THE EFFECT OF AMMONIA CONCENTRATION AND EXPOSURE TIME ON THE QUALITY OF MUSCLE TISSUES

By

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DEDICATION

This thesis as well as my life is dedicated to the glory of my Lord and Savior, ALLAH. May these works, as well as my future life works serve him well. With deep love this thesis dedicated to the great Messenger of ALLAH, MOHAMMED (THE MERCY FOR ALL CREATURES) peace be upon him.

Additionally, it is with great joy and heart felt love I dedicate this thesis to my parents, my father ABDULLAH MOHAMMED AL-SAHAL and my mother AMAT-ELMALEIK MOHAMMED AL-JUDAIRI. I can say with certainty that their steadfast love, prayer and supplication, encouragement, and advice was instrumental in allowing me to accomplish my educational goals. Words can not express my true feeling for these two wonderful and beloved people.

Special dedication is due to all my wonderful brothers and sisters for their love, unfailing encouragement, and support. With my heartfelt deep love I dedicate this work to my great and respectful sister MARIAM and her lovely daughters NAWAL and EL-HANOOF.

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Last but not least, this thesis dedicated to my lovely children, AMAL, MALEIKAH, ABDULLAH, and ABDULRAHMAN may ALLAH raise them with his guidance.

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CHAPTER I

INTRODUCTION

Since different activities are involved in the trading of meat, cold storage facilities are one of the most important segments in any meat business. Chilling and freezing are greatly acknowledged as important forms of heat reduction. Meat manufacturers have utilized chilling and freezing as steadfast methods to preserve and prolong the shelf-life of meat and meat products.

Ammonia is the oldest refrigerant utilized in the food industry today. Cold storage facilities using ammonia as a refrigerant are subjected to ammonia spill's from time to time depending on the level of awareness enhanced among the workers and the maintenance system advancement (Kramer et al, 1981). The frequency of ammonia spills is difficult to assess due to the differences in the concentration of every spill and the location as well as the danger of the situation. Small leaks of ammonia are rarely reported to the Environmental Protection Agency. Yearly, 100 incidents of ammonia leaks, in food cold storage warehouses, have been estimated in The United States of America and Canada alone (Smith, 1987).

Anhydrous ammonia (NH_3) is widely known as the best refrigerant because of its advantages including excellent heat reduction properties, economical cost, and environmental safety (Arnold, 1993). Quality of meat and meat products exposed to ammonia in cold storage facilities was effected when the concentration of ammonia was very high (200,000 ppm) (Anil, 1971; Herrmann, 1965; Kassem, 1965).

Contamination of meat and meat products is of great concern for both the processors and commercial cold storage warehouses. Changes in the quality of any meat exposed to ammonia have been estimated by the increases in the pH and ammonical nitrogen content of the meat surface as well as the acceptability of sensory evaluation scores. Increases of 1.0 pH unit and 0.15 % of nitrogen content due to ammoniacal nitrogen after ammonia contamination in meat and meat products have been a guide for condemnation (Anon., 1981; Goodfellow et al, 1978).

Even though, several methods were established and used to evaluate changes in the quality of foods exposed to high levels of ammonia, there is a paucity of information for meat exposed to low levels of ammonia. Furthermore, the time and concentration of exposure to ammonia that may affect the quality of meat have not been well clarified.

Demonstration of possible alterations in meat quality due to ammonia leakage has made it necessary to evaluate the merits of ammonia in meats and to determine the specific levels required to cause various meat items to be removed

from the food distribution channel. Accessing such information will lead to better decision making whenever a spill occurs. Therefore, the main purpose of this research was to determine the effect of ammonia concentration and length of exposure on some of the quality attributes of beef, pork and chicken musculature.

CHAPTER II

REVIEW OF LITERATURE

GENERAL CHARACTERISTICS OF AMMONIA

Physical & Chemical Properties

NH_3 is the chemical formula by which ammonia is identified with a relative molecular mass of 17.031. Approximately 1 ppm of ammonia (1 mg/liter) is equal to 0.70 mg/m^3, however, depending on the surrounding temperature and the atmospheric pressure, this number is changeable. Ammonia gas is easily detectable by the human nose because of its self alerted strong odor. Most people (least sensitive) can distinguish ammonia at concentration of 50 ppm and above in air. Trained people (most sensitive) may detect ammonia at concentration of 5 ppm (Raj, 1982).

Ammonia as a liquid is lighter than water (60% as heavy as water) and as gas is lighter than air under room temperature (25°C) and normal atmospheric pressure (760 mm of mercury = 1.01325 bars) (Ostner, 1986). Ammonia is a colorless gas which dissolves excessively in water, any

product containing water, or any solution formed by its reaction with water will form a strong alkaline solution. In other cases, when ammonia gas escapes, water and any product containing water will be the main target for ammonia. Corrosiveness of ammonia is a result of moisture content, therefore, dry ammonia (gas or liquid) is not corrosive to most materials. Ammonia is a very reactive chemical and easily reacts with a large group of substances. Oxidation is one of the most important reactions. Also, ammonia salts are the major products of the chemical reaction of ammonia with acids either gases or liquids and a white precipitate may form as Ammonium Carbamate which is highly corrosive to steel (Nat. Res. Council, 1979; Bogart, 1981; WHO, 1990).

Usually, storage and transportation of liquid ammonia at 25°C can be safe at a pressure of 10 atmospheres by using uncorrosive containers. Easily, ammonia gas could be compressed or cooled to a colorless liquid as in refrigeration systems. When liquid ammonia is spilled, due to its boiling point (-33.3°C), ammonia boils immediately and causes a cooling action (absorb heat) for the surrounding area by the vapor. A cloud of gas may formed after an ammonia spill due to the formation of an air-ammonia mixture which is dependent on the atmospheric pressure and temperature to become denser than air. Because

of the high density of the air-ammonia mixture, air saturated by ammonia may not dissipate effortlessly and it may remain close to the floor causing massive damages. Thirty minutes of exposure in 500 ppm of ammonia gas has been specified as being "Immediately Dangerous to Life and Health" (IDLH concentration) (Davis et al., 1987).

Some physical characteristics of ammonia include, the freezing point (-77.7°C), boiling point (-33.3°C), liquid density (681.9 kg/m^3 at -33.3°C and one atmosphere), and specific volume of vapor (1297 m^3/kg at 0°C and one atmosphere).

EFFECT OF AMMONIA ON HUMAN HEALTH

Ammonia Health Impact

Ammonia alkalinity when dissolved in body fluids is the major cause of irritation. Skin, eyes, and the respiratory tract are more susceptible to ammonia than other parts of the body. The degree of ammonia hazard on human health is dependent on three major factors: concentration of ammonia, length of exposure, and mechanism of that exposure (Lessenger, 1985). Obviously, breathing air containing ammonia as little as 5000 ppm causes death by suffocation in

a short time. Exposure to ammonia at 2000 ppm for a few seconds is enough to burn and blister the skin and may lead to serious lung edema. Unless treated immediately, exposure to ammonia concentration above 700 ppm will cause eye injury that can originate loss of sight (Slack and James, 1973; WHO, 1986).

Karplyuk et al.(1989) studied the possible harmful effects of meat exposed to ammonia (0.1% & 0.3% = 1000 & 3000 ppm) subsequently fed to three generations of experimental rats. In their conclusions, detrimental impacts were recorded after feeding the rats meat containing 0.3% ammonia. Damage to the body systems were observed during the first six months of each generation including: destruction of the fermentation function of the liver, reduction of the activity of cholinesterase (ChE) in the blood, and reduction in the level of liability for the central nervous system. Meat containing 0.1% ammonia (1000 ppm) generated smaller consequences on the rat systems. Activity of lactate dehydrogenase and alanineaminotransferase were affected and only the first generation experienced a functional disruption of the central nervous system. Furthermore, the greater the dosage of ammonia in feed the more pronounced the effect on the animal systems.

Fortunately, there is no evidence that exposure to ammonia causes any carcinogenic effect either in humans or in experimental animals. Ammonia may produce inflammatory injury of the colon and cellular proliferation, however, evidence is not available proving that ammonia is accountable for any kind of tumors. Life-time studies on mice demonstrated that tumors were not developed by the effect of ammonia and ammonia exposure does not increase the probability of cancer incidence (WHO, 1986).

Ammonia Removal from the Human Body

The Liver and kidney play a major role in eliminating ammonia from the human body systems via two mechanisms (Figure 1). The first, when ammonia reaches the blood and enters the liver, the liver transforms ammonia to carbamyle phosphate. In the urea cycle, carbamyle phosphate forms urea where it is transported via circulation to the kidney and excreted in the urine. The second way, ammonia formed or absorbed in human tissues is converted to glutamate, then to glutamine. Glutamine as a carrier for ammonia enters the blood circulation and is transported to the kidney where the ammonia ions are excreted in the urine (Ryer-Powder, 1991)

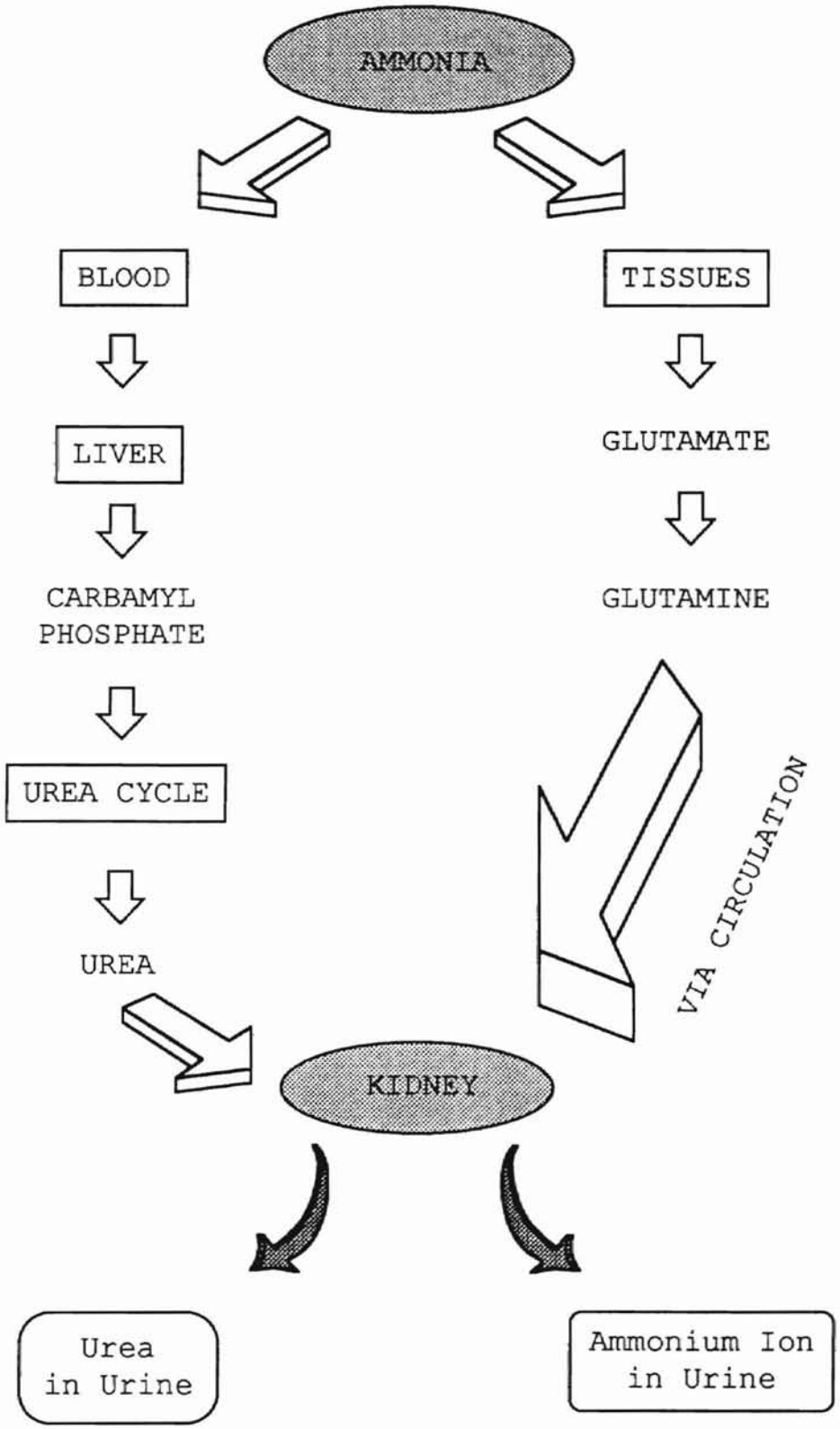

Figure 1. Ammonia Removal From the Body of Humans

MUSCLE CHARACTERISTICS

BEEF

Variations in color of muscles are dependent on many aspects including: species, age of the animal, sex (male vs female), mobility of muscle within the animal, and storage condition of the meat (Miller, 1994; Judge et al., 1989). Color references of steer, heifer, and cow beef lean are related specifically to the physiological maturity of the animal (Romans et al., 1994). Beef muscle color is typically bright, cherry red. Immature animals have less myoglobin pigment compared to fully grown or developed animals (veal vs beef A, B, C, D, or E Maturity) (Judge et al., 1989).

CHICKEN

Chicken production has gained importance from the shorter generation time of the animals and the higher feed conversion rate (FCR) compared to swine or beef. Also, high acceptance of chicken meat in the diet of humans (Henrickson, 1978) has attracted the attention of chicken growers. Even though the meat of poultry is considered a

white meat, the uniqueness of color variations in poultry meat (white to red) has made it significant to be used either as red meat or as white meat in manufacturing. Chicken muscles that are used more in animal movement tend to be darker and tougher (Labensky and Hause, 1995). The tenderness of chicken muscles is associated mainly with the age of the animal. The younger the animal the more tender the meat. Intramuscluar fat known in red meats as marbling is neither present in chicken meat nor is fat associated with chicken meat (Labensky and Hause, 1995); Instead, chicken fat is concentrated primarily in the skin.

PORK

Due to the high content of fat in pork, alot of research has been conducted to reduce it. The relationship between fat and some quality aspects of meat is of primary concern due to the effects of fat on some quality properties. Negatively, when the fat content of pork is reduced, flavor, juiciness and tenderness are affected.

DeVol et al.(1988) proved that tenderness, connective tissue amount, and Warner-Bratzler shear force (WBS) were more variable among pork carcasses than juiciness and flavor desirability. In addition, juiciness was more variable among different animals than flavor desirability. Lewis et

al.(1989) concluded that exercise produced leaner pork carcasses but with less tender muscles.

Quality of pork lean is estimated by the visual appraisal of the loin eye muscle at the 10th rib. Color, marbling, and firmness are the most noteworthy properties in quality of pork muscle (Boggs and Merkel, 1993; Romans et al., 1994). Five different color scores have been used to measure the color of pork muscle: pale pinkish gray (rejected), grayish pink (the most typical and desirable), reddish pink (acceptable), purplish red (acceptable), and dark purplish red (rejected) (Romans et al., 1994; Boggs and Merkel, 1993). High quality pork meat is a result of high firmness of exposed lean surface, fine-texture, and a uniform bright grayish-pink color (National Live Stock & Meat Board, 1988).

EFFECT OF AMMONIA ON THE QUALITY PROPERTIES OF FLESH FOODS

Generation after generation, the term meat quality has received different definitions. In general, quality of meat has different interpretations among meat animal producers, meat manufacturers, specialists, and meat consumers (Henrickson, 1978). Meat quality could be defined as the attractive feeling towards meat from a human being. Even though all quality properties of meat are identified

including color, tenderness, juiciness, water holding capacity, pH, flavor, taste, marbling, and firmness, there is a great possibility that some unknown or new properties may be identified or discovered in the future, as well as, other methods to indicate the quality properties of meat. The psychology of consumers regarding meat has been examined to specify their desires. Recently, the term quality of meat has been directed to some characteristics of meat which pleases consumer demand and increases the dollar gain for suppliers (manufacturers).

Ting and Henrickson (1986) summarized the effect of ammonia on the quality aspects of meat. In their conclusions, meats contaminated by high concentrations of ammonia showed high increases in pH, water holding capacity, and adversely affected the color of meat.

COLOR

Developing desirable eye appeal has been for centuries the major concern of the meat manufacturers. Usually, consumers judge meat quality by its color. Any change in the color of meat infers that it is unacceptable. Lawrie (1991) stated that the color of meat which attracted consumers is due not only to the level of myoglobin, but also to many other important factors such as the type of

myoglobin and its chemical form along with the characteristics of other meat components.

Bonne et al.(1993) studied a technical incident of ammonia gas leak in a cooler used for the holding of beef and sheep carcasses slaughtered that same morning and the consequence of ammonia contamination on carcasses. Bonne and his coworkers indicated that the color of meat contaminated by ammonia was influenced positively and appeared as an intensive red color. Further, the formed color was permanent and did not change even after the first 24 hours of contamination.

Shaw et al.(1992) studied the effect of ammonia exposure on the pink color of pork remaining after cooking by adjusting the pH of ammonia-treated samples to 5.4 and increasing the pH of free-ammonia (untreated) samples to pH 9.6. However, they concluded that the distinct pink color in pork muscle after ammonia treatment was not a result of pH increase. Also, the result indicated that the pink color which appeared in pork meat after exposure to ammonia was not the same as the pink color of fresh or cooked cured pork according to the spectra data collected using a Spectrophotometer.

Smolskiy et al.(1985) studied the properties of color formed in cooked sausages made from beef contaminated by ammonia (1000 to 1500 ppm) and concluded that sausages made

from contaminated beef had more a intensive pinkish color compared to controls in the data obtained either by sensory panel evaluation or by spectrophotometer analysis. Moreover, sausages that contained 7.5 mg sodium nitrite and ammonia at 1000 to 1500 ppm showed less color brightness in contrast to sausages containing 3.0 mg sodium nitrite and the same amount of ammonia. Thus, the color that formed in sausages made from ammonia contaminated beef is due mainly to the effect of the ammonia but not the sodium nitrite.

Tuengerthal (1979) discussed some considerations that should be contemplated on the sales values of stored frozen meat contaminated by ammonia. He suggested price reduction to reduce the uncertainty of buyers and due to the special services and additional expenditures that many buyers are enforced to invest. Also, Tuengerthal discussed the discoloration of the meat following the ammonia contamination and reported that some dark zones (5 cm in depth) appeared on the beef carcass surface. Furthermore, Tuengerthal summarized the most important factors that may contribute to ammonia effects on meat as: ammonia concentration, temperature, humidity, type of meat, type of cut and the condition of meat surface.

Anil (1971) used a sensory evaluation panel to assess color and showed no significant effects for ammonia on the color of cooked beef muscles exposed to ammonia (10

milliliters) for 72 hours. Anil also demonstrated that the product temperature either 0°F or 25°F under which the beef was stored did not have a significant effect on color. However, Anil (1971) did not evaluate the color of beef before cooking.

Herrmann (1965) used the Hunter Color Instrument in order to evaluate the color changes in beef and pork samples exposed to ammonia (10 milliliters) for 24 hours. However, a highly significant effect for ammonia on the color of pork was found by using the Analysis of Variance Test. Exposure of ammonia under different temperatures did not show any significant effect on the color of meat treated by ammonia as mentioned by Herrmann (1965).

Kassem (1965) conducted sensory evaluation for over-wrapped ground beef(Cryovac-, polyethylene-, regular-, wax-wrapper) exposed to ammonia (10 milliliters) for 60 hours at -10°F and aerated for 30 minutes at room temperature. In his conclusion, Kassem cited that a grayish color was found on the surface of the ground beef and this was most noticeable in the wax-wrapped and regular-wrapped samples while no changes in the color were detected inside the ground beef. Also, Hunter color values indicated a significant difference in the color between treated and untreated ground beef with no significant differences in color among treated ground beef samples.

Even though the ammonia effect on the color of meat has been the subject of many investigations, surprisingly little is known about the mechanism of ammonia effect on the color of meat.

FLAVOR

Flavor is the most obvious property that can be observed and affected after the color of meat. The oxidative rancidity process in meat has been the focus of many investigators. Gray and Crackel (1992) noted that the flavor of meat is influenced by many factors such as genetics, animal feed, processing, storage procedures, and growth of microorganisms.

Hagyard et al.(1993) studied the effects of exposure to a low concentration of ammonia on the development of flavor rancidity in lamb meat and, consequently, the effects on the shelf-life of meat. They removed the loins from the lamb carcasses and exposed them to a 2M ammonia solution (68,000 ppm) for 16 and 32 minutes at 10°C inside a 60 X 30 X 60 cm^3 glass chamber. Hagyard and his coworkers concluded that the meat exposed for 32 min. showed pH increases of 1.0 unit and developed a detectable rancid flavor after 3 months of storage. Moreover, the meat that was exposed for 16 min. which showed a 0.5 pH unit increase developed a detectable

rancid flavor after 6 months of storage. Thus, it is clear that the longer the exposure time and the higher the concentration of ammonia the more sever the reduction in the shelf-life of meat or meat products contaminated by ammonia. Further, freezing did not show any preventive effect on the rancidity after ammonia contamination.

Golovkin et al.(1969) estimated the changes in the quality of meat exposed to ammonia by the changes in the concentration of the aromatic substances present in meat which were extracted from meat by the vacuum distillation of boiled products in a flow of Nitrogen(N_2). The chromatographical analysis of the aromatic substances indicated that meat contaminated by ammonia is subjected to undesirable changes in the Normal Biochemical Processes (NBP) which developed in meat during storage. Also, the increase in some aromatic substances were attributed to ammonia effect. In addition, Golovkin and his coworkers suggested that meat exposed to ammonia vapor should not be stored for a long time, even under 0°C, because of the changes in the NBP that were initiated in meat by ammonia contamination.

Bonne et al.(1993) mentioned that the odor of ammonia was easily detectable on the surface of beef and lamb carcasses that was polluted by ammonia at the slaughterhouse. However, the ammonia odor disappeared from

the carcasses within several hours of aerating when the carcasses were placed in another cold room giving an economical solution for elimination of ammonia in such cases. Bonne et al.(1993) examined the possibility of using a Total Volatile Basic Nitrogen (TVBN) test as an appropriate and reliable method to detect ammonia contamination of meat after failure of ammonia refrigeration system. Contaminated ground meat samples (10 grams) were placed in 50 ML distilled water in a beaker, weak base (NaOH) was added, then the preparation was heated to allow evaporation of volatile nitrogen. A cooling column was used to condense the vapor and collect the liquid that contained the volatile nitrogen. Titration by using sulfuric acid (H_2SO_4) was carried out. Alizarin was used as an indicator of neutralization of sulfuric acid with volatile nitrogen compounds expressed as mg/100 grams of meat samples. Significant differences in the total volatile basic nitrogen content of contaminated meat samples were found compared to uncontaminated meat samples.

TENDERNESS

Tenderness of meat is an important aspect of palatability. Connective tissues, muscle fibers, and

adipose tissues each have a major influence in increasing or decreasing the tenderness of meat (Judge et al., 1989). Many research scientists continue to use the Warner-Bratzler Shear instrument as a common and very reliable device to measure the tenderness of meat and poultry.

Herrmann (1965) indicated that the tenderness of beef muscles exposed to ammonia for 24 hours and stored at 15°F and -20°F was significantly higher comparing to a control(not treated), while the samples that were stored at 0°F did not show any significant difference in tenderness. This result was unexpected and may be attributed to sampling error as Herrmann mentioned.

Anil (1971) concluded that the tenderness of meat samples (beef) exposed to ammonia (10 milliliters) for 72 hours was improved compared to control samples with no significant effect for both 0°F and 25°F storage temperatures. They also indicated that the outer surface of the meat directly exposed to ammonia was significantly more tender than the inner part of meat

pH

The pH is a measure of hydrogen ion (H^+) concentration. The range of pH in bases and acids vary from 0-14 units.

The majority of meat and meat products are located in the acidic side (below pH 7). After the slaughter moment, the pH of the meat is at equilibrium (pH 7). Postmortem glycolysis of glycogen in muscle produces an accumulation of lactic acid which, consequently, results in rapid decline in the pH of the meat (Greaser, 1986).

Hermann (1965) pointed out that the pH of beef and pork muscles exposed to high concentration of ammonia were significantly higher compared to unexposed muscles and the most effect was on the surface of the meat (exposed layer). Also, pH of exposed layer (first layer) was significantly higher than the internal layers (second, third, and fourth layers) with no significant effects of temperature on the pH of exposed samples. In addition, Hermann concluded that the first 1/4 inch layer surrounding muscles exposed to ammonia is the most affected of all other layers.

Kassem (1965) concluded that the increase in the pH of ground beef exposed to ammonia (unknown concentration due to leak in commercial company warehouse storage) was significantly higher than unexposed samples. Furthermore, whole chicken wrapped in a Cryovac container showed no significant effect of ammonia on pH, flavor, and ammonia odor after contamination, however, he attributed the results to the impermeability of the containers. Also, Kassem emphasized that, due to the buffering capacity of meat, the

pH determination is not an appropriate method to evaluate the amount of ammonia absorbed by the meat and suggested the use of titration with acid instead.

Anil (1971) found that there was a very significant increase in the pH of beef muscles exposed to ammonia (10 milliliters liquid ammonia) for 72 hours at 0°F and 25°F. Also, the penetration depth of ammonia was higher in first 1/4 inch layer compared to the second and the third layers with no significant difference between third layer and the control. Storage temperature did not influence the penetration of ammonia inside beef.

WATER HOLDING CAPACITY

Water binding capacity of meat could be defined as the capability of meat to hold its water during application of external actions or forces such as cutting, grinding, heating, centrifuging, or pressing (Judge et al., 1989; Jauregui et al., 1981). Quality properties of meat and meat products are influenced by the water holding ability of meat. Hamm (1986) discussed many factors affecting the water holding capacity of meat including pH levels, postmortem changes, freezing, thawing, and heating. "weep" in uncooked and unfrozen meat, "drip" in frozen and thawed uncooked meat, and "shrink" in cooked meat are different

names for the water content of meat that may altered due to physical or environmental surrounding changes (Lawrie, 1991).

Anil (1971) studied the alterations in the water holding capacity of frozen beef tissues contaminated by ammonia (10 milliliters) for 72 hours at a temperature of 0°F and 25°F. Polluted layers of raw and cooked beef muscles were evaluated for WHC according to Wierbicki and Deatherage (1958). Anil (1971) concluded that water holding capacity of beef muscle increased due to ammonia contamination. All contaminated raw and cooked beef layers displayed significantly higher ability to retain water compared to uncontaminated beef samples. Also, significant differences in WHC between layers were detected. According to Anil, water binding ability of second layer was significantly much more than the first layer (directly exposed), even the ammonia content of first layer was more than the second layer (pH 10.019 and pH 8.308 respectively). The interpretation of this unusual phenomena could be due mainly to the lower repulsion between positive charges of amino and imidazol groups because of an excessive alkaline medium. Hence, binding counter ions work as protective walls in order to separate those charged groups. Accordingly, water binding ability of the first layer of

beef is reduced due to the increase in free water content (Anil, 1971).

High water binding of ground beef was detected by Kassem (1965). Contaminated ground beef samples were difficult to form into patties due to the lack of the meat binding ability to hold together. This observable fact may be due to the effect of ammonia ions that replaced the sodium-calcium ions from the meat (Kassem, 1965).

EFFECT OF FREEZING AND FROZEN STORAGE ON MEATS

The effect of freezing and frozen storage on meat and chicken could be summarized as a permanent structural damage (the action of ice crystals size growth in rupturing muscle fibers and decreasing the water holding capacity of meat) and chemical property degradation. Muscle fibers, lipids, and proteins are the most affected portions.

Organoliptic properties of poultry meat were not affected significantly due to freezing or storage as Baker et al.(1976) concluded. Pikul et al.(1984) reported that lipid oxidation is accelerated with longer frozen storage for chicken meat stored for 6 months. Proteins transformation in frozen meat has been confirmed decades ago, especially sarcoplasmic and extractable proteins (Miller et al., 1980). Moreover, Miller et al.(1980)

indicated that there is no significant effect of pH on the drip amount either in beef or pork meat. The authers concluded that water holding capacity of pork and beef samples was decreased sharply with longer frozen storage periods. Furthermore, Igene et al.(1979) observed losses in the total lipid content of chicken meat during frozen storage that attributed to the changes in triglycerides. Marketing of restructured meat products in the frozen state has many disadvantages such as discoloration, rancidity development, and poor consumers appeal (Al-Joher and Clarke, 1993).

CHAPTER III

MATERIALS & METHODS

Experimental Design. Two steaks of each type of meat (beef, pork, and chicken) were assigned to various concentrations of ammonia (fixed volume of ammonia limited by the volume of the chamber) and different exposure times. Ammonia concentrations used were 5000, 10,000, 25,000, and 50,000 ppm. Exposure times were 0 (control), 3, 6, 12, 24, and 48 hours in a freezer at -18°C (0°F) temperature. This procedure was replicated three times under a safety hood.

Sample Preparation. Meats were obtained from approved food commercial industries. Vacuum-packaged, frozen beef (US choice) strip loins and pork (grade A) center cut loins were trimmed of external fat and sliced into steaks of 1.27 cm (1/2 inch) of thickness; frozen chicken breasts (grade A), skinless and boneless, were trimmed and shaped into steaks as well. Then, all samples were weighed, coded, and vacuum-packaged separately in Poly Vinyl Chloride (PVC) bags until treatment time.

Exposure Chambers. Five plastic dessicators (NALGENE Brand Products, ROCHESTER, NY 14602-0365 USA) were modified to serve as treatment chambers for the ammonia exposure. The volume of the desiccator (5 liters) when exposure ended is the fixed volume of ammonia required per treatment (5 liters). The modified desiccator has two stopcocks, one of them was connected to the ammonia cylinder via plastic tubing (TYGON S-50-HL, class VI, size 1/4 x 1/16) and the other stopcock is used to release the gas from the other side to get the required concentration surrounding the meat samples under the desired product temperature and pressure (Figure 2). A high vacuum stopcock grease was used to prevent any leakage of ammonia gas from the dessicator's lid. Four C-clamps were tightened to maintain pressure.

Ammonia Condition. The ammonia as gas was obtained in an aluminum cylinder (IWECO, INC.) through Sooner Airgas, INC., 3212 S. Boomer Drive, Stillwater, OK 74074. The cylinder was mounted with an aluminum regulator (Controls Corporation of America, Virginia Beach, Virginia 23454) to adjust the flow of the ammonia gas into the desiccator.

In a pilot experiment to get 50,000 ppm ammonia inside the desiccator, it was necessary to inject twice, each time with a 6.896 kilopascal (KPa) (1 pound per square inch) of

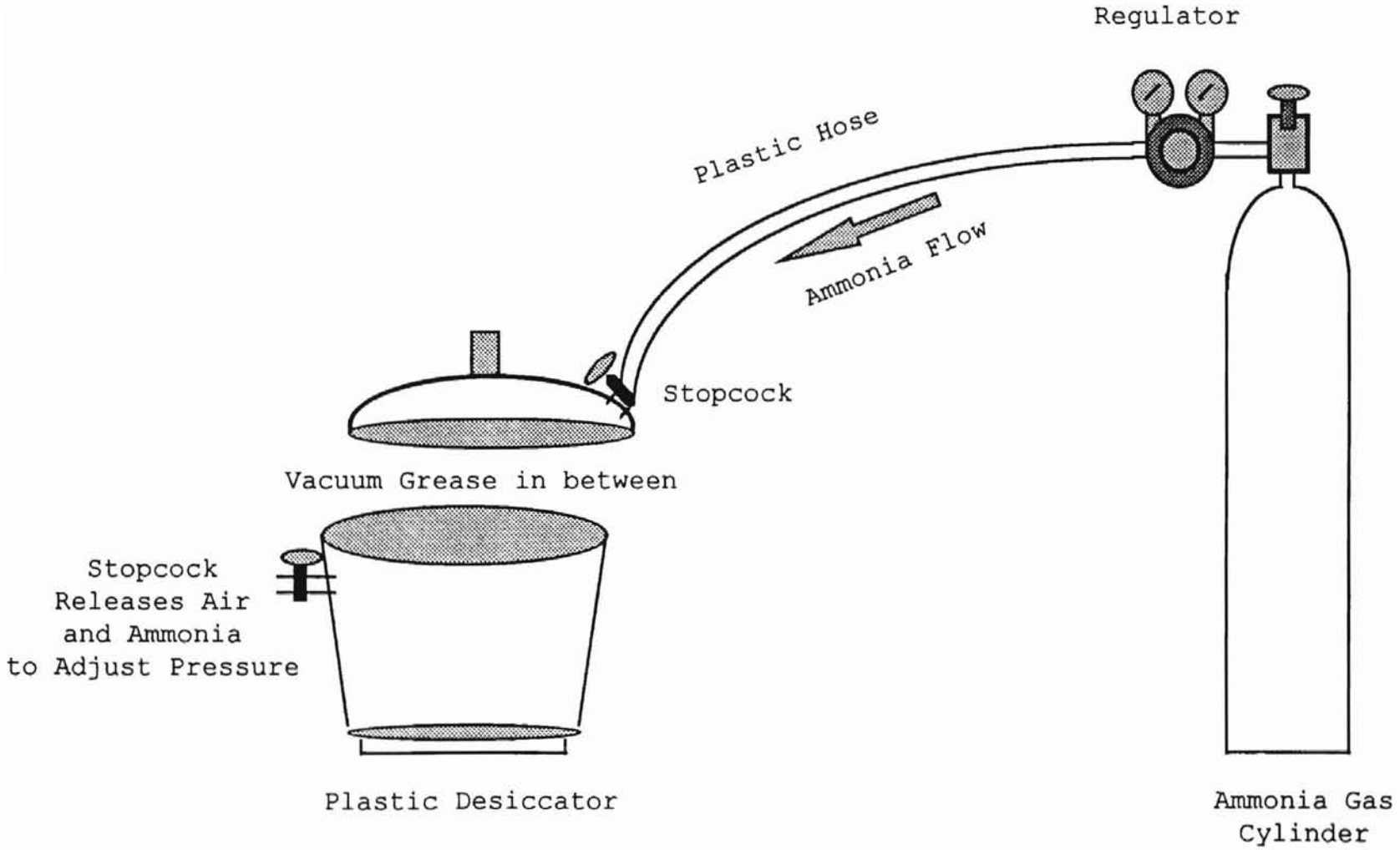

Figure 2. Materials and Flow Design

100,000 ppm of ammonia gas for 2 minutes. An interval of 5 minutes calibration was allowed between the two successive injections. In the same manner 25,000 ppm was achieved by injecting 50,000 ppm. However, to achieve 10,000 and 5000 ppm, 25,000 ppm ammonia was injected in a similar manner but the injection times were 90 and 30 seconds, respectively. The total time of preparation and exposure of steaks was approximately 10 minutes out of the freezer.

Ammonia Detection Method. The concentration of ammonia inside the desiccator was detected with a Drager Gas Detector Pump "accuro" using the tube specified for ammonia (Drager CH 31901 Ammonia 0.5%/a). Each tube contained a yellow pH indicating layer that changed to a violet color when air samples containing ammonia were sucked through the tube according to the following reaction:

$$NH_3 + \text{Acid} \xrightarrow{\text{Bromphenol blue}} \text{Violet reaction product}$$

One stroke was used to measure the concentration of ammonia inside the desiccator.

Upon completion of the treatment periods, one steak from each treatment and meat type was vacuum-packaged and designated for drip loss determination and color evaluation.

Color Evaluation. Color was tested objectively by using the Minolta Chroma Meter CR-300 that consisted of the measuring head and the Data Processor DP-301 to determine the difference in meat color before and after each ammonia treatment. The measuring head of the Chroma Meter CR-300 has an 8 mm-diameter viewing area and 0° viewing angle for accuracy. Color values (L*, a*, b*) were recorded after thawing (2 to 3 hours at room temperature) of all steaks. Three reads were measured from the surface of each steak. The measurements were replicated three times.

Drip Determination. The percentage weight lost during freezing and thawing was determined by weighing frozen steaks before exposure and reweighing the same steak thawed after exposure. Steaks were dried by paper towels then weighed.

Grinding & Sampling. The second steak from each treated and untreated meat type was ground three times through a fine blade (4 ml. diameter) using Rival electric grinder. Samples were divided into three small pouches for additional analysis.

pH Determination. pH values were measured by applying the methodology of AOAC, 1990 using a CORNING pH METER Model 130

(single electrode). Ground sample (5 grams) was diluted with 50 ml of distilled water inside a 100 ml plastic flask and homogenized to assure proper dispersion and uniform suspension of the sample in water by using a Brinkmann (Westbury, New York) polytron homogenizer. Duplicate samples were tested for each replicate.

Ammoniacal Nitrogen Analysis. Determination of ammonia nitrogen content of all meat samples was conducted by applying the AOAC, 1990 (Kjeldahl nitrogen) and using a new high performance LECO FP-428 device.

The LECO apparatus has three phases in the analysis cycle: purge, burn and analyze. In the purge phase, the encapsulated sample was placed in the loading head, then sealed, and the apparatus was purged of any atmospheric gases that may have entered during sample loading. The ballast volume and gas lines were also purged at this point. At the beginning of the burn phase, the sample was dropped into a hot furnace (850°C) and flushed with ultra-pure oxygen for rapid combustion. The products of combustion were passed through the thermoelectric cooler to remove most of the water, then collected in the ballast volume. The ballast volume has a free-floating piston, which moves up during collection of the gas products and was forced back down during gas removal. All the gas products in the

ballast volume were allowed to become a homogenous mixture at a pressure of 975 mm and a constant temperature. In the analyze phase, the piston was forced down, and a 10 cc aliquot of the sample mixture was collected. The sample aliquot is swept through hot copper to remove oxygen and change NO_x to N_2, then through Lecosorb and Anhydrone to remove CO_2 and water, respectively. The remaining combustion product (N_2)is measured by the thermal conductivity cell. The instrument was calibrated daily with ethylenediaminetetraacetic acid (EDTA) as a nitrogen standard. The final result is displayed as percent nitrogen or protein %. Results can also be calculated on a dry basis by entering a known moisture content.

Ground meat samples approximately 0.1 ± 0.03 gram were placed on preweighed foil. A plunger was used to get the appropriate sample amount. Then, the foil crimp was twisted, closed with tweezers and placed on attached balance and weighed. The foil capsule was placed on the LECO carousel sample holder in preparation for the automatic determination.

Water Holding Capacity(WHC) Determination. Modification of the method invented by Jauregui et al. (1981) and partially modified by DeLopez (1990) was used. Three pieces of Whatman # 50 circle filter papers (hardened 70 mm in

diameter) and two pieces of Whatman # 3 circle filter papers (qualitative 90 mm in diameter) were weighed on a Mettler AE 100 scale. Ground meat samples (1.5 ± 0.3 g) were weighed on the # 50 filter papers after zeroing the scale (run in duplicate per replicate). All three # 50 filter papers were folded on the sample as inner cover and covered by the two pieces of # 3 filter papers as outer cover. Covered samples were placed in a 50 Ml Nalgene High-Speed polycarbonate tubes and centrifuged using a Beckman Induction Drive Centrifuge Model J-6M for 45 min. at room temperature (25°C) and a speed of 4200 rpm (3640 X G). After centrifugation, the filter papers that contained meat samples were removed from the tubes with forceps, the meat removed from the filter papers using spatula, and the papers reweighed. The difference between the weight of the filter papers after centrifugation and the weight of dry filter papers is the weight of the expressible moisture. In order to calculate the percent of Water Holding Capacity (WHC%) of meat samples, moisture content of all meat samples were determined by applying the AOAC, 1990 methodology. Water Holding Capacity % was calculated by the following equation:

$$WHC\% = 1 - \left(\frac{\text{Moisture Loss \%}}{\text{Original Moisture Content of Sample \%}} \right)$$

Moisture Content Determination. In preweighed aluminum plates, 5 ± 0.2 grams of every meat sample was weighed and held in an Isotemp Oven Model 655F (Fisher Scientific) at 100°C for 6 to 8 hours. Plates were cooled in a glass desiccator for 10 minutes and then reweighed. Moisture Content was calculated by difference.

Statistical Analysis. The statistical design was a 3 X 4 X 6 factorial arrangement for specie (beef, chicken and pork), ammonia concentration (5,000, 10,000, 25,000 and 50,000 ppm) and exposure time (0, 3, 6, 12, 24 and 48 hours). Data were analyzed using the GLM procedure of SAS (1988). The statistical model included fixed effects of specie, ammonia concentration and exposure time as well as all possible interactions. Least squares means were used to determine significance when a significant F was obtained in the analysis of variance. Furthermore, contrasts were used to examine possible linear, quadratic, and cubic effects for independent variables on traits of interest.

CHAPTER IV

RESULTS & DISCUSSION

AMMONIA EFFECTS ON BEEF

The least squares means of the effect of different ammonia concentrations (5,000, 10,000, 25,000, and 50,000 ppm) over time (0, 3, 6, 12, 24, and 48 hours) on the quality aspects of unpackaged beef are shown in Tables 1 through 4, respectively.

pH

The pH of beef muscle increased ($P<.05$) as the ammonia concentration increased (Figure 3). The higher the ammonia concentration the greater the increase in pH. Also, there was increase in pH of samples treated with 5,000 ppm of ammonia gas (Figure 3. and Table 1) compared to the control (untreated) (0 hr.) with no significant difference within time treatments except at 12 hours which showed lower pH than others. On the other hand, samples treated with 50,000 ppm of ammonia showed a higher ($P<.05$) pH then at

Table 1. Least squares means for beef, chicken, and pork muscle traits stratified by exposure times at 5000 ppm ammonia gas.

Trait	Time (hr.)						Statistics[a]				
	0	3	6	12	24	48	SE	P	L	Q	C
Drip loss %											
Beef	9.26^{cd}	8.48^{cd}	7.99^{d}	9.94^{bc}	9.45^{bcd}	11.18^{b}	0.61	0.0380	0.9778	0.8180	0.8598
Chicken	9.30	8.12	6.27	8.35	7.64	9.02	0.94	0.3130	0.2607	0.3349	0.4071
Pork	13.40	11.60	11.41	12.36	12.79	12.61	0.58	0.2199	0.0952	0.0772	0.0798
pH											
Beef	5.14^{d}	5.37^{b}	5.34^{bc}	5.32^{c}	5.36^{bc}	5.38^{b}	0.01	0.0001	0.0071	0.0237	0.0374
Chicken	5.71	5.94	5.87	5.82	5.95	5.80	0.08	0.2770	0.5117	0.7501	0.8900
Pork	5.51^{d}	5.71^{c}	5.81^{b}	5.80^{b}	5.85^{b}	5.86^{b}	0.03	0.0001	0.0001	0.0015	0.0043
WHC %											
Beef	46.27^{d}	46.78^{d}	48.07^{cd}	49.53^{cd}	51.46^{bc}	53.83^{b}	1.25	0.0079	0.2569	0.7270	0.8451
Chicken	51.00	60.46	56.48	58.46	60.59	56.32	4.25	0.6312	0.4042	0.5894	0.6963
Pork	41.53	44.92	43.41	44.61	46.86	47.07	1.58	0.1931	0.4955	0.8538	0.9647
Nitrogen %											
Beef	3.86	3.93	3.94	3.85	4.03	4.06	0.05	0.0607	0.7685	0.4848	0.4576
Chicken	4.00	4.03	4.06	4.11	4.05	4.07	0.06	0.8787	0.2369	0.3026	0.3414
Pork	3.91	3.92	3.94	3.91	3.88	3.95	0.05	0.9094	0.6967	0.5441	0.4704

[a]SE=Standard Error, P=Probability values (P<.05), L=Liner, Q=Quadratic, C=Cubic.
[bcd]Means in the same row with different superscripts letters are different (P<.05).
Values represent the average of three replications (2 samples per replication).

Table 2. Least squares means for beef, chicken, and pork muscle traits stratified by exposure times at 10,000 ppm ammonia gas.

	Time (hr.)						Statistics[a]				
Trait	0	3	6	12	24	48	SE	P	L	Q	C
Drip loss %											
Beef	8.82^{b}	7.07^{d}	7.69^{cd}	7.71^{cd}	8.14^{bc}	8.04^{c}	0.27	0.0122	0.0364	0.0345	0.0390
Chicken	8.92	9.57	8.89	9.29	11.00	8.81	1.20	0.7867	0.7511	0.4962	0.4014
Pork	13.25	12.03	11.57	12.00	12.25	10.91	0.74	0.4117	0.1470	0.1624	0.1580
pH											
Beef	5.48^{c}	5.99^{b}	5.84^{b}	5.87^{b}	5.86^{b}	5.87^{b}	0.06	0.0010	0.0171	0.0347	0.0492
Chicken	5.73^{c}	6.16^{b}	6.24^{b}	6.18^{b}	6.16^{b}	6.14^{b}	0.09	0.0216	0.0056	0.0140	0.0226
Pork	5.68^{c}	6.05^{b}	6.05^{b}	6.09^{b}	6.17^{b}	6.18^{b}	0.06	0.0008	0.0043	0.0262	0.0503
WHC %											
Beef	44.30^{d}	53.83^{bc}	49.08^{cd}	52.90^{bc}	52.70^{bc}	54.87^{b}	1.77	0.0107	0.0626	0.1357	0.1712
Chicken	37.43^{c}	55.97^{b}	56.17^{b}	61.83^{b}	64.94^{b}	58.65^{b}	4.21	0.0087	0.0074	0.0491	0.1067
Pork	44.03^{d}	50.18^{cd}	49.87^{cd}	52.50^{bc}	55.94^{b}	56.40^{b}	2.22	0.0185	0.0850	0.3459	0.4963
Nitrogen %											
Beef	4.09^{b}	3.45^{d}	4.13^{b}	3.48^{cd}	3.97^{bc}	3.69^{bcd}	0.17	0.0408	0.2019	0.1937	0.1934
Chicken	4.11	4.07	4.06	4.12	4.08	4.10	0.05	0.9250	0.9056	0.9109	0.9229
Pork	4.11	4.07	4.08	4.14	4.11	4.09	0.04	0.7444	0.9557	0.8892	0.8145

[a]SE=Standard Error, P=Probability values (P<.05), L=Liner, Q=Quadratic, C=Cubic.
[bcd]Means in the same row with different superscripts letters are different (P<.05).
Values represent the average of three replications (2 samples per replication).

Table 3. Least squares means for beef, chicken, and pork muscle traits stratified by exposure times at 25,000 ppm ammonia gas.

	Time (hr.)						Statistics[a]				
Trait	0	3	6	12	24	48	SE	P	L	Q	C
Drip loss %											
Beef	9.93[b]	5.66[c]	6.33[c]	6.32[c]	5.90[c]	6.14[c]	0.56	0.0015	0.0124	0.0367	0.0591
Chicken	8.17	5.42	7.72	6.22	5.66	6.01	1.36	0.6350	0.6711	0.8529	0.9209
Pork	20.71[b]	12.91[c]	12.94[c]	12.41[c]	11.60[c]	12.19[c]	0.89	0.0001	0.0012	0.0073	0.0161
pH											
Beef	5.32[c]	6.40[b]	6.24[b]	6.39[b]	6.56[b]	6.50[b]	0.13	0.0002	0.0048	0.0266	0.0518
Chicken	6.14[d]	6.60[bc]	6.42[cd]	6.68[bc]	6.52[bc]	6.75[b]	0.09	0.0079	0.0114	0.0243	0.0304
Pork	5.65[d]	6.54[bc]	6.43[c]	6.74[bc]	6.77[bc]	6.87[b]	0.14	0.0005	0.0018	0.0122	0.0246
WHC %											
Beef	44.96[d]	65.42[c]	63.34[c]	67.23[bc]	71.39[b]	69.63[bc]	1.96	0.0001	0.0013	0.0136	0.0334
Chicken	52.67	58.68	60.61	63.8	61.38	64.54	2.89	0.1213	0.0184	0.0456	0.0629
Pork	41.49[c]	56.88[b]	54.88[b]	56.84[b]	60.04[b]	59.77[b]	3.42	0.0215	0.0345	0.1125	0.1709
Nitrogen %											
Beef	3.69	3.77	3.89	2.58	3.72	3.78	0.11	0.7651	0.1484	0.1408	0.147
Chicken	4.09	4.06	4.13	4.14	4.15	3.91	0.10	0.5616	0.8138	0.9796	0.8753
Pork	3.53	3.56	3.44	3.45	3.44	3.48	0.05	0.3961	0.2601	0.4868	0.6222

[a]SE=Standard Error, P=Probability values (P<.05), L=Liner, Q=Quadratic, C=Cubic.
[bcd]Means in the same row with different superscripts letters are different (P<.05). Values represent the average of three replications (2 samples per replication).

Table 4. Least squares means for beef, chicken, and pork muscle traits stratified by exposure times at 50,000 ppm ammonia gas.

Trait	Time (hr.)						Statistics[a]				
	0	3	6	12	24	48	SE	P	L	Q	C
Drip loss %											
Beef	8.69^{b}	2.32^{d}	3.27^{c}	2.36^{d}	2.11^{d}	2.21^{d}	0.23	0.0001	0.0004	0.0028	0.0064
Chicken	9.12^{b}	2.64^{c}	3.54^{c}	2.39^{c}	3.40^{c}	2.25^{c}	0.69	0.0001	0.0007	0.0022	0.0037
Pork	14.57^{b}	5.31^{cd}	6.33^{c}	5.26^{cd}	4.39^{d}	4.69^{d}	0.53	0.0001	0.0006	0.0044	0.0102
pH											
Beef	5.46^{d}	8.42^{b}	7.72^{c}	8.40^{b}	8.30^{b}	8.48^{b}	0.15	0.0001	0.0007	0.0037	0.0075
Chicken	5.95^{d}	7.86^{bc}	7.41^{c}	7.99^{bc}	7.72^{bc}	8.19^{b}	0.23	0.0002	0.0015	0.0054	0.0088
Pork	5.88^{d}	8.04^{bc}	7.82^{c}	8.39^{bc}	8.56^{b}	8.60^{b}	0.22	0.0001	0.0005	0.0050	0.0121
WHC %											
Beef	43.67^{c}	69.67^{b}	71.80^{b}	70.59^{b}	70.76^{b}	72.56^{b}	1.56	0.0001	0.0001	0.0007	0.0015
Chicken	48.66^{c}	64.19^{b}	64.19^{b}	58.79^{b}	62.51^{b}	63.94^{b}	3.20	0.0312	0.0752	0.1245	0.1511
Pork	44.20^{c}	60.60^{b}	64.13^{b}	63.89^{b}	60.23^{b}	61.65^{b}	1.59	0.0001	0.0001	0.0001	0.0002
Nitrogen %											
Beef	3.75^{c}	3.92^{b}	3.91^{b}	4.03^{b}	3.98^{b}	4.03^{b}	0.05	0.0130	0.0045	0.0189	0.0320
Chicken	4.14	4.23	4.28	4.22	4.25	4.27	0.05	0.4496	0.2145	0.3014	0.3402
Pork	4.13	4.12	4.18	4.19	4.13	4.13	0.04	0.8220	0.2303	0.2506	0.2796

[a]SE=Standard Error, P=Probability values (P<.05), L=Liner, Q=Quadratic, C=Cubic.
[bcd]Means in the same row with different superscripts letters are different (P<.05). Values represent the average of three replications (2 samples per replication).

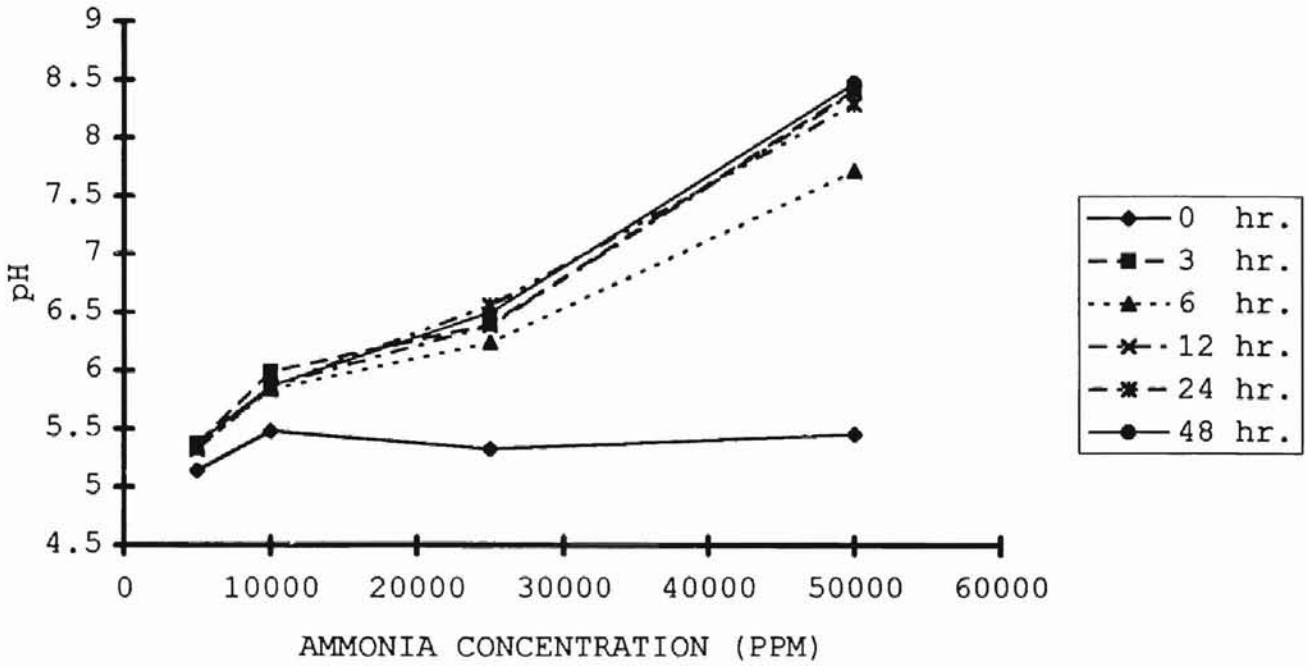

FIGURE 3. THE CHANGE IN pH OF BEEF STRIP LOIN STEAKS EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA OVER TIME

25,000 and 10,000 ppm of ammonia. In addition, there was an increase ($P<.05$) in the pH of all samples exposed for different periods 3, 6, 12, 24, 48 hr. compared to the control with no significant difference within exposure periods. Exposure at 25,000 ppm of ammonia gas for 3 hours or less was found to increase the pH of the beef by more than 1-unit (Table 3). These results agree with the work of Goodfellow et al. (1978) in that ammonia increased the pH of unpackaged beef muscles.

DRIP LOSS %

Percentage drip loss data are presented in Figure 4. The results show that the higher the concentration of ammonia the lower the percentage drip loss from beef muscle. Ammonia at 5,000 ppm did not affect the drip loss of beef while ammonia concentrations of 10,000, 25,000, and 50,000 ppm ($P<.05$) decreased the percentage drip loss. All samples exposed to ammonia for 3, 6, 12, 24, and 48 hours were similar ($P>.05$) in percentage drip loss except when compared to the control. Least squares means data showed that the greatest decreases in percentage drip loss were with the exposure to ammonia gas at 50,000 ppm (Table 4) followed by the 25,000 ppm ammonia (Table 3). This decrease in the

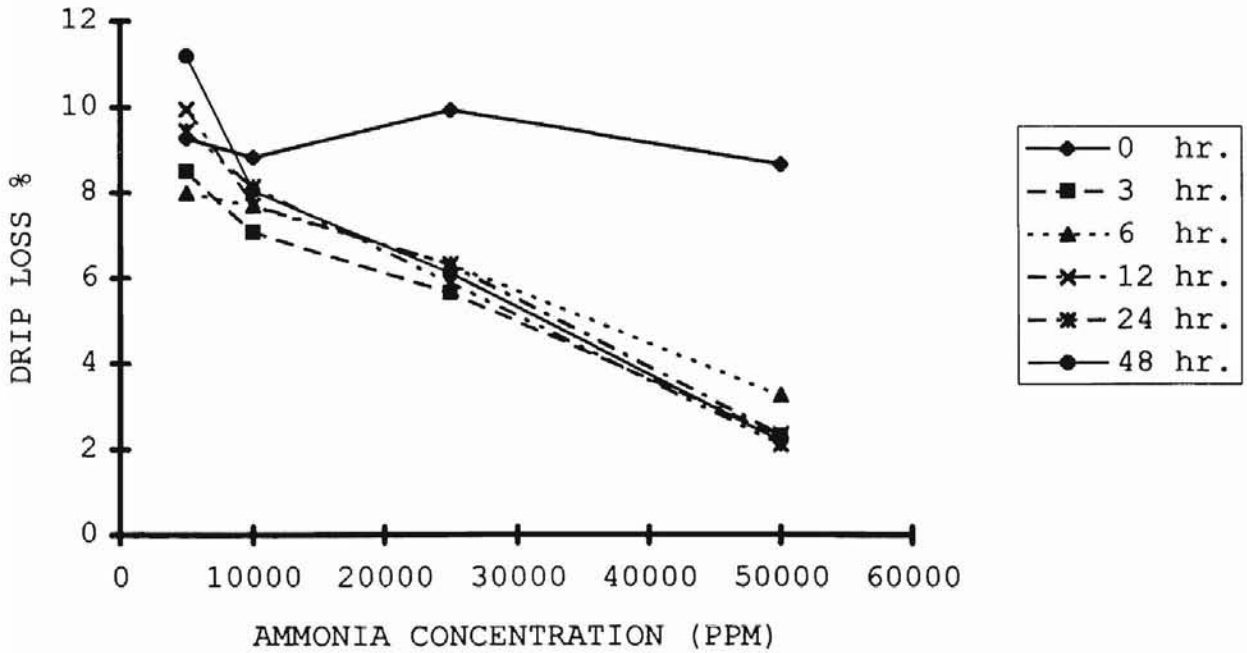

FIGURE 4. THE CHANGE IN DRIP LOSS % OF BEEF STRIP LOIN STEAKS EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA OVER TIME

percentage drip loss of beef is reflecting a changes in the ability of beef muscle to retain water.

WATER HOLDING CAPACITY % (WHC %)

The effect of different ammonia concentrations and exposure periods on the percent water holding capacity of beef is shown in Figure 5. Ammonia at 5,000 ppm increased ($P<.05$) the ability of beef to hold water. There was also a highly significant ($P<.05$) increase in water holding capacity of beef when exposed to 10,000, 25,000, and 50,000 ppm ammonia. Least square means data of all exposed samples for 3, 6, 12, 24, and 48 hours showed no significant difference in percent water holding capacity within them except when compared to the control.

These results indicated that 3 hours or less of exposure to 5,000, 10,000, 25,000, or 50,000 ppm ammonia is adequate to increase the percentage water holding capacity of beef. The greatest increase ($P<.05$) in percent water holding capacity was influenced by 50,000 ppm ammonia concentration, followed by 25,000 ppm, and 10,000 ppm ammonia gas.

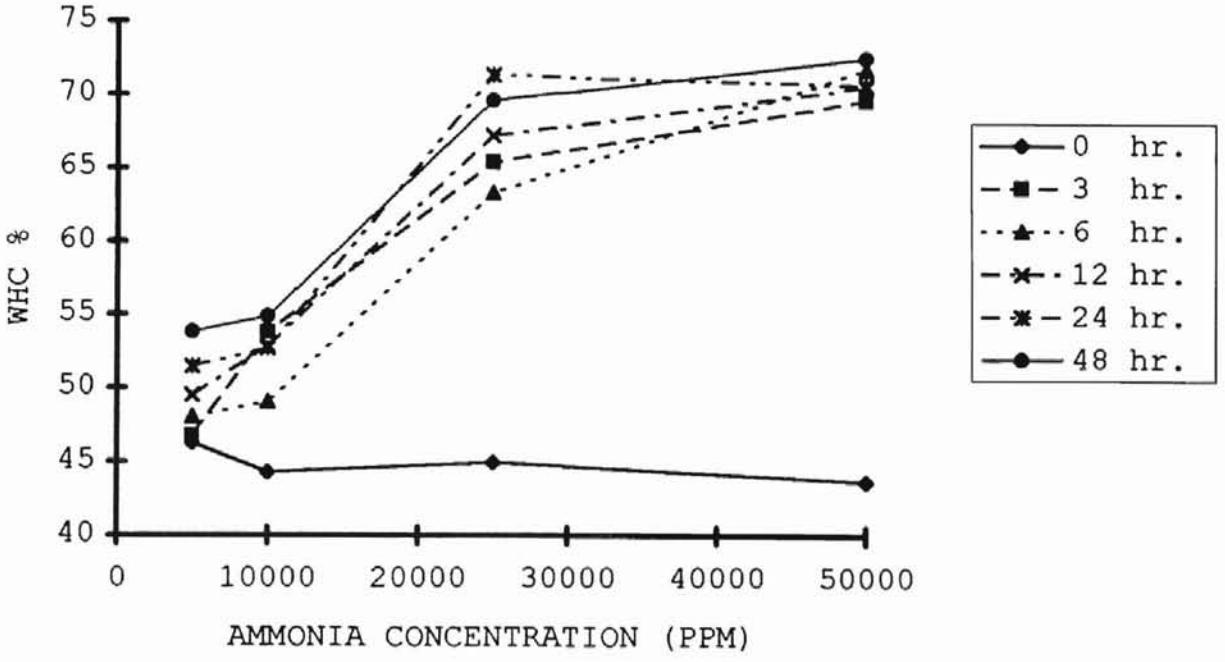

FIGURE 5. THE CHANGE IN WATER HOLDING APACITY % OF BEEF STRIP LOIN STEAKS EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA OVER TIME

AMMONIACAL NITROGEN %

The results of ammoniacal nitrogen determination are displayed in Tables 1 to 4. Essentially, beef exposed to small quantities of ammonia such as 5,000, 10,000, or 25,000 ppm did not affect ($P>.05$) the percent nitrogen content of beef muscles. Exposure to 50,000 ppm ammonia gas (Table 4) increased ($P<.05$) the ammoniacal nitrogen content of beef (Figure 6). Exposure times of 3, 6, 12, 24, and 48 hours at 50,000 ppm of ammonia gas did not affect ($P>.05$) the ammoniacal nitrogen content except when compared to the control (0 hr.).

COLOR

The summary of the least squares means of the Minolta Chroma Meter analysis of the changes in color of beef after exposure by different concentrations of ammonia over different time periods are presented in Table 5. Exposure periods with ammonia were similar ($P>.05$) in color except when compared to the control (0 hr.). Ammonia concentration of 10,000, 25,000, and 50,000 ppm increased ($P<.05$) the darkness (L* values) of beef steaks. At an ammonia concentration of 25,000 ppm over all time periods, Minolta Chroma Meter detected an increase ($P<.05$) in the redness (a*

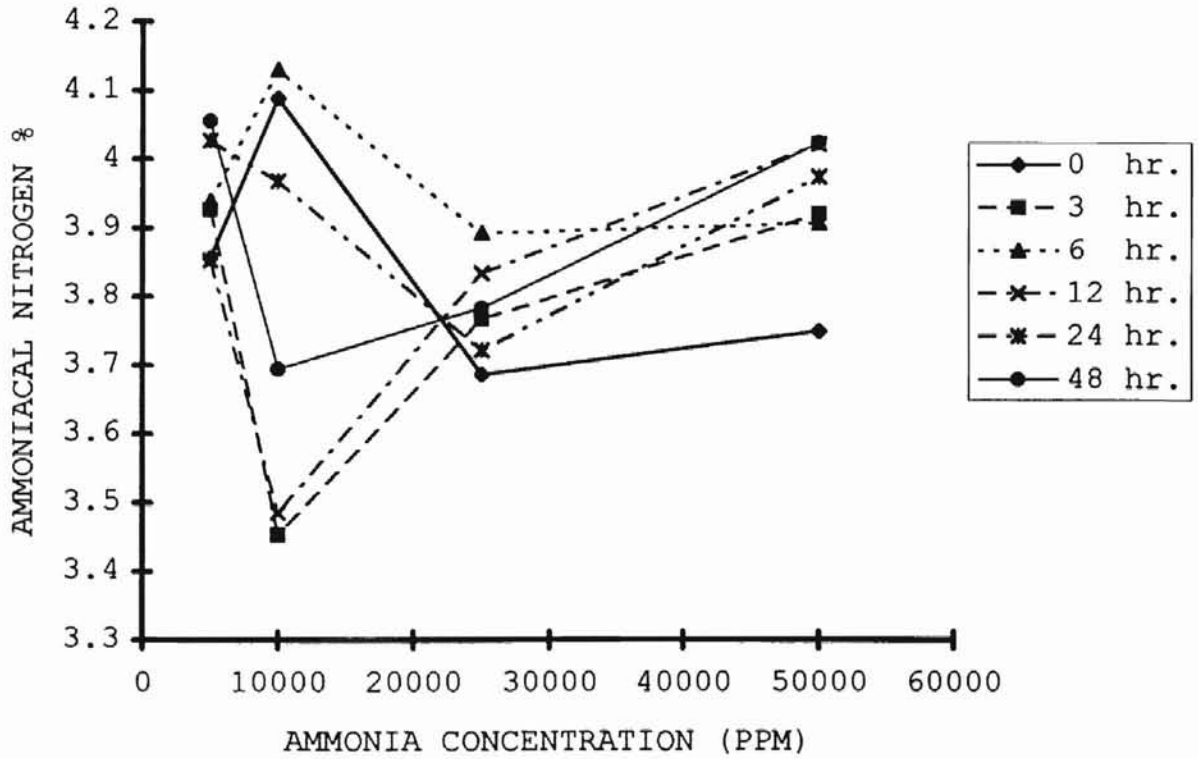

FIGURE 6. THE CHANGE IN AMMONIACAL NITROGEN % OF BEEF STRIP LOIN STEAKS EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA OVER TIME

Table 5. Least squares means for beef muscle color values stratified by different concentrations of ammonia gas at different times of exposure.

	Ammonia concentratin, ppm											
	5000			10,000			25,000			50,000		
Time,hr	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	34.93	16.40^{c}	7.45	34.23^{a}	17.14	7.90^{a}	33.54^{a}	18.44^{a}	8.00^{a}	32.79^{a}	19.02^{a}	7.14^{a}
3	32.81	18.31^{a}	6.24	30.48^{b}	17.74	5.51^{b}	28.38^{b}	15.48^{b}	4.73^{b}	28.25^{b}	13.55^{bc}	3.99^{b}
6	32.21	17.75^{ab}	6.17	30.35^{b}	16.97	5.51^{b}	27.95^{b}	15.93^{a}	4.62^{b}	27.86^{b}	13.83^{b}	4.02^{b}
12	31.69	18.20^{a}	6.09	31.20^{b}	17.20	5.97^{b}	27.91^{b}	14.43^{b}	4.37^{b}	28.00^{b}	12.66^{bcd}	3.45^{b}
24	31.38	16.84^{bc}	5.51	30.58^{b}	15.95	5.22^{b}	27.70^{b}	14.67^{b}	4.47^{b}	28.71^{b}	12.12^{d}	3.68^{b}
48	31.56	17.07^{abc}	5.38	29.72^{b}	17.01	5.42^{b}	28.14^{b}	13.73^{b}	4.17^{b}	28.47^{b}	12.20^{cd}	3.66^{b}

^{abcd}Means in the same column with different superscripts letters are different (P<.05). Color values using a Minolta CIELAB(L,a,b) scale: L*=lightness; a*=bluish-green/red-purple hue component;b*=yellow/blue hue component. Values represent the average of three replications (3 repeated measurements per replication).

values) of beef steaks when compared to controls but not at 5,000 or 10,000 ppm. No differences (P>.05) were noted across exposure times (3 hours of exposure expressed redness increase as much as 6, 12, 24, or 48 hours of exposure). Ammonia concentration of 50,000 ppm showed similar effects to 25,000 ppm ammonia on the redness of beef muscles.

AMMONIA EFFECTS ON CHICKEN

Least squares means of the effect of different ammonia concentrations (5,000, 10,000, 25,000, and 50,000 ppm) over exposure time (0, 3, 6, 12, 24, and 48 hours) on the quality properties of chicken muscles are shown in Tables 1 through 4, respectively.

pH

At 5,000 ppm, no differences (P>.05) in pH were detected among time treatments (Table 1). At 10,000 ppm no (P>.05) differences in pH (P>.05) were detected within time treatments, however, all time treatments were significantly higher in pH (P<.05) compared to the control (0 hr./untreated) (Table 2. and Figure 7). At 25,000 ppm ammonia (Table 3), no differences (P>.05) in pH were

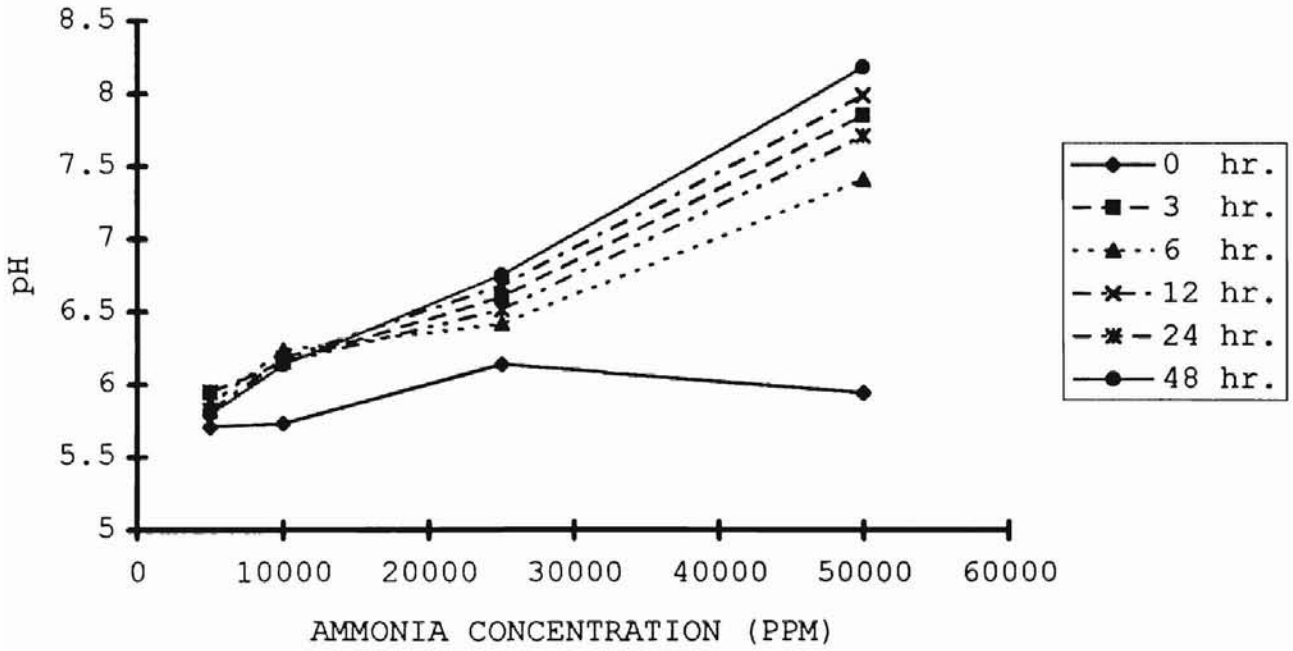

FIGURE 7. THE CHANGE IN pH OF CHICKEN BREAST EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA OVER TIME

observed within time treatments except between 6 and 48 hr. The pH of all time treatments was different (P<.05) from the control except at 6 hour exposure time. At 50,000 ppm (Table 4), all time treatments resulted in higher (P<.05) pH compared to the control, however, no significant differences were detected within time treatments except between 6 and 48 hr. with 48 having the highest pH value.

DRIP LOSS %

The changes in the percentage drip loss of chicken muscles are shown in Figure 8. At ammonia concentrations of 5,000, 10,000, and 25,000 ppm (Tables 1 through 3, respectively), no effects (P>.05) were detected on the percentage drip loss among all treatments. However, at 50,000 ppm, all time treatments resulted in lower (P<.05) percentage drip loss compared to the control, even though, there was no significant differences within time treatments.

WATER HOLDING CAPACITY %

Results of the least squares means of the percentage water holding capacity are presented in Tables 1 through 4. Ammonia concentration of 5,000 (Table 1) and 25,000 ppm (Table 3) did not show differences (P>.05) among treatments.

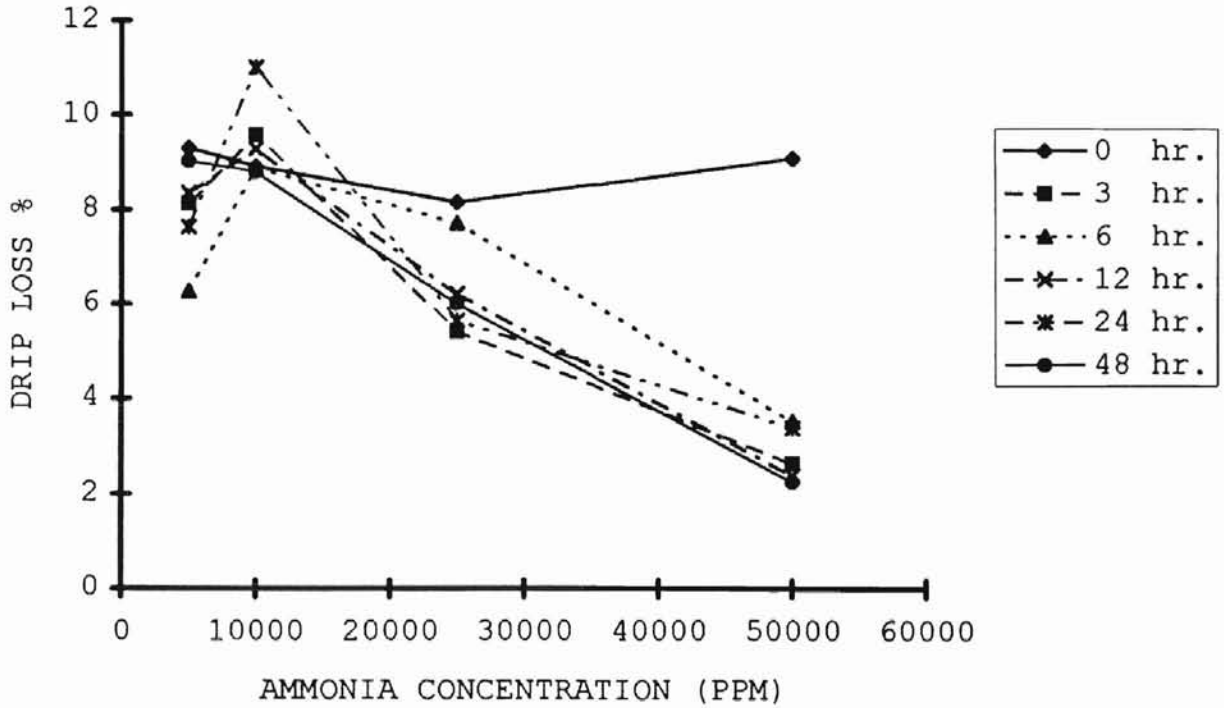

FIGURE 8. THE CHANGE IN DRIP LOSS % OF CHICKEN BREAST EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA OVER TIME

At 10,000 (Table 2) and 50,000 (Table 4) ppm ammonia, time treatments resulted in higher (Figure 9) ($P<.05$) water holding capacity compared to control with no differences ($P>.05$) within treatment times.

AMMONIACAL NITROGEN %

The ammoniacal nitrogen content of raw chicken muscles, after exposure to different ammonia concentrations over times, are presented in Tables 1 through 4 and Figure 10. Ammoniacal nitrogen content of chicken muscles was not affected ($P>.05$) due to ammonia exposure in all treatments.

COLOR

Color of chicken muscles exposed to different concentrations of ammonia over times is shown in Table 6. Ammonia did not affect ($P>.05$) the color of chicken muscles over all treatments probably due to the low level of pigments.

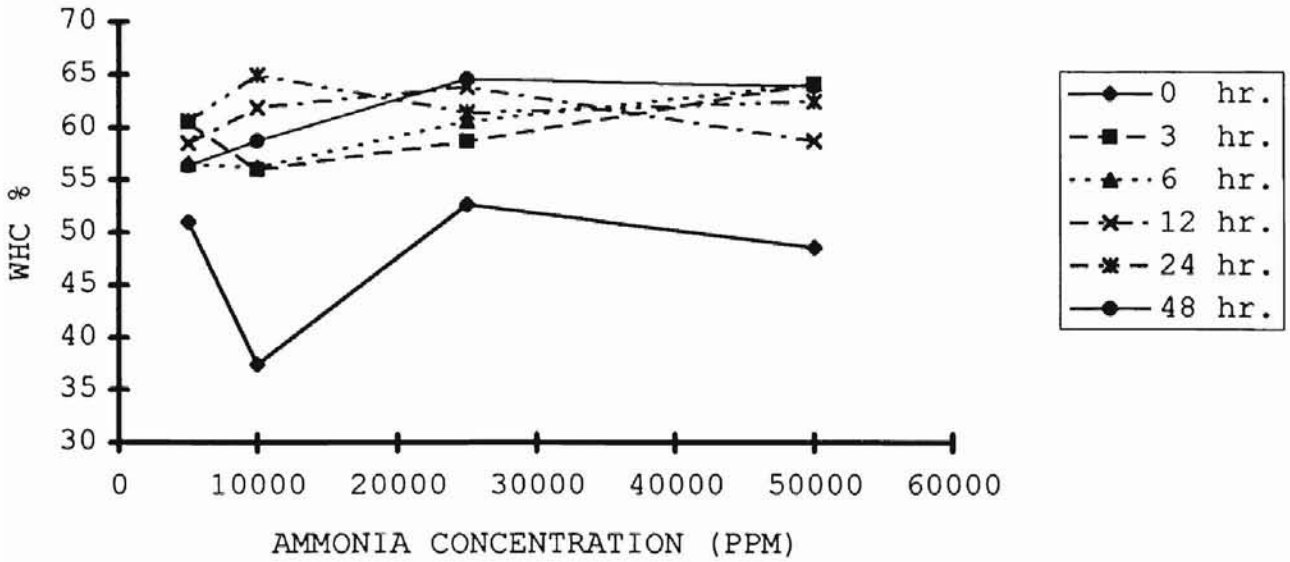

FIGURE 9. THE CHANGE IN WATER HOLDING CAPACITY % OF CHICKEN BREAST EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA OVER TIME

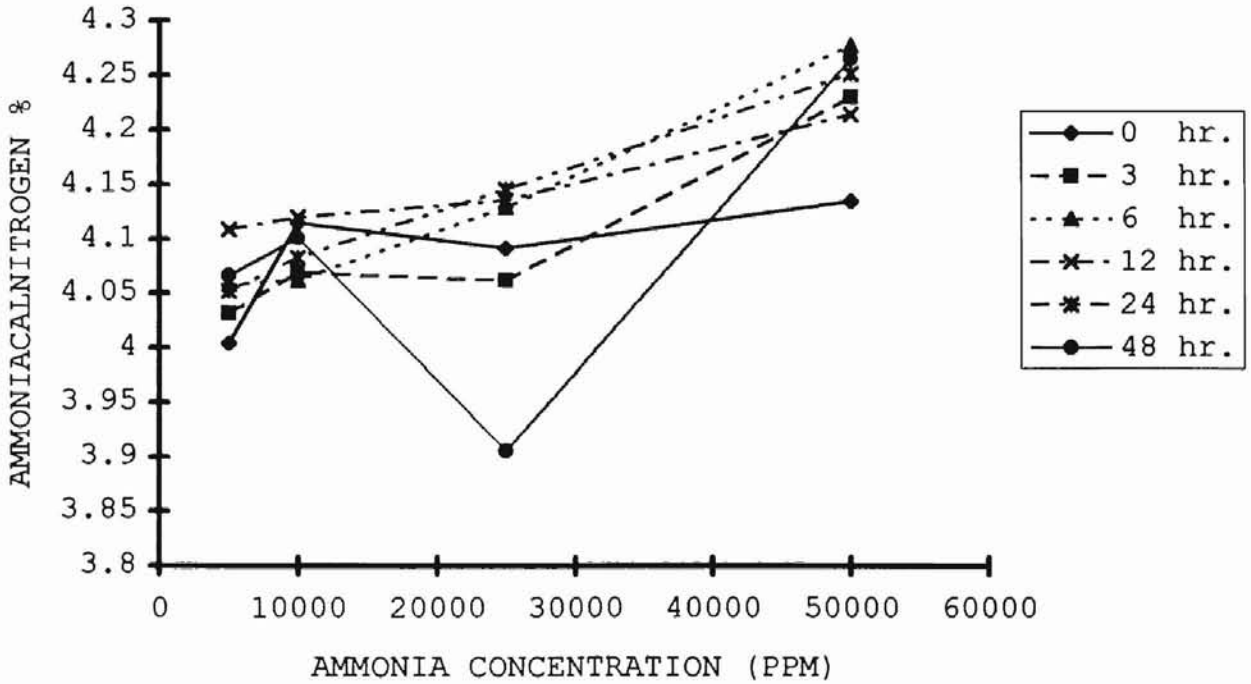

FIGURE 10. THE CHANGE IN AMMONIACAL NITROGEN % CHICKEN BREAST EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA OVER TIME

Table 6. Least squares means for chicken breast color values stratified by different concentrations of ammonia gas at different times of exposure.

	Ammonia concentration, ppm											
	5000			10,000			25,000			50,000		
Time, hr	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	45.41	3.17	9.36	44.88	2.73	7.90	43.08	3.06	8.42	46.81	2.61	10.55
3	43.82	3.30	9.70	44.65	2.29	8.90	43.04	2.86	9.72	42.11	2.47	7.94
6	44.55	2.60	8.44	43.32	2.19	8.00	44.08	2.84	9.33	42.17	3.34	7.65
12	44.76	3.60	8.73	44.57	3.17	10.37	42.25	3.47	8.41	45.40	1.05	6.46
24	44.97	2.49	8.74	43.56	2.40	9.84	42.80	2.88	9.52	43.99	2.48	6.50
48	43.34	2.50	8.51	46.04	2.34	9.79	41.99	2.84	7.92	44.09	2.24	6.30

Means in the same column without superscripts letters are not different (P>.05).
Color values using a Minolta CIELAB(L,a,b) scale: L*=lightness;
a*=bluish-green/red-purple hue component;b*=yellow/blue hue component.
Values represent the average of three replications (3 repeated measurements per replication).

AMMONIA EFFECTS ON PORK

The least squares means of the effect of different ammonia concentrations (5,000, 10,000, 25,000, and 50,000 ppm) over exposure time (0, 3, 6, 12, 24, and 48 hours) on the quality attributes of unpackaged pork muscles are shown in Tables 1 through 4, respectively.

pH

There was a difference ($P<.05$) in pH associated with ammonia concentration. On the other hand, samples treated with 50,000 ppm of ammonia showed a higher pH followed by 25,000, 10,000, with the least pH increase at 5000 ppm of ammonia. Also, there was significant increase in pH between the control (untreated) and all ammonia concentration treatments. At 5,000 ppm of ammonia, exposure time treatments were significantly higher in pH compared to the control (Figure 11 and Table 1) with no significant difference within time treatments except at 3 hr. (less pH than all time treatments). At 10,000 ppm ammonia level, similar results to 5,000 ppm were indicated with no difference across time treatments. In addition, there was a significant increase in pH of all pork samples exposed to 25,000 and 50,000 ppm of ammonia at all time treatments

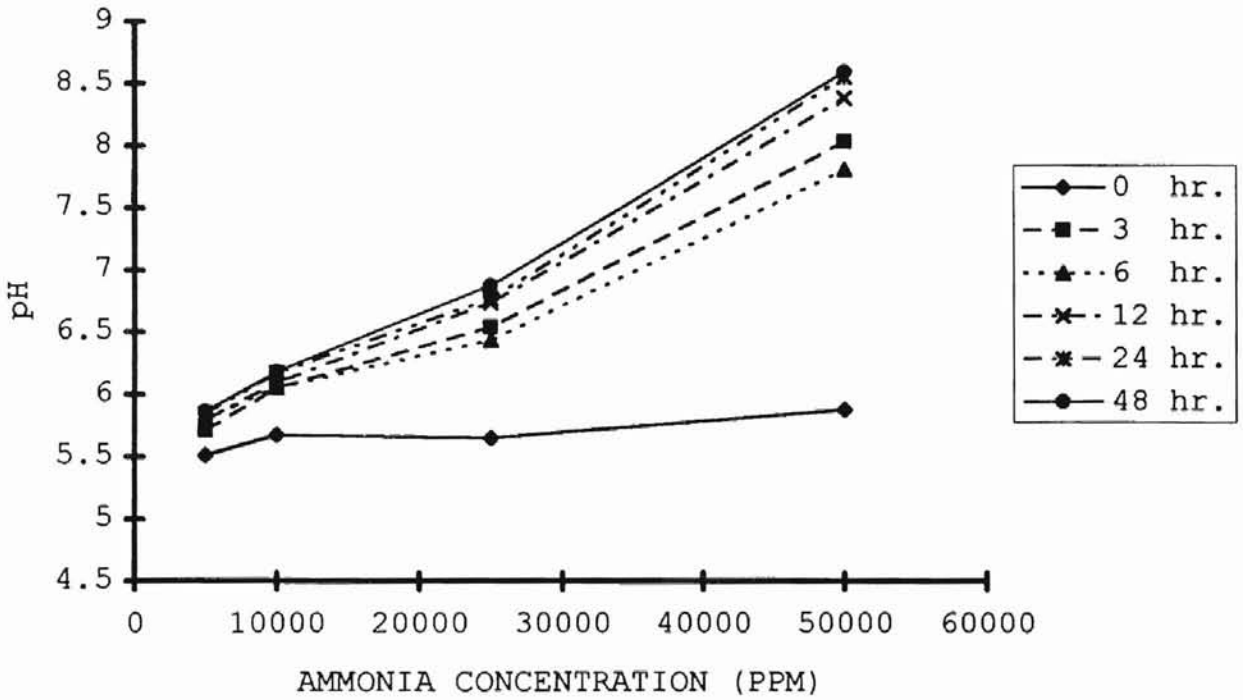

FIGURE 11. THE CHANGE IN pH OF PORK CENTER CUT LOIN CHOPS EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA OVER TIME

compared to the control. No differences (P>.05) were detected within time treatments except at 6 hours (significantly less pH than others) exposure time for both concentrations. Exposure at 50,000 ppm of ammonia gas for 3 hours or less was found to increase the pH of the pork by more than 1-unit (Table 4). This result agreed with Goodfellow et al. (1978), in that ammonia change the quality of unpackaged pork muscles.

DRIP LOSS %

The data for percentage drip loss of pork exposed to four ammonia concentrations over exposure time periods are shown in Tables 1 through 4 and Figure 12. At 5,000 and 10,000 ppm of ammonia, no (P>.05) effect was detected in percentage drip loss. The percentage drip loss of pork decreased (P<.05) at 25,000 and 50,000 ppm of ammonia. When pork was exposed to 25,000 ppm of ammonia there was a decrease (P<.05) in the percent drip loss over time treatments compared to the control; however, significant effect was detected across time treatments. At 50,000 ppm of ammonia similar results were noted with no significant change within time treatments except at 6 hours (higher drip loss compared to others).

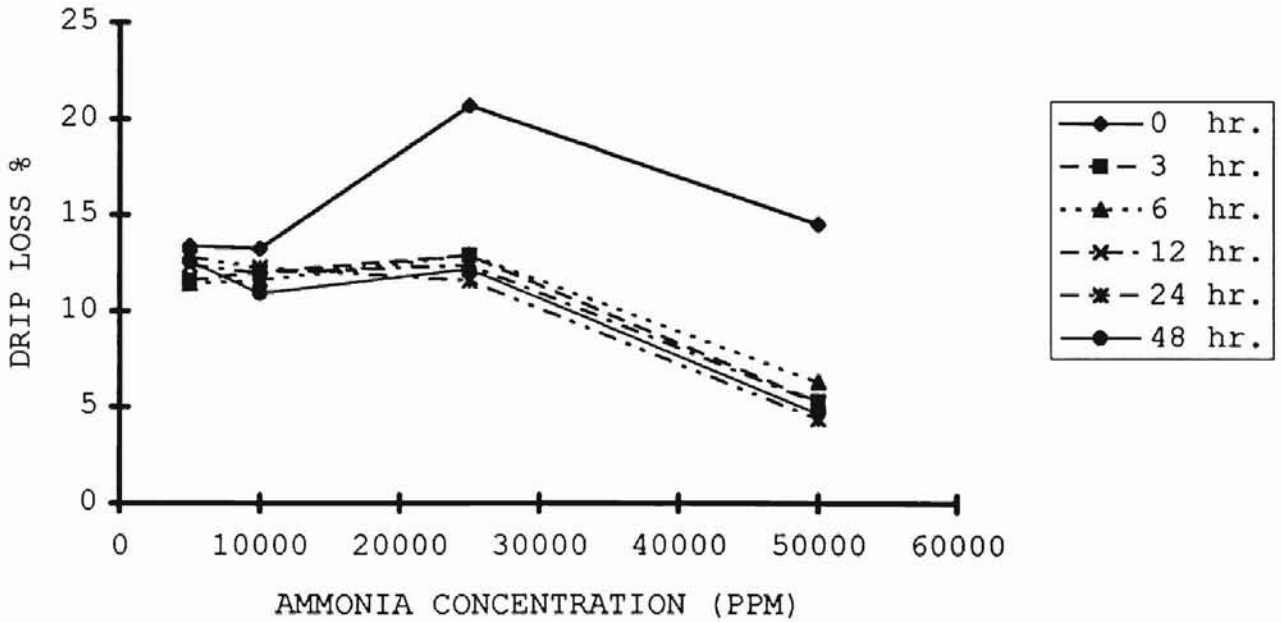

FIGURE 12. THE CHANGE IN THE DRIP LOSS % OF PORK CENTER CUT LOIN CHOPS EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA OVER TIME

WATER HOLDING CAPACITY %

The effect of different concentrations of ammonia over time treatments on the water holding capacity of unpackaged pork muscles are shown in Tables 1 through 4 and Figure 13. Exposure to ammonia 5,000 ppm did not indicate change ($P>.05$) in water holding capacity of pork. At 10,000 ppm of ammonia, an increase ($P<.05$) in water holding capacity of pork was detected. Time treatments of 24, and 48 hours exposure indicated pork possessed a greater ($P<.05$) ability to hold water than the control, 3, and 6 hours of exposure. Additionally, a 12 hr. exposure time was higher ($P<.05$) in water holding capacity compared to the control, 3, and 6 hours exposure with no difference ($P>.05$) when compared to 24 or 48 hours exposure times. The percent water holding capacity of pork increased ($P<.05$) at 25,000 and 50,000 ppm of ammonia. When pork was exposed to 25,000 ppm of ammonia there was an increase ($P<.05$) in the water holding ability over time compared to the control. No differences ($P>.05$) were detected across time treatments. At 50,000 ppm of ammonia similar results were observed as for 25,000 ppm.

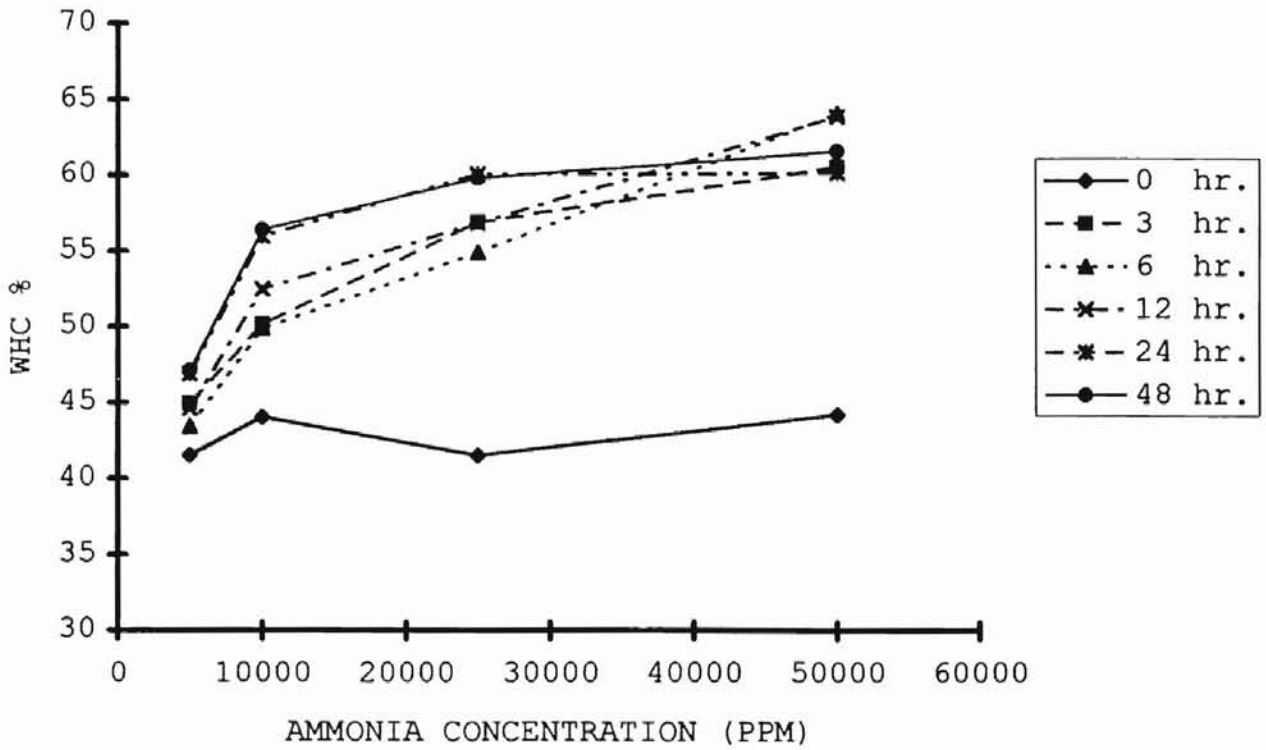

FIGURE 13. THE CHANGE IN WATER HOLDING CAPACITY % OF PORK CENTER CUT LOIN CHOPS EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONNIA OVER TIME

AMMONIACAL NITROGEN %

The ammoniacal nitrogen content of frozen pork muscles, after exposure to different ammonia concentrations over times, are presented in Tables 1 through 4 and Figure 14. Clearly, there was no significant effect of ammonia on the percent nitrogen in pork.

COLOR

Table 7 displays the Minolta Chroma Meter data of pork exposed to different concentrations of ammonia over times. No significant difference was detected in color of pork when exposed to 5,000 or 10,000 ppm of ammonia. Pork muscles subjected to 25,000 ppm of ammonia showed an increase ($P<.05$) in muscle darkness compared to control with no significant difference within period treatments. Pork redness data did not show any significant difference at 25,000 ppm of ammonia treatment. At 50,000 ppm of ammonia, pork redness increased significantly in all time treatments compared to control with no difference within treatments period except at 6 hours (less redness was observed).

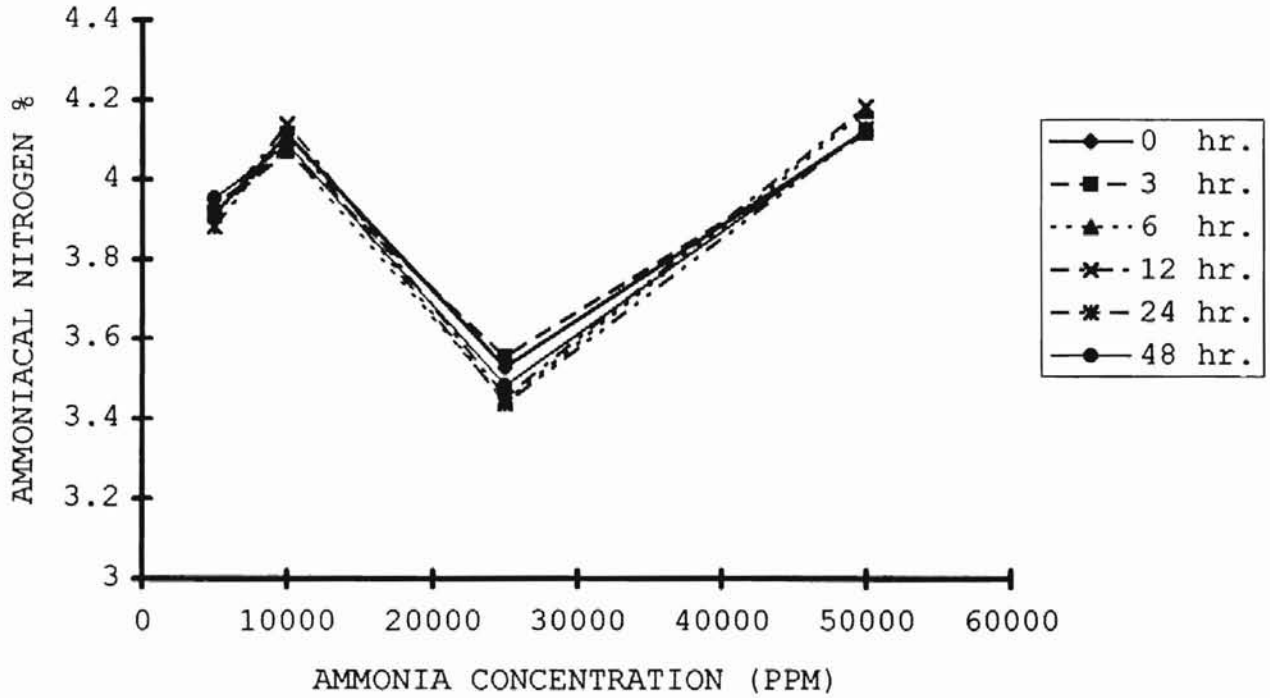

FIGURE 14. THE CHANGE IN AMMONIACAL NITROGEN % OF PORK CENTER CUT LOIN CHOPS EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA OVER TIME

Table 7. Least squares means for pork chop color values (L*a*b*) stratified by different concentrations of ammonia gas at different times of exposure.

	Ammonia concentration, ppm											
	5000			10,000			25,000			50,000		
Time,hr	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	46.65	5.97^{b}	6.10	46.57	6.91	6.18	52.87^{a}	6.36	8.19^{a}	43.86	9.40^{a}	5.54
3	44.74	5.88^{b}	5.86	43.13	8.03	5.47	46.45^{b}	7.12	5.75^{b}	39.20	8.31^{bcd}	3.98
6	43.45	7.49^{a}	6.37	44.00	7.89	5.83	47.99^{b}	6.92	5.97^{b}	39.26	9.12^{ab}	4.32
12	42.74	6.61^{ab}	5.88	43.27	8.03	5.70	48.48^{b}	6.19	5.82^{b}	38.27	8.38^{bd}	3.99
24	44.39	5.73^{b}	5.92	43.06	8.08	5.36	47.29^{b}	6.69	5.81^{b}	38.71	7.99^{cd}	3.74
48	44.53	6.05^{b}	5.56	44.99	7.07	5.56	47.19^{b}	6.52	5.49^{b}	38.79	7.87^{d}	3.85

^{abcd}Means in the same column with different superscripts letters are different (P<.05). Color values using a Minolta CIELAB(L,a,b) scale: L*=lightness; a*=bluish-green/red-purple hue component;b*=yellow/blue hue component. Values represent the average of three replications (3 repeated measurements per replication).

OVERALL DISCUSSION

General view of the experimental results on all meat traits indicated (with some exceptions) that there was no difference ($P>.05$) within exposure time 3, 6, 12, 24, or 48 hours except when compared to 0 hour exposure time (control/untreated). This findings imply that 3 hours or less of exposure was adequate for ammonia to achieve its effect on all three kinds of meat used.

pH

The pH of all three kinds of meat showed a dramatic increase when exposed to ammonia. The results indicated that 5,000 ppm of ammonia increased the pH of beef and pork significantly ($P<.05$) with no effect on chicken ($P>.05$) (Table 1). At 10,000 ppm of ammonia gas, pH of chicken breasts started to increase ($P<.05$) compared to the control. Buffer capacity of each type of meat may have affected the ability of each meat specie in absorbing ammonia which may be interpreted as the reason for the difference in pH between red meats and chicken breast at 5,000 ppm of ammonia exposure. At 25,000 ppm of ammonia concentration, beef and pork pH increased by more than 1 pH-unit while chicken reached that level at 50,000 ppm. Similar results of pH

increase were detected by Hermann (1965) on beef and pork, Kassem (1965) and Anil (1971) on beef as well. These findings are logical and may be attributed to the high alkalinity of ammonia gas.

WATER HOLDING CAPACITY & DRIP LOSS %

Ammonia at 5000 ppm did not have an effect (P>.05) on water holding capacity and drip loss % regardless of species. Starting at 10,000 ppm of ammonia concentration, beef muscles indicated a significant increase in the water holding capacity % with no effect on chicken or pork muscles. Pork WHC % started to increase significantly at 25,000 ppm of ammonia concentration, however, WHC % of chicken started to increase at 50,000 ppm ammonia level. Data for the effect of ammonia on the percentage water holding capacity of beef in this study were similar to the conclusions of Kassem (1965) and Anil (1971).

Percentage drip loss results indicated similar effects to WHC % for all meats and concentrations. These findings indicated that ammonia increased the pH of meats, hence improved the percentage water holding capacity of meat and decreased the percentage drip loss (Figures 15 to 20).

The relationship between the pH and water holding capacity of meat was explained by Wismer-Pederson (1987).

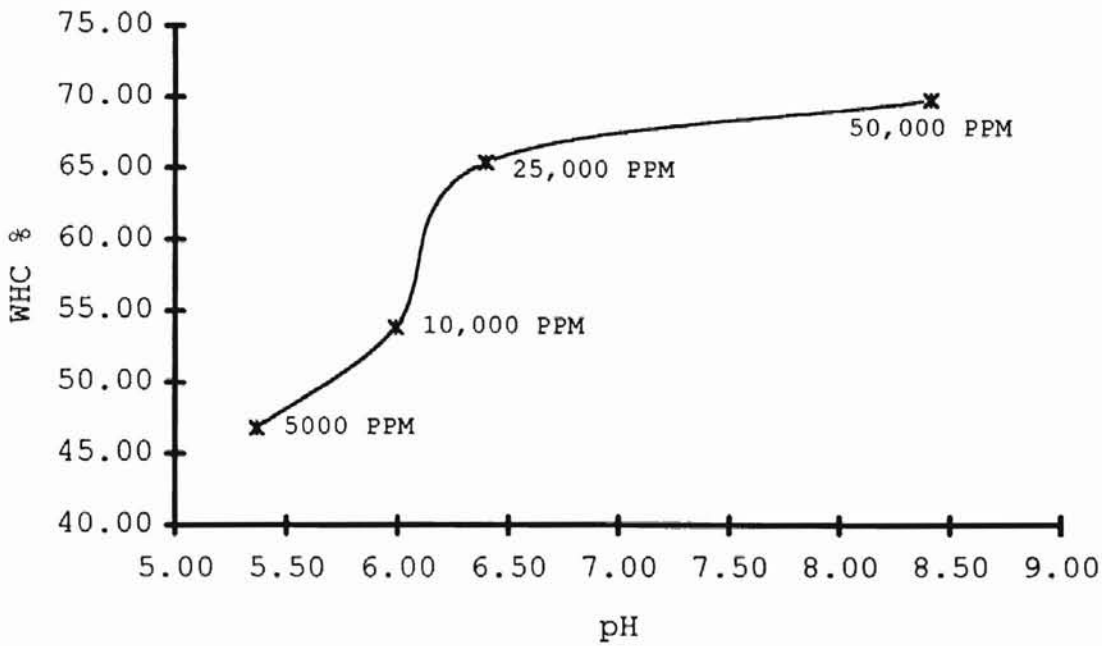

FIGURE 15. RELATIONSHIP OF WATER HOLDING CAPACITY TO pH IN BEEF STRIP LOIN STEAKS EXPOSED TO AMMONIA AT 3 HOUR EXPOSURE TIME

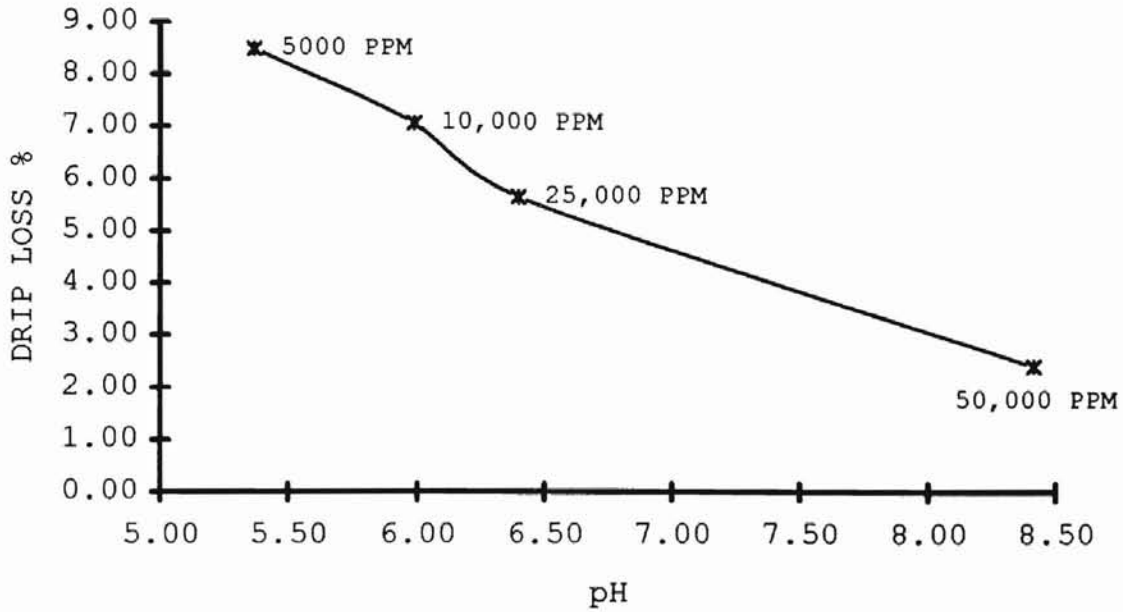

FIGURE 16. RELATIONSHIP OF DRIP LOSS % TO pH IN BEEF STRIP LOIN STEAKS EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA GAS AT 3 HOURS OF EXPOSURE TIME.

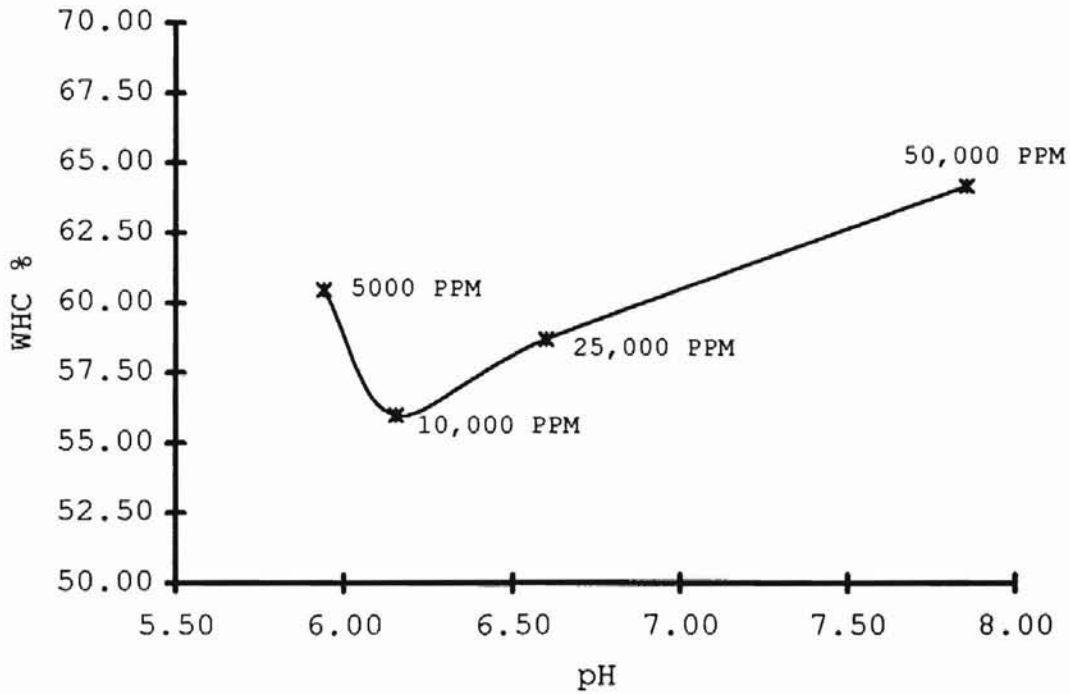

FIGURE 17. RELATIONSHIP OF WATER HOLDING CAPACITY % TO pH IN CHICKEN BREAST EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA AT 3 HOUR TIME

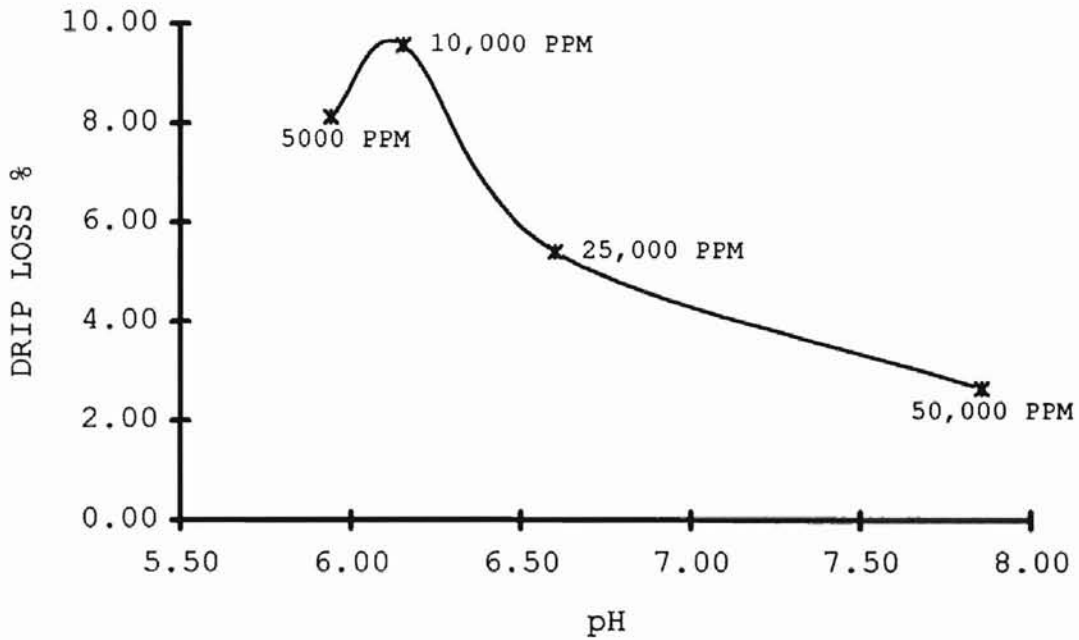

FIGURE 18. RELATIONSHIP OF DRIP LOSS % TO pH IN CHICKEN BREAST EXPOSED TO DIFFERENT AMMONIA CONCENTRATIONS AT 3 HOUR TIME

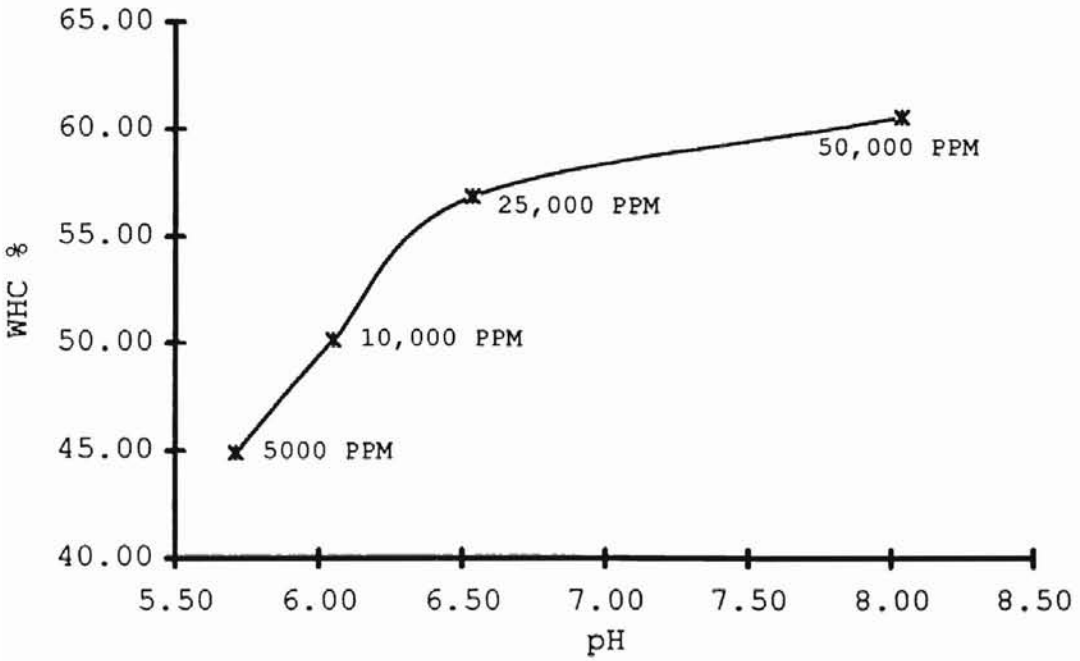

FIGURE 19. RELATIONSHIP OF WATER HOLDING CAPACITY % TO pH IN PORK CENTER CUT LOIN CHOPS EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA AT 3 HOUR EXPOSURE TIME

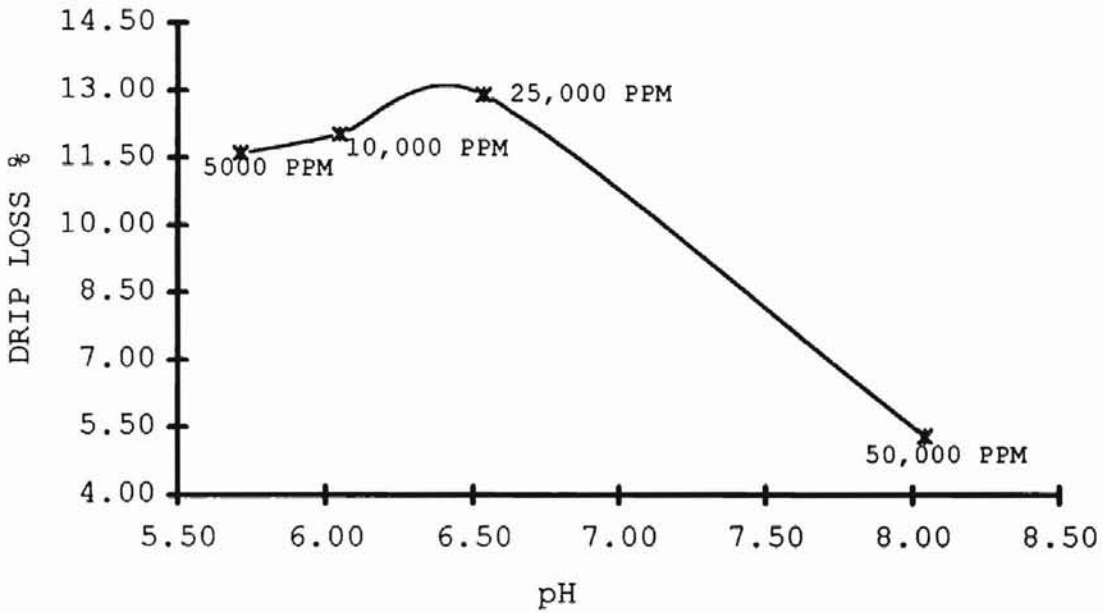

FIGURE 20. RELATIONSHIP OF DRIP LOSS % TO pH IN PORK CENTER CUT LOIN CHOPS EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA AT 3 HOUR EXPOSURE TIME

When the pH increased to the basic side, the distribution of the negative charge on the myofilaments is altered so as to cause a repulsion between myofilaments, causing more water to be held in between.

AMMONIACAL NITROGEN %

Even though all ammonia concentrations indicated changes in some quality attributes of beef, chicken, and pork muscles, the ammoniacal nitrogen content of these meats were not affected. This phenomenon may be due to the ammonia concentrations used in that they were too small to cause nitrogen increases with the volume of the chambers used (5 liters) (Odell, 1995). Relatively, Kassem (1965) concluded that the pH of contaminated meat is not a good way to assess the amount of ammonia absorbed by that meat. According to this study, a small amount of ammonia increased the pH of beef, chicken, and pork muscles by more than 2 pH units at 50,000 ppm of ammonia with no difference ($P>.05$) in ammoniacal nitrogen content of those meats.

COLOR

Beef, chicken, and pork color showed different responses to ammonia when exposed. In all treatments,

chicken showed the least ($P>.05$) change in color while, on other hand, beef and pork muscle color started to darken ($P<.05$) (L* values) at 10,000 ppm for beef (Figure 21) and 25,000 ppm for pork. Beef redness (a* values) started to increase ($P<.05$) at 25,000 ppm ammonia concentration, however, pork started at 50,000 ppm. Muscle pigments most likely are playing a major role in the color change of meats. Chicken muscle, due to low pigment content, did not show alteration in color. Beef and pork expressed formation of dark color as well as increase in redness due to ammonia contamination. These findings are essentially pointing out the relationship between the meat pigments and ammonia. Shaw et al.(1992) concluded that the pink color that formed on pork meat after exposure to ammonia was neither a result of pH increase nor the same as the color of cured or fresh meat. Kassem (1965) reported a similar color change between treated and untreated samples in darkness and redness due to ammonia exposure.

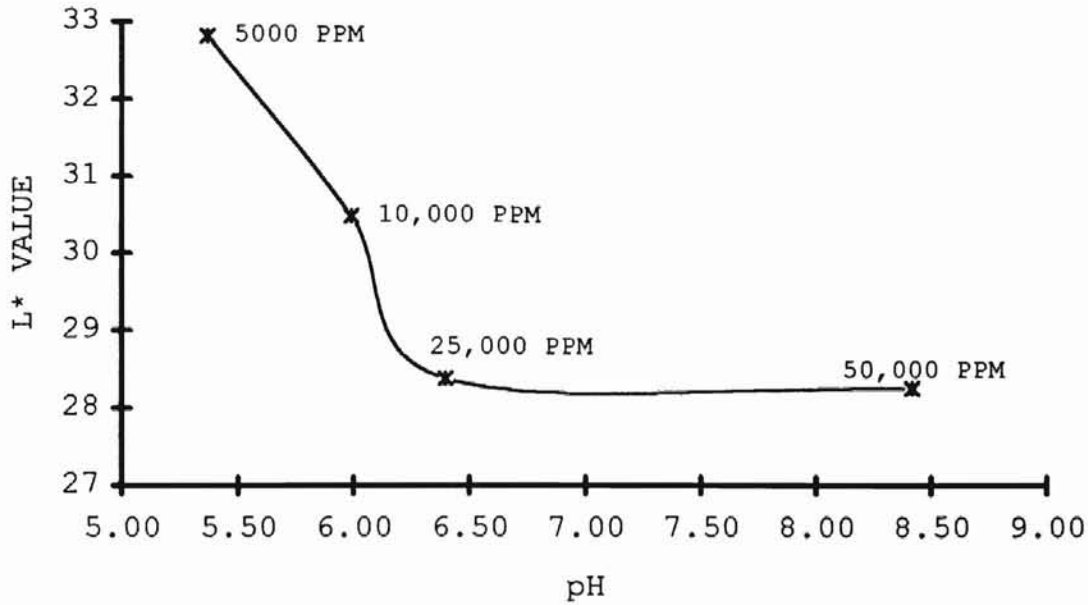

FIGURE 21. RELATIONSHIP OF COLOR DARKNESS (L* VALUE) TO pH IN BEEF STRIP LOIN STEAKS EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA AT 3 HOURE EXPOSURE TIME

CHAPTER V

SUMMARY & RECOMMENDATIONS

SUMMARY

This study was conducted to determine the effects of ammonia concentration and length of exposure on the quality of unpackaged beef, chicken, and pork muscles. Under the conditions of this experiment, the following conclusions may be drawn:

A general view of the experimental data across all specie types of meat, indicated (with some exceptions) that there was no difference between exposure times of 3, 6, 12, 24, and 48 hours except when compared to controls (0 hour/untreated). These findings indicated that 3 hours of exposure or less was adequate for ammonia to achieve its effect.

BEEF

1. The pH of beef steaks was significantly affected at all ammonia concentrations. The higher the ammonia concentration the greater the increase in pH of beef muscles.
2. Percentage water holding capacity of beef steaks increased at 10,000 ppm of ammonia gas exposure with no effect at 5,000 ppm of ammonia. Exposure to 25,000 and 50,000 ppm of ammonia significantly increased the percentage water holding capacity of beef.
3. Beef exposed to ammonia at 10,000, 25,000, and 50,000 ppm significantly decreased the drip loss. The higher the concentration of ammonia the lower the percentage drip loss in beef.
4. Percentage ammoniacal nitrogen in beef showed a significant increase at 50,000 ppm. No effect was detected at other concentrations.
5. Color of beef started to darken at 10,000 ppm of ammonia exposure and continued for 25,000 and 50,000 ppm. Beef redness started to increase when exposed to 25,000 ppm of ammonia gas.

CHICKEN

1. Ammonia at a level of 10,000 ppm and above significantly affected the pH of chicken.
2. Water holding capacity started to increase at 50,000 ppm of ammonia.
3. Drip loss of chicken decreased significantly at 50,000 ppm of ammonia but not at the lower concentration levels.
4. Ammoniacal nitrogen content of chicken muscles did not significantly change at any of the ammonia levels.
5. Color of chicken breasts was not affected at any ammonia level.

PORK

1. The pH of pork possessed similar changes to beef.
2. Water holding capacity of pork started to increase significantly at the 25,000 ppm ammonia level.
3. Drip loss of pork muscles decreased at 50,000 ppm of ammonia but not at the lower concentration levels.
4. Ammoniacal nitrogen content of pork muscles did not change regardless of ammonia level.
5. Color of pork muscles started to darken significantly at 25,000 ppm of ammonia exposure. Pork redness

significantly increased at 50,000 ppm ammonia level but not at lower levels.

Exposure to 25,000 ppm ammonia gas and higher for 3 hours or less was capable of altering the quality of unwrapped beef and pork muscles. Quality of unwrapped chicken breast was changed when exposed to 50,000 ppm ammonia gas for 3 hours.

RECOMMENDATIONS

Further investigation is suggested to be directed to:

1. The effect of ammonia on the quality of meats at times between 0 hour to 3 hours of exposure.
2. Effect of continuous accumulation of low concentrations of ammonia on meat quality.
3. Examination of the effect of different humidity levels on the ammonia absorption ability of meats.
4. An elucidation experiment on the mechanism of the effect of ammonia on the water holding capacity of meats whether it is a mechanical or a chemical effect.
5. The color of meat after ammonia exposure, its nature and causes.

6. Extensive studies leading to federal regulations in case of ammonia contamination of meats.
7. Study the effect of packaging on preventing ammonia contamination.
8. Study the effect of ammonia gas versus liquid ammonia.
9. The effect of a high initial exposure to ammonia for a period of time then held in low level for a similar period of time.

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APPENDIXES

Table A-1. Least squares means for beef, chicken, and pork muscle traits stratified by different concentrations at 0 time (control)

Trait	Concentration, ppm				Statistics[a]				
	5000	10,000	25,000	50,000	SE	P	L	Q	C
Drip loss %									
Beef	9.26	8.82	9.93	8.69	0.32	0.0869	0.2001	0.1317	0.1011
Chicken	9.30	8.92	8.17	9.12	0.77	0.7469	0.8763	0.9841	0.9536
Pork	13.40^{c}	13.25^{c}	20.71^{b}	14.57^{c}	0.98	0.0019	0.2581	0.0813	0.0430
pH									
Beef	5.14^{d}	5.48^{b}	5.32^{c}	5.46^{bc}	0.05	0.0041	0.0013	0.0015	0.0016
Chicken	5.71^{c}	5.73^{c}	6.14^{b}	5.95^{bc}	0.08	0.0176	0.6144	0.3286	0.2372
Pork	5.51^{d}	5.68^{c}	5.65^{cd}	5.88^{b}	0.05	0.0061	0.0621	0.0742	0.0709
WHC %									
Beef	46.27	44.30	44.96	43.67	1.23	0.4547	0.2700	0.2896	0.2930
Chicken	51.00	37.43	52.67	48.66	4.20	0.1179	0.0349	0.0286	0.0270
Pork	41.53	44.03	41.49	44.20	1.00	0.1564	0.0858	0.0735	0.0657
Nitrogen %									
Beef	3.86^{bc}	4.09^{b}	3.69^{c}	3.75^{c}	0.08	0.0400	0.0420	0.0278	0.0243
Chicken	4.00	4.11	4.09	4.14	0.10	0.8053	0.4891	0.5176	0.5281
Pork	3.91^{c}	4.11^{bc}	3.53^{d}	4.13^{b}	0.07	0.0006	0.0111	0.0039	0.0022

[a]SE=Standard Error, P=Probability values (P<.05), L=Liner, Q=Quadratic, C=Cubic.

[bcd]Means in the same row with different superscripts letters are different (P<.05).

Values represent the average of three replications (2 samples per replication).

Table A-2. Least squares means for beef, chicken, and pork muscle traits stratified by different concentrations at 3 hr. exposure time.

Trait	Concentration, ppm				Statistics[a]				
	5000	10,000	25,000	50,000	SE	P	L	Q	C
Drip loss %									
Beef	8.48[b]	7.07[c]	5.66[d]	2.32[e]	0.23	0.0001	0.0129	0.0436	0.0451
Chicken	8.12[b]	9.57[b]	5.42[c]	2.64[d]	0.85	0.0020	0.1296	0.0827	0.0804
Pork	11.60[b]	12.03[b]	12.91[b]	5.31[c]	0.67	0.0002	0.9451	0.8157	0.5638
pH									
Beef	5.37[e]	5.99[d]	6.40[c]	8.42[b]	0.12	0.0001	0.0146	0.0299	0.0232
Chicken	5.94[d]	6.16[cd]	6.60[c]	7.86[b]	0.15	0.0001	0.5751	0.7544	0.7010
Pork	5.71[d]	6.05[cd]	6.54[c]	8.04[b]	0.21	0.0002	0.4550	0.5953	0.5574
WHC %									
Beef	46.78[d]	53.83[c]	65.42[b]	69.67[b]	1.75	0.0001	0.1370	0.4505	0.6259
Chicken	60.46	55.97	58.68	64.19	4.16	0.5863	0.4760	0.4937	0.5228
Pork	44.92[d]	50.18[cd]	56.88[bc]	60.60[b]	2.92	0.0218	0.4296	0.6225	0.6919
Nitrogen %									
Beef	3.93[b]	3.45[c]	3.77[b]	3.92[b]	0.08	0.0101	0.0031	0.0034	0.0040
Chicken	4.03	4.07	4.06	4.23	0.06	0.1665	0.6683	0.6651	0.6265
Pork	3.92[c]	4.07[b]	3.56[d]	4.12[b]	0.04	0.0001	0.0024	0.0006	0.0003

[a]SE=Standard Error, P=Probability values (P<.05), L=Liner, Q=Quadratic, C=Cubic.

[bcde]Means in the same row with different superscripts letters are different (P<.05).

Values represent the average of three replications (2 samples per replication).

Table A-3. Least squares means for beef, chicken, and pork muscle traits stratified by different concentrations of ammonia at 6 hr. exposure time.

	Concentration, ppm				Statistics[a]				
Trait	5000	10,000	25,000	50,000	SE	P	L	Q	C
Drip loss %									
Beef	7.99[b]	7.69[b]	6.33[b]	3.27[c]	0.56	0.0012	0.9471	0.9051	0.9412
Chicken	6.27	8.89	7.72	3.54	1.29	0.0823	0.2101	0.2413	0.2861
Pork	11.41[b]	11.57[b]	12.94[b]	6.33[c]	0.58	0.0002	0.7776	0.5186	0.3206
pH									
Beef	5.34[e]	5.84[d]	6.24[c]	7.72[b]	0.08	0.0001	0.0060	0.0169	0.0140
Chicken	5.87[d]	6.24[cd]	6.42[c]	7.41[b]	0.12	0.0001	0.1069	0.1533	0.1387
Pork	5.81[d]	6.05[d]	6.43[c]	7.82[b]	0.11	0.0001	0.2857	0.4330	0.3588
WHC %									
Beef	48.07[d]	49.08[c]	63.34[b]	71.80[b]	1.20	0.0001	0.3405	0.0705	0.0490
Chicken	56.48	56.17	60.61	64.19	3.43	0.3618	0.8315	0.7571	0.7483
Pork	43.41[d]	49.87[d]	54.88[cd]	64.13[b]	2.35	0.0016	0.1765	0.2842	0.3071
Nitrogen %									
Beef	3.94	4.13	3.89	3.91	0.14	0.6055	0.2954	0.2694	0.2642
Chicken	4.06[d]	4.06[d]	4.13[cd]	4.28[b]	0.04	0.0259	0.9179	0.8392	0.8658
Pork	3.94[d]	4.08[c]	3.44[e]	4.18[b]	0.03	0.0001	0.0004	0.0001	0.0001

[a]SE=Standard Error, P=Probability values (P<.05), L=Liner, Q=Quadratic, C=Cubic.

[bcde]Means in the same row with different superscripts letters are different (P<.05).

Values represent the average of three replications (2 samples per replication).

Table A-4. Least squares means for beef, chicken, and pork muscle traits stratified by different concentrations of ammonia gas at 12 hr. exposure time.

Trait	Concentration, ppm				Statistics[a]				
	5000	10,000	25,000	50,000	SE	P	L	Q	C
Drip loss %									
Beef	9.94[b]	7.71[cd]	6.32[d]	2.36[e]	0.70	0.0004	0.1100	0.1797	0.1853
Chicken	8.35[b]	9.29[b]	6.22[bc]	2.39[c]	1.27	0.0205	0.4717	0.3994	0.4119
Pork	12.36[b]	12.00[b]	12.41[b]	5.26[c]	0.56	0.0001	0.5169	0.4115	0.2636
pH									
Beef	5.32[e]	5.87[d]	6.39[c]	8.40[b]	0.12	0.0001	0.0233	0.0550	0.0425
Chicken	5.82[e]	6.18[d]	6.68[c]	7.99[b]	0.06	0.0001	0.0177	0.0744	0.0632
Pork	5.80[d]	6.09[d]	6.74[c]	8.39[b]	0.20	0.0001	0.5627	0.7667	0.7202
WHC %									
Beef	49.53[c]	52.90[c]	67.23[b]	70.59[b]	2.45	0.0006	0.9396	0.6023	0.4781
Chicken	58.46	61.83	63.8	58.79	3.72	0.7106	0.6453	0.7357	0.7963
Pork	44.61[d]	52.50[cd]	56.84[bc]	63.89[b]	3.24	0.0175	0.2068	0.2989	0.3271
Nitrogen %									
Beef	3.85[b]	3.48[c]	3.83[b]	4.03[b]	0.10	0.0348	0.0292	0.0272	0.0296
Chicken	4.11	4.12	4.14	4.21	0.05	0.4996	0.9093	0.9268	0.9089
Pork	3.91[c]	4.14[b]	3.45[d]	4.19[b]	0.05	0.0001	0.0005	0.0001	0.0001

[a]SE=Standard Error, P=Probability values (P<.05), L=Liner, Q=Quadratic, C=Cubic.

[bcde]Means in the same row with different superscripts letters are different (P<.05).

Values represent the average of three replications (2 samples per replication).

Table A-5. Least squares means for beef, chicken, and pork muscle traits stratified by different concentrations of ammonia gas at 24 hr. exposure time.

Trait	Concentration, ppm				Statistics[a]				
	5000	10,000	25,000	50,000	SE	P	L	Q	C
Drip loss %									
Beef	9.45^{b}	8.14^{c}	5.90^{d}	2.11^{e}	0.34	0.0001	0.1356	0.3794	0.4044
Chicken	7.64^{bc}	11.00^{b}	5.66^{cd}	3.40^{d}	1.25	0.0146	0.0551	0.0410	0.0408
Pork	12.79^{b}	12.25^{b}	11.60^{b}	4.39^{c}	0.78	0.0002	0.6639	0.6678	0.5407
pH									
Beef	5.36^{e}	5.86^{d}	6.56^{c}	8.30^{b}	0.11	0.0001	0.0645	0.1812	0.1669
Chicken	5.95^{e}	6.16^{de}	6.52^{cd}	7.72^{b}	0.13	0.0001	0.4567	0.6154	0.5499
Pork	5.85^{e}	6.17^{d}	6.77^{c}	8.56^{b}	0.06	0.0001	0.0525	0.1932	0.1303
WHC %									
Beef	51.46^{c}	52.70^{c}	71.39^{b}	70.76^{b}	1.47	0.0001	0.2572	0.0392	0.0194
Chicken	60.59	64.94	61.38	62.51	1.57	0.2977	0.0736	0.0703	0.0701
Pork	46.86^{c}	55.94^{b}	60.04^{b}	60.23^{b}	2.26	0.0097	0.0525	0.1044	0.1393
Nitrogen %									
Beef	4.03	3.97	3.72	3.98	0.09	0.1563	0.9987	0.7465	0.6177
Chicken	4.05	4.08	4.15	4.25	0.08	0.3563	0.8828	0.9470	0.9500
Pork	3.88^{c}	4.11^{b}	3.44^{d}	4.13^{b}	0.03	0.0001	0.0001	0.0001	0.0001

[a]SE=Standard Error, P=Probability values (P<.05), L=Liner, Q=Quadratic, C=Cubic.

[bcde]Means in the same row with different superscripts letters are different (P<.05).

Values represent the average of three replications (2 samples per replication).

Table A-6. Least squares means for beef, chicken, and pork muscle traits stratified by different concentrations of ammonia gas at 48 hr. exposure time.

Trait	Concentration, ppm				Statistics[a]				
	5000	10,000	25,000	50,000	SE	P	L	Q	C
Drip loss %									
Beef	11.18^{b}	8.04^{c}	6.14^{d}	2.21^{e}	0.37	0.0001	0.0014	0.0048	0.0061
Chicken	9.02^{b}	8.81^{bc}	6.01^{cd}	2.25^{d}	0.91	0.0024	0.8293	0.6503	0.6501
Pork	12.61^{b}	10.91^{c}	12.19^{bc}	4.69^{d}	0.53	0.0001	0.0350	0.0261	0.0153
pH									
Beef	5.38^{e}	5.87^{d}	6.50^{c}	8.48^{b}	0.14	0.0001	0.1041	0.2162	0.1842
Chicken	5.80^{e}	6.14^{de}	6.75^{cd}	8.19^{b}	0.22	0.0003	0.5259	0.7087	0.6885
Pork	5.86^{e}	6.18^{d}	6.87^{c}	8.60^{b}	0.08	0.0001	0.1494	0.4355	0.3608
WHC %									
Beef	53.83^{c}	54.87^{c}	69.63^{b}	72.56^{b}	1.59	0.0001	0.4234	0.1222	0.0797
Chicken	56.32	58.65	64.54	63.94	4.31	0.4951	0.8852	0.9872	0.9360
Pork	47.07^{d}	56.40^{c}	59.77^{bc}	61.65^{b}	1.32	0.0002	0.0034	0.0093	0.0142
Nitrogen %									
Beef	4.06	3.69	3.78	4.03	0.12	0.1422	0.0754	0.0983	0.1207
Chicken	4.07^{cd}	4.10^{bc}	3.91^{d}	4.27^{b}	0.06	0.0118	0.3616	0.2175	0.1470
Pork	3.95^{c}	4.09^{b}	3.48^{d}	4.13^{b}	0.04	0.0001	0.0013	0.0002	0.0001

[a]SE=Standard Error, P=Probability values (P<.05), L=Liner, Q=Quadratic, C=Cubic.

[bcde]Means in the same row with different superscripts letters are different (P<.05).

Values represent the average of three replications (2 samples per replication).

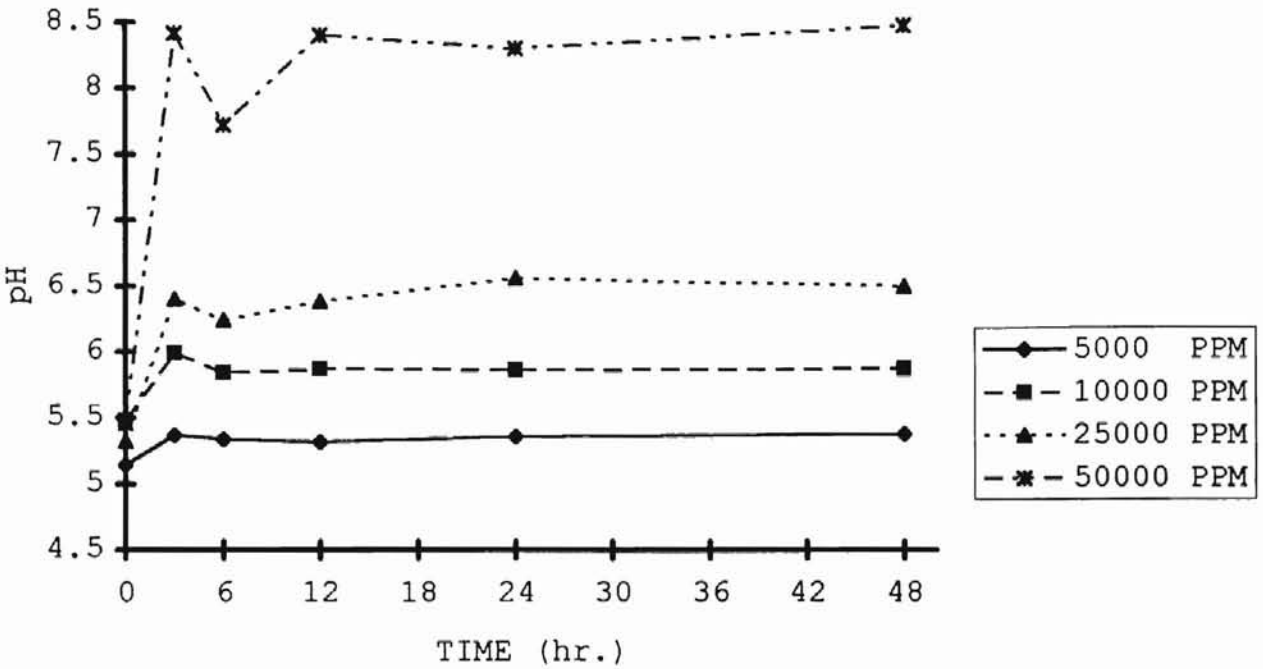

FIGURE A-1. THE CHANGE IN pH OF BEEF STRIP LOIN STEAKS OVER TIME WHEN EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA

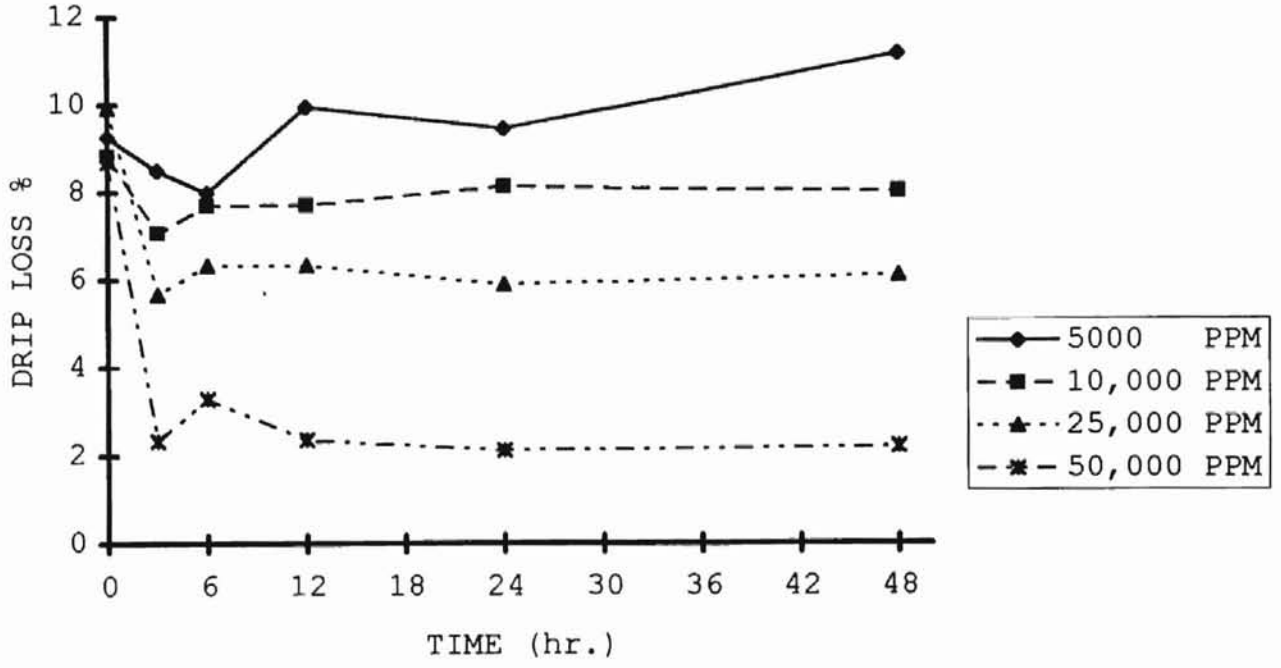

FIGURE A-2. THE CHANGE IN DRIP LOSS % OF BEEF STRIP LOIN STEAKS OVER TIME WHEN EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA

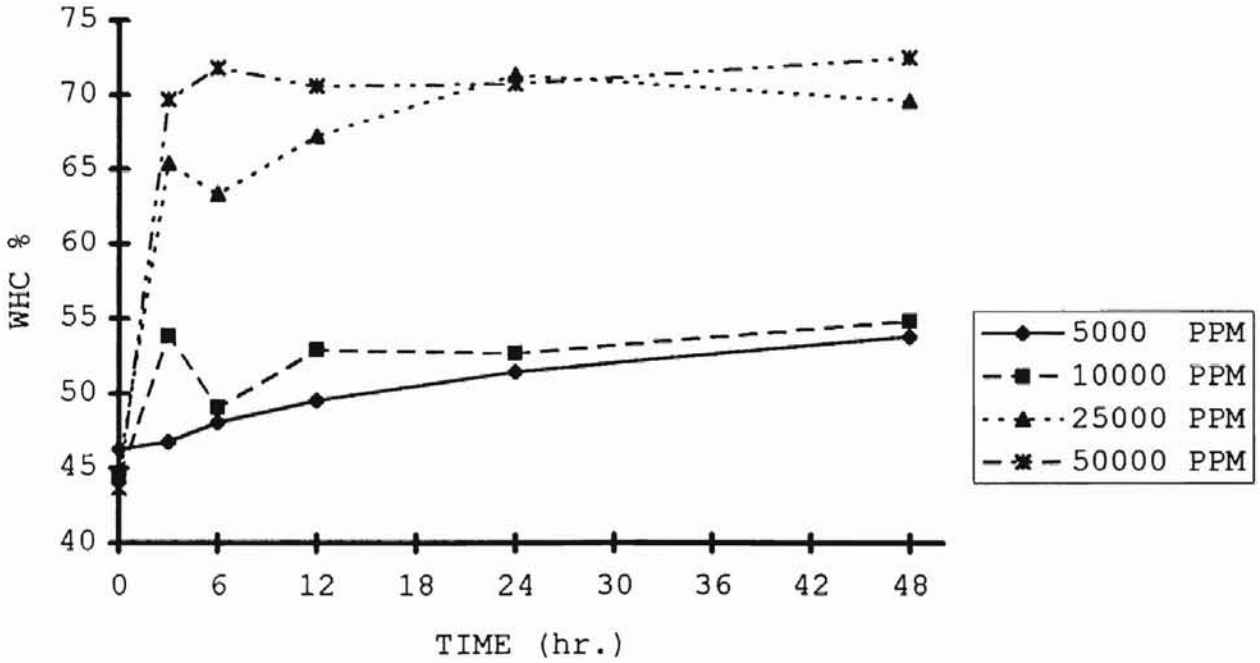

FIGURE A-3. THE CHANGE IN WATER HOLDING CAPACITY % OF BEEF STRIP LOIN STEAKS OVER TIME WHEN EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA

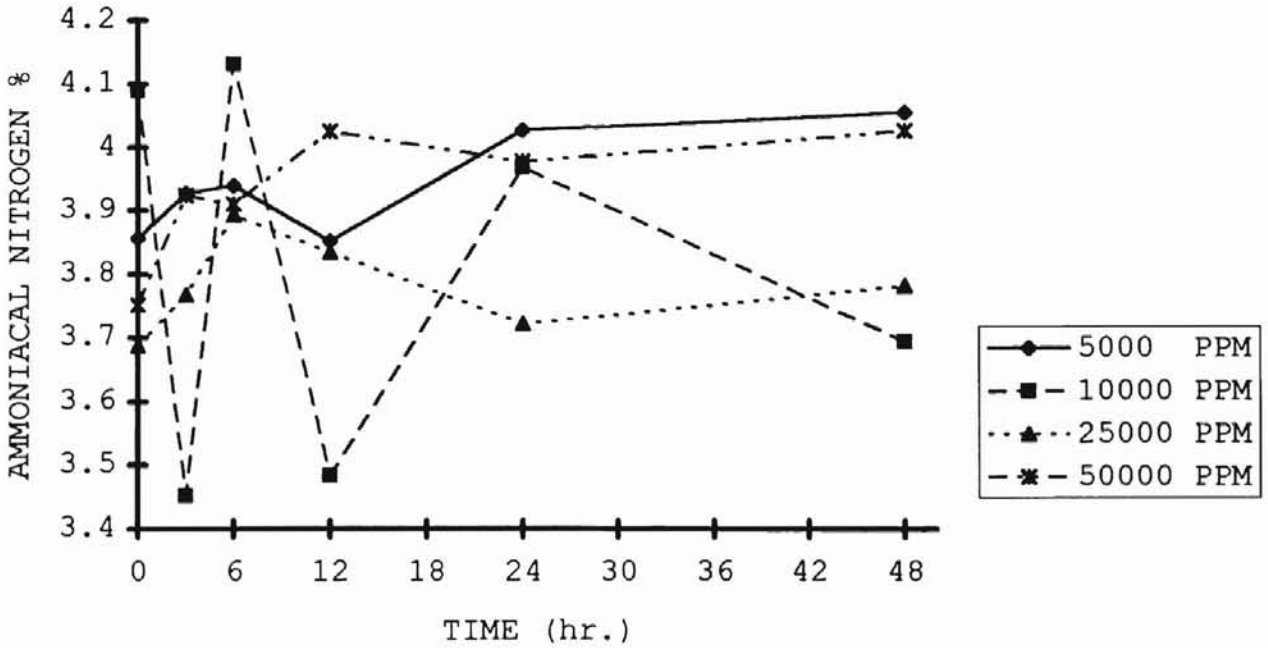

FIGURE A-4. THE CHANGE IN AMMONIACAL NITROGEN % OF BEEF STRIP LOIN STEAKS OVER TIME WHEN EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA

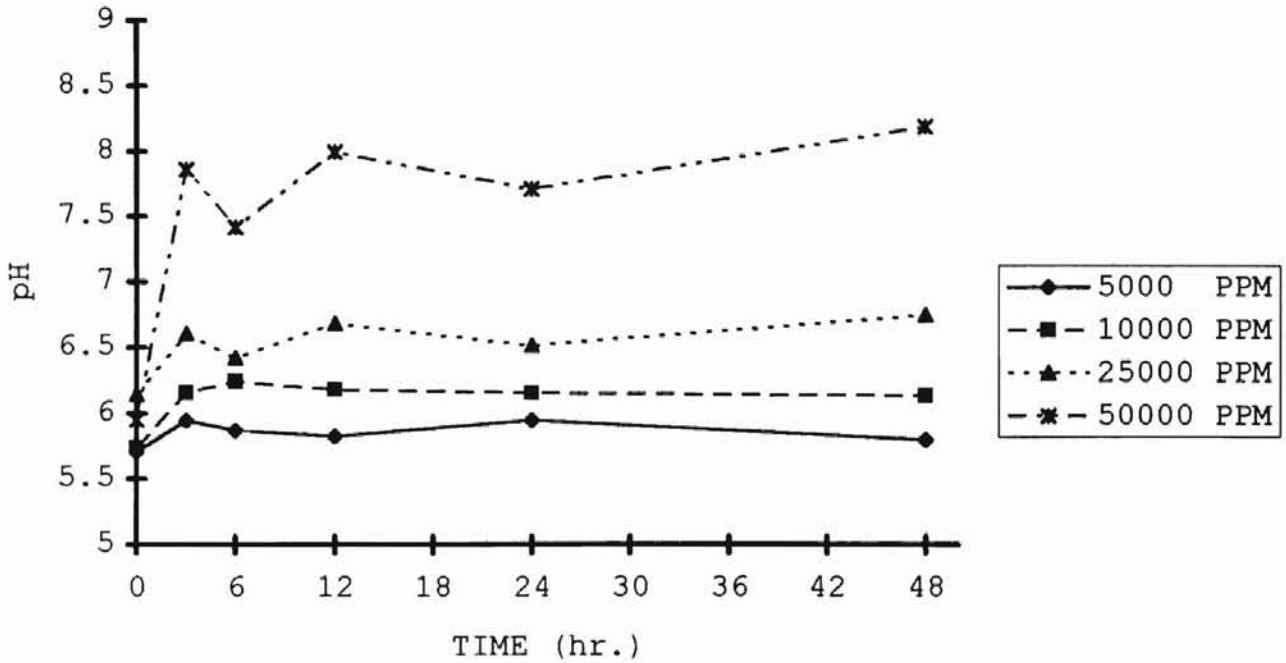

FIGURE A-5. THE CHANGE IN pH OF CHICKEN BREASTS OVER TIME WHEN EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA

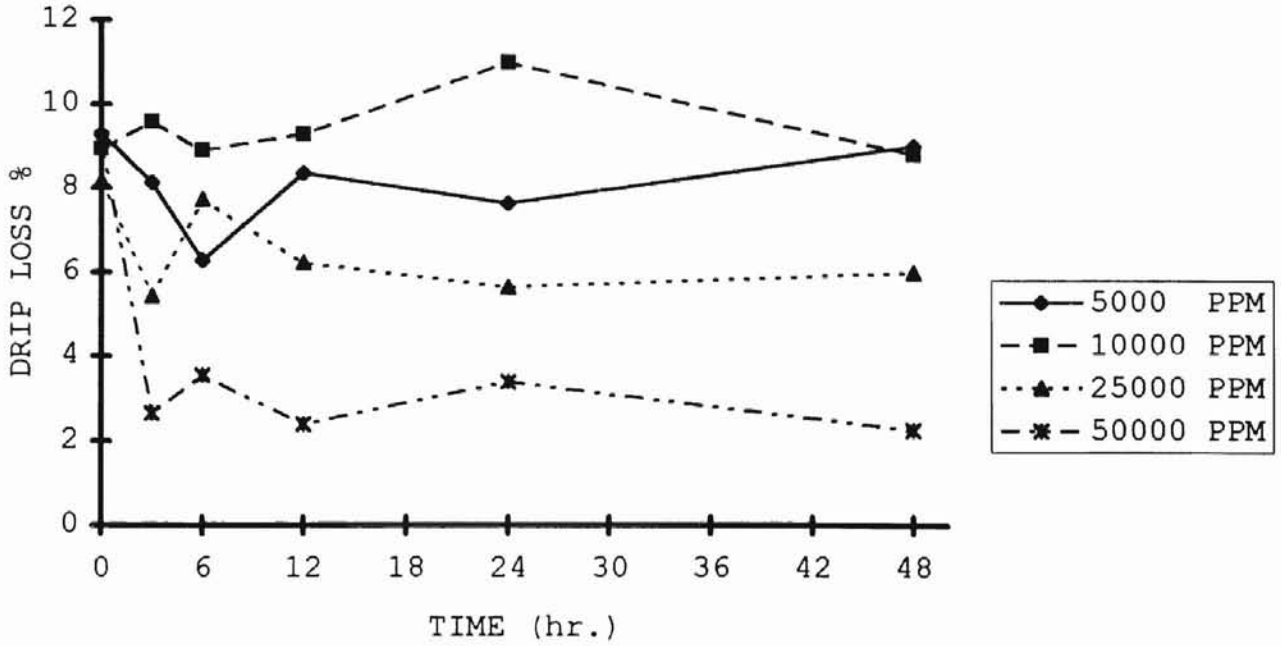

FIGURE A-6. THE CHANGE IN DRIP LOSS % OF CHICKEN BREASTS OVER TIME WHEN EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA

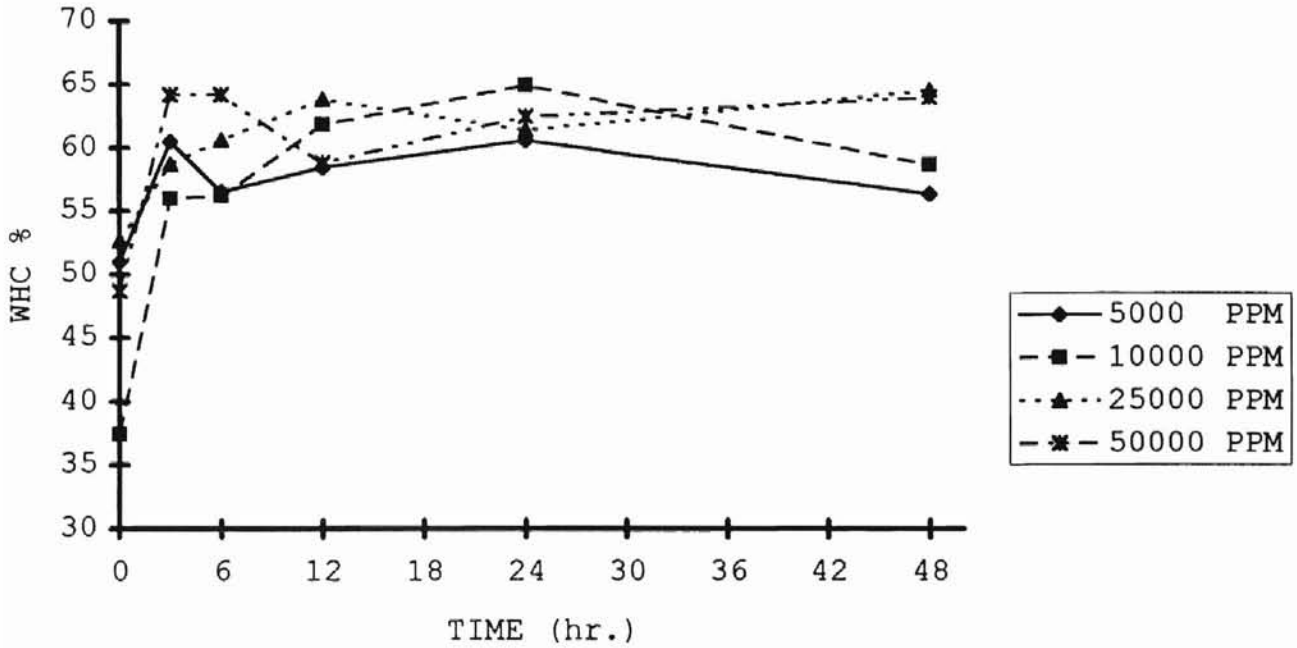

FIGURE A-7. THE CHANGE IN WATER HOLDING CAPACITY OF CHICKEN BREASTS OVER TIME WHEN EXPOSED TO DIFFERENT CONCENTRATION OF AMMONIA

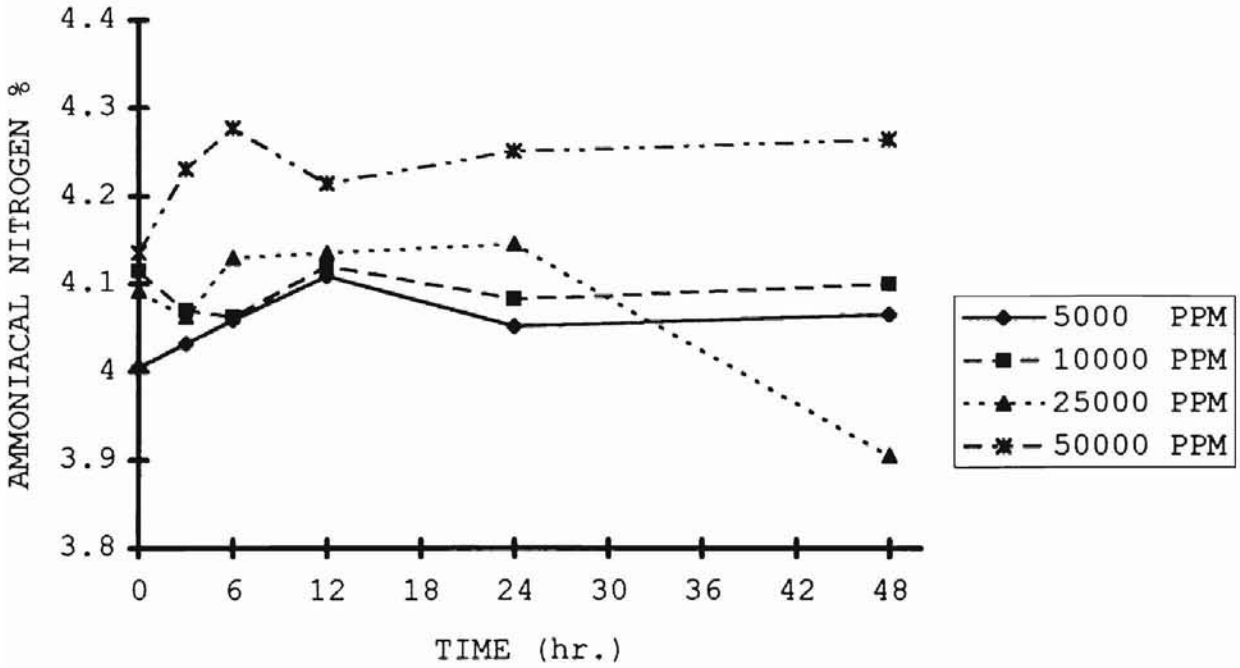

FIGURE A-8. THE CHANGE IN AMMONIACAL NITROGEN % OF CHICKEN BREASTS OVER TIME WHEN EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA

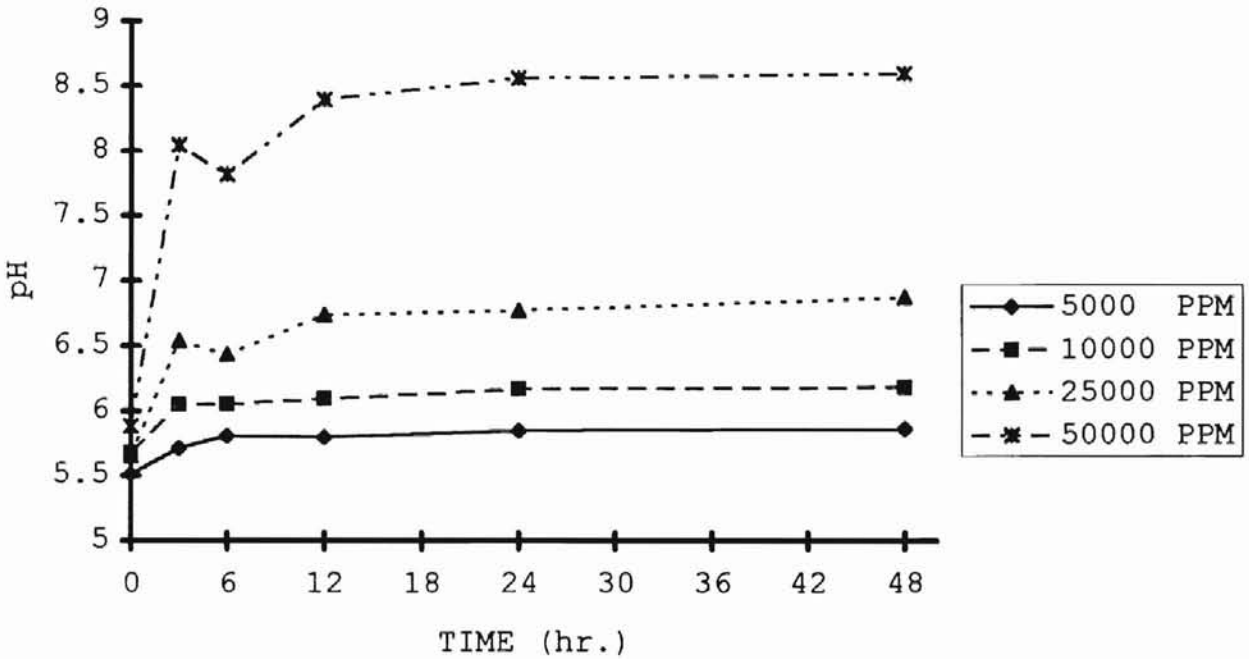

FIGURE A-9. THE CHANGE IN pH OF PORK CENTER CUT LOIN CHOPS OVER TIME WHEN EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA

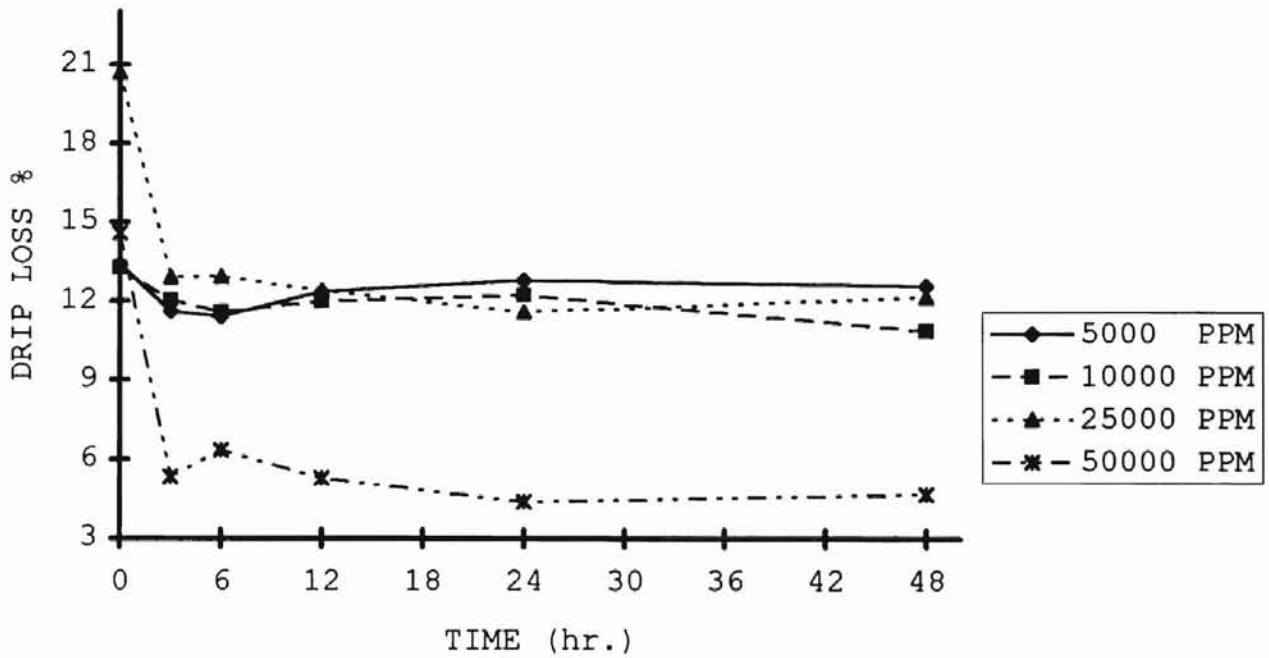

FIGURE A-10. THE CHANGE IN DRIP LOSS % OF PORK CENTER CUT LOIN CHOPS OVER TIME WHEN EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA

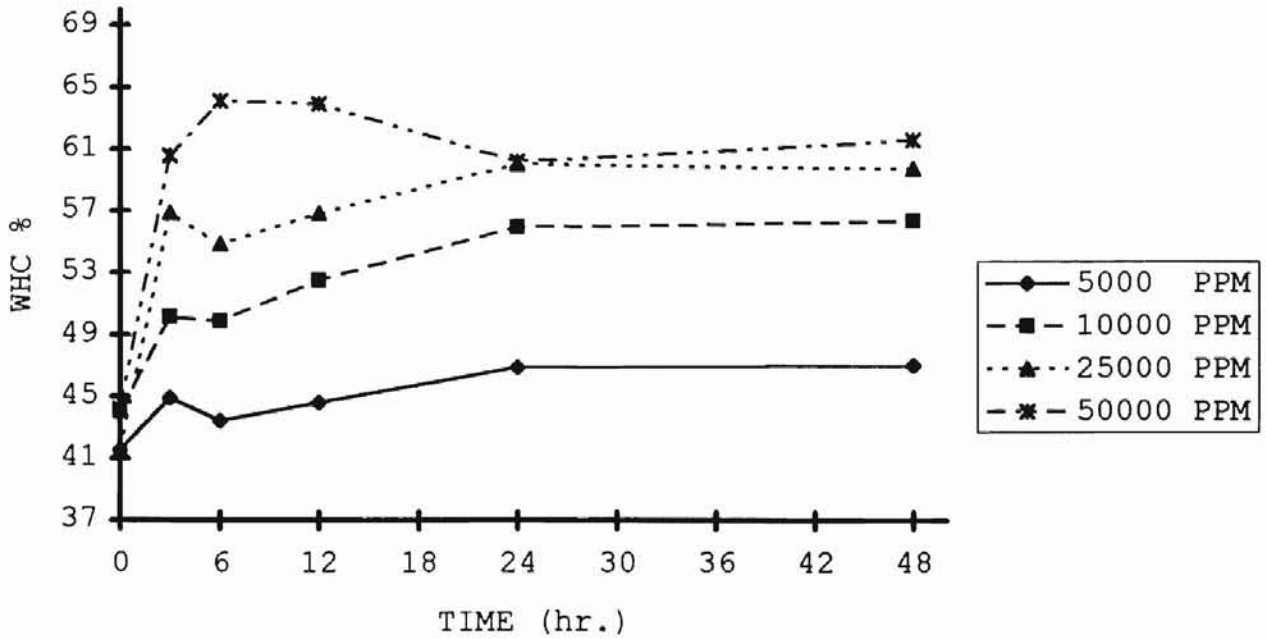

FIGURE A-11. THE CHANGE IN WATER HOLDING CAPACITY % OF PORK CENTER CUT LOIN CHOPS OVER TIME WHEN EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA

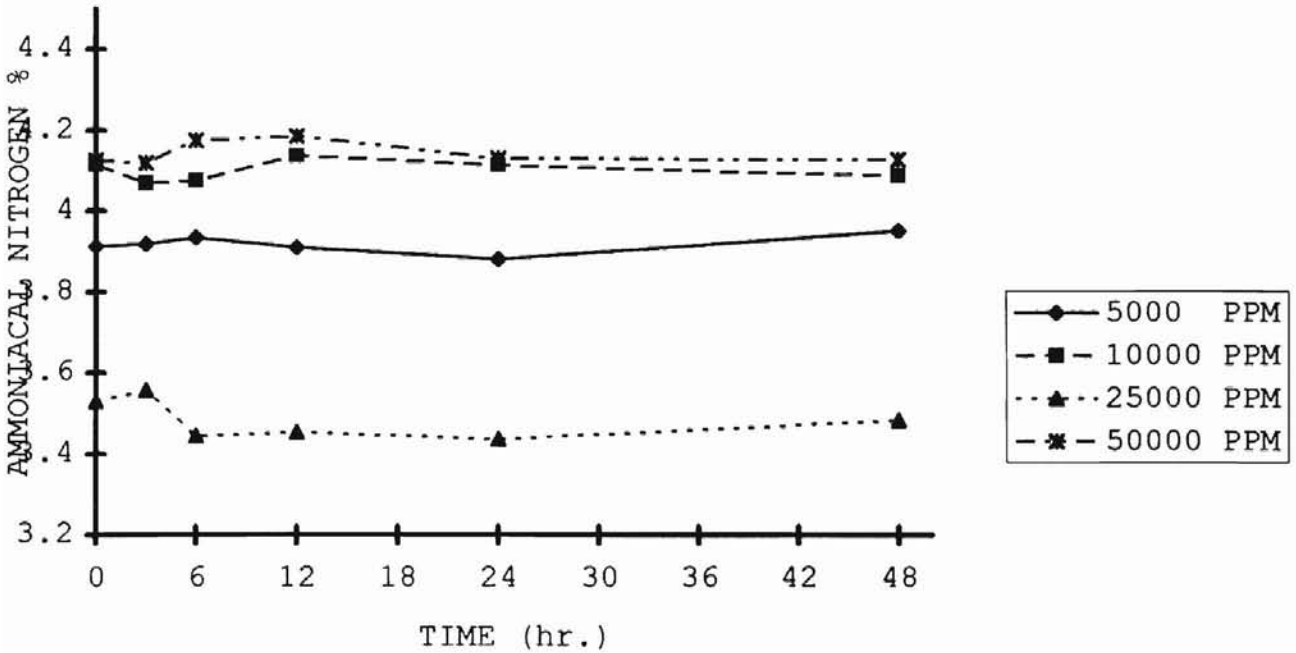

FIGURE A-12. THE CHANGE IN AMMONIACAL NITROGEN % OF PORK CENTER CUT LOIN CHOPS OVER TIME WHEN EXPOSED TO DIFFERENT CONCENTRATIONS OF AMMONIA

Table A-7. Least squares means for beef, chicken, and pork muscle color values stratified by times (hr.) at 5000 ppm ammonia gas.

	Beef			Chicken			Pork		
Time,hr	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	34.93	16.40^{c}	7.45	45.41	3.17	9.36	46.65	5.97^{b}	6.10
3	32.81	18.31^{a}	6.24	43.82	3.30	9.70	44.74	5.88^{b}	5.86
6	32.21	17.75^{ab}	6.17	44.55	2.60	8.44	43.45	7.49^{a}	6.37
12	31.69	18.20^{a}	6.09	44.76	3.60	8.73	42.74	6.61^{ab}	5.88
24	31.38	16.84^{bc}	5.51	44.97	2.49	8.74	44.39	5.73^{b}	5.92
48	31.56	17.07^{abc}	5.38	43.34	2.50	8.51	44.53	6.05^{b}	5.56

^{abc}Means in the same column with different superscripts letters are different ($P<.05$).
Color values using a Minolta CIELAB(L,a,b) scale: L*=lightness; a*=bluish-green/red-purple hue component;b*=yellow/blue hue component.
Values represent the average of three replications (3 repeated measurements per replication).

Table A-8. Least squares means for beef, chicken, and pork muscle color values stratified by times (hr.) at 10,000 ppm ammonia gas.

	Beef			Chicken			Pork		
Time,hr	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	34.23[a]	17.14	7.90[a]	44.88	2.73	7.90	46.57	6.91	6.18
3	30.48[b]	17.74	5.51[b]	44.65	2.29	8.90	43.13	8.03	5.47
6	30.35[b]	16.97	5.51[b]	43.32	2.19	8.00	44.00	7.89	5.83
12	31.20[b]	17.20	5.97[b]	44.57	3.17	10.37	43.27	8.03	5.70
24	30.58[b]	15.95	5.22[b]	43.56	2.40	9.84	43.06	8.08	5.36
48	29.72[b]	17.01	5.42[b]	46.04	2.34	9.79	44.99	7.07	5.56

[abc]Means in the same column with different superscripts letters are different ($P<.05$).
Color values using a Minolta CIELAB(L,a,b) scale: L*=lightness; a*=bluish-green/red-purple hue component;b*=yellow/blue hue component.
Values represent the average of three replications (3 repeated measurements per replication).

Table A-9. Least squares means for beef, chicken, and pork muscle color values stratified by times(hr.) at 25,000 ppm ammonia gas.

	Beef			Chicken			Pork		
Time, hr	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	33.54[a]	18.44[a]	8.00[a]	43.08	3.06	8.42	52.87[a]	6.36	8.19[a]
3	28.38[b]	15.48[b]	4.73[b]	43.04	2.86	9.72	46.45[b]	7.12	5.75[b]
6	27.95[b]	15.93[a]	4.62[b]	44.08	2.84	9.33	47.99[b]	6.92	5.97[b]
12	27.91[b]	14.43[b]	4.37[b]	42.25	3.47	8.41	48.48[b]	6.19	5.82[b]
24	27.70[b]	14.67[b]	4.47[b]	42.80	2.88	9.52	47.29[b]	6.69	5.81[b]
48	28.14[b]	13.73[b]	4.17[b]	41.99	2.84	7.92	47.19[b]	6.52	5.49[b]

[ab]Means in the same column with different superscripts letters are different ($P<.05$).
Color values using a Minolta CIELAB(L,a,b) scale: L*=lightness; a*=bluish-green/red-purple hue component;b*=yellow/blue hue component.
Values represent the average of three replications (3 repeated measurements per replication).

Table A-10. Least squares means for beef, pork, and chicken muscle color values stratified by times (hr.) at 50,000 ppm ammonia gas.

	Beef			Chicken			Pork		
Time, hr	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	32.79[a]	19.02[a]	7.14[a]	46.81	2.61	10.55	43.86	9.40[a]	5.54
3	28.25[b]	13.55[bc]	3.99[b]	42.11	2.47	7.94	39.20	8.31[bcd]	3.98
6	27.86[b]	13.83[b]	4.02[b]	42.17	3.34	7.65	39.26	9.12[ab]	4.32
12	28.00[b]	12.66[bcd]	3.45[b]	45.40	1.05	6.46	38.27	8.38[bd]	3.99
24	28.71[b]	12.12[d]	3.68[b]	43.99	2.48	6.50	38.71	7.99[cd]	3.74
48	28.47[b]	12.20[cd]	3.66[b]	44.09	2.24	6.30	38.79	7.87[d]	3.85

[abcd]Means in the same column with different superscripts letters are different (P<.05).
Color values using a Minolta CIELAB(L,a,b) scale: L*=lightness; a*=bluish-green/red-purple hue component;b*=yellow/blue hue component.
Values represent the average of three replications (3 repeated measurements per replication).

Table A-11. Least squares means for beef muscle color values (L*a*b*) stratified by different concentrations of ammonia gas at different times of exposure.

	Color values											
	L*				a*				b*			
Time, hr	5000	10,000	25,000	50,000	5000	10,000	25,000	50,000	5000	10,000	25,000	50,000
0	34.93	34.23	33.54	32.79	16.40	17.14	18.44	19.02	7.45	7.90	8.00	7.14
3	32.81	30.48	28.38	28.25	18.31^{a}	17.74^{a}	15.48^{b}	13.55^{c}	6.24^{a}	5.51^{ab}	4.73^{bc}	3.99^{c}
6	32.21^{a}	30.35^{ab}	27.95^{bc}	27.86^{c}	17.75^{a}	16.97^{ab}	15.93^{b}	13.83^{c}	6.17^{a}	5.51^{ab}	4.62^{bc}	4.02^{c}
12	31.69^{a}	31.20^{a}	27.91^{b}	28.00^{b}	18.20^{a}	17.20^{a}	14.43^{b}	12.66^{b}	6.09^{a}	5.97^{a}	4.37^{b}	3.45^{b}
24	31.38^{a}	30.58^{a}	27.70^{b}	28.71^{b}	16.84^{a}	15.95^{ab}	14.67^{b}	12.12^{c}	5.51^{a}	5.22^{ab}	4.47^{bc}	3.68^{c}
48	31.56^{a}	29.72^{b}	28.14^{c}	28.47^{c}	17.07^{a}	17.01^{a}	13.73^{b}	12.20^{b}	5.38^{a}	5.42^{a}	4.17^{b}	3.66^{b}

[abc]Means in the same row with different superscripts letters are different (P<.05).
Color values using a Minolta CIELAB(L,a,b) scale: L*=lightness; a*=bluish-green/red-purple hue component;b*=yellow/blue hue component.
Values represent the average of three replications (3 repeated measurements per replication).

Table A-12. Least squares means for chicken breast color values (L*a*b*) stratified by different concentrations of ammonia gas at different times of exposure.

Time, hr	Color values											
	L*				a*				b*			
	5000	10,000	25,000	50,000	5000	10,000	25,000	50,000	5000	10,000	25,000	50,000
0	45.41	44.88	43.08	46.81	3.17	2.73	3.06	2.61	9.36	7.90	8.42	10.55
3	43.82	44.65	43.04	42.11	3.30	2.29	2.86	2.47	9.70	8.90	9.72	7.94
6	44.55	43.32	44.08	42.17	2.60	2.19	2.84	3.34	8.44	8.00	9.33	7.65
12	44.76	44.57	42.25	45.40	3.60	3.17	3.47	1.05	8.73	10.37	8.41	6.46
24	44.97	43.56	42.80	43.99	2.49	2.40	2.88	2.48	8.74	9.84	9.52	6.50
48	43.34	46.04	41.99	44.09	2.50	2.34	2.84	2.24	8.51	9.79	7.92	6.30

Means in the same row without superscripts letters are not different (P>.05).
Color values using a Minolta CIELAB(L,a,b) scale: L*=lightness; a*=bluish-green/red-purple hue component;b*=yellow/blue hue component.
Values represent the average of three replications (3 repeated measurements per replication).

Table A-13. Least squares means for pork chops color values (L*a*b*) stratified by different concentrations of ammonia gas at different times of exposure.

	Color values											
	L*				a*				b*			
Time,hr	5000	10,000	25,000	50,000	5000	10,000	25,000	50,000	5000	10,000	25,000	50,000
0	46.65	46.57	52.87	43.86	5.97^{b}	6.91^{b}	6.36^{b}	9.40^{a}	6.10^{c}	6.18^{bc}	8.19^{a}	5.54^{c}
3	44.74	43.13	46.45	39.20	5.88^{c}	8.03^{a}	7.12^{b}	8.31^{a}	5.86	5.47	5.75	3.98
6	43.45^{ab}	44.00^{ab}	47.99^{a}	39.26^{b}	7.49	7.89	6.92	9.12	6.37	5.83	5.97	4.32
12	42.74^{b}	43.27^{b}	48.48^{a}	38.27^{c}	6.61^{b}	8.03^{a}	6.19^{b}	8.38^{a}	5.88^{a}	5.70^{a}	5.82^{a}	3.99^{b}
24	44.39^{ab}	43.06^{b}	47.29^{a}	38.71^{c}	5.73^{c}	8.08^{a}	6.69^{b}	7.99^{a}	5.92^{a}	5.36^{a}	5.81^{a}	3.74^{b}
48	44.53^{a}	44.99^{a}	47.19^{a}	38.79^{b}	6.05^{c}	7.07^{b}	6.52^{bc}	7.87^{a}	5.56^{a}	5.56^{a}	5.49^{a}	3.85^{b}

[abc]Means in the same row with different superscripts letters are different ($P<.05$).
Color values using a Minolta CIELAB(L,a,b) scale: L*=lightness; a*=bluish-green/red-purple hue component;b*=yellow/blue hue component.
Values represent the average of three replications (3 repeated measurements per replication).

VITA

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