Comparisons of Microbial Counts in Organic Chickens
and Commercially Processed Chickens

by

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ABSTRACT

The organic food industry is projected to reach sales of $32 billion by 2009. The basic tenets of organic food production involve production of food in a sustainable and environmentally friendly way without the use of chemicals; however, there may be food safety concerns associated with organic food production. For example, in organic production of chickens, processing takes place without any type of synthetic fertilizer, pesticides, or other chemicals like growth hormones and antibiotics and this may increase the prevalence of foodborne pathogens. The objective of this work was to compare the incidence and bacterial load of foodborne pathogens in organically and commercially processed chickens. Comparisons of incidence and average CFU/chicken of total aerobic bacteria, coliform bacteria, *Escherichia coli*, *Staphylococcus aureus*, yeast and molds, *Salmonella spp.* and *Listeria spp.* were made between organic and commercial chickens. Differences in incidence and bacterial load were detected between the two populations. Of particular interest were the higher levels of pathogenic

bacteria detected in the commercially raised chickens. Bacterial load of *E. coli* was significantly higher in the gut of the commercially raised chickens and bacterial load of *S. aureus* was higher in all locations tested in the commercial chickens. The results from this work indicate that differences in processing and handling practices between the organic and commercial industry may impact the safety of food products.

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Chapter I: Introduction

Statement of the Problem

A recent United States Department of Agriculture (USDA) task force concluded that there is a need to support small farm holdings in the United States such as organic farmers; however, to date there has been very little funding available to support research in this area. For example, there are many areas of organic poultry production that need to be addressed through research. Little is known about the impact of organic production practices on the populations of pathogenic bacteria. Foodborne pathogens in chicken cause over 1.1 million illnesses a year. The influence of organic production methods on foodborne pathogens is unknown.

Purpose of the Study

The purpose of this study is to compare the incidence and bacterial load of foodborne bacterial pathogens in organic and commercial chickens. This study will test the hypothesis that there is no significant difference in the microbial safety of organic chickens as compared with commercially grown chickens.

Assumptions of the Study

It was expected that the samples of organic chickens would have more microbial growth as compared with commercially grown chickens but that there would not be more pathogens.

Definition of Terms

100% organic.

> Organic food is produced by farmers who emphasize the use of renewable resources and the conservation of soil and water to enhance environmental quality for future generations. Organic meat, poultry, eggs, and dairy products come from animals that are given no antibiotics or growth hormones. Organic food is

> produced without using most conventional pesticides, petroleum-based fertilizers or sewage sludge-based fertilizers, bioengineering, or ionizing radiation. Before a product can be labeled "organic," a government-approved certifier inspects the farm where the food is grown to make sure the farmer is following all the rules necessary to meet USDA organic standards. (USDA, 2001-2002, para. 59)

All natural chickens. "A product containing no artificial ingredient or added color and is only minimally processed (a process which does not fundamentally alter the raw product) may be labeled natural. The label must explain the use of the term natural (such as - no added colorings or artificial ingredients; minimally processed)" (USDA, 2006c, para. 18).

Center for disease control (CDC).

> The Centers for Disease Control and Prevention (CDC) is one of the 13 major operating components of the Department of Health and Human Services (HHS), which is the principal agency in the United States government for protecting the health and safety of all Americans and for providing essential human services, especially for those people who are least able to help themselves (CDC, n. d., para. 1).

Commercially processed chickens. Chickens raised by a commercial grower and processed in a plant with appropriate Hazard Analysis Critical Control Point (HACCP) controls and sold to stores.

Critical control point (CCP). "A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level" (National Advisory Committee on Microbiological Criteria for Foods, 2000, para. 14).

Food safety and inspection service (FSIS). USDA agency with the mission to protect consumers by "ensuring that meat, poultry, and egg products are safe, wholesome, and accurately labeled" (USDA FSIS, 2006a, para. 1).

Free range chickens. "Producers must demonstrate to the USDA's food safety agency (FSIS) that the poultry has been allowed access to the outside" in order to be labeled Free Range or Free Roaming (USDA, 2006c, para. 8).

Hazard Analysis and Critical Control Point (HACCP).

> The Hazard Analysis Critical Control Point system is a scientific approach to process control. It is designed to prevent the occurrence of problems by assuring that controls are applied at any point in a food production system where hazardous or critical situations could occur. Hazards include biological, chemical, or physical contamination of food products (USDA FSIS, 1999, p. 3).

Petrifilm. A "ready made culture medium system that contains Standard Methods nutrients, a cold water soluble gelling agent and an indicator that facilitates colony enumeration" (3M, 2005, para. 1) made by 3M Microbiology Products. It replaces Petri dishes and media, reducing cost and disposal waste volume.

Limitations of the Study

Some of the limitations of this study were that the commercially processed chickens were refrigerated and the organic chickens were frozen because of the distance from the farm to the testing area. Also, the summer heat in the building where the testing was done was at two different temperatures at the different testing times. This study was able to run tests on 30 chickens due to the time limits and costs involved.

Chapter II: Literature Review

Food Safety

There are thousands of types of bacteria in the environment, but most of them do not cause harm. For example, there are some types of bacteria that are beneficial and keep the digestive tract healthy. When harmful bacteria, also known as pathogens, enter the food and water supply, they can cause food-borne illness and even death. Spoilage bacteria can cause foods to smell and taste bad. These bacteria can be harmful, but probably will not cause illness. Disease causing bacteria are more serious because they usually do not make the food smell or taste bad, but they can cause illness (Dewall, Alderton, & Liebman, 1999).

To ensure that the foods are safe to eat, they must be handled in such a way that the growth of pathogenic microorganisms is eliminated. Illness resulting from microbial growth in food arises by a contaminating microorganism that may infect the person who ingests the food or products of microbial growth in the food. Bacteria such as *Salmonella spp.*, *Listeria spp.* and other pathogens can be transported by poultry and poultry products to humans.

Estimating the costs of foodborne illnesses is a challenging task. However, based on data that has been reported to the CDC, evidence demonstrates that, "Each year foodborne pathogens cause 76 million human illnesses, 325,000 hospitalizations, 5,200 deaths, and an unknown number of chronic conditions..." (USDA Economic Research Service, 2001, para. 7). Breaking this information down by specific bacteria, the following data in Table 1 has been reported. (It should be noted that the data indicates only cases that have been reported). The CDC estimates that there are millions of cases of foodborne illnesses that go unreported each year (CDC, n. d.).

Table 1

Foodborne Illnesses, Hospitalizations, and Deaths Caused by Pathogens, U.S., Annually

Pathogens	Illnesses	Hospitalizations	Deaths
Campylobacter jejuni jejuni spp	1,963,141	10,539	99
Escherichia coli O157:H7	62,458	1,843	52
Listeria spp. spp. monocytogenes	2,493	2,298	499
Salmonella spp. typhi	659	494	3
Salmonella spp., nontyphoidal	1,341,873	15,608	553

Source: USDA Economic Research Service, 2001, para. 8

Safety Standards

In order to ensure that chickens are safe for human consumption, the USDA has put specific rules in place that must be applied to all chickens regardless of how they are raised or processed. Specifically, the USDA implemented pathogen reduction: Hazard Analysis and Critical Control Point (HACCP) Systems in 1996. The goal of this program is summarized as follows:

> The Food Safety and Inspection Service (FSIS) is establishing requirements applicable to meat and poultry establishments designed to reduce the occurrence and numbers of pathogenic microorganisms on meat and poultry products, reduce the incidence of foodborne illness associated with the consumption of these particular products and provide a new framework for modernization of the current system of meat and poultry inspection. (USDA, 1996, p. 38806)

Four specific rules were put in place under this legislation to help meet these goals. These include:

1. Require that all establishments develop standard operating procedures for sanitation.
2. Require regular microbial testing of the facility to establish the effectiveness of sanitation protocols.
3. Establish pathogen reduction protocols for *Salmonella spp.*
4. Require that all establishments establish preventative controls to limit the spread of foodborne illness.

With respect to the specific rules implemented, it is evident that reducing the presence of *Salmonella spp.* in chicken and other meats is critical for food safety. "FSIS verifies that establishments are meeting the standards by having federal inspection personnel collect randomly selected product samples and send them to FSIS laboratories for *Salmonella spp.* analysis..." (USDA FSIS, 1999). Baseline values for the presence of *Salmonella spp.* have been established through nationwide microbial baseline studies conducted by the USDA. Figure 1 below provides an overview of the results of *Salmonella spp.* testing for meat processing across all industries.

		Large Establishments		Small Establishments		Very Small Establishments		All Sizes Establishments	
Product	**Baseline Prevalence (%)**	**# Samp**	**% Pos**	**# Samp**	**% Pos**	**# Samp**	**% Pos**	**# Samp**	**% Pos**
Broilers	20.0	23,229	9.2%	7,757	13.7%	453	34.7%	31,439	10.7%
Market Hogs	8.7	5,701	3.5%	4,479	8.6%	6,393	4.9%	16,573	5.4%
Cows/Bulls	2.7	419	0.5%	4,164	2.0%	1,288	3.6%	5,871	2.2%
Steers/Heifers	1.0	766	0.1%	1,614	0.4%	1,403	0.7%	3,783	0.4%
Ground Beef	7.5	3,954	5.2%	48,595	3.8%	22,209	2.4%	74,758	3.4%
Ground Chicken	44.6	408	15.9%	536	16.0%	53	11.3%	997	15.7%
Ground Turkey	49.9	2,836	30.2%	812	25.6%	64	28.1%	3,712	29.2%

Figure 1. Salmonella spp. Testing 1998-2001

Source: USDA FSIS, 1999

The data clearly indicates that chicken processing plants of all sizes have been able to meet federal *Salmonella spp.* standards. With a baseline established at 44.6 percent, all establishments have been able to reduce *Salmonella spp.* presence to below 16%.

Summarizing the specific rules that have been implemented for ensuring the safety of processed chickens, the USDA FSIS (2006b) further notes that "All chickens found in retail stores are either inspected by USDA or by state systems which have standards equivalent to the Federal government. Each chicken and its internal organs are inspected for signs of disease" (para. 9). Once the poultry is inspected, it is provided with a seal from the USDA that "ensures the chicken is free from visible signs of disease" (para. 9). Chickens may also be graded based on guidelines established by the USDA Agricultural Marketing Service. Grading, unlike

inspection, is not mandatory. Grading provides an overall assessment of the chicken's meatiness, appearance and freedom from defects.

The Importance of Cooking Temperatures

In addition to establishing specific rules for inspection, the Department of Agriculture has also established specific rules for chicken handling and preparation. Bacteria on chicken is typically found in raw or undercooked products. According to the USDA, bacteria multiply rapidly between 40°F and 140°F—"out of refrigeration and before thorough cooking occurs" (USDA FSIS, 2006d, para. 17). The specific environment in which bacteria is present creates a situation in which most foodborne illnesses develop as a direct result of contamination from food handlers. Sanitary food handling and proper cooking and refrigeration should prevent foodborne illnesses. Cross contamination can occur when proper handling is not used—i.e. using a cutting board for chicken and then slicing tomatoes without properly cleaning the cutting board.

Processing Lines

The bacteria associated with chicken processing include: *Salmonella enteritidis*, *Staphylococcus aureus*, *Campylobacter jejuni*, and *Listeria monocytogenes*. Table 3, on the following page, provides an overview of the bacteria, the symptoms it causes, number of cases reported annually and available information on the target populations most affected by these bacteria. As reported in the table, *Salmonella enteritidis* is the most common infection reported in patients. However, it is important to note that the non-specificity of symptoms that occur in cases of *Staphylococcus aureus*, *Campylobacter jejuni* and *Listeria monocytogenes* has made it difficult for the CDC to effectively measure the total extent of the outbreaks that have occurred as a result of these bacteria in the United States.

Poultry are processed at plants designed to accept live birds and convert them to whole bird carcasses ready for packaging or for further processing. During the past 30 years, the average slaughter plant has increased in capacity from approximately 60,000 to 200,000 birds per day (Ollinger, MacDonald, & Madison, 2000). In 1972, approximately 25% of chicken and turkey slaughter plants employed over 400 employees. By 1992, plants employing over 400 people accounted for over 80% of poultry slaughter facilities. The continued shift towards large processing plants indicates that economies of scale are important.

Another major impact to the poultry processing industry has been in consolidation of poultry firms (Ollinger et al., 2000). To measure the rate of consolidation, a method called the four-firm concentration ratio is commonly used. The four-firm concentration ratio measures the percent share of the poultry industry output held by the four largest producers and is widely used as an indicator of structural change. In 1963, the four largest poultry firms controlled 14% of chicken slaughter plants and 23% of turkey plants. By 1992, those percentages had increased to 41% for chicken plants and 45% for turkey facilities.

HACCP

In 1996 the Federal government passed the final rule on Pathogen Reduction and Hazard Analysis and Critical Control Point (HACCP) Systems final rule (USDA, 1996). This targets pathogens that cause foodborne illness, strengthens the industry's responsibility to produce safe food, and focuses inspections and plant activities. The purpose of the HACCP rule was to provide a series of preventive controls based on seven principles. These seven principles include: (i) conducting a hazard analysis to determine where chemical, biological and physical hazards occur in a process; (ii) establishing critical control points (CCP's) that identify where a food safety hazard can best be controlled; (iii) setting critical limits to determine when a CCP is

no longer in control and becomes a food safety hazard; (iv) monitoring CCP's to ensure that they stay within the critical limit; (v) establishing corrective actions when CCP's breach the critical limit; (vi) keeping records to ensure compliance; and (vii) verification to ensure that the HACCP plan is working correctly (USDA, 1996; see Figure 2).

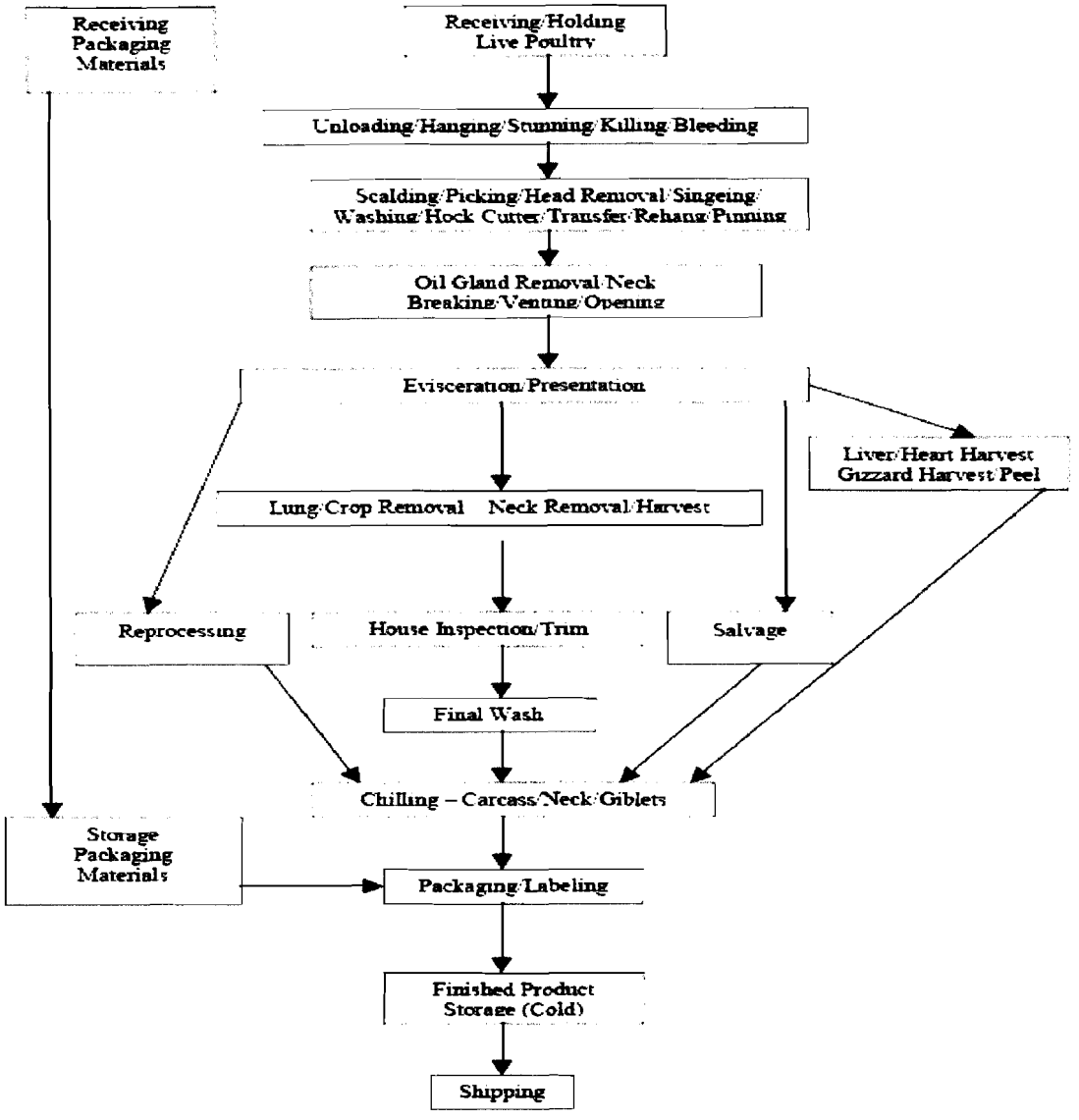

Figure 2. Processing Flow Chart for Poultry Processing Facility

Source: Stam, 2005, p. 24

Table 2

Overview of Bacteria Most Commonly Found in Poultry

Bacteria Name	General Description	Symptoms	Cases Reported	Target Populatio
Salmonella spp. Enteritidis[1]	*Salmonella spp.* is a rod-shaped, motile bacterium.	Acute symptoms—Nausea, vomiting, abdominal cramps, minal diarrhea, fever, and headache. Chronic consequences—arthritic symptoms may follow 3-4 weeks after onset of acute symptoms	It is estimated that from 2 to 4 million cases of salmonellosis occur in the U.S. annually	All age groups are susceptible, but symptoms are most severe in the elderly infants, and the infi AIDS patients suffe times more *Salmon spp.* outbreaks than healthy patients.
Staphylococcus aureus[2]	*S. aureus* is a spherical bacterium (coccus) which on microscopic examination appears in pairs, short chains, or bunched, grape-like clusters.	The most common symptoms are nausea, vomiting, retching, abdominal cramping, and prostration. Some individuals may not always demonstrate all the symptoms associated with the illness. In more severe cases, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur.	The true incidence of staphylococcal food poisoning is unknown for a number of reasons, including poor responses from victims during interviews with health officials; misdiagnosis of the illness, which may be symptomatically similar to other types of food poisoning; inadequate collection of samples for laboratory analyses; and improper laboratory examination	All people are belie to be susceptible to type of bacterial intoxication; howev intensity of sympto may vary

Table 2 continued

Bacteria Name	General Description	Symptoms	Cases Reported	Target Population
Campylobacter jejuni [3]	*Campylobacter jejuni jejuni*iosis is an infectious disease caused by bacteria of the genus *Campylobacter jejuni jejuni*.	Most people who become ill with *Campylobacter jejuni jejuni*iosis get diarrhea, cramping, abdominal pain, and fever within 2 to 5 days after exposure to the organism. The diarrhea may be bloody and can be accompanied by nausea and vomiting	*Campylobacter jejuni* is one of the most common bacterial causes of diarrheal illness in the United States. Virtually all cases occur as isolated, sporadic events, not as a part of large outbreaks. Active surveillance through FoodNet indicates about 15 cases are diagnosed each year for each 100,000 persons in the population	*Campylobacter jejuni jejur* can impact all individuals i the population.
Listeria spp. monocytogenes [4]	*Listeria spp.* monocytogenes is a gram positive bacterium motile by means of flagella	The manifestations of listeriosis include septicemia, meningitis (or meningoencephalitis), encephalitis, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion (2nd/3rd trimester) or stillbirth. The onset of the aforementioned disorders is usually preceded by influenza-like symptoms including persistent fever.	Incidence data prospectively collected by CDC suggests that there are at least 1600 cases of listeriosis with 415 deaths per year in the U.S. The vast majority of cases are sporadic, making epidemiological links to food very difficult.	The main target populatio for listeriosis are: pregnant women/fetus - perinatal and neonatal infections; persons immunocompromised by corticosteroids, anticancer drugs, graft suppression therapy, AIDS cancer patients - leukemic patients particularly

[1]http://www.cfsan.fda.gov/~mow/chap1.html
[2]http://www.seafoodhaccp.com/SeafoodData/BadBugBook/CHAP3.HTML
[3]http://www.cdc.gov/ncidod/dbmd/diseaseinfo/*Campylobacter jejuni jejuni*_g.htm
[4]http://vm.cfsan.fda.gov/~mow/chap6.h

Popularity of Organic Foods

The popularity of organic food has grown substantially in recent years. As reported by Bleasdale (2006), organic food has become the fastest growing segment of the food industry with more than a 20% increase in annual sales over the last few years. Organic foods are so popular that they are expected to reach $32 billion in sales by 2009. Bleasdale goes on to report that the popularity of organic foods stems from the basic context of their development: "the fundamental tenets of the organic movement are about producing food in a sustainable and ecologically friendly way without the use of synthetic fertilizers and pesticides" (p. 42).

As the popularity of organic foods continues to increase, interest in understanding this area of food production has increased as well. Currently, researchers examining organically grown foods have focused on a number of specific areas for investigation. In particular, researchers have examined whether or not organically grown and processed food is safer to eat (Magkos, Arvaniti & Zampelas, 2006), whether organic food is as nutritional as commercially processed food (Bourn, 2002), and the specific bacteriological quality of organically grown foods (Loncarevic, Johannessen & Rorvik, 2005). Using this as a foundation for a closer examination of the current literature, this review examines many of these issues in the context of organically processed poultry. Through a review of what has been noted on this subject, a more integral understanding of the history, development, and issues involved with organic poultry will be clarified.

History of Organic Foods and Poultry

In order to begin this investigation, it is first helpful to consider the specific issues that have given rise to the development of the organic food industry and organic poultry

in particular. Miller (2004) contends that "organic" labeling grew out of a need to provide consumers with healthier food choices. According to Miller, organic foods are grown "in pristine conditions" free from chemicals and toxins. Even though this movement was initiated by small regional farmers, the organic food industry has subsequently been taken over by large corporations, making organic food products a multi-billion dollar per year industry.

> Conan (2003) goes on to note that organically processed meat must come from sources that are raised on organic feed (which is free of genetically modified grain and antibiotics) and are not treated with hormones. Animals must also spend a specified amount of time outdoors, and ranchers are prohibited from using chemical pesticides or fertilizers on grazing fields (p. 154).

In most instances, it is the labor intensive process of raising organic meat that substantially increases the costs of these products. Although cost remains a pervasive issue when it comes to organically grown foods, popularity of this type of food continues to increase.

Issues with Organic Meat and Poultry

The decision to develop organic meat and poultry products stems from a larger concern about public health. Conan (2003) reported that in recent years, the American Medical Association (AMA) and the World Health Organization (WHO) have noted specific problems with commercially processed meats. Of particular concern is the non-therapeutic use of antibiotics in raising cattle and poultry. According to Conan, chickens are often raised in controlled indoor environments. To reduce the risk of infection caused

by injury, poultry farmers use antibiotics. These antibiotics also increase growth rate, making it cheaper for farmers to raise these animals.

As the use of non-therapeutic antibiotics increases, so too does the threat of antibiotic-resistant bacteria. Conan (2003) reports that this includes "such food-borne germs as *Salmonella spp.* and *Campylobacter jejuni*, are also the bacteria that cause urinary-tract infections and pneumonia" (p. 154). Lawn (2002) asserts that as the use of antibiotics for treating commercially processed meats continues, the ability of humans to fight disease and infection is compromised. As reported by Lawn, this is one of the essential issues impacting the development of organic meats and poultry.

Benefits of Organic Meat and Poultry

Overall, there is scant data which effectively demonstrates the true nutritional benefits of consuming organic poultry compared with commercially processed poultry. However, data ("Of Birds and Bacteria," 2003) demonstrates that there is a compelling argument for developing organic poultry processing. Researchers purchased broilers from all across the United States. Of these broilers, 75% were found to have either *Salmonella spp.* or *Campylobacter jejuni*. Even when these commercially processed chickens had been treated with antibiotics to remove these bacteria, "Many of the contaminated chickens harbored strains of *Salmonella spp.* and *Campylobacter jejuni* that are resistant to antibiotics commonly used against those bugs..."(para. 1). Thus, as the use of non-therapeutic antibiotics in commercial chicken processing increases, so to will the presence of antibiotic resistant bacteria.

The consequences of this situation are quite precarious. "...the estimated 1.1 million or more Americans sickened each year by undercooked, tainted chicken, or by

food that raw chicken juices have touched, may stay sick longer, possibly with more serious illnesses" ("Of Birds and Bacteria," 2003, para. 2). Further, physicians will have to prescribe more antibiotics to cure these diseases. This will result in higher healthcare costs for the individual. Although federal regulations developed under HACCP have served as the basis to reduce overall bacteria infections produced by poultry, it is evident that there are still gaps in the system that need to be addressed.

Given the history and development of organically processed foods and the pervasive issues that exist when it comes to commercially processed foods, the consumer's decision to seek out organic foods seems quite straightforward. However, it is important to consider whether these products have proven to have any notable benefits over their commercially processed counterparts. Critically reviewing the scant literature comparing organic and commercially processed foods, Magkos et al. (2003) assert that there is very little evidence which demonstrates the nutritional superiority of organically processed meats. According to these authors, "animal feeding experiments indicate that animal health and reproductive performance are slightly improved when they are organically fed. A similar finding has not been identified in humans" (p. 357). In terms of nutritional value—i.e. vitamins, minerals, protein and fat—there are no indications of substantial differences.

With the realization that organic meats and poultry offer no real nutritional advantage, the question that remains to be answered is why consumers continue to seek out these products. Shan (2006), in his examination of the overall appeal of organic food, reports that most consumers believe that these products have a higher nutritional value. Specifically, many consumers assume that by improving the conditions under which meat

and poultry are produced will, in turn, improve the overall quality of the product. As such, consumer belief in the nutritional value of organically developed and processed products is facilitating interest and growth in organic food products.

Organic Poultry—The Drawbacks

Despite the fact that interest in organic meat and poultry has grown substantially in recent years, research on this industry demonstrates that there are some pressing issues that have developed. Many of these issues involve the safety of this product with respect to certain bacteria. In an effort to provide a clear understanding of the specific problems engendered with organic chickens, it is first helpful to consider the bacterial-related problems that can arise in commercially processed chickens. With a clear understanding of these issues, an apparent comparison can be made between commercially processed and organically developed poultry.

Common Issues for Commercially Processed Poultry

A critical examination of what has been noted about bacteria in the development of commercially processed poultry demonstrates that there are a host of problems that must be addressed. According to Dinçer and Baysal (2004), "Meat and poultry carcasses and their parts are frequently contaminated with pathogens, which reach the carcasses from the intestinal tract or from fecal material on feet and feathers. Cross-contamination is a particular problem..." (p. 197). These authors go on to report that the pathogenic bacteria of most concern in poultry include *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Escherichia coli*, *Listeria monocytogenese*, *Salmonella servoars*, *Staphylococcus aureus* and *Yersinia enterolitica*. Even though protocols have

been designed to reduce the threat that these bacteria pose to human health, contamination of poultry from bacteria is a common problem for consumers.

Overall, research on the issue of bacteria in commercially processed poultry suggests that there are a host of methods and opportunities by which contamination can occur. For instance, Broadbent and Pattison (2003) report that ventilation for poultry in the winter can impact the spread of bacteria among poultry. Further, substantial risks have been noted in the processing of poultry. Because the intestines of poultry typically contain various bacteria, these pathogens are easily spread in the processing of these animals (Nauta, Van der Fels-Klerx, & Havelaar, 2005). While surface contamination of poultry with bacteria is a pervasive issue for poultry processors, researchers also report that pathogenic aerosols are also formed during this process. These aerosols create an air-born pathogen risk that can impact the quality of the poultry at any stage in the processing method (Heber, Peugh, Lutgring, Zimmerman, & Linton, 2006).

Common Issues for Organically Processed Poultry

Not surprisingly, many of the same pathogens that impact commercially processed poultry also impact organically processed poultry. Davies (2003) reports that *Salmonella spp. Enteritidis PT4* is a pervasive problem for organic/free range poultry farms. In a review organic poultry operations, Davies found significant contamination of the farm site, which was exacerbated by the presence of the pathogen in the soil. Fecal matter containing the pathogen is spread more substantially because of the manner in which the chickens are raised. As such, organic methods can engender novel problems for pathogen control.

Clearly, the development of organically processed chicken creates a unique situation. Organically developed poultry is supposed to be free of chemicals and drugs. While this is seen as the most viable benefit of organically produced meats, it does engender certain problems. For instance, Deumier (2004) reports that the presence of Enterobacteriaceae, total coliforms and *Escherichia coli* can be significantly reduced by washing poultry in mildly acidic solutions. Because organic chickens are not processed with any type of chemicals, these treatments are not used, thus increasing the prevalence of these types of pathogens.

Escherichia coli are not the only bacteria that are controlled through the use of chemicals in the poultry processing industry. Van Immerssel et al. (2006) reported that mild organic acids are typically used to reduce the presence of *Salmonella spp.* in poultry processing. According to these authors, "It is possible to decrease chicken carcass and egg contaminations by adding organic acids to the feed or drinking water at appropriate times" (p. 182). Although this process works well for reducing *Salmonella spp.* outbreaks in commercially processed chickens, the same methods cannot be used in processing for organic poultry. Here again, chemicals for organic poultry markedly limit the ability of processors to reduce the occurrence and spread of *Salmonella spp.*.

In addition to the fact that many of the same bacteria can be found in both commercially and organically processed poultry, Bojesen, Nielsen and Bisgaard (2003) report that organically processed chickens are susceptible to other pathogens, specifically Gallibacterium. In the research undertaken by these authors, the presence of Gallibacterium in several different processing methods was compared including: organic/free-range layer, batter-cage layer, layer parent, broiler parent and broiler

grandparent flocks. Tracheal and cloacal swabs were used for testing in each case. The results indicated the following: "All chickens from the broiler grandparent flocks sampled negative, whereas 28% of the broiler parents, 40% of the layer parents, 67% of the battery-cage layers and 96% of the organic/free range chickens sampled positive" (p. 503).

Petrifilms

Petrifilm plates are a thin film, sample ready, dehydrated, version of the conventional petri dish agar plate. They are ready to use immediately after taking them out of their packets and have several advantages over conventional agar plates, such as built in biochemical confirmation, ease of use and interpretation, no preparation, and smaller volumes of space used in incubation (all the plates use 1 mL of a sample). Petrifilm plates were developed for use in the food and beverage industry and are the Association of Official Analytical Chemists (AOAC) approved for each of these tests.

Petrifilm is a "ready made culture medium system that contains Standard Methods nutrients, a cold water soluble gelling agent and an indicator that facilitates colony enumeration" (3M, 2005) made by 3M Microbiology Products. It replaces Petri dishes and media, reducing cost and disposal waste volume. Various types of Petrifilm will be used to identify and count the number of: total aerobic bacteria present, yeast and molds, Coliforms, *Staphylococcus aureus*, *Escherichia coli*. Petrifilm is incubated at 37° C for 48 hours, except for the yeast and mold Petrifilm, which is placed in an incubator at room temperature between 24° C and 28° C for a period of 3-5 days. (*S. aureus* is also an exception.) Test tube media are used to screen for the presence/absence of *Salmonella*

spp. and *Listeria spp.* followed by selective plate media and Enterotubes to verify species.

After incubation, each of the colonies on the Petrifilms or plates are counted and recorded. The data is evaluated and the data from the organic chickens will be compared to the data for the commercially processed chickens. Statistical analysis is used to determine if there is any significant difference in the data from the two varieties of chickens.

Bacterial Detection-Petrifilm Aerobic Count Plates

The 3M Petrifilm Aerobic Count Plate is a ready-made culture medium system that contains Standard Methods nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates counting the colonies (3M Microbiology, 2006a). The indicator dye in the plate colors all colonies red so they show up on the yellow grided background.

Petrifilm Aerobic Count Plates were used for total aerobic population on each of the chicken's back, front and gut. This gives a general overview of the bacterial contamination. Each plate gets one milliliter of the sample and is incubated for 48 hours at 37° C. (3M Microbiology, 2006a). Aerobic plate count tests are done to indicate the total level of microorganisms in a particular product.

Petrifilm Rapid S. aureus Count Plates

The Petrifilm Rapid *S. aureus* (RSA) count plate consists of two parts: the Petrifilm RSA plate, which contains modified Baird-Parker nutrients with a cold-water-soluble gelling agent, and the Petrifilm Thermostable Nuclease reactive disk (TNase reactive disk), which contains DNA, Toluidine Blue-O, and a tetrazolium indicator that

facilitates colony enumeration and confirmation of the presence of a *staphylococcal* thermostable nuclease (3M Microbiology, 2006d). TNase is an enzyme produced by *S. aureus* that remains stable at high temperatures. Detection of TNase, like coagulase, is a confirmatory method for *S. aureus*. On the Petrifilm RSA plate, the TNase reaction is seen as a pink zone around a red or blue colony. The plate and disk is equivalent to the biological analytical manual (BAM) three-plate Baird-Parker agar and single tube-coagulase method.

The Petrifilm TNase reactive disk must be used with the Petrifilm RSA plates (3M Microbiology, 2006d). The Petrifilm RSA plate used alone will not show colonies because the indicator dye that facilitates enumeration of the colonies is in the Petrifilm reactive disk and not in the Petrifilm RSA plate.

Staphylococcus aureus is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents. The presence of this bacterium or its enterotoxins in processed foods or on food processing equipment is generally an indication of poor sanitation. *S. aureus* can cause severe food poisoning; it has been identified as the cause of many food poisoning outbreaks.

Petrifilm Rapid Coliform Count Plates

The 3M Petrifilm Rapid Coliform Count Plate is a ready-made culture medium system which contains Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, a pH indicator to detect acid and a tetrazolium indicator that facilitates colony enumeration (3M Microbiology, 2006c).

Petrifilm Rapid Coliform Count Plates are useful for the enumeration of coliform bacteria (3M Microbiology, 2006c). Early coliform results may begin to appear as soon

as six hours of incubation and appear as discreet, yellow acid zones, with or without colonies. Unlike traditional coliform tests, final coliform results appear as colonies associated with gas bubbles and may begin to appear as early as eight hours of incubation. A colony associated with gas within the 24-hour incubation period, is a confirmed coliform. Plates should be continually incubated after each reading to detect confirmed coliform growth. Total coliform count is determined at 24 hours.

Coliform bacteria include all bacteria in the Enterobacteriaceae family. Although most of them are not harmful, this count gives another indication of overall sanitation. Coliform bacteria originate as organisms in soil or vegetation and in the intestinal tract of warm-blooded animals (fecal coli; 3M Microbiology, 2006c). This group of bacteria has long been an indicator of the contamination of water and possible presence of intestinal parasites and pathogens.

Petrifilm E. coli/Coliform Count Plate

Coliform bacteria include the *E. coli* bacteria but this is a specific test to identify this bacteria. The presence of *E. coli* in raw food is an indicator of fecal contamination. Some is expected but if the count is large it indicates unsanitary processing conditions. Petrifilm E. coli/Coliform Count (EC) plates contain Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, and an indicator that facilitates colony enumeration (3M Microbiology, 2006b). Most *E. coli* (about 97%) produce beta-glucuronidase which produces a blue precipitate associated with the colony. The top film traps gas produced by the lactose fermenting coliforms and *E. coli*. About 95% of *E. coli* produce gas, indicated by blue to red-blue colonies associated with entrapped gas on the Petrifilm EC plate, within approximately one colony diameter.

Yeast and Mold Count

Yeast and Mold Count Plate is a ready-made culture medium system that contains nutrients supplemented with antibiotics, a cold-water-soluble gelling agent, and an indicator dye that makes colonies easier to see (3M Microbiology, 2006e). Petrifilm plates are manufactured with a grid background to facilitate counting colonies. Petrifilm Yeast and Mold plates can be used in place of standard fungal nutrient media such as Potato Dextrose Agar. Petrifilm Yeast and Mold Count Plates for yeast and mold population determination takes three to five days. An indicator dye stains yeast and mold colonies to provide contrast and facilitate counting. Yeasts are typically small, raised, blue-green colonies, with defined edges. Molds are often larger, variably colored, flat colonies with diffuse edges and central foci.

Both yeasts and molds cause various degrees of deterioration and decomposition of foods (U.S. Food and Drug Administration, 2006). They can invade and grow on virtually any type of food at any time; they invade crops such as grains, nuts, beans, and fruits in fields before harvesting and during storage. They also grow on processed foods and food mixtures. Their detectability in or on foods depends on food type, organisms involved, and degree of invasion; the contaminated food may be slightly blemished, severely blemished, or completely decomposed, with the actual growth manifested by rot spots of various sizes and colors, unsightly scabs, slime, white cottony mycelium, or highly colored sporulating mold. Abnormal flavors and odors may also be produced.

Salmonella spp.

Salmonella is a genus of the family *Enterobacteriaceae*. Like other *Enterobacteriaceae* genera, *Salmonella* consists of gram-negative flagellated rod-shaped bacteria (United States Meat Export Federation, 2003).

Listeria monocytogenes

Listeria monocytogenes is a Gram-positive rod-shaped bacterium (Todar, 2003). It is the agent of *Listeriosis*, a serious infection caused by eating food contaminated with the bacteria. *Listeriosis* has recently been recognized as an important public health problem in the United States. The disease affects primarily pregnant women, newborns, and adults with weakened immune systems.

Enterotubes

Enterotubes (Figure 2) were developed for clinical use to identify bacteria. They are only useful in identifying Gram negative bacteria. They contain 13 compartments, each with a different type of media, which will test for the presence of a different enzyme or set of enzymes in the unknown bacteria (Washington University, 2006).

Conclusion

When the data is summarized overall, it is clear that unique challenges are created in protecting humans from the threat of pathogens from organically processed chickens. Even though organically processed chickens have not been exposed to harsh chemicals, the absence of chemicals increases the presence of specific pathogens. The information presented here demonstrates that the specific pathogen threats that face commercial poultry producers are those that stem from the methods used in processing. Because organic poultry processors cannot utilize certain chemicals in the processing methods,

this may, in some instances, increase the presence of harmful pathogens. Poultry naturally contains a wide range of pathogens that can be easily spread when chickens are slaughtered and processed. The only effective method for controlling the spread of these pathogens is through the use of chemicals that can kill them. Hence, even though organically processed poultry may be free from harmful chemicals, removing the threat posed by harmful bacteria and other pathogens remains a pervasive concern.

Chapter III: Methodology

General Methodology

Thirty whole chickens (15 organic chickens and 15 commercial chickens) will be swabbed with sterile swabs on the breast, back and gut cavity inside the chicken. These swabs will then be used to determine the number of bacteria present in each individual chicken. Each swab will be placed in sterile phosphate buffered water to suspend the sampled bacteria, yeast and molds. Based on results from a pilot study, the bacterial suspension will be subjected to serial dilutions with a dilution factor of 1/10. A volume of 1.0 mL of each dilution will be spread over different Petrifilms. Various types of Aerobic Count Plate Petrifilm will be used to identify and count the number of total aerobic bacteria present. Coliform Petrifilm will be used to identify coliforms which will have gas associated with the colonies. *Escherichia coli* Petrifilm will be used to determine the number of confirmed colonies of *Escherichia coli.* These Petrifilms will be incubated at 37° C for 48 hours. *Staphylococcus aureus* Petrifilm will be used to identify *S. aureus* species and *S. aureus*. It will be incubated for 24 hours and when colonies are present, a TNase disk will be added to separate the species. The Yeast and Mold Petrifilm will be used for yeast and molds count and they will be incubated at between 24° C and 28° C for a period of 3-5 days.

Test tube media will be used to screen for the presence/absence of *Salmonella spp.* followed by selective plate media and Enterotubes to verify species. A 1 mL sample from each swab will be pre-enriched with Lactose broth for detection of *Salmonella spp.* species and *Listeria* Enrichment Broth for *Listeria spp.* The enrichment will be followed by appropriate plating as discussed below.

After incubation, each of the colonies on the Petrifilms or plates will be counted and recorded. The data will be evaluated and the data from the organic chickens will be compared with the data for the commercially processed chickens. Statistical analysis will be used to determine if there is any significant difference in the data from the two methods of processing the chickens.

Total Aerobic Bacteria

Aerobic Count Plate Petrifilm will be used to determine an overall count of bacteria. One mL of the sample from the dilution above will be used. The plates will be incubated for 48 hours at 35° C. All the colonies will be colored red because of the tetrazolium dye and will be counted with the Quebec Colony Counter. This count of the total population will indicate the cleanliness of the chicken carcasses. All of the counts will be recorded on a data sheet for the individual chickens.

Coliform Bacteria

Coliform bacteria include all bacteria in the Enterobacteriaceae family. Although most of them are not harmful, this count gives another indication of overall sanitation. One mL of each sample will be plated on a Coliform Petrifilm Plate. Red colonies with gas associated with them will be counted as positive coliforms. As mentioned in the review of literature, this media is equivalent to violet Red Bile Agar which is the Standard Method media for coliforms.

Escherichia coli

The *E. coli* Petrifilm will be spread with 1 mL of the sample diluted as above and incubated for 48 hours at 35° C. This Petrifilm has the glucorinidase indicator as well as the tetrazolium dye so the colonies counted will be blue and associated with a gas bubble.

The red colonies will not be counted because they could be any coliform. This process is not able to detect *E. coli* 0157:H7 because they are glucoronidase negative.

Staphylococcus aureus

The Rapid *S. aureus* Petrifilm plates will be used to determine the population of *S. aureus* on the chickens. One mL of the sample will be placed on the Petrifilm incubated for 24 hours at 35° C. Each Rapid *S. aureus* Petrifilm will then be examined for colonies. Each colony present represented one *Staphylococcus* bacterium. To determine if those colonies are *S. aureus* or another *Staphylococcus* species, such as *Staphylococcus epidermidis*, a blue TNase disk will be inserted and the plates returned to the incubator. The TNase disk detects an enzyme only present in *S. aureus*. After 1-3 hours of incubation, the plates will be re-examined for blue to pink colonies with pink halos.

Yeast and Mold Colonies

Yeast and Mold Petrifilm will be used to test for both yeasts and all kinds of molds. The Yeast and Mold Petrifilm will be spread with 1 mL of the sample diluted as above and incubated for 3-5 days at 20-25° C. Each film has a yellow background easy for identification a uniformed blue colony or blue spots for the yeast and a colony with uneven edges and variety of colors for the molds.

Presence test of Salmonella and Listeria

Enrichment is a critical step in enhancing the growth of certain bacterial species while inhibiting the development of unwanted microorganisms. One mL of each sample will be placed in a pre-enrichment Lactose broth with 1% brilliant green dye and incubated for 24 hours at 35° C. After the 24 hour time period, 1 mL from the pre enrichment will then be placed in Tetrathionate and 1 mL in Selenite cystine for a 24

hour time period as an enrichment phase. This allows *Salmonella* to grow, but there is no result after this step. Following the 24 hour time period the one loopful of the Tetrathionate and one loopful of the Selenite cystine will then be streaked on a petri dish of XLD (Xylose Lysine Desoxycholate), Bismuth sulfate and Hectoen enteric agar, incubated for 18-24 hours at 35° C. *Salmonella spp.* colonies are black on XLD, Dark blue to green on Hectoen Enteric Agar and Black metallic on Bismuth Sulfate. Results will be presumed positive at this step and these colonies will be put on an Enterotube for further identification.

Enterotubes

Enterotubes will then be used to interpret the results of the colonies, using the needle to inoculate the entire tube with a colony of the unknown bacteria. It will then be placed in the incubator for 24 hours at 37° C. After the 24 hour time period, each compartment will be examined for a color change and then recorded to compare to the reference book for a positive or negative result based on a color change of the media. This test includes a Triple Sugar Iron test as well as Indole, Methyl Red, Vogues Proskauer and Citrate tests which are standard methods of Gram negative identification. Each test will be assigned a number based on color changes in the media. The five-digit number will be looked up in the Enterotube code book, and the number corresponds to a species of bacteria that produced that particular combination of enzymes. Industry standards for the reading of Enterotubes can be obtained from Western Kentucky University (2006).

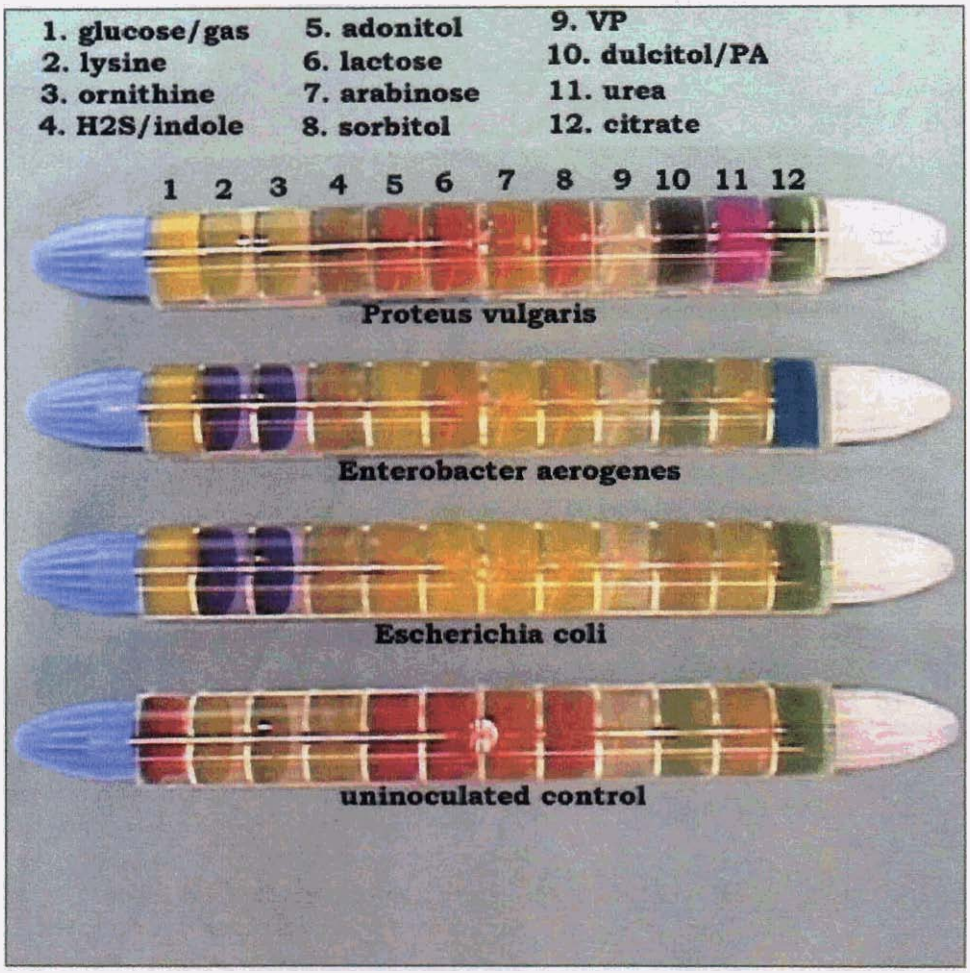

Figure 3. Enterotube II

Source: MicroVision, 1998, p. 1

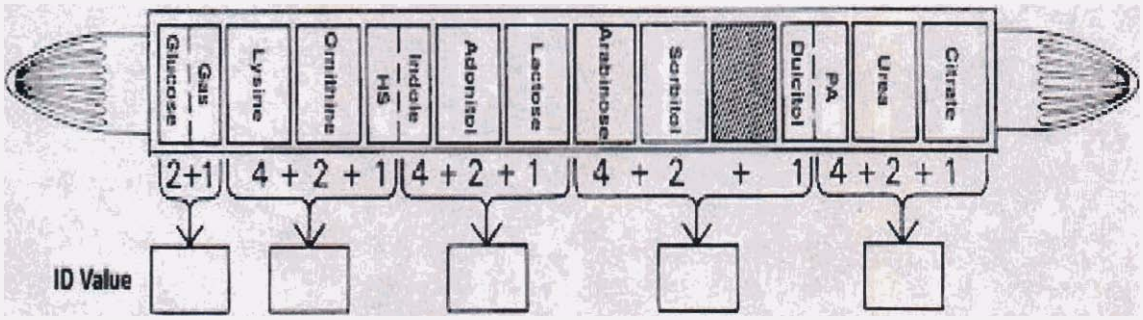

Figure 4. Enterotube Identification

Source: Western Kentucky University, n. d., p. 12-9

Listeria monocytogenes is the pathogen of concern but this test screened for *Listeria spp.* which includes the pathogenic species. A one mL sample of each of the swabs will be placed in *Listeria* enrichment broth with an antimicrobial supplement to remove all Gram negative bacteria and encourage the slow growing *Listeria* to grow. This will have no color change at this point of the testing. From the *Listeria* enrichment broth, 1 mL will be placed in Frazer broth with an esculin supplement and incubated at 35° C for 24 hours.

Frazer broth was chosen because the selective agents are Nalidixic acid that will inhibit Gram-negative bacteria and Acriflavin will suppress most other Gram-positive bacteria. If, after the 48 hours in the incubator, the culture turns black, 1 loopful will then be streaked out on Mox agar (Modified Oxford medium) and incubated for 24 hours at 35° C. After the 24 hours, any tiny black colonies that appear will be considered positive *Listeria spp. Listeria* species will not be identified.

Instrumentation

The instruments used in this experiment will be the Quebec Colony Counter, incubators, refrigerators, and autoclave. In addition, Petri dishes, test tubes, sterile pans, gloves and media 1.1 mL pipets, 99mL sterile dilution bottles, 3M Petrifilm, and sterile swabs are required to complete the study.

Data Collection Procedures

Each chicken will be numbered and all data from each number of chickens will come from the same chicken.

Statistical Analysis

A number of statistical analyses will be used in this study. The Statistical Program for Social Sciences, version 10.0, (SPSS, 2002) will be used to analyze the data. Data analysis will be completed using a program developed with Excel. Data will be examined using Comparison of Means methods.

Chapter IV: Results

Total Aerobic Bacteria

There was a high incidence of aerobic bacteria in both the organic and commercial chickens (see Figure 5). In the three locations tested for aerobic bacteria (breast, back and gut), the highest incidence of aerobic bacteria was observed in the gut cavity. All chickens in both the organic and commercial populations were colonized with aerobic bacteria in the gut cavity. At the other locations tested, there was a higher incidence of aerobic bacteria in the organic chickens than the commercial chickens. Aerobic bacteria were found on 93% of the backs of the organically grown chickens and only 53% of the backs of the commercially produced chickens. Eighty-seven percent of organically raised chickens were colonized with aerobic bacteria on the breast and only 47% of commercially grown chickens were colonized with aerobic bacteria on the breast.

The average aerobic bacteria CFU/chicken was highest at all locations in the organically grown chicken, however, there was no statistically significant difference in aerobic bacteria ($P > .05$) average CFU/chicken at any location on the chicken between the organic and commercial chickens (see Figure 6).

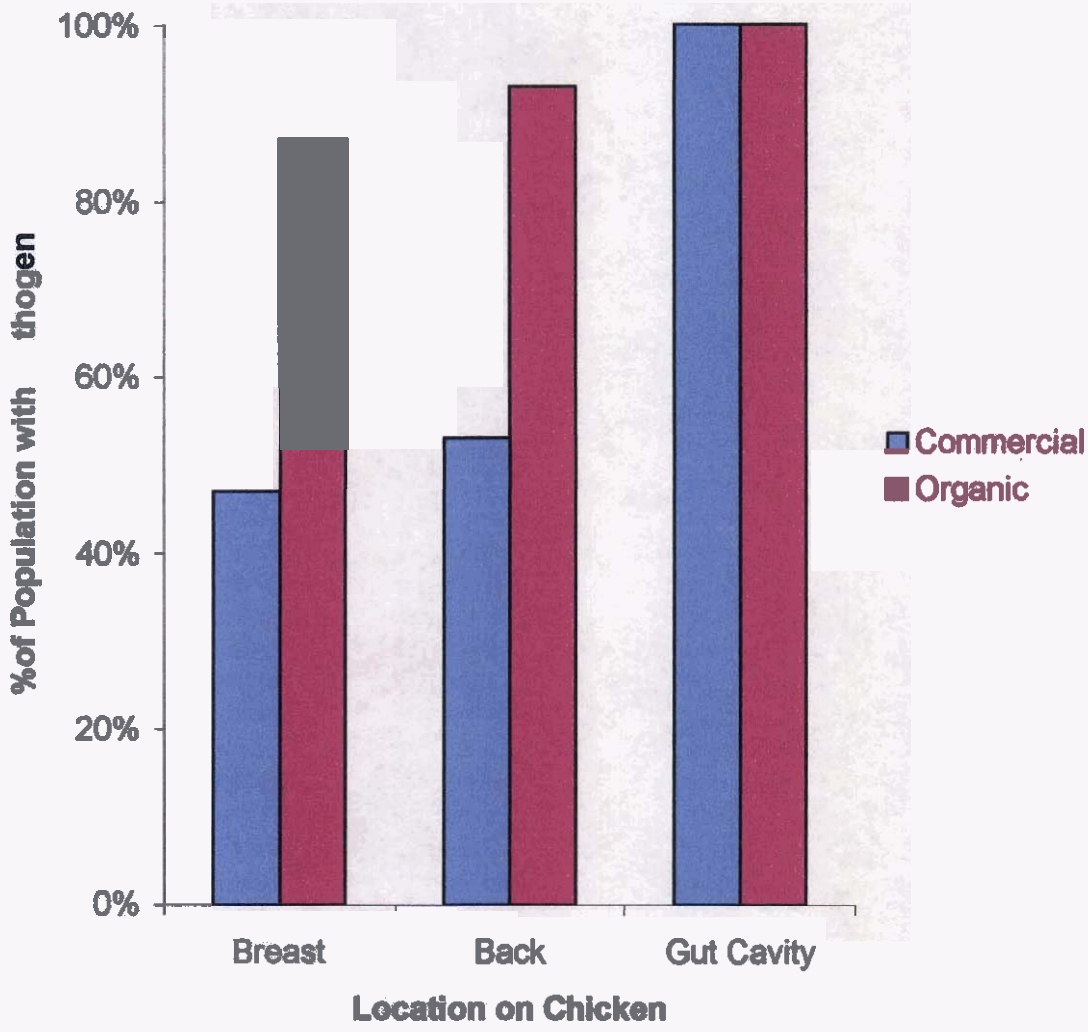

Figure 5. Incidence of aerobic bacteria in the population of organic or commercial chickens by location (breast, back, or gut cavity)

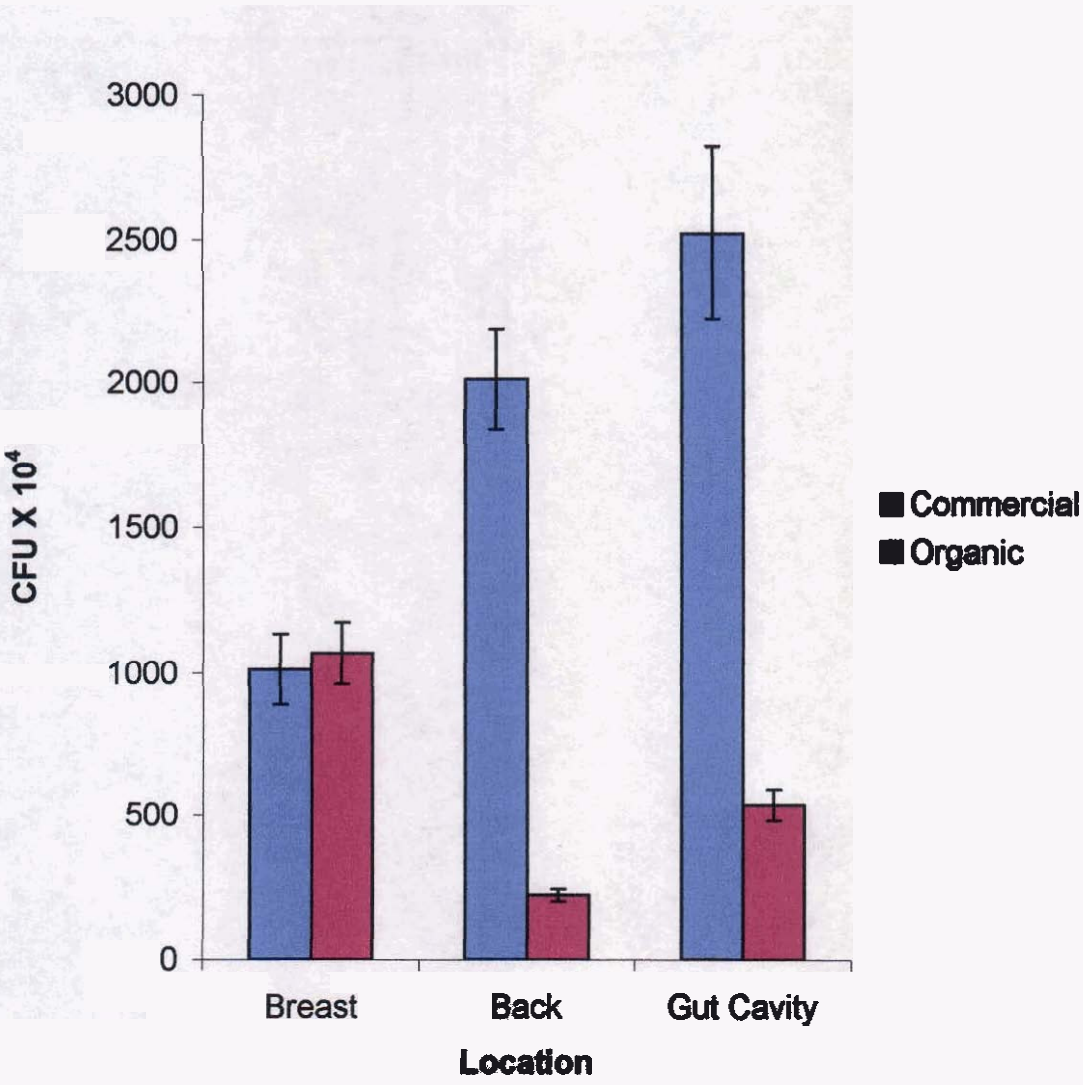

Figure 6. Average CFU/chicken detected in the organic and commercial chickens by location (breast, back, or gut cavity)

Coliform

Like aerobic bacteria, the highest incidence of coliform bacteria was noted in the gut cavity in both commercial and organic chickens (see Figure 7). Forty-six percent of chickens in both populations were populated with coliform bacteria in the gut cavity. In the other location's tests, there was a higher incidence of coliform bacteria in the organic chickens versus the commercial chickens. Coliform bacteria was found on the breast of 20% of the organically grown chickens and only 6% of the commercially produced chickens. Thirteen percent of the organically grown chickens compared to 6% of the commercially grown chickens showed coliform in the back portion of the chicken.

The average coliform bacteria CFU/chicken was highest at all locations in the organic chickens, however, there was no statistically significant difference in coliform bacterial load ($P > .05$) between the populations at any location (see Figure 7).

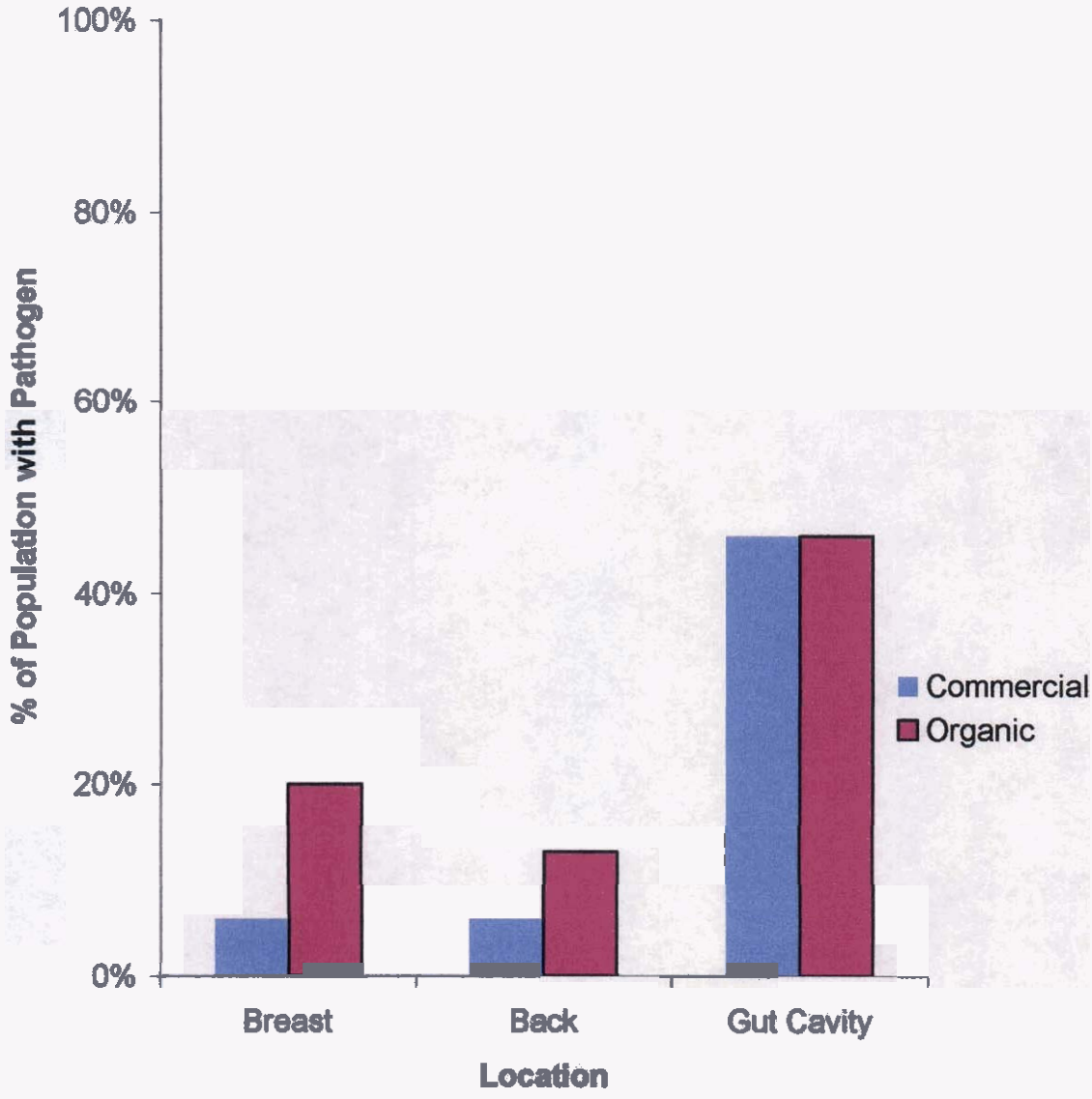

Figure 7. Incidence of coliform bacteria in the population of organic or commercial chickens by location (breast, back, or gut cavity)

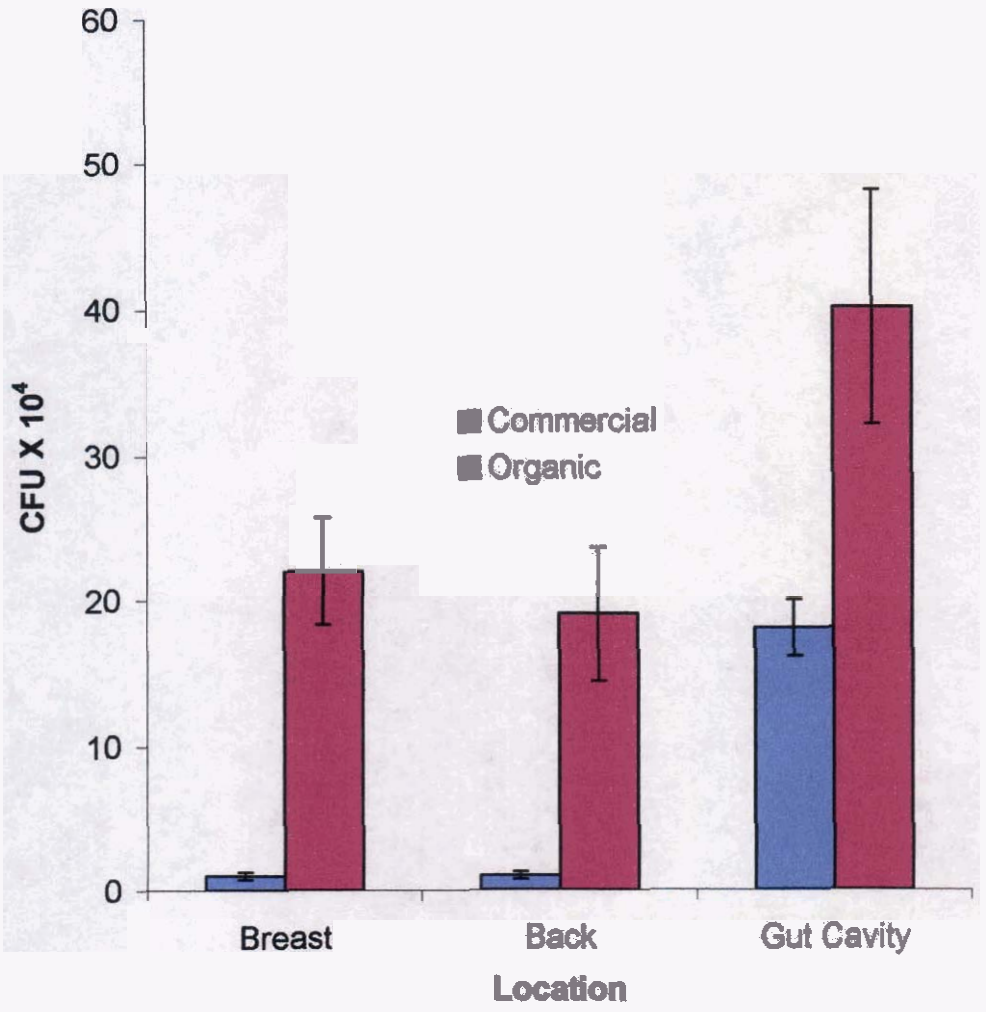

Figure 8. Average CFU/chicken detected in the organic and commercial chickens by location

Escherichia coli

In both populations, the highest incidence of *E. coli* was identified in the gut cavity (see Figure 9). *E. coli* was identified in 40% of the commercially grown chickens and 26% of the organically grown chickens. In the organically grown chickens, *E. coli* was only detected in the breast and gut. In the commercially raised chickens, *E. coli* was only detected in the back and gut.

The average CFU of *E. coli* bacteria /chicken was highest in the gut cavity of commercially processed chickens. There was a statistically significant difference in *E. coli* bacterial load ($P < .05$) in the gut cavity of the commercial and organically raised chickens. The commercial chickens had a bacterial load almost 10-fold greater than that detected in the organically raised chickens (see Figure 10).

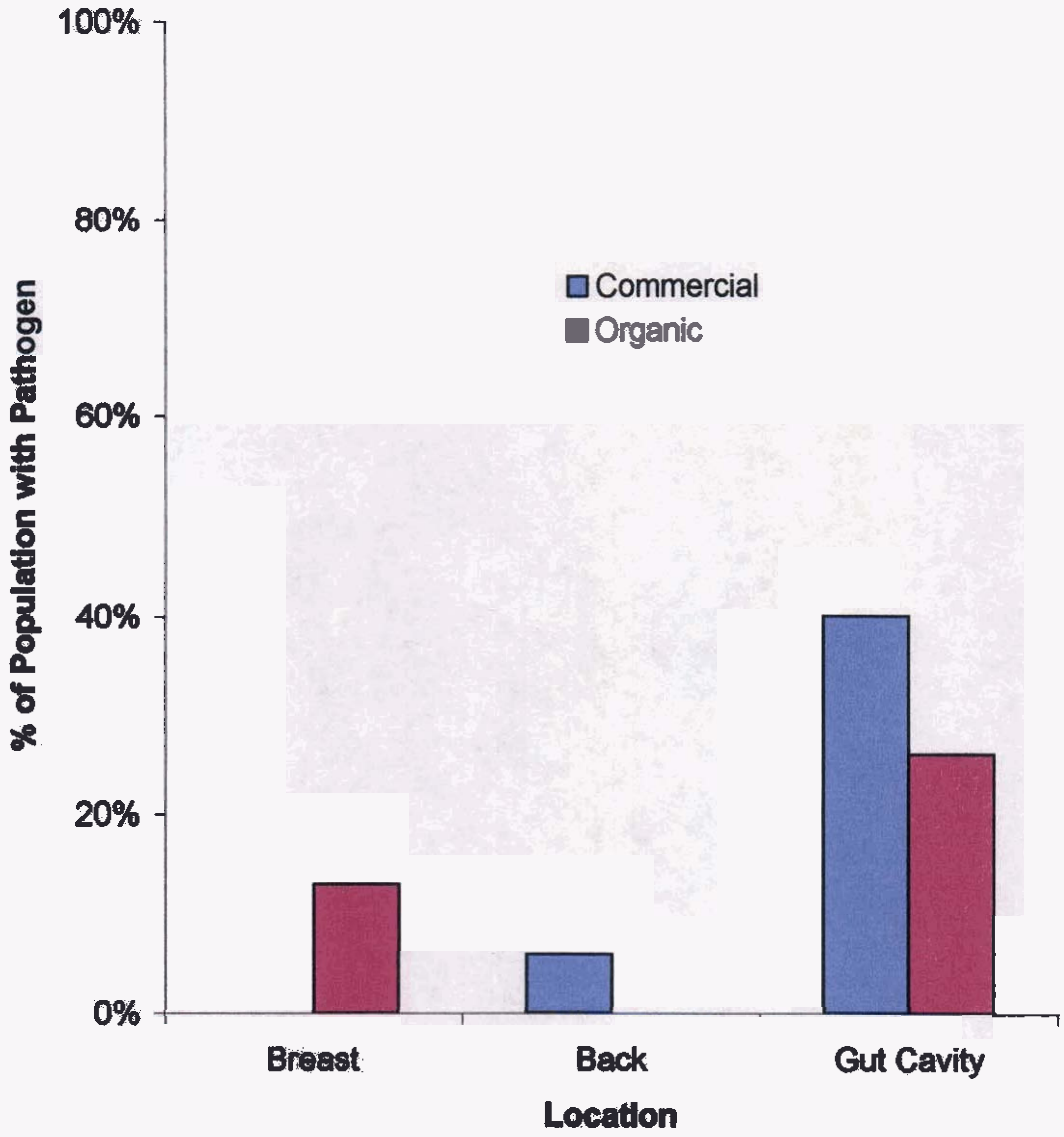

Figure 9. Incidence of *E-coli* bacteria in the population of organic or commercial chickens by location (breast, back, or gut cavity)

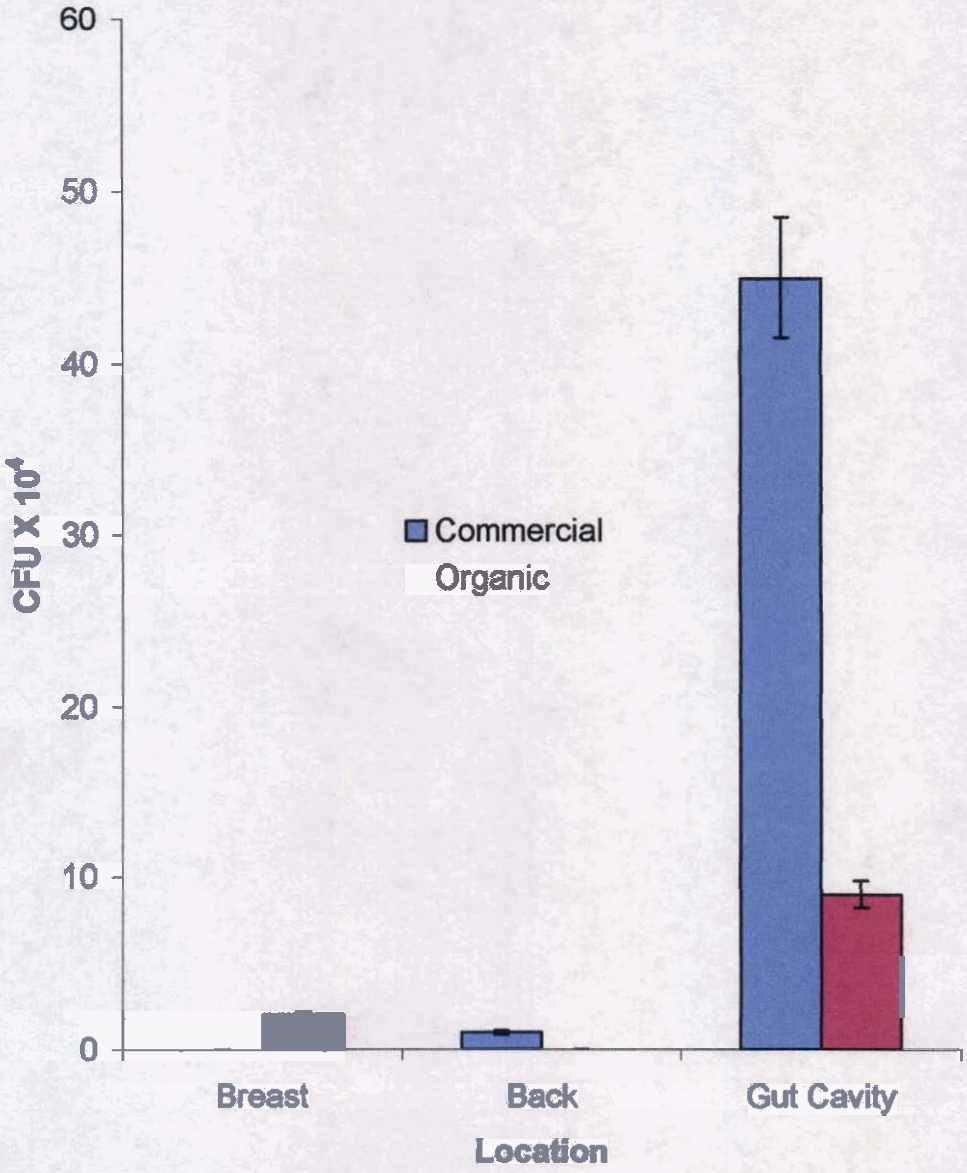

Figure 10. Average CFU/chicken detected in the organic and commercial chickens by location

Staphylococcus aureus

The highest incidence of *S. aureus* bacteria was noted in the back and gut cavity of commercial chickens (see Figure 11). The commercially processed chickens were populated with *S. aureus* bacteria in the breast, back and gut cavity. There was a higher incidence of *S. aureus* bacteria in the breast and back and gut cavity of commercially processed chickens versus the breast and back of organically grown chickens. *S. aureus* bacteria was found on 26% of the breasts of the commercially processed chickens and none of the breasts of the organically grown chickens. Forty-six percent of the commercially processed chickens were found to carry *S. aureus* bacteria while none of the backs of the organically grown chickens were populated with this bacteria. The gut cavity showed 46% of *S. aureus* on the commercially processed chickens and 20% on the organically grown chickens.

The average *S. aureus* bacteria CFU/chicken was highest at all locations in the commercially processed chicken and there was a statistically significant difference in *S. aureus* bacteria ($P < .05$) average CFU/chicken at all locations between organic and commercial chickens (see Figure 12).

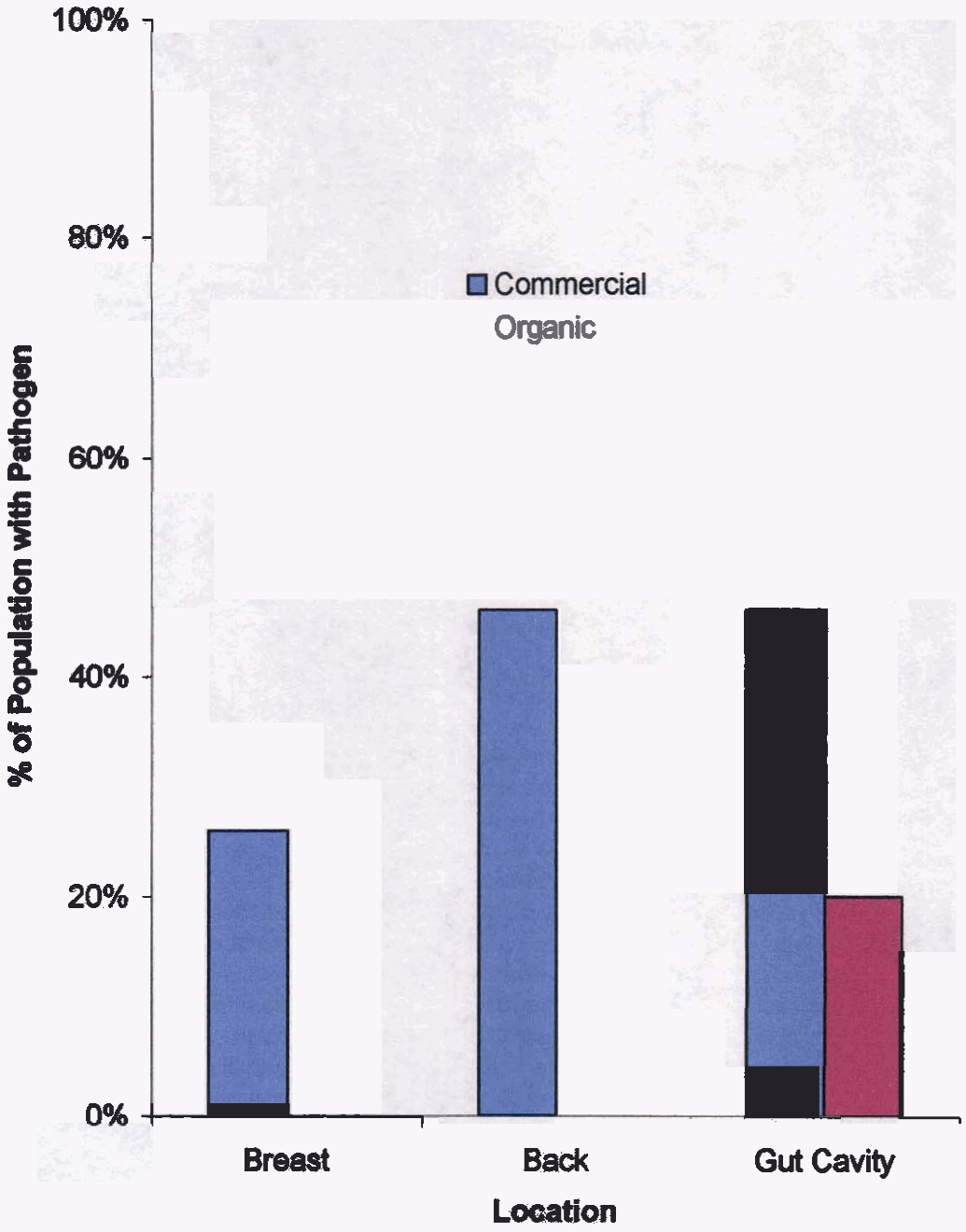

Figure 11. Incidence of *Staphylococcus aureus* bacteria in the population of organic or commercial chickens by location (breast, back, or gut cavity)

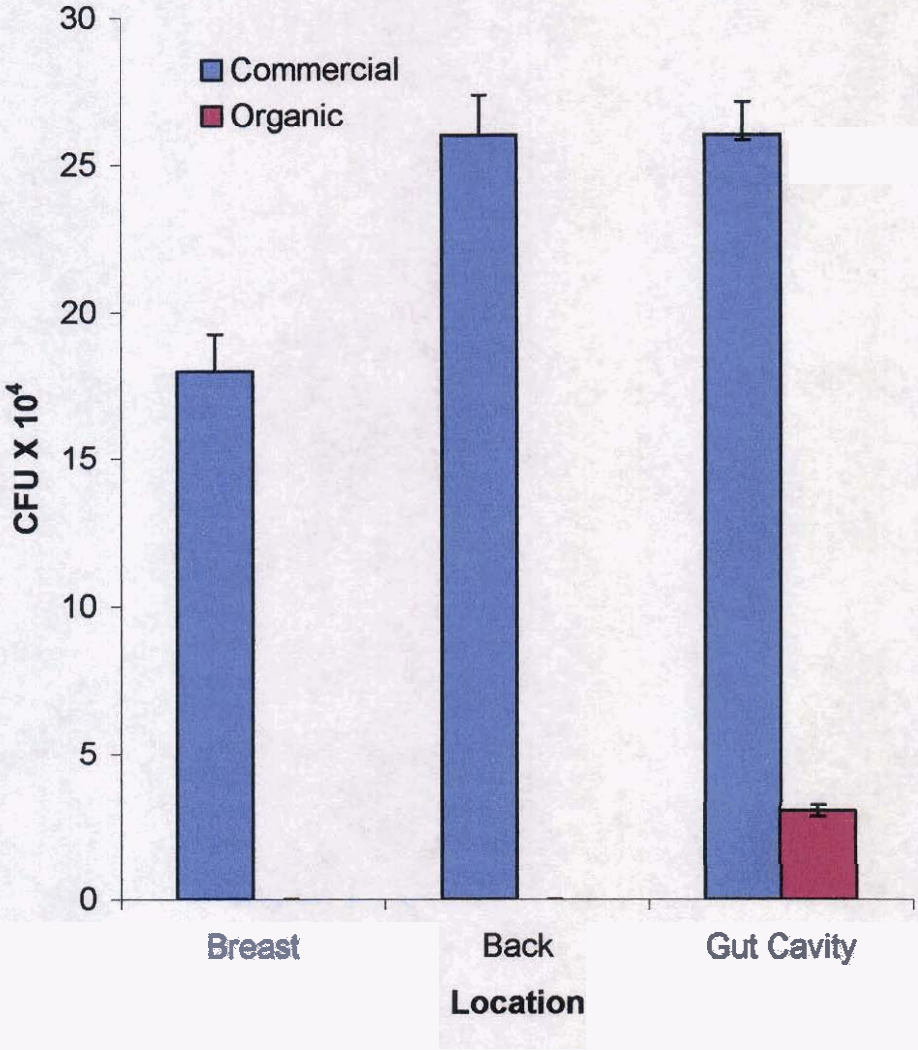

Figure 12. Average CFU/chicken detected in the organic and commercial chickens by location

Yeast and Mold Bacteria

There was a very low incidence of yeast and molds in both chicken populations (see Figure 13). There was a higher incidence of yeast and mold in the breast and gut cavities of commercially processed chickens versus the breast and gut cavities of organically grown chickens. Yeast and mold were found on 6% of the breasts of the commercially processed chickens and only one on the breast of the organically grown chickens. None of the organically grown chickens were found to carry yeast or mold and 6% of the gut cavities of commercially processed chickens were populated with this fungi.

The average yeast and mold CFU/chicken was highest at the breast and gut cavity locations in the commercially processed chicken, however, there was no statistically significant difference in yeast and mold ($P > .05$) average CFU/chicken on any location on the chicken between the organic and commercial chickens (see Figure 14).

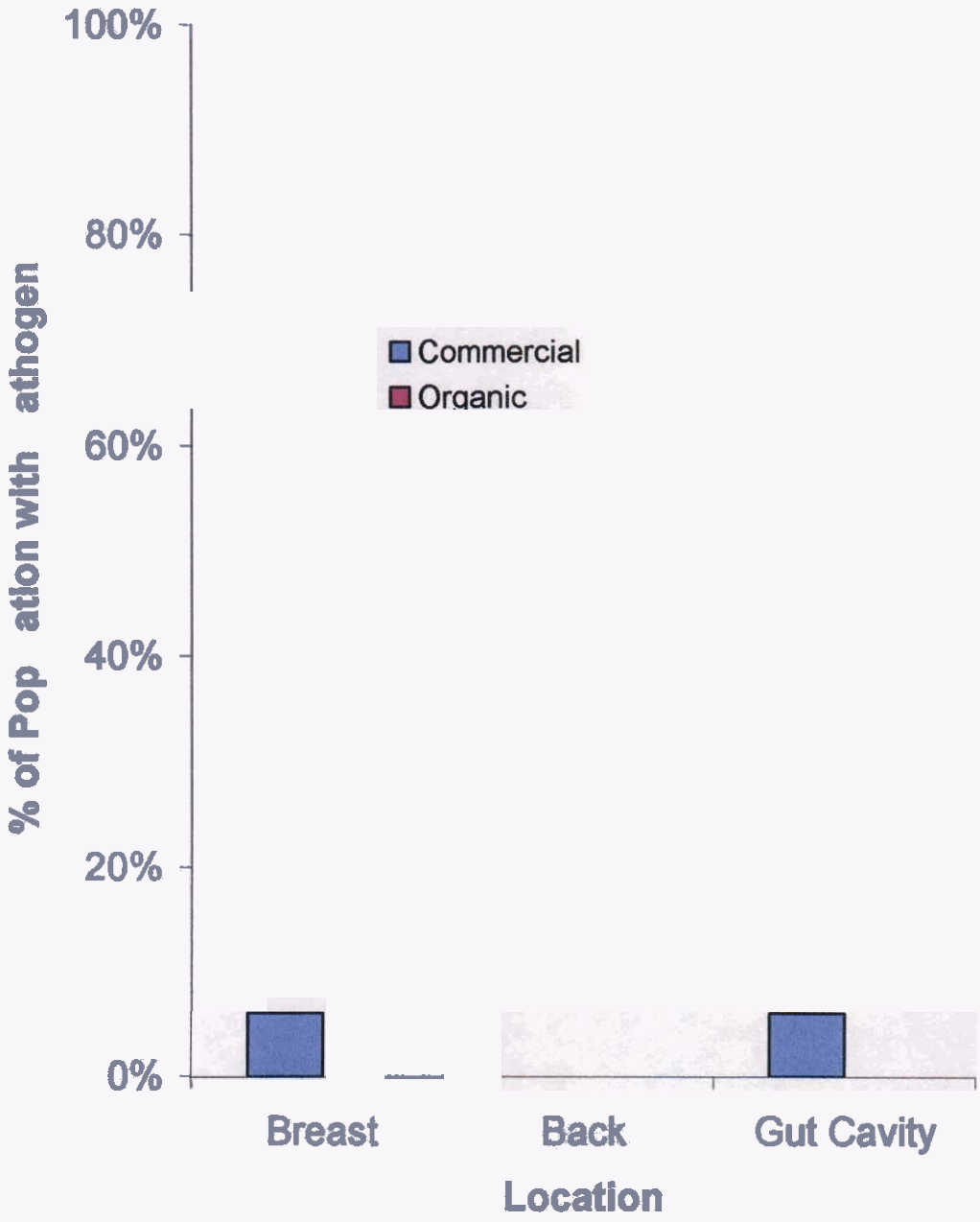

Figure 13. Incidence of yeast and mold bacteria in the population of organic or commercial chickens by location (breast, back, or gut cavity)

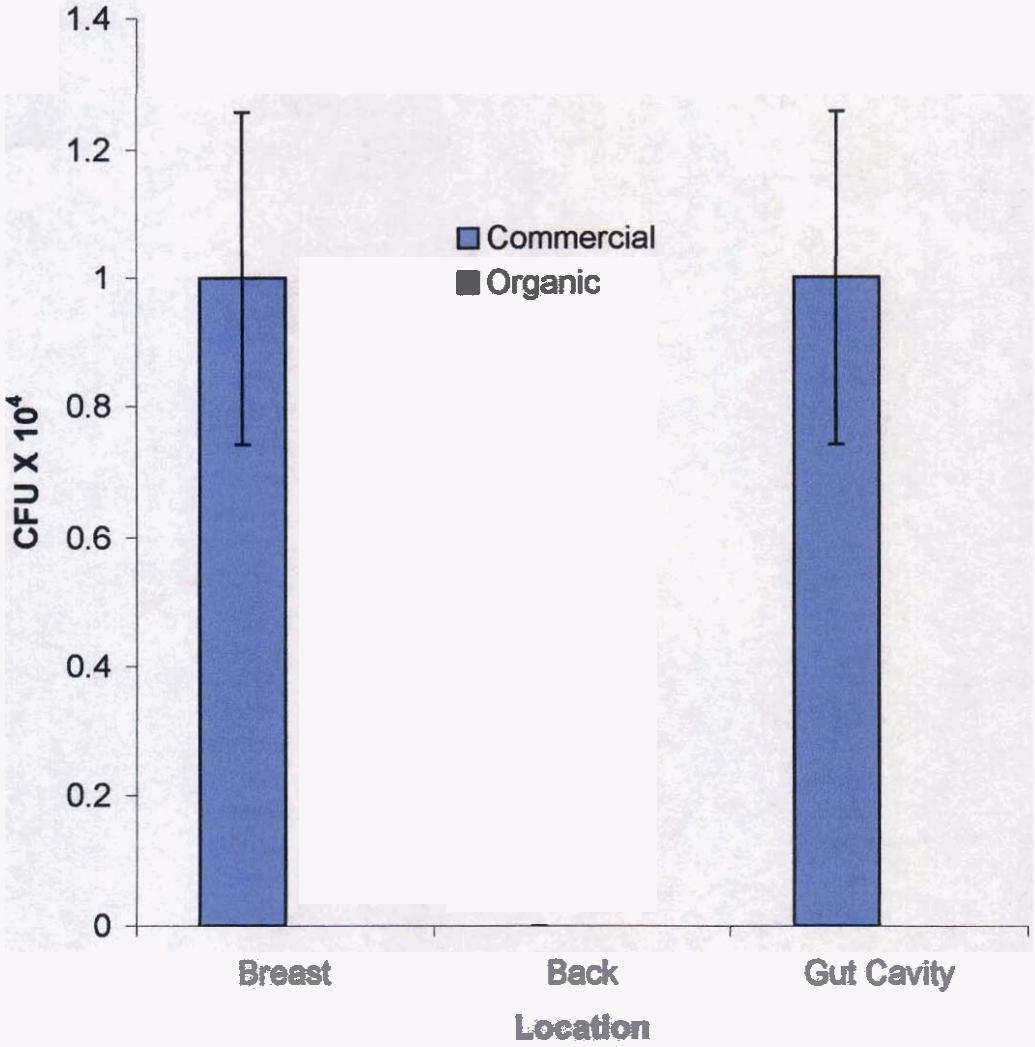

Figure 14. Average CFU/chicken detected in the organic and commercial chickens by location

Incidence of Listeria spp.

There was a higher incidence of *Listeria spp. (*in commercially processed chickens compared to organically grown chickens. *Listeria spp.* were present in 48.9% of all commercially processed chickens verses 11.10% of organically grown chickens (see Figure .15).

Listeria spp. were confirmed in 11.10% of the commercially processed population. They were either *Listeria monocytogenes* or *Listeria innocua* but species was not determined. There were no confirmed *Listeria spp.* in the organically raised chickens (see Figure 16).

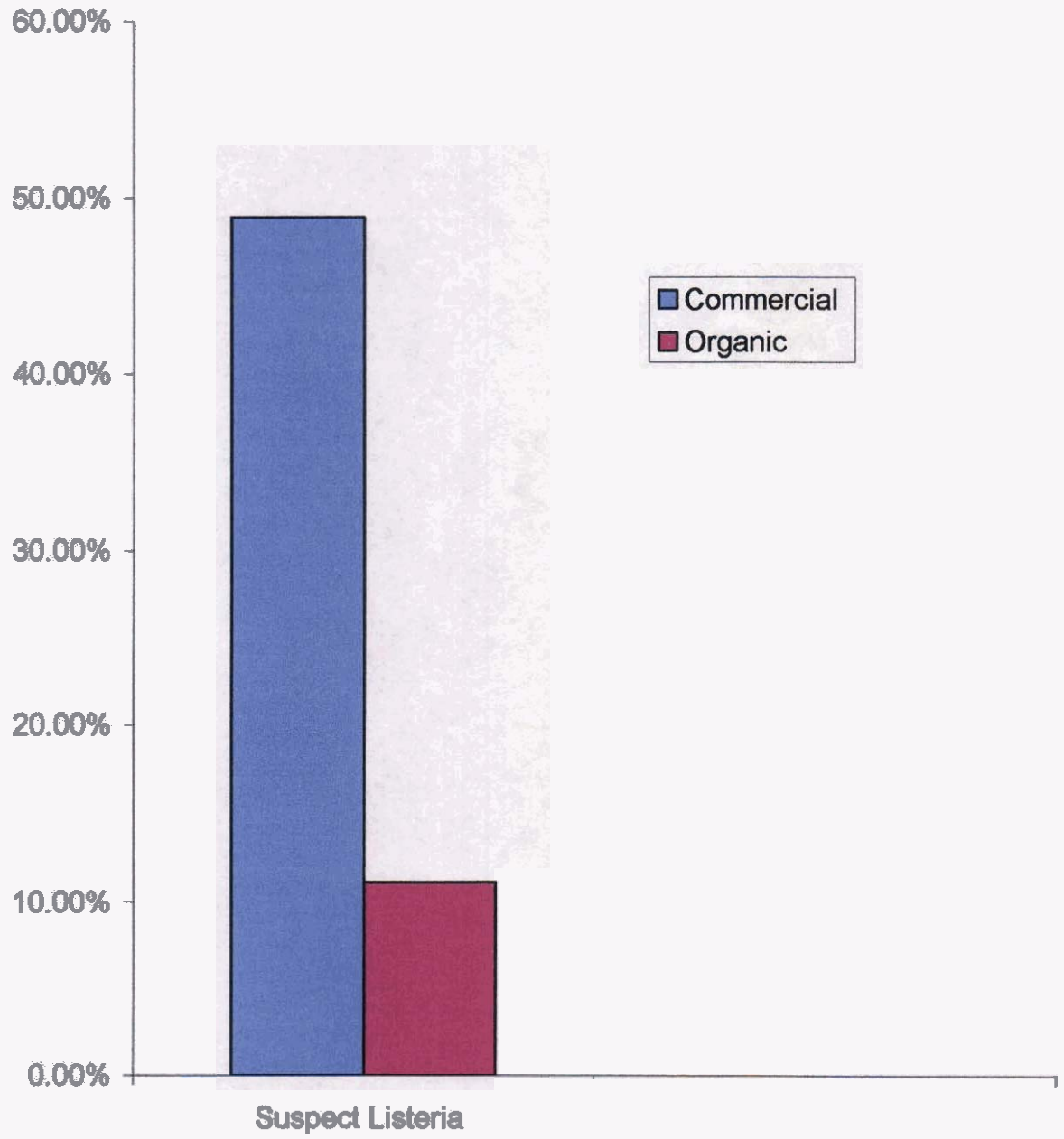

Figure 15. Screening for the presence/absence of Suspect *Listeria spp.* by chicken

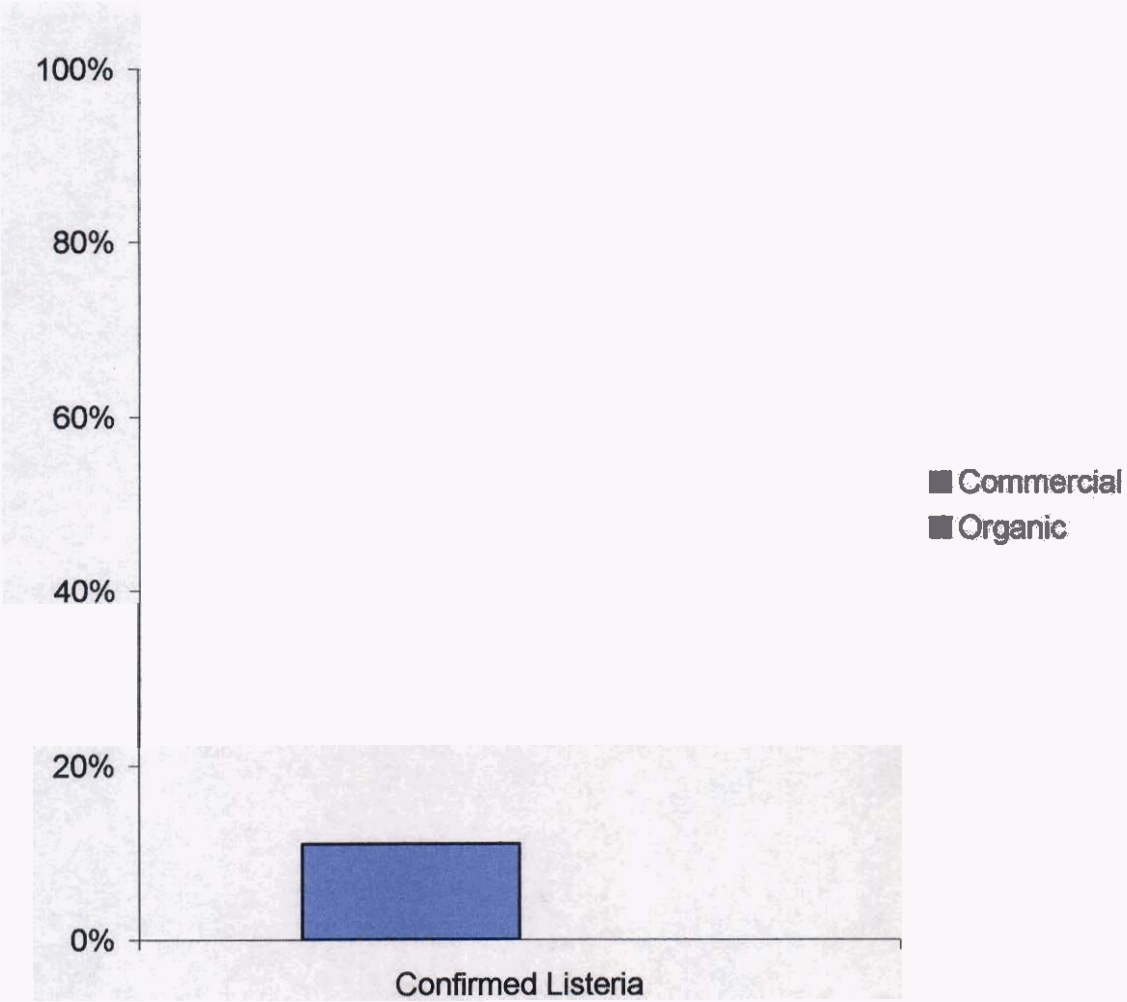

Figure 16. Confirmed *Listeria*

Incidence of Salmonella spp.

Suspect *Salmonella spp.* were present in 4.40% of all commercially processed chickens versus 17.70% of organically grown chickens (see Figure 17). There was a higher incidence of suspect *Salmonella* in organically grown chickens compared with commercially processed chickens.

Salmonella detection in commercially processed chickens was 4.40% versus the samples from the organically grown chickens were negative for confirmed *Salmonella spp.* (see Figure 18).

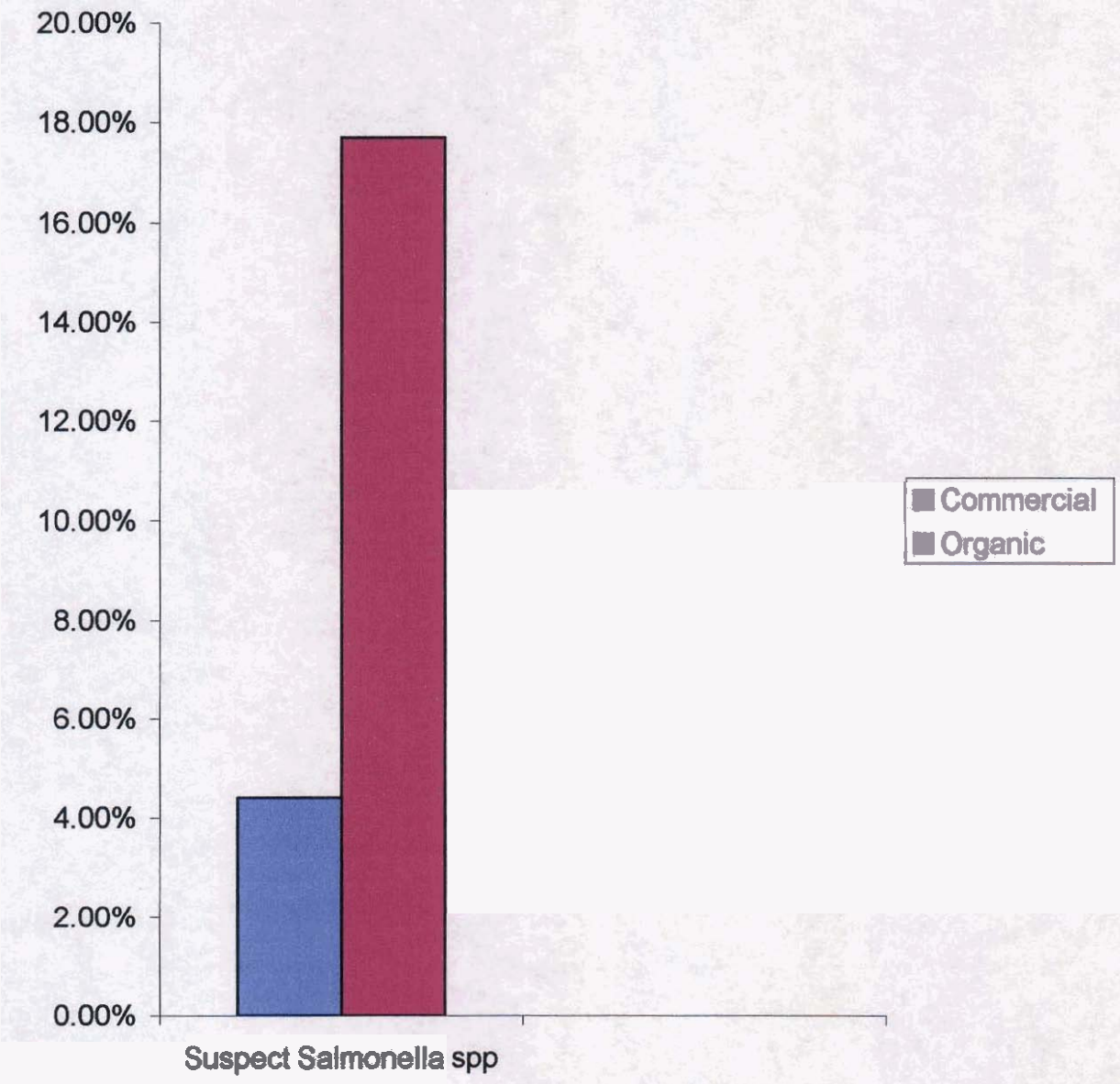

Figure 17. Incidence *of* suspect *Salmonella spp.*

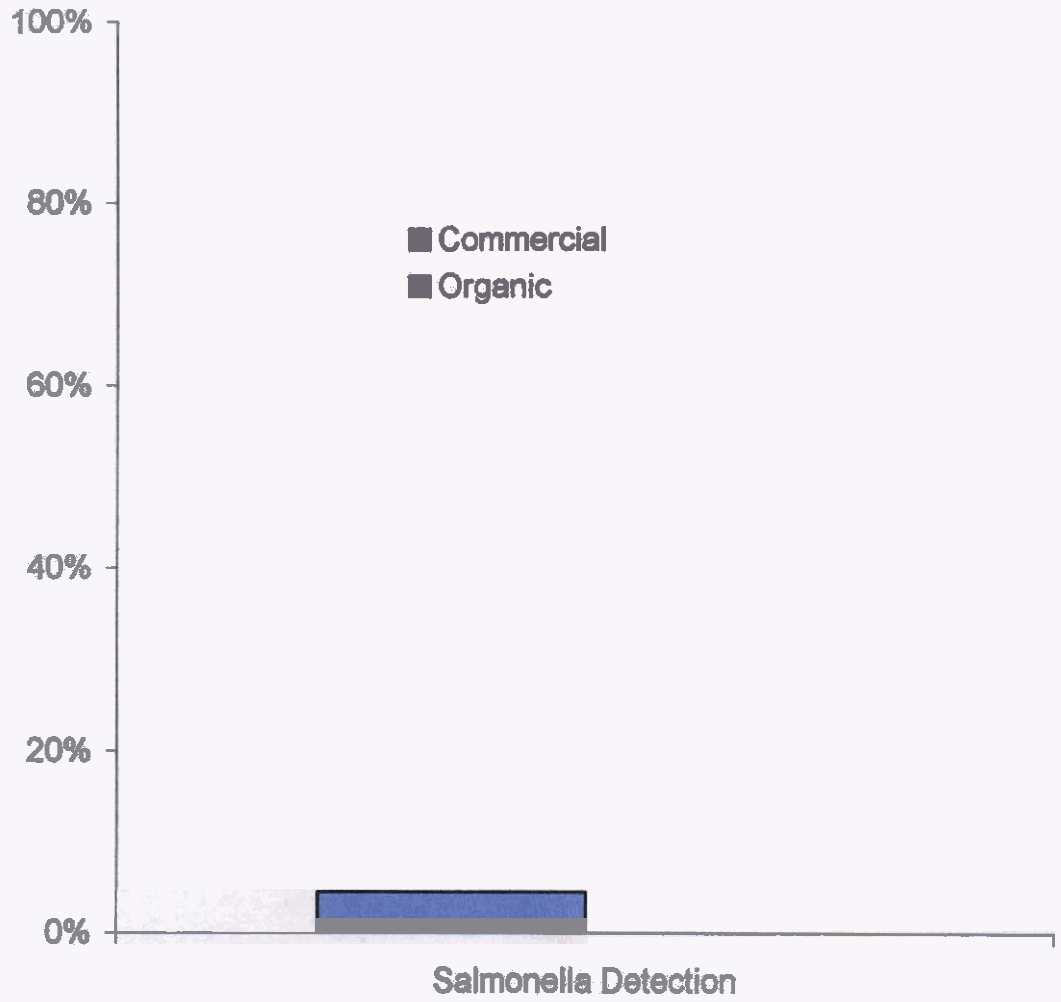

Figure 18. Confirmed Pathogenic *Salmonella*

Chapter V: Discussion

Aerobic Plate Counts

Although there are no government standards in place regarding aerobic plate counts, total aerobic plant counts (APC) are an indication of the bacterial load on a food product and may indicate how close food is to spoiling (Kohle, McClintock, Shukalek, McMullen, & Allison, n. d.). High incidence of aerobic bacteria and high APC could be attributed to poor sanitation and inconsistency in following HCCAP procedures at the processing plants.

In this work, incidence of aerobic bacteria and APC were determined in the organic chicken population and the commercial chicken population. There was no statistically significant difference in APC between the organic and commercially raised chickens; however, there were some differences in incidence of aerobic bacteria detected between the populations. In the organic and commercial chicken populations, there was no difference in incidence of aerobic bacteria in the gut cavity but there was a difference in incidence of aerobic bacteria on the backs and breasts. Commercial chickens were more likely to be colonized with aerobic bacteria on the backs and organically raised chickens were more likely to be colonized with aerobic bacteria on the breasts. These differences may indicate that there are differences in the handling during processing of organic and commercially raised chickens.

Coliform Bacteria

Coliform bacteria are associated with the intestinal tracts of humans and animals. Their presence outside of the intestines may be an indication of contamination with the fecal discharges of humans or animals. Numerous foodborne pathogens can be

transmitted through feces of humans and animals; the presence of coliforms may indicate the possibility that foodborne pathogens may also be contained in the food as well (Worobo, 1999). The presence of this bacteria in food results from contamination of food somewhere in the food chain; this is a good indicator of the hygiene of the production environment (Fanatico, 2003). Specifically, the presence of coliform bacteria in foods could be due to a lack of cleanliness in the food production area (Worobo).

The highest incidence of coliform bacteria was in the gut cavity of both commercially processed and organically grown chickens, and overall there was a higher incidence of coliform bacteria in all locations of the organic chickens. There was not a statistically significant difference in average coliform CFU/chicken between the organic and commercially raised chickens at any location. However, the higher incidence of coliform bacteria in the organically raised chickens may be indicative of differences in handling practices during processing.

E. coli

E. coli is a specific type of coliform which is in the intestinal tract of animals and is an indicator of fecal contamination. There was a higher incidence of *E. coli* in the gut of the commercially raised chickens, and there was a significantly higher *E. coli* bacterial load in the guts of the commercially raised chickens. This could be from the slaughtering process, when the contents of an animal's intestines and feces are allowed to come into contact with the chicken.

The slaughtering process includes several different steps which include pre-slaughter, catching and transporting. Broilers are usually processed at 4.5 lbs. live weight. Feed is withheld for 8 to 12 hours before the slaughtering process to reduce the amount of

feed in the gut and the possibility of tearing it during processing, which would cause fecal contamination of the carcass (Fanatico, 2003). Another important part of the slaughtering process is the washing of the chicken, the scalding/washing process; fecal contamination can also come into contact with the chicken carcass at this point of the operation. This could be one reason for the increase in *E-coli*. Standard practice has shown that chickens that are pre-washed with a chlorinated water of 50 ppm chlorine per bird can help reduce *E. coli*. Chlorine is effective at reducing *E. coli* and is safe if used correctly. Again, these results indicate that there are differences in the processing and handling of organically raised chickens and the commercially raised chickens that may result in higher bacterial loads.

Staphylococcus aureus

Staphylococci exist in air, dust, sewage, water, milk, and food or on food equipment, environmental surfaces, humans, and animals. Humans and animals are the primary reservoirs (USDA FSIS, 2006e). *S. aureus* is a bacterial pathogen that cause severe food poisoning (USDA FSIS, 2006b). It can be carried on human hands, in nasal passages, and throats. *S. aureus* is found in foods made by hand and improperly refrigerated foods. Food handlers are usually the main source of food contamination in food poisoning outbreaks, but equipment and environmental surfaces can also be sources of contamination with *S. aureus* from commercially processed chickens were populated with *S. aureus* bacteria in the breast, back and gut cavity. There was a higher incidence of *S. aureus* bacteria in the breast and back and gut cavity of commercially processed chickens than the breast and back of organically grown chickens. There was also a significantly higher *S. aureus* bacterial load in the commercially raised chickens versus

the organic chickens. This could be an indication of poor sanitation (USDA FSIS, 2006e). Poor sanitation would contribute to the higher counts in the commercially processed chickens, due to the fact that the employees handle multiple chickens for days at time, where as organically raised chickens are usually processed two to three times a year at a contracted processing plant (personal communication, organic chicken supplier, December 7, 2006). The organically raised chickens processing plants could also have better handling procedures and use careful standard protocols compared to commercially processed chickens.

Yeast and Mold

There was a very low incidence of yeast and molds in both chicken populations. The breast and gut cavities of commercially processed chickens had a higher incidence of yeast and mold then the breast and gut cavities of organically grown chickens. Mold spores can be carried by the wind, and can have easy entry to a food production facility which could explain the higher incidence in the commercially processed chickens since organically raised chickens are processed two to three times yearly depending on the processing plant.

Yeast and mold are a common contamination of food. While yeast does not result in food poisoning, it does cause food to spoil. Molds can produce mycotoxins, some of which can be harmful to humans.

Listeria spp.

Listeria spp. were present in 48.9% of all commercially processed chickens versus 11.10% of organically grown chickens. This finding could be from stainless steel

surfaces of processing equipment, conveyor belts, door handles or gloves from personnel in the processing facility.

Of the *Listeria spp.* found in the commercially processed population, 11.10% were either *Listeria monocytogenes* or *Listeria innocua*. Species was not determined. There were no *Listeria monocytogenes* in the organically raised chickens. *Listeria monocytogeneos* is the pathogen. The contamination on the commercially processed chickens could be from the lack of sanitation at the processing plant. If the organisms are not eliminated by sanitizing, the organisms have the potential to survive for extended periods under conditions found in many processing plants.

Salmonella spp.

There was a higher incidence of suspect *Salmonella spp.* in organically grown chickens compared to commercially processed chickens. This could be attributed to the presence of the pathogen in the soil or fecal matter.

The pathogen is spread more easily because of the manner in which the chickens are raised. Environmental sources of the organism can be from pre-wash water in processing plants or the late washes because chickens are washed both in the early and late stages of the slaughtering process.

Confirmed Salmonella detection in commercially processed chickens was 4.40%. The organically grown chickens were negative for confirmed *Salmonella spp.*

The organic and commercial chickens that tested suspect positive for *Salmonella species* turned out to be different bacteria. On the Enterotubes, they tested positive for *Cedecea lapagei, Cedecea spp, Enterobacter agglomerons* and *Shigella spp.*

Cedecea spp. has an optimum growth temperature of 37° Celsius but little information about this organism such as what can contribute to its presence is currently available. An unknown *Cedecea species* was found on the breast of the organically raised chicken and on the back the commercially raised chicken.

Cedecea lapagei can be found in different environmental sources associated with poultry processing. This bacteria was found on the back of the organically grown chicken.

One organic chicken had *Cedecea lapaga* or *Enterobacter agglomerons* on the breast as determined by the Enterotube test. *Enterobacter agglomerons* was also found on the back of a commercial chicken

The *Enterobacter agglomerans* that was found could be associated with lesions that are located in the skin between the thigh and midline. *E. coli* is most often isolated from the lesions, although *Pasteurella multocida* and *Enterobacter agglomerans* have also been isolated. Usually the lesions can be detected only after the feathers have been removed; no signs are visible in the living chickens (Derakhshanfar & Ghanbarpour, 2002).

Enterobacter agglomerans was found on the back of the commercially processed chicken. Commercially processed chickens are usually raised in very large poultry farms and the chickens are in cages in large farms which could cause these lesions.

One chicken had either *Enterobacter agglomerons* or a *Shigella spp.* on the breast. Both were listed as possibilities on the Enterotube test. *Shigella spp.* is frequently found in water polluted with human feces and with poultry plants using a large quantity of water and poultry farms close to or on the grounds of processing plants. This is one

possible source of the contamination of the chickens. Fecally contaminated water and unsanitary handling by food handlers are the most common causes of contamination (USDA FSIS, 2006f).

Limitations of the Study

Some of the limitations of this study were that the commercially processed chickens were refrigerated and the organic chickens were frozen because of the distance from the farm to the testing area. Also the summer heat in the building where the testing was done was at two different temperatures at the different testing times. This study was able to run tests on 30 chickens due to the time limits and costs involved.

Conclusions

The difference in microbial populations found in organic and commercial chickens implies significant differences in handling practices between the populations.

Recommendations

The recommended future research would be to do five trials of five commercially processed and five organic processed chickens. It may be interesting to weigh each chicken to see if there were differences size of chickens. The yeast and mold tests could be eliminated, but testing for *Campylobacter jejunei* and other species is suggested.

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Appendix A: Colony Counts of Commercial and Organically Chickens

Colony counts of Aerobic plate count of the Breast

Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	1	1	1000
2	0	2	1
3	0	3	1
4	0	4	1
5	0	5	2
6	0	6	9
7	0	7	2
8	1000	8	1
9	3	9	0
10	1	10	4
11	0	11	2
12	2	12	41
13	1	13	0
14	2	14	3
15	0	15	1

Colony counts of Aerobic plate count of the Back

Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	0	1	3
2	0	2	2
3	1	3	4
4	0	4	1
5	0	5	2
6	0	6	2
7	4	7	2
8	1000	8	2
9	2	9	1
10	1	10	5
11	1	11	4
12	0	12	4
13	0	13	0
14	1000	14	1
15	2	15	188

Colony counts of Aerobic plate count of the Gut Cavity

Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	4	1	19
2	15	2	34
3	13	3	9
4	16	4	25
5	67	5	14
6	7	6	14
7	11	7	36
8	1000	8	25
9	28	9	16
10	55	10	6
11	44	11	29
12	18	12	154
13	71	13	4
14	1000	14	6
15	171	15	141

Colony counts of *Coliform* of the Breast

Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	0	1	1
2	0	2	0
3	0	3	0
4	0	4	0
5	0	5	0
6	0	6	0
7	0	7	0
8	1	8	0
9	0	9	0
10	0	10	0
11	0	11	0
12	0	12	12
13	0	13	0
14	0	14	9
15	0	15	0

Colony counts of *Coliform* of the Back

*Coliform-Back*Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	0	1	0
2	0	2	0
3	0	3	0
4	0	4	0
5	0	5	0
6	0	6	0
7	1	7	0
8	0	8	0
9	0	9	0
10	0	10	1
11	0	11	0
12	0	12	0
13	0	13	0
14	0	14	0
15	0	15	18

Colony counts of *Coliform* of the Gut Cavity

Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	0	1	0
2	3	2	0
3	0	3	0
4	0	4	0
5	0	5	0
6	0	6	0
7	7	7	2
8	0	8	0
9	1	9	1
10	1	10	0
11	1	11	1
12	0	12	31
13	2	13	1
14	0	14	1
15	3	15	3

Colony counts of *E-coli* of the Breast

Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	0	1	0
2	0	2	0
3	0	3	0
4	0	4	0
5	0	5	0
6	0	6	0
7	0	7	0
8	0	8	0
9	0	9	0
10	0	10	0
11	0	11	0
12	0	12	1
13	0	13	1
14	0	14	0
15	0	15	0

Colony counts of *E-coli* of the Back

Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	0	1	0
2	0	2	0
3	0	3	0
4	0	4	0
5	0	5	0
6	0	6	0
7	1	7	0
8	0	8	0
9	0	9	0
10	0	10	0
11	0	11	0
12	0	12	0
13	0	13	0
14	0	14	0
15	0	15	0

Colony counts of *E-coli* of the Gut Cavity

Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	0	1	1
2	0	2	0
3	0	3	0
4	0	4	0
5	0	5	0
6	0	6	0
7	1	7	0
8	2	8	0
9	2	9	0
10	2	10	0
11	0	11	0
12	0	12	6
13	11	13	0
14	0	14	1
15	27	15	1

Colony counts of *Staphylococcus aureus* on the Breast

Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	0	1	0
2	0	2	0
3	4	3	0
4	0	4	0
5	0	5	0
6	6	6	0
7	6	7	0
8	2	8	0
9	0	9	0
10	0	10	0
11	0	11	0
12	0	12	0
13	0	13	0
14	0	14	0
15	0	15	0

Colony counts of *Staphylococcus aureus* on the Back

Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	0	1	0
2	0	2	0
3	3	3	0
4	5	4	0
5	0	5	0
6	6	6	0
7	2	7	0
8	0	8	0
9	0	9	0
10	5	10	0
11	0	11	0
12	0	12	0
13	4	13	0
14	1	14	0
15	0	15	0

Colony counts of *Staphylococcus aureus* of the Gut Cavity

Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	0	1	0
2	0	2	1
3	3	3	1
4	5	4	0
5	0	5	0
6	6	6	0
7	2	7	0
8	0	8	0
9	0	9	0
10	5	10	0
11	0	11	0
12	0	12	0
13	4	13	0
14	1	14	0
15	0	15	1

Colony counts of Yeast and Mold of the Breast

Commercial Chicken #	Colonies counted	Organic Chicken #	Colonies counted
1	0	1	0
2	0	2	0
3	0	3	0
4	0	4	0
5	0	5	0
6	0	6	0
7	0	7	0
8	0	8	0
9	1	9	0
10	0	10	0
11	0	11	0
12	0	12	0
13	0	13	0
14	0	14	0
15	0	15	0

Colony counts of Yeast and Mold of the Back

Commercial Chicken #	Colonies counted	Organic Chicken #	Colonies counted
1	0	1	0
2	0	2	0
3	0	3	0
4	0	4	0
5	0	5	0
6	0	6	0
7	0	7	0
8	0	8	0
9	0	9	0
10	0	10	0
11	0	11	0
12	0	12	0
13	0	13	0
14	0	14	0
15	0	15	0

Colony counts of Yeast and Mold of the Gut Cavity

Commercial Chicken	Colonies counted	Organic Chicken	Colonies counted
1	0	1	0
2	1	2	0
3	0	3	0
4	0	4	0
5	0	5	0
6	0	6	0
7	0	7	0
8	0	8	0
9	0	9	0
10	0	10	0
11	0	11	0
12	0	12	0
13	0	13	0
14	0	14	0
15	0	15	0

Screening for the presence/absence of *Salmonella spp* and *Listeria spp.* by chicken-Breast

Commercial Chicken #	Listeria	Salmonella	Organic Chicken #	Listeria	Salmonella
1			1	Yes	
2	Yes		2		
3	Yes		3		
4	Yes		4		
5			5		
6			6		
7	Yes		7		
8			8		
9			9		
10			10		Yes
11	Yes		11		
12			12		Yes
13			13		Yes
14			14		
15	Yes		15		

Screening for the presence/absence of *Salmonella spp* and *Listeria spp.* by chicken-Back

Commercial Chicken #	Listeria	Salmonella	Organic Chicken #	Listeria	Salmonella
1	Yes		1		
2	Yes	Yes	2		
3	Yes		3		
4			4		
5	Yes		5		
6			6		
7			7		
8	Yes		8		
9			9		Yes
10			10		
11	Yes		11		Yes
12			12		
13	Yes		13		
14			14		
15			15		

Screening for the presence/absence of *Salmonella spp.* and *Listeria spp.* by chicken-Gut Cavity

Commercial Chicken #	Listeria	Salmonella	Organic Chicken #	Listeria	Salmonella
1	Yes		1	Yes	
2	Yes		2	Yes	
3	Yes		3		
4			4		
5	Yes		5		
6	Yes		6		
7			7		
8	Yes		8	Yes	
9	Yes	Yes	9		Yes
10			10		
11	Yes		11	Yes	Yes
12			12		Yes
13			13		
14	Yes		14		
15			15		

Appendix B: Statistical Data

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PROJECT #3509

NOTE: The data was entered into an Excel spreadsheet by the researcher. The Research & Statistical Consultant had no control over its accuracy. The Excel spreadsheet was edited to eliminate headings, extra "sheets", and other extraneous information, and to standardize variable names.

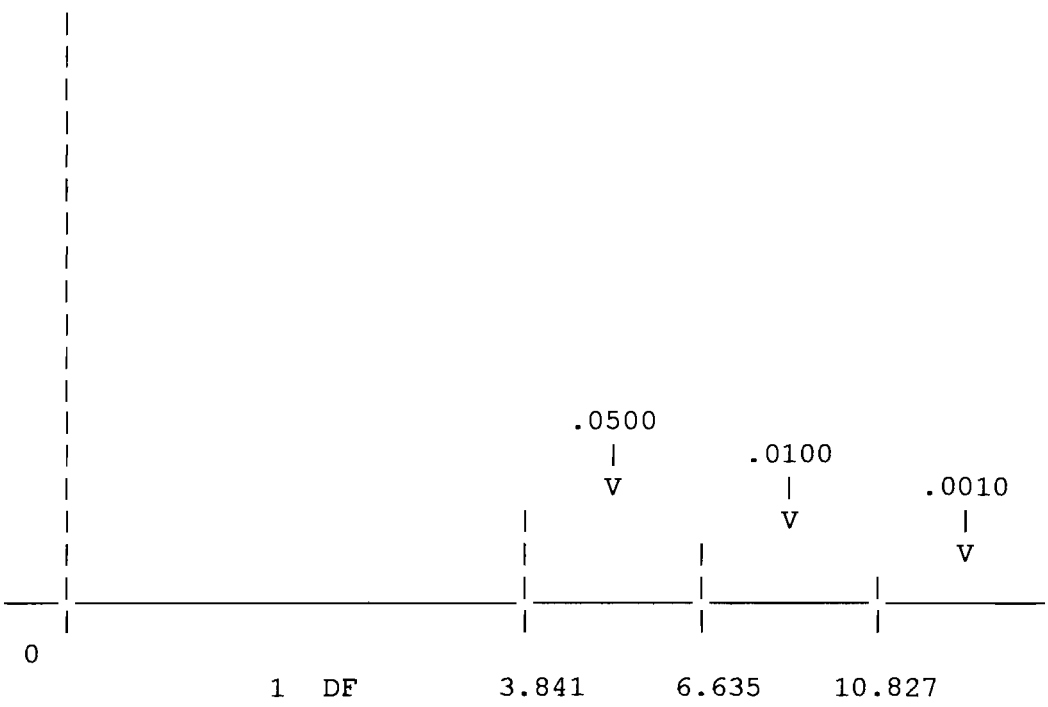
SPSS-X -- use GET TRANSLATE to import the Excel 5.0 workbook into an SPSS 14.0 data spreadsheet. All variables were RENAMED and had new VARIABLE WIDTHS, FORMATS, and VARIABLE LEVELS assigned to them when appropriate.

SPSS-X -- use FREQUENCIES to obtain frequency counts and percentages on the following sets of variables:
1 - COM_ORG, LOCATION, LISTERIA, MOX_AGAR, SALMONELLA, and XLD

SPSS-X -- use FREQUENCIES to obtain frequency counts, percentages, mean, median, and standard deviation on the following sets of variables:
2 - YEAST, STAPH, COLIFORM, AEROBIC, and E_COLI

SPSS-X -- use CROSSTABS to obtain frequency counts and percentages between the following combinations of variables:
3 - COM_ORG with LOCATION

SPSS-X -- use CROSSTABS, with a Chi Square analysis, to obtain
frequency counts and percentages between the following
combinations of variables:
4 - COM_ORG with LISTERIA and SALMONELLA

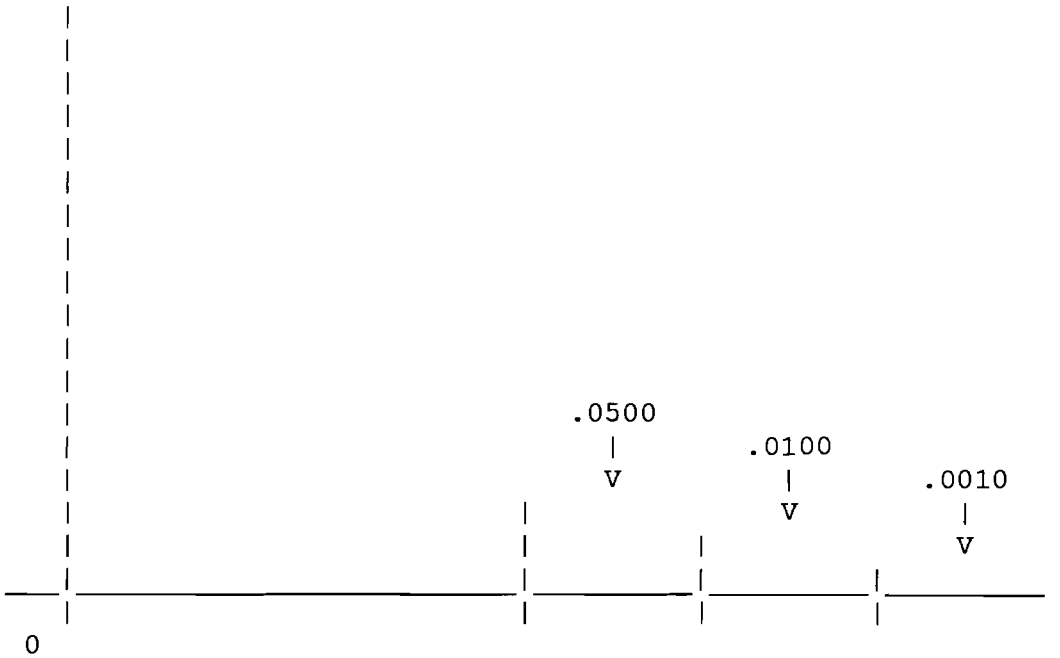


REFERENCE: Research Methods in Education: An Introduction
(4th Edition), William Wiersma, Allyn and Bacon,
Inc.,
Boston, Massachusetts, 1986, page 443.

SPSS-X -- use UNIANOVA to run a two-way analysis of variance
on YEAST, STAPH, COLIFORM, AEROBIC, and E_COLI
when separated by COM_ORG (commercial / organic)
and LOCATION (breast / back / gut)
using the following design:
NOTE: Options selected included:
/PRINT=DESCRIPTIVES (mean, standard deviation,
count)
/POSTHOC=LOCATION (SNK DUNCAN LSD)
(multiple comparison tests)
(Student-Newman-Keuls)
(Duncan)
(Least Significant Difference)

#5	BREAST	BACK	GUT
COMMERCIAL	N = 15	N = 15	N = 15
ORGANIC	N = 15	N = 15	N = 15

0 OMITS



1,80 - 1,89 DF	3.96	6.96
2,80 - 2,89 DF	3.11	4.88

REFERENCE: Statistical Analysis in Psychology and Education, George A. Ferguson, McGraw-Hill Book Company, New York, 1971, pages 452-455.