Characterization of Alkaline Hydroxide-Preserved Whole Poultry as a Dry Byproduct Meal[1]

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ABSTRACT Studies were conducted to examine the chemical preservation of whole broiler carcasses by using aqueous alkaline hydroxide solutions. Conversion of the preserved carcasses and solutions into an acceptable poultry byproduct meal was examined. Carcasses and alkaline solutions at a 1:1 ratio were blended and freeze-dried to produce a high fat whole poultry byproduct meal. The dry meal was analyzed for nutrient composition, true metabolizable energy, and amino acid content. Viable bacteria were not recovered after inoculation of the experimental meal with *Salmonella enteritidis*. The meal was incorporated at 5 and 10% of chick starter diets. Chicks found the meal-containing diets acceptable. Feed consumption, water consumption, BW, and mortality were not significantly different among the dietary treatments in either of the two feeding trials. Necropsy samples revealed no pathological or histological differences attributable to consumption of the alkaline poultry byproduct and blood serum evaluation found no variation in blood chemistry. Alkaline treatment of whole broiler carcasses was an effective preservation method and acceptable as a dry poultry byproduct meal.

(*Key words*: alkaline, hydroxide, byproduct, broiler, mortality)

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INTRODUCTION

The expansion of residential properties into once rural agricultural production areas has increased the need for prompt management of poultry production mortalities with emphasis on odor and pest control. The increase in feed protein costs and expansion of the poultry byproducts industry has increased the value of production mortalities. Research and development of carcass preservation and recovery technology may provide efficient mortality management and nutrient conservation.

An effective procedure should be able to preserve and stabilize mature fully feathered broilers, with minimal inputs of equipment, utilities, and labor. Effective handling of mortalities in the future should focus on the ultimate removal of poultry mortalities from the live production facility and their ultimate processing in a nutrient recovery facility. This procedure would allow the poultry industry to recoup protein, fats, and minerals while minimizing environmental impact and public concerns.

In response to these considerations, research investigations were undertaken to evaluate nutrient recovery of poultry by chemical preservation of whole broiler carcasses by using alkaline hydroxide (AH) in aqueous solutions. The use of broiler mortalities, as a quality source for poultry meal products, may be enhanced by preservation in aqueous AH solutions. An alkaline chemical preservation system could prove advantageous due to low resource input and ease of maintenance. The exclusion of putrefaction or rancidity would allow for holding of carcasses without excessive odors being generated. If feathers could be solubilized during the process before nutrient recovery by rendering, the prerendered or hydrolyzed material might be processed separately or rendered with other nonfeather-containing byproducts.

Alkaline Preservation of Poultry Meal

Poultry byproduct meals are produced to recover and conserve valuable protein and fat resources from the production and processing sectors of the poultry industry. Food grade AH are classified as generally recognized as safe (GRAS) chemicals when manufactured by nonmercury cell generation and meet the specifications for purity set forth in the Food Chemicals Codex of the Federal Registry (1981). Early methods to render feathers included the addition of sodium hydroxide (NaOH) as an accelerator to hydrolyze feathers, to lower temperature and time required for processing. Current guidelines for

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[1]The use of trade names in this publication does not imply endorsement by the Texas Agricultural Experiment Station nor criticism of similar ones not mentioned.

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Abbreviation Key: AH = alkaline hydroxide; APBPM = alkaline poultry byproduct meal; SE = *Salmonella enteritidis*.

hydrolyzed poultry feathers require meals to be free of additives or accelerators (AAFCO, 1994).

Latshaw (1990) reported that feather meal processing conditions did affect pepsin digestible protein and that, as pH or pressure were increased, cystine content decreased and lanthionine increased. The study also found that despite the changes due to processing, chicks in bioassay studies derived adequate sulfur amino acids from the different feather meals. Because treatment of four different protein isolates with NaOH was reported to reduce pepsin digestibility values, the authors theorized racemization of binding sites on the peptides prevented the attachment of enzymes (Hayashi and Kameda, 1980). Therefore, alkali treatments may not be accurately evaluated by using pepsin digestibility values. Fernon et al. (1978) reported on the toxicological aspects of alkali-treated proteins. Rats that were fed alkali-treated protein developed nephrocytomegaly. The authors related that this effect may have resulted from several factors in concert with the alkali treatment. These factors included the animal species, feeding duration, the chemical form and concentration of lysinoalanine, and the absence or presence of other high quality proteins within the diet.

Bland defibrillated proteins were extracted and recovered from insoluble beef connective tissue by treatment with heated alkali (Staackman and Furgal, 1962). Alkaline protein extraction was effective in extraction of protein from bovine and ovine offal (Young and Lawrie, 1974). Aqueous alkaline extraction processes were used to recover protein from mechanically deboned poultry meat (Young, 1975). Alkaline treatment of beef boning wastes produced a slurry, from which protein could be recovered by either procedure of acidification, freezing or heating (Jelen et al., 1979). G. W. Malone (1993, Research and Education Center, University of Delaware, Georgetown, DE 19947, personal communication) examined the ability of 17.5% hydrated lime (calcium hydroxide) in water-based solution to preserve carcasses as they were chopped by rotary blades. Whole birds with feathers were added at weekly intervals for 8 wk. Preserved chopped carcass analysis values for CP at 33.0% and fat content of 5.64% were reported. Samples were reported negative for salmonellae. The solution successfully digested feathers but produced calcium scale and ammonia odor at 7 wk. Shafer and Carey (1994) reported that 2.0 *M* of sodium hydroxide (NaOH) in aqueous solution produced extensive feather and carcass hydrolysis but prevented putrefactive odor production. Further research by the same investigators found that a 1:1 ratio of 2.0 *M* NaOH was adequate for carcass preservation beyond 60 d. The alkaline treatment produced an emulsified product with a pH of 13.5 and solids content of 41% (Shafer, et al., 2000). The objective of the present study was to characterize and gauge the acceptability of an experimental dry alkaline poultry by-product meal (APBPM).

MATERIALS AND METHODS

Alkaline-preserved poultry consisting of 2.0 *M* NaOH in aqueous solution and whole mature broiler carcasses with feathers at a 1:1 ratio (wt/wt) were allowed to react for 60 d to form a crude emulsion, then blended to a uniform consistency with a handheld blender.

Conversion to Dry Meal

The alkaline poultry product in the liquid state retained the foaming capability of AH, therefore conversion to APBPM was achieved by lyophilization. The alkaline product was placed in a commercial freezer at –14 C until solidified. The frozen material was transferred to a Thermovac FD-4[3] freeze-dryer at –50 C and dried under vacuum. Freeze-drying was performed on aliquots of 200 g until moisture content was less than 10% by weight. The dry APBPM product was ground to a uniform particle size, passable through a no. 10 (2 mm) standard wire mesh testing sieve.[4] The APBPM was placed in sealed polyethylene bags and held at –14 C until analyzed. Fat was not mechanically pressed from the meal product. The APBPM product was equivalent to a high fat whole poultry meal in color, density, with drier consistency.

Samples of the APBPM were submitted to outside laboratories for proximate composition,[5] true metabolizable energy,[6] and amino acid content.[7] The standard methodology of Sibbald (1986) was used for bioassay of the true metabolizable energy. Proximate composition and amino acid analyses were conducted according to AOAC (1984) acceptable methodology.

The dried ground APBPM was subjected to challenge by a primary poultry isolate of *Salmonella enteritidis* (SE) phage type 13 (SE89-8312) from confirmed biochemical serotype and of novobiocin- and nalidixic acid-resistant strain, obtained from the National Veterinary Services Laboratory.[8] Dry APBPM samples (5 g) in 50-mL sterile cap tubes were inoculated with 100 μL of preenriched stock culture containing 10^7 SE/mL. Tubes were incubated at 23 C for 24 h. Sterile distilled deionized water (5 mL) and concentrated hydrochloric acid were added to neutralize alkalinity. After neutralization, 35 mL of tetrathionate broth[9] was added, tubes were gently oscillated and incubated at 37 C for 24 h. No sample dilutions were conducted except the addition of distilled water, hydrochloric acid, and tetrathionate broth before plating and incubation. These additions resulted in a 1:9 dilution per gram of APBPM. Controls without APBPM, and with APBPM, inoculated after addition of tetrathionate and water, were simultaneously incubated. Each sample was streaked on brilliant green agar[9] plates (15 mL) containing 20 μg/mL naladixic acid and 25 μg/mL novobiocin.

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TABLE 1. Composition of diets for feeding Trial 1

	APBPM[1] level		
	0%	5%	10%
	(%)		
Ingredients			
Corn	55.44	54.85	54.27
Soybean meal (48%)	35.87	32.54	29.16
APBPM[1]	0.00	5.00	10.00
Vegetable oil	4.56	3.97	3.38
Calcium carbonate	1.55	1.32	1.38
Phosphate[2]	1.66	1.47	0.99
Salt	0.38	0.31	0.25
Vitamins[3]	0.25	0.25	0.25
Trace minerals[4]	0.05	0.05	0.05
MHA[5]	0.24	0.24	0.24
Calculated values			
ME (kcal/kg)	3,190	3,190	3,190
CP	22.00	22.00	22.00
Methionine	0.60	0.61	0.63
Cystine	0.34	0.32	0.30
Analyzed values			
CP	24.29	25.97	27.82
Fat	7.75	7.88	7.99
Fiber	2.83	2.76	2.61
Calcium	1.24	1.24	1.45
Phosphorus	0.80	0.79	0.85
Sodium	0.14	0.26	0.30

[1]Alkaline poultry byproduct meal.

[2]Mono-dicalcium phosphate source.

[3]Provides per kilogram of diet: vitamin A (retinyl acetate), 11,023 IU; vitamin D_3, 3,858 ICU; vitamin E (dl-α-tocopherol acetate), 46 IU; menadione, 1.47 mg; thiamine mononitrate, 3.19 mg; riboflavin, 5.8 mg; vitamin B_{12}, 0.165 mg; niacin, 45 mg; choline, 450 mg; d-pantothenic acid, 20.2 mg; pyridoxine, 7.17 mg; biotin, 0.55 mg; folic acid, 1.75 mg.

[4]Provides per kilogram of diet: manganese oxide, 68.2 mg; zinc oxide, 55 mg; ferrous sulfate, 26.4 mg; copper sulfate, 4.4 mg; calcium iodate, 1.1 mg; sodium selenite, 0.1 mg.

[5]Methionine hydroxy analog.

TABLE 2. Composition of diets for feeding Trial 2

	APBPM[1] level		
	0%	5%	10%
	(%)		
Ingredients			
Corn	55.33	56.86	57.68
Soybean meal (48%)	35.88	31.68	27.53
APBPM[1]	0.00	5.00	10.00
Vegetable oil	4.65	3.03	1.66
Calcium carbonate	1.55	1.47	1.39
Phosphate[2]	1.66	1.40	1.15
Salt	0.38	0.00	0.00
Vitamins[3]	0.25	0.25	0.25
Trace minerals[4]	0.05	0.05	0.05
MHA[5]	0.24	0.24	0.24
Calculated values			
ME (kcal/kg)	3,190	3,190	3,190
Crude protein	22.00	22.00	22.00
Methionine	0.60	0.61	0.63
Cystine	0.34	0.32	0.30
Analyzed values			
Crude protein	20.22	22.49	21.07
Fat	8.88	7.10	7.00
Fiber	2.22	2.38	2.25
Calcium	1.29	1.66	1.44
Phosphorus	0.77	0.84	0.75
Sodium	0.16	0.51	0.82

[1]Alkaline poultry byproduct meal.

[b]Monodicalcium phosphate source.

[3]Provides per kilogram of diet: vitamin A (retinyl acetate), 11,023 IU; vitamin D_3, 3,858 ICU; vitamin E (dl-α-tocopherol acetate), 46 IU; menadione, 1.47 mg; thiamine mononitrate, 3.19 mg; riboflavin, 5.8 mg; vitamin B_{12}, 0.165 mg; niacin, 45 mg; choline, 450 mg; d-pantothenic acid, 20.2 mg; pyridoxine, 7.17 mg; biotin, 0.55 mg; folic acid, 1.75 mg.

[4]Provides per kilogram of diet: manganese oxide, 68.2 mg; zinc oxide, 55 mg; ferrous sulfate, 26.4 mg; copper sulfate, 4.4 mg; calcium iodate, 1.1 mg; sodium selenite, 0.1 mg.

[5]Methionine hydroxy analog.

Plates were incubated for 24 h at 37 C. No detection limits were established as a result of visible confirmation of growth being the single parameter measured. Presence or absence of plate growth was visually confirmed and witnessed by a third party. The criterion for acceptable preservative action was absence of visible aerobic bacterial growth on the brilliant green agar plates.

APBPM was fed to broiler chicks to examine its acceptability as an ingredient in chick starter rations in two separate feeding trial experiments. Day-old Ideal[10] female broiler chicks were housed in a brooder battery within a temperature-controlled room. Feed and water were provided ad libitum.

Feeding Trial 1 had experimental design of three treatments with three replications per treatment, containing six chicks per replicate in random assignment. A total of 54 chicks were used in the trial. The three treatments consisted of a control diet without APBPM, a diet with 5% APBPM, and one with 10% APBPM (Table 1). Diets were computer formulated to be isonitrogenous, isocaloric, and to meet National Research Council (1994) recommendations. National Research Council poultry byproduct meal composition values and APBPM preliminary analyses were used to estimate the nutrient contribution of APBPM in the experimental diets for feeding Trial 1. Formulation parameters were adjusted between diets for sodium. Samples from the experimental diets were submitted to an outside laboratory[5] and analyzed for CP, crude fat, calcium, phosphorus, and sodium content (Table 1). Feed consumption, water consumption, BW, and mortality were recorded over a 10-d period. Three birds were randomly selected from each treatment and replicate group at 10 d and submitted for necropsy at the Texas Veterinary Medical Diagnostic Laboratory.[11]

Feeding Trial 2 experimental design and protocol were the same as for feeding Trial 1. APBPM was included at the same percentage level of diets as for Trial 1. Completed APBPM nutrient analyses were used for balanced dietary formulation. Diets were computer formulated to be isonitrogenous, isocaloric, and to meet or exceed NRC (1994) requirements. Samples from the experimental diets were analyzed for the same parameters as diets from feeding Trial 1 (Table 2). Feed consumption, water consumption, BW, and mortality were measured over a 24-d period. Three birds from each treatment and replicate

[10]Ideal Poultry Breeding Farms Inc., Cameron, TX 76520.

[11]College Station, TX 77841-3040.

TABLE 3. Proximate analysis of alkaline poultry byproduct meal

Nutrient	Percentage
Protein	37.29
Fat[1]	28.76
Fiber	0.35
Moisture	6.93
Calcium	1.85
Phosphorus	1.52
NaCl[2]	0.53
Sodium	12.30
Sulfur ppm	5,150
pH	10.2
TME[3] kcal/kg	4,013

[1]Acid hydrolysis with hexane extraction.
[2]Sodium chloride.
[3]True metabolizable energy.

group were submitted for the same necropsy evaluation given the chicks from Trial 1. Necropsy was conducted at 12 and 24 d of age. Blood chemistry values were evaluated for major and minor serum parameters at both ages. Pathological and histological examinations were performed on proventriculus, gizzard (ventriculus), and kidney samples. Right and left tibia were excised from the 27 birds at 24 d of age and dried at 105 C for 24 h. Tibia shear strengths (breaking force divided by bone weight expressed as kilograms per gram) were determined on both tibia by using an Instron universal testing machine[12] with a 50-kg-load cell at the 50-kg-load range with a crosshead speed of 50 mm/min with tibia supported on a 2.75-cm span. Tibia ash weights were determined by ashing in tared ceramic crucibles for 24 h at 615 C.

Statistical Analysis

Data from each experiment were analyzed independently in a one-way ANOVA. Statistical calculations were processed by computer by using the SAS statistical analysis software program, version 6.11.[13] Mean differences were separated by the PDiff option of the general linear model procedure. Pooled SEM were included within the data tables.

RESULTS AND DISCUSSION

The APBPM as a final dry product was stable at ambient temperature and had the necessary physical characteristics required to be incorporated into a corn-soy based broiler starter ration.

Nutrient Analyses

Proximate analysis, for the nutrient composition of APBPM, reported 37.29% protein and 28.76% fat (Table 3). Crude protein content was lower than poultry byproduct meal reported by NRC (1994) and Christmas et al. (1996). These composition values respectively represent poultry byproduct meals made from viscera with head and feet, and meal produced from whole commercial layer hen mortalities. The lower CP value of the APBPM represents the difference due to composition of the broiler carcasses used and the elevated crude fat content due to the experimental meal not being mechanically pressed. The moisture content of the APBPM was 6.93% and sodium content was reported at 12.3%. Analysis of the APBPM metabolizable energy, corrected for endogenous and excreta energy, resulted in a TME value of 4,013 kcal/kg. Results of the amino acid analysis were reported as grams per 100 g of protein or equivalent percentage (Table 4). The sulfur amino acids within the APBPM where found to be 0.75% for methionine and 0.09% for cysteine, with lanthionine reported at 0.17%. The methionine and cysteine values were lower than levels reported as methionine and cystine for poultry meal. These values may indicate some cysteine destruction or interference in the analysis from the sodium content or unknown alkalinity factors. However, the lanthionine value remained relatively low compared with what would be expected if cysteine had undergone conversion to form the lanthionine. Reported values of the other amino acids varied, but most were lower than those reported by NRC (1994) and Christmas et al. (1996). Comparison is difficult due to meal variation and the increased level of fat may produce a dilution effect.

Potential for Salmonellae Growth

Challenge by inoculation with SE produced no growth from APBPM test samples. Control samples that contained no APBPM were positive for growth, as were post-neutralization control samples inoculated after addition of acid, water, and tetrathionate broth. Absence of growth

TABLE 4. Amino acid analysis of alkaline poultry byproduct meal

Amino acid	Percentage
Alanine	2.70
Arginine	0.67
Aspartic acid	3.20
Cysteine	0.09
Glutamic acid	5.25
Glycine	3.28
Histidine	0.28
Hydroxylyine	0.11
Hydroxyproline	0.89
Isoleucine	1.18
Lanthionine	0.17
Leucine	2.85
Lysine	1.88
Methionine	0.75
Ornithine	0.93
Phenylalanine	1.53
Proline	2.47
Serine	0.92
Taurine	0.16
Threonine	0.48
Tryptophan	0.81
Tyrosine	0.98
Valine	1.98

[12]Instron Corp., Canton, MA 02021.
[13]SAS Institute Inc., Cary, NC 27511-8000.

TABLE 5. Feeding Trial 1, cumulative feed and water consumption, average BW, and mortality of 10-d-old chicks fed alkaline poultry byproduct meal[1]

APBPM[2]	Feed	Water	BW	Mortality
(%)	(g)	(mL)	(g)	(hd)
0	264	613	226	1
5	282	610	237	0
10	277	611	238	0
SEM[3]	7.4	2.0	8.5	NA[4]

[1]Means without superscripts are not significantly different ($P > 0.01$)
[2]Alkaline poultry byproduct meal.
[3]Pooled standard error of the mean.
[4]Not analyzed.

on the APBPM samples was confirmed visually and witnessed. Results indicate that the APBPM can resist potential growth when inoculated with viable SE.

Feeding Trials

Feeding Trial 1 established that APBPM could be fed to day-old broiler chicks in a starter ration without impacting bird performance. The only mortality was one chick in the control treatment at 9 d. Feed consumption, water consumption, and BW were not significantly different (Table 5). Brain, proventriculus, gizzard, intestine, and kidney were not observed to be different in integrity, appearence, tone, color, or incidence of lesions. Visually, gizzard lesions were described in control and both APBPM treatments, but no greater incidence among the different treatment samples was observed. Histological examination did not find treatment-induced variation from the normal pathology for chickens of the same age.

In feeding Trial 2, feed and water consumption levels were not significantly different among the treatments at 12 or 24 d (Tables 6 and 7). Body weights were not significantly different among treatments at 12 and 24 d (Tables 6 and 7). No mortality occurred in Trial 2. Blood serum chemistry values, including sodium, were not significantly different at 12 and 24 d (data not shown). Necropsy revealed no visible differences of internal organs at 12 and 24 d among the treatments. Kidney and gizzard samples were submitted for histological examination. Microscopic lesions were found in sections of gizzards from all three dietary treatments. Areas of thickening in the koilin lining of the gizzard were sectioned and described as having increased amounts of granular eosinophilic degenerate cellular debris. In addition, the tissues from the 24-d control birds were observed to have the areas of thickening that were found to contain colonies of bacterial cocci. No relation to consumption of the APBPM was determined from the pathological and histological examinations. No symptoms of sodium toxicity were observed in the 12- and 24-d birds while living or postmortem. Tibia from 24-d-old chicks had shear values and ash weights that were not significantly different between all dietary treatments (Table 7).

TABLE 6. Feeding Trial 2, cumulative feed and water consumption, and average BW of 12-d-old chicks fed alkaline poultry byproduct meal[1]

APBPM[2]	Feed	Water	BW
(%)	(g)	(mL)	(g)
0	433	840	355
5	431	827	336
10	419	852	341
SEM[3]	5.5	12.6	7.3

[1]Means without superscripts are not significantly different ($P > 0.01$).
[2]Alkaline poultry byproduct meal.
[3]Pooled standard error of the mean.

Carcasses and 2.0 *M* NaOH solution at 1:1 ratio were blended after 60 d, frozen, and freeze-dried to produce a high fat whole poultry byproduct meal. The dry meal was analyzed for nutrient composition, true metabolizable energy, amino acid content, and resistance to microbial inoculation. The dry meal was incorporated into feed at 5 and 10% of starter diets fed to day-old chicks. Chicks found the meal-containing diets as acceptable as controls. Feed consumption, water consumption, and mortality were not significantly different within two separate feeding trials. Histological and blood evaluation found no greater incidence of gizzard lesions or variation in blood chemistry than control birds consuming no poultry byproduct meal. Tibia parameters were not affected by dietary treatment. This study provides basic information for recovery and utilization of APBPM. Alkalinity and sodium contributed by the NaOH were not toxic to young poultry and provided adequate nutrition. Further investigations will provide greater understanding of the effects of alkaline preservation on proteins and fats recovered for nutritional and industrial applications. The hydrolysis of feathers and carcasses before heat rendering may allow lower temperature or shorter duration processing. Steam processing of APBPM will require neutralization of the foaming capability of the emulsified raw product. Dilution with other poultry byproducts could provide an acceptable route to introduce APBPM into a nutrient recovery process.

CONCLUSION

Analysis of costs to provide alkaline preservation for an individual broiler production unit was conducted by using calculations based on 2.0 *M* NaOH aqueous solution in a 1:1 ratio (wt/wt) with broiler mortalities. Assuming average mortality of 0.1% daily, over 49 d, a flock of 25,000 broilers will produce 1,134 kg (2,500 lb) of mortality (Blake et al., 1990). A poultry production unit of four houses, each with 25,000 broiler capacity, would generate 4,537 kg (10,000 lb) total mortality over a 49-d growout period. An equivalent weight of 2.0 *M* NaOH solution would be required, and the total mortality and solution weight would equal 9,074 kg (20,000 lb). Assuming that alkaline solution and carcasses will have a density equal to or greater than water, 9,074 kg (20,000 lb) of alkaline solution and mortalities would require, at most, a container of 9,074 L (2,398 gal). A fully enclosed top-loading

TABLE 7. Feeding Trial 2, cumulative feed and water consumption, average BW, tibia shear force, and tibia ash of 24-d-old chicks fed alkaline poultry byproduct meal[1]

APBPM[2]	Feed	Water	BW	Tibia shear force	Tibia ash
(%)	(g)	(mL)	(g)	(kg/g)	(g)
0	791	1,109	660	3.21	1.23
5	787	1,094	647	3.41	1.19
10	816	1,173	653	3.32	1.23
SEM[3]	26.0	19.0	15.3	0.19	0.007

[1]Means without superscripts are not significantly different ($P > 0.01$).

[2]Alkaline poultry byproduct meal.

[3]Pooled standard error of the mean.

tank with 9,462-L (2,500 gal) capacity, manufactured from food grade polyethylene, would have an estimated cost of $1,300.00.[14] Amortizing tank costs over 8 yr and 48 flocks, the tank cost per flock will equal $27.08. To produce the 4,537 (10,000 lb) of 2.0 *M* NaOH solution would require 377 kg (830 lb) of NaOH. Purchase of NaOH (97% purity) in bulk would cost $0.88/kg ($0.40/lb) for a total 49-d cost of $332.00.[15] Therefore, estimated costs (excluding cost of water) to preserve 4,537 kg (10,000 lb) of mortality over a 49-d period equal $359.08 or $0.0791/kg ($0.0359/lb) of mortality. These costs are lower than estimates projected by Crews et al. (1995) for a 100,000 broiler production unit, using refrigeration, incineration, large-bin composting, or fermentation.

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