UTILIZATION OF CRAB PROCESSING WASTE AND CHITIN ASSOCIATED WITH THE WASTE AS FEED FOR RUMINANTS

by

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(ABSTRACT)

Four experiments were conducted with crab processing waste. Addition of 1.5% propionic/formic acid prevented degradation up to 14 d. Desirable fermentation was achieved when 32% of the treated crab waste was ensiled with 32% straw, 16% molasses, and 20% water with or without .1% microbial inoculant. The pH of the silage was lowered and high concentration of lactic acid was observed. Use of .2% NaOCl or .4% H_2O_2 retarded spoilage of crab processing waste up to 7 d. Ensiling of the treated waste (32%), straw (32%), molasses (16%), and H_2O (20%) indicated that the chemical used in preservation enhanced fermentation. Addition of .4% NaOCl and 1.5% acetic acid to the crab processing waste retarded degradation up to d 4. The trimethylamine (TMA) concentration and NH_3 evolution were highest for the untreated waste while the lowest concentration for TMA and NH_3 evolution were observed for the NaOH-treated waste. Addition of 1% $NaNO_2$ or combination of .4% NaOCl/CaOCl (1:1, w/w) preserved the waste for up to 10 d. The evolution of NH_3 and H_2S,

and TMA concentration were lowest for the waste treated with 1% $NaNO_2$.

Apparent digestibility of DM, organic matter, CP, energy, NDF, ADF, cellulose and hemicellulose decreased linearly ($P<.01$) with level of crab waste-straw silage in diets containing 0, 50, and 100% crab waste-straw silage. Nitrogen retention increased linearly ($P<.05$) with level of crab waste-straw silage. The apparent absorption was higher ($P<.01$) and retention was positive ($P<.05$) for Ca, Mg, Na, K, Cu and Fe with sheep fed the highest level of crab waste-straw silage.

Daily gain,feed efficiency and carcass characteristics tended to be higher for steers fed 30% crab waste-straw silage, dry basis. The flavor and overall desirability of cooked meat were not adversely affected by feeding up to 30% crab waste-straw silage, dry basis.

Modifying the existing methods of quantifying chitin showed that initial decalcification or deprotinization of the samples with chemicals are not neccessary. Similar values were obtained for chitin when ADF/ashing method was compared to the ADF/deacetylation method. These methods prevented loss of chitin during preparation.

Digestibility of chitin was higher (58%) for sheep fed 100% crab waste-straw silage compared to the value of 37% in sheep fed 50% crab waste-straw silage. When ADF/ashing method was used in quantifying chitin, higher disappearances were

obtained for samples containing 100% crab meal kept in the rumen or incubated in vitro for 72 h.

DEDICATION

"For Paul planted and Apollos watered, but

God caused the growth"

To Him on the throne, be the glory, honor and strength.

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Chapter I

INTRODUCTION

The increase in human population has led to the prediction of a major food shortage in the foreseeable future. Protein is one of the most essential nutrients needed to solve the human food shortage. Efforts have been directed towards the use of marine animals to meet the human protein requirement. Seas cover about 71% of the surface of the earth, and provide an important source of high-quality protein. Increased emphasis has been placed on aquaculture to provide additional seafood. The availability of more seafood will help to provide for the nutritional needs of the world human and livestock population (Wignall and Tatterson 1976). The advent of technology in the seafood industry has improved processing methods. However, wastes result, which must either be discarded or utilized.

About 149,000 metric tons of crab are harvested from the sea annually (NMFS, 1975). Crab meat for human consumption constitutes approximately 10% of the tonnage (Brinsfield, 1980). The remaining 90%, which constitute the shell, viscera, water and some meat, is classified as crab processing waste (Mendehall, 1971). This byproduct presents some economical and enviromental problems (Brinsfield, 1980).

Crab processing waste is a highly perishable material, which can decompose within 5 h after processing (Brooks, 1980), hence, rapid and effective ways of disposal are needed. In the past, crab waste was dumped back into the sea (Olsen, 1980) or put in landfills (Brinsfield, 1980). The crab processing waste can also be converted into crab meal via dehydration (Brooks, 1980). Restricted measures by the Enviromental Protection Agency (EPA), odor emanating from the by-product, high energy cost of dehydration and variable price of the meal has made this process marginally profitable.

Fresh crab byproduct is high in crude protein, Ca and P. Previous work indicated that crab by-product can be ensiled successfully with straw in the presence of a moderate amount of molasses. However, the waste has to be preserved within hours after processing for effective fermentation.

The objective of this study was to develop effective methods of preserving crab processing waste, to evaluate the effects of these chemicals on fermentation characteristics and nutrient utilization by ruminants, and to develop a procedure for determining chitin digestibility.

Chapter II

LITERATURE REVIEW

Disposal of seafood waste is becoming more costly, and government regulations are becoming stricter. In 1976, the resource conservation and recovery act was passed to encourage the recovery of valuable materials and energy from solid wastes through the cooperation of federal, state, local government, and private enterprises (EPA, 1980).

The crab industry generates three marketable products, namely, whole crab, crab meat and crab byproduct. Crab byproduct consists of whole shells, chitin, protein extracts, meat and the viscera (Mendehall, 1971).

Methods of Disposal/Utilization of Seafood Byproducts

Crab Meal Production. Production of crab meal through dehydration is technically feasible. The crab meal generally contains approximately 35% crude protein (Conley, 1980) and a high level of Ca (Patton et al., 1975). The production of crab meal, however, has been hampered by high energy cost and severe environmental regulation. Competition from other protein sources, variable demand and irregular supply of crab due to seasonal changes have led to flunctuating demand for crab meal (Coale, 1980).

Landfill Disposal. Dumping of crab processing waste in nearby landfills is another method of disposal. The waste is dumped in dug up trenches and the content is covered with dirt to minimise odor problem (Brinsfield, 1980). However, the risk of polluting ground water has been cited (Bough, 1977). Bough (1977) stated that the landfill sites of many coastal areas of the United States are filling up, but alternative sites are scarce due to high water tables and limited land availability.

Overboard Discharge. In the past, dumping of crab processing waste into the ocean was a common practice. However, enviromental restrictions placed upon highly putrescible substances such as crab processing waste have made this practice unsuccessful (Bough, 1977). Dumping of the waste back into the ocean required proper docking facilities, equipment, and grinding of the waste to avoid floating of particles (Olsen, 1980).

Direct Farm Application. Crab processing waste is high in organic material, thus its use by the farmers as a fertilizer source is promising. At present, crab processing waste is spread on the land, and plowed into the soil prior to the planting season (Brinsfield, 1980). Crab processing

waste added at 10 to 40 g per 3.5 kg of sand medium has been shown to restrict the excessive vegetative growth of tomato plant (Aung et al., 1980).

Composting. Composting of crab processing waste has been shown as one method of achieving a stable product that can be stored without any odor or possible health hazards (Swartz, 1980). Composting is a biological process involving aerobic or anaerobic condition. In aerobic condition, air which is supplied to the compost material enhances decomposition, and reduces unpleasant odor. Also, less available land is required, and the high temperature obtained eliminates flies and pathogenic organisms. At present, composting may not be the most cost effective method.

Protein and Chitin Extraction. Soluble protein and chitin can be extracted from crab processing waste. On a large scale, Fryer (1980) showed that 5 to 6% of total chitin and 7 to 10% of soluble protein can be extracted by alkaline and enzymatic hydrolyses of crab processing waste. The soluble protein may be used as liquid fertilizer (Aung et al., 1980) or as protein supplement for pets and livestock (Simpson, 1978). The chitin can be processed into chitosan which is used as a floculating agent in the clarification of sewage water, as an adhesive, paper sizing agent or as a chelating agent for metal ions (McGahren et al., 1984).

Nutritional Value of Shellfish Waste.

Crab processing waste is an excellent source of crude protein, which ranges from 40 to 45%, dry basis (Johnson and Peniston, 1971; Samuels et al., 1982; Abazinge et al., 1986). The levels of essential amino acids in crab processing waste are similar to that found in fish processing waste (Johnson and Peniston, 1971). Camien et al. (1951) reported that the soluble NPN components of crustacea muscle consist of amino acids such as alanine, glycine and proline, but also taurine, betaine and trimethylamine oxide. These NPN components comprise 17% of the total N (Suyama et al., 1965). The crab processing waste is low in fat (.8%) and very high in ash (41.6%) (Lubitz et al., 1943). The carbohydrate content was estimated to be 17%, which is three times higher than fish processing waste (Johnson and Peniston, 1971). The level of riboflavin in crab meal is 2.9 ug/g (Lubitz et al., 1943).

Brundage (1986) reported the concentration of Ca and P in Tanner crab meal as 14.02 and 1.71%, respectively, and the calculated metabolizable energy as 1.30 mcal/lb. The crustacean raw material has also been shown to be a rich source of carotenoid (astaxanthin).

The high level of Ca in shellfish waste has been indicated as the limiting factor to its use as animal feed (Lubitz et al., 1943; Parkhurst et al., 1944a; Lovell et al.,

1968). Lovell et al. (1968) suggested inclusion of not more than 10% of shellfish waste in the animal diet.

Lovell et al. (1968) indicated that chitin and its cationic deacetylated form, chitosan are indigestible and have no protein value for monogastric animals. However, studies have shown that ruminants have the ability to degrade chitin (Patton et al., 1975).

Nutritional Value for Ruminants. Patton et al. (1975) showed that feeding 20% crab meal to ruminants resulted in no significant reduction in body weight gain, feed intake or feed effeciency. In one trial, N retention was not altered by crab meal substitution, however, in the second trial, N retention was reduced. The decreased retention was attributed to excessive amounts of Ca, Na, Cl, and Br, which increased the urinary output of the steers. In another study, Patton et al. (1975) showed that supplementing a protein-defficient diet with 10% crab meal resulted in increased daily gain, DM intake and feed effeciency, compared to calves fed a control diet.

Brundage et al. (1981) compared addition of crab meal or soybean meal at 2% of the diet of lactating cows. There was no significant difference with milk production, but inconsistence weight gains were observed with cows fed the crab meal diet. Adding up to 30% tanner crab meal to a high-concentrate diet did not significantly affect the accepta-

bility of the diet to either steers or heifers (Brundage 1986). However, steers receiving 7.5 to 22.5% of tanner crab meal weighed 27.3 kg less, and those receiving 30% crab meal weighed 36.4 kg less than steers fed the control diet.

Samuels (1983) added 16% acetic acid to the mixtures of crab processing waste and straw (1.5:1 or 1:1.5, wet basis) in order to lower the pH to 4.5. When silage containing the higher proportion of crab processing waste was fed to sheep, increased DM and CP digestibility, and improved N utilization were observed. In a similar study, Abazinge et al. (1986) reported an increase in apparent digestibility of CP and DM when sheep were fed crab processing waste and straw mixtures which were ensiled with 20% dry molasses, compared to sheep receiving either the crab waste-straw silage treated with 16% acetic acid, or those fed urea-treated straw.

Chitin has been considered as a potential energy source for ruminants. Patton et al. (1975) reported chitin digestibility ranging from 26 to 87% in steers fed diets containing 20% crab meal. Hirano et al. (1984) estimated the apparent digestibilities of N-acetylchitosan and crustacean shells fed to cattle. When 7% crustacean shells were fed, the hexosamine content, a product of chitin degradation, increased in the feces from .56% for the control animals to .80% for the cattle fed the crustacean shells.

Nutritional Value for Aquatic Animals. Dean et al. (1982) reported that addition of 10% blue crab waste to the diet of fingerling catfish improved growth rate and feed effeciency. However, feeding up to 15% of crab waste meal for 150 d resulted in lowest weight gain, compared to commercial feed or finfish waste diet. Simpson (1978) showed that protein and pigments recovered from shrimp waste and crab meal can be used for salmonids. The weight gains were not different among the fish, but there was a larger quantity of astaxanthin isolated from those fish receiving the shrimp waste.

Meyers and Perkins (1977) reported that carotenoids in shell fish may be transfered from the feed to the flesh of trout and salmon, thus enhancing the flesh coloration of these marine animals. In a feeding experiment, 29% shrimp meal fed to brook trout resulted in superior coloring and flavor, compared to the fish receiving the commercial diet (Saito and Regier, 1970). Spinelli et al. (1974) showed that feeding 10 and 25% red crab meal to rainbow trouts resulted in desirable flesh pigmentation. Campbell (1973) fed crustacean meal to crustacean stocked in an intensive farming condition. The chitinous meal significantly reduced cannibalism, and increased growth rate.

Nutritional Value for Laboratory Animals. Lubitz et al. (1943) fed purified chitin to rats and found 24% of the N was

digested. Watkins et al. (1982) reported that minks fed crustacean meal had lower final weight gains, and greater feed consumption than control animals. These results were attributed to low fat and high calcium contents in the diet. High Ca diets have been shown to result in decreased growth, reduced N retention (Goto and Sawamura, 1973a) and reduced fat absorption (Yacowitz et al., 1967) in rats.

Gordon (1984) fed chitin, chitosan and cellulose to rats at levels of 2.5, 5,10 and 20%. Chitosan, fed at levels between 2.5 and 10%, resulted in unthrifty appearance and reduced growth rate. At a level of 20%, all the rats died of toxicity. Feeding of chitosan resulted in reduced serum cholesterol, decreased iron absorption and significantly lower hemoglobin concentration. Reduction in growth of rats was noticed when 15% chitosan was fed (Landes and Bough, 1976).

Available crude protein with rats fed crab meal was 84.5% and the biological value was estimated as 76%, compared to the value of 100% for casein (Lubitz et al., 1943).

Nutritional Value for Poultry. Parkhurst et al. (1944b) reported that feeding of crab meal to chickens resulted in significantly less growth, compared to those receiving fish meal. Inclusion of crab meal gave more pigmentation, and induced no off flavors. Spreen et al. (1984) reported a change in the intestinal microflora of chickens fed chitin with or without whey and bacterial

inoculum. The bifidobacteria, isolated from the intestine of these chickens, had been shown to improve the utilization of whey in non-ruminants (Gyorgy et al., 1954; Poupard et al., 1973).

The digestibility of chitin in both the purified and natural form has been estimated in feeding experiments with mice, Japanese nightingales and chicken (Jeuniaux et al., 1978). The digestibility ranged from 19 to 58% in mice and chickens fed pure chitin and in Japanese nightingales fed meal worm larvae. Hirano et al. (1984) reported apparent digestibilities of 78 to 92 % for N-acetylchitosan and crustacean shells fed to chickens.

Chitin in Shell Fish Waste

Chitin is a colorless, amorphous compound which is insoluble in water, dilute acid, alkali, alcohol, and organic solvents (Hackman, 1962). However, it is soluble in concentrated mineral acids where it undergoes rapid and extensive degradation. Chitin is a major component of crab exoskeleton. It is a poly-N-acetylglucosamine which is analogous to cellulose, except the hydroxyl on the C-2 position of cellulose is replaced by a N-acetylamido group on the chitin molecule (White et al.,1968). The general structure of chitin is presented in figure 1. The chitin content of crab meal has been estimated to range from 12.7 to 13.6% (Lubitz et al., 1943; Welinder, 1974; Stelmock et al., 1985).

Rudall (1955) reported that chitin exists in three different forms (α,β, and γ), and each of these forms exhibit different functions. The α-chitin, the most stable form (Rudall,1965), is commonly found in crustacea, bound with protein and mineral deposits (Dennell 1960; Rudall and Kenchington 1973). The most abundant mineral, $CaCO_3$ is found in a complex with chitin where it serves as the cementing substance for crustacea (Lovell et al., 1968).

Commercial Utilization of Chitin and Chitosan.

Chitin and its deacetylated form (chitosan) have the same properties, and are comparable to cellulose or collagen (Perceval, 1978). Several studies have revealed the potentials of chitin, and chitosan and related enzymes in animal nutrition, biochemistry, medicine, pharmacology, enzymology, microbiology and general agriculture (Brine, 1984).

Chitosan has several desirable characteristics which make it unique and versatile. In addition to its hydrophylic property, chitosan possesses other features due to its amino groups, basicity, high capacity for transitional metal ions (Muzzarelli, 1978; Madhavan et al., 1978), fast binding rate, and flow rate for solutions penetrating through chitosan columns (Muzzarelli et al., 1969), buffering capacity for hydrogen ions and negligible swelling (Muzzarelli, 1978).

Chitin, due to its ability to dissolve in acid, and its renaturation into highly crystalline orientable films, be-

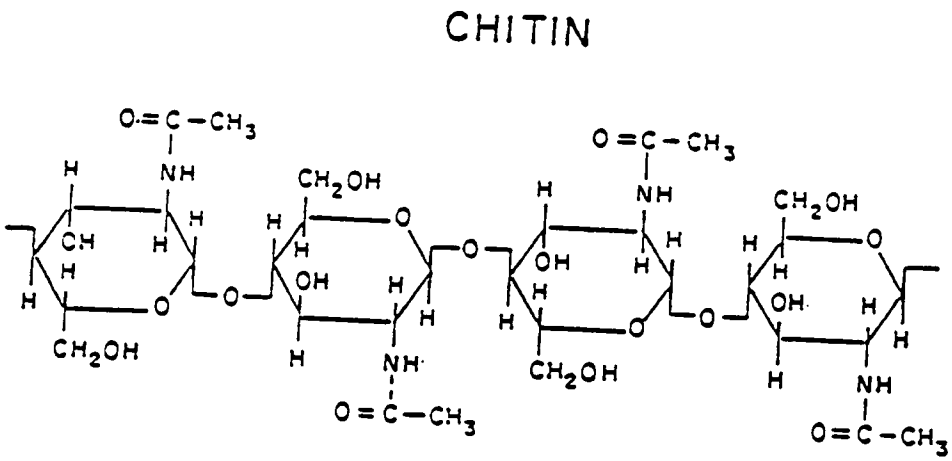

Figure 1. General Structure of Chitin
Adapted From Hackman (1973)

comes a suitable fiber for surgical suture and wound covering (Brine, 1984). Partially depolymerized and deacetylated chitin has been shown to accelerate the process of wound healing (Balassa et al., 1978), and is effective in the promotion of lactose digestion in animals (Austin et al., 1981; Austin et al., 1982; Zikakis et al., 1982). Bough et al. (1975) showed the effectiveness of chitosan to flocculate proteinaceous waste (egg breaking waste, brewery and cheese whey solids) from effluent water streams. In a study by Allan et al. (1978), the use of chitosan in the pulping process inhibited the disruptive action of water on paper cohesiveness.

Muzzarelli (1978) showed chitosan to be a reactive polymer, susceptible to a host of chemical modifications. Since then, several modified forms of chitosan have been produced. Glucosamine hydrochloride, a derivative form of chitin, has been shown to be an effective alternate to corticosteroid in the treatment of enteritis and colisitis (Prudden et al., 1970). Acetylated glucosamine was shown to be an effective antitumor agent (Bernacki et al., 1977), while D-glucosamine was found to be cytotoxic to malignant cells in vivo (Freidman et al., 1980), and effective against human influenza virus (Floch et al., 1976; Freidman et al., 1979).

Chitosan has been used as soil additive, seed treatment and in foliar spray to inhibit fungal growth and enhance host resistance to diseases (Hadwiger et al., 1984).

Methods for Determining Chitin

Several methods have been documented for quantifing chitin degradation and synthesis. Crude fiber analysis has been used extensively in the quantification of chitin (Lubitz et al.,1943; Black et al.,1950; Lovell et al.,1968). These authors concluded that this method gave a reliable estimate of chitin content of shellfish meal. Stelmock et al. (1985) postulated that since chitin had a similar molecular structure as cellulose, it can be treated as the fibrous component of the shellfish. In their study, the ADF method was used to determine the chitin content of the dungness crab meal. A value of 13.6% was reported. However, this assay can be used only to quantify chitin from samples of fresh tissue or scrubbed shell particles.

Chitin has also been quantified gravimetrically by weighing the residue remaining after the sample had been repeatedly digested with hot dilute aqueous alkali (Welinder, 1974). Harsh chemical treatments are usually required to remove $CaCO_3$ and protein from raw chitinous material (Richards, 1978). In crustaceans, $CaCO_3$ serves as the cementing substances and account for 18% of the Ca of the crustacean meals (Lovell et al., 1968).

Disodium ethylenediaminetetraacetic acid (EDTA), acetic acid, trichloroacetic acid (TCA) and HCl are commonly used for decalcification (Welinder, 1974; Andersen, 1978), while NaOH is used as a deproteinizing agent (Rutledge, 1971). Brown (1959) and Rutledge (1971) hydrolyzed shellfish samples with HCl or NaOH on a steam bath for 1 h. Lovell et al. (1968) compared the use of 90% formic acid, 1N HCL and 5% cold HCL. The demineralized materials were subjected to refluxing in 5% NaOH for 90 min. Clark and Smith (1936) removed the $CaCO_3$ with cold dilute HNO_3 and hydrolyzed the protein with boiling aqueous NaOH for 4 h. Franekel and Rudall (1940) used pepsin digestion to remove the protein.

Quantitative estimation of chitin, however, is difficult. Hackman et al.(1974) reported that some of the chitin is degraded during chemical treatment, while losses due to transfer of residue from one container to the other, and presence of impurity in the final product may affect the result.

Chitin can be treated with acids or alkali at high temperature. This resulted in the production of lower saccharides, glucosamine and acetic acid (Hackman, 1962). Glucosamine or hexosamine units can be quantified colorimetrically (Wieckowska, 1968). However, this procedure is not specific for chitin because other polysaccharides yield glucosamine on hydrolysis (Hackman et al., 1971; Richards, 1978). Approximately 1 g of purified chitin would

yield .22 g of acetate (Patton et al., 1975). This acetate can be quantified by gas-liquid chromatograph (Holan et al., 1971). The amount of chitosan produced is relative to the chitin present. Chitosan is subjected to color test (iodine) or spherocrystal formation (with acids) to determine the levels of chitin present (Campbell, 1929; Richards and Cutkomp, 1946; Prakasam and Azariah, 1975).

Other methods involve the use of X-ray diffraction technique, and the fluorescent conjugation of chitinase as stain for chitin. However, in order to use the X-ray method, the chitin has to be extracted and free of any contamination, while with the staining method, chitin is not necessarily accessible to chitinase (Benjaminson, 1969; Hackman et al., 1971).

Effects of Hydrolyzing Enzyme on Chitin Degradation

Generally, chitin is found in either free or bound form, and in association with protein. Free chitin in the presence of protein is readily hydrolyzed by enzymatic reaction, while chitin in a bound form can only be hydrolyzed after the protein has been removed (Jeuniaux 1963). Hackman (1960) has reported that protein is covalently attached to chitin to form glycoproteins. Certain chitinous membranes in the presence of protein molecules showed resistance to the attack of chitinase enzyme (Hackman, 1960). Jeuniaux (1959) reported that some chitinous membranes in a crustacean cuticle can be

degraded only by chitinase enzyme after alkaline digestion, which removed the protein molecules.

The chitinase system, namely, chitinolytic, chitinoclastic and chitosanolytic enzymes, are widespread in bacteria and fungi (Monaghan, 1973; Price et al., 1975; Berkeley,1978). Hackman (1960) showed that strips cut from the pro-ostracum of Sepia officianalis contained about 49% chitin. About 85% of this chitin is digestible by chitinase after extensive colonization and rapid degradation (Jeuniaux, 1968). However, under the same condition, no chitinase enzyme was noted with crab waste containing 75% chitin, in which 53% of the chitin was in a bound form with glycoprotein and other complex molecules.

Failure to obtain significant results from in vitro incubation of chitin material with the rumen fluid of cattle fed a diet containing no chitin sources was attributed to lack of chitinase enzyme (Patton et al., 1975). In a feeding trial, Patton et al. (1975) observed an increase in digestibility of chitin with steers fed 20% crab meal, after the steers were allowed 14 d to adapt to the chitin containing diet.

Jeuniaux et al. (1978) showed secretion of chitinase by gastric or pancreatic cells of animals that were adapted to chitin-containing diets. Berger et al. (1957) reported that chitinase randomly cleaves chitin, and the main product is chitobiose. N-acetyglucosamine can also be formed by the

action of chitinase, but at a very slow rate and in small quantities (Ohtakara et al., 1978). Chitinase did not attack chitobiose (Ohtakara, 1963) but chitobiase hydrolyzed this material into N-acetylglucosamine.

Insectivorous and omnivorous species secrete chitinases in their digestive tract, while carnivorous and herbivorous species only selectively secrete chitinase in the gastric mucosa (Frankignoul et al., 1965; Cornelius et al.,1976). Chitinase is secreted by the gastric mucosa only in insectivorous birds (chicken), however, chitinase enzyme was not detected in birds (pigeon and parrot) that were strictly adapted to grain (Jeuniaux et al., 1978). Jeuniaux et al.(1978) found no chitinase in the stomach or pancrease of sheep but reported pancreatic secretion of chitinase by the pig.

Techniques for Measuring Nutrient Degradation

In Vitro Method. The in vitro method can be very useful, at least as a mean of determining the relative digestibility of materials. The procedure of Tilley and Terry (1963) has been the method of choice in the determination of in vitro DM digestibility of feedstuff. Brown et al. (1987) used the first stage of Tilley and Terry (1963) procedure to determine in vitro fiber disappearance of limpograss hay. In this study, the in vitro fiber disappearance was determined

by refluxing the residue remaining after 4, 8, 12, 24, 48, 72, and 96 h incubation.

Patton et al. (1975) reported in vitro degradation of purified chitin incubated with rumen fluid for 3 h at 37 C. The chitin degradation, as quantified by acetate recovery, showed that chitin was degraded to a lesser extent, however, in the tubes containing rumen fluid but no chitin (control), greater amount of acetate was recovered, giving a false impression that chitin degradation occured. The authors attributed this result to lack of microbial adaptation, insensitivity of the assay used, and poor microbe to substrate ratio. Van Dyne (1962) reported that the type of diet consumed by an inoculum donor animal will affect in vitro digestibility of feed.

Nylon Bag (In Situ) Technique. The use of nylon bag technique for measuring the in vivo DM and nutrient disappearance has been well documented (Mehrez and Orskov, 1977; Nocek, 1985). The bags are used at a measurement and pore size that closely measured the rate of nutrient disappearance. Larger bags with bigger pore sizes are used to measure disappearance of fiber materials, compared to the smaller bags and pore sizes used for the concentrates (Nocek, 1985). Some factors affecting the in situ degradation of chitin include available organic matter, colonization or affinity of

bacteria to the substrate, particle size, and the initial concentration of chitin (Hood et al., 1978).

Hood et al. (1978) found that in situ degradation of chitin in an estuarine enviroment favored the untreated chitin while pure chitin, acid treated chitin, and molted chitin were degraded at a slower rate. Maximal degradation was observed in late summer when the average temperature was 30 C, and lowest degradation was obtained in mid-winter when the average temperature was 8 C. Patton et al. (1975) reported a DM disappearance of 42% for crab meal and 21.4% for purified chitin, incubated in the rumen for 48 h.

Neathery (1968) showed in his preliminary study that reliable results could be obtained between nylon bag technique and digestion trials with sheep, if a good quality roughage is fed to the animals designated for the in situ study. Weakley et al. (1983) reported a decrease in the in situ disappearance of DM and N in bags from cows fed a high concentrate, and low-hay diet. The authors attributed this to a large quantity of bacterial slime found in the rumen of concentrate-fed animals. This slime could block the bag pores, thus restricting the influx of digesting agents. Ganev et al. (1979) reported an increased cellulolytic activity in the rumen of forage fed animals which resulted in an increased protein digestibility.

Neathery (1968) compared alfalfa-orchardgrass to bermuda grass hay. The DM disappearance in cannulated steers

fed bermuda grass hay was higher than those on alfalfa-orchard grass hay. The author theorerized that bermuda grass hay may have encourage the increase in number and species of microorganisms that were specific for cellulose digestion, compared to steers fed alfalfa-orchard grass. Ohtakara et al. (1978) reported that addition of .2% glucose to a medium containing chitinase producing microbes increases production of chitinase, probably by stimulating the growth of the organism. However, an excess amount of glucose resulted in a rapid decrease in pH and enzyme production.

The homogeneity of the test diet used in the in situ study is very important. Mehrez et al. (1976) found a low percentage of DM disappearance with the bags containing whole grains, compared to the high percentage of DM disappearance when barley grain was incubated in a finer form. Disappearance of DM and N was greater in the in situ bags containing very fine or pulverized samples (Figroid et al., 1972). The decrease in particle size and increasing the surface area per unit weight of substrate allows increasing exposure of the substrate to the microbial attack in the bags. Particle sizes of 2 mm for protein and energy supplements and of 5 mm for whole cereal grains and fibrous products were suggested to represent the probable particle size entering the rumen (Nocek, 1985).

Ohtakara et al. (1978) found that chitinase activity increases with increase in the fineness of the incubated

chitin particles. Clumping of a small particle size substrate, however, has been observed with particles less than 6 mm (Figroid et al., 1972).

The effects of bag and pore sizes were examined by Mehrez and Orskov (1977). They reported that a bag size of 17x9 cm and pore size of about 1936 holes/cm^2 gave a reliable disappearance of DM compared to bag sizes of 25x15 cm or 8x5 cm. In a similar study, Rodriques (1968a) showed that pore sizes ranging from 1680 to 2550 holes/cm^2 gave similar values for DM disappearance. Uden et al. (1974) incubated guinea grass in the rumen of steers and found that a critical ratio exists between the sample size and the bag size. There was an increased digestibility with samples incubated in bags with pore size of 53 um, compared to the samples incubated in bags with pore sizes of 35 and 20 um. Increasing the sample size from 6.5 to 50 mg/cm^2 bag surface decreased cell wall digestibility. The authors suggested a bag size of 6 x 12 cm for a .5 g sample. Using a smaller bag size (5 x 8 cm) for a large sample (4.3 g D.M) resulted in decrease DM disappearance and variability between bags (Van Keuren et al., 1962; Rodriques 1968a. Mehrez and Orskov (1977) increased the bag size from 8 x 5 cm to 17 x 9 cm, and found an increase in the proportion of dry matter disappearance from 37.5 to 85%, and significant reduction in variability between bags. They concluded that large bag sizes allowed complete mixing and re-

moval of the end products of the incubated materials in the rumen.

Highfill et al. (1987) used a 8 x 13 cm nylon bag with the pore size of 42 um to measure DM disappearance of fescue hay or fescue hay/concentrate combination ground through a 1 mm mesh screen. Weakley et al. (1983) reported decreased N and DM disappearances when rip-stop nylon bags (acropore) with pore size of 5 um were used. However, using the dacron bags (6 x 10 cm) with pore size of 52 um resulted in greater disappearances of DM and N. Uden et al. (1974) also reported greater digestibility of guinea grass from nylon bags with 53 um pore sizes, compared to bags with 20 or 35 um porosity. The porosity of bags influences the influx of digesta and the microbes and the efflux of the digested residues (Weakley et al., 1983). A bag porosity of 40 to 60 um is commonly used, however, increasing the porosity of bags enhances the rate of degradation and microbial activity (Weakley et al., 1983; Nocek,1985). Mehrez et al. (1976) found no significant underestimation of the DM disappearance due to the influx of rumen contents into the bags.

Miles (1951) showed large variations in digestibility of feed incubated at different locations in the rumen. In similar studies, attachment of weights to the bags suspended in the ventral sac of the rumen, maintained the stability of the bags, and improved the DM disappearance. Balch and Johnson (1950) and Miles (1951) observed a rapid and precise

digestion in the ventral sac of the rumen. Rodriques (1968b) reported less variation in DM disappearance when the bags were attached to 50 cm rather than 30 cm strings. He suggested that the longer string allowed greater movement of the bags within the rumen of the steers. Poos-Floyd et al. (1985) suggested that the bags containing the samples should be soaked in warm water for 10 min to prevent floating in the rumen, and to eliminate lag time associated with hydration of dry feed particles prior to microbial attack. Mehrez et al. (1976) soaked the bags in H_2O for about 1 min before suspension in the rumen.

Large variations in DM disappearance were obtained between animals and bags when incubation time ranged from 1 to 24 h (Rodriques 1968a; Mehrez et al., 1976). Neathery (1968) found a close relationship between the values of DM disappearance obtained after 72 h incubation, and the values from conventional digestion trials.

After incubation, bags are removed from the rumen and washed thoroughly under running tap water until the rinsing water is colorless (Mehrez et al., 1976; Poos-Floyd et al.,1985). Crawford et al. (1978) suggested rinsing of the bag under tap water to remove ruminal materials from outside of the bags. This was followed by successive washings (3 times) in distilled water until the water squeezed out of the bag was colorless. Washing of the bags after incubation has been compared to instant drying and subsequent washing in

order to determine the dry matter loss (Mehrez et al., 1976; Van Keuren and Heineman 1962). These authors did not find significant losses in the DM due to direct washing of the bags.

The Use of Chemicals as Preservatives.

The effectiveness of an acid depends on the dissociation constant (pka) or the pH at which 50% of the total acid is dissociated (Doores, 1983). At this pH, which ranges from 3 to 5, the undissociated portion of the molecule is believed to be responsible for the antimicrobial effect (Raa et al., 1983). Thus, the use of organic and mineral acids in the preservation of feed materials, and agricultural and animal by-products became well established.

Alkali salt had been used in various ways to improve the nutritive value of plants and residues (Klopfenstein, 1978). However, little has been documented about the effectiveness of alkali salt in the preservation of agricultural by-products. Unlike acids, the antimicrobial effects of alkali can be achieved by their high buffer action and elevated pH (Greenhalgh et al., 1978). Greenhalgh et al. (1978) outlined the factors that may be contributing to the bactericidal effects of the alkali salt. These factors could be the direct effect of the Na^+ on the microorganisms, the soluble carbohydrate content of the feed, the water content and the osmotic pressure of the feed, or the degree of anaerobic

condition achieved in the silo. There is also a possibility that the increase in temperature following alkali treatment may be sufficient to kill some microorgarnisms.

Propionic and Formic acids. Propionic and formic acids have been used extensively in the ensiling processes of plant products (Wing et al., 1976; Stallings et al., 1981) and in the preservation of wet brewers grains (Allen et al., 1975). The chemicals are effective in maintaining shelf life of milk products (Muller et al., 1975; Rindsig et al., 1977). Their effectiveness in eliminating salmonella, total coliforms, fungi and aerobic bacteria in poultry waste has been reported (Evans et al., 1978b; Narasimhalu 1981).

Ensiling of chopped fresh Lolium multiforum with formic or propionic acid (.3 liter/100 kg) resulted in effective fermentation. The use of formic acid showed less organic matter loss, compared to propionic and untreated silages (Andrighetto et al., 1987). Michelena and Molina (1987) added .3, .6 and 1% propionic acid to an ensiled mixture of King grass. There was a reduction in pH, total VFA, NH_3-N, and an increase in lactic acid concentration.

The use of formic acid and commercial inoculant in treating grass silage with low DM (16%) and high water soluble carbohydrate (15%) has been reported by Haigh et al. (1987). These additives significantly decreased silage pH and NH_3-N. There was an increase in the residual soluble

carbohydrate with the above additives, when compared with the inoculant or untreated silages alone. These additives were also effective against clostridial fermentation. Muller et al. (1985) treated a livestock feed with .2% propionic acid, followed by inoculating the feed with strains of Penicillium bacteria. Onset of fungal growth was delayed.

Treating fish waste with formic acid produced a pH between 3.5 and 4 (Wignall and Tatterson, 1976) while propionic acid maintained a pH of about 4.5 (Gildberg and Raa, 1977). To achieve effective preservation of seafood by-products, a large amount of acid is needed to dilute the buffering effect of ash and protein content of many marine by-products (Kompiang et al., 1980).

Gilberg and Raa (1977) successfully preserved fish silage with propionic and formic acids. At a concentration of .75%, a stable pH of 4.3 and an increase in water soluble carbohydrates were observed. Addition of 3% (w/w) of 98% formic acid to minced cod viscera yielded a fairly stable silage with a low microbial count (Backhoff, 1976). The use of 1.5% formic acid in preserving silage of cod viscera resulted in decreased soluble carbohydrates, an increased pH and unpleasant odors of amines, with patches of mold growth (Gilberg and Raa, 1977). A mixture of propionic and formic acid at a concentration of 1.5 to 2% of both produced ensiled material with a pH of about 4.4 after 24 h. At this concentration, mold formation was prevented (Raa et al., 1983). The

use of 1.5% propionic/formic acid mixture in preserving crab waste has been investigated in our laboratory (Abazinge et al., 1986).

Acetic Acid. Acetic acid is a lipophilic antimicrobial agent which is considered to be more effective in limiting yeast and bacterial growth (Doores, 1983). This action is due to the lowering of pH below the optimum levels for bacterial growth (Freese et al., 1973).

Levine and Fellers (1939) showed that acetic acid has a broad range of inhibiting microorganisms. At a lower concentration of the acid, bacillus, salmonella, and staphylococcus species were more inhibited than saccharomyces and aspergillus. Owen (1946) showed that acetic acid effectively select against gram-positive organisms. Woolford (1975b) confirmed this report when acetic acid used to attain pH of 4, 5 or 6 showed higher resistant against the heterolactics than homolactics. Bacillii and gram-negative bacteria were more inhibited than lactic acid bacteria, yeast, molds, clostridia, and gram-positive bacteria. However, when the pH was lowered to 4, the latter five groups were similarly affected.

Kirby et al. (1937) reported that acetic acid at pH of 3.5 was effective against bread molds, Aspergilus niger, and Rhizopus nigrificans. Aspergillus fumigatus was inhibited with .2% acetic acid at pH of 5.0 or below. Pseudomonas aeruginosa was found to be sensitive to a 1% concentration

of acetic acid (Hedberg and Miller, 1969). A 1% concentration of acetic acid at pH of 4.5 completely inhibited growth and production of aflatoxin by Aspergillus parasiticus. Cruess and Irish (1932) showed that at pH of 3.5, .8 to 1.0% of acetic acid was required to inhibit Saccharomyces ellipsodeus and Penicillium glaucum. However, at pH of 7.0, a concentration greater than 4% was needed to achieve the same effect.

Mountney and O'Malley (1965) and Minor and Marth (1970) showed that acetic acid was a more effective antimicrobial agent than lactic, citric, fumaric, adipic and succinic acids. Vicini et al. (1983) reported that treating soybean meal with acetic acid prevented ruminal degradation of DM and CP, compared to the control treatment. Waltz and Loerch (1986) reported a reduction in nitrogen solubility and in vitro NH_3-N accumulation when 2.5 or 5% acetic acid was used in treating soybean meal.

Hydrogen Peroxide. Hydrogen peroxide is an oxidizing agent which has strong bactericidal properties (Branen, 1983). Foster et al. (1957) showed that when .2% of 30 to 35% H_2O_2 solution was added to milk preserved at 49 C for 10 to 15 min, 85% of the total bacterial flora in the milk were destroyed. Satta et al. (1947) determined the percent reduction of the bacterial count in milk treated with .20, .25 and .30% H_2O_2 at different temperatures. After 20 h, bac-

terial population was reduced by over 98% at 15 to 22 C, and 99.96% reduction was achieved at 32 C.

Nambudripad et al. (1952) reported that .2% of a 35% H_2O_2 solution used for 30 min at 49 C reduced the bacteria in treated raw milk from 304,280 counts/ml to 1090 counts/ml compared to 11,485 counts/ml obtained with pasteurized milk. Similar results were obtained with the reduction in counts of coliforms and aerobic sporeformers, while anerobic sporeformers were completely destroyed in the peroxide-treated milk.

Lipma and Owen (1943) showed that H_2O_2 inhibited strict and facultative anaerobic bacteria which were devoid of catalase. Bacteria which produce catalase are more resistant to destruction by H_2O_2 than coliform organisms. Roushdy (1959) determined survival periods of Streptococcus lactis, P. aeruginosa, S. aureus, and B. subtilis in milk treated with .05% H_2O_2, and stored at 30 C. Survival periods for the organisms were 2, 1, 1, and 3 h, respectively. These survival periods were reduced to 1, 1, .5 and 2 h when .075% H_2O_2 was used.

EL-Gerdy et al. (1980) showed that addition of .02% of H_2O_2 to the milk inhibited the growth and gas production by several species of clostridia. Von Ruden et al. (1967) and Walker and Harmon (1965) reported that addition of .05% H_2O_2 to milk heated to 49 C resulted in 99% decrease in staphylococci population. Heat application inactivates the

indigenous catalase in the milk and increases the activity of peroxide (Roundy 1958). Roundy (1958) concluded that H_2O_2 has a selective action on bacteria. Aerobic sporeforming organisms are most resistant to destruction by H_2O_2, coliforms are least, and lactic acid organisms are intermediate. Residual H_2O_2 may also inhibit the starter cultures.

Subramanian and Olson (1968) reported that a single strain of lactic streptococci and commercial lactic starter cultures can be inhibited by H_2O_2 at a concentration as low as 5 ug/ml.

Hydrogen peroxide can be produced naturally in dairy products through the action of lactobacilli (Gilliland, 1969). Gilliland and Speak (1975) reported that freshly picked crab meat was protected from psychrotrophs through the peroxide production of Lactobaccilus bulgaricus.

Hypochlorite. Chlorine compounds have been recognized as highly active antimicrobial agents with sporocidal properties (Dychdala, 1977a). Most of the studies with chlorine are connected with water treatment in which chlorination has been an effective method of preventing water-borne diseases. The oxidizing properties of chlorine compounds inactivate certain enzymes necessary for glucose utilization in bacteria, thereby causing their destruction (Green and Stumpf, 1946).

Mercer (1953) reported that the undissociated hypochlorus acid (HOCl) produced when chlorine is added to

water is considered to be the germicidal agent. This has been shown when death rate of bacteria is directly proportional to the concentration of the undissociated HOCl.

The germicidal properties of chlorine compounds can be influenced by the chlorine concentration, pH of the solution, temperature and level and type of organic matter (Dychdala, 1977a). Studies have shown that the germicidal effect of chlorine increases as the level added to the water increased (Zottola, 1973). Chlorine at a level of 1.2 ppm achieved the bactericidal effect within 10 min, while at a low level of chlorine, a greater portion of the germicidal properties of chlorine may be neutralized in the side reactions (Dychdala, 1977a).

Charlton and Levine (1937) reported that the pH was the most important single factor influencing the germicidal effect of hypochlorite solutions. The hydrogen ion concentration determines the fraction of the HOCl present as the undissociated molecule or as hypochlorite ion. As pH increases, the ionization of HOCl increases (Charlton and Levine, 1937). The stability of free available chlorine depends on the pH of the solution. As the pH becomes more alkaline, the concentration of available chlorine is reduced. Zottola (1973) reported that chlorine compounds are most effective at pH between 7.5 and 9.5. Rudolph and Levine (1941) showed that calcium hypochlorite (CaOCl) (1000 ppm) at pH of 7.3 took less than .33 min. to achieve 99% destruction of

Bacillus metiens spores while at pH of 11.3, 99% of the destuction was achieved in 70 min. Ito et al. (1968) indicated that as pH increases, the rate of bacteria destruction by CaOCl decreased.

Temperature has been shown to be a factor that affects the germicidal effect of chlorine compounds. Ito et al. (1968) showed that the time for 99.99% destruction of all types of Clostridium botulinum decreased with increasing temperature. However, a NaOCl solution held at 55 C for 3 h showed no appreciable loss of available chlorine.

The germicidal effect of chlorine compounds is greatly reduced in the presence of organic matter (Dychdala, 1977a). However, organic materials such as sugars and starches do not affect the germicidal activity of the chlorine compounds but peptone has been shown to combine with the free available chlorine, thus reducing its effectiveness (Lechowich, 1981). Calcium hypochlorite and NaOCl lose chlorine upon storage, high moisture or heat and light exposure, and there is tendency for hypochlorite resulting in increased salt deposition when added to hard water, thus reducing its germicidal effect (Branen, 1983).

Narasimhalu et al. (1981) reported that NaOCl reduced viable aerobic and coliforms numbers in caged layer and broiler excreta.

Sodium Hydroxide. It was shown that alkali treatment helps in the preserving of straw (Greenhalg et al., 1978).

Some microorganisms are known to be tolerant of high pH (Lie and March, 1968; Ohata et al., 1975). Ohata et al. (1975) reported that certain types of bacilli showed optimal growth at pH of 9 to 11. Lie and March (1968) found that Aspergillus flavus and A. parasiticus could grow and form aflatoxin in a casein substrate adjusted to pH of 9.3 and 9.9, respectively. Alkali treated materials do not always maintain a pH greater than 9.5, hence, satisfactory preservation does not depend on high pH.

Oji et al. (1977) ensiled maize stover treated with 20 kg NaOH/ton DM. The pH was reduced to 6.8 and no molds were detected on the silage. Molina et al. (1983) reported that ensiling of sugarcane bagasse treated with 2, 4 and 6% NaOH inhibited the development of bacteria and fungi. This inhibitory effect is suggested to be due to the reduced soluble carbohydrate content of the bagasse and the elevated pH. A subsequent exposure of the silage to air for 10 d showed a decline in total microbial counts, particularly fungi, compared to the untreated control. Bolsen et al. (1983) added NaOH at 50 g/kg DM to wheat and barley ensiled at low, medium, and high moisture levels for 60 d. Treatment with NaOH resulted in butyric acid production at low DM, and restricted fermentation at high DM levels. At medium and high DM, NaOH did not produce any butyric acid. The NH_3-N was low, and the insoluble N, expressed as percent of total N, was higher.

The pH remained above 9 at both medium and high DM levels of the ensiled materials, indicating a restricted fermentation.

Flipot et al. (1976) reported that the addition of 2, 3 or 4% of NaOH to corn plant or stover ensiled for 50 d resulted in high pH and increased lactic acid production. Total N of NaOH-treated silages tended to decrease. However, the alkali significantly reduced soluble and NH_3-N. Ensiling of chopped ryegrass straw with 4.5% NaOH (w/w) for 60 d showed a pH of 5.1, compared to 3.9 for the untreated straw. Overall production of acetic, propionic and butyric acids was higher for the treated silage, but lactic acid production favored the untreated straw (Shultz et al., 1974).

Wilkinson et al. (1978) reported that the use of NaOH at 1.05 to 10.0 g/100 g DM of the straw resulted in well-preserved silages. The pH of ensiled materials increased and was correlated with high residual water soluble-carbohydrates, and low lactic acid production in the treated silages. However, acetate production increased with addition of NaOH. This may suggest hydrolytic liberation of acetic acid from the acetyl groups attached to xylan chains in the polysaccharide units of the cell walls. Low levels of lactate and butyrate of the treated straw indicated some restricted bacterial fermentation. Upon exposure of the materials to air, total viable microorganisms increased progressively. Greenhalgh et al. (1978) treated barley straw with a 16% solution of NaOH/ton DM. The materials were aerobically or

anaerobically stored for 1 yr. There was an initial increase in temperature and pH, which later decreased. At 12 wk of storage, there were higher counts of mesophilic aerobic bacteria and fungi in the water-treated control, than the alkali treated straw.

Nitrite. Nitrite is known to have antimicrobial effects against a broad range of bacteria (Tanner et al., 1934; Tarr, 1941). The most important antimicrobial effect of nitrite is its action against the putrefactive and pathogenic clostridia (Tompkin et al., 1979a). Duncan and Foster (1968b) and Pivnick (1970) reported that nitrite at the minimum level inhibits the growth of spores rather than their germination. Benedict (1980) stated several possible mechanisms by which the antimicrobial activity of nitrite is achieved. He suggested that nitrite reacts with other components during heating to form an inhibitory substance. Also, nitrite may act as either an oxidant or reductant on cellular sites such as enzymes, enzyme cofactors, nuclei acids and cellular membranes. Nitrite may also react with cellular Fe, thereby interfering with the metabolism and repair mechanisms of the bacteria. Reaction of nitrite with thiols to form nitrosothiols is possible. This compound reacts with a spore membrane component thus inhibiting the metabolic or transport mechanisms of the bacteria.

Tompkin et al. (1979a) suggested that nitrite possibly reacts with the Fe in ferredoxin, thereby, rendering the

cofactor in the electron transport system of the bacteria inactive. Woods et al. (1981) reported that nitrite inhibited the growth of Clostridium sporogenes by inhibiting the phosphoroclastic system. Nitrite could also react with thiols and unsaturated lipids to form inhibitory oxidants (Tompkin et al., 1979a). Rowe et al. (1979) demonstrated that nitrite inhibits active transport, oxygen uptake, and oxidative phosphorylation of Pseudomonas aeruginosa, possibly by oxidizing ferrous ion of an electron carrier (cytochrome oxidase) to the ferric form.

Roberts et al. (1981a,b) reported that efficiency of nitrite is correlated to its undissociated HNO_2 concentration. A decrease in pH from 7 and up to 5 resulted in a five to ten fold decrease in the concentration of nitrite needed for an inhibitory action. Reducing pH from 7 to 5.5 lowered the salt tolerance of C. botulinum vegetative cells (Baird-Parker and Freame, 1967). Castellani and Niven (1955) reported that sensitivity of Staphylococcus aureus to nitrite increased as pH was reduced from 6.9 to 5.5. Henry et al. (1954) demonstrated that at pH of 7.5 or above, nitrite enhanced bacterial growth in curing brine. A pH of 5.6 to 5.8 was optimal for antimicrobial efficiency. At pH of 5.3 or below, nitrite rapidly disappeared and was ineffective.

Roberts et al. (1976) reported that the number of toxic samples due to bacterial growth was lower in materials incubated at 15 C than those incubated at 17 C. The optimum tem-

perature for growth of C. botulinum is 37 C; cell multiplication decreases with each reduction in temperature (Roberts et al., 1976). Christiansen et al. (1974) has shown a rapid depletion in level of residual nitrite when the treated product was subjected to a higher temperature (27 C) than when refrigerated.

Tucker (1930) reported that proteolytic activity of C. putrificum was inhibited when 12000 ug/g $NaNO_2$ was used. Tanner and Evans (1934) reported that 5900 ug/g of $NaNO_2$ inhibited 9 of 12 strains of clostridia in nutrient broth and all 12 strains in dextrose broth. Jensen and Hess (1941) showed that nitrite alone is effective in inhibiting the anaerobic putrefaction of canned ham for 30 d at temperature of 37.2 C. Tarr (1940, 1941) demonstrated the effectiveness of nitrite on stored fish muscle. The chemical delayed spoilage, and shelf life was extended when pH was reduced from 7.0 to 5.7. Two strains of Salmonella enteritis cultured in nutrient broth were used to study the effect of nitrite and NaCl on bacteria growth at 22 C. At a low pH of 5.0, 5% NaCl and 100 ug/g of $NaNO_2$ resulted in 30% reduction in growth of these bacteria. At a pH of 7.0, and 5% NaCl, nitrite was ineffective (Akman and Park 1974).

Duncan and Foster (1968b) reported that nitrite at a minimum level of 600 ug/g, and at a pH of 6.0 will allow emergence and elongation of vegetative and putrefactive anaerobe cells, but the cell division is inhibited. Thus,

these newly emerged cells are lysed. Nitrite at a level of 40 mg/g did not prevent germination or swelling of spores. However, nitrite at a level of 6% prevented germination of spores (Duncan and Foster 1968b).

Nurmi and Turunen (1970) studied the effect of adding nitrite to a previously autoclaved broth medium (pH 6.0). Lactobacilli (78 strains), micrococci and staphylococci (24 strains), and Pediococcus cerevisiae (1 strain) were examined for their tolerance to nitrite. Nitrite at levels of 1000 and 5000 ug/g either prevented or severely retarded the growth of all strains. At 200 ug/g growth was delayed or slowed. Nitrite was metabolized by S. aureus more under aerobic than anaerobic condition (Buchanan and Solberg, 1972). This suggested that nitrite was not used as an electron acceptor, or the nitrite reductase system had been blocked anaerobically.

Ensiling of Crab Processing Waste

Ensiling is a preservation method involving anaerobic fermentation (Barnett, 1954). The entire ensiling process requires 12 to 18 d (Muller, 1980), and is characterized by the production of heat, acetic and lactic acids, followed by quiescence during which the lactic acid concentration remains stable and the pH of the fermented mass becomes constant at 4 (Barnett, 1954).

Ensiling is a low-cost procedure, and eliminates or reduces pathogenic bacteria (McCaskey and Anthony, 1975). Due

to the perishability of crab processing waste, ensiling has been shown to be a rapid and effective method of utilizing this waste (Abazinge et al., 1986). Ensiling of crab processing waste will also alleviate the problem of disposal, water pollution and odor formation.

Ensiling of crab processing waste was attempted by Samuels (1983). Crab processing waste was ensiled with crop residues and chemical additives. The final pH was high for all ensiled mixtures except when 16% acetic acid was added to the mixtures. The silages were putrified with strong NH_3-like odor. Crab processing waste is high in CP and Ca (Cantor, 1980; Abazinge et al., 1986). These nutrients will, in turn, increase the buffering capacity of the ensiled mixture, thus resulting in a high pH. In the presence of high pH and low concentration of lactic acid, clostridial and other degrading types of bacteria invade the ensiling mass (McCullough, 1978). This action resulted in butyric type of fermentation, and silages with offensive odor (Samuels, 1983).

Groninger (1959) and Yamada (1967) showed that trimethylamine oxide is a major constituent of seafood animals. The salts of this compound have been shown to have a marked buffering action (Castell, 1949; Suyama, 1958), which consequently leads to prolonged fermentation. Bacteria which can use trimethylamine oxide as terminal electron acceptor are associated with marine animals such as crabs. These bac-

teria reduce this compound to trimethylamine in the abscence of appropiate substrates such as glucose and pyruvate (Watson, 1939). Thus, the offensive odor commonly found with shellfish waste may be due to the conversion of trimethylamine oxide to trimethylamine by microbes after the death of the marine animals (Beatty and Gibbons, 1937).

Silage Additives

Molasses. The majority of seafood byproducts contain low levels of fermentable sugars (Campbell, 1973; Raa et al., 1983). Thus,in order to achieve fermentation, additional sugar is neccessary. Raa et al. (1983) indicated the inability to achieve successful preservation of fish silage was due to the low level of fermentable sugars, coupled with high buffering capacity in the ensiled materials. Addition of sugar in the form of molasses, stimulated the lactic acid fermentation and produced proper silage (McDonald and Purves 1956; Anderson and Jackson, 1970). The presence of fermentable sugars, represses the production of deaminating enzymes by the spoilage microbes in the byproduct. Thus, NH_3 production and increase in pH are prevented (Raa et al., 1983).

Andrighetto et al.(1987) reported formation of large quantities of volatile compounds, with significant losses in organic matter when 4% molasses was added to ensiled lolium multiforum. Parigi-Bini et al. (1987) reported lactic acid

content of 1.16%, and total N of 7.05% when corn stover was ensiled, with 2% molasses for 3 mo at 32% DM.

Ensiling of alfalfa in laboratory silos with large amounts of molasses resulted in lower pH, increase lactic acid concentration and decrease in DM loss (Lanigan,1961). Abazinge et al. (1986) reported a decrease in pH, high lactic acid concentration and low residual water-soluble carbohydrates when 20% molasses was added to a mixture of crab waste and straw. Ayangbile et al. (1986) added 10% molasses to preserve caged layer waste. About 85% of the molasses was converted to lactic acid. This acid consequently lowered the pH and preserved the waste for several days.

Microbial Inoculant. The use of added microorganisms to convert the wastes and byproducts into a good quality protein for animal feed has been suggested (Wiseman and Cole, 1983). Thomas (1950) has stated that successful fermentation of the ensiled product depends upon the presence of anaerobic epiphytic microorganisms. Raa (1981) and Raa and Gildberg (1982) demonstrated that tropical fish waste can be preserved by lactic acid bacteria and the silage can be stored for at least 1 mo at 30 C without any loss of nutrient.

Luther (1986) concluded that in most large scale ensiling processes, the use of inoculant has not shown any significant improvement in the fermentation characteristics. Ohyama et al. (1973) found that inoculation with L. plantarum

did not affect silage quality, but attributed the fermentation to the presence of water-soluble carbohydrate and proper sealing of the silos. McDonald et al. (1964) showed that silages of good quality were obtained regardless of inoculation of Italian rye grass containing 16.2% water-soluble carbohydrate. However, addition of Lactobaccilus plantarum and glucose resulted in early production of acids, which prevented proteolysis.

Ely et al. (1981) reported lower pH, higher lactic acid concentration and greater DM recovery in alfalfa and wheat silage mixture inoculated with L. plantarum. However, addition of inoculant to corn or sorghum mixtures did not show any positive effect. Recovery of gross energy was greater but DM and CP recovery was lower when corn silage was treated with L. acidophilus and torulopsis, compared to the untreated silage (Buchanan-Smith and Yao, 1981). Whittenberg et al. (1983) reported no improvement in the preservation of DM, CP, ADF or gross energy when corn silage was treated with L. plantarum. Bolsen et al. (1984b) reported that the addition of L. plantarum and S. faecium improved DM recovery in a rapidly filled stave silos, but DM recovery was lower in silos where filling was delayed.

Chapter III

EFFECTS OF CHEMICALS ON PRESERVATION AND FERMENTATION CHARACTERISTICS OF CRAB WASTE-STRAW MIXTURE

ABSTRACT

Four experiments were conducted to study the value of certain chemicals to retard deterioration of crab processing waste. Addition of 1.5% propionic/formic acid (1:1) prevented degradation of crab processing waste up to 14 d. Trimethylamine (TMA) concentration increased from 1.30 mg N/100 g on day 0 to 30.76 mg N/100 g on day 28. Crude protein increased initially from 40.51% to 47.02%, dry basis. The increase of CP is a reflection of loss of other components. The preserved waste was ensiled with wheat straw, sugarcane molasses and water (32:32:16:20, wet basis) with or without .1% microbial inoculant. Desirable fermentation was achieved with significant reductions in pH and water-soluble carbohydrates, and an increase in lactic acid in silages made with or without inoculant. The highest pH and lowest lactic acid concentration was observed with silages made from crab waste stored for 2 d. Residual acids used in preservation may have restricted fermentation. Preserving crab processing waste with either .2% of sodium hypochlorite (NaOCl) or .4% hydro-

gen peroxide (H_2O_2) prevented deterioration up to d 7. Concentration of TMA increased ($P<.05$) from 3.70 to 12.85 mg N/100 g when NaOCl was used. However, no increase was found in TMA concentration when H_2O_2 was used (2.66 to 2.71 mg N/100 g). The reduction in pH, and high level of lactic acid indicated that the chemicals prevented deterioration, and enhanced fermentation of the crab waste-straw mixture. Addition of .4% NaOCl and 1.5% acetic acid retarded the degradation of crab waste for 4 d. Concentration of TMA was highest ($P<.01$) for the untreated waste (41.23 mg N/100 g) and lowest for the NaOH treated waste (2.42 mg N/100 g). On d 7, true protein content was highest for the acetic acid treated waste and lowest for the untreated waste. The use of 1.5% NaOH resulted in maggot infestation and production of NH_3. No H_2S was detected when waste was treated with NaOH, however, the untreated waste showed the highest concentration of H_2S (202 ppm). The use of 1% $NaNO_2$ and a combination of .4% sodium/calcium hypochlorite, (NaOCl/CaOCl) (1:1, w/w, or .2% of each) preserved the waste for a minimum of 10 d. Ammonia gas was first detected at d 12 and H_2S was not detected throughout the 15 d of preservation when 1% $NaNO_2$ was used. The TMA concentration was lowest for waste treated with 1% $NaNO_2$ and highest for the untreated waste. Ammonia and H_2S concentrations were highest for the untreated waste, while levels in waste treated with .2% NaOCl and a combination of

.1% NaOCl/.1% CaOCl (1:1, w/w) treated waste were intermediate.

(Key words: Trimethylamine, Chemicals, Hydrogen sulfide, Ammonia, and Degradation).

INTRODUCTION

In Virginia and Maryland, approximately 21 million metric tons of crabs are processed annually (Brinsfield, 1980). Crab processing waste amounts to 19 million metric tons. The waste undergoes degradation within 5 h after harvest (Brooks, 1980). In the past, efforts were made to maintain an aesthetic environment by dumping this waste into the ocean (Olsen, 1980) or putting into landfills (Brinsfield, 1980). However, restrictions by the EPA have made these practices unsuccessful. The conversion of the waste into crab meal as livestock feed has been a marginal situation, economically, due to the cost of energy and relatively low demand for the product (Coale, Jr. 1980). The use of the waste as fertilizer is not feasible due to the distance between the processing plants and farms, and the cost of hauling large amounts of this waste (Brinsfield, 1980; Coale, Jr. 1980).

Crab processing waste is an excellent source of crude protein (Abazinge et al., 1986) and minerals (Cantor, 1980). The waste has been ensiled with straw with the use of large

amounts of acetic acid (Samuels et al., 1982) or molasses and microbial inoculant (Abazinge et al., 1986). However, before any successful fermentation can be achieved, the waste must be kept in a fresh condition prior to ensiling.

Chemicals had been used extensively in the preservation of highly degradable products (Raa et al.,1983; Ayangbile et al., 1986). However, sufficient amounts of these chemicals must be added to lower the pH for effective preservation. Gildberg and Raa (1977) used .75% of propionic and formic acid in achieving successful preservation of fish silage. Backhoff (1976) reported a fairly stable silage and a low microbial count when 3% of 98% formic acid was added to minced cod viscera. Abazinge et al. (1986) reported a lower crude protein content and higher TMA concentration in crab waste treated with 1.5% propionic/formic (1:1, w/w) acid, compared to the other chemically-preserved waste. In the same study, the use of formaldehyde (HCHO) was effective in the preservation of crab processing waste. Narasimhalu et al. (1981) showed the effectiveness of HCHO (reducing agent) and NaOCl (oxidizing agent) in elimination of aerobic and coliform bacteria in treated caged layer waste. Lipma and Owen (1943) reported the inhibitory effect of H_2O_2 on obligatory and facultative anaerobic bacteria. Tompkin et al. (1979a) reported the antimicrobial effect of nitrite against the putrefactive and pathogenic clostridia. The antimicrobial efficiency of NaOCl, H_2O_2 and $NaNO_2$ may be due

to their ability to inactivate the bacterial enzymes and/or interfere with the metabolism and repair mechanisms of the bacteria (Benedict, 1980).

Four experiments were conducted to evaluate the effect of chemicals on the preservation of crab processing waste for extended period of time, and the residual effect of these chemicals on fermentation characteristics.

EXPERIMENTAL PROCEDURE

Experiment 1. Fresh crab waste was obtained from a crab-processing plant[1]. The material was mixed thoroughly with a shovel to achieve a homogenous mixture. A mixture of 1.5% propionic/formic acid (1:1, w/w) was applied with a hand sprayer after the flow rate had been determined. The crab waste was placed in 210 liter metal drums double-lined with polyethylene bags and sprayed with a known amount of chemical. After treatment, the tops of the polyethylene bags were closed, and holes were placed at the bottom of the bags and drums to allow drainage. The drums with the contents were left in an enclosed room which was maintained at about 65 C. Samples of the crab processing waste were obtained before and after spraying, and frozen.

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[1] Graham and Rollins, Hampton, Virginia

After 2, 5, 14, 21, and 28 d the preserved crab waste was ensiled with wheat straw, molasses and water (32:32:16:20), with or without .1% microbial inoculant[2]. After mixing, the mixtures were packed in 4-liter cardboard containers, double lined with polyethylene bags, which were sealed individually. The ensiled mixtures were opened after a minimum of 42 d. Samples were taken before and after ensiling for analyses.

Experiment 2. Fresh crab waste was obtained from the same source as given above. Approximately 114 kg of the crab waste were sprayed with .2% NaOCl or .4% H_2O_2, and shovel mixed. The treated wastes were placed into 210-liter metal drums double lined with polyethylene bags. Holes were placed at the bottom of the bags and drums to fascilitate drainage, and the material was not covered. After 7 d the preserved wastes were ensiled with straw (1:1), 16% molasses and 20% water, as described for experiment 1. Samples of the crab processing waste before and after 7-d preservation, the mixtures and silages were obtained and frozen for later analysis.

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[2] S. faecium and L. plantarum, Pioneer Hi-Bred International, Des Moines, Iowa

Experiment 3. Fresh crab waste was obtained during the spring of 1988 from the same source as in experiment 1. The waste was placed in a horizontal mixer, and treated with the following chemicals:control (untreated), 1.5% acetic acid, 1.5% sodium hydroxide (NaOH), and .4% NaOCl. After thorough mixing, the mixtures were placed into 210-liter metal drums double lined with polyethylene bags. Three 1 m polyvinyl chloride (PVC) pipes (1.5 cm inside diameter) with several holes at the bottom 43 cm were placed in each drum in order to measure temperature, NH_3 and H_2S. The bags were sealed tightly around the pipes, and rubber stoppers were placed at the opening. Holes were placed at the bottom of the drums and bags to allow adequate drainage and maintain partial aerobic conditions. The drums were placed in an enclosed shelter at approximately 18 C. Samples of the waste were taken initially, and once daily for 7 d. The volatile H_2S and NH_3 gases were measured with a gas sampler[3]. A thermometer placed inside the PVC pipe was used to determine change in temperature.

Experiment 4. During the summer of 1988, crab processing waste was obtained from the same source as in experiment 1. The waste was placed in a horizontal mixer and treated with the following chemicals: Control (untreated), .2% NaOCl,

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[3] Gastec Precision Gas Detector System, Japan

.2% NaOCl/CaOCl (1:1, w/w, or .1% of each), .4% NaOCl/CaOCl (1:1, w/w, or .2% of each), and 1% $NaNO_2$ After thorough mixing the mixtures wwere placed in 210-liter metal drums double lined with polyethylene bags. Two 1 m PVC pipes (1.5 cm inside diameter) with several holes at the bottom 43 cm were placed in each drum to measure the NH_3 and H_2S. The bags were sealed tightly around the pipes and rubber stoppers were placed at the opening. The drums and the content were stored in a semi-closed shelter. Holes were placed at the bottom of the drums to facilitate drainage. Samples of the treated waste were taken initially, and at d 1, 2, 3, and every other day until d 15. Samples of gases were also obtained at the same time.

Chemical Analyses

The samples of crab processing waste obtained for Exp. 1, 2, 3 and 4 were prepared for TMA determination by a colorimetric procedure (Dyer, 1959) after extraction with 7.5% trichloroacetic acid. Kjeldahl N was determined on wet samples (AOAC, 1984). Dry matter was determined by drying in a forced draft oven at a maximum of 60 C for 48 h. The dried samples were ground in a hammer mill and subsamples were taken for ash determination (AOAC, 1984). True protein N was determined on wet samples obtained from Exp. 3, by precipitation with tungstic acid, followed by N determination (AOAC, 1984). For the ensiled mixtures, the initial and final sam-

ples were prepared for analysis by homogenizing 25 g sample with 225 ml of distilled water in .5 liter jar in a Waring blender at full speed for 2 min. The homogenate was filtered through four layers of cheese cloth and the extract was used for determining pH (electrometrically), lactic acid (Barker and Summerson, 1941, as modified by Penninington and Sutherland, 1956) and water soluble carbohydrates (Dubois et al., 1956, as adapted to corn plants by Johnson et al., 1966).

Statistical Analysis

The data were tested by analysis of variance by the general linear model procedure of SAS (1982). For the preservation studies, day, treatment, and day x treatment interaction were included in the model, then linear and quadratic contrasts were made to test the data obtained for each period. For the propionic/formic acid treated crab waste-straw mixtures, effects of day, additive and day x additive interaction were included in the model and these data were tested by linear and quadratic contrasts. Only the treatment effect was included in the model for H_2O_2-and NaOCl-treated crab waste-straw mixtures. Comparisons of means were made for Exp. 3 and 4 by using Tukeys's HSD procedures in SAS (1982). All values given in the tables and graphs are least square means.

RESULTS AND DISCUSSION

Experiment 1. The DM of the propionic/formic acid-preserved crab waste decreased from 42.0% at d 0 to 37.4% on d 28 (Table 1). There was an increase in CP from 40.5% (d 0) to 47.0% (d 28). The increase in CP may be a reflection of decrease in DM content. Abazinge et al. (1986) reported a decrease in DM and CP contents when crab waste was treated with 1.5% propionic/formic acid (1:1, w/w) and stored for 56 d. The increase in ash value from 40.3% (d 0) to 47.4% (d 28) was an indication of degradation of the organic matter associated with crab processing waste. Ayangbile et al. (1986) reported an increase in ash content of caged layer waste stored for 42 d.

The TMA levels increased from 1.30 (d 0) to 30.8 mg N/100 g at d 28. Abazinge et al. (1986) reported a TMA concentration of 55.7 mg N/ 100 g with crab processing waste stored for a period of 56 d. In the present study, DM and pH of the initial mixtures (Table 2) increased ($P<.01$) gradually with time of storage. The elevated pH may be due to the buffer effect of the high ash and (or) TMA.

After ensiling, large reductions in water- soluble carbohydrate and pH, and an increase in lactic acid concentration were obtained. The reduction in pH and lactic acid production were less for the waste stored for 2 d prior to ensiling. The residue of propionic/formic acid used in pres-

Table 1. COMPOSITION OF PROPIONIC/FORMIC ACID-TREATED CRAB WASTE. EXP. 1.

Day	Dry matter[a]	Ash[a b]	Crude protein[a b]	Trimethyl-amine[a b c]
	%[b]			mg N/100 g
0	42.02	40.28	40.51	1.30
2	41.39	40.57	42.18	1.32
7	40.63	42.33	43.34	14.43
14	40.40	41.84	42.76	12.33
21	38.64	43.55	46.51	26.36
28	37.44	47.41	47.02	30.76
SE	.26	.24	.20	.67

[a] Linear effect of time ($P<.01$).
[b] Dry basis.
[c] Wet basis.

TABLE 2. FERMENTATION CHARACTERISTICS OF PROPIONIC/FORMIC ACID-TREATED CRAB WASTE-STRAW MIXTURE[a].EXP. 1.

Day	Dry matter[de]		pH[de]		Water sol. carbohydrates[be]		Lactic acid[be]	
	None	Add[c]	None	Add[c]	None	Add[c]	None	Add[c]
	------%------		---Pre-ensiling--		----------%----------			
2	55.47	55.02	7.09	6.97	10.87	10.41	0.38	0.35
7	57.27	57.53	7.31	7.32	10.55	9.89	0.40	0.38
14	57.10	57.49	7.34	7.48	9.64	9.85	0.40	0.39
21	54.97	55.16	7.84	7.83	10.70	10.26	0.32	0.36
28	57.17	56.57	8.01	8.03	9.90	10.13	0.21	0.23
SE	0.29		0.02		0.41		0.03	
			----Post ensiling--					
2	53.72	53.67	6.34	6.49	2.02	1.91	2.21	1.97
7	55.01	55.17	5.27	5.31	3.28	2.12	6.31	7.91
14	53.99	54.08	5.17	5.23	1.95	1.89	8.26	8.26
21	53.02	53.60	5.17	5.19	0.94	0.97	8.51	8.43
28	55.04	55.22	5.33	5.43	1.01	0.99	7.27	7.46
SE	0.20		0.06		0.11		0.27	

[a] Crab waste, straw, molasses and water (32:32:16:20, wet basis)
[b] Dry basis
[c] Additive (.1% Microbial inoculant)
[d] Linear effect of time for pre-ensiled mixtures ($P<.01$)
[e] Quadratic effect of time for post-ensiled mixtures ($P<.01$).

ervation may have restricted fermentation. There were no significant effects of microbial inoculant on any of the parameters measured. These results varied with those reported by Abazinge et al. (1986) who reported a lower pH and higher lactic acid concentration when fresh crab waste was ensiled with straw, molasses and water. Ohyama et al. (1973) showed that inoculation of ensiled ryegrass is not needed with a sufficient amount of water-soluble carbohydrate and proper sealing of the silo.

Experiment 2. A difference ($P<.05$) was found in DM content when crab processing waste was treated for 7 d with either H_2O_2 or NaOCl (Table 3). Ash content (39.60%) tended to be higher for NaOCl-treated waste, compared to the H_2O_2-treated waste. The TMA concentration increased from 3.70 to 12.85 mg N/100 g for NaOCl-treated waste, while the TMA concentration of the waste did not change (2.71 mg N/100 g), with crab processing waste preserved with H_2O_2 for 7 d ($P<.01$). However, the value of 12.85 mg N/100 g indicates minimal deterioration with NaOCl-treated waste (Abazinge et al., 1986). The effectiveness of H_2O_2 in controlling the odor production in feedlots, dairy cow and swine waste treatment operations has been reported (Ulich et al., 1975; Cole et al., 1975). The decrease in pH and water-soluble carbohydrate and the rise ($P<.05$) in lactic acid indicated good ensiling, regardless of the chemicals used in preserving

TABLE 3. COMPOSITION OF CRAB WASTE PRESERVED WITH CHEMICALS. EXP. 2.

Additive	Composition by day							
	Dry matter[a]		Ash[c]		Crude protein[c]		TMA[bde], mg N/100g	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
	%							
Sodium hypochlorite	39.24	39.35	36.39	39.60	44.29	44.01	3.70	12.85
Hydrogen peroxide	40.47	41.32	36.87	36.65	43.31	43.39	2.66	2.71
SE	.61		.84		.90		.46	

[a] Treatment differ (P<.05).
[b] Treatment differ (P<.01).
[c] Dry basis.
[d] Trimethylamine.
[e] Wet basis.

the waste (Table 4). Thus, these data showed similar effectiveness for both preservatives.

Experiment 3. On d 1, the temperature was highest for the untreated waste (34.8 C) and lowest for the waste treated with 1% acetic acid (Figure 2). Temperatures for each treatments remained constant throughout the storage period except the initial rise observed with acetic acid treated waste from d 1 to d 2.

Addition of 1.5% NaOH to the crab waste resulted in increase evolution of NH_3 (Figure 3). Concentration of NH_3 was 75 ppm or less for all other treatments. However, differences ($P<.01$) were observed with time for all treatments. The high level of NH_3 for the NaOH treated waste may have been due to the deamination of amino acids in crab waste. Overall, evolution of H_2S increased ($P<.01$) with time. However, evolution of H_2S was less when acetic acid, NaOH or NaOCl was used in treating the waste (Figure 4). The untreated crab waste showed the highest concentration of H_2S at d 3, 5 and 7.

The values obtained for DM were lowest ($P<.01$) initially for NaOCl-treated waste, compared to other treatments, and the effect persisted for 7 d. The low DM concentration for NaOCl-treated waste is a reflection of greater amount of chemical added to the waste. Overall, DM was higher ($P<.05$) for the NaOH and acetic acid treated waste (Table 5). The overall ash values were lowest ($P<.05$) for waste treated with

TABLE 4. FERMENTATION CHARACTERISTICS OF TREATED CRAB WASTE AND STRAW SILAGE[a]. EXP. 2.

Preservative	Time	pH	Dry matter	Water sol. carbohy.[bc]	Lactic acid[bc]
			-----%		
Sodium hypochlorite	Preens.	7.3	56.7	11.8	.6
	Postens.	5.5	54.5	1.0	8.1
Hydrogen peroxide	Preens.	7.1	57.6	10.9	.5
	Postens.	5.7	54.6	1.2	6.4

[a] Crab waste, wheat straw, molasses and water (32:32:16:20 wet basis).
[b] Dry basis.
[c] Treatment differ for post-ensiled mixtures ($P<.05$).

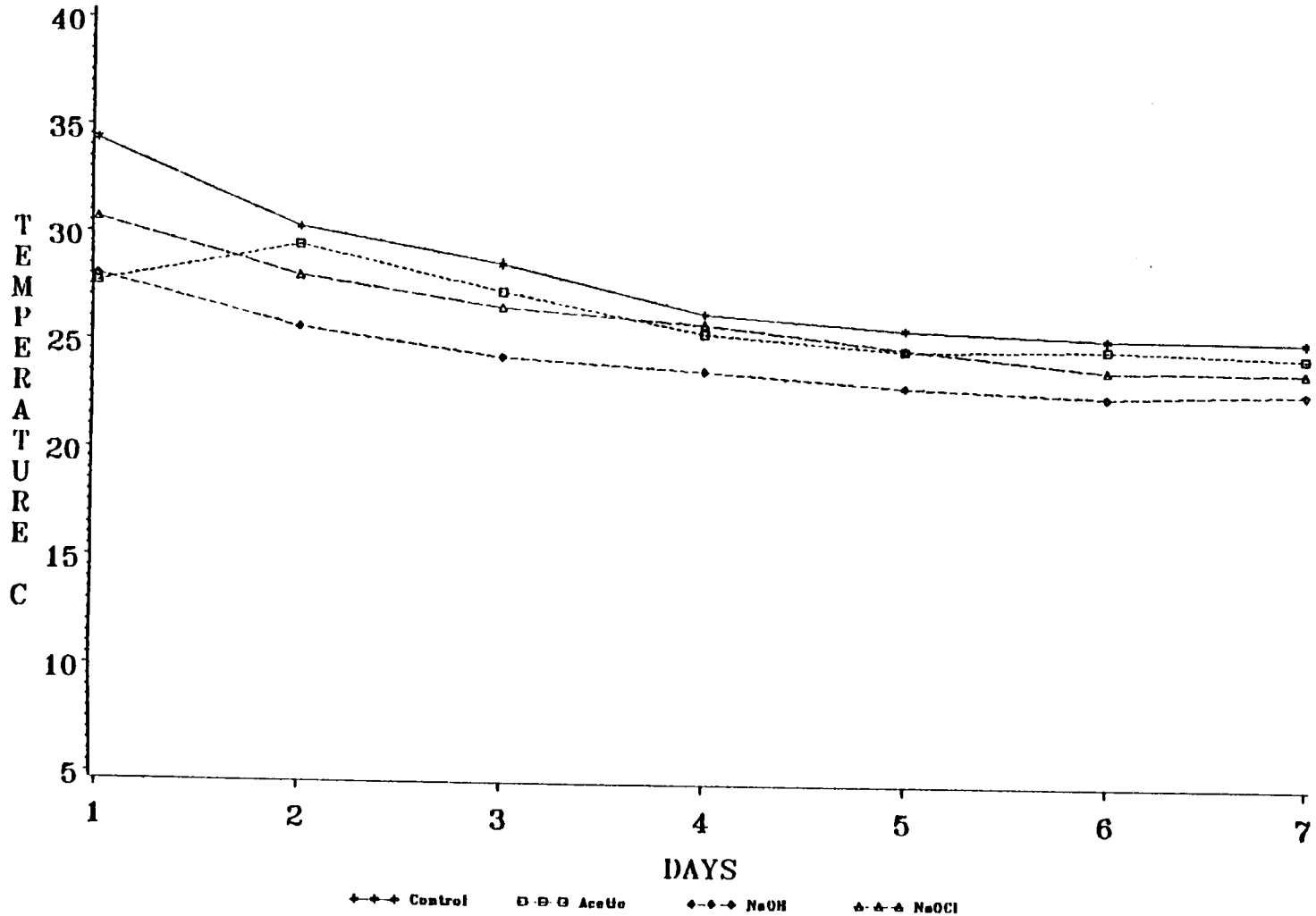

FIGURE 2. Effect of additives on temperature of preserved crab waste Experiment 3. Day*Treatment Interaction Linear ($P<.01$).

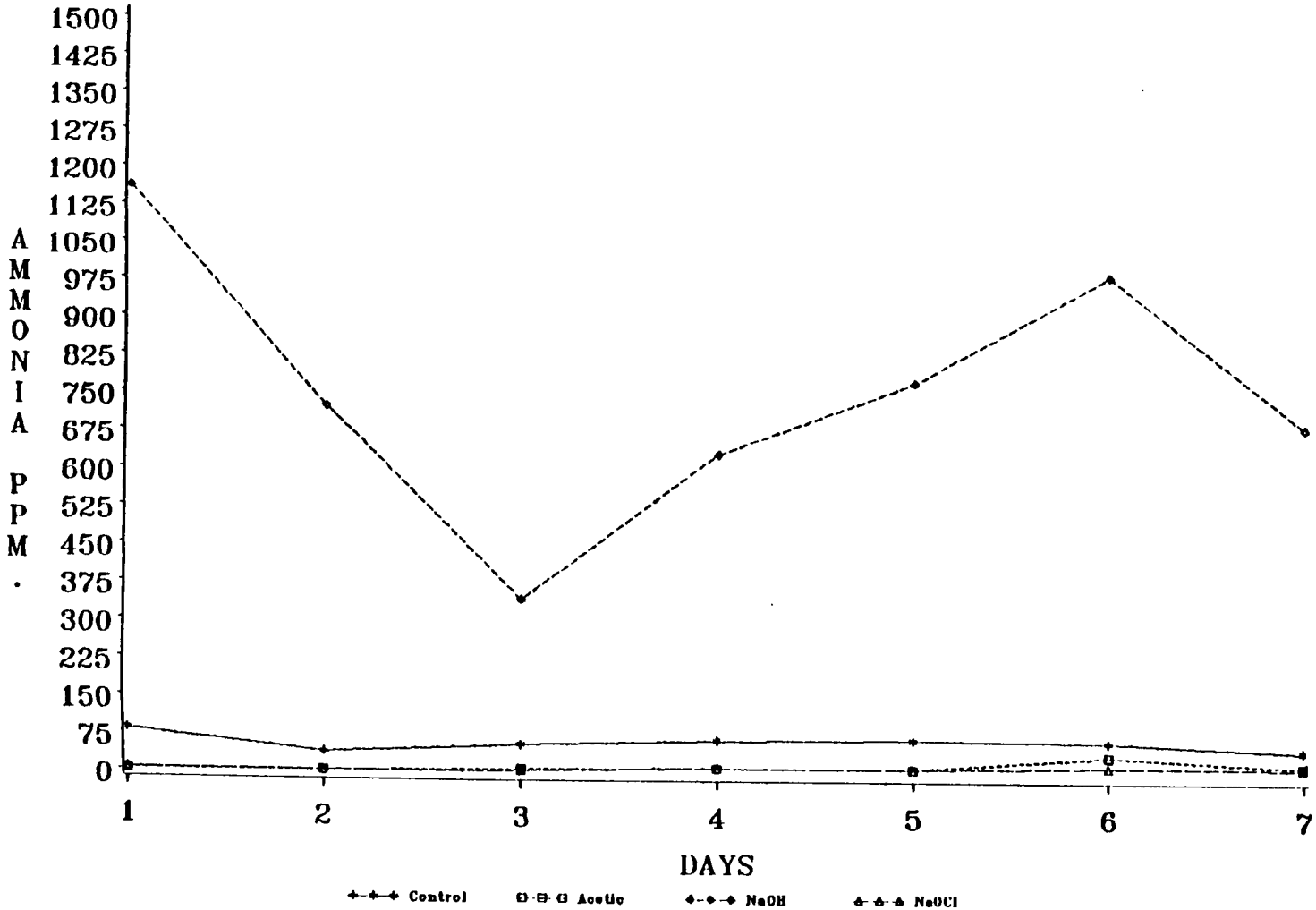

FIGURE 3. Effect of additives on ammonia concentration, ppm, wet basis. Exp. 3. Day*Treatment Interaction Linear (P<.01).

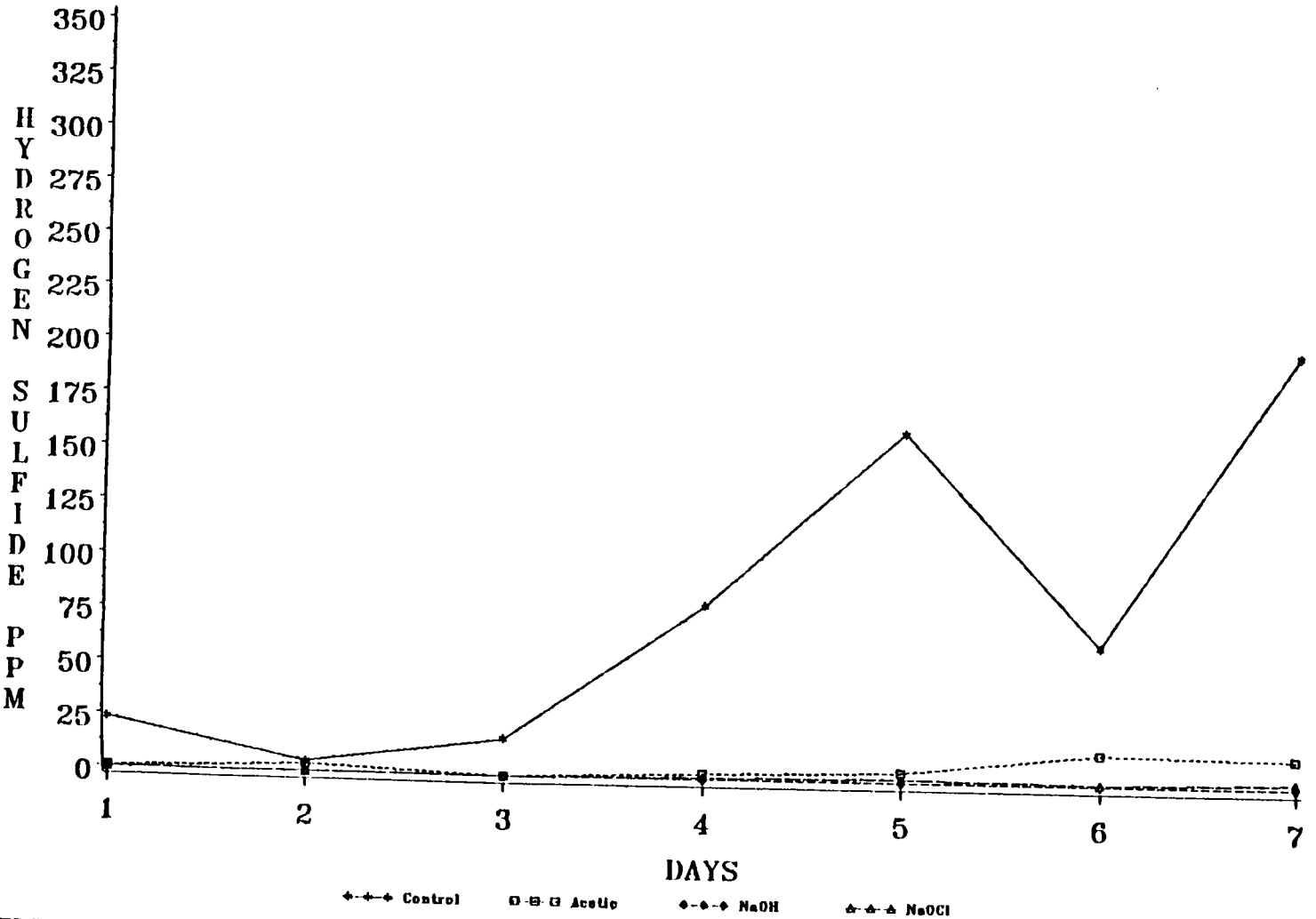

FIGURE 4. Effect of additives on hydrogen sulfide concentration, ppm, wet basis. Exp. 3. Day*Treatment Interaction Linear ($P<.01$).

TABLE 5. EFFECT OF CHEMICALS ON DRY MATTER[a,b] CONCENTRATION OF PRESERVED CRAB PROCESSING WASTE. EXP. 3.

Day	Chemical				SE
	None	Acetic acid	Sodium hydroxide	Sodium hypochlorite	
	%				
0	34.62	39.42	39.05	33.60	1.09
1	37.97	41.88	41.40	35.41	1.09
2	37.74	40.86	40.26	37.13	1.09
3	36.08	40.15	38.87	37.10	1.09
4	38.86	38.64	41.20	37.14	1.09
5	37.96	40.94	41.21	37.60	1.09
6	37.02	37.86	40.25	31.23	1.09
7	38.07	37.81	40.43	34.74	1.09
Avg[c,d]	37.29	39.69	40.33	35.49	2.38

[a] Crab waste, straw, molasses and water (32:32:16:20, wet basis).
[b] Linear effect of time ($P<.01$).
[c] Means of untreated vs treated differ ($P<.05$).
[d] Means of acetic acid and NaOH vs NaOCl treated waste differ ($P<.05$).

acetic acid, and highest (P<.05) for the untreated, while values for NaOH and NaOCl-treated waste were intermediate (Table 6). Apparently, the low value obtained for the acetic acid treated waste may be due to the addition of an organic preservative. Overall CP content was lowest (P<.05) when NaOH was used in treating the crab waste, probably due to loss of NH_3 (Table 7). Similar values were obtained from the untreated, acetic acid and NaOCl treated waste. However, values for the acetic acid and NaOCl treated waste tended to be lower (P<.05). True protein concentration tended to be lower for all treated wastes at d 0 (Table 8). A decrease was observed with time for all treatments except acetic acid treated waste. Raa and Gilberg (1982) showed that deamination of fish protein is suppressed under acid conditions. Vicini et al. (1983) reported that treating soybean meal with acetic acid was effective in reducing ruminal escape of N when this material was incubated in the rumen of cannulated steers for 12 h. The lowest (P<.05) true protein values were obtained for the untreated and NaOH treated waste, perhaps reflections of deterioration of the untreated waste, and deamination of the amino acids of the crab waste by NaOH treatment.

Concentration of TMA for all treatments increased linearly (P<.01) with time (Table 9). The highest overall level was observed with untreated waste (35.46 mg N/ 100g), however, the TMA concentrations for acetic acid and

TABLE 6. EFFECT OF CHEMICALS ON ASH[a,b] CONCENTRATION OF PRESERVED CRAB PROCESSING WASTE. EXP. 3.

Day	Chemical				SE
	None	Acetic acid	Sodium hydroxide	Sodium hypochlorite	
	%				
0	45.08	43.16	46.79	44.47	1.36
1	49.17	44.42	50.33	46.14	1.36
2	49.78	46.66	48.98	46.78	1.36
3	49.07	46.18	47.57	47.84	1.36
4	51.64	44.90	49.02	48.34	1.36
5	50.75	45.98	47.98	49.07	1.36
6	50.16	43.32	47.83	45.91	1.36
7	50.01	39.97	47.59	50.22	1.36
Avg[c,d]	49.46	44.32	48.26	47.34	3.73

[a] Crab waste, straw, molasses and water (32:32:16:20, wet basis).
[b] Linear effect of time (P<.01).
[c] Treatment means of untreated and NaOH vs acetic acid and NaOCl treated waste differ (P<.05).
[d] Treatment means of acetic acid vs NaOCl treated waste differ (P<.05).

TABLE 7. EFFECT OF CHEMICALS ON CRUDE PROTEIN CONCENTRATION OF PRESERVED CRAB PROCESSING WASTE[a]. EXP. 3.

Day	Chemical				SE
	None	Acetic acid	Sodium hydroxide	Sodium hypochlorite	
	%				
0	43.22	39.86	36.54	40.40	1.69
1	40.32	36.35	34.54	38.45	1.69
2	40.43	39.49	36.67	37.77	1.69
3	42.41	39.93	35.89	39.21	1.69
4	40.07	40.83	34.96	37.27	1.69
5	41.60	38.97	35.38	37.06	1.69
6	43.81	42.16	35.56	44.40	1.69
7	41.20	41.73	36.13	39.70	1.69
Avg[b,c]	41.63	39.91	35.71	39.28	5.71

[a] Crab waste, straw, molasses and water (32:32:16:20, wet basis).
[b] Treatment means of untreated vs NaOH and NaOCl treated waste differ ($P<.05$).
[c] Treatment means of acetic acid vs NaOH treated waste differ ($P<.05$).

TABLE 8. EFFECT OF CHEMICALS ON TRUE PROTEIN[a,b] CONCENTRATION OF PRESERVED PROCESSING CRAB WASTE. EXP. 3.

Day	Chemical				
	None	Acetic acid	Sodium hydroxide	Sodium hypochlorite	SE
			%		
0	35.91	30.97	31.05	34.00	1.50
1	27.45	30.25	25.49	29.28	1.50
2	26.88	32.18	27.36	27.95	1.50
3	26.45	33.09	26.17	31.47	1.50
4	23.51	31.58	25.03	28.62	1.50
5	22.95	29.27	26.61	27.93	1.50
6	24.22	32.09	24.91	28.98	1.50
7	23.24	30.62	23.90	27.52	1.50
Avg[c]	26.32	31.25	26.31	29.46	4.52

[a] Crab waste, straw, molasses and water (32:32:16:20, wet basis).
[b] Quadratic effect of time($P<.01$).
[c] Treatment means of untreated and NaOH vs acetic acid and NaOCl treated waste differ ($P<.05$).

TABLE 9. EFFECT OF CHEMICALS ON TRIMETHYLAMINE[a,b] CONCENTRATION OF PRESERVED CRAB PROCESSING WASTE. EXP. 3.

Day	Chemical				SE
	None	Acetic acid	Sodium hydroxide	Sodium hypochlorite	
	mg N/100 g				
0	7.56	1.94	1.33	2.22	2.05
1	39.51	5.83	1.55	10.33	2.05
2	35.19	10.02	2.36	10.31	2.05
3	40.23	21.13	1.79	17.86	2.05
4	37.50	24.49	1.74	28.07	2.05
5	42.20	22.90	2.12	27.86	2.05
6	40.28	25.81	1.85	28.93	2.05
7	41.23	25.87	2.42	30.28	2.05
Avg[c,d]	35.46	17.25	1.89	19.48	8.48

[a] Crab waste, straw, molasses and water (32:32:16:20, wet basis).
[b] Linear effect of time ($P<.01$).
[c] Treatment means of untreated vs treated waste differ ($P<.05$).
[d] Treatment means of acetic acid and NaOCl vs NaOH treated waste differ ($P<.05$).

NaOCl-treated waste were intermediate The concentrations of TMA for the NaOH-treated waste were lowest ($P<.05$) and did not change with time. A TMA concentration of 70.6 mg N/ 100 g was observed by Abazinge et al. 1986 in untreated crab processing waste stored for 56 d.

Experiment 4. Evolution of NH_3 increased linearly ($P<.01$) with time for all treatments. Concentration of NH_3 was highest ($P<.01$) for the untreated crab waste (Figure 5) while the value for waste treated with .2% NaOCl was intermediate. Crab waste treated with .2 or .4% NaOCl/CaOCl (1:1, w/w) showed a small increase in NH_3 concentration after 7 d. Treating crab waste with 1% $NaNO_2$ suppressed NH_3 production until d 12, after which a small increase was seen. No detectable H_2S was observed with crab waste treated with .2% NaOCl, .4% NaOCl/CaOCl (1:1, w/w) or 1% $NaNO_2$ (Figure 6.) The concentration was highest (28.2 ppm) for the untreated waste at d 4, and the level rapidly decreased to 6.3 ppm on d 8. The use of .2% NaOCl/CaOCl (1:1, w/w) resulted in a small increase in H_2S concentration which reached a peak (2.4 ppm) on d 6. Collins et al. (1973) reported that H_2O_2 effectively eliminated H_2S production in dairy manure. Hammond (1968) observed that when chlorine compounds and $NaNO_3$ were used in treating swine waste, lower H_2S and NH_3 productions were obtained.

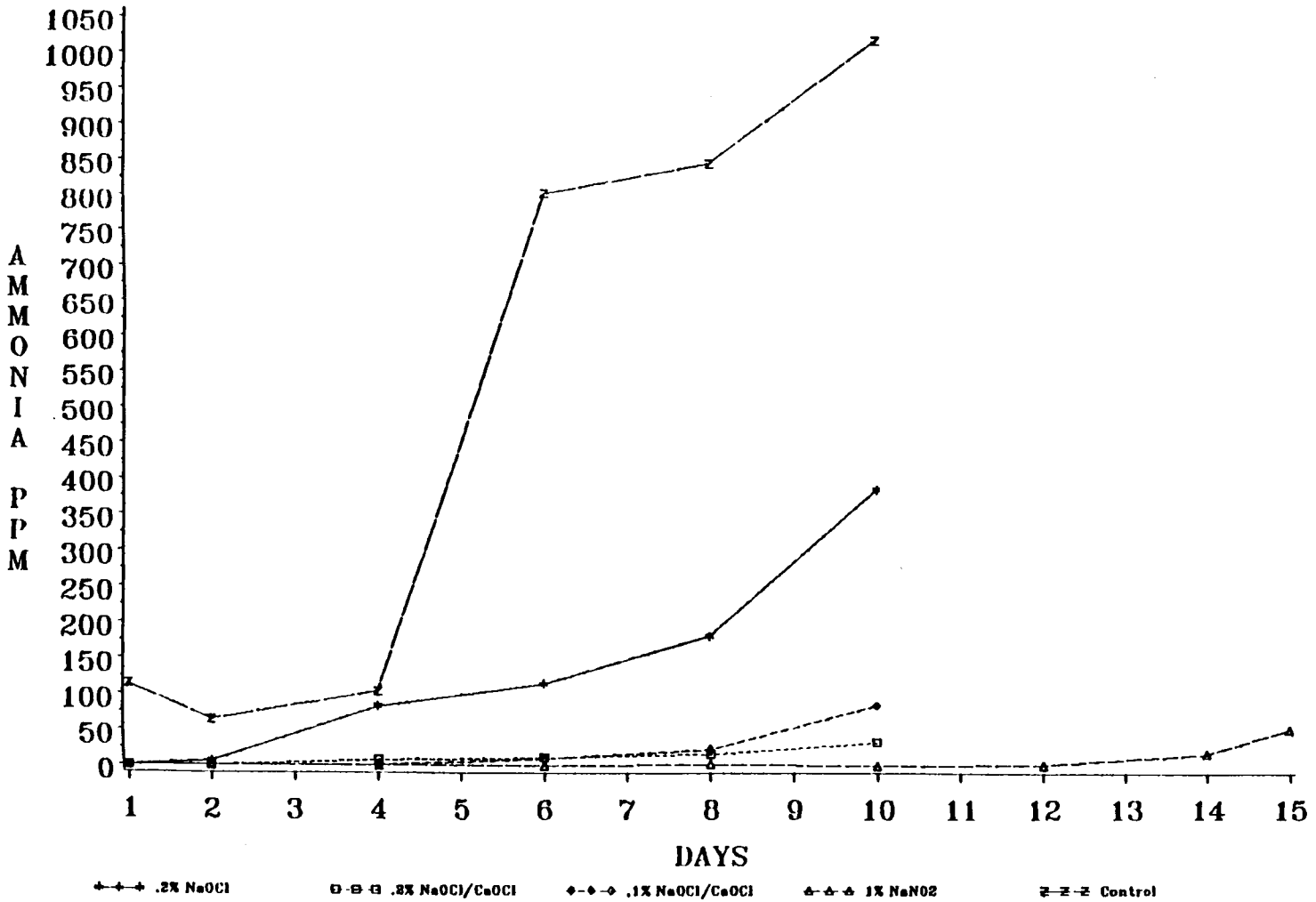

FIGURE 5.Effect of additives on ammonia concentration, ppm, wet basis. Experiment 4. Day*Treatment Interaction LINEAR (P<.01).

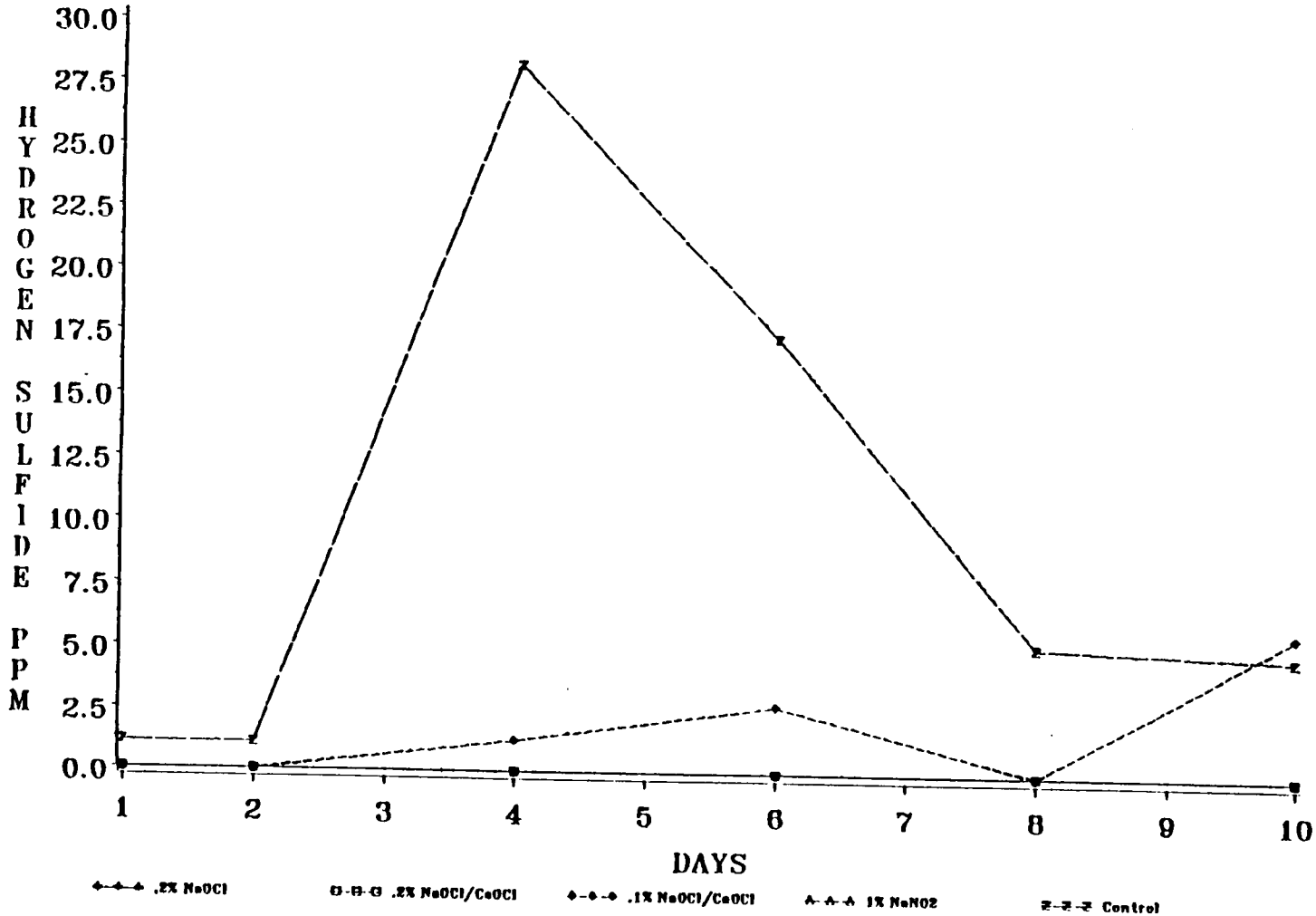

FIGURE 6. Effect of additives on hydrogen sulfide concentration, ppm, wet basis. Experiment 4. Day*Treatment Interaction LINEAR ($P<.01$).

The overall DM of the untreated waste was higher ($P<.05$), compared to the chemically treated-waste. The DM of the chemically treated crab waste was similar. However, the value of 32.0% was observed at d 0 for waste treated with .4% NaOCl/CaOCl (1:1, w/w), which may have resulted from a sampling error (Table 10). There was an increase in ash content during the storage period. Overall values for ash content were different ($P<.05$) for all treatments. However, the highest value was for the untreated waste.

Generally, overall CP content was higher ($P<.05$) for chemically treated waste than the untreated waste (Table 11). Abazinge et al. (1986) reported the lowest CP value of 35.83% in crab waste kept for 56 d without any preservative. In the present study, TMA values were low for all treatments at d 0. Increases were observed for all treatments at d 10. However, the overall increase was smaller ($P<.05$) for the crab waste treated with 1% $NaNO_2$. At day 10, the TMA concentration for crab processing waste treated with 1% $NaNO_2$ was lowest (2.23 mg N /100g).

TABLE 10. DRY MATTER AND ASH CONCENTRATION IN PRESERVED CRAB WASTE[a]. EXP. 4.

Day	Chemical					
	None	.2% NaOCl/CaOCl	.4% NaOCl/CaOCl	.2% NaOCl	1% $NaNO_2$	SE
	Dry matter,%					
0	39.32	38.11	31.97	36.26	37.37	1.25
5	37.26	36.13	36.88	35.35	37.52	1.25
10	38.26	35.77	36.32	36.67	39.01	1.25
15[d]	---	---	---	---	36.73	
Avg[e]	38.28	36.67	35.05	36.09	37.65	4.73
	Ash,%[bc]					
0	46.76	40.63	37.58	36.83	35.67	1.68
5	49.58	41.26	41.03	40.08	36.28	1.68
10	52.57	40.24	40.77	39.70	38.90	1.68
15[d]	---	---	---	---	36.42	
Avg[f g]	49.63	40.71	39.79	38.87	36.81	8.53

[a] Crab waste, straw, molasses and water (32:32:16:20, wet basis).
[b] Dry basis.
[c] Linear effect of time ($P<.01$).
[d] Measurement taken for $NaNO_2$ treatment only.
[e] Treatment means of untreated vs .4% NaOCl/CaOCl treated waste differ ($P<.05$).
[f] Treatment means of untreated vs treated waste differ ($P<.05$).
[g] Treatment means of .2% NaOCl/CaOCl vs 1% $NaNO_2$ treated waste differ ($P<.05$).

TABLE 11. CRUDE PROTEIN, AND TRIMETHYLAMINE CONCENTRATION IN PRESERVED CRAB WASTE[a]. EXP. 4.

Day	Chemical					
	None	.2% NaOCl/CaOCl	.4% NaOCl/CaOCl	.2% NaOCl	1% $NaNO_2$	SE
	Crude protein, %[b]					
0	37.76	42.22	49.20	44.15	46.86	1.85
5	43.36	45.60	46.08	45.66	47.54	1.85
10	40.16	48.00	45.87	46.49	46.19	1.85
15[d]	---	---	---	---	49.71	
Avg[e]	40.42	45.27	47.05	45.43	47.57	10.32
	Trimethylamine, mg N/100 g[b,c]					
0	1.06	0.82	0.99	0.86	0.62	.53
5	21.00	21.49	16.95	19.71	0.87	.53
10	31.95	26.17	19.93	26.00	2.23	.53
15[d]	---	---	---	---	7.24	
Avg[e,f,g]	18.00	16.16	12.62	15.52	2.74	.85

[a] Crab waste, straw, molasses and water (32:32:16:20, wet basis).
[b] Dry basis.
[c] Linear effect of time ($P<.01$).
[d] Measurement taken for $NaNO_2$ treatment only.
[e] Treatment means of untreated vs treated waste differ ($P<.05$).
[f] Treatment means of .4% NaOCl/CaOCl vs treated waste differ ($P<.05$).
[g] Treatment means of 1% $NaNO_2$ vs treated waste differ ($P<.05$).

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Chapter IV

DIGESTIBILITY AND NUTRIENT UTILIZATION BY SHEEP FED AN ENSILED CRAB WASTE-STRAW MIXTURE

ABSTRACT

Sodium hypochlorite at a level of .2% was used to preserve crab processing waste. Trimethylamine (TMA) production, an indication of putrefaction, in the preserved waste was 19.8 mg N/100 g. Proportions of the waste, straw, molasses, and water were 32:32:16:20, wet basis. A microbial silage inoculant at .1% was added and the mixture was ensiled for a minimum of 16 wk. A reduction in pH and water-soluble carbohydrates, and the higher concentration of lactic acid (4.9%, dry basis) indicated that effective fermentation had occured. The TMA concentration in the silage was 11.2 mg N/100 g. The silage was used in a digestion trial with 18 crossbred wethers with average weight of 43 kg. Diets fed were 1) basal (30% orchard grass hay, 65.1% corn grain, 4% soybean meal and .9% limestone) alone, 2) a 50:50 mixture, dry basis, of basal and crab waste-straw silage, and 3) 100% crab waste-straw silage. Apparent digestibility of DM, organic matter, CP, energy, NDF, ADF, cellulose, and

hemicellulose decreased linearly (P<.01) with increased level of crab waste-straw silage. Nitrogen retention increased linearly (P<.05) with level of crab waste-straw silage. The rumen VFA concentration was highest for sheep receiving 50% crab waste silage. Ruminal pH, NH_3-N and blood urea-N were higher (P<.01) for animals receiving 100% crab waste-straw silage. The apparent absorption was higher (P<.01) and retention was positive (P<.05) for Ca, Mg, Na, K, Cu and Fe with sheep fed the highest level of crab waste-straw silage. Negative absorption and retention values were obtained with Zn for the sheep fed all diets. The serum Mg and Zn concentrations were lower (P<.01) for animals fed 100% crab waste straw silage.
(Key words: Crab Waste, Sodium Hypochlorite, Digestibility, Absorption, Minerals and Trimethylamine).

Introduction

Disposal of crab processing waste is a major problem in the crab processing industry. Approximately 90% of the total catch is waste. This material is highly perishable (Cantor, 1980) except when processed immediately.

Crab processing waste can be processed into crab meal. Patton et al. (1975) reported no significant reduction in digestibility of DM, N and Ca when cattle were

fed 10 and 20% crab meal. Samuels (1983) observed a higher DM and organic matter digestibility when sheep were fed silage containing 60% of crab waste than those fed silage containing 40% of crab waste. Abazinge et al. (1986) observed increased digestibility of DM and CP in crab waste-straw mixture ensiled with molasses, compared to mixtures treated with 16% acetic acid or ensiled wheat straw alone. Higher N retention has been reported in cases where crab waste-straw silage is fed to sheep (Samuels, 1983; Abazinge et al., 1986). Lubitz et al. (1943) stated that quality of the shellfish protein from crab meal is higher compared to the fishmeal protein. Absorption and retention of Ca, P and Na were higher when sheep were fed crab waste silage containing 60% crab processing waste than silage containing 40% crab processing waste (Samuels, 1983).

This experiment was conducted to determine the digestibility and nutritional value of crab waste-straw silage when fed to sheep.

EXPERIMENTAL PROCEDURE

Crab waste was obtained from a processing plant[1]. where the meat was hand-picked. The waste was crushed as it was transfered into the horizontal mixer through a semi-vertical auger. The crab waste was treated with .2% NaOCl in the mixer. Approximately 30 min was allowed in order to achieve homogenous mixing. The treated waste was transfered to a truck lined with polytehylene plastic and the top was covered.

The treated crab processing waste was transported to Blacksburg for ensiling. The straw used in this study was ground in a tub grinder through a 2 cm screen. The treated waste, straw, liquid molasses, and water in proportions of 32:32:16:20, wet basis were treated with .1% microbial inoculant[4] (Streptococcus faecium and Lactobaccillus plantarum), and mixed for approximately 20 min. The mixture was then packed in a large polyethylene bag[5]. The mixture was allowed to ensile for a minimum of 16 wk before the metabolism trial began. Part of the silage sample and the initial mixture were prepared and stored for further analysis as described in chapter 3.

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[1] Graham and Rollins, Hampton, Virginia

[4] Pioneer Hi-Bred International, Des Moines, Iowa

[5] Silopress, Inc., Sioux city, Iowa

Eighteen crossbred wethers with an average weight of 43 kg were assigned to six blocks of three animals each based on weight and origin. Sheep within each block were randomly allotted to the following diets: 1) basal, consisting of 30% orchardgrass hay, 65.1% corn grain, 4% soybean meal and .9% limestone, 2) 50:50, ratio dry basis, of basal and crab waste-straw silage, and 3) 100% crab waste-straw silage. All animals were treated for internal parasites with Ivermectin[6] and given 500,000 I.U. of vitamin A and 75,000 I.U. of vitamin D intramuscularly.

The sheep were placed in metabolism stalls similar to those described by Briggs and Gallup (1949) which allowed for separate collection of urine and feces. A 5-d adaptation period to the stalls was followed by a 10-d transition to the experimental diets. Test diets were fed during a 10-d preliminary period followed by a 10-d collection period. Water was provided ad libitum except during feeding. The diets were fed twice daily, in equal portions at 0430 h and 1630 h. Lambs were fed 800 g of DM per day.

Samples of feed were obtained at each feeding 2 d prior to the beginning and 2 d prior to the end of the collection period. The silage samples were immediately

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[6] MSDAGVET, Div. of Merck & Co. Rahway, NJ

frozen in double thickness plastic bags and composited at the end of the trial.

Feces were collected each morning and dried in a forced draft oven at a maximum of 60 C for a minimum of 24 h. For each animal, the dried feces were composited in metal cans, which were loosely covered to allow moisture equilibration. At the end of the trial, fecal composites were weighed, mixed and subsampled. Urine was collected in plastic jars containing 15 ml of 1:1 (W/W) solution of concentrated H_2SO_4 and H_2O and about 500 ml H_2O. Each daily collection of urine was diluted to a constant weight with water and a 2% sample by volume was taken and placed in tightly capped bottles and refrigerated. The samples were subsampled at the end of the trial and frozen for N and mineral analyses. At the end of the trial, ruminal ingesta samples were collected 2 h post feeding via stomach tube with a metal strainer, and blood samples were taken 6 h post feeding by jugular puncture.

Samples of the diet components and feces were ground in a Wiley mill and analyzed for DM, ash (A.O.A.C., 1984), NDF (Van Soest and Wine, 1967), ADF (Van Soest, 1963), lignin and cellulose (Van Soest and Wine, 1968). Gross energy was determined with the use of an adiabatic bomb calorimeter. Nitrogen was determined on wet feed ingredients, the dry fecal samples and urine. The ruminal ingesta was strained through four layers of cheese cloth,

and the filtrate was used for determination of pH, and NH_3-N (Chaney and Marbach, 1962) and VFA (Erwin et al., 1961). Volatile fatty acids were determined with a Vista 6000 gas chromatograph. Blood urea-N was determined by the method of Coulombe and Favreau (1963).

The individual ingredients used in the silage mixture, feeds, fecal and urine samples were digested with a 3 to 1 (v/v) mixture of HNO_3 and $HCLO_4$ (Hern, 1979). The serum samples and digested feed, fecal and urine were analyzed for Ca, Mg, Zn, Cu by atomic absorption and Na and K by emission spectrophotometry. Lanthanum oxide was used in the dilution of the samples used for Mg and Ca analyses to prevent interference from P. The samples were analyzed for inorganic P by the colorimetric method of Fiske and Subbarow (1925).

Statistical Analyses

The data were tested by analyses of variance by the general linear model procedure of SAS (1982). Linear and quadratic contrasts were made.

RESULTS AND DISCUSSION

Chemical Composition

The DM of the silage was 51% (Table 12). Concentration of TMA was 11.2 mg N/100 g. The pH of 6.5, the

TABLE 12. COMPOSITION, FERMENTATION CHARACTERISTICS, AND TRIMETHYLAMINE OF ENSILED CRAB WASTE-STRAW

Item	Silage[a]
Dry matter, %	50.7
Trimethylamine, mg N/100 g[b]	11.2
pH	6.5
Water sol. carbohydrates,%[c]	
Initial	13.0
Final	1.5
Lactic acid, %[c]	4.9

[a] Crab waste, straw, molasses, water (32:32:16:20 wet basis) plus .1% microbial inoculant.
[b] Wet basis
[c] Dry basis

low residual water-soluble carbohydrates (1.5%) and lactic acid concentration of 4.9% indicated that good fermentation was achieved. Samuels (1983) and Abazinge et al. (1986) reported lower values for pH, than those obtained in the present study. Preserving the waste for approximately 3 d prior to completion of the ensiling process may have resulted in increase TMA concentration which subsequently contributed to the buffer effect. A TMA concentration of 7.96 mg/100 g silage was reported by Abazinge et al. (1986) when fresh crab waste was ensiled with straw and molasses.

The values for crude protein, NDF, ADF, and cellulose were higher for diets containing 100% crab waste-straw silage and lowest for the basal diet (Table 13). The CP of crab waste-straw silage (17.2%) was higher than the value (13.5%) reported by Abazinge et al. (1986), but the NDF, ADF and cellulose were lower.

Apparent Digestibility

Apparent digestibility of DM, organic matter, CP, energy, NDF, ADF, cellulose, and hemicellulose decreased linearly ($P<.01$) with increases in the level of crab waste-straw silage fed to the sheep (Table 14). Differences in apparent digestibility of CP were small (64.9, 61.5 and 59.2%). The digestibility values for crab waste-

TABLE 13. CHEMICAL COMPOSITION OF DIETS FED TO SHEEP

Item	Crab waste-straw silage,%[a b]		
	0	50	100
Dry matter,%	89.61	66.71	53.19
Crude protein,%[b]	10.06	13.31	17.25
Ash,%[b]	6.43	13.75	20.05
Acid detergent fiber,%[b]	19.57	28.56	38.27
Neutral detergent fiber,%[b]	40.70	48.80	50.75
Cellulose,%[b]	14.39	20.29	26.60
Gross energy,kcal/g[b]	4.26	4.00	3.91

[a] Crab waste, straw, molasses, water (32:32:16:20 wet basis) plus .1% microbial inoculant.
[b] Dry basis.

TABLE 14. APPARENT DIGESTIBILITY OF DIETS BY SHEEP

Component	Crab waste-straw silage, %[a,b]			
	0	50	100	SE
	----------%----------			
Dry matter[c]	77.1	63.6	44.4	.8
Organic matter[c]	78.1	67.0	46.9	1.0
Crude protein[c]	64.9	61.5	59.2	1.0
Energy[c]	78.3	66.5	48.8	1.3
Neutral detergent fiber[c]	68.9	63.4	44.6	1.5
Acid detergent fiber[c]	62.6	54.3	42.7	1.6
Cellulose[c]	64.4	59.5	46.6	2.1
Hemicellulose[c]	74.7	76.2	50.2	1.9
Lignin[c]	53.5	37.8	26.8	1.5

[a] Crab waste, straw, molasses, water (32:32:16:20 wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect ($P<.01$).

straw silage were lower than those reported previously (Abazinge et al., 1986).

The low digestion coefficient for energy (48.8%) for animals receiving 100% crab waste-straw silage may be due to the diluting effects of high ash content commonly found in shells. The ash content (20.05%) was highest for the 100% crab waste-straw silage. The increase in ash content with percent crab waste-straw silage was accompanied by a decrease in the gross energy (4.26, 4.00 and 3.91 kcal/g for diets with 0, 50 and 100% crab waste-straw silage, respectively). Watkins et al. (1982) showed that increasing the amount of shrimp waste in the diet of minks decreased the fat level, and hence lowered the energy level. Lovell et al. (1968) and Lubitz et al. (1943) reported that high level of Ca in shellfish waste lowers the energy available to the animals. Bull and Reid (1971) reported a decrease in energy value of poultry waste as livestock feed due to the high ash content.

Nitrogen Utilization

Nitrogen intake increased linearly with increase in the level of crab waste-straw silage in the diets (Table 15). Total N excretion increased linearly ($P<.01$) with level of crab waste-straw silage, a reflection of N intake. Van Soest (1982) stated that in situations where requirement for N is met, increase dietary N is generally

TABLE 15. NITROGEN UTILIZATION BY SHEEP FED DIETS WITH DIFFERENT LEVELS OF CRAB WASTE-STRAW SILAGE

Item	Crab waste-straw silage, %[a,b] 0	50	100	SE
Intake, g/d	12.9	17.0	22.1	.0
Excretion, g/d				
Fecal[c]	4.5	6.6	9.0	.1
Urinary[c]	5.2	6.2	8.0	.3
Total[c]	9.8	12.7	17.0	.3
Retention				
Gram/day[d]	3.1	4.3	5.0	.3
% of intake	24.3	25.2	22.8	2.1
% of absorbed	37.3	41.1	38.3	3.0

[a] Crab waste, straw, molasses, water (32:32:16:20 wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect ($P<.01$).
[d] Linear effect ($P<.05$).

balanced with an increase in the urinary output. Tagari et al. (1964) reported that high N intake is accompanied by lower efficiency of N utilization.

Nitrogen retention, expressed as g/d increased linearly ($P<.05$) with level of crab waste-straw silage. These values are in agreement with previous study by Abazinge et al., (1986). Lubitz et al. (1943) stated that the quality of N from shellfish waste is superior to that from fish waste. In the present study, N retention, expressed as percent of intake or absorbed, was similar among the sheep fed diets with different levels of crab waste-straw silage.

Ruminal and Blood Parameters

Ruminal pH and NH_3-N, and the blood urea-N increased linearly ($P<.01$) with increased amount of crab waste-straw silage fed (Table 16). The high ruminal pH found in the sheep fed crab waste-straw silage indicates the buffer effect of $CaCO_3$, which is the main constituent of the shellfish waste (Cantor, 1980). The levels of ruminal NH_3-N in sheep fed crab waste-straw silage, a reflection of the N intake, also contributed to the ruminal pH. In a similar study, Abazinge et al. (1986) reported higher pH, ruminal NH_3-N and blood urea-N for sheep fed crab waste-straw silage. The authors postulated that the high ruminal pH may be an indication of buffer effect produced

TABLE 16. RUMINAL pH AND AMMONIA-N AND BLOOD UREA-N OF SHEEP FED CRAB WASTE-STRAW SILAGE[a]

Item	Crab waste-straw silage, %[b] 0	50	100	SE
Rumen pH[c],	6.04	6.58	7.23	.07
Rumen ammonia-N, mg/100 ml[c],	13.72	21.15	22.53	1.24
Blood urea-N, mg/100 ml[c],	10.21	11.70	16.06	1.15

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis), plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect ($P<.01$).

by the high level of ruminal NH_3. The levels of blood urea-N is relatively proportional to the concentration of ruminal NH_3 (Preston et al., 1965).

Total VFA tended to be higher for sheep fed 50% crab waste-straw silage (Table 17). Expressed as moles/100 moles, only acetic acid was higher for the sheep fed 50% crab waste-straw silage (quadratic effect, $P<.05$). The propionic acid tended to be lower for animals fed the 50% crab waste-straw silage diet. Butyric and isovaleric acids decreased linearly with increases level of crab waste-straw silage. The high level of isovaleric acid may indicate ruminal proteolytic activity (Leng, 1973).

Mineral Utilization

The crab waste used in this study had high levels of Ca, P, Na, Zn and Fe (Table 18). Molasses contains high amounts of Mg, K and Cu. Patton (1971) reported higher levels of Ca, Na, Mg and Cu when crab meal was fed to steers, compared to the basal diet.

Calcium Utilization. Intake of Ca increased with level of crab waste-straw silage in the diet (Table 19). Fecal excretion of Ca increased linearly ($P<.01$) with level of crab waste-straw silage, a reflection of differences in intake. Urinary excretion of Ca was very low for sheep fed all diets. Apparent absorption and retention (g/d) of Ca were negative for animals receiving the con-

TABLE 17. RUMINAL VOLATILE FATTY ACIDS IN SHEEP FED CRAB WASTE-STRAW SILAGE[a]

Item	Crab waste-straw silage, %[b]			
	0	50	100	SE
Total VFA, umol/ml,	65.74	74.84	60.48	4.51
Moles/100 moles				
Acetic[d]	54.06	58.96	55.13	1.30
Propionic	25.68	22.35	26.71	1.75
Isobutyric	2.27	1.27	1.34	.84
Butyric	14.33	14.32	12.24	1.13
Isovaleric[c]	3.10	1.62	1.39	.22
Valeric	1.82	1.60	2.09	.19

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis), plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect (P<.01).
[d] Quadratic effect (P<.05).

TABLE 18. MINERAL CONCENTRATION[a] OF INGREDIENTS AND CRAB WASTE-STRAW MIXTURES

Item	Straw	Molasses	Crab waste	Silage[b]
Calcium, %	.49	1.12	14.26	4.32
Phosphorus, %	.16	.13	1.80	1.30
Magnesium, %	.11	.58	.53	.36
Sodium, %	.15	.12	1.39	.62
Potassium, %	1.83	3.36	1.18	2.94
Copper, ppm	3.50	55.44	20.94	23.00
Zinc, ppm	30.63	41.25	137.50	45.50
Iron, ppm	313.75	359.38	378.75	1085.00

[a] Dry basis.
[b] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.

TABLE 19. UTILIZATION OF CALCIUM BY LAMBS FED CRAB WASTE-STRAW SILAGE[a]

Item	Crab waste-straw silage, %[b]			
	0	50	100	SE
Intake, g/d	3.42	20.72	34.58	.01
Excretion, g/d				
fecal[c]	3.63	11.95	20.69	.75
urinary	.21	.21	.32	.04
total[c]	3.84	12.16	21.02	.75
Apparent absorption				
g/d[c]	-.21	8.76	13.88	.75
Retention				
g/d[c]	-.42	8.55	13.55	.75
% of intake[c]	-12.52	41.29	39.20	3.24
% of absorption	-52.99	97.41	97.61	11.30

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect ($P<.01$).

trol diet, but increased (P<.01) with level of waste. When expressed as percent of intake, absorption and retention were similar for sheep fed 50 and 100% crab waste straw silage. Samuels (1983) reported similar trends when sheep were fed a diet containing crab waste-straw silage compared to those fed the control diet.

Phosphorus Utilization. The P intake (Table 20) was related to level of crab waste-straw silage in the diet. The fecal and total excretion also increased linearly (P<.01) with the level of crab waste-straw silage in the diet. However, absorption and retention increased linearly (P<.01), indicating the P in the silage was utilized.

Magnesium Utilization. The Mg intake increased linearly with level of crab waste-straw silage fed to the sheep (Table 21). Fecal, urinary and total excretion of Mg increased linearly with level of crab waste-straw silage, a reflection of intake of Mg. Differences in apparent absorption and retention of Mg were not large.

Sodium Utilization. The Na intake also was related to level of crab waste-straw silage (Table 22). Excretion of Na increased linearly (P<.01) with level of crab waste-straw silage. Absorption and retention, expressed on g/d were also related to level of silage. However, when expressed as percent of intake or absorbed, retention decreased linearly (P<.01). Results for K followed similar pattern as for Na (Table 23).

TABLE 20. UTILIZATION OF PHOSPHORUS BY LAMBS FED CRAB WASTE-STRAW SILAGE[a]

Item	Crab waste-straw silage, %[b]			SE
	0	50	100	
Intake, g/d	5.13	7.75	10.47	.01
Excretion, g/d				
fecal[c]	3.21	4.00	4.87	.17
urinary[d]	.03	.03	.04	.00
total[c]	3.24	4.03	4.91	.17
Apparent absorption g/d[c]	1.91	3.74	5.60	.17
Retention				
g/d[c]	1.88	3.71	5.55	.17
% of intake[c]	36.73	47.89	53.07	2.87
% of absorption[c]	98.39	99.01	99.22	.17

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect ($P<.01$).
[d] Linear effect ($P<.05$).

TABLE 21. UTILIZATION OF MAGNESIUM BY LAMBS FED CRAB WASTE-STRAW SILAGE[a]

Item	Crab waste-straw silage, %[b]			
	0	50	100	SE
Intake, g/d	1.41	2.09	2.84	.01
Excretion, g/d				
fecal[c]	.35	1.02	1.59	.04
urinary[c]	.31	.34	.36	.02
total[c]	.66	1.37	1.95	.04
Apparent absorption g/d[c]	1.05	1.06	1.24	.04
Retention				
g/d[d]	.74	.71	.88	.04
% of intake[c]	52.83	34.29	31.04	2.01
% of absorption	70.63	67.44	70.32	2.21

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect ($P<.01$).
[d] Quadratic effect ($P<.07$).

TABLE 22. UTILIZATION OF SODIUM BY LAMBS FED CRAB WASTE-STRAW SILAGE[a]

Item	Crab waste-straw silage, %[b] 0	50	100	SE
Intake, g/d	.72	2.92	4.93	.01
Excretion, g/d				
fecal[c]	.06	.21	.47	.03
urinary[c]	.04	1.58	2.88	.07
total[c]	.10	1.79	3.36	.08
Apparent absorption				
g/d[c]	.65	2.70	4.45	.03
Retention				
g/d[c]	.61	1.12	1.56	.08
% of intake[c]	85.87	38.41	31.74	2.66
% of absorption[c]	94.07	41.48	35.06	2.89

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect ($P<.01$).

TABLE 23. UTILIZATION OF POTASSIUM BY LAMBS FED CRAB WASTE-STRAW SILAGE[a]

Item	Crab waste-straw silage, %[b]			
	0	50	100	SE
Intake, g/d	11.97	18.68	23.45	.01
Excretion, g/d				
fecal[c]	1.38	.41	.91	.11
urinary[d]	6.20	12.57	17.01	.26
total[d]	7.58	12.99	17.93	.31
Apparent absorption g/d[d]	10.58	18.26	22.53	.11
Retention				
g/d[e]	4.38	5.69	5.51	.31
% of intake[d]	36.63	30.46	23.52	1.97
% of absorption[d]	41.34	31.14	24.45	1.98

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Quadratic effect ($P<.01$).
[d] Linear effect ($P<.01$).
[e] Linear effect ($P<.05$).

Copper Utilization. Intake of Cu was related to level of crab waste-straw silage (Table 24). Differences in excretion, absorption and retention followed similar pattern as for Mg.

Zinc Utilization. The intake of Zn (Table 25) was likewise related to level of crab waste-straw silage. Excretion of Zn was related to intake. Apparent absorption and retention of Zn were negative among all treatments. However, lowest absorption, expressed as g/d, was for the animals fed 100% crab waste-straw silage. Negative interactions of Fe and Cu on Zn metabolism have been reported (Gipp et al., 1974). Underwood (1977) reported that high dietary Ca depresses Zn absorption. The Ca level (34.6 g/d) in the diet of animals fed 100% crab waste-straw silage may have interfered with Zn absorption.

Iron Utilization. The Fe intake (Table 26) also was related to level of crab waste-straw silage. The apparent absorption and retention were highest ($P<.01$) and positive only for sheep receiving 100% crab waste-straw silage. Absorption and retention were lower for sheep fed 50% crab waste-straw silage than for those fed the basal diet, although intake was over four-fold higher.

Blood Serum Minerals. The serum mineral concentration (Table 27) were within normal ranges (Underwood, 1977; Church, 1979). There was a decrease ($P<.01$) in serum Mg when sheep were fed 100% crab waste-straw silage.

TABLE 24. UTILIZATION OF COPPER BY LAMBS FED CRAB WASTE-STRAW SILAGE[a]

Item	Crab waste-straw silage, %[b]			
	0	50	100	SE
Intake, mg/d	3.20	11.60	18.40	.01
Excretion, mg/d				
fecal[c]	3.29	10.93	17.46	.35
urinary	.20	.18	.20	.01
total[d]	3.49	11.11	17.66	.35
Apparent absorption mg/d[d]	-.09	.66	.94	.35
Retention mg/d[d]	-.29	.48	.73	.35
% of intake[c]	-9.16	4.16	3.99	2.70

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect ($P<.01$).
[d] Linear effect ($P<.05$).

TABLE 25. UTILIZATION OF ZINC BY LAMBS FED CRAB WASTE-STRAW SILAGE[a]

Item	Crab waste-straw silage, %[b]			
	0	50	100	SE
Intake, mg/d	19.20	29.60	36.40	.01
Excretion, mg/d				
fecal[c]	25.70	35.57	52.70	2.31
urinary	1.17	.89	1.06	.18
total[c]	26.87	36.46	53.76	2.35
Apparent absorption				
mg/d[c]	-6.50	-5.97	-16.30	2.31
Retention				
mg/d[d]	-7.67	-6.86	-17.36	2.35
% of intake	-39.97	-23.19	-47.70	9.05

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect (P<.01).
[d] Linear effect (P<.05).

TABLE 26. UTILIZATION OF IRON BY LAMBS FED CRAB WASTE-STRAW SILAGE[a]

Item	Crab waste-straw silage, %[b] 0	50	100	SE
Intake, g/d	.070	.310	.860	.01
Excretion, g/d				
fecal[c]	.133	.415	.449	.04
urinary	.004	.006	.006	.00
total[c]	.137	.422	.456	.03
Apparent absorption				
g/d[c]	-.050	-.100	.410	.04
Retention				
g/d[c]	-.050	-.110	.410	.03
% of intake[c]	-73.55	-36.25	47.39	15.93

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect ($P<.01$).

TABLE 27. SERUM MINERAL CONCENTRATIONS OF SHEEP FED BASAL AND CRAB WASTE-STRAW SILAGE[a]

Item	Crab waste-straw silage, %[b] 0	50	100	SE
	----------mg/dl----------			
Calcium	13.97	13.99	14.26	.16
Inorganic phosphorus	6.08	5.38	5.95	.64
Magnesium[c]	2.61	2.61	2.39	.04
Potassium	26.66	26.67	30.00	.00
Sodium	340	338	335	.01
Iron	.30	.24	.28	.03
Zinc[c]	.12	.10	.10	.00
Copper	.10	.10	.08	.01

[a] Crab waste, straw, molasses water (32:32:16:20, wet basis), plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect (P<.01).

The effect may have been due to increased K intake with level of crab waste-straw silage (Newton et al., 1972). The presence of high Ca or P in the diet has been shown to increase fecal Mg excretion and decrease serum Mg (Chicco et al., 1973; Pless et al., 1973).

The serum Zn concentration was lower ($P<.01$) for sheep receiving crab waste-straw silage. Perry et al. (1968) reported that increasing the level of Ca in the diet of beef cattle from .25 to .50% decreased levels of serum Zn from 214 mcg/100 ml to 188 mcg/100 ml while the serum Ca tended to remain the same. Beeson et al. (1977) reported that supplemental Zn had virtually no effects on levels of Zn in the blood serum except when the dietary level was extremely high (300 or 620 mg/kg). As shown in table 25, it is apparent that the high dietary Zn present in the diet containing crab waste-straw silage was not available for absorption, possibly due to the interactions between the dietary Ca (Luecke et al., 1955; Underwood, 1977) and/or Fe and Cu (Gipp et al, 1974).

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Chapter V

PERFORMANCE AND CARCASS CHARACTERISTICS OF BEEF CATTLE FED THREE LEVELS OF CRAB WASTE-STRAW SILAGE

ABSTRACT

A 108-d trial was conducted with 30 yearling steers. The steers were fed finishing diets in which crab waste-straw silage was included in the diet at 0, 15, and 30%, dry basis. Average daily gain and feed efficiency tended to be highest for steers fed 30% crab waste-straw silage. Carcass weights were highest ($P<.05$) for steers fed 30% crab waste-straw silage. Average carcass quality grade was low choice, and yield grade averaged 2.3, with no significant differences among treatments. Cooking losses tended to be lower for roasts obtained from steers fed the high level of crab waste straw silage. The roasts from steers fed the high level of crab waste-straw silage tended to be more juicy and flavorable compared to the group fed the control diet. Results indicate that feeding crab waste straw silage as roughage and protein supplement did not adversely affect rate of gain, feed efficiency and carcass quality.

(Key words: Crab Waste, Carcass Quality, Feed Efficiency, Daily Gain and Cooking Losses).

INTRODUCTION

In Virginia and Maryland, about 21 million metric tons of crabs are processed annually. Crab processing waste amounts to 90% of the total catch (Brinsfield, 1980). In previous research at this station, crab waste and straw were successfully ensiled when a substantial amount of molasses was added to the mixture (Abazinge, 1986). Limited research has been conducted on feeding of crab waste silage to ruminants. However, studies have shown that crab meal can be successfully incorporated at a limited level in livestock diets (Lovell et al., 1968; Patton et al., 1975; Brundage et al., 1981). Brundage (1986) and Patton et al. (1975) did not observe any effect on daily gain when steers and heifers were fed 7 to 20% crab meal. Dean et al. (1982) reported improved growth rate and feed efficiency when 10% blue crab meal was fed to fingerling catfish. Reduced weight gains were noticed in rats (Watkins et al., 1982) and chickens (Parkhurst et al., 1944) fed crab meal.

An experiment was conducted to evaluate the effect of feeding different levels of crab waste-straw silage on

performance and carcass characteristics of finishing yearling steers.

EXPERIMENTAL PROCEDURE

Thirty Angus and Angus x Hereford crossbred steers were blocked according to breeding and weight, and were randomly allotted to six pens of five cattle each. The pens were alloted at random to diets containing 0, 15 and 30% crab waste-straw silage, dry basis. The basal diet consisted of 30% orchardgrass hay, 65.1% corn, 4% soybean meal, and .9% limestone. Crab waste-straw silage was substituted for protein supplement and roughage and corn grain was adjusted to maintain similar levels of protein and available energy (Table 28).

The crab waste used in this study was preserved with .2% sodium hypochlorite and transported from Hampton to Blacksburg for ensiling. Procedures for preservation have been described (chapter 3). The preserved crab waste (32%), 32% straw, 16% liquid molasses, 20% water and .1% microbial inoculant (S. faecium and L. plantarum) were mixed in a horizontal mixer for about 30 min, then ensiled in a large polyethylene bag[5] which was sealed, and the mixture was allowed to ensile for a minimum of 8 wk.

[5] Silopress, Inc., Sioux city, Iowa

TABLE 28. INGREDIENT AND CHEMICAL COMPOSITIONS OF DIETS FED TO FINISHING STEERS.

Item	Crab waste-straw silage, %[a,b]		
	0	15	30
Ingredient composition[c]	----------%----------		
Orchard grass hay	30.0	14.1	----
Corn grain	65.1	61.1	63.0
Soybean meal	4.0	1.9	----
Crab waste-straw silage	----	22.1	36.1
Limestone	.9	.8	.9
Chemical composition[b]			
Dry matter	87.6	82.8	76.7
Crude protein	10.7	11.2	11.2
Acid detergent fiber	17.6	14.8	14.3

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Wet basis.

Samples of the silage and other dietary ingredients were obtained, prepared, and stored for subsequent analyses as described in chapter 3.

The steers were housed in pens in an open front shed equipped with automatic waterers and block iodized salt was provided. Before the introduction of the test diets, the steers were dewormed with Ivermectin[6]. The silage and dry ingredients fed to the steers were mixed together in order to avoid selection by the steers.

At the beginning and end of the trial, the steers were weighed twice, and once every 2 wk during the trial before the morning feeding. The two successive weights obtained at the beginning and at the end of the trial were averaged for the initial and final weights. The trial continued for 108 d, after which the steers were slaughtered and carcass characteristics were obtained. Visual scoring of the carcass characteristics was done by trained panel of four. Rib roasts from 6th to 8th ribs of each carcass were cooked in a pressure cooker to an internal temperature of 70 C. The meat was randomized into either like or odd- samples, and definitions of characteristics to be evaluated were explained to a trained taste panel of 26. The panel went through orientation, followed by sensory evaluation of the meat at different periods during

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[6] MSDAGVET, Div. of Merck & Co. Rahway, NJ

the evaluation. The meat samples were scored according to flavor and overall desirability. The 8-point hedonic scale was used by the taste panel to evaluate the samples.

Statistical Analyses. Data were analyzed using the general linear model (GLM) procedure of the Statistical Analysis System (SAS, 1982). Linear and quadratic contrasts were used to test the responses of animals receiving the test diets.

RESULTS AND DISCUSSION

The composition, fermentation characteristics and the trimethylamine (TMA) concentration of the silage has been presented previously (chapter 3). The pH of 6.5, a reduction in initial water-soluble carbohydrates from 13.0 to 1.5, and the elevated lactic acid concentration indicated that effective fermentation was achieved. Abazinge et al. (1986) reported a pH of 6.0 and lactic acid concentration of 3.25 in fish waste-straw silage fed to steers. The TMA concentration of 11.21 mg N/100 g silage obtained in the silage did not affect the acceptability of the silage to the steers nor impart any deleterious characteristics to the silage. The CP values were similar among diets but ADF was lower for animals fed 15 and 30% crab waste-straw silage (Table 28).

Initially, steers receiving 0 and 15% crab waste-straw silage showed higher but gradually decreasing daily gain compared to the trend observed with steers fed 30% crab waste-straw silage. Beginning at d 56, steers fed 30% crab waste-straw silage showed an increased and higher daily gain which persist through the trial (Figure 7). The steers fed 30% crab waste-straw silage maintained the highest periodic gain at d 56, 70 and 108, compared to those fed 0 and 15% crab waste-straw silage (Figure 8). Overall, daily gain tended to be higher for steers receiving 30% crab waste-straw silage. Average DM intake per day was highest for the steers receiving 15% crab waste silage and lowest for the steers fed the control diet (Table 29).

Dry matter per kilogram gain tended to be lower for the cattle fed the higher level of crab waste-straw silage. Watkins et al. (1982) reported lower weight gains and poorer feed efficiency for minks fed 10 and 20% crustacean meal. Brundage (1986) reported a reduction in daily gain in steers fed 7.5 to 22.5% tanner crab meal. However, Patton et al. (1975) and Wignall et al. (1976) observed no reduction in weight gains, but improved feed efficiency with steers fed either crab meal or fish silage. Parkhurst et al. (1944) reported a rapid growth and positive feed efficiency with chickens fed either fish meal or crab meal. Lubitz et al. (1943) stated that 1 g

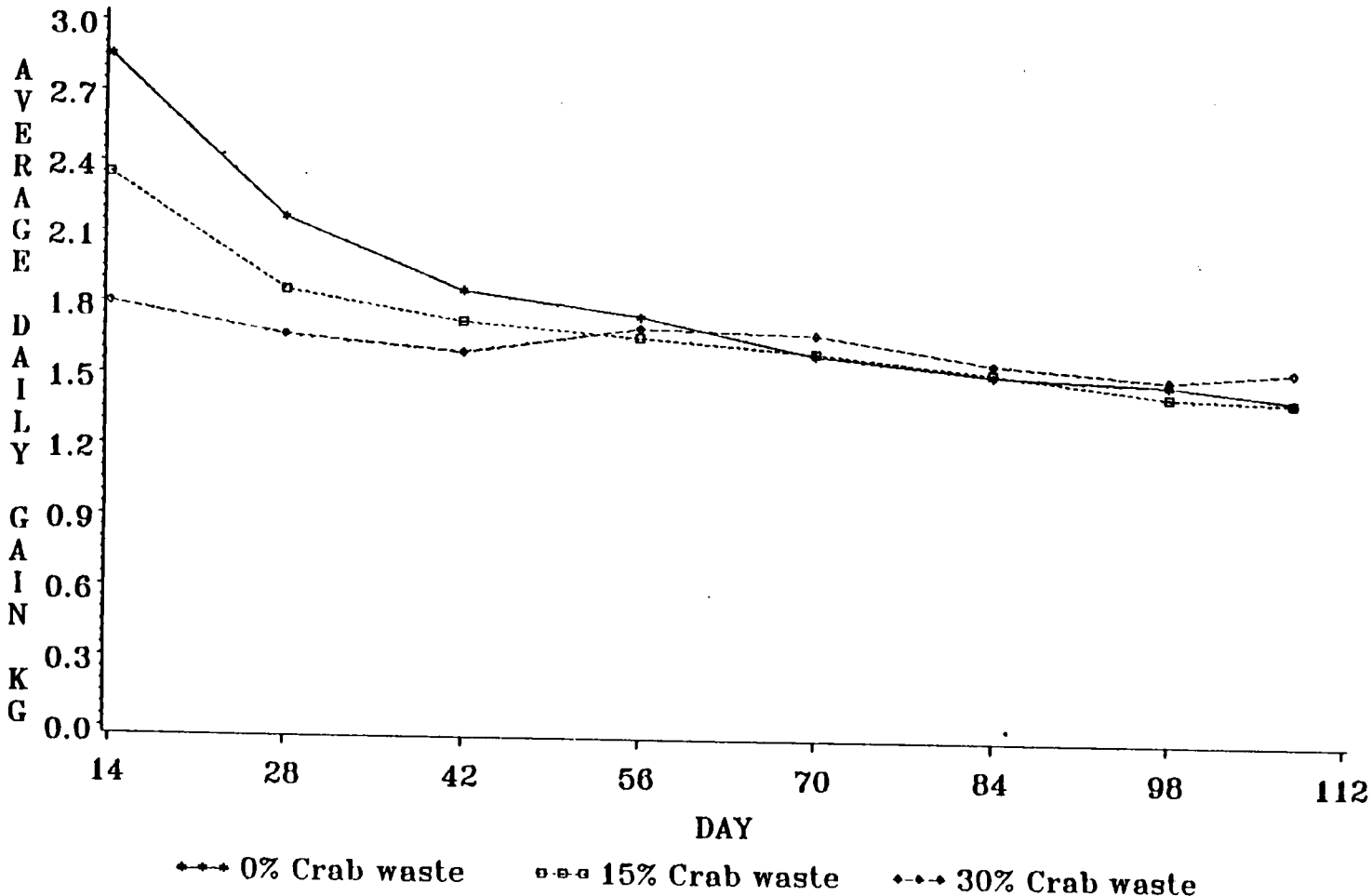

Figure 7. Cumulative daily gain of steers fed three levels of crab waste– straw silage (Dry basis).
Linear effect for 14 d. (P<.01). SE=.33.
Linear effect for 28 d. (P<.01). SE=.25.
Linear effect for 42 d. (P<.01). SE=.19.

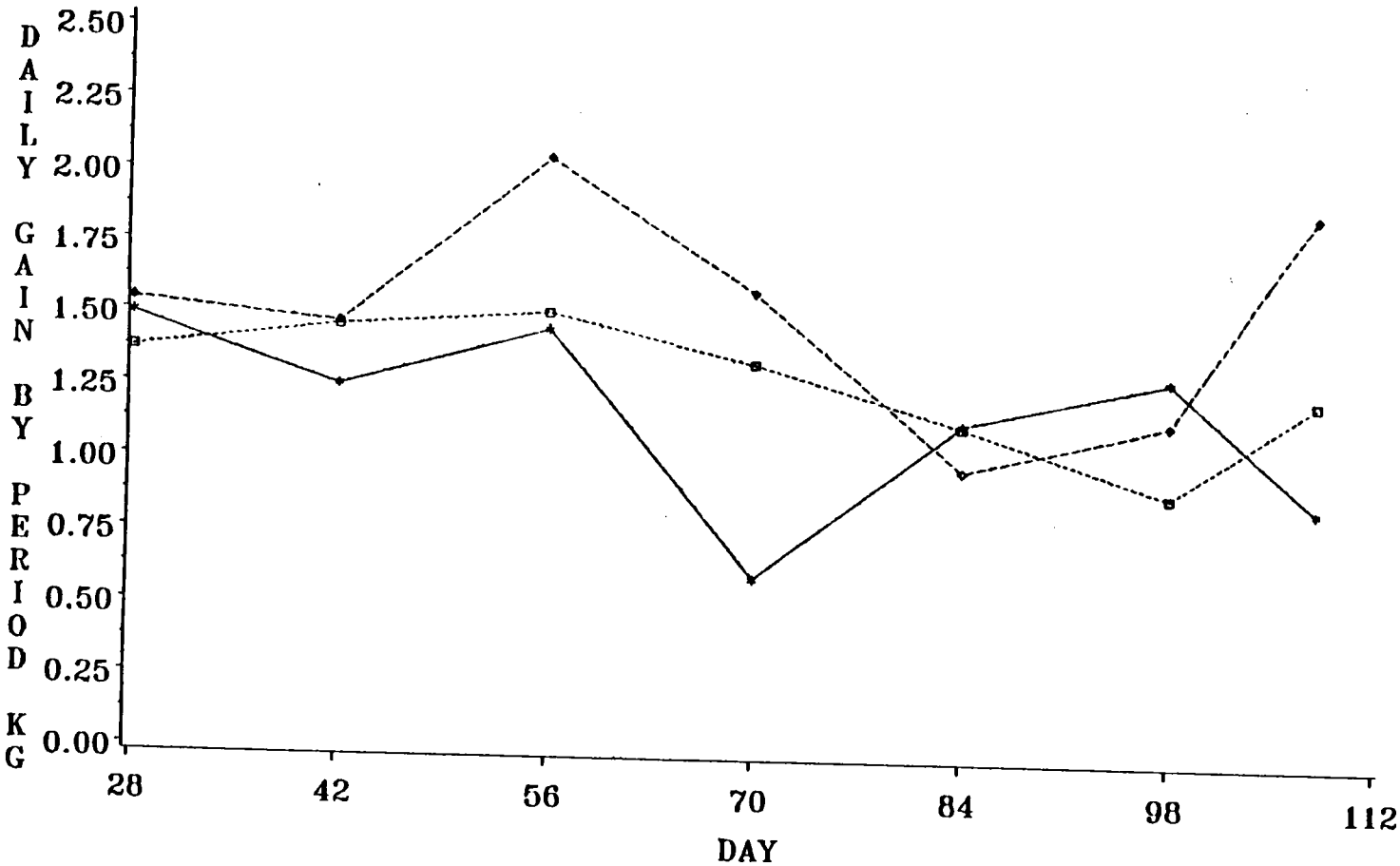

FIGURE 8. Periodic daily gain of steers fed three levels of crab waste– straw silage (Dry basis).
Linear effect for 56 d. (P<.05). SE=.40.
Linear effect for 70 d. (P<.01). SE=.28.
Linear effect for 108 d. (P<.01). SE=.46.

TABLE 29. PERFORMANCE OF STEERS FED TWO LEVELS OF CRAB WASTE SILAGE

Item	Crab waste-straw silage, %[a,b]			
	0	15	30	SE
Performance				
Initial weight.,kg.	373.9	379.1	378.3	10.0
Final weight.,kg.	529.0	533.1	545.9	15.9
Average daily gain,kg.	1.43	1.42	1.55	.1
Dry matter intake,kg/d.	11.7	12.3	12.0	.5
Dry matter/gain	8.1	8.7	7.8	.4
Carcass characteristics				
Carcass weight[e],kg.	303.6	304.1	319.4	10.2
Marbling[c]	4.6	4.7	5.1	.3
Quality grade[d]	12.4	12.6	12.9	.3
Dressing percent	57.4	57.1	58.5	.6
Ribeye area, sq cm.	76.1	78.7	81.9	.4
Backfat,cm.	1.2	1.0	1.2	.1
Kidney,pelvic-heart fat[f],%	2.3	2.5	2.1	.1
Yield grade	2.4	2.1	2.3	.2

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Code: 3-slight; 4-small; 5-modest, etc.
[d] Code: 11-high good; 12-low choice; 13-average choice;etc.
[e] Linear effect (P<.05).
[f] Quadractic effect (P<.05)

of crab meal protein has a supplementary growth promoting value equivalent to .76 g of casein protein for chickens.

Carcass weight was highest ($P<.05$) for steers fed 30% crab waste-straw silage, a reflection of the higher rate of gain. The dressing percent, marbling, quality grade and ribeye areas tended to be higher for steers fed 30% crab waste-straw silage. The kidney-pelvic-heart fat was highest ($P<.05$) for steers receiving 15% crab waste-straw silage.

The texture and firmness of lean tended to be higher for carcasses of steers fed the control diet (Table 30). The desirable characteristics of external fat was lower ($P<.05$) in carcasses of steers fed 30% crab waste-straw silage. The color of fat and lean was similar for carcasses of steers receiving the different diets. Total cooking losses (Table 31) were not significantly different among the roasts obtained from steers fed the diets.

Flavor and juiciness of roasts were not adversely affected by feeding crab waste silage. In fact, values tended to be higher for meat from steers fed 30% crab waste-straw silage. Luscombe (1973) reported good quality carcasses free of any taint in pigs fed fish silage. Parkhurst et al. (1944) did not observe any off-flavors in meat obtained from chicken fed crab meal diets. Watkins et al. (1982) reported that feeding crustacean waste to minks did not affect the pelt color or quality

TABLE 30. VISUAL SCORING OF THE CARCASSES FROM STEERS FED THREE LEVELS OF CRAB WASTE-STRAW SILAGE

Item	Crab waste-straw silage, %[a b] 0	15	30	SE
Texture[d]	4.02	3.62	3.88	.15
Firmness of lean[e]	3.90	3.77	3.88	.08
Color of lean[f]	3.67	3.62	4.00	.18
Quantity of exudate[g]	4.41	4.42	4.38	.15
Characteristics of external fat[c h]	4.15	4.02	3.78	.10
Color of fat[i]	4.18	4.02	4.22	.12

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Linear effect (P<.05).
[d] Code: 5-very fine, 4-fine, 3-slightly coarse etc.
[e] Code: 5-very firm, 4-firm, 3-moderately firm etc.
[f] Code: 5-cherry red, 4-moderately dark red, 3-dark red etc.
[g] Code: 5-moist, no exudate, 4-slight exudate etc.
[h] Code: 5-very firm and very brittle, 4-firm and brittle, 3-moderately firm etc.
[i] Code: 5-creamy white, 4-slightly creamy white, 3-slightly yellow etc.

TABLE 31. COOKING DATA AND TASTE PANEL EVALUATION OF BEEF FED THREE LEVELS OF CRAB WASTE-STRAW SILAGE

Item	Crab waste-straw silage, %[a b]			
	0	15	30	SE
Evaporative losses, %	20.35	20.34	20.58	.67
Drip losses, %	8.61	8.95	8.47	.56
Total cooking losses, %	28.96	29.29	29.06	.79
Flavor[c]	4.92	4.94	5.07	.12
Juiciness[d]	4.80	4.67	4.92	.15
Mouthfeel[e]	4.82	4.79	4.64	.11

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Dry basis.
[c] Code: 8 = extremely desireable to 1 = extremely undesireable
[d] Code: 8 = extremely juicy to 1 = extremely dry.
[e] Code: 8 = extremely firm to 1 = extremely soft.

of the mink carcasses. A recent study by Angelo et al. (1988) showed that the use of N-carborxymethyl (a derivative of chitin from crab shell) inhibited hexanal and 2-thiobarbituric acids, which are the main constituents responsible for the off flavors in meat. Feeding up to 30% fish waste-straw silage to finishing steers did not adversely affect taste of the meat (Abazinge, 1986).

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Chapter VI

METHODS OF DETERMINING CHITIN IN CRUSTACEAN MEAL

ABSTRACT

Treating crab meal for 24 h with 15% trichloroacetic acid (TCA) (w/v), 5% HCL (v/v) or 10% formic acid was effective in removing the minerals in the meal. Treating the meal with .1 M ethylenediaminetetraacetic acid (EDTA) did not remove any minerals. However, the initial demineralization of samples with HCl, TCA and/or deproteinization with NaOH resulted in degradation and loss of chitin. Chitin was estimated in mixtures of different proportions of crab meal and straw. The chitin values obtained with the ADF/ashing procedure were similar to those obtained with the ADF/deacetylation procedure. Overestimation of chitin content obtained when ADF/ashing procedure was used was eliminated by delignifying the residue obtained after acid detergent treatment. Treating the residue with $KMNO_4$ removed the lignin commonly found with plant fiber and the carotenoids found in association with crab shell. The ADF/deacetylation procedure is appropiate in determining the chitin content of mixed samples of unknown proportions of crab meal and straw,

while ADF/ashing procedure can be used to estimate chitin in known proportions of crab meal and straw mixtures.

(Key words: Chitin, Crab Meal, Acid Detergent Fiber, Ashing, Deacetylation and Potassium Permanganate).

INTRODUCTION

Chitin is a poly-N-acetylglucosamine and a structural component of crustacea (Rudall, 1965), silk worm pupae (Bergmann, 1938), insect cuticles (Rosedale, 1945; Patton et al., 1975), fungi and other microorganisms (Rammelberg, 1931; Smith, 1936). The molecular structure of chitin is (1-4)-2-acetamido-2-deoxy-β-D-glucan (Gardner and Blackwell, 1975). These authors stated that the chain that constitutes the structure of chitin is similar to that found in cellulose, except that the hydroxyl group on cellulose structure is replaced by the trans-planar acetyl group in chitin. There are three forms of chitin (α, β and ɣ), all with different functions (Rudall, 1955). The author reported that chitin found in plants serves similar purpose as cellulose while in animals the functions are similar to that of collagen. The α chitin is the most stable form commonly found in crustacea (Rudall, 1965). Different forms of chitin found in nature have made the methods of quantifying chitin difficult and inaccurate.

Chitin of crustacean origin is frequently sclerotized and encrusted with mineral deposits such as $CaCO_3$ (Lovell et al., 1968). Thus, harsh chemical treatment may be needed in order to obtain chitin in its free form (Richards, 1978). Hydrochloric acid (Rutledge, 1972), EDTA and TCA (Meenakshi et al., 1971; Welinder, 1974) have been used extensively as demineralizing agents. However, considerable loss of chitin is observed due to the use of these chemicals (Hackman et al., 1974). These authors stated that the losses occurred during initial separation of chitin into different components. Muzzarelli (1977) reported severe degradation of chitin during decalcification when crustacea shells were treated with concentrated acids. Similar degradation was reported when chitin was deprotinized with hot alkali.

The crude fiber method has been precise to determine chitin (Black et al., 1950), however, it is difficult to use this method in estimating chitin mixed with other fiber particles. Hackman (1962) reported the separation of chitin component into saccharides, glucosamine and acetic acid. Saccharides and glucosamine constitute chitosan (Muzzarelli, 1977), which can be quantified colorimetrically (Wieckowska, 1968a). Ride and Drysdale (1972) used alkali to deacetylate the chitin of fungi into chitosan. The glucosamine was obtained and later converted to aldehyde which was then measured colorimetrically.

Holan et al. (1971) and Patton et al. (1975) used hot alkali to deacetylate the isolated chitin into different components. The acetate recovered was then injected into gas-liquid chromatograph. The use of glucosamine to estimate chitin is not accurate since other polysaccharides yield glucosamine during acidic hydrolysis of chitin (Hackman et al., 1971). Chitin has been reported to yield different amounts of polyaminosaccharides (Muzzarelli, 1977). Krishnan et al. (1955) reported that chitin obtained from insect cuticles (Palamneus swammerdami) yield galactose instead of glucose, while chitin of cuttlefish yield galactose and mannose after hydrolysis (Stegemann, 1963).

These experiments were conducted to study the effectiveness of modification to the existing methods of determining chitin with minimal losses of the components during preparation.

MATERIALS AND METHODS

Experiment 1. Crab processing waste was obtained from a processing plant in Hampton, Va[1]. The waste was treated with .2% NaOCl as described in chapter 3. The treated waste was preserved for 5 d in 210 liter metal

[1] Graham and Rollins, Hampton, Virginia

drums, and a sample was dried in a forced-draft oven at 65 C for 48 h to produce crab meal. Dried waste and wheat straw were ground separately in a Wiley mill through a 2 mm screen. The ground materials were kept in air-tight containers. One gram of crab meal or wheat straw was placed in 75 ml polyethylene tubes with 20 ml of the following: .1M of EDTA, 2.5 or 5% acetic acid, 10 or 15% TCA, 10 or 15% formic acid, 3.2 or 5% HCL or water (control). The tubes were capped tightly and allowed to agitate for 24 h in a mechanical shaker at room temperature. Approximately 2 g of the untreated samples were weighed into crucibles and ashed in a muffle furnace at 600 C for 2 h (AOAC, 1984). The ash residue obtained represented the initial ash concentration in the samples.

At the end of 24 h the tubes were centrifuged at 800 x G for 15 min followed by rinsing with deionized water. This procedure was repeated twice. The treated material was washed into gooch crucibles with water. The water was filtered and the crucibles with the contents were placed in an oven and dried at 100 C for 12 h. The crucibles were then placed in a muffle furnace and ashed for 3 h at 500 C (AOAC, 1984). The initial ash concentration and the residual ash value of the treated samples were used to calculate the percent of minerals removed.

Experiment 2. The crab processing waste was obtained from the same source as in experiment 1. The crab waste and wheat straw were processed and stored as described in experiment 1. The following proportions of straw and crab meal were mixed until homogenous mixture were obtained: 100:0, 75:25, 50:50, 25:75, and 0:100. One gram of each sample was weighed into containers. The samples were subjected to three different methods to determine chitin concentration.

For method 1, the samples were weighed into 600 ml Erlenmeyer flasks. Approximately 200 ml of ADF solution were added to each flask. The samples were refluxed for 60 min. (Van Soest, 1963), then washed with hot water into weighed gooch crucibles. Rinsing of the treated material was repeated once with hot water and once with 80% ethanol, respectively. The crucible and the content were dried in the oven at 100 C for 24 h. The residual weight was obtained and the residue was kept for further analysis.

For method 2, the samples were weighed into 75 ml polyethylene tubes, and 20 ml of 5% HCl (v/v) were added. The tubes were tightly capped and shaked in a mechanical shaker, for 24 h followed by centrifugation at 800 x G, and the supernatant was discarded. Approximately 20 ml of water were added, the tubes were allowed to stand for about 1 min after which they were centrifuged, and the

supernatant was discarded. This step was repeated twice. The decalcified sample was then washed into 600 ml Erlenmeyer flask with about 15 ml of 5% NaOH. Additional NaOH solution was added, so a total of 100 ml of NaOH was used to reflux this material for 90 min at 100 C. Treatment of the samples with NaOH was necessary to remove the N commonly bound to the chitin. The material was filtered through the gooch crucible, followed by rinsing three times with hot water. The crucible and the contents were dried in the oven at 100 C for 24 h. The residue obtained was kept for further analysis.

For method 3, approximately 20 ml of 10% TCA were used in demineralizing the samples. The remaining procedure was as described in method 2, except the treated material was refluxed for 3 h with 8% NaOH.

The residues obtained from methods 1, 2 and 3 were subjected to either ashing at 500 C for 3 h (AOAC, 1984), or deacetylated with 10 ml of saturated KOH at 160 C for 30 min (Hackman, 1973; Patton et al., 1975). Exactly 5 ml of H_2O and 15 ml of 12N HCl were added to the deacetylated material in order to precipitate the chitosan (Muzzarelli, 1977; Okafor, 1978). The content was distilled by using a 125 ml round bottom flask placed in a heating mantle. The distillate was prepared for injection into the gas-liquid chromatograph (GLC) by adding 5 ml of the distillate with 1 ml of metamorphosphoric acid, and 5

ml of 4-methyl valeric acid solution (internal standard) (Erwin, et al., 1961). The sample was injected into the GLC (Vista 600 series) to quantify acetate. As a control treatment, samples from different proportions of straw and crab meal were either ashed (500 C for 3 h) or deacetylated directly without any prior chemical treatment. The equation used to calculate the percent chitin determined by ashing method is as follows:

$$\text{Percent chitin} = \frac{\text{Dried residue - ashed residue}}{\text{Initial sample (dry basis)}} \times 100$$

Proportion of straw in each sample was used as a factor. The proportional amount of straw in each mixture was multiplied by the residue obtained from straw alone. This value was then substracted from the total residue obtained from straw and crab meal mixture. The value obtained represents chitin content. The equations for determining chitin concentration via deacetylation procedure is as follows:

1) X um/g, as is basis = um/ml of acetate x dilution factor

2) X um/g, dry basis = X um/g as is basis - dry matter/100

1 um = 60 ug of acetate

3) X ug/g = X um/g x 60 ug

4) X percent of acetate = X ug/g x 10^{-4}

Theoretically, 1 g of chitin = .22 g of acetate.

Experiment 3. This experiment was conducted in order to improve on the recovery of chitin obtained in experiment 2. When ADF solution (Van Soest, 1963) was used, it was observed that the value obtained by difference for total chitin/cellulose may be overestimated due to the presence of lignin commonly found with fiber particles. Carotenoids with crab shell may also be a source of error. Samples used in this study were from the same source as in experiment 1 except the crab processing waste was not treated with any additives, but was frozen immediately. The waste was thawed and dried in a forced-draft oven at 65 C for 48 h to produce crab meal. Similar proportions of straw and crab meal were used as in experiment 2. The samples were weighed into 600 ml of Erlenmeyer beakers, and refluxed with 200 ml of ADF solution as described in experiment 2. A set of the refluxed samples was dried at 100 C for 24 h. Residual weight was obtained and the residue was subjected to ashing in a muffle furnace at 500 C for 3 h (AOAC, 1984). The other set was treated with $KMNO_4$ solution, (Van Soest and Wine, 1968) to remove the lignin and possibly the carotenoids. The residue was weighed and ashed as described above.

Further studies were also conducted to improve on the chitin estimation through deacetylation method. In this procedure, the deproteinization step was omitted due to the significant degradation of the chitin caused by

alkali. Also, effects of delignification on the chitin and the fiber particles were investigated. Samples of crab waste as previously described, and practical grade chitin[7]. were individually subjected to three different methods.

For method 1, 1 g samples were weighed into 600 ml Erlenmeyer flasks. For each sample, eight replicates were prepared. The samples were refluxed with ADF solution as previously described. The residues obtained after treatment with ADF solution were divided into two sets of four replicates. One set of replicates was further treated with $KMNO_4$ solution (Van Soest and Wine, 1968). The residues obtained after ADF and ADF/$KMNO_4$ treatments were deacetylated with saturated KOH and the distillate was prepared for acetate quantification as described in experiment 2.

For method 2, eight replicates of the same amounts of samples were weighed into 75 ml polyethylene tubes. Approximately 20 ml of 5% HCL (v/v) or 10% TCA (w/v) were added to the tubes containing the samples. The samples were treated as described in experiment 2 (methods 1 and 2). The treated samples were later refluxed in ADF solution as described above (method 1, experiment 2). One set of the residues (four replicates) was further treated with

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[7] Sigma Chemical Company St. Louis, MO

$KMNO_4$ solution (Van Soest and Wine, 1968). These residues and those obtained after the ADF treatment only were deacetylated with saturated KOH and the distillate was prepared for acetate quantification as described in experiment 2.

RESULTS AND DISCUSSION

Experiment 1. Prior to estimation of chitin from crustacea shells, the chitin has to be isolated from its bound form with minerals (Lafon, 1941). The use of TCA, formic and HCl at different levels enhanced the removal of minerals from the crab meal (Table 32). However, 15% of TCA and 5% of HCl were more effective in demineralization of the straw. Formic acid (Horowitz et al., 1957), HCl (Welinder, 1974) and TCA (Andersen, 1967) had been used extensively in decalcification of crab meal prior to chitin determination. In the present study, addition of .1M EDTA to the samples was not effective in removing the minerals associated with the crab meal. Foster and Hackman (1957) had stated that EDTA at pH of 9 will slowly decalcify the crustacea meal without any degradation of chitinous material. This reaction takes 2 to 3 wk. The lowest extraction of minerals associated with straw was observed with straw incubated for 24 h with water. The losses may reflect soluble portion of minerals

TABLE 32. EFFECT OF CHEMICAL TREATMENT ON DEMINERALIZING OF THE CRAB PROCESSING WASTE AND STRAW

Material	Water	EDTA[a] .1M	Acetic 2.5%	Acetic 5%	TCA[b] 10%	TCA[b] 15%	Formic 10%	Formic 15%	HCL[c] 3.2%	HCL[c] 5%
					%[d]					
Crab waste	9.2	.0	35.9	65.9	86.8	86.9	87.1	85.9	88.5	86.3
Straw	6.5	8.3	19.2	17.0	23.2	30.8	29.0	29.4	26.8	32.3

[a] Ethylenediaminetetraacetic acid.
[b] Trichloroacetic acid.
[c] Hydrochloric acid.
[d] Minerals removed.

found in straw. Lovell et al (1968) indicated that 1N or 5% (v/v) HCl was more effective in removing the inorganic materials associated with crustacean meal than formic acid.

Experiment 2. The schematic diagram of chitin procedure used for the initial chitin analysis is presented in figure 9. Recovery of chitin was highest when ADF residue was deacetylated or ashed, compared to the chitin values from residue of HCl/ADF and TCA/ADF pretreated samples (Table 33). The use of NaOH as a deprotinizing agent may have contributed more to the loss of chitin observed when HCl/ADF and TCA/ADF were used to treat the samples. Andersen (1967) and Welinder (1974) reported lower recovery of chitin when molluscs and crustacean meal were decalcified with acids. However, losses of chitin are greater with hot alkali, compared to acid treated samples (Foster and Hackman 1957; Whistler and BeMiller, 1962).

Chitin values obtained through ADF/ashing procedure tended to be greater than those obtained from ADF/deacetylation procedure. It is possible that the values obtained with ADF/ashing procedure were overestimated due to the presence of lignin complex commonly found with plant fiber. The chitin recovered from samples containing 100% crustacean meal may also have been overestimated due to the presence of carotenoids when samples were subjected to ADF/ashing procedure. When ADF/ashing procedure was

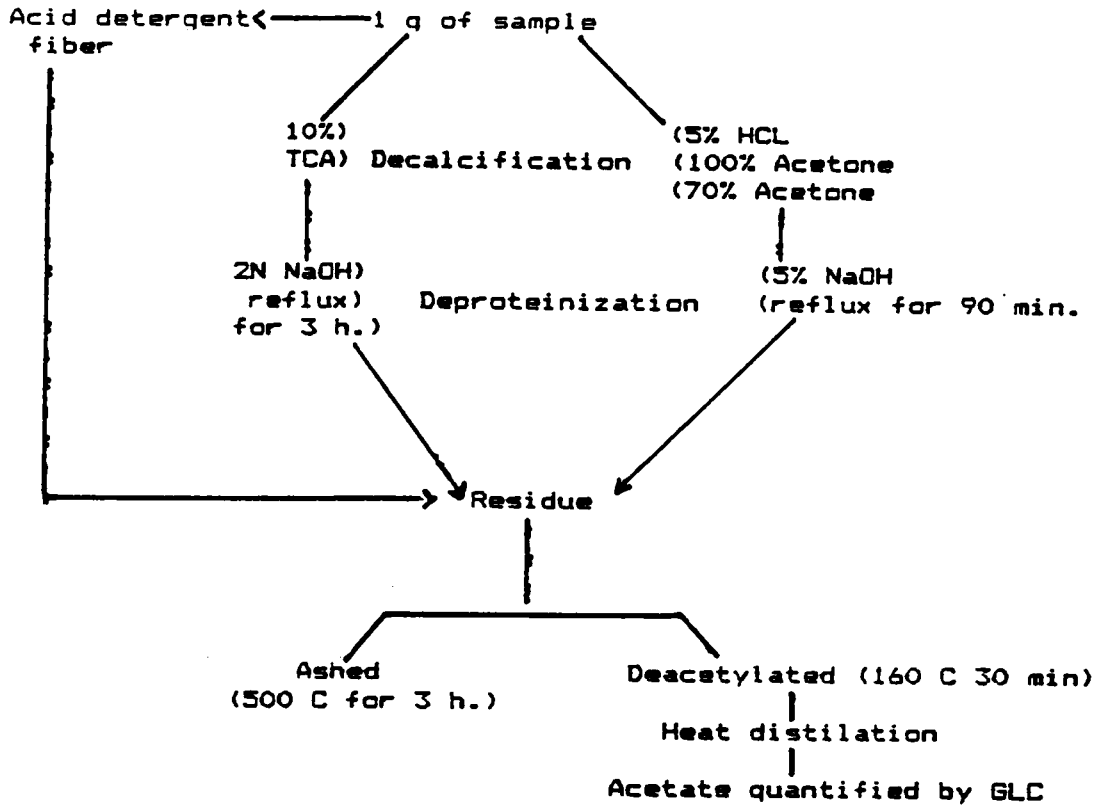

Figure 9. Procedures Used Initially for Chitin Assay.

TABLE 33. EFFECT OF CHEMICAL TREATMENT ON CHITIN ASSAY

Method[a]	Proportion of straw:crab waste[b]			
	75:25	50:50	25:75	100:0
	%[c]			
ADF-ashing	2.9	6.4	9.5	12.7
ADF-deacetylation	3.1	5.8	9.0	11.9
TCA-ashing	3.9	5.9	8.7	10.7
TCA-deacetylation	2.8	5.4	8.9	10.2
HCL-ashing	2.9	5.9	8.2	10.4
HCL-deacetylation	3.0	6.2	8.3	10.8
Untreated-ashing[d]	86.6	77.9	64.9	56.6
Untreated-deacetylation[d]	12.7	11.6	11.9	12.3

[a] NaOH solution was used to reflux residues obtained after treatment with ADF, TCA and HCL.
[b] Wet basis.
[c] Chitin concentration, dry basis.
[d] Samples used without any prior treatment.

used, a value of 12.7% chitin was found with sample containing 100% crab meal. Stelmock et al. (1985) reported a value of 13.74% chitin with tanner crab meal subjected to ADF/ashing method. The low value of 12.7% chitin may be a reflection of losses in chitin, carotenoids and organic matter as a result of refluxing the residue with NaOH. Perhaps, the carotenoids or organic matter associated with crab meal or straw need to be removed to avoid overestimation of chitin content obtained when ashing procedure is used. Muzzarelli (1977) had stated that carotenoids are found as conjugates with chitin and protein. Fox (1973) reported that carotenoids are combined with chitin amino groups by carbonylamino linkages. Removal of these carotenoids is possible after decalcification of the samples or when the chitin has been exposed as a result of harsh chemical treatment (Muzzarelli, 1977). The removal of carotenoids has been reported by treating the samples in warm ethanol or acetone, or .02% $KMNO_4$ at 60 C (Muzzarelli, 1977), or by immersing the decalcified sample in cold hypochlorite solution containing .3 to .5% available chlorine (Madhavan et al., 1974) or bleaching with H_2O_2 (Moorjani et al., 1975).

Experiment 3. The elimination of the deprotinization step reduced to a lesser extent the losses of chitin in all samples, but considerable losses were observed with

samples demineralized with TCA (Table 34). The elimination of protein bound to the chitin may not be necessary when chitin is to be determined via deacetylation procedure. Muzzarelli (1977) reported that attempts to remove protein from the crustacean meal is unneccessary if the samples are to be deacetylated to quantify chitosan. In the present study, delignification did not seems to be important for samples of crab meal or chitin. Treating samples containing different proportions of straw and crab meal with $KMNO_4$ solution resulted in losses of lignin with plant fiber and possible losses of carotenoids commonly found with crab shell. However, in the present study, delignification did not consistently affect the chitin recovered (Table 35). Presented in figure 10 is the modified chitin procedure. The demineralization and deprotinization steps have been omitted while the use of $KMNO_4$ which may improve chitin determined by ADF/ashing procedure was included. However, preliminary delignification of residues prior to deacetylation may not be necessary.

TABLE 34. EFFECT OF CHEMICALS AND DELIGNIFICATION ON CHITIN DETERMINATION BY DEACETYLATION PROCEDURE

Material	Delignification treatment	Pretreatment		
		ADF[a]	TCA[b] and ADF	HCL[c] and ADF
		%		
Crab meal	$KMNO_4$[d]	12.58	11.88	12.87
	None	12.23	11.15	12.77
Chitin[e]	$KMNO_4$[d]	64.05	66.26	64.47
	None	64.80	64.05	63.44

[a] Acid Detergent Fiber only.
[b] ADF and trichloroacetic acid.
[c] ADF and hydrochloric acid.
[d] Potassium permanganate.
[e] Practical grade, Sigma Chemical Company.

TABLE 35. EFFECT OF DELIGNIFICATION ON CHITIN RECOVERY AS MEASURED BY ACID DETERGENT FIBER/ASHING PROCEDURE

Straw:Crab meal	Total residue[a]		Chitin[b]	
	Lignified	Delignified[c]	Lignified	Delignified[c]
	%			
100:0	53.07	44.09	---	---
75:25	41.85	35.63	2.05	2.56
50:50	33.40	27.95	6.87	5.91
25:75	22.51	20.44	9.24	9.41
0:100	12.31	12.34	12.31	12.34

[a] Includes cellulose and chitin, determined by ADF/ashing procedure.
[b] Percent chitin estimated by using proportion of straw in the residue.
[c] Treated with potassium permanganate.

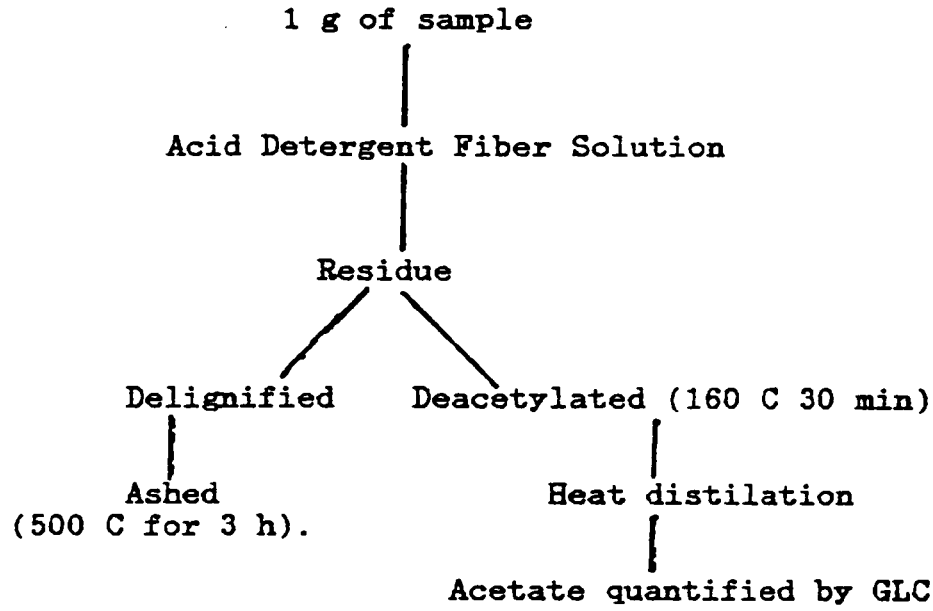

Figure 10. MODIFIED PROCEDURE FOR CHITIN ASSAY

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Chapter VII

CHITIN DIGESTIBILITY OF CRUSTACEAN MEAL AS MEASURED BY IN VITRO, IN SITU AND IN VIVO METHODS

ABSTRACT

The acid detergent fiber (ADF)/ashing and ADF/deacetylation procedures were used to estimate the disappearance of chitin in vitro, in situ and in vivo. Samples consisting of practical grade chitin and different proportions of straw to crab meal were placed in nylon bags in the rumen of a steer, or in tubes containing ruminal fluid incubated at 39 C. Greater disappearance of chitin was observed in samples kept in the rumen for 48 h or more. Highest ($P<.01$) disappearance (33 and 36%) was observed when 100% crab meal was kept in the rumen for 72 h. Similar disappearance of chitin was observed in the in vitro study. Keeping 100% practical grade chitin in the rumen for 72 h resulted in 26 and 35% disappearance for the in situ study, and 32 and 31% for the in vitro study, respectively, for the ADF/ashing and ADF/deacetylation methods of analysis. Lower disappearance of chitin was observed in samples containing large proportion of plant fiber. In a digestion trial, sheep were fed a basal diet,

100% crab waste-straw silage (32:32:16:20, of straw, crab waste, molasses, and water, wet basis) or a diet containing 50% crab waste-straw silage and 50% basal diet, dry basis. Chitin digestibility of 58% was observed with animals receiving 100% crab waste straw silage, compared to 37% ($P<.01$) for animals fed 50% crab waste straw silage. The amount of chitin digested by ruminants may depend on microbial preference for certain organic matter. Chitin in its natural form (crab meal) was more available to the ruminants than the isolated chitin (practical grade chitin).
(Key words: Chitin, Crab Meal, In Vitro, In Situ, In Vivo, Acid Detergent Fiber, Deacetylation and Disappearance).

INTRODUCTION

Chitin is an N-acetyglucosamine with a molecular structure, (1-4)-2-acetamido-2-deoxy-B-D-glucan), which is analogous to cellulose, however, the hydroxyl group on the C-2 position of cellulose is replaced by N-acetylamide group on the chitin molecule (White et al., 1968). Stelmock et al. (1985) reported that chitin content of crab meal ranges from 12.7 to 13.6%. Chitin serves as the cementing substance for crustacea (Lovell et al., 1968), thus, it is found in a complex bound with protein and $CaCO_3$ (Rudall et al., 1973).

Lovell et al. (1968) indicated that chitin and its deacetylated form chitosan, are indigestible and has no protein value for non-ruminants. However, studies have shown that chitin is a potential energy source and can be digested by ruminants (Patton et al., 1975; Oke et al., 1978; Hirano et al., 1984). The digestibility of chitin in mice, chickens and Japanese nightingales ranges from 19 to 58% (Jeuniaux and Cornelius 1978). Patton et al. (1975) showed that purified chitin is degraded after incubation in the rumen fluid for 3 h at 37 C.

Hood et al. (1978) reported in situ degradation of chitin in an estuarine environment. Rate of degradation was higher for the native chitin incubated for 24 h and lower for acid- treated and pure chitin. Patton et al. (1975) observed a DM disappearance of 21.4% for purified chitin incubated in the rumen for 48 h. The enzymes (chitinolytic, chitinoclastic and chitosanolytic) which are produced by the bacteria and fungi are needed for chitin degradation (Price et al., 1975; Berkeley, 1978). These enzymes are inducible if the diet containing chitin is fed to the animals (Patton et al., 1975; Jeuniaux et al., 1978).

Studies were conducted to estimate chitin digestibility in vitro, in situ and in vivo.

MATERIAL AND METHODS

Nylon Bag (In Situ) Experiment. Crab processing waste was obtained from a crab processisng plant in Hampton, Va.[1]. The waste was collected fresh, transported to Blacksburg, packed in ice and frozen. The waste was dried at 60 C for 24 h and then ground in a hammer mill. At the beginning of the experiment, the material was dried again in a forced-draft oven at 65 C for 48 h. The dried waste, practical grade chitin and straw were ground individually in a Wiley mill through a 5 mm screen and stored in air-tight containers until used. Practical grade chitin[7]. and the following proportions of straw to crab meal: 100:0, 75:25, 50:50, 25:75, 0:100, were mixed individually until homogenous mixtures were obtained.

A rumen fistulated Angus steer weighing approximately 691 kg was used in a repeated measure experimental design. The animal was isolated in a pen with access to water and feed that consisted of 70% alfalfa/orchardgrass hay (1:1 ratio), 20% crab meal and 10% corn grain (dry basis). The crab meal diet was introduced during a 5-d transition period, followed by a 7-d adaptation period.

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[1] Grahams and Rollings, Hampton, Virginia

[7] Sigma Chemical Company. St Louis, Missouri

The nylon bags[8]. used in this study were made of an industrial filter material with a standard pore size of 57 +/- 10 microns. The bags were 10 x 12 cm and were heat sealed on three sides. Prior to incubation, bags were labeled and dried for approximately 24 h. The weights of the bags were obtained, and 10 g of the prepared samples were placed in the bags. The bags were tied individually, and duplicates were tied together with Bar-Lok cable ties[9]. (diameter 7/8 in), and these were subsequently tied to steel rings with the nylon string at one end.

Approximately 15 min before incubation, the bags containing the samples were presoaked in warm water, after which they were carefully placed in the ventral sac of the rumen at exactly 1700 h for d 1, 2, and 3, which represent the 72, 48, and 24 h of incubation, and at 0500, 0900 and 1300 h on d 4, which represent 12, 8, and 4 h of incubation, respectively. All bags were removed from the rumen at approximately 1700 h on d 5. The bags were rinsed in cold water four times, and were covered with ice to prevent further microbial activity.

In the laboratory, the bags were rinsed for approximately 20 h in a continuous flow rinse, followed by individual washing and rinsing of the bags with the content

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[8] Ankom, Spencerport, New York

[9] Dennison Cable Tie Products, Framingham, MA

until the water was colorless. A set of bags containing the samples were also rinsed with water until colorless water was obtained. This set represented the control at 0 h incubation. The bags with the contents were dried at 60 C for 24 h. The dried bags with the contents were weighed and the residues and initial samples were obtained for subsequent analyses.

In Vitro Experiment. The in vitro procedure according to Tilley and Terry (1963) was used to determine the degradability of chitin. The experiment was repeated four times. The samples were prepared as in the in situ study. Before the morning feeding, ruminal fluid was obtained from the ventral portion of the rumen from the steer used for the in situ study. The fluid was filtered through eight layers of cheese cloth into a pre-warmed thermos flask. The fluid was transported to the laboratory where it was added to the buffer solution (4:1 ratio) which had been adjusted to 39 C. Carbon dioxide was passed through until the pH of 6.85 was reached.

The ruminal fluid was introduced into the tubes containing the test diets. For the control treatment, only ruminal fluid was placed in the tubes. The tubes were flushed with CO_2 in order to maintain anaerobic condition, and stoppered with rubber stoppers equipped with bunsen valves, which was designed to prevent accumulation of gas. The tubes were incubated in a shaking water bath kept at

39 C. After 4, 8, 12, 24, 48 and 72 h of incubation, two tubes containing each test diet were removed and frozen. The tubes were thawed and centrifuged at 800 x G for 25 min. The tubes containing the residue were dried at 60 C for 24 h. The residue and the initial samples were obtained and kept for further analysis.

In Vivo Experiment. The crab processing waste was obtained from the same source as above except the waste was treated with .2% sodium hypochlorite (NaOCl) and transported to Blacksburg for ensiling. The treated waste, straw, liquid molasses, and water (32:32:16:20 wet basis) with .1% of microbial inoculant were ensiled in a large polyethylene bag. The mixture was allowed to ferment for a minimum of 16 wk before the metabolism trial began. The procedure used in preserving the waste and subsequent ensiling with straw, molasses, water and microbial inoculant was described previously (chapter 4).

Eighteen crossbred wethers averaging 43 kg were assigned to six blocks of three animals each, based on weight and origin. Sheep within each block were allotted at random to the following diets: 1) basal diet (30% orchard grass hay, 65.1% corn grain, 4% soybean meal, and .9% limestone, dry basis), 2) 50:50 (dry basis) of crab waste-straw silage and basal diet, and 3) 100% crab waste-straw silage. The sheep were placed in metabolism stalls that allow separate collection of urine and feces.

The procedures used during the collection period for preparing diets and handling fecal samples were same as described in chapter 4.

Chemical Analyses. Samples of the straw, crab meal, straw and crab meal mixtures and practical grade chitin obtained from the above experiments were dried in a forced-draft oven at 60 C for 24 h. The dried samples were ground in a Wiley mill through a 1 mm screen. The samples were analyzed for chitin content according to the modified procedure for chitin assay (chapter 6). Samples were initially subjected to ADF treatments (Van Soest, 1963), followed by delignification (Van Soest and Wine, 1968) for chitin determination through the ADF/ashing procedure. For samples prepared for ADF/deacetylation procedure, the ADF residue was deacetylated with saturated KOH at 160 C for 30 min, followed by distillation. The VFA internal standards (5 ml) and 1 ml of 25% metamorphosporic acid were added to 5 ml of the distillate, and acetate was quantified with a Vista 6000 gas chromatograph.

Statistical Analysis. The disappearance of chitin over time with different levels of crab waste in the mixtures was tested by analysis of variance utilizing the general linear model procedure of SAS (1982). For the in situ and the in vitro methods, the period, treatment and period by treatment interaction were included in the model. Linear and quadratic responses for period were

tested. For the in vivo experiment, linear and quadratic responses for the levels of crab waste in the diet were tested.

RESULTS AND DISCUSSION

Nylon Bag (In Situ) Experiment. In situ disapperances of different proportions of straw and crab meal and practical grade chitin were determined by using the ADF/ashing and ADF/deacetylation procedures. The disappearance of chitin in the rumen was lower for samples containing greater levels of straw and highest for 100% crab meal (Table 36). This trend was similar for values obtained through ADF/ashing or ADF/deacetylation procedures. When ADF/deacetylation procedure was used, there tended to be a linear increase ($P>.1$) in chitin disappearance with time when crab meal or crab meal:straw mixtures were incubated in the rumen for 48 and 72 h. A similar trend was also seen in samples kept in the rumen for 72 h when ADF/ashing procedure was used. A linear increase ($P<.01$) with time was observed for all treatments.

The highest disappearance of chitin was observed when 100% crab meal was kept in the rumen for 72 h (33 and 36%, respectively). Disappearance of chitin from 100% practical grade chitin incubated for 72 h was intermediate (26 and 35%, respectively for ADF/ashing and ADF/deacetylation

TABLE 36. IN SITU CHITIN DISAPPEARANCES[a,b,c]

Period	Method[d]	Proportion of straw:crab meal 75:25	50:50	25:75	0:100	Chitin	SE
h		%					
4	a	.37	1.43	3.96	5.05	-1.79	1.28
8	a	1.89	1.37	4.73	5.69	-1.65	1.28
12	a	2.51	2.28	9.96	7.91	-.04	1.28
24	a	5.41	10.18	17.71	14.24	.72	1.28
48	a	10.01	17.78	26.85	23.25	14.51	1.28
72	a	11.08	21.94	31.68	32.98	25.95	1.28
Avg	a	5.21	9.16	15.81	14.85	6.28	
4	b	1.70	1.04	2.62	4.60	5.44	1.04
8	b	1.88	1.09	7.04	5.15	5.06	1.04
12	b	-3.99	3.47	8.72	6.04	7.35	1.04
24	b	5.28	11.06	17.93	13.84	15.06	1.04
48	b	9.67	14.55	20.99	22.30	26.04	1.04
72	b	9.74	26.46	35.83	36.32	34.70	1.04
Avg	b	4.04	9.61	15.52	14.70	15.60	
Avg a & b		4.62	9.38	15.66	14.77	10.94	

[a] Linear effect with time (P>0.1).
[b] Period * Treatment interaction (P<.01).
[c] Linear effect with treatment (P<.01).
[d] a=ADF/ashing procedure, b=ADF/deacetylation procedure

procedures). Patton et al. (1975) reported a DM disappearance of 21.4% when purified chitin was kept in the rumen of steers for 48 h. In the present study, when the ADF/ashing procedure was used, negative disappearance was observed with practical grade chitin incubated for 4, 8 or 12 h. These values may be a reflection of initial bacterial attachment prior to degradation. Overall, greater disapperances of chitin were observed when samples were kept in the rumen for more than 24 h.

In Vitro Experiment. In vitro disappearance of chitin in the rumen was lower in samples containing higher levels of plant fiber (Table 37), perhaps due to a preference by the microbes for the organic matter of plant origin than the crustacean meal. A similar trend was noticed for the chitin disappearances determined by the in situ method. Disappearances of chitin were greater for 100% crab meal incubated for 24, 48 and 72 h respectively. However, intermediate values were obtained during the same period for the practical grade chitin and the samples containing 75% crab meal. Negative disappearance was observed for practical grade chitin incubated for 4 and 12 h. The chitin disappearance of 4.98% obtained for 8 h may be due to sampling error.

Samples containing 50, 75 and 100% crab meal resulted in higher disappearance of chitin at 4, 8 and 12 h, compared to samples containing 25% crab meal. These values

TABLE 37. IN VITRO CHITIN DISAPPEARANCES[a,b,c]

Period	Method[d]	Proportion of straw:crab meal 75:25	50:50	25:75	0:100	Chitin	SE
h		%					
4	a	3.12	2.48	11.69	12.83	-2.17	1.32
8	a	4.48	8.10	18.68	10.31	4.98	1.32
12	a	5.69	11.15	17.62	13.74	-1.47	1.32
24	a	9.53	19.26	16.34	21.81	11.11	1.32
48	a	9.92	16.89	23.49	31.53	24.63	1.32
72	a	11.27	24.73	28.11	37.00	31.53	1.32
Avg	a	7.33	13.76	19.32	21.20	11.44	
4	b	1.70	8.85	15.25	15.49	5.46	1.19
8	b	1.54	12.99	19.11	16.03	6.60	1.19
12	b	.81	12.69	17.73	15.21	7.37	1.19
24	b	6.46	15.42	18.51	24.85	10.34	1.19
48	b	14.87	16.73	27.13	29.74	23.59	1.19
72	b	13.21	20.77	29.06	37.60	31.17	1.19
Avg	b	6.43	14.57	21.13	23.15	14.08	
Avg	a & b	6.88	11.71	20.22	22.17	12.76	

[a] Linear effect with time (P>0.1).
[b] Period * Treatment interaction (P<.01).
[c] Linear effect with treatment (P<.01).
[d] a=ADF/ashing procedure, b=ADF/deacetylation procedure.

were in contrast to the values obtained by the in situ method during the same period of time. Patton (1971) reported that chitin was 8.5% soluble in water and 21.4% soluble in the rumen fluid, hence, this may explained the higher disappearance of chitin observed with the in vitro method.

Chitin disappearance from practical grade chitin was positive and higher. A similar trend was observed with in situ method. These results may be a reflection of solubility of the finer particles of the chitin in the rumen fluid (Patton, 1971), and/or subsequent losses of the beta chitin (Rudall, 1955), and the non bound (free) chitin (Jeauniaux, 1978) which are highly susceptible to acid hydrolysis. Patton (1971) showed the highest recovery of acetate (an indication of chitin degradation) when 400 mg of purified or insect chitin were kept in the rumen fluid without any incubation.

In Vivo Experiment. The intake of chitin was 0, 11.76 and 25.12 g/d for animals fed 0, 50, and 100% crab waste-straw silage (Table 38). The chitin digested by the animals was lower ($P<.01$) for sheep fed a diet containing 50% crab waste-straw silage and 50% basal diet (37%) than for sheep receiving 100% crab waste-straw silage (58%), perhaps due to the associative effects and preference for the basal portion of the diets by the rumen microbes.

TABLE 38. CHITIN DIGESTIBILITY BY SHEEP FED CRAB WASTE-STRAW SILAGE

Item	Crab waste-straw silage, %[a]		
	0[b]	50[b]	100[b]
Intake, g/d	0	11.76	25.12
Digested, g/d[c]	0	4.38	14.53
Digested, %[c]	0	37.30	57.86

[a] Crab waste, straw, molasses, water (32:32:16:20, wet basis) plus .1% microbial inoculant.
[b] Percent dry basis.
[c] Linear effect ($P<.01$).

Patton (1971) has shown chitin digestibility of 59 and 69% respectively for steers receiving 10 and 20% crab meal. In the same study, higher digestibility of chitin (72%) was reported for the steers after they were adjusted to the crab meal diet. Hirano et al. (1984) reported apparent digestibilities of 78 to 92% for chickens fed N-acetylchitosan and crustacean shells, while the increase in hexosamine content, an indication of chitin degradation was seen in the feces of cattle fed 7% crustacean meal. Chitinase enzymes which are responsible for chitin digestion are produced by the bacteria (Ohtakara, 1978), and several fungi and actinomyces (Berkeley, 1978) which occupy the rumen and the large intestine (Akin, 1986).

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Chapter VIII

GENERAL DISCUSSION

Crab processing waste, amounting to over 125,000 metric tons annually, represents a valuable resource, which could be used for animal feed. However, in order to efficiently utilize these nutrients, the waste has to be used fresh. Hence, an apparently feasible method of processing the waste is to ensile it fresh with other ingredients for use as feedstuff for ruminants. Because the waste is highly perishable, it must be processed within hours of removing the edible portion.

In preservation studies a combination of organic acids (propionic and formic) at 1.5%, (1:1, wet basis) was effective in preserving the waste for several days. However, using these chemicals is not economically feasible. In other studies, oxidizing chemicals were effective in retarding degradation of crab processing waste. Dychdala (1977a) has shown that these chemicals exert their antimicrobial effect by rupturing the cell membranes of bacteria, while Tompkin et al. (1979a) have shown that these chemicals inhibited the electron transport system of the bacteria.

Sodium hypochlorite and H_2O_2 have been used extensively in preservation of edible products (Lechowich,

1981; El-Gerdy et al., 1980). The use of NaOCl in preserving crab processing waste seems to be technically and perhaps economically feasible. The chemical at .2% of active ingredient prevented putrefaction of waste for 5 d. Also, addition of .4% H_2O_2 kept the waste from degradation for up to 10 d, but its use as a preservative was discontinued due to the cost. The combination of NaOCl and CaOCl as preservatives was also tested. These chemicals extended the storage period of crab waste to 7 d.

Addition of 1% $NaNO_2$ retarded putrefaction of the waste for minimum of 15 d in the present studies. Nitrite has been used extensively in curing of meat (Henry et al., 1954), and this chemical can be metabolized by bacteria (Buchanan and Solberg, 1972) into products that are not toxic.

Desirable fermentation of silage was obtained when the NaOCl and H_2O_2-treated waste were ensiled with other ingredients. This shows that the initial preservation of the waste with chemicals did not impart any undesirable characteristics on the silage.

In a feeding trial, steers fed higher levels of crab waste-straw silage showed a higher daily gain and feed efficiency. Carcass quality grade was low choice and yield grade averaged 2.3. Abazinge (1986) reported similar quality grade but average yield grade of 1.8 when steers were fed different levels of fish-straw silage. In a di-

gestion trial with sheep, feeding 50 and 100% crab waste-straw silage improved digestibility of CP, which may have increased the daily gain in steers, especially when adequate amount of energy was supplemented. Reduced daily gain has been reported in steers fed 7.5 to 22.5% tanner crab meal (Brundage, 1986).

Flavor and juiciness of roasts were not adversely affected by feeding crab waste-straw silage. In fact, values tended to be higher for meat from steers fed 30% crab waste-straw silage. Abazinge (1986) reported a tendency for off-flavor in meat from steers fed higher level of fish waste-straw silage, compared to those fed low level of fish waste-straw silage.

Chitin of crustacea origin is found in a bound form with $CaCO_3$ (Lovell et al., 1968) and N (Muzzarelli, 1978), and is generally made up of α, β and γ structures. The β and γ structural forms of chitin are readily hydrolyzed in acidic medium. Thus, separation of chitin from minerals and N prior to quantification is a difficult task.

In the past, several methods have been used to quantify chitin. These methods include subjecting chitin-containing substances to harsh chemical treatment in order to separate the chitin from its bound form with $CaCO_3$ and N. Substantial amounts of chitin is lost during this separation. Black et al. (1950) used the crude fiber method to determine the chitin content of crustacean meal.

Stelmock et al. (1985) followed up on this procedure by using the ADF procedure to determine chitin content of crab meal. However, it is difficult to use these methods in estimating chitin mixed with other fiber particles, and also overestimation of the chitin values was unavoidable due to the other polymers that are not accessible to the hydrolyzing effects of the crude fiber or ADF solutions.

Modification of the previous procedures was done by eliminating the initial chemical treatments. Two methods were developed from this modification, the ADF/ashing and the ADF/deacetylation methods. The chitin values obtained by using the two methods were similar, indicating less degradation of chitin structures during preparation. The use of $KMNO_4$ eliminated the carotenoids (Muzzarelli, 1977) and the lignin polymer of plant origin. The ADF/ashing procedure is appropiate to determine chitin content in mixture with known proportions of crab meal or waste, while the ADF/deacetylation procedure is necessary for chitin values of unknown mixtures.

In in vitro and in situ studies greater digestion of chitin was observed when 100% crab meal was used, compared to practical grade chitin or samples containing different proportions of straw and crab meal. This indicates that chitin in its natural form is more available to the microbes. Hood et al. (1978) have shown that, in an in situ study, degradation of chitin was higher for the

native chitin and lower for the acid-treated and purified chitin. Disappearance of chitin from practical grade chitin and mixtures containing 50 and 75% of straw was intermediate, perhaps a reflection of preference by the microbes for the organic matter of plant origin.

Chitin digestibility was highest (58%) for sheep fed 100% crab waste-straw silage and lowest (37%) for those fed 50% crab waste-straw silage, perharps due to the associative effects and preference for the basal portion of the diets by the rumen microbes. Large colonization of native chitin by the bacteria has been reported by Hood et al. (1978) when this material was incubated in an estuarine environment, compared to the purified chitin which had less bacteria numbers.

In conclusion, the preservation of crab processing waste was achieved by using NaOCl, NaOCl/CaOCl, H_2O_2 and $NaNO_2$. At present, the use of H_2O_2 may not be feasible but further studies need to be conducted to lower the concentration, or possibly combine this acid with other additives to achieve similar or greater effects. The most promising additives are NaOCL and $NaNO_2$. These additives are currently used as preservatives in human food. The chemicals did not affect the fermentation characteristics nor the intake of silages by the animals. Crab waste silage can be used as a protein and roughage supplement for ruminants. The procedures used in determining chitin

are accurate and repeatable. Apparently, the chitin in crab meal is digested by ruminants.

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