

THE EFFECT OF PROCESSING METHOD OF BROILER LITTER
ON PASTEURIZATION, NITROGEN LOSS AND NITROGEN
UTILIZATION BY SHEEP

by

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INTRODUCTION

In the past decade broiler litter has been shown to be of potential economic importance as a feedstuff. When incorporated into ruminant rations, in addition to supplying nitrogen, it contributes an appreciable amount of available energy. Uric acid, which represents a substantial portion of the total nitrogen present in litter, is a satisfactory nitrogen source for rumen microorganisms.

Presently, the use of poultry litter as an animal feed is not sanctioned by the Food and Drug Administration due to apprehension concerning potential hazards to human and animal health from various drugs and pathogenic organisms in poultry litter. Several diseases exist in poultry that may possibly be transmitted to cattle, sheep, swine and man through the use of litter as a feed or feed constituent. No health problems have been reported, however, from feeding litter to cattle. Satisfactory daily gains and feed efficiency have been reported in fattening beef cattle when fed low levels of broiler litter, as compared to a conventional source of nitrogen such as soybean meal.

When considering the present position of animal wastes in the feeding of livestock, it becomes apparent

that broiler litter may never gain much impetus in the feed industry until methods are developed to alleviate potential hazards in litter.

Experiments were conducted to develop processing methods suitable for the destruction of pathogens in broiler litter. In all of the methods examined, pasteurization of the litter was the goal. The effect of pasteurization upon the nitrogen components of litter and upon the utilization of broiler litter by sheep were also investigated.

REVIEW OF LITERATURE

Presently the use of poultry litter as an animal feed is not sanctioned by the Food and Drug Administration due to apprehension concerning potential hazards to human and animal health from various drugs and pathogenic organisms in poultry litter as was emphasized by Kirk (1967).

Potential Infectious Microorganisms in Poultry Litter

Coliform bacteria are facultative anaerobes and show optimal growth at 37 C (Burrows, 1968). Since these bacteria thrive in the presence of bile they are numerous in the intestinal tract. According to Burrows (1968) coliforms are responsible for a number of disorders in animals, including cholera, accumulation of intraluminal fluid, cystitis of the urogenital tract, local infections such as abscesses and conjunctivitis, scours in young animals and gallstones since they possess the ability to precipitate cholesterol and other biliary constituents. Occasionally mastitis has been found to be due to certain coliform bacteria. Coliforms are gram-negative and do not form spores, facilitating ease of destruction. Most strains are killed by exposure to heat at 60 C for 30 min.

Pelczar and Reid (1965) list the classical species

of the coliform group as Escherichia coli and Aerobacter aerogenes. Salmonella, Shigella and Proteus spp also are included in the family Enterobacteriaceae (Burrows, 1968). These organisms are responsible for a wide variety of disorders including infections of the bones, central nervous system and other local infections, gastroenteritis and occasionally abortion. Members of these salmonella and proteus groups are commonly found in turkeys and broilers and are acquired by domestic fowl in a number of ways, particularly congenitally and from nondomesticated infected birds. Smith, Conant and Overman (1964) report that Shigella spp are found almost exclusively in man with a few rare exceptions in which shigella were found in the dog and the monkey. None of these organisms form spores.

Carpenter (1967) lists members of the Clostridium spp as being anaerobic spore - forming rods. The dehydrated endospore cytoplasm is not conducive to any kind of chemical activity and complexes formed between thermolabile substances within the endospore and peptides and calcium stores in the spore coat make Clostridium spp resistant to lethal chemicals. The diptheria organism is a member of the Corynebacterium genus and the organism which generates tuberculosis is classified within the genus Mycobacterium.

Bacteriological and Toxicological Studies
with Poultry Litter

Alexander, Carriere and McKay (1968) analyzed 44 field samples of poultry litter taken at different levels in three or four representative areas of the poultry houses sampled. Results were positive for 10 different organisms of the Clostridium spp, two Corynebacterium, three types of Salmonella and various Actinobacilli, Mycobacterium, Enterobacteriaceae (other than Salmonella) and yeasts. Of the ten Clostridium spp found, most frequently occurring were Clostridium perfringens which is responsible for "overeating disease" in sheep and cattle and Clostridium novyi which produces a nonhemorrhagic, gelatinous, local edema which may be fatal. Two of the Salmonella spp isolated were S. saint paul and S. typhimurium, both of which are responsible for enteric fevers. Bacilli were found in all 44 samples. Twenty-six of the 44 field samples collected in this study were from operations in which the litter was being fed to livestock. Four of the 44 samples were from salmonella - suspect flocks but all of these samples gave a negative test for Salmonella spp. Pathogenic bacteria were found to survive in litter for a significant period of time.

Zindel (1970) found Bacillus spp, Proteus spp, E. coli and other members of the Enterobacteriaceae family in 40% of fresh poultry fecal samples. Coliforms as a group

were present in 60% of the samples.

Halbrook, Winter and Sutton (1951) quantitated bacteria densities on 85 samples of wood shaving, bark and corn cob base litters. The litter samples varied in age from new, unused litter to that which had been in use for over 1 year. Broiler-type chicks were fed for 8 weeks in pens containing litter changed weekly, unchanged litter or built-up litter (used by hens for at least 1 year). All classes of bacteria, molds and yeasts studied were found to increase with litter use over the 8-week period. Built-up litter which was more than 1 year old, however, contained fewer coliforms than either litter changed weekly or unchanged litter and fewer lactobacilli and enterococci than litter which was not changed at weekly intervals. Unused litter was found to contain 1,000 to 200,000 bacteria of the lactobacilli and coliform groups per gram. This count increased to between 600,000 and 3,000,000 for litter used 1 to 8 weeks under the unchanged litter management system. When comparing unchanged litter with built-up litter, the former was found to contain 43 times more coliforms and 11 times more lactobacilli. Another group of bacteria may be present in greater abundance in built-up litter than in unchanged litter since the total bacteria count between the two litters was not appreciably different. Enterococci bacteria were not numerous in the litter samples, averaging

50 to 100 per gram. Bacteria counts were similar for litters containing various base materials.

Tucker (1967) reported the recovery of salmonellae from litter 19 weeks after the original infection had been introduced through oral inoculation of day-old chicks. The base material used in this study was sawdust and wood shavings. Maximum and minimum daily temperatures were recorded and the ammonia content and moisture percentage of the litter were measured regularly. Survival time was found to be independent of temperature and ammonia concentration. However, the moisture content of the litter did exert an effect upon survival time in that the higher the percentage moisture of the litter the shorter was the survival time of the salmonella. This correlation was observed in both old and new litter. In a particular area of one pen where the moisture content of the litter reached 44%, S. thompson disappeared in 8 weeks, as compared to recovery of the same organism after 19 weeks in a pen in which the moisture content of the litter averaged 21.6%.

Botts et al. (1952) orally administered a suspension of S. pullorum to day-old chicks and divided the chicks into four pens containing either old built-up litter or new corn cob litter. A pen having a wire floor was employed as a control. When the chickens reached 45 days of age one pen of each type litter was sprayed with a suspension of

S. Gallinarum. Salmonella pullorum and S. gallinarum ceased to be recovered from old built-up corn cob litter after 15 and 20 days, respectively. Survival times were 70 and 63 days, respectively, in the new litter.

Litter samples from four farms on which three different poultry enterprises were employed were examined by Lovett, Messer and Read (1971). Two of the farms produced pullets for laying stock. Each new flock reared on these farms was placed on fresh litter. Of the other two farms, one produced eggs and the other produced broilers on built-up litter. Wood chips were used as the base material on all farms. This study was designed to determine the relationship between microflora populations and the chemical and physical qualities of litter. Neither moisture content nor pH exerted any effect on bacterial or fungal population densities. Litter age was the only factor found to affect the densities of all organisms examined. Population densities increased with litter use. Bacterial population density did not change in litter stored in open and unprotected heaps for periods varying from 1 to 34 months. The densities for coliforms and E. coli remained nearly constant throughout the sampling period and approximately 10% of the total bacteria population following 1 week of litter use were coliforms. Of the coliform species present, one-third were E. coli. Of the litter samples collected from

the operation in which broilers were produced on built-up litter, the moisture content ranged from 9.1% to 19.1% in litter 27 months old. Anaerobe counts were of the magnitude of 10^5 to 10^8 organisms per gram, respectively. All litter samples tested negative for salmonella.

Lovett et al. (1971) did not show that alkaline litter favored fungal growth over that of any other category of organism whereas this had been demonstrated by Schefferle (1965). Schefferle (1966) found the actual counts of coryneform bacteria to be unaffected by type of litter or conditions such as temperature, moisture content or pH of the litter. She reported coryneform bacteria counts of 10^9 to 10^{11} per gram of fresh litter. Schefferle (1965) showed that these organisms accounted for 41 to 83% of the total viable count with an average of 68%. She found that a sample of unused litter material contained only 16% coryneform bacteria when expressed as a percentage of the viable bacteria. Strains grown on the surface of an agar medium to which uric acid had been added were found to decompose uric acid to urea and eventually to ammonia if decomposition was permitted to proceed. Only certain of the strains, those which were able to produce urease, could carry the decomposition as far as the production of ammonia, however. The ability of the coryneform bacteria to decom-

pose creatine and creatinine was found to be characteristic of many of the uric acid decomposers. This is of interest since creatine carries a small portion of the excretory nitrogen of the fowl.

Kraft et al. (1969) sampled freshly voided droppings and old wastes from one flock of turkeys and from the bottom-most levels of litter piled underneath caged laying hens. Of the 91 poultry houses sampled, 26 were positive when qualitatively tested for salmonella. In a quantitative study, densities of salmonella ranged from less than 1 to greater than 34,000 per gram of dry excreta, with the higher densities coming from caged layer operations while the lower densities came from houses where the hens were maintained on litter or wire floors.

Whitehead and Corson (1962) orally administered killed mycobacteria to four of eight guinea pigs. Two of the four received killed tubercule organisms of the mammalian variety and the other two were fed supernatant material from autoclaved tuberculous bovine and porcine glands. All four animals demonstrated sensitivity to injected tuberculin at a level comparable to the sensitivity exhibited by two guinea pigs which were fed unautoclaved supernatant material from cattle and swine glands suspected of being tuberculous. Upon necropsy no gross evidence of tuberculosis was found in the six animals that demonstrated tuber-

culin sensitivity.

The possibility of inducing tuberculin sensitivity through the feeding of poultry litter was investigated by Carriere, Alexander and McKay (1968) using guinea pigs. Samples of litter were homogenized in physiological saline and the resulting filtrate was treated with Zephiran chloride in order to eliminate other bacteria and favor the isolation of mycobacteria. Live virulent organisms were orally administered and injected intraperitoneally. Tuberculins were used to determine the degree of sensitivity by employing the comparative intradermal test. Tuberculin sensitivity was, in fact, produced whether the mycobacteria were ingested or injected. Animals displaying sensitivity were autopsied after a suitable period of time but no specific tuberculosis confirming lesions were found when various tissues were histologically examined. This work confirms the findings of Whitehead and Corson (1962) and lends insight into the potential health hazards that may be inherent when litter is incorporated into ruminant rations. Ray et al. (1963) were unable, however, to induce tuberculin sensitivity when a ration containing heat killed Mycobacterium bovis was fed to cattle. Carriere et al. (1968) produced sensitivity to live cultures of Mycobacterium spp when administered via milk to calves so it appears that mycobacteria, if killed by some means of litter processing,

may not present any real danger to the specificity of agents used in the tuberculin testing of cattle at the present. Survival tests conducted using M. avium seeded into autoclaved litter provided data showing recovery of the species for as long as 59 days post-seeding. M. avium, when seeded into a litter sample containing viable bacteria, were recovered at 32 but not at 47 days after seeding. Competition with other microorganisms may, therefore, be instrumental in the destruction of mycobacteria.

The existence of potentially harmful microbes in litter is well documented and a potential for the transmission of disease through the feeding of litter should be recognized.

Bactericidal Properties of Dry Heat

Messer et al. (1971) used samples of poultry litter ranging in moisture content from 6.5 to 53.6% with the average being approximately 23% to determine whether heat treatment of litter would effectively provide a barrier against transmission of diseases. Initial counts of total aerobes, E. coli and Salmonella and Arizona spp were performed on all samples before being placed in sterile 13 x 17 mm thermal death tubes for immersion in water baths at temperatures of 57.2, 62.8, 68.3 and 73.8 C for periods of 30 or 60 min. Heating of both woodchip and corn cob litters for 30 min. at 60 C completely eliminated E. coli.

The initial concentration of E. coli in the samples averaged 2×10^5 cells per gram. In a subsequent test, S. typhimurium was destroyed from artificially contaminated litter by heating at 47.2 C for 60 min. or 73.9 C for 30 min. This litter contained 24% moisture and was of a wood-chip base. Salmonella pullorum and an Arizona sp in corn cob litter were destroyed by heating at 62.8 C for 30 min. and 57.2 C for 30 min., respectively. The average initial count in the test involving the Salmonella and Arizona spp was 3×10^4 cells per gram.

Zindel (1970) recovered coliforms and Clostridium perfringens in 22% of the samples of poultry feces dried at 205 to 650 C. Bacillus and Streptococcus spp were found in 55 and 33% of the dried samples, respectively. He suggested that the presence of these bacteria in the dried fecal samples may indicate recontamination of the dried product by the caretaker of the drying unit. Drying times were not stated.

Connell and Garrett (1963) enumerated coliforms, spore formers and total bacteria on all or part of 189 samples of dried sludge collected from flash drying equipment at four different wastewater treatment plants. Sludge drying temperatures at the four plants were within the range of 88 to 143 C with the drying temperature most frequently being 118 C or above. Drying times used were not

stated. Coliform counts for the wet, unprocessed sludge ranged from 125 to greater than 100,000 per gram and spore former counts averaged approximately one billion per gram. Heat drying permitted the survival of coliforms in only 21 of the 189 samples and the highest count obtained was 17 per gram. Spore forming survivors in the heat dried samples ranged from 14 to 240,000 per gram. Total bacteria counts were reduced to as low as 30,000 per gram.

Akama et al. (1967) detected Clostridium perfringens in nearly all of the fecal samples obtained from healthy humans. The actual counts, ranging from 10^1 to 10^8 cells per gram of feces, were not reduced by heating the samples at 80 C. A significant reduction in the number of C. perfringens occurred when samples were heated at 90 C, although counts ranging from 10^1 to 10^6 clostridia per gram of feces were found in approximately 50% of the fecal samples following heating at 100 C for 60 min.

Wijewanta (1964) reported reduction of the viable spores of heat resistant Clostridium welchii to 0.1% of the original count in various soil suspensions by heating the suspensions for 2.5 hr. at 100 C. The soil samples used in this study were collected from pastures, zoological gardens, poultry yards and gardens which had been fertilized with excreta from one or more species of animals.

Riemann (1968) recorded a 10^6 -fold reduction in the

number of viable salmonellae added to sterilized meat and bone meal from heating the meal which contained about 10.3% moisture at 90 C for 20 min. Meal containing 7.0% moisture underwent a 10^4 -fold reduction in the number of viable cells when heated at 90 C for 40 min. Heating meal containing only 4.0% moisture at 100 C for 20 min. resulted in a 10^3 -fold decrease in the original count of salmonellae. Meal containing 10.8% moisture or higher underwent a 100-fold decrease in viable salmonellae during the first few days of storage at room temperature and a further 10^5 to 10^6 -fold reduction in cell number when stored at room temperature for an additional 4 to 6 weeks.

Liu and Snoeyenbos (1968) concluded that the heat resistance of salmonellae in feeds is a function of the water content of the feed. Their results showed that the heat resistance of S. senftenberg in both artificially and naturally contaminated samples of chick starter and meat and bone meal at all moisture levels studied was an exponential function of heating time (death was logarithmic).

Rasmussen et al. (1964) packed approximately 2 g of sterile animal by-product meal into glass tubes 40 cm in length having an internal diameter of 3 mm and submerged them in a water bath for either 7 or 15 min. at temperatures ranging from 52 to 77 C. The meal packed into the thermal death time tubes had been inoculated with Salmon-

ella senftenberg at a level of 2,000 cells per gram. The strain of salmonella used in this study is one of the most heat resistant strains known. All of the four samples heated at 69 C were free of salmonellae after 15 min. of heating. At 66 C two of the four samples heated still were positive when tested for salmonellae. Samples of naturally contaminated meat and bone meal were heated in the same manner as the inoculated meat and bone meal in a subsequent trial. Analysis of the naturally contaminated meal showed that it contained 10.7% moisture, 50.9% crude protein and 13.8% fat. Heating the meal for either 7 or 15 min. at 82 C was sufficient to insure destruction of the salmonellae. Chemical analysis of a naturally contaminated feather meal tested by the same procedure showed 8.2% moisture, 77.7% crude protein and 7.9% fat. Heating of the feather meal at 77 C for 15 min. was sufficient to provide sterilization. A naturally contaminated meat meal containing 13% fat required 7 min. of heating time at 91 C to sterilize six 10 g samples. The population of salmonellae present in the untreated meat meal was 25 per gram, some 50 times greater than that of the feather meal. A pugmill consisting of a chamber with a capacity for 450 g of meal was constructed such that rotating paddles stirred the meal during the heating process. A series of tests were conducted to determine the minimum temperature required to

kill salmonellae in the three naturally contaminated meals previously described. All of the salmonellae in the meat and bone meal were killed by heating the meal from room temperature to 71 C in 50 min. The moisture, fat and crude protein levels of the untreated meat and bone meal used in this experiment were 7.1, 6.4 and 46.0%, respectively. Heating the meal to 82 C changed the moisture content to 6.4% and the fat content to 5.3%. Crude protein content was not altered. A short bioassay using weanling rats indicated no significant change in the nutritive value due to heating meat and bone meal to 82 C as was evidenced by growth at a level of 98.4% of that of the rats fed the untreated meal. Heated meal made up to 8.6% of the diet fed to the rats.

Under most circumstances, coliform bacteria are effectively reduced in number or totally eliminated from various contaminated materials by heating at 150 C or less for periods of 60 min. or less. Other enteric bacteria are killed equally well by similar conditions of temperature and exposure time although the heat resistance of salmonellae appears to be more directly related to the moisture content of the contaminated material. Spore forming bacteria, of which clostridia are representative, require temperatures in excess of 100 C and prolonged exposure times as compared to coliforms in order to be destroyed.

Moist Heat Destruction of Microorganisms

Perkins (1957) indicated that moist heat in the form of saturated steam under pressure is the most dependable method known for the destruction of all forms of microbial life. The term "saturated steam" indicates that the steam is at the maximum pressure and density obtainable at a given temperature. Most commercial sterilization processes employ a pressure within the range of 0.70 to 1.05 kg/cm² and temperatures within the range 115 to 122 C.

Sykes (1958) reported that the United States Pharmacopeia quotes the use of steam under a pressure of 1.05 kg/cm² at 121 C for 20 to 30 min. for reliable surface sterilization. Death of microbes by dry heat is an oxidation process whereas death as a result of moist heat is due to the coagulation of some protein vital to the metabolism of the cell. Therefore, the rules which apply to one method will not necessarily work for the other as evidenced by the much shorter time required for destruction with the use of moist heat.

McCulloch (1945) recommended the use of an autoclave to sterilize feces since this is a material requiring positive disinfection in most instances where feces are being examined.

Warden and Schaible (1961) fed hen fecal material to broiler-type chicks at a level of 1% of the basal ration.

The hen feces were added to the diet as fresh, dried at 38 C for 72 hr. or autoclaved for 30 min. under steam pressure of 1.05 kg/cm². Chicks fed the control ration containing no feces were the heaviest at the end of 28 days and had a feed efficiency of 1.77. The chicks receiving diets containing fresh, dried or autoclaved feces had feed efficiencies of 1.92, 1.79 and 1.76, respectively. Growth depression in the chicks receiving fresh feces was significant. Total cocci, enterococci and E. coli counts were made on the intestinal contents sampled 5.08 cm above the cecum. In the chicks fed no fecal material the total cocci, enterococci and E. coli counts per gram of intestinal contents were 490, 210 and 5, respectively. For the other three treatments these counts increased to 24,000+, 3,500 and 478 for fresh feces consumers, 1,800, 1,800 and 12 for dried feces consumers and 9,200, 5,400 and 162 for autoclaved feces consumers, respectively.

Although the use of moist heat for the pasteurization of litter is not well documented, autoclaving has for many years been recognized as an effective means of achieving sterilization. It appears that autoclaving would lend itself to litter pasteurization on a large scale when certain adjustments in heating time are applied.

Paraformaldehyde as a Bactericide and its
Effect upon Nutritive Value

Walker (1944) defined paraformaldehyde (PFA) as a mixture of polyoxymethylene glycols containing 93 to 99% formaldehyde, with the formula, $(\text{CH}_2\text{O})_n$. According to Walker, commercial PFA, which is often erroneously termed "trioxymethylene," contains 95% or more formaldehyde, it is obtainable in a range of subdivisions from granular to fine powder and has a melting range of 120 to 170 C, depending upon the degree of polymerization; it dissolves slowly in cold water, but its solubility increases with an increase in water temperature. He stated that depolymerization takes place as it dissolves. A formaldehyde solution is obtained in this way which is the same as that obtained by dissolving gaseous formaldehyde in water. The degree of solubility reaches a minimum at pH 2 to 5, but increases rapidly on either side of this range. In general, reaction with formaldehyde hardens proteins and increases their resistance to the action of chemical reagents and enzymes. Formaldehyde most readily reacts with free amino groups of proteins but can also react with the amido nitrogen of peptide linkages. Reactivity with formaldehyde varies with different protein types and is dependent upon the relative proportion of amino and amido groups. Formaldehyde combines at the rate of 4.0 to 4.8 g per 100 g of gelatin and at the rate of 0.6 to 2.5 g per 100 g of casein.

According to Neely (1963), the mechanism by which microorganisms convert homocysteine to methionine is blocked in formaldehyde treated microbes since 1, 3-thiazane - 4 - carboxylic acid is formed. He suggested that if homocysteine and formaldehyde remain in a ratio of 1:1, or if homocysteine is in excess, the gross inhibiting effect of formaldehyde will not be expressed. No bacteria inhibition occurred at 1:1; it occurred if the concentration of formaldehyde exceeded that of homocysteine. Furthermore, the extent of growth inhibition due to formaldehyde was proportional to the extent to which formaldehyde exceeded homocysteine.

Seltzer, Moum and Goldhaft (1969) attempted to bind the ammonia released from fresh chicken feces in the form of hexamethylenetetramine and thereby control at least part of the odors emitted from poultry wastes. One hundred grams of fresh chicken feces were placed in each of six plastic bottles. Aerobic conditions were maintained in the bottles. Flake paraformaldehyde was added to the bottles at levels of 0, 0.5, 1, 3, 5 and 7 g, and 10 ml of sterile water was poured into each bottle to insure sufficient moisture. The bottles were maintained at room temperature. Daily measurement of the pH of the air above the feces in each bottle gave an accurate measurement of the amount of ammonia emitted from the feces. The untreated control bottle

reached a maximum pH of 11 within 48 hr. indicating over 100 ppm of ammonia gas. The bottles containing 0.5 or 1.0 g of PFA reached a maximum pH of 11 in 9 days. However, the bottle containing 1 g of PFA did not exhibit ammonia emission from the feces until 2 days after the bottle containing 0.5 g of PFA. The bottles containing 3, 5 and 7 g of PFA remained at pH of 6 throughout the 28 day duration of the test, indicating no production of ammonia. Bacterial counts performed on the twelfth day on a sample of feces from the bottles containing 0, 1, 3 and 7 g of PFA revealed counts of 2.2 billion, 164 million, 1,000 and 0 organisms per gram, respectively. At the completion of the 28 day test samples of feces were removed from the bottles containing 0, 1, 3 and 7 g of PFA and assayed for total nitrogen. The total nitrogen content of the four fecal samples were 1.39, 1.45, 1.75 and 2.32%, respectively. In subsequent tests under a variety of field conditions, similar results were obtained with respect to nitrogen retention. In this study, free sulfur retention was, likewise, directly proportional to the paraformaldehyde treatment level of the manure. Other observations showed that PFA treated manure did not possess as unpleasant an odor after drying, compared to untreated manure. Also, treated manure retained more of its original weight than untreated manure dried by the same method.

Starch - fermenting bacteria were found in 12 samples of non-fermenting drilling mud of the starch - base type (Myers, 1962). Of the 12 samples, 25% were found to contain very active starch - fermenting gram - positive spore formers believed to be Bacillus subtilis. Similar bacteria were found in fermenting starch - base drilling mud and in the corn starch and slough water used to prepare the mud. The bacteria isolated from drilling mud were not destroyed by 12 hr. exposure to 0.1% (w/w) PFA or 1 hr. exposure to 0.5% PFA as was evidenced by no diminution in their ability to ferment starch. Similarly, starch - fermenting ability continued to be exhibited by the bacteria after 3 hr. exposure to 0.5% PFA. Four hours exposure to 0.5% PFA was, however, sufficient to destroy the bacilli. Chemical analysis showed that at 22 to 23 C, PFA present in a concentration of 90 g per barrel of mud would completely disappear in 9 hr. In fact, a significant decrease in PFA concentration was detected as soon as 30 min. after preparation of the mud. It was suggested that the relatively high concentration of ammonia and chloride in the mud may facilitate the disappearance of PFA in that hexamethylenetetramine, a feeble antiseptic was formed.

Myers and McCready (1963) found the antibacterial properties of PFA solutions in concentrations from 0.1 to 0.4% (w/v) to be bacteriostatic rather than bactericidal.

Exposure time required to accomplish inhibition of the growth of Staphylococcus aureus by unbuffered solutions of PFA was significantly increased as the concentration of ammonium chloride increased from 0 to 3% (w/v) regardless of the concentration of PFA. More specifically, at a PFA concentration of 0.33% (w/v) the exposure time necessary to inhibit growth of the test organism was increased by 50% in the presence of 1% (w/v) ammonium chloride, by 75% in the presence of 2% ammonium chloride and by 100% in the presence of 3% ammonium chloride. Buffering of the PFA solutions caused a decrease in exposure time required for growth inhibition. An important relationship exists between PFA and ammonium chloride in an unbuffered solution. As reaction time increased from 0 to 360 min., the concentrations of PFA at 1, 2 and 3% ammonium chloride decreased by 0.054, 0.067 and 0.076 percentage units, respectively. Accompanying the disappearance of the PFA was a drop in pH by 1.2 to 1.4 pH units due to the production of hydrochloric acid. A significant decrease in PFA concentration occurred within 30 min. after preparation which substantiates the work of Myers (1962). Paraformaldehyde dissociation occurs to a greater extent in basic solutions. This therefore can account for the slightly greater antibacterial activity of the buffered PFA solutions. The above tests were performed at 25.8 C using 24-hour broth cultures of the test organism

to test the antibacterial activity of PFA.

Taylor, Barbeito and Gremillion (1969) nebulized 1 ml of a suspension of Bacillus subtilis containing 10^7 spores per ml onto samples of 15 types of surfaces commonly found in a laboratory. Also, filter paper patches, plastic vaccine storage tubes and various types of laboratory equipment were nebulized with suspensions containing various concentrations of B. subtilis and/or Serratia marcescens. Control swabs were taken on all nebulized materials to assure contamination. Contaminated surfaces and materials were then placed in a chamber in which the relative humidity was controlled at 60% and formaldehyde gas was released into the chamber by heating a known weight of PFA and causing depolymerization to take place. The temperature of the chamber was held at 24 C for 1 or 2 hr. following the complete depolymerization of 0.3 g of PFA per 0.028 cubic meter of chamber volume. Post-treatment viable bacteria recovery tests revealed complete destruction of B. subtilis and/or S. marcescens on all materials within the 1 and 2 hr. contact periods. Clostridium botulinum type A toxin was destroyed within 48 hr. by the same concentration of PFA at 45% relative humidity and 24 C.

Hall et al. (1971) compared soybean meal, DL - methionine fortified soybean meal and corn gluten meal when fed to steers as sources of supplemental nitrogen. The effect

of treating each nitrogen source with cane molasses, wood hemicellulose or formaldehyde was also studied. No differences in gain and feed efficiency between treatments were observed.

Schmidt et al. (1971) treated soybean meal with 1, 2, 3, or 4 ml of a 40% formaldehyde solution, 5, 10, 15, or 20 ml of a 40% glyoxal solution, 3, 6, 12, 18 or 36 ml of a 44.5% hexamethylenetetramine solution or water per 100 g of crude protein. Treated soybean meals were incorporated into semi-purified diets and fed to rats for nitrogen balance studies. Glyoxal treatment depressed gain. Formaldehyde treatment tended to lower nitrogen retention, as a percent of intake, as compared to the control, when 2 ml or more of the formaldehyde solution were used to treat 100 g of crude protein. Nitrogen retention in the hexamethylenetetramine treatment was superior to the control at all levels. In vitro rumen ammonia release was less than 10% of control for all levels of formaldehyde and glyoxal and was 50 to 94% of control for the hexamethylenetetramine treatment.

Schmidt, Jorgensen and Benevenga (1972) reported no difference in gains or blood urea nitrogen of lambs when 49% crude protein soybean meal which had been sprayed with 0, 1.5 or 3.0 ml of a 40% formaldehyde solution per 100 g of crude protein supplied half of the total dietary pro-

tein when used in rations with protein - energy ratios of 22, 18 or 15 g of digestible protein per mcal of digestible energy. Furthermore, neither formaldehyde nor protein - energy ratio caused any difference in daily nitrogen retention, fecal nitrogen or nitrogen apparently absorbed when all levels of formaldehyde and protein - energy levels of 18 and 15 g of digestible protein per mcal of digestible energy were fed in two nitrogen balance trials. Similar results were obtained when steers were fed conventionally and in a nitrogen balance trial except that soybean meal treated with formaldehyde at the 3.0 ml level significantly depressed daily gain. Ewes receiving soybean meal treated with 0 to 7.5 ml of formaldehyde solution showed a significant decrease in daily gain below the control at all levels of formaldehyde addition except for the 1.5 ml level. Release of rumen ammonia in vitro was significantly reduced with all levels of formaldehyde used.

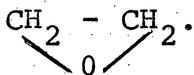
Nishimuta, Ely and Boling (1972) compared regular soybean meal to heat treated, formaldehyde treated and tannic acid treated soybean meals in conventional diets fed to wether lambs in a nitrogen balance trial. The heat treatment involved heating the soybean meal at 149 C for 4 hr. Preparation of the formaldehyde treated soybean meal involved the addition of 1% formaldehyde by weight. Dry matter digestibility and cellulose digestibility were sig-

nificantly lower in the formaldehyde treated soybean meal and heat treated soybean meal groups, respectively. Feeding of the formaldehyde treated soybean meal resulted in the lowest nitrogen digestion (46.45%), percent of nitrogen intake retained (17.36%) and blood urea nitrogen (6.97 mg/100 ml).

Paraformaldehyde, when permitted or caused to sublimate to formaldehyde gas, is an active chemical bactericide for sporulating and nonsporulating bacteria. Treatment of proteinaceous substances with paraformaldehyde or formaldehyde may affect the nutritive value of these substances for ruminants by interaction of aldehydes with proteins.

Ethylene Oxide as a Bactericide and its
Effect upon Nutritive Value

According to Phillips (1957) ethylene oxide (ETO) exists as a colorless gas at ordinary temperatures, liquefies at 10.8 C and freezes at - 111.3 C. The liquid is miscible with water and all common organic solvents in all proportions. Ethylene oxide has a pleasant, ethereal odor and is comparable to ammonia in inhalation toxicity. It is irritating to soft tissues. Ethylene oxide is vigorously flammable and a concentration of 3% in air will support combustion with explosive magnitude if it is confined. It has the formula



Phillips (1949) has linked the bactericidal properties of ETO to its chemical activity as an alkylating agent. He stated that according to an older theory the activity of ETO resulted from its hydrolysis to ethylene glycol. This theory was disproven by Yesair and Williams (1942) and Wilson and Bruno (1950) when they found ethylene glycol to be of lower bactericidal value. Attack of the carboxyl, sulfhydryl, hydroxyl and amino groups or, in other words, any site having a replaceable hydrogen atom appears to be the mechanism by which ETO kills microorganisms.

Messer et al. (1971) determined total aerobic bacteria, E. coli, salmonella and Arizona bacteria counts on samples of litter fumigated with ethylene oxide. Litter samples were spray inoculated with washed cells of Salmonella spp, E. coli and an Arizona sp while being mixed. Inoculated samples were allowed to equilibrate for 4 days at 18.3 C before being subjected to ETO fumigation. The litter was spread to depths ranging from 0.63 to 3.81 cm in metal pans 22.9 x 30.5 x 7.6 cm. Pans of litter were then treated with ETO at 1.24 to 1.41 kg/cm² pressure for 4 to 17 hr. at room temperature. Ethylene oxide reduced the bacterial population density but did not eliminate bacteria.

Pure liquid ETO was used by Hansen and Snyder (1947) at the rate of 1 ml per liter capacity of the container to sterilize 50 different plant and animal tissues including

alfalfa hay, pea pods, dry bean straw, wheat, dehydrated fruits and vegetables and insects. Soil, likewise, was sterilized. Fumigation time at room temperature was overnight or the equivalent. These workers suggested slight moistening of the material to be fumigated prior to initiation of fumigation.

McQuade, Atchison and Parisi (1969) used commercially prepared ETO ampules in a sterilizing canister to fumigate pea, sunflower and onion seeds for 18 hr. Water obtained from Petri dishes in which seed germination tests were conducted was streaked on several media and incubated, thus giving a qualitative test for sterility. Sunflower seeds and pea seeds were found to be sterile after fumigation with ETO, whereas untreated controls were not. Attempts to sterilize onion seeds were not always successful. Contaminating microorganisms, when in an environment having an abundance of thymidine, may convert thymidine to thymine after incorporating only a relatively small amount in the DNA. Sterilization of the sunflower and pea seeds was further proven since conversion of thymidine to thymine or intermediate products did not take place when treated seeds were placed in a radiothymidine solution.

Yesair and Williams (1942) performed total bacteria, total spore formers and aerobic spore formers counts on 127 samples of 40 different spices and herbs obtained from a

variety of locations including meat packers, canners and spice manufacturers. Pre-ETO fumigation counts on all samples showed black pepper to be most heavily contaminated, having total bacteria counts ranging from 1,200,000 to 16,300,000 per gram. Non-putrefactive anaerobes were found in 7 of the 127 samples. Black pepper and cinnamon were chosen as test spices because of their high contaminations. For ETO fumigation, 100 g lots of the two spices were placed in pint jars with loosely fitting lids and placed in an aluminum vacuum chamber immersed in a water bath maintained at 85 C. The chamber was evacuated to 68.6 cm of mercury and 15 ml of ETO were admitted, thereby reducing the vacuum in the chamber to 58.4 cm of mercury. The chamber was maintained under these conditions for 5 hr., then aerated with filtered air for 20 min. Total bacteria counts for the untreated samples of pepper and cinnamon were 16,000,000 and 36,400, respectively, with total spore counts being 48,000 and 10,000 per gram, respectively. Ethylene oxide fumigation reduced the total bacteria counts to a range of 380 to 1,480 for pepper and 20 to 50 for cinnamon. Spore former counts were reduced to less than 20 per gram for both spices. Five gram pepper samples from the jars of treated pepper and some black pepper that had been heat sterilized were inoculated with aerobic spores and stored overnight. Suspensions of each sample gave

approximately the same bacteria counts indicating no residual ETO in the fumigated sample after aeration.

Michael and Stumbo (1970) uniformly mixed washed cell suspensions of S. senftenberg and E. coli separately with whole eggs, lyophilized the inoculated slurry and ground it to a powder. This inoculated powder was then preconditioned overnight under refrigeration at 11% relative humidity. The sterilization chamber was a stainless steel anaerobic jar of about 3.17 liter capacity equipped for either vacuumizing or pressurizing. The jar was equipped with a humidity sensing element and, when in use, was immersed in a constant temperature water bath in order to maintain a temperature of 40 C within the chamber. Relative humidities of 11, 23, 33, 53 and 73% and exposure times of 0, 1, 2, 3, 4, 5 and 6 min. were tested. The sterilant gas was a mixture of 12% ETO and 88% dichlorodifluoromethane, by weight. This gas mixture was injected into the exposure chamber under pressure until a calculated concentration of 700 ± 20 mg/liter of ETO was reached. Lyophilized cells of both species displayed a greater resistance to the ETO fumigation as the relative humidity increased from 11 to 73%. Quantitation of surviving bacteria was performed using optical density techniques and establishment of growth curves after enrichment of the powdered egg mixture following fumigation. Cells of the

two bacteria possessed a greater resistance to ETO when mixed with the whole egg powder than when the same bacteria were exposed to ETO exclusive of the powdered egg. They suggested that protection of the bacteria from ETO in the inoculated powder may possibly have been due to competition for ETO by alkylatable components in the egg, many of which exist.

Roberts et al. (1943) used 0.8 atmosphere of pure ETO for 2 hr. to sterilize Crosby and 6 hr. to sterilize Warsaw silt loam soils. The length of time required to sterilize soil appeared to be related to the organic matter content. In this study the soil pH was slightly elevated as was the soluble organic matter level as a result of ETO fumigation. The increase in soluble organic matter was believed to be due to ethylene glycol formation. The levels of soluble salts in the treated soils were not appreciably changed.

Wilson and Bruno (1950) used 0.5 and 1.0% solutions of liquid ETO in a ratio of one part liquid ETO to 100 parts of the fluid to be sterilized, by volume, in an attempt to sterilize several types of microbiological media which could not be sterilized by heat since preservation of certain heat labile substances in the media was imperative. Heat sterilized portions of tryptose phosphate broth and milk were inoculated with Eberthella typhosa, Bacillus

subtilis, Clostridium tetani, Salmonella typhimurium, Sarcina lutea, Serratia marcescens and Lactobacillus casei. E. coli from a pyelonephritic urinary tract, Staphylococcus aureus from a patient's throat, other throat washings, feces, microbiological laboratory floor sweepings and soil were also used to thoroughly contaminate the media. Heavily contaminated media required 1.0% ETO to achieve sterilization. Clostridium tetani was totally eliminated by the ETO treatment, demonstrating the ability of this germicide to destroy anaerobic spore forming bacteria.

Spore formers known for their resistance to heat and chemical agents were subjected to gaseous ETO fumigation by Friedl, Ortenzio and Stuart (1956). An aluminum chamber was evacuated until a reading of 50.8 cm of mercury was recorded on a vacuum gauge. Exposure periods of 10, 20, 30 and 60 min. and 3, 6 and 18 hr. were used to fumigate five suspensions each of Clostridium botulinum, Cl. lentoputrescens, Cl. perfringens, Cl. sporogenes, Cl. tetani, Bacillus anthracis, B. coagulans, B. globigii, B. stearothermophilus and B. subtilis. Three hours of fumigation were required to sterilize the five suspensions for all organisms except B. subtilis and Cl. sporogenes. Complete destruction of the two above named bacteria required 18 hr. of exposure. Fumigation for 6 hr. sterilized 80% of the B. subtilis and 20% of the Cl. sporogenes suspensions.

Hawk and Mickelsen (1955) used ETO to treat a purified diet for rats. A stock diet of ground dog meal pellets likewise was treated using 10 ml of liquid ETO and an exposure chamber. Five hundred grams of each diet was spread to a depth of 1 cm in large Petri dishes and exposed to the ETO for 0 and 18 hr. for the purified diet and 0, 6, 12, 18 and 24 hr. for the stock diet. Thiamine hydrochloride dispersed in starch retained its full activity when exposed to ETO. Similar treatments in the presence of choline chloride destroyed the thiamine but destruction did not occur when choline dihydrogen citrate was substituted for choline chloride. They suggested that this is a reflection of pH elevation since an aqueous suspension of the starch mixture containing choline chloride was quite alkaline. This was not the case when choline dihydrogen citrate was substituted. Growth impairment was apparent after 2 days of feeding the treated purified diet and this response was even more severe when the stock diet fumigated for 12, 18 and 24 hr. was fed. When thiamine was supplemented to the diet in which it had been destroyed, the growth impairment response was not overcome indicating the possible destruction of other nutrients. Feed consumption of animals that had received the untreated stock diet for approximately 7 weeks was immediately reduced by 30% when placed on the ethylene oxide treated stock diet. This response was ob-

served in animals receiving diets that were permitted to aerate naturally and in diets to which a vacuum had been applied immediately following ETO fumigation. Seeding of the diets with B. globigii spores prior to treatment permitted evaluation of the spore killing power of ETO. All of the organisms were not destroyed by 18 hr. of fumigation, but the viable count was markedly reduced.

Oser and Hall (1956) performed bacteriological and chemical examinations on ETO treated and untreated samples of yeast which made up 10% of a purified diet for rats and samples of a natural, stock diet, fumigated in the complete form. The method of fumigation was injection of gaseous ETO into a chamber containing the material. The material was preheated in the chamber under a vacuum of 84 cm of mercury for 2.5 hr. prior to ETO injection. Ethylene oxide fumigation was carried on for 2.5 hr. at either 37 or 55 C making a total of 5 hr. of heating time at either 37 or 55 C. The samples were flushed with sterile air for 5 min. following ETO treatment and thermophilic spore and mesophilic bacteria counts were performed on the natural diet with only mesophilic bacteria counts performed on the yeast. Total viable mesophilic bacteria density was reduced by treatment from 1,000 per gram in the untreated yeast to less than 1 per gram in the fumigated yeast. Fumigation at 37 C reduced the viable thermophilic spore

count from "too numerous to count" in the untreated natural diet to 45 per gram in the treated samples. The reduction in spores at 55 C proceeded from "too numerous to count" in the unprocessed sample to 15 per gram in the treated sample. The untreated sample of the natural diet contained 2,000 mesophilic bacteria per gram while the treated sample contained less than 1 per gram, regardless of temperature. Treated yeast showed no significant loss of thiamine, riboflavin, niacin and choline. However, the loss of pyridoxine and folic acid due to treatment was significant. Similar results were obtained when the vitamin losses from the natural diet were assayed. During the first week of feeding the ETO treated natural diet to rats, a depression in intake below that of the untreated natural diet was observed. This response, however, failed to be significant after the first week. The moisture content of the yeast and the stock diet was reduced slightly, but not significantly, by treatment. Ethylene oxide fumigation caused no change in the ether extract content of either feed.

Rapid weight loss, stiffness of gait and severe nervous symptoms in weanling rats receiving a stock diet fumigated with ETO for 4.5 hr. was reported by Windmueller, Ackerman and Engel (1956). Their method of fumigation involved placing the material to be treated in a paper bag in a desiccator, evacuating the desiccator to approximately 7.6 cm of mercury and admission of gaseous ETO into the

evacuated desiccator until the pressure returned to ambient. Termination of the treatment period was performed by drawing off the gas in the desiccator and flushing the desiccator several times with filtered air. Subcutaneous injections of thiamine hydrochloride temporarily improved the condition of the rats receiving the treated stock diet, all of which had been observed in the comatose or convulsive state at least once by the eighteenth day of feeding, indicating that ETO treatment did exert some effect upon the nutritive value of the diet. Older rats fed the same diet fumigated for 6 hr. exhibited only a weight loss. Autopsy revealed the large intestinal and cecal contents of these rats to be more fluid, richer in mucous and lighter in color than that of control rats.

Weanling rats receiving a purified diet in which only the casein had been fumigated showed growth depression but gains in weight were observed immediately upon returning these rats to the untreated diet after 5 weeks on the fumigated casein diet (Windmueller et al., 1956). Application of the reduced sulfur test to fumigated and non-fumigated casein caused a greater precipitate of PbS to form in the non-fumigated sample indicating the possible effect of ETO fumigation on sulfur amino acids. However, neither cysteine nor methionine supplementation improved the growth of rats on the treated diet. Ethanol (95%) extraction of fumigated

casein likewise did not improve the biological value of the protein. The biological value of fumigated casein was, however, improved by the addition of non-fumigated casein or a mixture of the 10 essential amino acids. Subsequent studies showed growth improvement with methionine, cystine and threonine supplementation in combination if the ratio of fumigated casein to non-fumigated casein did not exceed 1:1 in favor of the fumigated casein in the diet. The supplementation of arginine and histidine also improved growth regardless of the fumigated to non-fumigated casein ratio. Other pairs of amino acids were not effective.

Mickelsen (1957) attempted to assess the extent to which ETO fumigation affects the nutritive value of a feed. Each component of a rat diet adequate for good rat growth in the complete and untreated form was fumigated with gaseous ETO. The salt mixture used in the diet was the only ingredient in which no reduction in the rate of weight gain was observed following ETO fumigation and incorporation into the complete diet. Other purified ingredients in the basal diet included vitamin-free casein, sucrose, cottonseed oil and vitamins. Treatment of the sucrose - cottonseed oil mixture produced only a slight reduction in growth of the experimental rats. Both male and female rats were maintained on the ration containing the treated sucrose - oil mixture since the cottonseed oil contained vitamins A,

D, and E. Chemical analyses and normal reproductive performance of the rats substantiated the stability of vitamin E to ETO. Some inhibition of growth was exhibited by the rats receiving the diet containing ETO treated casein. Microbiological assays of the purified diets containing fumigated casein showed destruction of 22% of the histidine and 17% of the methionine. Choline chloride, when added to the vitamin mixture, appeared to facilitate destruction of essentially all of the thiamine and large amounts of riboflavin, pyridoxine, niacin and folic acid when the mixture was treated with ETO. Pantothenic acid, biotin and vitamin B₁₂ were not affected.

A number of factors were found to influence the destructive effect of ETO upon certain vitamins. The type of choline compound present in the vitamin mixture was one of these. Choline citrate reduced the vitamin destructive potential of ETO below that of choline chloride. Choline bitartrate, however, was intermediate between choline citrate and choline chloride. The addition of B-vitamins to a ration treated with ETO in the complete form facilitated 50 g of gain in rats for the first 3 weeks it was fed. A loss in weight then became apparent, and the rats died at the end of the eighth week. Analysis of the ration revealed that thiamine had been gradually destroyed over a 2-week period. Other factors influencing growth rate of

rats on an ETO treated diet include moisture content of the ration, ETO concentration during treatment and length of exposure time. The growth of chickens fed an ETO treated ration displayed the same trends as that of the rats.

Mickelsen postulated that depressed growth in both species likely was not a result of toxicity attributable to residual ETO in the ration, since a reduced growth rate also accompanied the feeding of a diet which had been subjected to a vacuum in the exposure chamber following ETO fumigation.

Bakerman et al. (1956) subjected a choline chloride - starch - multivitamin mixture to ETO fumigation, using liquid ETO. The vitamin mixture was used in a purified diet at a level of 2% and contained 885 mg of corn, 200 γ of thiamine hydrochloride, 2,000 γ of nicotinic acid, 250 γ of pyridoxine hydrochloride, 300 γ of riboflavin, 83 γ of folic acid, 1,840 γ of calcium pantothenate, 8.5 γ of biotin, 10 γ of vitamin B₁₂, 100 γ of menadione and 100 mg of choline chloride per gram. In addition to the above vitamin mixture, various vitamins were singly suspended in starch and exposed to ETO. The vitamins studied individually were thiamine, pantothenic acid, niacin, biotin, folic acid, pyridoxine, and riboflavin. Folic acid and riboflavin were chosen for initial individual examinations because ETO treatment of the multivitamin mixture caused

a slight tinge in its pale yellow color. Vitamin K later was shown to be responsible for the color change. Thiamine was completely destroyed both in the multivitamin mixture and when treated singly. Niacin loss ranged from 50 to 80%; pyridoxine, 75%; riboflavin, 33 to 67%; folic acid, 33 to 50%. Losses were the same regardless of whether the vitamins were treated individually or in combination. Essentially no loss of pantothenic acid, vitamin B₁₂ or biotin occurred. In a subsequent experiment, exposure of a stock diet for dogs to ETO under the same conditions caused approximately 40% of the thiamine to be destroyed. A suspension of 2 g of the ETO treated multivitamin mixture in 10 ml of water had a pH of 9.0. They suggested that this elevation of pH along with the hygroscopic properties of choline chloride probably contributed to the destruction of the vitamins.

Ethylene oxide is an effective bactericide against a number of organisms, but its use for the large scale pasteurization of litter may be difficult to apply as the equipment and time involvement in its application is considerable. Furthermore, research has shown that ethylene oxide has a detrimental effect on a number of vitamins and amino acids.

Nitrogen Content of Broiler Litter, Metabolism
Studies With Litter and the Effect of Heat
on the Nutritive Value of Litter

Noland, Ford and Ray (1955) fed chicken litter to gestating - lactating ewes and to fattening steers. Analysis of the litters revealed 4.85 and 4.35% total nitrogen for the litters fed to the ewes and the steers, respectively. Uric acid nitrogen amounted to 0.93 and 0.86%, respectively, in the two litters. Expressed as a percentage of the total nitrogen, uric acid nitrogen was 19.17 and 19.77%, respectively.

Brugman et al. (1964) reported digestion coefficients of 77.82% for protein, 44.36% for fat, 91.04% for crude fiber and 59.15% for gross energy for laying house litter fed to Hereford bulls. Digestion coefficients were determined by the chromic oxide indicator method. The chemical analysis of the litter showed 80.5% dry matter, 2.30% total nitrogen, 0.38% non-protein nitrogen and 0.39% ammonia nitrogen. Non-protein nitrogen and ammonia nitrogen expressed as a percentage of the total nitrogen were 16.52 and 16.95%, respectively.

Nitrogen fractions of peanut hull broiler litter were reported by Bhattacharya and Fontenot (1965). The litter contained 85.54% dry matter. The nitrogen fractions expressed as a percentage of the total nitrogen were:

true protein nitrogen, 46.25%; non-protein nitrogen, 53.75%; uric acid nitrogen, 30.49%; ammonia nitrogen, 13.24%; urea, 2.69%; creatine, 4.48%; and others, 2.85%. Wethers were fed the litter at levels to replace 0, 25, 50 or 100% of the nitrogen supplied by soybean protein in a purified diet. A significant depression in crude protein digestibility was obtained with each increase in litter nitrogen level above 25%. Regardless of the manner in which it was expressed, nitrogen retention was significantly lower in the animals receiving the 100% litter nitrogen level as compared to the 0% litter nitrogen level. Treatment did not affect blood urea nitrogen levels. Ammonia nitrogen levels in the ruminal fluid displayed no particular trend.

In a later trial, Bhattacharya and Fontenot (1966) compared the protein and energy value of autoclaved peanut hull and wood shaving litters when fed to wethers in a conventional diet at levels of 25 and 50% of the diet. The nitrogen fractions content of the wood shaving litter were: true protein nitrogen, 44.38%; non-protein nitrogen, 55.62%; uric acid nitrogen, 28.80%; ammonia nitrogen, 15.40%. The above values were expressed as a percentage of the total nitrogen. Total nitrogen in the same litter amounted to 4.89% of the dry matter. Dry matter amounted to 88.90%. Analysis of the peanut hull litter gave similar

results. Calculated digestible protein and TDN values for the peanut hull litter were 23.4 and 60.7%, respectively, when peanut hull litter made up 25% of the ration. When peanut hull litter made up 50% of the ration, digestible protein was unchanged while TDN was 59.3%. Digestible protein values for the wood shaving litter were 22.5 and 21.6% when litter accounted for 25 and 50% of the ration, respectively. Values for TDN for the same levels of litter were 61.0 and 58.0%, respectively.

Fontenot et al. (1971) collected broiler litter samples from 13 houses located in three major broiler producing areas of Virginia. Chemical analysis of the samples indicated considerable variation in composition substantiating the need for detailed analysis of broiler litter before incorporation into rations. Average values with standard errors for crude protein, protein nitrogen, non-protein nitrogen and ammonia nitrogen were $29.98\% \pm 2.572$, $2.87\% \pm 0.209$, $1.92\% \pm 0.257$ and $1.02\% \pm 0.066$, respectively.

In a metabolism trial, Smith and Calvert (1972) fed wethers ad libitum rations in which dehydrated poultry waste was substituted for soybean meal in order to supply 0, 50 or 100% of the crude protein content of the rations. Mean digestibilities for dry matter and nitrogen were 65.3 and 55.5%, respectively, and were not significantly differ-

ent between treatments. Nitrogen retention likewise was not significantly different between rations.

Harmon, Fontenot and Webb (1971) studied the nitrogen loss from dry heat and moist heat processed litter and the relative effects of processing upon litter nitrogen utilization, ration digestibility and ruminal and blood parameters in metabolism trials with lambs. The total nitrogen content of untreated, autoclaved, dry heat processed and acidified dry heat processed litters were reported as being 6.22, 5.55, 5.45 and 5.96%, respectively. The results of this study indicated that acidification of litter to a pH of approximately 6 with sulfuric acid prior to heat treatment reduced nitrogen loss by about 50%, as compared to dry heated litter which had not been acidified. In the balance trial, method of processing had no significant effect on nitrogen utilization. Apparent digestion coefficients likewise were not affected by treatment with the exception of a significant depression in crude fiber digestibility for the ration containing acidified dry heated litter, as compared to dry heating only. Blood urea levels, ruminal fluid pH, ruminal fluid ammonia nitrogen levels and VFA's were normal and were not significantly affected by treatment.

El-Sabban et al. (1970) processed poultry waste obtained from a caged layer operation by three different

methods: autoclaved for 30 min. at a steam pressure of 2.11 kg/cm²; cooked for 30 min. using steam at atmospheric pressure; and dried for 1 min. in a continuous flow forced-air dryer at temperatures ranging from 425 C at the entrance of the dryer to 150 C at the end of the drying process. Autoclaved poultry waste, cooked poultry waste or soybean meal were used as supplemental nitrogen sources in semi-purified isonitrogenous rations fed to wethers in metabolism stalls. Dry matter digestibility was not significantly different among rations, but the digestibility of protein was significantly higher for the ration containing soybean meal as the nitrogen source. Fecal nitrogen excretion was significantly lower for the ration containing soybean meal than for either of the rations containing poultry waste. The autoclaved poultry waste fed to the sheep contained 93.15% dry matter and 7.70% nitrogen. The dried poultry waste contained 91.17% dry matter and 3.98% nitrogen while the cooked poultry waste contained 93.20% dry matter and 7.67% nitrogen.

Buyss and Potgieter (1959) added various levels of uric acid to uric acid - free poultry excreta in an attempt to evaluate the effectiveness of lithium carbonate extraction of uric acid from poultry feces. The instability of uric acid to heat was established when recoveries of 44 to 75% were obtained for the added uric acid when extrac-

tion took place using an 80 C solution of lithium carbonate. Extraction using a lithium carbonate solution cooled to room temperature resulted in recoveries ranging from 97.2 to 100.9%.

Certain nutrients present in litter make it a potential source of nitrogen for ruminants. Nitrogen containing components are, however, subject to destruction by processing and this must be considered when developing methods of pasteurization.

OBJECTIVES

Experiments were conducted to determine the effect of different processing methods on pasteurization and nitrogen loss of broiler litter and to study the relative effects of processing method on broiler litter nitrogen utilization, ration digestibility and blood and ruminal parameters when litter was fed to sheep.

EXPERIMENTAL PROCEDURE

Experiment 1. The following broiler litter processing methods were used to study effectiveness in the pasteurization of litter and the effect upon nitrogen loss from litter:

1. Dry heating in a forced-draft oven at 150 C for 10, 15 and 20 minutes with the litter at a depth of 0.63 cm.
2. Autoclaving at 121 C under steam pressure of 1.05 kg/cm² for 5, 10, 15 and 30 minutes with the litter at a depth of 5.08 cm.
3. Dry heating in a forced-draft oven at 150 C for 15 minutes at litter depths of 0.63 and 2.54 cm following the addition of 0, 1, 2 and 4 g of paraformaldehyde per 100 g of litter.
4. Fumigation with ethylene oxide at 22 C for 30, 60 and 120 minutes.

Metal pans with dimensions 15 x 15 x 5 cm, with perforated bottoms were used for the litter processed by the two dry heat processes and the autoclave process. The litter processed by dry heating alone was evenly spread to a depth of 0.63 cm in a metal pan, and the pan was placed approximately in the center of the oven.

Litter to be autoclaved was evenly spread to a depth level with the top of the metal pan (5.08 cm), and the pan was placed in the center of the autoclave.

In the dry heat plus paraformaldehyde treatment, 0, 1, 2 or 4 g of dry paraformaldehyde were mixed with 100 g of litter by rotating in a 1.9 liter glass jar held horizontally. The paraformaldehyde treated litter was then evenly spread to a depth of 0.63 or 2.54 cm in the metal pans, and the pans were randomly placed in the approximate center of the oven.

In the ethylene oxide fumigation process, a glass sample jar with an approximate volume of 120 ml was filled to a depth of 7.62 cm with litter prior to subjection to treatment. Each sample jar of litter processed by the ethylene oxide method was placed in a desiccator devoid of desiccant, and the desiccator was evacuated using a vacuum pump until a reading of 2.5 mm mercury was recorded on a McLeod vacuum gauge connected between the desiccator inlet and the vacuum pump. Ethylene oxide was then injected into the evacuated desiccator until the pressure inside the desiccator equalled that of the atmosphere. A mercury-filled U-tube 61 cm in length constructed from glass tubing having an outside diameter of 1.12 cm was connected to the evacuated desiccator between the desiccator inlet and the ethylene oxide source. Through this arrangement it was

assured that the exact volume of air removed from the desiccator was replaced with an equal volume of ethylene oxide.

In all cases, measurement of processing time was begun when all of the specified conditions for the appropriate processing method were satisfied.

All the broiler litter¹ processed in this study was obtained from one commercial, insulated, humidity-controlled broiler house and was ground through a 0.63 cm mesh screen and thoroughly mixed in a horizontal mixer prior to initiation of the study.

A modification of the procedure used to test pasteurized milk (Anonymous, 1967) was adopted to perform total bacteria and coliform counts on each litter sample. The criteria for determining the effectiveness of each processing method for the pasteurization of litter were less than a total of 20,000 bacteria and less than 10 coliforms per gram by plate count. These are essentially the standards used for pasteurized milk.

A qualitative test for the presence of the enteric bacteria genera of Salmonella, Shigella and Proteus was also performed on each sample (Lewis, 1964). Bacterial determinations, both quantitative and qualitative, included in all cases a sample of unprocessed litter.

¹Obtained from Rockingham Poultry Marketing Cooperative, Broadway, Va.

The effect of vacuum alone on pasteurization of litter was examined in connection with the process involving ethylene oxide fumigation. Formaldehyde solutions are known to have a bactericidal effect, and, since the plating procedure by which total bacteria and coliforms were enumerated recommends homogenization of 1 g of litter in 99 ml of sterile distilled water to make an initial 1:100 dilution, a test was conducted in order to determine the extent to which bacterial numbers would be reduced by the formation of a formaldehyde solution caused by dissolution of residual paraformaldehyde in water. Residual paraformaldehyde, if existing in litter after heating, could result in lower than actual bacterial counts. Therefore, samples containing 1, 2 and 4 g of paraformaldehyde per 100 g of litter were plated without being heated in order to ascertain whether the bactericidal effect of the paraformaldehyde through heating at 150 C or whether the bacteria were killed by the solution containing formaldehyde which was created when the litter samples were homogenized in distilled water.

Samples of litter from processes which proved to be effective for the pasteurization of litter and all unprocessed samples were finely ground through a 1 mm mesh screen and subjected to analysis for nitrogen fractions by the following methods: total nitrogen, A.O.A.C. (1970); am-

monia nitrogen, magnesium oxide method for ammonia in fertilizer (A.O.A.C., 1970); true protein, nitrogen determination following precipitation with tungstic acid; uric acid, Buys and Potgieter (1959). Standard errors were calculated on the total bacteria and coliform counts (Ostle, 1963). Nitrogen fraction averages for pasteurized samples were statistically analyzed within processing methods by least squares analysis of variance (Ostle, 1963) and significant differences among treatment means were tested by the multiple range test of Duncan (1955).

Experiment 2. Eighteen wethers, averaging 49.8 kg initially, were used in a metabolism trial to study the relative effects of processing method of broiler litter on nitrogen utilization, ration digestibility and blood and ruminal parameters. The sheep were assigned to six outcome groups (blocks) of three on the basis of weight and breed, and the sheep within each outcome group were randomly allotted to three rations. Internal parasites were controlled by drenching all sheep with thiabendazole² prior to the initiation of the metabolism trial.

Broiler litter used in the metabolism trial was obtained from an insulated, humidity-controlled, commercial broiler house. One group of broilers had been raised on the

²Merck Chemical Division, Merck & Co., Inc., Rahway, N.J.

wood shaving base. Approximately equal amounts of litter were processed by the following three methods:

1. Dry heating in a forced-draft oven at 150 C for 20 minutes.
2. Dry heating in a forced-draft oven at 150 C for 15 minutes immediately following the addition of 2 g of paraformaldehyde (PFA) per 100 g of litter.
3. Ethylene oxide (ETO) fumigation at 22 C for 60 minutes.

In the process involving dry heat and dry heat plus paraformaldehyde the litter was evenly spread to depths of 0.63 cm and 2.54 cm, respectively, in perforated bottom metal pans with dimensions 47 x 46 x 5 cm. Four pans of each litter were processed during each processing period. The position of the pans in the oven was rotated between periods. The paraformaldehyde was manually mixed with the litter in a vertical bucket immediately before each processing period. In the ethylene oxide fumigation treatment, the processing was performed exactly as it was in Experiment 1 with the exception that the litter was evenly spread to a depth of 15.24 cm in a desiccator before fumigation was started. Total bacteria and coliform counts and the test for Salmonella, Shigella and Proteus were conducted on samples of each of the processed litters prior to mixing of the

litters into rations.

The composition of the rations used in the experiment is presented in table 1. Proximate analysis and calcium and phosphorus determinations were performed on the ground corn cobs, ground ear corn, ground hay and processed litters before formulation of the experimental rations. Ground corn cobs, ground ear corn and ground hay were present in equal amounts in all rations. The levels of other ingredients were altered in an attempt to equalize crude protein, crude fiber, calcium and phosphorus among rations. An additional stipulation in formulating the rations was that half of the total ration nitrogen was to be contributed by processed broiler litter. Vitamins A and D were supplemented in the rations at the level of 800 IU and 150 IU per kilogram of total ration, respectively. In an attempt to equalize dry matter intake the sheep were fed 500, 505 and 508 g of the dry heat, dry heat plus PFA and ETO rations, respectively, plus 5 g of trace mineralized salt at 7:30 am and 7:30 pm (total of 1000, 1010 and 1016 g/day, respectively). Water was available ad libitum except during the 2-hour feeding periods.

Preparation of the three litters for processing and feeding involved grinding in a small hammer mill fitted with a 0.63 cm mesh screen. The ear corn was also ground through a 0.63 cm mesh screen, and the corn cobs and hay

TABLE 1. COMPOSITION OF EXPERIMENTAL RATIONS

Item	Litter processing methods		
	Dry heat	Dry heat + paraform- aldehyde	Ethylene oxide
Ingredient composition, %			
Ground corn cobs	40.00	40.00	40.00
Ground ear corn	24.00	24.00	24.00
Ground hay	5.00	5.00	5.00
Dry heat litter	18.62	---	---
Dry heat + PFA litter	---	17.56	---
ETO litter	---	---	17.11
Corn sugara	12.38	12.49	12.55
Cellulose ^b	---	0.74	1.09
Defluorinated phosphate	---	0.21	0.25
Vitamins A ^c & D ^d	++	++	++
Chemical composition			
Dry matter, %	91.07	89.58	88.77
Composition of dry matter, %			
Crude protein	10.18	9.88	9.56
Ether extract	1.70	1.96	1.73
Crude fiber	20.58	22.89	22.42
Ash	4.08	4.18	3.83
NFE	63.46	61.09	62.46

^aCerelose, Corn Products Refining Co., New York, N.Y.

^bSolka-floc, BW-20, Brown Co., Berlin, N.H.

^cFed to supply 800 I.U. per kilogram of ration.

^dFed to supply 150 I.U. per kilogram of ration.

were ground through a 1.9 cm mesh screen. The ground hay, processed litter, corn sugar, cellulose, defluorinated phosphate and vitamins A and D were mixed into premixes for the respective rations. The premixes, ground corn cobs and ground ear corn were mixed by hand at each feeding to insure uniform intake. Feeds were sampled at each feeding starting 2 days prior to the beginning and ending 2 days prior to the end of the collection period.

The metabolism trial consisted of a 10-day preliminary period followed by a 10-day collection period. During both the preliminary and collection periods the sheep were maintained in metabolism stalls similar to those of Briggs and Gallup (1949) facilitating separate collection of urine and feces. Urine was collected in glass jars to which had been added approximately 250 ml of water and 15 ml of a 1:1 (w/w) mixture of concentrated sulfuric acid and water. Each 24-hour urine collection was diluted to a constant weight, and a 2% sample by volume was taken and stored under refrigeration in air-tight 1 liter plastic bottles. The total fecal excretion was collected daily and dried for 24 hr. in a forced-draft oven at a maximum temperature of 60 C. Daily collections were composited by animal in metal cans with loosely fitting lids and allowed to equilibrate with atmospheric moisture after being dried. Composites were weighed and sampled at the end of the collection period.

Samples of litters, feeds, premixes and air equilibrated feces were finely ground through a 1 mm mesh screen in preparation for proximate analysis and calcium and phosphorus determinations. Crude fiber in these samples was determined by the method of Whitehouse, Zarrow and Shay (1945). Determination of urinary nitrogen and moisture, nitrogen, ether extract and ash in litters, feeds, premixes and feces was performed using A.O.A.C. (1970) procedures. Calcium analysis of litters and feeds was performed by Perkin-Elmer 403 atomic absorption spectrophotometric methods and phosphorus analysis by the method of Fiske and Subbarow (1925) after dry ashing by the procedure of Chapman and Pratt (1961).

At the end of the collection period rumen samples were taken via a stomach tube 2 hr. after the morning feeding. The rumen contents were strained through four layers of cheesecloth, and pH of the strained fluid was determined electrometrically. Analyses for ammonia nitrogen (Conway, 1958) and volatile fatty acids (Erwin, Marco and Emery, 1961), were performed on the strained rumen fluid.

Blood samples were taken on the same day as the rumen ingesta samples by jugular vein puncture 6 hr. after the morning feeding. The blood samples were analyzed for blood urea by the method of Coulombe and Favreau (1963).

The data from the trial were analyzed by analysis of

variance (Ostle, 1963). Significant differences among treatment means were tested by using the multiple range test of Duncan (1955).

RESULTS AND DISCUSSION

Experiment 1

As shown in table 2, unprocessed litter was found to be highly contaminated.

Dry heating at 150 C for 10, 15 or 20 min. completely eliminated coliforms but total bacteria were reduced to an acceptable count only by heating for 20 min. Similarly, Connell and Garrett (1963) reported reductions in bacterial counts for sludge dried by flash drying equipment at temperatures ranging from 88 to 143 C, and Messer et al. (1971) found E. coli to be destroyed and total aerobes to be reduced by heating poultry litter at temperatures ranging from 57.2 to 73.8 C for periods of 30 or 60 min.

Autoclaving for 5, 10, 15 or 30 min. also eliminated coliforms, but total bacteria counts were reduced to less than 20,000 per gram only by autoclaving for 10 min. or longer. Warden and Schaible (1961) reported reduced counts of total cocci, enterococci and E. coli in intestinal contents of chicks fed autoclaved hen fecal material, as compared to chicks receiving fresh, unprocessed hen feces. Although their method of measuring bacterial count reduction was indirect, the bactericidal properties of moist

TABLE 2. EFFECT OF PROCESSING ON BROILER LITTER PASTEURIZATION

Processing method and temperature	Processing time	Litter depth cm	Other conditions	Coliforms per gram ^{a,b}	Total bacteria per gram ^a
Unprocessed				>30,000	>30,000
Dry heat (150 C)	10 min.	0.63		0	61,750± ^c
	15 min.			0	20,059±
	20 min.			0	5,247±
Autoclave (121 C)	5 min.	5.08	Steam pressure of	0	49,198±
	10 min.		1.05 kg/cm ²	0	9,348±
	15 min.			0	873±
	30 min.			0	185±
Paraformaldehyde added followed by dry heating (150 C)	15 min.	0.63	0g ^d /100 g litter	0	26,267±
			1g/ "	0	3,463±
			2g/ "	0	1,929±
			4g/ "	0	1,630±
	2.54		0g/ "	0	89,090±
			1g/ "	0	18,837±
			2g/ "	0	2,607±
			4g/ "	0	352±
Ethylene oxide fumigation (22 C)	30 min.	7.62	Desiccator evacuated	1.25	18,117±
	60 min.		to 2.5 mm Hg pressure	0	5,147±
	120 min.			0	700±

^aMeans of six replications per treatment.

^bAll samples tested negative for Salmonella, Shigella and Proteus.

^cStandard errors of means.

^dLevel of paraformaldehyde.

heat was demonstrated.

The addition of 1 g of PFA to 100 g of litter and heating for 15 min. at 150 C at a depth of 2.54 cm produced a marginal response. The addition of higher levels of PFA appeared to be quite effective. Although heating the litter in a forced draft oven for 15 min. at 150 C and a depth of 0.63 cm without PFA treatment was not effective in reducing the total bacteria count to less than 20,000 per gram, the addition of 1 to 4 g of PFA to litter processed under similar conditions did, however, depress the total bacterial count to acceptable levels at thicknesses of 0.63 or 2.54 cm. Seltzer et al. (1969) found that the addition of 1, 3 and 7 g of PFA to 100 g of fresh chicken feces reduced total bacterial counts from 2.2 billion per gram for the untreated feces to 164 million, 1,000 and 0 organisms per gram, respectively.

It was found that the addition of 1, 2 or 4 g of PFA without heating gave total bacteria counts of greater than 300,000 bacteria per gram of litter for 1 and 2 g of PFA per 100 g of litter and a count of 63,125 bacteria per gram of litter for 4 g of PFA per 100 g of litter. One gram of PFA per 100 g of litter also permitted the survival of 2,992 coliforms per gram of litter. From this it can be concluded that heating of the litter was necessary in order for PFA to manifest its pasteurizing effect.

Fumigation with ethylene oxide for 30 min. showed marginal effectiveness with respect to total bacterial count, but the counts obtained with the longer fumigation periods were more desirable. The 30 min. fumigation treatment also permitted the survival of 1.25 coliforms per gram. Fumigation for 60 min. or longer was quite effective. This is in agreement with work by Messer et al. (1971) in which bacterial counts for Salmonella spp., E. coli and an Arizona sp in poultry litter were reportedly reduced by ETO fumigation under pressure for 4 to 17 hours at room temperature. Vacuum alone was not effective in pasteurizing the litter in the present study.

Thus, the minimum effective treatments included heating in a forced draft oven at 150 C for 20 min. at a thickness of 0.63 cm; autoclaving for 10 min. or longer; heating in a forced-draft oven at 150 C with litter at a depth of 0.63 or 2.54 cm following the addition of 1 to 4 g of PFA to 100 g of litter; and fumigation with ethylene oxide for 30 min. or longer.

All of the processed and unprocessed samples of litter examined gave a negative test for Salmonella, Shigella and Proteus spp. A negative test for Shigella in poultry litter is to be expected according to Smith et al. (1964). Salmonella and proteus have been found in poultry excreta by Alexander et al. (1968), Kraft et al. (1969) and

Zindel (1970). Since the litter used in this study was maintained in burlap bags from the time it was removed from the house until it was ground in preparation for processing, ample time was available for the litter to go through a heating period.

Table 3 shows the dry matter and nitrogen composition of pasteurized and unprocessed (control) samples of litter. Dry matter of the pasteurized samples was significantly ($P < .01$) increased over the control samples by both dry heat alone or following PFA treatment. Depth of litter heated following PFA treatment also significantly ($P < .01$) influenced the dry matter content of samples.

In the dry heat process, total nitrogen was significantly ($P < .01$) depressed by heating for 20 min. These data substantiate the work of El-Sabban et al. (1970) and Harmon et al. (1971) in that a loss of total nitrogen accompanied dry heating of poultry litter. Autoclaving did not cause a loss in total nitrogen in the present litter pasteurizing study. Harmon et al. (1971), however, did show some loss of nitrogen as a result of autoclaving litter. Total nitrogen was significantly ($P < .01$) depressed by heating PFA treated litter for 15 min. at a depth of 0.63 cm. Fumigation with ETO for 30, 60 and 120 min. resulted in significantly ($P < .05$) lower levels of total nitrogen.

TABLE 3. EFFECT OF PROCESSING BROILER LITTER ON DRY MATTER AND NITROGEN COMPOSITION

Process	Dry Basis						
	Dry matter %	Total N %	Protein N %	Non-protein N %	Uric acid N %	Ammonia N %	
Dry heat							
Control	84.11 ^a	3.97 ^a	2.35 ^a	1.63	0.37 ^a	0.88 ^a	
20 min.	97.13 ^b	3.49 ^b	2.16 ^b	1.34	0.24 ^b	0.36 ^b	
Autoclave							
Control	85.26	4.04	2.38 ^a	1.66 ^a	0.38 ^{d,e}	0.87 ^a	
10 min.	84.31	4.14	2.74 ^b	1.40 ^{a,b}	0.41 ^d	0.84 ^a	
15 min.	85.32	4.14	2.81 ^b	1.33 ^b	0.35 ^e	0.76 ^b	
30 min.	86.18	4.11	2.79 ^b	1.31 ^b	0.34 ^e	0.75 ^b	
Paraformaldehyde added							
Control	84.35 ^a	4.21 ^a	2.22 ^{a,b}	2.00 ^a	0.39 ^a	0.83 ^a	
0.63cm ¹ , 1g	96.52 ^b	3.74 ^b	2.25 ^{a,b}	1.49 ^b	0.28 ^b	0.48 ^b	
0.63cm, 2g	96.44 ^b	3.74 ^b	2.18 ^a	1.56 ^c	0.23 ^b	0.44 ^b	
0.63cm, 4g	96.12 ^b	3.88 ^b	2.39 ^b	1.49 ^b	0.26 ^b	0.46 ^b	
2.54cm, 1g	88.26 ^c	4.02 ^{a,b}	2.75 ^c	1.26 ^b	0.37 ^a	0.72 ^{a,b}	
2.54cm, 2g	88.32 ^c	4.03 ^a	2.77 ^c	1.26 ^b	0.39 ^a	0.62 ^{a,b}	
2.54cm, 4g	89.07 ^c	4.04 ^a	2.79 ^c	1.25 ^b	0.42 ^a	0.50 ^b	

TABLE 3 - CONTINUED

Process	Dry matter	Dry Basis				
		Total N	Protein N	Non-protein N	Uric acid N	Ammonia N
	%	%	%	%	%	%
Ethylene oxide fumigation						
Control	87.25	4.12 ^d	2.23 ^a	1.89	0.40	0.85 ^a
30 min.	89.34	3.88 ^e	1.98 ^b	1.90	0.40	0.71 ^b
60 min.	88.90	3.97 ^e	2.03 ^b	1.93	0.37	0.65 ^{b,c}
120 min.	89.40	3.98 ^e	2.11 ^{a,b}	1.87	0.41	0.62 ^c

a,b,c Means within one process with different superscript letters are significantly different (P < .01).

d,e Means within one process with different superscript letters are significantly different (P < .05).

f Litter depth.

g Amounts of paraformaldehyde.

Protein nitrogen was significantly ($P < .01$) lowered by dry heat for 20 min., but autoclaving the litter for 10, 15 and 30 min. significantly ($P < .01$) increased the protein percentages as was the case when litter was heated at a depth of 2.54 cm after the addition of 1, 2 or 4 g of PFA to 100 g of litter. Fumigation with ETO for 30 and 60 min. significantly ($P < .01$) reduced protein nitrogen.

There is no easy explanation for the increase in protein nitrogen in the litters processed by autoclaving and dry heating following the addition of PFA. Likewise, the loss of protein nitrogen from the samples fumigated with ETO for 30 and 60 min. is difficult to explain.

Autoclaving generally decreased non-protein nitrogen. The differences were significant ($P < .01$) for the litter autoclaved for 15 or 30 min. A significant ($P < .01$) decrease in non-protein nitrogen was observed for the addition of PFA at levels of 1, 2 and 4 g per 100 g of litter followed by heating for 15 min. at a depth of 2.54. The effects on non-protein nitrogen were less severe at the lower litter thickness.

A significant ($P < .01$) loss in uric acid nitrogen accompanied dry heating for 20 min. and heating at a depth of 0.63 cm following the addition of 1, 2 or 4 g of PFA to 100 g of litter. Also, litter pasteurized by autoclaving for 15 or 30 min. resulted in lowered uric acid nitrogen

levels ($P < .05$). Uric acid loss as a result of heat, regardless of whether moist or dry, does agree with the findings of Buys and Potgieter (1959). They found that uric acid in poultry excreta was destroyed upon extraction with hot lithium carbonate.

Ammonia nitrogen was significantly affected by all four processes. A significant ($P < .01$) reduction in ammonia nitrogen levels below that of the respective controls was observed for dry heating for 20 min.; autoclaving for 15 or 30 min.; heating at a depth of 0.63 cm following all levels of PFA treatment and heating at a depth of 2.54 cm following the addition of 4 g of PFA to 100 g of litter; and ETO fumigation for 30 or 120 min.

The data of the present experiment showing that there was generally a loss of ammonia nitrogen in the PFA treated litter do not agree with the results of Seltzer et al. (1969). They found improved ammonia retention by poultry feces as the level of added PFA increased from 1 to 7 g per 100 g of fresh feces. Ammonia loss in the present study was likely a function of heating. It appears that the PFA treatment may have reduced ammonia loss since the loss in the PFA treated samples appeared to be less than in the dry heat samples. Seltzer et al. (1969) applied no heat to PFA treated fecal samples, and the PFA was permitted to bind with the ammonia present in the fresh feces to form hexa-

methylenetetramine. Heating of the litter in the present experiment may have expedited the conversion of PFA to formaldehyde gas, thus preventing binding of ammonia since the ammonia and formaldehyde gas were probably simultaneously driven off by the heating.

Ammonia, and consequently, total nitrogen loss from ETO fumigation can be explained as a physical rather than a chemical phenomena as the evacuation of the desiccator exposure chamber prior to injection of gaseous ETO would tend to draw off ammonia from the litter.

Experiment 2

The ETO fumigation process used to pasteurize litter used in the metabolism trial was altered from the one employed in Experiment 1 in order to be able to process a relatively large quantity of litter in a short period of time. Since data obtained in Experiment 1 indicated that dry heating at 2.54 cm following the addition of 1 g of PFA to 100 g of litter and fumigation with ETO for 30 min. provided only marginal effectiveness with respect to pasteurization, the 2 g PFA level and 60 min. ETO fumigation period were chosen to pasteurize litter used in the metabolism trial.

Total bacteria and coliform counts and the chemical composition of the dry heat (control), dry heat plus PFA

and ETO processed litters are presented in table 4. Bacterial counts were within the limits of pasteurized litter. Dry matter was increased, and crude protein was decreased by both processes involving heat. Changes in dry matter, crude protein and ether extract were greatest in litter pasteurized by dry heating alone. Crude fiber and ash were not appreciably affected by processing method.

Three of the six wethers fed the diet containing ETO processed litter had feed refusals during the first 2 days of the preliminary period. Two of the three animals continued to refuse part of the feed allowed through the eighth day of the preliminary period, but all palatability problems ceased beyond that time. No attempt was made to draw off residual ETO from the pasteurized litter after processing, and the odor of ETO could be detected even after incorporation of the ETO processed litter into the ration at a level of 17.11%. During fumigation of the litter, ETO was observed to have an irritating effect on skin abrasions. Refusals may therefore have been due to irritation of soft tissues by ETO. Depressed intake was reported by Hawk and Mickelsen (1955) and Oser and Hall (1956) when ETO fumigated diets were fed to rats.

Nitrogen Utilization. Nitrogen utilization data are presented in table 5. Nitrogen intakes, expressed as grams per day, were 14.83, 14.30 and 13.78 for the dry heat, dry

TABLE 4. TOTAL BACTERIA AND COLIFORM COUNTS AND CHEMICAL COMPOSITION OF LITTERS FED TO SHEEP

Item	Litter processing methods		
	Dry heat	Dry heat + paraform- aldehyde	Ethylene oxide
Total bacteria per gram	6,858	10,574	5,825
Coliforms ^a per gram	0	0	0
Chemical composition			
Dry matter, %	98.84	88.60	83.33
Composition of dry matter			
Crude protein	24.44	28.92	31.55
Ether extract	2.71	4.82	4.19
Crude fiber	21.60	20.90	20.42
Ash	14.45	14.42	14.47
NFE	36.80	30.94	29.37

^aAll samples tested negative for salmonella, shigella and proteus.

TABLE 5. NITROGEN UTILIZATION AND APPARENT DIGESTIBILITY BY SHEEP FED RATIONS CONTAINING PROCESSED BROILER LITTER

Item	Litter processing methods		
	Dry heat	Dry heat + paraform- aldehyde	Ethylene oxide
Nitrogen utilization			
Nitrogen intake, g/day	14.83	14.30	13.78
Nitrogen excretion, g/day			
Fecal	6.94	6.65	6.31
Urinary	5.79	5.38	5.90
Total	12.73	12.03	12.21
Nitrogen retention			
Grams per day	2.10	2.27	1.58
Percent of intake	14.2	15.9	11.4
Percent of absorbed	25.0	29.4	21.1
Apparent digestibility, %			
Dry matter	67.6	66.5	67.1
Crude protein	55.9	53.9	54.2
Ether extract	77.2	72.8	76.2
Crude fiber	54.5	58.8	57.8
NFE	75.4	73.4	74.3

heat plus PFA and ETO treatments, respectively.

Fecal, urinary and total nitrogen excretion did not differ significantly among the three treatments. Fecal nitrogen excretion was greatest for the control group and least for the ETO group, possibly a reflection of differences in nitrogen intake. Urinary nitrogen excretion tended to be higher for the ETO treatment. Total nitrogen excretion was lowest in the dry heat plus PFA group and highest in the control group.

Nitrogen retention was not significantly affected by treatment, regardless of the manner in which expressed. Harmon et al. (1971) obtained retention values similar to the values found in this study for rations into which they incorporated heat processed litter.

The trend toward higher nitrogen retention values for the dry heat plus PFA group may have resulted from partial binding of the litter ammonia nitrogen in the form of hexamethylenetetramine as well as hardening of proteins by the formaldehyde, thus accomplishment of rumen bypass. Schmidt et al. (1971), Schmidt et al. (1972) and Nishimuta et al. (1972) reported no improvement in nitrogen retention as a result of aldehyde treatment of protein. Schmidt et al. (1971) did, however, report improved nitrogen retention in rats when soybean meal protein treated with hexamethylenetetramine was fed.

Apparent Digestibility. The apparent digestibility data are also presented in table 5. Processing method did not significantly affect the apparent digestibility of dry matter, crude protein, ether extract, crude fiber and NFE. Apparent digestibility coefficients were lowest for all components except crude fiber in the dry heat plus PFA group. Crude fiber digestibility was, in fact, higher in this group than in either of the others. Dry matter digestibility was quite uniform between treatments as was the apparent digestibilities for crude protein and NFE.

The apparent digestibility coefficients obtained in this trial are approximately the same as those obtained by Harmon et al. (1971) for conventional rations in which litter nitrogen supplied 50% of the ration nitrogen. Nishimuta et al. (1972) reported depressed nitrogen digestion in lambs fed formaldehyde treated soybean meal. In the same study, heat treatment of soybean meal lowered cellulose digestibility. These findings agree in part with the results observed in this trial since dry matter digestibility and nitrogen digestibility were lowest for the ration containing PFA processed litter. Apparent crude fiber digestibility was lowest for the ration containing heat treated litter. El-Sabban et al. (1970) reported no significant difference in dry matter digestibilities among

rations when autoclaved, cooked and dried poultry wastes were compared to soybean meal as supplementary nitrogen sources for wethers. Mean digestibilities for dry matter and nitrogen were 65.3 and 55.5%, respectively, as reported by Smith and Calvert (1972) when dried poultry wastes were fed to wethers at levels sufficiently high to supply 0, 50 or 100% of the crude protein content of the ration. These values are in close agreement with the values obtained for dry matter and nitrogen digestibilities for the dry heat and dry heat plus PFA treatments used in this study.

Ruminal Fluid and Blood Parameters. Average ruminal fluid pH values are presented in table 6. The value for the dry heat plus PFA treatment was the lowest while the value for the ETO treatment was highest, but no significant differences were observed. Harmon et al. (1971) found no significant difference in ruminal fluid pH values of sheep fed autoclaved or dry heated litter or soybean meal as the supplementary nitrogen source.

Ruminal fluid ammonia nitrogen levels are also presented in table 6. The value of 21.0 mg/100 ml for the control group was significantly ($P < .05$) higher than the level of 16.7 mg/100 ml for the ETO group. The value of 19.8 mg/100 ml for the dry heat plus PFA group was intermediate. Harmon et al. (1971) found ruminal fluid ammonia

TABLE 6. RUMINAL FLUID pH AND AMMONIA AND BLOOD UREA OF SHEEP FED RATIONS CONTAINING PROCESSED BROILER LITTER

Item	Litter processing methods		
	Dry heat	Dry heat + paraform- aldehyde	Ethylene oxide
Ruminal fluid			
pH	6.56	6.42	6.62
NH ₃ -N, mg/100 ml	21.0 ^a	19.8 ^{a,b}	16.7 ^b
Blood urea, mg/100 ml	17.4	14.3	11.8

^{a,b} Means with different superscript letters are significantly (P < .05) different.

nitrogen levels to be elevated by the inclusion of litter in rations for lambs, and his values ranged from 16.0 to 16.6 mg/100 ml which agrees with the value of 16.7 mg/100 ml obtained for the group which received ETO fumigated litter.

All ruminal ammonia nitrogen values obtained in this trial are lower than those obtained by Bhattacharya and Fontenot (1965). Ruminal fluid ammonia nitrogen levels obtained in their trial were 51.0 and 42.7 mg/100 ml when litter nitrogen replaced 25 and 50%, respectively, of the soybean protein nitrogen. They, however, fed purified diets containing greater than 11% crude protein, compared to approximately 10% crude protein used in the present trial. Furthermore, they used isolated soy protein which would be more easily degraded than the protein used in this trial.

The lower ruminal ammonia nitrogen levels obtained in the present metabolism trial are in agreement with the results of Schmidt et al. (1972), who, reported a depression in the release of rumen ammonia nitrogen in vitro by formaldehyde treatment of soybean meal. Since nitrogen retention (table 5) for the dry heat plus PFA tended to be higher than for dry heat, perhaps some rumen bypass was accomplished by processing the litter with the addition of PFA.

Blood urea levels (table 6) were within a normal

range and followed the same general trend as the rumen ammonia nitrogen levels but were not significantly different among treatments. The values for the dry heat and dry heat plus PFA groups were similar to those obtained by Harmon et al. (1971) but were higher than those obtained by Bhattacharya and Fontenot (1965). The value of 11.8 mg/100 ml for the ETO treatment is very close to the value of 12.0 obtained by Bhattacharya and Fontenot (1965) when autoclaved litter nitrogen replaced 50% of the soybean protein nitrogen.

Depressed blood urea nitrogen levels were recorded when formaldehyde treated soybean meal was fed to lambs by Nishimuta et al. (1972).

Table 7 shows the average ruminal fluid volatile fatty acid concentrations of the three groups of wethers fed pasteurized litter. When expressed as μ mole/ml, the only VFA that was significantly affected by treatment was acetic. It was significantly ($P < .01$) higher for the dry heat plus PFA treatment than for the control. Similarly, total VFA's were significantly ($P < .05$) greater for the dry heat plus PFA treatment than for the control. In terms of moles/100 moles, acetic was significantly ($P < .05$) higher for the dry heat plus PFA group than for either of the other groups, but propionic was significantly ($P < .05$) lower for the dry heat plus PFA treatment than

TABLE 7. RUMINAL FLUID VOLATILE FATTY ACID CONCENTRATIONS OF SHEEP FED RATIONS CONTAINING PROCESSED BROILER LITTER

Item	Litter processing methods		
	Dry heat	Dry heat + paraform- aldehyde	Ethylene oxide
Volatile fatty acids, μ mole/ml			
Acetic	41.0 ^a	52.1 ^b	46.3 ^{a,b}
Propionic	15.5	16.8	19.1
Butyric	8.7	11.0	8.3
Valeric	1.5	1.5	1.4
Isobutyric	1.9	2.2	1.9
Isovaleric	1.4	1.5	1.5
Total	70.0 ^c	85.0 ^d	78.5 ^{c,d}
Volatile fatty acids, moles/100 moles			
Acetic	58.6 ^c	61.3 ^d	58.9 ^c
Propionic	22.1 ^{c,d}	19.7 ^c	24.3 ^d
Butyric	12.5	12.8	10.6
Valeric	2.2	1.7	1.8
Isobutyric	2.7	2.6	2.4
Isovaleric	2.0	1.8	1.9

^{a,b} Means with different superscript letters are significantly ($P < .01$) different.

^{c,d} Means with different superscript letters are significantly ($P < .05$) different.

it was for the ETO treatment. No other VFA's were significantly affected by treatment when expressed as moles/100 moles. Harmon et al. (1971) showed no significant difference in VFA concentrations regardless of the manner in which expressed when litter was fed to lambs.

Acetic acid, expressed as μ mole/ml, was highest for the two treatments in which a chemical sterilant was employed to pasteurize the litter. There is a possibility of residual PFA or ETO being present in the litter incorporated into the final rations. The difference in concentrations of acetic among treatments may be a reflection of rumen microbial population alteration as a result of bactericidal properties exerted by ingested residual PFA or ETO, or their derivatives.

It may be concluded from data obtained in this study that versatility exists among the numerous methods by which contaminated broiler litter can be pasteurized. It should be kept in mind, however, that certain of the methods which were satisfactory for the pasteurization of litter result in a significant loss of nitrogen from nitrogen containing components within litter, and these losses must be minimized in order for litter to attain a position as an economically important feedstuff in the livestock feeding industry. Certain pasteurizing methods involving the chemical bactericides, paraformaldehyde and ethylene oxide, appear to

exert no profound effects upon nitrogen utilization, ration digestibility or blood and ruminal parameters, when compared to the dry heat pasteurization of broiler litter.

SUMMARY

Various broiler litter processing methods were evaluated. The treatments effective for litter pasteurization were dry heating at a depth of 0.63 cm for 20 min.; autoclaving for 10 min. or more; dry heating following the addition of 1 to 4 g of paraformaldehyde (PFA) to 100 g of litter at depths of 0.63 and 2.54 cm; and ethylene oxide (ETO) fumigation for 30 min. or longer. Moisture and uric acid nitrogen were lowered ($P < .01$) for dry heat and the PFA processes. Autoclaving and PFA addition followed by dry heating significantly ($P < .01$) lowered NPN. Ammonia nitrogen was significantly ($P < .01$) reduced by all pasteurizing treatments except autoclaving for 10 min.

A metabolism trial was conducted to study the effects of different litter processing methods. Litter pasteurized by dry heating at a depth of 0.63 cm for 20 min., dry heating at a depth of 2.54 cm after the addition of 2 g of PFA to 100 g of litter or fumigation with ETO at a depth of 15.24 cm for 60 min. supplied 50% of the nitrogen in the respective rations. Method of processing had no significant effect on nitrogen utilization and apparent digestion coefficients. Ruminal fluid ammonia nitrogen was significantly ($P < .05$) higher for the dry heat treat-

ment than for the ETO treatment, but ruminal fluid pH and blood urea levels were not significantly affected. Acetic acid and total VFA's, expressed as μ mole/ml were significantly ($P < .05$) higher in the PFA treatment than in the dry heat treatment. When expressed as moles/100 moles, acetic was significantly ($P < .05$) higher for the PFA treatment than the other treatments, and propionic was significantly ($P < .05$) higher for the ETO treatment than for the PFA treatment.

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THE EFFECT OF PROCESSING METHOD OF BROILER LITTER
ON PASTEURIZATION, NITROGEN LOSS AND NITROGEN
UTILIZATION BY SHEEP

by

Larry Forrest Caswell

(Abstract)

Various broiler litter processing methods were evaluated. Treatments effective for litter pasteurization were dry heating at a depth of 0.63 cm for 20 min; autoclaving for 10 min. or longer; dry heating following the addition of 1 to 4 g of paraformaldehyde (PFA) per 100 g of litter at depths of 0.63 and 2.54 cm; and ethylene oxide (ETO) fumigation for 30 min. or longer. Moisture and uric acid nitrogen were lowered ($P < .01$) for dry heat and the PFA processes. Autoclaving and PFA addition followed by dry heating lowered ($P < .01$) NPN. Ammonia nitrogen was reduced ($P < .01$) by all pasteurizing treatments except autoclaving for 10 min. A metabolism trial was conducted with sheep. Litter pasteurized by dry heating at a depth of 0.63 cm for 20 min., dry heating at a depth of 2.54 cm after the addition of 2 g of PFA per 100 g of litter or fumigation with ETO at a depth of 15.24 cm for 60 min. supplied 50% of the nitrogen in the respective rations. Processing method had no significant effect on nitrogen utilization

and apparent digestion coefficients. Ruminal ammonia nitrogen was higher ($P < .05$) for the dry heat than for the ETO treatment. Acetic acid and total VFA's, expressed as μ mole/ml were higher ($P < .05$) for the PFA than the dry heat treatment. Expressed as moles/100 moles, propionic was higher ($P < .05$) for the ETO than for the PFA treatment.