STUDIES OF METHODS OF PRESERVING AND ENHANCING FERMENTATION, NUTRITIONAL VALUE AND PALATABILITY OF SEAFOOD WASTE FOR FEEDING RUMINANTS,

by

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In memory of our beloved Blair whose tragic death occurred on 1985.

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Chapter I

INTRODUCTION

The most valuable seafood products in the U.S.A. are shrimp, salmon, tuna and crabs in descending order of importance (Warner, 1977). Total crab catch amounts to about 149,000 metric tons annually (NMFS,1975).

It has been estimated that waste amounts to 80 to 90% of processed crabs (Brinsfield, 1980). In the past, the crab processing industry disposed of this waste by dumping it into natural waters adjacent to the plants. Environmental concerns heightened, and in 1972, offshore dumping of crab processing wastes was banned. Current information on overboard dumping of shellfish waste has been summarized by the environmental protection agency (EPA,1980). Most of the objection to offshore dumping of crab processing waste was based on odor problems, floating debris and increased turbidity of the water.

Current disposal methods include crab meal production, landfilling and agricultural land application (Olsen, 1980). Of these methods, crab meal production is attractive in terms of nutrient recovery for livestock. However, the economic feasibility of this method is questionable.

An alternative method of disposal that requires minimum energy input is ensiling. This method has previously been investigated in our laboratory with little success due to odor, high pH and low levels of lactic acid in the silages. Since crab waste is high in moisture and minerals and low in fermentable sugars, addition of a source of fermentable carbohydrates may enhance fermentation and produce an acceptable silage.

These studies were undertaken to investigate development of practical methods of ensiling crab processing waste and crop residues, and to evaluate the potential feeding value of the ensiled mixture for ruminants. The effect of feeding ensiled finfish waste and straw to finishing cattle was studied also.

Chapter II

LITERATURE REVIEW

The waste from fish processing consists primarily of heads, fish offal and whole fish. Shellfish processing waste consists primarily of the inedible shell and viscera. Approximately one-half of the crab harvest in the U.S.A. is comprised of the blue crab, Callinectes sapidus (Warner, 1977).

Characteristics of Crab Waste

Crab processing waste includes the exoskeleton, egg masses, viscera and the fluids. Cantor (1980) reported that blue crab meal consists of 47% calcium carbonate, 25.7% protein, 22.3% chitin and 5% fat, dry basis. The shell, anatomically, consists of the epicuticle, exocuticle, endocuticle and a membranous layer. The epicuticle is composed of lipoprotein and is flexible due to the low level of calcification (Hickman,1967). The exo-and endocuticles are highly calcified and are made up of chitin-protein microfibril matrices (Muzzarelli, 1971). The membranous layer is cellophane-like and uncalcified (Hickman, 1967).

Chitin and its derivative chitosan are white solids. Chemically, chitin is N-acetyl-D-glucose- amine and chitosan

is deacetylated chitin. These organic compounds form the interguments of many insects and crustaceans.

Muzzarelli (1971) stated that crabs and lobsters are 20 to 50% chitin, dry basis. The main dorsal carapace of the crab averages about 15% chitin, dry basis, while the claws contain 12% (Brine, 1978). The cuticle of edible crab contains the highest proportion of chitin to protein of all the arthropods (Muzzarelli, 1971). The viscera of crabs is comprised of the gills, heart, stomach, intestines, bladder and hepatopancreas. These are all organic in nature and are susceptible to degradation and putrefaction (Warner, 1977).

Chitin and chitosan are polymers that occur abundantly in the marine environment. These polymers are important chelators in both fresh and ocean waters, but they are readily degraded (Martin, 1970). Many of the organic compounds in fresh and ocean water form strong complexes with metal ions in solution. This is important in the ecological cycling of metals (Siegel, 1971; Lerman and Childs, 1973). The collection rate, however, depends on several factors, including the surface area of the polymer, temperature, stirring or shaking, pH and competition with other ions (Muzzarelli, 1971).

Chitin and chitosan do not contract, swell, shrink or change color upon contact with alkali or alkaline earth me-

tals (Muzzarelli,1971). These organic ligands eventually break down in solution. The rate of decomposition is controlled by bacterial degradation, oxidation and photo-dissociation (Lerman and Childs, 1973).

The chelation of metals in natural habitats may serve two functions: 1) to provide trace metal nutrients that can be easily assimilated by the microorganisms and 2) to protect the organisms from the toxic effects of metals. These phenomena are evidence of the importance of organic chelators in the cycling of metals in aqueous environments (Engel and Sunda, 1981).

Shellfish Waste Disposal

The major disposal methods for crab processing waste include agricultural land application, offshore disposal, burial or landfilling and crab meal production (Titus, 1981; Champ et al, 1981).

Agricultural Land Application. Waste from crab processing plants is spread on the land and disked into fields as a form of fertilizer for forages and crops. If properly integrated with the crop cultivation, the waste may be valuable (Titus, 1981). The waste must be turned under rapidly after application to minimise odor problems. This problem precludes the use of crab waste as a fertilizer on farms close to residential areas.

Offshore Dumping. There is no permit required by the Environmental Protection Agency (EPA) for the offshore dumping of crab wastes (EPA, 1980). However, in South Carolina a permit is required by the national pollutant discharge elimination system (Titus, 1981). The EPA excludes fish wastes from the federal ocean dumping and permit requirements. Fish waste is defined by the EPA as the returning to the sea of any unadulterated seafood wastes. The main support for this method of disposal lies in the contention that the process recycles products taken from the sea in a manner similar to the natural process of death and decay in the sea (Champ et al., 1981). Environmental impacts of this method of disposal include high levels of ammonia, hydrogen sulfide, phosphorus and low levels of dissolved oxygen, and accumulations of waste and debris (EPA, 1980).

Burial or Landfilling. This would appear to be an economical method of disposal since the only cost involved is the cost of hauling to suitable sanitary sites. However, the moisture dripping from trucks causes insect and odor problems along the route (Miles, 1981). Also, the decomposition of the waste at these sites adds to the odor problem.

Crab Meal Production. Crab meal production from crab processing waste involves dehydration and pulverization of the waste to form a meal, which is sold primarily as a pro-

tein supplement (Kirk et al., 1967). This procedure is more of a resource recovery, and appears environmentally sound and profitable. However, energy costs have continued to increase, and crab meal is not competitive with other supplements. Crab meal production is also variable as a result of seasonal variability in the crab catch (Murray, 1981). This method of disposal is obviously not feasible in areas where crab meal production facilities do not exist.

Problems of Ensiling Crab Waste

Two major problems are encountered in the ensiling of crab waste: 1) inability to lower the pH of the ensiled mass to acceptable levels and 2) offensive odor (Samuels, 1983). The offensive odor is due to the production of volatile gases particularly trimethylamine (Beatty and Gibbons, 1937), as a result of bacterial reduction of trimethylamine oxide (TMAO). The high pH of the ensiled material is probably associated with the mineral content, which averages about 35%, on a dry basis. This high level of ash contributes to a high level of buffering activity.

Trimethylamine oxide has the following structural formula: $(CH_3)_3$-N=O. It is solid at room temperature with a melting point of 257 C (Ronald and Jakobsen, 1947). The salts of the oxide have a marked buffering action (Castell,

1949; Suyama, 1958). Trimethylamine oxide is a constituent of marine animals, including both fish and invertebrates (Groninger, 1959; Yamada, 1967). After death of the organism, TMAO is reduced to TMA, which gives the characteristic off-flavor of marine fish in the early stages of decay (Beatty and Gibbons, 1937; Tarr, 1954). Bacteria, particularly the gram negative rods, reduce TMAO according to the general equation: $(CH_3)_3\text{-}N{=}O + AH_2 \longrightarrow (CH_3)_3\text{-}N + A + H_2O$ (Watson, 1939), where A represents any hydrogen donor.

There is variation in the content of TMAO among species of animals, but it is frequently 2 to 4% of the dry weight of a teleost (bony fishes) and 3 to 7% of an elasmobranch (cartilaginous fishes) (Yamada, 1967). The TMAO in these marine species is involved with the maintenance of tissue osmotic pressure (Cohen et al., 1958).

The occurrence of TMAO in higher animals has been reported by Barrenscheen and Pantlitschko (1950). They showed that TMAO acts as a methyl donor for the synthesis of choline in the muscle of guinea pigs (cholinephrase). In mammals, Norris and Benoit (1945) have reported the formation of TMAO as a result of choline degradation. This is caused by bacterial breakdown of choline to TMA in the intestinal tract. Very small amounts of TMAO and TMA are present in mammalian tissues, however, no function has been reported (Dyer and Wood, 1947).

Nutritional Value of Shellfish Waste

The general composition of shellfish waste is 25 to 40% crude protein, 20 to 40% ash, 10 to 30% chitin and 2 to 10% ether extract, dry basis (Watkins, 1976). The exoskeleton of all crustaceans is composed primarily of chitin, a polymer of an acetylated glucosamine (Penniston et al., 1969). Thus, the N and glucose moieties are in close association, which may suggest a potential for degradation by the ruminal microbial population. The other major component of crustaceans is ash, mainly $CaCO_3$. The Ca has nutritional value in livestock feeding programs, especially young growing animals and dairy cows, which have a particularly high requirement for Ca.

Crustacean wastes have value as feed supplement for swine, poultry and cattle primarily for its protein content, if offered at low levels in combination with traditional feedstuffs (Bray et al., 1932; Parkhurst et al., 1944a,b; Kirk et al., 1967; Patton et al., 1975).

Data concerning feeding of chitinous material to ruminants is limited. Early experiments were conducted with oyster shells as a roughage replacer for fattening beef animals (Perry et al., 1968). Crossbred steer calves averaging 225 kg were self-fed fattening diets and oyster shells replaced 2.5% of the ground corn cobs. Steers not fed oyster

shells gained faster, and the carcasses graded higher than steers fed the oyster shell. Steers on the oyster shell diet also had a characteristic frothy condition of the rumen contents, and the rumen wall was characterized by hyperkeratosis and rumenitis. The authors suggested that the feeding of antibiotics was beneficial in alleviating these conditions.

In another study (Williams et al., 1970) the addition of oyster shells to 20% roughage and all-concentrate diets resulted in lower gain, feed consumption and feed efficiency. The incidence of bloat was increased by addition of oyster shells but ruminal fluid pH and concentration of volatile fatty acids (VFA) were not altered.

Patton and Chandler (1975) investigated chitinous products by in vivo fermentation. Various products were used, including crab meal, which contains about 13% chitin and shrimp meal which has about 8% chitin. Samples of shrimp meal, crab meal and purified chitin were placed in the rumen of fistulated steers and the average solubilities were 17.4, 35.7 and 21.5%, respectively. In vitro dry matter, organic matter and nitrogen disappearance were 75, 58 and 62% respectively (Brundage et al.,1979) in studies using crab meals.

Patton and Chandler (1975) fed blue crab meal at 10 and 20% of a basal diet to young calves and reported that di-

gestibility of chitin ranged between 26 and 87%. These workers observed no differences in apparent digestibility and retention of N. Feed intake and weight gains were not adversely affected by feeding crab meal.

Mangold and Hock (1938) and Mangold and Damkobler (1938) reported crude protein digestibilities of 80 and 81%, respectively, for crab meal and shell-free crab meal in chickens. The amino acid content of crab meal was reported by Johnson and Peniston (1971). On the basis of these data Meyers and Rutledge (1973), concluded that the digestibility and essential amino acid content of crustacean proteins were adequate to meet the protein requirements of chickens and rats. However, they suggested that crustacean protein should be combined with conventional protein sources for optimum utilization and efficiency. Rutledge (1971), suggested that the high mineral content of shellfish meals may limit incorporation into feeds to about 10%.

The use of shellfish meal in the diets of rats was evaluated by Goto and Sawamura (1973). They reported that high Ca diets resulted in decreased growth, reduced nitrogen retention and reduced fat absorption. Fat utilization is decreased by the formation of insoluble soaps (Gacs and Barltrop, 1977; Yacowitz et al., 1967). Chitosan has been found to reduce growth in rats (Landes and Bough, 1976).

Watkins et al. (1982) evaluated shrimp and king crab processing by-products as feed supplements for minks. The waste products replaced 10 and 20% of the protein in a 33% protein diet. Weight gains were lower, and feed consumption higher for minks fed the waste diets, compared to the control group. They attributed the lower performance to the high Ca content, which interfered with the utilization of other nutrients.

Meyers and Perkins (1977) reported that carotenoids in shellfish may be transferred from the feed to the flesh of trout and salmon, thus giving the desired flesh coloration in these fishes. Campbell (1973) reported that intensive farming of crustaceans in ponds and lagoons frequently leads to high levels of cannibalism. Feeding diets containing chitin resulted in reduced cannibalism and increased growth rate.

More recently, Samuels (1983) evaluated the feeding value of crab processing waste by sheep. Crab waste and straw mixtures were stabilized with glacial acetic acid and fed with a basal diet (1:1, dry basis). Dry matter digestibility of about 60% was reported. Nitrogen retention, as grams per day, was slightly higher for lambs fed the crab waste diet compared to control animals fed a basal diet (4.4 vs 3.0).

Fish Waste Utilization

The by-products produced from rendering plants are fish meal, fish oil and fish solubles. The total fish meal produced in the United States was estimated to be 257 million kilograms (Pennington and Husby, 1979). The fish meal is utilized extensively in animal feeding programs. Condensed fish solubles are concentrated from the water removed at fish meal and oil plants. The total production of fish solubles was estimated to be 65 million kilograms (Soderquist and Williamson, 1975), and most of this is used in feeds and fertilizers. In the U.S.A., the fish oil produced from rendering plants is used for various purposes but not for human food.

Fish has a high content of water (about 75%), and is subject to chemical changes by enzymes in the fish and by bacterial action (Pike and Tatterson, 1980). Fish also contains oil and virtually no carbohydrates. Since there is very little free sugar, which is the preferred energy substrate for growth of most microorganisms involved in spoilage, degradation of proteins becomes an active process and results in the production of amines and ammonia (Tatterson, 1980).

The methods currently available for the utilization of fish by-products include: 1) rapid drying to produce fish meal, 2) ensilage-anaerobic storage with acid addition, 3)

feeding fresh (Hike and Patterson, 1980), and 4) ensiling with low quality roughages with the addition of a small amount of dry molasses (Samuels, 1983).

Renewed interest in the ensiling of fish waste is mainly due to the low input of energy and the unavailability of meal production plants in some areas (Raa and Gilberg, 1982).

Production of Fish Silage

Fish silage at the present time can be produced by three processes: 1) Liquifaction by addition of acid, 2) bacterial fermentation and 3) ensiling with dry material.

Liquifaction of the Fish by Acid Addition. This process involves chopping or mincing of the waste, which is necessary for the distribution of the acid, thorough mixing of the acid into the material, digestion and storage (Tatterson, 1976). The digestion process is accomplished by indigenous proteolytic enzymes. The amount of acid required to lower the pH sufficiently in the fish homogenate depends on the concentration of protein and ash in the raw material (Raa and Gilberg, 1982). Although mineral acids such as sulfuric and hydrochloric acids have been used, they are corrosive (McDonald and Whittenbury, 1973) and must be neutralized prior to feeding (Tatterson and Windsor, 1974). Organic

acids such as formic acid (Backhoff, 1976) and propionic acid (Strom et al., 1980) have been used to produce fish silage. The concentration of acid used varies, but 3% is commonly used by most researchers.

Bacterial Fermentation. This is accomplished with the addition of acid, sugars and lactic acid bacteria (Roa, 1965). In this type of silage, the free glucose will repress the production of deaminating enzymes by the spoilage microbes in the raw fish material and in this manner suppress ammonia production and prevent an increase in pH. The lactic acid bacteria will simultaneously convert the glucose to acid which lowers the pH. Under these conditions, coliforms, enterococci and typhoid bacteria are destroyed (James and Nair, 1977).

Ensiling of Fish Waste. Ensiling of fish waste and crop residues with the addition of a small amount of dry molasses was shown to produce acceptable silage for feeding ruminants (Samuels, 1983). This may become more important in developing countries for the combined utilization of two by-products.

Nutritive Value of Fish Waste

Fish silage maintains good nutritional value during long-term storage (Nilson and Rydin, 1963). Lactic acid production during fermentation may serve as an active antioxi-

dant, and the presence of antibiotic substances produced by the lactic acid bacteria may be involved in preservation (Schroder et al., 1980). Fish silage produced by fermentation has been shown to have a significantly higher nutritional value for chickens than fish silage produced by acid preservation (Kompiang et al., 1980). It has also been shown that uptake and incorporation of Ca into the eggshell is facilitated by fermented fish silage (Raa and Gilberg, 1982). Despite the good nutritional value, ammonia formation is usually higher in fermented silage than in acid preserved silage (Raa and Gilberg,1982).

Fish Meal. Fish meal made from offal (white fish meal), normally has about 65% crude protein, less oil and a higher mineral content than meal from whole fish (Pike and Tatterson, 1980). The energy value of white fish meal is slightly lower but the the amino acid makeup is similar.

Extensive feeding trials with pigs and poultry indicate that fish meal in the diet improves animal performance (Pike, 1975). Pike (1975) also reported that fish meal contains unknown growth factors (UGF), and inclusion of fish meal in the diet usually results in increased efficiency of feed utilization. Another new possible use of fish meal may be in the feeding of high-producing ruminants. Drying of forages usually causes the protein to have a lower degrad-

ability in the rumen, yet remains digestible in the small intestine (Klopfeinstein et al., 1979). This may be true with fish waste. From calculations based on provisional data from rumen degradability, coupled with amino acid make-up, it has been shown that fish meal supplies about five times as much methionine and three times as much lysine in the small intestine of ruminants, compared to an equivalent amount of soybean meal (Watkins et al., 1982). Subsequent trials with lambs, calves and lactating cows, showed improved growth and milk yield when fish meal was included in the diet.

Fish Silage. Fish silage preserved by adding acid (formic acid, hydrochloric acid, sulfuric acid or their combinations), is an acceptable feed for fish, particularly for salmonids (Austreng, 1982). Work by Whittemore and Taylor (1976) with de-oiled fish silage produced from herring offal, showed the product to be equivalent in nutritional value (dry basis) to fish meal, based on digestibility and nitrogen retention in pigs when the fish products were fed with barley. Luscombe (1973) and Smith and Adamson (1976) compared fish silage and fish meal by feeding the products to pigs along with barley. Weight gains were similar in both groups, however, Smith and Adamson (1976) reported that feed conversion was slightly poorer for pigs receiving a

fish silage. Carcass quality was not different (Disney et al., 1978). Raa and Gilberg (1982) indicated that a fishy off-flavor to the carcass may be a potential problem in the feeding of fish silage. However, this problem is not limited to fish silage, but is related to the amount of oil in the product. Potter et al.(1980) recommended that the level of fish oil be less than 1% and that conventional feed meals should be fed during the last few days before slaughter. Also, the addition of vegetable oil to the diet a few weeks before slaughter has been shown to reduce the off-flavor caused by fish lipids (Opstvedt, 1971).

Edin (1940) investigated the effects of H_2SO_4 preserved silage on performance of chickens and reported that growth rates were identical to those on control basal diets. Barlow and Pike (1977), in their studies with layers and broilers, reported that feeding fish silage did not impart an off-flavor to the eggs but tended to taint the broiler carcasses. Raa and Gilberg (1982) reported that factors such as protein to lipid ratio and the freshness of the material being fed, are important considerations when feeding fish silage. Kompiang et al. (1980) recommended that 20% (dry basis) should be considered the upper limit for inclusion of fish silage in broiler diets.

About 1.3% of the amino-N was released as ammonia in silage after 3 wk at tropical temperatures (Kompiang et al., 1980). This may imply a reduction in nutritive value if the ammonia was derived from essential amino acids. Free tryptophan decomposes in acid silage (Backhoff, 1976) and there are reports that methionine (Atkinson et al.,1974) and histidine (Disney et al., 1978) are also unstable. Storage for 40 d at 30 C resulted in loss of 30% of the tryptophan in silages preserved with formic acid with a pH of about 4.0 (Backhoff, 1976). The exact mechanism of degradation is unknown. Histidine may be a limiting amino acid in fish silage (Disney et al., 1978), particularly if the silage is prepared from partly spoiled fish, since this amino acid is quickly degraded by putrefying bacteria.

Most fish contain an enzyme, thiaminase that degrades thiamine (vitamin B_1), so there is some risk of thiamine deficiency when feeding fish silage (Disney and Hoffman, 1976). Poor growth on fish silage has sometimes been attributed to a deficiency of this vitamin (Disney et al., 1978).

Another possible effect of fish silage in feeding programs is related to the peroxidation of lipids and decomposition of vitamin E (Kompiang et al., 1980). The free radicals have detrimental effects on animal performance. Vitamin

E supplementation has been shown to improve growth in chickens fed fish silage.

The inclusion of acid-preserved fish silage in the diets of dairy cows resulted in slightly lower milk yield, compared to cows on a commercial high-protein diet (Raa and Gilberg, 1982). Ferreiro et al. (1977) also reported decreased feed intake in cattle on a diet containing formic acid preserved fish silage.

Samuels (1983) ensiled various proportions of fish waste and crop residues with the addition of a small amount of dry molasses. Apparent digestibility of dry matter and crude protein of 60 and 70% respectively, were reported when fish waste silages were fed with a basal diet on a 1:1, dry basis. Nitrogen retention was not significantly different for sheep fed the fish-waste silages, compared to those on a basal diet.

Silage Fermentation

General Principles. Forage material can be stabilized and conserved through the ensiling process (Watson and Nash, 1960), which is controlled fermentation. The main objective is to achieve and maintain anaerobic conditions, allowing lactobacilli to proliferate and produce lactic acid. This subsequently prevents the growth of spore-forming anaerobes, especially clostridia which produce CO_2, NH_3 and amines.

McDonald (1981) enumerated three interrelated factors that govern the ensiling process: 1) the composition of the plant material placed in the silo, 2) the amount of air allowed into the ensiled mass and 3) the bacteria on the material. Adequate compaction is essential since silage quality and temperature development have been related to the extent of consolidation (Langston et al., 1958; McDonald and Whittenbury, 1973).

In ensiled forage, the obligate aerobic flora will vanish quickly, yeasts after a few days and the facultative anaerobes more slowly (Pedersen, 1976). There is a simultaneous rapid increase in the number of lactic acid bacteria, which after 1 wk become the dominant bacterial group, present in numbers of about 10 billion/g of wet silage. Streptococcus species dominate among the lactic acid bacteria during the early phases of fermentation; later, lactobacillus species take over (Nilsson and Nilsson, 1956; Stirling and Whittenbury, 1963). A "clostridial" type of fermentation associated with poor quality silage can best be prevented by increasing the dry matter to above 30% and encouraging the formation of lactic acid to reduce the pH. Clostridia are inhibited more by a lack of moisture than by acid, hence, wilting crops above 30% DM is desirable (Wieringer, 1960). Proteolysis also occurs more in wetter than in drier silages.

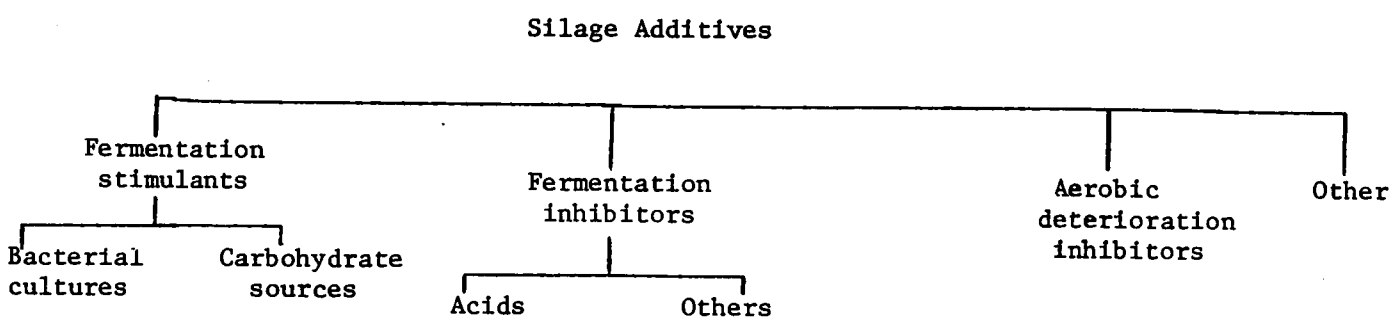
The production of good quality silage involves the controlled fermentation of water-soluble carbohydrates by microbes with the production of organic acids, mainly lactic acid, resulting in a pH of 4.5 or less (Barnett, 1954). Langston et al. (1958) reported that a well preserved forage contains less than .2% butyric acid, less than 11% ammoniacal nitrogen and lactic acid content of 3 to 13%, dry basis. Factors in silage fermentation include effective acidity (Watson and Nash, 1960), and antimicrobial effects of organic acids (Woolford, 1975).

Stimulation of lactic acid formation is the natural way to ensure a suitable and stable silage (Langston et al., 1958; McDonald and Whittenbury, 1973). The ensiling process begins with the laceration of the crop during harvesting and the release of cell sap containing readily-available carbohydrates required for microbial growth. Forages that are low in available carbohydrates may benefit from carbohydrate addition. Also, forages high in cations and protein content, such as legumes, tend to have a high buffering activity and may prevent a large decrease in pH.

Silage Additives

The general classification of silage additives is shown in figure 1.

Fig. 1. Classification of silage additives[a]



Bacterial cultures	Carbohydrate sources	Acids	Others	Aerobic deterioration inhibitors	Other
Lactic acid bacteria	Glucose	Mineral acids	Formaldehyde	Propionic acid	Urea
	Sucrose	Formic acid	Paraformaldehyde	Capróic acid	Ammonia
	Molasses	Acetic acid	Sodium nitrite	Sorbic acid	Biuret
	Cereals	Lactic acid	Sulphur dioxide	Pimaricin	Minerals
	Whey	Benzoic acid	Sodium metabisulphite	Ammonia	
	Beet pulp	Acrylic acid	Ammonium bisulphate		
	Citrus pulp	Glycollic acid	Sodium chloride		
	Potatoes	Sulphamic acid	Antibiotics		
	Cellulases	Citric acid	Carbon dioxide		
		Sorbic acid	Carbon bisulphide		
			Hexamethylenetetramine		
			Bronopol		
			Sodium hydroxide		

[a]Adapted from McDonald (1981)

Molasses. Molasses contains over 50% sucrose and has been widely used as an additive. In experimental silos, the lactic acid content increased as the amount of molasses increased from 0 to 4%, while pH decreased (Lanigan, 1961). Silage made from grass and added molasses was equal to wilted silage for milk production, and produced silage with lower pH and NH_3-N, with more lactic acid (Dijkstra, 1954). Control silages incubated at 30 C were inferior to those incubated at 15 C, but addition of molasses improved both silages and Dijkstra (1951) and Colovos et al.(1957) reported that DM losses decreased with addition of molasses. Lanigan (1961), using alfalfa, demonstrated in laboratory silos that increasing the level of molasses decreased pH, increased lactic acid and decreased DM loss.

In early studies, Reed and Fitch (1917) reported that alfalfa silage preserved with molasses was consumed by steers in preference to alfalfa treated with straw, ground corn or sweet sorghum stover. Allen and Fitch (1942) and McCullough and Neville (1960) reported that performance of steers fed molasses-treated alfalfa silage was equal to or greater than that of control animals on alfalfa and corn silage. Pratt et al. (1958) and Mather et al. (1960) fed molasses-preserved alfalfa silage using corn silage as control. They found no differences in milk production over a 2-yr period. Similar

results were reported by King (1944) in studies with soybean silage made with 4% added molasses and corn silage as control.

Inoculants. Early work with microbial cultures produced results which were variable among experiments (Wieringa, 1960; McDonald et al., 1964). These workers concluded that the results were expected because of the many factors influencing the response, such as types and number of bacteria present, type and variability of the cultures used, availability of fermentable sugars and the moisture level of the ensiled mass. Baker and Voelker (1958) and Lesins and Schultz (1968) studied the effects of lactobacillus cultures on clover and crown vetch silages, and found that microbial treatment resulted in lower pH and higher lactic acid, compared to control silages with no inoculants. Kirov (1962) treated alfalfa (30% dry matter) with .5% lactobacillus culture and 1.5% molasses. The culture plus molasses-treated silage had higher total titrable acidity, lower pH and lower total bacterial numbers than untreated silage. The effect of inoculation in combination with the addition of sugar was studied by Wieringa (1961) and Lesins and Schultz (1968). These workers reported that in most cases the combination of inoculant and sugar produced positive results. Ohyama et al. (1975) showed that glucose and Lactobacillus plantarum ino-

culation resulted in large amounts of lactic acid, while the addition of glucose alone produced variable results. Untreated silages were of poor quality.

Watson and Nash (1960) reported that the success of inoculation depends on many factors, of which the choice of species to be used seems the most important. Whittenbury (1961) defined criteria which a potential organism should satisfy for use in silage: the organism 1) must have a high growth rate and be able to compete and dominate other organisms likely to occur in silage, 2) must be acid tolerant and produce pH drop quickly, 3) must be homofermentative, 4) must be able to ferment glucose, fructose, sucrose and preferably fructosans and pentosans and 5) should have no action on organic acids. No single organism is expected to satisfy all these criteria but combinations may be satisfactory. This is one reason for the commonly used and recommended combination of streptococcus species with lactobacilli (Stirling and Whittenbury, 1963). Streptococci are dominant during the early phase of fermentation (near neutral pH) and later lactobacilli take over as the pH drops (Nilsson and Nilsson, 1956; Pedersen, 1976). Heterofermentative bacteria are active at pH above 5.5 (Raa, 1981).

Acids. Addition of acid is an alternative to fermentation for the preservation of silages. The first information

concerning additives which have nearly total inhibitory effects on silage microflora were reported by Virtanen (1929). The acids used were a mixture of sulfuric and hydrochloric acids (AIV acid). The production of silage by the AIV process was found useful and acceptable. It reduced pH to below 3.0, prevented fermentation and proteolysis but animal acceptability was very low. There were also mineral feeding problems associated with the silage and the acid also corroded silos and equipment (Watson and Nash, 1960). Studies by Disney et al. (1977) suggests that sulfuric acid or hydrochloric acid can be replaced by phosphoric acid.

The addition of formic acid allows limited proteolysis and production of lactic acid (Backhoff 1976). Thus, it acts as a partial sterilant (Barker et al., 1973). Waldo (1977) reported that addition of formic acid to direct-cut forage when ensiled improved DM recovery from storage, animal intake, efficiency of animal performance and energy digestibility. No effects on milk production were observed. Formic acid is metabolized during the ensiling process and has little effect on restricting yeast numbers or stabilizing silage when removed from the silo (Henderson and McDonald, 1971). However, for high-moisture forage that is difficult to ensile, formic acid addition produces better preservation and greater feed consumption than direct-cut silage without additive (Castle and Watson, 1973).

Woolford (1975) reported that propionic acid inhibited mold growth but did not inhibit the growth of lactic acid producing bacteria, when tested in pure cultures. Thomas (1976) and Goering and Gordon (1973) indicated that treating forage with propionic acid will reduce the temperature of the ensilage and retard aerobic fermentation of the silage after removal from the silo at the time of feeding. Yu Yu and Thomas (1975) treated haylage with propionic acid and reported reduced acid detergent fiber (ADF) and lignin, compared to untreated controls. This is probably a reflection of reduced fermentation and the retention of more water-soluble carbohydrates.

Formaldehyde. Formaldehyde has been successfully used as a bacteriostat in silage preservation (Watson and Nash, 1960). Wilkins et al. (1974) studied the inhibiting effects of formaldehyde on 34 pure cultures of bacteria by adding various amounts of formalin. The bacteria were not inhibited at a concentration of .07%, but were inactivated at .3%. Barry and Fennesy (1972) treated grass-legume and legume forages with .6 to 4.4% formaldehyde, dry basis. These workers reported that there was a marked reduction in NH_3-N and total titrable acidity. A similar observation was made by Valentine and Radcliffe (1975) with subterranean clover (Trifolium subterranean) treated with 1.2% formaldehyde.

Many researchers have reported that the pH of formaldehyde-treated forage was higher than control silages, indicating that formaldehyde acts as a sterilant reducing bacterial fermentation (Valentine and Brown, 1973; Waldo, 1977).

In feeding trials with alfalfa treated with .9% formaldehyde, Valentine and Brown (1973) obtained increased digestibility of both crude protein and DM, compared to controls. Voluntary intake and wool growth of sheep were not affected. Beaver et al. (1977) compared untreated perennial ryegrass and .74% formaldehyde-treated ryegrass in sheep digestion trials. They reported that over 71% of the protein entering the small intestine was of microbial origin when the sheep were fed untreated silage, compared to 17% for sheep fed the formaldehyde-treated silage.

Mann and McDonald (1976) compared the effectiveness of formaldehyde, formic acid, acetic acid and propionic acid as sterilants by treating Italian ryegrass (Lolium multiflorum) with .45% of each chemical and ensiling in 3 kg capacity silos. All additives restricted bacterial fermentation. However, acetic and propionic acids were less effective than formic acid and formaldehyde.

Crop Residues

The United States produces annually about 44 million metric tons of wheat (Trifolium aestivum), and 186 million metric tons of coarse feed grains, dry basis (USDA, 1979). Grain producing plants usually produce an equal or greater weight as vegetative material than as grain (Vetter and Boehlje, 1978). Thus, a total of at least 300 million metric tons of straws, stalks and stubbles are produced each year. This amount of residue has been estimated to have sufficient energy to meet the entire needs of the beef cattle industry (Anderson, 1978). Corn crop residue is estimated to be about 162 million metric tons of dry matter per year (Keys and Smith, 1981) which is over one half the total available residue supply. The estimated quantity of wheat straw (44 million metric tons) per year constitutes only about 15% of the total crop residue supply.

Nutritive Value of Corn Stover and Wheat Straw. Colenbrander et al. (1971) reported that corn stover contains considerable energy for ruminants and contains about 69% neutral detergent fiber (NDF), but is low in CP (about 6%). The cornstalks decrease in digestibility and soluble cell contents with time after physiological maturity (Berger et al., 1979; McDonnell and Klopfenstein, 1980). Digestibility is also influenced by moisture, variety and temperature

(McDonnell and Klopfenstein, 1980). Different havesting systems will also produce corn crop residues with varying feeding value. Koers et al. (1970) obtained .3 kg/d weight gains in calves fed supplemented corncobs and Berger et al. (1979) reported .65 kg/d weight gains with growing calves fed supplemented stalklage. However, Paterson et al. (1979) reported digestibilities as low as 37%, which would not be sufficient for maintenance.

Braman and Abe (1977) reported that wheat straw contained about 3% crude protein (CP) and 82% cell walls, dry basis. Variation in nutritional value has been attributed to variety, cultural practices and time of harvesting (Jackson, 1978). Feeding trials with straw have been evaluated by Acock et al. (1979) and Dinusson (1969). These workers have shown that feeding one- third alfalfa hay with wheat straw can meet the protein needs of gestating beef cows. Coombe et al. (1979) and Lesoing et al. (1980) have demonstrated that chopped wheat straw alone has little value for feeding growing calves.

Chemical Treatment of Crop Residues. It has been established that as plants mature, the lignin content increases (Kamstra et al., 1958) and lignin has very low digestibility. Digestibility can be improved by treating with chemicals. Four chemicals which have been routinely used in ex-

perimentation with the various straws are NaOH, $Ca(OH)_2$, KOH and NH_3 (Klopfeinstein, 1978).

The modes of action for chemical treatment of crop residues have been established (Waller, 1976). In general, chemical treatment solubilizes some of the hemicellulose while not changing the cellulose content. The reaction breaks the ether linkages between lignin and cellulose or hemicellulose (Lau and Van Soest, 1980). There has also been suggestions of a swelling effect of chemical treatment on cellulose and hemicellulose (Waller, 1976).

Ammonia appears to react in a manner similar to NaOH, but the reaction time is much longer, up to 20 d (Waiss et al., 1972), compared to 24 h for NaOH treatment. Ammonia treatment obviously will increase the CP content and eliminate the detrimental effects of mineral residues observed with the other chemical treatments.

Treatment of crop residues has been shown by many researchers to improve digestibility and animal performance, if the mineral imbalance created by alkali treatment is corrected (Paterson et al., 1978). Treated wheat straw, untreated straw and corn silage were fed to growing calves and their performance compared. Chemical treatment increased rate and efficiency of gain, compared to untreated straw (Lesoing et al., 1980). Acock et al. (1979) also showed that

treated straws plus protein supplementation used in gestating beef cow diets resulted in improved weight gains over controls. Improvement in digestibility of chemically treated crop residues has been shown to be 10 to 20 percentage units, over untreated residues (Klopfenstein, 1978).

Chapter III

JOURNAL ARTICLE 1

ENSILING CHARACTERISTICS OF CRAB WASTE AND WHEAT STRAW TREATED WITH VARIOUS ADDITIVES

ABSTRACT

The crude protein of the crab (Callinectes sapidus) waste and wheat (Triticum aestivum) straw were 44.1 and 4.0%, dry basis, respectively. In all experiments crab waste, and wheat straw were ground and ensiled in 1:1 proportions. In expreiment 1, 0, 10 or 20% dry molasses; 0 or .1% microbial silage inoculant; and 0 or 5.4% phosphoric acid were added. In a second experiment, silages were prepared with 10, 15 or 20% molasses, 0 or .1% microbial silage inoculant and 0 or 20% water. For these two experiments 3 x 2 x 2 fatorial arrangement was used. For the third experiment, the treatments were 0 or 20% water and 0 or .1% of three different microbial silage inoculants in a 2 x 4 factorial arrangement. After ensiling, the pH of the mixtures without added molasses or acid was higher than the initial pH. Lactic acid levels increased linearly ($P<.05$) with increasing levels of molasses. Trimethylamine (TMA) which indicates offensive odor in marine products was lower ($P<.01$) for molasses- and inocu-

lant-treated silages. Generally, TMA was lower for mixtures in which the pH was low and contained appreciable quantities of lactic acid. Acetic acid was the predominant volatile fatty acid in all the ensiled mixtures. Acetic, propionic and isobutyric acid concentrations were higher ($P<.01$) in the mixtures with added water and molasses. Total VFA were increased ($P<.01$) by addition of water, molasses and inoculant. Addition of 20% dry molasses to crab waste-straw mixtures prior to ensiling resulted in silages with substantial amounts of lactic acid. In a large study, pH was lower and lactic acid was higher ($P<.05$) for ensiled mixtures with added molasses, compared to molasses alone. Treating with inoculant resulted in greater effects than treating with only molasses ($P<.05$).

(Key words: Crab waste, Wheat straw, Sugarcane byproduct, Silage, Lactic acid).

Introduction

Crabs represent the fourth most important seafood product in the U.S.A., with a total annual catch of about 149,000 metric tons (NMFS, 1975). After processing, 80 to 90% remains as waste material (Brinsfield, 1980). Disposal of the waste presents problems due to the high moisture content (Miles, 1981). In the past, the major disposal outlet was the production of crab meal (Olsen, 1980), which was attrac-

tive in terms of nutrient recovery for livestock. However, the economic feasibility of this method is questionable under current energy prices. An alternative method of disposal that requires minimal energy input is ensiling. Previous efforts in this laboratory to ensile crab waste were unsuccessful due to offensive odor and increased pH (Samuels, 1983). However, crab waste and wheat straw mixtures were stabilized by adding 16% glacial acetic acid, but this practice is not economical.

Crab waste is high in minerals and protein (Cantor, 1980), which contribute buffering activity to the mixtures. Trimethylamine, a volatile product, buffers and imparts offensive odor to the mixtures (Beatty and Gibbons, 1937). In order to successfully ensile crab waste, substantial amounts of fermentable sugars are reqiured to produce large quantities of lactic acid to lower the pH and stabilize the product (Raa et al., 1982). There is evidence to suggest that ammonia is produced early in the fermentation process before the pH has dropped (Raa and Gilberg, 1982). This may be avoided by combined acidification and sugar addition. Lactic acid bacteria are natural inhabitants of seafood products (Schroder et al., 1980), but they are present in low numbers.

This study was undertaken to investigate the level(s) of additives necessary to produce acceptable silages of crab waste and straw.

Materials and Methods

Small Silo Studies. Crab waste was obtained from a processing plant[1] in which meat was hand picked from steamed blue crabs. The crab waste was ground in a high speed hammermill without screen. Wheat straw was ground in a tub grinder[2] through a 2 cm screen.

Three experiments were conducted. In the first experiment, ground crab waste and wheat straw were mixed in 1:1 proportions, wet basis, with the addition of 0, 10 or 20% dry blackstrap molasses, 0 or 5.4% phosphoric acid and 0 or .1% micrbial silage inoculant[3] composed of Streptococcus faecium and Lactobaccillus plantarum. Thus a 3 x 2 x 2 factorial arrangement of treatments was used. The amount of acid added was calculated to lower the pH of the mixtures to 7.0. Water was added at 20% to all mixtures.

In the second experiment, molasses at 10, 15 or 20%, water at 0 or 20% and microbial silage inoculant at 0 or .1% were added in a 3 x 2 x 2 factorial arrangement of treat-

[1] Graham and Rollins, Inc., Hampton, Va.

[2] Sperry-New Holland, New Holland, Pa.

[3] Obtained from Pioneer Hi-Bred International, Inc., Des Moines, IA

ments. In the third experiment, water at 0 or 20% and microbial silage inoculants at 0 or .1% were added. Three inoculants were tested: silagenie[4] 13 and 16 (the composition of these are unknown) and an inoculant consisting of S. faecium and L. plantarum. Molasses was added at 20% to all mixtures. Thus a 2 x 4 factorial arrangement of treatments was used.

For all experiments, the mixtures were prepared by slowly adding the wheat straw to the crab waste in a horizontal mixer. The inoculant was hand-mixed in the molasses and the molasses was slowly added to the crab waste-straw mixtures and allowed to mix for 30 min. In the first experiment, three laboratory silos, consisting of 1 liter cardboard containers double lined with polyethylene bags, each containing approximately .7 kg of the initial mixture were prepared for each treatment. Six silos, consisting of 3.8 liter cardboard containers double lined with polyethylene bags and each containing approximately 2 kg of the initial mixtures were prepared for each treatment in the second experiment. Initial samples were taken for dry matter (DM) determination. Mixtures were firmly packed and each bag was individually sealed after elimination of air. The silos were weighed before and after ensiling.

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[4] Obtained from Silopress, Inc., Sioux City, IA.

After a minimum of 42 d in an enclosed barn during September and November, the silos were weighed, opened and observed for appearance and odor. The top 5 cm of the ensiled material were removed prior to sampling. Water extracts of the initial and fermented mixtures were prepared by homogenizing duplicate 25 g samples with 225 ml of deionized water in .5 liter jars in a Waring blender at full speed for 2 min. The homogenate was filtered through four layers of cheesecloth. The extracts of the initial and fermented mixtures were used for measurement of pH (electrometrically), lactic acid (Barker and Summerson, 1941, as modified by Pennington and Sutherland, 1956), water soluble carbohydrates (Dubois et al., 1956, as adapted for corn plants by Johnson et al.,1966), and VFA (Erwin et al., 1961). Trimethylamine was determined on initial and fermented mixtures by a colorimetric procedure (Dyer, 1959) after extracting in 7.5% trichloroacetic acid. Dry matter of the mixtures was determined by drying duplicate 200 g samples in a forced-draft oven at a maximum of 60 C for 48 h. The samples were allowed to air equilibrate, composited and ground through a 1 mm sieve and analyzed for DM and ash (AOAC, 1980). Kjeldahl N was determined on wet silage samples (AOAC, 1980).

Large Silo Study. Based on data from the small silo studies and previous work in this laboratory, a study was con-

ducted to further determine the fermentation characteristics, nutrient metabolism and palatability of ensiled crab waste and wheat straw. Crab waste and wheat straw (1:1 proportions) were ensiled with the following additives: 1) 20% dry molasses, 2) 20% dry molasses and .1% silage inoculant containing a blend of Streptococcus faecium and Lactobacillus plantarum[3] and 3) 16% (v/w) glacial acetic acid. Wheat straw was also ensiled alone. Water was added at 20% to the mixtures prior to ensiling.

Crab waste and wheat straw were ground and mixed in a horizontal mixer and the various additives were slowly added. After mixing for about 30 min, the mixtures were augered into 210 liter metal drums double lined with .08 mm polyethylene bags. All mixtures were firmly packed by trampling and the bags were individually sealed after exclusion of air. Initial samples of the ingredients and mixtures were collected, composited, subsampled and frozen.

The mixtures were allowed to ensile in a closed barn January and February for a minimum of 42 d. The top 5 cm were removed upon opening the silos and samples were taken from several areas of the silo, subsampled and frozen for subsequent analyses. Procedures used for the determination of fermentation characteristics, DM and N were the same as described for the small silo studies. Samples were also ana-

lyzed for neutral detergent fiber (NDF) (Van Soest and Wine, 1967), acid detergent fiber (ADF) (Van Soest, 1963), lignin and cellulose, hemicellulose (Van Soest and Wine, 1968), and ash (AOAC, 1980).

Statistical Analyses. Analyses of variance were performed using the general linear model (GLM) procedure of the Statistical Analyses System (SAS, 1982). For the small silo experiments, the results were analysed as 3 x 2 x 2 (experiment 1 and 2) and 2 x 4 (experiment 3) factorial arrangements of treatments. For the first two experiments, the effect of level of molasses was tested by linear and quadratic contrasts. For the third experiment, three orthogonal contrasts were made: 1) no additive vs inoculants, 2) Pioneer Hi-Bred International microbial silage inoculant vs Silopress microbial silage inoculants and 3) silagenie 13 vs silagenie 16.

For the large silo study, the following comparisons were made : 1) Straw vs crab waste-straw, 2) acetic acid treatment vs molasses, 3) molasses vs molasses plus inoculant.

Results and Discussion

Chemical Composition. The DM of the crab waste was 45.2% (table 1), which is slightly higher than the value of 40.2 reported by Samuels (1983). The crude protein of the crab waste and straw were 44.1 and 4.0%, respectively. The pro-

Table 1. Chemical Composition of the Crab Waste and Wheat Straw Used in Experiments

Components	Crab waste			Wheat straw		
	Small silos			Small silos		
	Experiment 1	Experiment 2	Large silos	Experiment 1	Experiment 2	Large silos
Dry matter, %	45.22	44.80	44.92	89.20	89.50	90.12
Crude protein[a]	44.13	44.52	44.36	4.02	4.04	3.94
Neutral detergent fiber[a]	22.53	21.82	22.40	86.72	85.90	86.62
Acid detergent fiber[a]	17.05	16.94	16.85	58.15	58.10	58.30
Cellulose[a]	0.14	0.16	0.18	46.52	46.48	46.60
Hemicellulose[a]	5.48	5.24	5.36	28.57	28.62	28.70
Lignin[a]	0.01	0.03	0.03	10.38	10.28	10.34
Ash[a]	39.25	39.15	39.18	5.57	5.62	5.54
Water-soluble carbohydrates[a]	3.42	3.38	3.46	4.08	4.13	4.14

[a]Percent of dry matter

tein content of the crab waste was higher than the value of 28% reported by Cantor (1980) but similar to those reported by Watkins (1982) and Samuels (1983). The crab waste used in the present study was from the same source as the waste used by Samuels (1983). Neutral detergent fiber and ADF were 22.5 and 17.0%, respectively, for the crab waste and the ash content was 39.2%. Water-soluble carbohydrates for the crab waste and wheat straw were low.

Small Silos. The silages with pH in the range of 5.0 to 6.0 had pleasant aroma. The control mixture had a pungent and offensive odor after ensiling. The initial pH in the first experiment was similar for all mixtures, averaging approximately 7.0 (table 2). After ensiling, all mixtures showed a decrease in pH except for those with no additives and the inoculated treatment alone. The pH for these increased to about 8.0 after ensiling. Adding molasses decreased ($P<.05$) post ensiled pH, suggesting enhanced fermentation.

Initial WSC levels reflected the amount of molasses added. There was a decrease in WSC after ensiling. Post ensiled WSC was higher $P<.05$) for mixtures with added acid, suggesting decreased microbial activity. Addition of acid probably inactivated the silage microbes. Adding a microbial silage inoculant had no effect $P>.05$) on post ensiled pH.

Table 2. Fermentation Characteristics of Crab Waste - Wheat Straw Silages, Small Silos, Experiment 1

Item	Treatments[a]												
	None	Acid[b]	Inoculant[c]	10% molasses	20% molasses	Acid[b] + 10% molasses	Acid[b] + 20% molasses	Acid[b] + inoculant	Inoculant + 10% molasses	Inoculant + 20% molasses	Acid[b] + inoculant + 10% molasses	Acid[b] + inoculant + 20% molasses	S.E.
pH													
Pre-ensiled	7.34	7.14	7.36	6.87	7.13	7.06	6.96	6.86	7.18	6.95	6.81	6.99	.06
Post-ensiled[def]	8.30	6.81	8.23	6.83	5.31	6.49	6.47	6.55	6.07	5.34	6.64	6.42	.08
Water soluble carbohydrates[g]													
Pre-ensiled[efh]	2.25	2.53	2.16	10.73	22.70	10.40	20.76	2.84	12.11	19.65	10.87	20.71	.33
Post-ensiled[dei]	2.10	2.42	1.99	3.62	4.81	5.82	4.39	2.68	3.84	3.79	3.99	6.71	.11
Lactic acid[g]													
Post-ensiled[defh]	.47	.41	.59	3.03	6.85	1.55	2.80	.69	4.88	11.07	1.19	2.67	.07

[a]Crab waste and wheat straw (1:1, wet basis)

[b]Phosphoric acid (5.4%)

[c]Silage inoculant, S. faecium and L. plantarum (.1%)

[d]Acid effect ($P < .05$)

[e]Molasses effect ($P < .05$)

[f]Acid x molasses interaction ($P < .05$)

[g]Percent of dry matter

[h]Inoculant x molasses interaction ($P < .05$)

Substantial levels of lactic acid were observed only for the ensiled mixtures with molasses. Addition of acid decreased ($P<.05$) lactic acid in the the post ensiled mixtures, indicating that fermentation was decreased by addition of acid. A large decrease occurred when it was added to mixtures with added molasses (acid x molasses interaction, $P<.05$) Adding molasses and microbial silage inoculant increased ($P<.05$) lactic acid of the ensiled mixtures, compared to molasses alone, but the effect was noted only when the acid was not added.

The effect of different levels of molasses on fermentation are presented in table 3. The post ensiled pH of the mixtures decreased linearly ($P<.05$) with increasing levels of molasses. Water soluble carbohydrate disappearance was highest for the mixtures with added 20% molasses, indicating a greater extent of fermentation. Lactic acid levels increased linearly ($P<.05$) with level of molasses.

The change in TMA in the crab waste-straw silages after ensiling, which indicates offensive odor is presented in table 4. Initial mixtures had concentrations of about 7.0 to 8.0 mg/100g silage. After ensiling, essentially no change occurred in TMA only for the mixture with 20% molasses. Addition of microbial inoculant further decreased ($P<.05$) TMA levels. The high levels of TMA in the silages without mo-

Table 3. Effect of Different Levels of Molasses on Fermentation Characteristics of Crab Waste - Wheat Straw Silages, Small Silos, Experiment 1[a]

Item	Level of molasses, %			S.E.
	0	10	20	
pH				
Pre-ensiled[b]	7.17	6.98	7.01	.06
Post-ensiled[b]	7.47	6.51	5.88	.08
Water soluble carbohydrates[c]				
Pre-ensiled[b]	2.44	11.03	20.96	.33
Post-ensiled[bd]	2.30	4.32	4.93	.11
Lactic acid[c]				
Post-ensiled[bd]	.54	2.66	5.85	.07

[a]Averaged over additives.

[b]Linear effect ($P < .05$)

[c]Percent of dry matter

[d]Quadratic effect ($P < .05$)

Table 4. Effect of Ensiling upon Trimethylamine Production, Small Silos, Experiment 1

Item	Treatments[a]												
	None	Acid[b]	Inoculant[c]	10% molasses	20% molasses	Acid + 10% molasses	Acid + 20% molasses	Acid + Inoculant	Inoculant + 10% molasses	Inoculant + 20% molasses	Acid + Inoculant + 10% molasses	Acid + Inoculant + 20% molasses	S.E.
	mg/100 g												
Pre-ensiled	8.57	7.86	7.65	7.32	7.18	7.29	7.11	7.48	7.19	7.16	7.23	7.11	.18
Post-ensiled[def]	20.55	14.97	18.84	16.84	8.37	14.44	15.15	15.16	12.08	8.11	15.52	14.85	.56

[a]Crab waste and wheat straw (1:1, wet basis)

[b]Phosphoric acid (5.4%)

[c]Silage inoculant, S. faecium and L. plantarum (.1%)

[d]Molasses effect ($P < .01$)

[e]Inoculant effect ($P < .01$)

[f]Acid x molasses interaction ($P < .05$)

lasses is congruent with the report by Tatterson (1980), where it was suggested that in the abscence of free sugar, putrefying microbes actively degrade proteins, resulting in the production of amines. Watson (1939) reported that microbes in marine animals including crabs, used trimethylamine oxide as a terminal electron acceptor, and reduced it to TMA in the abscence of appropriate substrates such as pyruvate and glucose.

Addition of molasses, water and microbial inoculant decreased ($P<.05$) the pH of the mixtures after ensiling (table 5), but the effect of inoculant was small. Water decreased pH only in mixtures with 15 and 20% molasses (molasses x water interaction, $P<.05$). Water addition increased lactic acid at all levels of molasses but the absolute effect was greater at the higher levels of molasses. Molasses had little effect if moisture was not added. The effect of water may have been to permit more effective packing or making the soluble carbohydrates more readily available to the bacteria. Adding inoculant decreased pH and increased lactic acid at all levels of molasses with or without added water. Differences in the level of the pre-ensiled WSC is a reflection of the amount of molasses added.

The predominant volatile fatty acid (VFA) in all mixtures was acetic acid (table 6). Addition of water to the mixtures

Table 5. Fermentation Characteristics of Crab Waste - Wheat Straw Mixtures, Small Silos, Experiment 2

Item	Treatments[a]												
	10% Molasses				15% Molasses				20% Molasses				
	None	H_2O[b]	Inoculant[c]	H_2O + inoculant	None	H_2O	Inoculant	H_2O + inoculant	None	H_2O	Inoculant	H_2O + inoculant	S.E.
Dry matter, %	71.82	49.04	70.30	50.12	72.15	59.61	71.80	60.14	73.52	58.40	73.15	59.54	
pH													
Pre-ensiled[defgh]	7.55	7.25	7.32	7.29	7.24	7.19	7.22	7.23	7.13	7.21	7.12	7.22	.17
Post-ensiled[defgh]	6.97	6.75	6.81	6.65	6.77	5.74	6.78	5.49	6.27	5.35	6.41	5.17	.06
Water soluble carbohydrates[i]													
Pre-ensiled[dg]	12.02	12.32	12.46	11.98	18.39	18.76	18.18	18.27	22.31	22.28	23.44	23.57	.26
Post-ensiled[defgh]	10.22	6.82	9.59	8.39	12.49	10.37	12.14	7.84	14.62	6.94	13.75	5.50	.12
Lactic acid[i]													
Post-ensiled[defgh]	.94	3.04	1.17	4.46	.88	4.46	1.27	5.13	.80	6.97	1.05	11.06	.11

[a] Crab waste and wheat straw (1:1, wet basis)

[b] Water (20%)

[c] Silage inoculant, S. faecium and L. plantarum (.1%)

[d] Molasses effect ($P < .05$)

[e] Water effect ($P < .05$)

[f] Inoculant effect ($P < .05$)

[g] Molasses x water interaction ($P < .05$)

[h] Molasses x inoculant interaction ($P < .05$)

[i] Percent of dry matter

Table 6. Effect of Ensiling Crab Waste and Various Additives upon Volatile Fatty Acid Production, Small Silos, Experiment 2

Volatile fatty acids	Treatments[a]												
	10% molasses				15% molasses				20% molasses				
	None	H_2O[b]	Inoculant[c]	H_2O + inoculant	None	H_2O	Inoculant	H_2O + inoculant	None	H_2O	Inoculant	H_2O + inoculant	S.E.
	% dry basis												
Acetic[defghijk]	1.55	2.75	1.57	2.87	1.39	2.72	2.14	3.70	1.18	2.58	1.10	2.39	.14
Propionic[eghij]	.06	.11	.06	.13	.05	.07	.12	.08	.05	.11	.05	.11	.01
Isobutyric[deghij]	.08	.04	.06	.13	.06	.14	.06	.09	.06	.07	.07	.07	.01
Total[defghijk]	1.69	2.90	1.69	3.13	1.50	2.93	2.32	3.87	1.29	2.76	1.22	2.57	.14

[a] Crab waste and wheat straw (1:1, wet basis)

[b] Water (20%)

[c] Silage inoculant, S. faecium and L. plantarum (.1%)

[d] Molasses effect ($P < .01$)

[e] Water effect ($P < .01$)

[f] Inoculant effect ($P < .01$)

[g] Water x inoculant effect ($P < .01$)

[h] Water x molasses effect ($P < .01$)

[i] Molasses x inoculant effect ($P < .01$)

[j] Linear effect of molasses ($P < .05$)

[k] Quadratic effect of molasses ($P < .05$)

increased (P<.01) acetic, propionic and isobutyric acid concentrations, and subsequently resulted in higher total VFA concentration. The high levels of lactic acid and total VFA concentration in silages with added water, suggests that fermentation occurred to a greater extent in these silages. Adding microbial silage inoculant increased (P<.01) the levels of acetic and total VFA in the mixtures. Addition of water and molasses increased (P<.01) propionic and isobutyric acids. The abscence of butyric acid in these silages is in contrast to the findings of Samuels (1983) in which appreciable quantities were reported for the crab waste silages. Butyric acid is associated with 'clostridial' type of fermentation and usually indicates the breakdown of amino acids. The abscence of butyric acid in the present study agrees with the findings of Roa (1965) that free glucose represses the production of deaminating enzymes by putrefying microbes. Propionic acid increased linearly (P<.05) with increasing levels of molasses. Quadratic (P<.05) effects were observed for acetic acid and total VFA. The effects of the different microbial silage additives on ensiling parameters and TMA production are presented in appendix tables 29 and 30.

Large Silo Study. The crude protein content of the crab waste-straw silage mixtures averaged between 12 and 13%, dry

basis, while that of the wheat straw averaged 3.9% (table 7). Fiber components were higher for the wheat straw silage, compared to the crab waste-straw silages, a reflection of low fiber of the crab waste and additives. Ash content was high in the crab waste mixtures.

The initial pH values were lower ($P<.05$) for the acetic acid treated silage (table 8), a reflection of added acetic acid. There was no apparent change in pH for the acetic acid treated silage after ensiling. Other silages showed marked decreases in pH and were characterized by pleasant aroma. The molasses-inoculant treated silage had a lower ($P<.01$) post ensiled pH, compared to molasses alone. There was also a high disappearance of WSC in the crab waste-straw silages, suggesting a high rate of fermentation in these mixtures. Addition of .1% microbial silage inoculant resulted in higher ($P<.01$) levels of lactic acid, compared to molasses alone. Lactic acid levels were higher ($P<.05$) for the crab waste-straw silages, compared to ensiled wheat straw silage.

The predominant VFA, as in the small silos, was acetic acid (table 9). Acetic acid was higher ($P<.01$) in the acetic acid treated silages, a result of the acetic acid addition. There was a higher ($P<.05$) level of acetic acid in the 20% molasses mixture, compared to the molasses-inoculant

Table 7. Chemical Composition of Initial Mixtures of Crab Waste - Wheat Straw, Large Silos

Item	Additives to mixtures[a]			Wheat straw
	16% acetic acid	20% molasses	20% molasses + .1% inoculant[b]	
Dry matter, %	49.30	60.82	60.58	53.90
Crude protein[c]	12.04	12.68	12.74	3.92
Neutral detergent fiber[c]	60.04	55.74	54.84	86.72
Acid detergent fiber[c]	43.08	40.54	41.98	58.12
Cellulose[c]	33.02	31.39	33.21	46.50
Hemicellulose[c]	16.96	15.20	12.86	28.60
Lignin[c]	8.53	8.32	8.15	10.40
Ash[c]	20.28	15.89	15.82	5.57

[a]Crab waste and wheat straw (1:1, wet basis)

[b]S. faecium and L. plantarum

[c]Percent of dry matter

Table 8. Fermentation Characteristics of Crab Waste - Wheat Straw Silages

Item	Additives to mixtures[a]				
	16% acetic acid	20% molasses	20% molasses + .1% inoculant[b]	Wheat straw	S.E.
pH					
Pre-ensiled[c]	4.35	6.97	7.02	7.05	.03
Post-ensiled[cd]	4.36	5.28	4.70	4.75	.04
Water soluble carbohydrates[e]					
Pre-ensiled[cf]	6.42	26.82	27.12	4.04	.22
Post-ensiled[df]	5.47	6.42	5.12	1.28	.10
Lactic acid[e]					
Post-ensiled[cdf]	.55	10.84	12.76	2.46	.16

[a] Crab waste and wheat straw (1:1, wet basis)

[b] S. faecium and L. plantarum

[c] Acetic acid and molasses treatments differ ($P < .05$)

[d] Molasses and inoculant treatments differ ($P < .01$)

[e] Percent of dry matter

[f] Wheat straw and crab waste silages differ ($P < .05$)

Table 9. Effect of Ensiling Crab Waste and Wheat Straw upon Volatile Fatty Acid Production, Large Silos

Volatile fatty acids	Additives to mixtures[a]			Ensiled wheat straw	S.E.
	16% acetic acid	20% molasses	20% molasses + .1% inoculant[b]		
Acetic[cde]	34.68	3.65	1.78	1.49	.47
Propionic[cd]	.07	.18	.16	.25	.02
Isobutyric[cd]	.02	.03	.03	.00	.02
Butyric[c]	.00	.00	.00	.04	.00
Total[cde]	34.77	3.86	1.97	1.78	.47

[a]Crab waste and wheat straw (1:1, wet basis)

[b]S. faecium and L. plantarum

[c]Wheat straw and crab waste silages differ ($P < .01$)

[d]Acetic acid and molasses treated silages differ ($P < .01$)

[e]20% molasses and inoculated silages differ ($P < .05$)

mixture. This observation suggests that inoculation may be beneficial in directing fermentation to lactic acid formation. Propionic acid was higher ($P<.01$) in the wheat straw, compared to the crab waste-straw silages, although all values were low. Isobutyric acid was detected in only the crab waste-straw silages and butyric acid in only the wheat straw silage.

There were no significant differences ($P>.05$) in TMA between the crab waste-straw silages (table 10). The levels remained approximately the same after ensiling, and are in the range for acceptable smell. However, there was a tendency for the mixtures with added molasses-inoculant to have lower levels of TMA. The molasses-inoculant mixture also had the highest level of lactic acid. Crab waste and wheat straw mixtures ensiled satisfactorily with addition of moderate amount of molasses. Moisture was critical in the ensiling process. It appears from this study that the production of TMA depends on an adequate energy source for microbial fermentation, as well as the development of a low pH within the ensiled mass.

Table 10. Effect of Ensiling on Trimethylamine (TMA) Production, Large Silos

Item	Additives to mixtures[a]			Ensiled wheat straw	S.E.
	16% acetic acid	20% molasses	20% molasses + .1% inoculant[b]		
	mg/100g				
Pre-ensiled[c]	7.82	7.78	7.80	.00	.08
Post-ensiled[c]	8.12	8.14	7.96	.00	.07

[a]Crab waste and wheat straw (1:1, wet basis)

[b]S. faecium and L. plantarum

[c]Wheat straw and crab waste silages differ ($P < .01$)

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Chapter IV

JOURNAL ARTICLE II

DIGESTIBILITY, NITROGEN UTILIZATION AND PALATABILITY OF ENSILED CRAB WASTE-WHEAT STRAW MIXTURES FED TO SHEEP

ABSTRACT

Crab waste and wheat straw mixtures, ensiled with different additives, were evaluated in metabolism and palatability trials. Thirty crossbred wethers were fed a 1) basal diet consisting of 75% orchardgrass (Dactylis glomerata) hay and 25% concentrate, 2) crab waste-wheat straw ensiled with 16% glacial acetic acid 3) crab waste-wheat straw ensiled with molasses, 4) crab waste-wheat straw ensiled with molasses and a microbial inoculant and 5) ensiled wheat straw supplemented with urea. Crab waste and straw were mixed in proportions of 50:50, wet basis and 20% water was added to all mixtures. The inoculant[3] was a blend of S. faecium and L. plantarum. The mixtures were ensiled in 210 liter metal drums double lined with polyethylene bags. Apparent dry matter digestibility was higher (P<.01) for the basal diet, compared to the crab-waste silages. Apparent digestibility of dry matter and crude protein was lower (P<.05) for acetic

[3]Obtained from Hi-bred International, Inc, Des Moines, IA.

acid treated silage, compared to silages containing molasses. Values were higher (P<.05) for the molasses-inoculant treated silage than the molasses-treated silage alone. Values were lower (P<.01) for acetic acid treated silage, compared to the silages containing molasses. Utilization of N was similar for lambs fed the basal diet and crab waste silages. Among the lambs fed the crab waste silages, fecal N excretion, as g/d, was 5.2 for lambs fed the acetic acid-treated silage, compared to 4.2 for those receiving the molasses-treated silages (P<.05). Percent N retained was lower (P<.05) for lambs fed the acetic acid-treated silages. Nitrogen retention was higher (P<.05) for lambs fed the molasses-inoculant treated silage, compared to the molasses treated silage (5.4 vs 3.9 g/d). Ruminal NH_3-N and blood urea-N were higher (P<.05) for lambs fed the molasses-treated silages, compared to those receiving the acetic acid treated crab waste mixture. Total ruminal volatile fatty acids (VFA) were higher (P<.05) for the sheep fed the crab waste silages, compared to those on ensiled wheat straw diet. Dry matter intake was higher (P<.01) for lambs fed the crab waste silages, compared to sheep fed the ensiled straw diet. Among the crab waste silages, intake was lower (P<.01) for lambs receiving the acetic acid treated silage than for those fed the molasses treared mixtures (47 vs 57

g/kg of metabolic size). Treatment of crab waste-straw mixtures with molasses produced a palatable silage which was utilized satisfactorily by lambs.

(Key words: Crab Waste, Sugarcane Byproduct, Acetic Acid, Digestibility, Palatability).

Introduction

Large quantities of waste are produced in seafood processing, which in the use of crabs amount to 85% of the catch (Brinsfield, 1980). The small size of most of the crab processing plants reduces the feasibility of processing the waste into by-products such as crab meal. Furthermore, substantial fossil fuel is required to produce crab meal. The high moisture content and perishability of the waste precludes transportation for long distances for processing.

Ensiling has been shown to be effective in the preservation and subsequent utilization by ruminanats of various types of animal wastes (Fontenot et al., 1971). Ensiling has been shown by various workers to be effective in destroying coliforms (Caswell et al., 1975), salmonella (Creger et al., 1971) and staphyllococci (McCaskey and Anthony, 1973). Ensiling of crab waste presents special problems because it is low in soluble carbohydrates, the primary substrate of fermentative bacteria (Barnett, 1954).

Crop residues represent another potential source of feed for ruminants if properly supplemented (Klopfeinstein, 1978). Samuels (1983) reported that mixtures of crab waste preserved satisfactorily with addition of 16% glacial acetic acid. It is questionable if this practice would be economically feasible. However, adding of moderate amounts of blackstrap molasses resulted in satisfactory ensiling (chapter III). This experiment was conducted to determine the nutritional value and palatability of crab waste and wheat straw ensiled with different additives.

Methods and Materials

Metabolism and palatability trials were conducted with sheep to evaluate crab processing waste[1] [6] ensiled with ground wheat straw in 210 liter metal drums double lined with .08 mm polyethylene bags. Detailed procedures were given in chapter III, so only a brief description will be given here. The crab waste and wheat straw were ensiled in proportions of 50:50, wet basis, with the following additives 1) 16% glacial acetic acid, 2) 20% dry molasses and 3) 20% dry molasses plus .1% silage inoculant. Wheat straw was also ensiled alone. Water was added at 20% to all mixtures. The mixtures were tightly packed and the bags individually sealed after excluding air. The mixtures ensiled for a minimum

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[6] [1]Obtained from Graham and Rollins, inc., Hampton, Va.

of 42 d before the trials were initiated.

Metabolism Trial. Thirty crossbred wethers, averaging 40 kg, were blocked by weight and breed and randomly allotted to the following diets: 1) basal diet; crab waste-wheat straw ensiled with 2) 16% acetic acid 3) dry molasses, 4) dry molasses and a microbial inoculant and 5) ensiled wheat straw supplemented with urea. Urea was added to the wheat straw silage to raise the crude protein level to about 10%, dry basis. The basal diet consisted of 75% orchardgrass hay (IFN 1-03-438), 20% ground corn (IFN 4-02-935), 4.5% soybean meal (IFN 5-04-604) and .5% limestone. The diets were calculated to be isonitrogenous.

All lambs were treated for internal parasites and administered 2000 1. U. of vitamins A and D by intramuscular injection prior to the start of the trial. The lambs were fed 700 g of dry matter (DM)/d of each diet plus 10 g iodized salt daily in equal portions during 2 h feeding periods at 12 h intervals. Water was available at all times except during the 2-h feeding periods.

The sheep were placed in false bottom metabolism stalls similar to those of Briggs and Gallup (1949), which permitted separate collection of urine and feces. A 2-d adaptation period to the stalls and a 5-d transition to the experimental diets were followed by a 10-d preliminary period and

a 10-d period during which urine and feces were collected. Two days before the start until 2-d prior to the end of the collection period, the experimental diets were sampled and refusals (if any) were collected at each feeding. Refusals were frozen, composited daily by animal, and were weighed and subsampled at the end of the trial.

Feces were collected each morning, dried in a forced-draft oven at a maximum of 60 C for 24 h, and composited by animal in metal cans with loose lids. At the end of the trial, fecal composites were weighed, mixed and subsampled. Procedures for collecting and handling the urine were similar to those described by Bhattacharya and Fontenot (1965).

Duplicate 200 g samples of the diets were dried in a forced-draft oven at a maximum of 60 C for 48 h for DM determination. These samples were allowed to air equilibrate. Feed, refusal and fecal samples were ground through a 1 mm screen and analyzed for DM and ash (AOAC, 1980), neutral detergent fiber (NDF) (Van Soest and Wine, 1967), acid detergent fiber (ADF) (Van Soest, 1963), lignin and cellulose (Van Soest and Wine, 1968). Hemicellulose was determined by difference. Nitrogen (AOAC, 1980) was determined on the feeds, urine, feces, and refusals.

At the end of the trial, ruminal fluid samples were taken via stomach tube 2 h after feeding. The samples were

strained through four layers of cheesecloth and the pH was determined (electrometrically). The ruminal fluid was analyzed for NH_3-N (Beecher and Whitten, 1970) and VFA (Erwin et al., 1961). Blood samples were taken 6 h after feeding by jugular puncture and analyzed for urea-N (Coulombe and Favreau, 1963).

Palatability Trial. Thirty crossbred wethers with an average weight of 40 kg were blocked by breed and weight and randomly allotted within blocks to the five diets used in the metabolism trial. The lambs were placed in individual 1.2 x 4 m stalls in a semi-enclosed barn. The animals were provided with fresh feed every 12 h at about 10% in excess of intake. Water was provided ad libitum.

The trial consisted of a 2-d adaptation period to the stalls, a 5-d transition to the experimental diets, a 7-d preliminary and a 7-d measurement period. Refusals were collected once daily during the measurement period and dried at 100 C in a forced-draft oven. Diet samples were taken during the trial, frozen and later composited, subsampled and analysed for DM.

The average of the weights taken before the start and at the end of the trial was used to determine metabolic size.

Statistical Analyses. Statistical analyses were performed using the analyses of variance by the general linear

model (GLM) procedure described by the Statistical Analyses System (SAS, 1982). The following orthogonal contrasts were tested: 1) Basal vs silages, 2) straw silage vs crab waste-straw silages, 3) Acetic acid silage vs molasses treated silages and 4) Molasses alone vs molasses plus inoculant crab waste-straw silages.

Results and Discussion

Chemical Composition. The DM of the basal was 91.6% and the CP averaged 12.6%, dry basis (table 11). Respective values for the crab waste mixtures were 57 and 13%. Dry matter of the wheat straw silage was 52.9%. Neutral detergent fiber and hemicellulose were higher and ash was lower in the basal than the crab waste-straw mixtures.

Apparent Digestibility. Apparent digestibility of DM was highest ($P<.01$) for the basal diet (table 12). Apparent digestibility of CP was similar ($P>.05$) between the basal diet and silages. Digestibility of DM and CP was higher ($P<.01$) for molasses-treated, compared to the acetic acid-treated mixtures. Although differences were small, digestibility of DM and CP were higher for the molasses-inoculated silage, compared to molasses alone. Digestibilities of both DM and CP were lower ($P<.05$) for the wheat straw-urea diet, compared to the crab waste silages. The digestibility of DM for the crab waste mixtures was slightly lower than those

Table 11. Chemical Composition of Basal Diet and Silages Fed to Sheep in the Metabolism and Palatability Trials

Component	Basal	Additives to mixtures[a]			Ensiled wheat straw	S.E.
		16% acetic acid	20% molasses	20% molasses + .1% inoculant[b]		
Dry matter, %	91.62	56.60	58.41	57.58	52.94	1.20
Crude protein[c]	12.57	12.13	13.44	13.53	12.82	1.05
Neutral detergent fiber[c]	69.01	55.84	59.30	60.30	84.72	0.94
Acid detergent fiber[c]	40.51	40.57	44.66	45.53	59.68	1.09
Cellulose[c]	29.66	28.92	32.78	33.55	41.88	1.22
Hemicellulose[c]	28.50	15.27	14.64	14.77	25.04	1.14
Lignin[c]	10.41	9.36	10.56	11.54	16.71	1.32
Ash[c]	6.94	21.02	16.94	16.75	5.25	1.02

[a]Crab waste and wheat straw (1:1, wet basis)

[b]Obtained from Pioneer Hi-Bred International, Inc. (S. faecium and L. plantarum)

[c]Dry basis

Table 12. Apparent Digestibility of Diets by Sheep

Component	Basal	Additives to mixtures[a]				S.E.
		16% acetic acid	20% molasses	20% molasses + .1% inoculant[b]	Ensiled wheat straw	
	%					
Dry matter[cdef]	61.98	51.05	54.20	57.74	44.37	1.09
Crude protein[def]	70.17	64.75	70.34	74.36	66.90	1.20
Neutral detergent fiber[cdef]	59.79	39.47	42.07	49.06	49.67	1.76
Acid detergent fiber[cde]	60.10	41.74	47.34	53.76	55.73	3.28
Cellulose	54.59	46.78	45.54	52.61	53.32	2.82
Hemicellulose[cdef]	59.34	17.03	26.03	34.57	35.25	2.90
Lignin[cdef]	64.24	42.77	44.69	50.23	57.04	2.35

[a] Crab waste and wheat straw (1:1, wet basis)

[b] S. faecium and L. plantarum

[c] Basal diet and silages differ ($P < .01$)

[d] Acetic acid treatment and 20% molasses and inoculant treatments differ ($P < .01$)

[e] 20% molasses treatment and inoculant treatment differ ($P < .05$)

[f] Wheat straw and crab waste silages differ ($P < .05$)

reported by Patton et al. (1975) when 10 and 20% crab meal was included in the diet of ruminating calves. Bohman et al. (1954) and Hamilton et al. (1948) reported that molasses depressed digestibility of DM in poor quality roughage rations, possibly because rumen bacteria use the soluble sugars in molasses in preference to the less available fibrous material.

The digestibilities of NDF, hemicellulose and lignin followed similar trends as DM digestibility. The digestibility of cellulose, tended to be lower for the crab waste-straw silages, compared to the basal and wheat straw silage diets. Adding molasses prior to ensiling increased the digestibility of all the cell wall components except cellulose ($P<.01$), compared to adding acetic acid. Use of the bacterial inoculant resulted in an increase in digestibility of NDF, hemicellulose and lignin.

Nitrogen Utilization. Nitrogen intake was similar for all diets (table 13). Nitrogen utilization was not significantly different between lambs fed the basal and those fed the crab waste silages. Fecal N excretion was higher ($P<.05$), and urinary N similar for sheep fed the acetic acid-treated crab waste silage, compared to those fed the molasses-treated crab waste silages. The net result was higher ($P<.05$) N retention for lambs fed the molasses-treat-

Table 13. Nitrogen Utilization by Sheep Fed a Basal Diet and Crab Waste and Wheat Straw Silages

Item	Diets[a]					
		Additives to mixtures[b]				
	Basal	16% acetic acid	20% molasses	20% molasses + .1% inoculant[c]	Ensiled wheat straw	S.E.
Intake, g/d	14.28	14.70	15.40	15.19	14.63	.38
Excretion, g/d						
Fecal[de]	4.26	5.18	4.57	3.89	4.84	.36
Urinary[ef]	5.89	6.90	6.90	5.87	7.64	.36
Total[ef]	10.15	12.08	11.47	9.76	12.48	.30
Retention						
G/d[def]	4.13	2.62	3.93	5.43	2.15	.41
% of intake[def]	28.92	17.81	25.57	35.75	14.69	2.80
% of absorbed[def]	41.15	27.03	36.38	48.06	21.80	3.95

[a] Each value represents the average of six animals

[b] Crab waste and wheat straw (1:1, wet basis)

[c] S. faecium and L. plantarum

[d] Acetic acid treatment and molasses treatments differ ($P < .05$)

[e] Molasses alone and with inoculant treatments differ ($P < .05$)

[f] Wheat straw and crab silages differ ($P < .05$)

ed silages. Urinary N excretion was highest for lambs on the wheat straw silage. This is probably a reflection of the lower efficiency of N utilization from urea than from crab waste and soybean meal. Fecal and urinary N excretion were lower ($P<.05$) and N retention was higher ($P<.05$) for the molasses-inoculum treated silage, compared to the silage treated only with molasses.

Ruminal and Blood Parameters. Ruminal fluid pH was lower ($P<.01$) for lambs fed the basal diet than for those receiving the silages (table 14). The difference in ruminal pH between the basal and crab waste silages may be due to the high ash content of the crab silages. The major component of the ash in crab waste is $CaCO_3$ (Cantor, 1980) and this may have buffered the rumen contents of lambs fed the crab waste silages. Ruminal pH of lambs fed the acetic acid crab waste silage was lower ($P<.05$) than that of lambs receiving molasses crab waste silages, probably a reflection of appreciable amounts of acetic acid added to the initial mixture.

Ruminal fluid NH_3-N levels were higher ($P<.01$) for sheep fed the silages than those receiving the basal diet. This high level of ammonia for lambs on the crab waste silages may have also contributed to buffer the rumen contents of lambs receiving these silages. Rumen fluid NH_3-N was lower ($P<.05$) for sheep that were fed the acetic acid treated crab

Table 14. Ruminal pH, Ammonia Nitrogen and Blood Urea Nitrogen of Sheep Fed a Basal Diet, and Crab Waste and Wheat Straw Silages

Item	Diets[a]					
		Additives to mixtures[b]				
	Basal	16% acetic acid	20% molasses	20% molasses + .1% inoculant[c]	Ensiled wheat straw	S.E.
Ruminal fluid pH[de]	6.76	6.86	7.29	7.08	6.99	.08
Ruminal fluid NH_3-N, mg/dl[def]	26.87	28.65	34.37	30.87	42.27	1.27
Blood urea-N, mg/dl[def]	24.32	25.42	32.13	28.82	40.45	1.47

[a]Each value represents the average of six animals

[b]Crab waste and wheat straw (1:1, wet basis)

[c]S. faecium and L. plantarum

[d]Basal and crab waste silages differ ($P < .01$)

[e]Acetic acid treatment and 20% molasses and inoculant treatments differ ($P < .05$)

[f]Wheat straw and crab silages differ ($P < .01$)

waste silage, compared to those on molasses treated crab waste silages. This suggests a lower rate of ruminal degradation and/or deamination of the acetic acid-treated crab waste silage. Raa and Gilberg (1982) showed that deamination is suppressed under acid conditions and the lower ruminal pH of lambs fed the acetic acid-treated crab waste silage may have reduced the extent of deamination. The highest ($P<.01$) level of ruminal NH_3-N was observed for sheep fed the wheat straw silage supplemented with urea. This reflects the rapid ruminal degradation of urea to ammonia, which was likely responsible for low efficiency of N utilization (table 13). Blood urea-N followed a similar trend as the ruminal ammonia-N (Preston et al., 1965).

Total VFA was higher ($P<.05$) for lambs fed crab waste silages, compared to those on wheat straw silage (table 15). Total VFA also tended to be higher for lambs receiving the acetic acid treated crab waste silage. This level of VFA probably contributed to the lower ruminal pH observed for sheep fed this silage. Acetic acid concentration was higher ($P<.01$) for sheep fed the acetic acid-treated silage than for those fed the molasses treated silages, undoubtedly, a reflection of the large difference in acetic acid in the silage. Acetic and propionic acid concentrations were higher ($P<.06$) for sheep fed the crab waste-straw mixtures, com-

Table 15. Rumen Fluid Volatile Acid Concentrations, Metabolism Trial

Volatile fatty acids	Diets[a]					
		Additives to mixtures[b]				
	Basal	16% acetic acid	20% molasses	20% molasses + .1% inoculant[c]	Ensiled wheat straw	S.E.
	μ mole/ml					
Acetic[d]	47.90	80.90	51.56	55.63	50.41	6.35
Proponic[d]	17.38	11.09	27.34	26.72	17.20	1.92
Isobutyric[d]	.93	.56	1.13	.91	.81	.09
Butyric[d]	5.57	3.62	6.53	6.50	4.74	.52
Isovaleric[de]	1.10	.53	1.53	1.10	.94	.11
Valeric[defg]	.55	.47	1.64	1.21	.47	.11
Total[fg]	73.43	97.17	89.73	92.07	74.57	7.75

[a] Each value represents the average of six animals

[b] Crab waste and wheat straw (1:1, wet basis)

[c] S. faecium and L. plantarum

[d] Acetic treatment and molasses treatments differ ($P < .01$)

[e] 20% molasses treatment and inoculant treatment differ ($P < .05$)

[f] Straw and crab waste silages differ ($P < .05$)

[g] Basal and crab waste silages differ ($P < .05$)

pared to those on the wheat straw silage diets. Propionic acid was twice as high (P<.01) for lambs fed the molasses-treated crab waste silages, compared to those fed the acetic acid-treated silage. Chappell and Fontenot (1968) reported a tendency for total VFA concentration in the rumen to be higher when readily-available carbohydrate sources were fed, and this increase was due primarily to increased levels of propionate and butyrate. In the present study, this trend was observed for the molasses treated crab waste silages. Isobutyric acid tended to be higher and isovaleric and valeric acids were higher (P<.05) for sheep fed the molasses treated crab waste mixture alone, compared to those fed the inoculated crab waste silage. Leng (1973) reported that the branched chain fatty acids are indicative of proteolysis and/or deamination. Decreased production of isobutyrate and isovalerate in fermentations is indicative of reduced proteolysis and/or deamination (Leng, 1973). This may be one reason for the higher N retention observed for the lambs fed the molasses-inoculum treated crab waste silage, compared to those receiving the molasses treated silage.

Palatability Trial. Dry matter intake expressed as g/d or as grams per unit of metabolic size was similar for lambs fed the basal and crab waste silages (table 16). Dry matter intake expressed as g/d, was higher (P<.01) for lambs fed

Table 16. Dry Matter Intake of Sheep Fed a Basal Diet, Crab Waste and Wheat Straw Silages

Item	Diets[a]					
		Additives to mixtures[b]				
	Basal	16% acetic acid	20% molasses	20% molasses + .1% inoculant[c]	Ensiled wheat straw	S.E.
G/d^{de}	908.2	779.0	936.0	955.0	709.0	19.0
$G/W_{kg}^{.75}/d^{de}$	56.1	47.1	56.1	58.0	42.3	1.1

[a]Each value represents the average of six animals

[b]Crab waste and wheat straw (1:1, wet basis)

[c]S. faecium and L. plantarum

[d]Wheat straw and crab silages differ ($P < .01$)

[e]Acetic acid treatment differs from molasses treated silages ($P < .01$)

the crab waste silages, compared to those fed the straw silage diet. Lambs fed the molasses treated crab waste silages consumed more ($P<.01$) feed than those fed the acetic acid-treated crab waste silage. Wilkins et al. (1971) and Brown and Radcliffe (1972) reported low voluntary dry matter intake and poor performance in feeding trials with silages in which acetic acid was the additive. The exact mode of action of acetic acid in depressing intake is, however, still not known (Osborn and Wilkins, 1967).

Dry matter intake expressed per unit of metabolic size followed the same trend as intake, being higher ($P<.01$) for sheep fed the molasses treated crab waste silages, compared to those fed the acetic acid treated crab waste silage. Dry matter intake was lowest for the sheep fed the wheat straw silage diet. The high level of intake observed for lambs receiving the molasses treated crab waste silages may be due to an enhancement in palatability (Conrad, 1966; Silvestre et al., 1978).

Ensiling crab waste-wheat straw mixtures with added molasses produced palatable silages which were utilized satisfactorily by lambs.

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Chapter V

JOURNAL ARTICLE 111

PERFORMANCE AND CARCASS CHARACTERISTICS OF BEEF CATTLE FED DIFFERENT LEVELS OF FINFISH-WHEAT STRAW SILAGE

ABSTRACT

Finishing beef steers were fed different levels of finfish-straw silage. Fish waste-wheat straw silage was made by mixing fish waste and chopped wheat straw in 1:1 proportions and adding 5% dry molasses. Corn silage was made by ensiling chopped corn forage. Fish waste-wheat straw mixtures ensiled satisfactorily as indicated by the pH and lactic acid level. The fish waste-straw silage was included as 25 and 50% of the diets, dry basis, of individually-fed finishing beef cattle (10/diet). Dry matter (DM) intake and rate of gain were not affected by including fish waste-wheat straw silage as 25% of the diet DM. However, at the higher level (50% of DM) of fish waste-straw silage, intake was lower and this was reflected in lower ($P<.05$) rate of gain, compared to steers on the other two diets. Feed efficiency (DM intake/gain), was similar between cattle fed fish waste-straw silage and control diets. Quality grades for the steers were low to average good, with no differences among the animals fed the different diets. Carcass weights re-

flected differences in liveweights. Cooking losses tended to be lower for roasts from cattle fed the high level of fish waste-straw silage, probably reflecting the lower level of fat. The meat from cattle fed the fish waste- straw silage was more juicy than meat from the control cattle. Meat from cattle fed the high level of fish waste-straw silage tended to have some off-flavor. It is concluded from this study that fish waste-straw silage is satisfactory to feed to finishing cattle.

(Key words: Fish scrap, pH, Lactic acid, Feed efficiency, Carcass quality).

Introduction

Fish silage has been produced by addition of acids (Sumner and James, 1977) and bacterial fermentation (James, 1977). The silage is produced by autolysis and results in a liquified product. Incorporation of the fish silage in the diets of pigs has been shown to improve weight gains and feed efficiency, compared to those fed lysine-enriched soybean meal diet (Hillyer et al., 1976). Tibbetts et al. (1981) fed weanling pigs diets containing 0, 3, 6, and 9% fish silage and reported no differences in feed conversion. Hansen (1959) reported that feeding acid preserved fish silage to chickens produced similar growth rates and overall performance, compared to other protein sources. Finfish

waste and wheat straw have been satisfactorily ensiled with the addition of a small amount of molasses (Samuels, 1983). Digestibility and palatability trials with sheep indicated that the silage has potential value in the diets of ruminants and may be a satisfactory feed in beef cattle diets. However, data are not available concerning level of fish waste-straw silage that can be incorporated into ruminant diets without adversely affecting performance and carcass quality. Thus, this study was conducted to determine the effect(s) of feeding different levels of finfish waste-straw silage to beef cattle on performance, carcass quality and taste of the meat.

Experimental Procedure

An experiment was conducted with 30 steers, fed in individual stalls. The cattle consisted of 27 Angus and 3 Hereford steers with an average initial weight of 268 kg. The cattle were allotted at random within blocks of the same breed and similar weight to the follwing diets: 1- Control diet (corn silage and soybean meal), 2- Low level of fish waste-straw silage, and 3- High level of fish waste-straw silage. The low level of fish waste-straw silage consisted of 25% and the high level consisted of 50% of the diet, dry basis.

Fish waste-wheat straw silage was made by mixing ground fish waste and chopped wheat straw in a 1:1 ratio and adding 5% dry molasses. The fish waste, straw and molasses were mixed in a horizontal mixer for about 30 min, then ensiled in large Silopress polyethylene bags.[a] Corn silage was made by chopping corn forage and ensiling in an upright concrete-stave silo.

The ingredient composition of the diets is given in table 17. The control diet consisted of corn silage and soybean meal. The diets were calculated to be isonitrogenous and isocaloric (DE). At the high level of fish waste, corn grain was used as the energy source. A small amount of corn grain and soybean meal were used at the low level of fish waste-straw silage to increase the energy and protein, respectively.

Each animal was administered 2,000,000 I.U. of vitamin A by intramuscular injection prior to the start of the trial. The cattle were fed in individual stalls twice daily, at about 730 am and 1500 h. The animals were kept in stalls from about 1500 h until about 900 h, and were in large exercise lots with acess to water from 900 h to 1500 h. Refusals were collected once daily. Weights of the cattle taken on two consecutive days were used to calculate the initial and

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[a]Obtained from Silopress, Inc., Sioux city, IA.

Table 17. Ingredient Composition[a] of Diets Fed in Finishing Trial

Item	Diets		
		Fish-straw silage	
	Control	Low level	High level
	%	%	%
Corn silage	93	62	0
Fish waste-straw silage	0	21	62
Soybean meal	7	4	0
Corn grain	0	13	38

[a]As fed basis.

final weights. The animals were weighed at 14-d intervals during the trial. All steers were slaughtered at the end of the trial which lasted 120 d and carcass data were collected. Rib roasts (6 to 8th ribs) from each carcass were cooked to an internal temperature of 70 C. The meat was evaluated by a 11-member sensory panel. The panel was familiar with the definitions of the characteristics to be evaluated and proper procedure for evaluation of the meat. A 37-member panel evaluated the meat for flavor and overall desirability. The panel used an 8-point hedonic scale to evaluate the samples.

Silage samples were obtained and water extracts were prepared by the method described by Harmon et al. (1975). The extract was used to measure pH (electrometrically), water soluble carbohydrates (WSC) (Dubois et al., 1956, as adapted for corn plants by Johnson et al., 1966) and lactic acid (Barker and Summerson, 1941, as modified by Pennington and Sutherland, 1956). The samples were also analyzed for DM, ash, N (AOAC, 1980), neutral detergent fiber (NDF) (Van Soest and Wine, 1967), acid detergent fiber (ADF) (Van Soest, 1963), lignin and cellulose (Van Soest and Wine, 1968).

Statistical Analyses. Results of the experiment were analyzed using the general linear model (GLM) procedure of the

Statistical Analysis System (SAS, 1982). The following comparisons were made: 1) Control vs Fish waste-straw silage and 2) 25% level vs 50% level of fish waste-straw silage.

Results and Discussion

The initial pH of the fish waste-straw silage was 7.40 (table 18). Satisfactory ensiling occurred in both silages and were characterized by pleasant aroma. Appreciable amounts of lactic acid were measured in the silages. The pH and lactic acid of the fish waste silage were similar to those reported by Samuels (1983).

The chemical composition of the ingredients used in the trial is presented in table 19. Corn forage was ensiled at about 42% DM while the fish waste-straw mixture was ensiled at about 60% DM.

The initial weight of the control group was slightly higher than that of steers fed the other diets (table 20). Average DM intake per day was lower ($P<.05$) for steers fed the high fish waste-straw silage diet than for those fed the low level of fish waste-straw silage and control diets. The reduced DM intake of steers on the high level of fish waste-straw silage was reflected in a lower ($P<.05$) average daily gain, compared to steers receiving the other diets. Feed efficiency (DM/gain), was not different ($P>.05$) among the steers consuming the different diets. Kompiang et al.

Table 18. Fermentation Characteristics of Corn Forage and Fish Waste-Straw Silages

Item	Corn silage	Fish waste-straw silage
pH[a]	4.60	6.00
Water soluble carbohydrates[b]		
Initial	-	4.63
Final	5.00	1.40
Lactic acid[a,b]	7.24	3.25

[a]After ensiling

[b]Percent of dry matter

Table 19. Chemical Composition of Ingredients Used in Feeding Trials

Item	Ingredient			
	Fish waste-straw silage	Corn silage	Corn grain	Soybean meal
Dry matter, %	59.22	41.90	93.46	92.16
Composition of dry matter, %				
Crude protein	14.55	7.99	10.39	43.09
Neutral detergent fiber	66.18	50.99	21.32	23.12
Acid detergent fiber	44.47	26.58	4.63	14.79
Cellulose	35.89	23.06	3.82	13.59
Lignin	8.65	3.55	0.89	1.44
Ash	6.59	3.44	2.11	5.78

Table 20. Performance of Cattle Fed Fish Waste - Straw Silage

Item	Diets		
	Control	Fish-straw silage	
		Low level	High level
Initial wt, kg	271	264	268
Final wt, kg[ab]	400	392	365
Daily gain, kg[b]	1.16	1.15	0.88
Dry matter/day, kg[ab]	7.70	7.32	5.79
Dry matter/kg gain, kg	6.64	6.36	6.58

[a]Control and fish waste cattle differ ($P < .05$)

[b]Low and high fish waste cattle differ ($P < .05$)

(1980), demonstrated with broilers that poor growth resulted when fish silage made up more than 20% of the diet. Feed consumption and feed conversion of chickens were negatively affected by preserved fish silage in the diet (Disney et al., 1978). Tibbetts et al. (1981) fed weanling and finishing pigs diets containing 0, 3, 6 and 9% fish silage and reported no differences in average daily gain and feed conversion due to treatment. Batterham and Gorman (1980) reported that feed efficiency and daily liveweight gains of pigs fed a cereal diet supplemented with fish silage were higher than for pigs on a soy-protein based diet. The advantage of fish silage in pigs may be due to quality of protein in fish. Also, Pike (1975) reported that fish products contain unidentified growth factors (UDG).

Carcass weight was lower ($P<.05$) for steers fed the high level of fish waste-straw silage (table 21), reflecting the lower liveweights of these steers. Carcasses graded slightly better than low good and grade was similar between the steers fed the three diets. Backfat was lower ($P<.05$) for steers fed the high level of fish waste-straw silage than for those fed the other diets. Kidney-pelvic-heart fat and yield grade were not significantly different among steers fed the different diets. These results appear to be in agreement with those reported by Disney et al. (1978), in

Table 21. Carcass Characteristics of Steers Fed Fish Waste - Straw Silage

Item	Diet		
	Control	Fish-straw silage	
		Low level	High level
Carcass wt, kg[ab]	227	220	201
Dressing percent	56.2	56.0	54.8
Quality grade[c]	9.3	9.5	9.2
Backfat, cm.[ab]	.61	.56	.33
Ribeye muscle area, sq cm[b]	61.3	62.6	53.5
Kidney-pelvic-heart fat, %	1.75	1.95	1.75
Yield grade	1.9	1.8	1.8

[a]Control and fish waste carcasses differ ($P < .05$)

[b]Low and high fish waste carcasses differ ($P < .05$)

[c]Code: 8 = high standard; 9 = low good; etc.

their studies with pigs, who reported no adverse effects on carcass quality when pigs were fed silage made from tuna offal. Hillyer et al. (1976) also reported similar results with pigs fed fish silage and soybean meal as protein supplements.

Cooking losses were not different between roasts from steers fed the different diets (table 22). However, losses tended to be lower for the roasts from cattle fed the high level of fish waste-straw silage probably reflecting the lower fat content of these roasts. Meat from cattle fed the fish waste-straw silage was more juicy than from control cattle ($P<.05$). Meat from cattle fed the high level of fish waste-straw had more ($P<.05$) off-flavor than meat from those fed the low level of waste, but no trends were noted between cattle fed the lower level of waste and basal diet.

Inclusion of fish waste-straw silage in the diets of beef cattle did not adversely affect animal performance, efficiency of gain and quality of the meat, especially at the lower level.

Table 22. Cooking Data and Taste Panel Evaluation of Beef Fed Fish-Straw Silage

	Control	Fish Silage	
		Low	High
Total cooking losses, %	21.6	21.5	20.5
Evaporative losses, %	18.1	17.8	17.2
Drip losses, %	3.6	3.7	3.3
Juiciness[ab]	4.9	5.7	5.4
Tenderness[c]	5.0	5.2	5.1
Beef flavor[d]	5.6	6.12	5.5
Off-flavor[ef]	1.5	1.4	1.9
Connective tissue[g]	5.3	5.4	5.1

[a]Code: 8 = extremely juicy to 1 = extremely dry

[b]Control and fish waste roasts differ ($P < .05$)

[c]Code: 8 = extremely tender to 1 = extremely tough

[d]Code: 8 = extremely intense to 1 = none

[e]Code: 8 = extremely intense to 1 = none

[f]Low and high fish waste roasts differ ($P < .05$)

[g]Code: 8 = none to 1 = abundant

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Chapter VI

EFFECT OF VARIOUS ADDITIVES ON THE PRESERVATION AND ENSILING CHARACTERISTICS OF CRAB WASTE

ABSTRACT

Crab waste was stored in 210 liter metal drums with the following treatments: 1) none, 2) 1.5% formaldehyde and 3) .75% propionic/.75% formic acid and 4) 10% liquid molasses. The mixtures were kept for 4 wk in an open, semi-enclosed barn. The crab waste was then evaluated by a panel. The waste which was not decomposed severely was ground and mixed with wheat straw, 1:1, wet basis, and 20% dry molasses and ensiled in small laboratory silos (six/treatment) for about 6 wk. Each silo consisted of a 4 liter cardboard container double lined with polyethylene bags, which were sealed individually after expelling air. The control crab waste was putrefied after 4 wk but the preserved crab wastes were acceptable for ensiling. The molasses treated crab waste lost the least ($P<.05$) ammount of moisture, compared to the chemical additives. The crude protein (CP) of the control crab waste was lower ($P<.05$) than that of the preserved crab waste. Among the chemical preservatives, the formaldehyde treated crab waste had a higher ($P<.05$) CP and a lower ($P<.05$) concentration of trimethylamine (TMA). The level of TMA was higher ($P<.05$) in the molasses preserved crab waste,

compared to the chemical preservatives. After ensiling, mixtures with molasses-preserved waste had lower ($P<.05$) pH than those with the chemical preservatives. Disappearance of water-soluble carbohydrates (WSC) was greater ($P<.05$) in the mixtures with molasses preserved waste, compared to mixtures preserved with chemicals. This trend resulted in higher ($P<.05$) levels of lactic acid in the ensiled mixtures which contained waste preserved with molasses, compared to the other treatments. Among the chemical preservatives, fermentation occurred to a greater extent in the mixtures containing crab waste preserved with propionic/formic acid, resulting in higher ($P<.05$) levels of lactic acid.

(Key words: Crab waste, Preservation, Spoilage, Silage).

Introduction

Seafood is a perishable product which must be refrigerated soon after harvest from the water to retard deterioration. Even with reasonable handling and storage bacterial growth tends to impart undesirable odors and flavors to the product. Crab waste has been ensiled successfully (chapter III), but ensiling must occur within hours to prevent deterioration. This is unpractical under present farming conditions and precludes long distance transportation of the waste.

Treating forages with sulfuric and hydrochloric acids (Virtanen, 1929), formic acid (Barker et al., 1973), propionic acid (Woolford, 1975) and formaldehyde (Watson and Nash, 1960) has been shown to be effective in preserving these. Seafood waste has been successfully preserved with acids (Gilberg and Raa, 1977). Inorganic acids cannot be successfully used in preserving seafood products unless the pH is lowered to 2 or below (Edin, 1940). However, with organic acids, a stable product may be obtained at higher pH (Tatterson and Windsor 1974).

This study was undertaken to test the effectiveness of various additives in retarding and/or preventing microbial deterioration of crab processing waste to be used for ensiling.

Materials and Methods

The following additives were applied to fresh crab processing waste[1] immediately after processing at Hampton, VA. 1) Control, consisting of crab waste and 1.5% water, 2) crab waste plus 1.5% formaldehyde 3) crab waste plus .75% formic acid and .75% propionic acid and 4) crab waste plus 10% liquid molasses. The treated waste was placed in 210 liter metal drums lined with polyethylene bags. An average of 45 kg of crab waste was put in each of six drums per

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[1]Obtained from Graham and Rollins, Inc., Hampton, Va

treatment. The chemical additives were diluted with water in a 1:1 ratio and applied with a spray pump. The chemicals were applied by spraying as the waste was added to the drums. The molasses was applied with a perforated polyethylene container to disperse the molasses onto the waste material. Initial samples were obtained for each treatment and immediately frozen in liquid nitrogen. The samples and drums containing the waste were transported to Blacksburg on the same day the additives were applied. The samples were subsequently placed in freezers for later analyses. The drums were left open and stored in a partially open barn for about 4 wk.

After the preservation period, the crab wastes were evaluated for appearance and odor and overall quality by a panel of six persons. The waste materials that appeared unacceptable were discarded. Acceptable waste materials were ground and mixed with wheat straw in a 1:1 ratio, plus 20% dry molasses and 20% water, and ensiled in small laboratory silos for a minimum of 6 wk. A detailed description of sampling and analyses of the silages was as given in chapter III.

Statistical Analyses. Statistical analyses were performed using the general linear model (GLM) procedure of the statistical analysis system (SAS, 1982). The following comparisons were made: 1) control vs additives, 2) chemical

treatment vs molasses treatment and 3) formalin treatment vs propionic/formic acid treatment.

Results and Discussion

At the end of 4 wk the control crab waste was putrefied and consequently discarded. The preservatives resulted in products with some odor but were acceptable, with an average quality score of about 3.0 (table 23). For all treatments a small amount of the waste at the bottom of the container were putrefied which appeared to be due to accumulation of water. Perhaps if provisions had been made for drainage, no spoilage would have occurred in the waste treated with preservatives. The dry matter (DM) of the molasses preserved crab waste was lower ($P<.05$) than that of the chemically preserved crab waste.

The CP of the preserved crab wastes was higher ($P<.05$) than that of the control, reflecting differences in loss of N, probably as NH_3 from the control crab waste. The average CP content of the control treatment was 35.8%, compared to about 41.0% for the preserved crab waste. The control crab waste also had the highest ($P<.05$) level of TMA, averaging 71 mg/100 g of waste. Among the chemical preservatives, the CP was higher ($P<.05$) and the TMA was lower ($P<.05$) for the crab waste treated with formaldehyde, compared to the propionic/formic acid treatment. Crude protein was similar

Table 23. Effect of Additives on the Preservation of Crab Waste

Item	Control	.75% propionic + .75% formic acid	1.5% formaldehyde	10% molasses	S.E.
Dry matter, %[abc]	53.57	54.78	50.85	48.03	.13
Crude protein[ac], % dry basis	35.83	40.32	43.07	41.18	.10
Trimethylamine[abc], mg N/100g	70.57	55.74	42.95	51.94	.22
Quality score[d]	1.3	3.1	3.2	2.8	.0

[a]Control and preservatives differ ($P < .05$)

[b]Molasses and chemical treatments differ ($P < .05$)

[c]Propionic/formic acid and formalin treatments differ ($P < .05$)

[d]Code: 1 = putrefied; 2 = strong odor/moldy; 3 = acceptable; 4 = good/some odor/ 5 = good/pleasant

between the chemically preserved and molasses preserved crab waste.

After ensiling with wheat straw, all mixtures had acceptable aroma and showed decreased pH (table 24). The post ensiled pH of the molasses preserved crab waste was 6.03. This was lower ($P<.05$) than the pH of the other mixtures but differences were small. There was also a greater ($P<.05$) loss of WSC from the molasses-preserved silages, compared to the mixtures preserved with chemicals. This trend was accompanied by higher ($P<.05$) levels of lactic acid, which averaged 5.5% in the molasses-preserved mixtures. The microbial population in the chemically-preserved crab waste may have been severely reduced or inactivated, resulting in lower levels of acid production. Initial TMA concentration was similar in all mixtures, although it tended to be higher in crab waste preserved with molasses. After ensiling, there was little change in TMA level, which may explain the pleasant aroma in the mixtures.

Perhaps a microbial silage inoculant would have been beneficial (Chapter III) in these mixtures since most of the pH values were around 6.0 after ensiling. Appreciable levels of WSC (over 10%) were still available after ensiling in all mixtures, suggesting that fermentation was not extensive enough to produce adequate amounts of lactic acid which will

Table 24. Fermentation Characteristics and Trimethylamine Levels of Preserved Crab Waste and Wheat Straw Silages

Item	Crab waste and wheat straw[a]			S.E.
	10% molasses	.75 propionic + .75 formic acid	1.5% formaldehyde	
pH[b,c]	6.03	6.23	6.24	.05
Water soluble carbohydrates[d]				
Initial[c]	20.70	18.68	18.63	.59
Final[c]	13.05	14.53	15.03	.30
Lactic acid[b,d]	5.46	4.45	3.45	.13
Trimethylamine, mg/100g silage				
Initial	7.92	7.88	7.82	.30
Final	8.20	8.24	8.15	.20

[a] Crab waste and wheat straw (1:1, wet basis)

[b] Postensiled

[c] Molasses and chemical treatments differ ($P < .05$)

[d] Percent of dry matter

[e] Propionic/formic acid and formalin treatments differ ($P < .05$)

lower the pH to acceptable levels. Crab waste was stabilized with certain additives and ensiled satisfactorily by addition of a moderate amount of molasses.

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Chapter VII

GENERAL DISCUSSION

Previous work in this laboratory has indicated that seafood processing waste has potential feeding value for ruminants (Samuels, 1983). Ensiling of crop residues and fish waste but not crab waste was satisfactory with the addition of a small amount of molasses. Crab waste-straw mixtures were stabilized only with addition of substantial amounts of acetic acid. The economic feasibility of this practice is however, questionable. In the present study, crab processing waste was satisfactorily ensiled with addition of a substatial amount of molasses. Use of this ingredient is more economically feasible than use of acetic acid.

With the addition of a mixed silage inoculant in which the microbes complement each other, the fermentative pathway can be redirected to produce higher levels of lactic acid (Whittenbury et al., 1967). This was the basis for the inclusion of Streptococcus faecalis and Lactobacillus plantarum mixture in the present study. Whittenbury et al. (1967) reported that a mixed culture of S. faecalis and L. plantarum was effective in lowering silage pH. The inoculation (Lesins and Schultz, 1968) of silage with resulting increased production of lactic acid has a number of advantag-

es: Firstly, the energy of the feed is maintained. In heterofermentation, loss of CO_2 constitutes a loss of the C skeleton of the sugar, and hence, loss of energy. Secondly, acid conditions are detrimental to the growth of putrefying microbes that reduce trimethylamine oxide to trimethylamine, the volatile base responsible for the offensive odor in the waste. Thirdly, the production of silage with appreciable amounts of lactic acid may be beneficial in certain instances since this acid may promote the transport of some minerals across the gut mucosa (Metson et al., 1966). In the present study, the addition of a mixed microbial silage inoculant to crab waste-wheat straw mixtures was beneficial in increasing lactic acid level. This may be one reason for the improved utilization of the mixtures with added molasses and inculant, compared to mixtures with molasses alone.

Seafood waste has traditionally been used to produce meals, used primarily for feeding nonruminants. However, present economic and energy trends, suggest that meal production is rapidly becoming less cost effective. Thus, alternative uses in the form of fish silage will become of increasing importance. Producers have been apprenhensive of using fish meal or silage because of the fear of fishy-flavor imparted onto the finished product. In the present study, no off-flavor was detected in the meat of steers fed

fish waste-straw silage. If a fishy flavor occurs, it may be eliminated by including vegetable oils to the diet a few weeks before slaughter (Opstvedt, 1971), or revert to conventional protein supplements a few days before slaughter (Sumner, 1978).

Crop residues represent another major source of underutilized resource. A total of over 400 million metric tons are produced annually in the U.S. (Larson, 1979). The nutritive value of these residues has generally been improved by various chemical treatments particularly ammonia, or urea supplementation. The use of fish waste with wheat straw has been shown to improve dry matter and protein digestibility and N retention, compared to ammonium hydroxide-treated straw (Chirase et al., 1985). This trend may be partly explained by the fact that the fish waste contains amino acids and oligopeptides (Raa and Gilberg, 1982). The C skeleton of the amino acids serves as an important source of the branched chain VFA, which are required by some rumen microbes. Some microbes also require amino acid-N which is absent when ammonia or urea is used as supplement. It is clear from this that seafood waste and crop residues complement each other and enhancement in animal performance may result if the two sources of waste can be preserved cheaply. Ensiling is generally more cost effective in this regard.

A potential limitation to the use of seafood waste is the fact that it has to be ensiled soon after processing or it must be refrigerated to avoid deterioration. This is a serious problem since it dictates that the waste can only be used in close proximity to its production. The preservation of the waste with additives will permit storage and transportation to other areas prior to ensiling. Formaldehyde, formic/propionic acids and molasses have been investigated as possible preservatives. The results indicate that these additives have potential use as preservatives. The putrefactive changes occurring with these additives tended to be less severe for the formaldehyde, but the smell was better for the formic/propionic acid and molasses-treated waste. If drainage had been provided in the drums, spoilage may have been eliminated entirely, since spoilage seemed to have been restricted to the bottom of the containers. It is apparent from this study, that a combination of formaldehyde and propionic, formaldehyde and formic and possibly molasses and propionic acid may result in a more stable product that can be stored prior to ensiling.

In conclusion, the ensiling of crab waste was achieved in this experiment by the addition of a moderate level of molasses. Digestion and palatability studies indicate that the ensiled product is well digested and consumed readily by

ruminants. The addition of 20% molasses to the mixtures did not reduce digestibility of the fiber components. Karalazos and Swan (1976) suggested that molasses can comprise 20% of the dietary dry matter without depressing the digestibility of the dietary energy. Addition of 20% molasses and microbial silage inoculant to crab waste-straw mixtures prior to ensiling was beneficial in increasing the level of lactic acid in the ensiled product and improving utilizating of fiber and N by sheep.

Satisfactory ensiling of the crab waste-straw mixture is of scientific interest since it was demonstrated conclusively from the results of this experiment, that fermentation in the mixtures is curtailed primarily from lack of soluble sugars. Crab waste is produced only at the coastal areas where the price of molasses is generally low. Thus, it is likely that it would be practical and economically feasible to stabilize the waste by ensiling. Preservation of the crab waste with additives was achieved with some success, but further studies should be conducted to determine the most appropriate combination of additives that will improve the overall quality of the stored product.

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APPENDIX

Table 25. Effect of Various Additives on Fermentation Characteristics and Trimethylamine (TMA) Production of Crab Waste - Wheat Straw Silages, Small Silos, Experiment 1

Additive	Level, %	pH		Water soluble carbohydrates[a]		Lactic acid[a]	TMA mg N/100g silage	
		Pre-ensiled	Post-ensiled	Pre-ensiled	Post-ensiled	Post-ensiled	Pre-ensiled	Post-ensiled
Phosphoric acid	0	7.13	6.68	11.60	3.36	4.48	7.51	15.02
	5.4	6.97	6.56	11.31	4.38	1.55	7.35	14.21
Inoculant[b]	0	7.08	6.70	11.54	3.89	2.52	7.55	15.05
	0.1	7.02	6.54	11.36	3.85	3.51	7.30	14.18
Molasses	0	7.18	7.47	2.38	2.37	.54	7.89	17.38
	10	6.98	6.51	11.03	4.32	2.66	7.26	14.72
	20	7.00	5.80	20.95	4.92	5.85	7.14	11.74

[a] Percent of dry matter

[b] Silage inoculant (.1%)

Table 26. Effect of the Addition of Molasses and Inoculant on Fermentation of Crab Waste - Wheat Straw Silages, Small Silos, Experiment 1

Molasses, %	Inoculant[a]	pH		Water soluble carbohydrates[b]		Lactic acid[b]
		Pre-ensiled	Post-ensiled	Pre-ensiled	Post-ensiled	Post-ensiled
0	0	7.44	6.86	12.24	8.52	1.99
0	+	7.27	6.73	12.14	8.99	2.81
10	0	7.23	6.26	18.28	11.42	2.67
10	+	7.21	6.13	18.51	9.99	3.20
20	0	7.13	5.81	22.87	10.78	3.89
20	+	7.22	5.70	22.92	9.63	6.05

[a]Silage inoculant (.1%)

[b]Percent of dry matter

Table 27. Effect of the Addition of Water and Molasses on Fermentation of Crab Waste - Wheat Straw Silages, Small Silos, Experiment 2

Molasses, %	Water, %	pH		Water soluble carbohydrates[a]		Lactic acid[a]
		Pre-ensiled	Post-ensiled	Pre-ensiled	Post-ensiled	Post-ensiled
10	0	7.39	6.89	12.17	9.91	1.05
10	20	7.31	6.70	12.22	7.60	2.75
15	0	7.21	6.77	18.57	12.31	1.07
15	20	7.22	5.62	18.22	9.10	4.80
20	0	7.17	6.34	22.29	14.18	.93
20	20	7.17	5.26	23.51	6.22	9.01

[a]Percent of dry matter

Table 28. Effect of the Addition of Water on Fermentation Characteristics and Trimethylamine (TMA) Production, Small Silos, Experiment 2

Level of water, %	pH		Water soluble carbohydrates[a]		Lactic acid[a]	TMA[b], mg N/100 g silage	
	Pre-ensiled	Post-ensiled	Pre-ensiled	Post-ensiled	Post-ensiled	Pre-ensiled	Post-ensiled
0	7.26	6.67	17.98	12.13	1.02	8.07	15.47
20	7.23	5.80	17.68	7.64	5.85	8.06	12.30

[a]Percent of dry matter

[b]Trimethylamine

Table 29. Effect of Different Inoculants on the Ensiling Characteristics of Crab Waste - Straw Silage, Small Silos, Experiment 3

Item	Treatments[a]								
	No additive		Hi-bred inoculant[b]		Silagenie 13[b]		Silagenie 16[b]		
	None	+ H_2O	None	+ H_2O	None	+ H_2O	None	+ H_2O	S.E.
pH									
Initial	7.13	7.12	7.21	7.22	7.17	7.13	7.18	7.13	.08
Final	6.27	5.35	6.41	5.17	6.46	5.07	6.60	4.95	.04
Lactic acid, % dry basis[cde]	0.80	6.97	1.05	11.06	1.21	11.40	1.38	12.42	.05
Water soluble carbohydrates, % dry basis									
Initial	22.31	23.44	22.28	23.57	21.99	22.56	22.26	22.51	.33
Final[c]	14.62	6.94	13.75	5.50	13.56	5.93	12.56	4.98	.12

[a] Crab waste and wheat straw (1:1, wet basis)

[b] Silage inoculant (.1%)

[c] No additive and inoculant treatments differ ($P < .05$)

[d] Hi-bred and Silagenie inoculant treatments differ ($P < .05$)

[e] Silagenie 13 and Silagenie 16 treatments differ ($P < .05$)

Table 30. Effect of Different Inoculants on TMA Production, Small Silos, Experiment 3

	Treatments[a]								
	No additive		Hi-bred inoculant[a]		Silagenie 13[a]		Silagenie 16[a]		
	None	+ H_2O	None	+ H_2O	None	+ H_2O	None	+ H_2O	S.E.
	mg/100 g								
Initial	7.92	7.86	8.19	8.19	7.96	7.81	7.97	7.82	.08
Final	12.68	8.58	13.03	8.83	13.29	8.37	13.66	7.29	.10

[a]Crab waste and wheat straw (1:1, wet basis)

[b]Silage inoculant (.1%)

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