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Treatment of Alfalfa Haylage with Propionic Acid and Physiological Alterations Resulting From Feeding Heat Damaged Forage to Cows, Heifers, and Voles

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TREATMENT OF ALFALFA HAYLAGE WITH PROPIONIC ACID AND PHYSIOLOGICAL ALTERATIONS RESULTING FROM FEEDING HEAT DAMAGED FORAGE TO COWS, HEIFERS, AND VOLES

By

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TREATMENT OF ALFALFA HAYLAGE WITH PROPIONIC ACID AND PHYSIOLOGICAL ALTERATIONS RESULTING FROM FEEDING HEAT DAMAGED FORAGE TO COWS, HEIFERS, AND VOLES

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Alfalfa haylage was treated with 1% (wet basis) propionic acid at ensiling (Experiment I). Propionate increased ($p < .05$) DM recovered after ensiling. Temperature and acid detergent insoluble nitrogen in haylage were not reduced by propionate, but water soluble nitrogen (indicative of reduced proteolysis) was. Acetic acid concentration was less in propionate treated haylage, but butyric and isobutyric acid concentrations were very low in both treated and untreated. Ad libitum forage DM intake was increased ($p < .025$) 2.1 KG/day for cows consuming propionate treated haylage as the only forage compared with those consuming untreated haylage. Corn silage added to haylage rations did not affect forage consumption. Fat corrected milk production and milk fat production were not different between treated and untreated haylage or between rations with or without corn silage. Milk fat percent was reduced ($p < .05$) about .3 percentage units in cows receiving propionate treated haylage with or without corn silage compared with cows receiving control haylage.

In Experiment II alfalfa-grass mixtures were ensiled in glass jars for 40 days at different DM contents. Water soluble nonprotein nitrogen increased during wilting, and water soluble protein decreased. Water soluble ammonia did not increase during wilting. The pH and water soluble nitrogenous compounds were reduced ($p < .025$) during ensiling with propionate below untreated material. During ensiling ammonia showed the largest increase of the water soluble N fractions, but all fractions were increased above unensiled values.

In Experiment III unheated and heated (80 C) alfalfa based diets were fed to immature meadow voles for 9 days (only alfalfa was heated). Gains and protein efficiency ratios (PER) were reduced ($p < .05$) on negative control (low protein) and 72 hr. heated alfalfa diets. After removal from experimental diets compensatory gains occurred for voles fed diets with restricted gains during the experimental period. Mortality was not treatment related. In the second part of this experiment diets were formulated with and without supplemental casein. Gain and PER both unadjusted and adjusted for intake differences appeared to be increased by addition of casein to the heated diet, and the PER of the supplemented heated diet approached values for unheated controls. Therefore, supplemental protein overcame detrimental effects (reduced gains and intake) of heating.

In a longer term study mature females were fed these diets for 60 days (Experiment IV). No hypertrophy of liver, kidneys, or

intestine occurred on the heated diets. Mortality due to cannibalism occurred on the unsupplemented heated diet, but supplementation with casein alleviated this. At day 0, 10, and 16 of lactation pups per litter, weight per pup and total litter weight were reduced for females fed the unsupplemented heated diet compared with those fed the supplemented heated diet. Crude protein intakes for the supplemented heated diet approached those fed unheated positive control. This indicates supplemental protein added to a heated ration is utilized for gestation and lactation, and no overt detrimental effects were evident.

In Experiment V heat damaged haylage (50% ADIN) was fed to growing heifers during a 20 day trial. Intake of DM was not affected by switching from a "good" quality (low ADIN) to a "poor" quality haylage. Digestibility of DM in heat damaged haylage averaged 48.9%. One-half of the animals were fed a protein supplement equal to 12-20% of the consumed protein from haylage. Albumin was reduced ($p < .05$) and globulin increased ($p < .05$) in heifers supplemented with protein. Serum sodium, phosphorus and uric acid concentrations were elevated ($p < .05$) after a switch from "good" to "poor" haylage. GOT, urea and glucose were reduced ($p < .05$) after this switch.

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INTRODUCTION

Since the beginning of animal agriculture there has been a need for a year round supply of feed. In more tropical latitudes with adequate moisture this could be accomplished by year round grazing, but in temperate areas that is not possible. Therefore a system of forage preservation allowing feed storage for prolonged periods became a necessity. To accomplish this feed was either dried or ensiled. In the dry form no microbial activity and consequent decomposition occurs and the forage or cereal is stable for long periods. On the other hand ensiling involves formation of an anaerobic atmosphere in a sealed enclosure. Microbial activity occurs and a drop in pH results from formation of organic acids. This drop in pH if drastic enough will retard any further microbial activity resulting in a stable feed with limited transformations occurring.

Alfalfa or grass forage can be stored at varying dry matter (DM) concentrations ranging from about 90% for hay to about 20% for direct cut material (no wilting) with haylage being intermediate (40-60%). Less total nutrient losses results with a haylage system compared with hay (large field losses) or direct cut silage (large storage losses). Also haylage can be handled mechanically with a minimum input of labor whereas hay requires considerable manual labor.

Although haylage has become more frequently used by farmers, problems still occur such as excessive heating due to improper storage conditions. Also mold growth and spoilage can occur on occasion if the storage system is not anaerobic. With this in mind I have attempted to use propionic acid as a preserving agent to increase the nutritive value of haylage. An examination of changes in nitrogen fractions as a result of drying and ensiling with and without propionate was undertaken in an attempt to relate nitrogen transformations to changes in quality.

Heat damaged forage has been shown to have excessive protein bound to other components resulting in a reduction in digestible protein. Experiments with heated casein-glucose systems indicate this might not be the only detrimental effect since addition of extra protein does not always overcome deleterious signs. Also certain physiological processes have been affected by feeding this type of browned product. With this in mind I undertook the present study to ascertain if these same problems might be encountered with browning of forages.

LITERATURE REVIEW

Conservation of Forages

Forage is handled primarily in three ways: hay (80-90% DM), haylage (40-60% DM) or direct cut silage (25-40% DM). Extent of material harvested as haylage has increased during the past several years.

Dry matter recovery during harvesting and storage appears greatest for wilted silage or haylage (85%) when compared with field dried hay (79%) or direct cut silage (80%) (Dijkstra, 1957). Waldo (1977) in a review summarizes DM recoveries from several experiments and concludes field cured hay averaged 75%, haylage 85% and direct cut silage 80%, thus giving an advantage to haylage. Hoglund (1964) considered field losses to be greatest in making field cured hay and storage losses to be greatest for direct cut silage, and was consistent with Dijkstra (1957) above.

Haylage has another advantage over hay because it can be handled mechanically with a small amount of manual labor input. This is not an advantage over direct cut silage.

Hay does have certain advantages, such as greater intakes when compared with ensiled feeds (Demarquilly and Jarrige, 1970). This indicates that transformations during ensiling may be responsible for reduced intakes since the actual presence of water in the forage is not the cause illustrated by the fact intake of fresh

forage is greater than that of hay. This positive relationship between DM content during storage and intake has been observed by Shepard et al. (1953), Hillman et al. (1958), Thomas et al. (1961), Gordon et al. (1961), and Clancy et al. (1977).

Another problem associated with haylage is a tendency for overheating especially with higher DM material. Pierson et al. (1971), Thomas et al. (1972), and Goering and Adams (1973) conducted field surveys and found 30-40% of haylage samples are "heat damaged," and a consequent reduction in nitrogen digestibility would be expected. This points out the potential for problems associated with excessive heating in haylage systems.

Transformations of Nitrogenous Compounds in Forages During Drying and Ensiling

Nitrogenous Compounds in Fresh Forage

According to Hegarty and Peterson (1973) most of the nitrogen in fresh forage is in true protein form. The nonprotein nitrogen fraction is composed primarily of amides (glutamine and asparagine) and free amino acids, but there are also low molecular weight peptides, nucleotides, amines, ureides, chlorophyll, nitrates and ammonia.

True protein in fresh forages has been found to vary with species and stage of maturity (Kolousek and Coulson, 1954). Protein nitrogen as a percent of total nitrogen averaged 83% for mature red clover, 70% for mature alfalfa, 60% for mature orchard grass and 80%

for mature timothy. Pepsin insoluble nitrogen appeared greatest for those species with the most true protein.

Solubility of nitrogen is a commonly used procedure for fractionation of nitrogenous compounds. Solubility has also been related to degradability in the rumen of cattle giving an indication of nitrogen available to rumen microbes. Wilson and Tilley (1965) determined the water soluble nitrogen content of fresh alfalfa and certain grasses, and found alfalfa to average about 32% of the total nitrogen as water soluble while grasses averaged only 15%. This difference might reflect the greater concentration of cell contents found in legumes. Brady (1960) observed that a large part (48%) of the soluble nitrogen in fresh grass forage was protein in nature.

Transformations During Drying

With wilting a certain amount of proteolysis occurs. Kemble and Macpherson (1954) demonstrated during a 3 day wilting period over 20% of the protein was degraded to nonprotein nitrogen, and rate of proteolysis depended on rapidity of dehydration being least on the most rapidly dehydrated. They also observed an increase in proline with reductions in other monoamino monocarboxylic acids. Proline may play a role in ammonia detoxification when plants are water starved. In ryegrass soluble amino, volatile and amide nitrogen increased during wilting from 0 to 26.5 hr. coinciding with a reduction in protein nitrogen (Brady, 1960).

Transformations During Ensiling

The ensilage process results in a redistribution of nitrogen after fermentation. Kemble (1956) anaerobically ensiled direct cut rye-grass with glucose at 30°C in jars. Soluble nitrogen increased from 18.2% of total nitrogen to 59.2% by day 147 of ensiling. Volatile bases (ammonia) also increased from .5 to 3% and alpha-amino nitrogen from 5 to 20.6%. Another group of jars were inoculated with Clostridia, pH increased upon ensiling indicating poor quality silage. With this increased pH was observed a greater increase in soluble nitrogen than in uninoculated silage. Also large amounts of volatile bases were present in poor quality silage indicating excessive protein degradation in improperly ensiled material. McDonald and Whittenbury (1973) considers clostridial fermentation to result in ammonia and undesirable nitrogenous compound formation which can be prevented by proper ensiling techniques. Kemble in the above experiment observed a considerable excess of alanine above that which could be explained by proteolysis alone in the "bad" silage, and after 8 weeks alpha-amino butyric acid began to appear. Another series of jars ensiled without microbes (sterile) underwent extensive proteolysis, but no ammonia was present. Kemble interpreted this to imply that microbial activity is not necessary for proteolysis which is probably a result of plant proteases, but microbes are necessary for amino acid breakdown to ammonia. Contrary to this Brady (1960) indicated deamination of amino acids to ammonia can result from plant enzyme activity during early ensilage.

Plant leaf proteases appear to have a pH optimum between 5 and 6 (Tracy, 1948). Most fresh forages will be within this range. After ensiling, pH will decrease and the rapidity will affect the extent of protein breakdown and at a pH of 4.3 or below most of the proteolytic activity will be inhibited (Macpherson, 1952). Addition of acids at ensiling would be expected to reduce proteolysis and subsequent formation of nitrogenous compounds implicated in reducing intakes in ruminants.

Brady (1960) observed an increase in soluble amino, volatile and amide nitrogen during wilting of ryegrass from 0 to 26.5 hrs. This increase in nonprotein nitrogen coincided with a reduction in protein nitrogen. When this material was ensiled at differing DM contents soluble amino nitrogen increased as DM increased, but volatile and amide nitrogen decreased. This indicates that protein degradation to amino acids was most predominant in higher DM silages, but subsequent breakdown to ammonia did not occur as readily as in the lower DM silages. The unidentified nitrogen fraction was about the same regardless of initial DM. The author did note this unidentified fraction increased early in the ensiling process, but the increase was not apparent during wilting indicating a different degree or type of transformation.

Barry et al. (1978) observed alanine and alpha and gamma butyric acids to be increased in poor quality silages, probably a result of decarboxylation by proteolytic clostridia. Ammonia formation, on the other hand, was a result of amino acid

deamination. The authors present the idea that silage nutritive value is better related to decarboxylation than deamination because decarboxylation results in amines which are potentially toxic to animals. Deamination yields only volatile fatty acids and ammonia which are commonly present in the gastro-intestinal tract of ruminants and are non-toxic under physiological conditions. If this is true analysis of silage for presence of alanine, alpha and gamma butyric acid would give a much more precise indication of nutritive value than analysis for volatile fatty acids or ammonia. The authors conclude that this amino acid analysis might be abbreviated by using short-column procedures which measure alanine, alpha and gamma butyric acid.

Ohshima and McDonald (1978) presented the idea that ammonia formation during ensiling results mainly from deamination of arginine, serine and amides and the reduction of nitrate by lactic acid bacteria.

Hughes (1970) ensiled ryegrass in 7ft. high silos and determined the composition of nitrogen compounds in the water soluble fraction. In unensiled material peptide nitrogen (includes soluble proteins) composed 60% of the water soluble nitrogen, amino nitrogen 7.3%, amide 10%, volatile amine (ammonia) 1.0% and unknown nitrogen 21.7%. After ensiling for 2 mo. these values were 11.0, 45.5, 3.8, 13.4 and 27.3% respectively. By 18 mo. of ensiling these values were 5.0, 33.2, 3.0, 22.8, and 36.0% respectively. No water soluble protein was found in any of the silages. Total soluble nitrogen

of initial forage was 53.1% of the total nitrogen and increased to 66% by 2 mo. and did not change much thereafter. These results demonstrate a decrease in peptide and amide nitrogen with ensiling while ammonia and the unknown nitrogen fraction increases. The amino nitrogen fraction was greatest at 2 mo. then decreased, but was above initial material at 18 mo. From a practical point of view the author feels these changes are probably not sufficient to influence nitrogen utilization since ruminants are animals which degrade a portion of the nitrogen before utilization by microbes. No relationship with intake was considered, nor was the desirability of insoluble protein.

Hughes went one step further and characterized amino acid and non-volatile amine changes during ensiling. Cadeverine was found to compose 6-7% of the nonprotein nitrogen and would account for a majority of the lysine losses (about 80%) during ensiling. Putrescine composed 4-5% of the nonprotein nitrogen and accounts for 60-70% of argine losses. Histamine or tryptamine were not present in these silages except in bound form, and only low concentrations of ethanolamine, phenylethylamine and tryamine were present. The presence of these amines demonstrates decarboxylases exert a major role in nitrogen transformations during ensiling. A selective degradation of amino acids occurred and certain ones were quickly degraded while others were not. Losses of aspartic acid, methionine, tyrosine, lysine and arginine occurred during the first 2 mo. of ensiling. Lesser losses of threonine, serine, glutamic acid and histidine were noted. Reduction in amount of proline, glycine,

cystine, valine, isoleucine, leucine and phenylalanine was not observed during the first 2 mo., but proline, glycine, cystine and leucine were degraded during further storage.

In a subsequent paper Hughes (1971) examined composition of nitrogen components in poor quality grass silages collected from farmers. Three high pH silages (4.9-5.7) were analyzed. Losses of amino acids coincided with increases in ammonia, but the lower aliphatic amines were not present. Putrefaction products (resulting from decarboxylation) putrescine, cadaverine and histamine were present only in small amounts. The author concluded nitrogen changes in high pH silages are similar to those in good quality silages. Since high pH silages are likely to have high numbers of clostridia the results of this experiments are surprising because Kemble (1956) found excessive protein breakdown in silages with large clostridia numbers. Therefore,even though these silages were of poor quality with a high pH, clostridia probably did not dominate secondary fermentation. The water soluble nitrogen fraction of a heat damaged silage was composed of 23% peptide nitrogen, but both high pH and good quality silages contained only 6%. Also the free amino acid content of the heat damaged silage was low. This implies that the water soluble peptide fraction may be of some nutritional importance in heat damaged silage.

Fermentaion reduces the true protein fraction in silages and increases soluble nonprotein nitrogen and may also increase unavailable insoluble nitrogen (acid detergent insoluble nitrogen).

The greater the degree of heating, the greater will be the proportion of insoluble nitrogen. The true protein fraction can be divided into protein readily degraded when incubated with proteolytic enzymes versus protein not readily degraded. Kinetic studies reveal readily degradable protein has a half-life of about 10 min. when incubated with a protease. The less degradable protein fraction has a half-life of about 4 hrs. (Pichard and Van Soest, 1977).

Hawkins et al. (1970) ensiled alfalfa at 4 DM contents (22, 40, 45, 80%) and found water soluble nitrogen decreased from 68 to 29.1% of the total nitrogen as DM increased from 22 to 80%. Water soluble nonprotein nitrogen components (ammonia, alpha amino nitrogen and undetermined nitrogen) all showed an inverse relationship with forage DM indicating proteolysis decreased as DM increased. Sheep DM intake increased from 49.1 at 22% DM to 63.3g/Kg body wt. at 80% DM. Demarqiully (1973) fed 87 silages to sheep and found a 33% reduction in intakes compared with fresh forage. Degree of reduction varied from 1 to 64%. It is tempting to speculate this reduction in intake at lower DM's is due to products of proteolysis, but other parameters are also different such as pH and organic acid concentration.

Addition of Propionic Acid at Ensiling

The nitrogen fraction considered most important and given the most attention in haylages is the nitrogen found in the acid detergent fiber fraction and is termed acid detergent insoluble nitrogen (ADIN). Thomas (1976) summarized several experiments using

forage conserved in cement stave silos, small experimental silos and wet baled hay and noted a positive correlation (.7 or above) between temperatures produced during ensiling and ADIN concentrations.

A negative relationship between temperatures during ensiling and nitrogen utilization in vivo has been demonstrated by several investigators (Gordon et al. 1961; Roffler et al. 1967; Sutton and Vetter, 1971; Yu and Thomas, 1975).

Addition of propionic acid at ensiling has been advantageous for high moisture grains such as corn (Jones 1970), corn silage (Britt et al. 1975) and haylage (Yu and Thomas, 1975). Thomas (1976) found more feedable halage, reduced temperatures, and reduced fungal numbers in material ensiled with propionic acid using 55 gal. barrels. Therefore, propionic acid appears to reduce molding and heating which would increase silage quality and limit ADIN formation.

Propionic acid may also be acting to improve the quality of ensiled material by reducing the degree of fermentation. Yu and Thomas (1975) treated alfalfa with .4 and .8% propionate as it was entering the silo. Lactic acid concentrations, indicative of degree of fermentation, were reduced. Britt et al. (1975) added 1% propionic acid to corn silage at ensiling and found lactic acid concentrations reduced from 8% of the DM on the untreated to less than 1% for the treated material. Reduced fermentation would indicate less degradation of compounds found in the original unensiled forage.

Haylage treated with .4 and .8% propionic acid was fed ad libitum to lactating dairy cows (Yu and Thomas, 1975). Cows consuming haylage treated with .8% propionic acid consumed 17.7 Kg/day compared with 13.6 Kg/day for those consuming untreated haylage, but milk production was not stimulated. Therefore, propionic acid appears to alter ensiling processes and reduce formation of compounds that limit intake.

One of the reasons treated haylage was consumed in greater quantities may be a reduced concentration of proteolytic byproducts. Few experiments have determined nitrogen distribution after propionic acid addition. Lichtenwalner et al. (1979) reconstituted sorghum grains by adding water to produce a 70% DM product. The grains were ensiled with or without 2% propionic acid for 21 days. Propionic acid reduced proteolytic activity by 75% by day 3 when compared with the untreated control. Buffering did not prevent reduction in activity indicating reduced pH was not responsible. Therefore, propionic acid appears to exert an effect on proteases not due to pH.

Formation of Heat Damaged Proteins

Carbonyl-Amine Reactions (The Maillard Reaction)

Amino groups ($-NH_2$) of amino acids, peptides and proteins are capable of reacting with other compounds especially those containing a carbonyl group (-COH). These processes most often occur during feed or food processing, but can also occur during storage.

Maillard in 1912, using a glucose-glycine solution, characterized the carbonyl-amine interaction, and this reaction is now known as the Maillard Reaction. The browning reaction or nonenzymatic browning are other synonyms.

This carbonyl-amine reaction is really a complex set of many reactions involving several types of basic amino compounds. Amine groups can be components of amino acids, peptides or proteins. When a protein is altered the first group affected is the N-terminal amino group; next are the basic amino acids (their side chains) especially lysine followed by the sulfur containing amino acids (cystine and methionine) (Adrian, 1974). Alpha-amino groups of free amino acids are available for reaction, but those already in the peptide bond of proteins are not free to participate. The epsilon-amine group of lysine is especially susceptable to this reaction, and this group is available even if the amino acid is in a peptide form (Carpenter, 1960). Dworschak and Orsi (1977) found that -NH group of the indole ring of tryptophan was available for the reaction.

Reducing sugars can be a source of carbonyl groups involved in the Maillard Reaction and different sugars have differing rates of participation (Adrian, 1974). Pentoses undergo browning faster than hexoses, and this is related to the rate of ring opening. When a sugar is in a cyclic form the carbonyl group is part of another bond, and is therefore unavailable until opened. Rate with which the sugar ring opens is related to rate of initial browning (Overend et al. 1961). Also sugar isomers can vary in rate of reaction.

Other compounds in addition to reducing sugars can produce carbonyl groups for participation in the Maillard Reaction. Oxidation of fatty acids produces a product called malonaldehyde capable of reacting with free amine groups (Dugan, 1976). Degree of unsaturation increases susceptibility to oxidation and thus ability to react (Deuel, 1951). Buchanan (1969) demonstrated the relationship between lipid content and digestibility when leaf protein was heated at 103 C. Protein digestibility was reduced from 70 to 17%. Yanagita and Sugano (1978) confirmed this using casein and oxidized lipids.

Phenolic compounds can be converted to reactive quinones, capable of reacting with thiol and free amino groups, by oxidase enzymes (Synge, 1975). Diphenolic compounds when incubated in the presence of diphenol oxidase from orchard grass and red clover decreased digestibility, biological value and available lysine of casein (Horigome and Kandatsu, 1968).

Middleton (1978) incubated various plant cell fractions with protein in order to determine which were more reactive. The acid detergent fiber fraction did not appear to bind nitrogen when heated, in contrast to the neutral detergent fraction indicating the hemicellulose was responsible for binding of protein.

Hodge in 1953 presented the idea of carbonyl-amine reactions occurring in 3 stages and involving 7 basic types of reactions (Figure 1). The first stage is characterized by carbonyl-amine condensation, enolization and amadori rearrangement. No color is

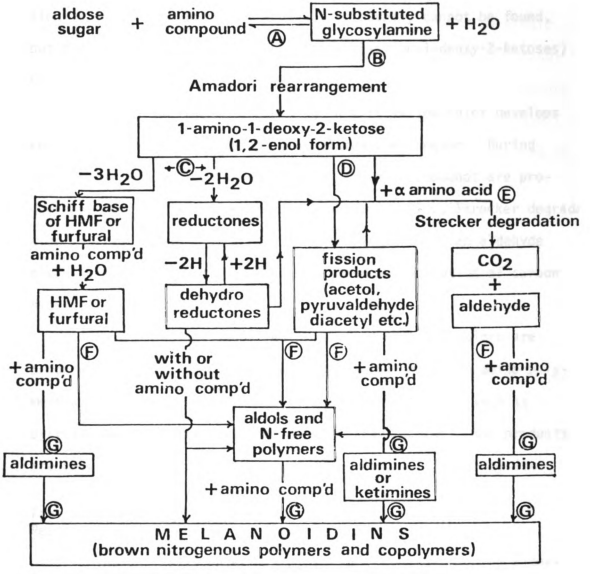

Figure 1.--Summary of reactions leading to browning in sugar amine systems (Hodge,1953).

developed during this stage, but altered amino acids are formed and have a reduced nutritive value (Lewis and Lea, 1950). Condensation usually occurs as the material is heated or dehydrated. Enolization is very rapid and the glycosylamine usually cannot be found, but the Amadori compounds (N-substituted 1-amino-1-deoxy-2-ketoses) have been isolated (Mills et al. 1969).

During the intermediate stage a buff yellow color develops which strongly absorpts in the near-ultraviolet region. During this stage reductones, furfural and dicarbonyl compounds are produced by sugar dehydration and fragmentation. Also, Strecker degradation of amino acids occurs resulting in formation of an aldehyde having one less carbon than the amino acid and evolution of carbon dioxide occurs.

From the final stage red-brown to dark brown colors are produced as a result of aldol condensation and aldehyde-amine polymerization. Also, formation of heterocyclic compounds such as pyrroles, imidazoles, pyridines and pyrazines occurs. End products are brown nitrogenous polymers and copolymers called melanoidins.

Factors Affecting the Maillard Reaction

The pH exerts an effect on the Maillard Reaction by altering the rate of ring openings of sugars. As pH increases from acid (1.7) to basic (8.4) rate of color formation also increases which is related to sugar conversion to the acyclic form (Wolfrom et al. 1953). Lea (1950) demonstrated linearity between degree of heat damage and pH, increasing from 3 to 8 and probably up to 10. The first rate

limiting reaction in browning appears to be opimum at an alkaline pH, but later reactions proceed more readily at an acid pH (Katchalsky and Sharon, 1953).

Temperature increases rate, but browning can occur slowly at room temperature (Haugaard et al. 1951). Lea and Hannon (1949) found a linear increase in rate of browning when casein and glucose was heated at temperatures ranging from 0 to 90 C. Other investigators have observed that heated forages (40 to 100 C) increased in concentration of insoluble nitrogen as temperature increased (Goering et al. 1973).

Moisture content can exert an effect by diluting reactants. Wolfrom and Rooney (1953) studied a system ranging in moisture from 0 to 100% and found 30% to be optimum for color development. Miller et al. (1965) heated cod muscle with glucose and found carbonyl-amine compounds to be greatest at 14% moisture. In forage systems greatest heat damage occurs between 20-70% moisture and varies with forage type (Goering et al. 1973). Loncin et al. (1965) considers water to be necessary to allow mobility of initial reactants, but becomes an inhibitor at dehydration stages of the Millard Reaction.

Oxygen is not necessary for browning, but it does enhance the reaction (Webb, 1935). Middleton (1978) using alfalfa ensiled in jars found insoluble nitrogen increased with increasing aeration rates at constant temperatures.

During heat treatment reactions other than the Maillard Reaction can occur resulting in a loss of amino acids. Chatelus (1964) observed decarborylation of amino acids in the absence of sugars. Autoclaved soybeans can form lysine-aspartic and lysine-glutamic acid bonds which are resistent to mild hydrolysis (Evans et al. 1961).

Sensitivity of Feeds and Foods to the Maillard Reaction

Intensity of the Maillard Reaction in a feed or food appears to depend on type of carbohydrate and to a lesser degree type of amino acid or protein (Adrian, 1974). Therefore, type of material processed has a large influence on degree of browning.

Dairy products are very susceptible to heat damage because of a high concentration of lactose and fragility of the proteins (Cook et al. 1951). Pastuerization and spray-drying result in only a mild denaturation of whey proteins, but evaporation, sterilization, condensation and roller drying result in lysine destruction (Mauron et al. 1955; Mauron, 1964).

Fish and meat are relatively stable during heating compared with milk products. The sulfur containing amino acids are generally more affected by oxidation, than lysine (Adrian, 1974). Because fishmeal is high in lysine, but low in sulfur containing amino acids lysine is not normally limiting, and moderate lysine modification during heating will not change this relationship.

Cereal products are somewhat sensitive to heat treatment, but not as susceptible as dairy products. Lysine is usually the

amino acid affected and is limiting on such diets. Other amino acids can be destroyed, but are usually not as important. Peters et al. (1950) found toasted flakes (treated 2 min. at 200 C) and puffed cereals (preheated 120 C and treated under pressure at 200 C for 2 min.) to have reduced efficiencies of protein utilization.

Leguminous seeds appear resistant to heat treatment, but if alterations do occur, they are in the sulfur containing amino acids. The reason for this resistance is a low concentration of reducing sugars. If sugar is added this can be reversed (Evans and Butts, 1949).

Goering et al. (1973) incubated several forages at 53% moisture for 24 and 48 hrs. Using acid detergent insoluble nitrogen (ADIN) as indicative of heat damage they found degree of heat damage varied regardless of plant species, initial ADIN or total N.

Ingestion of Heat Damaged Proteins

Synge (1976) listed four possible effects resulting from the ingestion of altered proteins: (1) modified proteolysis in lumen of intestine; (2) a modified peptide might be absorbed but excreted in the urine; (3) modified free amino acid may not be in a form acceptable for protein synthesis and therefore must be excreted; (4) intrinsic toxicity of altered protein. The first process results in reduced nitrogen digestibility due to reduced proteolysis in the gastro-intestinal tract. The second and third processes would not affect apparent digestibility, but biological value would be reduced due to unavialability of amino acids at the ceullular level.

The fourth process would result in reduced physiologcal performance and efficiency of metabolism. A combination of all of these processes may be involved in reduced performance by animals fed heat damaged proteins.

Absorption and Utilization of Heat Damaged Proteins and Amino Acids

Mori and Nakatsuji (1977) studied utilization of browned casein and glucose (37 C for 20 days) by labeling with C-14 lysine. Three hrs. after ingestion by rats the stomach, small intestine, large intestine and cecum contained 49.4% of ingested dose for those fed unheated casein vs. 62.0% for those fed heated casein. This decreased to 1.7% and 12.2% respectively by hr. 22 after ingestion, but very little was found in the feces in either treatment. Rats fed unheated casein excreted 2.5% of the total radioactivity in the urine by 22 hrs. after ingestion, but rats fed heat damaged diets excreted 22.1%. Therefore, excessive heating results in a reduced rate of absorption of labeled lysine from the gastro-intestinal tract, but much is eventually absorbed and excreted in the urine which would reduce biological value. The absorption delayed material was identified as fructose-lysine and accounted for 70% of total radioactivity in the small intestinal lumen TCA soluble fraction 7 hrs. after feeding (Mori, 1978).

Tanaka et al. (1974) heated a mixture of egg albumin and glucose at 37 C from 0 to 40 days. Essential amino acid index, protein score, chemical score, available lysine, biological value,

protein efficiency ratio and true digestibility decreased as length of heating increased. Protein efficiency ratio was found to be the most sensitive indicator of heat damage. Dry matter and nitrogen absorption rate was reduced in rats receiving browned material. Peptides 4-10 residues long were found in the feces of rats fed the browned products, and lysine, arginine, histidine, glutamic acid, isoleucine and alanine were found to be the amino acids present.

Tanaka et al. (1975) used radioactive fructose-tryptophan to study absorption of Maillard products in rats. The cecal microflora could degrade fructose-tryptophan in vitro, but autoclaving cecal contents prevented degradation. In vivo introduction of labeled fructose-tryptophan into the cecum revealed 20% of administered radioactivity was recovered in the urine within 24 hrs. Only small amounts of fructose-tryptophan were found in feces indicating this compound was degraded by intestinal microbes and absorbed or was absorbed unaltered. This experiment was different than that of Tanaka et al. (1975) in that a modified amino acid was used instead of a modified protein. Therefore, digestion to small peptides or free amino acids could not be evaluated.

Johnson et al. (1977) orally administered fructose-phenyalanine to chicks receiving a phenyalanine deficient diet. No response in growth occurred indicating phenyalanine was not available at the cellular level. Liver tissue from chicks fed fructose-phenyalanine demonstrated an in vitro rate of C-14

phenyalanine incorporation lower than livers from chicks not fed this compound. If fructose-phenyalanine was added directly to the tissue in vitro no reduction in phenyalanine incorporation occurred demonstrating an in vivo process occurred that reduced protein synthesis. Metabolism to another molecule could occur in the gastrointestinal tract. Results from this in vitro system could be misleading if the browned compound caused lipid infiltration of the liver since data is expressed on a per unit weight basis. In the presence of increased lipid less protein synthesis would occur per unit of tissue since more of that unit would be fat. An accumulation of lipid in the liver has been observed in rats when casein and oxidized lipids were incubated and fed (Yanagita and Sugano, 1978).

In a subsequent study Johnson et al. (1979) administered labeled fructose-phenyalanine either via stomach tube or by intraperitoneal injection to rats. Excretion of radio-activity in expired air and urine peaked within 2 hrs. when unaltered phenyalanine was administered, but it took 48 hrs. for the same quantity to be excreted when phenyalanine was altered. Antibacterial agents did not affect unaltered phenyalanine absorption and excretion, but drastically reduced urinary excretion of the altered compound. These results together with those of Sgarbieri et al. (1973) and Tanaka et al. (1975) demonstrate the necessity of the intestinal microflora prior to absorption of altered amino acids.

Nesheim and Carpenter (1967) surgically altered chicks to allow separate collection of urine and feces. Cod muscle was

heated at 116 C for 27 hrs. (14% moisture) and then fed to cecectomized and intact chicks. Three hrs. after a test meal chicks receiving heated muscle had 3 times more nitrogen in their small intestine than chicks receiving freeze dried cod muscle (control). Apparent nitrogen digestibility of intact chicks fed control muscle averaged 90%, and was not reduced in cecectomized chicks (89%). Intact chicks fed heat damaged muscle averaged 77% of the nitrogen digestible, but was reduced to 68% in cecectomized chicks. This demonstrates that the cecum is necessary for complete digestion of heated but not unheated, proteins, probably via microbial alteration as discussed above. This study tested a heated protein not heated amino acids as did others (Tanaka et al. 1975; Johnson et al, 1978). Although apparent nitrogen digestibility was higher in intact chicks this does not necessarily mean the extra absorbed nitrogen is used by the animal. In fact this is probably responsible for increased urinary nitrogen observed in other experiments. The authors theorize that most of this nitrogen is absorbed as ammonia.

Ford and Shorrock (1971) heated freeze-dried cod fillets at 135 C for 20 hrs. before feeding to rats. During a 48 hr. feeding trial urinary excretion of peptide bound amino acids increased 2.6 times due to heating, and lysine comprised a large percentage. Lysine, aspartic acid and glutamic acid composed 70% of the urinary amino acid residues. Urinary excretion of free amino acids increased 2.1 fold due to heating. The authors propose that the episilon-amino group of lysine and the amide groups of asparagine

and glutamine are responsible for the majority of binding during heating which results in large concentrations of these amino acids in the urine. This agrees with observations of Bjarnason and Carpenter (1970). Heat damage at low carbohydrate concentration as in this experiment is probably different than when they are present in greater quantities. Since peptides were found in urine in the previous study, this implies that peptides can be absorbed directly from the gastro-intestinal tract and do not have to be in free amino acid form. However, the increase in free amino acids in the urine could result from peptide hydrolysis within the kidney or the peptide might have lowered the renal threshold for free amino acids by saturating reabsorption sites at the renal tubules. The authors calculate only .6% as free amino acids leading them to conclude this loss is only of marginal nutritional significance. The possibility exists that more peptide is absorbed from the gut than is recovered in urine and is slowly available to the animal.

Aspartyl-lysine and glutamyl-lysine cross-links have been found in chicken muscle autoclaved at 116 C (Hurrell et al. 1976). No lanthionine was found in chicken muscle, but was present in bovine plasma albumin heated at 121 C. No aspartyl- or glutamyl-lysine were found in heated bovine albumin. Lactalbumin, zein, egg albumin and casein contained both types of cross-links when heated at 115 C. Neither lysinoalanine or ornithinoalanine were found in any of the heated proteins. Apparent nitrogen digestibility of heated chicken muscle was 89% vs. 98% for unheated, and

ileal digestibilities were 76 vs. 88% respectively indicating some digestion occurred in the large intestine probably due to bacterial action. Lysine isopeptides were as digestible as the rest of the protein. Reduced utilization of absorbed nitrogen is unclear since only a small portion of that absorbed is excreted in urine (Ford and Shorrock, 1971). The modified amino acid which cannot be used for protein synthesis may be broken down in the body, and the carbon skeleton used as an energy source and the nitrogen excreted.

Rivera et al. (1978) dried corn at temperatures ranging from 50 to 125 C, and found amino acid availabilities to weanling rats decreased as severity of heat treatment increased. Lysine, threonine, isoleucine, methionine, valine, tryptophan, phenyalanine and leucine were most affected although total nitrogen digestibility was not affected. The authors conclude the loss of protein quality is not the sole cause of reduction in rat gains and probably energy values are also affected.

Physiological Consequences Resulting from Consumption of Damaged Material

Products of heating can be classified as soluble (termed premelanoidins) or insoluble (Adrian, 1974). The insoluble polymerized compounds are usually inert and not available nutritionally or pharmacologically. The premelanoidins on the other hand are reactive and may be able to alter physiological processes.

If amino acid destruction is the only cause of reduced performance supplementation with the more severely affected amino

acids should revert physiological processes to normal. When casein and glucose are heated protein efficiency ratio decreased from 2.6 to .7, but addition of lysine and methionine to the ration returns it only to 2.2 (Rao et al. 1963). Donoso et al. (1962) noted a 15% loss of lysine and a 35% loss of methionine after heating and a subsequent 50% loss of net protein utilization. This incomplete reversal of deliterious effects may be explained if other amino acids in the vicinity of an altered amino acid were also rendered unavailable or if an interference with utilization of other unaltered amino acids occurred.

Hurrell and Carpenter (1977) heated cake-mix at 200 C for 30 minutes which was then fed to rats. Protein efficiency ratio of unheated was 3.9 and this declined to .8 after heating. Addition of 6.3 g lysine per Kg of heated diet (sufficient to meet animals needs) resulted in an increase to 2.6. Since lysine is affected most by baking and appears to be the limiting amino acid then supplementation with lysine would be expected to overcome detrimental effects. Again this was not the case, therefore the heated material must be affected in other ways. Cross-linking with other amino acids may have occurred.

Adrian and Frangne (1973) introduced premelanoidins into casein-based diets so that 17% of the nitrogen came from this soluble nitrogen form. Based on calculations and assumptions from rats on nitrogen free diets the authors calculated an increase in fecal nitrogen from casein when premelanoidins were included, but

urinary nitrogen decreased. The net result was a reduction of retained nitrogen as well as biological value of unheated casein when soluble heat damaged products were included in diets. Heated glucose added to this ration does not exert this effect. Reduced molecules formed during the Maillard reaction does not appear to be the cause since oxidation after heating does not correct this interference with protein utilization (Adrian, 1974).

At low doses premelanoidins (8.5 to 50 mg degraded nitrogen per Kg basal ration) tend to stimulate intake and therefore growth (Adrian et al. 1966). This stimulation is probably due to an agreeable flavor. At larger doses (1500 to 2400 mg nitrogen per Kg) protein efficiency ratios can be reduced 20-40% with no affect on intakes.

Adrian and Susbielle (1975) used a casein based diet containing 16% protein for rats. Premelanoidins (glucose-glycine heated 1 hr. at 90 C) were added (165 ml per Kg diet) to rations of one half the females on a reproduction trial. Number of females pregnant after 31 days on experimental diets were 76% for control ration vs. 56% for females receiving premelanoidins. Intakes were not affected by treatment, but number of implants, number of live births, litter weights, number per litter and number weaned were reduced. Number of resorptions were increased from .42 per female to 2.41 when premelanoidins were present. These observations indicate a nitrogen deficiency as a result of having these soluble compounds in the ration. Comparing rate of resorptions to other

experiments differing in protein contents it appears these diets with added soluble nitrogenous compounds corresponds to a 9% protein diet. Birth weights correspond to a 11.5% protein diet. If this is true one third of the protein in the diet was not utilized. This assumes no other effect other than reduced utilization of nitrogen. These authors had no concurrent rats fed 9 or 11.5% protein.

In vitro digestion of proteins incubated with premelanoidins revealed reduced lysine and methionine in the soluble fraction, but the concentration of these amino acids in small peptides increased indicating interference with the final stages of hydrolysis (Adrian and Frangne, 1973). Zabrodskii and Viktovskaya (1960) found even the melanoidins (insoluble) were capable of inhibiting amylolytic activity of malt.

Lee et al. (1977) heated apricots at 12% moisture and 45 C for 3 mo. The resulting product comprised 71% of a ration using casein as the protein source. The control ration was composed of unheated apricots and pair-fed to rats fed heated apricot based diets for 2 mo. Lactase activity in the intestinal mucosa was reduced 30%, sucrase 48% and maltase 35% when fed heated apricot ration. Body weight was reduced 13%. The water soluble, not the ether soluble, fraction of the heated apricot was responsible for these effects. In a similar experiment browned egg albumin was used as the source of heat damaged material. Reductions in disaccharidase activities were not as pronounced being 43% for lactase,

31% for sucrase and 22% for maltase. Supplementation of the browned diet with amino acids did not return activities to normal, but sucrase and maltase activities were higher after supplementation of browned diet indicating available protein might have an effect on enzyme activity. This theory contrasts with other reports where protein free or protein-deficient diets with no heat damage were fed with no reduction in intestinal disaccharidase activity (Solimano et al. 1967; Prosper et al. 1968; Troglia et al. 1970).

The kinetics of absorption of amino acids was studied using an in vitro everted gut sac and in vivo using a catherized portal vein (Lee et al. 1977). Both in vitro and in vivo fructose-tryptophan competitively inhibited absorption, but the browned product did not appear to be absorbed in large quantities. The authors theorize that altered amino acids may saturate absorption sites for unaltered amino acids thus reducing availability of unheated protein. This might explain certain studies that observed incomplete growth recovery after supplementation of heated rations with unheated protein.

Amaya (1975) observed that the Maillard dipeptide fructosyl-leucine was not hydrolyzed by leucine-amino-peptidase in vitro, and this peptide was capable of preventing hydrolysis of a normal, unaltered peptide.

Shorrock and Ford (1978) heated cod fillets at 135 C for 20 hrs. Unavailable small peptides were isolated from an enzymatic digest, and found to inhibit leucine uptake using everted sacs in vitro.

However, neither glucose uptake nor metabolism to lactate were affected in the wall of the everted intestinal sac. The authors speculated that this peptide attached to binding sites adjacent to sites for amino acid uptake and exerted an allosteric effect inhibiting uptake. Another idea presented was the peptides might be absorbed into mucosal cells and accumulate interferring with normal function.

Percival and Schneeman (1978) heated casein (121 C for 24 hrs.) and found a 46% reduction in digestibility when measured in vitro. Rats were fed heated or unheated casein based diets (24%) for 8 days. After three and a half hrs. the pancreatic contents of rats fed heat damaged casein based diets contained less chymotrypsin and amylase, and normal concentrations of protein and trypsin. Intestinal contents contained greater quantities of trypsin, chymotrypsin and amylase activities as well as increased protein. The authors concluded that increased enzyme secretion in animals fed heat damaged material was compensating for a reduction in digestibility. A follow up experiment revealed the gut mucosal enzyme leucine-amino-peptidase was not affected by heat damaged casein (Percival and Schneeman, 1979). Since this enzyme increases after a meal and is greater in fed animals it is logical to assume the intestinal mucosa is receiving amino acids from the heated casein since no reduction in activity was observed.

Toxicity of Maillard products has been implicated in several reports. A protein-free diet caused a weight loss of 1.03 g per

day in rats, but when premelanoidins were added the animals lost 1.36 g per day (Adrian, 1974). A large protion of the heated nitrogen product was retained by the animals, but did not appear to benefit the physiological state.

Krug et al. (1959) deomnstrated that an intraperitoneal administration of 1 part amino acid and 3 parts glucose mixture had an LD 50 greater than 29 g per Kg body weight. However, after heating 10 min. at 160 C the LD 50 of this mixture decreased to 11.2 to 4.1 g per Kg depending on the amino acid used. Lysine products appeared the most toxic. Since lysine is the amono acid usually affected by heating, this relationship could be of significant consequence.

Fink et al. (1958) found animal death by liver necrosis to be .95% when fed unheated liquid milk but increased to 40% for spray dried milk powder and 76% for a roller-dried product. Heat treatment during processing appeared to be the cause of this increase.

In an attempt to detect physiological changes occurring after feeding heat damaged material, Lee et al. (1974) used heat damaged apricot based diets (70%) with unheated casein being the protein source. Rat weight gains and feed efficiences were reduced on the heated diet. Glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities in serum were increased indicating changes in hepatic metabolism even though no change in hepatic morphology was observed. These enzyme activities

remained elevated even after return to a control diet for 2 mo. indicating damage might be irreversible. Blood total protein was slightly reduced, whereas urea and albumin were not changed. Blood glucose and liver glycogen were reduced. This could be a result of either reduced disaccharidase activity or reduced available carbohydrate in the heated ration. No indication of an alteration of glomerular or tubular function was noted. Relative liver and kidney weights were increased when fed the browned diet while spleen, intestine, heart, and lung weight were not affected. Diarrhea resulted when animals were fed heated apricots. This was probably a result of increased lumen osmolarity due to the undigested substances remaining in the intestine. Diarrhea was accompanied by an enlarged cecum indicating that fermentation acids may have enhanced the already increased osmolarity.

Tanaka et al. (1977) heated 3 parts egg albumin and 2 parts glucose at 15% moisture and 37 C for varying periods up to 10 days. This heated material served as the protein source for rats. Weight gains and protein efficiency ratios were increasingly reduced with increased heating time. After feeding for 3 mo. relative liver and kidney weights were increased in male rats on the heat damaged diet, but this was not true in the females. Addition of non-essential amino acids to the heated rations of male rats reversed the hypertrophy. Serum glucose was increased on the heated diet in contrast to what was noted before indicating heated protein did not interfere with glucose absorption. No data was presented on

intakes making it difficult to draw conclusions. Blood urea nitrogen, GOT, GPT and alkaline phosphatase were elevated indicating liver damage might have occurred. Serum protein was reduced, and was not corrected by supplementation. This might be a result of reduced food intake and not a result of heating. Even though the browned material made up only 10% of the diet this was sufficient to cause a change in the physiological state of the animals.

Adrian and Susbielle (1975) found a slight hypertrophy of the liver and considerable hypertrophy of the growing rat kidney when premelanoids were incorporated into a basal diet. In the mature female the cecal weight was increased as well.

Cooking ground beef at 200 C resulted in formation of mutagens (Commoner et al. 1978). "Well done" hamburgers contained mutagens at .14 parts per million that were extractable via organic solvents. Treatment with nitrous acid resulted in increased mutagenic activity. A nitroso group might cause this increased mutagenicity.

Stegink et al. (1975) noticed that when heated sugar-amino acid complexes were fed to infants they appeared in blood and urine, in conjunction with increased fluid losses. A 2 to 5 fold increase in urinary Zn, Cu, and Fe but not Mg or Mm resulted. In another report Stegink and Pitkin (1977) infused glucose-amino acid complexes into monkeys. These compounds accumulated in maternal plasma and were transported into the fetal circulation. A non-heated glucose-amino acid mixture showed no accumulation in plasma or fetus.

Although there are several reports implicating browning products with adverse physiological effects over and above reduced nitrogen digestibility when fed to animals, some reports do not agree. Atkinson and Carpenter (1970) heated several kinds of meats at 90 C for 44 hrs. Several of the heat-damaged preparations stimulated rat growth when fed as a supplement to a casein and amino acid basal diet. Another study added to a basal 18% casein diet, 2, 4 or 9% autoclaved egg albumin and found no depression in weight gain or feed efficiency (Boctor and Harper, 1968). No analytical data are presented for extent of heat damage in several of these studies.

Heat Damaged Forages

Occurrence in Forages

Heat damage can occur in forages and can produce results similar to those observed in other food/feed systems. Damage can occur during ensiling of the forage especially when the material is too dry allowing penetration of oxygen and excessive heating. Forage harvested as hay can undergo excessive heating if harvested before complete drying (greater than 20% moisture) indicating some moisture is necessary for the reaction. Heat damage has also occurred during dehydration where moisture is removed at a high temperature over a short interval. These are the more common causes of heat damaged feeds in animal agriculture. Frequency of occurrence, extent of damage and physiological effects have been examined, but not as extensively as for human foods.

Unpublished data by Thomas indicates brewers grains have a fairly high concentration of ADIN (about 18% of total nitrogen). Goering (1976) demonstrated the frequency of heat damage in dehydrated alfalfa samples obtained from retail outlets in 15 states. When acid detergent insoluble nitrogen was used as a measure of heat damage over 89% of the samples contained .29% (% of DM) (minimun heat damage). Dehydration at 120 and 145 C has been demonstrated not to affect nitrogen digestibility in contrast to 180 C (Goering and Lindahl, 1975). Most samples of dehydrated alfalfa contain more than 10% of total nitrogen as ADIN.

Goering and Adams (1973) collected hay, haylage and corn silage samples from farms and assayed for excessive heating. About 40% of the haylage samples were heat damaged (over .29% of DM as ADIN), compared to only 12% of the hays and 0% of the corn silages. Therefore, haylage appears much more prone to heat damage than hay or corn silage. Michigan (Thomas et al. 1972) workers reported 33% of haylage samples contained .36% ADIN (% of DM).

Consequences of Heated Forages

Bechtel et al. (1943) observed brown sorgo silages when fed to dairy cows had lower digestion coefficients for crude protein and nitrogen-free extract than corresponding greener material. In a later study green, brown and black hays were fed to dairy animals and protein digestibilities were 67, 16 and 3% respectively. Intakes were also reduced (Bechtel et al. 1945).

Hill and Noller (1963) noticed brown haylage with a carmelized odor had reduced nitrogen digestibilities. Therefore, the relationship between heat damage in forages and reduced digestibilities has been recognized for several years, but observations beyond an effect on digestibility have not been measured.

Goering et al. (1972) determined the relationship between various measurements of heat damage in forages and in vivo digestibility. Nitrogen digestibilities ranged from 6 to 85%. Acid detergent insoluble nitrogen and pepsin soluble nitrogen expressed as a percent of the dry matter explained 89 and 93% respectively of the variation in nitrogen digestibility, but a lesser amount of the variation in energy digestibility (81 and 63% respectively). Yu and Thomas (1976) showed acid detergent insoluble nitrogen expressed as percent of total nitrogen was the best predictor of nitrogen digestibility. This relationship was greater in forages with no heat damage compared with those that were heated. Evidently fresh forages contain a small amount (<8%) of nitrogen as ADIN. This percentage increases with length and extent of heat and oxygen exposure (Middleton, 1978). Consequently, 2 equations should be used to predict nitrogen digestibility based on acid detergent insoluble nitrogen.

One of the more in-depth studies on heat damaged haylage was done by Yu and Veira (1977). They heated alfalfa haylage (about 48 dry matter) in model silos at 88 C for 24 and 48 hrs. in a

force-draft oven. Acid detergent insoluble nitrogen expressed as percent of total nitrogen was 7.7% for unheated, and increased to 15.2% by 24 hrs. of heating and 24.1% by 48 hrs. Sheep, when given a choice, tended to discriminate against the heated material, but intake was not reduced when fed as the only forage. Cheeke and Myer (1975) suggest reduced palalability arising from heating of alfalfa due to bitter Maillard products. Apparent dry matter digestibility decreased from 61.3% for unheated to 56.5% to 24 hr. to 49.4% at 48 hrs. of heating. Nitrogen digestibilities were 69.8, 55.8, and 47.6% respectively. This corresponds to a reduction of 19.4% in dry matter digestibility and 31.8% for nitrogen digestibility by heating for 48 hrs. Acid detergent fiber and nitrogen-free extract digestibilities were also reduced, but crude fiber, ether extract and acid detergent insoluble nitrogen digestibilities were not. At least 11.3% of the acid detergent insoluble nitrogen was digested and at most 22.4% indicating some degradation and possibly absorption had occurred on passage through the gastro-intestinal tract. This relationship did not appear to be temperature related. Urinary nitrogen decreased with increased heating. Overall, TDN digestibility was reduced 7.0% by heating for 24 hrs. and 19.4% by heating for 48 hrs. Mean retention time in the gastro-intestinal tract was not affected by heating. At similar nitrogen intakes sheep excreted 34% more nitrogen in feces when fed the 24 hrs. heated forage and 74% more for the 48 hrs. heated forage. Nitrogen retention was correlated negatively with acid detergent insoluble

nitrogen, but reduced retention would probably be a result of both a reduction of TDN and digestible nitrogen.

The cited studies indicate that heat damaged forage is frequently encountered on farms and can have a detrimental effect on animal nutritive status. Speculations, not proven, are that heating of forages results in the Maillard reaction similar to that which has been observed in food systems. If this is true certain modified peptides and amino acids would be expected to be present in heated forages, probably in the ADIN fraction. Yu and Veira (1977) demonstrated that a part of the ADIN is digested and, though small, might contribute to the physiological status of the animal. Also unabsorbed compounds in the intestine could effect utilization of other diet components. With these ideas in mind, I set out to evaluate effects of heat damaged diets on an animal's physiological state.

MATERIALS AND METHODS

Experiment I

First cutting alfalfa was ensiled in four 3.7 by 6.1 m silos. Propionic acid (diluted 1:1 with water) was applied at 2% of the fresh weight of the forage to two of the silos giving a final propionate concentration of 1%. The remaining two silos were untreated. After 5 to 7 weeks, silos were opened and top spoilage was removed and weighed.

Determination of Dry Matter Disappearance During Ensiling

A weighed amount of haylage representative of each silo was placed into nylon pantyhose and secured in position when each silo was two-thirds full. Another pantyhose filled with haylage was buried .6 m under the top surface of each silo. Recovery of DM during ensiling was estimated by haylage DM disappearance from the pantyhose.

Temperatures During Ensiling

Temperatures were monitored daily during the first week and weekly thereafter via a portable potentiometer with thermocouples buried in the center of each silo at the top, middle, and bottom areas.

Determination of Nitrogen Fractions

Nitrogen fractions in the samples from the pantyhose before and after ensiling were determined. Samples were frozen until analyzed. Total nitrogen was determined by Kjeldahl analysis on a finely chopped fresh sample. Water soluble nitrogen was determined by Kjeldahl analysis on the supernatant after sample homogenization (10 g forage and 190 ml water) for 3 min., in a Sorvall Omnimixer, strained through four layers of cheesecloth, and centrifuged at 27,000 times g for 5 min. Water soluble nonprotein nitrogen was measured by Kjeldahl analysis on the supernatant after precipitation of protein with 50% sulfosalysilic acid and centrifugation at 27,000 times g for 20 min. Water insoluble nitrogen was calculated by difference between total nitrogen and water soluble nitrogen. Water soluble true protein was calculated by difference between water soluble nitrogen and water soluble nonprotein nitrogen. Acid detergent insoluble nitrogen was determined by Kjeldahl on the acid detergent fiber fraction.

Laboratory Analysis

Dry matter of feed composites was determined by oven drying at 100 C for 24 hr., and acid dergent fiber on an air dry sample by methods of Goering and Van Soest (1970). For determination of pH haylage was placed in a beaker, saturaged with water .5 hr before determination using a portable meter equipped with glass electrode.

Concentrations of volatile fatty acids of haylage composites were determined by gas chromatograph on the water soluble fraction,

as prepared for water soluble nitrogen. Five parts supernatant and 1 part orthophosphoric acid (85% vol/vol) were mixed, and 3 ul were injected into the column. The column was glass (2.7 m length and 2 mm inside diameter) packed with carbopack B. Oven temperature was maintained at 160 C with injection port and detector temperatures at 200 C. A hydrogen flame ionization detector was used. Nitrogen (30 ml/min) was the carrier gas. Peak areas were measured via an electronic intergrator and unknowns calculated by comparing peak areas to standard mixtures of fatty acids.

Lactation Trial

Forty-four lactating Holstein cows were blocked by milk production, stage of lactation, number of lactations, and genetic group and assigned randomly to receive control haylage (C), control haylage plus corn silage (C+CS), propionate treated haylage (P) or propionate treated haylage plus corn silage (P+CS). Cows receiving corn silage (37% DM and 8.4% CP) were fed a mixture of 70% haylage and 30% corn silage on an as-fed basis. Concentrate (16% CP) was fed at 1 Kg per 3 Kg of milk produced.

After a preliminary 14 days during which untreated haylage was fed, cows were placed on treatments for 50 days. Intakes and milk production were monitored daily. The amount of forage fed was adjusted weekly to allow 10% refusal. Milk for determination of fat was sampled once biweekly at the AM and PM milkings. Samples of silage were taken three times per week and refrigerated. These were composited each week, then frozen until analyzed.

Experiment II

Third cutting alfalfa-grass mixtures were manually cut and passed through a forage harvester to allow normal chopping (3/8 in. chop) and microbial inoculation. Chopped forage was then taken to the lab and spread on trays to air dry.

This experiment was divided into two trials. Experiment IIa compared untreated to propionate treated forage ensiled in 1 liter air tight glass jars to simulate silos. Propionic acid (diluted 1:1 with water) was applied at 2% of the fresh weight of the forage and thoroughly mixed to give a final propionate concentration of 1%. Forage was taken at 0, 3.5, and 9 hr. of drying and packed tightly into jars. Samples were taken before and after ensiling 40 days. There was one jar per treatment at each DM content.

In Experiment IIb forage was ensiled at 0, 4, 12 and 24 hr. after drying. Treatments were the same as in Experiment IIa plus another containing 1% chloroform. All other procedures were the same except all treatments were in duplicate at each DM content.

Determination of Nitrogen Fractions

Homogenization of samples were the same as in Experiment I immediately after sampling. The liquid supernatant of the homogenate was frozen for further analysis. The same fractionation scheme was followed as in Experiment I plus water soluble ammonia and alpha-amino nitrogen.

For ammonia determination 50 ml of supernatant was placed in a 100 ml beaker with a magnetic stirrer. An ammonia probe (Orion Specific Ion Electrode) connected to a Beckman digital pH meter was placed into the sample and ten drops of 10M NaOH was added to convert NH_4^+ to NH_3 to which the electrode is sensitive. Standard solutions of ammonium chloride containing 14 to 140 mg per liter ammonia were used for construction of a standard curve.

Alpha-amino nitrogen was determined by the method of Palmer and Peters (1969). Dry matter and pH were determined as in Experiment I.

Experiment III

A breeding colony of meadow voles (Microtus pennsylvanicus) was maintained on the campus of Michigan State University. Immature voles used in this experiment were obtained from this source. All experimental animals were maintained in the same environment as the regular colony. Temperature was stabalized at about 16 C year round and incandescent lights were on 24 hrs. a day.

Composition and Preparation of Diets

This experiment was divided into parts IIIa and IIIb. The composition of diets used in IIIa and b are presented in Table 1. Vole diets were formulated based on studies by Shenk et al. (1971).

Sources of ingredients were: corn starch (A. E. Stanley Co., Oakbrook, IL), vitamin free casein (source unknown), sucrose (Monitor Sugar Co., Bay City, MI), corn oil (Mazola Corn Products Co.,

TABLE 1.--Composition of vole diets used in Experiments III and IV

Ingredient	Experiment IIIA and IVa	Experiment IIIb and IVb	
		Basal Diet	Supplemented
Alfalfa	59.4% (29.7)*	58.7% (29.4)*	55.4% (27.7)*
Casein	1.0%	2.0%	7.7%
Corn starch	21.8%	21.6%	20.3%
Sucrose	9.8%	9.7%	9.1%
Corn oil	2.0%	2.0%	1.9%
Minerals	3.0%	3.0%	2.8%
Vitamins	2.0%	2.0%	1.9%
Cellulose gum	1.0%	1.0%	.9%
Cellulose	---- (29.7)*	----- (29.4)*	----- (27.7)*

*Numbers in parenthesis are for the negative control which had half of the alfalfa replaced by cellulose.

Englewood Cliffs, NJ), Rogers-Harper Mineral Mix (Teklab Test Diets, Madison, WI), Vitamin Fortification Mix (Taklab Test Diets, Madison, WI), alpha-cellulose (Sigma Chemcial Co., St. Louis, MO) and cellulose gum (Hercules Inc., Wilmington, DE).

Wilted, chopped alfalfa (50% DM) was obtained from the farm at Michigan State University as material was being prepared for ensiling. Forage was placed in plastic bags and frozen until used. Heated alfalfa used in Experiment IIIa was prepared by placing in plastic bags and heating in a forced-air drying oven at 80 C for 8, 24, or 72 hrs. Only 72 hr. heated material was used in Experiment IIIb. In both trials negative and positive controls contained unheated alfalfa. All alfalfa was air dried and ground through a 2 mm Wiley mill screen. Diet ingredients were thoroughly mixed in a Hobart mixer, placed in bags, and frozen until used.

Growth Trials

Immature voles were weaned, maintained on a standard colony ration for 10 days or less, then randomly assigned to a test diet after blocking by litter. In Experiment IIIa test diets contained either unheated alfalfa (positive and negative controls), or alfalfa heated for 8, 24, or 72 hr. In Experiment IIIb basal diets contained alfalfa unheated (positive and negative controls) or heated for 72 hr. Another set of diets were composed by adding supplemental protein (casein) to these basal diets. Experiment IIIa initially contained eight voles per treatment and IIIb ten. Each vole after placement on test diets was caged individually. Diets

were offered ad libitum and intakes monitored daily for seven days after a two day adaptation period. In Experiment IIIa water intakes were also monitored. Body weights were recorded initially and every two days thereafter for the duration of the experiment. After nine days on the test diets voles were changed to a standard colony ration of steam rolled oats (La Crosse Milling Co., Cochrane, WI) containing 15% crude protein and a rat chow (Peerless Pet Foods, Battle Creek, MI) containing 20% crude protein. In Experiment IIIa voles were weighed 14 days after placement on this ration. Recovery gain in g per day was calculated from this period.

Laboratory Analysis

Each diet was sampled and determinations made for DM, total nitrogen, acid detergent fiber and acid detergent insoluble nitrogen as in Experiment I.

Experiment IV

Meadow voles used in this experiment were obtained and maintained as those described in Experiment III.

Composition and Preparation of Diets

Experiment IVa and b diets were composed of the ingredients and proportions shown in Table 1. All diets were prepared as described in Experiment III.

Reproduction Trials

In Experiment IVa mature females were randomly assigned to diets containing alfalfa unheated (positive and negative control) or heated for 72 hr. There were 7 voles per treatment. Females were kept in harems with 3 or 4 per cage. Two males (full brothers) were placed in separate harems and rotated every two days. All females were exposed to both males (1 male per 10 females).

In Experiment IVb the basal diets were the same as in IVa. In addition three diets were added containing supplemental protein. Ten females were randomly assigned per treatment. One male was maintained for every 10 females and these were full brothers. Harems were maintained similar to those in IVa.

Body weights were obtained at five day intervals. Pregnant females were removed from harems before parturition and placed in individual cages. At parturition, day 10 and 16 number per litter and litter weight were recorded. After day 16 of lactation mothers were returned to harems and pups placed on the colony ration.

Diets and water were offered ad libitum for the duration of the trials. To add succulence a small amount of lettuce was made available. Group intakes were monitored twice weekly in the harems. During lactation individual intakes were monitored daily for the first 10 days.

Status at Termination

After 60 days on these diets voles were euthanized by decapitation after being rendered unconscious by ether. Blood was drained

into a beaker, transferred to a 5 ml test tube, let stand for at least one hr. at room temperature to allow clotting, and then entrifuged at 1300 times g for 10 mins. Due to the small volume of blood collected, serum from voles on each treatment was composited. Serum was placed in a 7 dram plastic vial, maintained on ice and transported to a clinical lab. Total protein, albumin, blood urea nitrogen, calcium, phosphorus, glucose, cholesterol, bilirubin and uric acid were determined using a Technicon Sequential Multiple Analyzer (SMAC).

Liver, kidney and the gastro-intestinal tract were removed and weighed. The cecum was removed from the gastro-intestinal tract and weighed full and empty. Contents were frozen and pH determined at a later time. The small intestine was removed and weighed after removal of contents.

Status of the reproductive tract was observed at termination. Number of fetuses was recorded.

Experiment V

Six Holstein heifers ranging in weight from 132 to 350 Kg were housed in tie stalls. During a 14 day pre-experimental period only haylage was fed. Haylage was an alfalfa-grass mixture considered to be of good quality low in ADIN (not heat damaged). After adaptation to an all haylage ration heifers were placed on a heat damaged haylage high in ADIN during a 20 day experimental period. Heat damaged haylage was obtained from a bunker silo on a farm in Michigan. This material was relocated to the Michigan State

farm and ensiled with propionic acid to prevent molding. One half of the heifers received a corn gluten-soy bean meal-fish meal protein supplement equilivant to about 25% of the haylage protein intake. Five grams of trace mineralized salt was mixed with the forage daily. Haylage was offered ad libitum and intakes determined daily. During the last seven days of the experimental period haylage was offered at 90% of ad libitum determined during the previous four days.

Body weights were taken before the start of the pre-experimental period, before start of experimental period and after 20 days on the heat damaged forage.

Digestion Trial

During the last five days of the experimental period feces were collected. Total fecal weights were obtained for each heifer, daily. Fecal material was mixed thoroughly and a 10% sample taken, placed in a plastic bag and frozen. At the end of the experiment the five day samples were thawed, mixed and a representative sample taken for later analysis.

Laboratory Analysis

Haylage was sampled daily during feeding and composited weekly. Analysis for DM, total nitrogen, acid detergent fiber and acid detergent insoluble nitrogen was conducted on haylage composites as in Experiment I. Fecal samples obtained in the digestion trial were analyzed for DM by drying at 60 C and total DM excreted during the trial calculated.

Blood Collection and Analysis

Blood was collected at approximately 0900 hr. twice weekly via the tail vein using a 20 guage 25 mm vacutainer needle and 10 ml vacutainer tubes. Blood was allowed to stand at room temperature for about one hr. before centrifugation for 20 min. at 1000 times g. Serum was removed and a portion frozen.

Serum from heifers on the seventh day of pre-experimental period, sixth and seventeenth day of the experimental period was placed in a plastic vial, placed on ice and transported to a clinical lab. Serum components measured were the same as in Experiment IV plus triglyceride, alkaline phosphatase, lactate dehydrogenase, creatine phosphokinase, glutamic oxalacetic transaminase, glutamic pyruvic transaminase, sodium, potassium and chloride.

Statistical Analysis

Data in Experiment I were analyzed by analysis of variance. In the lactation trial interaction of hyalage treatment and addition of corn silage to the ration was tested. If an interaction existed means were tested for difference by Bonferoni t test. If no interaction was detected, Tukey's test was used. In Experiment II data were analyzed by randomized complete block analysis using each DM content as a block. This assumes no propionate-DM interaction. Overall means were tested for differences by Dunnett's t test.

Data in Experiment III and IV were analyzed by simple one-way analysis of variance. In Experiment III covariate analysis was

used to eliminate intake differences. All means were tested for differences using Dunnett's test.

In Experiment V blood parameters were analyzed by 2-way analysis of variance with no interaction. Animals served as blocks and days as treatments to test changes with time of haylage feeding. Dunnett's t was used to test mean differences.

RESULTS AND DISCUSSION

Experiment I

This experiment was undertaken in an attempt to quantitate ensiling losses and transformations as a result of propionate addition. Relationship of transformations with ad libitum intakes and milk production was observed when haylage was fed to lactating dairy cows. Also corn silage addition to a haylage ration was attempted in order to detect any associative effects that might exist.

Recovery of Dry Matter

A greater ($p < .05$) percentage of dry matter (DM) placed in pantyhose was recovered for propionate treated haylage compared with untreated (96.4 vs. 88.1%; Table 2). Differences in recovery between control and propionate treated haylage were greater for pantyhose in the upper portion of the silos than those in the bottom. A 20 percentage unit (99.8 vs. 79.1%) advantage existed for treated haylage in the upper silo area while the lower ones had only a difference of 2.1 percentage units (94.7 vs. 92.6%). This indicates propionate was most effective in preventing losses from the top silo areas. Reduction in DM loss with propionate treatment of haylage has been observed by Thomas (1976), Yu and Thomas (1975) and Larsen et al, 1976.

Greater DM loss would indicate greater CO_2 formation (Mo and Fyrileiv, 1979) possibly as a result of respiration by microbes

TABLE 2.--Dry matter recoveries of haylage placed in pantyhose and buried at two depths during ensiling (Experiment I)

Depth	Control	1% p[a]	X
.6M	79.1	99.8	89.5
2.0M	92.6	94.7	93.7
X	88.1[b]	96.4[b]	

[a]P = propionic acid, added as forage entered blower

[b]Overall means differ ($p < .05$).

during the early stages of eniling. Top areas of silos near the air interface are more prone to undergo respiration due to infiltration of oxygen, and propionate may be acting by reducing microbial respiration. If more respiration occurred in untreated than in treated haylage then heating would be expected, but this was not observed since temperatures for both treatments were about the same (Table 3). Dexter (1966) observed that DM loss due to respiration in an airtight silo was relatively unimportant compared with losses caused by anaerobic fermentation perhaps indicating propionate was reducing the amount of fermentation. An important difference was the use of an airtight silo by Dexter while the silos in this study were not airtight.

Temperatures During Ensiling

Temperatures during ensiling are presented in Table 3. Temperatures (C) increased from week 1 to week 2 and then remained

TABLE 3.--Temperatures of control and propionate treated haylage at three depths in silos (Experiment I)[a]

	Control			1% Propionate		
	Bottom[b]	Middle[b]	Top[b]	Bottom[b]	Middle[b]	Top[b]
	^{0}C					
Week						
1	32	40	43	31	31	33
2	35	48	48	32	44	47
3	31	46	46	32	50	50
4	34	47	46	37	49	54
5	36	46	45	33	47	53
$\bar{X}$	34	46	46	33	44	47
Maximum Temperature	39	57	57	41	51	59

[a]Each value an average of two silos except maximum temperature which is the maximum value reached in either of the two.

[b]Relative position of thermocouples in the silos.

stable. More heating occurred in the top vs. bottom for both treated and untreated haylage (47 vs. 33 for treated; 46 vs. 34 for untreated). Also the maximum temperature observed was in the top for both treated and untreated corresponding to increased ADIN values in Table 4. Therefore propionate addition did not reduce mean temperatures or maximum temperatures in these silos.

Changes in Nitrogen Fractions

Table 4 contains values for nitrogen fractions before and after ensiling of haylage in pantyhose. Water soluble nitrogen (WS-N) was determined on supernatants from homogenates of haylage from each silo, and water insoluble nitrogen calculated by difference from total nitrogen. About 46% of the total nitrogen was water soluble in the wilted, unensiled forage, and 54% was insoluble. After ensiling the WS-N in the untreated haylage increased to 60% in the top (buried .6M) and 64% in the bottom (buried 2.0M) pantyhose, while propionate treated haylage contained 45 and 57% WS-N respectively. Decreased WS-N values for protionpate treated haylage coincides with increased DM recoveries in Table 2. A reduction in WS-N is indicative of reduced proteolysis as a result of adding 1% propionate, and probably reflects reduced fermentation.

In the unensiled forage 8 to 9% of the total nitrogen was in the acid detergent insoluble (ADIN) fraction. This fraction increased to 11 and 10% in the top and bottom pantyhoses in

TABLE 4.--Nitrogen fractions of haylage placed in pantyhose and buried at two depths during ensiling (Experiment 1)[a]

	Control		1% pb	
	% of DM	% of Total N	% of DM	% of Total N
As ensiled				
Total N[c]	2.76		2.88	
Water insoluble N	1.49	54	1.55	54
ADIN[d]	.25	9	.24	8
Water soluble N	1.27	46	1.33	46
WS-NPN[e]	.98	36	.99	34
WS-Protein	.29	10	.34	12
Depth = .6 M				
Total N	2.90		2.71	
Water insoluble N	1.15	40	1.50	55
ADIN	.32	11	.43	16
Water soluble N	1.75	60	1.21	45
WS-NPN	1.68	58	1.17	43
WS-Protein	.07	2	.04	2
Depth = 2.0 M				
Total N	2.98		2.87	
Water insoluble N	1.07	36	1.23	43
ADIN	.31	10	.25	9
Water soluble N	1.91	64	1.64	57
WS-NPN	1.85	62	1.61	56
WS-Protein	.06	2	.03	1

[a]Means of two silos.
[b]P = propionic acid.
[c]N = nitrogen
[d]ADIN - acid detergent insoluble nitrogen.
[e]WS-NPN = water soluble nonprotein nitrogen.

untreated haylage and to 16 and 9% respectively in propionate treated haylage. None of these values would indicate extreme "heat damage," but using Georings' (1976) criteria of .29% of DM as ADIN three out of four sample averages would be classified as heated. Propionate did not reduce heating or ADIN values as might be expected from previous reports (Thomas, 1976; Yu and Thomas, 1975). Slightly more heating occurred in the upper areas of the silos and more ADIN was present in the top pantyhoses.

Unensiled alfalfa contained 74 to 77% of the WS-N or 34 to 36% of the total nitrogen as nonprotein nitrogen (NPN). True protein in this material was 23 to 26% of the WS-N or 10 to 12% of the total nitrogen. After ensiling, NPN in haylage in the top pantyhoses increased to 96 to 97% of total soluble nitrogen, and true protein composed only 3 to 4%. In the bottom pantyhose 97 to 98% of this WS-N was NPN and 2 to 3% true protein. These percentages did not seem to be treatment related. When expressed as a percent of total nitrogen top pantyhose contained 58 and 43% WS-NPN for control and propionate treated haylage, and bottom contained 62 and 56% respectively. Propionate treated haylage contained less WS-NPN mainly due to a reduced amount of total WS-N but as a percent of the water soluble extract no difference was detected.

True protein in the WS-N fraction was degraded regardless of addition of propionate even though total WS-N was reduced.

Therefore, propionate prevents protein breakdown of the water insoluble fraction during ensiling. Soluble protein would probably be the first degraded due to the ease of access by bacterial and plant proteases. Propionate effects on proteolysis will be pursued further in Experiment II.

Analytical Values for Composites

Haylage composites from silos ranged in DM content from 43.1 to 53.6% (Table 5). Silo 3 containing treated haylage was the driest of the four haylages. Crude protein averaged 17.9% of DM for both treated and untreated haylage. Acid detergent fiber was slightly lower (37.6 vs. 39.1%) for the propionate treated haylage. Treated haylage averaged 13.4% of total nitrogen as ADIN and untreated contained 11.4%. These values are similar to those of haylage in pantyhose (Table 4). Haylage pH averaged 4.9 for both treatments, and appeared highest in the drier silos (silos 2 and 3).

Acetate concentration in haylage was reduced from 30.9 to 17.6 m moles/100g DM with propionate addition at ensiling (Table 5). This indicates a reduction in fermentaion due to acid treatment, and is in agreement with Britt et al. (1975) who found reduced lactic acid concentrations in corn silages treated with 1% propionate. Yu and Thomas (1975) also found lactic acid reduction in treated haylage, but no reduction in acetate resulted. A reduction in fermentaion would normally be expected to coincide with reduced protein degradation (Table 4) and DM losses (Table 2).

TABLE 5.--Analysis of control and propionate treated haylage composites (Experiment 1)

	Control			Propionate (%)		
	Silo 1	Silo 2	$\bar{X}$	Silo 3	Silo 4	$\bar{X}$
Dry matter (%)	43.4	47.4	45.4	53.6	43.1	48.4
Crude protein (% DM)	17.7	18.1	17.9	17.6	18.1	17.9
ADF (% DM)[a]	38.9	39.2	39.1	38.4	36.7	37.6
ADIN (% TN)[b]	12.1	10.6	11.4	15.8	11.3	13.4
pH	4.7	5.0	4.9	5.1	4.7	4.9
Volatile fatty acids (m moles/100 g DM)						
Acetate	25.6	36.3	30.9	11.9	23.2	17.6
Propionate	.369	1.688	1.029	15.541	20.394	17.968
Iso-butyrate	.028	.268	.148	.028	.028	.028
n-butyrate	.012	.065	.034	.547	0	.273
TOTAL	26.0	38.3	32.1	28.0	43.6	35.9

[a]ADF = acid detergent fiber.

[b]ADIN = acid detergent insoluble nitrogen; TN = total nitrogen.

Propionate concentration of control haylage averaged 1.03 mM/100g DM compared with 17.97 for propionate treated. If we assume 1.03 mM/100g DM was a result of fermentation in propionate treated haylage, 16.94 mM would be of exogenous nature or 94% of the total propionate. If fermentation was reduced, we would expect even more propionate to be from an exogenous source.

If all of this propionate was of exogenous origin, silo 3 retained 61.5% of the added acid and silo 4 65.1%. Therefore, a good protion was not recovered. Part of the reason for this could be volatilization during filling of silos. Also propionate can be metabolized and converted to other compounds during fermentation. These recovery values are similar to those of Yu and Thomas who found retention of added propionate to range from 52 to 70%.

Butyric and iso-butyric acid concentrations were very low in both treated and untreated haylage. Butyric acid can be made during initial fermentation or as a result of protein degradation in silages due to secondary fermentaion resulting in reduced forage intakes (Murdoch, 1966). Concentrations found in these haylages would not be expected to be a factor in animal response and were probably of a primary rather than secondary origin.

Total volatile fatty acid (VFA) concentration averaged 32.1 mM/100 g DM in the untreated haylage vs. 35.9 in the treated. Therefore, propionate did not reduce the total VFA concentration. If 16.94 mM/100 g DM in the propionate treated haylage is from an

exogenous source, 47% (16.94 ÷ 35.9 x 100) of the total VFA concentration would be of an exogenous nature leaving 53% from endogenous sources. Therefore, 19.0 m moles/100 g DM (35.9 x .53) would come from fermentation of plant components in propionate treated haylage vs. 32.1 (100% of VFA's) in untreated haylage. These calculations indicate less total fermentaion and, thus, less loss of energy with acid treatment.

Lactation Trial

This trial was undertaken to evaluate propionate treatment of alfalfa haylage as well as the associative effects of feeding corn silage with haylage. Cows were blocked and assigned to a treatment with one-half receiving corn silage plus haylage and the remainder receiving haylage as the only forage. Grain was fed at 1 Kg per 3 Kg of milk produced.

Cows receiving propionate treated haylage as the only forage ad libitum consumed more ($p < .025$) total forage than those receiving only untreated haylage (11.0 vs. 13.1 Kg/day; Table 6). Consumption of treated and untreated haylage with corn silage was intermediate (11.8 and 11.9 Kg/day respectively). Cows receiving untreated haylage consumed 11.5 Kg vs. 12.5 Kg ($p < .025$) for those receiving propionate treated haylage, and forage intake persistencies (experimental/pre-experimental period) were .82 and .88 respectively ($p < .025$). Corn silage did not improve forage consumption when added to either treated or untreated haylage.

TABLE 6.--Intakes of control and propionate treated haylage with and without corn silage to lactating dairy cows (Experiment I)

	C[a]	C+CS[a]	P[a]	P+CS[a]	Propionate -	Propionate +	Corn Silage -	Corn Silage +
N	11	11	11	11	22	22	22	22
Forage DM intake (Kg/Day)	11.06[b]	11.9[bc]	13.1[c]	11.8[bc]	11.5[b]	12.5[c]	12.0	11.8
SE[d]	.35	.35	.35	.35	.35	.25	.25	.25
Forage DM intake persistency[e]	.81[b]	.84[b]	.94[c]	.82[b]	.83[b]	.88[c]	.88	.83
SE[d]	.03	.03	.03	.03	.02	.02	.02	.02
Total DM intake (Kg/day)	16.2[b]	17.0[bc]	18.3[c]	17.1[c]	16.6[b]	17.7[c]	17.2	16.9
SE[d]	.46	.46	.46	.46	.33	.33	.33	.33
Total DM intake persistency[e]	.85[b]	.87[b]	.95[c]	.86[b]	.86[b]	.91[c]	.90	.87
SE[d]	.02	.02	.02	.02	.01	.01	.01	.01

[a]C = control haylage; C+CS = control haylage + corn silage; P = propionate treated haylage; P+CS = proionate treated haylage + corn silage.

[b,c]Means within rows within groupings having unlike superscripts differ ($p < .025$)

[d]SE = standard error of the mean

[e]persistency = experimental period/pre-experimental

Total DM intakes include both forage fed ad libitum plus grain fed at 1 Kg for 3 Kg of milk produced (Table 6). Total DM intakes reflect values of forage intakes because grain consumption was constant across all treatments. Consumption of total DM on propionate treated forage rations alone or with corn silage was greater ($p < .025$) than that of control haylage rations. Cows receiving corn silage consumed 16.9 Kg DM/day vs. 17.2 for those receiving none. This effect was not significant ($p > .1$) and agrees with data by McGuffey et al. (1976).

Cows consuming control haylage, control plus corn silage, propionate treated haylage and propionate treated haylage plus corn silage had energy balances of -2, +.3, -.2 and -.1 MCal per day based on NRC requirements (NRC, 1971). About 60% of the energy came from the forage.

Interaction of haylage treatment and feeding with corn silage had a significant ($p < .05$) effect on feed intake. Forage DM consumption increased when corn silage was added to untreated haylage rations, but then decreased when added to treated haylage rations. An explanation for this is not evident since both haylages appeared to be of good quality.

Barry et al. (1978) observed that sheep decreased voluntary intake, apparent biological value and nitrogen retention linearly with increasing concentration of acetic acid and ammonia in alfalfa silage. This implies a product of protein degradation might limit

intake. Data by Hawkins et al. (1970) supports this concept. If this is true it could help explain stimulation of intakes in this study since we observed decreased WS-N (proteolysis) and acetic acid in treated haylage. Ammonia would also be expected to be reduced, but was not measured. Haylage acidity could not explain consumption differences since pH values were similar for both haylages. Also no reduction in total VFA concentration was observed in the treated haylage.

Yu and Thomas (1975) found a stimulation of intake (4-5 Kg/day) in lactating dairy cows when alfalfa haylage was treated with .8% propionate, but no increase in milk yield after adjustment by covariance. Soluble nitrogen fractions were not quantitated in their experiment, but lactic acid levels were reduced indicating reduced fermentation and perhaps proteolysis. A review by Thomas (1978) presents data showing a stimulation of intakes in sheep receiving propionate treated haylage. These studies indicate propionate added at ensiling is exerting some effect that results in greater consumption of treated material. This action might be due to either of the following variables: altered fermentation or reduced proteolytic end products.

Corn silage addition to haylage rations did not exert a significant effect on DM consumption, but tended to reduce them in animals fed propionate treated haylage. This effect was significant if forage DM intake persistency is considered (.94 vs. .82).

Perhaps corn silage has enough fermentation byproducts to overcome the effects of the propionate treated haylage, but this question cannot be answered with the current data.

Production means are presented in Table 7. No differences were observed in total milk or milk persistency as a result of haylage source or addition of corn silage to the ration. Cows fed propionate treated haylage produced slightly more milk (19.6 vs. 18.8 Kg/day), but when expressed as a fraction of pre-experimental period differences appeared less (.96 vs. 94). Differences were not significantly different ($p > .1$).

When milk production was adjusted to a 4% fat basis differences were even less. Cows receiving control haylage produced 18.2 Kg/day and those receiving propionate treated produced 17.9 Kg/day. Cows receiving haylage with or without corn silage produced 18.0 Kg/day fat corrected milk again indicating no advantage of adding corn silage to a haylage ration. Fat corrected milk persistencies reflect actual production values, and no differences were detected.

Total production of milk fat and persistency was not affected ($p > .1$) by treatment of haylage or type of forage, but tended to be less for cows receiving propionate treated haylage (709 vs. 671 g/day). Fat production persistency was .96 for cows fed untreated haylage and .92 for those fed treated haylage.

Milk fat percent was greatest for cows containing only control haylage (3.8%), followed by those receiving untreated plus

TABLE 7.--Production means during feeding of control and propionate treated haylage with or without corn silage to lactating dairy cows (Experiment I)

	C[a]	C+CS[a]	P[a]	P+CS[a]	Propionate -	Propionate +	Corn Silage -	Corn Silage +
N	11	11	11	11	22	22	22	22
Milk (Kg/day)	18.8	18.9	19.7	19.8	18.8	19.6	19.1	19.3
SE[e]	.74	.74	.74	.74	.52	.52	.52	.52
Milk (persistency)[e]	.93	.96	.96	.95	.94	.96	.94	.95
SE	.02	.02	.02	.02	.02	.02	.02	.02
4% FCM (Kg/day)[f]	18.2	18.1	17.9	18.0	18.2	17.9	18.0	18.0
SE	.69	.69	.69	.69	.49	.49	.49	.49
4% FCM (persistency)	.95	.94	.93	.92	.95	.93	.94	.93
SE	.01	.01	.01	.01	.01	.01	.01	.01
Fat (g/day)	714	704	673	668	709	671	694	686
SE	33	33	33	33	24	24	24	24
Fat (persistency)	.98	.94	.92	.90	.96	.92	.95	.92
SE	.04	.04	.04	.04	.03	.03	.03	.03
Fat (%)	3.8	3.7	3.5	3.5	3.8[b]	3.5[c]	3.6	3.6
SE	.17	.17	.17	.17	.12	.12	.12	.12
Fat (persistency)	1.04	.98	.95	.89	1.01[b]	.92[c]	.99	.93
SE	.05	.05	.05	.05	.03	.03	.03	.03
Body weight change (Kg/50 day)	+11.6	+7.6	+9.8	+12.5	+9.6	+11.2	+10.7	+10.1
SE	6.4	6.4	6.4	6.4	4.5	4.5	4.5	4.5

[a]C=control haylage; C+CS=control haylage + corn silage; P=propionate treated haylage; P+CS=propionate treated haylage + corn silage

[b,c]Means within rows within groupings having unlike superscripts differ ($p < .05$)

[d]SE=standard error of the mean

[e]Persistency = experimental ÷ pre-experimental period

[f]FCM = fat corrected milk

corn silage (3.7%) and least for those receiving propionate treated haylage with or without corn silage (3.6%). The four values were not statistically different but when grouped by haylage source the cows receiving control haylage had greater ($p < .025$) milk fat percents than those receiving propionate treated haylage (3.8 vs. 3.5%). Also milk fat percent as a fraction of pretrial value was greater for those animals fed control haylage (1.01 vs. 192; $p < .025$). This is in contrast to observations by Yu and Thomas (1975) and McGuffey et al. (1976) who showed no effect on milk fat test of haylage treated with 1% or less propionate. If propionate does reduce milk fat when fed these quantities, an economic loss would result since payment for milk is based partially on fat percentage.

Cows consuming propionate treated haylage was the only forage consumed 13.1 Kg/day. Calculations reveal this intake supplied 172 g/day propionate whereas cows consuming control haylage alone (11 Kg/day) received 9 g/day of propionate. Yu and Thomas (1975) fed 126 g/day propionate from treated haylage and considered this to be far below that needed to reduce milk fat percent. Balch et al. (1967) showed a 7.3% reduction in fat with infusing 725 g propionate into the rumen. Our study shows about an 8% reduction with only 172 g. The reasons for these wide differences in response to varying concentrations of propionate are not evident.

Body weight changed during the 50 day feeding trial did not appear to be treatment related and gain appeared to be greatest (+ 12.5 Kg) for those cows fed propionate treated haylage plus corn silage and least (+ 7.6) for control haylage plus corn silage. These differences were not significant. Those cows receiving propionate treated haylage gained 11.2 Kg vs. 9.6 Kg for those receiving untreated haylage. Blaxter and Wainman (1964) observed the efficiency for fattening decreased as the molar percentage of acetic acid increased in the rumen of sheep. Untreated haylages in this experiment had acetate/propionate ratios of 30/1 and propionate treated had ratios of 1/1. Therefore, due to this difference more fattening might have been expected to occur with those cows receiving propionate treated haylage, but this was not observed. Actual rumen fatty acid concentrations were not quantitated.

Summary

Treatment of alfalfa haylage at ensiling with 1% propionate improved DM recovery, decreased soluble nitrogen compounds and probably proteolysis, and reduced fermentation. No reduction in pH or ADIN was observed. When propionate treated haylage was fed to lactating dairy cows more total forage DM was consumed compared to untreated haylage, but no improvement in total milk or fat corrected milk production resulted. Milk fat

percent was reduced in cows fed propionate treated haylage. No differences in weight changes during this period were detected. Corn silage did not improve intakes, milk production or fat percent when added to haylage rations.

Experiment II

In order to confirm changes in the nitrogen fractions during ensiling with propionate observed in Experiment 1, quart jars were packed with chopped forage and sealed for 40 days before opening.

Changes in Nitrogen Fractions During Drying

During drying WS-N decreased slightly from 1.6% of the DM at 25% DM to 1.4% at 89.3% DM (Table 8). This indicates that drying slightly reduced the solubility of nitrogen. Nonprotein nitrogen (NPN) comprised 38% (.61% of DM) of the WS-N at 25% DM (direct cut) and the remainder or 62% (1.04% of DM) is true protein. At 89.3% DM this changed to 54% (.76% of DM) and 46% (.66% of DM) respectively. At all DM contents ammonia composed less than 1% of the WS-N. Alpha-amino nitrogen made up about 3% (.05% of DM) of the WS-N at 25% DM and less than 1% (.01% of DM) after drying to 89.3%.

This data indicates water soluble protein declines with drying due to proteolysis with a consequent increase in water soluble NPN. This increase in NPN is not reflected in increased ammonia or alpha-amino nitrogen fractions indicating larger prptides probably comprise the majority of this increased NPN. Brady (1960) found

TABLE 8.--Changes in nitrogen fractions of chopped alfalfa plants during drying (Experiment IIa)

DM[a]	WS-N[a]	WS-NPN[a]	WS-protein[a]	WS-NH_3[a]	WS-αNH_2[a]
%			% of DM		
25.0	1.6	.61	1.03	.01	.05
27.7	1.5	.64	.89	.01	.04
33.1	1.4	.68	.68	.01	.03
89.3	1.4	.76	.66	.02	.01

[a]DM = dry matter; WS = water soluble; N = nitrogen; NPN = nonprotein nitrogen; NH_3 = ammonia; αNH_2 = alpha-amino nitrogen.

soluble amino, volatile and amide nitrogen to increase during wilting coinciding with a reduction of protein nitrogen.

Kemble and Macpherson (1954) found 20% of the total plant protein was degraded during a 3 day wilting period. In this study 36% of the water soluble protein was degraded and/or disappeared from the water soluble fraction during 70 hours of drying. This confirms the idea that protein is degraded during wilting before ensiling probably due to plant proteases, but the end product does not appear to be ammonia. Therefore, intermediate sized peptides must be formed.

Changes in Nitrogen Fractions During Ensiling

Across all DM contents the pH of the untreated ensiled haylage averaged 5.0 vs. 4.4 for propionate treated material (Table 9).

TABLE 9.--Nitrogen fractions and pH of alfalfa ensiled for 40 days at different dry matter contents with and without propionic acid (Experiment IIa)[a]

DM[b]	pH	WS-N[b]	WS-NPN[b]	WS-NH_3[b]	WS-αNH_2[b]
%			% of DM		
			Control		
23.5	4.0	3.0	3.0	.47	.12
25.0	5.0	3.3	3.3	.53	.10
29.3	5.1	2.6	2.6	.53	.08
X	5.0±.02[c]	3.0±.11[e]	3.0±11[e]	.51±.01[c]	.10
			1% Propionate		
24.4	4.3	2.1	2.1	.19	.10
26.2	4.5	2.4	2.4	.27	.06
30.1	4.6	2.3	2.3	.26	.07
X	4.4±.02[d]	2.3±.11[f]	2.3±.11[f]	.24±.01[d]	.08

[a]Each value an average of duplicates taken from one jar at each dry matter content.

[b]DM = dry matter; WS = water soluble; N = nitrogen; NPN = nonprotein nitrogen; NH_3 = ammonia; α-NH_2 = alpha amino nitrogen

[c,d]Means differ ($p < .001$) assuming no propionate-DM interaction.

[e,f]Means differ ($p < .025$) assuming no propionate-DM interaction.

This is in contrast to what was observed in the large silos of Experiment I where no differences were detected due to propionate treatment. Since Experiment I and other reports (Britt et al. 1975; Yu and Thomas, 1975) demonstrated a reduction in fermentation due to propionate, the lower pH would probably be a result of the added acid. The difference between Experiments I and II might be explained by the fact acid was added as material was being blown into the silos in Experiment I giving time and opportunity for volatilization of propionate resulting in less acid remaining on the forage in the large silos. Recoveries of acid ranged from 61.5% to 65.1% in Experiment I. In Experiment II forage and acid were mixed by hand in a container in the lab then immediately ensiled in sealed glass jars. No quantitation was made in Experiment II so no recovery could be calculated. Also less propionate was added per unit of DM in Experiment I because it was dryer forage than in Experiment II.

The WS-N (% of DM) averaged 3.0 across all DM contents for untreated ensiled haylage vs. 2.3 for propionate treated ($p < .025$). This confirms observations in Experiment I showing reduced WS-N as a result of propionate. All of the WS-N in both treated and untreated haylages was NPN demonstrating that propionate does not prevent hydrolysis of soluble protein. A small amount of water soluble protein was found after ensiling in Experiment I (Table 4). One reason for this might be the fact forage in Experiment I was higher in DM content resulting in less proteolysis.

Water soluble ammonia (% of DM) averaged .51 across all DM contents for untreated haylage vs. .24 for propionate treated ($p < .001$). When ammonia was expressed as a percent of WS-N, untreated haylage contained 17.2% vs. 10.5% for treated. Therefore, reduction of ammonia by propionate was not just a result of reduced WS-N, but also due to the concentration of ammonia in this fraction. Since ammonia is a major end product of proteolysis a reduction in concentration indicates reduced protein breakdown.

Alpha-amino nitrogen values were low in this experiment compared with values of other investigators (Hawkins et al. 1970), and the reason for differences is not known. Across all DM contents alpha-amino nitrogen averaged .1% of DM for untreated haylage vs. .08% for treated. Expressed as a percent of WS-N these values were 3.4 and 3.5% respectively indicating propionate had no effect on alpha-amino nitrogen concentration.

To illustrate changes from unensiled material during ensiling, the ensiled value from Table 9 was divided by the unensiled value from Table 8. These are presented in Table 10. The WS-N doubled during ensiling of untreated-alfalfa while treated alfalfa increased only 1.5 times. Water soluble NPN increased 4.6 times for untreated and 3.5 for treated. Water soluble ammonia increased 51 times in untreated haylage vs. only 24 times in untreated, and water soluble alpha-amino nitrogen increased 2.5 times in untreated vs. 1.9 times in treated. All of these values show a reduction in protein degradation when haylage is treated with propionate. The ammonia

TABLE 10.--Relative concentrations of nitrogen fractions in silage compared to that in forage before ensiling (Experiment IIa)

DM[a]	WS-N[a]	WS-NPN[a]	WS-NH_3[a]	WS-αNH_2[a]
%	Ensiled values ÷ unensiled values			
	Control			
23.5	1.9	4.9	47	2.4
25.0	2.2	5.1	53	2.5
29.3	1.9	3.9	53	2.7
X	2.0	4.6	51	2.5
	1% Propionate			
24.4	1.3	3.5	19	2.0
26.2	1.6	3.7	27	1.5
30.1	1.7	3.4	26	2.3
X	1.5	3.5	24	1.9

[a]DM = dry matter; WS = water soluble; N = nitrogen; NPN = nonprotein nitrogen; NH_3 = ammonia; αNH_2 = alpha amino nitrogen

fraction had the largest increase over unensiled values, mainly because of low ammonia in fresh material. Propionate acted to reduce increases in the above fractions indicating more true protein would be present in the insoluble fraction due to reduced proteolysis.

To increase the range of DM's studied I undertook another trial similar to the previous one (Table 11). As was observed in Table 9, pH was reduced ($p < .001$) from an average of 5.2 to 4.6 when haylage was ensiled with 1% propionic acid.

The WS-N (% DM) averaged 2.0 for untreated haylage vs. 1.4 for treated ($p < .001$). Again no protein was found in the water soluble fraction.

Water soluble ammonia (% DM) averaged .46 for untreated vs. .16 for treated ($p < .005$). If ammonia is expressed as a percent of WS-N untreated haylage average 23.0% vs. 11.4% for treated which is similar to data from Table 9. This again demonstrates the ability of propionate to reduce ammonia concentration and therefore proteolysis.

Alpha-amino nitrogen values were again low, but similar to those in Table 9. Untreated haylage contained .08% of the DM as alpha-amino nitrogen vs. .06 for treated. When expressed as a percent of the WS-N these values were 4.0 and 4.3% respectively. Again, as in Table 9, no effect of propionate on alpha-amino nitrogen concentration was observed.

TABLE 11.--Nitrogen fractions and pH of alfalfa ensiled for 40 days at different dry matter contents with and without propionic acid (Experiment IIb)[a]

DM[b]	pH[b]	WS-N[b]	WS-NPN[b]	WS-NH_3[b]	WS-αNH_2[b]
%			% of DM		
			Control		
21.6	5.2	2.0	2.0	.41	.12
23.8	5.1	2.1	2.1	.50	.11
33.4	5.3	2.3	2.3	.48	.05
42.8	5.3	1.6	1.6	.44	.03
X	5.2±.09[c]	2.0±.11[c]	2.0±.11[c]	.46±.06[e]	.08
			1% Propionic Acid		
22.5	4.4	1.5	1.5	.15	.09
25.6	4.6	1.3	1.3	.13	.06
33.0	4.5	1.6	1.6	.17	.05
40.3	4.7	1.3	1.3	.18	.03
X	4.6±.09[d]	1.4±.11[d]	1.4±.11[d]	.16±.06[f]	.06

[a]Each value an average of two jars at each dry matter content done in duplicate.

[b]DM = dry matter; WS = water soluble; N = nitrogen; NPN = nonprotein nitrogen; NH_3 = ammonia; αNH_2 = alpha amino nitrogen

[c,d]Means differ ($p < .001$) assuming no propionate-DM interaction

[e,f]Means differ ($p < .005$) assuming no propionate-DM interaction

Data from Tables 9, 10, and 11 indicate that proteolysis is reduced by addition of 1% propionic acid at ensiling. This is indicated by reduced WS-N, water soluble NPN and ammonia. A consequent increase in insoluble protein would be expected. These results support those values in Table 4 where alfalfa was ensiled in large silos (Experiment I) and a reduction in WS-N was observed. Values from Table 4 are from a higher DM forage than those in Experiment II. Therefore, actual values are not comparable, but relative values are.

Certain trends can be observed with increasing DM contents (Tables 9 and 11). The pH gradually increased as DM content increased in both treated and untreated forage. This is consistent with other studies (Hawkins et al. 1970; Yu and Thomas, 1975) that have demonstrated reduced fermentation and acid production at higher DM contents. Another reason pH might increase with increasing DM in treated haylage is because acid added per unit of DM is less in the dryer material than in the wetter because application was per unit of wet weight. This would not explain differences in untreated haylage.

The WS-N tended to increase slightly after ensiling as DM content increased reaching a maximum at 30 to 42% DM (Table 9 and Table 11). No obvious trends were observed for water soluble ammonia, although less ammonia would be expected due to reduced proteolysis at higher DM contents (Hawkins et al. 1970). This was not observed however, as the range in DM contents may not have been

sufficient to observe trends. Alpha-amino nitrogen showed a decline in concentration with increasing DM regardless of treatment.

Reduction of proteolysis and proteolytic end products has been observed in reconstituted sorghum with 2% propionate added (Lichtenwalner et al. 1979). This effect was not due to pH reduction since buffering did not prevent reduction in proteolytic activity. In the quart jars in this experiment, pH could have been a factor, but no pH differencies were observed in the large silos of Experiment I and decreased proteolysis still appeared to occur. Propionate might have reduced the initial pH sufficient to inhibit proteolysis during the early ensiling phase, but after fermentation acid production reduced pH of untreated haylage to a similar degree. Therefore, pH may have a role in reducing protein degradation in acid treated haylage, but also there might be other modes of action resulting in reduced proteolysis (Lichtenwalner et al. 1979).

Summary

Treatment of alfalfa haylage at ensiling in jars with 1% propionate decreased pH, WS-N, water soluble NPN and water soluble ammonia indicating a reduction in proteolysis. This supports data from Experiment I. Drying of alfalfa also resulted in degradation of water soluble protein.

Experiment III

This experiment was undertaken in order to evaluate possible detrimental effects that may result from feeding heat damaged

forage. Observations indicate that there is reduced nitrogen digestibility when heat damaged forages were fed, and any adverse effects could be attributed to a reduced protein status of the animal. The intent of this study was to detect any adverse effects that could not be overcome when supplemental protein was added to a heat damaged forage ration. Meadow voles were utilized due to their ability to digest high fiber rations, and therefore tolerate large amounts of alfalfa in their diets (Shenk et al. 1971). Studies by Shenk et al. (1974) demonstrate voles are very good assay animals for detection of antiquality or toxic compounds in forages. Immature voles were used in this study to examine the effect of feeding heat damaged alfalfa on growth rate during a relatively short term (9 days) and compensatory gain after removal from these diets.

Analytical Values

Diets fed to voles in Experiment IIIa had crude protein contents (% DM) of 12.8% for positive control (PC), 7.5% for negative control (NC), 12.9% for 8 hr. heat damaged (8 HD), 12.7% for 24 hr. heat damaged (24 HD) and 12.5% for 72 hr. heat damaged (72 HD) (Table 12). Acid detergent fiber (% DM) values were 17.8, 40.8, 18.7, 21.1 and 24.6% respectively. Added cellulose to the NC diets was responsible for the elevated acid detergent fiber (ADF) values. Also increased time of heating resulted in increased ADF values indicating formation of acid insoluble compounds and/or volatilization of soluble compounds. Acid detergent insoluble nitrogen (ADIN)

TABLE 12.--Analytical values of meadow vole diets

Diet	Crude Protein	Acid Detergent Fiber	Acid Detergent Insoluble N
	% of DM		% of TN
	Experiment IIIa and IVa		
Postiive Control	12.8	17.8	6.9
Negative Control	7.5	40.8	7.0
8 hr. heated	12.9	18.7	9.6
24 hr. heated	12.7	21.1	18.5
72 hr. heated	12.5	24.6	40.5
	Experiment IIIb and IVb		
Positive Control	13.0	17.2	6.6
Positive Control plus Casein	17.8	15.9	2.9
Negative Control	7.2	43.0	7.0
Negative Control plus Casein	12.3	36.4	5.9
72 hr. heated	13.4	26.7	38.3
72 hr. heated plus Casein	17.9	21.1	23.3

increased from 6.9 and 7.0% of total nitrogen on PC and NC respectively to 9.6% in 8 HD, 18.5% in 24 HD and 40.5% in 72 HD.

Analytical values for Experiment IIIb are in Table 12. The basal positive control (PC) diet contained 13.0% crude protein, and casein (PC + C) increased this to 17.8%. The basal negative control (NC) contained 7.2% and increased to 2.3% after casein addition (NC + C). The basal heat damaged diet (72 HD) contained 13.4% and increased to 17.9% with casein (72 HD + C). Acid detergent fiber for basal and supplemental casein diets were, respectively, 17.2 and 15.9% for positive control, 43.0 and 36.4% for negative control, and 26.7 and 21.1 for 72 hr. heated diets. Addition of casein to the 3 diets diluted the ADIN and reduced it from 6.6 to 2.9% for positive control, 7.0 to 5.9% for negative control and 38.3 to 23.3% for 72 hr. heated diets.

Growth Trials

Data from Experiment IIIa are presented in Table 13. This trial was of 9 day duration with the first 2 days considered an adaptation period. Mortality did not appear to be treatment related, and was 11% on PC, 14% on NC, 13% on 8 HD, 10% on 24 HD and 0% on 72 HD diets. This indicates no overt toxicity of the more heat damaged forage, and even though nitrogen digestibility would be expected to be reduced on the more severely heated diets, all voles lived.

TABLE 13.--Body weight changes and intakes during feeding of alfalfa based diets to immature meadow voles for 9 days (Experiment IIIa)

	Unheated		Hours heated		
	Positive Control	Negative Control	8	24	72
N	8	7	7	9	8
Mortality (%)	11	14	13	10	0
Body weights (g)					
Day 0	19.1	21.2	24.6	22.9	24.0
Day 2	18.2	20.3	23.5	20.7	21.9
Day 9	21.5	19.9	25.7	22.1	20.4
Intakes					
Dry matter (g/day)	5.4± .45	6.2± .48	5.6± .48	5.4± .42	5.2± .45
Dry matter (% BW)[c]	27.9	29.7	22.5	24.3	23.4
Water (g/day)	12.4±2.9	15.6±3.1	18.8±3.1	20.0±2.7	16.1±2.9
Crude protein (g/day)	.69	.47	.72	.69	.65
Acid detergent Insoluble Protein (g/day)	.05	.03	.07	.13	.26
Available protein (g/day)	.64	.44	.65	.56	.39
Body weight changes (g/day)					
Last 7 days	.47±.13[a]	-.05±.14[b]	.27±.14[a,b]	.20±.12[a,b]	-.22±.13[b]
Covariate adjusted for intake	.48±.10[a]	-.10±.15[b]	.25±.11[a,b]	.22±.10[a,b]	-.19±.11[b]
Post experiment gain	.78±.20	1.25±.23	1.33±.21	1.02±.19	1.30±.20
Protein efficiency ratio (g gain/g protein intake)					
Last 7 days	.64±.21[a]	-.23±.23[b]	.39±.23[a,b]	.26±.20[a,b]	-.50±.21[b]
Covariate adjusted for intake	.66±.17[a]	-.32±.22[b]	.36±.19[a,b]	.29±.17[a,b]	-.44±.19[b]

[a,b]Means within rows with different superscripts are different ($p < .05$).

[c]BW = body weight.

Average body weights ranged from 19.1 g for voles on PC to 24.6 for those on the 8 HD diet. Although an attempt was made to balance for body size deaths prevented this from being effective, and consequently, weights were not exactly equal on all treatments. Voles lost body weight for the first 2 days when placed on all 5 diets. This was probably due to low consumption due to poor acceptability when diets were first introduced. Unheated (positive and negative controls) and 8 HD diets when fed to voles produced a loss of .9g during this 2 day period while the 24 HD and 72 HD fed voles lost 2.2 and 2.1g respectively. Therefore, initial weight loss appeared to be treatment related and was greater for the more severely heated diets. Only NC and 72 HD fed voles had further weight reductions by day 9.

DM intakes as g/day or as % body weight were not significantly different ($p > .1$), but tended to be higher for voles fed the NC (6.2g/day). There was a stepwise decrease of 5.6, 5.4 to 5.2 g/day with increasing degree of heat damage, and perhaps was related to low palatability and/or reduced gastro-intestinal transit time due to reduced digestibility of heated material. Water intakes (g/day) were not statistically different ($p > .1$), but the average consumption for all heat damaged fed voles was about 6 g/day more than those fed PC. Lee et al. (1974) observed increased water intakes on browned apricot diets and increased diarrhea. Increased intestinal osmolarity may be responsible for this stimulation in water intake as well as increased diarrhea.

Crude protein intakes were about the same (.65 g/day or above) except for voles fed NC (.47 g/day). Acid detergent insoluble protein intakes (g/day) were low for voles consuming the unheated positive and negative control diets (.05 and .03 respectively) and 8 HD (.07) diets, but increased to .13 on the 24 HD and .26 on the 72 HD diets. Available protein intakes were estimated by subtracting the acid detergent insoluble protein consumed from the total crude protein. These values were .64, .44, .65, .56 and .39 g/day for PC, NC, 8 HD, 24 HD, and 72 HD fed animals respectively.

Body weight changes during the last 7 days of the experiment were greatest ($p < .05$) for these voles fed the PC (.47 g/day), but those fed NC and 72 HD diets lost .05 and .22 g/day respectively. When gains were adjusted for intake by covariance the same pattern was observed.

After removal from experimental diets all voles were placed on a standard colony ration. Voles that gained the most during the experimental period (PC) did not appear to gain as much relative to voles on the other diets during the post-experimental period (.78 vs. 1.02 to 1.33 g/day), although differences were nonsignificant ($p > .1$). This indicates any physiologcal or biochemical alteration that might have occurred on the heat damaged diets can be reversed or alleviated by changing to a nonheated ration. Lee et al. (1974) demonstrated compensatory growth after removal from a heated apricot diet, but elevated serum enzymes indicative of

tissue damage and liver hypertrophy did not return to normal indicating certain metabolic changes may be irreversible. The study by Lee was conducted over several months whereas the duration of our study was only 9 days.

Protein efficiency ratio (PER) is the g of gain divided by g of protein consumed and was greatest ($p < .05$) for PC (.64) compared to NC (-.23) or 72 HD (-.50). Values for 8 HD and 24 HD were not reduced ($p > .1$) significantly. Adjustment of PER for intake by covariance did not change relative values. The PER reflects the efficiency with which the protein in the diet is being utilized for gain. With increasing heat damage the PER was decreased indicating the protein is altered in such a way as to interfer with utilization and this corresponds to increased ADIN's.

Data from Experiment IIIb are presented in Table 14. The purpose was to determine if detrimental effects could be overcome by addition of supplemental protein to a heat damaged ration. Mortality on PC, PC+C and NC was 0. Voles fed NC+C had 25% mortality, and those fed 72 HD had 17% and 9% for basal and the supplemented diet respectively.

Initial body wieghts were slightly greater than in Experiment IIIa, and ranged from 23.9 to 28.5 g. Most of the voles lost weight during the first 2 days except for those receiving PC+C and 72 HD+C. The greatest amount of weight loss during this 2 day adaptation period occurred on the PC and 72 HD diets, and did not appear to be treatment related. Average weights were increased by day 9.

TABLE 14.--Body weight changes and intakes during feeding of alfalfa based diets to immature meadow voles for 9 days (Experiment IIb)

	Positive Control		Negative Control		72 hr. Heated	
	Basal	Plus Casein	Basal	Plus Casein	Basal	Plus Casein
N	10	10	9	9	9	10
Mortality (%)	0	0	0	25	17	9
Body Weights (g)						
Day 0	28.5	23.9	25.9	25.8	26.3	25.7
Day 2	26.3	25.3	24.9	24.1	24.3	25.7
Day 9	30.5	29.7	27.9	27.9	25.1	31.3
Intakes						
Dry matter (g/day)	8.8± .56[a]	8.0±.56[a,b]	10.7± .56[a]	9.1± .59[a]	6.4± .59[b]	9.5± .56[a]
Dry matter (% BW)[e]	31±1.9[a]	29±1.9[a]	41 ±2.0[b]	34±1.9[a]	26±2.0[a]	34± .9[a,b]
Crude protein (g/day)	1.14	1.42	.77	1.12	.86	1.70
Acid detergetn Insoluble Protein (g/day)	.08	.04	.05	.07	.12	.40
Available Protein (g/day)	1.06	1.38	.72	1.05	.53	1.30
Body weight change (g/day)						
Last 7 days	.59±.14[a]	.63±.14[a]	.44±.14[a,b]	.54±.15[a,b]	.12±.15[b]	.80±.14[a]
Covariate adjusted for intake	.58±.12	.74±.24	.16±.56	.49±.16	.46±.67	.69±.24
Protein efficiency ratio (g gain/g protein intake)						
Last 7 days	.49±.11[c]	.42±.11[c,d]	.63±.11[c]	.39±.11[c,d]	.11±.11[d]	.48±.11[c,d]
Covariate adjusted for intake	.49 ±11	.47±.44	.49±1.11	.37±.23	.28±1.33	.43±.44

[a,b]Means within rows with different superscripts are different ($p < .05$)

[c,d]Means within rows with different superscripts are different ($p < .1$)

[e]BW = body weight

Dry matter intakes (g/day) averaged 8.8 for voles fed PC and 8.0 on PC+C. NC without and with supplemental protein averaged 10.7 and 9.1 respectively. This was not a significant increase in intake on the NC unless expressed as a % of body weight ($p < .05$). Voles fed 72 HD diet consumed 6.4g which was less ($p < .05$) than consumption on PC, NC, NC+C and 72 HD+C. Addition of C to 72HD stimulated intake to 9.5. Therefore, the heat damaged diet was discriminated against, but added protein overcame this. Casein may act to flavor the diet to increase palatability and/or increase transit time through the gastro-intestinal tract. Another possibility would be to improve the nutritive status and indirectly increase intake.

Voles consumed 1.14g/day crude protein on the PC and 1.42 on PC+C. On the NC without and with C voles consumed .77 and 1.12 g/day respectively. On the 72 HD diets without and with C voles consumed .86 and 1.70 g/day respectively. Acid detergent insoluble protein was consumed in small quantities on positive and negative controls ranging from .04 to .08 g/day. On the 72 HD diet insoluble protein consumption increased to .33, and up to .40 g/day when C was added. Available protein intakes were 1.06, 1.38, .72, 1.05, .53 and 1.30 g/day for PC, PC+C, NC, NC+C, 72HD and 72HD+C respectively.

Body weight gain (g/day) during the last 7 days of the experiment was .59 for voles fed PC and .63 on the PC+C diet. Voles consuming the NC gained .44 which increased to .54 on NC+C. Voles gained .12 on the basal 72 HD diet vs. .80 on 72 HD+C ($p < .05$).

Addition of supplemental protein to this heat damaged diet caused a marked increase in gain, and part of this stimulation must result from increased intake. All voles in this part of the experiment gained weight (Table 14) unlike Experiment IIIa (Table 13). Voles in IIIa were smaller and therefore probably more susceptable to poor quality diets.

To eliminate differences due to intake weight gains were covaried with intake and adjusted means calculated. This allows comparison of gains at a constant intake. After adjustment, no significant ($p > .1$) differences were observed, but certain trends existed. Voles fed, respectively, diets without and with C had adjusted gains of .58 and .74 for PC, .16 and .49 for NC and .46 and .69 g/day for 72 HD. Negative control appeared to respond most markedly to added C, mainly due to the larger intake on the basal diet, and therefore adjustment for constant intake would result in a large relative reduction in prediced gain. The important point is that the 72 HD+C adjusted gain is almost as much as gain on PC+C and available protein intake was about the same. This indicates most of the added protein is utilized on the heat damaged diet.

When NC and 72 HD adjusted gains are compared, the advantage appeared to lie with 72 HD diet (.16 vs. .46 respectively). This indicated that the available protein on the 72 HD diets is greater than on the NC, but estimation of available protein indicates the opposite. Efficiency of utilization may vary with level of intake and comparison of the two extremes (NC vs. 72 HD) should only be

done with caution especially since no significant differences were recorded.

Voles fed basal and supplemental casein diets respectively had PER's of .49 and .42 for PC, .63 and .39 for NC and .11 and .48 for 72 HD. For the diets containing unheated alfalfa (negative and positive control) addition of casein to the basal diet resulted in no improvement in protein efficiency for gain, and slight reductions were detected. The PER of the 72 HD diet was less ($p < .1$) than those for the PC, NC and 72 HD+C. Addition of casein to 72 HD improved PER to .48 which is similar to those for PC. Therefore, on unheated diets PER was not improved by addition of casein, but was improved for the heat damaged diet. Adjustment of PER by covariance with intake did not change relative values, but reduced differences. Supplemental protein added to a heat damaged diet is available for gain, and efficiency approaches that on unheated forage diet.

Energy intakes on these two experiments appeared to be sufficient to support much greater weight gains. Assuming energy from sucrose, starch and casein is equal to 4 Kcal/g (100% available), unheated alfalfa 4 Kcal/g (60% available), heated alfalfa 4 Kcal/g (55% available) and corn oil 9 Kcal/g (100% available) the following digestible energy (DE) intakes were calculated. Cellulose was considered to be unavailable. For Experiment IIIa PC, NC, 8 HD, 24 HD, and 72 HD diets supplied 15.5, 13.4, 15.4, 14.9 and 14.3 Kcal DE/day respectively. In Experiment IIIb voles consumed 25.0

on PC, 21.4 on PC+C, 22.9 on NC, 18.3 on NC+C, 17.5 on 72 HD and 24.3 Kcal DE/day on 72 HD+C. Shenk (1976) reported a DE intake of 13 Kcal would support weight gain of .8 g/day. Only one group average approached this value (72 HD+C) indicating energy was probably not a limiting factor in these trials.

Observations from this study do not agree with a previous report which demonstrated incomplete restoration of PER after addition of amino acids to a heat damaged diet (Rao et al. 1963). Donoso et al. (1962) noticed protein utilization decreased during heating more than could be accounted for by the loss of lysine and methionine. This observation was also made by Amadi and Hewitt (1975). These results might be explained by an alteration of several amino acids in the vicinity of an affected amino acid so that a greater reduction in amino acid availability would result than would be expected from amino acid analysis. The present study involved addition of a complete protein to a heat damaged diet not just amino acids as the above experiments used.

Shorrock and Ford (1978), Lee et al. (1977) and Amaya (1975) found certain products of the Maillard reaction to interfere with amino acid uptake from the intestine. One mode of action could be competition for absorption between altered and unaltered amino acids. If this process was occurring in our present study, it was not in sufficient magnitude to affect response to added casein on the heat damaged diet. Therefore, alteration of amino acid uptake may have occurred, but was not sufficient to prevent utilization of

supplemental protein on these short term studies. Growth rates as used here may not be sensitive enough to detect subtile differences in absorption and metabolism, but no overt toxicity was observed.

Summary

Immature voles when fed heat damaged diets demonstrated reduced growth with increased degree of heating of the diet, and voles fed the most severely heat damaged diet responded similarly to negative control. DM intakes were not significantly different between treatments, but tended to decline with increased heating. After voles were removed from experimental diets and placed on standard rations, they responded with compensatory growth, indicating reversibility of detrimental effects. In a second experiment (IIIb) voles fed 72 HD+C consumed and gained more than did those fed only 72 HD. When gains were corrected for intake differences, a response to added casein to the heat damaged diet was observed. Efficiency of protein utilization for gain (PER) was increased on 72 HD+C over 72 HD fed voles. The PER for 72 HD+C approach those fed positive control diets. This indicates supplemental protein added to a heated diet is being utilized in sufficient quantity to normalize PER.

Experiment IV

As a continuation of Experiment III a longer term trial was designed in order to test long term effects of consuming heat

damaged forage. Reproductive performance and maintenance of lactation were quantitated in mature voles fed alfalfa based diets.

Reproduction Trials

Diets used in the a and b part of this experiment are the same as those used in Experiment IIIa and b respectively except the b part does not conatin PC (Table 12).

Table 15 contains data from Experiment IVa. Mortality of voles fed alfalfa based diets was 14% on PC, 0 on NC, and 33% on 72 HD. The two dead voles on the 72 HD diet were cannibalized indicating death might have been by fighting. Voles fed the heat damaged diet appeared to be aggressive and acted strangely as if stressed. None of the losses on the PC were cannibalized.

The average initial weight (g) for voles fed the PC was 43.5 vs. 40.0 on NC and 35.3 on 72 HD. Body weights were balanced at the beginning, but deaths caused the imbalance. Final weights (60 day) were all greater than initial. PC, NC, and 72 HD fed voles weighed 48.5, 48.1 and 39.3 g respectively. Several of the females were pregnant at termination and this contributed to the increase in body weight. On PC 50% were pregnant at termination, 80% on NC and 25% on 72 HD. Therefore, no profound weight losses were observed in the long term feeding of female voles on any treatment. Intakes were not monitored so protein status could not be estimated.

Liver weights were larger ($p < .1$) on PC than on 72 HD diets (2.54 vs. 1.92 g). NC was intermediate at 2.03 g. Since body

TABLE 15.--Body and organ weights of female voles fed alfalfa based diets for 60 days (Experiment IVa)

	Positive Control	Negative Control	72 hr. heated
n	6	5	4
Mortality (%)	14	0	33
Initial weight (g)	43.5	40.0	35.3
Final weight (g)	48.5	48.1	39.3
Liver weight (g)	2.54± .17[a]	2.03± .19[a,b]	1.92± .21[b]
Liver weight (%IBW)[c]	6.02± .59	5.39± .54	5.58± .66
Kidney weight (g)	.56± .04	.48± .04	.50± .05
Kidney weight (%IBW)[c]	1.32± .11	1.26± .10	1.45± .13

[a,b]Means having no common superscript differ ($p < .1$)

[c]IBW = initial body weight

n = number slaughtered

weights at termination were confounded by pregnancy, liver weights were also presented as percent of initial body weight. No significant differences were detected, but PC fed voles appeared to have the larger livers (6.02%) compared with NC (5.39%) and 72 HD (5.58%) fed voles. Increased protein status and/or pregnancy can cause hypertrophy of the liver, either of which could have caused the observed differences between treatments (Poo et al. 1940). Therefore, the heat damaged diet did not cause an increase in liver weight as has been reported in other studies (Adrian and Susbielle, 1975). An increase in liver weight might be expected if large quantities of compounds were being detoxified. No gross abnormalities or changes in liver color were observed on any of the treatments. Lee et al. (1974) found no changes in hepatic morphology when heated apricot diets were fed even though there were increased activities of liver enzymes (SGOT and SGPT) in the serum.

Kidney weights (g) were not significantly affected by diet, and were .56, .48, and .50 on PC, NC, and 72 HD respectively. Adjustment to a percent of initial body weight resulted in values of 1.32, 1.26, and 1.45% respectively. Adrian and Susbielle (1975) found kidneys to hypertrophy when soluble Maillard products were incorporated into basal rations. Perhaps compounds in the forage diet were not absorbed in sufficient quantities to exert this effect.

Reproductive efficiency and litter performance data are in Table 16. On the PC diet all (6/6) of the females underwent

TABLE 16.--Reproductive efficiency and litter status of female voles fed alfalfa based diets for 60 days (Experiment IVa)

	Positive Control	Negative Control	72 hr. heated
Litters/total females	6/6	1/5	1/4
Parturition (day 0)			
pups/litter	5.5± .8	2.0±1.9	3.0±1.9
weight/pup (g)	2.9± .1	3.0± .3	3.1± .3
litter weight (g)	16.0±1.7	6.0±4.2	9.3±4.2
Day 10			
pups/litter	5.0± .4[a]	1.0± .9[b]	3.0± .9[a,b]
weight/pup (g)	7.0± .2[c]	9.9± .4[d]	6.4± .4[c]
litter weight (g)	35.0±2.7[a]	9.9±6.0[b]	19.4±6.0[a,b]
Day 16 (weaning)			
pups/litter	6.0± .4[a]	1.0± .9[b]	3.0± .9[a,b]
weight/pup (g)	10.6± .8	14.9±1.8	10.1±1.8
litter weight (g)	56.3±2.7[a]	14.9±6.0[b]	30.3±6.0[b]
Females pregnant at termination	3	4	1
number fetuses/female	5.3±1.1[a]	2.8± .9[b]	4.0±1.9[a,b]

[a,b]Means having no common superscript differ ($p < .1$)

[c,d]Means having no common superscript differ ($p < .05$)

parturition, but only 1 out of 5 on the NC and 1 out of 4 on the 72 HD diet gave birth. This statistic reveals nothing about conception rate, only that they did not produce a litter. Reduced number of litters could be attributed to either reduced available protein or to toxicity on the heated diet. Live pups per litter were 5.5 on PC and reduced to 2.0 on NC and 3.0 on 72 HD. The corresponding weights per pup were 2.9, 3.0 and 3.1 g respectively. Total litter weight would be an indicator of ability to maintain and deliver total fetal mass. Although no significant ($p > .1$) differences were detected, the total litter weight for voles fed PC averaged 16.0 but only 6.0 and 9.3 g on NC and 72 HD, respectively.

At day 10 after parturition the number of pups per litter on PC was greater ($p < .1$) than on NC (5.0 vs. 1.0), but not different from 72 HD fed voles (3.0). Weight per pup was greater ($p < .05$) on the NC compared with PC and 72 HD (9.9 vs. 7.0 and 6.4 respectively). This is somewhat misleading since the NC had only 1 litter with 1 pup and PC averaged 5 per litter. Total litter weight (g) was 35.0 g on PC, 9.9 on NC and 19.4 on 72 diets. Since numbers per litter were drastically different between treatments at birth comparison of litter weights at various times of lactation is of limited significance.

At day 16 PC fed females had 6.0 pups per litter vs. 1.0 and 3.0 on NC and 72 HD diets respectively. Females on the PC diet weaned 36 young while the others weaned only 1 and 3 respectively. Weight per pup (g) was not significantly different between

treatments, and averaged 10.6, 14.9, and 10.1 respectively. Total litter weight was greater ($p < .1$) on PC than on NC or 72 HD (56.3 vs. 14.9 and 30.3 g). Therefore, voles fed PC maintained more pups per litter than females on the other 2 diets. The ability of females fed NC and 72 HD diets to maintain large litters was not tested. Reproductive efficiency did appear to be reduced on both the low protein NC and 72 HD diet, and indicates reduced available protein on the heat damaged diet might be responsible.

Females pregnant at termination of the experiment were 3 out of 6 on PC, 4 out of 5 on NC, and 1 out of 4 on 72 HD. Since fewer voles fed heat damaged forage were pregnant, this could indicate a reduction in conception rate. The negative controls were conceiving, but the data indicate they have decreased ability to carry litters to term. When slaughtered the number of fetuses per female was greater ($p < .1$) on PC (5.3) compared with NC (2.8). The 72 HD fed voles were intermediate (4.0).

The second part of this experiment involved feeding alfalfa diets with and without supplemental protein. Table 17 presents mortality, body weights, and intakes of mature female voles fed these diets for 60 days. Mortality was 0 on all diets except the 72 HD on which 20% were lost. All carcasses were cannibalized as in Experiment IVa and aggressive behavior was noticed. The 72 HD+C group had no mortality or aggressive behavior. Therefore, stress due to feeding heat damaged forage was apparently responsible for the deaths, and this could be alleviated by addition of casein to this diet.

TABLE 17.--Mortality, body weights and intakes of females fed alfalfa based diets for 60 days (Experiment IVb)

	Positive Control	Negative Control		72 hr. heated	
	Plus Casein	Basal	plus Casein	Basal	plus Casein
n[c]	10	9	9	8	10
Mortality (%)	0	0	0	20	0
Initial weight (g)	52.6	52.3	49.0	48.6	52.6
Final weight (g)	45.3	43.5	48.9	43.7	49.6
Nonlactating part of experiment					
Dry matter intake (g/day)	8.3 ± .8[a]	11.5 ± .8[b]	10.1 ± .8[a,b]	11.0 ± .8[b]	8.3 ± .8[a]
Crude protein intake (g/day)	1.5	.8	1.2	1.5	1.5
Acid detergent insoluble protein intake (g/day)	.04	.06	.07	.57	.35
Available protein (g/day)[d]	1.46	.74	1.13	.93	1.15
Lactating part of experiment					
Dry matter intake (g/day)	15.0±2.6	23.7±2.2	23.5±4.5	15.5±2.2	15.2±3.2
Crude protein intake (g/day)	2.7	1.7	2.9	2.1	2.7
Acid detergent insoluble protein intake (g/day)	0.8	.12	.17	.80	.63
Available protein intake (g/day)[d]	2.62	1.58	2.73	1.30	2.07

[a,b]Means having no common superscript differ ($p < .1$)

[c]n = voles per treatment

[d]Available protein = (crude protein - acid detergent insoluble protein)

Initial weights of voles fed these diets ranged from 48.6 to 52.6 g. Animals lost weight on all treatments by day 60, and this loss was greater on PC + C. Percent pregnant at termination for PC + C, NC, NC + C, 72 HD and 72 HD + C was 0, 30, 56, 38, and 30%. This is in contrast to Experiment IVa where voles gained during the feeding of these diets. Since voles were at various physiological states (pregnant, lactating) at termination, any direct relationships of diet to weight change is difficult.

Nonlactating voles consuming PC + C or 72 HD + C (8.3 g/day) consumed less ($p < .1$) total DM than voles on NC (11.5) or 72 HD (11.0). NC + C (10.1) appeared to be consumed in greater quantity than PC + C or 72 HD + C, but not significantly so. Crude protein intakes averaged 1.5g/day on PC + C, 72 HD and 72 HD + C. Voles consuming NC without and with supplemental protein consumed .8 and 1.2 g/day respectively. Acid detergent insoluble protein intake (g/day) was .07 or less on all unheated diets, but increased to .57 and .35 for 72 HD and 72 HD + C respectively.

Consumption of available protein was estimated by subtracting intake of acid detergent insoluble protein from intake of total crude protein. This assumes only the protein in the acid detergent fiber fraction is unavailable and the remainder is not affected. During the nonlactating state voles consumed 1.46, .74, 1.13, .93 and 1.15 g/day available protein on PC + C, NC, NC + C, 72 HD and 72 HD + C respectively. Therefore, less available protein was consumed on the NC and 72 HD diets. More available protein was

consumed on the PC + C than on NC + C or 72 HD + C diets. Intakes during the nonlactating state are best estimates obtained twice a week from each harem (1 to 4 females).

Individual intakes of lactating females were obtained. No significant differences were observed in total DM intakes, but voles receiving either NC or NC + C (23.7 and 23.5 g/day) appeared to consume more than PC + C (15.0), 72 HD (15.5) or 72 HD + C (15.2). Cellulose in the NC may have increased the transit time through the gastro-intestinal tract or the lactating voles attempted to consume more energy resulting in greater DM intakes on the negative controls. All lactating voles consumed about double the DM consumed during the nonlactating state except those fed the 72 HD diet. Cole and Hart (1938) found rats were able to about double their intakes from the pregnant state to second half of lactation. If DM digestibility is reduced on the 72 HD diet more material would remain in the intestine, therefore gut capacity may be reached before a doubling of intake on this diet compared with the unheated diets. This appears to be a contradiction because the negative controls containing slowly digestible cellulose appeared to stimulate intake, but due to the purified crystaline nature of the cellulose compaction and increased transit could result. The 72 HD + C diet was consumed in lesser quantity during nonlactation than the 72 HD diet allowing for more of a percentage increase during lactation before gut capacity is reached.

Lactating voles fed PC + C consumed 2.7 g protein/day vs. 1.7 on NC, 2.9 on NC + C, 2.1 on 72 HD and 2.7 on 72 HD + C. Total

protein intake was reduced on the NC and 72 HD diets, but the diets with supplemental protein were about the same. Acid detergent insoluble protein intakes were .08, .12, .17, .80 and .63 g/day respectively. Available protein intakes were 2.62, 1.58, 2.73, 1.30, and 2.07 g/day respectively.

Assuming energy from sucrose, starch and casein is equal to 4 Kcal/g (100% available) unheated alfalfa 4 Kcal/g (60% available), heated alfalfa 4 Kcal/g (55% available) and corn oil 9 Kcal/g (100% available) the following DE intakes were calculated. Cellulose in NC was considered unavailable. During the nonlactating part of the experiment intakes were 22.7, 25.2, 21.1, 30.2 and 21.8 Kcal DE/day for PC + C, NC, NC + C, 72 HD and 72 HD + C respectively. Therefore, all energy intakes were about the same except voles fed 72 HD. During the lactating part of the experiment intakes were 44.8, 52.6, 54.5, 43.3, and 40.1 Kcal DE/day respectively.

Liver weights were 2.25, 2.12, 2.52, 2.09 and 2.71 g for PC + C, NC, NC + C, 72 HD and 72 HD + C respectively (Table 18). None of these differences were significant. Addition of casein to the NC and 72 HD diets appeared to result in slightly larger liver weights. No changes in liver color were evident by gross (visual) observation. Since liver size can be altered by the physiological state of the animal (Fell, 1972) values for all voles pregnant or lactating 1 week before termination were omitted and termed nonlactating and nonpregnant. Such averages for liver weight (g) were 1.38 for PC + C, 2.03 for NC, 2.55 for NC + C, 1.35 for 72 HD and

TABLE 18.--Organ weights of female voles fed alfalfa based diets for 60 days (Experiment IVb)

	Positive Control	Negative Control		72 hr. Heated	
	Plus Casein	Basal plus Casein		Basal plus Casein	
Liver weight (g)	2.25±.23	2.12±.25	2.52±.26	2.09±.26	2.71±.23
Nonlactating and nonpregnant liver weight (g)	1.38±.30	2.03±.33	2.55±.43	1.35±.33	1.50±.33
Kidney weight (g)	.55±.07	.47±.07	.48±.07	.52±.08	.53±.07
Nonlactating and nonpregnant kidney weights (g)	.53±.05	.47±.06	.48±.07	.53±.06	.50±.06
Empty small intentine weight (g)	1.41±.13	1.49±.14	1.54±.14	1.47±.15	1.54±.13
Empty cecal weight (g)	.61±.05	.69±.05	.65±.05	.66±.06	.65±.05
Cecal pH	7.29±.08[a]	7.49±.09[a]	7.34±.09[a]	7.27±.09[a]	7.01±.08[b]

[a,b]Means having no common superscript differ ($p < .05$).

1.50 for 72 HD + C. When lactating and pregnant vole values were removed from the analysis most of the treatment averages were reduced from overall values. This is consistent with other studies demonstrating increased liver weights during pregnancy and lactation in the rat (Poo et al. 1940; Souders and Morgan, 1957). As was the case in Experiment IVa no hypertrophy was detected when heat damaged forage was fed, and no gross abnormalities or color changes were observed.

Kidney weights (g) were .55 for PC + C, .47 for NC, .48 for NC + C, .52 for 72 HD and .53 for 72 HD + C. When nonlactating and nonpregnant voles are considered the average values are similar to overall values and no differences were detected. Therefore, kidney weights do not appear to change with physiological state of the animal as does the liver. Since no hypertrophy occurred on the heat damaged diets, no excessive excretion of absorbed compounds was suspected. This was similar to Experiment IVa.

The empty small intestine weights (g) were 1.41, 1.49, 1.54, 1.47 and 1.54 on PC + C, NC, NC + C, 72 HD and 72 HD respectively. Cecal weights ranged from .61 to .69 g and were not significantly different. Lee et al. (1974) found diarrhea to be accompanied by an enlarged cecum when heat damaged material was fed to rats, and this was probably a result of increased fermentation. Cecal pH was lower ($p < .05$) on the 72 HD + C diet (7.01) compared with 7.29 on PC + C, 7.49 on NC, 7.34 on NC + C, and 7.27 on 72 HD. Therefore,

cecal acid production may have been more on the 72 HD + C diet than on the 72 HD and other diets.

Reproductive efficiency and litter performance are in Table 19. On the PC + C diet 8 out of 10 females gave birth compared with 6 out of 10 on NC, 6 out of 9 on NC + C, 7 out of 8 on 72 HD and 4 out of 10 on 72 HD + C. Therefore, voles fed the 72 HD + C diet produced fewer litters. Intakes of available protein and energy were similar during the nonlactating part of the experiment for NC + C and 72 HD + C, and greater for the 72 HD + C fed voles than those consuming NC or 72 HD diets. This rules out the possibility that intake of available protein or energy was responsible for reduced reproductive efficiency on the 72 HD + C diet. If some compound due to heating was affecting reproduction, voles on the 72 HD diet would also be expected to be affected which was not the case.

The average number of live pups per litter at parturition was 4.8 for PC, 5.2 for NC, 4.5 for NC + C, 3.4 for 72 HD and 4.5 for HD + C. Therefore, the voles fed the unsupplemented heat damaged diet (72 HD) appeared to have fewer live pups per litter than voles on the other diets, but supplementation with protein (72 HD + C) overcame this increasing numbers similar to controls. Voles consuming the 72 HD diet consumed about 9 Kcal/day more than those consuming 72 HD + C illustrating the fact energy was probably not limiting and available protein was. More pups may have been produced than were recorded since dead young are usually eaten by the mother.

TABLE 19.--Reproductive efficiency and litter status of female voles fed alfalfa based diets for 60 days (Experiment IVb)

	Positive Control	Negative Control		72 hr. Heated	
	Plus Casein	Basal	plus Casein	Basal	plus Casein
Litters/total females	8/10	6/10	6/9	7/8	4/10
Parturition (day 0)					
Live pups/litter	4.8± .7	5.2± .8	4.5± .8	3.4± .7	4.5± 1.0
Weight/pup (g)	3.0± .1	2.7± .1	3.0± .1	2.7± .1	2.9± .2
Litter weight (g)	14.4±1.8	14.1±2.1	13.5±2.1	9.2±2.0	13.1± 2.6
Day 10					
Pups/litter	4.4± .7[a]	4.8± .7[b]	4.8± .8[a]	3.0± .7[b]	4.0± .7[a]
Weight/pup (g)	9.0± .6[a]	6.9± .7[a,b]	9.1± .8[a]	6.8± .7[b]	8.9± .9[a]
Litter weight (g)	39.6±4.3[a]	33.1±5.0[a,b]	43.7±5.5[a]	20.4±5.0[b]	35.6± 6.1[a,b]
Day 16					
Pups/litter	4.3± .6	4.8± .7	4.8± .7	2.6± .7	4.0± .8
Weight/pup (g)	13.4±1.1	10.9±1.3	13.8±1.4	10.0±1.4	14.6± 1.6
Litter weight (g)	61.6±7.1[a]	52.3±8.2[a,b]	66.2±9.0[a]	26.0±9.0[b]	58.4±10.1[a,b]
Females pregnant at termination	0	3	5	3	3
Number fetuses/female	0	3.7± .8[a]	6.2± .6[a,b]	5.7± .8[a,b]	7.0± .8[b]

[a,b]Means differ $p < .05$.

Weight per pup (g) was 3.0, 2.7, 3.0, 2.7 and 2.9 on PC + C, NC, NC + C, 72 HD and 72 HD + C respectively. Available protein intake was lowest on NC and 72 HD during the nonlactating state (Table 17), and a corresponding decrease in pup weight was observed. Weight per pup was increased by addition of casein to the heat damaged diet. Total litter weight appeared to be reduced only on the 72 HD diet (9.2 vs. at least 13.1 g). This reduction of litter weight was a result of reduced pups per litter as well as weight per pup. Therefore, even though 7 out of 8 of the voles fed 72 HD conceived and gave birth, their ability to produce large litters was adversely affected, but supplemental protein corrected this.

Adrian and Susbielle (1975) found soluble Maillard products reduced the percentage of females conceiving during a reproduction trial with rats. This is in contrast to this trial since the 72 HD diet did support reproduction. The authors also found number of live births, number per litter and litter weights to be also reduced. This was also observed on the 72 HD diet, but supplemental protein appeared to alleviate these effects. The NC fed voles consumed less available protein than those fed 72 HD (.74 vs. .93 g/day) and less energy (25.5 vs. 30.2 Kcal/day) during the non-lactating state but produced a larger litter. This indicates the estimate of available protein may be an over estimation for the 72 HD diet and/or other problems may be affecting reproduction. Since casein alleviated these problems, the former would probably be the case.

Pups per litter at day 10 averaged 4.4 on PC + C, 4.8 on NC, 4.8 on NC + C, 3.0 on 72 HD and 4.0 on 72 HD + C. Weight per pug (g) was lower ($p < .05$) on NC (6.9) and 72 HD (6.8) compared with PC + C (9.0), NC + C (9.1) and 72 HD + C (8.9). Voles receiving the supplemental protein appeared to consume more available protein during lactation than those fed NC and 72 HD (Table 17). This is partially responsible for stimulation in pup weight. On the 72 HD + C diet voles appeared able to utilize the supplemental protein, and pup weight was almost the same as those fed PC + C.

Total litter weight (g) at day 10 was lower ($p < .05$) on 72 HD (20.4) compared with PC + C (39.6) and NC + C (43.7). A reduction in pup weight and number per litter appears to be responsible. NC and 72 HD + C averaged 33.1 and 35.6 respectively.

At day 16 (weaning) there were 4.3 pups on PC + C, 4.8 on NC, 4.8 on NC + C, 2.6 on 72 HD and 4.0 on 72 HD + C. Weight per pup was 13.4, 10.9, 13.8, 10.0 and 14.6 g respectively. Total litter weight (g) was lower ($p < .05$) on 72 HD (26.0) than on PC + C (61.6) or NC + C (66.2). NC (52.3) and 72 HD + C (58.4) were intermediate, and not different from either extreme. This again demonstrates that supplemental protein added to a heat damaged diet will be utilized and pup liveability and gain are similar to those on PC + C. During lactation crude protein intakes (g/day) averaged 2.7 on both PC + C and 72 HD + C, but estimated available protein intake was 2.62 g/day on PC + C and 2.07 on 72 HD + C. Since pups per litter, weight per pup and total litter weights were similar

for these two diets the available protein on the 72 HD + C diet must have been as or more efficiently used as that on the PC + C.

Number of females pregnant at termination was 0, 3, 5, 3, and 3 for PC + C, NC, NC + C, 72 HD and 72 HD + C respectively. The number of fetuses per female was greater ($p < .05$) on 72 HD + C (7.0) than on NC (3.7). Number of fetuses for NC + C (6.2) and 72 HD (5.7) were intermediate. Since none of the PC + C fed voles were pregnant there were 0 fetuses per female.

Blood Parameters

Blood profiles of voles at termination are presented in Table 20. Serum from 4 to 5 voles was composited in order to have enough sample for analysis. Therefore, only 2 values are present for each treatment, and the means are in parenthesis. These values are from voles at various physiological states, and therefore interpretation must be done with caution. The main objective was to characterize concentrations of some blood constituents for adult voles and determine if any large change from normality occurs due to feeding heat damaged forage.

Total serum protein appeared to be highest on the PC + C (6.1 g/dl) compared with 5.7 on NC, 5.3 on NC + C, 5.6 on 72 HD and 5.6 on 72 HD + C. This could possibly indicate protein status was improved on the PC + C diet, but no improvement could be detected when casein was added to either NC or 72 HD diets. Albumin was 3.9, 3.4, 3.2, 3.4 and 3.4 g/dl respectively, and was slightly

TABLE 20.--Blood profiles of female voles fed alfalfa based diets for 60 days (Experiment IVb)[a]

	Positive Control	Negative Control		72 hr. Heated	
	Plus Casein	Basal plus	Casein	Basal plus	Casein
Total protein (g/dl)	(6.1) 6.3,5.9	(5.7) 5.9,5.4	(5.3) 5.3,5.2	(5.6) 5.7,5.5	(5.6) 6.0,5.1
Albumin (g/dl)	(3.9) 4.2,3.5	(3.4) 3.4,3.3	(3.2) 3.0,3.3	(3.4) 3.1,3.6	(3.4) 3.5,3.2
Globulin (g/dl)	(2.3) 2.1,2.4	(2.3) 2.5,2.1	(2.1) 2.3,1.9	(2.3) 2.6,1.9	(2.2) 2.5,1.9
Albumin/Globulin (ratio)	(1.8) 2.0,1.5	(1.5) 1.4,1.6	(1.5) 1.3,1.7	(1.6) 1.2,1.9	(1.6) 1.4,1.7
Blood urea N (mg/dl)	(22) 21,23	(15) 14,16	(20) 19,20	(17) 13,20	(17) 19,15
Glucose (mg/dl)	(104) 109,98	(101) 94,108	(96) 88,103	(111) 109,112	(109) 113,104
Calcium (mg/dl)	(11.9) 12.3,11.4	(9.5) 9.0,10.2	(10.5) 9.9,11.0	(11.1) 9.5,12.6	(11.9) 12.5,11.3
Phosphorus (mg/dl)	(8.4) 9.3,7.5	(8.4) 8.3,8.4	(7.8) 7.7,7.9	(8.1) 9.0,7.1	(8.4) 9.0,7.7

[a]Each value an analysis of a composite from 4 to 5 animals. Number in parenthesis is the mean.

elevated on the PC+C diet. Since albumin serves as a major amino acid pool a depression in serum concentration would indicate increased utilization by tissues of the body (Dimopoullos, 1970).

Globulin concentrations were relatively constant across all treatments and were 2.3, 2.3, 2.1. 2.3 and 2.2 g/dl for PC+C, NC, NC+C, 72 HD and 72 HD+C respectively. Globulin concentration would be increased due to infection or disease, but this was not observed. Albumin to globulin ratios were 1.8, 1.5, 1.5, 1.6, and 1.6 respectively. Lee et al. (1974) found total protein to be slightly reduced after feeding a heat damaged apricot diet even though the casein in the diet was unheated. Serum albumin was not affected. Tanaka et al. (1977) also observed reduced serum protein when a heated albumin-glucose mix was fed as the protein source, and supplementation with unheated protein did not correct this. These two studies indicate interference of nitrogen absorption and/or utilization when heated material is fed even when the diet protein source is not heated. Serum nitrogen parameters measured in this study would not contradict this.

Blood urea nitrogen was 22 mg/dl on PC + C, 15 on NC, 20 on NC + C, 17 on 72 HD and 17 on 72 HD + C. Blood urea nitrogen is produced in the liver as a result of amino acid deamination. Generally, the greater the intake of protein, the greater the serum urea nitrogen concentration. Therefore, this value would be indicative of available protein. PC + C appeared to be highest as would be expected, and supplementation of the NC resulted in an

increase in blood urea nitrogen. Supplementation of the 72 HD diet resulted in no increase. Tanaka et al. (1977) found blood urea nitrogen to increase after feeding heated albumin-glucose and was considered indicative of liver damage as serum enzyme activities were also elevated. Their observations are in opposition to findings in this study.

Glucose was 104 mg/dl on PC + C, 101 on NC, 96 on NC + C, 111 on 72 HD and 109 on 72 HD + C. Sucrose and starch would probably be the primary sources of glucose on these diets. Since concentrations were similar in each diet, no differences would be expected if absorption was not affected. Absorption of glucose might be affected if sucrase activity was inhibited as was observed by Lee et al. (1974) on heat damaged diets. Blood glucose could also be affected by liver damage on heated diets which might reduce the amount of gluconeogenesis. No reduction in blood glucose resulted on these diets so any alterations were probably not sufficient to cause problems.

Calcium concentration was 11.9 mg/dl on PC + C, 9.5 on NC, 10.5 on NC + C, 11.1 on 72 HD and 11.9 on 72 HD + C. All values appeared to be within normal ranges for rodents (Simesen, 1970). Phosphorus concentration was 8.4, 8.4, 7.8, 8.1 and 8.4 mg/dl respectively. Heating of alfalfa potentially could result in binding of minerals with a resulting decrease in absorption. All diets in this experiment had supplemental minerals, therefore, any reduction in mineral availability from the forage would probably be masked.

Discussion

Results from this experiment as well as Experiment III support the idea that supplemental protein added to a heated forage ration can overcome detrimental effects. If intestinal proteolytic activity was being inhibited by consumption of heat damaged forage, no increase in gain over and above an increase in intake would be expected in Experiment III. Because there was a stimulation in gain after adjustment for intake differences, we can conclude supplemental protein was being utilized. Also competitive inhibition of amino acid absorption was probably not an important factor as has been observed in vitro (Shorrock and Ford, 1978; Lee et al. 1977). If these intestinal alterations were occurring on heated diets, they were not of sufficient magnitude to affect growth response. Mortality during Experiment III did not appear to be treatment related, and therefore, overt toxicity was not a problem on heated diets.

Longer term feeding trials were conducted in Experiment IV. Liver weights appeared to be related to protein status because supplementation with casein of the negative control and the heat damaged diets produced a larger liver weight. No hypertrophy of livers, kidneys, or cecums was detected on heat damaged diets as other studies have observed (Adrian and Susbielle, 1975; Lee et al. 1974). Reproductive efficiency was reduced on the supplemented heat damaged diet, but not on the unsupplemented diet. Litter size and weight at parturition as well as day 10 and 16 was improved by addition of protein to the heated diet. Ability to support the

litter, indicated by total litter weights, was about the same for PC + C and the supplemented heat damaged diet even though available protein was reduced on the 72 HD + C diet. Therefore, supplemental protein was utilized for lactation.

All forage used in Experiment III and IV was heated at 80 C, which for haylage would be considered severe although not impossible to attain in a silo. Lower temperatures might produce different compounds having different effects on animal metabolism than those observed in these experiments. There are not data to support this theory, but more severe heating could produce greater amounts of chemical binding and polymerization resulting in compounds that may have been soluble if heated at lower temperatures but not solubilized at elevated temperatures. The net result could be reduced absorption.

Summary

Mortality was increased on the unsupplemented heat damaged diet probably a result of cannibalism due to a lack of available protein in the diet. DM intakes were greater on negative controls during the nonlactating and lactating part of the experiment than on positive control and 72 HD + C. Voles fed 72 HD were not able to compensate during lactation to the same magnitude as the voles fed other diets. Liver weights appeared to be stimulated on diets with more available protein regardless of the source of the basal diet. No hypertrophy of liver, kidneys, cecum or intestine was observed on heated diets. Total litter weights were reduced on

unsupplemented heated diets at birth, day 10 and 16 of lactation due to reduced number per litter and weight per pup. Supplementation with casein improved these values, and values were similar to unheated controls. Available protein intake was reduced on the supplemented heat damaged diet during lactation compared with PC + C, but litter gains were similar indicating utilization of available protein. Blood profiles were not drastically altered for nitrogen components, glucose and minerals for voles on all diets.

Experiment V

Six Holstein heifers were used in this experiment in order to observe responses in ruminants during feeding of heat damaged haylage. Haylage was obtained from a farmer in Michigan who had ensiled his alfalfa in a bunker silo. Four tons of material was transported to the Michigan State University Dairy Barns and placed in an upright silo until fed.

Feeding Trial

Table 21 contains compositional data of feeds consumed during this trial. During the preliminary period (14 days) a "good" quality haylage (low in ADIN) was the only feed consumed, and contained 14.4% of DM as crude protein (CP) and 12.1% of the total nitrogen as ADIN. The "poor" quality haylage (high in ADIN) averaged 15.8% CP and 51% ADIN during the first week of feeding, and decreased to 14.7 and 47.9% respectively during the third week. The protein supplement contained 54.7% CP (% DM).

TABLE 21.--Analytical values, intakes, digestibility and weight changes during feeding of haylage to growing heifers (Experiment V)

	Preliminary Period	Week 1	Week 2	Week 3[a]
Haylage				
Crude protein (% DM)[b]	14.4	15.8	16.1	14.7
ADIN (%TN)[c]	12.1	51.0	49.8	47.9
Supplement				
Crude protein (% DM)[b]	--	54.7	54.7	54.7
Intake				
Dry matter (Kg/day)	5.39	5.99	6.11	5.73
Dry matter (% BW)[d]	2.15	2.30	2.45	2.20
Haylage crude protein (Kg/day)	.78	.95	.98	.84
Haylage Digestible protein (Kg/day)	.47	.22	.23	.23
Digestibility				
Dry matter (%)	--	--	--	48.9
Weight Change (Kg/day)	+.88	+.40	+.40	+.40

[a]Intakes restricted to 90% of ad lib during wk. 3.

[b]DM = dry matter.

[c]ADIN = acid detergent insoluble nitrogen; TN = total nitrogen

[c]BW = body weight

Total DM intake (Kg/day) during feeding of the "good" quality haylage averaged 5.39. After change to the heat damaged haylage consumption increased to 5.99 during wk 1 and 6.11 during wk. 2. Consumption during wk. 3 was limited to 90% of ad libitum. Expressed as a percent of body weight, intakes were 2.15 on "good" haylage, 2.30 during wk 1 of heat damaged haylage feeding and 2.45 during wk 2. Therefore, consumption of heated forage was not reduced as was observed with meadow voles in Experiments III and IV. Yu and Veira (1977) observed that sheep did not reduce consumption of heated forage as ADIN (% of total N) increased to 24%. Haylage in this study contained about 50% ADIN and no reduction in intake resulted.

Consumption of CP from haylage during the preliminary period averaged .78 Kg/day and increased to .95 and .98 during wk 1 and 2 respectively. Digestible protein (DP) from haylage was calculated using the equation, apparent N digestibility (%) = 71.67 - .96 (ADIN, % total N), developed by Yu (1974). During the preliminary period all of the consumed protein came from haylage and .47 Kg DP/day was consumed. During wk 1 and 2, .22 and .23 Kg DP/day respectively was consumed from haylage. Requirements (NRC, 1971) for DP ranged from .30 to .46 Kg/day for .73 Kg/day gain. One half of the heifers receiving heat damaged haylage also received 12-20% of their total nitrogen from a protein supplement. Although total CP intakes were more than requirements, calculated DP intakes during feeding of heat damaged haylage were below requirements.

Apparent DM digestibility determined during wk. 3 ranged from 40.5 to 55.1% and averaged 48.9%. This indicates DM digestibility was not affected as drastically as predicted nitrogen digestibility. Using the above equation and analytical values from the heat damaged haylage (50% ADIN) predicted nitrogen digestibility would be about 24% compared with 60% (12% ADIN) for the "good" haylage. Yu (1974) also developed equations using acid detergent fiber (ADF) as a predictor of DM digestibility. His equation was: apparent DM digestibility (%) = 93.79 - .9 (ADF; %DM). Using this a DM digestibility of 44.3% would be expected when 55% ADF was present. This value is similar to the one determined during the present trial (48.9%).

Heifers gained .88 Kg/day during the preliminary period, but gain was reduced to .40 during feeding of the "poor" quality forage. Even on this "poor" quality forage gains in body weight occurred.

Table 22 contains serum enzyme activities during the feeding of heat damaged haylage with and without supplemental protein to heifers. Lactate dehydrogenase (LDH), creatine phosphokinase (CPK), glutamic pyruvic transaminase (GPT) and glutamic oxalacetic transaminase (GOT) activities were similar when fed heat damaged haylage with or without supplemental protein.

Serum protassium, chloride, sodium, carbon dioxide, calcium and phosphorous cencentrations are also in Table 22. Supplementation with protein did not affect any of these values.

TABLE 22.--Blood parameters of 6 Holstein heifers fed heat damaged haylage with and without supplemental protein (Experiment V)

	Day 6			Day 17		
	Unsupplemented	Supplemented	SE[a]	Unsupplemented	Supplemented	SE[a]
Lactate Dehydrogenase (IU/l)	1427	1485	95	1363	1412	74
Creatine Phosphokinase (IU/l)	54	141	18	76	109	40
Glutamic Pyruvic Transaminase (IU/l)	43	34	2.0	46	40	3.5
Glutamic Oxalacetic Transaminase (IU/l)	59	56	7.0	60	52	7.3
Potassium (mEq/l)	5.5	5.2	.21	5.4	4.7	.29
Chloride (mEq/l)	105	103	2.0	105	104	.9
Sodium (mEq/l)	147	147	1.0	146	143	1.3
CO_2 (mM/l)	23	25	1.2	24	24	.5
Electrolyte Gap (Na- ($CI+CO_2$)	19	19	1.50	16	16	.70
Calcium (mg/dl)	9.3	9.0	.40	8.9	9.1	.20
Phosphorous (mg/dl)	7.6	7.4	.40	6.4	6.8	.54

TABLE 22.--Continued

	Day 6			Day 17		
	Unsupplemented	Supplemented	SE[a]	Unsupplemented	Supplemented	SE[a]
Total Protein (g/dl)	6.9	7.0	1.7	6.7	7.0	.10
Albumin (g/dl)	3.7	3.4	.15	3.6[b]	3.4[c]	.05
Globulin (g/dl)	3.2[b]	3.6[c]	.07	3.1[b]	3.6[c]	.13
Albumin/Globulin (ratio)	1.15[b]	.94[c]	.05	1.15[b]	.94[c]	.05
Blood Urea N (mg/dl)	5.7	7.3	1.0	7.0	6.0	.08
Glucose (mg/dl)	83	87	2.6	80	87	3.7
Triglyceride (mg/dl)	17.7	15.3	2.5	13.0	11.0	2.1
Cholesterol (mg/dl)	107	90	11.5	111	91	12
Creatinine (mg/dl)	1.2	1.0	.06	1.3	1.1	.07
Uric Acid (mg/dl)	1.0	1.2	.20	.9	1.0	.17

[a]Standard Error

[b,c]Means with no common superscript differ ($p < .05$)

Supplementation of protein did not significantly ($p > .1$) increase total serum protein (Table 22). At day 6 supplemented heifers averaged .1 g/dl (7.0 vs. 6.9) more than unsupplemented, and at day 17 this difference was .3 (7.0 vs. 6.7). Albumin concentration (g/dl) was significantly ($p < .05$) increased at day 17 in unsupplemented heifers (3.6 vs. 3.4), but not at day 6. Globulin concentration (g/dl) increased ($p < .05$) on the supplemented at day 6 (3.6 vs. 3.2) and day 17 (3.6 vs. 3.1). Because of this increase albumin/globulin ratio was reduced ($p < .05$) on the supplemented ration at both day 6 and 17 (.94 vs. 1.15 on both days). This is in contrast to what would be expected because albumin normally would increase with increasing protein status (Dimopoullos, 1970).

Blood urea nitrogen, glucose, triglyceride, cholesterol, creatinine or uric acid concentrations were not affected by protein supplementation. Therefore, with the exception of albumin and globulin no changes in the metabolic profile was detected due to protein supplementation.

To further examine the type of changes in blood chemistry resulting from feeding of heat damaged haylage, values for the 6 heifers were averaged after 6 days (-8) on the "good" quality haylage and after 6 and 17 days on the heat damaged haylage (Table 23). Lactate dehydrogenase activities appeared to be above normal (Vet Path Animal Lab, Hackensack, NJ) at all days of haylage feeding, but was not related to the feeding of heat damaged haylage. Creatine

TABLE 23.--Blood parameters of 6 Holstein heifers fed Normal Haylage (-8) followed by heat damaged haylage for 17 days (Experiment V)

	Normal Range	Day of Feeding			Standard Error
		-8	6	17	
Lactate Dehydrogenase (IU/l)	357-756	1516	1456	1389	54
Creatine Phosphokinase (IU/l)		247	103	93	103
Glutamic Pyruvic Transaminase (IU/l)	1-48	36	39	43	3.3
Glutamic Oxalacetic Transaminase (IU/l)	9-67	67^{a}	$58^{a,b}$	56^{b}	3.1
Potassium (mEq/l)	2.8-5.6	5.0	5.4	5.1	.19
Chloride (mEq/l)	91-105	103	104	105	.8
Sodium (mEq/l)	133-143	143^{c}	147^{d}	$145^{c,d}$	.5
CO_2(Mm/l)		25.5	24.0	24.2	.57
Electrolyte Gap [Na-(Cl+CO_2)]		14.2^{c}	19.2^{d}	$15.8^{c,d}$	.49
Calcium (mg/dl)	9.9-12.5	9.2	9.2	9.0	.17
Phosphorous (mg/dl)	3.4-6.7	5.8^{a}	7.5^{b}	6.6^{b}	.23

TABLE 23.--Continued

	Normal Range	Day of Feeding			Standard Error
		-8	6	17	
Total Protein (g/dl)	4.9-7.7	7.2	7.0	6.8	.08
Albumin (g/dl)	2.4-3.8	3.6	3.5	3.5	.05
Globulin (g/dl)	1.8-4.6	3.6	3.4	3.4	.06
Albumin/Globulin (ratio)	.45-1.33	1.00	1.04	1.05	.02
Bood Urea N (mg/dl)	5-21	13.7^{c}	6.5^{d}	6.5^{d}	.71
Glucose (mg/dl)	73-123	91^{a}	$85^{a,b}$	83^{b}	1.8
Triglyceride (mg/dl)	14-56	17.8	16.5	12.0	2.1
Cholesterol (mg/dl)	20-147	99	99	101	5.1
Creatinine (mg/dl)	.9-1.9	1.1	1.1	1.2	.05
Uric Acid (mg/dl)	0-1.6	$.8^{a}$	1.1^{b}	$1.0^{a,b}$	.08

^{a,b}Means having no common superscript differ ($p < .05$)

^{c,d}Means having no common superscript differ ($p < .01$)

phosphokinase and glutamic pyruvic transaminase activities were within normal ranges and were not treatment related. Glutamic oxalacetic transaminase activity (IU/l) decreased ($p < .05$) from 67 at -8 days to 56 after 17 days of heat damaged haylage feeding. A reduction in overall protein status of the animals might be responsible because less protein would be available for enzyme synthesis. This does not explain why other enzyme activities were not affected. Lee at al. (1974) observed both GOT and GPT activities to be elevated in rats fed a heated apricot diet, and these changes were considered indicative of alterations in liver metabolism. No treatment related increased activities were observed in these heifers. Therefore, heat damaged forage probably does not affect metabolism the same way or degree as do other heated materials. Species differences may also have an effect on response.

Potassium and chloride concentrations were not affected by heat damaged haylage feeding, and values were within normal ranges. Sodium concentration (mEq/l) was elevated ($p < .01$) over preliminary period values on day 6 of heat damaged haylage feeding (143 vs. 147), but was not at day 17 (145). Blood carbon dioxide was not affected by the treatments imposed. Electrolyte gap (Na- ($Cl+CO_2$)) was increased ($p < .01$) on day 6, but not day 17. The reason for this was the increased sodium because chloride and carbon dioxide were not altered. Most of the sodium (75%) is normally reabsorbed in the kidney tubules, therefore, no reduction in this active process was observed since sodium concentrations were above normal during

feeding of heated haylage (White, Haudler and Smith, 1973). One of the explanations for increased blood sodium might be reduced water intakes, but no such measurements were made.

Total serum protein (g/dl) appeared to be greatest (7.2) during feeding of "good" haylage, and decreased slightly to 7.0 at day 6 and 6.8 at day 17 (Table 23). All values were within normal ranges. Albumin, globulin and albumin/globulin ratio were not affected by feeding of heat damaged haylage, and values appeared normal. Blood urea nitrogen was reduced ($p < .01$) from 13.7 mg/dl on day -8 to 6.5 on days 6 and 17. Reduced urea nitrogen reflects the reduced available nitrogen in the heat damaged haylage. Blood urea nitrogen has been shown to be related to protein intake in lactating dairy cows (Lee et al. 1978).

Glucose concentration (mg/dl) was reduced ($p < .05$) from 91 on day -8 to 83 on day 17. This probably reflects a decreasing available energy supply to these heifers due to reduced DM digestibility. Lee et al. (1978) found glucose to be related to energy intake in lactating dairy cows.

Triglyceride concentration (mg/dl) tended to be reduced with time on heat damaged haylage being 17.8 and 12.0 on day -8 and 17 respectively. Cholesterol and creatinine concentrations were not affected by feeding of heated haylage. Uric acid was increased ($p < .05$) from .8 mg/dl on day -8 to 1.1 on day 6 and 1.0 on day 17. Increased nucleic acid turnover can be responsible for increased uric acid concentrations. Perhaps a change in nucleic acid

metabolism occurred as a result of feeding heated haylage, but all values were within normal ranges.

These results indicate no drastic alterations in metabolism duirng short term feeding of heat damaged haylage to ruminants other than reduced nitrogen and DM availability.

Summary

Heifers consumed slightly more DM and CP when fed ad libitum after changing from a "good" quality to a heat damaged haylage, but estimated digestible protein consumption was reduced to about half. DM digestibility of heat damaged haylage averaged 48.9%. Heifers receiving supplemental protein (12-20% of haylage protein consumed) during feeding of heat damaged haylage had elevated serum globulin and reduced albumin/globulin ratio at day 6 and 17 of feeding. Sodium, phosphorus and uric acid were elevated in serum during feeding of heat damaged haylage, and glutamic oxalacetic transaminase activity, urea nitrogen and glucose were reduced. Reduced blood urea nitrogen and glucose reflect reduced protein and energy status respectively.

SUMMARY

1. DM recovery was increased and proteolysis (water soluble nitrogen) and fermentation (acetate concentration) decreased when alfalfa haylage was ensiled with 1% propionate.

2. Temperatures and acid detergent insoluble nitrogen values were not reduced by propionate.

3. More propionate treated forage was consumed by lactating dairy cows than untreated haylage when fed ad libitum with no corn silage, but no increase in fat corrected milk resulted.

4. Milk fat percent was reduced when cows consumed propionate treated haylage with or without corn silage.

5. Corn silage addition to haylage rations did not improve ad libitum consumption of forage or fat corrected milk production.

6. During drying of alfalfa water soluble NPN increased and water soluble protein decreased indicating proteolysis occurred during drying.

7. After 40 days of ensiling in model silos, pH, water soluble nitrogen, NPN and ammonia were reduced when ensiled with 1% propionate compared with untreated silage.

8. Immature voles fed negative control and a 72 hr. heat damaged diet lost weight when fed for 9 days, but were able to compensate and increase gain during a 14 day post-experimental

period during which an unheated diet was fed. Therefore, detrimental effects appear to be reversible.

9. DM intakes were reduced below those on unheated diets when alfalfa was heated for 72 hrs., but supplementation with casein alleviated this.

10. Gains and protein efficiency ratios adjusted for intake differences were restored to near normal when casein was added to a heated diet. This indicates supplemental protein is being utilized by these growing voles.

11. Cannibalism occurred on the unsupplemented heat damaged diet, but not on the supplemented. This indicates aggressive behavior was probably a result of a lack of available protein.

12. No hypertrophy of livers or kidneys resulted from feeding heat damaged diets for 60 days.

13. Pups per litter, weight per pup and total litter weights at day 0, 10 and 16 of lactation were increased when casein was added to a heat damaged diet.

14. Heifers fed ad libitum haylage did not reduce consumption when switched from a "good" quality to a heat damaged haylage.

15. Heifers fed heat damaged haylage supplemented with protein had elevated serum globulin and reduced albumin/globulin ratios than those not supplemented.

16. Serum GOT, urea nitrogen and glucose were reduced when heifers were switched from "good" quality to a heat damaged haylage. This indicates reduced protein and energy available to these heifers.

17. Sodium, phosphorus and uric acid concentrations were elevated after a switch from "good" quality to a heat damaged haylage.

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