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METHODS OF PRESERVING AND ENHANCING FERMENTATION AND
NUTRITIONAL VALUE OF CAGED LAYER WASTE-WHEAT STRAW SILAGES
FED TO SHEEP

by

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DEDICATION

In honor, glory and adoration to my heavenly father, this thesis is dedicated to the memory of my father and to my mother who assumed the full responsibility for my success.

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To the members of his graduate committee,

, , and

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Chapter I

INTRODUCTION

Disposal of caged layer waste is a major problem in large scale intensive poultry operations. The operations located in heavily populated areas are nuisances to neighboring residents, especially during the warmer seasons. Removing of the waste on a daily basis or more frequently has been a means of reducing odors. Dumping of these wastes in landfills, spreading on land as fertilizer or incineration have not been consistently economical.

During the last two decades, attention has been directed towards nutrient recovery from caged layer waste for feeding. Caged layer waste is a source of energy, crude protein and minerals. Much of the N is in the form of uric acid and ammonium salts. These non-protein sources of N and fiber make caged layer waste better suited for ruminants than non-ruminants.

Ensiling has been effective in processing caged layer waste to be fed to ruminants. However ensiling must be done frequently to maintain quality of the waste with minimum loss of nutrients. Ensiling for feeding would be much more feasible if the waste could be accumulated for a few weeks prior to ensiling. Use of effective preservatives would allow less frequent ensiling.

These studies were conducted to investigate the effects of chemicals in stabilizing and preserving caged layer waste for an extended period of time, and to investigate the ensiling characteristics of preserved waste and wheat straw mixture ensiled alone and with silage additives, and the nutrient utilization and digestibility of the mixtures when fed to sheep.

Chapter II

LITERATURE REVIEW

The two main kinds of waste collected from poultry enterprises are poultry litter and caged layer waste. Poultry litter includes broiler and turkey litters which are composed of bedding materials, excreta, wasted feed and feathers. Caged layer waste consists of excreta, wasted feed, feathers and broken eggs.

Nutritive Value of Caged Layer Waste.

The nutrient content and digestibility of caged layer waste depends largely on the age of the birds (White et al., 1944), feeding regime (Evans et al., 1978b), environmental conditions (Forsht et al., 1974) and periods between excretion and collection of the waste (Flegal et al., 1972).

Nitrogen.

The crude protein content of caged layer waste has been shown to range from 18 to 40% (Liebholtz, 1969; Bull and Reid, 1971). True protein ranges from 37 to 45% of the total N; uric acid, ammonia, and urea range from 28 to 55%, 8 to 15% and 3 to 10%, respectively (Liebholz, 1969; Muller, 1980).

Blair (1972) and Bare (1964) showed that non-protein nitrogen (NPN) in poultry waste is of little value to nonruminants. However, studies showed that uric acid can be used by rumen microorganisms to synthesize protein (Belasco, 1954; Jurtshuk et al., 1958). Uric acid is more efficiently utilized by ruminants than urea, (Oltjen and Dinius, 1976) perhaps, because it is less soluble in water (Muller, 1980). Oltjen et al. (1968) attributed the efficient utilization of uric acid to its slower rate of degradation than urea. In-vitro studies by Koenig et al. (1978) showed that rumen microbes required 2 to 3 d adaptation to uric acid. However, Oltjen et al. (1968,1976) suggested a period of 21 to 33 d. Ammonia and urea can be utilized by rumen microorganisms (Virtanen, 1966).

Energy.

The energy value of caged layer waste is influenced by the level of structural carbohydrates and other indigestibles in the diets of the layers (Smith et al., 1970). Poultry waste containing about 69% neutral detergent solubles will provide a high percentage of total digestible nutrients for ruminants (Smith et al., 1973; Muller, 1980).

The high ash content (28%) in caged layer waste may lower its energy value (Bhattacharya and Taylor, 1975). Evans et

al. (1978) reported that a 33% increase in ash content of caged layer waste resulted in a 14% decrease in gross energy value. The total gross energy of 3,533 kcal/kg for layer waste, dry basis, was reported by Polin et al. (1971). Tin-nimit et al. (1972) reported a TDN value of 52.3%, dry basis, for dehydrated poultry waste. This compares with the value of 59.8%, dry basis, for broiler litter (Bhattacharya and Fontenot, 1966). Bhattacharya and Taylor (1975) reported a digestible energy of 2000 kcal/kg when dehydrated poultry waste was fed to sheep and cattle. Brugman et al. (1964) found an apparent energy digestibility of 59.2% for layer litter fed to cattle. A value of 2.22 mcal/kg of metabolizable energy was reported when dehydrated layer waste was fed to sheep (Parigi-Bini 1969). Feeding 100% poultry waste resulted in 60.3% digestibility of energy (Lowman and Knight, 1970). Salo et al. (1975) estimated the metabolizable energy value of layer waste to be 1.58 mcal/kg. This is lower than the value of 1.74 mcal/kg dry matter, reported by Lowman and Knight (1970).

Minerals.

Caged layer waste supplies high levels of Ca and P, thus, supplementation of these minerals may not be necessary. Bhattacharya and Taylor (1975) reported that caged layer

waste contained 8.8 and 2.5% Ca and P, dry basis, respectively. Respective values of 9% Ca and 1.5% P were reported by Brugman et al., (1964) and Lowman and Knight, (1970). Bull and Reid (1971) found that Ca and P in the poultry waste were 95 and 75% available as the only source of minerals in ruminant diets.

Effects of Processing Poultry Waste on Nutrient Utilization, and Performance in Ruminants.

Application of heat resulted in loss of nitrogen (Caswell et al., 1975; El-Sabban et al., 1970; Fontenot et al., 1971; Shannon and Brown, 1969; Manoukas et al., 1964). The use of chemicals may reduce loss of nutrients (Caswell et al., 1975; Koenig et al., 1978) while overprotection with formaldehyde may render the N less available for ruminants (Seltzer et al., 1969).

Utilization of Nitrogen.

Smith and Calvert (1972) substituted dried poultry waste for 0, 50, and 100% of the soybean meal in sheep diets. They found no differences in digestibility of crude protein among treatments. Substituting 45% of soy protein N with dried poultry waste N resulted in a 10% reduction in protein digestibility. However, nitrogen retention, expressed as percent of absorbed, was 20% higher than for the control

diet (Tinnimit et al., 1972). Gihad (1976) reported a 15% increase in N retention in sheep fed dehydrated poultry waste, compared to those fed soybean meal. El-Sabban (1970) replaced soy protein with cooked or autoclaved poultry waste, and found improved N retention in sheep. Crude protein digestibility of 53% was reported by Tinnimit et al. (1972) when 90% of the dietary N was supplied by dried poultry waste fed to sheep. Crude protein digestibility of 73% had been reported by Bull and Reid (1971), which was similar to the value of 77% reported by Lowman and Knight (1970). A digestion coefficient of 77.8% for crude protein was reported by Brugman et al. (1964) when laying house litter was fed to bulls. Smith et al. (1976) found a 10 to 58% increase in nitrogen digestibility when 19 and 38% dehydrated broiler excreta were incorporated into the diet of sheep. Smith et al. (1979) showed a 71% N digestibility when pelleted diets of corn meal were supplemented with dehydrated caged layer excreta supplying 40% of the dietary N. However low N intake and retention were observed, compared to a cottonseed meal supplemented diet.

Flipot et al. (1975) reported a reduction in apparent digestibility of N when ensiled caged layer waste was treated with paraformaldehyde, compared to treatment with tannic acid. Arvat et al. (1978) showed that sheep consuming silag-

es containing 22.7% caged layer waste did not show significant difference in apparent digestibility of N but lower N retention, compared to the control diet. Autoclaving or treating with ethylene oxide, H_2SO_4 or paraformaldehyde prior to dry heating had no significant effect in utilization of N in broiler litter fed to sheep (Caswell et al., 1975; Fontenot et al., 1971; Harmon et al. 1974).

Digestibility of Organic matter.

Drying of poultry waste at 315.5 C for 40 min resulted in high ash content due to loss of over 50% of the organic matter (Silva et al., 1976). High ash content will limit the energy value of the waste (Brugman et al., 1964; Liebholtz, 1969; Bull and Reid, 1971).

Smith and Calvert (1976) showed that feeding of 57 and 100% dehydrated broiler excreta to sheep reduced organic matter digestibility. Kristensen et al. (1976) reported that digestibility of organic matter in dehydrated poultry waste fed to dairy cows was 60 to 65%. Lowman and Knight (1970) reported organic matter digestibility of 66.5% when 100% dehydrated poultry waste was fed as total diet to sheep. Increasing the level of dehydrated caged layer waste fed to sheep from 20 to 80% resulted in reduction of organic matter digestibility from 77 to 68% (Tinnimit et al., 1972).

Salo et al. (1975) reported that feeding dehydrated layer waste to sheep resulted in the organic matter digestibility of 63%. Smith and Calvert (1976) reported that feeding 38% dehydrated broiler excreta to sheep resulted in organic matter digestibility of 70%. Goering and Smith (1977) found digestibility of organic matter of 65% from feeding dehydrated layer waste ensiled with corn forage to sheep. Muller (1980) concluded that ensiling of poultry waste with corn and hay may yield organic matter digestibility of 70 to 74%.

Performance in Ruminants.

Thomas et al. (1972) observed a reduction in milk production when 20 to 32% of dried poultry excreta was fed. This reduction was attributed to a low energy value in the diet rather than a direct effect of the waste. Smith et al. (1976) reported that milk production of dairy cows was not affected by including 23% of the total dietary N from dried poultry excreta. Van Horn and Silva (1976) fed dairy cows 0, 10, 20, and 30% of dehydrated poultry excreta diets containing 60% ash and 14% calcium. Diets containing 20 and 30% levels of poultry waste resulted in reduced milk production. Based on a summary of several experiments, Smith and Wheeler (1979) reported average daily gains of 1.10 vs 1.07 kg when cattle were fed dehydrated poultry excreta and con-

trol diets. Arvat et al. (1978) reported a daily gain of 100 to 120 g when sheep were fed silages containing 22.7% caged layer waste, ground corn and hay, compared to a daily gain of 160 g for sheep fed a control diet containing soybean meal and corn silage.

El-sabban et al. (1970) showed that steers fed diets containing dehydrated or autoclaved poultry waste did not show any significant differences in average daily gain, feed efficiency or carcass characteristics, compared to those fed a control diet. Smith and Lindahl (1977) observed that lambs fed dehydrated poultry excreta containing 12% crude protein converted the organic matter of the diet for growth 32% more efficiently than lambs fed diets supplemented with alfalfa. Rusnak et al. (1966) reported an average daily gain of .98 kg in steers fed diets containing hydrolyzed poultry waste. Goering and Smith (1977) showed a higher average daily gain in lambs fed corn silage supplemented with dehydrated poultry excreta than in lambs fed silages with urea or soybean meal supplementation. Kali and Merrill (1975) fed dairy cattle corn silage containing 12% dried caged layer waste. Average daily gain was increased by 50%, compared to the control silage. Clark and Dethrow (1975) found no significant differences in weight gains by steers fed corn silage diets supplemented with dried caged layer waste or a 50:50 mixture

of soybean meal-dried caged layer waste. However, steers on waste diets had higher gains than those fed soybean meal-corn silage diets.

Cullison et al. (1976) found a significant reduction in the rate of gain in steers fed dehydrated caged layer waste, compared to steers fed broiler excreta. Dairy cattle were fed dried caged layer waste at 0, 10, 20 and 30%, dry basis. Reduction in body weights and milk yields was observed at 20 and 30% levels. Oliphant (1974) found no difference in average daily gain in steers fed diets containing dehydrated poultry waste, fish meal or soybean meal.

Evans et al. (1978) reported average total weight gains of 16 and 11 kg respectively in sheep fed caged layer waste treated with 2% molasses or 1% propionic acid. Koenig et al. (1978) observed lower average daily gain in steers fed 10% waste treated with 37% formaldehyde than those fed a control diet. Shannon et al. (1978) reported an average daily gain of .73 kg in steers fed silage containing 27% (w/w) caged layer waste and chopped corn plant, compared to the control silage. Studies have shown that ensiled poultry litter can be utilized more efficiently, compared to dehydrated poultry waste (Meregalli et al., 1973).

Methods of Processing Poultry Waste.

Yushok and Bear (1943) reported loss of moisture and organic matter, increased ash content and nitrogen loss of 30 to 35%, when caged layer waste was stored for 240 d. Flegal et al. (1972) observed that storage of hen droppings for a period of 98 d without processing resulted in a decrease in crude protein from 30.3 to 18.3%, dry basis.

Dehydration.

Silva et al. (1976) reported that Flegal and Zindel (1971) suggested that dehydration of poultry waste may be an attractive processing system. Surbrook et al. (1971) reported that a dehydrater reaching temperature of 370 to 750 C will reduce or eliminate pathogenic organisms. These workers observed that this method reduced the bulkness of animal waste by 20 to 30% of the original volume. However, this process may not be economically feasible due to high operational and energy costs.

Dehydration may be effective against pathogens but the method showed a considerable loss of nutrients (Caswell et al., 1975; Silva et al., 1976; Shannon and Brown, 1969; Caldertone and Wilson, 1976). Shannon and Brown (1969) reported that drying of poultry manure at 60 C and 120 C resulted in a 4.6 and 10.6% loss in nitrogen and 5.5 and 2.8%

loss of energy, respectively. Manoukas et al. (1964) found N and gross energy loss ranged from 7.1 to 15.2% and 1.2 to 20.2%, respectively, when poultry excreta were dried in a conventional oven at 65 C for 24 h. Higher drying temperatures and longer drying periods increased loss of N from broiler excreta (Kubena et al., 1973). Caldertone and Wilson (1976) found that drying of poultry waste at 45 C resulted in 20.8% loss of N and 5.9% loss of P. Drying of poultry waste renders the P less available for intestinal absorption (Tagari et al., 1981). Silva et al. (1976) noted an increase in ash content and over 50% loss of organic matter due to dehydration of poultry waste. Fontenot et al. (1971) reported that drying of broiler litter at 150 C for 3 h resulted in a 20% loss of total N. Harmon et al. (1974) investigated the effect of acidifying broiler litter with H_2SO_4 to a pH of 6 on nitrogen loss. A 50% reduction in nitrogen loss was reported when the acidified litter was dried at 150 C for 4 h.

In-house drying has been investigated by some workers. Bressler and Bergman (1971) suggested that pre-drying of caged layer waste reduces dehydration cost. This method involved the exposure of mechanically stirred poultry waste to a high velocity air movement. Waste with a moisture content of approximately 83% was removed, followed by mechanical

drying. Level of NH_3 and odors were reduced. A similar method has been described by Ostrander (1975), which involved collection of waste on timber slats of different widths, suspended by metal straps or ropes and the wastes removed after 60, 120 and 180 d. The loss in moisture content ranged from 7.5 to 12.7%, with subsequent reduction in odor.

Autoclaving.

Caswell et al. (1975) reported that autoclaving of broiler litter at 121 C under steam pressure of 1.05 kg/cm for 5, 10, 15 or 30 min at a depth of 5 cm resulted in the lowest losses of non-protein nitrogen (NPN) compared to dry heat, paraformaldehyde and ethylene oxide treatments. Studies by Long et al. (1969) showed autoclaving of hen manure resulted in crude protein and ether extract losses of 35.4 and 3.6%, respectively, compared to 24.9 and 2.2% when hen manure was air dried at 427 C. Harmon et al. (1974) reported a nitrogen loss of 10.8% when litter was autoclaved for 40 min. Autoclaving of poultry waste is effective against pathogens (Harmon et al., 1974; Caswell et al., 1975).

Fumigation.

Messer et al. (1971) reported that fumigation with ethylene oxide reduced counts of enteric bacteria in poultry litter. Caswell et al. (1975) showed that fumigation with ethylene oxide from 30 to 120 min at 22 C and 1 atmospheric pressure was effective against coliforms. The treated litter lost a significant amount of total- protein-and $\text{NH}_3\text{-N}$. Harry et al. (1973) found that fumigation with methyl bromide was effective in the destruction of *Salmonella typhimurium*.

Deep Stacking.

Deep stacking of poultry waste involves storage of the materials in an open shed while the inner parts undergo anaerobiosis in the absence of oxygen. Dana et al. (1978) investigated changes in litter deep stacked for a period of 42 d. The upper part of the litter underwent an aerobic process due to a rise in temperature. After 14-d, the upper and lower parts maintained a fairly constant temperature. Fecal coliforms, *Salmonella* and *Shigella* were eliminated by this process.

Oxidation Ditch.

The oxidation ditch is a technologically advanced aerobic process, consisting of a continuous open-channel ditch and

an aeration motor that circulates the liquid in the ditch and supplies oxygen (Muller, 1980). Dunn and Robinson (1972) found a N loss of up to 80% over 138 d when poultry manure was treated in an oxidation ditch. A similar study by Stewart and McIlwain (1971) showed a reduction of approximately 60% of the N and 55% of the organic matter. Hashimoto (1971) reported a 60 to 70% loss of N in a diffused-air aeration system under caged laying hens, and this was attributed to denitrification. Poelma (1974) reported that use of a floating aerator in an under-floor manure storage system resulted in approximately 50% N loss. Muller (1980) suggested that the system may be technically feasible but is unreliable for nutrient recovery because about 80% organic matter is mineralized or converted into gases.

Anaerobic Ponds.

Papanos and Brown (1950) reported a loss of 62% N from poultry manure slurries stored in pits at room temperature for 24 d, compared to 77% loss from dropping pits stored for 120 d. Eno (1962) reported that 50 to 75% of N may be lost from poultry dropping pits in 24 to 60 d. This was attributed to the conversion of N to $(\text{NH}_4)_2\text{CO}_3$, which is subsequently lost by volatilization.

Ensiling.

Ensiling is a preservation method involving anaerobic fermentation (Barnett, 1954). The entire ensiling process requires 12 to 18 d (Muller, 1980), and is characterized by the production of heat, and acetic and lactic acids, followed by quiescence during which the lactic acid concentration remains stable and the pH of the fermented mass becomes constant at 4 (Barnett, 1954). The ensiling process prevents the loss of crude protein, converts part of the NPN into true protein (Muller, 1980), and increases the solubility of N (Goering and Waldo, 1974). Ensiling is a low-cost procedure, and eliminates or reduces pathogenic bacteria (McCaskey and Anthony, 1975). Albert (1977) ensiled caged layer waste which had accumulated for 3 yr with corn forage at 20 and 40% of the dry matter. Final pH increased with an increase in the level of waste. Total bacteria counts were reduced, and coliforms and proteus organisms were eliminated.

Saylor and Long (1974) ensiled caged layer waste and grass hay in proportions of 100:0, 90:10, 80:20, 70:30 and 60:40. Putrefaction was observed in silages containing 100 and 90% poultry waste. A higher crude protein (20%) was reported for the 60:40 mixture, the only mixture which ensiled satisfactorily. Harmon et al. (1975) suggested that the

production of lactic and acetic acids from corn forage-broiler litter silage was responsible for destroying the bacteria. They suggested that ensiling broiler litter with corn forage is more economical and convenient than use of artificial heat. Caswell et al. (1978) reported that ensiling of broiler litter at 20 to 50% moisture eliminated coliform organisms and reduced total bacteria count. They found that a minimum moisture of 40% was needed to attain a low pH, high lactic acid concentration and total elimination of pathogens. In another study, Caswell et al. (1977) showed that ensiling high moisture corn grain and broiler litter in a ratio of 2:1 did not eliminate coliforms and proteus. This was attributed to high dry matter content (76.6%) for the corn-litter silage. The importance of moisture level on ensiled materials was further elucidated by Duque et al. (1978). Whey or water was added to broiler litter to obtain the moisture levels of 30, 40, 50, 60 and 70%, respectively. Addition of whey markedly lowered the pH and increased the lactic and acetic acid production in litter which had been deep stacked, but not in fresh litter, compared to adding water. Both whey and water effectively eliminated coliforms at the various moisture levels.

Flipot et al. (1975) ensiled chopped alfalfa hay with 64% caged layer excreta treated with 3% tannic acid or 2% para-

formaldehyde. The material was allowed to ferment for 42 d at room temperature. The pH was higher for the waste silages than the soybean silage. Ammonia nitrogen, as percent of total nitrogen, was elevated in waste silage, but lactic acid concentration was lowered for the paraformaldehyde treated silage.

The Use of Chemicals as Preservatives.

Smith et al. (1978) found that the danger of transmitting salmonella and coliforms from feeding wet poultry excreta can be reduced or eliminated with organic acid treatment. Jones et al. (1974) suggested that any chemicals used as preservatives should be easily metabolized by the organisms or host animal; possess no toxic, mutagenic, carcinogenic or teratogenic properties; not adversely affect the nutritive value of wastes; and leave no residue in animal tissue or products.

Woolford (1975) studied the microbiological screening of the straight chain fatty acids (C1 to C12) as potential silage additives. He found that the acids reduced the pH of the silages. Formic, acetic and propionic acids were effective against yeast, molds, sporebearing and lactic acid bacteria, and propionic acid was effective as an antimycotic agent. He found that formaldehyde had a wide antimicrobial

spectrum that would suppress all groups of microorganisms, thereby producing a non-fermented silage. Surbrook et al. (1971) found that alkalinity of poultry waste is responsible for the nitrogen loss. At the pH levels of 7.2 to 8.6, 26% of N was lost from dairy manure, compared to 6% at pH of 4.5 to 6.5. Formaldehyde prevents loss of nitrogen by forming a cross link with the protein molecule (McGilliard, 1972). Horton and Richardson (1985) reported that treating of soybean meal with 1% formaldehyde resulted in over-protection of the protein and less was available for microbial fermentation. Wing et al. (1976) investigated the effects of formaldehyde, propionic and formic acids on protein degradation in alfalfa haylage. The chemicals were effective in reducing NH_3 and non-protein and soluble N. Increasing the levels of the preservatives also increased hemicellulose.

The effects of formaldehyde and propionic acid in preservation and fermentation of colostrum have been investigated (Muller et al., 1977; Rindsig et al., 1977). The chemicals used were effective in retarding mold and yeast growth and degradation of protein. In another study, Muller et al. (1975) investigated the effects of propionic and formic acids and formaldehyde on the preservation and fermentation of colostrum at 21, 32 and 39 C. Formic and propionic acids maintained low pH after 18 and 23 d. Formaldehyde main-

tained the initial pH of the colostrum. At 21 and 32 C, the three treatments reduced protein degradation, compared to the control. The addition of 1 ml of 37% formaldehyde to 4 kg of reconstituted milk replacer retarded bacterial growth and prevented souring of the milk for 24 h at 25 C, and 72 h at 7 C (Lindhahl, 1974). Addition of a mixture of formaldehyde and formic acid markedly reduced carbohydrate fermentation and protein degradation of wilted perennial rye-grass in the silo (Siddons et al., 1979).

Evans et al. (1978) indicated that the addition of 1.0, 1.5 or 2% (w/w) propionic acid lowered N loss in caged layer waste at 70 and 80% dry matter when stored for 90 d. Smith et al. (1978) found that adding .5% (w/w) of 80% propionic-20% acetic acid to caged layer waste eliminated salmonella and total coliforms within 7 d. Davies et al. (1975) described the organiform process which involves complexing of manure with urea and formaldehyde. This method increased the N content of the manure from 2 to 14%, dry basis. Caged layer waste was treated with an 80% propionic-20% acetic acid mixture, formaldehyde or a combination of both at .25, .5 and 1% (Anon, 1977). The untreated waste was heavily decomposed with marked N losses. The chemicals at .5 and 1% preserved the waste for 7 and 14 d, respectively. A study by Smith et al. (1978) showed that .5 and 1% of acetic-pro-

pionic acid mixture, formaldehyde and combination of the mixture and formaldehyde, retarded N losses up to 7 and 14 d, respectively. The acetic-propionic acid mixture appeared most effective in retarding mold growth and discoloration.

Narasimhalu (1981) incubated broiler or layer excreta for 24 h with the addition of sodium metabisulfite, calcium hypochlorite, sodium hypochlorite, propionic acid, tannic acid and formaldehyde at .01 .05 and .5% (w/w). Effectiveness against fungi, coliforms and aerobic bacteria increased with level of chemicals. Caswell et al. (1975) showed that the addition of sulfuric acid and paraformaldehyde to broiler litter prior to heat treatment reduced NH_3 losses and pathogenic organisms.

Allen et al. (1975) investigated the effect of formic, propionic and a formic-propionic acid mixture on uncovered piles of wet brewers grains stored for 14 d. The low (.20%) and medium (.30%) levels of additives did not prevent extensive mold growth, discoloration and dry matter reduction. In another study Allen et al. (1975) showed that .5 and .75% formic acid and .75% formic-propionic mixture were effective in preserving wet brewers grains ensiled for 18 d.

A mixture of propionic and formic acids for preserving silage of cod viscera was investigated by Gildberg et al. (1977). At a concentration of .75%, a stable pH of 4.3 and

an increase in water-soluble carbohydrates were observed. At 220 d, NH_3 level corresponding to 8% loss of protein N was reported. Addition of 3% (w/w) 98% formic acid to minced cod viscera yielded a fairly stable silage with a low microbial count (Backhoff, 1976). The use of 1.5% formic acid in preserving silage of cod viscera resulted in decreased soluble carbohydrates, an increased pH and unpleasant odors of amines, with patches of mold growth (Gildberg et al. 1977).

Ensiling chopped barley with formic acid, formic-formaldehyde and propionic acid reduced lactic acid production, which indicates restricted microbial activity (Candlish et al. 1973). The treatment of high-moisture corn with propionic acid has been shown to be effective against fungal growth and aflatoxin contamination, when stored for a period of 180 d (Utley et al., 1977). A decrease in proteolysis and proteolytic end products has been observed in reconstituted sorghum with additions of 2% propionate (Lichtenwalner et al., 1979).

Stallings et al. (1981) investigated the effects of .2% formic acid and 1% propionic acid on alfalfa grass haylages stored with slight exposure to air. At day 56, mold contamination was 18 and 75%, respectively. Formic acid treated silage had an increase in pH from 5 to 7 and in soluble N

from 41 to 68%. However, 1% propionic acid resulted in a reduction of pH, soluble N and NH_3 concentration. Henderson and McDonald (1971) had shown that up to 50% of formic acid used in silage making is lost through vaporization.

Zelter (1960) reported that .4% sodium metabisulfite has bacteriostatic action on the organisms producing butyric acid, but is less protective against proteolysis and loss of nutrients. Murthy (1969) showed that the use of .2 to 1 g of sodium metabisulfite per liter of whole milk had no bactericidal effect, and high protein instability was observed within a 7 d storage period. Owens et al. (1970) investigated the effect of sodium metabisulfite and crude protein sources on fermentation characteristics of ensiled corn plants and stalkage. Addition of bisulfite and urea increased the dry and wet weight losses of silage. Hydrogen sulfide production was greater and pH was elevated in silages containing bisulfite and urea. In a similar study, Owens et al. (1970b) showed that addition of sodium bisulfite and urea to silages increased the ammonia concentration and the crude protein content. This was attributed to the increase synthesis of bacteria protein and dry matter losses. Meiske et al. (1965) observed that the addition of .4% (w/w) sodium bisulfite inhibited nitrogen dioxide production in a large field silo. The bisulfite-treated silages contained more

residual nitrate, which resulted in higher pH and lower concentrations of acetic and lactic acids. Scalleti et al. (1961) reported that sodium metabisulfite- treated silages inhibited the toxic gas production in laboratory silos. Sodium metabisulfite has been shown to inhibit the formation of volatile fatty acids (VFA) but has less effect on lactic acid production (Murdoch et al., 1956).

Effects of Silage Inoculants.

Studies have shown that inoculation of ensiled materials with lactic acid producing bacteria may or may not influence the characteristics of the silage (Lesins et al., 1968). McDonald et al. (1964) showed that silages of good quality were obtained regardless of inoculation of Italian rye grass containing 16.2% water-soluble carbohydrates. Ohyama (1973) found that inoculation with *L. plantarum* did not affect silage quality, but attributed the fermentation to the presence of water-soluble carbohydrates and proper sealing of the silos. Whittenbury (1961) outlined the qualities for a potential silage inoculant. The organisms must grow rapidly to compete effectively with undesirable bacteria; be tolerant to acid and low moisture content; be capable of producing a final pH of 4.0; be non-proteolytic; and have no adverse action on organic acids.

The microbial cultures of *Aspergillus oryzae* and *Bacillus subtilis* were investigated by Sherrod et al. (1975). Addition of the inoculant to corn silage had no appreciable influence on composition and in vitro digestibility of the silage. Sherrod et al. (1971) observed that addition of .003 and .012% of the inoculant did not influence the nutritive value of sorghum silage. Addition of .05% commercial "silo grain" on alfalfa, orchard grass and whole corn silages resulted in good quality silage. Waldo et al. (1976) and Black et al. (1980) reported that the addition of commercial "silo grain", "ensila", "silo guard" and "super silozyme" to sorghum silage resulted in well preserved silages, with no differences among treatments. Ohyama et al. (1975) inoculated Halina rye grass and cocksfoot with 10^6 /g of *Lactobacillus plantarum*, with and without addition of glucose. A rapid growth of the inoculum reaching 10^9 /g was observed on the second day of ensiling. The increase resulted in higher production of lactic acid, low pH and good quality silage. Macpherson et al. (1966) investigated the effect of low pH on protein degradation. Addition of *Lactobacillus plantarum* and glucose resulted in early rapid production of acid, which prevented proteolysis, especially the release of free arginine and lysine. The ensiling characteristics of inoculated alfalfa silage have been studied by Lesins and Schulz

(1968). The materials were inoculated with rods and cocci lactic acid bacteria and incubated at 30 or 43 C. Bacterial inoculation increased the acidification of sedge (*Carex ath-erodes*). Inoculation of alfalfa had less effect on pH but prevented putrefactive silage spoiling bacteria to thrive.

Woolford (1972) observed that some species of lactic acid producing bacteria grow slowly, and will not produce acid until the pH falls below 5.0. McDonald (1981) suggested that mixed inocula of *Streptococcus faecalis* and *Lactobacillus plantarum* can be used. *Streptococcus faecalis* is not tolerant to acid; thus, it dominates the fermentation in the early stage of ensiling, while *Lactobacillus plantarum* takes over as pH falls below 5.0. The addition of mixed cultures of *S. faecalis*, *L. plantarum*, and *leuconostoc mesenteroides*, and glucose to ryegrass-clover mixtures resulted in well preserved silages. At d 4, pH of inoculated silages fell to 3.79 and control silage remained at 4.34. The reduction in pH resulted in higher protein and lower $\text{NH}_3\text{-N}$ levels in all inoculated silages (Carpintero et al., 1979).

Wood (1961) showed that homolactic acid bacteria protect amino acids from being degraded more than the heterolactic species. A non-proteolytic homofermentative bacteria, *Lactobacillus acidophilus* has been isolated from feces of infants. The organism does not produce NH_3 from arginine, and

its addition to ensiled wheat resulted in a rapid decline in pH (McDonald, 1981).

McDonald et al. (1964) investigated the effects of eight strains of homofermentative *Lactobacillus* on *Lolium multiflorum* containing 16.2% soluble carbohydrates. Addition of inoculum, molasses and molasses plus inoculum produced well preserved silages with pH from 3.9 to 4.1, and high lactic acid. Addition of molasses, whey and six strains of *Lactobacillus* bacteria to sorghum and corn silages resulted in significant reductions in pH and higher lactic acid (Neekakantan, 1976). McDonald et al. (1965) studied ensiling red clover containing 11.8% water-soluble carbohydrates with 3% molasses, .2% *Lactobacillus* or no additive. The pH values were 4.1, 3.8, and 4.2, respectively. Molasses-treated silage showed a loss of dry matter but lactic acid production was similar in both treated silages.

Health Hazards and Safety Considerations.

In 1967, the Food and Drug Administration (FDA) stated that the agency did not sanction the use of poultry waste as animal feed due to the potential hazard of drugs and disease organisms (Kirk, 1967). In 1980 the FDA entrusted the regulation guiding the use of animal waste as feedstuff to the individual states (FDA, 1980).

Pathogenic Organisms.

Biester et al. (1959) reported that cattle and swine are susceptible to *Salmonella pullorum* and *Erysipelothrix rhusiopathia* found in poultry excreta. *Listeria monocytogene* is another disease communicable from poultry to cattle and sheep (Biester and Schwartz, 1959). Samples of fresh excreta from 91 poultry houses were tested for *Salmonella* (Kraft et al, 1969). They reported that 26% of the samples contained salmonella. Alexander et al. (1968) reported that 23 out of 44 samples of poultry litter were positive for *Clostridium* species, including eight *Clostridium perfringens*, the agent of bovine enterotoxemia; *Corynebacterium pyogenes* and *equi* which cause abortion, cystitis and pyelonephritis in cattle and horses. Three types of salmonella noted as potential agents of serious enteric disease in livestock have been recovered in poultry litter (Alexander et al., 1968). Zindel (1970) reported that 40% of fresh layer waste examined contained *Bacillus* spp., *Proteus* spp., *E. coli* and other enterobacteria, while 60% of the samples were positive for coliforms.

Drug and Chemical Residues.

Antibiotics and other antimicrobial drugs which are used in disease control in poultry are sources of potential ha-

zards in waste recycling. Muller (1980) reported that the various metabolic actions of these drugs in the intestine determine the potency in the excreta. Brugman et al. (1964) reported no drug residues except arsanilic acid in the litter of layers fed diets containing arsanilic acid, zoalene, unistat, micarbazin, furan and sulfaquinoxaline. Another study by Brugman et al. (1968) investigated the effects of feeding lambs diets supplemented with poultry litter containing amprolium plus and 3-nitrohydroxyphenylarsonic acid. No residual drugs were found in the heart, spleen, 12th rib, kidney, kidney fat, liver and brain of the lambs. Webb et al. (1975) found no residues of neomycin, zinc bacitracin, nicarbazin and amprolium in tissues of steers fed diets containing these feed additives. Low levels of chlortetracycline residues were found in kidney fat of 3 out of 20 steers slaughtered. Bruggemann et al. (1963) reported a consistent accumulation of amprolium, zoalene, arzene and arsanilic acid in spite of a steady increase in dietary intake of the drugs.

Moody and Williams (1964) and Overby and Straube (1965) noted that arsanilic acid appears to be excreted unchanged following oral administration to hens. Detectable amounts of arsenic were found in litter from birds fed organoarsenicals (Morrison, 1969). Webb et al. (1975) found a consis-

tent increase in liver arsenic when cattle were fed diets containing 50% broiler litter. El-Sabban et al. (1970) showed low levels of arsenic accumulation in liver samples from steers fed diets supplemented with soybean meal, urea or processed caged layer waste.

Pesticide Residues.

Messer et al. (1971) reported no detectable traces of DDT or DDE in poultry litter samples obtained from six commercial farms. Wasti et al. (1970) detected residues of Rabon, an orally administered pesticide used to control parasite and fly larvae, in hen manure. Ivey et al. (1968) found Rabon to be non hazardous to cattle. A similar study by Miller and Gordon (1973) showed no health or reproductive problems, and no accumulation of Rabon in milk of dairy cows fed 252 ppm of Rabon. Smith et al. (1976) detected a PCB level of 5 ppm in milk fat of dairy cows fed a diet containing 32% dried poultry excreta for 50 d. Steers fed the processed caged layer waste showed no significant differences in the accumulation of chlorinated hydrocarbon pesticide in backfat, compared to controls. El-Sabban (1970) showed that feeding a diet containing 28% dried caged layer waste to fattening cattle did not result in pesticide residue accumulation in backfat of the steers. Fontenot (1971a) reported

that broiler litter contained low levels of pesticide but when fed to cattle no accumulations were found in the liver and fat.

Mineral Residues.

Copper and Se are used as feed additives, while Cd, Pb and Hg are not added to feeds but occur naturally in feedstuffs (Fontenot et al., 1979). Fontenot et al. (1971) suggested that Cu content of waste fed to sheep should be controlled due to sensitivity to Cu in the diet. Suttle and Price (1976) showed that 15 mg/kg dry matter of Cu in the diet may cause problems in sheep. Webb et al. (1979) fed cows high broiler litter diets containing up to 320 mg/kg Cu. Performance was not affected but liver Cu levels increased from 58.8 mg/kg in the control to 561.3 mg/kg in the test diet in the spring, but the levels were reduced in the subsequent fall, after grazing during the summer. Blair (1974) reported a range of 28 to 109 mg/kg dry matter of Cu in dehydrated layer waste. Calvert and Smith (1976) reported a range from 48 to 150 ppm with an average value of 94 ppm for Cu in poultry excreta. In a feeding trial, substituting dehydrated poultry waste containing 94 ppm of Cu resulted in liver Cu levels of 333 ppm, compared to 158 ppm in control animals. Copper toxicity in ewes fed broiler litter

containing 57.1 and 109.1 ppm of Cu have been reported (Fontenot et al., 1972c). Bruhn et al. (1977) reported no significant differences in Cu content of milk from cows fed a diet supplemented with 9.9% dehydrated poultry waste containing 51 ppm of Cu, compared to cows fed control diets. Vijchulata et al. (1980) reported an increase from 55 to 490 mg/kg Cu in livers of steers fed diets containing up to 25% caged layer waste. Webb et al. (1975) conducted two feeding trials with steers fed 25 or 50% broiler litter containing 230 or 289 mg/kg copper. Increases in liver and muscle copper levels were reported in both trials.

Westing et al. (1985) reported lower liver Se for fattening heifers fed corn-broiler litter silage (70:30,w/w, dry basis) compared to a control diet of corn silage and protein supplement. Bruhn et al. (1977) detected 6.24 ug/kg of Cd in raw milk of cows fed dehydrated poultry waste containing 1.3 ppm of Cd, compared to 3.71 ug/kg in the control group. Feeding diets containing 7 ppm of Pb resulted in 49.4 ug/kg of Pb in raw milk, compared to 56.2 ug/kg for the control.

Hormone Residues.

Mathur and Common (1969) detected measurable levels of estrone and estradiol-17B in layer waste. The levels were significantly lower than in waste from non laying hens.

Calvert et al. (1978) reported the presence of androgenic and estrogenic activity in poultry waste. Hertenlendy et al. (1965) reported the presence of 17B estraiol in hen urine. Griel et al. (1969) reported abortion in cows fed broiler litter containing 10 mg/100 g of estrogenic activity. The effect was attributed to the presence of dienesterol diacetate in the poultry diet.

Chapter III
JOURNAL ARTICLE I

PRESERVATION OF WET CAGED LAYER WASTE AGAINST PUTREFACTION
AND NUTRIENT LOSSES PRIOR TO ENSILING

ABSTRACT

Fresh caged layer waste which had accumulated on a concrete floor was collected within 24 h. Three preservation studies were conducted during the fall and spring seasons of 1984-85. Different chemicals and feed additives were added to the homogenized waste, and the mixtures were stored in polyethylene lined 210 liter metal drums which were exposed to air for a maximum of 42 d. In experiment 1, the treatments were: untreated (no additive), 1.0 and 1.5% formaldehyde, 1.5 and 2% sodium metabisulfite, and 1.5 and 2%; propionic/formic (1:1,w/w). In the second experiment, the low levels of the chemicals applied in experiment 1 were used, plus two treatments, 10% dry molasses with and without 2% sodium chloride. Based on the results obtained from the two previous experiments, the third study was designed with four treatments: untreated (no additive); 1% formaldehyde; 10% dry molasses and 10% dry molasses plus 2% sodium chloride. Water was added as needed to obtain similar dry matter con-

tent among treatments. In experiment 1, massive putrefaction, maggot infestation and dark color were observed in untreated waste. The sodium metabisulfite and formaldehyde treatments were extensively covered with mold. In experiment 2, the untreated waste had the lowest ($P<.01$) dry matter content. The ash values increased for all treatments; untreated waste had a 19% increase which was higher ($P<.01$) than for the treated waste. The additives used in the third study were effective in preventing spoilage and nutrient losses. An increase ($P<.01$) in dry matter and a decrease ($P<.01$) in ash were observed with formaldehyde treatment. The crude protein increased for all treatments. A decrease in water-soluble carbohydrates and pH was observed in the molasses-treated waste. The pH of the mixture with molasses alone and with salt decreased ($P<.01$) from 7.45 and 7.62% to 6.64 and 6.38%, respectively, after storage. In the waste treated with molasses 85% of the available water soluble carbohydrates were converted to acids, compared to 28 and 20% ($P<.01$) for the control and formaldehyde treatments.

(Key words: Caged layer waste, Chemicals, Feed additives, Putrefaction, Preservatives).

INTRODUCTION.

Disposal of caged layer waste is a major problem in large scale intensive poultry production. The operations located in heavily populated areas constitute nuisances to neighboring residents, especially during the warmer seasons. Land utilization has been the most popular means of disposing the waste. Attention has been directed towards nutrient recovery of caged layer waste. Caged layer waste is a potential source of energy, crude protein and minerals. A large part of the N is in the form non-protein (Liebholz, 1969), which can be utilized by ruminants (Oltjen et al., 1976).

There is a need to prevent deterioration and loss of nutrients in caged layer waste prior to ensiling. Organic acids and oxidizing agents can be used in preserving caged layer waste. Flegal et al. (1972) showed a progressive deterioration and loss of crude protein when poultry waste was stored for 93 d. Treating caged layer waste with 80% propionic-20% acetic acid mixture would prevent N degradation (Anon, 1977). Smith et al. (1978) showed that a combination of acetic-propionic acid mixture decreased N losses up to 14 d, with total elimination of salmonella and coliform organisms. Organic acid production can be induced by direct involvement of lactic acid bacteria, which may be present in the caged layer waste. This can be achieved by incorporation

of readily fermentable carbohydrates in the caged layer waste.

Studies were conducted to investigate the effects of various chemicals and feed additives in stabilizing and preventing degradation of nutrients in stored caged layer waste.

EXPERIMENTAL PROCEDURE

Three experiments were conducted with caged layer waste¹ which had accumulated on concrete floors for a maximum of 24 h. The hens received feeds containing not less than 14% crude protein, 4.5% crude fiber, 3.4% calcium and .55% phosphorus respectively. The waste was transferred into an electronic auger mixer and was thoroughly mixed to obtain homogenized mixture.

Experiment I.

For this experiment, 159 kg waste were placed into 210 liter metal drum single lined with .08 mm polyethylene bags. A small auger was used to mix the contents as chemicals were added initially and at 7-d intervals thereafter. The following chemicals were used: no additive, 1 and 1.5% formaldehyde, 1.5 and 2.0% propionic/formic (1:1, wet basis), and

¹ Obtained from Green Valley Poultry Farm, Abingdon, Virginia.

1.5 and 2.0% sodium metabisulfite. Chemical concentrations were calculated on wet basis. Water was added to the sodium metabisulfite, propionic/formic and the untreated waste in order to equalize the dry matter content of all preserved waste. The drums were exposed to air and stored for 42 d in a shelter at ambient temperature. Samples were taken initially, at 7-d intervals after mixing and at the end of the trial. The extent of visible changes such as discoloration, mold and yeast growth and putrefaction were examined at 7-d intervals. These changes were scored on a scale of 0 to 5.

Experiment 2.

Waste was treated with no additive, 1% formaldehyde, 1.5% propionic/formic (1:1, w/w), 1.5% sodium metabisulfite, 10% dry molasses, and 10% dry molasses and 2% sodium chloride (salt). Procedures were similar as for experiment 1 except the mixtures were mixed only initially. Initial and final samples were taken for subsequent analyses.

Experiment 3.

Treatments consisted of untreated waste (no additive), 1% formaldehyde and 10% dry molasses with and without 2% sodium chloride (salt) were used. Procedures were the same as for experiment 2.

Chemical Analyses.

Samples for experiments 2 and 3 were prepared for analysis by homogenizing 25 g sample with 225 ml of distilled water in .5 liter jar in a Waring blender at full speed for 2 min. The homogenate was filtered through four layers of cheese cloth and the extract was used for determining pH (electrometrically), lactic acid (Barker and Summerson, 1941, as modified by Penninington and Sutherland, 1956) and water-soluble carbohydrates (Dubois et al., 1956, as adapted to corn plants by Johnson et al., 1966). Kjeldahl N was determined on wet samples (A.O.A.C., 1980). Dry matter was determined by drying in a forced draft oven at a maximum of 60 C for 24 h. The dried samples were ground in a hammer mill and subsamples were taken for ash determination (A.O.A.C., 1980)

Statistical Analysis.

The data for experiment 2 and 3 were treated by analyses of variance by the general linear model procedure of SAS (1982). In experiment 2, the following contrasts were made: 1) untreated vs treated waste, 2) chemical vs molasses treated wastes, 3) formaldehyde vs sodium metabisulfite and propionic/formic treated waste, 4) sodium metabisulfite vs propionic/formic treated waste and 5) molasses vs molass-

es plus salt treated waste. For experiment 3, the following comparisons were made: 1) untreated vs treated waste, 2) formaldehyde vs molasses treated wastes and 3) molasses vs molasses plus salt treated waste.

RESULTS AND DISCUSSION

Experiment 1.

Color change from gray to dark was observed with the untreated waste at d 7, and putrefaction and extensive maggot infestation became progressive with time.

The mixture with 1.5% sodium metabisulfite did not show any deterioration up to d 21, however, after this time the waste became infested with yeast and mold throughout the storage period. The high level of sodium metabisulfite prevented putrefaction up to d 35 (table 1) followed by a characteristic smell of H_2S , which may be due to gradual oxidation of sodium metabisulfite (Wastson and Nash, 1960) as a result of increased temperature (Lanigan, 1961). There was a partial darkening of the waste at the sides which were in contact with the polyethylene bags.

Propionic/formic acid at a level of 2%, prevented spoilage, putrefaction and mold infestation up to d 35. Evaporative losses may be responsible for the effectiveness of propionic/formic acid. Burnell (1973) showed that a large

amount of propionic acid used in treating of high-moisture corn was lost during storage. Allen et al.(1975) reported massive aerobic deterioration when wet brewers' grain was treated with propionic acid and stored for 14 d. The low level of propionic/formic acid was less effective against color change. There was a slight darkening, and extensive mold and yeast growth.

Sodium metabisulfite and formaldehyde-treated waste showed firm consistency, however, the waste treated with 1% formaldehyde was covered with patches of mold and yeast, which is in agreement with the results of Smith et al. (1978) and Yu Yu and Thomas (1975). The mixture with 1.5% formaldehyde did not show any color change.

Experiment 2.

The dry matter content of the untreated waste was lower ($P<.01$) than that of the treated waste (table 2). Dry matter content of the sodium metabisulfite and formaldehyde treated waste increased from 24.82 and 25.36% to 25.69 and 26.99%, respectively, during the preservation period. Values for the untreated waste decreased from 24.45 to 22.21%.

Initial ash values for the molasses and molasses plus salt treated waste were lower ($P<.01$), than for the chemically-treated waste. By d 42, all the stored wastes showed

TABLE 1. VISIBLE CHANGES^a IN PRESERVED CAGED LAYER WASTE (EXPERIMENT 1)

Preservative	Concentration of preservative	Days after treatment					
		7	14	21	28	35	42
None		1	4	4	4	4	4
Sodium metabisulfite	1.5	0	0	0	2	2	2
	2.0	0	2	3	3	5	5
Formaldehyde	1.0	0	2	2	2	2	2
	1.5	0	0	0	0	0	0
Propionic/formic	1.5	0	1	1	1	3	3
	2.0	0	0	0	0	0	3

^aCode:

0 = no visible change.

1 = color change.

2 = mold and yeast growth.

3 = color change + mold and yeast growth.

4 = color change + putrefaction.

5 = color change + mold and yeast growth + putrefaction.

TABLE 2. EFFECTS OF ADDITIVES ON COMPOSITION AND FERMENTATION
CHARACTERISTICS OF STORED CAGED LAYER WASTE (EXPERIMENT 2)

Item	Additives ^a						SE ^b
	None	1.5% sodium bisulfite	1.5% prop/ formic	1% formal- dehyde	10% dry molasses	10% dry mol + 2% salt	
Dry matter, %							
Initial ^{c,d}	24.45	24.82	24.45	25.36	31.62	33.63	.59
Final ^{c,d,e,f,g}	22.21	25.69	23.73	26.99	30.44	32.43	.14
Ash, %							
Initial ^d	30.67	33.26	28.98	29.21	23.71	23.94	1.65
Final ^{c,d,e,h}	38.01	36.78	35.76	30.64	27.88	26.42	.41
Crude protein, %							
Initial ^{c,d,i}	33.54	29.25	31.30	30.10	22.28	23.10	.56
Final ^{c,d,e}	36.28	35.26	36.89	30.95	25.67	24.00	.75
pH							
Initial ^{c,d,e,g}	8.08	7.54	6.11	7.11	7.65	7.67	.03
Final ^{c,d,e}	7.83	7.81	7.82	7.35	5.97	5.70	.02
WSC.% ^j							
Initial ^{c,d,g}	3.28	4.93	6.41	4.03	15.88	14.64	.72
Final ^{c,d,e,g}	2.27	4.01	2.55	4.86	2.94	3.10	.11

^aLevels of chemicals and molasses are on wet basis.

^bStandard error of means.

^cNone vs treated waste (P<.01).

^dChemical vs molasses treated waste (P<.01).

^eFormaldehyde vs sodium bisulfite and propionic/formic (P<.01).

^fMolasses vs molasses + salt (P<.01).

^gSodium metabisulfite vs propionic/formic (P<.01).

^hMolasses vs molasses + salt (P<.05).

ⁱSodium metabisulfite vs propionic/formic (P<.05).

^jWater soluble carbohydrates.

increases in ash, with the untreated waste showing the highest level ($P < .01$). This increase suggests decomposition of organic matter during storage, which is in agreement with findings by White et al. (1944).

The initial crude protein content of the untreated waste (33.53%, dry basis) was higher than the average value of 28% reported by previous workers (Liebholz, 1969; Tinnimit et al., 1972 and Flegal and Zindel, 1971), but was lower than value of 36.9 and 35.7% reported by Samuels (1980) and Smith et al., (1978), respectively.

The molasses and molasses plus salt treated waste had the lowest ($P < .01$) initial crude protein contents of 22.28 and 23.10%, respectively, a reflection of dilution. By d 42, crude protein increased for all stored wastes except for formaldehyde treated waste. The increases in crude protein probably reflect concentration in the dry matter, caused by loss of carbohydrates by fermentation. Similar observation was reported by Moore and Anthony (1970). These workers found a significant increase in crude protein from 16.99 to 43.26% when fresh cattle manure was fermented for 3 d.

The highest ($P < .01$) initial pH (8.08) was for the untreated waste, while the propionic/formic treated waste had the lowest pH (6.11). The final pH remained relatively unchanged for the sodium metabisulfite and formaldehyde treat-

ed waste. Muller and Syhre (1974) reported that .25% formaldehyde maintained a stable pH in colostrum for 18 d. An increase in pH was observed with the propionic/formic acid treated waste to 7.82. Allen et al. (1975) showed that propionic/formic acid used for open storage of wet brewers' grain increased the surface pH from 4.36 to 7.67 by d 14. The molasses and molasses plus salt treated waste had final pH values of 5.97 and 5.70, respectively, indicating that fermentation had occurred.

The addition of 10% dry molasses increased the initial water-soluble carbohydrates to 15.88 and 14.64%, dry basis, respectively, for molasses and molasses plus salt treated waste. The lowest ($P < .01$) initial value (3.28%) was for the untreated waste. This compares with a value of 3.3%, dry basis, reported by Samuels (1980). The reduction in final pH indicated that fermentation had occurred in all the treated waste except the sodium metabisulfite treatment which showed only a small decrease in water-soluble carbohydrates (4.93 vs 4.01) and formaldehyde treatment which showed an increase from 4.03 to 4.86%.

Experiment 3.

The visible observation in this study showed that the additives were effective against putrefaction and spoilage.

The untreated waste had mold, and maggot infestation, which was evident up to d 28. The top of the formaldehyde-treated waste was covered with patches of mold, but the waste had a firm consistency. A characteristic sweet smell was observed for the molasses-treated waste. Allen et al. (1975) showed that addition of 2% molasses to wet brewers' grain was less effective against discoloration, mold and putrefaction than a high concentration of formic and propionic acid.

The effects of various additives upon initial and final composition and fermentation characteristics are presented in table 3. Formaldehyde treated waste showed a small increase in dry matter content by the end of storage period, while the other wastes showed a small decrease. By d 42, the ash contents increased among all treatments, except for a reduction from 27.44 to 21.66% for formaldehyde-treated waste. Untreated waste showed the highest ($P<.01$) level. Initially, the crude protein content was highest ($P<.01$) for the untreated waste and lowest for the molasses treatments. The final crude protein content showed increases among all treatments, perhaps reflecting a concentration due to fermentation of carbohydrates.

During the preservation period the pH of the molasses and molasses plus salt treated wastes was lowered from 7.45 and 7.62 to 6.64 and 6.38, respectively. The pH of untreated

TABLE 3. EFFECTS OF ADDITIVES ON COMPOSITION AND FERMENTATION CHARACTERISTICS OF STORED CAGED LAYER WASTE USED IN LARGE SILO STUDY EXPERIMENT 3

Item	Additives ^a				SE ^b
	None	1% Formaldehyde	10% dry molasses	10% dry mol + 2% salt	
Dry matter, %					
Initial ^{c,d}	25.73	27.00	28.21	28.16	.17
Final ^{c,d,f}	24.68	27.82	26.75	27.35	.16
Ash, %					
Initial ^{c,d,e}	30.28	27.48	21.49	26.19	.35
Final ^{c,d,e}	35.12	21.66	26.51	29.63	.35
Crude protein, %					
Initial ^{c,d}	33.80	29.71	23.57	22.46	.79
Final ^{c,d,e}	37.25	33.76	29.55	26.24	.45
pH					
Initial ^{c,d}	7.94	7.27	7.45	7.62	.02
Final ^{c,d}	7.80	7.52	6.64	6.38	.09
WSC.% ^h					
Initial ^{c,d}	3.39	5.50	15.93	16.69	.43
Final ^{d,g}	2.43	4.43	2.49	2.42	.19

^aLevels of chemicals and molasses were on wet basis.

^bStandard error of means.

^cUntreated vs treated waste differ (P<.01).

^dFormaldehyde vs molasses treated waste differ (P<.01).

^eMolasses vs molasses plus salt treated waste differ (P<.01).

^fMolasses vs molasses plus salt treated waste differ (P<.05).

^gUntreated vs treated waste differ (P<.05).

^hWater soluble carbohydrates.

and formaldehyde-treated waste remained high (7.80 and 7.52, respectively). For the molasses and molasses plus salt treated waste 84.4 and 85.5% of the water-soluble carbohydrates were fermented. These values were higher ($P < .01$) than those for the untreated and formaldehyde treated waste, in which 28.4 and 19.5% of the available water soluble carbohydrates present were fermented.

This study shows that loss of nutritional value of caged layer waste can be prevented with the use of preservatives. Formaldehyde and dry sugar cane molasses are effective against odor and discoloration during a 42-d storage period.

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Chapter IV

JOURNAL ARTICLE II

ENSILING CHARACTERISTICS AND NUTRITIONAL VALUE OF PRESERVED CAGED LAYER WASTE AND WHEAT STRAW

ABSTRACT

Caged layer wastes was treated with different preservative and stored for a minimum of 42 d prior to ensiling. In a small silo study, the preservatives used were: none, 1% formaldehyde, 1.5% sodium metabisulfite, 1.5% propionic/formic acid (1:1,w/w), 10% dry sugar cane molasses and 10% dry molasses plus 2% sodium chloride (salt). Each of the wastes and wheat straw (60:40,wet basis) were ensiled with the following additives: none, silage inoculant, 10% dry molasses, or 10% dry molasses plus inoculant. The mixtures were placed in 4-liter cardboard containers double lined with polyethylene bags. The waste used for a large silo study had been treated with no additive, 1% formaldehyde, 10% dry molasses and 10% molasses plus 2% salt. These were ensiled with wheat straw and dry molasses (55:35:10,wet basis) in 210 liter metal drums double lined with polyethylene bags. The silage from the large silo was used for a digestion trial with 30 wethers with average weight of 31.8 kg.

Diets fed were 1) basal (77% wheat straw and 23% concentrates) alone or in a 50:50 ratio (dry basis) with waste-straw silage containing waste treated as described above. In the small silo study, inclusion of inoculant alone to the untreated and sodium metabisulfite treated waste produced silages with characteristics pungent smell. Molasses alone or with inoculant improved the fermentation, as indicated by a decrease ($P < .05$) in pH, and increase ($P < .01$) in lactic acid. In the large silos, control silage had higher ($P < .01$) volatile fatty acids (VFA) than the treated waste silages. The formaldehyde silage showed a stable ($P < .01$) pH, and lower ($P < .01$) concentration of lactic acid. Among the waste silages, apparent digestibility of crude protein was higher ($P < .01$) for the molasses-treated waste silages than the control silage. Dry matter digestibility, calculated by difference, tended to be higher for molasses treated waste silages. Nitrogen retention of lambs fed the molasses-treated waste silages was higher ($P < .01$), compared to the basal diet.

(Key words: Silage Additives, Preservatives, Ensiling, Caged Layer Waste, Digestibility).

INTRODUCTION

Caged layer waste is high in crude protein (Bhattacharya and Taylor, 1975), organic matter and energy (Lowman and Knight 1970). Satisfactory results have been obtained from feeding dehydrated caged layer waste to ruminants (Bhattacharya and Taylor, 1975). However, the cost in dehydrating the waste is a deterrent to the economic feasibility of using caged layer waste as a feedstuff.

Caged layer waste has been ensiled successfully with crop residues (Fontenot, 1984). However, to produce satisfactory silage it is essential that the waste be collected at frequent intervals for storage. Some success was achieved in preserving caged layer waste with certain additives (Chapter III).

Studies were designed to investigate the fermentation characteristics, nutrient utilization and digestibility by sheep of ensiled preserved waste-wheat straw mixtures.

EXPERIMENTAL PROCEDURE

Small Silo Study.

The caged layer waste¹ had been preserved with 1.0% formaldehyde, 1.5% sodium metabisulfite, 1.5% propionic/formic (1:1,w/w), 10% dry molasses and 10% dry molasses with 2.%

¹ Obtained from Green Valley Poultry Farm, Abingdon, Virginia.

sodium chloride (salt). The procedure used for the preservation study was presented earlier (chapter III). Briefly, the waste was obtained within 24 h of excretion, mixed with the preservatives and stored in polyethylene lined 210 liter metal drums for 42 d. After 42 d of preservation, the wastes were ensiled with wheat straw (60:40, wet basis) 1) alone, and with 2) 10% dry molasses, 3) .1% silage inoculant^{2 1} (wet basis), or 4) combination of 10% molasses plus .1% inoculant (wet basis). The wheat straw used was ground in a hammer mill through a 2.5 cm screen. Six laboratory silos per treatment, each containing about 2 kg of mixture, were prepared by firmly packing the mixtures in 3.8 liter cardboard containers, double lined with polyethylene bags. Each bag was individually sealed after expelling air. Samples of initial mixtures were taken for analyses.

At the end of 42 d the silos were opened, and the visible appearance and odor were examined. The top 5 cm of the silage were removed and samples were taken from the center. Water extracts of the pre-and post- ensiled samples were prepared by homogenizing 25 g sample with 225 ml of distilled water in .5 liter jar in a Waring blender at full speed for 2 min. The homogenate was filtered through four layers of cheese cloth and the extract was used for deter-

^{1 2} Obtained from Pioneer Hi-Bred International, Inc., Des Moines, IA.

mining pH (electrometrically), lactic acid (Barker and Summerson, 1941, as modified by Pennington and Sutherland, 1956) and water soluble carbohydrates (WSC) (Dubois et al., 1956, as adapted to corn plants by Johnson et al., 1966). In addition, samples were aseptically taken from the center of the silage and with the use of sterilized materials, and the extracts obtained were subjected to quantitative tests for total coliforms (Anonymous, 1967) and fecal coliforms (Millipore, 1973).

Kjeldahl N was determined on wet samples (A.O.A.C., 1980). Dry matter was determined by drying in a forced draft oven at a maximum of 60 C for 48 h. The dried samples were ground in a Wiley mill and subsamples were taken for determination of ash (A.O.A.C., 1980).

Large Silo Study.

The caged layer waste was preserved with no additive, 1% formaldehyde, 10% dry molasses, 10% dry molasses with 2% sodium chloride (salt). Procedures for preservation were as described in chapter 111.

The wheat straw was ground in a hammer mill through a 2.5 cm screen. Waste, straw and dry molasses (55:35:10, wet basis) were mixed in the horizontal mixer and augered into 210 liter metal drums double lined with .08 mm polyethylene

bags. Initial samples were taken, composited, subsampled and frozen for subsequent analyses. The mixtures were packed by trampling and the bags were individually sealed. All silos were allowed to ensiled for a minimum of 42 d. When silos were opened, the top 5 cm of the silages were removed. Sterilized materials were used to obtain samples from different areas of the silos. Samples were prepared for extracts which were used for total and fecal coliforms, pH, water soluble carbohydrates and lactic acid determination as previously described. Some of the dried samples were ground in a Willey mill and subsamples were taken for determination of ash (A.O.A.C., 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). The remaining samples were frozen for subsequent analyses.

Metabolism Trial.

Thirty crossbred (Rambouillet*Suffolk*Hampshire) wethers with an average weight of 31.8 kg were assigned to six blocks of five animals each based on the weight and origin. Sheep within each block were randomly allotted to the following diets: 1) basal (76.9% wheat straw, 9.9% corn, 7.6% soybean meal, 1.4% urea and 4.2% liquid molasses) alone or in a

50:50 ratio (dry basis) with waste-straw silage containing waste treated with the following preservatives: 2) no additive, 3) formaldehyde, 4) dry molasses and 5) dry molasses plus sodium chloride . The supplemental urea and soybean meal were calculated to represent 50% each of non-protein and true protein supplemented N, the approximate ratio in poultry waste (Liebholz et al., 1969). All animals were treated for internal parasites and 500,000 I.U. of vitamin A and 75,000 I.U. of vitamin D were injected intramuscularly.

The sheep were placed in metabolism stalls similar to those described by Briggs and Gallup (1949) which allow for separate collection of urine and feces. The diet was fed twice daily, one half at 0400 h and other half at 1600 h. Lambs were fed 640 g of dry matter per day before the test diets were introduced. A 5-d adaptation period to the stalls was followed by a 10-d transition to the experimental diets. Test diets were fed for a 10-d preliminary period followed by a 10-d collection period. Water was provided except at feeding.

Samples of feeds were obtained at each feeding 2 d prior to the beginning and 2 d prior to the end of the collection period. The silage samples were frozen daily in double thickness plastic bags and composited at the end of the trial. Refusals for each feeding period were collected sepa-

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

Samples of the diet components, refusals and feces were analysed for dry matter, ash and cell wall components. Nitrogen was determined on wet feed ingredients, the dry samples and urine. The methods of analyses were same as described for the small silo study. The rumen fluid was strained through four layers of cheese cloth. The filtrate was used for pH and ruminal ammonia-N (Beecher and Whitten, 1970) and volatile fatty acid (Erwin et al., 1961) determi-

nation. Volatile fatty acids were determined with a Vista 6000 gas chromatograph. Blood urea nitrogen was determined by the method of Coulombe and Favreau, (1963).

Statistical Analyses.

The data were tested by analyses of variance by the general linear model procedure of SAS (1982). In the small silo study, the following orthogonal contrasts were made: 1) untreated vs treated waste silages, 2) chemical vs molasses treated waste 3) formaldehyde vs sodium metabisulfite and propionic/formic, 4) sodium metabisulfite vs propionic/formic silages and 5) molasses vs molasses plus sodium chloride. The effect of silage additives on fermentation was analyzed as follows: 1) no additives vs additives, 2) molasses, 3) inoculant, and 4) molasses * inoculant interaction. In the large silo study contrasts were: 1) untreated vs treated waste, 2) formaldehyde vs molasses treated waste and 3) molasses vs molasses plus salt treated waste silages. In the metabolism trial contrasts were: 1) basal vs silages, 2) untreated vs treated waste silages, 3) formaldehyde treated waste silage vs molasses treated waste silage and 4) molasses vs molasses plus sodium chloride treated waste silage.

RESULTS AND DISCUSSION.

Small Silo Study.

A dark color and pungent smell were observed for silages made from sodium metabisulfite and propionic/formic treated, and untreated wastes. The top surfaces of formaldehyde silages were covered with molds. The effect of the preservatives on composition and fermentation characteristics of the ensiled mixtures is presented in table 4. The dry matter content of the initial silages reflected the dry matter content of the waste (chapter III). Crude protein varied among silages. The main difference was the lower value for the molasses-treated waste silages, which reflected lower protein of the waste (chapter III). The reduction in pH and WSC indicated that fermentation had occurred in all silages. The molasses treated waste silages had lower ($P<.01$) pH values of 4.7 and 4.8, respectively, compared to the untreated (5.1) and formaldehyde (6.4)- treated waste silages. Values for pH were higher for the silage made with formaldehyde-treated waste, compared to silages made with waste treated with sodium metabisulfite and propionic/formic acid. Lactic acid increased after ensiling for all silages, except the formaldehyde- treated waste silages. Highest values were for silages made with molasses- treated waste.

TABLE 4. EFFECTS OF PRESERVATIVES ON COMPOSITION AND FERMENTATION
CHARACTERISTICS OF CAGED LAYER WASTE-WHEAT STRAW MIXTURES^a
(SMALL SILO STUDY)

Item	Waste preservatives					
	None	1.5% sodium bisulfite	1.5% prop/ formic	1% formal- dehyde	10% dry molasses	10% dry mol. + 2% salt
Dry matter, %						
Initial ^{b,c,d,e}	52.55	55.85	55.88	56.45	57.61	59.32
Final ^{b,c}	50.43	53.66	52.03	54.44	56.29	57.19
Crude protein, %						
Initial ^c	11.52	11.43	11.78	11.76	10.78	10.63
Final ^{b,c,d}	13.09	12.93	13.90	12.63	11.80	11.61
pH						
Initial ^{b,c,d,e,f}	8.00	8.01	7.98	7.25	6.16	5.92
Final ^{b,c,d}	5.06	5.04	5.02	6.40	4.74	4.80
Lactic acid						
Initial ^{b,c}	.02	.38	.43	.34	2.57	2.61
Final ^{b,c,d,f,h}	1.23	2.69	1.48	.01	4.27	4.74
Water-soluble carbohydrates, %						
Initial ^{c,f,g}	6.06	6.50	5.66	6.42	5.93	5.60
Final ^{b,c,f}	2.90	3.42	2.39	4.15	3.96	3.72

^aCaged layer waste, wheat straw and molasses (55:35:10, wet basis).

^bNone vs treated waste silages (P<.01).

^cChemical vs molasses treated waste silages (P<.01).

^dFormaldehyde vs sodium bisulfite and propionic/formic (P<.01).

^eMolasses vs molasses + salt (P<.01).

^fSodium metabisulfite vs propionic/formic (P<.01).

^gFormaldehyde vs sodium bisulfite and propionic/formic (P<.05).

^hMolasses vs molasses + salt (P<.05).

A distinct sweet smell was detected in all silages with molasses added at time of ensiling. Initially, pH was similar for all mixtures (table 5). After ensiling, the silages containing molasses alone or with inoculant showed a rapid reduction in pH from 7.17 and 7.20 to 4.95 and 4.94, respectively. Final pH values differed ($P < .01$), from the values for the control silage and the silage treated only with inoculant.

The lactic acid contents were similar for all pre-ensiled mixtures. The levels in these mixtures may be attributed to the residual lactic acid produced when the wastes were stored for 42 d. Lactic acid levels were higher ($P < .01$) in silages containing molasses alone or with inoculant. The levels did not change in silages containing the inoculant, or in the control silage. Initial water soluble carbohydrates were higher ($P < .01$), for mixtures containing molasses and molasses plus inoculant. However, the residual WSC showed that 52.5 and 57.4% of the available carbohydrates in the two treatments had been converted to acids. This is reflected in the lower pH observed in these silages. Silages with no additive, and with inoculant alone had only slight reductions in WSC levels.

Acetic acid was the main VFA in all silages (table 6). The lowest ($P < .01$) levels of acetic acid were observed in

TABLE 5. EFFECTS OF SILAGE ADDITIVES ON COMPOSITION AND FERMENTATION CHARACTERISTICS OF PRESERVED WASTE-WHEAT STRAW MIXTURES (SMALL SILO STUDY)

Item	Silage additives ^a			SE ^b
	None	Dry molasses + inoculant	Dry molasses Inoculant	
Dry matter, % ^c				
Pre-ensiled ^{d, e}	54.96	57.73	58.37	.68
Post-ensiled ^f	52.49	55.03	55.65	.81
Crude protein, % ^c				
Pre-ensiled ^{d, e}	11.40	11.09	10.65	.18
Post-ensiled ^{e, g}	13.01	12.27	12.16	.27
pH				
Pre-ensiled	7.26	7.17	7.20	.27
Post-ensiled ^f	5.44	4.95	4.94	.14
Lactic acid, % ^c				
Pre-ensiled	1.24	1.07	.93	.34
Post-ensiled ^{d, e}	.82	3.93	3.87	.47
Water-soluble carbohydrates, % ^c				
Pre-ensiled ^{d, e}	3.18	8.71	8.61	.16
Post-ensiled	2.57	4.14	3.67	.25

^aThe molasses and inoculant added at 10 and .01% (w/w) respectively.

^bStandard error of means.

^cDry basis.

^dControl vs inoculant, molasses and molasses + inoculant (P<.01).

^eInoculant vs molasses and molasses + inoculant differ (P<.01).

^fInoculant vs molasses and molasses + inoculant differ (P<.05).

^gControl vs inoculant, molasses and molasses + inoculant (P<.05).

TABLE 6. EFFECTS OF SILAGE ADDITIVES ON VOLATILE FATTY ACIDS OF PRESERVED WASTE-WHEAT STRAW MIXTURES (SMALL SILO STUDY)

Volatile fatty acid ^b	Silage additives ^a				SE ^c
	None	Dry molasses	Dry molasses + inoculant	Inoculant	
	-----%-----				
Acetic ^{d,e}	3.34	2.58	2.37	3.49	.21
Propionic	.82	.68	.70	.82	.08
Isobutyric	.04	.05	.05	.04	.01
Butyric	.50	.42	.44	.53	.05
Isovaleric ^{f,g}	.05	.04	.04	.06	.01
Valeric	.01	.00	.00	.00	.00

^aThe molasses and inoculant added at 10 and .01% (w/w) respectively.

^bDry basis.

^cStandard error of means.

^dControl vs inoculant, molasses and molasses + inoculant (P<.01).

^eInoculant vs molasses and molasses + inoculant differ (P<.01).

^fControl vs inoculant, molasses and molasses + inoculant (P<.05).

^gInoculant vs molasses and molasses + inoculant differ (P<.05).

silages containing molasses. The lower level of total VFA in silages containing molasses suggested that most of the available WSC were fermented into lactic acid. Total and fecal coliforms were absent in all silages containing treated wastes. Silages containing untreated waste showed insignificant counts of total coliforms.

Large Silo Study.

At the end of 42 d distinctive sweet smell was observed in silages made from formaldehyde, molasses and molasses plus salt treated wastes. The control silages had dark color and a noticable pungent smell.

The initial pH of the mixtures containing the untreated and formaldehyde treated wastes (7.75 and 7.52 respectively) differed ($P < .01$) from the values of 6.64 and 6.54 for the silages containing molasses and molasses plus salt treated waste (table 7). After ensiling, pH of the silages containing the formaldehyde treated waste remained stable (7.52 vs 7.21), indicating limited fermentation. Valentine and Brown (1973) showed that the pH of formaldehyde-treated alfalfa silage was stable, indicating that formaldehyde acts as a sterilant, reducing bacterial fermentation. In the present study the pH of the silage made with untreated waste and molasses-treated waste decreased, indicating that fermentation had occurred.

Initially, no lactic acid was detected in control silage but molasses and molasses plus salt treated waste silages contained 1.38 and 1.64% lactic acid, dry basis, respectively. Silages containing molasses and molasses plus salt treated waste contained 8.86 and 8.32% lactic acid, which differ ($P < .01$) from the value of .71%, dry basis, observed with the formaldehyde-treated waste silage. A decrease in WSC was noted for all silages after ensiling for 42 d. The large decrease for the silage made with formaldehyde-treated waste cannot be explained on the basis of lactic acid production.

The main VFA in the silages was acetic acid (table 8). Highest ($P < .01$) levels of acetic and propionic acid were observed in control silage. The lowest level of acetic acid (1.81%) was observed with the formaldehyde treated-waste silage. Ohyama et al. (1975) reported that abnormal silages usually contain large amounts of total VFA, especially propionic and butyric acid, with little or no lactic acid. In the present study, the high residual WSC, stable pH, and low lactic acid and VFA suggest that fermentation was limited in silages containing formaldehyde-treated waste. Test for total and fecal coliforms were negative in all silages made with preserved wastes.

TABLE 7. FERMENTATION CHARACTERISTIC OF THE SILAGES^a USED
IN LARGE SILO STUDY

Item	Waste preservatives				SE ^b
	None	Formaldehyde	Dry molasses	Dry mol + salt	
pH					
Pre-ensiled ^{c,d}	7.75	7.52	6.64	6.54	.01
Post-ensiled ^{c,d,e}	5.15	7.21	4.91	4.85	.02
Lactic acid, % ^g					
Pre-ensiled ^d	0.00	0.26	1.38	1.64	.05
Post-ensiled ^{c,d,e}	5.37	0.71	8.86	8.32	.33
Water-soluble carbohydrates, % ^g					
Pre-ensiled ^{c,d}	10.41	10.72	9.82	9.05	.22
Post-ensiled ^{d,f}	3.46	5.70	4.03	4.21	.21

^aCaged layer waste, wheat straw and molasses (55:35:10, wet basis).^bStandard error of means.^cNone vs treated waste silages differ (P<.01).^dFormaldehyde vs molasses silages differ (P<.01).^eMolasses vs molasses + salt silages differ (P<.01).^fMolasses vs molasses + salt silages differ (P<.05).^gDry basis.

TABLE 8. EFFECTS OF WASTE PRESERVATION OF SILAGES^a
ON VOLATILE FATTY ACID (LARGE SILO STUDY)

Volatile fatty acid	Waste preservatives				SE ^b
	None	Formaldehyde	Dry molasses	Dry mol + salt	
	-----% ^c -----				
Acetic ^{d,e,f}	3.59	1.81	2.66	2.11	.08
Propionic ^d	1.15	.63	.51	.80	.13
Isobutyric ^d	.06	.03	.02	.02	.01
Butyric ^{d,e,f}	.60	.32	1.01	.91	.02
Isovaleric ^d	.10	.04	.05	.03	.01
Valeric ^{e,g}	.02	.01	.04	.02	.00

^aCaged layer waste, wheat straw and molasses (55:35:10, wet basis).

^bStandard error of means.

^cDry basis.

^dNone vs treated waste silages differ ($P < .01$).

^eFormaldehyde vs molasses silages differ ($P < .01$).

^fMolasses vs molasses + salt silages differ ($P < .01$).

^gMolasses vs molasses + salt silages differ ($P < .05$).

Chemical Composition.

The dry matter content varied from 54 to 56% for ensiled mixtures (table 9 and 10). The average crude protein and ash contents were higher for the control and formaldehyde treated waste silages.

The preservatives used in storing the caged layer waste prior to ensiling did not consistently alter the cell wall composition of the silages. The basal diet was similar in composition to the waste-straw silages, except for lower ash content (table 10).

Apparent Digestibility.

Apparent digestibility of dry matter, organic matter, crude protein and cell wall components was higher ($P < .01$) for the basal diet (table 11). The digestibility of crude protein was higher ($P < .01$) for the diet containing silages made from waste treated with molasses with and without salt, than for the diet containing silage made from waste treated with formaldehyde. Digestibility of most components was lower ($P < .05$) for the control silage, compared to silages made with treated waste. These results may indicate that some of the more easily degraded components had been degraded during storage of the untreated waste.

TABLE 9. COMPOSITION^a OF INITIAL MIXTURES OF ENSILED CAGED LAYER WASTE AND WHEAT STRAW^b (LARGE SILO STUDY)

Component	Waste preservatives				SE ^c
	None	Formaldehyde	Dry	Dry	
			molasses	mol + salt	
-----%-----					
Dry matter ^{d,e,f}	54.44	56.01	54.40	55.83	.07
Crude protein ^{d,g}	13.71	13.15	12.42	11.44	.25
Ash ^{d,e}	13.44	12.68	10.87	11.51	.22
Neutral					
detergent fiber ^{d,e}	62.05	61.63	60.12	59.53	.29
Acid detergent fiber	37.18	36.29	37.09	37.07	.32
Cellulose ^{d,e,g}	27.91	27.76	28.68	29.23	.16
Hemicellulose	24.87	25.34	23.03	22.46	
Lignin	7.49	7.26	7.15	7.48	.29

^aDry basis.

^bCaged layer waste, wheat straw and molasses (55:35:10, wet basis).

^cStandard error of means.

^dNone vs treated waste mixture differ ($P < .01$).

^eFormaldehyde vs molasses treated waste mixture ($P < .01$).

^fMolasses vs molasses + salt treated waste mixture ($P < .01$).

^gMolasses vs molasses + salt treated waste mixture ($P < .05$).

TABLE 10. COMPOSITION OF BASAL DIET AND SILAGES^a
FED IN SHEEP METABOLISM TRIALS.

Component	Basal diet	Waste preservatives				SE ^b
		None	Formaldehyde	Dry molasses	Dry mol+salt	
		-----%-----				
Dry matter ^{e, f}	80.66	56.54	55.98	56.98	57.75	.27
Crude protein ^{d, h, i}	12.04	13.42	13.96	12.52	11.38	.13
Ash ^{e, g}	5.15	12.91	12.79	11.47	12.10	.22
Neutral						
detergent fiber ^d	65.67	60.91	64.29	61.00	60.73	.57
Acid detergent fiber	40.33	36.27	38.58	37.04	37.62	.78
Cellulose	33.25	28.67	29.94	29.07	29.51	.52
Hemicellulose	25.34	24.64	25.95	23.96	23.11	
Lignin	5.91	6.64	6.92	6.38	6.75	.21

^aCaged layer waste, wheat straw and molasses (55:35:10, wet basis).

^bStandard error of means.

^cDry basis.

^dFormaldehyde vs molasses silages differ ($P < .01$).

^eFormaldehyde vs molasses silages differ ($P < .05$).

^fMolasses vs molasses + salt silages differ ($P < .05$).

^gNone vs treated waste silages differ ($P < .05$).

^hNone vs treated waste silages differ ($P < .01$).

ⁱMolasses vs molasses + salt silages differ ($P < .05$).

TABLE 11. APPARENT DIGESTIBILITY OF PROXIMATE COMPONENTS AND CELL WALL FRACTIONS BY SHEEP FED BASAL DIET AND CAGED LAYER WASTE-WHEAT STRAW SILAGES

Component	Basal diet and silages ^{a, b}					SE ^d
	Basal	None ^c	Formal-	Dry ^c	Dry mol.	
	diet		dehyde ^c	molasses	+ salt ^c	
-----%-----						
Dry matter ^{e, f}	53.45	42.11	44.39	48.75	46.04	1.58
Organic matter ^{e, f}	54.52	43.58	46.40	49.93	47.12	1.53
Crude protein ^{e, g, h}	64.95	53.53	58.38	64.26	63.28	1.52
Neutral detergent ^{e, f, i}	51.72	41.82	48.05	48.85	43.37	1.84
Acid detergent ^{e, f}	48.12	37.93	43.61	46.13	40.45	2.01
Cellulose ^{i, j}	58.90	49.50	54.98	56.86	49.88	1.89
Hemicellulose ^j	57.45	47.99	55.11	53.50	48.35	1.86
Lignin ^{f, h, j}	-2.05	-8.45	-8.21	8.00	6.78	3.54

^aBasal plus silages (1:1, dry basis).

^bCaged layer wastes, wheat straw, and molasses (55:35:10, wet basis).

^cPreservatives used to treat waste.

^dStandard error of means.

^eBasal vs silages differ (P<.01).

^fControl vs treated waste silages differ (P<.05).

^gControl vs treated waste silages differ (P<.01).

^hFormaldehyde vs molasses silages differ (P<.01).

ⁱMolasses vs molasses plus salt differ (P<.05).

^jBasal vs silages differ (P<.05).

TABLE 12. APPARENT DIGESTIBILITY^a OF CAGED LAYER WASTE-WHEAT STRAW SILAGES^b BY SHEEP

Component	Waste preservatives				SE ^c
	None	Formaldehyde	Dry	Dry mol.	
			molasses	+ salt	
-----%					
Dry matter	33.31	36.01	44.42	39.39	2.9
Organic matter ^d	33.56	38.90	45.71	40.48	2.7
Crude protein ^{e, f}	43.08	52.31	63.63	61.79	3.2
Neutral detergent fiber ^{d, h}	32.75	44.66	46.21	35.88	3.3
Acid detergent fiber ^{d, h}	28.61	39.44	44.29	33.57	3.5
Cellulose ^{d, h}	40.89	51.36	54.99	41.79	3.3
Hemicellulose	39.32	52.94	49.87	40.19	3.6
Lignin ^{d, g}	-15.01	-14.60	16.56	13.28	6.9

^aCalculated by difference (Crampton and Harris, 1969).

^bCaged layer waste, wheat straw and molasses (55:35:10, wet basis).

^cStandard error of means.

^dNone vs treated waste silages differ (P<.05).

^eNone vs treated waste silages differ (P<.01).

^fFormaldehyde vs molasses silages differ (P<.05).

^gFormaldehyde vs molasses silages differ (P<.01).

^hMolasses vs molasses plus salt silages differ (P<.05).

The digestibility of caged layer waste-wheat straw mixtures, calculated by difference (Crampton and Harris, 1969) is presented in table 12. Digestibilities of organic matter, crude protein, NDF, ADF, cellulose and lignin were lower ($P < .05$) for the silage made from untreated waste, compared to treated wastes. Silages containing molasses and molasses plus salt treated wastes had higher ($P < .05$) crude protein digestibility, compared to silage containing formaldehyde-treated waste. The digestibility of hemicellulose (52.94%) was numerically higher for silage made from formaldehyde treated waste, compared to other silages.

Nitrogen Utilization.

Nitrogen intake (table 13) was lower for the diets containing the silage made with untreated waste, attributed to the degradation of the waste. Generally, fecal N followed similar trends as N intake; an exception was the relatively high fecal excretion for sheep fed silage made with untreated waste. Urinary-N was higher for the sheep fed the basal diet, compared to those fed the diet with ensiled caged layer waste. Total N excretion was higher for sheep receiving silage with formaldehyde-treated waste than for those fed silages with the molasses-treated waste, resulting in higher N retention for sheep fed the molasses-treated waste silage.

TABLE 13. NITROGEN UTILIZATION BY SHEEP FED BASAL AND CAGED LAYER WASTE-WHEAT STRAW SILAGES

Component	Basal diet and silages ^a (1:1,dry basis)					SE ^c
	Basal diet	None ^b	Formaldehyde ^b	Dry ^b molasses	Dry mol. +salt ^b	
Nitrogen intake ^{c,d} g/d	10.81	8.65	11.52	11.94	10.88	.50
Nitrogen excretion g/d						
Fecal ^{e,f}	3.79	3.96	4.77	4.26	3.97	.15
Urinary	6.27	5.70	5.37	5.02	4.85	.37
Total	10.06	9.66	10.14	9.28	8.82	
Nitrogen retention						
Grams per day ^{c,d}	.75	-1.00	1.37	2.65	2.07	.44
Percent of intake ^{c,d}	6.98	-12.90	11.83	21.92	17.73	4.26
Percent of absorbed ^{cd}	10.81	-28.32	20.18	34.14	27.65	8.74

^aCaged layer waste, wheat straw and molasses (55:35:10, wet basis).

^bPreservative used to treat waste.

^cBasal vs silages differ (P<.01).

^dNone vs treated waste silages differ (P<.01).

^eNone vs treated waste silages differ (P<.05).

^fFormaldehyde vs molasses silages differ (P<.01).

The higher N retention may have been due to the presence of higher levels of readily-available carbohydrates in the molasses- treated waste silages (Stone and Fontenot, 1965).

Ruminal and Blood Parameters.

Ruminal pH (table 14) was similar for sheep fed the basal and silage diets. The ruminal fluid NH_3 and blood urea-N tended to be higher for sheep receiving the basal diet, compared to those fed silage. Among the silages containing the treated wastes, the total ruminal VFA was similar among the sheep fed the different diets (table 15). Ruminal fluid propionic acid was higher ($P < .01$) for the molasses treated waste silage diets than for the formaldehyde-treated waste silage diet. Covey et al. (1977) showed that glucose and formaldehyde formed a highly resistance complex which is not degradable in the rumen or abomasum. Barry (1976) reported a low concentration of VFA in the ruminal fluid of sheep fed formaldehyde treated lucerne hay.

This study shows that inclusion of silage additive (molasses) may improved the fermentation characteristics of waste-straw silages. Higher digestibilities of N and cell wall components for silages made with molasses treated waste may suggest benefits of readily available carbohydrates in the silage.

TABLE 14. RUMINAL FLUID pH, AMMONIA NITROGEN AND BLOOD UREA NITROGEN OF SHEEP FED A BASAL AND CAGED LAYER WASTE-WHEAT STRAW SILAGES

Item	Basal diet and silages ^a (1:1, dry basis)					SE ^c
	Basal diet	None ^b	Formaldehyde ^b	Dry ^b molasses	Dry mol. ^b + salt	
Ruminal fluid pH	6.68	6.85	6.74	6.74	7.04	.11
Ruminal fluid NH ₃ -N, mg/dl	22.19	10.41	11.57	14.17	11.54	2.26
Blood urea-N, mg/dl	23.75	18.74	20.47	18.31	19.23	1.12

^aCaged layer waste, wheat straw and molasses (55:35:10, wet basis).

^bPreservatives used to treat waste.

^cStandard error of means.

TABLE 15. RUMINAL FLUID VOLATILE FATTY ACID CONCENTRATION OF SHEEP

	Basal diet and silages ^a (1:1,dry basis)					SE ^c
	Basal diet	None ^b	Formal- dehyde ^b	Dry molasses ^b	Dry mol. +salt ^b	
Volatile fatty acids						
Total, umole/ml	77.20	77.63	75.85	79.75	75.79	5.58
Moles/100moles						
Acetic ^d	71.00	69.07	71.23	67.18	66.58	.38
Propionic ^{d,e,f}	19.65	20.50	13.97	23.00	22.84	.15
Isobutyric ^{e,g,h}	.64	.59	.66	.58	.63	.01
Butyric ^{d,f,h}	7.51	7.97	7.81	8.32	8.70	.09
Isovaleric ^{d,e,f}	.75	.59	.76	.54	.73	.02
Valeric ^{d,e,f,h}	.57	.65	.71	.56	.55	.01

^aCaged layer waste, wheat straw and molasses (55:35:10, wet basis).

^bPreservatives.

^cStandard error of means.

^dFormaldehyde vs molasses treated waste silages (P<.01).

^eBasal vs silages (P<.05).

^fUntreated waste vs molasses treated waste silages (P<.05).

^gFormaldehyde vs molasses treated waste silages (P<.05).

^hMolasses vs molasses plus salt (P<.05).

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Chapter V

GENERAL DISCUSSION

In the present studies, the average crude protein content was 34%. This value was higher than the value (28%) reported by Liebholz (1969). Smith et al. (1970) reported that caged layer waste contained high energy value. However, this value may be lowered by the ash content in the manure. In our studies, the average ash value obtained was 31%, compared to the lower value of 28% reported by Bhattacharya and Taylor (1975). Supplemental Ca and P may not be necessary when caged layer waste is fed to ruminants due to the presence of these minerals (8.8 and 2.5%, respectively) (Bhattacharya and Taylor, 1975).

Smith and Calvert (1972) reported a higher (20%) N retention when 45% of dried poultry excreta was fed to sheep. Smith et al. (1976) found with sheep a 10 to 58% increase in nitrogen digestibility with diets containing 19 and 38% dehydrated broiler excreta. Smith and Wheeler (1979) reported average daily gains of 1.10 vs 1.07 kg with cattle receiving dehydrated caged layer waste and control diets. No negative effects were shown in body weights and milk yields when dairy cattle were fed diets containing 10% dehydrated caged layer waste (Cullison et al., 1976).

Feeding of caged layer waste has not consistently affected the quality of the animal products. Caged layer waste do not contain detectable levels of Cu and drugs commonly found in broiler litter, however, the presence of pathogens may constitute a danger. Chemical treatments of caged layer waste prior to feeding may reduce the pathogens (Evans et al., 1978). In these studies, the tests for fecal and total coliforms were negative, perhaps, due to the use of chemicals as preservative and the ensiling process that followed.

Different processing methods had been reported, however, most of the methods are not economically feasible. Dehydration of caged layer waste has been one of the common method, this method is expensive and usually results in loss of nutrients. Loss of N, organic matter and increase in ash content had been reported (Silva et al., 1976; El-Sabban et al., 1970). Ensiling appears to be the most economical processing method. Caged layer waste and crop residues had been ensiled successfully (Samuels, 1980). However, frequent collection and ensiling of the waste is needed in order to prevent the loss of nutrients.

In our laboratory, we achieved some success in the preservation of fresh caged layer waste for minimum of 42 d prior to ensiling. Among the preservatives used, propionic/formic acid was effective against mold and yeast growth for

up to 35 d. Dry sugarcane molasses and formaldehyde prevented any visible deterioration during the storage period. These preservatives were effective against pathogens and coliforms. These studies showed that caged layer waste can be stored for weeks with minimum nutrients loss. The chemicals and molasses used are less expensive, compared to other processing methods. The formaldehyde and formic acid may be cheaper than molasses especially when 85% of molasses added as preservative was fermented to acids. Using molasses as preservative may be more affordable in developing countries where molasses can be produce cheaper than chemical additives.

Following the preservation, the stored waste, wheat straw and molasses fermented satisfactorily. The best silages were obtained from the wastes that were successfully preserved, but the silages made with untreated waste had a characteristics pungent smell indicating undesirable fermentation. In the metabolism trial, higher digestibilities of N and cell wall components were for silages made with molasses treated waste.

Currently, the use of formaldehyde is not approved due to its carcinogenic effect, but future research can be directed towards determining the effective and safe level for the use of formaldehyde in preserving caged layer waste. The use of

dry molasses at levels lesser than 10% and the addition of sodium chloride (salt) as the only preservative should be considered. The appropriate combination of molasses, propionic with and without formic acid also need further investigation.

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METHODS OF PRESERVING AND ENHANCING FERMENTATION AND
NUTRITIONAL
VALUE OF CAGED LAYER WASTE-WHEAT STRAW SILAGES FED TO SHEEP

by

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Animal Science

(ABSTRACT)

Three experiments were conducted with fresh caged layer waste collected within 24 h after excretion. The waste was stored uncovered for 42 d in polyethylene lined 210 liter metal drums. In the first experiment, waste was treated with no additive, 1 and 1.5% formaldehyde, 1.5 and 2% sodium metabisulfite and 1.5 and 2% propionic/formic acid (1:1, w/w). In the second experiment, the additives used were: none, 1% formaldehyde, 1.5% sodium metabisulfite, 1.5% propionic/formic (1:1, w/w), 10% dry sugar cane molasses and 10% dry molasses plus 2% sodium chloride (salt). In this experiment, the wastes were ensiled with wheat straw (60:40, wet basis) in 4-liter cardboard containers double lined with polyethylene with the following additives: control, 10% dry molasses, silage inoculant or 10% dry molasses plus inoculant.

In the third study, the preservatives were: untreated, 1% formaldehyde, 10% dry molasses or 10% dry molasses and 2% salt. After 42 d, treated wastes and straw (60:40) were ensiled with 10% dry molasses in 210 liter metal drums doubled lined with polyethylene. A metabolism trial was conducted with 30 crossbred wethers fed a basal diet alone or with silages containing the wastes which had been treated with the preservatives (1:1, dry basis).

In all studies, putrefaction, maggot infestation and dark color were observed for untreated waste. The tops of the wastes treated with sodium metabisulfite and formaldehyde were covered with mold. Formaldehyde-treated waste maintained a stable pH, and water-soluble carbohydrate level. The higher level of propionic/formic acid was effective against visible deterioration.

The pH of the the silages containing molasses-treated waste was lower than for silages containing control or chemically-treated wastes. Adding molasses at ensiling reduced pH and increased lactic acid. Digestibilities of organic matter, crude protein and neutral detergent fiber were lower ($P < .05$) for the diet containing silage made from untreated waste, compared to diets containing silage made from treated waste. In all studies, putrefaction, maggot infestation and dark color were observed for untreated waste. The tops of

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