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In Vitro and In Vivo Evaluation of Protecting Whey Protein Concentrate for Ruminants

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IN VITRO AND IN VIVO EVALUATION OF PROTECTING WHEY PROTEIN CONCENTRATE FOR RUMINANTS

BY

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Abstract

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Under the supervision of Associate Professor Lawrence D. Muller

In experiment I a series of in vitro buffer and rumen fermentation studies were conducted to evaluate protein solubility at pH 6.8 and pH 2.5 (with pepsin) and ammonia production from whey protein concentrate (WPC, 55% protein) treated with 0, .25, .5, 1.0, and 3% formaldehyde; 0, .5, 1.0, 2.0, 3.0, and 6% tannic acid; and 0, 1, 2, and 3 hr of heat treatment at 104 C. Protein solubility and ammonia production from casein treated with 0, .5, 1.0, and 3% formaldehyde and 0, .5, 1.0, and 3% tannic acid were also studied. All levels of formaldehyde treatment of WPC depressed ($P<.01$) protein solubility to less than 10% of the control at pH 6.8, and solubility at pH 2.5 was about 33% ($P<.01$) of the control. All the levels of formaldehyde reduced ammonia production ($P<.01$) indicating protection of WPC from degradation in the rumen. Protein solubility of WPC at pH 6.8 was depressed by 2.0, 3.0, and 6.0% tannic acid ($P<.01$) compared to the control, but all the levels of WPC-tannic acid treatment were highly soluble at pH 2.5. Ammonia production from WPC-tannic acid did not differ from the control, although 6% tannic acid reduced ($P<.05$) ammonia production. All the WPC-heat treatments greatly reduced ($P<.01$) protein solubility at pH 6.8 and reduced solubility by 50% at pH 2.5. Ammonia production was reduced to about 20% of the control in all the heat treatments. All levels of

formaldehyde reduced ammonia production but only 1 and 3% differed ($P < .01$) from the control. All casein-tannic acid treatments were highly soluble at both pH 6.8 and pH 2.5 and did not differ from the control. Only 3% tannic acid reduced ($P < .01$) ammonia production from the control. Mice were fed similarly treated WPC or casein in a 17% protein ration to evaluate the extent of protein protection. Mouse growth was comparable to the control when .25 and .5% formaldehyde treated rations were fed, but mouse growth was decreased ($P < .01$) with the 1% formaldehyde treated ration. Although feed consumption of WPC-tannic acid treatments (except 6% tannic acid) was similar to the control, growth decreased as the levels of tannic acid increased in the ration. All the mice fed heat treated rations consumed similar amounts of feed as the control group. Mice fed WPC-heat treatment (1 hr) gained weight at about 60% of the control. Mice fed rations heated 2 and 3 hr gained less weight as the heating time increased.

In experiment II four lactating Holstein cows were fed iso-nitrogenous rations of urea-corn silage and a 15% crude protein, pelleted grain ration containing whey protein concentrate (34% protein) either untreated (U-WPC) or treated (T-WPC) with 1% formaldehyde on a protein basis. The trial design was a three period double reversal with 12 days per period during which milk and digestibility parameters were measured the last 4 days of each period. Apparent nitrogen digestibility (%), productive nitrogen retained (milk plus retained, g/day), and dry matter digestibility were 60.0 and 53.9 ($P < .05$), 89.0 and 103.8, and 67.4 and 63.2 for cows fed U-WPC and T-WPC rations,

respectively. Productive nitrogen as a percent of absorbed was greater for cows fed the T-WPC ration, suggesting more efficient utilization of absorbed nitrogen. Milk production (kg/day), fat (%), faty yield (kg/day), and 4% fat-corrected milk (kg/day) were 27.6 and 29.4, 3.1 and 3.4, .86 and 1.00 ($P<.05$), and 23.7 and 26.9 for cows fed the U-WPC and T-WPC rations, respectively. Total milk nitrogen (g/day), true protein nitrogen (g/day), and casein nitrogen (g/day) were 135.84 and 140.90, 132.84 and 137.30, and 118.95 and 124.13 for cows fed U-WPC and T-WPC rations, respectively. No differences were found in rumen ammonia or blood urea. Rumen volatile fatty acids were higher in cows fed U-WPC ration at 4 and 6 hr postfeeding. Only milk fatty acid 16:0 was greater ($P<.05$) in cows fed U-WPC ration than in cows fed T-WPC ration. Differences in total and most essential amino acids between tail and mammary blood were greater for cows fed T-WPC ration.

IN VITRO AND IN VIVO EVALUATION OF PROTECTING

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, 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.

/Date/

Head, Dairy Science Department / Date

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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INTRODUCTION

Protein is one of the most important and limiting nutrients in human and animal nutrition and also one of the most expensive. Because of these factors, it has been necessary to study this nutrient in detail to discover methods to improve its utilization in both human and animal nutrition. Animal protein has been consumed by the human population for centuries but is becoming more limited and expensive.

Ruminant animals have been one of the major sources of high quality animal protein for humans. Ruminants have generally been considered to be more efficient than nonruminants in the conversion of dietary protein to animal protein for human consumption, primarily because of their unique digestive system. More research is necessary in order to find methods to further improve the utilization of dietary protein, primarily high quality dietary protein.

In ruminants a major portion of the dietary protein is degraded in the rumen to peptides and amino acids and then converted into ammonia. The microorganisms in the rumen utilize this ammonia to synthesize their own protein which is then utilized by the host animal. The amount of protein degraded by the rumen microorganisms depends on the amount of energy available in the diet, the solubility of this protein in the rumen fluid, and numerous other factors.

This scheme of protein utilization by ruminants has certain advantages and disadvantages, depending on the quality of this protein. If low quality protein is fed in the diets of ruminants, the microorganisms in the rumen transform this low quality protein into a

protein of high biological value that is better utilized by the animal. The main disadvantage of this utilization scheme is, when high quality protein is fed to ruminants, most of this protein is inefficiently utilized because the microorganisms synthesize a protein which may be of lower quality than the original dietary protein. Because of this disadvantage in protein utilization, studies are being conducted to determine methods to improve utilization of high quality dietary protein through bypassing the rumen and preventing the usual ruminal degradation of proteins. These methods may ultimately prove beneficial in reducing the amount and cost of dietary protein fed to ruminants and improve the production of animal protein for human consumption.

The present study was conducted to investigate the feeding of whey protein concentrate (WPC), a high quality protein, treated with 1% formaldehyde solution to protect it from ruminal microbial degradation to dairy cattle. The general objectives of this study were (1) to study methods and optimum conditions for protecting WPC from ruminal degradation using in vitro techniques and (2) to study the influence of feeding rations containing protected WPC to dairy cattle on milk production and composition and nitrogen utilization and metabolism.

REVIEW OF LITERATURE

Nitrogen Metabolism

The utilization of dietary nitrogen and protein has been studied by many investigators and reviewed recently (39, 80, 81). A general scheme of protein and nonprotein nitrogen (NPN) utilization and metabolism by ruminants is presented in Fig. 1.

Dietary proteins and NPN compounds are the main nitrogen sources for ruminants. Approximately 60% of the dietary protein is degraded in the rumen by the rumen microbial population to amino acids which may be partially utilized by the microbes to synthesize microbial protein. Most of the dietary protein is converted to ammonia during the ruminal fermentation process and then resynthesized into microbial protein. Approximately 40% of the dietary protein may bypass the rumen, some of which is utilized in the abomasum and lower digestive tract and the remainder excreted in the feces. The proportion of dietary protein that is degraded to ammonia or bypasses the rumen depends on numerous dietary and animal factors.

The NPN in the diet of ruminants is converted to ammonia by the rumen microbes, much of which is later utilized by the bacteria for microbial protein synthesis. Ammonia which is not utilized for microbial protein synthesis may pass through the rumen wall to the liver where it is utilized to form nonessential amino acids or metabolized in the liver to urea which may be excreted in the urine or recycled to the rumen via saliva and blood.

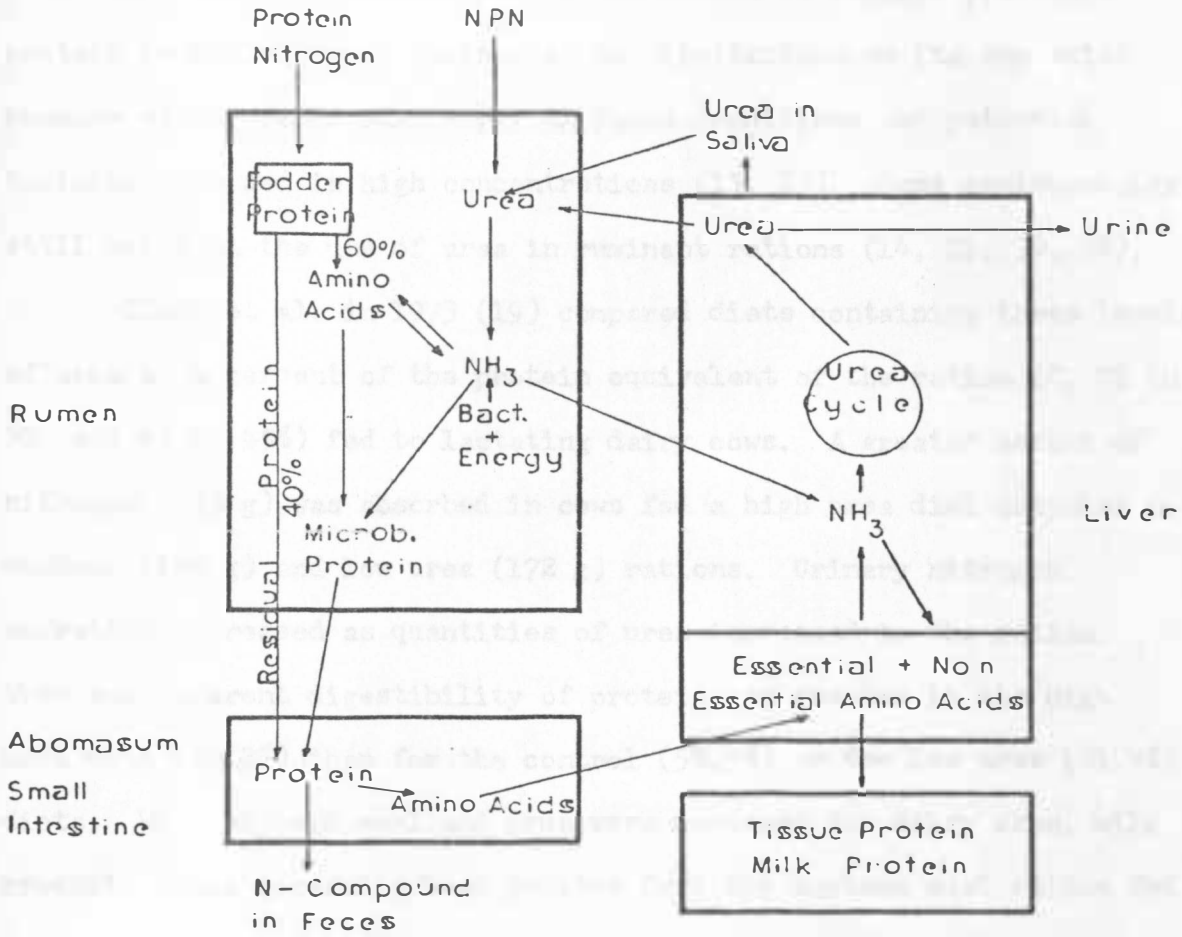

Fig 1 Scheme of utilization and metabolism of protein and non-protein nitrogen by ruminants

The use of NPN compounds in feeding programs has been widely accepted, but their use is restricted by the degree of solubility of each compound. In the United States and in other parts of the world, urea has been the most widely used NPN source to replace preformed protein in the diets of ruminants, but limitations on its use exist because of its rapid solubility in rumen conditions and potential toxicity when fed in high concentrations (13, 15). Some controversies still exist on the use of urea in ruminant rations (14, 21, 37, 64).

Clark et al. in 1973 (19) compared diets containing three levels of urea as a percent of the protein equivalent of the ration (0, 28 to 30, and 40 to 45%) fed to lactating dairy cows. A greater amount of nitrogen (203 g) was absorbed in cows fed a high urea diet compared to control (145 g) and low urea (172 g) rations. Urinary nitrogen excretion increased as quantities of urea increased in the ration. True and apparent digestibility of protein was greater in the high urea diet (66.3%) than for the control (58.9%) or the low urea (61.4%) diets. When soybean meal and urea were compared for dairy cows, milk production has generally been greater from the soybean meal ration fed than when urea replaced soybean meal in the diet. The addition of urea to a basal diet containing 11.4% crude protein improved the performance of the cows, suggesting that the addition of urea to a low protein ration was beneficial under those conditions (78). Similar conclusions were drawn by Clark et al. in 1973 (19) when soybean meal was fed to dairy cattle.

Plant proteins presented to the rumen microbes which do not bypass the rumen are converted to amino acids which in relatively small numbers are absorbed across the rumen wall (29), but most amino acids are further degraded to ammonia, carbon dioxide, and volatile fatty acids (30, 71). Lewis and Emery (43), Looper et al. (46) and Schelling et al. (67) in their studies observed that the ruminal infusion of amino acids produced different quantities of ammonia at different times, suggesting that dietary amino acids like dietary proteins are also broken down to ammonia in the rumen by the microorganisms.

Rumen Bypass of Proteins and Amino Acids

Naturally insoluble protein. Many dietary proteins and all non-protein nitrogen compounds, are degraded by the rumen microorganisms to ammonia depending on the degree of solubility (17). McDonald (48) compared casein, gelatin, and zein diets in their degree of solubility when fed to ruminants. He found casein and gelatin more soluble at rumen pH than zein. Consequently, the production of ammonia increased with increased solubility of the proteins. Other researchers (12, 45, 49) obtained similar results when casein or soybean meal were compared with other proteins for their degree of solubility.

Tagari (74) compared the efficiency of utilization of proteins contained in alfalfa hay and soybean meal for sheep. He observed that soybean meal produced less rumen ammonia and free amino acids than alfalfa hay, but alfalfa hay was more effectively utilized by the rumen bacteria to synthesize bacterial protein. When soybean oil meal was fed to ruminants, Nikolic et al. (52) observed that rumen ammonia

increased rapidly 1 hr after feeding compared with no ammonia increase when the basal diet without soybean oil meal was fed.

Abomasal infusion of proteins and amino acids. The failure of orally administered amino acids and proteins to obtain maximum utilization is attributed to their degradation in the rumen by the rumen microbes. Thus, it has been observed that nitrogen from high quality proteins has been more efficiently utilized when these proteins were infused into the abomasum rather than being fed or introduced into the rumen (6, 9, 26, 44, 62).

Reis and Schinckel (62) infused 60 g of casein into the abomasum of sheep and observed an increase in wool growth of 123 to 181% compared to gelatin which only increased wool growth by 17 to 24%. The addition of sulfur-containing amino acids further increased wool growth by 16 to 37%. Similar responses in wool growth were found by Colebrook and Reis (20) when 100 g of the high quality proteins casein, whole egg, or egg albumen were infused into the abomasum of sheep. The infusion of corn gluten and gelatin into the abomasum resulted in much less wool growth than that obtained with casein, suggesting that bypassing the rumen with high quality proteins was beneficial.

Orskov and Fraser (53) investigated the feeding of protein supplements to weaned lambs via the esophageal groove. They infused directly into the abomasum via the esophageal groove a basal diet of barley and various sources of proteins (fish meal, yeast, soybean meal, and sunflower meal). Nitrogen intakes in the control, fish meal, yeast, soybean meal, and sunflower meal diets were 14.28, 20.16, 20.53, 19.00,

and 18.87 g/day, respectively. Nitrogen retention was greater in fish meal and yeast diets (9.39 and 8.19 g/day) than in the control, soybean meal, and sunflower meal diets (5.09, 7.80, and 7.86 g/day, respectively). The same researchers found that, when fish meal, blood meal, and casein were fed as liquid supplements to lambs via esophageal groove, nitrogen intake was similar and nitrogen retention was greater in the casein diet than in fish meal and blood meal diets. In another experiment, Orskov et al. (59) observed that feeding proteins in liquid suspension to weaned lambs resulted in a better utilization of these proteins than when fed in a dry state.

Schelling and Hatfield (66) observed an increase in feed consumption and in nitrogen retention when casein was infused into the abomasum of lambs. But, when acid hydrolyzed casein as a mixture of L-essential amino acids was infused into the abomasum, nitrogen retention increased but to a lesser extent than when only casein was infused. These same researchers found that the infusion of a mixture of arginine, histidine, lysine, phenylalanine, and methionine into the abomasum increased nitrogen retention but less than when all the ten essential amino acids were infused. The infusion of methionine or phenylalanine did not result in an increase in nitrogen retention, but the infusion of either lysine or glutamic acid increased nitrogen retention similar to that found with the mixture of the five amino acids.

Contrasting results were found by Boila and Devlin (8) when 3.6 and 9.0 g lysine per day were infused into the abomasum of steers. The low levels of lysine had no effect on nitrogen retention, whereas 9.0 g lysine per day decreased nitrogen retention. This may suggest that the infusion of single amino acids into the abomasum caused an amino acid imbalance in the animal.

Research on ruminal and abomasal infusion of proteins and amino acids was conducted to determine their effects on milk yield and milk constituents of dairy cows. Broderick et al. (9) infused 800 g per day of a 10% w/v solution of sodium caseinate plus 24 g per day of methionine into the abomasum of lactating cows producing 31 kg milk per day. The infusion of casein increased milk production by 6.2% and protein production by 11.6%, while grain consumption decreased 10.0%. Tyrrel et al. (77) and Derrig et al. (26) found an increase in milk production and milk protein production when casein was infused into the abomasum, but this increase in milk production varied from 4.3 to 12.5% according to the amount of casein infused. Similar results in milk production were found by Vik-Mo and Huber (79) when 270 to 300 g casein were abomasally infused in lactating cows. When isocaloric diets (35) consisting of 2% urea, 2% urea plus 3% casein, 1% urea, and 1% urea plus 3% casein were infused into the abomasum of lactating cows, it was observed that the combinations of urea plus casein increased milk production; but 1% urea plus 3% casein resulted in higher milk production compared to the other diets. No significant differences were

observed among the diets on milk nitrogen yield, but fat percent decreased in casein-urea mixtures.

The influence of casein into the abomasum increased plasma levels of lysine, histidine, isoleucine, leucine, threonine, tyrosine, phenylalanine, alanine, and serine but did not change the levels of arginine, valine, methionine, cystine, aspartic acid, glutamic acid, and glycine (26). Other researchers (1, 9, 15, 53, 54) reported that plasma amino acid levels were affected by nitrogen in the diet and by amino acids or proteins infused into the abomasum.

Physical and chemical treatment. Since it may be beneficial for high quality dietary protein to bypass the rumen, considerable research has been conducted investigating methods of treating proteins to allow them to bypass the rumen. Physical methods such as heat treatments and chemical methods such as aldehydes and tannins have been studied. In many cases these methods or techniques have been effective.

Tagari et al. (75) applied three heating methods to soybean meal; namely, (1) evaporation of solvent from the flakes at room temperature avoiding additional heating, (2) removal of the solvent from flakes at 80 C for 10 min, and (3) steaming the flakes at 120 C for 15 min. When these diets were fed to ruminants, treatment 3 reduced the solubility of the soybean meal more than treatments 1 and 2. Ruminal ammonia and blood urea nitrogen decreased, and apparent digestibility of nitrogen increased more on treatment 3 than on treatments 1 and 2. Similar results were found by Glimp et al. (34) when soybean meal was heated at 149 C for 4 hr and fed to weaned lambs

in two levels of protein rations. Contrary to results reported by Tagari et al. (75), Chalmers et al. (11) compared different heat treatments to peanut meal. They found no differences among the treatments in digestibility of proteins, nitrogen balance, and milk production, but they observed a reduction in rumen ammonia production when the toasted ration was fed compared to the air dried and basal rations.

Nishimuta et al. (56) compared treatments of heat, 2% formaldehyde, and 9% w/w tannic acid to soybean meal. When these diets were fed to crossbred wether lambs averaging 35 kg, heat treatment of soybean meal significantly decreased cellulose digestion and suppressed ammonia production in the rumen. At 0, 2, 4, and 6 hr postfeeding, ruminal ammonia was greatest for the untreated ration, followed by the 2% formaldehyde solution treatment, 9% tannic acid, and finally the heat treatment. Plasma urea levels were not different with time between treatment groups. The heated soybean meal group was found to have higher plasma levels of valine, isoleucine, and lysine than all the groups except the formaldehyde soybean meal treatment. Plasma levels of glycine and histidine were lowest in the heat treatment group.

Tannic acid treatment has been also evaluated as a method of protecting dietary proteins from ruminal degradation, but its effectiveness varies depending on the protein physico-chemical properties and the heat treatment (85). The protective effect of tannins on different proteins has been confirmed in vivo and in vitro (22, 23, 42).

Driedger and Hatfield (28) evaluated soybean meal in vitro treated with 0.5, 10, 20, and 25% allepo tannin. All the treatments were found to depress ruminal ammonia. The 5% tannin treatment reduced ammonia to 57.6%, the level produced from the untreated soybean meal. The same researchers evaluated urea and soybean meal, both untreated or treated with 10% tara tannin, for lambs. The treated soybean meal produced higher daily gains (217 g/day) compared to urea (112 g/day) and untreated soybean meal (117 g/day) diets. Gain to feed ratios were higher for the soybean meal treatment, but nitrogen retention for lambs receiving urea differed from the two soybean meal rations. The addition of carob pod (76), a tannin substance, to proteins showed that protein biosynthesis in vitro was not affected when up to 0.7 mg/ml of carob tannin was used, but a concentration of 2.1 mg/ml of this tannin blocked protein biosynthesis and increased the amount of ammonia.

Nishimuta et al. (56) found that crude protein digestibility was highest in untreated soybean meal, lowest for 2% formaldehyde treated soybean meal, and intermediate for the heat and 9% tannic acid treated soybean meal. Nitrogen retention was greatest for the heat treated soybean meal, followed by the tannic acid treatment and untreated soybean meal, and finally the formaldehyde treated soybean meal. Plasma free amino acids in the tannic acid treatment were intermediate among the treatments, with only aspartic acid, arginine, and phenylalanine at higher levels compared to the other diets.

Among the various treatments, formaldehyde appears to be the most practical for protecting high quality protein from ruminal

degradation. The mechanism by which formaldehyde protects proteins is presumably related to the formation of methylene bridges and other cross-linkages between the protein chains (82).

Ferguson et al. (33) fed 80 g/day commercial grade casein treated with 4% formaldehyde solution to sheep for 9 wk and observed an increase of wool growth of 70% compared to the group fed untreated soybean meal. Barry (3) observed an increase of 34% in wool growth when casein treated with 1% formaldehyde solution was fed to sheep. Nitrogen retention increased as the casein intake increased. Plasma urea nitrogen also followed the same pattern. An increase in wool production was also found by Wright (84) when casein treated with 2.25% formaldehyde was fed to sheep.

The addition of 0.3% methionine to the basal diet resulted in a 26% increase in rate of gain and a 19% increase in feed efficiency, but the addition of methionine to the formaldehyde treated diet did not increase wool growth.

Some differences in response from formaldehyde treatment of protein were found by Faichney (31) when casein was treated with 1.5% formaldehyde solution. In the first experiment, an improvement of 7% in weight gain and feed conversion was observed when the treatment diet was fed to sheep, whereas organic matter digestion was three times lower in sheep fed the treatment diet. In a second experiment, no differences were observed. Although a lower nitrogen digestibility was found when the same treated ration was fed to sheep, sheep excreted more nitrogen in feces and less nitrogen in urine (2.3 and 1.7 g/day)

than from the untreated (1.4 and 3.2 g/day). No significant differences were found in plasma ∝-amino nitrogen and plasma urea nitrogen. Similar results were observed by Offer et al. (57) when casein treated with 1% formaldehyde solution was fed to sheep. The treatment of formaldehyde led to an increase in total amino acids in the digesta (14.83 g/day) compared to the untreated ration (10.40 g/day). A decrease in ammonia production was also observed, but no significant difference in nitrogen in feces was found between the treated and untreated rations.

Peter et al. (61) using in vitro techniques treated soybean meal with several aldehydes (acetaldehyde, acrolein, butyraldehyde, formaldehyde, glutanaldehyde, glyoxal, and propionaldehyde). Incubation of the treated soybean meal with rumen fluid reduced ammonia production with acrolein, formaldehyde, glyoxal, or glutaraldehyde treated compared to the untreated soybean meal. The other aldehydes had no significant reduction on ammonia production. In an in vivo study, lambs receiving formaldehyde or glyoxal treated soybean meal gained significantly faster and more efficiently than lambs receiving the control diet. Plasma urea nitrogen was depressed with the glyoxal treatment. No significant differences were found in wool growth among the diets. Hughes and Williams (38) in an in vitro study also found a decrease in solubility from 90.0% to 4.9% when casein was treated with 1% formaldehyde solution. The same researchers also observed in an in vitro experiment a reduction in solubility of peanut meal from 70% to 20% and a reduction in ammonia production of about 10% compared to that found with untreated meal.

Nishimuta et al. (56) compared untreated soybean meal to soybean meal treated with heat, tannic acid, and formaldehyde on nitrogen utilization by sheep and found similar results to those of the other researchers (31, 32, 55, 69). They found that formaldehyde treatment significantly reduced protein and dry matter digestibility and pointed out that, although protein digestibility was reduced, total concentration of plasma free amino acids did not differ among treatments. Soybean meal treated with formaldehyde was found to produce higher levels of lysine and histidine but lower levels of glutamic acid, arginine, and serine in comparison to the untreated diet and the other treatments.

When calves were fed rapeseed meal treated with 0.7% formaldehyde solution (69), calves gained more weight ($P > .05$) than calves fed the commercial untreated rapeseed meal. A significant reduction in rumen ammonia was observed in calves fed the treatment diet. Plasma urea levels and rumen volatile fatty acids did not differ significantly between the treatments. The treatment of rapeseed with formaldehyde tended to reduce dry matter, nitrogen, fiber, and energy digestibility. When the amount of formaldehyde increased to 5.6% (70), plasma urea nitrogen and rumen ammonia levels were reduced significantly compared to calves fed untreated rapeseed meal. No differences in dry matter consumption, daily gain, or feed efficiency were observed. Total volatile fatty acids in the rumen were higher ($P < .05$) in calves fed the untreated ration compared to calves fed the treated ration. No significant effects were found on nitrogen consumption and nitrogen

retention. Contrary to these results (69, 70), Faichney and Davies (32) found a 9% increase in weight gain when peanut meal was treated with formaldehyde and fed in a 12% protein ration to calves, but no increase in weight gain was observed when this treated protein was fed to calves in 15% or 19% protein rations.

The results found by Reis and Tunks (63) are in agreement with those reported by Derrig et al. (25). Reis and Tunks (63) found that, when casein treated with 4% formaldehyde was fed to mature wethers, the small increase in the concentration of plasma amino acids doubled when this casein was infused into the abomasum. Both treatments increased plasma essential amino acids; namely, valine, leucine, isoleucine, and phenylalanine, while glycine was markedly depressed.

The addition of formaldehyde to forages when fed to beef cattle has improved their utilization and improved carcass characteristics (10, 83).

When a daily supplement of 1 kg of formaldehyde treated casein-soybean meal was fed to lactating Jersey, Sahiwal, and Sahiwal x Jersey cows, an increase of 15% and 6% in milk production and milk fat was observed, but a decline of 5% in nonfat solids was found (60).

Treatment of soybean meal with 3% formaldehyde (40) depressed milk production in goats (1.3 kg/day) compared to the control ration (1.5 kg/day). Fat percent was found to be higher in the treatment ration (2.6%) than in the control ration (2.5%), but fat yield was depressed due to the decreased milk production. Total fatty acids in milk were comparable in both rations and medium chain saturated fatty

acids tended to lower, while long chain fatty acids tended to increase in both rations. Clark et al. (18) found no significant difference in milk production and milk constituents when cows were fed untreated and 0.9% formaldehyde treated soybean rations.

EXPERIMENT I

EVALUATION OF METHODS FOR PROTECTING WHEY PROTEIN AND CASEIN FROM RUMINAL DEGRADATION

Abstract

A series of in vitro buffer and rumen fermentation studies were conducted to evaluate protein solubility at pH 6.8 and pH 2.5 with pepsin and ammonia production from whey protein concentrate (WPC, 55% protein) treated with 0, .25, .5, 1.0 and 3% formaldehyde; 0, .5, 1.0, 2.0, 3.0 and 6.0% tannic acid; and 0, 1, 2 and 3 hr of heat treatment at 104 C. Protein solubility and ammonia production from casein treated with 0, .5, 1.0 and 3% formaldehyde and 0, .5, 1.0 and 3% tannic acid were also studied.

All levels of formaldehyde treatment of WPC depressed ($P<.01$) protein solubility to less than 100% of the control at pH 6.8 and solubility at pH 2.5 was about 33% ($P<.01$) of the control. All the levels of formaldehyde reduced ammonia production ($P<.01$) indicating protection of WPC from degradation in the rumen. Protein solubility of WPC at pH 6.8 was depressed by 2.0, 3.0 and 6.0% tannic acid ($P<.01$) compared to the control, but all the levels of WPC-tannic acid treatment were highly soluble at pH 2.5. Ammonia production from WPC-tannic acid did not differ from the control, although 6.0% tannic acid reduced ($P<.05$) ammonia production. All the WPC-heat treatments greatly reduced ($P<.01$) protein solubility at pH 6.8 and reduced solubility by 50% at pH 2.5. Ammonia production was reduced to about 20% of the control in all the heat treatments. All levels of formaldehyde reduced ($P<.01$) protein solubility of casein at pH 6.8. Solubility was reduced by 80% ($P<.01$) compared to the control at pH 2.5. All the levels of casein-formaldehyde reduced ammonia production,

but only 1 and 3% differed ($P<.01$) from the control. All casein-tannic acid treatments were highly soluble at both pH 6.8 and pH 2.5 and did not differ from the control. Only 3% tannic acid reduced ($P<.01$) ammonia production from the control. Mice were fed similarly treated WPC or casein in a 17% protein ration to evaluate the extent of protein protection. Mouse growth was comparable to the control when .25 and .5% formaldehyde treated rations were fed, but mouse growth was decreased ($P<.01$) with the 1% formaldehyde treated ration. Although feed consumption of WPC-tannic acid treatments (except 6% tannic acid) was similar to the control, growth decreased as the levels of tannic acid increased in the ration. All the mice fed heat treated rations consumed similar amounts of feed as the control group. Mice fed WPC-heat treatment (1 hr) gained weight at about 60% of the control. Mice fed rations heated 2 and 3 hr gained less weight as the heating time increased.

Introduction

The microflora in the rumen utilize dietary protein and synthesize their own microbial protein which is utilized by the animal. This utilization scheme is beneficial when the dietary protein is of low quality. When high quality proteins are fed to ruminants, these proteins are also degraded. High quality protein may be more efficiently utilized if the protein was allowed to bypass the rumen.

Methods of protecting proteins from ruminal degradation such as coating the protein with formaldehyde and tannic acid have been

examined (27, 31, 33, 56). Heat treatment has been used to protect proteins from microbial degradation (11, 34, 75).

In vivo and in vitro studies with high quality proteins have been conducted (3, 27, 36, 61) to find appropriate methods to protect proteins from ruminal degradation. In many of the studies sodium caseinate and plant proteins have been used. Whey protein, a by-product of cheese-making, is of similar high biological value to casein with regard to amino acid patterns (24). No previous research has been reported on protection of whey protein to bypass the rumen.

The objective of the present investigation was to study methods of protecting whey protein concentrate and sodium caseinate from ruminal degradation which would allow utilization in the abomasum and lower digestive tract of the ruminant.

Materials and Methods

In vitro. Several in vitro studies were conducted to compare ammonia release and protein solubility of whey protein concentrate[1] (WPC) containing 55% protein treated with formaldehyde[2] (0, .25, .5, 1.0 and 3.0%), tannic acid[3] (0, .5, 1.0, 3.0, and 6.0%) and heat (1, 2, and 3 hr). Casein treated with formaldehyde (0, .5, 1.0, and 3.0%)

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[1]Whey protein concentrate was produced in the South Dakota State University dairy plant by ultrafiltration of cheddar cheese whey.

[2]Commercial formaldehyde (37%), Fisher Scientific Company, Fair Lawn, New Jersey.

[3]Tannic acid, Mallinckrodt Chemical Works, St. Louis, Missouri.

and tannic acid (0, .5, 1.0, and 3.0%) was also evaluated. The number of in vitro studies for each treatment varied depending on results obtained in the preceding trial.

Ten g of WPC and casein were mixed with 100 ml of different solutions of formaldehyde and tannic acid. The mixture was stirred continuously for 2 hr, dried in an oven at 60 C, and ground in a mortar. Whey protein concentrate samples were heated in an autoclave at 2.04 kg of pressure and 104 C for the specified time and handled similarly to the previously described samples.

The in vitro studies were done according to the procedure described by Peter et al. (61) and modified to our conditions. The in vitro system utilized 100 ml tubes fitted with gas release valves in a water bath. One-half g of the various WPC and casein treatments were incubated in 50 ml of buffer solution at pH 6.8 and at pH 2.5 with .14 g pepsin. Also, .5 g of each treated sample was incubated in 50 ml of a mixture of strained rumen fluid-buffer solution (1:2 v/v) at pH 6.8. All samples were incubated at 39 C for 24 hr. After 24 hr, samples incubated in rumen fluid were acidified to pH 2 to stop bacterial action, centrifuged at 8000 x g for 4 min and 10 ml aliquots collected and stored in a refrigerator at 10 C until later analysis.

The samples incubated in buffer solutions were analyzed for soluble protein using the micro-Kjeldahl method (2), and samples incubated in rumen fluid were analyzed for ammonia by the phenol method (16).

In vivo. Eight 21-day-old weaned mice were assigned to each ration treatment shown in Table 1 in a series of in vivo assays designed to determine protein utilization. The protein portion of the ration was WPC or casein according to the previously described treatments. The number of feeding trials varied according to the response found in preceding studies. Feed consumption was determined daily by weighing the feed fed and subtracting the estimated feed refused in the feeders. The weight of feed refusals was not always accurate because some feed was scattered and some feces were mixed in the feed. Weight gain was determined by differences in weight at the start and end of the trials.

TABLE 1. Composition of diets fed to mice.

Ingredient	Amount, %
Salt mixture[a]	4.0
Vitamin mixture[b]	2.2
Corn oil	5.0
∝-cellulose[c]	1.5
Glucose[d]	70.3
Protein[e]	17.0
	100.0

[a] Wesson modification of Osborne-Mendel formula, Nutritional Biochemical Corporation, Cleveland, Ohio.

[b] Vitamin diet fortification mixture, Nutritional Biochemical Corporation, Cleveland, Ohio.

[c] Nutritional Biochemical Corporation, Cleveland, Ohio.

[d] Dextrose, J. T. Baker Chemical Corporation, Phillipsburg, New Jersey.

[e] Whey protein concentrate untreated or treated with .25, .5, or 1.0% formaldehyde; 1, 2, 3, or 6% tannic acid; or 1, 2, or 3 hr heat treatment or sodium caseinate (Nutritional Biochemical Corporation, Cleveland, Ohio) untreated or treated with .5 or 1.0% formaldehyde or 1.0% tannic acid.

Statistical analyses. The data were analyzed by the least square analysis of variance described by Steel and Torrie (73). Dunnett's test was used to compare the means to the control.

Results and Discussion

In vitro. Fig. 2 shows the protein solubility in pH 6.8 and pH 2.5 buffer (rumen and abomasum conditions, respectively). Fig. 3 shows in vitro rumen ammonia levels of WPC treated with different levels of formaldehyde, tannic acid, and heat. All formaldehyde treatments (Fig. 2) substantially reduced protein solubility of WPC ($P<.01$) at pH 6.8 compared to the control. At pH 2.5, WPC treated with all levels of formaldehyde was about 33% as soluble ($P<.01$) as the control. Rumen ammonia levels were reduced in all treatments, but only .5, 1, and 3% formaldehyde differed ($P<.01$) from the control. These results show that formaldehyde protected WPC from ruminal degradation as shown by the rumen ammonia values. These results indicate that all levels of formaldehyde protected WPC from ruminal degradation accompanied by low solubility at pH 2.5 (abomasum conditions).

Peter et al. (61) found that treatment of soybean meal with 0.6% formaldehyde or 1.5% glutaraldehyde, glyoxal, or 5% acrolein reduced ammonia production after 24 hr incubation in vitro. They also found protein solubility depressed ($P<.01$) when these same aldehydes were incubated in buffer solution.

Whey protein concentrate treated with .5 and 1% tannic acid was highly soluble in both pH 6.8 and 2.5 (Fig. 2). Solubility of

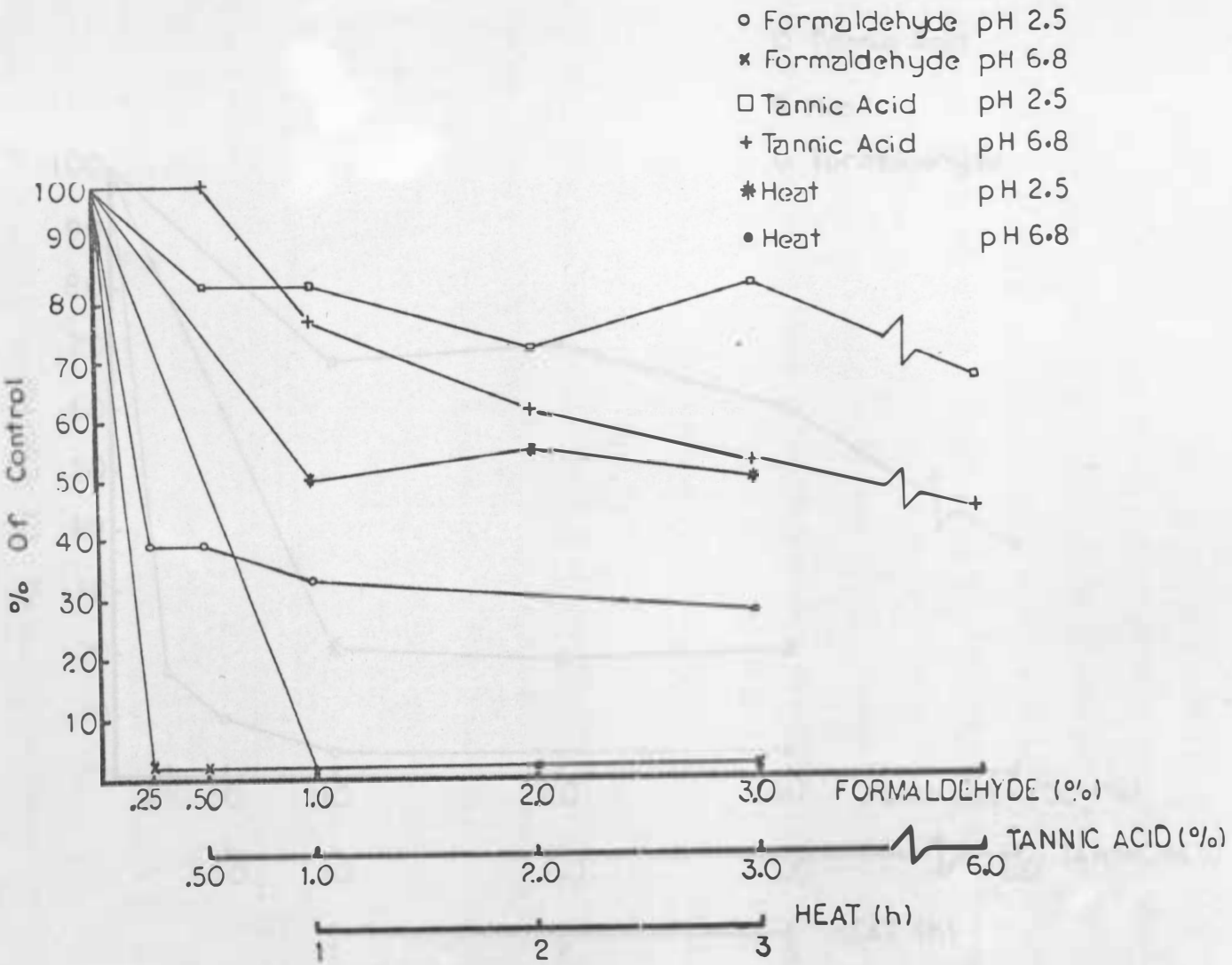

FIG. 2. Protein solubility of whey protein concentrate treated with different levels of formaldehyde, tannic acid, and heat under in vitro buffer conditions pH 6.8 and pH 2.5.

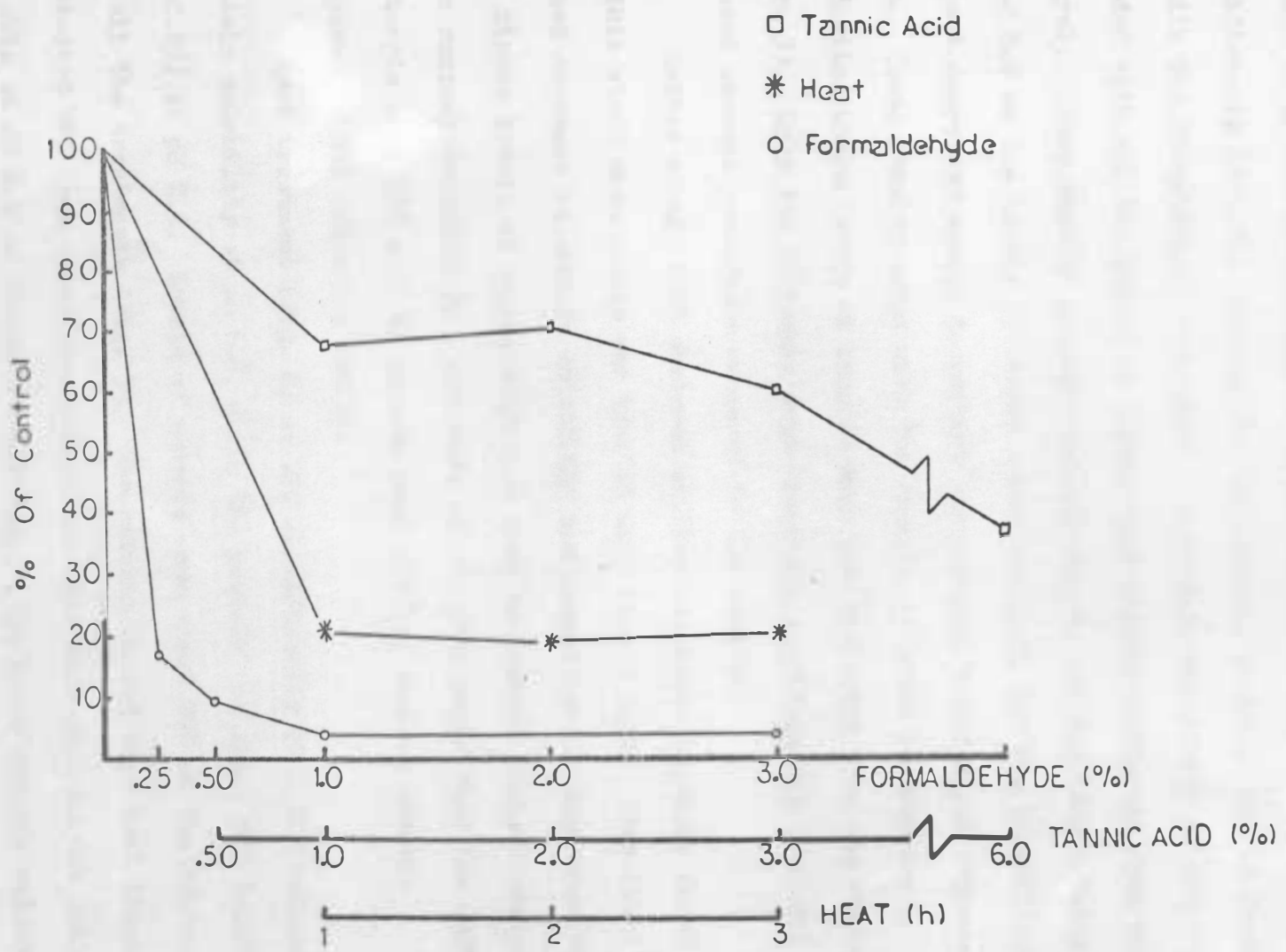

FIG. 3. Ammonia release of whey protein concentrate treated with different levels of formaldehyde, tannic acid, and heat under in vitro rumen conditions pH 6.8.

protein at pH 6.8 treated with 2, 3, and 6% tannic acid was reduced significantly ($P < .01$) compared to the control, although not as great as with the formaldehyde treatment. At pH 2.5, solubility of WPC treated with all the levels of tannic acid was not different from the control. These results suggest that protein in WPC was less soluble at pH 6.8 as the levels of tannic acid increased, but the solubility was not depressed enough to protect the protein from ruminal degradation. These results agree with the results obtained in ammonia production where levels of ammonia were not different from the control (Fig. 3). Only the 6% tannic acid treatment significantly ($P < .05$) reduced ammonia production compared to the control.

Zelter et al. (85) observed similar responses to those found in this study when casein was treated with tannic acid. They found a marked decrease in protein solubility and ammonia production when 4% and higher levels of tannic acid were used to protect dietary proteins from ruminal degradation. Nishimuta et al. (56) found that the addition of tannic acid (9% w/w) to soybean meal reduced ruminal ammonia compared to the untreated ration.

Heat treatment (Fig. 2) of WPC significantly ($P < .01$) reduced protein solubility at pH 6.8, while the protein was about 50% soluble ($P < .05$) at pH 2.5. Levels of ammonia were about 20% of the control in all the treatments (Fig. 3). The results showed that heat treatment protected WPC from rumen degradation and that this protein was quite soluble at pH 2.5 or abomasum conditions. The lower ammonia values

support the solubility results obtained under pH 6.8 buffer conditions where WPC was greatly protected.

Fig. 4 shows that all levels of formaldehyde reduced ($P < .01$) casein solubility at pH 6.8 and at pH 2.5 compared to the control. Fig. 5 shows that all the formaldehyde treatments decreased ammonia production, but only 1 and 3% formaldehyde treatments were statistically different ($P < .01$) from the control. These results suggest that casein was protected from degradation in the rumen, but the formaldehyde levels were not low enough to allow protein solubilization at pH 2.5 or abomasum conditions.

All the tannic acid treatments of casein failed to decrease protein solubility (Fig. 4) at both pH 6.8 and pH 2.5 compared to the control. Ammonia production (Fig. 5) was significantly reduced ($P < .01$) only with 3% tannic acid. There is no apparent explanation for the discrepancy between protein solubility at pH 6.8 and ammonia levels with the 3% tannic acid treatment.

In vivo. Feed consumption of mice fed WPC and casein treated with several levels of formaldehyde (Tables 2 and 3) was similar to the control group. Only the mice fed casein treated with 1% formaldehyde consumed less ($P > .05$) feed. Mice receiving WPC treated with .25 and .5% formaldehyde gained more weight than mice receiving WPC treated with 1% formaldehyde but gained less weight ($P > .05$) than the control group (Table 2). Mice consuming casein diets treated with .5% formaldehyde gained more weight than mice consuming casein diets treated with 1% formaldehyde but gained less ($P < .05$) than the control

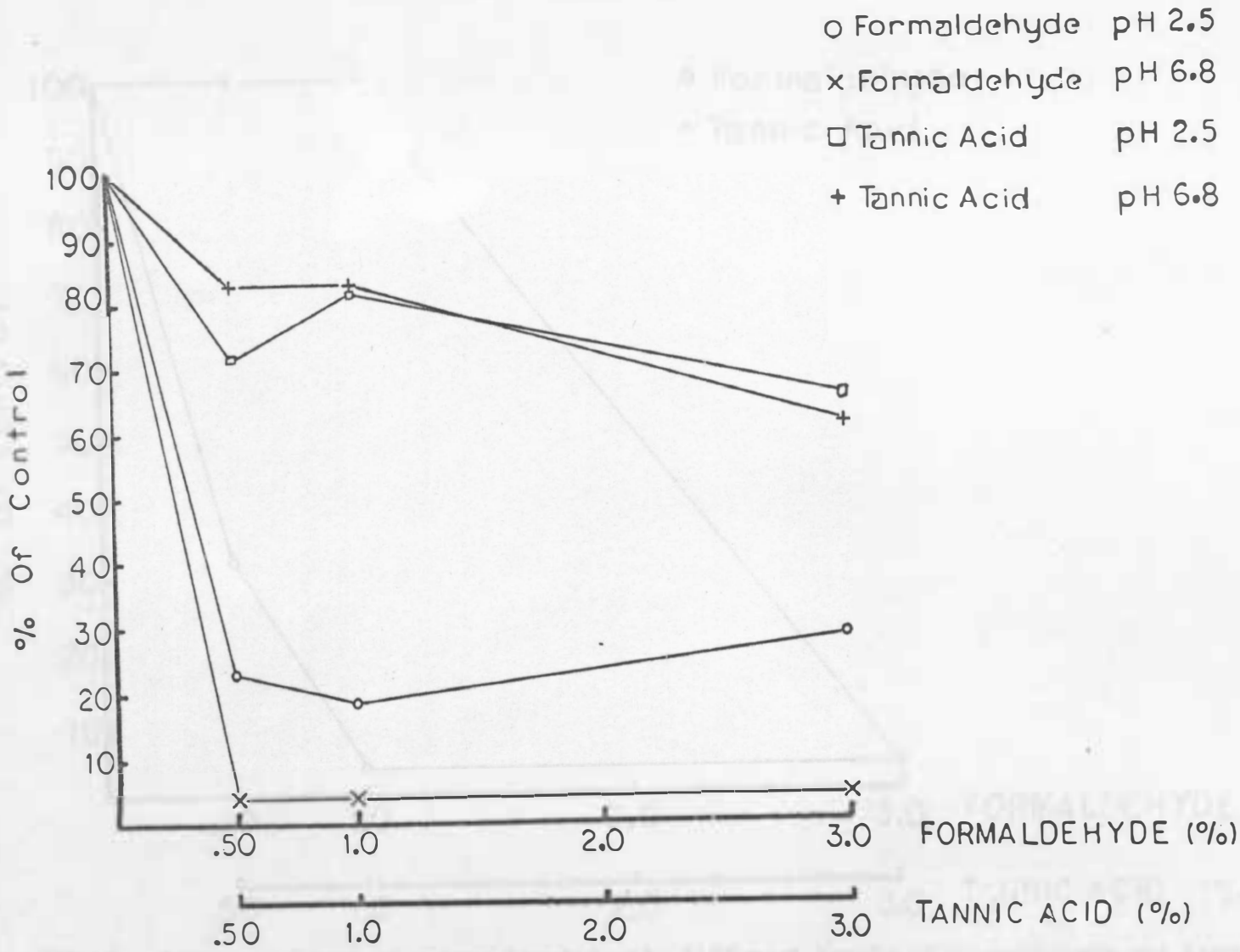

FIG. 4. Protein solubility of casein treated with different levels of formaldehyde and tannic acid under in vitro buffer conditions pH 6.8 and pH 2.5.

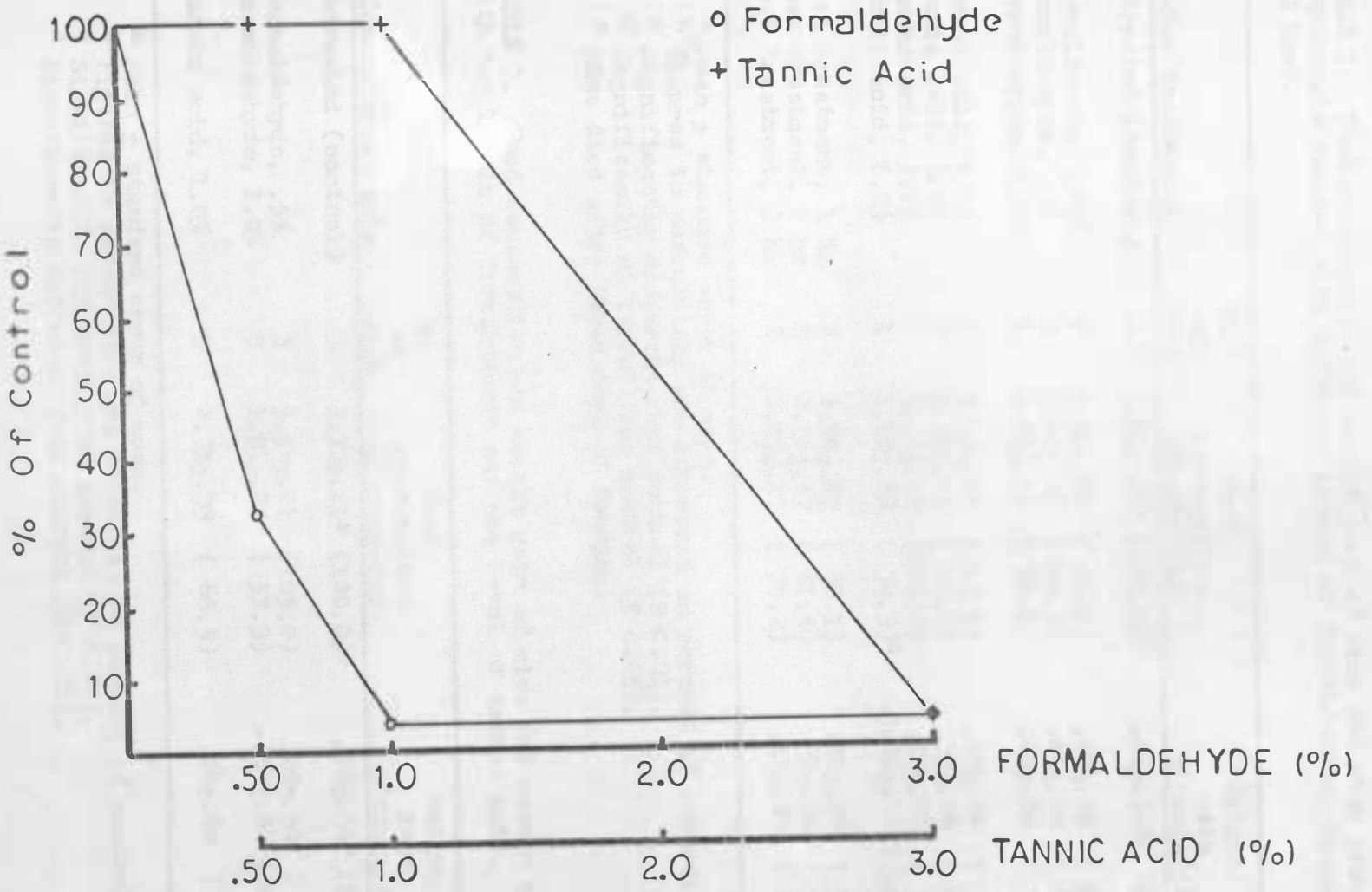

FIG. 5. Ammonia release of casein treated with different levels of formaldehyde and tannic acid under in vitro rumen conditions pH 6.8.

TABLE 2. Feed consumption and weight gain of mice fed whey protein concentrate treated with different levels of formaldehyde, tannic acid, and heat.

Whey treatments	No. of runs	Feed consumption g/mouse/day	Weight gain g/mouse/day
Untreated (control)	3	3.41±.21[a] (100.0)[b]	.66±.04[a] (100.0)[b]
Formaldehyde, .25%	2	3.34±.27 (97.9)	.60±.04 (90.9)
Formaldehyde, .5%	3	3.40±.21 (99.7)	.45±.04 (68.2)
Formaldehyde, 1.0%	1	2.46±.33 (72.1)	.21±.06 (31.8)[c]
Tannic acid, 1.0%	2	3.30±.27 (96.8)	.49±.04 (74.2)
Tannic acid, 2.0%	2	3.32±.21 (97.4)	.32±.04 (48.5)[d]
Tannic acid, 3.0%	2	3.58±.27 (105.0)	.24±.04 (36.4)[c]
Tannic acid, 6.0%	1	1.17±.28 (34.3)[c]	-3.02±.12 (Negative)[e]
Heat treatment, 1 hr	2	2.80±.27 (82.1)	.38±.04 (57.6)[d]
Heat treatment, 2 hr	2	2.78±.27 (81.5)	.20±.04 (30.3)[c]
Heat treatment, 3 hr	2	2.53±.27 (74.2)	.16±.04 (24.2)[c]

[a] Mean ± standard error of mean.
[b] Figures in parenthesis are expressed as percent of control.
[c] Significantly different from control ($P < .01$).
[d] Significantly different from control ($P < .05$).
[e] Mice died after three days of feeding.

TABLE 3. Feed consumption and weight gain of mice fed casein treated with two levels of formaldehyde and one level of tannic acid.

Casein treatments	No. of runs	Feed consumption g/mouse/day	Weight gain g/mouse/day
Untreated (control)	3	3.37±.21[a] (100.0)[b]	.66±.04[a] (100.0)[b]
Formaldehyde, .5%	3	3.54±.21 (105.0)	.40±.04 (59.7)[c]
Formaldehyde, 1.0%	3	1.93±.21 (57.3)	-.64±.04 (Negative)[d]
Tannic acid, 1.0%	1	2.31±.33 (68.5)	.64±.04 (95.5)

[a] Mean ± standard error of mean.
[b] Figures in parenthesis are expressed as percent of control.
[c] Significantly different from control ($P < .05$).
[d] Significantly different from control ($P < .01$).

group. The results in weight gain suggest that 1% formaldehyde over-protected WPC and casein, and mice were unable to utilize these proteins efficiently for growth. The results obtained here do not completely agree with the in vitro results observed in Fig. 2 and 4 in which both WPC and casein treated with formaldehyde were soluble at pH 2.5.

Ferguson et al. (33) found a 70% increase in wool growth when casein treated with 4% formaldehyde was fed to sheep. The change from treated to untreated ration decreased wool growth to 15% above the control group. Similar results were observed by Peter et al. (61) when soybean meal treated with .6% formaldehyde or 1.5% glyoxal was fed to sheep.

Feed consumption of mice fed WPC treated with several levels of tannic acid (Table 2) did not differ compared to the control. Only mice fed WPC treated with 6% tannic acid consumed less ($P<.01$) feed. Although mice in the treatment groups consumed similar amounts of feed as the control, they did not gain equal weight as the control group. Feed consumption and weight gain in mice fed casein treated with 1% tannic acid (Table 3) were similar to the same treatment of WPC. This decrease in weight gain may be due to a certain degree of toxicity caused by the tannic acid. Tannic acid intake of 6.0 g/kg body weight by mice (50) is considered lethal.

When WPC rations treated with heat (Table 2) were fed to mice, feed consumption was about 80% of the control, but weight gain decreased as heat time increased. Weight gain was highest in WPC treated with

heat for 1 hr. Weight gain on the 2 and 3 hr heated diets were significantly lower ($P<.01$) from the 1 hr and control groups. These results agree quite closely with the results observed in Fig. 2 in which WPC treated with heat was about 50% soluble at pH 2.5 compared to the control. Improved weight gains were found when heated dietary proteins were fed to ruminants (34, 56).

Conclusions

The conclusions that can be made from the results of this study are:

1. Formaldehyde treatment of whey protein at increasing levels (.25 to 3.0%) decreased ruminal degradation of the protein as indicated by in vitro incubation. Although these treatments did not show great solubility at pH 2.5, mice gained weight satisfactorily when fed rations containing .25 and .50% formaldehyde. Rations with WPC-formaldehyde levels greater than 1% appeared to overprotect the protein based on in vitro and mice trial results.

2. All levels of WPC-tannic acid were quite soluble at both pH 6.8 and pH 2.5. Ammonia production was significantly reduced ($P<.05$) only with 6% tannic acid. Based on in vitro and mice trial results, tannic acid was not effective in protecting whey protein.

3. All heat treatments protected whey protein from microbial degradation, but the protein was still soluble at pH 2.5. Heat treatment for 2 and 3 hr resulted in a large reduction in mice weight gains, indicating overprotection of the protein.

4. Tannic acid treatment of casein at the levels studied was not effective in protecting the protein.

5. All formaldehyde treatments protected casein from ruminal degradation. Based on mice weight gains, formaldehyde treatments greater than .5% overprotected the protein.

EXPERIMENT II

FORMALDEHYDE TREATED WHEY PROTEIN CONCENTRATE FOR LACTATING DAIRY CATTLE

Abstract

Four lactating Holstein cows were fed isonitrogenous rations of urea-corn silage and a 15% crude protein, pelleted grain ration containing whey protein concentrate (34% protein) either untreated (U-WPC) or treated (T-WPC) with 1% formaldehyde on a protein basis. The trial design was a three period, double reversal with 12 days per period during which milk and digestibility parameters were measured the last 4 days of each period. Apparent nitrogen digestibility (%), productive nitrogen retained (milk plus retained, g/day), and dry matter digestibility were 60.0 and 53.9 ($P < .05$), 89.0 and 103.8, and 67.4 and 63.2 for cows fed U-WPC and T-WPC rations, respectively. Productive nitrogen as a percent of absorbed was greater for cows fed the T-WPC ration, suggesting more efficient utilization of absorbed nitrogen. Milk production (kg/day), fat (%), fat yield (kg/day), and 4% fat-corrected milk (kg/day) were 27.6 and 29.4, 3.1 and 3.4, .86 and 1.00 ($P < .05$), and 23.7 and 26.9 for cows fed the U-WPC and T-WPC rations, respectively. Total milk nitrogen (g/day), true protein nitrogen (g/day), and casein nitrogen (g/day) were 135.84 and 140.90, 132.84 and 137.30, and 118.95 and 124.13 for cows fed U-WPC and T-WPC rations, respectively. No differences were found in rumen ammonia or blood urea. Rumen volatile fatty acids were higher in cows fed U-WPC rations at 4 and 6 hr postfeeding. Only milk fatty acid 16:0 was greater ($P < .05$) in cows fed U-WPC rations than in cows fed T-WPC rations. Differences in total and most essential amino acids between tail and mammary blood were greater for cows fed T-WPC rations.

Introduction

In ruminants a large portion of the dietary proteins are degraded in the rumen to peptides and amino acids (29) and then to ammonia. The ammonia is utilized by the rumen microorganisms to synthesize microbial protein. When low quality proteins are fed, the rumen microbes transform this dietary protein into a protein of high biological value. The opposite occurs, namely, high quality dietary proteins are converted by the rumen microbia into protein of lower biological value than the original protein. Thus, feeding high quality protein to ruminants may be wasteful unless the protein is permitted to bypass the rumen.

Infusion of high quality protein into the abomasum resulted in better protein utilization in terms of reduced rumen ammonia production and increased milk production (9, 26). Physical and chemical methods have been evaluated to protect high quality proteins from ruminal degradation. Formaldehyde is known to protect high quality proteins at normal rumen pH and allow the protein to be solubilized at the lower pH in the abomasum and lower digestive tract. Formaldehyde treatment of dietary protein improved protein utilization compared to feeding the same protein untreated (33, 37, 55, 60). No previous research has been conducted on bypassing the rumen with whey protein.

The present study was conducted to investigate the feeding of whey protein concentrate (WPC), a high quality protein, treated with 1% formaldehyde solution on milk production and composition and on nitrogen utilization and metabolism in dairy cattle.

Materials and Methods

Four lactating Holstein cows in similar stages of lactation were used to evaluate whey protein concentrate[4] (WPC) untreated (U-WPC) or treated with formaldehyde (T-WPC). A double reversal design with 12 days per period was employed. The cows were producing 28 to 30 kg milk per day at the beginning of the trial. The four cows were fed isonitrogenous rations of corn silage containing .5% urea and a 15.0% crude protein, pelleted grain ration containing WPC (34% protein). The total ration averaged 13.5% crude protein on a dry matter basis. The composition of the grain ration is shown in Table 4.

TABLE 4. Composition of grain rations containing untreated and formaldehyde treated whey protein concentrate (WPC).

Ingredient[a]	Ration kg
Whey protein concentrate (34% CP)[b]	19.50
Ground shelled corn	36.50
Ground oats	36.50
Dry molasses	5.00
Dicalcium phosphate	.70
Ground limestone	.30
Trace mineralized salt	1.00
Percent CP (N x 6.25)	15.00

[a] Vitamin A, 1200 IU/kg; vitamin D, 120 IU/kg.
[b] WPC either untreated or treated with formaldehyde.

The formaldehyde treated WPC was prepared by the addition of 1.22 l of commercial grade formalin (37%) to 132 kg of WPC in 160 l

[4]The whey protein concentrate was produced from the ultrafiltration of whey and was donated by Frank Thomas, Pollock, South Dakota.

of water. Formaldehyde was added at a rate of 1 g per 100 g protein in WPC. Untreated WPC was handled in a similar manner except the formaldehyde was omitted. Both T-WPC and U-WPC were dried in a forced air dryer on polyethylene beds for 16 days at 60 to 70 C until dryness. The WPC was stirred twice daily during the drying process. After drying, the WPC was ground and mixed into the respective rations.

Cows were adjusted to rations and environment for 6 days prior to initiation of the trial. During the trial, cows were fed 9 kg of grain ration daily and corn silage free choice. The cows were fed individually twice daily, and refusals were measured once daily. Feed and refusal samples were collected every other day, frozen, and later composited for laboratory analysis.

Individual milk weights were recorded daily. The last 4 days of each 12-day period were used for statistical analyses in order to eliminate any possible carryover effect. Milk samples were taken twice daily during the last 4 days of each period and composited for analysis. Samples were evaluated organoleptically by a panel composed of five persons. Milk samples were analyzed for fat by the Babcock test, total solids by the Mojonnier procedure, and nitrogen by the standard Kjeldahl procedure (2). Nitrogen components were determined by the Rowland procedure (65). Milk fatty acids were determined by gas liquid chromatography after methylation according to the technique described by Metcalf et al. (51). Prepared samples were injected into

a 1.83 m x 3.17 mm stainless steel column containing 10% EGSS[5] coated on 100/120 Gas Chrom P. Milk fatty acids were calculated by triangulation.

Digestibility of ration components and nitrogen balance were determined the last 4 days of each period. A sterile Bardex Foley urinary catheter[6] using 75 ml of sterile water was inserted into the bladder of each cow. Urine was collected into containers with 2 ml toluene added as a preservative. Individual urine and feces output were measured daily, frozen and later composited by period according to the proportion excreted daily by each cow.

Total nitrogen determinations of urine, feces, feed, and feed refusals were analyzed by the Kjeldahl procedure. Fecal and feed samples were analyzed on a wet basis. Dry matter content was determined in a forced air oven and energy content was determined with a Parr oxygen bomb calorimeter[7].

On the last day of each period, rumen samples were collected via esophageal tube at 0, 2, 4, and 6 hr postfeeding and pH immediately determined. Mercuric chloride was added (0.5 ml) to 10 ml of rumen fluid to stop bacterial action. Rumen samples were centrifuged at 7000 xg for 6 min, the supernatant collected and later analyzed for

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[5]Applied Science Lab, Inc., State College, Pennsylvania.

[6]C. R. Bard, Inc., Murray Hill, New Jersey.

[7]Parr Instrument Company, Moline, Illinois.

ammonia and urea using the method described by Chaney and Marbach (16). A portion of the supernatant was acidified for rumen volatile fatty acids determination (4) by gas liquid chromatography using a flame ionization detector. Samples were injected into a 1.52 m x 3.17 mm stainless steel column with 20% NPGS[8] plus 2% H_3PO_4 on 60/80 firebrick.

Jugular blood samples were collected from each cow at 0, 2, 4, and 6 hr postfeeding on the last day of each of the three periods. Blood samples were collected from tail artery and mammary vein at 6 hr postfeeding. All blood samples were allowed to clot, centrifuged at 1100 x g for 20 min and about 10 ml serum transferred into small plastic tubes and frozen. Serum samples were analyzed for ammonia and urea (16).

Serum amino acids were determined using the Beckman spinco amino acid analyzer Model 121 B[9] after precipitation of serum proteins with picric acid (7, 72).

Statistical analyses of all data were performed according to the method described by Lucas (47) for a double reversal design.

Results and Discussion

No significant differences were found in dry matter and energy intake or dry matter, crude protein, and energy digestibility between cows fed the U-WPC or T-WPC rations (Table 5). Although the differences were not statistically significant ($P > .05$), lower digestibility values

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[8]Applied Science Lab, Inc., State College, Pennsylvania.

[9]Beckman Instrument, Inc., Palo Alto, California.

TABLE 5. Dry matter, crude protein, and energy digestibilities of cows receiving untreated and formaldehyde treated whey protein concentrate (WPC) supplemented rations.

Variable	Ration		
	Untreated WPC	Formaldehyde treated WPC	SEM[a]
DM intake, kg/day	19.8	20.7	1.6
Apparent digestibility, %			
Dry matter	67.4	63.2	3.2
Crude protein	60.0	53.9	3.3
Gross energy intake (Mcal/day)	76.2	79.6	6.0
Fecal energy (Mcal/day)	25.7	30.3	3.9
Digestible energy (Mcal/day)	50.5	49.2	4.0
Digestible energy, %	66.2	61.7	3.2

[a] Standard error of mean.

were observed in cows fed the T-WPC ration. The decrease in protein digestibility probably accounted for the decrease in dry matter and energy digestibility.

Results in the literature (18, 31, 68) show that dry matter intake and dry matter digestibility were not affected when low levels of formaldehyde (.7 to 1.0% on protein basis) were used. Higher levels of formaldehyde (2.27%) added to soybean meal (54) decreased dry matter digestibility.

Barry (3) observed that the treatment of casein with 3% formaldehyde solution did not affect energy digestibility in the whole ration, but the digestibility was lower when casein consumption was low and digestibility increased as the casein consumption increased. Infusion of a liquid diet (5) providing 62% of the total energy into the abomasum lowered energy digestibility compared to when it was infused into the rumen. In trial I when WPC was treated with several

levels of formaldehyde, treatment with .25 to 3.0% formaldehyde solution (dry matter basis) protected protein from microbial degradation. The protein was approximately 50% soluble at abomasum conditions. Whey protein concentrate treated with .5% formaldehyde (dry matter basis) resulted in greater weight gain in mice as compared to higher levels of formaldehyde treatment. Feeding this level of formaldehyde treated WPC to dairy cattle appeared to decrease protein digestibility, possibly through overprotection of the protein.

Nitrogen utilization data from cows receiving U-WPC or T-WPC rations are presented in Table 6. Nitrogen absorbed was lower for cows fed the T-WPC than fed the U-WPC ration ($P>.05$). Urinary nitrogen excreted tended to be lower for cows fed the T-WPC ration compared to the U-WPC ration. Milk nitrogen tended to be greater in cows fed T-WPC than fed the U-WPC ration. No statistically significant differences were found in any of these parameters between cows fed the rations, but trends toward improved nitrogen utilization were apparent in cows fed the T-WPC ration. A small number of animals and large standard errors were associated with these means.

Nitrogen excreted in feces, urine, and milk and productive nitrogen expressed as a percent of N intake or percent of absorbed N were not different ($P>.05$) between cows fed the two rations. Higher values in terms of milk and productive N as a percent of absorbed were found in cows fed the T-WPC ration. Urinary N was lower in cows fed the T-WPC ration. These results reveal a tendency toward an improved

TABLE 6. Nitrogen utilization in cows receiving untreated and formaldehyde treated whey protein concentrate (WPC) supplemented rations.

Component	Ration: Untreated WPC	Ration: Formaldehyde treated WPC	SEM[a]
N intake, g/day	440.81	458.19	53.64
N absorbed, g/day[b]	264.89	246.77	23.58
N excreted, g/day			
Feces	175.92	211.42	23.69
Urine	175.85	142.98	50.20
Milk	135.84	140.90	21.07
N retained	-46.80	-37.11	30.94
Productive N[c]	89.04	103.79	49.39
N, % intake			
Feces	39.91	46.14	3.30
Urine	39.89	31.20	12.27
Milk	30.86	30.75	2.72
Productive N[c]	20.20	22.65	10.22
N, % absorbed			
Urine	66.39	57.94	20.18
Milk	51.28	57.10	7.90
Productive N[c]	33.61	42.06	20.17

[a] Standard error of mean.
[b] Absorbed = N intake - N feces.
[c] Productive nitrogen = N milk plus N retained.

utilization of the absorbed N for the T-WPC ration, possibly because of the high quality of the dietary protein bypassing the rumen.

Similar results in nitrogen digestibility, fecal and urinary nitrogen excretion, and utilization of absorbed N have been reported by researchers who bypassed the rumen with soybean and casein protein (1, 54, 55). Derrig et al. (26) also found an improved utilization of absorbed N when casein was infused into the abomasum.

The 1% formaldehyde treatment did not affect rumen pH, rumen ammonia, or plasma urea levels (Table 7). Ruminal ammonia levels tended to decrease slightly at 2 hr postfeeding ($P>.05$) in cows fed

TABLE 7. Rumen pH, ruminal ammonia, and plasma urea in cows fed untreated and formaldehyde treated whey protein concentrate (WPC) supplemented rations.

Variable (hours postfeeding)	Ration		SEM[a]
	Untreated WPC	Formaldehyde treated WPC	
Rumen pH			
0 hr	7.08	7.07	.11
2 hr	6.65	6.50	.30
4 hr	6.65	6.58	.36
6 hr	6.67	6.61	.22
Average	6.76	6.69	.05
Ruminal ammonia, mg/100 ml			
0 hr	4.64	4.94	2.38
2 hr	11.49	9.81	5.92
4 hr	5.60	7.35	5.56
6 hr	7.72	5.41	2.81
Average	7.36	6.88	1.92
Plasma urea, mg/100 ml			
0 hr	10.94	12.24	2.30
2 hr	11.05	12.03	1.21
4 hr	9.35	10.68	1.35
6 hr	9.19	9.63	1.66
Average	10.13	11.14	1.10

[a] Standard error of mean.

the T-WPC ration compared to cows fed the U-WPC ration. Plasma urea levels tended to be greater in cows fed the T-WPC ration than fed the U-WPC ration, although not significantly ($P > .05$).

Treatment of soybean meal with 2.27% formaldehyde (55) reduced rumen ammonia and plasma urea levels compared to controls. Schmidt et al. (68) observed that rumen ammonia levels decreased when soybean meal was treated with 1.6% formaldehyde and fed to steers. They observed higher blood urea levels in cows fed the treated ration than in cows fed the control ration, but this increase was not significant. Their results were similar to the results found in the present study.

There is no apparent explanation for the results obtained on serum urea levels in cows fed the T-WPC ration. The decrease in protein digestibility of cows fed the T-WPC ration indicated that overprotection of protein may have occurred which should have resulted in decreased serum urea levels.

Rumen VFA results (Table 8) show that at 0 hr (prior to feeding) rumen concentrations of individual and total VFA's were higher for cows fed the T-WPC ration than fed the U-WPC ration. Total VFA's at 0 hr were significantly different ($P<.05$). At 2 hr postfeeding, rumen VFA's were higher in cows fed the T-WPC ration, but only valeric acid was significantly different ($P<.05$) between rations. Volatile fatty acid levels were higher at 4 to 6 hr in cows fed the U-WPC ration, but these differences were not significantly significant ($P>.05$). Only valeric acid was found significantly different ($P<.05$) at 4 and 6 hr. Probably the differences in VFA concentrations between rations at 0 hr exerted a carryover influence on the concentration of VFA's at 2 hr. A change in VFA's from lower concentrations in cows fed the T-WPC ration to higher concentrations in cows fed the U-WPC ration occurred. At 6 hr the concentration of VFA's in the rumen remained higher in cows fed the U-WPC ration.

Similar results in rumen VFA's were found by Hutjens and Schultz (40) when soybean meal was treated with 2.7% formaldehyde and by Langlands (41) when wheat was treated with 0, 5, 10, 20, or 30% formaldehyde. Langlands (41) observed a slight decrease in rumen VFA's in cows fed the treated rations, and this decrease was related to the

TABLE 8. Rumen volatile fatty acids of cows fed untreated and formaldehyde treated whey protein concentrate (WPC) supplemented rations.

Volatile fatty acids	Ration: Untreated WPC	Ration: Formaldehyde treated WPC	SEM[a]
	m moles/L		
0 hr, before feeding			
C_2	58.24[b]	74.06[c]	7.72
C_3	28.50[b]	32.24[c]	.99
C_4	5.45	7.20	1.62
Ci_5	.66	.96	.23
C_5	.63	.67	.10
Total	93.48[b]	115.13[c]	6.81
2 hr postfeeding			
C_2	102.96	126.31	48.31
C_3	53.82	65.15	20.46
C_4	14.77	20.23	9.72
Ci_5	1.47[b]	1.84[c]	.17
C_5	2.10	2.78	.71
Total	175.12	216.31	78.58
4 hr postfeeding			
C_2	109.25	92.63	26.68
C_3	59.58	57.00	7.93
C_4	17.84	14.86	3.81
Ci_5	1.25[b]	.90[c]	.08
C_5	2.62	2.24	1.09
Total	190.54	167.63	29.87
6 hr postfeeding			
C_2	111.98	71.83	25.37
C_3	60.61	39.39	27.71
C_4	18.06	10.57	4.89
Ci_5	1.70[b]	.73[c]	.04
C_5	2.86	.74	1.79
Total	195.21	123.31	57.02

[a] Standard error of mean.
[b,c] Means in the same row with unlike superscripts differ ($P < .05$).

ammonia levels in the rumen. The decrease in rumen VFA's was not statistically significant between cows fed the treated and untreated rations.

Results in Table 9 show milk production and composition from cows fed U-WPC and T-WPC rations. Fat-corrected milk and milk fat percentage were not significantly different between cows fed both rations, but fat percentage tended to be higher in cows fed the T-WPC ration. Milk fat yield (kg/day) was higher ($P < .05$) in cows fed the T-WPC ration than fed the U-WPC ration, a result of the higher milk fat percentage and milk yield. Milk protein percentage (total N x 6.38) was higher ($P < .05$) in cows fed the U-WPC ration, but no difference in milk protein yield (kg/day) was observed between rations. Total solids percentage and yield were not statistically different between cows fed the two rations. Although the number of cows and the experimental periods were not large enough to reliably measure total milk production, cows fed the T-WPC ration showed a tendency toward increased milk production, milk fat percentage, and total solids percentage and yield.

Hutjens and Schultz (40) found that treatment of soybean meal with 2.7% formaldehyde decreased milk production and milk fat yield in goats, but fat percentage increased compared to the untreated ration. Reports in the literature (9, 26, 78) indicate increased milk production and milk production and milk protein but no effect on milk fat percentage when high quality proteins were infused into the abomasum.

TABLE 9. Dry matter intake, milk production, and milk composition of cows fed untreated and formaldehyde treated whey protein concentrate (WPC) supplemented rations.

Variable	Ration		SEM[a]
	Untreated WPC	Formaldehyde treated WPC	
Dry matter intake, kg/day			
Grain	8.50	8.50	2.58
Silage	11.28	12.18	1.52
Total	19.78	20.68	1.56
Milk yield, kg/day	27.62	29.36	4.12
4% fat-corrected milk, kg/day	23.73	26.89	2.31
Milk fat, %	3.10	3.42	.31
Milk fat yield, kg/day	0.86[b]	1.00[c]	.04
Milk protein (N x 6.38), %	3.14[b]	3.09[c]	.02
Milk protein yield, kg/day	.87	.90	.11
Total solids, %	12.08	12.22	.59
Total solids yield, kg/day	3.34	3.59	.34

[a] Standard error of mean.
[b,c] Means in same row with unlike superscripts differ ($P<.05$).

The results of nitrogen distribution in milk (Table 10) show that total nitrogen, protein nitrogen, and whey nitrogen percentages were higher ($P<.05$) in cows fed the U-WPC ration than fed the T-WPC ration. Nonprotein nitrogen and casein nitrogen were not different between the cows fed the two rations. None of the yields (g/day) of milk nitrogen fractions were different between rations, although a trend existed for higher yields of all nitrogen components on cows fed the T-WPC ration. Clark et al. (18) found similar results in total nitrogen in milk as those found in this study when soybean meal treated with .9% formaldehyde was fed to lactating cows. Derrig et al. (26) found that the continuous infusion of 5 l of a 9% (w/v) solution of sodium caseinate into the abomasum increased total nitrogen in milk. No detailed results of N components were reported.

TABLE 10. Nitrogen distribution in milk of cows fed untreated and formaldehyde treated whey protein concentrate (WPC) supplemented rations.

Component	Ration		
	Untreated WPC	Formaldehyde treated WPC	SEM[a]
Milk nitrogen fractions, %			
Total nitrogen	.493[b]	.485[c]	.004
Nonprotein nitrogen	.011	.012	.002
Protein nitrogen	.482[b]	.473[c]	.004
Casein nitrogen	.432	.428	.006
Whey nitrogen	.050[b]	.045[c]	.002
Milk nitrogen yield, g/day			
Total nitrogen	135.84	140.90	17.6
Nonprotein nitrogen	3.00	3.60	.55
Protein nitrogen	132.84	137.30	17.0
Casein nitrogen	118.95	124.13	14.8
Whey nitrogen	13.89	13.17	1.73

[a] Standard error of mean.
[b,c] Means in same row with unlike superscripts differ significantly ($P < .05$).

Milk fatty acids (Table 11) expressed as molar % did not differ ($P > .05$) between cows fed the U-WPC and T-WPC rations, except 16:0 was higher ($P < .05$) in cows fed the U-WPC ration. A depression in milk fatty acids 14:0 and 16:0 was observed by Hutjens and Schultz (40) when soybean meal treated with 2.7% formaldehyde was fed to goats. They found a slight increase in unsaturated long chain fatty acids. Similar results were observed in the present study in cows fed the T-WPC ration.

Table 12 shows the results of concentration of serum amino acids in tail artery and mammary vein and the difference or the apparent amino acid uptake by the mammary gland. In the present study more emphasis should be directed toward total amino acids rather than individual amino acids. The results showed a higher total essential amino acid uptake in cows fed the T-WPC than fed the U-WPC ration.

TABLE 11. Milk fatty acids from cows fed untreated and formaldehyde treated whey protein concentrate (WPC) supplemented rations.

Milk fatty acids	Ration		SEM[a]
	Untreated WPC	Formaldehyde treated WPC	
	Molar %		
4:0	4.88	5.03	2.09
6:0	6.04	6.34	1.51
8:0	2.88	3.11	.82
10:0	5.59	6.49	3.00
12:0	5.01	5.00	.41
14:0	14.17	14.24	1.04
16:0	27.85[b]	25.84[c]	1.23
16:1	2.02	2.12	.29
18:0	8.13	8.22	.90
18:1	21.64	22.54	3.16
18:2	.94	1.08	.35
18:3	.34	.33	.20

[a] Standard error of mean.
[b,c] Means in same row with unlike superscripts differ significantly ($P < .05$).

This increase in total essential amino acid uptake is also shown with most of the individual essential amino acids. This may be related to the previously improved utilization of the absorbed nitrogen when high quality protein bypassed the rumen and, consequently, an improved amino acid balance at the mammary gland. The present results are in agreement with those observed by Derrig et al. (26) when casein was infused into the abomasum.

TABLE 12. Concentration of amino acids in tail artery and mammary vein of cows fed untreated and formaldehyde treated whey protein concentrate (WPC) supplemented rations.

	Ration					
	Untreated WPC			Formaldehyde treated WPC		
Amino acid	Tail	Mammary	Difference	Tail	Mammary	Difference
	uM/100 ml					
Essential						
Lysine	8.0	4.3	3.7	7.7	3.5	4.2
Histidine	.4	2.5	--	1.2	1.6	--
Arginine	7.7	5.9	1.8	7.1	3.9	3.2
Threonine	7.6	4.7	2.9	5.7	2.9	2.8
Valine	9.2	8.4	.8	10.9	3.8	7.1
Methionine	.9	.2	.7	3.5	.3	3.2
Leucine	8.6	4.9	3.7	8.1	3.1	5.0
Isoleucine	5.5	3.5	2.0	5.3	2.1	2.2
Phenylalanine	1.8	1.3	.5	2.5	1.0	1.5
Nonessential						
Aspartic acid	4.9	2.5	2.4	3.5	3.0	.5
Serine	9.8	7.4	2.4	7.1	4.4	2.7
Glutamic acid	6.4	3.0	3.4	5.2	3.2	2.0
Glycine	24.9	21.8	3.1	18.7	9.3	9.4
Alanine	16.1	14.1	2.0	13.4	7.5	5.9
Cystine	.4	2.1	--	Tc	1.2	--
Tyrosine	2.3	1.4	.9	1.8	1.2	.6
EAA[a]	49.7	35.7	14.0	52.0	22.2	30.0
NEAA[b]	64.8	52.3	12.5	49.7	29.8	17.9
EAA:NEAA[a,b]	.8	.7		1.0	.7	

[a] Essential amino acids.
[b] Nonessential amino acids.
[c] Trace.

Conclusions

The conclusions that can be made from the results of this study are:

1. Formaldehyde treatment of WPC to allow rumen bypass showed a tendency toward increased milk production, milk fat yield, and total solids.

2. Formaldehyde treatment did not greatly alter nitrogen content of milk (percent and yield).

3. Formaldehyde treatment lowered nitrogen digestibility, but absorbed nitrogen tended to be more efficiently utilized for productive purposes.

4. Serum amino acid patterns indicate that bypassing the rumen with high quality protein may be associated with the improved utilization of absorbed nitrogen.

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