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EFFECTS OF RUMEN PROTEIN DEGRADABILITY ON RUMEN CHARACTERISTICS, MILK PRODUCTION AND REPRODUCTIVE PERFORMANCE IN HOLSTEIN DAIRY COWS

by

Mario Raul Figueroa

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Animal Science

Approved:

Major Professor	Committee Member
Committee Member	Committee Member
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Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

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Finally, I wish to dedicate this work to my mother Juana Figueroa, and to the memory of my father Dr. Raul E. Figueroa Lopez, who have always been my inspiration.

Mario Raul Figueroa F.

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ABSTRACT

Effects of Rumen Protein Degradability on Rumen Characteristics, Milk Production and Reproductive Performance in Holstein Dairy Cows

by

Mario Raul Figueroa, Doctor of Philosophy
Utah State University, 1992

Major Professor: Dr. Michael J. Arambel
Department: Animal Dairy and Veterinary Sciences

Three non-lactating Holstein cows fitted with rumen cannula were used to determine crude protein and dry matter rate of disappearance of two protein supplements: 1) soybean meal and 2) bypass protein blend by using the in situ bag technique. Rate disappearance (%/hr) was higher for soybean meal. Two collection periods were completed using 6 cows with a minimum of 21 d adaptation to the treatment top dressed on to the total mixed ration. Ruminal concentration of ammonia N, blood ammonia, and urea did not differ between treatments. Total concentration of volatile fatty acids was higher for bypass protein blend-fed cattle as well as percent molar concentration of propionate, butyrate, and valerate, while pH was lower. Total protozoa, and total and cellulolytic viable bacteria populations did not differ. Four of the cows

received a dose of 5 ml of Prostaglandin $F_{2\alpha}$. Blood and cervical mucus samples obtained showed no difference in blood ammonia and urea concentration . Forty-six Holstein cows were assigned to one of the two treatments (top dressed on the total mixed ration), according to parity during the following 125 d postpartum. Daily dry matter intake and milk production were recorded. Feed, orts, and feces were sampled. Milk samples were collected weekly and analyzed for components. Percent lactose and solid non-fat showed higher for cattle fed the bypass prorein blend. Starting on day 10 postpartum, cows were observed for signs of estrus and bred at first estrus observed after 45 d postpartum. Cervical mucus and blood collected at first standing estrus, and first, second, and third service, did not show a significant difference in urea concentration between rations. Twice-weekly collected blood samples showed similar monthly mean concentration of ammonia, urea, and progesterone profile for both treatment groups. Percent pregnancy, services per pregnancy, first service pregnancy and embryo mortality showed no significant difference. Motility and survivability of bull sperm were evaluated by incubating thawed semen in different levels of previously observed physiological concentrations of urea and/or ammonia. There were no significant treatment differences observed. Detrimental effects of treatment on sperm were not detected. (109 pages)

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Protein Requirements of High-Producing Dairy Cows

Intake of net energy determines optimum amounts of protein intake and the fraction of the protein that should be ruminally undegradable by directly affecting bacterial protein synthesis and indirectly by influencing milk production (7). Net energy balance, the difference between net energy intake and net energy output for maintenance and production, changes from negative to positive as lactation proceeds and dry matter intake increases. Crude protein intake must supply nutrients for ruminal microflora as well as nutrients for the cow for maintenance, mandatory metabolic fecal protein and milk production (37). Energy derived from body stores is only available for tissue metabolism, whereas dietary energy is used in the synthesis of protein by ruminal microorganisms and requirements of the animal for mammary gland productivity.

Therefore, changes in energy intake and milk production determine requirements for crude protein and undegradable intake protein throughout lactation (18). During the first 6 weeks of lactation, requirements for crude protein and undegradable crude protein are 18.0 and 45.0%, respectively, (39). From wk 6 to 12 of lactation, requirements for crude protein are 17.0 to 18.0 with an undegradable intake protein of 40.0% (37). As dry matter intake increases and production decreases, requirements for crude and undegradable protein

also decrease to 15.0 and 36.0%, respectively. Ruminal protein undegradability of common feedstuffs ranges from 30.0 to 85.0% (37). Because rations are formulated to contain specified amounts of energy and fiber, there are limitations to the maximum amount of some feed ingredients that can be included in dairy cow rations. It is difficult to have diets that contain more than 50.0 and less than 30.0% undegradable intake protein (38). Most protein sources are ruminally undegradable to some extent; however, some are more undegradable than others. Conventional rations mainly comprise legume haylage, corn silage, corn, and soybean meal and contain about 30.0% ruminal undegradable protein (31). In order to supply adequate protein for absorption by the host animal, increasing the undegradable protein fraction is necessary through the feeding of byproduct protein sources such as distillers dried grains, corn gluten meal, blood meal, meat and bone meal, and fish meal.

Another means of providing undegradable intake protein is to increase the total amount of crude protein fed. However, overfeeding crude protein is not efficient, since the amount of degradable intake protein also increases. The added dietary protein that is not used for production purposes is usually excreted in the urine and/or feces and is considered wasted protein. To achieve maximum protein economy, rumen efflux protein should be minimized (18). Research has proven that rumen degradability of protein sources is directly

intestine (43). Therefore, the most desirable protein balance would highlight those combinations of more degradable protein sources that reach the small intestine partially intact. Meat and bone meal are a good source of lysine (3), whereas corn gluten meal is low in lysine and high in sulfur amino acids (37). Together with sufficient energy, these two protein sources exemplify complementary feeds. A combination of blood meal and corn gluten meal fed by Roth et al (42) improved performance in steers. This may be due to a more optimum amino acid pattern that reaches the small intestine through a complementary effect. Methionine and lysine, both important in milk production, have been shown as limiting amino acids in ruminant rations (40).

Factors improving the amino acid profile reaching the small intestine have been examined. Chemical treatment and physical processing have been used to alter degradability of dietary proteins in the rumen. Ahrar and Schingoethe (2) and Mielke and Schingoethe (35) examined the effects of feeding heat-treated soybeans and soybean meal to lactating cows. Dry matter intake and milk yield were similar in both studies. Ahrar and Schingoethe (2) showed no difference in rumen ammonia or VFA concentration. Amino acid profile in venous or arterial samples or uptake of amino acids by the mammary gland was unaffected by heat treatment. Annexstad et al. (6) compared the effect of feeding three combinations of extruded whole soybeans and corn gluten meal or soybean meal on milk

production. Milk yield of cows consuming 75% of their supplemental protein from corn gluten meal was similar to other treatments. One of the chemical treatments examined was formaldehyde, which is used to treat dietary protein to increase the fraction that escapes ruminal degradation. This practice was proven beneficial for weight gain in young ruminants (47). However, results have not produced significant increases in milk production when fed to lactating dairy cows (9). Feeds that have been analyzed for their rumen undegradability potential are, whole cottonseed, extruded whole cottonseed, directly heated whole cottonseed, soybean meal, heated soybean meal, and corn gluten meal (45). From these analyses, different combinations of degradable and undegradable protein sources have been studied to improve milk production and milk components in lactating dairy cows. Drackley and Schingoethe (12) examined the effect of an extruded blend of soybean meal and/or sunflower seeds on milk yield and components in cattle during early lactation. Feeding the sunflower ration did not increase milk yield, but decreased milk solids non-fat. Zerbine et al (49) fed a high degradable (soybean meal) and a low degradable protein source (fish meal) to determine their effect on nitrogenous compounds entering the small intestine of dairy cows. Results showed that substituting fish meal for soybean meal resulted in a lower ruminal protein breakdown and microbial synthesis.

Amino acid results did not reveal a difference in duodenal

amino acid flow to the small intestine. Urea fed in combination with a protein source of low rumen degradability, such as brewers grains, distillers grains, and blood meal, improved performance compared to a soybean meal-supplemented ration fed to beef cattle (41).

Effect of Protein Intake on Reproduction in Dairy Cows

Results from numerous studies involving the evaluation of productive and reproductive parameters in dairy cattle have indicated a close relationship between protein nutriture and fertility. Many of these results have demonstrated that excessive protein intake in early lactation may be detrimental to postpartum fertility. In addition, dietary protein solubility and degradability are highly important subjects to consider. The results obtained from several studies are summarized in Table 1 (18). Most studies show that as crude protein intake increases, conception rate decreases and services per conception increase. However, increases were not consistent in magnitude, and in some instances, services per conception were not affected. One of the studies did not show a difference in any parameter measured (24).

Proposed Mechanisms for the Negative Influence of Crude Protein on Postpartum Fertility

Local Toxic Effect. Feeding excess rumen degradable protein can lead to an elevation of ammonia, urea, and other unidentified nitrogenous compounds in body tissue (26). Most,

but not all, of the ammonia absorbed from the digestive tract is metabolized to urea by the liver. Maximum hepatic capacity to detoxify ammonia is about 2 mmol/min per kg of liver tissue (46). Thus, diets that result in high ruminal ammonia production can cause elevated blood concentrations of ammonia, as well as urea. This can lead to elevated urea and ammonia levels in the reproductive tract (23). It has been suggested that ammonia exerts a direct impairment on sperm and ova function (16). Dietz at al. (11) showed the presence of urea cycle enzymes in the sperm cell, suggesting the existence of the urea cycle in the cell. When ammonia is excessive, the urea cycle becomes less functional. Aerobic respiration of the sperm cell is then obstructed by the inhibition of the tricarboxylic acid cycle. Sperm motility can be reduced in an aqueous solution containing as little as .006% urea, suggesting its use as a potential contraceptive (10). In an analogous study, Umezaki and colleagues (48) obtained similar results, but with higher levels of added urea. Eighty g of urea dissolved in 210 ml of 5% dextrose have been successfully used to exert abortion in women at about the 17th week of pregnancy when injected intraamniotically (21).

Cows fed rations containing 35% crude protein had more unfertilized eggs than cows fed a 25% crude protein diet, suggesting that fertilization failure may be the mechanism effecting fertility in cows fed rations not balanced to provide optimal amounts of rumen degradable protein (16). The

other aspect of the local toxic effect of high dietary degradable protein on reproduction involves tissue and cell damage. Most mammalian cells are highly permeable to NH_3, and because of rapid equilibration between NH_3 and NH_4^+, specific transport systems for either form are unlikely (22). Gradient formation, however, can occur when there is a pH difference across membranes. Under such circumstances NH_3 equilibrates between the extracellular spaces and NH_4^+ rises within cells by trapping H^+, causing the more acidic intracellular environment (30). Mammalian cells can only tolerate modest concentrations of ammonia due to disruptions in intracellular pH (25). Interference in the tricarboxylic acid cycle is inhibited at the isocitrate dehydrogenase step, because of elevated urinary citric acid when an animal has suffered from ammonia intoxication (36). Ammonia can alter DNA synthesis. Blood ammonia concentrations in the 1 to 3 mmol range can result in a decrease in thymidine incorporation in mucosa of the ileum or colon, spleen, and testes (50). Results from in vitro studies indicate that elevated systemic ammonia may reduce the immunoresponsiveness of animals against infectious diseases. Klucinski and Targowsky (32) found subtoxic (5 to 10 µg/ml) concentrations of ammonia lowered bovine lymphocyte responsiveness to mitogens. One or a combination of the previously described actions of ammonia on tissue cells could account for the ciliary loss of bronchial mucous membrane of chickens exposed to an atmospheric

concentration of ammonia (5). In a similar study, ammonia inhalation in chickens significantly increased the infection rate of chickens subsequently exposed to an aerosol of Newcastle disease virus (4). Stalheim and Gallager (44) cultured bovine oviductal tissues in a 0.1M concentration of ammonia and obtained a 100 % cilia-stopping-effect after 96 h of incubation. Through scanning electron microscopy, they encountered deciliation and the remaining cilia were very disorganized.

Systemic Toxic Effect. High protein rations have also been shown to reduce blood progesterone levels in dairy cows by reducing luteinizing hormone binding to ovarian receptors (27). Low levels of progesterone in the cycle prior to breeding have been correlated with low fertility (20). Progesterone is required for maintenance of pregnancy. Benefits of high circulating concentrations prior to insemination have been associated with higher conception rates (15). Naturally occurring steroids such as estrogen, progesterone, and testosterone (34) are oxidatively metabolized by NADPH-dependent enzymes found in the liver microsome. These enzymes are a complex of relatively non-specific enzymes known collectively as Mixed Function Oxidases (MFO). Evidence that steroid hormones could serve as substrates for MFO enzymes has been documented (33). Consequently, an increase in MFO activity would be expected to reduce circulating levels of steroids. A reduction in dietary

protein can result in decreased MFO activity in rodents (29). Increased dietary protein has been shown to reduce the concentration of progesterone in pregnant swine (13) and dairy cattle (27); therefore, steroid production may be altered by changing the level of CP fed and thus affect fertility.

Dividing crude intake protein into degradable and undegradable fraction characterizes fertility and protein interaction better than crude protein itself. Additional analyses are required which elucidate the interaction of protein excess and conception rate. Animals used to test these effects need to be adequately categorized into groups balanced in terms of age, production, body weight, and reproductive potential. How homeostatic regulation of metabolism may be influenced by protein nutrition and by the effect on fertility needs to be investigated. Local toxic factors versus systemical toxic effects influencing fertility need to be further described.

TABLE 1. Comparative effects of dietary protein intake on fertility.[1]

Study	No. cows	CR[2]	S/C[3]	DO[4]	CP[5]	UIP[6]
Folman et al. (19)	20	69	1.45	..	16	34
	19	56	1.79	96	16	30
	20	44	2.25	100	20	30
Edwards et al. (14)	9	44	2.28	123	13	36
	9	39	2.60	141	15	36
	9	37	2.70	140	17	36
Jordan et al. (26)	15	68	1.50	71	13	25
	15	54	1.90	98	16	26
	15	41	2.60	108	19	28
Aalseth at al. (1)	32	65	1.50	82	15	32
	31	58	1.71	80	20	30
Kaim et al. (28)	33	63	1.80	..	16	28
	20	44	2.25	..	20	28
Carrol et al. (8)	28	64	1.50	72	13	..
	28	56	1.80	82	20	..
Ferguson et al. (17)	60	23	..	..	18	33
	60	47	..	..	16	38
Howard et al. (24)	55	86	1.39	80	15	36
	55	85	1.40	80	20	37

[1]Adapted from Ferguson and Chalupa (18).
[2]Conception rate (%).
[3]Services/conception.
[4]Days open.
[5]Crude protein (%).
[6]Undegradable intake protein (% of total protein).

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CHAPTER II

THE EFFECT OF DEGRADABLE AND UNDEGRADABLE INTAKE PROTEIN ON RUMINAL AND REPRODUCTIVE TRACT METABOLITES IN FISTULATED NON-LACTATING HOLSTEIN COWS

ABSTRACT

Three mature non-lactating Holstein cows fitted with rumen cannula were used to determine rumen disappearance of DM and CP of two protein supplements (soybean meal, SBM, and bypass protein blend, BP) by using the in situ polyester bag technique. Basal ration feeding was offered at 0700 and 1800 h. The supplements used in the in situ trial (SBM or BP) were top dressed by hand during the am feeding. Rate of DM and CP disappearance (%/hr) was higher for SBM than BP (2.05 vs 1.03 % and 2.38 vs 1.04 %, respectively).

Two collection periods were completed with a minimum of 21 d adaptation. The effects of treatment on blood plasma, rumen fermentation characteristics, digesta rate of passage, liquid dilution rate, and total tract apparent nutrient digestibility were measured using six fistulated non-lactating cows.

Ruminal concentration of ammonia N did not differ between DIP and UIP fed cattle (22.03 vs 22.47 mg/dl, respectively). Total concentration of volatile fatty acids (VFA) was higher for UIP fed cattle (124.1 vs 117.1 mmol/ml). Percent molar concentration of propionate, butyrate, valerate was also higher for UIP than DIP fed cattle while pH was lower (6.36 vs

6.42). Total protozoa and total and cellulolytic viable bacteria populations did not differ. Blood ammonia and urea levels did not differ between treatments. Four cows were determined suitable for estrus synchronization and received a dose of 5 ml of Prostaglandin $F_{2\alpha}$ after an adaptation period of 21 d. Cows were observed three times a day during the next four days. When a cow was determined to be in standing estrus, blood samples were obtained and cervical mucus was aspirated with an insemination pipette and syringe. Blood was centrifuged and the plasma separated. Plasma and mucus samples were frozen (-20°C) in 25 ml scintillation vials for later analysis of ammonia and urea. Blood ammonia and urea concentration did not differ between treatments, although urea concentration in cervical mucus tended to be higher for UIP fed cattle (18.2 vs 15.0 mg/dl).

INTRODUCTION

Degradable dietary protein entering the rumen is converted to ammonia, which is necessary for microbial growth and protein synthesis, and for production of other metabolites such as volatile fatty acids (VFA), which are necessary for animal production. Microbial protein passes through the abomasum and small intestine where it is enzymatically digested into amino acids and absorbed for utilization by the host (3). Dietary protein sources need to be maintained to provide the appropriate ratio of degradable and undegradable protein, so that demands for increased milk production can be met. It is known that microbes themselves cannot provide sufficient protein for fast growth and the later stage of pregnancy (37) or maintain the increased demands of high milk production (23, 34). Rate of passage of digesta, feed intake, ration density, particle size, and source of degradable protein are some factors that affect protein degradation (2, 37). The amount of amino acids that reaches the small intestine is affected as well. If rate of passage of digesta is increased, feed intake increases above maintenance tolerance of the animal, and digestibility of the ration can decrease (2). If the diet fed is of greater specific gravity (is denser), rumen and liquid dilution rates become lower (5). Ration particle size can increase rate of passage through the rumen, especially if the particles are small, therefore

decreasing the amount of nutrient digested. Dietary protein breakdown also depends upon rate of rumen degradability. Slower rumen degradable proteins, such as meat and bone meal, fish meal, and corn gluten meal, are called bypass proteins since they can escape rumen degradation by rumen microorganisms to be utilized more efficiently in the lower gut (41). Protein that escapes ruminal fermentation can be used to the animal's advantage to improve milk production and growth rate or to reduce the amount of a conventional protein source required (9, 32, 43).

To achieve the greatest flow of amino acids to the small intestine, dietary protein must escape ruminal degradation without decreasing the efficiency of microbial protein synthesis (2). Dietary proteins that are both degradable and undegradable in the rumen should be included in the diet, but an excess of either can decrease efficiency of feed utilization and milk production (11). An excess of ruminally degradable protein will result in an accumulation of ammonia in the rumen, which will be absorbed, converted to urea in the liver, and excreted via the kidney, resulting in a waste of nitrogen (8). Feeding an excessive amount of ruminally undegradable protein and a deficient amount of ruminally degradable protein will result in a shortage of ammonia for rumen bacterial incorporation as well as in a reduced rate of degradation of fiber and organic matter (47, 41). The ratio between degradable and undegradable dietary protein in the

rumen is a critical factor affecting the quantity and pattern of amino acids flowing to the small intestine. This ratio must be considered if feed efficiency and milk production are to be maximized (10). Establishing the optimum ratio of degradable and undegradable dietary protein should reduce the amount of protein required without decreasing milk production or a feed efficiency. The suspension of feed materials in the rumen via the in situ bag technique allows intimate contact of test feed with the ruminal environment. Therefore, this in vitro procedure is a common methodology used for the estimation of ruminal protein degradability (35, 7) as well as for the determination of intestinal availability of rumen undegradable protein (13).

Potential toxicants may be derived from ruminal protein catabolism. Increases in dietary crude protein fed increase rumen (18, 26), blood ammonia (25), and serum urea nitrogen (SUN) (22, 24, 25, 26, 36). Serum urea nitrogen levels increase with an increased CP level and decreased level of energy fed (26). Urea is a normal constituent of uterine fluid (31). Tissue levels reflect blood levels. An increased urea nitrogen level in uterine fluid occurs as the level of protein fed is increased (25). A fermentation trial using six cannulated non-lactating Holstein cows was conducted to evaluate the effect of feeding a high protein ration containing two sources of rumen degradable protein on ruminal fermentation characteristics, blood plasma, and estrual

cervical mucus metabolite changes. Likewise, a study was performed to determine the rate of dry matter and crude protein disappearance of these two dietary protein sources.

MATERIALS AND METHODS

In Situ Trial

Three non-lactating Holstein cows equipped with rumen cannula were used for in situ estimation of rumen dry matter and crude protein disappearance. Cows were randomly assigned to one of two rations (treatments), degradable intake protein (DIP) or undegradable intake protein (UIP), using a single reversal Latin square design. The principal sources of protein in the treatments (DIP, UIP) were soybean meal (SBM) and a ruminal bypass protein combination (BP), respectively (Table 2 and 3). Nutrient analysis of the treatments is presented in Table 4. Cows received 9 kg basal ration (100% dry matter) plus 1 kg SBM (DIP) or 1.5 kg BP (UIP). Basal ration feeding was divided into two daily feedings (0700 and 1800 h) and supplements were top dressed by hand during the am feeding. Rumen disappearance of dry matter and crude protein were determined using the in situ bag technique (35). White polyester monofilament (Bar, Diamond Inc., Parma, ID) bags with an average pore size of 32 μ and dimensions of 10x20 cm were used. Approximately 5 g of SBM or BP were placed in each bag before sealing. After a 21 d adaptation being fed the assigned treatment, each cow received 16 bags containing SBM,

collecting two bags at 0.5, 1, 2, 4, 8, 12, 24, and 36 h postfeeding. The same procedure was later used for the bags containing BP. Once removed from the rumen, each bag was frozen (-20 °C), and after all bags were removed, they were rinsed in cold tap water and lyophilized (Freeze Dry System, Lyph-Lock 12, Labconco, Kansas, MO). The dried residue was then removed from each bag, ground through a cyclone mill (Cyclotec 1093, Tecator, Hoganas, Sweden), fitted with a 1 mm screen, and analyzed for dry matter (52) and crude protein (21).

Rumen Fermentation

Six mature non-lactating Holstein cows fitted with rumen cannula were randomly assigned to one of two treatments in a single reversal Latin square design. The treatments were degradable intake protein (DIP) or undegradable intake protein (UIP). The principal sources of protein in the treatments (DIP, UIP) were soybean meal (SBM) and ruminal bypass protein combination (BP), respectively (Table 2 and 3). Nutrient analysis of the treatments is presented in Table 4. Cows received 9 kg basal ration (100 % dry matter) plus 1 kg SBM (DIP) or 1.5 kg BP (UIP) twice daily, with SBM and BP being topdressed during the am feeding.

A 12 d adaptation was provided prior to the collection period. On day one of the collection period, cows were ruminally dosed with 150 g chromium mordanted straw (49) (5 to

10 mm particle size) and subsequent fecal grab samples were collected at 0, 24, 27, 30, 33, 36, 48, 60, 72, 84, 96, and 108 h after dosing. Samples were immediately oven dried at 60° C for 3 days and ground through a 1 mm screen using a Wiley mill (Thomas-Wiley Laboratories, Swedesboro, NJ). Dried and ground fecal samples were then divided into two bags; one was reground through a Cyclone mill (Cyclotec 1093, Tecator, Hoganas, Sweden) to improve sample homogeneity for chromium analysis (54). The remaining bag was used to composite fecal samples within cow and collection period. Composited samples were then analyzed for DM, ADF, NDF (52), CP (21), and AIA (50). AIA was used as an internal marker to determine total tract apparent nutrient digestibility. At the beginning of each collection period, basal ration and supplement sources were collected, oven dried 60°C for 3 d, ground and analyzed for DM, ADF, NDF, CP, and AIA. On day five, cows were dosed with 50 g of NaCo-EDTA (dissolved overnight in 250 ml DI H_2O) directly into the rumen through the cannula. Rumen fluid samples from the ventral sac were collected at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 h postfeeding. Rumen sampling was prolonged to include 26, 36, and 50 h samples after Co-EDTA was dosed to calculate the liquid dilution rate (39,40). The pH was recorded using fresh rumen samples (Fisher Accumet model, 425 digital pH/ion meter, Pittsburgh, PA). Rumen fluid was strained through two layers of cheese cloth, centrifuged at 20,000 X g using a Sorvall RC-

5B Centrifuge (Dupont, Wilmington, DE) for 20 min at 4°C, preserved with 6 N HCl (2 ml HCl + 18 ml rumen fluid), and frozen in plastic 25 ml scintillation vials for later analysis. Cobalt was determined by atomic absorption spectrophotometry (48)(Buck Scientific, Northwalk, CT). Ammonia nitrogen (NH_3-N) was determined spectrophotometrically using Nessler's reagent (20). Volatile fatty acids were determined by gas chromatography (HP 5890 Hewlett-Packard, Co. San Fernando, CA) using a 1.83 X 2mm ID glass column packed with GP 10% SP-1200/1% H_3PO4 on 80/100 120 C mesh Chromosorb W-AW (Supelco, Bellefonte, PA). At 2 h postfeeding additional rumen fluid samples were collected and used in the enumeration of total and cellulolytic bacteria (28) and protozoa (14). Blood samples were collected from a coccigeal vessel at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 h post-feeding, using heparinized 10 ml vacutainer tubes. Blood was kept on ice after collection and immediately centrifuged for 30 min. Plasma was then removed, stored, and frozen (-20°C) in plastic 25 ml scintillation vials for later analysis of ammonia (Ammonia kit Assay, Sigma Diagnostics, St. Louis, MO) and urea (BUN Rate Assay, Sigma Diagnostics, St. Louis, MO).

Reproductive Tract Metabolites

The six cows used in the fermentation trial were palpated to detect the presence of corpora lutea and observed for behavioral estrual activity. Four cows were determined

suitable for estrus synchronization and assigned to one of two treatments (DIP and UIP) in a single reversal Latin square design. After a 21 d adaptation, cows were first estrus synchronized by receiving a dose of 5 ml of Prostaglandin $F_{2}\alpha$ (Lutalise, Upjohn, Kalamazoo, MI) intramuscularly (17). Cows were observed for signs of estrus three times a day during the next four days. When a cow was observed in standing estrus, cervical mucus was aspirated with an insemination pipette (45 cm long 2mm i.d.) and syringe. Mucus was collected at 0, 1, 2, 3, 4, 6, 8, and 10 h post am feeding and samples were immediately stored in 25 ml scintillation vials and frozen (-20°C) for later analysis of urea. Two hundred and fifty µl of thawed mucus (5°C) were withdrawn using a digital varimetric micropipettor (DV-1000, Labindustries, Berkeley, CA) and diluted in 1 ml of double distilled water, vortexed for 10 sec, and allowed to set for 10 min (5°C). The liquid fraction was used for urea determination (BUN Rate Assay, Sigma Diagnostics, St Louis, MO).

Statistical Analysis

Data were analyzed by using general linear model, including dietary treatment, cows, and time (42). When main effect means were significant ($P < .05$), treatment means were separated using least significant difference (45).

RESULTS AND DISCUSSION

In Situ Trial

The in situ technique has been used for several years and is the basis for predicting digestion in several feeding systems (4). In this study, DM and CP ruminal disappearance rates of the protein sources used were analyzed using this technique. Dry matter and crude protein rate of disappearance are presented in Table 5 and Figure 1. Dry matter disappearance rate (%/hr) of SBM was significantly ($P < .05$) higher than BP (2.05% vs 1.03%, respectively). Crude protein rate of disappearance was significantly ($P < .05$) higher for SBM when compared to BP (2.38% vs 1.04%, respectively). Similar results have been found by others (1, 30). SBM is recognized as a more degradable protein source than the BP combination, because single ingredients tend to have a higher rumen undegradability (34). Rumen protein degradability is important to the ruminant because of the supply of ammonia to microorganisms that require protein in the form of amino acids and peptides (5). Protein available to the host ruminant is known as dietary and microbial bypass or escape protein. Bypass dietary protein escapes ruminal degradation and then is chemically and enzymatically degraded postruminally where it is absorbed as amino acids (27).

Rumen Fermentation

Treatment effects on ruminal fermentation characteristics

are shown in Table 6. Undegradable intake protein (UIP)-fed cows had higher ($P < .05$) total VFA (124.1 vs 117.1 mmol/ml, respectively) concentration, as well as higher molar percent propionate, butyrate, and valerate, when compared to the DIP-fed cattle. Consequently, the acetate to propionate ratio was lower in UIP-fed cattle. Similar results were noted by Mielke and Schingoethe (33). They found ruminal pH was lower ($P < .05$) for UIP than the DIP-fed cattle (6.36 vs 6.42, respectively). Treatment ration fed had no effect ($P > .05$) on cellulolytic or total viable bacteria (Table 7). This agrees with results by Stern et al. (46) where no effect was found on bacterial population from animals fed corn gluten meal as a UIP source. Cellulolytic bacteria, however, were numerically higher in cattle fed the UIP diet. This could be a response of the bacterial population to a ration containing a higher biological value protein source, resulting in an increased ruminal fermentative activity and a higher VFA production, and acid byproducts of ruminal fermentation such as VFA-reduced pH (51). Proteolytic enzymes must contact protein through an interaction involving water so that soluble protein is more quickly degraded. Insoluble protein is often degraded slowly (6). SBM is approximately 65% rumen degradable (34); therefore, if energy is sufficient, CP can be maximally utilized (5, 27), or if CP is in excess of the capability of the microbes, an increased ruminal ammonia nitrogen can occur as shown by Jordan et al. (25). Protozoal

populations were also unaffected ($P > .05$) by treatment (Table 7). The flow of liquid and solids through the digestive tract represents separate pools. Water may pass through the rumen wall as a result of osmotic pressure (51). The washout rate of liquids influences rumen microbial population and the outflow of nutrients. Soluble substances moving with the liquid phase are more apt to pass through the rumen at higher liquid dilution rates (3). Therefore, faster washout can enhance the microbial population by reducing generation time and increasing the number of young and more metabolically active rumen microbes (51). Liquid dilution rate (Figure 2) and dry matter rate of passage (Figure 3) results are presented in Table 6. In our study, liquid dilution rates in cattle fed DIP and UIP rations were not different. The rate of DM passage between treatments was not statistically ($P > .05$) different. Average ammonia nitrogen concentration was not different in rumen fluid samples for the DIP treatment when compared with UIP (22.03 vs 22.47 mg/dl, respectively). This disagrees with other studies where the addition of SBM increased rumen ammonia nitrogen (1,29,30). Satter and Roffler (44) reported that ruminal ammonia concentration rose from 0.8 to 56.1 mg/100 ml as dietary CP-fed cattle increased from 8 to 24% in the diet. It is important to recognize that feeding a less rumen degradable protein source could create a counterbalance allowing for less microbial mass to be produced in the rumen (47). Excess ammonia produced is

absorbed either in the reticulo-rumen or in the lower gastrointestinal tract, transported to the liver, and converted to urea. Hepatic concentration of the urea cycle enzymes, arginine synthase, ornithine carbamyltransferase, and arginase increase when cattle are fed high protein diets (38). Plasma ammonia concentration averaged 0.95 µg/ml for DIP and 1.02 µg/ml for UIP-fed cattle; however, this difference was not significant ($P > .05$). Blood ammonia concentration in cattle fed both treatments suggest that enzymes in the urea cycle converted all excess ruminal ammonia into urea-N, possibly because uptake into hepatic cells was sufficient, since plasma urea was unaffected ($P > .05$) by treatment (14.6 mg/dl for DIP and 14.8 mg/dl for UIP-fed cattle). Another possibility is that ammonia in the intestines, which is dependent upon dietary protein, may diffuse across the peritoneal cavity to peripheral circulation without passing through the liver (53). Total tract apparent digestibility of crude protein, acid detergent fiber, and neutral detergent fiber were unaffected by treatment (Table 8). This agrees with previous work (12, 19).

Reproductive Tract Metabolites

Response to the synchronizing dose of $PGF_{2}\alpha$ (onset of estrus) ranged from 48 to 72 h. Although ammonia and urea concentration in plasma analyzed during the fermentation trial were unaffected by treatment, urea concentration in cervical

mucus was significantly higher ($P < .05$) for cows fed the UIP treatment (18.2 vs 15.0 mg/dl, respectively). The results are summarized in Table 9. The lack of a significant difference between urea and plasma ammonia among treatments suggests that urea concentration should not have been different for cervical mucus secretion. Nevertheless, UIP-fed cattle presented a higher sustained mean level of urea. A possible explanation is the time in which the estrual mucus sampling took place. In cows, preovulatory, ovulatory, and postovulatory estrual mucus has been reported as being quite variable with regards to nitrogenous mucal compounds (15, 16).

CONCLUSIONS

Results obtained from the in situ trial indicated a very marked difference in DM as well as CP rate of disappearance among the two protein sources used. Nevertheless, ruminal fermentation characteristics were affected minimally with regards to protein source fed. The analysis of estrual cervical mucus urea revealed concentrations similar to those previously found in reproductive tract secretions of cows fed similar levels of CP in the ration.

TABLE 2. Ingredient composition of treatments.

Item 100% dry matter	DIP	UIP
Corn silage	8.6	8.4
Alfalfa hay	22.3	21.9
Alfalfa silage	5.8	5.7
Rolled barley	21.5	21.0
Dist. corn grain	12.8	12.5
Cottonseed meal	11.9	11.7
Beet pulp	8.3	8.2
32% dairy mix[1]	2.1	2.0
Protected fat	1.9	1.8
Mineral vitamin premix[2]	1.0	1.0
Soybean meal	3.9	0.0
Bypass protein	0.0	5.7

[1]Crude protein, 32%; crude fat, 15%; calcium, 2.5%; phosphorus,1.0%; salt, 2.5%; vitamin A (USP units/kg), 66,000; vit D3 (USP units/kg), 16,500; vitamin E (IU/kg),66.

[2]Calcium, 8%; potassium, 3.26%; magnesium, 2.2%; sodium, 6%;phosphorus, 5.5%; sulfur, 3.2%; copper, 400 ppm; iron, 3730 ppm; manganese, 2000 ppm; and zinc, 2000 ppm.

TABLE 3. Added protein source composition.

Soybean meal	Bypass protein
100% SBM	35% Canola Meal
	25% Distillers grain
	10% Fish meal
	10% Blood meal
	10% Feather meal
	10% Meat and bone meal

TABLE 4. Treatment ration nutrient analysis.

Nutrient (DM basis)	DIP	UIP
	-------%-------	
CP	20.0	20.0
DIP[1]	65.0	60.0
UIP[1]	35.0	40.0
DM	68.6	69.5
ADF	25.2	25.2
NDF	45.0	43.1
$NE_{(L)}$ Mcal/kg[1]	1.74	1.74

[1]NRC (34).

TABLE 5. Dry matter and crude protein disappearance of soybean meal (DIP) and bypass protein (UIP).

Item	DIP	UIP	SEM
DM disappearance	------%	------	
12 hr	57.2[a]	25.9[b]	1.31
24 hr	78.5[a]	31.3[b]	1.67
Percent /hr	2.05[a]	1.03[b]	0.22
CP disappearance			
12 hr	43.25[a]	12.75[b]	0.34
24 hr	77.72[a]	26.18[b]	1.13
Percent /hr	2.38[a]	1.04[b]	0.17

[a,b]Means in the same row with the different superscript differ ($P < .05$).

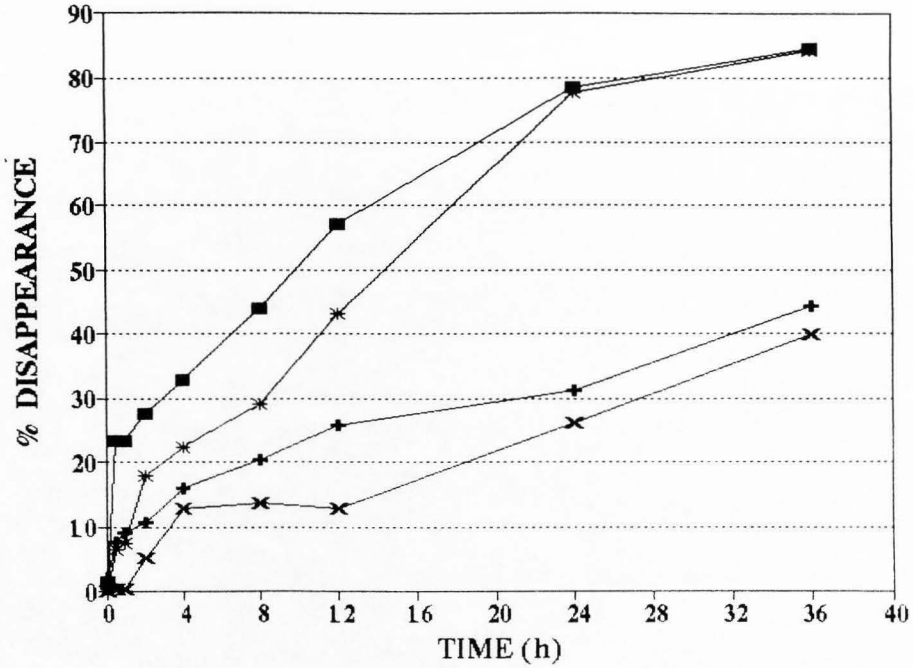

Figure 1. Rate of in situ DM and CP disappearance (%/h) of protein sources. Protein sources consisted of DIP (DM ▪, CP +) and UIP (DM *, CP x).

TABLE 6. Effect of treatment on ruminal fermentation parameters.

Item	DIP	UIP	SEM
pH	6.42[a]	6.36[b]	0.02
Ammonia -N (mg/dl)	22.03	22.47	5.4
Liquid dilution rate (%\hr)	2.06	1.84	0.56
Rate of DM passage (%\hr)	1.08	1.09	0.08
Total VFA (mmol/L)	117.1[a]	124.1[b]	2.37
Acetate, molar %	66.9	65.2	1.59
Propionate, molar %	16.9[a]	17.8[b]	0.44
Isobutyrate, molar %	1.2	1.1	0.03
Butyrate, molar %	11.9[a]	12.7[b]	0.32
Isovalerate, molar %	1.7	1.7	0.04
Valerate, molar %	1.4[a]	1.5[b]	0.03
Ac/Pr ratio	3.96[a]	3.66[b]	0.03

[a,b]Means in the same row with different superscripts differ ($P < .05$)

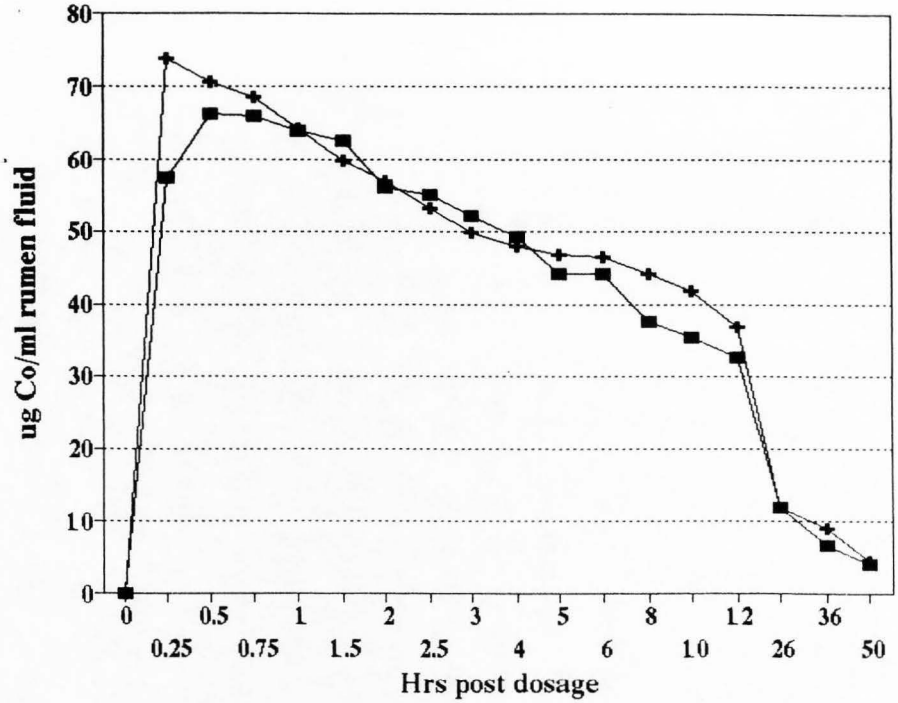

Figure 2. Liquid dilution rate (%/h). DIP (▪) and UIP (+).

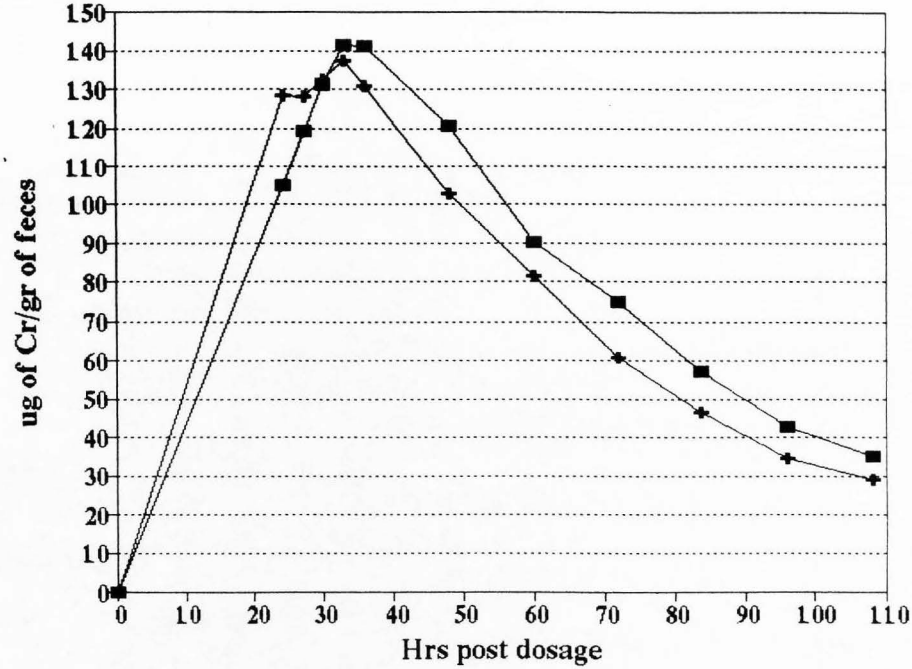

Figure 3. Rate of DM passage (%/h). DIP (▪) and UIP (+).

TABLE 7. Treatment effect on ruminal microbial population.

Item	Treatment		
	DIP	UIP	SEM
Bacteria (ln/ml)			
Total	22.49	22.47	0.34
Cellulolytic	13.74	16.09	1.62
Protozoa (ln/ml)	12.41	12.17	0.15

TABLE 8. Treatment effect on percent total-tract apparent nutrient digestibility.

Item	Treatments		
	UIP	DIP	SEM
	-------%	--------	
CP	77.13	75.17	2.49
ADF	61.19	53.61	3.55
NDF	68.63	63.68	2.60

TABLE 9. Effect of treatment on plasma urea, ammonia and estrual cervical mucus urea concentration.

	Treatments		
Item	DIP	UIP	SEM
Plasma ammonia (µg/ml)	0.95	1.02	0.06
Plasma urea (mg/dl)	14.6	14.8	0.34
Estrual cervical mucus urea (mg/dl)	15.0[a]	18.2[b]	0.79

[a,b]Means in the same row with different superscript differ ($P < .05$).

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CHAPTER III

THE EFFECT OF UNDEGRADABLE AND DEGRADABLE INTAKE PROTEIN ON PRODUCTION AND REPRODUCTIVE PARAMETERS IN POSTPARTUM HOLSTEIN COWS

ABSTRACT

Forty-six Holstein cows were assigned to one of two treatments according to lactation number (primiparous or multiparous) in a 2 X 2 factorial arrangement, during the following 125 d postpartum. Treatment diets contained: 1) total mixed ration (TMR) plus soybean meal (DIP), or 2) TMR plus bypass protein combination (UIP). Cattle had ad libitum access to treatment and water.

Mean daily dry matter intake (DMI) and milk production were recorded. Feed, orts, and feces were sampled and composited for analysis. Milk samples were collected weekly and analyzed for percent fat, protein, lactose, and solids-not-fat (SNF). Percent lactose and solid non-fat were higher ($P < .05$) for cattle fed the UIP versus DIP treatment (4.99 vs 4.86 and 8.68 vs 8.56%, respectively). Starting on day 10 postpartum, cows were observed twice daily for 30 min at 0600 and 1900 h for signs of estrus. Reproductively, healthy cows were bred at first estrus observed after 45 d post partum. Pregnancy was confirmed by rectal palpation 45-60 d postinsemination. Cervical mucus and blood collected at first standing estrus, and first, second, and third AI service did not show a

significant difference in urea concentration between rations fed. Twice-weekly collected blood samples showed similar monthly mean concentration of ammonia and urea for both treatments. Days to first standing estrus, first luteal activity, first progesterone peak, peak progesterone concentration, and mean progesterone concentration were similar ($P > .05$) for both animal treatment groups. Chi-square analysis did not show any significant ($P > .05$) difference when percent pregnancy services per pregnancy, first service pregnancy, and embryo mortality were evaluated between treatments. Pregnant and nonpregnant cows were paired with their respective plasma and cervical mucus urea concentration within treatment and no significant difference was observed, indicating no detrimental effects on cows fed either treatment ration.

INTRODUCTION

Intensive genetic selection via artificial insemination has brought about a large increase of milk production per cow in the national herd over the last 30 years (> 3,000 kg/lactation). During this period, first service pregnancy rate (PR) has decreased from 66% in 1951 to 53% in 1987 (2). Reduction in PR has occurred despite remission of infectious diseases as a cause of infertility through competent vaccination and herd health programs. Reduction in PR is often attributed to herd size, and more intense management of raised heifers, yet PR has not decreased in these animals (8). Researchers have associated a negative relationship between higher milk production and fertility (21), while others have not (29). Hanson et al. (19) and Faust et al. (11) found milk yield to be antagonistic to fertility in first lactation animals. Selection for higher milk production does not appear to be directly antagonistic to fertility, as evidenced by similar PR in virgin heifers now and in 1952 (2). Reproductive traits have a low heritability; therefore, any relationship between increased milk yield and fertility must be phenotypic. Many factors may combine the relationship between milk yield and fertility, such as days in milk when inseminated, age of cow, season, number of services (17), and nutrition, especially crude protein levels fed (1). Increasing crude protein (CP) intake leads to increased milk

production (3). Because profits are related to reproductive performance as well as milk production, a negative relationship between dietary CP intake and fertility would be important to know. Previous studies have shown that pregnancy rate may decrease and number of services/conception and days open may increase as the percentage of crude protein fed increases (24). Protecting protein from ruminal degradation can improve conception rates and decrease the number of services per conception and days open (14). The percentage of dietary protein in the ration may affect fertility more in older cows (fourth lactation or greater) than in younger cows (24). Therefore, cow maturity and protein degradability may influence the effect of dietary CP on fertility. In some cases improvements in conception rate (greater than 20%) have been obtained by decreasing protein degradability of a ration by 5% (13).

The primary determinant of microbial growth is energy (31). Because ruminal ammonia not utilized for microbial growth is converted to urea in the liver, economical use of nitrogen in ruminants depends on the energy content of the diet and a proper balance of dietary protein that is degraded and undegraded in the rumen (5). Fertility may be reduced because excess protein degraded in the rumen may lead to increased concentrations of ammonia, urea, or other nitrogenous compounds in the blood and in reproductive tract fluids (23). There is evidence that suggests that these compounds are toxic

to spermatozoa, ova, or embryos (12). Lactating cows fed rations high in dietary crude protein can reduce blood progesterone levels (22). Low levels of progesterone in the estrous cycle prior to breeding have been correlated with low fertility (15). In the mouse and rat, naturally occurring steroids such as estrogen, progesterone, and testosterone (28) are oxidatively metabolized by NADPH-dependent enzymes, which are a complex of relatively nonspecific enzymes known collectively as MFO, found in liver microsomes. Evidence that steroid hormones could serve as substrates for microsomal-oxidizing enzymes have been documented (27) by demonstrating that Km values for progesterone, testosterone, and estradiol were 10 times lower than those for several drug oxidations. Therefore, an increase in MFO activity would be expected to reduce circulating levels of steroids. Reduction in dietary protein can result in decreased MFO activity in rodents (25), and increased dietary protein has been shown to reduce the concentration of progesterone in pregnant swine (9) and dairy cattle (22). Therefore, an increased MFO-induced activity due to a high level of protein fed may result in a reduction of circulating blood progesterone. Thus, hormone production may be altered by CP level in the diet and affect fertility. Potential toxicants produced by rumen metabolism according to protein degradability may also be a factor impairing fertility.

The objectives of this study were to investigate the effect

of feeding a high crude protein ration differing in rumen degradability on reproductive performance of dairy cows in early lactation, and to measure the N concentration as either ammonia or urea in the rumen, blood plasma, and reproductive tract as influenced by CP degradability.

MATERIALS AND METHODS

Production Parameters

Forty-six Holstein cows at day one postpartum were assigned to one of two treatment groups (n= 23) according to lactation number and mean milk production calculated from previous lactation. Experimental period was from day one postpartum through 125 d of lactation. Treatment diets contained: 1) total mixed ration (TMR) plus soybean meal (DIP), or 2) TMR plus bypass protein blend (UIP). Components of the treatment rations and protein supplements are detailed in Tables 10 and 11, while nutrient analysis of the treatments is presented in Table 12. The basal ration was balanced according to NRC (32) requirements for 600 kg cows producing 40 kg milk/d. Cows were individually fed ad libitum through Calan gates (American Calan, Inc., Northwood, NH) twice a day (0530 and 1800 h). A minimum 10% weighback was used to ensure ad libitum intake. Feeding was performed by top dressing supplements and mixing by hand during the morning feeding. Ten percent orts were allowed with intake adjustments performed daily. One kg/d of long-stem alfalfa

hay at 2300 h was offered to all cows in both treatments.

During the 125 d duration of the trial, dry matter intake (DMI) and milk production were recorded daily. Feed samples and orts were sampled weekly and later composited for laboratory analysis. Feces were sampled from each cow twice daily during the last three days of trial and later composited within cow for laboratory analysis. Feed and feces samples were dried at 60°C for three d, ground through a Wiley mill (Thomas Wiley Laboratories, Suedesboro, NJ) equipped with a 1 mm screen, and analyzed for DM, CP (18), ADF, NDF (40), and acid insoluble ash (AIA)(38). Milk samples were collected weekly and analyzed for fat, protein, lactose, and solids-not-fat (SNF) by the Dairy Herd Improvement laboratory (DHIA, Logan, UT) using a Multispec M Infrared Analyzer (Wheldrake, York, England). Feed, orts, milk, and body weights were sampled and recorded the same day weekly during the trial before the morning feeding.

Apparent total tract nutrient digestibility values were calculated using nutrient data obtained from laboratory analysis using acid insoluble ash (AIA) as an internal marker (38).

Reproductive Parameters

Starting on day 10 postpartum, all cows were observed twice daily for 30 min at 0600 and 1900 h for signs of estrus in the exercise lot in accordance with the farm management program.

Estrus was determined when a cow was either observed standing to be mounted by a herd mate or displayed at least two secondary signs of estrus. Reproductive health of all nonpregnant postpartum cows was monitored by weekly rectal palpation of the reproductive tract via the rectum. Occurrence of parturient disorders such as milk fever, dystocia, and retained placenta were recorded as well as postpartum disorders such as ovarian cyst, uterine infection, and displaced abomasum. A retained placenta was not treated systemically unless the cow did not consume feed and had an elevated temperature. Standard treatment consisted of two or three intrauterine flushings with tetracycline until placenta was expelled. When some of these abnormalities were too severe, the cow was replaced with an alternate animal. Three cows were not considered for reproductive data due to cystic ovary or anestrus. This situation reduced the number to 43 animals (DIP N=21, UIP N=22).

Reproductively, healthy cows were bred at first estrus observed after 45 d postpartum. Pregnancy was confirmed by rectal palpation 45-60 d postinsemination. Cervical mucus was collected nonsurgically at first standing estrus and first, second, and third service. Prior to artificially inseminating the cow, an insemination plastic pipette and syringe were used to collect cervical mucus. The plastic pipette (45 cm long, 2mm i.d) was passed through the vagina into the cervix without using any flushing medium. Blood was collected at the same

time from a coccygeal vessel using a vacutainer heparinized 10 ml tube. Mucus was kept on ice upon collection and then stored and frozen (-20°C) in 25 ml scintillation vials. Blood was also kept on ice and then centrifuged for 20 min (Centrific centrifuge, Fisher Scientific, Pittsburgh, PA). Plasma was separated and frozen until analyzed for urea (BUN Rate Assay, Sigma, Diagnostics, St. Louis, MO). Cervical mucus was frozen (-20°C) and stored in 25 ml scintillation vials until analyzed for urea. Two hundred fifty µl of thawed mucus (5°C) were withdrawn using a digital varimetric micropipettor (DV-1000, Labindustries, Berkeley, CA) and diluted in 1 ml of double distilled water, vortexed for 10 sec, and allowed to set for 10 min (5°C). The liquid fraction was used for urea determination (BUN Rate Assay, Sigma Diagnostics, St Louis, MO). Thereafter, blood samples from a coxigeal vessel were collected twice a week starting day ten postpartum using vacutainer EDTA tubes until 125 d postpartum or the cow was confirmed pregnant. Blood samples were kept on ice and then centrifuged for 20 min (Centrific centrifuge, Fisher Scientific, Pittsburgh, PA). Plasma was separated and immediately analyzed for ammonia (Ammonia kit Assay, Sigma, Diagnostics, St. Louis, MO). Remainder of the sample was frozen at -20°C and later analyzed for urea and progesterone. Progesterone was determinated by radioimmunoassay (Coat-A-Count, Progesterone. Diagnostic Products Corporation, Los Angeles, CA) using a gammacounter (Micromedic 4/200 Plus.

Automatic Gammacounter. ICN, Micromedic Systems, Inc, Horsham, PA). The first ovaric cyclic activity was estimated from plasma analysis when serum progesterone increased >/ 1 ng/ml (37). Cows maintaining raised progesterone (>/ 4 ng/ml) 21 d post service that had a subsequent estrus were determined as having had an embryonic death (20). Services per conception were the total number of inseminations for cows that conceived during trial period divided by the number of cows confirmed pregnant. First service conception rate was the percentage of cows confirmed pregnant from first service. Pregnancy rate was the percentage of cows pregnant during the 125 d trial.

Statistical Analysis

Production, plasma, and cervical mucus metabolite data were evaluated by a 2x2 factorial analysis using a general linear model (30), where the two factors were treatment (DIP vs UIP) and lactation number (1 vs >/2 lactation). Pregnancy rate was evaluated by means of a chi-square analysis.

RESULTS

Production Parameters

Production yield and percent body weight change of lactating cows fed DIP or UIP were not different ($P > .05$). Dry matter intake, milk yield, 4.0% FCM, production efficiency, and percent change in body weight are displayed in Table 13. Treatments did not affect mean daily milk yield.

Production efficiency (4.0% fat-corrected milk/DM intake) was not statistically ($P > .05$) different between treatments. Milk components analyzed are presented in Table 14. Percent milk fat and protein were not statistically different ($P > .05$) between treatments. Percent lactose as well as percent solids non-fat were significantly ($P < .05$) higher for the UIP fed cattle when compared with DIP fed animals (4.86 vs 4.99 %, 8.56 vs 8.68 %, respectively). Apparent total tract nutrient digestibilities were unaffected by treatment (Table 15). Average monthly concentration of plasma metabolites, ammonia and urea did not differ ($P > .05$) between treatments throughout the duration of the trial. Urea concentration in blood plasma and estrual cervical mucus at artificial insemination were unaffected ($P > .05$) by treatment (Table 16).

Reproductive Parameters

Chi-square analysis did not reveal any significant differences ($P > .05$) between treatments in percent pregnancy, services/pregnancy, first service pregnancy, and embryo mortality (Table 18). The concentration of urea in blood plasma and estrual cervical mucus was evaluated at artificial insemination for all cows; however no significant differences ($P > .05$) were found when comparing pregnant and nonpregnant cows within treatment (Table 18). Other reproductive parameters evaluated, which include: days to

first standing estrus, progesterone profile and length of days of first estrual cycle, were also unaffected ($P > .05$) by treatment (Table 17).

DISCUSSION

Dietary treatment had no effect on DM intake or body weight change, although as expected, there was an interaction between lactation number and time ($P < .05$) during the 125 d experimental period. Milk production, percent milk fat and protein, 4% fat corrected milk, and feed efficiency were unaffected by treatment. There appeared to be no advantage of feed selection based on protein source degradability during the first 125 d postpartum. One reason for lack of milk production response to degradable protein source may be that specific amino acids rather than protein per se limit milk protein production (35, 6). Orskov et al. (33) noted that milk production response to fish meal was only observed in early lactating cows consuming small amounts of feed. They attributed this to a slower rate of passage of feed, which reduced differences in rumen degradability between fish meal and soybean meal. Propionate is undoubtedly the most important single source of carbon for dairy cow gluconeogenesis, accounting for 30 to 60% of blood glucose produced (39). Comparison of the net daily glucose turnover with propionate production suggests a regression slope near the theoretical one mole of glucose per two moles of

propionate (10). Propionate is the main source for synthesis of lactose in milk secretions. Approximately 1.5 units of glucose are required for the synthesis of one unit of milk lactose. Net daily requirements for glucose may be as high as 2.8 to 3.0 kg of glucose in the case of a high-producing cow (1). In this study an increase ($P < .05$) in milk lactose was observed for UIP-fed cattle when compared to the DIP treatment (4.99 vs 4.86%, respectively). As a result of the increase in this component, solids non-fat were also increased ($P < .05$)(8.68 vs 8.56 %). These results agree with Oskov et al. (33), where SNF were increased when adding to the diet fish meal, a source of undegradable intake protein. However, Santos et al. (34) and Kung et al. (26) reported no effect on individual ruminal VFA's when feeding diets with different protein degradabilities.

Apparent total tract digestibility of CP, ADF, NDF, and DM was unaffected by source of dietary protein. These similarities are in accordance with previous work where other sources of degradable intake protein have been fed (7, 16).

One mechanism proposed for the negative influence of CP on fertility is that excessive intake of CP leads to increased ruminal and blood ammonia and urea concentration. This results in a local toxic effect on sperm, ovum, or developing embryo (12). To assess the influence of increased circulating N directly on the reproductive tract, cervical mucus urea levels were determined. There was no difference in cervical

mucus urea concentration upon artificial insemination between DIP and UIP-fed cattle (21.56 vs 25.23 mg/dl, respectively). In our study, a positive relationship between pregnant and non-pregnant cows within treatment according to plasma and/or cervical mucus urea concentration was not noted. This demonstrates that a relationship between degradable intake protein and urea levels in reproductive tissue may not exist. Our results conflict with field trial studies reported by Ferguson et al. (13), where cows with a serum urea concentration greater than 20 mg/dl at time of breeding were three times less likely to become pregnant. Previous work has suggested that high-producing dairy cows fed rations containing high levels of crude protein had increased basal blood concentrations of luteinizing hormone (LH)(21). Elevated levels of LH may be related to a low serum concentration of progesterone or an exaggerated pituitary release of LH in response to a LH-releasing hormone (LHRH) injection (22). In the same manner, a lower progesterone concentration in the estrous cycle has been reported in cows fed high dietary levels of crude protein (36). These levels are similar to those found in our study. No significant difference ($P > .05$) by treatment was observed in day to first luteal activity and length of first estrual cycle. First estrous cycle and mean peak progesterone concentration were similar between treatments. Days to first standing estrus and first service did not vary between treatments (69.5 d vs 72.31

d for DIP and UIP, respectively). Pregnancy rate and services per pregnancy, as well as first service pregnancy, were similar between treatment groups. Carrol et al. (4) observed an earlier first estrus in cows fed 20% crude protein diet.

CONCLUSIONS

The results obtained in this study suggest that milk production performance at a high level of intake protein (20%) was unaffected by degradable protein source. Our study suggests that under a controlled reproductive management program, feeding a ration containing high levels of CP, regardless of rumen degradability, to dairy cattle in early lactation does not significantly affect general reproductive performance. Since blood plasma levels of urea and estrual cervical mucus did not differ between pregnant and non-pregnant animals, our results do not support the theory that urea from more degradable protein sources has a local toxic effect on gametes or hinder overall reproductive performance.

TABLE 10. Ingredient composition of treatments.

Item 100% dry matter	DIP	UIP
Corn silage	8.6	8.4
Alfalfa hay	22.3	21.9
Alfalfa silage	5.8	5.7
Rolled barley	21.5	21.0
Dist. corn grain	12.8	12.5
Cottonseed meal	11.9	11.7
Beet pulp	8.3	8.2
32% dairy mix[1]	2.1	2.0
Protected fat	1.9	1.8
Mineral vitamin premix[2]	1.0	1.0
Soybean meal	3.9	0.0
Bypass protein	0.0	5.7

[1]Crude protein, 32%; crude fat, 15%; calcium, 2.5%; phosphorus,1.0%; salt, 2.5%; vitamin A (USP units/kg), 66,000; vit D3 (USP units/kg), 16,500; vitamin E (IU/kg),66.

[2]Calcium, 8%; potassium, 3.26%; magnesium, 2.2%; sodium, 6%;phosphorus, 5.5%; sulfur, 3.2%; copper, 400 ppm; iron, 3730 ppm; manganese, 2000 ppm; and zinc, 2000 ppm.

TABLE 11. Added protein source composition.

Soybean meal	Bypass protein
100% SBM	35% Canola Meal
	25% Distillers grain
	10% Fish meal
	10% Blood meal
	10% Feather meal
	10% Meat and bone meal

TABLE 12. Treatment ration nutrient analysis.

Nutrient (DM basis)	DIP	UIP
	-------%-------	
CP	20.0	20.0
DIP[1]	65.0	60.0
UIP[1]	35.0	40.0
DM	68.6	69.5
ADF	25.2	25.2
NDF	45.0	43.1
$NE_{(L)}$ Mcal/kg[1]	1.74	1.74

[1]NRC (34).

TABLE 13. Effect of treatment on production parameters, dry matter intake and body weight change.

Parameter	DIP	UIP	SEM
DM Intake (kg/d)	19.07	18.96	0.37
Milk yield (kg/d)	32.91	33.11	0.55
4% FCM Milk (kg/d)	32.29	32.72	0.75
Production efficiency (4% FCM/DMI)	1.74	1.77	0.06
Body weight change % (kg/16 weeks)	15.8	19.8	6.07

TABLE 14. Effect of treatment on milk composition.

Item	UIP	DIP	SEM
Fat, (%)	3.91	3.95	0.12
Protein, (%)	3.00	3.01	0.02
Lactose, (%)	4.86[a]	4.99[b]	0.05
Solids non-fat, (%)	8.56[a]	8.68[b]	0.04

[a,b]Means in the same row with different superscripts differ ($P < .05$)

TABLE 15. Treatment effect on total tract apparent nutrient digestibility.

Nutrient	DIP	UIP	SEM
	-------%	--------	
DM	55.09	57.65	3.29
CP	64.49	70.32	2.52
ADF	34.38	38.39	4.56
NDF	36.25	41.56	4.59

TABLE 16. Effect of treatment on ammonia and urea concentration in plasma and /or estrual cervical mucus.

Item	DIP	UIP	SEM
Plasma ammonia (monthly avg µg/ml)	0.59	0.57	0.01
Plasma urea (monthly avg mg/dl)	23.16	22.08	0.80
Urea concentration at AI (mg/dl avg all services)			
Plasma	22.88	23.18	0.85
Estrual cervical mucus	21.56	25.23	1.90

TABLE 17. Effect of treatment on reproductive parameters.

Item	DIP	UIP	SEM
Days 1st standing estrus	69.5	72.31	3.94
Days to 1st luteal activity	3.20	34.86	4.82
1st P_4[1] peak, d	52.03	41.50	4.80
1st cycle peak P_4[1] conc., ng/ml	4.37	4.27	0.23
1st cycle mean P_4[1] conc., ng/ml	1.96	2.02	0.96
Length of 1st estrual cycle, d	21.23	21.33	0.80

[1]P_4=Progesterone.

TABLE 18. Effect of treatment on plasma and estrual cervical mucus urea concentration as it affects pregnancy rate.

Item	DIP			UIP		
	P[1]	NP[2]	SE[3]	P	NP	SE
Plasma urea conc. (mg/dl)	22.37	23.39	1.20	23.39	22.96	1.20
Estrual cervical mucus urea conc. (mg/dl)	22.42	20.71	2.70	24.16	23.30	2.70
Pregnancy % *		62.0			57.0	
Services / pregnancy *		1.15			1.33	
1st service pregnancy % *		57.0			36.0	
Embryo mortality % *		37.5			40.0	

*None of the values were found significant under chi-square analysis ($\alpha = .05$).

[1]P=Pregnant
[2]NP=Nonpregnant
[3]SE=Standard error

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CHAPTER IV

EFFECT OF VARIOUS LEVELS OF ESTRUAL MUCUS UREA AND/OR PLASMA AMMONIA ON IN VITRO BOVINE SPERM PROGRESSIVE MOTILITY AND SURVIVABILITY

ABSTRACT

Estrual cervical mucus and blood samples from cows fed a 20% crude protein ration were taken at insemination and evaluated for urea and ammonia concentration. Based on these results, four levels of these metabolites were subjectively selected to study the effects on sperm: in cervical mucus; 25mg/dl, medium urea (MU), and 55mg/dl, high urea (HU); blood plasma, 0.55µg/ml, medium ammonia (MA), and 0.95µg/ml, high ammonia (HA). To re-create physiological conditions, stock solutions of urea and/or ammonia were diluted into tissue culture media (TCM). Five treatments where then prepared: 1) TCM, 2) TCM+MU, 3) TCM+HU, 4) TCM+MU+MA, 5) TCM+HU+HA. Progressive motility (PM) of frozen semen from two bulls was evaluated. Two straws were thawed and incubated in duplicate in petri dishes by using each of the five treatments. Samples were inoculated at 37°C, with data collection occurring at 1, 3, and 6 h post inoculation. At each incubation time, two counts per dish were recorded using a microscope and hemacytometer. Cell survivability (CS) was analyzed using polystyrene culture tubes. At each incubation time samples were immediately frozen in dry ice. For CS analysis, a

bioluminescent somatic assay was conducted. Fifty µl of the cell dilution were thawed and assayed through a luminometer. The results, in relative light units (RLU), were then transferred into ATP units and the proportion of live cells in each sample was determined. There were no significant treatment differences in PM or CS. However, statistical differences due to bull semen and incubation time were observed. Detrimental effects of treatment on sperm PM or CS were not detected.

INTRODUCTION

Dairy cows fed excess crude protein (19.2% dry matter) have been reported as having greater number of days open and requiring more services per conception than cows fed a lower CP ration (12.7%, dry matter) (8). Excess crude protein fed in the ration decreases serum progesterone concentration (9) and uterine magnesium concentration and elevates plasma ammonia, urea, potassium, phosphorus, and uterine urea levels (10). Urea is toxic to sperm and ova (2,15) and can cause abortion when injected intra-amniotically (7). The existence of the urea cycle in bovine spermatozoa has been documented (3) where increasing amounts of ammonia have reduced oxygen uptake by up to 33%, suggesting an interference with cell aerobic respiration. Concentration changes in such plasma and uterine constituents have been suggested to either indirectly or directly reduce fertility of cows fed high levels of dietary crude protein (12). It is not known whether reduced fertility is due to fertilization failure or early embryonic mortality. Ayalon (1) has reported that fertilization failure and embryonic death contribute equally to fertility losses in normal cows. In the case of nonparous beef females, Maurer and Chenault (13) have reported fertilization failure and early embryonic death contribute equally to reproductive failure. In parous females, however, early embryonic death was the suggested cause of reproductive failure. To resolve

the problem of reduced fertility in dairy cows fed high levels of crude protein, both aspects of fertility loss, fertilization failure, and embryonic mortality require investigation. Cervical mucus that accumulates in the vaginal pool may contain endometrial, oviductal, follicular, and peritoneal fluid (11). Therefore, the objective of this study was to determine if cervical mucus concentration of urea and plasma ammonia concentration in cows fed a high level crude protein were detrimental to spermatozoal progressive motility and survivability.

MATERIALS AND METHODS

Two different levels of ammonia in blood plasma, 0.95µg/ml (high), 0.55µg/ml (medium); and two different levels of urea in cervical mucus, 55 mg/dl (high), 25 mg/dl (medium) were found during the lactation trial (chapter III) and subjectively assigned to be used to perform a sperm assay using tissue media culture (TMC) (Dubelcco's Modified Eagle's Medium, DME/F12 1:1 mixture, plus L-Glutamine, Sigma Diagnostics, St Louis, MO). Either urea (Urea carbamide, Fisher Scientific, Co., New Jersey, NJ) and/or ammonia (Ammonia control 5µg/ml, Sigma Diagnostics, St. Louis, MO) was added into solution in order to recreate the physiological environment in which sperm survive. Five treatments were prepared using petri dishes (kimax, Fisher Scientific, Co. Pittsburgh, PA): Treatment 1= Control (TMC), treatment 2=

Medium urea (TMC+MU), treatment 3= High urea (TMC+HU), treatment 4= Medium urea + medium ammonia, (TMC+MU+MA) treatment 5 = High urea + high ammonia (TMC+HU+HA).

Experiment 1. Progressive Motility Assay

The effect of incubating bovine spermatozoa at different levels of urea and ammonia was evaluated. A straw of frozen semen (Select Sires, Logan, UT) from each of two bulls was thawed at 37°C for 30 sec and diluted 1:10 into TMC, 100 µl mixed into previously prepared treatments and incubated at 37°C. One hundred microliters were evaluated for percent progressive motility (PM), assaying replicate counts per duplicate sample (400X) per treatment by using a microscope (Micromaster, model CK, Fisher Scientific, Pittsburgh, PA) and a hemacytometer (Neubauer, American Optical, Co., Buffalo, NY). Incubation times were 1, 3, and 6 h post inoculation.

Experiment 2. Survivability Assay

For this assay, steps similar to the motility assay were taken, using polystyrene culture tubes (Fisher Scientific, Co, Pittsburgh, PA). When incubation times were completed, however, samples were immediately frozen in dry ice and kept at -20° C until all samples had undergone incubation periods. To perform the cell survivability (CS) analysis, a bioluminescent somatic assay was used. The assay was conducted using a bioluminescent assay kit (FL-ASC, Sigma

Diagnostics, St. Louis, MO) designed for the determination of Adenosine 5'-triphosphate (ATP) released from a suspension of viable cells. Fifty microliters of the dilute cell sample were thawed and assayed through a luminometer (LB 9501 Lumat, Messrs Berthold, Germany). The results, relative light units (RLU), were then transferred into ATP units and the proportion of live cells in each sample was determined according to the method of Tung et al. (15).

Statistical Analysis

Treatment effects on sperm progressive motility (PM) and cell survivability (CS) rates were analyzed by one-way analysis of variance. Mean separation was accomplished by Duncan's multiple range test (12).

RESULTS AND DISCUSSION

Percent motility and survivability of bovine spermatozoa incubated at different physiological concentrations of urea in cervical mucus and plasma ammonia are presented in Table 19. Bovine spermatozoa incubated in the five treatments did not show any significant difference ($P > .05$) in PM or CS. However, semen used and duration of incubation were highly significant ($P < .05$) in the reduction of PM and CS (Table 20). These results were expected, since semen from a proven bull and a younger sire were used and there are differences in semen concentration from puberty until mature age (6). A

similar incubation time effect has been reported by others (4, 14). Although concentrations of urea as low as 0.006% have spermicidal properties (2), Umezaki and colleagues (1975) required much higher urea concentrations to obtain a reduction in cell survivability. The greater amount of urea required does not negate the potential usefulness of urea as a contraceptive agent. Solutions with up to 30% urea have been used intravenously for an extended time to treat human cerebral edema without major detrimental effect (16). More recently, 40% urea solutions have been employed for intra-amniotical abortion in the second trimester of pregnancy in humans (7). There have been other studies that have attempted to determine if the uterine environment of cows fed high protein rations is hostile to sperm. Williams et al. (18) found no difference in percent cell motility and/or intact acrosome of sperm incubated in uterine flushings of cows fed 12 or 23% crude protein. Ferguson et al. (4) found no effect of urea concentration on progressive motility of bull sperm in extender using up to 200 mg/dl as a final concentration.

CONCLUSIONS

In summary, at this time the available data are still unclear. Chalupa and Ferguson (5) have indicated that solubility and degradability of protein sources fed in ruminant rations are important aspects that require proper management. As observed in the fermentation trial of this

study and others (5, 10), dietary protein has a major effect in rumen, plasma, and reproductive tract metabolites, such as ammonia and urea. We were not able to determine estrual mucus ammonia concentration accurately. However, ammonia is known to be harmful to mammalian tissue, depressing ciliary movement and creating mucosal degeneration (17). In vitro incubation of bovine sperm in TCM failed to elicit any harmful effect at medium and high physiological levels of estrual mucus urea and plasma ammonia.

TABLE 19. Mean percent progressive motility (PM) and cell survivability (CS) of bovine spermatozoa incubated at different levels of estrual mucus urea and/or plasma ammonia.

	Treatments					
Incubation time (h)	TCM[1]	TCM+MU[2]	TCM+HU[3]	TCM+MU+MA[4]	TCM+HU+HA[5]	SEM
	%					
PM						
1	46.08	52.3	53.44	45.85	47.94	0.88
3	21.83	29.24	29.85	23.13	27.09	0.88
6	14.11	6.43	12.79	9.22	11.97	0.88
CS						
1	79.19	78.94	75.95	71.61	77.23	0.48
3	56.69	58.22	56.43	56.01	56.69	0.48
6	39.13	40.15	39.21	38.95	39.13	0.48

[1]TCM=Tissue culture media.
[2]TCM+MU=Tissue culture media + medium urea.
[3]TCM+HU=Tissue culture media + high urea.
[4]TCM+MU+MA=Tissue culture media + medium urea + medium ammonia.
[5]TCM+HU+HA=Tissue culture media + high urea + high ammonia.

TABLE 20. Mean percent semen and incubation time effect on progressive motility (PM) and cell survivability (CS) on bovine spermatozoa incubated in different levels of estrual mucus urea and/or plasma ammonia.

	PM %	SEM	CS %	SEM
Semen[1]				
1	65.7[a]	6.4	61.3[a]	0.89
2	32.7[b]	6.4	54.4[b]	0.89
Incubation time (h)				
1	72.2[a]	7.8	76.6[a]	1.09
3	45.9[b]	7.8	57.7[b]	1.09
6	29.1[c]	7.8	39.8[c]	1.09

[1]Semen provided by Selects Sires, Co. Logan Utah.
1= Bull # 7H3088
2= Bull # 7H3189
[a,b,c]Means in the same column with the different superscript differ ($P < .05$).

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CHAPTER V

CONCLUSIONS

Results obtained from the in situ trial indicated a very marked difference in dry matter as well as crude protein rate of disappearance between the two protein sources used (soybean meal and bypass protein blend). Nevertheless, when the fermentation trial was performed, ruminal fermentation characteristics were affected minimally regarding protein source fed. It seems as though a 5% difference in ruminal protein degradability does not greatly affect rumen traits regarding microfloral fermentational byproducts, other than some of the volatile fatty acids produced and overall pH. The analysis of estrual cervical mucus urea of those cows synchronized for estrus revealed concentrations similar to those previously found in reproductive tract secretions of cows fed similar levels of crude protein in the ration.

High ruminally degradable protein intake has been implicated in increasing days to first estimated ovulation, services per conception, first service conception, and overall conception rate in high-producing cows. Since blood plasma levels of urea and estrual cervical mucus obtained prior to artificial insemination did not differ between pregnant and non-pregnant animals, our results do not support the theory that urea from more degradable protein sources has a local toxic effect on gametes or hinders overall reproductive performance. Progesterone profile obtained from plasma

analysis on blood obtained throughout the entire trial period did not show significant difference regarding items such as first luteal activity, first estrus, and peak and cyclic progesterone concentration; therefore, these results fail to indicate that there is a systemic effect of protein ruminal byproducts on ovaric metabolism.

Other results obtained during the lactation (production) trial suggest that milk production performance at a high level of intake protein (20%) was unaffected by degradable protein source. There was a significant increase in solids-nonfat, possibly due to the increase in milk lactose percent for those cows fed the bypass protein blend. This agrees with the results obtained in the fermentation trial where high propionate was observed, and as is known, propionate is the most important gluconeogenic source from rumen metabolism.

A study comparing the effect of degradable crude protein intake on cows with or without aggressive reproductive management would help to differentiate whether treatment of disorders and programs to detect estrus accurately prevent possible complications from high degradable crude protein intake. It has been indicated that solubility and degradability of protein sources fed in ruminant rations are important aspects that require proper management; therefore, feeding high crude protein should also be accompanied by a sound reproductive management program.

As observed in the fermentation trial of some studies,

dietary protein has an effect in rumen, plasma, and reproductive tract metabolites, such as ammonia and urea. We were not able to determine estrual mucus ammonia concentration accurately. However, ammonia is known to be harmful to mammalian tissue, depressing ciliary movement and creating mucosal degeneration. In vitro incubation of bovine sperm in a tissue culture medium failed to elicit any harmful effect at medium and high physiological levels of estrual mucus urea and plasma ammonia observed during the production trial.

Effects of crude protein nutrition on reproduction appear complex. Confounding factors such as age, energy, protein degradability, and uterine health may influence responses to varying protein supply. Although protein effects on fertility may be minor within a herd of cows, adverse impacts may happen within some groups, such as older cows or cows with post-partum complications. This may influence culling decisions due to the problem-breeding animals, which eventually affects genetic improvement within the herd. To minimize economic impacts of inefficient protein feeding on production and reproduction, rations should be formulated to provide proper amounts of ruminally degradable and undegradable protein. This study suggests that under a controlled reproductive management program, feeding a ration containing high levels of crude protein, regardless of rumen degradability, to dairy cattle in early lactation does not significantly affect general reproductive performance.

APPENDICES

Appendix A.

Abreviations:

* .....P < .1

** .....P < .05

DM .....dry matter

CP .....crude protein

h .....hours

Bac.....bacteria

ADF.....acid detergent fiber

NDF.....neutral detergent fiber

L .....lactation

T .....treatment

W .....week

AI .....artificial insemination

Std .....standing

Prog.....Progesterone

Conc.....concentration

Appendix B.
Analysis of Variance Tables.

1. DM and CP disappearance (Table II4, Figure II1).

DIP

DM at 12 h			DM at 24h	
Source	DF	MS	Source	MS
Treatment	1	1471.40**	Treatment	3346.00**
Error	4	10.30	Error	16.70
Total	5		Total	

UIP

CP at 12 h			CP at 24 h	
Source	DF	MS	Source	MS
Treatment	1	1395.70**	Treatment	3984.00**
Error	4	0.70	Error	7.70
Total	5		Total	

2. Ruminal fermentation parameters (Table II5, Figure II2 and II3).

Liquid dilution rate

Source	DF	MS
Treatment	1	474.90
Timextreatment	2	12603.25**

Rate of DM passage

Source	DF	MS
Treatment	1	10.40
Timextreatment	2	6298.64**

	1		2
Source	DF	MS	MS
Treatment	1	0.01**	1851.41
Period	1	0.07	25086.23
Cow	5	0.22**	8956.22
Time	1	0.37**	487316.37
Timextreatment	1	0.04	6837.76
Total	9		

		3	4
Source	DF	MS	MS
Treatment	1	633.33**	2.55
Period	1	28.52	28.03
Cow	5	4683.07**	2108.00
Time	1	21881.96**	7386.30
Timextreatment	1	50.47	168.89
Total	9		

Source	DF	5 MS	6 MS
Treatment	1	159.35**	0.07
Period	1	3.26	0.06
Cow	5	156.34**	0.72
Time	1	1387.11**	5.40**
Timextreatment	1	7.82	0.34**
Total	9		

Source	DF	7 MS	8 MS
Treatment	1	77.14**	1.67
Period	1	2.51	0.88**
Cow	5	113.25**	1.35
Time	1	239.39**	20.48**
Timextreatment	1	2.28	0.79**
Total	9		

Source	DF	9 MS	10 MS
Treatment	1	0.45**	3.66
Period	1	0.38	0.02**
Cow	5	0.57**	0.25
Time	1	8.73**	
Timextreatment	1	0.02	0.66**
Total	9		

1 pH, 2 Ammonia-N, 3 Total VFA, 4 Acetate, 5 Propionate, 6 Isobutyrate, 7 Butyrate, 8 Isovalerate, 9 Valerate, 10 Acetate/propionate ratio.

3. Ruminal microbial population (Table II6).

Source	DF	Protozoa MS	Cellulolytic Bac. MS
Treatment	1	0.17	16.62
Period	1	0.52	121.42
Cow	5	0.05	27.09

Source	DF	Total Bac. MS
Treatment	1	0.00
Period	1	67.50
Cow	5	0.31

4. Tract apparent digestibility (Table II7).

Source	DF	ADF MS	NDF MS
Treatment	1	172.74	73.55
Period	1	14.76	12.95
Cow	5	101.47	67.97
Total	7		

Source	DF	CP MS
Treatment	1	11.60
Period	1	113.09
Cow	5	25.88
Total	7	

5. Fermentation trial plasma urea, ammonia and estrual cervical mucus urea conc (Table II8).

Source	DF	Plasma urea MS	Plasma ammonia MS
Treatment	1	2.58	0.21
Period	1	38.72**	1.27**
Cow	5	124.76**	9.70**
Time	1	50.26	0.08
Timextreatment	1	3.27	1.12
Total	9		

Source	DF	Mucus urea MS
Treatment	1	137.65**
Period	1	42.69
Cow	5	76.67**
Time	1	26.32
Timextreatment	1	21.78
Total	9	

6. Production parameters, dry matter intake and body weight (Tables III4 and III5).

Source	DF	DM intake MS	Milk yield MS
Lactation	1	838.62**	5713.74**
Treatment	1	2.22	6.98
LxT	1	195.75	23.81
Cows/TxL	42	74.76	323.23
Week	15	277.58**	768.7**
LxW	15	3.84	46.04**
TxW	15	3.26	11.38
LxTxW	15	6.9	12.76
Error	630	6.15	14.12
Total	735		

Source	DF	4 % FCM Milk MS	Prod. Efficiency MS
Lactation	1	5555.17**	2.56*
Treatment	1	31.74	0.24
LxT	1	9.20	1.36
Cows/TxL	42	383	0.68
Week	15	495.8**	2.03**
LxW	15	67.41**	0.37**
TxW	15	21.88	0.14
LxTxW	15	27.39	0.14
Error	630	26.13	0.16
Total	735		

	Body weight % change		Milk fat %
Source	DF	MS	MS
Lactation	1	3183.90**	0.00
Treatment	1	155.82	0.33
LxT	1	156.22	0.22
Cows/TxL	42	509.974	2.31
Week	15	66.74**	5.6**
LxW	15	80.92**	0.66
TxW	15	8.43	0.2
LxTxW	15	24.54	0.29
Error	630	19.65	0.62
Total	735		

	Milk protein %		Milk Lactose %
Source	DF	MS	MS
Lactation	1	0.97*	3.04**
Treatment	1	0.00	1.60**
LxT	1	1.12*	0.17
Cows/TxL	42	0.33	0.38
Week	15	0.94**	0.41**
LxW	15	0.16	0.11
TxW	15	0.06*	0.12
LxTxW	15	0.1	0.08
Error	630	0.102	0.09
Total	735		

	Milk solids non-fat %	
Source	DF	MS
Lactation	1	8.03**
Treatment	1	4.01**
LxT	1	1.74
Cows/TxL	42	1.02
Week	15	0.22**
LxW	15	0.05
TxW	15	0.08
LxTxW	15	0.1
Error	630	0.07
Total	735	

7. Lactation tract apparent digestibility (Table III6).

		ADF	NDF
Source	DF	MS	MS
Treatment	1	176.30	308.60
Lactation	1	708.40	892.70
TreatxLact	1	922.20	411.80
Error	42	955.40	971.10
Total	45		

		CP	DM
Source	DF	MS	MS
Treatment	1	371.90	71.90
Lactation	1	743.40	311.50
TreatxLact	1	190.40	1048.60
Error	42	292.20	496.4
Total	45		

8. Lactation, ammonia (/month) and urea conc. in plasma (/month and at AI) and mucus (at AI) (Table III7).

Plasma ammonia (/month)

Source	DF	MS
Treatment	1	0.02
Cow(treat)	28	0.01
Month	3	0.11
Treatxmont	3	0.01

Source	DF	Mucus urea (at AI) MS	Plasma urea (at AI) MS
Treatment	1	468.60	0.8
Pregnancy	1	126.00	0.78
Treaxpreg	1	268.00	4.78
Error	36	145.50	28.85
Total	39		

Plasma urea (/month)

Source	DF	MS
Treatment	1	189.72
Lactation	1	1257.64**
LxT	1	0.54
Cows/TxL	42	86.26
Month	15	195.96**
TxM	15	13.15
LxM	15	45.53*
LxTxM	15	5.3
Error	630	14.56
Total	735	

9. Reproductive parameters (Table III8).

Source	DF	Days to 1st std. Heat MS	Days to 1st Luteal activity MS
Treatment	1	81.7	669.1
Lactation	1	1695.3	202.6
Treatxlact	1	1071.8	857.8
Error	39	667.8	332
Total	42		

Source	DF	Days to 1st Prog. peak MS	Peak Prog. conc. MS
Treatment	1	1018.8*	0.09
Lactation	1	204.2	0.35
Treatxlact	1	866.7	0.33
Error	39	307.6	2.13
Total	42		

	Mean Prog. Conc.		Days first cycle
Source	DF	MS	MS
Treatment	1	0.03	0.1
Lactation	1	0.01	6.98
Treatxlact	1	0.36	65.64
Error	39	0.36	25.34
Total	42		

10. Mean % motility and survivability of incubated bovine sperm (Tables IV1 and IV2).

	% motility		% survivability
Source	DF	MS	MS
Treatment	4	15.2	5.5
Error	10	377.8	349.7
Total	14		

	% motility		% survivability
Source	DF	MS	MS
Treatment	4	90.7	10.46
Time	2	4832.1**	3401.88**
Bull	1	8166.69**	363.63**
Treatxtime	8	223.35	4.61

CURRICULUM VITAE

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CURRENT ADDRESS

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Place of birth: Tegucigalpa, Honduras, C.A.
Birthdate: August 21, 1959.

Marital Status: married
Norma - spouse
Bernie - son
Norma L. - daughter

EDUCATION

Ph.D. Animal Nutrition. Expected completion fall 1992. Major area of study: Dairy cattle nutrition.

M.S. Animal Reproductive Physiology. 1983-1985. Mississippi State University.

Thesis title: Synchronization of estrus in early diestral dairy heifers with prostaglandin $F_{2}\alpha$ and estradiol benzoate.

B.S. Animal Science. 1979-1980. University of Florida.
Agronomy. 1976-1978. Escuela Agricola Panamericana. Honduras. Central America.

WORK EXPERIENCE

1980-1983 Extension officer for the Ministerio de Recursos Naturales, Honduras. Responsibilities: Technician in the artificial insemination program.

1985-1989 Sub director of the Regional Livestock Program of the Ministerio de Recursos Naturales at San Pedro Sula, Honduras. Responsibilities: Organization and execution of artificial insemination training program for farmers and technicians.

1987-1989 Teaching position at the University of San Pedro Sula, Honduras. Courses taught; animal physiology, dairy cattle, and animal nutrition.

1989- Currently working at Skaggs Nutrition Lab in the Animal, Dairy and Veterinary Science Dept. at Utah State University, Logan Utah. Responsibilities include: Design, collection and analysis of data from studies with cows, and forage. Served as teaching assistant for courses in mineral metabolism and animal nutrition laboratory.

PUBLICATIONS

Figueroa, M. R., D. P. Dawson, C. E. Batallas, D. Y. Kim, M. J. Arambel and J. L. Walters. 1992. Nutritional and physiological effects of feeding two levels of rumen degradable and undegradable protein. J. of Dairy Sci. 75 (supplement 1). (Abstract).

Figueroa, M. R., D. P. Dawson, D. Y. Kim, C. E. Batallas, B. A. Kent, M. J. Arambel and J. L. Walters. 1992. Effect of rumen undegradable intake protein on reproductive parameters in post-partum lactating cows. J. of Dairy Sci. 75 (supplement 1). (Abstract).

Kim, D. Y., M. K. Wandersee, C. E. Batallas, D. P. Dawson, B. A. Kent, M. R. Figueroa, M. J. Arambel and J. L. Walters. 1992. Effect of feeding yeast culture with or without Aspergillus oryzae on dry matter disappearance in three different forage types. J. of Dairy Sci. 75 (supplement 1). (Abstract).

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Batallas, C. E., D. P. Dawson, M. R. Figueroa, D. Y. Kim, B. A. Kent, M. J. Arambel, and J. L. Walters. 1992. Effect of feeding a high level of rumen protected fat with rumen undegradable protein and niacin on rumen fermentation characteristics. J. Dairy Sci. 75 (supplement 1). (Abstract).

Figueroa, M. R., B. Kent, D. P. Dawson, C. E. Batallas, and M. J. Arambel. 1991. Evaluation of an enzyme containing feed additive for dairy cattle rations on ruminal fermentation characteristics and nutrient digestibility. J. of Dairy Sc. 74 (supplement 1):255. (Abstract).

Figueroa, M. R., J.W. Fuquay, and S. K. Shipley. 1988. Synchronization of early diestral dairy heifers with prostaglandin $F_{2}\alpha$ and estradiol benzoate. Theriogenology. Dec. VOL. 30 NO. 6 :1093.

Figueroa, M. R. 1987. Folleto practico; Practicas en Inseminacion Artificial. Ministerio de Recursos Naturales. Honduras.

Bunch, T. D., R. J. Callan, A. Maciulis, M. R. Figueroa, J.C. Dalton. 1991. True Hermaphroditism in a wild sheep: A clinical report. Theriogenology. Aug. VOL. 36 NO. 2:185.

PROFESSIONAL ORGANIZATIONS

American Dairy Science Asociation, (1992).
American Society of Animal Science, (1992).
Asociacion Hondurena de Produccion Animal (APHA, 1982).
Asociacion de Graduados de la Escuela Agicola Panamericana (AGEAP, 1978).

PRESENTATIONS

1991 American Dairy Science Association Meeting (Utah State University, Logan, Utah).
1992 American Dairy Science Association Meeting (Ohio State University, Columbus, Ohio).