abrasion of the epithelial tissue of the omasum and abomasum, and (5) enzyme secretions into the abomasum. Although, most of the first three sources probably are degraded by ruminal microorganisms, and consequently do not contribute substantially to protein passage to the small intestine (Dairy NRC, 2001).

Digestion and absorption in the small intestine

Proteins are exposed to series of metabolic events between ingestion and the time protein constituents become substrates for tissue metabolism. A complex series of biological interactions referred to simply as digestion and absorption precede these amino acids becoming available substrates for protein synthesis in animal tissues. Digestion embraces a synchronized series of hydrolytic events involving chemicals and enzymes of animal origin and of hydrolytic processes originating from the microbial population inhabiting the gastrointestinal tract (Webb and Matthews, 1994). Digestion of protein that leaves the rumen starts in the abomasum with acid-pepsin digestion and is completed in the small intestine with pancreatic and intestinal proteases (Stern et al, 1997). The end products of protein digestion that become available for absorption include amino acids,

small peptides and ammonia (Webb and Matthews, 1994). Under normal circumstances, virtually all protein that is solubilized by gastric juice (pepsin plus HCl) is thought to be digested in the small intestine (Owens and Zinn, 1993).

The small intestine is the principal site of absorption of amino acids, but the duodenal, jejunal, and ileal regions of the small intestine appear to have different abilities to absorb amino acids. In sheep and most likely in other ruminants, the major capacity for amino acid absorption appears to be associated with the more distal region, the ileum (Webb and Matthews, 1994). Although the sources of nitrogen entering the duodenum can be variable, the apparent absorption of amino acids and nonammonia N does not vary greatly. This situation, constancy of intestinal N absorption, implies similar absorbabilities for N in microorganisms, undegraded feed, and endogenous secretions (Chalupa, 1984).

Another characteristic particular of ruminants is an abundant secretion of pancreatic ribonuclease (Owens and Zinn, 1993). This enzyme is capable of splitting nucleic acids eventually to mononucleotides (Smith, 1979). A small proportion of the absorbed pyrimidines is used by animal tissues though the purines are largely excreted in urine. RNA digestion helps to conserve N and can increase N recycling as pyrimidines are catabolized in the liver. However, the major benefit of ribonuclease activity is the conservation and recycling of phosphorus; for which other enzymes remove the phosphate group from the nucleotides to form nucleosides (Owens and Zinn, 1993).

Fate of protein metabolites in the large intestine

Some of the protein reaching the small intestine is digested, but the remaining is passed into the feces. Another major source of N in the feces is the fecal metabolic

protein, which comes from digestive enzymes secreted into the intestine and the rapid replacement of intestinal cells. The fecal metabolic protein must be accounted for because it represents a loss of protein from the body (Chalupa, 1984; Wattiaux, 1995).

In the cecum, proteins are degraded and amino acids deaminated. Also, in the cecum microbial protein synthesis is probably regulated by the fermentable energy availability. However, most of the readily fermentable substrate in the diet is removed before the cecum. Thus, protein synthesis in this lower portion of the tract is much less than in the rumen. It seems likely that no significant amounts of essential amino acids are absorbed from the large intestine, and microbial protein synthesis at this point merely increases the output of crude protein in the feces (Hogan, 1975).

Nitrogen recycling

Movements of N across the gut can either be associated with net losses or as a means by which N products can be salvaged and used for anabolic purposes. Between 40 and 80 percent of urea-N synthesized by the liver is returned to the gut, and 35 to 55 percent of this is converted to further anabolic use. While some of this anabolic usage may occur in the small intestine most involves the rumen, a process that is dependent on energy supply under conditions in which the efficiency of conversion of digested N to amino acids is low. As much as 40 percent of ammonia absorbed from the gut is derived from endogenous urea-N and forms part of a cycle that conserves N within the body. Loss of N also occurs from intrarumen recycling due to the presence of proteolytic bacteria and protozoa. Net inflows due to endogenous protein secretions amount to 30 to 40 percent of apparent absorption across the small intestine, and 30 to 70 percent of this may be lost through oxidation (Lapierre and Lobley, 2001).

Metabolisable protein

Metabolisable protein (MP) is defined as the total digestible true protein (amino acids) available to the animal for metabolism after digestion and absorption of the feed in the animal's digestive tract (AFRC, 1993). MP has three components: digestible microbial true protein, digestible RUP, and digestible endogenous protein (Dairy NRC, 2001).

Predicting passage of metabolizable protein

Microbial crude protein (MCP) is considered to contain 80 percent true protein (Dairy NRC, 1989); the remaining 20 percent is present as nucleic acids, which cannot be directly used by the ruminant for the synthesis of body tissue or milk. The true protein of MCP is assumed to be 80 percent digestible. Thus, the conversion of MCP to MP is 64 percent (Dairy NRC, 1989).

Ruminally undegraded feed CP is assumed to be 100 percent true protein (Dairy NRC, 1989); however, estimates of intestinal digestibility assigned to the RUP fraction of individual feedstuffs vary from 50 to 100 percent. Therefore, the contribution of RUP to MP is variable and dependent on feed type, its composition, and processing (Dairy NRC, 2001).

The true protein content of endogenous crude protein (ECP) passing to the duodenum is assumed to be 50 percent. The true protein of ECP is assumed to be 80 percent digestible; therefore, the conversion of ECP to MP is assumed to be 40 percent (Dairy NRC, 2001).

Metabolizable protein requirements

The protein requirement includes that needed for maintenance and production. The maintenance requirement consists of urinary endogenous N, scurf N (skin, skin secretions, and hair), and metabolic fecal N which consists of bacteria and bacterial debris synthesized in the large intestine, keratinized cells, and a host of other compounds. The requirements for production includes the protein needed for the conceptus, growth, and lactation (Dairy NRC, 2001).

Estimation of ruminal protein degradation

Kinetics of ruminal protein degradation

Ruminally degraded (RDP) and ruminally undegraded (RUP) protein are the two components of dietary feed CP that have separate and distinct functions. The most used model to estimate these components uses in situ ruminal protein degradation and divides feed crude protein into three fractions (A, B, and C). Fraction A is the percentage of total CP that is NPN (assumed to be instantly degraded), and a small amount of true protein that rapidly escapes from the in situ bag because of its high solubility or very small particle size. Fraction C is the percentage of CP that is completely undegradable; this fraction is generally determined as the feed CP remaining in the bag at a defined end point of degradation. Fraction B is the remaining of the CP and includes the proteins that are potentially degradable. The amount of fraction B that is degraded in the rumen is determined by the fractional rate of degradation (k_d) and the fractional rate of passage (k_p) (Dairy NRC, 2001).

Ruminal fractional rate of passage (k_p) is the ruminal output to the omasum divided by ruminal volume (Owens and Goetsch, 1993). Ruminal fractional rate of

degradation (k_d) refers to the quantity of feed that can be digested per unit of time (Van Soest, 1994).

The most widely used model for computing RDP and RUP values for feedstuffs (as a percent of CP) is the one proposed by Orskov and McDonald (1979). Using this method the potential degradability of the feed CP is measured by incubating feedstuffs in nylon bags in the rumen, and relating degradation to time of incubation (Orskov and McDonald, 1979).

Theoretical assumption for the degradation model

The application of in situ systems involves: (1) there exists one protein fraction which disappears very rapidly within the period before the earliest removal of a bag from the rumen, (2) that a second protein fraction disappears at a constant fractional rate per unit of time, (3) in some protein supplements there is a third protein fraction that does not disappear over the period of the observations. In addition, some supplements may have a time lag before the onset of disappearance, which changes the interpretation of the parameters but not the form of the equation (Orskov and McDonald, 1979).

In calculating RDP it is assumed that the rapidly disappearing fraction is completely degraded in the rumen, which seems a reasonable approximation since most water soluble proteins are known to be degraded very rapidly (Orskov and McDonald, 1979).

Microbial contamination

During ruminal incubation there is an intimate contact of the test feed particles with ruminal microflora, and consequently there is potential contamination with microbial constituents. However, no significant differences were detected between N

degradation rate constants with or without correction for bacterial N contamination (Nocek, 1985). On the other hand, during RDP quantification microbial contamination should be considered, since it may have an important effect.

Bacterial contamination increases curvilinearly with time of ruminal incubation, which suggest that bacteria continually attach to particles up to a particular time of ruminal exposure, after which attachment appears to be a function of surface area or substrate availability (Nocek, 1988). Bacteria attach to feed particles at a fast rate up to 4 hours. Then, the rate of contamination and/or attachment proceeds at a slower rate until 12 hours, then declines (Nocek, 1985). Concentrate ingredients generally contain little microbial contamination (5 to 10 percent of the residual N), while forage residues tend to have more contamination. Therefore, at least low protein forages and coarse feedstuffs should be corrected for microbial contamination (Nocek, 1988)

Some sources of variation with in situ measurements

Diet composition. Any dietary factor that has an influence on the ruminal microbial population, such as starch or fiber content, will potentially affect the rate and/or extent of digestion within synthetic bags. In situ incubations ideally would be conducted in rumens of animals that are consuming the diet of interest; however, this is not always practically feasible and it may constitute an important source of variation. Since there is a wide range of feedstuffs that can be evaluated in situ, and in order to maximize the diversity of the microbial population and to ensure that individual nutrients do not limit ruminal digestion, diets ranging from 50 to 70 percent forage have been recommended for in situ studies (Vanzant et al, 1998).

Feeding level. Nocek (1988) and NRC (2001) recommended ad libitum feeding, but AFRC (1993) suggested feeding animals at maintenance level during in situ studies. At present the relationship between intake and in situ degradation is not clear. If intake does not have an important effect on in situ disappearance, either approach is adequate. However if intake level significantly affects in situ degradation, then more research will be needed to establish degradation values (Vanzant, 1998).

Frequency of feeding. Diurnal fluctuations in rumen microbial population have been noted in animals that are fed once a day. Such fluctuations decrease with increasing feeding frequency. Furthermore, it has been found that bacteria entered synthetic bags more rapidly and in larger numbers with more frequent feeding. Therefore, more frequent feeding should enhance in situ fermentation. For these reasons a minimum of two daily feedings is recommended (Vanzant, 1998).

Variation among animals, days, and bags. Even when controllable sources of variation are accounted for, substantial variation still exists with in situ degradability measurements. Variation exists among animals, across days within the same animals, and between replicate bags within these animals on a given day. In order to minimize variation from these sources and to provide greater repeatability of estimated values, the number of animals, days, and bags should have replication (Vanzant, 1998).

Measuring intestinal protein digestion

Estimates of the CP digestibility in the small intestine can be determined by using the "In situ mobile-bag technique." This technique, originally introduced to study digestibility in swine, has been modified to study postruminal digestion of feedstuffs by ruminants. Using this method, sample feeds are incubated in the rumen during 16 h for

concentrate feeds and 24 h for forages, then a small amount of feed that had been preincubated in the rumen is placed in nylon bags, and introduced into the duodenum and subsequently collected from the feces (Broderick and Cochran, 2000; Stern et al, 1997). Therefore, total tract measurement using this technique can be considered as estimates of true, rather than apparent digestibility (Stern et al, 1997).

Protein rich concentrates such soybean meal are characterized by high RUP digestibility, whereas protein concentrates relatively high in fiber (canola meal) or ash (meat and bone meal) are characterized by lower digestibility. The RUP digestibility of concentrates is usually greater than that of forages (Stern et al, 1997).

Mobile bag technique assumptions

Estimated digestibility obtained from fecal collection of bags assumes that neither bags nor feed residues are contaminated with microbial protein from large intestinal fermentation. Also it is assumed that protein leaving the ileum is not further digested by microorganisms in the hind gut. For practical purposes fecal collection is considered more convenient (Stern et al, 1997).

The influence of energy on protein supply and utilization

Energy-protein interrelationships

Balch (1967) concluded that dietary protein and energy are interdependent in ruminants; the higher the energy supply the greater the performance response to protein. This researcher described the N balance response to increasing levels of protein supply when energy is not limited as having a positive linear slope. Whereas, at any given energy intake above maintenance, protein output responds curvilinearly to protein intake. Over the normal range of intakes, the anabolic effect (N retention) from protein intake

depends on the level of energy supplied, and vice versa, (Balch, 1967). However, one limitation with this approach is that feed protein does always not reflect the protein reaching the small intestine (Chowdhury and Orskov, 1997).

Andrew and Orskov (1970) fed diets at different intake levels and obtained results that clearly reflected a family of curves, in which responses to protein depends on supply of energy, which agrees with the principle proposed by Balch . However, these results were questioned when Orskov and Fraser (1973), using the same diets, demonstrated that at low feeding levels N flow did not increase with an increased protein supply, while at incremental levels of feeding, N flow increased. Yet, when the results were expressed in terms of microbial crude protein and RUP the same curve response was observed; in other words, the higher the level of N supply at the intestinal level the greater the N balance response (Chowdhury and Orskov, 1997).

These results do not necessarily represent the requirements of the animals but represent responses to supply. At higher levels of feed intake, more protein presumably by passed the rumen and may have resulted in an animal response. In addition, these results suggest that Balch's proposal does not reflect changes in protein needs, but rather changes in protein supply (Chowdhury and Orskov, 1997).

Fasting protein metabolism

Fasted animals fed protein alone can utilize protein with the same efficiency as fed animals, which implies that an animal can have protein accretion at the cost of endogenous energy, such as body fat (Orskov et al, 1983). Consequently, it can be stated that fat could be utilized to fuel protein deposition.

Trials using abomasal casein infusions at different rates along with mineral and vitamin supply, but no additional energy, showed a curvilinear relationship between casein N infusion and N retention. This means that ruminant animals can mobilize body reserves to fuel protein accretion, at different rates depending upon the N supply to the abomasum (Chowdhury and Orskov, 1997).

Chowdhury et al (1991) continuously infused different levels of casein into the abomasum, either with or without energy input from volatile fatty acids (qtd. in Chowdhury and Orskov, 1997). Except for a reduction in fasting N excretion when exogenous energy was supplied there were no differences in N retention whether the animals received volatile fatty acids (exogenous energy) or not, as long as sufficient endogenous energy (body reserves) was available. The absence of the effect of VFA infusion on N balance at higher levels of casein infusion could mean that VFA-unsupplemented animals can efficiently utilize endogenous energy to fuel protein retention. This may also mean that body fat can be mobilized to maintain the energy requirement for protein accretion, provided that sufficient amino acids are available. However, at lower levels of casein infusion, additional energy improved the N balance probably by sparing some amino acid oxidation from gluconeogenesis (Chowdhury and Orskov, 1997).

The mechanisms by which protein is accreted and fat is oxidized simultaneously are not well understood. In human subjects, it was suggested that insulin levels are lower when protein is given in absence of carbohydrates, allowing for more energy to be derived from endogenous fat (Chowdhury and Orskov, 1997). In contrast, in ruminants, Oldham (1984) pointed that amino acid supplementation increased concentration of

growth hormone in blood. Growth hormone is a lipolytic hormone known to be associated with cell differentiation, net protein anabolism, net fat catabolism, and insulin resistance (Chowdhury and Orskov, 1997).

Tissue protein synthesis depends upon insulin-dependent intracellular uptake of amino acids and energy intermediate (ATP). During dietary energy undernutrition (fasting), when the amount of protein is adequate to provide the required intracellular amino acid concentrations, an animal can maintain protein accretion by generating ATP through oxidation of adipose tissue under the influence of GH-IGF1 control (Chowdhury and Orskov, 1997). However, during lactation-induced negative energy balance, extensive oxidation of fat to provide energy for milk protein synthesis can contribute to ketosis (Orskov et al, 1987).

Unilateral increase of amino acid flow to the lower digestive tract can be accomplished by the use of undegradable protein supplements, protected amino acids, or postruminal infusions, which under the proper circumstances may increase protein deposition. For instance, the addition of undegraded protein to a diet deficient, but not severely so, in energy results in a significant increase in N retention. This appears to be the result of the animal's ability to utilize body fat to provide the energy necessary to sustain tissue synthesis in an energy deficit, the lipotropic effect. However, this is a metabolic effect and not a dietary one (Asplund, 1994).

Protein to energy ratios

The Agricultural Research Council (1980) recommended a ratio of 1.87 grams of RDP per Mcal of metabolizable energy (0.3 g RDP/MJME). However, Nocek and

Russell (1988) concluded that this ratio does not result in maximal ruminal protein synthesis.

Van Horn et al, (1985) suggested that the ratio of energy to protein (TDN:CP) more accurately described protein requirements than percent CP. In addition, they found a tendency for greater production responses if the ratio was decreased by limiting energy (Van Horn et al, 1985). However, this ratio should be requalified especially for higher producing cows since a decrease in energy will probably reduce microbial protein synthesis and consequently metabolizable protein.

Paquay et al (1973) proposed an optimal ratio of digestible CP to metabolizable energy intake, according to the stage of lactation; 2.32 for early lactation, 1.94 for mid lactation, and 1.64 for late lactation (grams of CP/McalME). However, digestible CP could be misleading for all protein sources since this term does not differentiate clearly the CP fractions (RUP and RDP).

Pattarajinda (2001) suggested to use a ratio of 75 to 83 grams metabolizable protein (MP)/ Mcal NE_l. The MP value used was calculated based on RUP and microbial protein (MCP); the latter one was calculated based on non fat NE_l using an adjusted NRC (1989) equation. With this approach, it was found improved performance in lactating dairy cows during summer time. However, the new dairy NRC (2001) considers MP to be the sum of RUP, MCP, and endogenous CP; in addition, it is also recommended that the energy values used for the calculation of MCP be expressed at maintenance level, and be adjusted for intake over maintenance, without considering the energy coming from fat above 3 percent. Therefore, this values may need to be reconsidered.

Influence of energy in amino acid metabolism

To better understand the undeniable influence of energy in amino acid metabolism, there are some factors that need to be consider. The energy status of the animal as one of the major determinants of the metabolic pathway to be followed by a given amino acid. Also it needs to be pointed out that both the level and the nature of the energy supply are critical (Asplund, 1994).

If glucose is in limiting supply, the glucogenic amino acids will be metabolized for energy to meet the animal's requirements for glucose precursors, with the result that the adequacy of the amino acid supply for protein synthesis will be substantially impaired. Furthermore, protein synthesis and degradation continue in the absence of dietary sources of amino acids, and that the supply of energy largely determines the proportion of the amino acids released by degradation that will be recaptured for necessary protein synthesis. Therefore, the nature of the energy supply controls much more than simply the disposition of absorbed amino acids, but rather controls the size and dynamics of all amino acid pools in the body (Asplund, 1994).

Relationships of protein to microbial synthesis

Microbes can be starved for N when RDP is low (<30%). However, if RDP is greater than 60 percent, N losses are excessive, even if rumen available carbohydrate is also high (Nocek and Russell, 1988).

Increasing RUP increases milk production when CP intake is marginal (<14% CP), but responses are diminished if CP is more than 16%. Furthermore, adding RUP appears to be most beneficial when cows are in negative energy balance, and sufficient body fat is available for energy demands (Nocek and Russell, 1988).

Ruminally undegradable protein supplementation

Different approaches have been proposed to increase the post ruminal amino acid supply. A common practice is the inclusion of animal protein sources in the diet; one of the most used is fish meal (FM) at rates that vary from 2-6 % DM of the diet. Blending of various protein supplements offers opportunity to better supply RUP. Blends of RUP feeds are commonly used in commercial feeding situations, by which it is thought the cow would receive a better intestinal amino acid profile (Liu et al, 2000). However, not all studies resulted in positive responses using either animal protein sources or protein blends. Researchers have thought that in some cases the by-pass protein supplements cause an imbalance between N-ammonia requirement and RUP. Furthermore, even though protein supplements have high RUP values they can have at least one limiting amino acid (Santos et al, 1998) . Therefore, it is greatly recommended that a protein blend of various protein sources be fed so that the ingredients will complement each other in their amino acid profiles.

A review of the literature showed that there are three main types of protein supplements: (1) animal/marine protein, where generally fish meal (FM) is considered as the marine source; (2) high CP high RUP animal protein, usually containing blood meal (BM) and feather meal (FHM); and (3) animal-plant protein blends.

Menhaden fish meal is the most used type of FM; however, there are alternatives. Menhaden FM has an average 68.5% CP and 65.8% RUP (Dairy NRC, 2001). It is a good source of lysine, and has an amino acid profile closely related to milk protein. The first limiting amino acid in FM based blends is thought to be leucine (Abu-Ghazaleh, 2000).

A search in the literature showed that the average composition of the high RUP animal protein blends is 93% CP, 69% RUP. The average ratios for BM and FHM are 50:50 to 15:85. These animal protein blends have shown a complementary effect in different studies. However, to achieve a better complementary effect and provide a better ratio of RUP and RDP, it seems that an animal-plant protein blend is the best alternative.

The most used feeds for this purpose are: heated soybean meal (HSBM), soybean meal (SBM), corn gluten meal (CGM), BM, FHM, FM, meat and bone meal (MBM), and poultry protein meal (PPM) (Sloan et al, 1990). The proportions vary a lot but it seems that a mixture containing about 30 to 50% plant protein sources, and 50 to70% of animal/marine protein sources was most effective.

There are many possible combinations but a blend of FM, CGM, BM, and FHM, might be a good combination due to the complementary effect between FM-CGM, and BM-FHM. FM is high in lysine, but deficient in leucine; on the other hand CGM is high in methionine and leucine, but lysine deficient. BM and FHM are also complimentary, provably due to the fact that FHM's most limiting amino acid is histidine and BM is a good source.

Lipid metabolism in the rumen

Microbial metabolism of lipids in the rumen

Fatty acids in conventional diets are mainly fed in the form of triglycerides. There are two major metabolic transformations of fatty acids in the rumen: lipolysis and biohydrogenation of fatty acids. Fatty acids are hydrolyzed rapidly by rumen lipolytic bacteria; however, protozoa may not be capable of lipolytic activity. Biohydrogenation

of unsaturated fatty acid is dependent upon a free carboxyl so that lipolysis as the initial step is mandatory.

Some dietary fatty acids are incorporated readily into cellular lipids of rumen bacteria and protozoa, and may inhibit de novo synthesis of fatty acids. As rumen microbes do not contain storage triglycerides, predominant cellular fatty acid is membrane phospholipid and unsterified fatty acid. Both bacteria and protozoa are capable of de novo synthesis of long chain fatty acids. Fatty acid precursors may have either even or odd number of carbons, or may have branched chains (Palmquist and Jenkins, 1980).

Rumen and caloric effects of lipids

The energy value of dietary fat is changed little as it passes through the rumen. On average, 87 percent of fatty acid intake is recovered at the duodenum. This small fatty acid loss is often compensated for by de novo lipid synthesis by ruminal microorganisms, causing a net gain of fatty acids through the rumen. Ruminal loss of dietary fatty acids occurs from limited lipid metabolism by ruminal epithelial cells, along with minimal fatty acid absorption into blood and degradation by microbes (Jenkins, 1994).

Rumen and regulatory effects of lipids

It was thought ruminant diets were limited to relatively small quantities of added fat to avoid digestive disturbances. However, levels of supplementation are most limited for unsaturated fatty acids, which have a more potent antimicrobial effect and are more inhibitory of ruminal fermentation (Jenkins, 1993). Dietary unsaturated fatty acids

provide a hydrogen sink for ruminal microbial metabolism and are extensively hydrogenated to saturated fatty acids by rumen microorganisms (Jenkins, 1994).

Duodenal lipids in ruminants can be divided into three fractions: dietary lipid that escapes microbial transformation, dietary lipid susceptible to microbial transformation, and microbial lipid. Transformed dietary lipid and microbial lipid contain primarily saturated fatty acids and unique lipids of microbial origin contributed by ruminal fermentation (Jenkins, 1994).

Dietary control of rumen fatty acids

Changing the grain content of the diet seems to have some influence in biohydrogenation. The reduced number of lipolytic bacteria in the rumen associated with grain feeding appears to explain the diminished capacity for biohydrogenation, since a free carboxyl group on a fatty acid is a prerequisite for the initial isomerization step in biohydrogenation (Jenkins, 1994).

Other characteristics can reduce rates of lypolysis and biohydrogenation and include the amount and type of added fat. The two major properties of fat that may influence its effect on digestion in the rumen are unsaturation and esterification. As stated earlier, unsaturated fats are more toxic to rumen microbes than are saturated. Esterification may also be a factor in utilization of fat in the rumen even though microbial lipolytic activity is high (Palmquist and Jenkins, 1980). Oilseeds can be fed without observable ruminal inhibition, probably because of a slow release of the oil into the ruminal contents. A number of commercial fat supplements are available that have little effect on ruminal fermentation but are highly digestible postruminally (Coppock and Wilks, 1991).

Effect of dietary fat on fiber digestibility

The addition of fats to ruminant diets depresses fiber digestibility. The theories that try to explain this effect are: (1) physical coating of the fiber with fat preventing microbial attack, (2) a modification of the rumen microbial population from possible toxic effects of fat on certain microorganisms, (3) inhibition of microbial activity from surface-active effects of fatty acids on cell membranes, and (4) reduced cation availability from formation of insoluble complexes with long chain fatty acids. Although opinions may vary, most data support an inhibitory effect on microbial activity, perhaps sufficient to change viability of certain bacteria and, therefore, microbial populations (Palmquist and Jenkins, 1980).

Intestinal digestion and absorption

Apparent digestibility of fat in typical ruminant diets is low, due to a high content of nonfatty acid material and to the small proportion of fat in the total diet. With most diets endogenous secretions of fatty acids are relatively high. Biohydrogenation in the rumen is a major factor in causing uniformity whereas the increased digestibility of saturated fats is likely due to the unique interactions of particulate matter, bile acids, lysolecithin, oleic acid, and acidity in the upper small intestine. Digestibility of moderate amounts (3 to 5 %) of added fat is about 80%, but fatty acid in excess of 5 to 6% of the diet (up to 10% added) is absorbed less efficiently (about 56%) (Palmquist and Jenkins, 1980).

The pH of the proximal one half of the ruminant intestine remains relatively acid due to the low bicarbonate content of pancreatic secretions. Although this low pH decreases solubility of fatty and bile salts, it may solubilize calcium soaps allowing

higher absorption of both fatty acid and calcium than would be possible or alkaline pH. Ruminal inert fats that are saponified fatty acids or calcium soaps of fatty acids are used to by pass the rumen fermentation. In addition to pH, bile salts are absolutely required for fatty acid absorption in ruminants, dispersing fatty acids in the small intestine by their detergent effect (Palmquist and Jenkins, 1980).

Metabolic limits to fat utilization

Lactating cows utilize fatty acids by direct secretion into milk, deposition in tissues as membrane or storage lipids, and oxidation. However, there are factors, other than inhibition of rumen fermentation, that limits utilization of large amounts of fat by ruminants. These factors include lower intestinal absorption at high intake, and sensitivity to nutrient balance, causing reduced energy intake (Palmquist, 1994).

Benefits and properties of fats as supplements

Fats have some properties that make them attractive to dairy nutritionists. First, the net energy of lactation of a calcium soap of 85 percent palm fatty acids is about 6.52 Mcal/kg of dry matter, which is 3.33 times the net energy of corn. Second, long chain fatty acids are used with a high efficiency for lactation because they can be transferred directly to milk fat. Third, other benefits are that fiber intake could be maintained while increasing energy density, improved reproductive performance, greater persistency in milk yield, less ketosis, and less dust in feeds (Coppock and Wilks, 1991).

Negative effects of fat supplementation

Ruminal microbes are inhibited by some fatty acids released into the rumen, particularly medium size (C_8 to C_{14}) fatty acids and unsaturated longer chain fatty acids. This may decrease fiber digestion, change fatty acid ratios in rumen fluid, and decrease

milk fat percentage. Moreover, total dry matter intake could be reduced, especially if the amount of dietary fat required for milk fat synthesis is exceeded (Coppock and Wilks, 1991).

Effect of supplemental fat on milk yield and composition

Chalupa (1991) compiled the results of 10 studies with calcium salts of palm fatty acids and the report was (1) + 2.40 kg/d of milk; (2) milk fat percentage, + 0.05; (3) 3.5% fat corrected milk, +2.64 kg/d; and (4) milk protein percentage, - 0.16 (qtd in Coppock and Wilks, 1991).

Feeding supplemental fat invariably reduces milk protein content, nevertheless milk protein yield is usually still increased but not as much as the increase in milk yield. This reduction in milk protein percentage occurs with all sources of supplemental fat fed (Schingoethe, 1996). Four causes of the lower milk percentage have been proposed: (1) there is a reduced microbial protein production; (2) there is a restricted availability of glucose; (3) insulin resistance by the mammary gland impairs amino acid transport and milk protein synthesis; (4) a reduced release of bovine somatotropin reduces mammary gland uptake of amino acids. Furthermore, lower concentration of branched chain amino acids in plasma often indicated a diminished absorption of protein from the small intestine. This indicates a lower microbial protein synthesis (Cant et al, 1993; Coppock and Wilks, 1991).

Schingoethe (1996) quotes that a 7% reduction in mammary blood flow was observed with high fat diets which prevented an increase in uptake of critical amino acids needed to improve efficiency of milk synthesis. This coupled with no change or slight

reduction in arterial amino acid concentrations, leads to reduced amino acid uptake by the mammary gland.

Conclusion

Dairy producers in the Southeast are confronted with lower forage quality and heat stress. Both of these problems can depress intake and performance of lactating dairy cattle. Low quality forages are generally high in fiber and low in energy and CP, which decreases the nutrient density of the ration. Lower intake causes decreased nutrient intake which greatly lowers performance. Reduced energy intake may cause energy undernutrition and consequently a negative energy balance. Energy intake affects microbial protein synthesis by reducing the amount of fermentable organic matter. Feeding RUP supplements may be of benefit to counteract the intake related depression in microbial protein synthesis. In addition, in order to improve the energy status of the cow utilization of supplemental fat is recommended because it increases the energy density of the diets, and may counteract caloric insufficiency associated with low dry matter intakes. However, fat does not provide energy to support the growth of ruminal bacteria. Thus producers in the Southeast have justification for using rations that are relatively high in fat and fiber that should accentuate the need for RUP.

RUP sources are relatively expensive especially FM. However, there are other alternatives like poultry protein meal (PPM) that may provide a lower cost alternative but there is a lack of information about efficacy of such alternative feeds. Although it is thought that the RUP value of PPM is high, as is that of most heat-processed animal feed protein feeds, this value has not been clearly established.

Therefore, the focus of this thesis was to study RUP supplementation in cows fed high fat and high fiber diets and determine the ruminal kinetic and digestibility of certain feedstuffs with special attention to PPM.

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CHAPTER 3

ESTIMATING RUMINAL PROTEIN DEGRADATION AND RUMINALLY UNDEGRADED PROTEIN DIGESTIBILITY, USING THE IN SITU AND MOBILE BAG TECHNIQUE[1]

[1]Canseco, M.A., M.A. Froetschel, and H.E. Amos. To be submitted to Journal of Dairy Science.

Abstract

Two ruminally cannulated Holstein steers and two duodenally cannulated Hereford steers were used to determine the in situ degradation kinetics and the in situ intestinal digestibility of certain feedstuffs. Ruminally undegraded protein (RUP) was determined for eight feeds: wheat silage, corn grain, soybean meal, whole cotton seed, soybean hulls, an RUP-blend (fish meal 60% and dry distillers grains 40%), porcine blood meal, and pet food grade poultry protein meal. Nylon bags containing approximately 10 mg/ cm^2 of each feed sample were presoaked and then incubated in duplicate in the rumen for 0, 2, 4, 8, 16, 24, 48, and 72 h. An extra set of 6 bags was incubated for 16 h for concentrates and 24 h for wheat silage. Intestinal digestibility was also determined on some feed ingredients: wheat silage, soybean meal, whole cotton seed, RUP-blend, blood meal, and poultry protein meal. These samples were placed in smaller nylon bags and introduced to the duodenum. The bags were recovered from the feces after 15 to 20 h.

Blood meal was the least degradable ruminally but the most degradable intestinally. RUP-blend and the PPM had very similar characteristics in both ruminal degradation and intestinal digestibility. Based on our results it appears that pet food grade poultry protein meal is a good substitute for other animal protein feeds. This study also showed that its intestinal digestibility is relatively high even though it has lower ruminal degradation.

Introduction

It has been accepted that feeding ruminants based simply on quantity of crude protein alone is inadequate. Proteins digested in the small intestine of the cow are a combination of feed protein passing through the rumen, microbial protein synthesized in the rumen and endogenous protein. Current guidelines recommend formulation of diets for ruminally undegraded protein **(RUP)** and ruminally degraded protein **(RDP)** in order to meet the requirement of metabolizable protein **(MP)** through a greater amino acid flow to the duodenum. However, positive responses to **RUP** supplementation have not always been found (Santos et al., 1998). Lack of animal response to **RUP** supplementation has been attributed to various factors, such as poor amino acid profile of the **RUP** source or depression in microbial nitrogen **(N)** flow. However, possibly the greatest reason for lack of response is because too much protein is fed in control diets and/or lower producing cows were used as experimental subjects that do not require higher levels of **RUP**. In addition, poor protein digestibility arising from over processing can minimize animal responses. Furthermore, dairy NRC (1989) crude protein **(CP)** requirements are too high because they were based on low RUP concentrate feeds (soybean meal and corn). Paradoxically, the 1989 Dairy NRC recognized that when cows were fed based on UIP and DIP (same as RUP and RDP) they require less CP.

In order to formulate diets for **RUP** one must first adequately predict the degree to which nutrients are made available in the rumen from a variety of feedstuffs, as well as the proportion of feed protein that passes to the small intestine.

In recent years, the in situ procedure has been often used to evaluate ruminal **CP**, dry matter **(DM)** and fiber degradation, due to its ability to expose feedstuffs to ruminal

digestive conditions thought to be similar to those existing in vivo (Dairy NRC, 2001). However, feeds are not subjected to the total ruminal experience: i.e. mastication, rumination, and passage (Nocek, J.E. 1988). Furthermore, the in situ procedure has emerged as the most widely used approach for estimating RUP (Stern et al, 1997), and most importantly it was adopted by for the Dairy NRC 2001.

The objective of this study was to estimate the RUP value and the intestinal RUP digestibility of dairy feed ingredients that are to be used in a feeding trial with lactating dairy cattle.

Materials and Methods

Animals

For the estimation of ruminal protein degradation by the in situ technique, two ruminally cannulated Holstein steers weighing approximately 400 kg were utilized. The RUP intestinal digestibility measured by the in situ mobile nylon bag technique was done in two Hereford steers weighing approximately 500 kg, fitted with proximal duodenal cannulae.

Feeding and housing

Steers received total mixed rations (Table 3.1) twice a daily, and had free access to water. The diet fed to steers for the degradability measurements was approximately 45% forage, whereas that fed for the digestibility study was approximately 50% forage. The estimated dry matter intake was approximately 14.5 kg/d. Animals used for the RUP study ate at 3.5 X maintenance. Steers were housed indoors in individual tie stalls under continuous lighting.

Feedstuffs

The RUP value and the RUP digestibility were estimated for six feedstuffs (wheat silage, whole cotton seed, corn, soybean meal, soy hulls, blood meal, and poultry protein meal) and one RUP-blend (60% fish meal, and 40% dry distiller grains). The estimated nutrient composition of the samples is presented in Table 3.2.

In situ procedure for RUP determination

Ruminal crude protein degradability was determined following current recommended procedures (Appendix 1). All feedstuffs were subjected to in situ incubation in an as fed state. Forage samples were frozen using 1 part of dry ice per 3 parts of forage, and then ground through a 6 mm screen Wiley Mill (Arthur H. Thomas, Philadelphia). The rest of the feedstuffs were ground through a 2 mm screen Willey Mill. The equivalent of 4 g of DM were weighed and were placed into 10 X 20 cm polyester bags (R1020, Ankom Technology, Fairport, NY, USA) with a pore size of 50 ± 15 F. Polyester bags were sealed with an electric impulse heat sealer (Ankom Technology, Fairport, NY, USA). Duplicate samples of each feed for each incubation period were placed in the rumen of two steers. Incubation periods were 2, 4, 8, 16, 24, 48, and 72 h. In situ samples were grouped according incubation period and steer, placed into 36 X 50 cm polyester mesh bags, one per each incubation period, and incubated in the ventral sac of the rumen. The mesh bags were maintained in the ventral sac of the rumen by a 600 g metal piece. Mesh bags were tied to a 50 cm line and anchored to the rumen cannula. Prior to incubation, the bags were soaked in warm water (aprox. 39°C) for 20 min, using 1 l of water/250 mg N. Samples were placed in the rumen in a reverse order beginning with the 72 h sample so that all bags were removed from the rumen at one time for

uniformity in removal and washing. Zero time disappearance values (0 h) were obtained by washing pre-soaked samples along with in situ samples.

Immediately after removal from the rumen, bags were immersed in ice-water to stop or minimize microbial activity and then they were rinsed under running tap water to remove rumen debris. Posteriorly, bags were hand washed until rinse water running from the bags was clear. Washed bags were air dried over screens for approximately 8 h, and then dried at 55°C to constant weight (approximately 48 h) for DM determination.

Special considerations

Ratio of sample to bag surface area (SS:SA). Since SS:SA is relatively simple to control and significant differences can be expected across the wide range of SS:SA (10 to 15 mg/cm^2), it is recommended to use the lower value, 10 mg/cm^2 (Vanzant et al., 1998). The number 16 h (for concentrates) and 24 h (for wheat silage) incubation period samples were increased in order generate enough incubation residue for the digestibility study. The SS:SA ratio was calculated with the following equation:

SS:SA = sample size (mg)/[bag width (cm) x bag length (cm) x 2]

Microbial contamination correction. After retrieving the mesh bags from the rumen a sample of rumen fluid was taken from each steer. A bacterial fraction was isolated by differential centrifugation, and its RNA content was determined as described by Zinn and Owens (1986). In addition, RNA content of the 16 h residues (for concentrates) and 24 h residue for wheat silage was determined. RNA contents of the bacterial isolates and residues were used to correct the C fraction for bacterial contamination. Degradation constants were calculated without correcting for bacterial

contamination since it was demonstrated that contamination does not affect this calculation (Nocek, 1985).

Crude Protein fractions determinations

The most widely used model to describe in situ ruminal protein degradation divides feed CP into three fractions (A, B, and C): soluble protein (fraction A), potentially degradable (fraction B), and undegradable (fraction C). Only the B fraction is affected by relative rates of degradation; all of fraction A is considered to be degraded and all of the fraction C is considered to pass to the small intestine.

CP fraction A. Fraction A includes NPN, rapidly solubilized protein, and protein in particles of smaller size than the porosity of the polyester bags into which the feedstuffs are placed during rumen incubation. The different forms of N in fraction A cannot neither be separated by using the in situ procedure, nor can the rate be determined at which fraction A is degraded (England et al, 1997; NRC, 2001).

The a fraction was determined via solubility in an aqueous solution. Zero h bags attached to weights (about 150 g) were soaked in 39 ± 3 °C tap water (1 l of water/250 mg of feed nitrogen) for 20 minutes prior to the placement in the rumen to remove water soluble and material filterable at 53 μ. Thus, water-soluble pool will be assumed to be eliminated by presoaking (Nocek, 1988).

A zero time washout value obtained after soaking the samples in warm water was used as the 100 % DM value. Incubated zero h bags were rinsed immediately after soaking, then residues were dried at 55 °C; however, the residues that were intended to be used for in situ digestibility were freeze dried.

CP fraction C. Fraction C is estimated by a defined end-point of degradation, which corresponds to the lowest percent residual beyond which no further ruminal degradation occurs (Nocek, 1986; NRC, 2001); this end point occurs about 48 h for concentrates and 72 h for forages.

CP fraction B. Fraction B consists of the proteins that are potentially degradable. Only the B fraction is influenced by the relative rate of degradation. It can be estimated by using the following equation (Broderick and Cochran, 2000; England et al, 1997; NRC, 2001):

$$B = 100 - A - C$$

Mathematical model to estimate rate of degradation (K_d) of the CP fraction B, %h

The nonlinear approach described by Orskov and McDonald, (1979) was used to estimate k_d. Using this model, ruminal CP disappearance follows first-order kinetics defined by the equation:

$$P = A + B(1 - e^{-KdT})$$

Where: P = CP disappearance

A = Protein fraction which disappears very rapidly within the period before the earliest removal of a bag from the rumen (%CP)

B = A second protein fraction that disappears at a constant fractional rate k_d per unit of time (%CP)

K_d = Rate of degradation (h-1) of the B fraction

T = Time in the rumen (h)

Statistical method

In situ rumen degradation rates for DM and CP were estimated using nonlinear regression (PROC NLIN) methods of SAS (SAS, 1990) (Appendix 3.2). The model for describing DM and CP degradation kinetics was the one proposed by Orskov and McDonald (1979).

Rate of passage (K_p) estimation

According to the dairy NRC (2001), three prediction equations can be used to estimate rate of passage and adjust in situ degradation data:

Equation for estimating K_p of wet forages (i.e., silages and fresh forages)

$$K_p = 3.054 + 0.614X_1$$

Where: K_p = rate of passage from the rumen, %/h

X_1 = DMI, percentage of BW

Equation for estimating K_p of dry forages

$$K_p = 3.362 + 0.479X_1 - 0.007X_2 - 0.017X_3$$

Where: K_p = rate of passage from the rumen, %/h

X_1 = DMI, percentage of BW

X_2 = concentrate, percentage of diet DM

X_3 = NDF of feedstuffs, percentage of diet DM

Equation for estimating K_p of concentrates

$$K_p = 2.904 + 1.375X_1 - 0.020X_2$$

Where: Kp = rate of passage from the rumen, %h

X_1 = DMI, percentage of BW

X_2 = concentrate, percentage of diet DM

RUP estimation

The portion of fraction B that is not degraded, plus fraction C, is assumed to be RUP. This component of the dietary feed CP is the second most important source of absorbable AA to the animal. As stated earlier, microbial protein, a function of RDP, is greatest fraction of metabolizable protein. An important assumption with the in situ method is that "disappearance" from the bag is synonymous with degradation and that any N that has disappeared from the bag, including N associated with rapidly degradable proteins is likely to be hydrolyzed as peptides (Broderick and Wallace, 1988), and used by ruminal microorganisms. However, the efficiency of converting RDP to microbial protein depends on the readily fermentable carbohydrate supply to provide energy for protein synthesis. Under conditions where readily fermentable carbohydrates limit microbial protein synthesis the RUP fraction may be more important.

RUP value for a feedstuffs (percent of CP) was computed using the following equation:

$$RUP = B[K_p/(K_p + K_d)] + C$$

RDP estimation

RDP provides a mixture of peptides, free amino acids, and ammonia for microbial growth and synthesis of microbial crude protein. The extent of RDP degradation (% of CP) was calculated as follows:

$$RDP = A + B[K_d/(K_d + K_p)]$$

or

$$RDP = 100 - RUP$$

In situ procedure for RUP intestinal digestibility

Measurement of CP disappearance during intestinal passage was determined for six feedstuffs residue derived from the un situ rumen fermentation study. They were wheat silage, corn, soybean meal, whole cotton seed, poultry protein meal, and the RUP mix (60% fish meal and 40% dry distillers grains), (Table 3.3).

Concentrate samples were incubated in situ 16 h and wheat silage 24 h, to correspond to mean ruminal retention time of concentrates (Beckers et al 1996) and forages, respectively (Broderik and Cochran, 2000). After in situ incubation, bags were immediately immersed in ice-water, rinsed, air dried and subsequently freeze dried. Residues incubated in each steer were composited for each feed and ground using a mortar and pestel. A small portion (approx. 1 g) of the sample was used for lab DM determined from drying at 105°C in a forced air oven. Samples were also analyzed for N (Leco FP 528 N analyzer).

Feed residues were weighed into 5.0 X 6.0 cm polyester bags with 0.6 g each one for concentrates (10 mg/cm^2) and 0.3 g for wheat silage (5 mg/cm^2). Polyester bags had a pore size of 50 ± 15 F (Ankom Technology, Fairport, NY, USA), and were sealed with an electric impulse heat sealer (Ankom Technology, Fairport, NY, USA).

Sample residues were inserted into the duodenal cannula in quadruplicate in each steer. Prior to intestinal insertion, bags were preincubated in an HCl solution (0.004 M HCl) for 1 h and 2 h in a pepsin/HCl solution (100 mg of pepsin per liter of 0.004 M HCl) at 40°C. During the meal, 12 bags were inserted into the duodenum of each steer via a T-cannula. Starting at 1500 h, at 15 min interval each bag was introduced. The four bags were divided over 2 days. Bags were collected every day from the feces 15 h

after the first bag had been introduced to into the duodenum, until 20 h after. Bags that were not recovered 20 h after introduction were discarded. Bags were rinsed under tap water until rinse water running out of the bags was clear. After rinsing, bags were dried at 55°C, and residues were pooled to represent each feed sample and each steer, and subsequently analyzed for N (Leco FP 528 N analyzer).

Estimation of the RUP intestinal digestibility

Estimates of RUP digestibility obtained using this technique are considered to be estimates of true digestibility. To calculate the RUP digestibility in small intestine the following equation was used:

$$TD = (UDN - TU)/UDN$$

Where: TD = true digestibility of rumen undergraded dietary protein in the small intestine

UDN = fraction of undegraded dietary nitrogen

TU = fraction of true indigestible nitrogen in the feed

The assumptions behind this equation are that a feed contains a protein fraction, which is both undegradable in the rumen and indigestible in the intestine.

Results and Discussion

All the feed components were evaluated to determine the CP and DM in situ degradation constants. Parameters of degradation kinetics of DM and CP are shown in Table 3.4 and Table 3.5, respectively . Dry matter degradation constants range from 0.04 (blood meal) to 7.37 (corn). Feedstuffs high in readily fermentable carbohydrates (corn) and feeds high in degradable protein (soybean meal) have higher DM degradation, followed by those samples higher in fiber (soy hull, wheat silage, and whole cotton seed),

and the lowest values belong those feeds considered as RUP supplements (Blood meal, RUP mix, and PPM). Since fiber is not highly digestible it was expected that high fiber feeds would have lower degradation constants than concentrate feeds, like RUP supplements. It appear that most of protein content in high RUP feeds was slowly degradable even more so than fiber.

CP degradation constants (Table 3.5) range from 0.05 (blood meal) to 19.32 (whole cotton seed). Most of these constants compared closely to values listed in the dairy NRC (2001). Blood meal had a very low degradation rate, which indicates that it was practically undegraded in nylon bags; these results agree with Maiga (1996) who found a zero degradation rate for blood meal. It is suspected that during processing heat treatment may have denatured protein and contributed to the lack of degradation of BM. One value that did not seem to compare to NRC values was the CP degradation constant for wheat silage. The laboratory value for wheat silage CP k_d was determined to be 2.44%/h, as compared with the NRC value of 29.0 %/h. This discrepancy may result from the reported NRC value being more a function of the most degradable fraction. Since the soluble fraction (A) in wheat silage is high it is reasonable that the potentially degradable fraction (B) has a low degradation rate. However, it is also known that forages are highly variable usually associated with maturity at harvest and this difference may be due to associated variability.

Protein fractions (Table 3.5 and Figure 3.1) varied according to the type of feed. Wheat silage had the highest A fraction (78.38 %) and blood meal had the lowest (1.06 %). Soybean meal had the highest B fraction (82.31 %) and wheat silage the lowest (2.67 %). On the other hand, soybean meal had the lowest (0.66 %) C fraction and blood meal the

highest (79.31 %). It appears that RUP supplements have either evenly distributed CP fractions (i.e. RUP-blend and PPM) or a relatively small B fraction but a high C fraction (i.e. blood meal).

An NRC (2001) value for pet food grade poultry protein meal is not available. Our results indicate that its CP kd value is 2.45 %/h. This degradation rate indicates that poultry protein meal is more degradable than blood meal and fish meal, but less degradable than dry distiller grains.

Protein degradability values were estimated for all feedstuffs used in the ration Table 3.5 and Table 3.2. RUP values varied from 20.8% for wheat silage to 97.5% for blood meal. NRC (2001) values (Table 3.5) for the same feeds varied from 23.2% for wheat silage to 77.5% for blood meal. Poultry protein meal was estimated to contain 58.49% RUP, and compares well with other research that have found feed grade poultry protein meal to contain 55.2% (Bohnert et al, 1998).

Intestinal digestibilities (Table 3.6) for most of the feeds were within the ranges previously reported (Maiga et al, 1996). However, blood meal was above the higher end, since it appears that is 100% digestible. Reports have not been consistent with regard to blood meal. This variation could be attributed to processing methods, and the specie from which the raw material comes. Most of the reported results are based on bovine blood meal, while in our experiment the blood meal was from porcine origin. The values range between 74.91 % for whole cotton seed and 100 % for blood meal. In addition, PPM has a digestibility as high as the RUP blend, 85 and 86% respectively. Although there were differences in rumen disappearance, intestinal digestion was similar for both ruminally degraded protein sources and undegraded sources (Figure 3.3 and 3.4). In

general, it seems that intestinal digestion is very efficient, and the main difference in digestive utilization occurs primarily in the rumen digestion process.

Conclusions

The results of the present study indicate that RUP values can be reliably determined by using the in situ technique. Based on these results it appears that pet food grade poultry protein meal is a good substitute for other animal protein feeds. In addition, PPM like the RUP blend has protein that is evenly distributed across the different protein fractions as characterized by degradability.

This study also showed that intestinal digestibility of the by pass protein is generally high and independent of lower ruminal degradation. The study also showed that wheat silage (and probably most of the fermented forages) may have a lower degradation rate, especially if we consider that its A fraction is high. Also, it appears that blood meal, despite its high CP content, has a very variable response to in situ determinations.

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Table 3.1 Ingredient and nutrient composition of the diets[1]

	Diet	
Item	**In situ study**	**Digestibility study**
Ingredients	---------- % ----------	
Wheat silage	44.90	-
Grass hay	-	12.00
Alfalfa pellets	-	38.00
Whole cotton seed	12.50	-
Corn	24.50	47.05
Soybean meal	9.18	1.55
Soy hulls	3.00	-
Fish meal	2.27	-
Dry distillers grain	1.52	-
Vitamin - mineral premix	0.11	0.12
Limestone	1.22	0.70
Phosphate	0.37	-
Urea	0.27	0.45
Salt	0.16	0.13
Nutrient composition[2]	---------- % ----------	
Dry matter	49.28	88.98
Crude protein	17.55	15.07
NDF	33.88	29.09
ADF	21.68	18.63
Fat	6.00	3.35
Ash	7.23	5.96
NE_l (Mcal/kg)	1.74	1.51
Forage-concentrate ratio	45 F - 55 C	50 F - 50 C

[1] Dry matter basis
[2] Estimated values

Table 3.2 Feedstuffs composition[1]

Ingredient	DM	CP	NDF	ADF	Ash
	---------- % ----------				
Wheat silage	32.21	9.72	64.93	38.90	3.98
Corn	86.31	9.45	14.78	4.45	1.32
Soybean meal	88.05	54.22	11.02	5.46	6.76
Whole cotton seeds	89.55	20.71	54.90	39.84	3.28
Soy hulls	89.28	12.52	64.17	44.70	5.09
RUP mix[2]	91.27	49.19	32.80	7.45	14.02
Blood meal[3]	88.93	98.66	0.46	0.19	1.46
Poultry protein meal[4]	95.10	69.86	49.25	3.75	16.90

[1] Dry matter basis. DM = dry matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber.

[2] Blend of 60% fish meal and 40% dry distillers grains.

[3] Porcine blood meal.

[4] Pet food grade poultry protein meal (PPM).

Table 3.3 Dry matter degradation kinetics[1]

Feedstuffs	Laboratory values		
	DM	K_d	SEM
	%	%/h	
Wheat silage	32.21	4.68	0.004
Corn	86.31	7.37	0.006
Soybean meal	88.05	7.21	0.006
Whole cotton seed	89.55	4.67	0.005
Soy hull	89.28	4.99	0.005
RUP blend[2]	91.27	3.27	0.005
Blood meal	88.93	0.04	0.004
Poultry protein meal	95.10	3.26	0.006

[1] Kd = degradation constant calculated using the method proposed by Orskov and McDonald.

[2] Fish meal 60%, and Dry distillers grains 40%.

Table 3.4 Crude protein degradation kinetics: Comparison b/w laboratory values and NRC 2001[1]

Feedstuffs	Laboratory values			NRC 2001 values	
	CP %	K_d %/h	SEM	CP %	K_d %/h
Wheat silage	9.72	2.44	0.022	12.0	29.0
Corn	9.45	4.8	0.007	9.4	4.9
Soybean meal	54.22	5.41	0.005	53.8	7.5
Whole cotton seed	20.71	19.32	0.002	23.5	15.7
Soy hull	12.52	4.45	0.009	13.9	6.2
RUP blend[2]	49.19	1.69	0.006	-	-
Fish meal	-	-	-	68.5	1.4
Dry distillers grains	-	-	-	29.7	3.6
Blood meal	98.66	0.05	-	95.5	1.9
Poultry protein meal[3]	69.86	2.45	0.007	1.40	55.3

[1] Kd = degradation constant calculated using the method proposed by Orskov and McDonald (1979).

[2] Fish meal 60%, and Dry distillers grains 40%.

3 NRC 2001 does not have values for poultry protein meal. The values shown in the table were taken from Bohnert et al, 1998.

Table 3.5 Degradability of crude protein[1]

Feedstuffs	CP	Fraction			k_d	k_p	RUP	RDP
		A	B	C				
Laboratory values						3.5 X Maintenance		
Wheat silage	9.72	78.38	2.67	18.95	2.44	5.24	20.77	79.23
Corn	9.45	31.68	63.86	4.46	4.80	6.70	41.66	58.34
Soybean meal	54.22	17.03	82.31	0.66	5.41	6.70	46.19	53.81
Whole cotton seed	20.71	37.32	54.62	8.05	19.32	6.70	22.11	77.89
Soy hulls	12.52	42.70	43.71	13.59	4.45	6.70	39.85	60.15
RUP blend[2]	49.19	29.92	32.88	37.20	1.69	6.70	63.46	36.54
Blood meal	98.66	1.06	19.63	79.31	0.53	6.70	97.49	2.51
Poultry protein meal	69.86	31.99	35.54	32.46	2.45	6.70	58.49	41.51
NRC 2001 values						4 X Maintenance		
Wheat silage	12.00	69.50	8.70	21.80	29.0	-	23.20	76.80
Corn	9.40	72.50	3.60	4.90	4.90	-	47.30	52.70
Soybean meal	53.80	15.00	84.40	0.60	7.50	-	42.60	57.40
Whole cotton seed	23.50	45.40	46.70	7.90	15.70	-	22.90	77.10
Soy hulls	13.90	22.50	72.20	5.30	6.20	-	44.60	55.40
Fish meal	68.50	22.80	72.00	5.20	1.40	-	65.80	34.20
Dry distillers grains	29.70	28.50	63.30	8.20	3.60	-	50.80	49.20
Blood meal	95.50	10.10	60.90	29.00	1.90	-	77.50	22.50
Poultry protein meal[3]	-	28.80	-	-	-	-	55.20	44.80

[1] Kd = degradation constant calculated using the method proposed by Orskov and McDonald; kp = passage rate; RUP = Ruminally undegraded protein; RDP = Ruminally degraded protein.
[2] Fish meal 60%, and Dry distillers grains 40%.
[3] NRC 2001 does not have values for poultry protein meal. The values shown in the table were taken from Bohnert et al, 1998.
[4] Value of reporting data to 0.01.

Table 3.6 Crude protein, RUP, and estimated intestinal digestibility[1]

Feedstuffs	CP	RUP	I.D.	SEM[2]
	---------- % ----------			
Wheat silage	9.72	4.32	84.56	5.48
Soybean meal	54.22	89.21	99.88	0.17
Whole cotton seed	20.71	6.82	74.91	1.59
RUP blend[3]	49.19	56.78	85.81	0.61
Blood meal	98.66	99.10	100.00	-
Poultry protein meal	69.86	70.42	85.37	0.36

[1] CP = crude protein as a % of DM; RUP = as a % of CP; ID = intestinal digestibility.

[2] Intestinal digestibility standard error of the mean.

[3] Fish meal 60%, Dry distillers grains 40%.

[4] Value of reporting data to 0.01.

Figure 3.1 Values for crude protein fractions, lab values from table 3.8

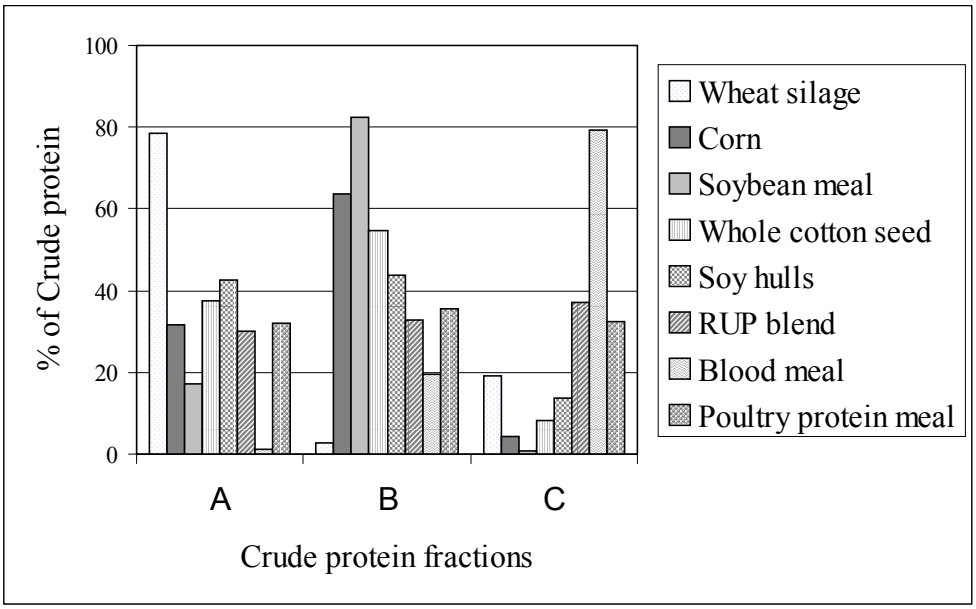


Figure 3.2 Values for RUP and RDP, laboratory values Table 3.8

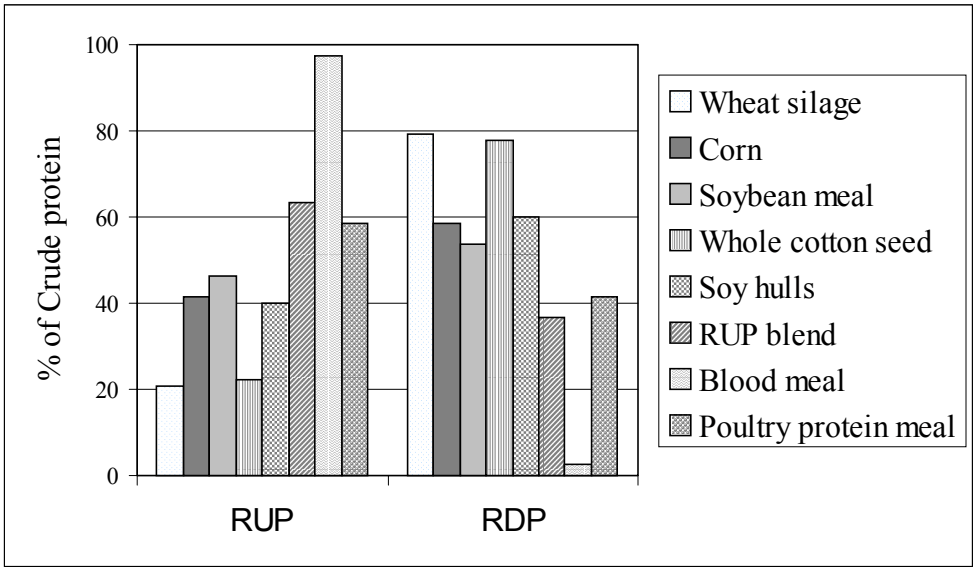


Figure 3.3 Crude protein concentration before and after incubation in rumen

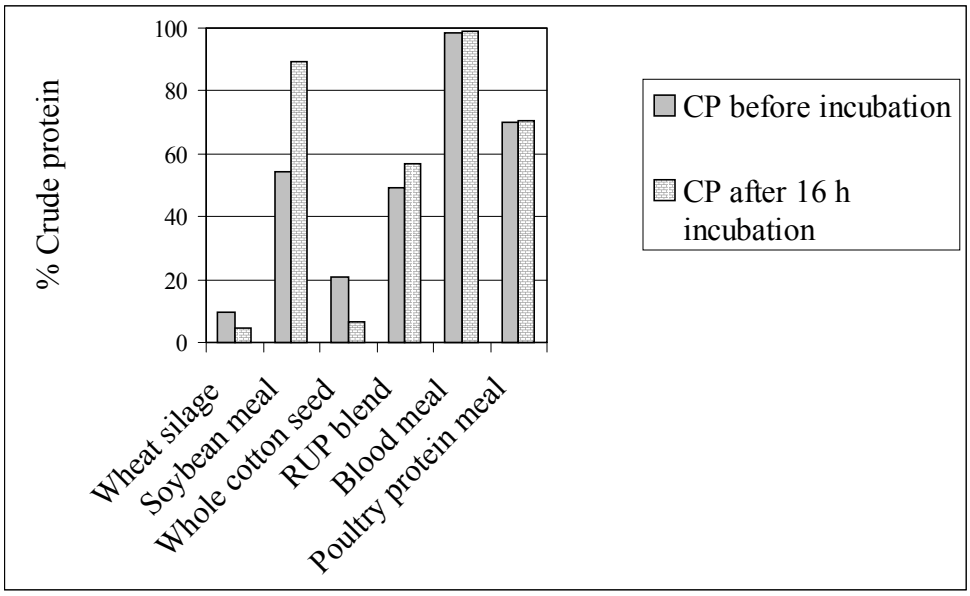
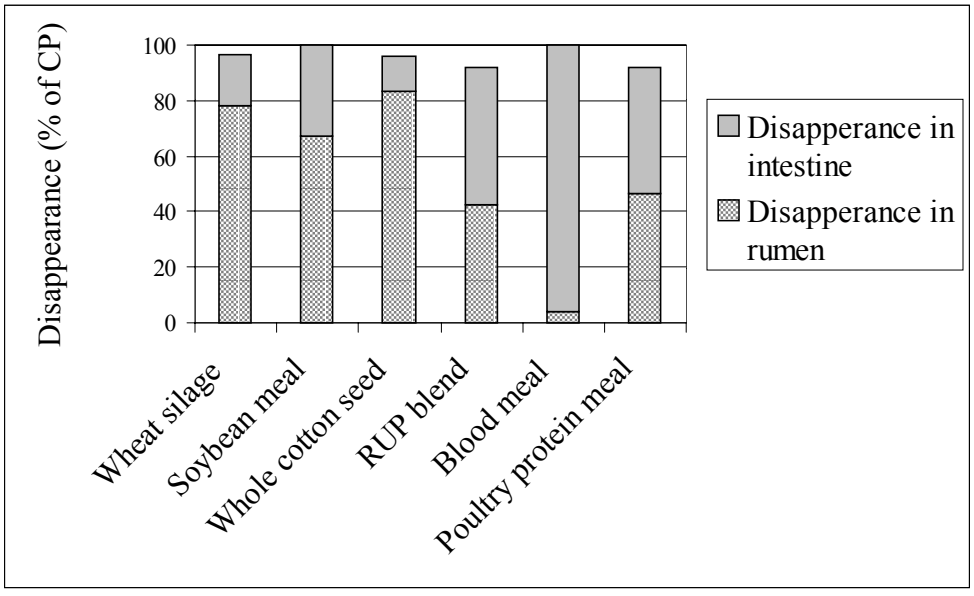


Figure 3.4 Disappearance of CP in the rumen and intestine measured by the mobile nylon bag technic



CHAPTER 4

EVALUATION OF PET FOOD GRADE POULTRY PROTEIN MEAL AS A PROTEIN SUPPLEMENT FOR LACTATING DAIRY COWS FED HIGH FAT AND FIBER RATIONS[1]

[1]Canseco, M.A., M.A. Froetschel, H.E. Amos, and J.K. Bernard to be submitted to Journal of Dairy Science.

Abstract

Twenty-four high producing Holstein lactating dairy cows were used in a randomized complete block design to determine the utilization of pet food grade poultry protein meal as a protein supplement. All cows were fed diets relatively high in fat and high fiber to accentuate the need for ruminally undegraded protein **(RUP)**. Cows in early lactation ranging between 30 and 120 DIM, were blocked into six groups based on their level of milk production. One cow from each block was assigned to each treatment group. The basal diet contained approximately 55% wheat silage and 6.5% fat. Treatments varied due to protein supplementation and were described as: 1) positive control-soybean meal (SBM), plus a ruminally undegraded protein (RUP) blend of fish meal (FM), blood meal (BM), and dry distillers grains (DDG); 2) negative control-only SBM; 3) 50% PPM-50% pet food grade poultry protein meal (PPM) substituted for the RUP blend in treatment 1; and 4)100% PPM-100% substitution PPM for RUP blend in treatment 1. Cattle were fed behind Calan gates a common diet for two weeks and treatment diets for 12 weeks. Intake (DMI) was lowest with cows fed the positive control diet and fat corrected milk production was highest in cows fed the 100% PPM diet. Fat corrected milk, milk fat, fiber digestibility and body weight loss were all lower in cattle fed the negative control diet. Cows received more digestible energy per unit of diet with RUP supplement. Efficiency of net energy usage did not differ among treatments. Back fat change detected from ultrasonography was greater in cattle fed the negative control diet. Insulin and blood urea nitrogen (BUN) were influenced by treatment especially during the first 4 weeks. This results indicate that PPM is an economic alternative for more expensive animal based sources of RUP.

Introduction

Dairy nutritionists in the southeast have a greater challenge in meeting nutritional requirements of high producing lactating dairy cows because intake is often compromised by of heat stress and forage quality. Heat stress reduces feed intake as animals compensate reduced heat load associated with energy expenditure due to heat dissipation and heat increment (Grummer, 1992). Whereas, low quality forages reduce intake due to ruminal distention as related to particle size reduction and passage; in addition, high fiber forages can contribute to higher heat increment that further limit intake. This situation is even more critical in early lactation cows, since intake is already depressed subjecting the cow to the risk for undernutrition and further negative energy balance.

One method to overcome these problems is to feed high levels of supplemental fat, which has approximately two times the energy value of carbohydrates and increases the energy density of the diet (Coppock and Wilks, 1991). However, until recent years limited amounts of fat could be practically fed to ruminants. Feeding natural sources of fat was restricted to 5% fat in the ration in order to prevent inhibition of rumen cellulolytic bacteria, fiber digestion, and caloric intake (Dairy NRC, 1989). In the last 20 years sources of fat have been chemically modified to be inert in the rumen. These fats are Ca soaps of fatty acids, and can be used to increase levels of fat from 5 to 10% without decreasing performance of lactating dairy cows (Doreau et al, 1991). Even with these new fat sources, high levels of fat feeding have not consistently improved lactating dairy cow performance (Dairy NRC, 2001). Although fat increases energy density of the diets and may alleviate problems associated with energy undernutrition, it acerbates the protein shortage caused by decreased intake and limited substrate for microbial growth.

Thus, protein supplementation becomes more important in feeding high fat rations to lactating dairy cow. Furthermore, RUP is the type of protein supplementation that becomes greater in requirement with high fat diets.

Protein supplementation of dairy cows cannot be investigated properly unless fat, fiber, and protein are considered simultaneously. Fiber is required to maintain a proper digestive function. Fat affects fiber digestion and, when it replaces fermentable carbohydrate, lowers microbial protein synthesis. Abomasal protein infusions have shown increased milk yield (Orskov et al, 1977); however, it works better when fermentable carbohydrates or supplemental fat are also supplied (Maiga and Schingoethe, 1997).

In addition, when the proportion of dietary protein as ruminally undegraded protein is increased, the quality of the protein becomes increasingly more important (Palmquist, 1993). Therefore, evaluation and selection of proteins from various sources in necessary to ensure a better protein supplementation.

Under the assumption that high fat and high fiber rations should even more accentuate the need for RUP, a basal ration, as such, was formulated with the objective to test the utilization of pet food poultry protein meal (PPM) in lactating dairy cows as a protein supplement compared to other protein sources.

Materials and Methods

Twenty-four multiparous high producing lactating Holstein dairy cows, at the UGA-Athens Dairy Center, were used in a completely randomized design to evaluate the utilization of pet food grade poultry protein meal as a ruminally undegraded protein supplement. Cows were handled and managed under guidelines approved by the UGA

animal care and use commitee. The basal ration was formulated to be relatively high in fat and high in fiber to accentuate the cow's requirement for RUP. Cows in early lactation ranging between 35 and 126 days in milk, were allocated into six groups according to their days in milk and level of milk production using data collected during the two weeks prior to the beginning of the experiment, with all cows receiving the same ration. Within groups, cows were randomly allocated to one of the four treatments. Rations were based on all cows being offered a basal diet containing approximately 55% wheat silage and 45% concentrate (Table 4.1). Diets were formulated to supply approximately 1.1 X the energy requirement. The intention of this formulation was to make protein rather than energy the main treatment variable. Dietary treatments varied due to protein supplementation and were: 1) ruminally undegraded protein (RUP) blend as a positive control (Pos-control), which consisted of fish meal (FM), dry distillers grains (DDG), and blood meal (BM); 2) no RUP supplementation as a negative control (Neg-control), all supplemental CP from SBM ; 3) substitution of 50% pet food grade poultry protein meal (PPM) for the RUP blend in treatment 1 (PPM-50%); and 4) PPM as only RUP supplement in treatment 1 (PPM-100%) (Table 4.1).

The experiment consisted of a 2-wk covariance period, during which all cows were offered the same diet, followed by a 12-wk treatment period when cows were offered one of the four treatment diets.

Feeding and management

Cattle were group housed in an open sided free stall barn, and individually fed using Calan gates (American Calan, Inc., Northwood, NH) allowing individual feed intakes to be determined. Concrete walkways were flushed twice daily and free stalls

were bedded with saw dust. Cows were milked twice daily at approximately 4:00 and 16:00 h. Throughout the experiment, cows were fed treatment diets as a TMR twice daily at 8:00 and 16:00 h, allowing for 10% feed refusal. During the last two days of wk 0, 4, 8 and 12, cows were weighed on 2 consecutive d, after the am milking, for determination of BW.

Sampling and laboratory analysis

Feed intake was measured for each cow daily during the entire experiment, and averaged by week for statistical analysis. Samples of diets, silage, whole cotton seed, and concentrate were collected 3 times per week (~ once every other day), frozen and composited into one weekly sample per treatment. Samples were dried to constant weight in forced air oven at 55°C for 72 h to estimate DM (farm dry matter). These dried samples were air equilibrated and stored for subsequent nutrient analysis. Concentration of all nutrients except for DM were expressed as a percentage of the DM (lab dry matter) determined by drying at 105°C in a forced air oven. Feed samples were assayed for nutritional composition (Table 4.2) including CP (Leco FP 528 N analyzer, Leco Inc. St. Joseph, MI), NDF and ADF (Ankom200, Fairport, NY), total fat (acidified ether extract, AOAC, 1990), ash and DM (AOAC, 1990).

Milk yield was recorded at each milking for each cow, and averaged weekly for statistical analysis. Milk samples were taken the last week of each 4-wk experimental period. Individual milk samples from each cow were collected twice and frozen daily, during 3 d per sampling week. Samples were thawed, composited by cow and subjected to milk compositional analyses: CP (Leco FP 528 N analyzer), fat (Babcock method, .AOAC 1990), and total solids (lyophilized) (Labconco Kansas City, MO).

Apparent digestibility

During wk 8 through 9, 25 g Cr_2O_3 was included in the daily diet as an external digestibility marker. On d 1 to 4 of wk 9, fecal grab samples were collected twice daily every 12 h, advancing sampling times 3 h between each d to represent a 24 h interval. Individual fecal samples were dried separately in an forced air oven at 55°C for 72 h and composited by cow for digestibility measurements. Feed samples were taken every day during the digestibility study, dried and composited by treatment. Each composite of fecal and feed samples were analyzed for chromium. Apparent digestibilities of DM, CP, NDF, ADF, and OM were calculated by the marker ratio technique using the following equation (Merchen, 1993):

$$AD = 100 - [100*(Md/Mf)*(Nf/Nd)],$$

where Md (%) is the concentration of the marker in the diet, Mf (%) is the concentration of the marker in the feces, Nf (%) is the concentration of the nutrient in the feces, and Nd (%) is the concentration of the nutrient in the diet.

Gross energy (GE) of diets and feces were obtained using a bomb calorimeter (Parr Instrument company, Ltd. Moline, IL). Digestible energy (DE_p) was calculated as a difference between GE intake and fecal GE excreted. All net energy values pertaining to conversion of dietary energy to milk, maintenance, and BW gain were estimated from NRC 2001 methods. The equations used were:

$$ME_p \text{ (Macl/kg)} = [1.01 * DE_p - 0.45] + 0.0046 * (EE - 3)$$

$$NE_{Lp} \text{ (Mcal/kg)} = 0.703 * ME_p - 0.19 + \{[(0.097 * ME_p + 0.19)/97] * (EE - 3)\}$$

Blood samples

Blood was sampled 3 h before and after feeding on the last day of each 4-wk period. Cattle were held in a holding pen for 3 h, then stanchioned and blood was sampled from coccygeal vessels in the tail immediately prior to feeding. Three h after initiation of feeding the bleeding procedure was repeated. Blood was prepared as plasma, and frozen. Samples were analyzed for insulin (ImmuChemTM125I RIA kit., ICN Pharmaceutical., Inc., costa Mesa, CA), glucose (Sigma Diagnostics kit, procedure number 315, St. Louis, MO), and urea nitrogen (Sigma Diagnostics kit, procedure number 640, St. Louis, MO Costa Mesa).

Body condition score and ultrasound measurements

Body condition score (BCS) and ultrasound measurements were obtained at the last d of period 1 and the last d of period 3, both on the same d. Utilizing the technique developed by Edmonson et al (1989), cows were assigned an BCS as estimated by two trained individuals.

An ultrasound machine with a 5-MHz linear array transducer was used to determine the amount of subcutaneous fat at two different locations, rib eye area and rump area. A trained individual operated the ultrasound machine for all cattle and measurements were obtained without clipping the hair coat. Ultrasound measurements were collected using an Aloka 500-V ultrasound unit (Corometrics Medical Systems, Wallingford, CT) witha 17.2-cm, 3.5-MHz linear probe and interpreted using Beef Information Manager^{TM} software, version 3.0 (Critical Vision, Inc., Atlanta, GA).

Statistical analysis

Data with replication in time (milk yield, DMI, plasma metabolites, and milk components) were analyzed using the mixed model procedure (PROC MIXED) of SAS for a completely randomized design (SAS, 2000). The statistical model was.

$$Y_{ijkl} = F + T_i + P_j + C_{k(j)} + TP_{ij} + E_{ijkl}$$

Where, Y_{ijk} = dependent variable; F = overall mean; T_i = effect of treatment i (i = 1 to 4); P_j = effect of period (j = 1 to 3); $C_{k(j)}$ = effect of cow k within period j (k = 1 to 24); TP_{ij} = interaction between treatment i and period j; and E_{ijkl} = Subplot error. All terms were considered fixed except $C_{k(j)}$ and E_{ijkl}, which were considered random. Least square means for treatments are reported. Milk yield and dry matter intake, average values from the two-week covariance period were used as covariates and included in the model ($b⇳PM_{ijk}$ = Pre-milk covariate effect).

Variables that did not have repeated measures (apparent digestibility, energy balance, body composition, and body condition score) were analyzed using the general linear model procedure (PROC GLM) of SAS for a completely randomized design (SAS, 2000). The statistical model was.

$$Y_{ij} = F + T_i + E_{ij}$$

Where, Y_{ij} = dependent variable; F = overall mean; T_i = effect of treatment i (i = 1 to 4); E_{ij} = residual error.

Treatment effects were compared with orthogonal comparisons to first demonstrate that there was a difference between the negative control and all the other diets. Also Pos-control was compared with the PPM containing treatments, and the third comparison was between PPM-50% an PPM-100%.

One cow in the Pos-control and another in the PPM-50% treatments had to be removed from the experiment because they were not properly trained to use only their gates. Another cow for Neg-control had also to be removed from the experiment due to health problems unrelated to treatment. All results from these cows were treated as missing values in the statistical analyses.

Results and Discussion

The objective of this experiment was to test a blend of various protein supplements against PPM with a basal ration that would accentuate the need for RUP. Experimental diets (Table 4.1) contained more fat and fiber than typically fed to cows at this level of production. Diets were formulated to contain approximately 6.5% total fat mainly from supplemental Megalac® and whole cotton seed. Laboratory analysis of experimental diets reported in Table 4.2 show that the estimated values of CP closely relate with compositional analyses of the TMR. However, NDF values were 4 to 5% higher than estimated values. Ration NDF varied from 46.5 to 48.2%. Also fat was higher than estimated values. The forage to concentrate ratio ranged from 46 to 51%.

The intent of making this comparison was to prove that these cows required more RUP than that supplied by negative control. The second comparison was made to contrast the positive control against the diets that contained PPM. This comparison should demonstrate the suitability of substituting PPM for a FM, BM, DDG RUP blend. The third comparison contrasted the two PPM containing diets.

Dry matter intake (DMI)

Dry matter intake and intake as a percentage of body weight are reported in Table 4.3. There was a main treatment effect on DMI ($P<0.05$). Dry matter intake of cows fed

the negative control, containing primarily soybean meal as a protein supplement, was 16.4% higher than the positive control that contained a RUP blend of animal and plant protein supplement. Orthogonal comparisons showed there was no difference in intake response between cows fed the negative control as compared to the RUP supplemented diets. However, comparing rUP-supplemented diets, there was a difference ($P<0.01$) in DMI between positive control and PPM supplemented diets. Cows fed diets containing PPM consumed 13.8% more DM than cows fed the positive control.

The negative control ration contained approximately 5% less forage, 1 to 2% less NDF, and 2.5 to 3.0% less forage NDF. It is well recognized that fiber will depress intake of lactating dairy cattle fed diets formulated to meet both their energy and DM intake requirements (Mertens, 1991; Nichols et al, 1996). Nichols et al (1996) found that an approximately 7 percentage unit change in forage NDF resulted in a 12% decrease in DM intake of lactating dairy cattle fed tropical corn and sorghum silage based diets.

The difference in intake of DM between cows fed the positive and negative control diets was of such a magnitude (16.4%) that it appears protein supplementation rather than fiber was more responsible for this effect. In addition, the PPM-100% diet had higher NDF content than the negative control treatment but no difference was observed in DM intake between cattle fed these two treatments. The influence of RUP on DM intake was only negative for the positive control diet.

It appears that the RUP provided in the positive control diet had a distinct effect on intake as compared to that provided in the 50 and 100% PPM diets. The feed components used to provide RUP in the positive control diet may be responsible for this effect and include fish meal, dried distillers grains, and blood meal.

Milk production and milk composition

Milk production, 4% fat corrected milk (FCM), and milk composition (milk fat, milk CP, and milk total solids) were not statistically affected by the main effect of treatment ($P<0.05$). However, there was a statistical trend ($P<0.09$) for cows fed RUP supplements to produce more FCM than those fed the negative control diet. Cows fed RUP supplements produced 9.55% more FCM than those fed the negative control. Although there were no significant differences in milk composition among treatments, the percentage of CP was low for all treatments. Similar milk composition results were obtained when high fat diets were fed to dairy cows (West and Hill, 1990; Schaufff et al, 1992; Maiga and Schingoethe, 1997). The rationale for dietary fat depressing milk protein content is a matter of debate. Wu and Huber (1994) indicated that milk protein depression associated with high fat diets might be due to insufficient essential AA to meet requirements for increased milk production. Schingoethe (1996) suggested that higher RUP diets may be needed with high fat rations since fat replaces fermentable carbohydrates and may lower microbial protein synthesis. However, in this experiment, high RUP diets did not increase milk CP percentage. On the contrary, they increased yield but decreased the percentage of milk protein even more than diets low in RUP. It is possible that RDP and rumen ammonia limited microbial protein synthesis in cattle fed high RUP diets, as suggested by Rodriguez et al (1997). Cant et al (1993) provided an alternative theory that may have some credibility based on our results. He suggested that high fat diets lower mammary blood flow and thereby inhibit AA uptake. This author indicated that a slightly decreasing arterial amino acid concentration along with

increasing in energy supply to mammary tissue are responsible for reduction of mammary blood flow rates.

Treatment by period interactions ($P<0.05$) were observed for both fat corrected milk and milk fat. Both parameters were influenced to a greater effect by treatment during the first period of the feeding trial. During this period FCM was 12.28% higher for RUP supplemented diets ($P<0.05$). This response (Figure 4.1) corresponds with the time the cows had highest levels of production and highest requirements for protein and energy. However, this difference disappears afterwards as related to decreasing milk yield and with stage of lactation, likely due to a decreased need for RUP supplementation. Differences in milk fat percentage were most apparent in the first two periods of the feeding trial ($P<0.05$), as shown in Figure 4.2. Also in period 1, RUP supplemented diets increased 12.85% milk fat percentage compared with the negative control. In period 2, PPM-100% resulted in the lowest milk fat (2.52%), a 21.7% decrease as compared to period 1. It is difficult to explain these effects that are not consistent with results from periods 1 and 3 this, where this treatment resulted in the highest values. Although dietary protein is considered to have minor influence on percentage milk fat, it appears that milk fat can be influenced by protein supplementation (Jaquette et al, 1988; Sutton et al, 1989).

Apparent digestibility

Dry matter intake (DMI) and digestibility results from week 8-9 of the lactation trial are reported in Table 4.4. Digestibility of DM, OM, NDF ($P<0.05$), and fat ($P<0.01$) were greater in cows fed the RUP supplemented diet as compared to those fed the negative control diet. Pos-control and PPM-100% had the highest digestibilities

followed by PPM-50% and Neg-control. Digestibilities of DM, OM, and NDF were 5.55%, 6.12%, and 12.3% ($p<0.05$) greater in cows fed RUP supplemented diets as compared to those fed the negative control diet (Figure 4.3). Also a trend was observed for the PPM-100% diet ($p<0.1$) to be more digestible in OM, NDF, and ADF as compared to the PPM-50% diet. Fat digestibility was the highest in the PPM-100% (71.57 %) followed by Pos-control (66.56 %), PPM-50% (54.99 %), and Neg-control (50.87 %). RUP supplemented diets had 26.5 % higher digestibility as compared those fed negative control diets. A variety of digestibility responses have been reported when high fat diets were fed to lactating dairy cows. In an experiment where cows were fed different levels of RUP with 4% fat, Volden et al (1999) observed increased apparent digestibilities of OM, NDF, and ADF and attributed these results to reduced feed intake. This results agree with our results for Pos-control, which was associated with lowered intake and increased digestibility of DM, NDF, ADF, OM, and CP as compared with the diet low in RUP (Neg-control). On the other hand, the PPM-100% diet had higher RUP than Neg-control and lower than Pos-control, but the intake is higher and the digestibility is equal to Pos-control. Orthogonal comparisons between PPM-100% and PPM-50% showed there is a trend ($p<0.1$) for PPM-100% to have higher digestibilities. Research that had evaluated the use of calcium salts of fatty acids (West and Hill, 1990) reported that this type of fat did not alter digestibility of DM, CP, ADF, NDF or fat. However, these researchers reported 76.96 % total fatty acid digestibility, which is higher than our values. On the other hand, results of including fat and also RUP (Nianogo et al, 1991; Christensen et al, 1994; Goodling and Grummer, 1998) observed that DM, OM, and CP digestibilities were increased. These data support our results since digestibilities in RUP

supplemented diet were found to be higher. Although it appears that RUP increases digestibility, the mechanisms by which RUP affects digestibility are not clear. It is possible that less degradable proteins provide N substrate for bacterial growth and activity at a time that cellulose digestion is occurring.

Energy balance and energy efficiency

Energy content of the experimental rations and parameters used to estimate energy utilization are reported in Table 4.5. Energy intake (Mcal/d) was not statistically different for any of the treatments. Gross energy intake and digestible energy intake were similar among treatments. However, when digestible energy (DE) was expressed as Mcal/kg of diet (Figure 4.4), RUP supplemented diets had 6.13% more energy ($P<0.05$). This effect was maintained even when energy was expressed as metabolizable energy (ME) and net energy (NE). Previous research (Christensen et al, 1994) demonstrated that rumen protected amino acids increased the DE concentration of diets low in protein (14.2% CP) but reduced DE in diets high in protein (17.5% CP); however, the RUP values of the experimental diets were not reported.

In our research, the difference found in DE and NE values due to RUP supplementation probably reside in the higher digestibility of the diets supplemented with RUP, as it was shown earlier. Higher DM, NDF, and OM digestibilities suggest that there might be a more efficient fiber and fat digestion and consequently higher release of energy yielding products. Having fed high fiber diets the rate of passage was probably slower, as fiber generally increases ruminal retention time of particulate matter. Another reason could be a decrease of the intestinal rate of passage due to the influence of fat; however, fat was high in all treatments and therefore its effect was for all diets. In

addition, high RUP diets have higher proportion of the B protein fraction, which is the potentially degradable protein if sufficient time is provided. By having a higher proportion of fraction B, RUP supplemented diets may have had a sustained release of N that was more synchronous with fiber digestion and consequently increased energy value of the diet.

The heat increment associated with RUP supplemented diets were estimated as 36.65% of ME and for Neg-control is 37.5%. This difference although small per kg of diet may need to be addressed for high producing and heat stressed dairy cows.

By pass protein supplementation tended to increase fat corrected milk production and increased body weight loss. Dairy cattle fed the Neg-control diet actually gained more weight. Similar results were observed by Nianogo et al (1991). This agrees with the results obtained by Orskov et al (1987) who postulated and demonstrated that it is possible to increase milk yield by stimulating fat mobilization through feeding RUP protein.

Body weight and body weight change

The average body weight (BW) was 589, 608.53, 614.9, and 601.32 kg for Pos-control, Neg-control, PPM-50%, and PPM-100% respectively (Table 4.6). All RUP supplemented dairy cows lost weight, but cattle fed the Pos-control diet lost the most weight. Body weight change has a trend for treatment effect ($P<0.09$). Furthermore, orthogonal comparisons showed that there was a treatment difference ($p<0.06$) between treatments with RUP as compared to the Neg-control. Cattle fed RUP treatments had a BW loss 3.7 times greater than those fed lower RUP treatment. As discussed earlier, it seems that cows fed RUP supplements lost body reserves to support increased milk

production. With this respect, Whitelaw et al (1986) concluded that body reserves mobilization is a secondary response to an increased amino acid supply. Body condition score ranged between 2.83 and 3.28 but it was not influenced in a statistical manner by treatment. Probably because of the subjective nature of this measurement as indicated by its SEM being relatively high (SEM = 0.869).

There was a treatment by period interaction ($p<0.05$) observed for BW change as shown in Figure 4.5. During the first period animals fed PPM-50% diet lost 33.48% more than the rest of the treatments. In period two the treatment one Pos-control lost 72.97% more that Neg-control and 9% more than PPM supplemented diets. During period three all cattle gained weight.

Body composition parameters

Effects of the diets on body composition are presented in Table 4.7. Cows fed experimental diets containing RUP supplements showed a change in back fat between period 1 and 3 compared to Neg-control. These results goes along with the BW change data described earlier. Back fat change was more negative for Pos-control, which once again indicates that body reserves were used for milk yield when RUP was fed. Furthermore, this denotes that body fat accounts for most of the energy lost/gain. Although not statistically different, back fat change for PPM-50% and PPM-100% were positive, indicating that cows in these treatments were gaining weight. In addition, at the end of the trial cows fed Neg-control and PPM-100% had higher marbling score, which indicated that cows fed lower RUP diets milk less, but gained body condition back faster.

Blood metabolites

Table 4.8 contains data on insulin, glucose and BUN from samples taken 3h before feeding and 3h after feeding. Concentration of insulin was not affected by diet . However, there is a treatment by period interaction for insulin 3h after feeding. This interaction is mainly due to differences during the first period, during the time cows had the highest milk production. PM insulin was 7.5% higher than AM insulin. Whitelaw et al (1986) suggested that insulin concentration increased with an increase of protein supply; however, in our data there was no clear pattern for insulin concentration before or after feeding to be influenced by protein supplementation. However, within certain periods and the insulin rise after feeding did appear to be related to RUP. There was a treatment by period interaction observed for insulin concentration after feeding (Figure 4.6). In period one insulin concentration was 60.8 to 109.2% higher in cows fed the 50% PPM diet as compared to the other rations. Insulin is generally negatively correlated with milk production in lactating cows and this results may help to explain the relatively lower performance of cows fed PPM-50% diet. In addition, there was a treatment by period interaction with the rise in insulin after AM feeding (Figure 4.7). In period one insulin rise was 2 to 4 fold higher in lactating cows fed Pos-control or the PPM-100% diet. In period two insulin rise was approximately 4 fold higher in cows fed PPM-100% as compared to those fed other treatments. It appear that although insulin concentration may be negatively correlated to performance the rise in insulin after feeding may positively related to performance.

Glucose was inversely related to insulin, in other words, the higher the insulin the lower the glucose. Glucose levels were fairly constant which may be due to gluconeogenesis occurring at a rate adequate for glucose utilization for milk synthesis.

Blood urea nitrogen concentration before feeding tended to be ($P<0.13$) higher for cows fed the low RUP diet as compared to those fed higher RUP. However, there were no other main effects of treatment for BUN. However, there are treatment by period interactions which seems to be related to decreased milk production between periods. There were treatment by period interaction observed for serum BUN before and after feeding, and rise associated with feeding. In period one, BUN levels before feeding were approximately 20% lower in cattle fed the PPM-50% and PPM-100% diets as compared to those fed Pos-control diet (Figure 4.8). In period two, BUN levels before feeding cows fed PPM-100% was 6.4% lower than BUN levels of cows fed the other diets. BUN levels after feeding (Figure 4.9) in period one were 16.7% lower in cattle fed PPM-100% as compared to those fed other treatments. There were no differences in BUN levels in periods 2 and 3. BUN rise after feeding (Figure 4.10) in period one was lowest for lactating cows fed PPM-100% while the BUN rise for PPM-50% had the highest, about 7 fold compared to PPM-100%, 5.5 fold compared with Pos-control, and 2.2 fold compared with Neg-control.

Conclusions

Overall, there was a general trend for improved performance of lactating dairy cattle fed the RUP supplemented diets versus the cattle fed the negative control diet. However, this effect cannot be just explained due to differences in the supply of protein.

Intake (DMI) was lowest with the positive control diet and highest with the negative control diet. Fat corrected milk production was higher with cattle fed the higher RUP diets and especially those fed PPM-100% diet. Fat corrected milk, milk fat, fiber digestibility and body weight loss were all lower in cattle fed the negative control. Back fat change detected from ultrasonography indicates that cattle fed RUP diets mobilized more fat to support lactation. Insulin and blood urea nitrogen (BUN) were influenced by treatment especially during the first 4 weeks of the trial.

The most interesting response of practical value is the increase in DE concentrations of diets supplemented with RUP sources. This corresponded with an increase in diet NE content. This effect relates to higher fat, fiber and OM digestibilities that are likely responsible for the increased DE. The PPM-50% did not improve performance as compared to the Pos-control or PPM-100%. It is possible that this combination of RUP supplements did not result in an essential amino acid complementary effect.

These results indicate that PPM is a very good alternative for more expensive animal based sources of RUP. The current prices of these feeds are as follows: PPM (pet food grade) $300.00 (personal communication, Kerry Courchaine), PPM (feed grade) cost $195/ton compared with soybean meal $233/ton or FM $560/ton (Feedstuffs July 22, 2002; Atlanta price). Pet food grade PPM generally costs $75-$100 above feed grade PPM (personal communication, Kerry Courchaine). Thus, PPM is not only an alternative RUP supplement, it is economical and recognized as a safe product for ruminants considering that so far there are no restrictions for feeding PPM to ruminants.

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Table 4.1 Ingredient composition of experimental diets[1]

	Treatment[2]			
Ingredients	**Pos-control**	**Neg-control**	**PPM-50%**	**PPM-100%**
	----------%----------			
Wheat silage[3]	54.98	49.26	55.14	55.29
Whole cotton seed	8.69	10.02	8.72	8.74
Soy hulls	6.52	7.52	6.54	6.56
Corn	15.21	15.04	15.26	15.30
Cane molasses	2.17	2.51	2.18	2.19
Soybean meal	4.35	11.52	4.36	4.37
Megalac®	2.17	2.51	2.18	2.19
RUP blend[4]	2.61	0.00	1.31	0.00
Blood meal	1.74	0.00	0.87	0.00
Poultry protein meal	0.00	0.00	2.18	4.37
Dicalcium phosphate	0.17	0.23	0.02	0.00
Magnesium oxide	0.12	0.08	0.13	0.14
Limestone	0.45	0.52	0.30	0.05
Mineral premix[5]	0.05	0.05	0.05	0.05
Vitamin premix[6]	0.05	0.05	0.05	0.05
Zinpro®[7]	0.17	0.17	0.17	0.17
Salt	0.54	0.54	0.54	0.55

[1]Ingredient concentration are expressed on a DM basis.
[2]Pos-Control = RUP mix plus blood meal as rumen undegradeable protein sources;
Neg-Control = Soybean meal is main protein source; PPM-50% = replace 50% of pos-control with poultry protein meal; PPM-100% = replace 100% of pos-control with poultry protein meal.
[3]Wheat silage was harvested at an early head stage of maturity from two separate fields, and approximately 100 tn were stored in a 2.42 by 30 m plastic bag. Wheat silage was analyzed to contain 40.38% DM, 9.73% CP, 58.89% NDF, 33.42% ADF, and 4.26 ash.
[4]Blend of 60% fish meal and 40% dry distiller grains.
[5]Mineral premix contained Ca (min) 10.35%, Cu 5%, Fe 5%, Mn 12%, Zn 12%, Co 600 ppm, I 2500 ppm, and Se 600 ppm.
[6]Vitamin premix contained vitamin A 8000000 IU, vitamin D3 1000000 IU, and vitamin E 15000 IU.
[7]Zinc methionine.

Table 4.2 Nutrient composition of experimental diets

Nutrient	Treatment			
	Pos-Control	Neg-Control	PPM-50%	PPM-100%
Estimated values[1]	---------- % ----------			
CP	15.28	15.99	15.21	15.14
RUP	38.88	34.19	37.33	35.77
RDP	60.99	65.81	62.55	64.23
NDF	43.12	41.25	43.22	43.31
aNDF[2]	39.43	37.31	39.66	39.88
FNDF[3]	32.47	29.01	32.55	32.56
Starch	18.87	18.19	18.91	18.94
Calcium	0.71	0.70	0.71	0.71
Phosphorus	0.38	0.38	0.38	0.40
Magnesium	0.23	0.23	0.23	0.23
Fat	6.45	6.58	6.55	6.67
NE_l (Mcal/kg)	1.75	1.78	1.75	1.74
Forage	55.14	49.37	55.28	55.42
Concentrate	44.86	50.63	44.72	44.58
Laboratory values[4]	---------- % ----------			
DM	52.77	54.60	53.30	53.66
CP	14.89	15.69	15.76	14.92
NDF	47.36	46.49	47.32	48.21
FNDF[3]	32.10	28.96	31.48	31.92
ADF	28.34	27.75	28.02	27.55
Ash	5.36	5.48	5.64	5.22
Fat	8.15	8.44	8.00	8.50
Forage[5]	51.00	46.00	50.00	51.00
Concentrate	49.00	54.00	50.00	49.00

[1]Wheat silage and poultry protein meal values for DM, CP, and NDF were based on laboratory analysis, NE_l value was calculated based on TDN, which was based on NDF.
TDN = 105.2 - (0.68*NDF), Ag. Serv. Lab. UGA; NE_l = (0.0245*TDN) - 0.12, Dairy NRC 1989;
Other feedstuffs values were obtained from 2001 Dairy NRC.
[2]aNDF = Adjusted NDF for concentrates. For any ground high fiber feed with NDF less than 40% assign the value of 12% aNDF or its own NDF if it is less than 12%. The aNDF of any ground, high fiber feedstuff with more than 40% NDF is calculated as 0.30 X %NDF (Mertens, 1992).
[3]FNDF = Dietary NDF supplied by forage only. Laboratory values based on NDF = 62.95%.
[4]The University of Georgia, Nutrition Lab.
[5]Determined by daily (am and pm) observation of the TMR's during one week. TMR samples were collected to obtain dry matter values and express the forage and concentrate ratio on a dry matter basis.

Table 4.3 Dry matter intake, milk yield and composition of milk from lactating dairy cattle fed high fat and fiber diets with different types of protein supplementation.[1]

Item[3]	Treatment				SEM[2]	Orthogonal Contrasts (Pr>F)		
	Pos-Control	Neg-Control	50%-PPM	100%-PPM		Contrast 1 2 vs others	Contrast 2 1 vs 3 and 4	Contrast 3 3 vs 4
DMI, kg/d	19.71[a]	22.95[b]	21.69[ab]	23.17[b]	0.79	0.13	0.01	0.20
BW, kg	579.07	598.09	607.31	585.06	24.99	0.80	0.59	0.53
DMI, % BW	3.41	3.94	3.60	3.98	0.21	0.26	0.16	0.19
Milk Yield, kg/d	35.91	34.1	35.61	36.35	1.05	0.14	0.96	0.63
4% FCM, kg/d	30.91	28.91	31.21	31.38	1.07	0.09	0.78	0.91
Milk Fat, %	3.11	3.07	3.19	3.14	0.11	0.57	0.71	0.78
Milk Fat, kg/d	1.11	1.03	1.13	1.13	0.05	0.12	0.74	0.96
Milk CP, %	2.69	2.82	2.70	2.54	0.12	0.22	0.64	0.37
Milk CP, kg/d	0.98	0.94	0.96	0.89	0.06	0.99	0.50	0.37
Milk Solids, %	12.18	12.27	12.03	11.76	0.22	0.28	0.30	0.38

[1]All values are least squares means estimated from approximately 6 cows per treatment over a twelve week period.
[2]Standard errors of the mean.
[3]Abbreviations: DMI = dry matter intake, BW = body weight, FCM = 4% fat corrected milk, CP = crude protein.
[4]Superscripts (a,b) are used to separate individual treatment means that were significantly different (P<.05).

Table 4.4 Apparent digestibility of ration nutrient components fed to lactating dairy cattle receiving high fat and fiber diets with different sources of protein supplements.[1]

Item[4]	Treatment				SEM[2]	Orthogonal Contrasts (Pr>F)		
	Pos-Control	Neg-Control	50%-PPM	100%-PPM		Contrast 1 2 vs others	Contrast 2 1 vs 3 and 4	Contrast 3 3 vs 4
DMI[3], kg/d	19.84	21.28	20.59	22.71	1.08	0.86	0.19	0.17
DM DIG,%	63.34[a]	59.05[b]	60.51[ab]	63.15[a]	1.23	0.04	0.34	0.14
OM DIG, %	65.18[a]	60.66[b]	62.51[ab]	65.42[a]	1.19	0.02	0.42	0.10
NDF DIG, %	53.52[a]	46.39[b]	48.84[ab]	53.89[a]	2.09	0.03	0.41	0.10
ADF DIG,%	54.53	48.64	46.72	54.29	3.13	0.39	0.31	0.10
CP DIG, %	63.19[a]	60.70[ab]	59.71[b]	62.74[ab]	1.14	0.39	0.18	0.07
FAT, DIG %	66.56[a]	50.87[b]	54.99[b]	71.57[a]	2.79	0.001	0.35	0.001

[1]All values are least squares means estimated from approximately 6 cows per treatment. Composited samples of feed and feces collected during week 9 of the feeding trial, along with chromic oxide as an external marker was used for these estimations.
[2]Standard errors of the mean.
[3]DMI = dry matter intake during digestibility study.
[4]Abbreviations: DIG = digestibility, DM = dry matter, OM = organic matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, CP = crude protein.
[5]Superscripts (a,b) were used to separate individual treatment means that were significantly different (P<.05).

Table 4.5 Parameters used to estimate energy balance and energy efficiency of lactating dairy cattle fed high-fat and high-fiber diets with different sources of protein supplementation.[1]

Item[2]	Treatment				SEM[3]	Orthogonal Contrasts (Pr>F)		
	Pos-Control	Neg-Control	50%-PPM	100%-PPM		Contrast 1 2 vs others	Contrast 2 1 vs 3 and 4	Contrast 3 3 vs 4
GE, Mcal/kg	4.63	4.60	4.62	4.63	-	-	-	-
GE intake, Mcal	91.80	97.90	95.09	98.41	3.23	0.46	0.23	0.48
DE,Mcal/kg	2.95[a]	2.72[b]	2.82[ab]	2.89[ab]	0.07	0.05	0.26	0.52
DE,intake, Mcal	58.96	57.87	58.19	61.12	2.01	0.55	0.66	0.32
DE/kg $BW^{0.75}$, Mcal	0.531	0.505	0.497	0.532	0.03	0.62	0.61	0.36
ME_P, Mcal/kg	2.55[a]	2.32[b]	2.42[ab]	2.48[ab]	0.07	0.05	0.26	0.51
NE_{lp}, Mcal/kg	1.62[a]	1.45[b]	1.53[ab]	1.57[ab]	0.05	0.05	0.27	0.51
NE_l/kg $BW^{0.75}$	0.291	0.269	0.269	0.289	0.01	0.44	0.52	0.34
Prod. Level, X NE_l maint.	3.63	3.37	3.36	3.61	0.18	0.44	0.52	0.34
NE_{lp} intake, Mcal	32.06	30.89	31.45	33.24	1.2	0.34	0.85	0.31
NE_l gain/loss, Mcal	-1.58[a]	0.43[b]	-0.78[ab]	-0.61[ab]	0.52	0.03	0.18	0.82
NE_l Total, Mcal	33.64	30.46	32.22	33.84	1.46	0.12	0.74	0.44
NE_l maint., Mcal	8.84	9.28	9.39	9.21	0.32	0.72	0.25	0.69
NE_l production, Mcal	24.79	21.17	22.83	24.63	1.54	0.12	0.58	0.42
NE_l from milk, Mcal	22.91	20.32	21.95	21.74	1.23	0.21	0.49	0.91
NE_l from milk+maint., Mcal	31.76	30.22	31.49	31.09	1.06	0.33	0.72	0.79
Energy Balance %	94.98	98.38	99.6	92.22	5.17	0.73	0.95	0.39
Gross Efficiency, %	68.54	65.82	69.53	64.54	4.65	0.69	0.75	0.52
Net Efficiency, %	93.62	97.35	100.2	89.54	7.17	0.83	0.95	0.38

[1]All values are least squares means estimated from 6 cows per treatment during week 9 of the lactation trial.
[2]Abbreviations: GE = gross energy, DE = digestible energy, ME_P = Metabolizable energy for production, NE_{LP} = Net energy for lactation at production level, Energy balance = energy in milk + maintenance divided by energy intake, Gross Efficiency = energy in the milk divided by energy intake, Net Efficiency = energy in milk divided by energy intake.
[3]Standard error of the mean.
[4]Unique superscripts (a,b) were used to separate individual treatment means that were significantly different (P<.05).

Table 4.6 Body weight (BW), BW change and body condition score (BCS) of lactating dairy cattle fed high-fat and high-fiber diets with different sources of protein supplementation[1].

Item[3]	Treatment				SEM[2]	Orthogonal Contrasts (Pr>F)		
	Pos-Control	Neg-Control	50%-PPM	100%-PPM		Contrast 1 2 vs others	Contrast 2 1 vs 3 and 4	Contrast 3 3 vs 4
Body weight, kg	589.00	608.53	614.90	601.32	24.83	0.82	0.55	0.70
BW change, kg	-19.32[a]	3.81[b]	-8.48[ab]	-3.10[ab]	5.97	0.06	0.08	0.52
BCS (5)	2.83	2.85	3.275	2.94	0.87	0.53	0.31	0.28

[1]All values are least square means estimated from 6 cows/treatment.
[2]Standard error of the mean.
[3]BW = body weight, BCS = body condition score.
[4]Unique superscripts (a,b) indicate that individual means are statistically significant (P<.05).

Table 4.7 Body composition parameters measured using ultrasound in lactating dairy cattle fed high-fat and high-fiber with different protein supplements[1].

Item[3]	Treatment				SEM[2]	Orthogonal Contrasts (Pr>F)		
	Pos-Control	Neg-Control	50%-PPM	100%-PPM		Contrast 1 2 vs others	Contrast 2 1 vs 3 and 4	Contrast 3 3 vs 4
Body weight PI, kg	598.91	591.61	619.14	602.87	25.33	0.59	0.71	0.66
Rib eye area PI, cm^2	50.58	53.74	54.06	56.65	4.13	0.99	0.37	0.67
RBA CWT PI, %	0.59	0.67	0.61	0.62	0.05	0.30	0.73	0.92
Back fat PI, cm	0.21	0.23	0.30	0.19	0.07	0.93	0.69	0.31
Rump fat PI, cm	0.25	0.36	0.45	0.21	0.12	0.69	0.61	0.17
Beef QOM PI, %	4.95	5.59	6.28	4.59	0.46	0.57	0.40	0.02
Body weight PII, kg	579.59	610.43	610.43	599.77	24.83	0.64	0.41	0.76
Rib eye area PII, cm^2	55.87	58.84	58.39	60.77	3.70	0.91	0.43	0.65
RBA CWT PII, %	0.68	0.68	0.67	0.72	0.04	0.84	0.71	0.43
Back fat PII, cm	0.18	0.36	0.33	0.23	0.10	0.32	0.45	0.45
Rump fat PII, cm	0.25	0.51	0.48	0.25	0.17	0.39	0.59	0.34
Beef QOM PII, %	5.78^{a}	6.85^{b}	7.33^{b}	6.44^{ab}	0.33	0.40	0.01	0.07
BW change, kg	-19.32^{a}	3.81^{b}	-8.70^{ab}	-3.10^{ab}	5.97	0.06	0.08	0.52
REA change, cm^2	5.29	3.87	4.26	4.13	1.99	0.76	0.66	0.95
RBA CWT chg, %	0.08	-0.01	0.06	0.10	0.05	0.13	0.92	0.60
Back fat change, cm	-0.03^{a}	0.11^{a}	0.03^{ab}	0.03^{ab}	0.04	0.05	0.30	0.95
Rump fat change, cm	0.01	0.12	0.02	0.05	0.06	0.25	0.61	0.93
Beef QOM change, %	0.83	2.31	-0.41	1.86	1.12	0.25	0.94	0.16

[1]Values are least squares means from approximately 6 cows per treatment.
[2]Standard error of the mean.
[3]BW = body weight, REA = rib eye area, Beef QOM = intramuscular fat.
[4]Unique superscripts indicate differences between treatment means (P<.05).

Table 4.8 Plasma metabolites of lactating cows fed high-fat and high-fiber diets with different protein supplements[1].

Item [3]	Treatment				SEM[2]	Orthogonal Contrasts (Pr>F)		
	Pos-Control	Neg-Control	50%-PPM	100%-PPM		Contrast 1 2 vs others	Contrast 2 1 vs 3 and 4	Contrast 3 3 vs 4
Insulin am, FIU/ml	6.73	5.95	6.21	6.38	0.41	0.32	0.4	0.77
Insulin pm, FIU/ml	8.05	9.19	10.22	8.4	0.91	0.78	0.27	0.16
Insulin rise, FIU/ml	1.32	3.24	4.02	2.03	0.97	0.50	0.17	0.16
Glucose am, mg/dl	65.3	62.46	65.55	65.43	1.85	0.19	0.94	0.96
Glucose pm, mg/dl	56.47	55.1	56.02	57.96	2.15	0.51	0.85	0.52
Glucose rise, mg/dl	-8.83	-7.36	-9.53	-7.47	1.77	0.55	0.88	0.41
BUN am, %	22.77	23.41	22.27	21.94	0.58	0.13	0.36	0.68
BUN pm, %	25.19	25.99	25.87	24.73	1.16	0.60	0.94	0.49
BUN rise, %	2.41	2.58	3.6	2.79	1.06	0.78	0.56	0.59

[1]All values are least square means from 6 cows per treatment. Cows were bled from the jugular vein immediately before and approximately 3h after the morning feeding.
[2]Standard error of the mean.
[3] FIU/ml = micro international units per milligram, BUN = blood urea nitrogen.

Figure 4.1 Fat corrected (4%) milk production (kg/d) of lactating dairy cattle fed high-fat and high-fiber diets with different protein supplements

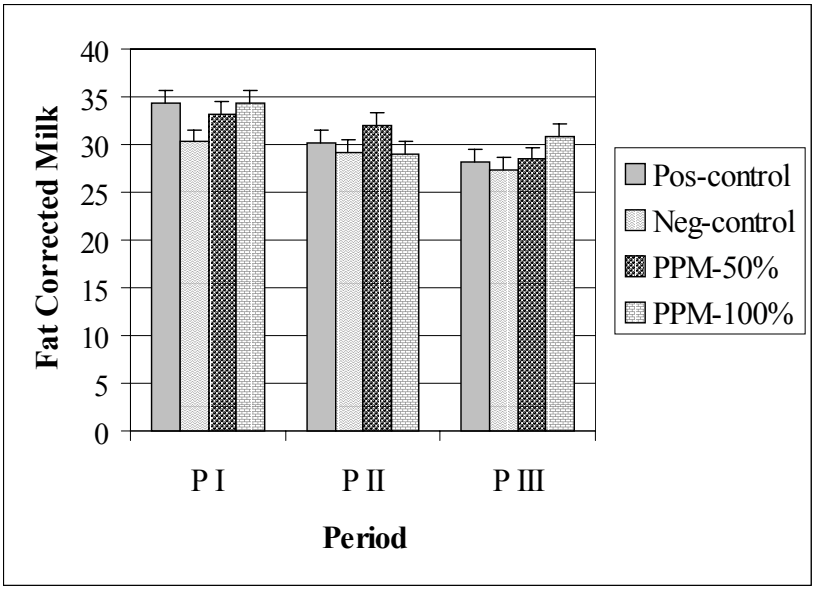


Figure 4.2 Fat percentage of milk from lactating dairy cattle fed high-fat diets and high-fiber diets with different protein supplements

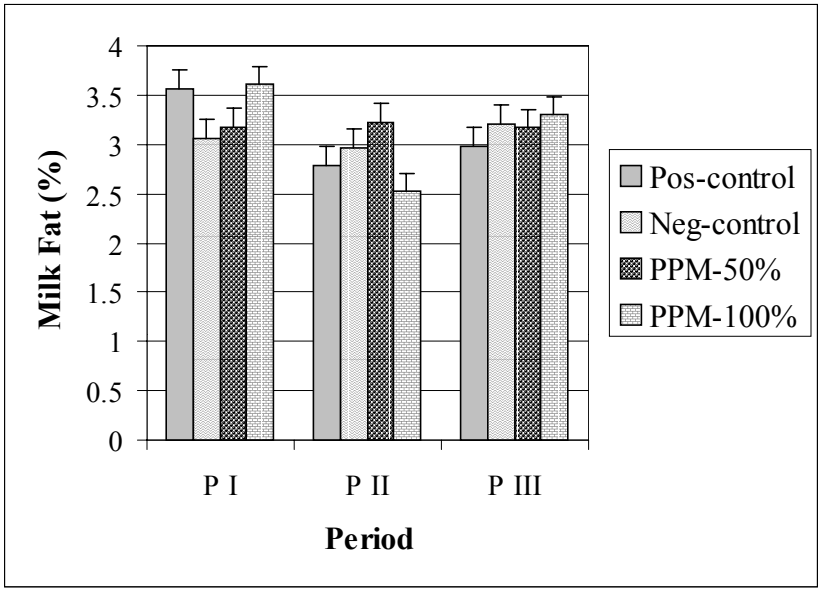


Figure 4.3 Apparent digestibility of lactating dairy cattle fed high fat and high fiber diets with different protein supplements

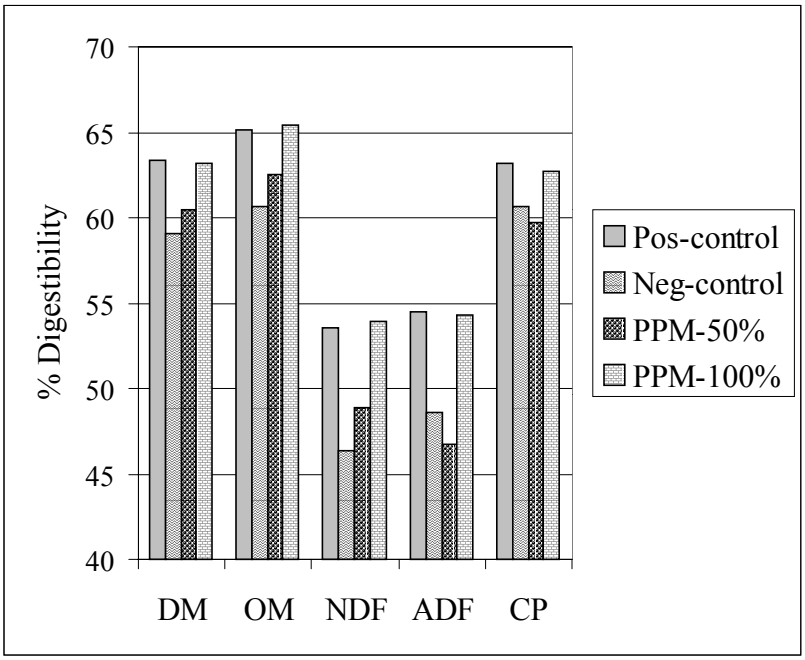


Figure 4.4 Gross energy, digestible energy, metabolizable energy, and net energy of high fat and high fiber diets with different type of supplements

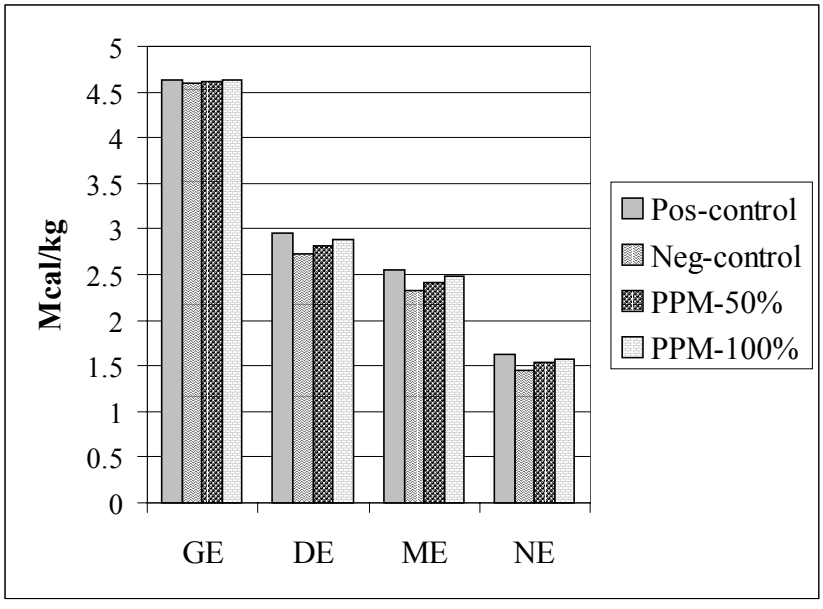


Figure 4.5 Changes in body weight of lactating dairy cattle fed high-fat and high-fiber diets with different protein supplements

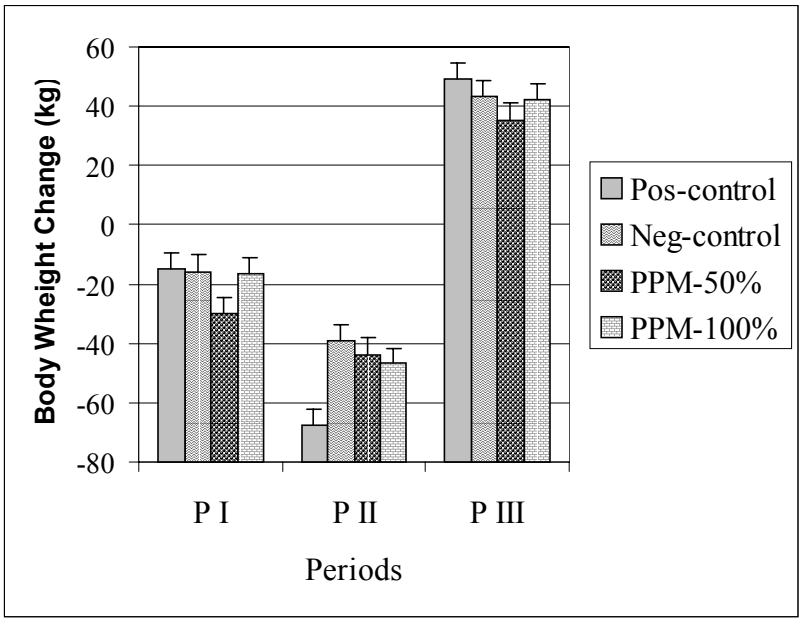


Figure 4.6

Circulating insulin concentrations after feeding in serum of lactating dairy cattle fed high-fat diets and high-fiber diets with different protein supplements

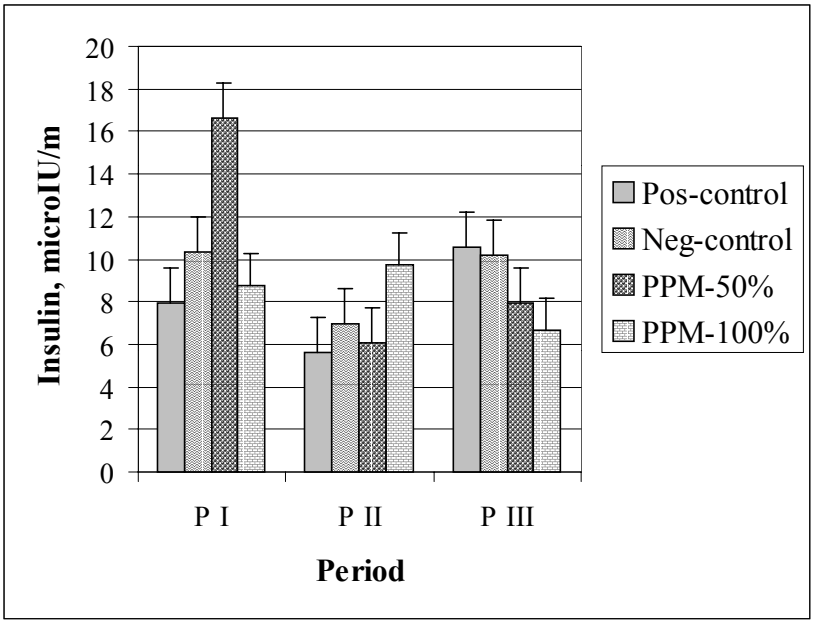


Figure 4.7 Circulating insulin concentrations rise after feeding in serum of lactating dairy cattle fed high-fat diets and high-fiber diets with different protein supplements

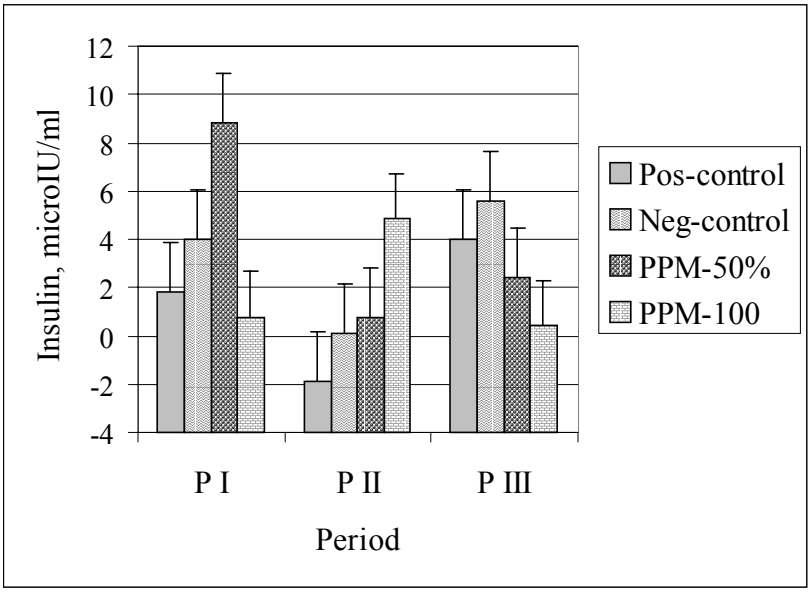


Figure 4.8 Circulating blood urea nitrogen concentration before feeding in serum of lactating dairy cattle high-fat and high-fiber diets with different protein supplements

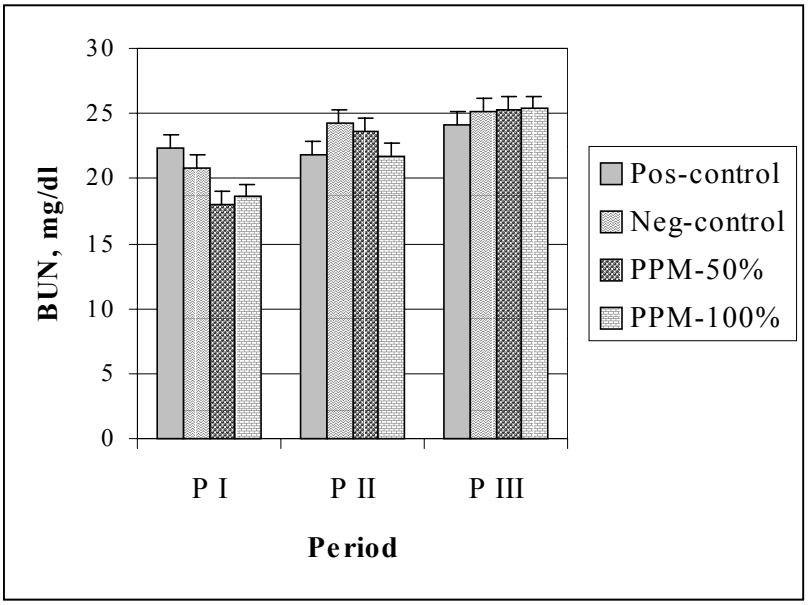


Figure 4.9 Circulating blood urea nitrogen concentration after feeding in serum of lactating dairy cattle high-fat and high-fiber diets with different protein supplements

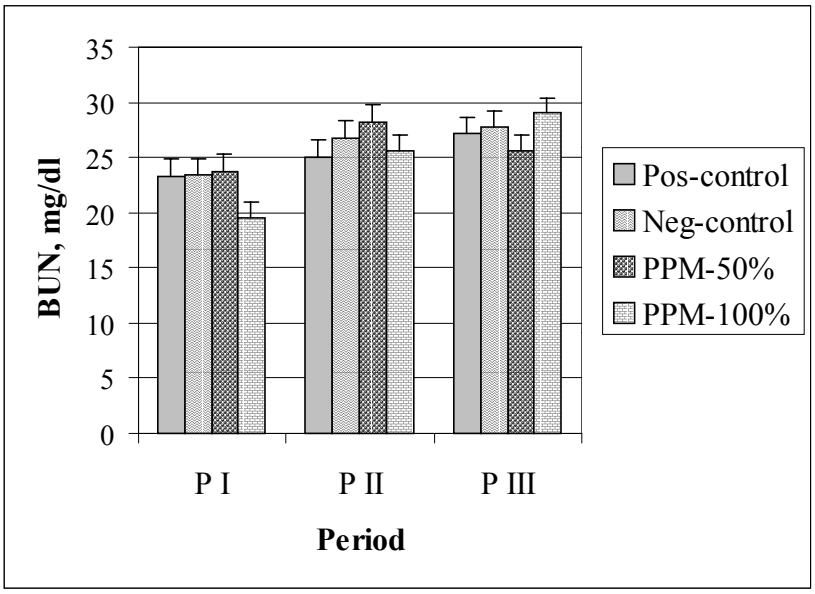
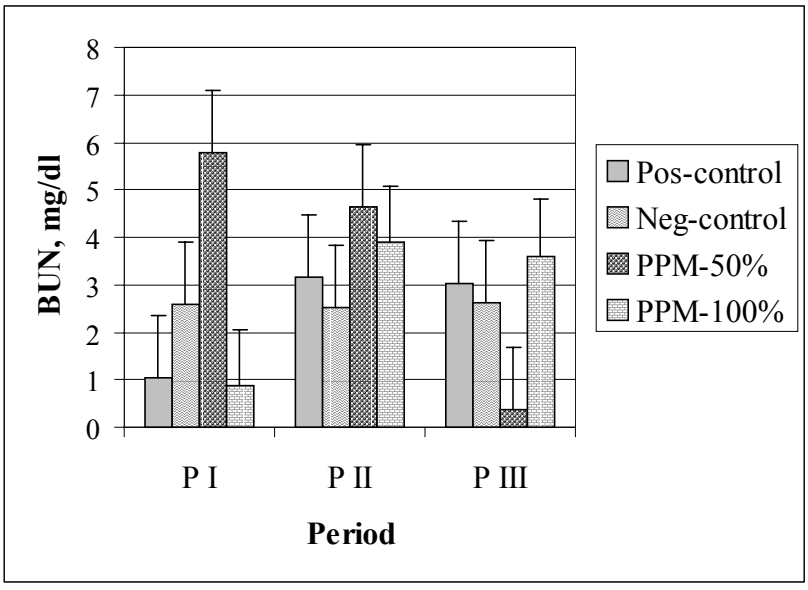


Figure 4.10 Circulating blood urea nitrogen concentration rise after feeding in serum of lactating dairy cattle high-fat and high-fiber diets with different protein supplements



CHAPTER 5

CONCLUSIONS

The response to protein supplementation in dairy cows fed high fat and high fiber diets was study doing two experiments. In the first experiment the ruminal protein degradation and intestinal digestibility of certain feedstuffs were evaluated using the in situ and mobile technique. Based on these results it appears that pet food grade poultry protein meal, due to its high RUP value, is a good substitute for other animal protein feeds. In addition, PPM like the RUP-blend has protein that is evenly distributed across the different protein fractions as characterized by degradability. This study also showed that intestinal digestibility of the by pass protein is generally high and independent of lower ruminal degradation.

The second experiment different feedstuffs were evaluated as protein supplements for lactating dairy cows fed high fat and high fiber rations. Animals fed the RUP supplemented diets had a trend for improved performance. However, this effect cannot be just explained due to differences in the supply of protein. Intake (DMI) was lowest with the positive control diet and fat corrected milk production was higher in the 100% PPM diet. Fat corrected milk, milk fat, fiber digestibility and body weight loss were all lower in cattle fed lower RUP diet. Back fat change detected from ultrasonography indicates that cattle fed the RUP diets mobilized more fat to support lactation. Insulin and blood urea nitrogen (BUN) were influenced by treatment especially during the first 4

weeks. The most interesting response of practical value is the increase in DE concentrations of diets supplemented with RUP sources. This corresponded with an increase in diet NE content. Effect that is complemented with higher fiber and OM digestibilities that appears to be the main responsible for the increased DE. The PPM-50% did not improve performance as compared to the Pos-control or PPM-100%. It is possible that this combination of RUP supplements did not result in an essential amino acid complementary effect.

This results indicate that PPM is a very good alternative for more expensive animal based sources of RUP. PPM is not only an alternative RUP supplement, it is also economical and recognized safe product for ruminants considering that so far there are no restrictions for feeding PPM to ruminants.

APPENDICES

Appendix 1. Recommended procedures and reporting details for standard ruminal in situ trials for determining ruminal protein degradability in dairy cattle[1]

Diet	
Type	Total mixed ration, similar to that of desired application. Report ingredient and chemical composition (minimum of).
Feeding level	Ad libitum.
Feeding frequency	2 times/day.
Evaluated feedstuffs	
Chemical composition	Report (minimum) DM, CP, NDF, and ash. These values are the initial content (no soaking, ruminal incubation or washing). The disappearance of N from the bags are expressed as a percentage of the initial N in the feedstuffs.
Physical characteristics	Report specifics about processing of feedstuffs.
Sample preparation	
Grinding	Forages: Freeze forages samples with dry ice (3:1 forage: dry ice ratio), and grind through a 6 mm screen. Concentrate: grind to 2-mm screen size. If ground, describe particle size (sieve).
Sample size: surface area	10 mg/cm^2 (acceptable range 10 to 15 mg/cm^2).
Sample size	4 grams (+/- 0.2) DM basis. Weigh feed as fed basis, but weight is adjusted according to DM content.
Labeling	Label bags with a acetone resistant marker and weigh after drying at 55 °C for 24 hours.
Blanks	Empty sealed in situ bags incubated for 24 hours and use to correct residue weights for microbial and feed contamination.
Sealing	Polyester bags are sealed with an electric impulse heat sealer (twice).
Bag	
Material	Polyester bags.

Pore size	Recommended range of 40 to 60 m.
Incubation procedure	
Number of animals	2; report BW.
Number of days	2 minimum.
Number of replications	2
Presoaking	In 39 $\pm$ 3 °C tab water for 20 minutes, 1 liter/250 mg of feed nitrogen.
Ruminal position	Ventral rumen.
Insertion/removal	Sequential entry (in reverse sequence), at specific time interval, followed by removal and rinsing as a group.
Incubation times, h	0, 2, 4, 8, 16, 24, and 48 (include 72 for forages).
Rinsing	In order to stop bacterial degradation, immediately after removal from the rumen, bags should be soaked in ice water.
	If machine available: five 1-minute cold water rinses (delicate setting), and 2-minute spin, at low water setting (about 45 liters).
	If manual: Rinse in tab water until rinse water is clear, about 90 seconds/bag with moderate manipulation.
Drying	Drying to a constant weight at 55 °C.
	Freeze-drying, for amino acid analysis samples.
Microbial correction	A rapid procedure for purine measurement.
Mathematic model	Non-linear.

[1] Adapted and modified from Armentano et al, 1997; Bohnert et al, 1998; Broderick and Cochran, 2000; Coblentz et al, 1997; Crooker et al, 1978; Dairy NRC, 2001; Madsen et al, 1995; Nocek, 1988; Vanzant et al, 1998.

Appendix 2. SAS program obtain crude protein degradation constant (k_d), non linear approach.

```
title1 'IN SITU CRUDE PROTEIN DEGRADATION';
title2 'Nonlinear Model ORSKOV and MCDONALD 1979';
title3 'Kd: Crude Protein Degradation Constant';
proc sort;
by feedstuffs;
proc nlin method=dud noitprint;
parameters a=0 to 100 by 10 b=0 to 100 by 10 k=0 to 1 by 0.01;
model cpd=a+b*(1-exp(-k*time));
by feedstuffs;
output out=preds predicted=pcpd;
proc plot;
plot cpd*time = '+' pcpd*time = '*';
by feedstuffs;
run;
```

Appendix 3 Recommended standard mobile bag technique procedure for determining intestinal digestibility of rumen undegraded protein[1]

Diet	
Type	Total mixed ration, similar to that of desired application. Report ingredient and chemical composition.
Feeding level	Feed requirements of test animals, according to their ADG.
Feeding frequency	2 tiimes/day (ad libitum).
Evaluated feedstuff	
Chemical composition	Report (minimum) DM, CP, NDF, and ash.
Physical characteristics	Report specifics about processing of feedstuffs.
Samples	Residues of rumen undegraded material after 16 and 24 h in the rumen for concentrate and roughage, respectively.
Sample preparation	
Drying	Freeze-drying of samples.
Grinding	1-mm screen size
Sample size: surface area	Concentrates: 10 mg/cm^2 (acceptable range 10 to 15 mg/cm^2). Roughage: 5 mg/cm^2 (acceptable range 5 to 7 mg/cm^2).
Bag	
Material	Polyester.
Pore size	50 μ, ideally 11 μ
Dimensions	5 x 6 cm
Preincubation	
Step 1	Bags containing Ruminally undegraded feed residues will preincubated in 0.004 M HCl solution at pH = 2.4 for 1 h.
Step 2	In a pepsin/HCl solution (100mg pepsin per liter of 0.004 M HCl solution, pH = 2.4) for 2 h at 40°C in a shaking water bath.
In vivo procedure	
Number of animals	2; report BW.
Number of days	2
Number of replications	at least 2 per steer.

Insertion/recovery	Preincubated samples are placed into the duodenum, and then recovered from the feces. The upper time limit for bag appearance will be 20 hours; bags that are not recovered within the next 20 hours after insertion should be discarded, and rerun. 6 to 12 samples per day, after feeding. 15 minutes between the insertion of individual bags to ensure unimpeded movement within the duodenum.
Rinsing	If machine available: five 1-minute cold water rinses (delicate setting), and 2-minute spin, at low water setting (about 45 liters). If manual: Rinse in tab water and subsequently wash in a sieve basket in cold running water for one hour.
Drying	Drying to a constant weight at 55 °C.

[1]Adapted and modified from Broderick and Cochran, 2000; Madsen, 1995; and NRC, 2001.

Appendix 4 Poultry Protein Meal: Feed grade

Definition

Poultry Protein Meal (PPM) consist of the ground, rendered, clean parts of the carcass of slaughtered poultry, such as necks, feet, undeveloped eggs, and intestines, exclusive of feathers, except in such amounts as might occur unavoidably in good processing practices. The label should include guarantees for minimum crude protein, minimum crude fat, maximum crude fiber, minimum phosphorus (P), and minimum and maximum calcium (Ca). The Ca level shall not exceed the actual level of P by more than 2.2 times (AAFFCO, 1988).

Poultry Protein Meal (PPM) is the product produced from the clean parts of the carcasses of slaughtered poultry. By wet or dry rendering and removal of most of its oil, a meal is produced for animal feeding (AAFFCO, 1983).

However, in common practice PPM often includes hatchery wastes, birds found dead on arrival at the processing plat, and perhaps dead breeders. Recently, the material called dissolved air flotation (DAF) sludge, a high fat product, has been incorporated into PPM by some companies as means of disposing it (Escalona, R.R. and Pesti, G.M., 1987).

Alternative Names

Poultry by-product meal.

Qualities: Feed grade and pet food grade.

Chemical Composition.

Table 1. Poultry by-product meal composition (a)

Sample number	Plant	Water	Gross energy	Crude protein	Ether extract	Ash	Calcium	Phosphorus	Curde fiber
		%	(kcal/kg)	%					
1	A	6.47	4863.26	62.94	13.04	14.14	4.39	2.49	1.32
2	A	6.76	4751.81	63.88	12.15	14.42	4.37	2.45	1.19
3	A	6.07	4874.72	63.56	12.56	14.40	4.14	2.54	1.25
4	B	5.28	4631.47	60.50	11.42	18.32	5.94	2.48	1.45
5	B	7.63	5004.00	62.25	14.75	11.91	3.60	2.04	1.50
6	B	3.03	5356.40	60.31	18.48	14.58	4.75	2.15	1.43
7	B	4.21	4489.39	54.00	13.38	24.88	8.84	2.17	1.67
8	C	4.09	4762.68	62.19	11.32	16.28	5.08	2.55	2.13

(a) Adapted from Pesti (1986) and others.

Table 2. Amino acid and protein composition of poultry by-product meal samples collected compared to National Research Council (NRC, 1984) table values (b)

	Sample No.									NRC
	1	2	3	4	5	6	7	8	Avg[1]	Table 25
	(%)									
Alanine	4.21	4.25	4.21	3.82	3.98	3.82	3.57	4.13	4.00 ± .09	
Aspartic acid	4.92	4.90	4.85	4.45	4.79	4.49	4.10	4.78	4.66 ± .10	
Arginine	4.42	4.43	4.37	3.96	4.37	3.93	3.53	4.26	4.16 ± .11	4.00
Glycine	6.92	6.89	6.89	5.89	6.51	5.91	5.55	6.71	6.41 ± .19	5.90
Serine	2.52	2.50	2.50	3.00	3.30	2.71	2.52	2.58	2.70 ± .10	3.68
Glutamic acid	7.98	7.96	7.95	7.06	7.65	7.09	6.48	7.76	7.49 ± .20	
Histidine	1.30	1.33	1.28	1.18	1.14	1.15	1.09	1.26	1.22 ± .03	1.50
Isoleucine	2.13	2.15	2.09	2.03	2.26	2.07	1.77	2.08	2.07 ± .05	2.00
Leucine	3.97	4.01	3.92	3.97	4.21	3.88	3.51	3.94	3.93 ± .07	3.70
Lysine	3.50	3.54	3.46	2.93	3.10	3.01	2.77	3.40	3.21 ± .10	2.70
Hydroxylysine	0.33	0.33	0.32	0.23	0.25	0.23	0.22	0.31	0.28 ± .02	
Methionine	1.21	1.23	1.15	0.99	1.07	1.08	0.94	1.25	1.12 ± .04	1.00
Cystine	0.69	0.71	0.68	1.14	1.31	0.95	0.86	0.78	0.85 ± .10	0.69
Phenylalanine	2.35	2.37	2.30	2.38	2.52	2.38	2.24	2.32	2.36 ± .03	2.10
Tytosine	1.95	1.96	1.92	1.82	2.01	1.91	1.65	1.90	1.89 ± .04	0.54
Proline	4.34	4.32	4.33	4.25	4.72	4.03	3.81	4.37	4.27 ± .09	
Hydroxyproline	2.81	2.96	2.89	2.09	2.37	2.18	2.08	2.73	2.51 ± .13	
Threonine	2.26	2.27	2.23	2.24	2.36	2.17	1.97	2.23	2.22 ± .04	2.00
Tryptophan	0.57	0.57	0.56	0.62	0.81	0.59	0.58	0.62	0.62 ± .03	0.53
Valine	2.66	2.71	2.64	2.79	3.06	2.74	2.43	2.65	2.71 ± .06	2.60
Taurine	0.39	0.38	0.37	0.30	0.38	0.31	0.21	0.40	0.34 ± .02	
Protein	62.94	63.88	63.56	60.50	62.25	60.31	54.00	62.19	61.2 ± 1.20	58.00

(b) Adapted from Escalona (1986) and others

[1] Mean ± SEM.

The highly variable nature of PPM, even from the same producer, may make nutrient compositions of batches of the same feed formulation quite different. Thus, it would be the best if the consumer could measure the crude protein and ash of each lot of PPM received.

Table 3. Tag guarantee vs. NRC 94

	SPPM[1]	PFPPM[1]	PBPM[2]
CP, %	62.37	65.10	60.00
ME, kcal/kg			2950.00
Fat, %	11.85	11.50	13.00
CF, %	>3	2.20	1.50
Ash, %		14.00	
Ca, %	4.47	4.80	3.00
P, %	2.40	2.30	1.70
Moisture, %	5.16	5.00	7.00
Lysine	3.51	3.68	3.10
Methionine	1.13	1.18	0.99
Cystine	0.76	0.65	0.98
Tryptophan	0.53	0.47	0.37
Histidine	1.37	1.25	1.07
Arginine	4.08	4.35	3.94
Aspartic ac.	4.93	4.89	
Threonine	2.57	2.34	2.17
Serine	2.57	2.66	2.71
Glutamic ac.	7.68	7.72	
Proline	4.03	4.52	
Glycine	5.75	7.31	6.17
Alanine	4.12	4.36	
Valine	2.92	2.51	2.87
Isoleucine	2.25	2.02	2.16
Leucine	4.39	4.02	3.99
Tyrosine		1.84	1.68
Phenylalanine	2.46	2.25	2.29

[1] American Proteins, Inc.

[2] National Research Council, 1994

Nutrient availability

Table 4. Protein and amino acid concentrations (g/kg) in the poultry by-product meal ('c)

Component	Total[1]	Digestible[2]
Protein	700.00	-
Threonine	25.40	20.10
Cystine	8.90	4.10
Valine	27.60	23.50
Methionine	12.70	11.30
Leucine	45.80	40.10
Isoleucine	22.40	19.50
Tyrosine	18.90	14.70
Phenylalanine	24.80	20.40
Histidine	13.90	11.30
Lysine	37.90	30.60
Arginine	46.00	39.50
tryptophan	6.00	4.90

(c) Adapted from Wang and Parson (1998)
[1]Protein and amino acid values are presented on en air-dry or as fed basis. The dry matter of the PBPM was 964 g/kg.
[2]Determined using the precision-fed caecectomised cockerel assay. Values are means of 4 cockerels.

Nutritive Value

PPM is a very good source of protein, rivaling meat meal, meat and bone meal, blood meal, and fish meal. The caloric value is moderately low, despite a high fat (ether extract) content. Ca, Fe, K, and Zn are in good supply, as well as Se. However, it has moderately deficient essential amino acids.

Considering the requirements of the meat-type chicken that has a very demanding growth requirement, the data in Table 4 shows that the most limiting amino acids are cystine and tryptophan.

Table 5. Limiting order of amino acids (AA) in poultry by-product meal determined by deletion and addition assays in broiler chickens (d)

Amino acid	Limiting order
Cystine	1
Tryptophan	2
Lysine	3
Threonine	4
Valine	5
Isoleucine	6
Histidine	7
Methionine	8

(d) Adapted from Wang and Parson (1998)

When considering for the laying hen, there are four deficient essential amino acids, namely, phenylalanine, methionine, isoleucine, and leucine. Total sulfur amino acids (met+cys) are also moderately deficient for the laying hen (Polin, D. 1990?).

Palatability: Poor

Effect of PBPM on animal performance

When PBPM is incorporated at 5% level into corn-soy meal-based practical diets, no differences in gain or feed efficiency can be detected in comparison with all-plant-based diets. However, when PBPM is included into a diet at the 10% level chick growth and feed efficiency are significantly depressed (Escalona, R. and Pesti, G. 1987).

Table 6. Composition of experimental diets (f)

Ingredients	Plant-based control	Poultry by-product meal sample				
		B[1]	B[1]	B[2]	B[2]	C[3]
				%		
Corn grain	***54.23***	***58.59***	***63.01***	***58.35***	***61.05***	***63.45***
Soybean meal	***37.17***	***29.96***	***22.67***	***30.85***	***24.77***	***22.60***
Poultry by-product meal	***---***	***5.00***	***10.00***	***5.00***	***10.00***	***10.00***
Poultry oil	***5.19***	***3.53***	***1.85***	***3.57***	***2.47***	***1.88***
Limestone ground	0.67	0.66	0.65	---	---	0.50
Phosphate, defluorinated	1.98	1.49	0.99	1.44	0.90	0.71
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix	0.05	0.05	0.05	0.05	0.05	0.05
Selenium premix	0.05	0.05	0.05	0.05	0.05	0.05
DL-Methionine	0.21	0.20	0.20	0.20	0.21	0.20
L-Lysine	---	---	0.04	---	0.02	0.04
Salt	0.21	0.22	0.24	0.23	0.25	0.27
Composition by calculation:						
ME, kcal/g	***3.20***	***3.20***	***3.20***	***3.20***	***3.20***	***3.20***
CP, %	***23.00***	***23.00***	***23.00***	***23.00***	***23.00***	***23.00***
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00
Av. P, %	0.50	0.50	0.50	0.50	0.50	0.50
Methionine, %	0.58	0.59	0.60	0.59	0.59	0.59
Lysine, %	1.31	1.24	1.20	1.25	1.20	1.20
Met+Cystine, %	0.93	0.93	0.93	0.93	0.93	0.93

(f) Adapted from Wang and Parson (1998)

[1] Plant B: Broiler waste only

[2] Plant B: Broiler, hatchery, waste and disolved air flotation sludge

[3] Plant C

Concentrate inclusion rates

Table 7. Concentrate inclusion per species (g)

	Inc %		Inc %		Inc %
Calf	5.0	Pigs:		Chick	5.0
Dairy	7.5	Weaner	7.0	Broiler	5.0
Beef	7.5	Grower	7.5	Breeder	7.5
Lamb	5.0	Finisher	10.0	Layer	7.5
Ewe	7.5	Sow	7.5		

(g) Ewing (1997)

Deleterious factors

Biogenic amines in spoiled animal by-product feeds have been implicated in causing poor performance and intestinal lesions in broilers. The amines usually found in those areas with reported problems attributed to biogenic amines are: phenylethylamine, putrecine, cadaverine, and histamine. However, these four amines, at the concentrations typically found in animal by-products in the United States, do not pose a serious health concern for the broiler industry (Bermudez and Firman, 1998).

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