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Effects of Water Acidification on Turkey Performance

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INSIDE

Introduction

Acidification of the drinking water has become very popular in the broiler industry as a tool for improving bird performance. However, little is known about the exact effects of water acidification on weight gains, feed conversion efficiency and livability for turkey production. In addition, little documentation exists which compares different drinking water pH adjustment products for turkeys. Therefore a trial was conducted to determine how turkeys respond to different products used to adjust the drinking water pH.

Materials and Methods

Nine hundred and sixty turkey hen poults (day-old) were randomly placed in 48 floor pens to give 20 birds/pen and six replications per treatment. Each pen was equipped with one hanging tube feeder and a water plasson. Each pen had its own water supply via a 5 gallon sealed bucket. Plassons were cleaned every day and water usage was measured for the first 28 days. This measurement involved accounting for the water added to each pen as well as the water removed each time the plassons were cleaned. Seven treatments were compared to a control (Fayetteville city water). The treatments (outlined in Table 1) included PWT (Jones-Hamilton Co., Walbridge, OH) added to the control water to an adjusted pH of 4 and 6, I.D. Russell Citric Acid (Alpharma, Fort Lee, NJ) added to the water to adjust the pH to 4 and 6, Dri Vinegar (BVS, (Willmar, MN)) added to the water to adjust the pH to 6, Acid Sol (BVS, Willmar, MN)) added to the water to adjust the pH to 6 and Ema-Sol (Alpharma, Fort Lee, NJ) added to the water to adjust the pH to 4. Each solution was prepared in a 50 gallon container and then dispersed to the corresponding replicate pens. Each container was filled with Fayetteville city water and allowed to sit over night to allow residual chlorine to dissipate. Prior to the preparation of each solution a hand-held pH meter was first standardized using pH 4, 7 and 10 buffer solutions. The pH was continuously checked as each solution was slowly mixed to the desired pH. To enhance the dissolving of the dry products, PWT and citric acid, concentrated stock solutions of each was prepared in room temperature water. This concentrated solution was slowly stirred into the appropriate treatment container until the desired pH was achieved. Fresh solutions were made at lease twice weekly and more frequently during the last four weeks of the trial. The pH was verified and recorded, as each batch was prepared. All water and feed added to the pens was weighed. Birds received a commercial diet regime supplied by Cargill. Diets were changed every two weeks.

The birds were group weighed by pen at day 1 and then individually weighed on days 14, 28, 42, 56, 70 and 84. Feed consumption was measured for each period. Pens were checked twice daily for mortality. The weight of all dead and cull birds was recorded for use in determining an adjusted feed

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conversion rate. At week six and twelve, one bird per pen was weighed and sacrificed by suffocation with carbon dioxide. The pH of the crop and gizzard was measured by emptying approximately 20 grams if the contents and blending with an equal amount of distilled, de-ionized water.

Results were analyzed using the GLM procedure of SAS. Pens served as the experimental unit. The mortality percentage data was transformed using square root transformation to normalize the distribution. All means which were statistically significant at the P<. 05 level were separated using the repeated t-test. The feed-conversion rates were calculated as cumulative values. The mortality was calculated for each weigh period.

Table 1. Water Treatments

Treatment Number	Treatment	Water pH
1	Control	8
2	PWT	6
3	PWT	4
4	Citric Acid	6
5	Citric Acid	4
6	Dri Vinegar	6
7	Acid Sol	6
8	Ema-Sol	4 then 6

Treatments 1 through 7 were started at day of age. Each bucket of solution was monitored for solubility on a daily basis. Treatment 8 was pH 4 for days 0-14, then adjusted to pH 6 through the remainder of the trial.

Results

The average body weights of the hens are shown in Table 2. At day 14 the hens receiving the Acid Sol were significantly heavier and the hens receiving the Ema-Sol adjusted to a pH of 4 were significantly lighter than all of the birds receiving the other treatments and the control water. At this time the decision was made to raise the Ema-Sol treatment pH to 6. By day 28 there were no significant differences in body weight and this trend remained throughout the remainder of the trial. Though not significant, the hens receiving the Ema-Sol water lagged behind slightly in weight through day 56 but by day 70 the Ema-Sol birds had similar body weights to the other treatments. Again while not significant, it is interesting to note that the birds receiving the PWT 4, Citric acid 4 or Dri Vinegar 6 treatments had the highest numerical body weights at day 84. No statistical differences were seen for feed conversions for any of the periods measured (Table 3). Birds receiving the Ema-Sol treatment had a significantly higher mortality rate for the first fourteen days. However, overall mortality remained very low and after fourteen days there were no additional losses of Ema-Sol birds until day 56 (Table 4).

Water usage was measured through day 28. However, since the drinkers were plasson and were cleaned daily, this measurement can only be considered an estimation of water usage (Table 5). For the first fourteen days water usage for the Ema-Sol birds significantly lagged behind all other treatments. This trend continued through day 28 and even after raising the Ema-Sol treatment pH to 6 the birds receiving this treatment still lagged slightly behind in consumption. At the time that the pH of the gizzard and crop contents were to be measured, only a small amount of dry material was found in these organs, so an equal weight of distilled de-ionized water (pH 6.68) was added to each sample (Table 6.). While this addition probably influence final pH, the same amount of water added to each

Table 2. Impact of Drinking Water Acidification on Average Hen Weight.

Treatment Name	14 Days (lbs)	28 Days	42 Days	56 Days	70 Days	84 Days
Control	0.819b	2.009	4.883	8.581	12.394	16.361
PWT 6	0.828b	2.009	4.872	8.553	12.445	16.355
PWT 4	0.825b	2.004	4.859	8.577	12.469	16.456
Citric Acid 6	0.826b	2.018	4.894	8.572	12.366	16.333
Citric Acid 4	0.819b	2.018	4.861	8.513	12.440	16.507
Dri Vinegar 6	0.810b	1.991	4.830	8.443	12.187	16.498
Acid Sol 6	0.859a	2.062	4.954	8.714	12.520	16.449
Ema-Sol	0.775c	1.984	4.799	8.566	12.504	16.434
SEM	0.006	0.019	0.041	0.072	0.092	0.132
P Value	.0001	.2549	.3096	.3622	.2573	.2534

Table 3. Impact of Drinking Water Acidification on Average Hen Feed Conversion

Treatment Name	14 Days	28 Days	42 Days	56 Days	70 Days	84 Days
Control	1.086	1.414	1.492	1.588	1.793	1.985
PWT 6	1.098	1.467	1.528	1.607	1.778	1.969
PWT 4	1.075	1.389	1.497	1.576	1.769	1.971
Citric Acid 6	1.090	1.428	1.489	1.595	1.795	2.013
Citric Acid 4	1.080	1.389	1.485	1.585	1.792	1.966
Dri Vinegar 6	1.101	1.465	1.517	1.613	1.803	1.987
Acid Sol 6	1.101	1.454	1.532	1.610	1.780	1.995
Ema-Sol	1.107	1.415	1.546	1.642	1.795	1.988
SEM	0.016	0.024	0.022	0.016	0.020	0.020
P Value	.8486	.1493	.3396	.1833	.9455	.9061

Feed conversion totals are cumulative

sample so that the effect would be the same across all treatments. As seen in the broiler trial, the pH of the gizzard was in the 3 to low 4 range while the crop pH was higher but did not necessarily reflect the pH of the water treatments.

Conclusion

The results of this trial indicate that lowering the pH of the drinking water with PWT, citric acid, Dri vinegar, Acid Sol and Ema-Sol resulted in turkey hen performance similar to the birds receiving the control water. Starting the poults on Ema-Sol adjusted to a pH of 4 resulted in a significantly higher mortality and reduced weights through day 14. The pH of the Ema-Sol treatment was then raised to 6 for the remainder of the trial and the birds had final weights statistically similar to the birds receiving the other treatments.

Table 4. Impact of Drinking Water Acidification on Average Hen Mortality

Treatment Name	0-14 Days (%)	14-28 Days (%)	28-42 Days (%)	42-56 Days (%)	56-70 Days (%)	70-84 Days (%)
Control	0.88b	0.00	0.00	0.00	0.06	0.00
PWT 6	0.00b	0.88	0.87	0.00	0.00	0.30
PWT 4	0.00b	0.88	0.00	0.00	0.06	0.00
Citric Acid 6	0.92b	0.88	0.00	0.00	0.11	0.00
Citric Acid 4	0.88b	0.00	0.00	0.00	0.01	0.83
Dri Vinegar 6	1.85b	0.00	0.00	0.00	0.00	0.00
Acid Sol 6	0.00b	0.00	0.88	0.00	0.01	0.83
Ema-Sol	9.83a	0.00	0.88	0.00	0.83	0.98
SEM	1.58	0.589	0.41	….	0.29	0.54
P Value	.0012	.7746	.5489	….	.4456	.6581

Mortality totals are cumulative

Table 5. Impact of Drinking Water Acidification on Average Water Usage

Treatment	Days 0 - 14 Usage (kg)	Day 14-28 Usage (kg)	Day 0 - 28 Usage (kg)
Control	1.85a	1.82	3.67a
PWT 6	1.92a	1.95	3.87a
PWT 4	1.99a	2.04	4.04a
Citric Acid 6	1.85a	1.61	3.46a
Citric Acid 4	1.83a	1.60	3.43a
Dri Vinegar 6	1.53a	1.86	3.39a
Acid Sol 6	1.95a	1.84	3.79a
Ema-Sol	0.96b	1.44	2.41b
SEM	.184	.13	.180
P Value	.0055	.0572	.0001

Table 6. Impact of Drinking Water Acidification on Crop and Gizzard pH

Treatment Name	Day 42 Crop pH	Day 42 Gizzard pH	Day 84 Crop pH	Day 84 Gizzard pH
Control	5.79	3.87	5.35	3.41
PWT 6	5.56	3.84	5.58	3.18
PWT 4	5.86	3.71	6.18	3.56
Citric Acid 6	5.89	3.82	5.83	3.24
Citric Acid 4	5.87	3.85	6.10	3.25
Dri Vinegar 6	5.95	3.65	5.65	3.20
Avid Sol 6	6.05	4.13	6.24	3.33
Ema-Sol	5.78	3.78	6.12	3.61
SEM	.19	.16	.25	.17
P Value	.7411	.6234	.1366	.5177

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Two New Programs: Premises Identification and the National Animal Identification System

Introduction

The last 10 years has seen an increase in the number of disease outbreaks around the world. In the United States there have been several foreign animal disease outbreaks in the last 4 years (Low Pathogenic Avian Influenza - Virginia. 2000, Exotic Newcastle-California, Nevada, Arizona, Texas 2003-04, High Pathogenic Avian Influenza-Texas 2003 and Bovine Spongiform Encephalopathy-USA and Canada 2003). These outbreaks have caused tremendous interest in developing a method to quickly identify animals for the purposes of protecting animal health and easily tracking animals. Many countries (Australia, Canada, and the European Union to name a few) have some system of animal identification already in place. The United States Department of Agriculture has made the development of a National Animal Identification System (NAIS) a top priority to respond to the national and international concerns regarding protecting animal health and quickly identifying and tracking animals. The first step toward this system is a premises identification/registration program.

Premises Identification

The National Premises Identification System (NPIS) is the first step towards a National Animal Identification System (NAIS) and will be established before animals can be tracked. The registration of premises and thus knowing where animals are located is a key component of accurately tracking animal movement in the case of a disease investigation. The premises involved in the commerce of livestock and poultry will be identified with a unique identification number assigned by the Animal Plant and Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) who will closely working with state and/or tribal agencies/authorities involved with animal health. A premise is defined as any geographically unique location that is associated with the commerce, movement or commingling of poultry and/or livestock. This definition will thus include farms, ranches, livestock auctions, feedlots, county or state fairs, and livestock and/or poultry exhibits. There are three components of the NPIS. The premises number allocator will be how a unique number is assigned by USDA to a premise. Each premise must have a valid address and/or a verifiable description of the location where animals are commingled or have some association with the animal industry (such as a veterinary clinic or diagnostic laboratory). Only one number will be allocated to a premise regardless of the number of species associated with the premise. The premise number allocator will be maintained at the national level only. The premise registration system is the second component and is a database program for storing the information necessary for the premise. Since the information stored is unique to a premise this allows animal health officials to rapidly contact the appropriate owner or supervisor of the premise in the event of a disease investigation. The plan is to maintain the data for 20 years and it will include the date the premise was initiated or deactivated so the appropriate people can be contacted for a specific time frame if needed. The state and/or tribal animal health agencies/authorities are responsible for handling and maintaining the premise

...knowing where animals are located is a key component of accurately tracking animal movement in the case of a disease investigation

registration under their jurisdiction. A standardized registration system is to be provided to them by APHIS for use if desired or they can use a system developed by them or some other party. The third component of the system is the national premise information repository. This is a very important component of the system and contains data forwarded from the premise registration system. This repository will be a centralized system maintained by USDA/APHIS and will contain data that is necessary to support the NAIS such as the unique numbers to be assigned to animals at a specific premise.

The numbers assigned for premise identification will be of two types, both of which will consist of sever alphanumeric characters (7 letter/number combinations). One number is a unique national number that will be assigned to any location or premise that is involved in livestock and/or poultry agriculture. This number will be permanently assigned by the state or tribal registration system to the premise. The number does not change if the property is sold. The second type of number is a unique number that is assigned to entities that do not manage or hold livestock or poultry (such as animal identification services, veterinarians, or breed registries), but are still involved in the NAIS. Once premises are identified, animal identification will be the second step of the NAIS.

Animal Identification

The goal of the NAIS is to be able to identify any animal or premise that has had contact with a disease of concern (foreign or domestic) within 48 hours after discovery of the disease. This can be done with identification of the premise and animal or animal group. The first phase of the NAIS is to uniquely identify a premise; when this is complete the second phase is to uniquely identify an animal or animal/poultry group or lot associated with the premise. This will be done via a unique number for each animal. A 15 character number will be used for individual animals. A 13 character number may be an option for those species such as poultry and pigs that move as one group in the chain of production. The exact technology for uniquely identifying an animal does not exist as a "one size fits all." The technology that works best for one specie may not work well for others. Because of this the USDA focus is on the design of the data system as to what information should be collected and when it should be reported with the belief that once the system is designed the most appropriate technology for the system needs will be market determined.

When development is complete the NAIS will be a standardized system of animal identification that will allow rapid tracing in the event of a disease concern (foreign or domestic). The system will allow identification of cattle, bison, deer, elk, llamas, alpacas, goats, horse, sheep, pigs, and poultry. Participation in the program will be voluntary while it is under development. But USDA will continue to assess the program while it is developed and tested to see if parts or all of it should be mandatory. Currently, there is no timeframe for the system to be in place. However, USDA is now moving the program forward using a phase approach with the first priority being the premise identification. Once premises are identified, animal identification systems will be tested. Naturally, there has been concern about confidentiality issues. The information contained in the system (premise and animal identification) will be accessible by federal, state, and tribal authorities when needed for administration of animal health programs. The need to access data is an important part of conducting an animal health and disease control program designed to prevent disease spread and to protect the public health. USDA/APHIS is very concerned about confidentiality issues and as such is exploring effective means of collecting data and options for protecting the data from public access. The national repository will only contain information as it relates to the purpose of tracking animals and diseases.

What Can Producers Do Now

Livestock and poultry producers should check with their state or tribal animal health authorities about the availability of the program in their area. In Arkansas the Arkansas Livestock and Poultry Commission (ALPC) is the agency responsible for animal health concerns. If the premise registration system is operational in their area, a producer can obtain a unique identification number for their premise. The information needed for a number will include: name, address, and phone number of person in charge of the location, contact name, and type of premise. Once the premise is registered a producer may participate in the animal identification program if it is available in the state or tribal reservation. Currently, there has been no defined budget for the program by USDA. The intent of USDA is to minimize cost as possible; however, some expenses may be associated with the program. The decision for costs for registering a premise are in the jurisdiction of the state or tribe.

Summary

Disease outbreaks can be costly. Time is valuable when it comes to controlling disease outbreaks. Preventing death losses, market loss, and reducing treatment costs depends on prompt disease diagnosis and rapid identification of exposed animals. Changing markets, trade issues, disease outbreaks, and ease of worldwide travel necessitate the need for a method to identify and track animals as quickly as possible. These two programs will allow the animal industries of the USA to be able to do just that. Additional information about the programs can be obtained from the University of Arkansas, Division of Agriculture, Cooperative Extension Service, your county agent, the listed references, or the NAIS website (http://www.aphis.gov/lpa/issues/nais/nais.html)

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What are Bacteriophages?

Introduction

Don't let the big word (bacteriophage) scare you. Bacteriophages (sometimes called phages) are viruses that infect bacteria. The word "phage" means to eat, so the literal meaning of the word bacteriophage is "bacteria eater" (Anonmyous3, ND). It may seem strange that creatures as small as bacteria could be infected with a virus, but bacteriophages are about 40 times smaller than bacteria (Anonmyous1, ND) and have apparently been around about as long as bacteria have. This article will provide an outline of how bacteriophages function and their possible benefits.

Bacteriophage – Structure and Function

Bacteriophages have been compared to "space ships that are able to carry genetic material between susceptible cells and then reproduce in those cells" (Kutter, 1997). Bacteriophages are, in fact, very simple organisms that consist of genetic material (DNA or RNA) surrounded by a protein coat, a hollow protein tail and tail fibers. The general structure of a bacteriophage is shown in Fig. 1.

Figure 1. Bacteriophage Structure

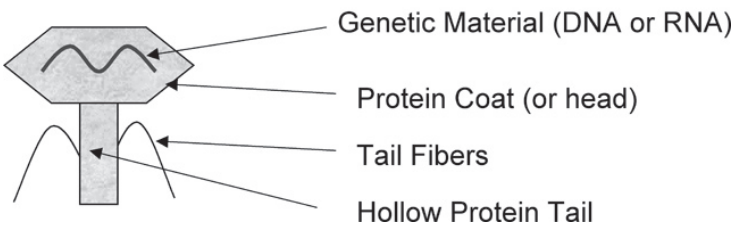
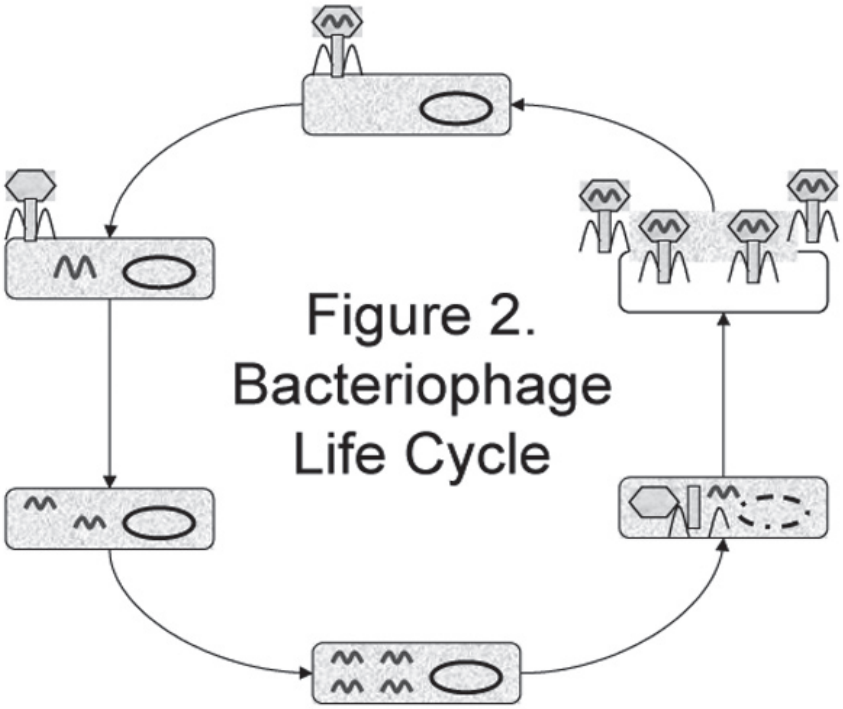


Figure 2 outlines the bacteriophage life cycle. Bacteriophages cannot reproduce without a bacterial cell. The bacteriophage particle attaches to a bacteria and binds to the cell. The particle then injects genetic material into the cell. The genetic material seizes control of the cell causing it to make additional bacteriophage genetic material. In addition, the bacteriophage genetic material forces the cell to make protein coats, hollow protein tails and tail fibers, which are then assembled into new bacteriophage particles. Finally, when no more bacteriophage particles can be made, the cell breaks open, releasing the new bacteriophage particles into the environment to repeat the process with other bacterial cells. This process of infection, replication and release of new bacteriophage particles continues until there are no more cells to infect. However, the description of the bacteriophage life cycle may prompt questions. If this process happens with bacterial cells, what's to keep it from happening with plant, animal or human cells?

The surface of each cell contains a unique blend of proteins, carbohydrates, fats and other organic compounds. The organic compounds on the surface of bacterial cells allow bacteriophages to recognize and attach only certain bacterial cells. If bacteriophages do not recognize the characteristic blend of proteins, carbohydrates and fats, they will not attach to the cell. This means that bacteriophages will not attach to cells unless they are bacteria. The organic compounds on the surface of plant, animal and human cells are not recognized by bacteriophages and they do not attach. In addition, the genetic material injected into cells by a bacteriophage is only capable of acting on bacterial internal contents. Since the internal contents (that is, the structure and chemistry) of plant, animal and human cells is different from that of bacterial cells, bacteriophage genetic material cannot seize control of the cell. This means that even if a bacteriophage attached and injected genetic material into a plant, animal or human cell, the material could not take over the internal machinery of those cells (Kutter, 1997). Because of the specificity of bacteriophages, they are considered safe and, indeed, bacteriophages have not been reported to infect plant, animal or human cells.

In fact, bacteriophages tend to be very specific in the bacteria they infect. For instance, a bacteriophage that infected an *E. coli*, would not infect a *Salmonella*. This specificity can be an advantage and a disadvantage. Specificity could mean that specific pathogenic organisms are

knocked out, while beneficial organisms are left unharmed. However, when several organisms are responsible for a problem or infection within an animal, bacteriophages would have to be directed at each organism. Bacteriophages may be beneficial in treating human, animal and even plant diseases. In fact, it may surprise you to learn that bacteriophages (or phages) have been used to treat bacterial diseases for over 80 years in Eastern Europe (Anonymous1, ND). Indeed, in the 1970s and 80s the Soviet Union produced thousands of gallons of phage each month and every Soviet soldier carried a powder containing bacteriophage in his emergency medical pack (Anonymous1, ND). A brief examination of the history of bacteriophages may be helpful here.



A Very Brief History of Bacteriophage

In 1896 a researcher reported that when the waters of the Ganges and Jumna Rivers in India were filtered to remove the bacteria something in the waters was antibacterial. About 20 years later other researchers demonstrated that a virus was involved and named the virus "bacteriophage" (Anonmymous3, ND). In view of the fact that at the time sulfa drugs and antibiotics were not yet discovered, bacteriophages were explored as disease treatments. The first reported use of bacteriophage to treat a bacterial disease came from France in 1921 (Anonymous2, ND). Bacteriophages were used to treat a variety of diseases. They were taken orally, put on wounds, applied as aerosols, given as injections and used in eye drops. Success rates for bacteriophage therapy were reported to be 75 to 100%, depending on the pathogen involved (Anonmyous3, ND, Kutter, 1997). Indeed, bacteriophage products were produced by United States pharmaceutical companies and licensed for sale in the 1930s (Anonymous3, ND). However, in the 1940s, new "miracle" drugs (antibiotics) became widely available and bacteriophage (or phage therapy) was largely abandoned by the western world (Kutter, 1997). However, current difficulties with antibiotic resistant bacteria have prompted researchers to re-examine bacteriophage.

Summary

Bacteriophages are viruses that infect only bacterial cells. Because of the specificity of bacteriophages, they are considered safe and have not been reported to infect plant, animal or human cells. Bacteriophages (or phages) have been used to treat bacterial diseases for over 80 years in Eastern Europe. Current difficulties with antibiotic resistant bacteria have prompted researchers to re-examine bacteriophage.

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Bacteriophage: A Replacement for Antibiotics?

Introduction

Antibiotics, miracle drugs of the 20th century, have saved millions of human and animal lives, and contributed to efficient animal production to feed a hungry world. Antibiotics are used in poultry production in high doses to treat poultry diseases and at low doses in feed to prevent poultry diseases, as well as reduce the levels of food borne pathogens on poultry products. However, over the last decade the emergence of bacteria resistance has made it increasingly difficult to treat human and animal diseases with antibiotics. Whether the use of antibiotics in animal production poses a threat to human health has been debated for decades and remains undecided. Yet concern over the failure of antibiotics to effectively treat human diseases has led the European Union to ban the use of low doses of antibiotics in animal feeds and encouraged government officials to seriously consider drastically restricting the use of antibiotics in animal production in the United States. Concerns over antibiotic resistance prompted many researchers around the world to look for alternatives to antibiotics. However, to date none of these alternatives consistently provide improved animal production comparable to the growth promoting effects of antibiotics.

Research into Antibiotic Alternatives

Over the past several years we have been looking at the potential of bacteriophage as an alternative to antibiotics to prevent and treat poultry diseases, and reduce food borne pathogens on poultry products.

Bacteriophages are viruses that infect and kill bacteria. Bacteriophages are nature's own way of controlling bacteria, and they are safe, because they have no known effects on animal or plant cells. Therefore, it would appear possible to use bacteriophage to prevent and treat bacterial diseases of animals and humans.

Colibacillosis (airsacculitis) is a serious infection of poultry caused by the bacteria *Escherichia coli*. This disease starts as a respiratory infection in poultry, then enters the blood stream and, when severe, kills chickens through infection of the liver and heart. We were able to isolate a bacteriophage to an *E. coli* that causes colibacillosis in chickens. Over the last several years we have tested the bacteriophage we isolated to see it was possible to prevent or treat colibacillosis in poultry.

In trial 1 we determined whether or not the bacteriophage could inactivate *E. coli* and protect birds from death by *E. coli* infection. We had three treatment groups and all were infected with 10,000 *E. coli* cells, but the cultures used to infect the groups were treated in different ways. The culture used to infect birds in treatment group 1 contained only *E. coli*, no bacteriophage. The culture for group 2 had 10,000 bacteriophage particles added to the *E. coli* and group 3 had 100,000,000 bactriophage particles added to the *E. coli* culture. The results of the trial are shown in Fig. 1. As expected, most birds in group 1 died. However, birds in groups 2 and 3 were partially or completely protected by the bacteriophage (Huff et al, 2002a).

To further test how bacteriophage could prevent colibacillosis we sprayed the birds with bacteriophage prior to infecting them with *E. coli*. There were four treatment groups in this trial. Birds in treatment group 1 were infected with *E. coli* but had no bacteriophage spayed on them. Birds in group 2 were sprayed with bacteriophage and infected with *E. coli* on the same day. Birds in group 3 were sprayed with bacteriophage and infected the following day and birds in group 4 were sprayed with bacteriophage and challenged three days later. The results of this trial are presented in Fig. 2 (Huff et al., 2002b). As expected, most birds in group 1 died, but birds sprayed with bacteriophage were protected from colibacillosis even when the birds were challenged 3 days after

the being sprayed with bacteriophage.

We also took a look at whether bacteriophage could be used to treat a severe outbreak of colibacillosis. In this trial birds were infected with *E. coli* and then injected with bacteriophage. There were four treatment groups in the trial, with birds in group 1 being infected, but receiving no injection of bacteriophage. Group 2 birds were injected with bacteriophage on the day they were infected. Birds in group 3 were injected with bacteriophage one day after infection and group 4 birds were injected two days after infection. The results of this work can be seen in Fig. 3 (Huff et al., 2003). While most birds in group 1 died, significantly fewer birds injected with bacteriophage died, even when the injections were delayed for 48 hours.

What Does This Research Mean?

This research is preliminary research that is designed to identify possible alternatives to antibiotics. Years of further research may be required before bacteriophage are used commercially against poultry diseases. However, our research suggests that bacteriophage could be developed as an effective alternative to antibiotics to prevent and treat bacterial diseases in poultry. Bacteriophage might be used to spray birds at the hatchery to prevent the early onset of colibacillosis (airsacculitis) at placement. Bacteriophage might also be sprayed in a house with a severe outbreak of colibacillosis to prevent the bird to bird transmission. However, bacteriophage treatment may not be practical since it would require injection of each bird.

A number of laboratories throughout the world are taking a look at bacteriophage as an alternative to antibiotics. Bacteriophage are also being examined to reduce human food borne pathogens (*Salmonella, Campylobacter, Listeria*, and *E. coli*) in the intestinal tract of animals. Bacteriophage kill bacteria and have enormous potential to be used in a variety of applications as an alternative to antibiotics and disinfectants. However, it remains to be seen if bacteriophage products can be developed to provide effective, practical and cost effective uses in our agricultural production systems.

Figure 1. The Effect of Mixing Bacteriophage with an *E. coli* culture to Prevent Colibacillosis in Broiler Chickens

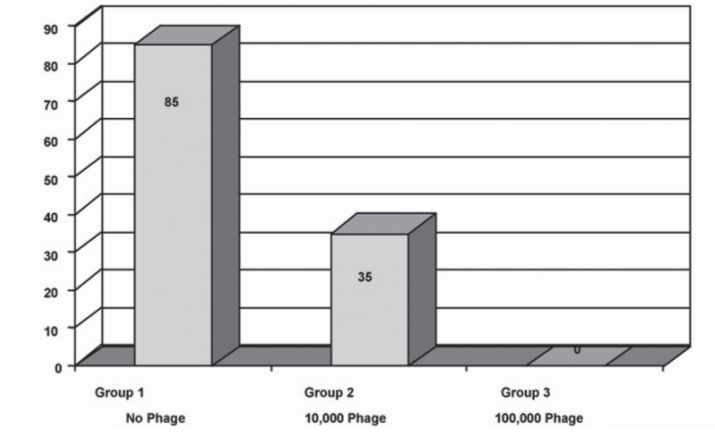


Figure 2. The Effect of Spray Administration of Bacteriophage Prior to Infection with E. coli to Prevent Colibacillosis in Broiler Chickens.

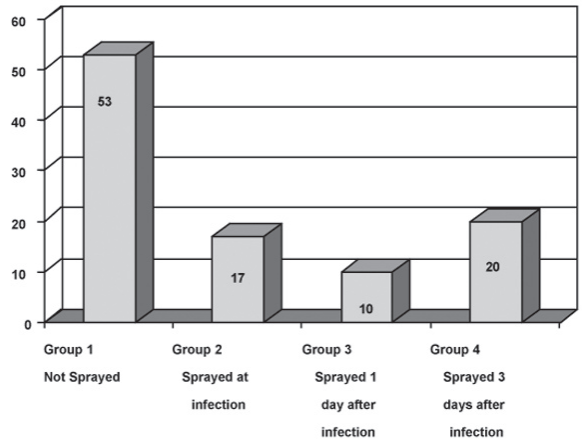
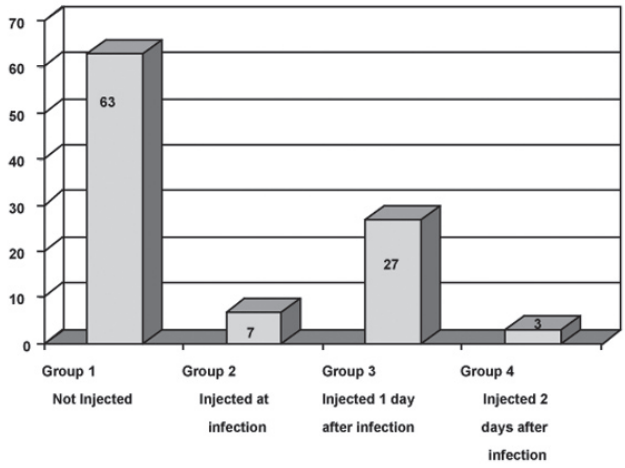


Figure 3. The Effect of Injection with Bacteriophage after Infecting Broiler Chickens with Colibacillosis



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Evaluation of Litter Treatments on *Salmonella* Recovery in Poultry Litter

Introduction

Pathogenic bacterial populations can have a negative effect on the production and health of birds if concentrations are too high. Bacteria cause numerous disease conditions including necrotic enteritis, botulism, gangrenous dermatitis, airsacculitis, and cellulitis. In addition, pathogenic bacterial populations are also linked to current food safety concerns at the processing plant. Because of these concerns, USDA –Food Safety Inspection Service (FSIS) has mandated that poultry processing plants follow HAACP programs to control pathogenic bacteria. FSIS is now evaluating the feasibility of implementing food safety regulations at the farm level. Should pathogen control begin at the farm level, integrators and growers will be challenged to reduce pathogen production during grow-out. Corrier et al. (1999) reported that the incidence of *Salmonella* increased in the crop of broilers at the end of the feed withdrawal period as compared to the level of *Salmonella* in the crops at the beginning of the feed withdrawal period (10% versus 1.9%). The researchers speculated that the increased incidence of *Salmonella* was associated with an increased tendency for the broilers to consume contaminated litter in the broiler house during the withdrawal period. Trampel et al. (2000) reported that *Salmonella* recovered from carcasses in poultry processing plants could be due to fecal shedding onto the litter which may lead to heavy contamination of the bird's feathers and feet.

Many integrators and growers are currently faced with disposal problems of used litter. This leads to the re-use of litter over an extended time frame which could compromise the poultry producer's ability to follow proper sanitation procedures and best management procedures (BMP's). Growers then may rely on the use of litter amendments and disinfectants as their sole source of solving any problems associated with diseases caused by high bacterial levels. Unfortunately, in order to cut costs, growers may apply litter amendments below manufacturer's recommendations with the hope of accomplishing somewhat of an improvement from current conditions of the poultry house.

Litter amendments are commonly used in poultry houses for the reduction of harmful ammonia levels by lowering litter pH. It has been shown that by lowering pH levels, reduction occurs in bacterial concentrations. A study was conducted to determine if the application of Poultry Guard at different levels would effectively reduce the incidence of *Salmonella* in used litter (Trial 1). A separate study (Trial 2) was conducted to determine if the application of Poultry Guard and PLT (Poultry Litter Treatment) would effectively reduce the incidence of *Salmonella* as well as determine at what application rate reduction would occur.

Should a litter treatment be an effective method of reducing food pathogens in the litter, then the potential for crop and possibly carcass contamination could be significantly reduced through the application of a litter treatment prior to implementing feed withdrawal programs. With reduced pathogens in the bird's environment, contamination of the exterior body should be lowered, thus reducing pathogen recovery at the processing plant.

Materials and Methods

Bedding material was obtained from one of the University of Arkansas' commercial broiler houses that serves as a contract production facility for a local poultry integrator. Prior to the experiment, the litter had been exposed to one flock for Trial 1 and three flocks for Trial 2. The original bedding material was kiln dried pine shavings. Litter was placed at a depth of 2 inches in one square foot baking pans. All pans were then covered with aluminum foil and autoclaved for 45 minutes at 121^{o}C to sterilize the litter. Pans were then removed from the autoclave and allowed to cool to room temperature.

TRIAL 1

Inoculation: All pans were inoculated with 100 ml of 104 CFU/ml nalidixic acid-resistant *Salmonella typhimurium* (NAL-SAL). The application rate of 100 ml was chosen due to its ability to create a good coverage on the litter surface.

Treatments: There were 4 replicate pans of litter per treatment. The two treatments were top-dressed onto the litter as recommended by the manufacturer. The four control pans remained untreated. The treatments consisted of Poultry Guard at 100 and 150 lb/1000 ft^2 application rates. A total of twelve pans of litter were used.

Sampling techniques: Surface and core samples were collected from each pan 24 hours after application. Surface samples were collected using a sterile cellulose sponge hydrated with sterile skim milk. Core samples measuring one inch in depth and weighing 25 grams were collected. All samples were then placed into Butterfield's Phosphate Diluent and enumerated onto XLT 4 agar containing nalidixic acid, which was incubated at 35^{o}C. Litter pH and moisture content was determined in all groups 24 hours post application.

TRIAL 2

Inoculation: All pans were inoculated with 50 ml of 105 CFU/ml NAL-SAL.

Treatments: Each treatment was assigned to 16 pans with 4 application rates of 25, 50, 75, and 100 lbs/1000 ft^2. Replicates of 4 were used for each rate along with 4 untreated pans serving as the control. The treatments consisted of Poultry Guard and PLT. Both treatments were top dressed onto the litter as recommended by the manufacturer. Recommended rates were 75-100 lbs/1000 ft^2 for Poultry Guard and 50-100 lbs/1000 ft^2 for PLT.

Sampling techniques: Core samples measuring half an inch in depth and weighing 25 grams were collected 24 hours post treatment. All samples were then placed into Butterfield's phosphate diluent and enumerated onto XLT4 agar containing nalidixic acid, which was incubated at 35°C for 24 hours. Litter pH and moisture content was determined in all groups 24 hours post application.

Analysis Results: were analyzed using the GLM procedure of SAS. All counts were converted to log10 values prior to analyses. Significantly different means were separated using the repeated t-test.

Results

In Trial 1, the application of Poultry Guard at 100 and 150 lb/1000 ft^2 resulted in lowering NAL-SAL to undetectable levels when compared to the control pans. This reduction was observed in both core and surface samples. Significant reductions were observed on litter pH, compared to the control, when both rates were applied (P=0.0001) (Table 1).

In Trial 2, as compared to the untreated control pans, both litter amendments resulted in significantly lower levels of NAL-SAL versus the control when used at the rate of 100 lbs/1000 ft^2 (P=0.0075) (Table 2). Also compared to the control pans, significant differences of NAL-SAL levels were not observed for either litter amendment when used at rates of 25, 50, and 75 lbs/1000 ft^2. When both treatments were applied at the 25 lbs/1000 ft^2 level, *Salmonella* recovery was higher than the control pans. All application rates used for both treatments significantly lowered pH levels, versus the control, with the highest application rate having the most significant effect. Moisture content remained consistent for all treatments including the control.

Table 1. Effect of Poultry Guard on pH and NAL-SAL Counts Obtained from Inoculated Litter

Litter Treatment	Level (lbs/ 1000ft^2)	NAL-SAL Log_{10}/sponge Surface	NAL-SAL Log_{10}/sponge Core	pH
Control	-	3.64a	4.4a	6.47a
Poultry Guard	100	0b	0b	1.95b
	150	0b	0b	1.53b
P-value		0.0001	0.0001	0.0001

Table 2. Effect of Different Levels of Poultry Guard and PLT on pH and NAL-SAL Counts Obtained from Inoculated Litter

Litter Treatment	Level (Lbs./ 1000ft2)	NAL-SAL Log_{10}/ Sponge	pH	Moisture (%)
Control	-	2.77abc	8.300a	23.60
Poultry Guard	25	3.435a	5.825bc	25.40
	50	2.843abc	4.425d	24.30
	75	2.281bcd	3.550e	23.75
	100	1.727d	2.675f	23.80
PLT	25	3.011ab	6.233b	23.27
	50	2.091cd	5.475c	23.32
	75	2.164cd	4.425d	25.60
	100	1.471d	3.475e	24.97
	SEM	.36	.272	.813
	P-value	.0075	.0001	.7610

Discussion

Litter amendments are often times applied below the manufacturer's recommended levels to save costs. When this practice is used on older litter with high pH levels, lesser amounts of treatment may only be lowering the litter pH to ideal levels for bacterial growth. Another consideration is the possibility of creating litter pathogens somewhat tolerant to litter treatments by exposing the pathogens to sub lethal amounts of treatment. According to Trial 2, rates of 100 lbs/1000 ft^2 for the two litter treatments tested are required to significantly lower levels of NAL-SAL in litter. In Trial 1, Poultry Guard at application rates of 100 and 150 lbs/1000 ft2 reduce NAL-SAL to undetectable levels, although this was not observed for the 100 lb. application rate in Trial 2. A possible explanation for this occurrence could be the difference in inoculation rates for both trials. Trial 1 received a higher inoculation rate of 100 ml while Trial 2 received a 50 ml inoculation rate. The higher inoculation rate would increase the litter moisture content, possibly causing an increased activation of the litter amendment. This may explain why we observed a complete reduction of NAL-SAL in Trial 1. Litter amendments are not the sole solution for disease problems. BMP's and a good sanitation program must be in place in order to maintain a successful operation. With this in mind, *Salmonella* found on carcasses in processing plants could potentially be reduced with proper sanitation procedures and the correct use of litter treatments.

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