

**DEVELOPMENT OF A NEW FOAM VACCINATION METHOD FOR
POULTRY IN THE HATCHERY**

by

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ABSTRACT

To protect poultry flocks from diseases, individual or mass vaccination methods are utilized in the industry. Mass vaccination methods are commonly used in hatcheries and the field to vaccinate large numbers of poultry. In the hatchery *in-ovo*, gel droplets, and spray cabinet are used to protect against Marek's Disease, coccidiosis, infectious bronchitis (IBV) and Newcastle disease. The failure to deliver a consistent vaccine dose to each bird warrants a new vaccination method. Due to various uses in the pharmaceutical, culinary, and agricultural industries, foams were investigated as a potential vaccine administration method. Different foaming materials were evaluated on their characteristics, including expansion rate, liquid drainage, and deterioration rate. The foaming materials included egg white (EW), egg white with guar gum (EWGG), sodium stearyl lactylate (SSL), whey protein isolate, and Polysorbate 20, 60, and 80. Whipping and sparging methods were used to generate foams. The whipping method used CO₂ and N₂O gases at 75 PSI. The sparging method used 1.0, 2.5, and 5.0 L/min airflow rates with a 10 µm sparging disc during testing. Compared to the whipping method, the sparging method had a significantly higher expansion rate ($P < 0.01$) and lower drainage rate at 10 min ($P < 0.01$). There was no significant effect among deterioration rates between 1.0 and 2.5 L/min airflow rates ($P > 0.1$) or 5 and 10 min time points ($P > 0.1$) regardless of the material. Foams produced with egg white (EW) and sodium stearyl lactylate (SSL) by sparging and whipping generating methods were then applied to chicks under laboratory conditions that mimicked the field conditions in the hatchery. Chick behavior and the ingestion of the foam and

spray treatments by the chicks were evaluated. Six total treatments and 5, one-day-old chicks per treatment were used. Chick ingestion behaviors included the chick preening itself or another chick, pecking, and drinking. The chicks were exposed over a 10-min period to the treatments and a fluorescent tracer was added to the treatment to quantify the ingestion of the foams by the chicks. The foams generated by sparging had the highest ingestion volume over the 10-min period ($P < 0.01$). The foam ingestion volumes from the sparging methods were 2.3 times of the spray and 2.6 times of the whipping over the 10-min exposure period. Chicks administered foams from the whipping method demonstrated more (107% higher) ingestion related behaviors compared to the average of spray and sparging methods ($P < 0.05$). The volumes of ingestion by chicks did not directly correlate to ingestion related behaviors. The foams from the sparging method had a higher expansion rate and increased the ingestion rate of the chicks. The viabilities of infectious bronchitis virus (IBV) in foams produced with egg white (EW) and sodium stearoyl lactylate (SSL) by sparging and whipping generating methods were tested. Specific-pathogen-free (SPF) embryonated chicken eggs were inoculated at 11-days of age at IBV dilution levels from 10^0 to 10^{-3} at 60 and 120 min after producing the foams. Eggs were candled and mortality was recorded for 8 days. Virus recovery was determined by examination of the embryos for lesion characteristics of IBV and titers were calculated for each foam treatment using the Reed and Muench method. The results showed the viability of IBV was not affected by foams generated from EW and SSL with both whipping and sparging methods when eggs were inoculated after 120-min of foam generation. However, EW foams from both methods caused a lower viral viability at the 60-min inoculation.

Key words: Vaccination, foam, chicken, hatchery, behavior

Chapter 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Vaccinations play an important role in improving the health and immunity in production animals. It is critical to properly administer vaccines to prevent the spread of disease. Within flocks and herds in the agricultural community, vaccination aids with the survival rate of the animals. There are several diseases that have the potential to infect poultry throughout their life stages, and it is important to vaccinate against these diseases. Effective vaccination protects hundreds of millions of poultry worldwide and results in improved flock health and production efficiency (Cobb-Vantress, 2013, 1-34). Every commercial poultry type, such as broilers, turkeys, and layer hens, have their own schedules of vaccination. Broiler chickens are the type of birds that are grown for meat, while layer hens are the birds used to produce commercial eggs. Some diseases may infect turkeys more readily compared to broilers, causing the vaccination schedule to differ in timing and agents. Layer hens require a long-lasting vaccination program since they have a longer life-span (80-120 weeks) compared to broilers (7 weeks) (Cutler, 2002b, 451-461). In addition, vaccination schedules vary by company. Proper vaccination can induce an immune response in poultry, create a protective immunity and protect the birds against the diseases, which is an effective means to prevent and reduce the adverse effects of specific diseases in poultry (Sharma, 2011, 4-6; Jacob, 2015, 1-4). Broilers are susceptible to diseases such as Marek's disease (MD), Newcastle disease (ND), infectious bronchitis (IB),

infectious bursal disease (IBD), avian influenza (AI), and infectious laryngotracheitis (ILT).

The three major vaccinations in hatcheries include MD, ND, and IB. Marek's disease virus (MDV) is a double-stranded, enveloped DNA virus part of the family *Herpesviridae*. In diameter, the particles range from 100-200 nm. This virus causes tumors in the organs of poultry, which is considered a neoplastic disease (Nair, 2008, 258-267). MDV can infect chickens, quail, turkeys, pheasants, and jungle fowl (Wakenell and Sharma, 2008, 99-105). It is spread by indirect or direct contact, and 2-4 weeks after infection viral shedding occurs. Forms of the disease include classical, acute, acute cytolitic disease, and transient paralysis and all can cause different clinical signs. A common sign of classical MD is paralysis of the legs and wings (Nair, 2008, 258-267).

ND is in the family *Paramyxoviridae* which is divided into *Paramyxovirinae* and *Pneumovirinae*. *Paramyxovirinae* is divided into five genera. Newcastle disease virus (NDV) and other avian viruses are categorized under the genus *Avulavirus*. NDV has distinct clinical signs ranging from respiratory infection to intestinal tract lesions. Transmission occurs through air droplets or the oral-fecal route. The mortality of the flock is dependent on the virulence of the strain, where a highly virulent strain may cause up to 100% mortality (Alexander, 2008, 294-305). Birds of all ages can be affected and in rare cases, mammals can contract conjunctivitis (Butcher, 2015, 1-15).

Infectious bronchitis virus (IBV) is a member of the family *Coronaviridae* and a member of the order *Nidovirales*. Members of this family are enveloped and spherical, usually with a diameter of 120-140 nm. Distinct, club-shaped projections are an important feature of these viruses. This virus infects poultry, primarily chickens,

causing a highly contagious respiratory disease. Clinical signs include sneezing, watery nasal discharge, facial swelling, diarrhea, dehydration, and depression. Gross lesions may be observed in the nasal cavities and the trachea. The air sacs may be cloudy and the lungs could be congested (Cook, 2008, 340-349). As with many respiratory viruses, the birds are susceptible to infection by secondary bacterial pathogens. A unique characteristic of the virus is the short incubation time of 24-72 hours. More than twenty serotypes of IBV are recognized (Gelb and Jackwood, 2008, 146-149). Within the United States commercial poultry industry, Arkansas (Ark), Connecticut (Conn), Delaware (DE/072/92), and Massachusetts (Mass) are the commonly isolated serotypes. While morbidity may reach 100%, mortality is usually under 5% (Gelb and Jackwood, 2008, 146-149). Kidney lesions are caused by some strains, causing a higher flock mortality (Cutler, 2002c, 473-542).

Due to modern management systems, which usually have high densities, these diseases have the potential to readily spread (Jacob, 2015, 1-4). The spread of these diseases within a commercial poultry farm can be detrimental for the birds and growers. All of these diseases have vaccines available that can help to prevent the spread from bird to bird. Vaccine administration methods can either be per individual bird or mass administration. The goal of conducting mass application techniques is to reach the same efficiency as individual vaccination, which could be to tens of thousands of birds at a time (Cserep, 2002, 1-12).

Two types of vaccines are used in the poultry industry, live attenuated and inactivated. Live attenuated vaccines are naturally or deliberately modified to be less virulent, which induces a milder form of the disease. These vaccines typically require a smaller dose since they rapidly replicate, especially in the target organs of the host

(Cserep, 2008, 66-81). Inactivated vaccines have been done so during production and formulated as an injectable form (Cobb-Vantress, 2013, 1-34). Inactivated vaccines are most effective when administered with an adjuvant; a compound that will enhance the host immune response after injection. A high dose of inactivated antigens is combined with an oil emulsion or aluminum hydroxide adjuvant for this type of vaccine. A long and high level of immunity is produced once administered, and it must be injected to each individual bird (Cserep, 2008, 66-81).

1.2 Immunological Response

Both live and inactivated vaccines induce an immune response. The bursa of Fabricius and thymus are important in protecting chickens against diseases. T-lymphocytes mature in the thymus, which is an organ in the neck, and form lymphokines, but not antibodies. T-lymphocytes create cell-mediated immunity (Cutler, 2002a, 443-450). B-lymphocytes are associated with the bursal system and mature within the bursa of Fabricius. Once matured, the cells move to secondary lymphoid organs such as the spleen (Sharma, 2011, 4-6). B-lymphocytes create plasma cells that produce antibodies and create humoral immunity (Cutler, 2002a, 443-450). Antibodies are immunoglobulin (Ig) molecules that are divided into three classes for chickens (IgM, IgG, and IgA) (Sharma, 2011, 4-6). Once the immune response is initiated, memory cells are primed to recognize the type of virus or bacteria (RUMA, 2006, 1-31). Vaccines are used to stimulate infection and activate the immune system of the bird. The effectiveness of the vaccine is dependent on the virulence of the vaccine, the number of virus particles per individual dose, and environmental factors such as the stress or genetic background of the bird (Cutler, 2002b, 451-461). The first vaccination administered to the birds produces a short immune response, which is

called the primary immune response. The primary immune response produces more IgM and some IgG antibodies. The first vaccine delivered is usually a live vaccine (RUMA, 2006, 1-31). More IgG antibodies are produced once administering a second immunization, which is also a live vaccine (Sharma, 2011, 4-6). If a killed vaccine is used for the second immunization, it is important to administer several live vaccines in three week intervals beforehand (Cutler, 2002b, 451-461). When the B-system produces antibodies, memory cells that are primed to recognize a second exposure to the antigen responds to the exposure, creating a faster immune response. This is called an anamnestic response, which allows the body to defend and produce cells faster than the first response (Cutler, 2002a, 443-450).

1.3 Vaccination Methods

Numerous mass vaccination methods are utilized in the industry to administer vaccines to poultry, which vary in timing and administration route depending on the type of poultry (Table 1.1). In the hatchery, *in-ovo* and coarse spray cabinets are commonly utilized methods for administering live vaccines. Administration of live vaccines in the field, such as in drinking water, are also necessary methods. The methods have the capability to be used for different types of vaccinations.

1.3.1 Hatchery Vaccination Methods

1.3.1.1 *In-Ovo*

In-ovo is a vaccination procedure performed in the hatchery when the eggs are transferred from the setter to the hatcher, vaccinating the embryos when still growing in the egg between days 18 and 19 (Cobb-Vantress, 2013, 1-34). In the United States, this procedure is commonly used to prevent Marek's disease (Cutler, 2002b, 451-461).

Timing, inoculation site, mixture of vaccine, and sanitization of machinery all impact the efficacy of the vaccination (Cobb-Vantress, 2013, 1-34). If there is contamination of the egg at the injection site, livability may be decreased due to bacterial or fungal infection (Marangon and Busani, 2006, 265-274). Improvement of vaccination using this method remains of interest with viral, bacterial, and coccidial strains (Vermeulen et al., 2001, 13-20).

1.3.1.2 Subcutaneous or Intramuscular

Live vaccines, such as Marek's disease (MD), may be administered in hatcheries through subcutaneous or intramuscular injection (Davison et al., 1999). Day-old chicks are administered 0.2-0.5 ml of the vaccine subcutaneously under the skin in the back of the neck or intramuscularly in the leg. Machines are designed for the subcutaneous neck injection, vaccinating 1,600-2,000 chicks per hour (Cobb-Vantress, 2013, 1-34).

If a skilled operator is working, this method can be very efficient and time effective. This method provides a uniform administration of the vaccine while avoiding a respiratory reaction when utilized properly (Marangon and Busani, 2006, 265-274). Disadvantages include insufficient application of the vaccine by a bent needle or improper calibration. Sterilization of the needles and machines are necessary, and needle replacements are mandatory for at least every 1,000 chicks (Cobb-Vantress, 2013, 1-34).

1.3.1.3 Coarse Spray Cabinet

To vaccinate against ND and IB, live-attenuated vaccines diluted in water are administered by coarse spray droplets to chicks (Cobb-Vantress, 2013, 1-34). The

vaccine travels through hydraulic nozzles at a particular pressure, which produces the spray droplets. Maintaining constant pressure is an important aspect of this method to have consistent homogenous droplet sizes (Soares and Paniago, 2007, 1-3). Two systems are available, the Desvac hatch spray and automatic sprayer (Paniago, 2006, 1-3). The aerosol, ideally 100-300 μm and 7-15 ml per tray, lands on ocular and nasal mucous membranes or is inhaled into the respiratory tract of the day-old chicks (Cobb-Vantress, 2013, 1-34; Paniago, 2006, 1-3). The size of the droplet is important in initiating a local immunity in the respiratory tract and eyes (Paniago, 2006, 1-3).

This type of vaccine administration method is the most commonly used in the hatchery. The automatic sprayer has the chick trays pass under the nozzles on a conveyer belt, reducing labor costs and chick tray handling. If all aspects of the nozzles are correctly prepared, fewer birds are missed and there is a lower vaccination reaction (Paniago, 2006, 1-3). If using the manual hatch sprayer, labor costs are increased since an employee must insert the chick tray, activate the nozzles, then remove the tray from the box. This type of sprayer is most commonly seen worldwide (Paniago, 2006, 1-3).

The coarse spray cabinet can vary the droplet size, which may not distribute the same amount of vaccine to each chick tray. If the cabinet has more than one nozzle, an uneven dispersion of pressure between the nozzles can cause irregular spray patterns (Soares and Paniago, 2007, 1-3). It is important to check the volume of the vaccine being administered, air pressure, nozzle orientation and spray pattern, and chick box height. These aspects could affect the amount of vaccine being delivered to the box of chicks and ultimately reduce the impact of the vaccine (Cobb-Vantress, 2013, 1-34). Unequal droplet size may cause too mild or too severe of an immune response, which would affect the productivity of the chicks (Soares and Paniago, 2007, 1-3). Using

spray as an administration route does not guarantee exposure of the vaccine to the chick and may create a reduction in the dose delivered (Caldwell et al., 2001, 99-106). Due to the small surface area of the chicks' eyes, contact with other birds may be an important component in vaccine uptake (Purswell et al., 2010, 1310-1315). Specifically, the Ark-DPI IBV strain was found to have a decrease in efficacy using the coarse spray cabinet method (Roh et al., 2015, 149-152). There is also a limit of the amount of day-old chicks that should be vaccinated in a day due to quality issues (Paniago, 2006, 1-3).

1.3.1.4 Intraocular

Broilers can be protected against ILT or ND using live vaccines (Marangon and Busani, 2006, 265-274). Successful conjunctival sac vaccination route occurs if the drop (0.03 ml) is inserted into the eye and absorbed (Cobb-Vantress, 2013, 1-34). This is the most effective method, but very time and labor intensive (Cserep, 2008, 66-81). Each bird must be handled to ensure the drop has been successfully absorbed through the lacrimal duct (Cutler, 2002b, 451-461). The exposed eye surface comprises a very small proportion of the total exposed surface area of the body of a chick. If the drop is not fully absorbed and accurately placed, an immunological response may not occur (Cobb-Vantress, 2013, 1-34; Cserep, 2008, 66-81).

1.3.1.5 Gel Droplet

Using live coccidial vaccines in the form of a gel is another method utilized in hatcheries. The gels are formed in a casing of 2,000 doses. By uniformly delivering the vaccine, the chicks will have better immunity (Dasgupta and Lee, 2000, 613-616). Previously the coccidial vaccine was administered by the coarse spray method. A new

gel technology was invented, which involves mixing a diluent with the vaccine and forming gel droplets that adhere to the chicks. Chickens vaccinated by the gel droplets compared to the spray method had up to a 100-fold greater oocyst output (Jenkins et al., 2012, 306-309). The gel vaccine improves uniformity and viability of the oocysts with an enhanced immune response to the chicks (Dasgupta and Lee, 2000, 613-616; Danforth et al., 1997, 445-451). A disadvantage is that this technology is new and difficult to find a cost-effective way to have the best protection for the birds (Jenkins et al., 2012, 306-309).

1.3.2 Field Vaccination Methods

Administering vaccinations in the field is necessary to induce a secondary immunological response in a poultry house. This is accomplished through mass vaccination, typically by water or spray administration. Methods are determined by labor availability, house type, and the type of water systems in the particular house (Cobb-Vantress, 2013, 1-34).

1.3.2.1 Drinking Water

ND and IB vaccines are mixed with water through a pump system or water tanks, then distributed throughout the drinkers. A proportioner is a dosing machine that is used to administer the vaccinated water quickly and evenly throughout the drinking lines (Cserep, 2002, 1-12). The water consumption by each bird and the overall amount of water used to mix with the vaccine are two very important aspects of this procedure. If administering at two weeks of age, about 14 liters per 1,000 broilers should be added (Cserep, 2008, 66-81). Live attenuated vaccines are normally utilized in the drinking water application (Cobb-Vantress, 2013, 1-34).

Before administering the vaccine into the drinking lines, the correct amount of vaccine to water ratio must be calculated. Dye tablets can be added to the water to visualize that the vaccine was administered to the chicks. Due to the mass administration, this method is the least labor intensive. Disadvantages include inconsistent water consumption per bird, incorrect calculation of vaccine to water ratio, water quality affecting the vaccine, and dead space in the drinker system. Stress could be induced by dehydration since water is withdrawn from the birds for hours to ensure they drink the vaccine water (Cserep, 2002, 1-12).

1.3.2.2 Wing-Web

Fowl pox, fowl cholera and avian encephalomyelitis are three diseases that can be prevented using the wing-web administration method. Broiler breeders and layers are commonly vaccinated to protect against these diseases, while broilers are not (Davison et al., 1999). The two-pronged needle is placed into the center of the wing web and inoculates twice the area to provide increased protection (Cserep, 2008, 66-81). Disadvantages are the time and labor costs due to handling each chicken. Precision is important when vaccinating to avoid feathers, blood vessels and bones. Needles should be changed every 500 birds or immediately if a blood vessel was struck (Cobb-Vantress, 2013, 1-34).

1.3.2.3 Coarse Spray

This administration is similar to coarse spray method in the hatchery, but is applied using backpack or hand-held sprayers (RUMA, 2006, 1-31). Live vaccines in aerosol form at about 5 μm in diameter are administered. This method could create an inconsistent immune reaction. Open-sided houses may be less efficient than a

controlled house (Cserep, 2008, 66-81). It is important to maintain constant pressure of 65-75 PSI while having the nozzle 1 meter above the birds' heads (Cobb-Vantress, 2013, 1-34). Since this method mimics the coarse spray cabinet, there are similar advantages and disadvantages.

1.3.2.4 Subcutaneous or Intramuscular

A different type of injection mechanism can be used in the field using killed vaccines with an oil-adjuvant. Automatic syringes have preset dosages that are injected intramuscularly into the breast or leg or subcutaneously into the neck. Inaccuracy or needle malfunctions may cause harm to the birds depending on the injection site. Equipment should be checked regularly to ensure correct dosage (Cserep, 2008, 66-81). Using this type of vaccine may lead to lesions, such as granulomas, forming in the muscle (Cobb-Vantress, 2013, 1-34; Droual et al., 1990, 473-478). These lesions may cause dissatisfaction of the consumer due to blemishes on the meat (Droual et al., 1990, 473-478). If the human administering the vaccine accidentally injects themselves, this poses a health concern and medical attention should be sought immediately (Cobb-Vantress, 2013, 1-34).

1.3.3 Industrial Use of Foam

Foams have numerous applications across different industries including culinary, pharmaceutical, and agricultural. Mousse, whipped cream, beer, bread, meringues, and chocolate are categorized as aerated food products. Numerous methods have been used in the food industry to produce foam products including fermentation, whipping, and gas injection (Campbell and Mougeot, 1999, 283-296). Products used in this industry are generally regarded as safe. Factors affecting foaming properties as

well as the stability of egg whites (Lomakina and Mikova, 2006, 110-118; Radvanyi et al., 2012, 412-420) in comparison to whey protein (Davis and Foegeding, 2007, 200-210) have been previously studied.

Foams have been formerly used as a drug delivery method. Delivery systems of drugs are formed using different types of materials such as lipids, proteins, polymers, and surfactants (Fanun, 2010, xiii-xix). Surfactants are commonly used in foams since they lower surface tension, which increases stability (Walstra, 1989, 1-15). The traits of these substances, specifically surfactants and polymers, have been manipulated to correspond to desired drug release and absorption characteristics. The application methods include oral, topical, intravenous and intramuscular. Within the last 25 years, drug delivery techniques associated with these materials have had significant developments, with great improvements in efficacy and decreases in toxicity (Misra et al., 2010, 1-53).

In agriculture, foam has been utilized as an emergency depopulation method for poultry. Benson et al. (2007, 219-224) investigated a water-based foam (WBF) as a depopulation method, which forms a blanket to block air and induces mechanical hypoxia of poultry. The WBF is a mixture of gas, water, and foam concentrate that ejects from nozzle systems produced by a foam generator (Benson et al., 2007, 219–224). This foam has a medium expansion rate and sufficient stability to recover itself (Benson et al., 2012, 891-896). Expansion rate equals the ratio of solution to foam volume produced. Foam recovery is the time it takes a foam to return to its original shape after being disturbed.

1.4 Objective

An improved system for vaccination in the poultry industry is needed. With a new method of vaccinating poultry, the industry has the potential to become more efficient and effective in terms of production and health. Foam has been effectively used in numerous industries as a stable substance. The overall objective was to design a new method to administer a foam vaccination to chicks in hatcheries. This objective included testing foaming materials and investigating their characteristics, which determined preferred foaming agents (Chapter 2). The behaviors associated with the chicks' reactions and interactions with the foams would indicate their interest level, which would be important with determining the efficiency of the foam vaccination (Chapter 3). Lastly, ensuring the efficacy of the vaccine virus would not be negatively impacted due to hatchery conditions or the foaming materials were two vital pieces of information (Chapter 4). The purpose of these experiments was to determine the fundamental background of the foam vaccination method, while deciding what aspects to further investigate.

Table 1.1: Description of vaccinations administered in the poultry industry*

Operation	Route	Vaccine Type	Disease	Age (days)	Advantages	Disadvantages
Hatchery	<i>In-Ovo</i>	Live-attenuated	Marek's disease, infectious bursal disease	18-19 day of incubation	Improved efficacy, high production, early protection	Machine sanitation and upkeep, embryo infection
	Subcutaneous/Intramuscular	Live-attenuated	Marek's disease	1	Efficient, time effective, uniform administration	Machine sanitation and upkeep, needle malfunction
	Coarse Spray	Live-attenuated	Newcastle disease, infectious bronchitis, coccidiosis	1	Reduced labor and handling, low vaccination reaction possible, inexpensive	Machine upkeep and inspection, severe respiratory reaction possible
	Gel Droplet	Live	Coccidiosis	1	Doesn't chill chicks, uniform	New technology, cost, storage temperatures
	Intraocular	Live-attenuated	TRT ¹ , ILT ² , Newcastle disease	1	Most effective protection if properly administered	Precise volume, labor intensive, time consuming
Field	Drinking Water	Live-attenuated	Infectious bursal disease, infectious bronchitis, Newcastle disease	9-14	Reduced labor and handling, easy administration	Improper distribution, inconsistent water quality, stressed birds due to dehydration
	Coarse Spray	Live-attenuated	Infectious bursal disease, infectious bronchitis, ILT, Newcastle disease	14-126	Reduced labor and handling, inexpensive	Machine upkeep and inspection, respiratory reaction dependent on particle size
	Wing-Web	Live-attenuated or inactivated	Fowl pox, fowl cholera, AE ³	70-126	Efficient and uniform administration	Machine sanitation and upkeep, labor-intensive, needle malfunction
	Subcutaneous/Intramuscular	Inactivated	Newcastle disease infectious bursal disease, infectious bronchitis	70-126	High protection	Labor intensive, confirmation of injection site

*Adapted from Marangon and Busani, 2006, Cutler, 2002b, and Stewart-Brown, 2016.

¹Turkey avian rhinotracheitis

²Infectious laryngotracheitis

³Avian encephalomyelitis

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Chapter 2

CHARACTERISTICS OF FOAMING AGENTS

2.1 Abstract

In poultry hatcheries, various vaccination methods are used to deliver vaccines to chicks. Some of the methods may not be cost effective for mass vaccine administration in hatcheries. Coarse spray in a cabinet is a cost effective and widely used method in hatcheries although it does not provide maximum coverage. In this study, foams were investigated as a potential vaccine administration method. Different foaming materials were evaluated on their characteristics, including expansion rate, liquid drainage, and deterioration rate. The foaming materials included egg white (EW), egg white with guar gum (EWGG), sodium stearyl lactylate (SSL), whey protein isolate, and Polysorbate 20, 60, and 80. Whipping and sparging methods were used to generate foams. For the whipping method, CO₂ and N₂O gases were used at 75 PSI. For the sparging method, 1.0, 2.5, and 5.0 L/min airflow rates with a 10 µm sparging disc were tested. Compared to the whipping method, the sparging method had a significantly higher expansion rate ($P < 0.01$) and lower drainage rate at 10 min ($P < 0.01$). There was no significant effect among deterioration rates between 1.0 and 2.5 L/min airflow rates ($P > 0.1$) or 5 and 10 min time points ($P > 0.1$) regardless of the material.

Key words: Foam, sodium stearyl lactylate, egg white, chicken, vaccination

2.2 Introduction

Hatcheries within the poultry industry utilize routine vaccinations to protect chicks from infection of common viruses (Breytenbach, 2013, 17-21). Mass applications methods, such as the coarse spray cabinet, are commonly used in the industry to induce a rapid immune response while saving time and labor costs. Unequal droplet size may cause too mild or too severe of an immune response, which would affect the productivity of the chicks (Soares and Paniago, 2007, 1-3). Uptake of larger droplets into the upper respiratory tract creates a proper reaction while fine droplets penetrate the lower respiratory tract, causing a higher immunological response and more disease severity. The spray will also land on the feathers of the birds, stimulating the birds to preen and uptake the vaccine through contact with the ocular, nasal, and oral mucous membranes (Breytenbach, 2014, 7-9). Efficacy of the vaccination can be greatly reduced if the vaccine is not stored, handled and prepared correctly. Thus, a more effective and efficient method of vaccination would be helpful for the industry.

Foams have been used as a drug delivery method. Delivery systems of drugs are formed by different types of materials such as lipids, proteins, polymers, and surfactants (Fanun, 2010, xiii-xix). Surfactants are commonly used in foams since they have lower surface tension (Walstra, 1989, 1-15). The characteristics of these substances, specifically surfactants and polymers, have been manipulated to correspond to desired drug release and absorption characteristics. The application methods range from oral and topical to intravenous and intramuscular. Within the last 25 years, drug delivery techniques associated with these molecules have had significant developments, with great improvements in efficacy and decreases in toxicity (Misra et al., 2010, 1-53).

Foam has the potential to be used as a vaccine administration method for chickens. Chicks vaccinated by foam vaccine administration could cause pecking or preening itself or another bird, leading to ingestion and development of immunity to the vaccine virus. Therefore, the foam and the foaming process used for vaccine purposes should be safe to the virus and the birds. The characteristics of the foam are also very important. Foams with a higher expansion rate have a larger volume and would increase the contact between the chicks and the foam vaccine and affect chick behaviors. The stability of the foam is also important and can affect the vaccination efficiency. The foam creating process and foaming materials could affect the characteristics of the foam. Foam can be created by gas dissolved within a liquid escaping when the liquid is subjected to a sudden pressure drop, such as the whipping technique used to generate culinary foam (Wilson, 1996, 243-274). Pressures higher than 570 MPa can inactivate viruses, which would be ineffective for vaccines (Dusing et al., 2002, 355-359). Foam can also be produced mechanically by injecting gas through openings submerged in a liquid, such as bubbling gas with a sparging disc in a liquid (Walstra, 1989, 1-15). Numerous types of materials are capable of producing foam without potentially harming chicks. Egg whites, whey protein, sodium stearoyl lactylate (SSL), and Polysorbates (Tweens™) are considered as safe foaming agents widely utilized in the food and cosmetic industries. The objective of this study was to identify safe foaming agents and generation methods for a foam based vaccine delivery system.

2.3 Materials and Methods

2.3.1 Foam Materials

Two types of materials, proteins and small molecule surfactants, were used as foaming agents. The materials used included egg whites (Egg Beaters: All Natural 100% Egg Whites, Conagra Brands, Chicago, IL), whey protein isolate (Piping Rock Whey Protein Isolate, Ronkonkoma, NY), Polysorbate 20 (Making Cosmetics, Snoqualmie, WA), Polysorbate 60 (Making Cosmetics, Snoqualmie, WA), Polysorbate 80 (Making Cosmetics, Snoqualmie, WA), sodium stearyl lactylate (Aroma Alternatives, Austin, TX), and guar gum (NOW Real Food, Bloomingdale, IL). Egg white and whey protein isolate were protein based, while the other materials were small molecule surfactants. Guar gum was added to egg white to create an egg white guar gum mixture. Different concentrations of the materials were used during trials (Table 2.1). All foaming materials were prepared under room temperature (23°C) and homogenized for 1 minute with distilled H₂O (dH₂O) using a kitchen hand mixer (Sunbeam Mixmaster® hand mixer, Sunbeam Appliance Co, Oakbrook, IL) set to “3” or “6” before they were used to generate foam.

Table 2.1: The components and concentrations of materials used throughout foam characterization trials

Material Name	Components	Concentrations of Materials
Egg White (EW)	EW and dH ₂ O	75% EW, 25% dH ₂ O
Egg White Guar Gum (EWGG)	EW, GG and dH ₂ O	30% EW, 0.25% GG, 69.75% dH ₂ O 30% EW, 0.125% GG, 69.875% dH ₂ O
Whey Protein Isolate (WPI)	WPI and dH ₂ O	5% WPI, 95% dH ₂ O 10% WPI, 90% dH ₂ O
Sodium Stearyl Lactylate (SSL)	SSL and dH ₂ O	1.5% SSL, 98.5% dH ₂ O 0.5% SSL, 99.5% dH ₂ O

Table 2.1 continued

Polysorbate (P)	P and dH ₂ O	80% P, 20% dH ₂ O
		60% P, 40% dH ₂ O
		50% P, 50% dH ₂ O
		40% P, 60% dH ₂ O
		20% P, 80% dH ₂ O

2.3.2 Foam Generation Methods

2.3.2.1 Whipping Dispenser

A 0.5-L whipping dispenser (Chef-Master Whipped Cream Dispenser, Mr. Bar-B-Q Inc., Old Bethpage, NY) was modified and used to generate foams (Figure 2.1). Forty (40) ml of foam materials were added into the dispenser and nitrous oxide (N₂O) or carbon dioxide (CO₂) gases were used to charge the pressure. A pressure gauge and valve were added to the dispenser to control the pressure, reaching up to 80 PSI. The dispenser was shaken up to 120 seconds. Foams were generated when the pressurized foam materials were dispensed.



Figure 2.1: Whipping generator with added pressure gauge

2.3.2.2 Sparging

A sparging disc with 10 μm holes (20 mm diameter) was used to create foam by sparging air into a liquid with the foaming agents. The sparging disc was connected to a compressed air tank with an airflow meter for flow adjustment. Airflow rates were set at 1.0, 2.5, and 5.0 L/min. To start foam generation, the liquid materials and sparging disc were placed into a transparent carbonate plastic column (69 mm diameter and 406 or 508 mm height).

2.3.3 Foam Characteristics

Foam expansion rate (ER), liquid drainage (LD), and deterioration rate (DR) were used to assess the characteristics of each foam candidate.

2.3.3.1 Expansion Rate

Expansion rate was calculated by the following equation:

$$ER = \frac{V_f}{(Vl_{initial} - Vl_0)} \quad (1)$$

Where:

V_f is the volume of foam (ml)

$Vl_{initial}$ is the volume of liquid before generating foam (ml)

Vl_0 is the volume of liquid after generating foam where $t = 0$ min (ml)

For the whipping method, the initial volume of the liquid foam materials was recorded and the foams were dispensed into a transparent carbonate plastic column (51 mm diameter and 152 mm height or 69 mm diameter and 508 mm height). The height of the foams in the column was measured to determine the expansion rate. All materials were tested with CO_2 while only EW, EWGG, and SSL were tested with N_2O . For the sparging method, 40 ml of the materials were added into a plastic column (69 mm diameter and 406 mm height) and sparged until foam reached the top of the column at the flowrates of 1.0, 2.5, and 5.0 L/min. The heights of the remaining liquid foam materials and foam were recorded to calculate the expansion rate. The difference between the initial and final liquid volumes was the liquid volume used to generate foam.

2.3.3.2 Liquid Drainage

Liquid drainage was derived using the following equation:

$$LD = \frac{Vl_t}{(Vl_{initial} - Vl_0)} \quad (2)$$

Where:

V_t is the volume of liquid after generating foam where $t = 2-10$ min (ml)

V_{initial} is the volume of liquid before generating foam (ml)

V_0 is the volume of liquid after generating foam where $t = 0$ min (ml)

For the whipping method, the initial volume of the liquid foam materials before foam was generated was recorded and the foams were dispensed into a transparent carbonate plastic column (69 mm diameter and 305 mm height) with a drainage hole at its bottom. For the sparging method, 40 ml of foam materials were placed into a transparent carbonate plastic column (69 mm diameter and 305 mm height) with a drainage hole at its bottom. Foam was produced until reaching the top of the plastic column. Both methods had a 10-ml graduated cylinder placed underneath the column collected the drained liquid at five time points (2, 4, 6, 8, and 10 min) (Figure 2.2). The volume of liquid collected at each time point was used to calculate LD.

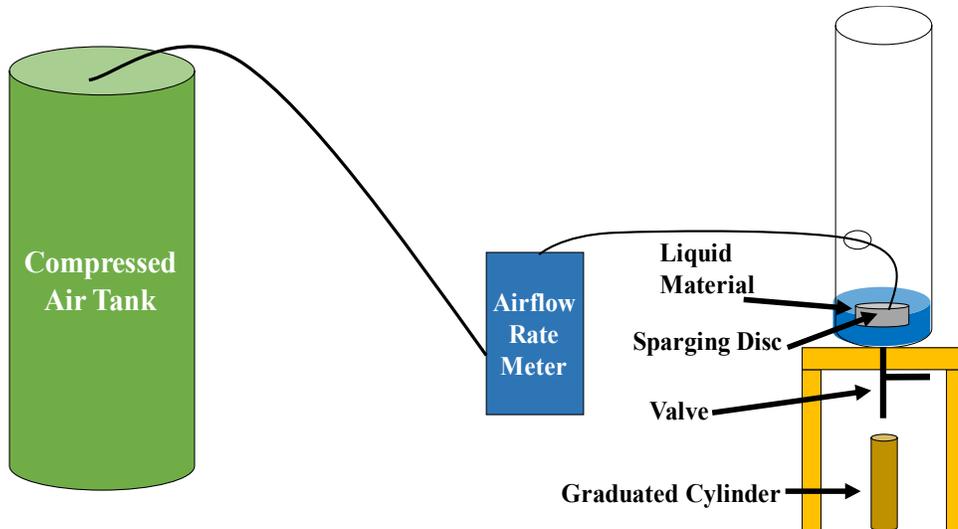


Figure 2.2: Schematic of the liquid drainage system for the sparging generator

2.3.3.3 Deterioration Rate

Deterioration rate (DR) was derived using the following equation:

$$DR = \frac{CBW_{final} - CBW_{initial}}{foam\ added} \quad (3)$$

Where:

CBW_{final} is the final weight of cotton ball (grams)

$CBW_{initial}$ is the initial weight of cotton ball (grams)

Foam added is the amount of foam initially added to the cotton ball (grams)

Deterioration rate (DR) was evaluated to determine the stability of the foams when the foams were applied to chicks and rested on the chicks' body. Foams were created and applied to cotton balls (Healthy Accents Jumbo cotton balls, DZA Brands,

Salisbury, NC) mimicking the chicks' down coat. Each cotton ball (51 mm width x 840 mm length x 10 mm thickness) was covered by the different foams. The amount of foam added (grams) to each replicate was determined by the expansion rate of the foam generated. After 5 and 10 min, the remaining foams on the cotton balls were removed and the weight gains of the cotton balls were used to determine the DR of the foams. For the sparging method, foams were generated using airflow rates of 1.0 and 2.5 L/min. The bubbles generated with an airflow of 5.0 L/min were too large to test.

2.3.3.4 Bubble Size

Images of the wall of the transparent column were taken after foams were produced to quantify the bubble size. The diameters of 30 bubbles were measured for each treatment and the average of the bubble size was calculated.

2.3.4 Statistical Analysis

Expansion rate, liquid drainage, and deterioration rate were used in the analysis. One-way ANOVA and Tukey-Kramer HSD tests were performed using the software JMP Pro[®] (Version 12.1.0, SAS Institute Inc., Cary, NC, 1989-2007). Factors included time, generator type, and material. Within the generator type, gas and airflow rate were factors for the whipping and sparging generator, respectively. All statistical analysis was conducted at a 5% significance level ($P < 0.05$). Expansion rate replicates ranged from 3 to 12 depending on the material. Three replicates for liquid drainage and deterioration rate trials were conducted.

2.4 Results

2.4.1 Foam Expansion Rate

Foams from the whipping method had lower average expansion rates than the sparging method for all materials ($P < 0.01$) (Tables 2.2 and 2.3). For the whipping method, the expansion rates of all materials were similar and there was no significant difference among them ($P > 0.05$). Neither N_2O nor CO_2 affected the expansion rates of the materials ($P > 0.05$) (Table 2.2). For the sparging method, the expansion rate of SSL was 88% higher than the average of EW and EWGG ($P < 0.05$). SSL was significantly different than EWGG at airflow rates of 2.5 and 5.0 L/min ($P < 0.01$). At an airflow rate of 2.5 L/min, SSL was different than EW ($P < 0.05$). Polysorbate materials were labeled as mild skin, ingestion and eye irritant to humans, therefore it would not suitable as a vaccination base for chicks. Whey protein and polysorbate materials did not produce a stable foam with the sparging method, therefore no measurement of expansion rate was recorded.

Table 2.2: Mean expansion rates (\pm standard errors) of the different foaming materials with the whipping generator and gases (n=3 to 12)

Gas	Egg White	Egg White + Guar Gum	Sodium Stearoyl Lactylate	Polysorbate 20	Polysorbate 60	Polysorbate 80	Whey Protein Isolate
CO ₂	4.49 \pm 0.63	3.17 \pm 0.22	3.69 \pm 0.22	3.51 \pm 0.45	3.66 \pm 0.61	10.1 \pm 6.4	3.74 \pm 1.02
N ₂ O	3.01 \pm 0.12	2.30 \pm 0.19	3.22 \pm 0.19	-	-	-	-

Table 2.3: Mean expansion rates (\pm standard errors) of the different foaming materials with the sparging generator at different airflow rates (n=3)

Airflow Rate (L/min)	Egg White	Egg White + Guar Gum	Sodium Stearoyl Lactylate
1.0	109.87 ^A \pm 18.10	81.42 ^A \pm 22.75	184.62 ^A \pm 33.43
2.5	93.63 ^A \pm 11.68	102.71 ^A \pm 12.94	181.46 ^B \pm 15.35
5.0	107.25 ^{AB} \pm 19.01	87.23 ^A \pm 13.99	180.31 ^B \pm 20.80

Note: Materials were compared within each airflow rate, different superscript letters in that material distinguishes a significant difference ($P < 0.05$)

2.4.2 Foam Liquid Drainage

For the whipping method, the LD of foams increased with time ($P < 0.01$) (Figures 2.3 and 2.4). The type of gas had a significant effect on the liquid drainage at 2 min for EWGG and SSL materials ($P < 0.05$) (Table 2.4). At 6 min, gas type for EWGG had a significant effect on drainage ($P < 0.05$) (Table 2.5). Neither CO₂ nor N₂O had a significant effect on the average liquid drainage of the materials at 10 min. The type of material used with either CO₂ or N₂O did not have an effect on liquid drainage at 10 min (Table 2.6).

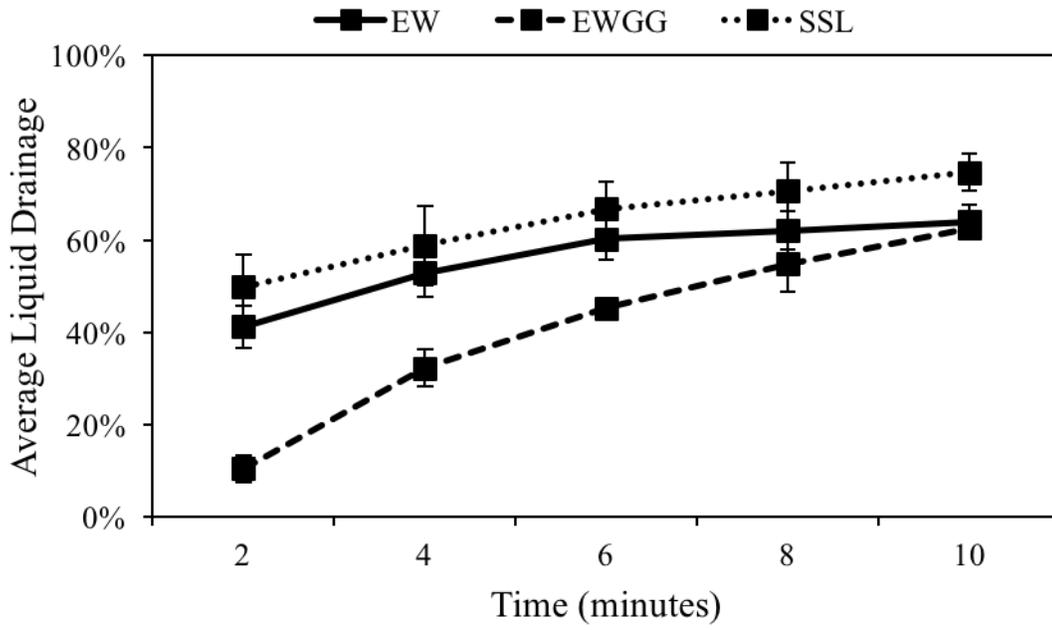


Figure 2.3: Average liquid drainage (%) over time (min) for each material with CO₂ gas with the whipping dispenser generator (\pm standard errors)

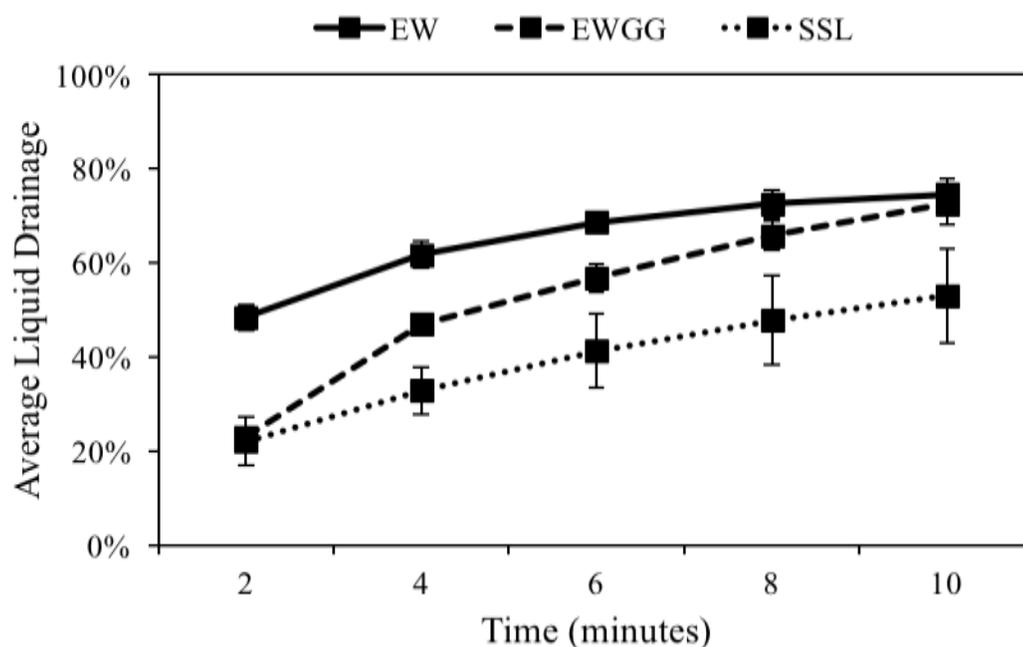


Figure 2.4: Average liquid drainage (%) over time (min) for each material with N₂O gas with the whipping dispenser generator (\pm standard errors)

Table 2.4: Mean liquid drainage (\pm standard errors) at 2 min of the different foaming materials with the whipping dispenser generator (n=3)

Material	Egg White	Egg White + Guar Gum	Sodium Stearoyl Lactylate
CO ₂	0.41 ^A \pm 0.05	0.10 ^A \pm 0.03	0.50 ^A \pm 0.07
N ₂ O	0.48 ^A \pm 0.03	0.23 ^B \pm 0.02	0.22 ^B \pm 0.05

Note: Gases were compared within each material, different superscript letters in that material distinguishes a significant difference ($P < 0.05$)

Table 2.5: Mean liquid drainage (\pm standard errors) at 6 min of the different foaming materials with the whipping dispenser generator (n=3)

Material	Egg White	Egg White + Guar Gum	Sodium Stearoyl Lactylate
CO ₂	0.60 ^A \pm 0.04	0.45 ^A \pm 0.02	0.67 ^A \pm 0.06

Table 2.5 continued

N ₂ O	0.69 ^A ± 0.02	0.57 ^B ± 0.03	0.42 ^A ± 0.08
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Note: Gases were compared within each material, different superscript letters in that material distinguishes a significant difference (P < 0.05)

Table 2.6: Mean liquid drainage (± standard errors) at 10 min of the different foaming materials with the whipping dispenser generator (n=3)

Material	Egg White	Egg White + Guar Gum	Sodium Stearoyl Lactylate
CO ₂	0.64 ± 0.04	0.63 ± 0.02	0.75 ± 0.04
N ₂ O	0.75 ± 0.03	0.73 ± 0.04	0.53 ± 0.10

As time increased, the average liquid drainage increased for 1.0, 2.5, and 5.0 L/min (Figures 2.5, 2.6, and 2.7). At 2 and 6 min, the airflow of 1.0 L/min was significantly different than 2.5 and 5.0 L/min on liquid drainage for EW (P < 0.05) (Tables 2.7 and 2.8). The airflow rates of 1.0 and 2.5 L/min were significantly different from each other, but not different from 5.0 L/min for EW at 10 min (Table 2.9). At specific time points for EW (2, 4, 6, and 8 min), airflow rate had a significant effect on liquid drainage (P < 0.05). For EWGG, airflow rates of 1.0 and 5.0 L/min had a significant effect on the liquid drainage at 4 min (P < 0.05). The airflow rate did not have a significant effect on liquid drainage at the different time points for SSL (P > 0.05). For different airflow rates at 10 min, the type of material did not influence drainage (P > 0.05). The drainage for all materials was affected by the type of generation method used at 10 min (P < 0.05) (Table 2.10). Disregarding the type of material, the type of generation method had a highly significant effect on liquid drainage (P < 0.01).

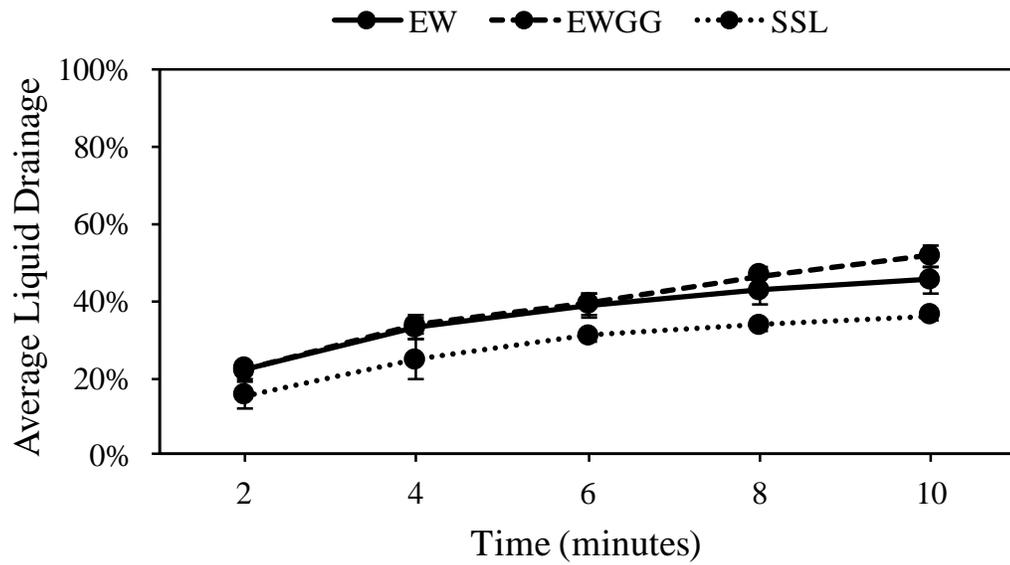


Figure 2.5: Average liquid drainage (%) over time (min) for each material with the sparging generator at 1.0 L/min (\pm standard errors)

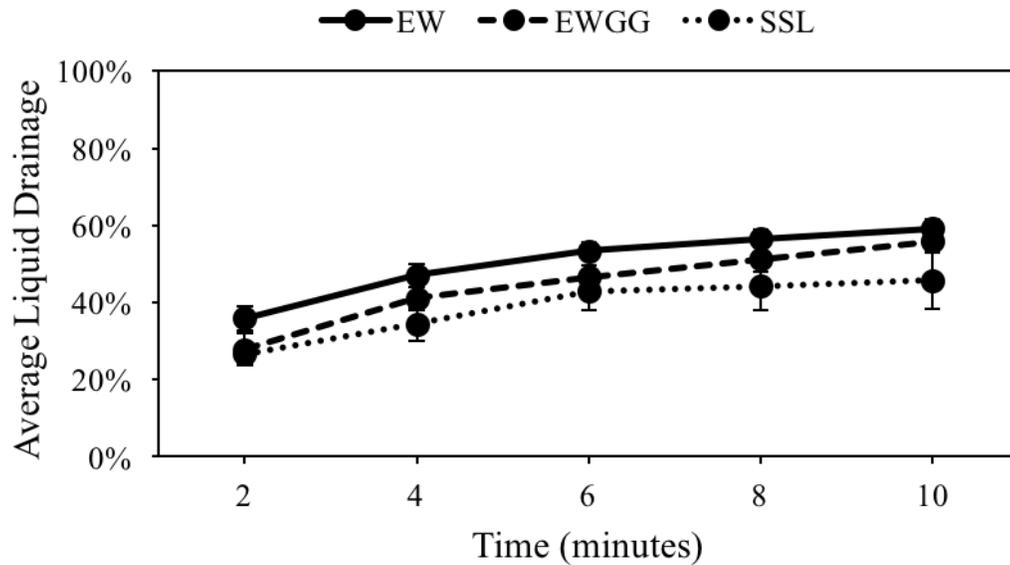


Figure 2.6: Average liquid drainage (%) over time (min) for each material with the sparging generator at 2.5 L/min (\pm standard errors)

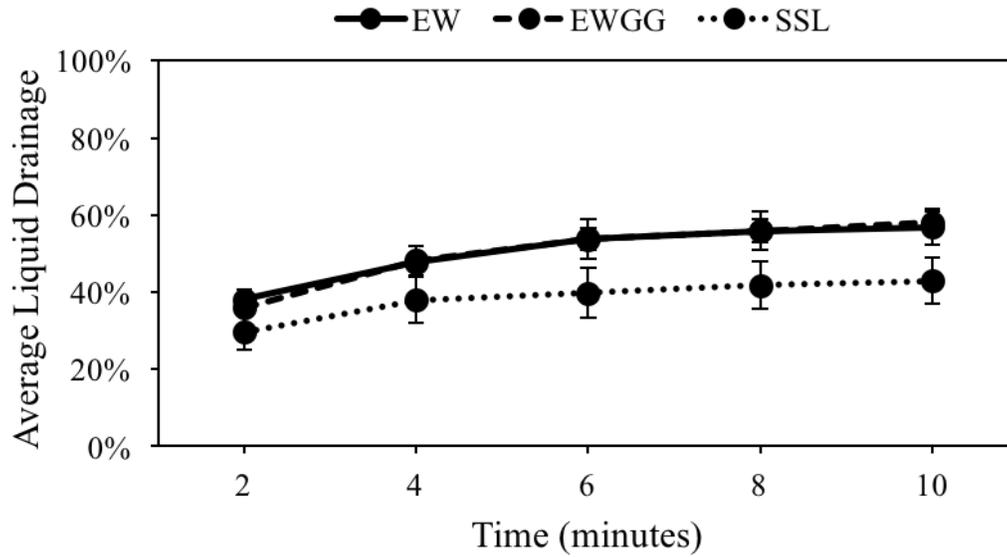


Figure 2.7: Average liquid drainage (%) over time (min) for each material with the sparging generator at 5.0 L/min (\pm standard errors)

Table 2.7: Mean liquid drainage (\pm standard errors) at 2 min of the different foaming materials with the sparging generator (n=3)

Airflow Rate (L/min)	Egg White	Egg White + Guar Gum	Sodium Stearoyl Lactylate
1.0	0.22 ^A \pm 0.03	0.22 ^A \pm 0.02	0.15 ^A \pm 0.04
2.5	0.36 ^B \pm 0.04	0.28 ^A \pm 0.04	0.27 ^A \pm 0.02
5.0	0.38 ^B \pm 0.02	0.36 ^A \pm 0.05	0.30 ^A \pm 0.04

Note: Airflow rates were compared within each material, different superscript letters in that material distinguishes a significant difference ($P < 0.05$)

Table 2.8: Mean liquid drainage (\pm standard errors) at 6 min of the different foaming materials with the sparging generator (n=3)

Airflow Rate (L/min)	Egg White	Egg White + Guar Gum	Sodium Stearoyl Lactylate
1.0	0.39 ^A \pm 0.03	0.39 ^A \pm 0.02	0.31 ^A \pm 0.05
2.5	0.53 ^B \pm 0.03	0.46 ^A \pm 0.03	0.43 ^A \pm 0.03

Table 2.8 continued

5.0 0.54^B ± 0.01 0.54^A ± 0.05 0.40^A ± 0.07

Note: Airflow rates were compared within each material, different superscript letters in that material distinguishes a significant difference (P < 0.05)

Table 2.9: Mean liquid drainage (± standard errors) at 10 min of the different foaming materials with the sparging generator (n=3)

Airflow Rate (L/min)	Egg White	Egg White + Guar Gum	Sodium Stearoyl Lactylate
1.0	0.45 ^A ± 0.04	0.52 ^A ± 0.02	0.36 ^A ± 0.05
2.5	0.59 ^B ± 0.03	0.56 ^A ± 0.03	0.46 ^A ± 0.03
5.0	0.57 ^{AB} ± 0.01	0.58 ^A ± 0.08	0.43 ^A ± 0.06

Note: Airflow rates were compared within each material, different superscript letters in that material distinguishes a significant difference (P < 0.05)

Table 2.10: Mean liquid drainage (± standard errors) at 10 min comparing the two generation methods with different foaming materials (Whip n=6, Sparg n=9)

Method	Egg White	Egg White + Guar Gum	Sodium Stearoyl Lactylate
Whipping	0.69 ^A ± 3.39	0.67 ^A ± 3.06	0.64 ^A ± 6.89
Sparging	0.54 ^B ± 0.03	0.55 ^B ± 0.03	0.42 ^B ± 0.03

Note: Methods were compared within each material, different superscript letters in that material distinguishes a significant difference (P < 0.05)

2.4.3 Foam Deterioration Rate

The average deterioration rates for each material with the sparging generator were similar (Figure 2.8). There was no significance of airflow rate on deterioration rate among any of the materials (Tables 2.11, 2.12, and 2.13).

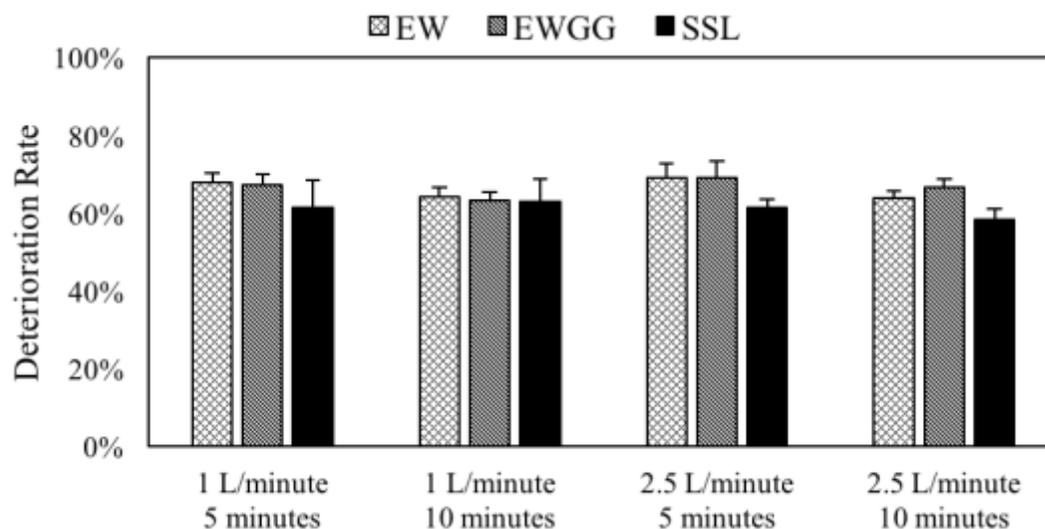


Figure 2.8: The sparging generator deterioration rate (%) for each material over 5 and 10 min in combination with changing airflow rate (\pm standard errors)

Table 2.11: Mean deterioration rate (\pm standard errors) at 5 and 10 min of egg white (EW) with the sparging generator (n=3)

Airflow Rate (L/min)	5 min	10 min
1.0	0.68 \pm 0.03	0.64 \pm 0.02
2.5	0.69 \pm 0.04	0.64 \pm 0.02

Table 2.12: Mean deterioration rate (\pm standard errors) at 5 and 10 min of egg white and guar gum (EWGG) with the sparging generator (n=3)

Airflow Rate (L/min)	5 min	10 min
1.0	0.67 \pm 0.03	0.64 \pm 0.02
2.5	0.69 \pm 0.04	0.67 \pm 0.02

Table 2.13: Mean deterioration rate (\pm standard errors) at 5 and 10 min of sodium stearoyl lactylate (SSL) with the sparging generator (n=3)

Airflow Rate (L/min)	5 min	10 min
1.0	0.62 ± 0.07	0.63 ± 0.06
2.5	0.61 ± 0.02	0.58 ± 0.03

2.4.4 Bubble Size

For each airflow rate (1.0, 2.5, and 5.0 L/min) with EW, EWGG, and SSL materials, diameter of bubbles were recorded. As the airflow rate increased, the bubble size increased (Figure 2.9). For all materials, the airflow rate had a significant effect on bubble size ($P < 0.05$).

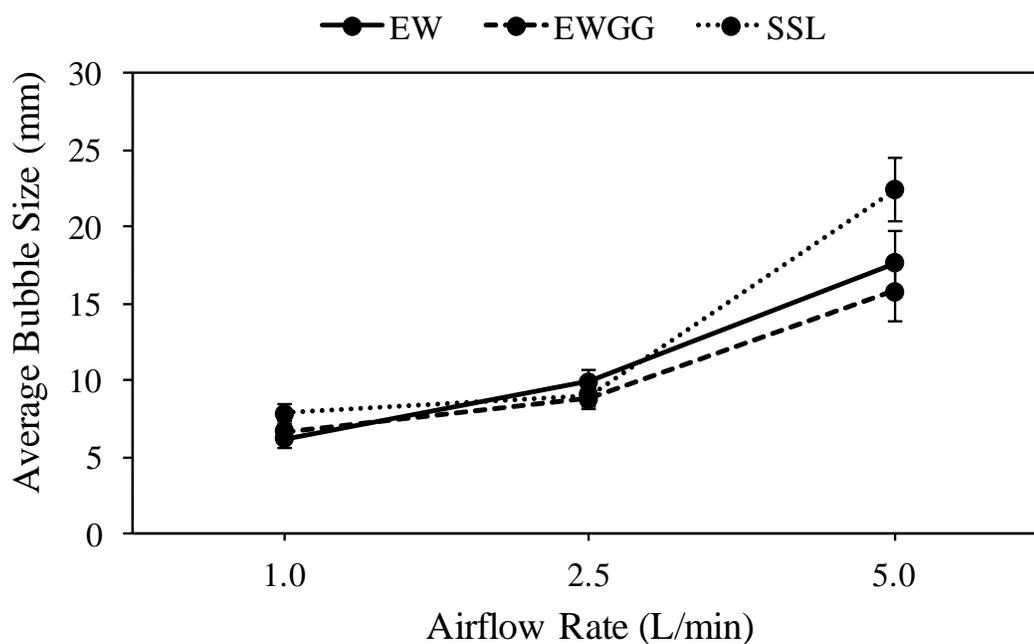


Figure 2.9: Average bubble size with different airflow rate with the sparging generator (n=30)

2.5 Discussion

Foegeding et al. (2006, 284-292) found that foams produced from whey protein isolate with the sparging generator were lacking stability. Tween™ products (Tween™ 20, 60, and 80) failed to produce a stable foam due to high surface tension (Kothekar et al., 2007, 477-484) and degradation of their functional groups (Kishore et al., 2011, 1194-1210). Due to lower stability, whey protein isolate and the Tween™ materials are not suitable as a foaming agent for vaccination delivery.

Lomakina and Mikova (2006, 110-118) found egg white produced 44% drainage when used in a high pressurized system. When compared to whey protein isolate at equal bubble size, egg white protein had higher stability (Yang and Foegeding, 2011, 1687-1701). Chudzikowski (1971, 43-60) found that at low concentrations guar gum forms high viscosity solutions. In this current study, egg white (EW) had at least a 64% of liquid drainage (LD), which was higher than previous studies. With the addition of guar gum (GG) to EW, there was lower average drainage and higher stability.

Rand and Kraynik (1983, 152-154) determined that smaller bubble size and distribution resulted in decrease of LD and increase of stability. When airflow increased, the bubble size increased. This relationship was evident in this current study when calculating bubble size. As the airflow rate increased from 1.0 to 5.0 L/min, the bubble size with EW increased from 6.2 to 17.6 mm in diameter. A lower airflow rate resulted in a lower liquid drainage. The airflow rates of 1.0 and 2.5 L/min had a marginally significant effect on liquid drainage ($P < 0.1$). The sparging generator had a higher expansion rate compared to the whipping dispenser generator. SSL had a significantly higher expansion rate compared to EW and EWGG mixture ($P < 0.01$).

Using foam as a vaccine administration method could potentially increase contact between the vaccine and chicks, thus improving vaccination efficiency and uniformity in commercial poultry hatcheries. The coarse spray method generates small droplets that dry in 1 to 2 min. A small amount of the vaccine is inhaled or ingested by the chicks due to its size. Foam with a high expansion rate would increase the chance of contact between the chick and foam vaccine due to longer stability and a slower drying rate compared to the coarse spray droplets. Therefore, foams generated by the sparging method with a lower airflow rate would be a better candidate due to its high expansion rate and low drainage rate over a 10-min period. The 10-min period provides longer exposure compared to the coarse spray method. Further studies are warranted to evaluate how chicks behave with foams that exhibit different characteristics, and the consumption and ingestion of foams by chicks. These studies would help determine the optimal foams that could be used to deliver vaccines in hatcheries.

2.6 Conclusions

Compared to the whipping method, sparging had a significantly higher expansion rate ($P < 0.01$) and lower drainage rate at 10 min ($P < 0.01$). The type of gas (CO_2 or N_2O) used for the whipping dispenser had no statistical effect on liquid drainage for any material at 10 min ($P > 0.1$). At 10 min, the type of material used with the whipping generator did not have a significant effect on liquid drainage regardless of gas type ($P > 0.1$). The type of material used for the sparging generator did have a significant effect on drainage at 10 min regardless of airflow rate ($P < 0.01$). Neither time or airflow rate had a significant effect on deterioration rate ($P > 0.1$). For future

studies, egg white (EW), egg white guar gum mixture (EWGG), and sodium stearyl lactylate (SSL) are foam candidates due to their stability characteristics.

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Chapter 3

ASSESSMENT OF CHICK BEHAVIOR AND INGESTION VOLUMES OF DIFFERENT FOAMS COMPARED TO COARSE SPRAY

3.1 Abstract

Using foams as a method for delivering vaccines for chicks in hatcheries was investigated. Foams produced with egg white (EW) and sodium stearyl lactylate (SSL) by sparging and whipping generating methods were applied to chicks under laboratory conditions that mimicked the field conditions in the hatchery. Chick behavior and the ingestion of the foam and spray treatments by the chicks were evaluated. Six total treatments and 5, one-day-old chicks per treatment were used. Chick ingestion behaviors included the chick preening itself or another chick, pecking, and drinking. The chicks were exposed over a 10-min period to the treatments and a fluorescent tracer was added to the treatments to quantify the ingestion volumes of the foams by the chicks. The foams generated by sparging had the highest ingestion volume over the 10-min period ($P < 0.01$). The foam ingestion volumes from the sparging methods were 2.3 times of the spray and 2.6 times of the whipping over the 10-min exposure period. Chicks with foams from the whipping method demonstrated more (107% higher) ingestion related behaviors compared to the average of spray and sparging methods ($P < 0.05$). The volumes of ingestion by chicks did not directly correlate to the ingestion related behaviors. The foams from the sparging method had a higher expansion rate and increased the ingestion rate of the chicks.

Key words: Foam, vaccine, behavior, hatchery, chicken

3.2 Introduction

In the hatchery, mass vaccination methods include *in-ovo*, coarse spray cabinet, or gel droplet. The goal of mass vaccination methods is to reach the same efficiency as individual vaccination (Cserep, 2002, 1-12). Coarse spray cabinets target day-old chicks' mucous membranes and the upper respiratory tract through inhalation and ocular uptake of the droplets. Absorption of the vaccine through the eye stimulates the Harderian gland, activating the immune system (Soares and Paniago, 2007, 1-3).

The behaviors that chicks exhibit when vaccines are administered play an important role in developing immunity. Many of the first behaviors exhibited by chicks are innate, such as pecking at stimuli around them, raising their head and swallowing or drinking (Appleby et al., 2004, 45-69; Hogan, 1973, 355-356). Day-old chicks do not have the ability to distinguish food from other items. Chicks learn that pecking leads to food ingestion around 3 days' post-hatch (Hogan, 1984, 360-376). They learn how to drink by pecking at shiny stimuli. When around others in the flock, chickens can learn new behaviors by observation (Appleby et al., 2004, 45-69).

For chicks to remain well-groomed, they often preen their feathers (Appleby et al., 2004, 45-69). Enhancing preening activity is an important aspect of spray-applied products (Caldwell et al., 2001a, 99-106). The spray lands on the chicks' coat, stimulating preening behaviors and increased ingestion of the vaccine. Caldwell et al. (2001a, 99-106) determined ingestion volumes increased due to an increase of preening behaviors. The success of the vaccine also depends on the equipment used for the delivery method. Proper air pressure, nozzle settings, and environmental conditions are necessary for optimal vaccine delivery. Higher pressure causes larger spray

particles to be released (Soares and Paniago, 2007, 1-3). When administering too large of a particle, the chick will not inhale the correct dose of the vaccine into their respiratory system. When a smaller particle is administered, too severe of a reaction occurs due to deep penetration into the respiratory tract. Using the correct pressure and nozzle size together creates a coarse spray particle of 100-150 μm .

Using foams as a vaccine delivery method for chicks has not been researched. Our research group conducted a pilot research study to screen potential edible foams that could be produced and used as a vaccine delivery method. Egg white (EW) and sodium stearoyl lactylate (SSL) were suitable candidates to be used as foaming agents for foams with both low and high expansion rates and reasonable stability. It was necessary to investigate how chicks react to foams with different characteristics and the behaviors associated with their interactions with different foams. The objective of this study was to analyze ingestion volumes and the chicks' behaviors with different foams and coarse spray.

3.3 Materials and Methods

3.3.1 Ingestion Volume Calibration Curve Determination

A fluorescent tracing method using fluorescein (Sigma-Aldrich, St. Louis, MO) was used to determine the volume of foam ingested by chicks. First, a calibration curve was developed to determine the amount of ingested foam by the chicks to the measured optical density units. The calibration curve was generated by gavaging chicks with known volumes of fluorescein water solution. Nine, day-old Cobb 500 pullet chicks from a local hatchery were used and administered a 0.1% fluorescein water solution at three different volumes (1 μl , 10 μl , and 100 μl) by gavaging. Three (3) chicks were

used per volume. Ten (10) minutes after administering the fluorescein mixtures, the chicks were euthanized by an approved method and the upper gastrointestinal tract, including esophagus, crop, proventriculus and gizzard, was removed (Figure 3.1).

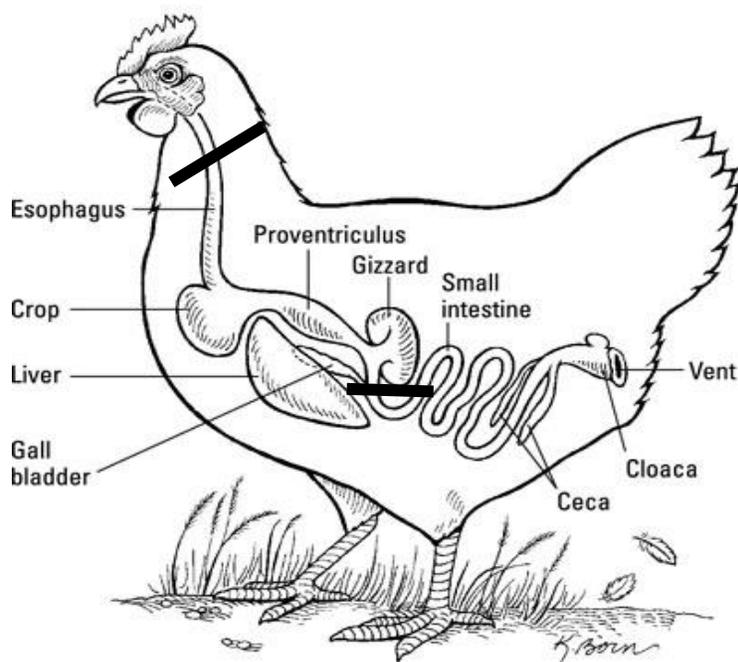


Figure 3.1: Diagram of the sample removed from the chicks, from the esophagus to the gizzard. This figure was adapted from Born, K., 2013

The removed upper gastrointestinal tract was placed into an 80-ml Seward filter bag with 1 ml dH₂O and double-bagged with a 118-ml black Whirl-Pak bag. The samples were stomached for 1 min at a speed of 120 rpm and liquid samples in the bags were then placed onto a 96-well polystyrene plate at a 1:2 dilution to determine the optical density units using an absorbance reader (ELx800, Bio-Tek Instruments. Inc., Vermont, USA) at 490 nm excitation level. A standard curve was created using the data from the absorbance reader.

3.3.2 Foam Generation and Administration

From preliminary studies conducted, whipping and sparging were generation methods used to form a stable foam. A 0.5-L whipping dispenser (Chef-Master Whipped Cream Dispenser, Mr. Bar-B-Q Inc., Old Bethpage, NY) produced foam by attaching carbon dioxide (CO₂) gas charger to the dispenser and rapidly releasing the pressure. A sparging disc with 10 µm holes (20 mm diameter) was used to create foam by sparging air in a liquid with the foaming agents. The sparging disc was connected to a compressed air tank with an airflow meter for flow adjustment.

Ten (10) ml of a 0.4% fluorescein H₂O solution was added to five treatments. Thirty (30) ml of egg white (EW) was added to EW treatments. For sodium stearoyl lactylate (SSL) treatments, 0.2 grams of SSL was added to 30 ml of distilled water (dH₂O). All components of the treatments totaled 40 ml (Table 3.1). Spray treatment materials were added to an 8-oz. spray bottle with a fixed spray volume of 1.25 ml. CO₂ gas and the materials were mixed in the whipping dispenser for 1 min and 2.5 grams of foam was dispensed. For the sparging treatments, the sparging disc was added to the transparent carbonate plastic columns (51 mm diameter and 152 mm height) with an airflow rate of 1.0 L/min to create 1.5 grams of foam. The amount of foam administered to the chicks was determined by the foams' expansion rate.

Five (5) chicks were held in a square cardboard tray (37.5 in²) and administered 1.25 ml (spray), 2.5 grams (whipping) or 1.5 grams (sparging) to the top of the chicks. The volume of treatment administered to the chicks was similar to coarse spray in a commercial hatchery. Using a spoon, the foams from whipping and sparging methods were administered to the chicks from the top so the chicks could see the foams. The foams landed on the chicks or on the cardboard floor since the whole floor was not fully covered by chicks.

Table 3.1: Composition of each treatment administered to the chicks

Number	Treatment	Generator	Components
1	Spray Control	Spray	dH ₂ O ^A , green food dye
2	Spray Dye	Spray	dH ₂ O, fluorescein stock solution
3	Whipping EW ^B	Whipping	EW, fluorescein stock solution
4	Whipping SSL ^C	Whipping	dH ₂ O, SSL, fluorescein stock solution
5	Sparging EW	Sparging	EW, fluorescein stock solution
6	Sparging SSL	Sparging	dH ₂ O, SSL, fluorescein stock solution

^AdH₂O: distilled water (H₂O)

^BEW: egg white

^CSSL: sodium stearyl lactylate

3.3.3 Chick Behavior Monitoring and Necropsy

A video recording system was used to capture the behaviors and the response of the chicks exposed to the different treatments over a 10-min period. Each chick within the treatment was dyed with a different color to aid with coding. An ethogram was developed to analyze the different behaviors using coding software (Solomon Coder, Version beta 16.06.26). This software determined the duration and frequency of each behavior over the 10-min monitoring period for each individual chick. The behaviors included resting, walking, shaking, aggression, pecking, preening, and drinking. Pecking, preening, and drinking were grouped as ingestion related behavior. Ten (10) minutes after administering either the foam or spray, the chicks were sacrificed and the upper gastrointestinal tract was removed and analyzed with an absorbance reader (ELx800, Bio-Tek Instruments. Inc., Vermont, USA), conducting the same method used in the previous experiment. The volumes ingested were determined with the standard curve generated previously.

3.3.4 Statistical Analysis

Duration of the chick behaviors and ingested volume levels were analyzed (each chick was 1 replicate with 5 chicks per treatment). A log transformation was used for the chick behavior analysis and ingestion volume readings. Statistical tests conducted included one-way ANOVA and Tukey-Kramer HSD using the software JMP Pro[®] (Version 12.1.0, SAS Institute Inc., Cary, NC, 1989-2007). Treatment type was a factor for the ingestion analysis. Treatment type and type of behavior were factors for analysis of duration. All statistical analysis was conducted at a 5% significance level ($P < 0.05$).

3.4 Results

3.4.1 Ingestion Calibration Curve

With known amounts of fluorescein solution administered to the chicks, a calibration curve was developed to correlate the optical density with the amount of the solution to determine the amount of the solution ingested by the birds (Figure 3.2). As the volume administered increased, the optical density readings increased (Table 3.2). As more volume of the solution was administered to the chick, the higher the reading of optical density units.

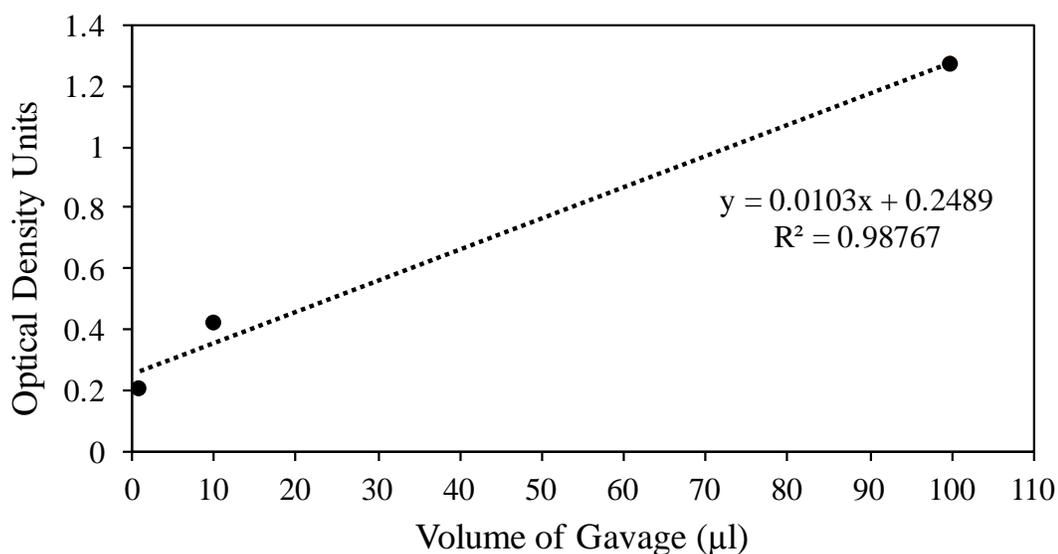


Figure 3.2: Curve created from gavage trial when administering known fluorescein solution volumes of 1, 10, and 100 µl to the chicks

Table 3.2: Mean values (\pm standard error) of fluorescence signal units when administering the specific amount of volumes to the chicks (n=3)

Volume of Gavage (µl)	Optical Density
1	0.20 ± 0.06
10	0.42 ± 0.11
100	1.27 ± 0.45

3.4.2 Foam Ingestion by Chicks

Using the previously generated standard curve, values were calculated to determine the amount of foam ingested by the chicks. The more foam ingested by the chick, the more fluorescein within the sample, which meant higher optical density units. Sparging SSL and sparging EW treatments were significantly different than whipping EW ($P < 0.05$) (Table 3.3). Compared to other treatments, the volumes of

SSL and EW foams generated by the sparging method were ingested by the chicks in a shorter percentage of the total monitoring period (Figure 3.3).

Table 3.3: Mean values (\pm standard error) of amount ingested treatment (μl) when administering treatments to the chicks (n=5)

Treatment	Average Amount Ingested ($\mu\text{l}/\text{bird}$)
Spray: Dye	37.00 ^{AB} \pm 7.89
Whip: EW	28.34 ^B \pm 17.09
Whip: SSL	45.83 ^{AB} \pm 9.94
Sparg: EW	91.16 ^A \pm 20.24
Sparg: SSL	103.04 ^A \pm 30.23

Note: Ingestion volumes were compared within each treatment, different superscript letters in the ingestion volumes distinguishes a significant difference ($P < 0.05$)

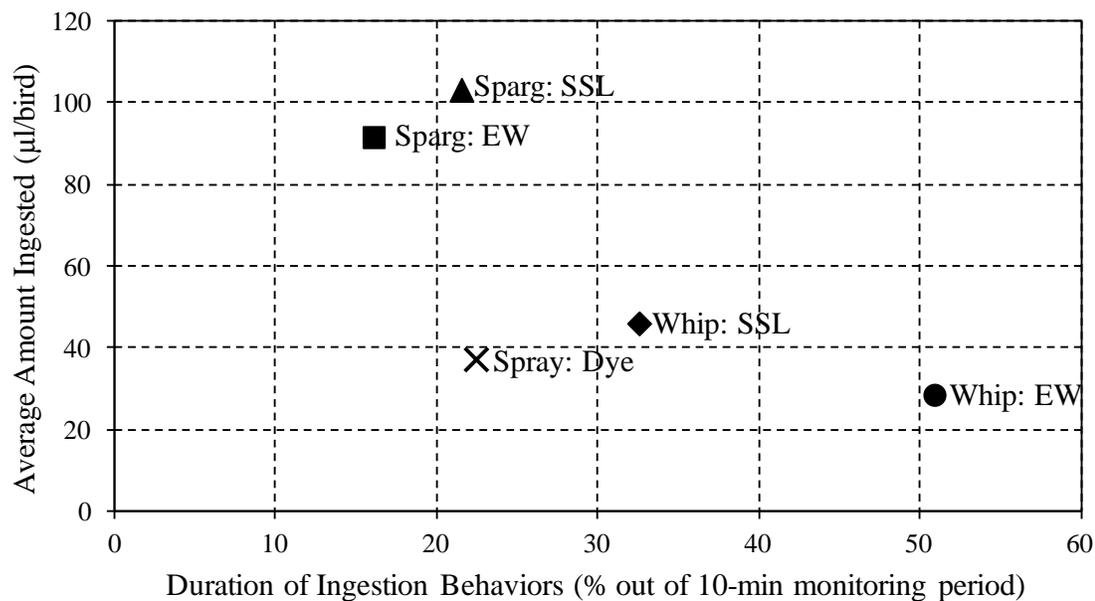


Figure 3.3: Percentage of ingestion behaviors over 10-min monitoring period by average amount ingested ($\mu\text{l}/\text{bird}$)

3.4.3 Chick Behavior

To analyze the amount of treatment ingested, pecking, preening self, preening another, and drinking behaviors were considered. The type of treatment administered had a significant effect on the duration of all four ingestion behaviors ($P < 0.05$).

The drinking behavior was exhibited for the longest amount of time with whipping EW (Figure 3.4). Sparging SSL was significantly different than the two spray treatments (Table 3.4). The treatment administered had a significant effect on the duration time for pecking ($P < 0.01$). After administering the treatments, pecking was the most observed out of all ingestion behaviors. Chicks administered the whipping EW treatment had the longest duration of pecking (Figure 3.5). Whipping EW had a significantly higher pecking behavior than spray dye and sparging EW ($P < 0.01$) (Table 3.4). Treatment type had a significant effect on duration time for preening another chick ($P < 0.01$). Chicks were preening another chick the most when administered either whipping SSL or spray dye treatments (Figure 3.6). The type of treatment had a marginally significant effect on the preen self-behavior ($P = 0.05$). Preen self was the least frequently observed behavior of all four ingestion behaviors. Over the total time, chicks preened themselves the most after being administered whipping EW (Figure 3.7).

The average of all four ingestion behaviors was highest for the two whipping treatments (Table 3.4). Sparging treatments had the highest quantitative ingestion volumes (Table 3.3). Therefore, increased behaviors related to ingestion did not directly contribute to the increasing amount of ingested foam or liquid of the treatment.

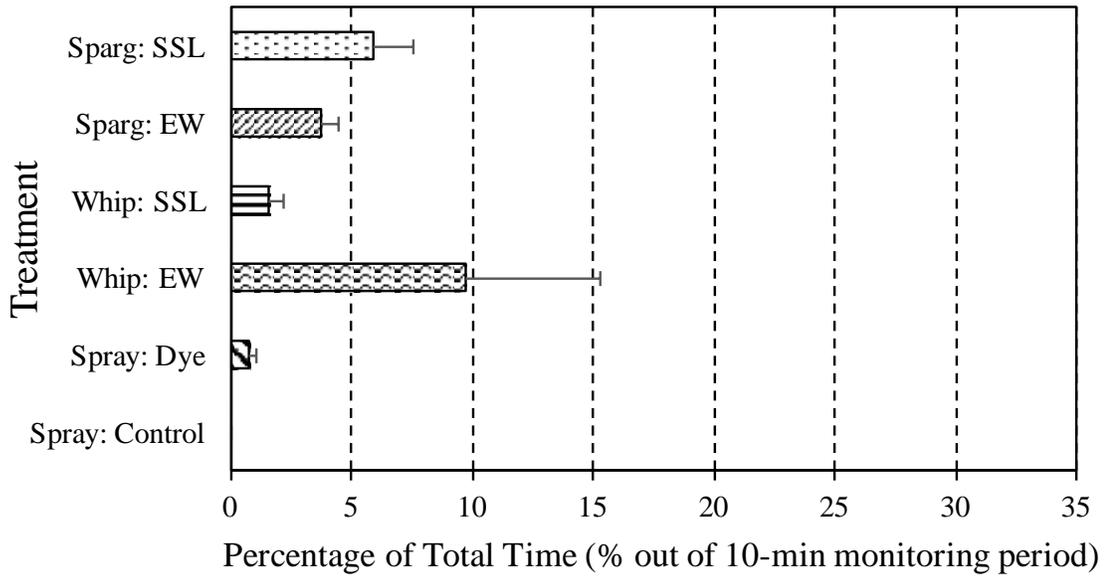


Figure 3.4: Percentage of the drinking behavior over 10-min monitoring time per treatment

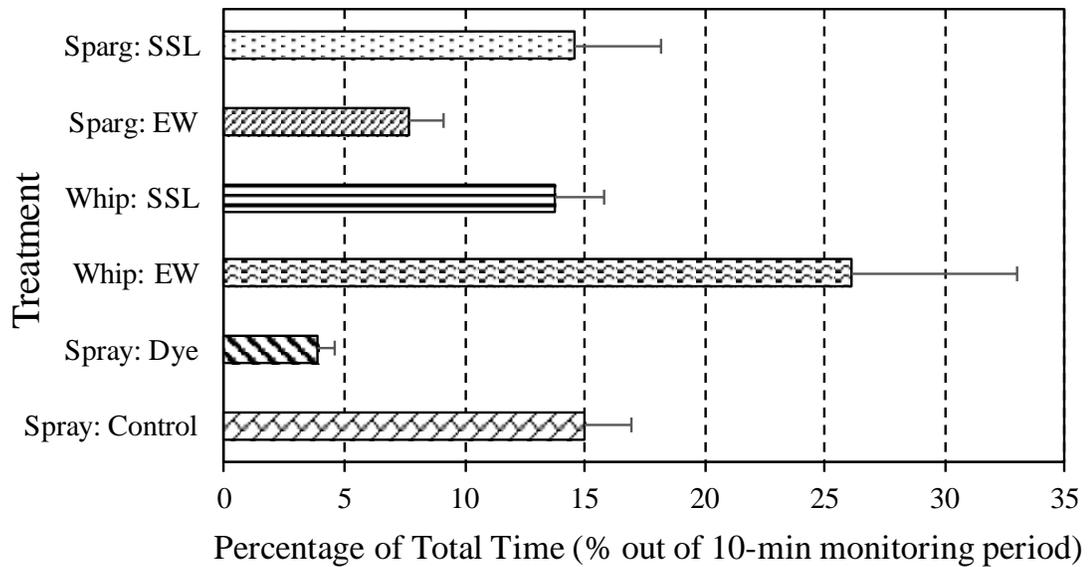


Figure 3.5: Percentage of the pecking behavior over 10-min monitoring time per treatment

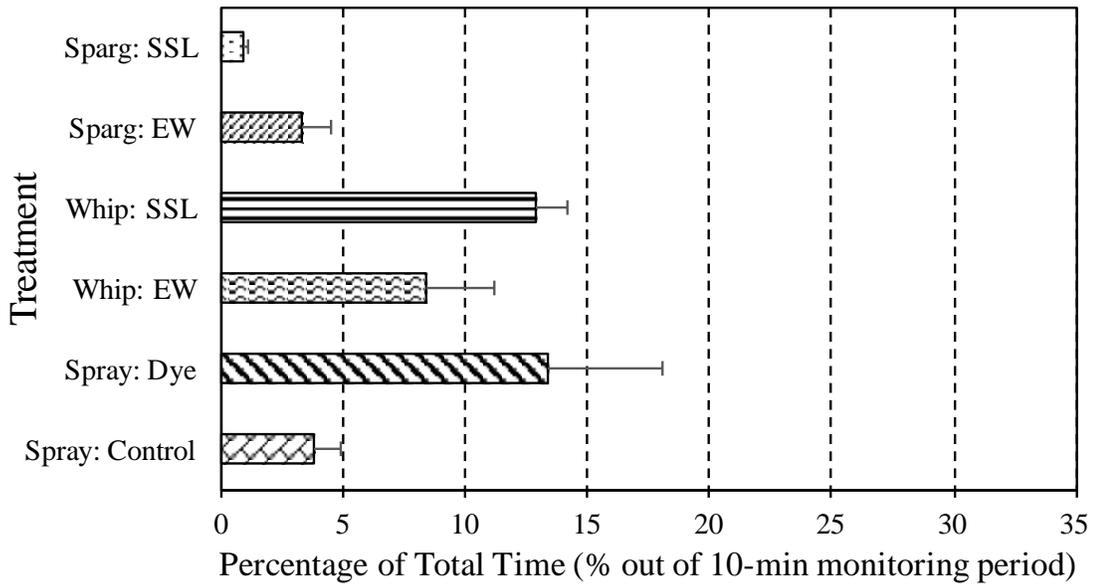


Figure 3.6: Percentage of the preening another behavior over 10-min monitoring time per treatment

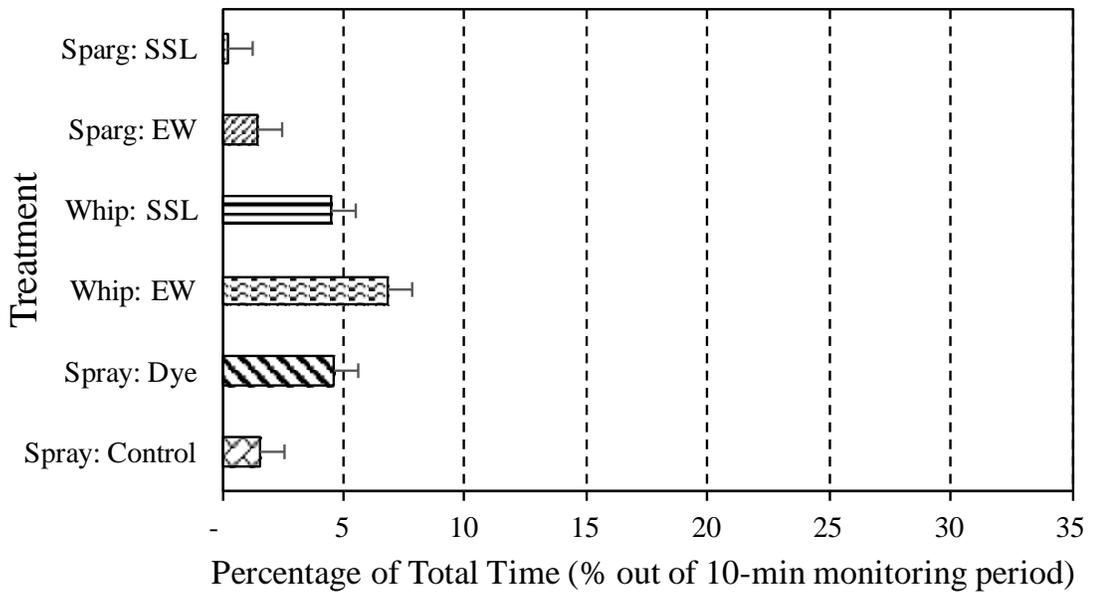


Figure 3.7: Percentage of the preening self behavior over 10-min monitoring time per treatment

Table 3.4: Mean values (\pm standard error) of chicks' behavior duration (seconds) over 10-min monitoring period after the chicks were administered with different foams and coarse sprays (n=5)

Treatment	Drinking	Pecking	Preen Another	Preen Self	Total Average Ingestion Behaviors
Spray: Control	0.00 ^C \pm 0	89.80 ^{AB} \pm 12.05	22.78 ^{ABC} \pm 6.54	9.46 ^{AB} \pm 2.28	122.04 ^B \pm 15.95
Spray: Dye	4.34 ^B \pm 1.88	23.26 ^C \pm 4.25	80.18 ^{AB} \pm 28.28	27.52 ^{AB} \pm 22.55	135.30 ^B \pm 39.32
Whip: EW	58.38 ^{AB} \pm 33.16	156.54 ^A \pm 41.36	50.12 ^{AB} \pm 16.88	40.92 ^A \pm 22.20	305.96 ^A \pm 16.95
Whip: SSL	9.02 ^{AB} \pm 3.78	82.30 ^{AB} \pm 12.48	77.42 ^A \pm 7.42	26.98 ^{AB} \pm 17.71	195.72 ^B \pm 25.58
Sparg: EW	22.28 ^{AB} \pm 4.54	46.18 ^{BC} \pm 8.32	19.76 ^{BC} \pm 6.98	8.72 ^{AB} \pm 4.08	96.94 ^B \pm 13.35
Sparg: SSL	35.42 ^A \pm 9.84	87.62 ^{AB} \pm 21.73	5.10 ^C \pm 1.01	1.54 ^B \pm 1.07	129.78 ^B \pm 29.74

Note: Treatments were compared within each behavior, different superscript letters in that behavior distinguishes a significant difference ($P < 0.05$)

3.5 Discussion

Caldwell et al. (2001a, 99-106) measured ingestion of spray-applied products with fluorescein solution. As the volume gaged to the chicks increased (0.1, 1, 10, and 100 μ l), the units of fluorescent signaling increased. These results correspond to the results found in this current study. When gaged incremental volumes (1, 10, and 100 μ l), the amount of optical density units increased (Figure 3.3). In the current study, there was a 1,000-fold difference in the amount of signaling units read compared to Caldwell et al. (2001a, 99-106). For both studies this curve was used to measure the voluntarily ingested treatments.

There was a higher ingestion rate for the sparging treatments compared to the whipping EW treatment ($P < 0.01$). Whipping EW took about 51% of the total time to ingest an average of 28 μ l/bird of foam. Sparging EW took about 16% of the total time for an average of 91 μ l/bird to be ingested and sparging SSL 22% of the total time for an average of 103 μ l/bird to be ingested (Figure 3.3). Caldwell et al. (2001a, 99-106) used a calibration curve equation to determine unknown ingestion values from spray-applied treatments. Chicks in the Caldwell et al. (2001a, 99-106) study ingested 9 μ l of fluorescein labeled water when under similar environmental conditions, while each chick ingested an average of 37 μ l of spray applied fluorescent treatment in this current study. The variation in the volumes of ingested materials may be due to the differences in chicken breed or the environmental factors.

When comparing the two spray treatments, spray dye had a high duration of preening behaviors and low ingestion intake. This suggests that an increase of preening behavior did not correlate to an increase of ingestion. Due to the fine particles of the coarse spray, pecking, and preen another were the top behaviors exhibited (Table 3.4).

Caldwell et al. (2001a, 99-106) found that increased preening behavior resulted in increased ingestion of spray applications. Environmental conditions may have impacted the number of preening events.

When analyzing foam characteristics previously, it was evident that there was an average liquid drainage of 67% for whipping treatments. Due to the higher liquid drainage of the whipping foam, increased drinking and pecking behaviors were observed.

Previous studies investigated behaviors associated with administering new objects to chicks (Marples and Roper, 1996, 1417-1424; Murphy, 1977, 335-349). Fear and exploratory responses are used to measure reactions to novel objects. The type of response varies depending on the intensity of the object. Exploratory responses are directed towards the stimulus, while fearful are away. (Murphy, 1978, 422-431). Murphy (1977, 335-349) presented chicks with novel objects and recorded fear and exploratory responses. When presented with a novel colored ball, both fear and exploratory responses were observed (Murphy, 1977, 335-349). Administering a foam substance to chicks was not been previously studied. When administering the foam treatments for this current study, the behaviors observed were considered exploratory. For all treatments, the chicks were interested in the materials by exhibiting behaviors including pecking, preening another or itself. When administering the spray treatments, the chicks ran away from the stimulus, exhibiting a fearful response.

The characteristics of the materials could explain the chicks' interest in the foam. Poultry sense using vision, hearing, taste, olfaction and chemoreceptors (Scanes et al., 2004, 153-161). When administering black colored spray, more preening events were recorded (Caldwell et al., 2001b, 107-111). Roper and Marples (1997, 207-213)

found that adding red and green coloring to drinking water had a higher latency. The coloring of the control spray was green while the other treatments were a light yellow. The color did not affect behavior since the number of preening events did not significantly differ between spray treatments.

The chicken has 24 taste buds, detecting only salt, acid, and sugars (Scanes et al., 2004, 153-161). These senses may contribute to the interest associated with the foams. Chemically, egg white is composed of water (88%), protein (11%), carbohydrates, ash, and lipids (1%) (Abeyrathne et al., 2013, 3292-3299). For the EW treatments, 75% of the total foam mixture was EW. SSL treatments consisted of 0.5% SSL of the overall foam mixture. For both treatments, majority of the mixtures were water.

3.6 Conclusions

The behaviors of chicks and ingestion of foam were evaluated in this study. When the chicks were administered with foam as vaccine delivery method, the following conclusions were drawn:

1. The ingestion volume of foams from the sparging method was 2.3 times of the spray. The ingestion volume of foams from whipping method was the same as the spray over the 10-min monitoring period.
2. Chicks exposed to foams produced by the whipped method demonstrated more ingestion related behaviors compared to the spray (95% higher) and sparging (121% higher) methods ($P < 0.05$).
3. The ingested volumes of foams ingested by the chicks did not increase as ingestion behaviors increased. The foams from the sparging method had a higher expansion rate and increased the ingestion intake of the chicks.

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Chapter 4

EFFECTS OF FOAMING MATERIALS AND PROCESSES ON VIRUS VIABILITY OF INFECTIOUS BRONCHITIS VIRUS VACCINE

4.1 Abstract

Vaccinations are a vital aspect of the poultry industry by reducing morbidity and mortality and increasing flock health. Numerous research papers focus on the viability and efficacy of particular vaccine strains to determine the most beneficial and immunologically protecting method for poultry. The viabilities of infectious bronchitis virus (IBV) in foams produced with egg white (EW) and sodium stearyl lactylate (SSL) by sparging and whipping generating methods were tested. Specific-pathogen-free (SPF) embryonated chicken eggs were inoculated at 11-days of age at IBV dilution levels from 10^0 to 10^{-3} at 60 and 120 min after producing the foams. Eggs were candled and mortality was recorded for 8 days. Virus recovery was determined by examination of the embryos for lesion characteristics of IBV and titers were calculated for each foam treatment using the Reed and Muench method. The results showed the viability of IBV was not affected by foams generated from EW and SSL with both whipping and sparging methods when eggs were inoculated after 120-min of foam generation. However, EW foams from both methods caused a lower viral viability at the 60-min inoculation.

Key words: Foam, chicken, vaccine, infectious bronchitis virus, inoculation

4.2 Introduction

Evaluating the efficacy of vaccination methods is an important aspect of the viability of the attenuated vaccines. There are guidelines with specific vaccines to determine the effects of the vaccine. For example, anticoccidial vaccinations criteria for efficacy include weight gain measurements, feed conversion efficiency, oocyst production and lesion presence (Chapman et al., 2005, 279-290). Vaccination and challenge is a common assessment to test the amount of protection an animal species develops from a specific vaccine. When conducting these tests, a strain of virus is given to the animal and after a period of time, observations of clinical signs and sampling of the animal are conducted (Deville et al., 2012, 85-92). Studies to test the efficacy of coarse spray or gel administration methods have been conducted (Deville et al., 2012, 85-92; Price et al., 2016, 82-93). Purswell et al. (2010, 1310-1315) analyzed the coarse spray method and how different nozzle sizes and pressure levels corresponded to the amount of absorption of the spray through the ocular method. To test infectious bronchitis virus (IBV), comparisons of the immunological response between *in-ovo* vaccination and spray vaccination were conducted (Wakenell et al., 1995, 752-765). A better understanding of how to improve poultry flock health can be determined by researching different aspects of virus strains and vaccine administration methods.

It is a common technique to inoculate specific-pathogen-free (SPF) chicken eggs with a desired virus for viral isolation (Gelb and Jackwood, 2008, 146-149). This method allows the investigator to examine the growth and development of the embryo by studying the effects of the specific virus on the embryo. This type of experiment may precede vaccine efficacy tests to determine if the virus dose is viable under certain parameters. Chicken embryos are readily available, economically friendly, and

convenient (Cunningham, 1973). There are numerous methods of inoculation that are specific to the virus being studied. Yolk sac, allantoic sac, amniotic sac or chorioallantoic membrane (CAM) are all different and common types of inoculation routes. Since inoculating via CAM route is less likely to be affected by bacterial contamination, it is preferred for many viruses (Senne, 2008, 204-208). Factors that can influence the growth of the virus and embryos include the age of the embryo, inoculation route, concentration and volume of inoculum, temperature of incubation, and time of incubation following the inoculation (Cunningham, 1973).

IBV was chosen for this experiment due to its vaccination use in the hatchery. Broilers are vaccinated against this disease on the hatch day, commonly by the spray route. Due to the availability and distinct embryonic characteristics associated with the virus, IBV was chosen to inoculate eggs for this experiment. The objective of inoculating SPF eggs with IBV was to determine if the materials of the foam or the processes of creating the foam would impact the virus. It was important to determine if the foaming materials or processes would alter the viability of the virus since the overall goal is to deliver a viable vaccine virus to chicks.

4.3 Materials and Methods

4.3.1 Foam Generation

From preliminary studies, whipping and sparging were the selected generation methods because of their ability to form a stable foam. A 0.5-L whipping dispenser (Chef-Master Whipped Cream Dispenser, Mr. Bar-B-Q Inc., Old Bethpage, NY) produced foam by attaching carbon dioxide (CO₂) gas source to charge the dispenser and to rapidly releasing the pressure by dispensing the foam. A sparging disc with 10

µm holes (20 mm diameter) was used to create foam by sparging air in a liquid with the foaming agents at an airflow of 1.0 L/min. The sparging disc was connected to a compressed air tank with an airflow meter for flow adjustment.

4.3.2 Inoculation of Treatments

Whipping and sparging methods were used to generate foams using egg white (EW) and sodium stearyl lactylate (SSL) mixed with IBV vaccine Arkansas (Ark) AviPro and tested in SPF chicken eggs (Charles River Avian Vaccine Services, Norwich, CT). A 10,000 dose IBV Ark vaccine was re-suspended in 30 ml of sterile diluent to create a more concentrated dose. There were nine different treatments including negative controls, positive controls and treatments (Table 4.1). A total of 258 SPF eggs were used: 5 eggs per negative control (NC) and 19 eggs per positive control (PC) and each of the treatments (T).

Table 4.1: Composition of each treatment (NC n=5; PC and T n=19)

Number	Treatment Name	Generation Method	Components
1	Negative Control 1 (NC1)	No generator	No materials, No virus
2	Negative Control 2 (NC2)	No generator	EW, No virus
3	Negative Control 3 (NC3)	No generator	SSL, No virus
4	Positive Control 1 (PC1)	No generator	Water, Virus
5	Positive Control 2 (PC2)	Whipping	Water, Virus
6	Positive Control 3 (PC3)	Sparging	Water, Virus
7	Treatment 1 (T1)	Whipping	EW, Virus
8	Treatment 2 (T2)	Sparging	EW, Virus
9	Treatment 3 (T3)	Sparging	SSL, Virus

Virus and foam materials were manually mixed at a 1:5 dilution and added to either the sparging or whipping methods to generate foam. Once foam was generated, the time was recorded. Over time the foam broke down into 100% liquid. The foam liquid (10^0) was mixed at a 1:2 dilution with media and antibiotics (sterile tryptose phosphate broth (TPB), Lonza Biowhittaker Penicillin/Streptomycin). Ten-fold dilutions of each treatment from 10^0 to 10^{-3} were prepared and incubated at room temperature (22°C) for a minimum of 30 min to reduce bacterial contamination.

After 60 and 120 min from the recorded time of foam generation, each dilution of treatment was inoculated via chorioallantoic fluid (CAF) route into the 11-day-old eggs. The same sample was used for both time periods. Before inoculation, the eggs were sprayed with 70% ethanol and inoculated with 0.2 ml per egg using 26-gauge needle, sealed with Duco glue and set to incubate at 37.5°C . The eggs were candled over the next 8 days to check for mortality. At day 21 of age the eggs were placed at 4°C . Embryos from any treatments that survived from day 4 of candling onward were evaluated for IBV-induced lesions. External characteristics of IBV infectivity include stunting and curling of the embryo, clubbing of the feet, or hemorrhagic traits (Gelb and Jackwood, 2008, 146-149). Internally, urates of the mesonephros of the kidney indicate viral infectivity. After analyzing each chick and determining the infectivity endpoint, the virus titer was calculated. The embryo infectious dose at 50% (EID_{50})/ml was computed using the Reed and Muench method:

$$\text{Index} = \frac{\% \text{ infected at dilution above } 50\% - 50\%}{\% \text{ infected at dilution above } 50\% - \% \text{ infected at dilution below } 50\%} \quad (4)$$

4.4 Results

The EW and SSL materials alone did not reduce the viability of the virus. When EW foam was generated by the whipping and sparging methods, there was an endpoint reached and decrease in viral viability. No other treatments reached an endpoint (Table 4.2). The sparging and whipping EW foams had an increase in viability at the 120-min inoculation time. None of the treatments reached an endpoint except the whipping method with water and virus (Table 4.3). Overall, as the inoculation time increased from 60 to 120 min, the viability of the virus increased (Table 4.4).

Table 4.2: Percent of embryonated chicken embryos infected at the 60-min inoculation time

60 min	Percent Infected (%) of Each Treatment					
Dilution	PC1 ^A	PC2	PC3	T1 ^B	T2	T3
10 ⁰	92	100	100	100	100	100
10 ⁻¹	90	100	100	100	100	100
10 ⁻²	86	100	100	100	50	100
10 ⁻³	75	75	100	50	0	100

^APC: positive control

^BT: treatment

Table 4.3: Percent of embryonated chicken embryos infected at the 120-min inoculation time

120 min	Percent Infected (%) of Each Treatment					
Dilution	PC1 ^A	PC2	PC3	T1 ^B	T2	T3
10 ⁰	100	100	100	100	100	100
10 ⁻¹	100	100	100	100	100	100
10 ⁻²	100	100	100	86	100	100
10 ⁻³	100	0	100	60	75	100

^APC: positive control

^BT: treatment

Table 4.4: Viral titers of the treatments at both time periods

60 min		120 min	
Treatment	Virus Titer	Treatment	Virus Titer
PC1 ^A	> 10 ³	PC1 ^A	> 10 ³
PC2	> 10 ³	PC2	10 ^{2.5}
PC3	> 10 ³	PC3	> 10 ³
T1 ^B	10 ³	T1 ^B	> 10 ³
T2	10 ²	T2	> 10 ³
T3	> 10 ³	T3	> 10 ³

^APC: positive control

^BT: treatment

4.5 Discussion

Egg whites are composed of ovomucin and anti-microbial proteins such as ovotransferrin. Ovomucin has carbohydrate chains (O and N-linked) that can operate as viral-ligand receptors. Tsuge et al. (1996, 1503-1504) suggests that the O-linked carbohydrate chains of ovomucin can aid in the binding of viruses. Van der Plancken et al. (2007, 1410-1426) and Hayakawa et al. (1996, 756-762) found that treatments with pressure above 400 MPa creates a loss of secondary structure of egg whites. With pressures between 100-400 MPa, the denaturation of egg whites structure may be reversible (Hayakawa et al., 1996, 756-762). Previous literature suggests that the ovomucin component of the egg white may interact and bind with IBV. Along with the composition of the egg whites, the processing of the foam may affect the viability. Although pressures in this study (80 PSI/0.55 MPa) were lower than previous studies, a potential loss of secondary structure at the 60-min inoculation may have occurred. At the 120-min inoculation, the proteins may have reversed its' structure, causing the increase in virus viability.

4.6 Conclusions

The results showed that viability of IBV was not affected by foams generated from EW and SSL with both whipping and sparging methods when eggs were inoculated 120 min after foam generation. However, EW foams from both methods caused a lower viral viability of IBV at the 60-min inoculation.

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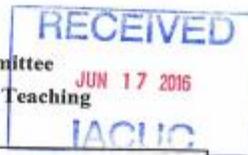
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Appendix

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE LETTER

University of Delaware
 Institutional Animal Care and Use Committee
 Application to Use Animals in Research and Teaching



Title of Protocol: Analyzing the Behavior of Day Old Chicks Under Foam Vaccination Administration Method	
AUP Number: 61R-2016-0	← (4 digits only — if new, leave blank)
Principal Investigator: Hong Li	
Common Name: Broiler chicken	
Genus Species: <i>Gallus gallus domesticus</i>	
Pain Category: (please mark one)	
USDA PAIN CATEGORY: (Note change of categories from previous form)	
Category	Description
<input type="checkbox"/> B	Breeding or holding where NO research is conducted
<input checked="" type="checkbox"/> C	Procedure involving momentary or no pain or distress
<input type="checkbox"/> D	Procedure where pain or distress is alleviated by appropriate means (analgesics, tranquilizers, euthanasia etc.)
<input type="checkbox"/> E	Procedure where pain or distress cannot be alleviated, as this would adversely affect the procedures, results or interpretation

<p>Official Use Only</p> <p>IACUC Approval Signature: <u><i>Ann Tuller, DVM</i></u></p> <p>Date of Approval: <u>7/24/2016</u></p>
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