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David Paul Casper

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EVALUATION OF UREA AND DRIED. WHEY IN DIETS *Of* COWS DURING EARLY LACTATION

By

DAVID PAUL CASPER

A thesis submitted in partial fulfillment of the requirement for the degree Master of Science South Dakota State University 1985

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EVALUATION OF UREA AND DRIED WHEY IN DIETS OF COWS DURING EARLY LACTATION

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

David J. Schingoethe
Thesis Advisor

.Date

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DPC

ABSTRACT

Previous research indicated that soluble nitrogen may be utilized more efficiently for rumen microbial production in diets containing dried whey. To further evaluate this, 33 Holstein ,cows (30 multiparous and 3 primiparous) were fed one of three concentrate mixes containing all natural proteins (SBM) , 1% urea (U), or 1% urea and 30% dried whey (UDW) from wk 3 through 16 postpartum. Urea replaced portions of the soybean meal in the SBM mix while dried whey replaced portions of the corn and soybean meal. Cows were fed total mixed rations consisting of 40% (dry matter basis) corn silage, 10% alfalfa hay, and 50% of respective concentrate mix. Diets were formulated to be isonitrogenous at 16% crude protein, but soluble nitrogen was formulated to be approximately 23, 30, and 42% of the total nitrogen for SBM, U, and UDW diets. Milk production and composition were adjusted by analysis of covariance using the second week milk production and composition as covariates. Milk kg/day) for **cows** fed SBM, U, and UDW, respectively, yield was similar (33.8, 33.4, and 33.2 indicating that diets high in soluble nitrogen can support milk production equal to that of natural protein supplemented rations. · Production of 4% fat-corrected milk (29.9, 28.0, and 29.2 kg/day) was lower for cows fed U diet because of lower milk fat percentages (3.23, 2.94, and

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3.23%). Milk protein percentages (3.10, 3.04, and 3.04%) and solids-not-fat percentages (8.74, 8.79, and 8.81%) were not affected by type of concentrate fed. Milk solid percentages (12.02, 11.70, and 12.01%) and production of solids-corrected milk (30.3, 28.6, and 29.6 kg/day) were higher for cows fed SBM and UDW versus U. Dry matter intakes $(22.0, 20.2, and 23.1 kg/day)$ were highest for cows fed UDW and lowest when fed U. Rumen parameters showed a decrease in molar percentages of acetate (56.6, 50.3, and 50.2%) for cows fed U, and UDW, but cows fed U had higher molar percentages of propionate (24.8, 28.6, and 25.0%). Molar percentages of butyrate were higher (13.6, 14.4, and 18.4%) for cows fed UDW. Ratios of acetate+butyrate:propionate were highest (2.95, 2.40, and 2.93) for cows fed SBM and UDW. Concentrations of rumen ammonia (11.8, 20.3, and 13.5 mg/dl) and serum urea (19.5, 22.9, and 16.5 mg/dl) were highest for cows fed U. In conclusion production was improved by adding dried whey to stimulate rumen microbial protein synthesis to diets of early lactation cows. utilization of urea nitrogen for milk

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INTRODUCTION

Feeding adequate amounts of protein to lactating cows is one of the most expensive and important aspects of dairy cows nutrition. Protein requirements of these cows can be met by feeding both natural proteins and nonprotein nitrogen (14, 17, 35, 38, 68, 69, 77, 78). As more high quality natural proteins are used for direct human consumption, .finding alternate sources of protein and utilization of nonprotein nitrogen in ruminant rations becomes increasingly important politically and economically.

Whey, a highly nutritious by-product of the cheese manufacturing industry, can be effectively utilized as a feedstuff for livestock (79). Dried whole whey contains mainly (70%) lactose, which is readily fermented in the rumen to various short chain acids. Indirect evidence indicated that the utilization of ammonia nitrogen was being increased when diets contained lactose by stimulating rumen microbial protein synthesis (52, 74, 90, 98).

Several studies (17, 18, 69, 75, 77) showed increased milk production when cows were fed diets containing less soluble or natural proteins over that of high soluble protein sources. However, other studies (24, 33, 47, 48, 57, 92) suggest that highly soluble nonprotein nitrogen sources such as urea will support adequate milk

production when compared to all natural protein sources. No studies have evaluated the combination of urea as a soluble source of nitrogen with a highly readily fermentable carbohydrate as dried whole whey in diets fed to lactating cows.

The objective of the research was to ascertain the lactational response of cows during early lactation to diets containing a greater proportion of nonprotein nitrogen and a readily fermentable source of energy.

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LITERATURE REVIEW

Feeding Whey

Whey, a highly nutritious by-product of the cheese manufacturing industry, can be effectively utilized as a feedstuff for livestock. Whey may be fed as liquid whey $(2, 3, 4, 51, 61, 70, 96)$, condensed whey (97) , fermented ammoniated condensed whey (21, 23, 28, 36, 45, 66), dried whey $(37, 40; 73, 81, 82, 83, 84, 98)$, and dried whey products $(11, 49, 54, 86, 87)$. The use of whey and whey products in animal feeding was recently reviewed (56, 79). The uses of lactose in animal and human nutrition were reviewed earlier (7). Therefore, I will not review all aspects of feeding whey, but will emphasize only those areas related to feeding of ruminants.

Liquid Whey. Liquid whey is mostly (93%) water but the highly nutritious whey solids contain 77% lactose and 14% protein (80). Two types of liquid whey are available: 1) sweet whey results from Cheddar cheese production and 2) acid whey is obtained from cottage cheese production.

Several researchers have successfully fed liquid whey to ruminants (3, 4, 50, 61, 96, 97). Anderson et al. (4) reported that one dairy cow can consume the whey produced from three to five cows. Milk production and composition were not affected by liquid whey intake even when fresh water was not provided. Recent data by Pinchasov et al. (61) showed that feeding liquid acid whey to cows in early lactation tended to have lower milk production but a higher production of 3.5% fat corrected milk. These trends of lower milk production were also observed in other studies (4, 59). However, production of 3.5% fat-corrected milk was higher (P<.01) during days 15 to 69 postpartum.

whey. was the only source of liquid, cows consumed about 90 kg/day. However, Welch and Nilson (96) reported intakes as high as 136 kg/day for extended periods of time while Cows can readily consume large amounts of liquid Anderson et al. (4) reported that when liquid whey maintaining production. Pinchasov et al. (61) reported liquid whey intakes rose from 35 to 54 liters/day with some cows consuming as high as 86 liters/day. Increasing intakes were attributed to the increased nutrient requirements as production rose, however cows maintained uniform whey intake proportional to total dry matter consumption. Increased urination and manure handling can be a problem at these high levels (70, 96).

The consumption of liquid whey can significantly reduce the consumption of hay or grain. Welch et al. (97) reported a mixture of condensed whey and molasses fed at 5 kg/cow/day resulted in a reduction of hay consumption with

no effects on milk production and health. Hay refusals were also reported by (4) , but indicated refusals were higher in leaf content probably indicating a need for fiber to maintain gut fill (16). Restricting grain to less the 1 per 4 kg milk produced did not increase whey consumption, but actually resulted in a 10% decrease of feed intake and milk production (3). This probably indicated that. gut fill was limiting feed intake. With cattle fed high concentrate rations (83:17 concentrate:forage), feeding large amounts of whey caused a decrease in concentrate intake and an increase in hay consumption (61).

Ration digestibility of dry matter was increased from 54.5 to 61.3% by addition of liquid whey to a basal diet in cows and steers (70). Although cows were offered more hay and less soybean meal than steers, there was no difference in digestibility due to sexes. Acid detergent fiber digestion tended to be lower for the liquid whey diet due to the source of readily fermentable carbohydrates. Increasing the amounts of readily fermentable sources of carbohydrates or the grain to forage ratio decreased fiber digestibility in (52, 58, 90). Earlier work by Anderson (2) reported that the digestibility of liquid whey dry matter was approximately 87% when whey solids constituted 29% of the ration. Protein digestibility ranged from 73 to

81.6%. These results were later confirmed by (70) indicating that liquid whey is a readily available source of nutrients.

Liquid whey can be used as a satisfactory feedstuff for growing ruminants weighing over 250 kg (60). Nilson and Welch (60) showed that calves over 250 kg had very satisfactory gains with hay and liquid whey but also recommended the limitation for whey to calves under 200 kg. Calves weighing approximately 150 kg will consume only enough whey to satisfy their liquid requirement indicating need for proper diet formulation (50). Holstein steers fed a restricted grain finishing diet were able to consume 57% of their total dry matter intake from acid whey (51). However, weight gains of steers were only 65% of the gains of those fed grain ad libitum due to reduction of dry matter intake to 81% of ad libitum fed steers. Carcass dressing percent, rib fat cover, and loin eye area were lower, but over all carcass tenderness and desirability were not affected by whey consumption. Heifers fed liquid whey had faster weight gains than heifers fed control diets (4, 96). Heifers fed whey and grain gained 19% faster than those fed whey or grain alone (4).

whey feeding, but none have been insurmountable to prevent th~ Several problems have been encountered with liquid economical advantages of liquid whey feeding (61). All

animals, regardless of age, required an adjustment period to adapt the digestive system before whey was consumed readily (4, 50). Slight diarrhea occurred in some animals that consumed too much whey until fully adjusted (4). Lynch et al. (51) observed several cases of bloat in Holstein steers fed acid whey but prevented bloat by feeding timothy hay at .4% of body weight. This probably resulted in slowing down fermentation of readily available nutrients· by maintaining a more normal rumen population thereby giving animals sufficient time for eructation of gas (16).

When palatability problems as long as it was fresh (97). Palatability of whey is lowered as the whey becomes more feeding liquid whey, there were no acidic. Whey kept over 36 hours at ambient temperatures was not consumed readily (4, 60, 97), although 24 hour old whey was acceptable (4). Acid resistant tanks, (such as plastic, stainless steel, or glass-lined milk tanks), should be used for storage and feeding of whey to prevent corrosion (60, 79). Proper sanitation is needed in feeding whey or whey products to animals, to prevent growth of bacteria and attraction of flies especially during warm weather (4, 59, 79).

Dried Whey And Whey Products . Dried whole whey and whey products appear to be acceptable ruminant

feedstuffs (79). Advantages of using dried whey instead of liquid whey include the ease of transportation and the ability for longer storage times. However, the cost of drying the liquid whey must be considered.

Adding , dried whey or whey products to high grain-low roughage, milk fat depressing rations will return milk fat . percentages back toward normal (37, 40, 73, 86). Whey minerals . are most likely responsible for maintaining milk fat percent, although lactose may be responsible to a lesser extent (86). Rosser et al. (73) postulated that maintaining milk fat percentage on high concentrate diets by the addition of whey or whey products could be enhancing triglyceride transport into the mammary gland. An advantage of using dried whey or whey products as a feed additive to prevent milk fat depression on high concentrate rations is that whey is very palatable (79).

With rations containing adequate amounts of roughage, actual milk production was similar or slightly lower when fed a concentrate mix containing small amounts (i.e. 5% or less of concentrate mix) of dried whole whey or whey products (81, 83). However, 4% fat-corrected milk and fat percentages were higher $(P₀, 05)$ for cows receiving the whey rations $(81, 83)$.

Feeding large amounts of dried whole whey (65% of concentrate mix) to lactating cows resulted in lower milk

production; however, 4% fat-corrected milk and solids-corrected milk were similar due to increases in milk fat percent and milk protein percent (84). Slight diarrhea was observed in some of the cows. Steers consuming large amounts of dried whey (65% to 86% of concentrate mix) had dry matter intakes and weight gains similar to control animals (46, 85). Urine output increased when steers were fed 45 to 60% dried whey. This was also observed when feeding liquid whey (4) due to increased mineral consumption from whey, but with no apparent harm to the animals (4, 85). Dry matter content of feces was reduced when rations contained 30% or more lactose as lactose or as dried whey in several studies (8, 84, 85), although King and Schingoethe (46) observed no decrease in fecal dry matter in steers when fed 60% of the total dry matter as dried whole whey. Ruminants have the capability to consume large amounts of dried whey or whey products without experiencing any serious health or production problems.

Fermented ammoniated condensed whey. The basic idea underlying fermented ammoniated condensed whey (FACW) is to ferment the lactose in whey to lactic acid with Lactobacillus bulgaricus and to maintain the pH nearly neutral with continuous infusion of anhydrous ammonia, producing ammonium lactate (5, 66). The ammonium lactate would then serve as a nonprotein nitrogen source for

ruminants (45). Hazzard et product was unpalat~ble to **cows** when fed at 10% of the al. (28) reported that the concentrate Henderson et al. (30) and Henderson et al. (31) reported mix. However, Crickenberger et al. (20), that FACW stimulated daily dry matter consumption when fed at normal required levels and had only a slight depressing effect when fed at twice the required level (20). Welch et al. (97) showed that acceptability of a condensed whey product equal amounts of molasses. This would suggest that improved substantially when it was mixed with condensed whey products are unpalatable fed at high levels unless fed with a flavoring agent to increase palatability.

Huber et al. (36) fed FACW at 9 and 18% of the concentrate mix in place of soybean meal to lactating dairy cows averaging 130 days postpartum. Results showed that FACW was equal to soybean meal or urea in maintaining milk yields and feed efficiency when FACW furnished 27% of the nitrogen in well-mixed rations. They observed no palatability problems as intakes were not different from the soybean protein which is adequate during later lactation; however, meal diet. These rations contained 13% crude since FACW is a nonprotein nitrogen source, one might question if results would be as favorable during early lactation. Erdman et al. (23) reported that increasing FACW to 100% replacement of soybean meal in a

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40%concentrate-60% corn silage ration reduced dry matter intakes and milk production of cows during early lactation. However, it was pointed out that FACW was fed at 3 times that reported earlier (36) where intake was not reduced by FACW addition at 18% of concentrate mix.

Juenst (45) listed some advantages of converting whey to FACW as a feed supplement for ruminants. They include: 1) all the whey solids are used with no further processing required, 2) concentration of whey to 60-70% solids requires less energy than spray drying, 3) FACW has an elevated crude protein to about 45% versus 7% for condensed whey, 4) FACW contains true protein in form of whey proteins and microbial cells, 5) fermentation results in little energy loss of product, and 6) FACW is superior to urea as a source of nonprotein nitrogen because of a readily available energy source which makes FACW much less toxic.

butyrate increased, and propionate usually decreased when whey or whey products were fed to cattle or sheep (2, 11, 37, 40, 46, 61, 73, 81, 84, 85, 86, 87, 91, 98). Changes Rumen metabolism . Ruminal molar percentage of in acetate concentrations were not consistent when whey was fed. Schingoethe and Skyberg (84) noted decreased molar percentages of acetate when lactating cows were fed dried whole whey. This observation was also reported by

Pinchasov et al. (61) , and Anderson (2) when lactating cows were fed liquid whey. Addition of a dried whey-fat blend product at 30% of grain mix also resulted in decreased molar percentages of acetate as well (87). In contrast, Windschitl and Schingoethe (98), and Schingoethe et al. (86) observed no change in rumen acetate when dried whey or whey products were fed. King and Schingoethe (46) observed an increased molar percent of acetate when steers were fed dried whey.

The increased butyrate observed when whey or whey products are fed may be attributed to lactose fermentation (40). Data of Schingoethe et al. (85) also support this, since butyrate was highest in rations that contained the highest lactose. Satter and Esdale (76) indicated that butyrate is the ultimate end product of lactate metabolism. The oxidation of lactate to pyruvate resulted in butyrate synthesis from acetate in an attempt to maintain an oxidation-reduction balance. The production of butyrate rids the rumen of excess hydrogen ions and reduces acidity which may be detrimental to rumen fermentation. Total rumen volatile fatty acid (VFA) concentrations generally are not affected by addition of whey or whey products (40, 46, 63, 81, 84, 85, 86, 87, 98), although total VFA production may be increased (98).

Rumen ammonia concentrations tended to be lower in

animals fed whey (46, 84, 85, 91, 98). Thivend and Ehoninsou (91) observed a lowered rumen ammonia concentration when sheep were fed a urea lactose diet. Poncet and Rayssiguier (63) reported decreased rumen ammonia and blood urea concentrations when sheep were fed a lactose-hay diet. The lowered ammonia concentrations observed would suggest more ammonia was being utilized for rumen microbial protein synthesis, since lactose is a readily fermentable energy source. Windschitl and Schingoethe (98) reported increased rumen microbial protein synthesis in cows fed a diet containing 38% dried whole whey as measured indirectly by use of bacterial and protozoa fermentable markers. sources of energy will stimulate rumen This would indicate that readily microbial protein synthesis. Stern et al. (90) reported that as the level of nonstructural carbohydrates was increased by addition of starch, there was a decrease in ammonia concentrations resulting in more microbial protein synthesized. Whey would be classified as a nonstructural carbohydrate. Another in vitro experiment (74) reported that ammonia production was always inversely proportional to rate of carbohydrate additions and that cell growth was limited by the amount of carbohydrates fed. MacGregor et al. (52) observed lower rumen ammonia concentrations in lactating dairy cows fed diets containing higher amounts of 414138

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nonstructural controls. carbohydrates (32.9%) versus (24.9%) for

Huber et al. (40) reported that pH of rumen contents was not significantly affected by type or amount of whey fed. They stated that partially delactosed whey may exhibit a buffering effect due to the high mineral content. Schingoethe et al. (86) also suggested that whey minerals play a role in maintaining rumen pH at an acceptable level. The mechanism for maintaining rumen pH is thought to be that the high sodium concentration of whey may stimulate increased salivary flow and water consumption (71, 72). Rogers et al. (71) reported that cows fed a diet supplemented with 2.0% sodium chloride or sodium bicarbonate had higher water intakes and higher ruminal $pH's$. FACW generally does not elevate rumen pH (45) which may make it superior to urea as a nonprotein nitrogen source. Pinchasov et al. (61) observed a decrease in rumen pH when lactating cows (in early lactation) were drinking acid whey. Trials done by (65, 91) resulted in a decrease in pH when lactose was fermented in the rumen of sheep. Lactate concentrations were elevated in both trials (61, 65).

Poncet and Rayssiguier (63) observed improved total ration organic matter digestibility when lactose was added to lucerne hay diets. But, a decrease in acid detergent

fiber digestibility of hay portion was also observed. Boman and Huber (11) also observed decreased digestibilities of crude fiber and ether extract when lactose was included in the ration. Lynch and Bond (49) reported increased feed efficiency and improved nitrogen retention and utilization in calves fed liquid lactose. Schingoethe et al. (85) reported higher digestibilities of energy and dry. matter in steers fed diets containing 10 to 40% lactose either as lactose or dried whole whey versus controls. Ration fiber digestibility was not different. When diets contained only small amounts of lactose as supplied by a 5% dried whey product in concentrate mix; no effect on digestibility of dry matter, nitrogen, or energy versus controls (82).

Absorption and retention of calcium, phosphorus, and magnesium were not affected by addition of lactose or dried whey to rations of steers or cows (82, 85). Adding small amounts of dried whey may increase mineral absorption and retention in nonruminants (79), but may not in ruminants because the lactose in dried whey is fermented in the rumen and unavailable for aiding absorption from the small intestine (46, 82). Rayssiguier and Poncet (65) reported 400g lactose addition to a diet of lucerne hay for sheep increased (P<.05) apparent absorption of magnesium, calcium, and phosphorus. Absorption of potassium was

slightly decreased. They concluded that readily fermentable carbohydrates increase mineral absorption as related to changes in fermentation patterns.

Metzger et al. (54) observed the effects of whey products on high grain, restricted roughage rations to determine the effect on rumen microflora. Protozoal numbers were simllar for all types of whey products except demineralized whey where numbers tended to be lower. Bacterial numbers increased during the high-grain feeding period. The number of lactose. fermenters increased on diets containing whey or whey products. No differences in numbers of starch digesters were observed among experimental treatments. This would support data by King and Schingoethe (46) that ruminants can ferment large amounts of dried whey and that little or no lactose escapes degradation in rumen. Grummer et al. (27) reported that when defaunation of steers fed a diet containing 45% dried whole whey, rumen fermentation changed from a high butyrate to a high propionate fermentation. They attributed this to the fact that ciliated protozoa are major high butyrate producers, and their disappearance resulted in a loss of butyrate production.

Utilization Of Nonprotein nitrogen and the manufacturer

Future world food demands may dictate that ruminants consume nonprotein nitrogen (NPN) to their

maximum utilizable capacity; however, for the past two decades the incorporation of NPN into dairy cattle rations has been based on economics (18, 35). The profitability of substituting NPN as a crude protein source for natural protein supplements in dairy cattle rations depends on a ,. number of factors: 1) cost of the NPN source, 2) relative price of the energy and the natural protein which is being replaced, and 3) the animal response to substitution reflected in changes in milk yield. Extensive excellent reviews have been written on the types of NPN compounds and their utilization in ruminant nutrition (18, 29, 34, 35, 38, 68, 69, 77). Due to the quantity and diversity of material, I will review only those articles pertaining to urea utilization in lactating dairy rations.

Benefit to cows from urea is totally dependent upon its use for making microbial protein (35). Urea is readily hydrolyzed to ammonia by ruminal ureases (16), which is the central compound for microbial protein biosynthesis in the rumen. If rumen bacteria do not use NPN for protein synthesis, it is of no benefit to the animal and represents a waste of nitrogen (18).

Allison (1) reported that 82% of the bacterial strains isolated grew with ammonia as their main nitrogen source, especially the cellulolytic bacteria (12). The amounts of NPN that can be synthesized into microbial

protein is fermentable energy in the ration (77). Studies of Satter dependant on the availability of readily and Slyter (78) using in vitro rumen fermentors concluded that microbial protein synthesis· was maximized when rumen ammonia concentrations reached 2 to 5 mg/dl. In contrast, Miller (55) and Mekrey et al. (53) suggested that microbial protein synthesis *was* maximum when rumen ammonia concentrations were 20 to 29 mg/dl. In vivo studies indicate that microbial protein synthesis is not maximized until rumen ammonia concentrations are 10 (43) to 29 (55) $mg/d1$.

Roffler and Satter (69) summarized data from ³⁹ experiments on nonammonia nitrogen entering the small intestine. Nonammonia nitrogen passage increased as long as the crude protein in rations increased, but there was a plateauing effect as level of NPN was raised. The strong dependence of microbial nitrogen utilization on the energy content of rations was the basis for development of models to recommend NPN incorporation based primarily on dietary energy (13, 14, 68, 77).

The maximum dietary protein at which NPN additions may benefit lactating dairy cattle is still highly controversial. Huber and Kung (38) suggested NPN additions are not beneficial when dietary crude protein exceeds 15% of the ration dry matter at even high energy

concentrations, providing proteins have not been selected or treated for low ruminal solubility. However, Roffler and Satter (69), after reviewing 39 experimental comparisons, stated that typical dairy rations above 12 to 13% crude protein do not warrant addition of NPN. In contrast, Jones et al. (44) reported a trend in increased milk yield· when raising the crude protein from 14.5 to 15.8% by addition of NPN. Virtanen (95) reported that cows fed a purified protein free diet supplemented with urea supported milk production of 24 kg/day indicating sufficient production. protein synthesis for maintenance and

Satter and Roffler (77) and Clark and Davis (18) suggested that rations for cows in early lactation be supplemented with only natural proteins. They further proposed that supplemental NPN should be used in rations containing 12% crude protein after 14 weeks postpartum. Kwan et al. (48) reported that cows in early lactation can use urea nitrogen when high-energy complete rations are fed and attain milk production levels similar to controls. Holter et al. (33) reported that feeding 1.5% urea in the concentrate mix to high producing Holstein cows over a complete lactation obtained the same milk and fat production as the control diet. Several studies have suggested that urea feeding is compatible to high milk

production in early lactation (24, 33, 47, 48, 57, 92). In contrast (99), production of 4% fat-corrected-milk was higher when cows were fed higher levels of crude protein. The increased response was less when the concentrate contained NPN versus soybean meal, indicating that at higher crude protein levels NPN is of less value as a protein supplement than natural proteins.

Wohlt et al. (99) reported decreased milk yields in cows fed a 14.5% crude protein ration containing 50% of the supplemental nitrogen as urea. However, the results might be partially explained by the fact that concentrate was fed at 1 kg/2.75 kg milk and initial milk production was lower for the urea supplemented group. Huber et al. (36) fed a diet supplemented with 2.5% urea in the concentrate mix to mid lactation dairy cows and observed no difference in production over controls. In numerous experiments, cows producing at moderate production have shown no differences in milk yields (33, 39, 42, 48) indicating that NPN is equivalent to natural protein supplements when diets contain approximately 12% crude protein.

There are at least three reasons why lactating cows response to NPN might be suboptimal (18): 1) fewer amino acids may be synthesized by the microflora to reach absorption sites in the small intestine, 2) excess ammonia caused by rapid degradation of NPN may reduce feed intake,

and 3) excess ammonia may overload the detoxifying systems in the liver causing derangements in metabolism in the liver and other tissues. A decrease in synthesis of amino acids by supplementation with NPN has yet to be proven; however, a reduction in feed intake is not observed if ·· recommended levels of NPN are fed (35). Spires and Clark (88) reported that addition of ammonium chloride inhibited glucose synthesis of liver tissue slices in vitro by 25 to 30%. Feeding practices that increase efficiency of NPN utilization may do so by minimizing the detrimental effects of ammonia on glucose synthesis. This may be especially pertinent for lactating cows since lactose synthesis requires 60 to 80% of the available glucose (17).

Reports of depressed milk production by supplementation of urea are usually the result of NPN fed in excess of recommended limits of 225 to 250 g/day (35), which translates to 1.5% of the concentrate or 1.1% of a total mixed ration (18, 41, 62, 93, 94). Thus, the lactating dairy cow should be able to support high levels of milk production by using nonprotein nitrogen at recommended levels for the synthesis of microbial protein in early lactation.

A review of the literature, has indicated that increasing the amount of readily fermentable carbohydrates may enhance the utilization of NPN for microbial protein

synthesis in the diets of high producing early lactation dairy cows (52, 74, 90, 98). The state of the computation of the state of the s

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MATERIALS AND METHODS

A 14 wk lactation trial utilized 33 Holstein cows (30 multiparous and 3 primiparous) randomly assigned at approximately 2 wk postpartum to 1 of 3 treatments to measure the response to higher levels of soluble nitrogen and readily fermentable carbohydrates. Treatment 1, designated SBM, consisted of soybean meal as the main protein supplemeat in the concentrate **mix.** Treatment 2, designated U, contained 1% urea in the concentrate mix replacing a portion of the soybean meal in the concentrate mix. Urea increased the proportion of soluble nitrogen in the diet. Treatment 3, designated UDW, consisted of 1% urea and 30% dried whole whey in replacing portions .of corn and soybean meal in the concentrate mix. Urea and dried whole whey increased the proportions of soluble nitrogen and readily fermentable carbohydrates in the concentrate mix. Composition of experimental concentrate mixes is given in Table 1. Total mix rations were formulated to be isonitrogenous at 16% crude protein and consisted of 40% (dry matter basis) corn silage, 10% alfalfa hay, and 50% of the respective concentrate mix.

Cows were housed in a free stall barn and individually fed the respective total mixed ration ad libitum once daily using Calan feeding doors. Cows were gradually switched to their respective treatment rations the last few days of the

Table 1. Composition of concentrate mixes containing soybean meal (SBM), urea (U), and urea dried whey (UDW).¹

 1 Plus 8818 IU of Vitamin A, 1764 IU of Vitamin D, and .88 IU of Vitamin E/kg of concentrate mix.

second wk postpartum with the third wk postpartum as initiation of the 14 wk experimental period.

Cows were milked twice daily with milk weights recorded at each milking throughout experimental period. Two 24-h (p.m. plus a.m.) milk samples were collected from ·each cow during wk 2 postpartum (pretreatment) and one 24-h sample was taken every 2 wk throughout the trial. Samples were analyzed fot fat by Babcock (6), total solids by Mojonnier (6), and crude protein by Kjeldahl procedure (6). Body weights were recorded three eonsecutive days at the start and end of experimental period as well as once every two weeks during experimental period.

Concentrate mixes, corn silage, and alfalfa hay were sampled weekly throughout duration of trial. Four weekly samples were combined into a monthly composite for analyses of dry matter, crude protein, ether extract, and ash according to the procedures of Association of Official Analytical Chemists (6). Acid detergent fiber and lignin were determined by the procedures of Goering and Van Soest (25) and neutral detergent fiber by the procedure of Robertson and Van Soest (67). Determination of soluble nitrogen of feedstuffs was done by two methods. The first method was the modified Burroughs' mineral buffer solution (22) as further modified (64) for determination of residue nitrogen to eliminate correction for background ammonium

sulfate to obtain an estimate of protein solubility. The second method described by Sahlu et al. (75) was used to obtain an estimate of protein solubility and also protein degradability. Protein solubility and degradability values were also obtained on a total mixed ration sample made from monthly composites to be used as a comparison with calculated values from individuals measurements.

Rumen contents were sampled via esophageal tube two to four hours after feeding into a 250 ml sample bottle containing .5 ml of saturated mercuric chloride. Samples were analyzed for pH by a glass electrode pH meter. Samples were then strained through four layers of cheese cloth. A 10 ml aliquot was centrifuged at 1500 rpm for 10 minutes • The supernatant was decanted and acidified with • 5 ml of .1 N HCL and frozen until analyzed for rumen ammonia (15). An additional 10 ml aliquot was acidified with 2 ml of 25% metaphosphoric acid, centrifuged at 1500 rpm for 10 minutes. The supernatant was decanted and frozen until analyzed for volatile fatty acids (VFA) by gas-liquid chromatography according to the method of Baumgardt (9) using a 10% SP-1200/1% phosphoric acid on 80/100 Chromosorb **W AW in** a 183 cm x 2 mm ID glass column (Supelco ' Inc., Bellefonte, PA.). Venous blood was also obtained at same time as rumen sampling via jugular puncture and vacuum tube. Tubes were centrifuged for 20

minutes at 2000 rpm, the supernatant was decanted and frozen until analyzed for serum urea (15).

Milk production and composition were adjusted by covariance analysis using the second week postpartum milk production and composition as covariates. Body weight ·changes were also analyzed by . covariance analysis using an average of the first three consecutive weighings as the covariant. All data were subjected to least square analysis of variance by the General Linear Model Procedure (SAS Institute Inc., Cary, NC) and all results are expressed as least square means. Whenever significant differences were detected, the Waller-Duncan K ratio t test (89) was used to separate treatment means.
RESULTS AND DISCUSSION

Chemical composition of feeds and total diets is in Table 2. Concentrate mixes and computed diets contained similar concentrations of dry matter, crude protein, degradable crude protein, and cellulose. Diets **were** formulated to be isonitrogenous, but with varying proportions of protein solubility. Solubilities of nitrogen were highest for UDW and lowest for SBM by both solubility procedures. The difference in solubility between the two procedures was probably due to differences in incubation time and buffer systems used (64, 75). The amount of degradable protein did not differ between diets fed (P>.05), thus allowing conclusions drawn from experimental responses to be related to differences in protein solubility.

Solubility and degradability estimates of total ration composite samples are in Table 3. Sahlu et al. (75) stated that analyses of samples of mixed diets may give a better indication of nitrogen solubility and degradability than analyses of individual feeds. Solubilities in 10% Burroughs' buffer (Table 3) were generally the same as calculated from individual measurements (Table 2) for SBM and U, but were higher than expected for UDW (50.3 versus 41.1). Solubilities of nitrogen by Ficin protease assay (Table 3) were lower for the total diet than expected, from

Table 2. Chemical composition of forages and concentrate mixes containing soybean meal (SBM), urea (U), and urea dried whey (UDW).

a, b, CHeans with unlike superscript differ, P<.05.

1_{Computed.}

 2 Solubility in 10% Burroughs' solution (22).

 3 Ficin protease assay (75) .

4
Soluble nitrogen plus degradable insoluble nitrogen.

 5 Neutral detergent fiber - acid detergent fiber.

 6 Acid detergent fiber - lignin.

 7_{100} - (crude protein + neutral detergent fiber + ether extract + ash).

Table 3. Solubility and degradability estimates for nitrogen (N) in respective total mixed rations containing soybean meal (SBM), urea (U), and urea dried whey (UDW).

 $^{\text{1}}$ 50:40:10 (concentrate:corn silage:alfalfa hay, dry $\texttt{matter basis}.$

calculated values (Table 2) although solubilities of diets containing urea were closer to expected values than were solubilities of the all natural protein supplemented (SBM) diet. Data would suggest that different feed proteins may solubilize or degrade at different rates when combined as a complete diet as opposed to individual measurements. Since urea was added to the concentrate mix, diets containing urea would be expected (Table 3) to be closer to calculated values (Tabie 2) than diets containing natural protein supplements, due to more dietary nitrogen in a readily soluble form. In further supporting the theory, the author would expect no protein (nitrogen) solubility antagonism between the addition of urea and natural feed proteins.

Concentrate mixes contained different (P<.05) concentrations of NDF, ADF, lignin, hemicellulose, ether extract, ash, and soluble residue (Table 2). Differences in concentrate composition can be attributed to ingredients used for formulation (Table 1). Soluble residue was calculated to estimate the amount of material that would be readily available for microbial growth. indirect measure of the amount of nonstructural This was an carbohydrates or those carbohydrates not associated with cell wall components. By trying to ascertain the amount of soluble residue, one can estimate the amount of energy or

carbon skeletons that are readily available to the microflora for microbial protein synthesis. The urea dried whey concentrate mix and thus the UDW diet contained more soluble residue which may suggest that more readily fermentable carbohydrates were available for rumen ·microbial protein synthesis.

Covariate adjusted milk production and composition for cows fed SBM, U, and UDW are listed in Table 4. Milk production was similar (P>.16) for cows fed all three diets; however, production of 4% fat-corrected milk and solids-corrected milk was lower (P<.05) for cows fed U. Data indicated that cows fed diets containing nitrogen in a more readily soluble forms can support milk production equal to that of all natural protein supplements if the diet also contains more fermentable carbohydrates. Grummer and Clark (26) reported that cows fed diets ranging from 21.7 to 34.4% soluble nitrogen showed no difference in milk yield, fat-corrected milk, or composition.

When production was plotted by week as in Figure 1, **COWS** fed different However, consistent milk were not Schingoethe and SBM had graphically higher, but not statistically (P>.50) peak production than cows fed U or UDW. cows as fed UDW had higher initial and more production through peak production, but persistent in maintaining production. Skyberg (83) and Schingoethe et al. (81)

Table 4. Milk yield and composition from cows fed rations containing soybean meal (SBM), urea (U), and urea dried whey
(UDW).

a, b_{Means} on the same line with unlike superscripts differ, P<.05.

1Adjusted by covariance for production and composition during wk 2 postpartum.

Figure 1. Covariate adjusted milk yield of cows fed rations containing soybean meal (SBM), urea (U), or urea dried whey (UDW).

reported similar milk production to that of controls in late lactation cows fed 5% dried whole whey in the concentrate mix. In contrast, several research trials (10, 40, 84) reported a decrease in milk production when cows were fed diets containing various concentrations (10, 60, or 65%) dried whole whey in the concentrate mix. Cows fed U did not peak as high and were later in reaching peak milk production than cows fed SBM or UDW. Huber et al. (41) reported decreased milk yields when urea furnished 21 to 48% of the ration nitrogen, but no effects were detected with 11% of the nitrogen from urea. This would be consistent with our results since, urea furnished approximately 10% of total dietary nitrogen in U and UDW diets. Virtanen (95) fed a purified diet where NPN furnished 100% of ration nitrogen and achieved relatively high milk production, but milk production was still far below what would be considered high production by today's standards. 38, 68, 69, 77, 95, 99) indicated that, although urea can A survey of the literature (17, 18, 32, 34,35, be an acceptable nitrogen supplement, the cow still needs preformed natural protein sources. Wohlt and Clark (99) reported that cows fed a ration supplemented with soybean meal produced more milk then when the diet was supplemented with urea to raise the crude protein level from 11 to 14.5%.

Cows fed SBM and UDW produced more (P<.05) 4% fat

corrected milk than cows fed U because of higher $(P<.01)$ milk fat percentages (Table 4). Others (10, 11, 37, 73, 81, 83, 84, 86) reported higher milk fat percentages when dried whey or dried whey products were added to rations. Schingoethe and Skyberg (84) reported similar 4% fat-corrected milk production to controls due to an increase in· milk fat percentages by addition of 65% dried whey to concentrate mix. Schingoethe et al. (81) also noted higher milk fat percentages in late lactation **COWS** fed dried whey or dried whey product. Similar results have been reported by Bishop and Bath (10) when feeding 3.7% partially delactose whey and Bowman and Huber (11) when feeding 56% lactose in the concentrate mix.

Figure 2 illustrates that milk fat percentages were not consistent and apparent trends were not detected possibly due to biweekly analysis of milk composition throughout the experimental period. However, cows fed U had consistently lower milk fat percentages and 4% fat-corrected milk production (Figure 3) versus cows fed SBM and UDW. At 16 weeks postpartum (Figure 3) all cows, regardless of treatment, had similar production of 4% fat corrected milk. This reflected that the dietary responses being evaluated in this study may occur during early lactation, but not in mid or late lactation. The apparent milk fat depression seen for cows fed U can not be

Figure 2. Covariate adjusted milk fat percentages of cows fed rations containing soybean meal (SBM), urea (U), or Urea dried whey (UDW).

Figure 3. Covariate adjusted production of 4% fat-corrected milk (FCM) for cows fed rations containing soybean meal (SBM), urea (U), or urea dried whey (UDW).

attributed to reported decreases of milk fat percentages from cows fed urea. Trials by percentages due reported here may possibly be due to inherent differences of diet fed. No previous studies have (41, 92) found no difference in milk fat to urea supplementation. Responses in how cows were randomly assigned to experimental treatments because, . no previous lactational performance data was used for basis of assignment to treatments.

Milk protein percentages were similar (P>.14) for cows fed all diets (Table 4), but the amount of protein produced was less (P<.05) for cows fed U than SBM due to small differences in milk production and protein percentages between treatments. Cows fed SBM and UDW produced more (P<.01) solids-corrected milk than cows fed U because of U. **Cows** percentages and solids-corrected milk production throughout the trial decreases (P<.05) of protein and solids-not-fat percentages lower total solids percentages $(P \langle .05 \rangle)$ when fed fed U had consistently lower total solids (Figures 4 and 5). Huber et al. (41) reported from cows supplemented with urea; this was attributed to a lower energy intake as amount of urea supplied up to 48% of dietary nitrogen. They indicated that energy was the main factor limiting milk yield and composition when urea is added as a crude protein supplement to the diet.

Dry matter intakes (Table 5) were highest for cows

Figure 4. Covariate adjusted total solids percentages of cows fed rations containing soybean meal (SBM), urea (U), or urea dried whey (UDW).

Figure 5. Covariate adjusted production of solids-corrected milk (SCM) for **. cows** fed rations containing soybean meal (SBM), urea (U), or urea dried whey (UDW).

. *I*

Table 5. Feed comsumption for cows fed rations containing soybean meal (SBM), urea (U), and urea dried whey (UDW).

a, b, CMeans with unlike superscripts differ, P<.05.

110% Burroughs' buffer solution.

 2 Ficin protease assay.

fed UDW and lowest for cows fed U. Some of the differences in feed intake may have . been due to inherent differences in appetites of some cows assigned to various diets, or the differences may have reflected true dietary treatment responses. When dry matter intake was plotted by week (Figure 6) cows fed U were ·slow in increasing their dry matter intake possibly indicating a problem with adapting to the U diet. When dry matter intake as a percent of body weight was plotted by week (Figure 7) the same trends were seen as in Figure 6. Urea is known to be unpalatable at high concentrations. Van Horn et al. (92) observed a decreased (P<.01) feed intake when urea was added at 2.2 and 2.7% of concentrate **mix.** lowered dry matter intake Other data suggest that is from taste (35), but the complete mechanism of intake depression is not understood (38). Conrad et al. (19) reported that when cows consumed 58% of their nitrogen from urea or soybean meal, length of initial meals was markedly reduced for cows fed urea supplemented diets. However, total feed dry matter intake was similar (12.0 versus 11.6 kg/day) and the decrease in meal length was compensated for by an increase in number of meals per day or an increase in eating rate. This may help explain the fact that cows fed urea need time for proper rumen adaptation (16, 35, 38, 68, 69). This would explain the constant dry matter intake for the U diet during first

Figure 6. Daily dry matter intakes (DMI) of cows fed rations containing soybean meal (SBM), urea (U), or urea dried whey (UDW).

Figure 7. Dry matter intakes (DMI) as a percent of body weight for cows fed rations containing soybean meal (SBM), urea (U), or urea dried whey (UDW).

two weeks on experiment (Figures 6 and 7).

Cows fed UDW had consistently higher daily dry matter intakes on a kg basis or when expressed as % of body weight. Dried whole whey is palatable (79) and effectively masked the undesirable effect of intake depression of urea addition to concentrate mix. King and Schingoethe (46) reported higher dry matter intakes for steers fed dried whey at 86% of the concentrate mix. No differences in dry matter intakes were reported for lactating cows fed dried whey or whey products (81, 83, 84, 85).

Due to variations in intake and ration composition cows, fed U had lower (P<.05) intake of crude protein, acid detergent fiber, neutral detergent fiber, hemicellulose, cellulose, ash, and soluble residue. Consumption of lignin and ether extract did not differ between rations. Cows fed SBM and UDW had similar intakes of crude protein, degradable crude protein, acid detergent fiber, cellulose and ash. Differences in soluble crude protein intake can be attributed to diet formulation and variations in dry matter intakes.

Conversion of feed to milk did not differ between SBM and UDW (Table 6), but efficiency was higher for U because of maintaining acceptable milk production with lower dry matter intakes. The conversion of crude protein to milk protein did not differ between SBM and UDW

Table 6. Body weights and feed efficiencies for cows fed rations containing soybean meal (SBM), urea (U), and urea dried whey(UDW).

a, b, c_{Means} with unlike superscripts differ, P<.05.

¹ 10% Burroughs' buffer solution.

 2 Ficin protease assay.

indicating that highly soluble nitrogen diets with readily fermentable carbohydrates can support maximum microbial protein synthesis to provide sufficient amino acids for milk protein synthesis equal to that provided by soybean meal.

Covariate adjusted body **weights** are in Table 6. Cows fed U did not achieve body weights similar to controls probably due to reduction in feed intake (Table 5) associated with the unplatability of urea.

Table 7 contains data for rumen volatile fatty acids, ammonia and pH. Cows fed higher soluble nitrogen diets (U and UDW) had lower (P<.05) mole percentages of acetate and cows fed UDW had higher (P<.05) concentrations of butyrate. Previous trials indicated that acetate, on a molar percentages basis, may increase over controls (46), remain unchanged from controls (86, 98), or decrease from controls (2, 61, 84) when dried whey or whey products were fed. Although acetate may be variable, butyrate concentrations are almost always higher than that of controls when dried whey is fed (2, 11, 37, 40, 46, 73, 81, 84, 85, 86, 87, 91, 98). Satter and Esdale (76) reported that butyrate is the end product of lactate fermentation. By converting two molecules of acetate into one molecule of butyrate, the oxidation-reduction balance of the rumen is maintained. Propionate was higher for cows fed U which may

TABLE 7. Rumen volatile fatty acids (VFA), ammonia, and pH
for cows fed rations containing soybean meal (SBM), urea (U), and urea dried whey (UDW).

 a, b Means with unlike superscripts differ, $P \le 0.05$.

indicate altered rumen fermentation conducive to low milk fat percentages. Low dietary fiber and the addition of corn in place of portions of the soybean meal in the concentrate mix, may have provided a more than ample supply of starch which . may have lead to a proliferation of propionate-producing microorganisms. When data were analyzed on a umole per ml basis, the same trends were apparent as when. data were expressed on a mole percent basis. Total amount of volatile fatty acids did not differ across rations.

Cows fed U had a lower ratio of acetate to propionate than cows fed SBM or UDW. The ratio of acetate plus butyrate to propionate may give a better explanation of the effect of rumen fermentation on production of precursors for milk fat synthesis since, butyrate can also be used as a precursor for milk fat synthesis. That ratio was also higher (P<.05) for cows fed SBM and UDW than for **COWS fed U.**

Cows of ammonia (Table 7), but cows fed UDW had similar concentrations to those fed SBM (13.53 versus 11.81 mg/dl) fed U had the highest ruminal concentrations despite the fact that the UDW diet contained more soluble nitrogen than the U diet (Table 2). Satter and Slyter (78) reported that an ammonia concentration of 5 mg/dl was sufficient for maximum microbial protein synthesis in

vitro, but Miller (55) and Mekrey et al. (53) reported that 20 to 29 mg/dl may be necessary for maximum ammonia utilization in vivo. Serum urea concentrations tended to follow the same trends as rumen ammonia concentrations (Table 7), but cows fed UDW had numerically lower serum urea concentrations than cows fed SBM rations.

Data of ruminal ammonia and serum urea concentrations indicated that the UDW diet increased nitrogen utilization by stimulating microbial protein synthesis. Windschitl and Schingoethe (98) reported that dried whey increased nitrogen utilization through stimulating increased microbial protein synthesis. The increased nitrogen utilization by feeding dried whey could be due to increased amounts of readily fermentable lactose for a energy source or carbon skeleton for protein synthesis. concentration of starch Stern et al. (90) reported that as increased in vitro, the concentration of ammonia decreased with an increase in microbial protein synthesis. Russell et al. (74) stated that ammonia concentration was inversely proportion to the level of readily soluble carbohydrates. MacGregor et al. (52) reported that diets with similar nitrogen solublities (35.5 versus 36.3% of nitrogen), but higher levels of nonstructural carbohydrates (32.9 versus 24.9%) produced more milk than cows fed low nonstructural carbohydrates

diets. nitrogen, ruminal ammonia concentrations were reduced by Although those diets were high in degradable rapid availability of ·readily fermentable carbohydrates. Data from this lactation trial and literature cited above suggest that diets high in nitrogen solubility or degradability may benefit from addition of a readily fermentable carbohydrate source. The data would further substantiate the conclusion that diets high in soluble nitrogen can support milk production equal to all natural plant protein supplements. The plant of the protein

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