EFFECTS OF TIME SPECIFIC F-STRAIN *MYCOPLASMA GALLISEPTICUM* INOCULATION OVERLAYS ON PRE-LAY TS-11-STRAIN *MYCOPLASMA GALLISEPTICUM* INOCULATION ON PERFORMANCE, EGG, BLOOD, AND VISCERAL CHARACTERISTICS OF COMMERCIAL EGG LAYING HENS

By

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Vaccination programs using live whole cell vaccines are presently being used to control outbreaks and to help protect flocks from field strains of *Mycoplasma gallisepticum*. The primary goal of the present study was to investigate the effects of a ts11-strain M. *gallisepticum* inoculation alone, and in conjunction with F-strain *M. gallisepticum* overlays at 22 or 45 weeks of age during lay.

In this study, it was shown that a pre-lay ts11 vaccination had a broad range effect on the physiology of the commercial layer without affecting overall performance. The timing of an overlay vaccination using F-strain *M. gallisepticum* during lay also had varied effects on physiological parameters without affecting overall performance. This study indicates that using ts-11-strain *M. gallisepticum* in conjunction with F-strain *M. gallisepticum* does not negatively affect laying hen

Key words: *Mycoplasma gallisepticum* vaccines, commercial egg production,

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CHAPTER I

INTRODUCTION

Mycoplasma gallisepticum, the pathogen responsible for chronic respiratory disease in chickens is primarily a respiratory pathogen of meat-type chickens and turkeys, and a reproductive pathogen of table egg chickens. Due to the ease of disease transmission resulting systemic infection results in great economic loss worldwide. Decreased egg production (**EP**) and hatchability, downgrading and condemnation of carcasses, and reduced feed conversion can result from *M. gallisepticum* infections (Yoder and Hofstad, 1964; Domermuth et al., 1967; Patterson, 1994).

Vaccination programs using live whole cell vaccines are presently being used to control outbreaks and to help protect flocks from field strains of *M .gallisepticum*. The F-strain *M. gallisepticum* vaccine strain is less virulent than many of the field strains and has a lower bird to bird transmission rate, yet is able to displace the more virulent stains of *M. gallisepticum* (Levison and Kleven, 1981; Kleven et al., 1990). The F-strain *M. gallisepticum* vaccine, however, is not totally apathogenic and has been reported to infect *M. gallisepticum* free birds and turkeys (Evans et al., 1992, Ley et. al., 1997). More recently, apathogenic whole cell live vaccines ts11-strain *M. gallisepticum* and 685-strain *M. gallisepticum* have been licensed for use in layer chickens. These vaccines show

virtually no bird to bird transmission, but have not been proven to displace wild type *M. gallisepticum* (Kleven et al., 1998). Furthermore, these strains may not confer continued protection as does the F-strain throughout lay (Yoder, 1978, 1991; Mohammed et al., 1987).

There are possible benefits of using a prelay ts11 *M. gallisepticum* inoculation in conjunction with F–strain *M. gallisepticum* inoculations during lay to effectively protect flocks against field strain MG infections, while reducing possible subsequent depressions in EP due to a pre-lay F–strain *M. gallisepticum* inoculation. Commercial operations may acquire flocks already inoculated with ts11 *M. gallisepticum*, but desire to continue using the F–strain *M. gallisepticum* inoculation regime familiar to them. Therefore, the goal of this study was to investigate the effects of a ts11 *M. gallisepticum* .inoculation pre-lay overlaid with F–strain *M. gallisepticum* inoculation during lay on the performance, blood, internal egg and eggshell, and reproductive and digestive organ characteristics of commercial layers.

CHAPTER II

REVIEW OF LITERATURE

The Avian Reproductive System

The avian reproductive system initially begins with both left and right ovaries and oviducts; however, the primordial germ cells to the ovaries begin the process of asymmetrical distribution by day four of incubation. This is followed by initiation of regression of the right oviduct by Day 10. By the time the bird is an adult, (except for rare occasions); only the left ovary and oviduct are functional (Romanoff and Romanoff, 1949; Kinsky, 1971). A chick will produce approximately 280,000 oocytes of which only approximately 200-500 will mature and be ovulated during a bird's life span. Oogenesis is terminated at hatch. These oocytes (follicles) are governed by what is known as a follicular hierarchy. This follicular hierarchy functionally produces only 4-6 follicles (ova) which become large yolk filled mature follicles measuring approximately 40 mm in diameter, a larger number of smaller yellow follicles and numerous small yellow white follicles.

Follicle production is driven by the follicle stimulating hormone (**FSH**), while the follicle hierarchy is under the control of estrogen which is secreted by the mature ovary. Secretion of estrogen induces the secretion of lutenizing hormone (**LH**), which then

controls the ovulation of mature follicle from the ovary. Follicles are ovulated in the order of the follicular hierarchy. The avian oviduct has five distinct regions; infundibulum, magnum, isthmus, shell gland, and vagina. The infundibulum is a funnel shaped part of the oviduct, (not attached to the ovary), which catches the ovulated ova. The ova remain in the infundibulum for approximately 18 minutes (Warren and Scott, 1935).

The infundibulum is the site where fertilization can occur and where the first layer of albumen is deposited (Gilbert, 1979). The ovum then proceeds to the magnum, which is the largest portion of the oviduct, measuring approximately 33 cm. The magnum is highly glandular consisting of tubular glands that are responsible for the production of ovotransferrin and ovomucoid, and the site where the chalazae and the majority of albumen are formed (Aitken, 1971; Tuohimaa, 1975). Albumen is secreted in the magnum in response to the pressure of the yolk and the amount of albumen is believed to be related to the size of the yolk suggesting that the smaller yolk stimulates less albumen resulting in smaller egg size (Sturkie and Mueller, 1976). The ovum remains in the magnum for approximately 2-3 hr where the initiation of calcium secretion occurs (Eastin and Spaziani, 1978). Haugh Unit scores are a means to evaluate this inner egg quality, specifically albumen. This suggests that the inner egg quality is determined by the health and functionality of the magnum (Branton et al., 1988) the ovum continues moving into the isthmus which is more muscular and less glandular, containing tubular gland cells overlaid with epithelial cells, where it will remain for approximately 2 -3 hr. The isthmus is the site of formation of both the inner and outer shell membranes. The ovum then enters the uterus, a highly muscular structure also known as the “shell gland” which is

lined with both tubular and unicellular goblet cells (Johnson, 1986). This is where the egg begins what is termed the "plumping" process where salts are absorbed. The egg remains in the uterus for approximately 26 hr. Calcification takes approximately 15 hr and pigmentation approximately 5 hr (Warren and Conrad, 1942). Thus, the uterine function/dysfunction should be reflected by ESS, shell thickness, and pimpling (Branton et al., 1988)

Blood and meat spots, sometimes observed in the yolks are attributed to intrafollicular hemorrhage; possibly resulting from increase in either blood pressure or ovarian capillary fragility. The final portion of the oviduct is the vagina where ovipositioning of the egg occurs (Johnson, 2000).

Hormonal Influences on Avian Reproduction

The hormones progesterone and estrogen can found in their highest concentration in the preovulatory follicles, conversely concentrations of testosterone decrease in the larges preovulatory follicles 6-8 hours before ovulation (Shahabi et al., 1975). Approximately 4 -6 hours prior to ovulation, a peak occurs in plasma concentrations of lutenizing hormones (Lague et al., 1975; Johnson and van Tienhoven, 1980; Etches and Cheng, 1981). This LH peak coincides with peak in progesterone (Etches and Cheng, 1981), and is thought to be a necessary part of ovulation. Etches and Cheng (1981) asserts that there is a second peak of LH at 14–16 hours prior to ovulation. Testosterone, which is secreted from at least four of the largest follicles, returns to the peak preovulatory concentration 6-10 hours before ovulation.

Ovulation can occur without testosterone or DHT indicating that they are not directly involved in ovulation (Johnson, 1986). Cortecosterone has both a daily and peaks with ovoposition, but does not increase in relationship with ovulation. (Johnson and van Tienhovek, 1981). The follicle stimulating hormone (FSH) concentration and the binding to ovarian tissue peaks 15 hours prior to ovulation (Seanes et al., 1877; Etches and Cheng, 1981).

Two different kinds of calcium can be found in circulating blood; non-diffusible protein-bound calcium (unionized) and diffusible ionized calcium. Plasma calcium-binding proteins, vitellogenin, and albumen bind with the non-diffusible calcium (Guyer et al., 1980). Estrogen increases the amount of these binding proteins; thus, increasing blood calcium levels (Bacon et al., 1980). Adequate amounts of calcium must be provided in the diet or a significant decrease in egg production can occur. Ovary regression also occurs 6-9 days necessary dietary calcium is not provided (Taylor et al., 1962; Luck and Scanes, 1979). Layers will voluntarily increase calcium consumption when the egg enters the uterus and when the egg-shell calcification process occurs (Mongin and Sauveur, 1974).

Photoperiods are necessary for layer egg production. Although hens can continue to lay in the complete darkness, a decrease in egg production will occur (Wilson and Woodward, 1958; Morris, 1968). The ovarian development is most impacted by 12-14 hours of light; however, optimal egg production has been achieved by up to 18 hours of light.

Composition of the Avian Egg

As describe above, the mature egg consists of 3 main parts; eggshell, a multilayer albumen, and yolk. There is a thick inner layer of albumen is surrounded by a thin inner and outer layer of albumen. The thick albumen layer consists of ovomucin which increases the viscosity of the mucin. Approximately 60% of the total egg weight is albumen weight. There are approximately 40 different proteins in the albumen; however, the ovalbumen is the major protein involved. The egg yolk is surrounded by the vitellin membrane which separates it from the albumen. The egg yolk also is made up of two layers. The deep yellow layer, which is formed during the daylight hr and the light yellow layer, is formed during the night hr. The egg yolk consists of lipoproteins and rich lipids.

These lipids make up approximately 60% of the total yolk weight. Lipids found in the yolk are triglycerides, phospholipids, cholesterol and cerebroside. The major fatty acids found in the egg yolk are oleic, palmic, linoleic, and predominantly stearic and palmic fatty acids. They make up approximately 38% of the egg, the calcified portions, can be divided into three layers; the mammilary knob layer formed from the outward crystallization of the mammilary core, the palisade layer and the shell matrix. The egg is then covered by a thick waxy covering that is believed to protect the egg from moisture loss.

Avian Lipid Metabolism

Fatty acids can be characterized as saturated and unsaturated, as well as, essential or nonessential. Avian lipid requirements are relatively small only requiring a small

amount of the few fatty acids that cannot be synthesized by the bird's body (arachadonic, linolenic, and linoleic). These fatty acids that cannot be synthesized are considered to be essential fatty acids. Dietary supplements of linoleic acid can satisfy the need for both linoleic acid and arachodonic acid since synthesis of arochodonic acid is possible from linoleic acid. Fatty acid synthesis mostly occurs in the liver. Synthesis of fatty acids in the bird is under two major enzyme systems. Acetyl-Co A carboxylase (a biotin dependent enzyme) is first and the multienzyme system fatty acid synthetase is the second (Parkhurst and Mountney, 1987; Kleven, 2003).

Lipase activity occurs in the small intestine, as well as, the stomach where triglycerides are hydrolyzed to diglycerides, monoglycerides, fatty acids, and glycerol (Johnson, 1986). During digestion, micelles are formed and eventually absorbed into the small intestine Cholecystokinin induces pancreatic enzyme secretion in response to lipid entrance into the digestive tract (Dockray, 1975). The small intestine has villi which absorb fatty acids while the lipids are transported by the portal blood system as portomicrons after being esterfied (Bensadoun and Rothfield, 1972; Frazer et al., 1986). Various hormones regulate lid metabolism.

Glucose is the end product of carbohydrate digestion and becomes a major precursor of lipids. Glucagon and insulin are essential factors in lipogenesis regulation. Glucagon affects lipolytic and antilipogenic hormones and insulin stimulates lipogenesis. Avian pancreatic peptide reduces the concentration of circulating free fatty acids and partially suppresses lipolysis by glucogon. Insulin inhibits the release of glycerol and frees fatty acids in adipose tissue and also stimulates the conversion of glucose to fat by

stimulating the lipoprotein lipase, incorporation of circulating triglycerides into cells increases.

Mycoplasma gallisepticum Structure and Pathology

Mycoplasmas are members of the taxonomic class *Mollicutes,* and are among the smallest and simplest known pleiomorphic microorganisms (Panangala et al., 1992; Wise et al., 1992) capable of self-replication (Razin, 1981). *Mycoplasma gallisepticum* is an infectious gram negative bacterium (Razin and Freundt, 1984) that is bound by a plasma membrane, lacks a cell wall (Kleven, 1998) , and is able to infect entire flocks. Colonies are approximately 1 mm in diameter and have a characteristic "fried-egg" appearance when viewed through a microscope (Kleven, 1998) with a genomic size of about 5×10^8 daltons (ranging from about 600 to 1300 kbp). This small genome size allows very limited metabolic capabilities.

Researchers have examined the mechanism(s) of *Mycoplasmas* adherence to the host cells in order to understand the initiation of disease pathogenesis and develop a treatment that would prevent the adherence and thus inhibit infection. This mechanism is well described by Razin (1985). Using electron microscopy, it was determined that an attachment organelle or tip structure (Uppal and Chu, 1977; Tajima et al., 1979; Razin et al., 1980; Kirchhoff et al., 1984; Levisohn, 1984; Maniloff and Quinlan, 1973), was present which allows *Mycoplasma* to bind to sialoglycoproteins (Glasgow and Hill, 1980; Bredt et al., 1981; Kahane et al., 1984). These extracellular parasites attach to the host epithelium via the external bleb (attachment organelle) for nutritional support which can cause disease by causing host cell injury. *Mycoplasmas* have a small amount of motility

that may contribute to their adherence to various structures *in vivo* (Razin, 1984). Although adherence to epithelial cells is not singularly the cause of pathogenicity, it tends to play an important role. Research has proven that MG organisms are able to adhere to the epithelial surface of the respiratory tract of chickens by their terminal bleb structures (Maniloff and Quinlan, 1973). Although it has been determined that the body is able to recognize and mount an immune response to *Mycoplasmas*, (Kerr et al., 1970), they can evade the host immune. *Mycoplasma* has an antigenic diversity mechanism (Panangala, et al., 1992; Wise, 1992) Antigenic diversity of *M. gallisepticum* strains has also been reported (Panagala, et al., 1992).

This mechanism of antigenic diversity allows *M. gallisepticum* to circumvent the host immune response to specific epitopes; thereby, leading to chronic infection characteristic of *M. gallisepticum*. This directly impacts the virulence of *Mycoplasmas* as organisms evade nonspecific defense mechanisms (Howard and Taylor, 1979; Davidson et al., 1988). Different antigenic profiles in MG are due to the differential expressivity of major surface antigens (Garcia et al., 1994). Upon entering the host and multiplying, these organisms evade the host's immune system by phenotypic switching (phase shifts), then escape to infect new hosts.

Although, the primary location of *M. gallisepticum* infection colonization is the respiratory system, the invasion of other organs is possible due to their ability to migrate through the blood stream from the mucosal membrane of the respiratory system to other organs via attachment to erythrocytes (Winner et al., 2000). *M. gallisepticum* is capable of infecting entire flocks and tends to be more severe in young birds and in cold weather (Ley and Yoder, 1997).

Due to its minimal genomic size, and lack of cell wall, *Mycoplasma* cannot survive outside its host for long periods of time and are susceptible to cleaning and disinfecting agents (Kleven, 2003). Viability of *M. gallisepticum* is dependent on the strain and the conditions at which MG contaminated materials are held (Chandiramani et al., 1966). It can be transmitted bird to bird through horizontal and vertical transmission, as well as, through the hatching egg which is the major route of infection for the next generation (Kleven, 1981; Glisson and Kleven, 1984).

It has been shown that maternal antibodies inhibit multiplication of *M. gallisepticum* organisms in the egg in the beginning of the incubation period, but do not kill the organisms. Eggs from effected chickens had the highest rates of mortality at the end of the incubation period, suggesting the multiplication of the *Mycoplasmas* inhibit the embryo's development (Levinsohn et al., 1985). Once a bird is infected, it is considered to be infected for life and is able to infect others in a flock (Brown et al., 1995)

Respiratory infections caused by *Mycoplasma* are often mild unless accompanied by secondary infections (Kerr and Olson, 1967). Although *M. gallisepticum* infections are more prevalent in the respiratory system, they can become systemic over time, and are not fatal unless a secondary infection is present. Symptoms of *M. gallisepticum* are often confused by symptoms from secondary infections. Respiratory disease in poultry has a complex etiology in which various species of *Mycoplasmas* are only a single factor. Chronic respiratory infections associated with *M.gallisepticum* become more severe and adversely affect producer profit if the disease is complicated with Newcastle's Disease or Infectious Bronchitis exposure (Mohammed et al., 1987; Patterson, 1994). *Mycoplasma*

gallisepticum infection is one of the costliest diseases confronting the poultry industry (Yoder, 1978). Many birds may have *M .gallisepticum* present but the infection remains dormant until these birds are exposed to a stressor, the organism is then likely to spread throughout the flock.

As mentioned, the colonization and infection with *M. gallisepticum* is most often occurs in the air sacs of the avian respiratory tract causing air sacs to become cloudy and filled with mucus. As the disease persists, this mucus develops a yellow color and a cheesy consistency and may result in development of lesions in the respiratory tract in birds (Trampel and Fletcher, 1981; Nunoya et al., 1987, 1995). If *M. gallisepticum* infection is complicated by *E. coli*, extensive pneumonia and air sacculitis are common, along with fibrinopurulent pericarditis and perihepatitis (Gross, 1990). Young birds are more likely to show signs of clinical disease after exposure to more virulent strains of *Mycoplasmas* (Jordan, 1972).

***Mycloplasma* Isolation and Identification**

Researchers have documented that *M. gallisepticum* can be cultured from tracheal, air sac, lung, and sinus exudates (Kleven and Yoder, 1989),as well as choanal cleft (Branton et al., 1984; Brown et al., 1995) brain (Chin, 1991) oviduct (Yoder and Hofstad, 1964), liver ,spleen, uterus, vagina, (Suhu and Olson, 1976) and cloaca (Amin and Jordan, 1979; MacOwan, 1983) of birds. Isolation, identification, and serological tests, such as serum plate agglutination (**SPA**), hemagglutination inhibition (**HI**), and enzyme linked immunosorbent essay, ELISA and fluorescent antibody (**FA**) tests are used to diagnose *M. gallisepticum* infections. Because the SPA test is quick, relatively

inexpensive, and sensitive, it has been widely used as an initial screening test for flock monitoring and serodiagnosis. The HI test has been commonly used to confirm reactors detected by SPA or, more recently, ELISA. It is vital that *M. gallisepticum* infection diagnosis is accurate, because false positives or negative results can lead to great economic loss.

Effects of *M. gallisepticum* on Layer Performance

Egg producers experience lost revenues from poor feed conversion, decrease body weight (**BW**), and increased medication costs, in addition to drops in EP and poor egg quality (Mohammed et al., 1987; Patterson, 1994). It is thought the reduced feed consumption of infected layer hens may affect the necessary components of essential dietary factors necessary to sustain adequate egg formation and egg production (Burnham et al., 2002a). Birds infected with wild type *M. gallisepticum* have a drastic decrease in EP; therefore, vaccination programs and intense bio-security measure are vital to prevent substantial economic loss to egg facilities. Naturally infected flocks are shown to produce as many as 16 fewer eggs per hen per year than *M. gallisepticum* -negative flocks (Carpenter et al., 1981).

Control of *Mycoplasma* Infection

Eradication of *M. gallisepticum* is the ultimate goal for the poultry industry; however, in multi-age layer complexes, it is not economically feasible (Yoder, 1978). Although the poultry industry has been able to reduce the number of *Mycoplasma* infected birds, the ubiquitous nature of *M. gallisepticum* results in continuation of high

percentages of infected layers. A number of antibiotics have been used treat *M. gallisepticum* infected birds to minimized EP losses. *M. gallisepticum* is susceptible to a number of antibiotics: tylosin, tiamulin, gentamicin, tetracycline, streptomycin, erythromycin, spiromycin, valnemulin, enrofloxacin, lincomycin, and spectinomycin (Ose et al., 1979; Jordan et al., 1998; Timms et al., 1989).

Total eradication of *Mycoplasma* is the ideal; however, depopulation and disinfection of multi-age layer houses is too costly and impractical, the best solution in this case may be to establish an *M. gallisepticum* vaccination program (Bermudez and Kalbac, 1988). Vaccination programs, which stimulate the development of immunity to *Mycoplasmas*, are commonly practiced for protection in animals (Barile, 1985). Presently *M. gallisepticum* strains F, 685, and ts11 are the three live-attenuated vaccines in use. Use of live vaccines helps prevent large production losses when used on multiage layer facilities where *M. gallisepticum* is known to be present.

Effects of F- strain *M.gallisepticum* Vaccine on Layer Performance

Vaccination with F-strain *M. gallisepticum* has been predominantly used by commercial layers facilities maintaining multi-age layer facilities for the following reasons: it was the first live vaccine, USDA licensed, (Branton et. al., 1999), it has been proven to displace more virulent strains and wild-type *M. .gallisepticum*, and it has a lower transmission rate (Ley et al., 1997). Vaccination of young layers which have the potential to contract *M. gallisepticum* may increase their production rates. F-strain *M. gallisepticum* has been evaluated as a means of counteracting reductions in EP in

multiple-age egg-layer complexes caused by infections with wild or field strains of *M. gallisepticum* (Carpenter et al., 1981; Lin and Kleven, 1982; Hildebrand et al., 1983).

Inoculations with F-strain *M. gallisepticum* between 8 and 18 wk of age allow a pullet to receive a mild infection and recover before coming into EP (Yoder et al., 1984). Birds vaccinated with F-strain *M. gallisepticum* appear to be protected against wild field strains which cause greater EP losses (Levisohn and Kleven, 1981; Kleven et al., 1990). *M. gallisepticum* F-stain vaccine has strengths; however, it is not completely apathogenic (Lin and Kleven, 1982) and the spread to *M. gallisepticum*-free turkey and chicken farms has been reported. In situations where eradication appears unobtainable, controlling the production loses appears to be the only viable alternative (Mohammed et al., 1987; Patterson, 1994). Branton et al., (1997) reported that EP and other egg characteristic in birds inoculated with F-strain *M. gallisepticum* at 10 wk of age were not significantly different than controls. It has, however, been proven that F-strain *M. gallisepticum* given late in lay does reduce EP. In a more recent report by Burnham et al., (2002a), using birds inoculated at 12 wk of age, EP was reported to be significantly different in the F-strain vaccinated birds compared to the controls. Burnham et al., (2002a) reported that onset of lay was delayed and that a significant decrease in EP occurred. It was reported that inoculated hens had fewer mature ovarian follicles and a decreased magnal, isthmal and vaginal proportion of the reproductive tract at trial termination (60 wk) compared to F-strain *M. gallisepticum* free hens (Burnham et al., 2002a). Burnham et al., (2003b) suggested that the establishment of systemic F-strain *M. gallisepticum* infections and long term changes were the result of the disease rather than the F-strain *M. gallisepticum*

vaccination. To avoid careless use of F-strain *M. gallisepticum* vaccine, its use is under the supervision of official state veterinarians or regulatory agencies.

Effects of ts11 and 685- strain *M. gallisepticum* Vaccines on Layer Performance

More recently apathogenic ts11 and 685 *M. gallisepticum* vaccines have been licensed for use in the United States. These strains are not vertically (bird to egg) or horizontally (bird to bird) transmissible; however, they have not been proven under commercial conditions to have the ability to displace wild type or more virulent strains of *M. gallisepticum* (Turner and Kleven, 1998).

Branton et al., (2000) reported that under ideal conditions when ts11 vaccine given was given pre-lay ovaries and oviducts functioned similarly to control hens. Kleven et al., (1998) reported that all three of the live vaccine strains colonized the upper respiratory tract, but that re-isolation of 685 strains was inconsistent. They reported that serological response to F-strain *M . gallisepticum* was strongest and ts11 strain responses were intermediate, and that 685 responses were very weak. Kleven et al., (1998) also reported that F-strain vaccine readily displaces the more virulent R-strain *M. gallisepticum*, while ts11 and 685 vaccine strains show no ability to displace the more virulent R strain under the conditions of the experiment.

Effects of ts11 and F -strain *M. gallisepticum* on the Blood, Egg Yolk, Digestive, and Reproductive Organ Characteristics of Laying Hens

No information is available in the literature for blood, digestive and organ characteristics for birds inoculated with ts11- strain *M. gallisepticum*; however, Burnham

et al.,(2003a) in trials where birds were vaccinated with F-strain vaccine at 12 wk of age whole blood hematocrit (**HCT**) increased at 8 wk after challenge suggesting a compensatory polycythemic response to the F-strain vaccination but levels returned to those of the controls, indicating that the birds adjusted through other physiological means to the F-strain vaccination stressor. When a stressor such as F-strain *M. gallisepticum* is introduced, triglycerides (**ST**) may become elevated in response to the infection. An elevation in ST is known to be a common response to the presence of infectious disease agents (Guyton and Hall, 1996). Burnham et al., (2000) reported significant changes in HCT, plasma protein (**PP**), and serum cholesterol (**SC**), ST, and calcium (**SCA**) at some point when F-strain vaccine was given alone. *M. gallisepticum* has also been shown to affect the eggshell forming mechanisms (Domermuth et al., 1967; Burnham et al., (2003b) has also asserted that F-strain *M. gallisepticum* infection affects yolk lipid (significantly higher at wk 32 and 44, and lower at wk 48) and cholesterol concentrations (decreased at wk 22) as well as fatty acid composition. Yolk lipid concentration differences may be due to adaptations to infection, where the yolk fatty acid concentrations are indicative of changes in liver lipid synthesis. While the effects of F-strain *M. gallisepticum* on performance are noted by Burnham (Burnham et al., 2002a) among others, 685-stain *M. gallisepticum* is lacking in literature documenting its effect on performance (Branton et al., 2002).

In a study by Peebles et al., (2007), also using the F-strain a significant increase in fatty liver hemorrhagic syndrome (**FLHS**), as well as, increase in ovarian follicular regression, and decrease in isthmal and vaginal properties of the reproductive tract were observed for birds vaccinated with F-strain vaccine at 12 verses 22 wk Burnham et al.,

(2002b). Peebles et al., (2007) reported lower magnum weight in birds vaccinated with F-strain vaccine at 12 verses 22 wk. Relative duodenal length was also decreased in bird given the F-strain at 22 wk verses 12 wk.

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CHAPTER III

EFFECTS OF TIME SPECIFIC F-STRAIN *MYCOPLASMA GALLISEPTICUM* INOCULATION OVERLAYS ON PRE-LAY TS11-STRAIN *M. GALLISEPTICUM* INOCULATION ON PERFORMANCE CHARACTERISTICS OF COMMERCIAL LAYING HENS

ABSTRACT

Mycoplasma bacteria are virtually ubiquitous in layer chicken flocks and *M. gallisepticum* is the species of greatest concern to commercial egg producers. Live *M. gallisepticum* vaccines were initially approved by the USDA for use in commercial layers in 1988 to help control *M. gallisepticum* outbreaks. In the present study, 2 trials were conducted to determine the effects of 2 currently available live *Mycoplasma* vaccines (ts11- and F-strains) when used together. The following 4 inoculation treatments were utilized: 1) sham inoculation at 10 wk of age, 2) ts11 at 10 wk, 3) ts11 at 10 wk overlaid by F at 22 wk, and 4) ts11 at 10 wk overlaid by F at 45 wk. In each trial at various ages between 18 and 57 wk of age, hen mortality; BW; egg weight; egg production; eggshell breaking strength; incidences of egg blood spots, egg meat spots, and eggshell pimpling; and eggshell weight per unit of surface area were assessed. The effects of inoculation treatment on egg weight at 27, 37, and 38 wk were inconsistent and variable. Eggshell

pimpling and egg blood spot incidences at 56 wk were highest in eggs belonging to the ts11 at 10 wk / F at 45 wk group. Despite increases in pimpling and blood spot incidences very late in production due to the tsll at 10 wk F at 45 wk treatment, performance in layers was not adversely affected by a 10 wk ts11 inoculation alone or in conjunction with subsequent overlay inoculations of F during lay. It is, therefore, suggested that the 10 wk inoculation of commercial layers with ts-11 may reduce the negative impacts of a pre-lay F inoculation on performance, as reported in earlier studies, while providing protection against subsequent field strain *M. gallisepticum* infections. Furthermore, the ts-11- and F-strain *M. gallisepticum* treatment combinations may overcome some of the inadequacies that prelay ts-11- or F-strain *M. gallisepticum* vaccines may have when given independently.
(Key words: commercial layer, egg production, egg quality, F-strain *Mycoplasma gallisepticum*, ts11-strain *Mycoplasma gallisepticum*)

INTRODUCTION

Mycoplasma gallisepticum, the pathogen responsible for chronic respiratory disease in chickens, continues to be a concern to commercial table egg producers maintaining multi-age layer houses. Once a bird is infected with *M. gallisepticum*, it is considered to be chronically infected for life (Brown et al., 1995). Primarily a respiratory pathogen of meat-type chickens and turkeys and a reproductive pathogen of table egg chickens, *M. gallisepticum* results in great economic loss worldwide due to its ability to systemically infect individual birds and the ease at which it is transmitted between birds. Decreased egg production (**EP**) and hatchability, downgrading and condemnation of

carcasses, and reduced feed conversion can result from *M. gallisepticum* infections (Yoder and Hofstad, 1964; Domermuth et al., 1967; Patterson, 1994).

Vaccination programs are presently being used to help protect flocks from field strains of *Mycoplasma*. Three live vaccines are commercially available for use in the United States. The first live *M. gallisepticum* vaccine, referred to as F-strain, was approved for use in commercial table egg chickens by the USDA in 1988 (Branton et al., 1999). The F-strain *M. gallisepticum* vaccine strain is less virulent than many of the field strains of *M. gallisepticum* and has a lower vertical transmission rate, yet it is able to displace the more virulent stains of *M. gallisepticum* (Levisohn and Kleven, 1981; Kleven et al., 1990). Continuous use of F-strain *M. gallisepticum* vaccines for replacement flocks in multi-age commercial layer facilities has been shown to protect these flocks from field strains (Kleven et al., 1990). The F-strain *M. gallisepticum* vaccine, however, is pathogenic to turkeys, and is not approved or licensed for use in poultry other than commercial layers. In a controlled study in biological isolation units, early vaccination of commercial layers with F-strain *M. gallisepticum* did not adversely affect EP (Branton et al., 1997). Anecdotal evidence from producers that have used F-strain *M. gallisepticum* has also shown that no adverse effects on EP occur when F-strain *M. gallisepticum* vaccinations are provided pre-lay (personal communication, Jack Self, VP of operations, Cal Maine Foods, Inc.). However, some field studies have determined that F-strain *M. gallisepticum* vaccination can reduce EP when compared to *Mycoplasma*-free birds (Carpenter et al.,1981; Mohammed et al., 1987; Branton et al., 1988). More recently, apathogenic ts11-strain *M. gallisepticum* and 685-strain *M. gallisepticum* live vaccines have been licensed for use in layer chickens. These vaccines show virtually no

bird to bird transmission, but have not been proven to displace wild type *M. gallisepticum* (Kleven, 1998). Furthermore, these strains may not confer continued protection as does the F-strain throughout lay (Yoder, 1978, 1991; Mohammed et al., 1987). More testing is needed to determine if combinations of vaccines can lessen the adverse impact of prelay F-strain *M. gallisepticum* vaccinations on layer performance when given alone. Therefore, the objective of the current study was to determine the effects of pre-lay ts11-strain *M. gallisepticum* inoculations and time specific F-strain *M. gallisepticum* inoculation overlays administered during lay on the performance characteristics of commercial laying hens.

MATERIALS AND METHODS

Pullet Housing and Management

Two trials were performed using Hy-Line W-36 pullets that were obtained at 1 d of age from a commercial source that was monitored and certified free of both *M. gallisepticum* and *M. synoviae* (USDA-APHIS-VS, 2003). Chickens were vaccinated at 10 d of age for infectious bursal disease via the drinking water. At 5 wk of age, 20 randomly selected chickens were tested for antibodies to both *M. gallisepticum* and *M. synoviae* using both the serum plate agglutination and the hemagglutination-inhibition tests (Yoder, 1975) and swabs were obtained from the choanal cleft (Branton et al., 1984) and placed into tubes containing Frey's broth medium (Frey et al., 1968) supplemented with an additional 0.15 thallium acetate and 10^6 IU penicillin-G/mL. Tubes were incubated at 37 C for 30 d or until the phenol red indicator reaction occurred in the

media. A sample from those that changed color was then inoculated onto Frey's-based agar and incubated at 37 C. Colonies with morphology suggestive of *Mycoplasma* species were examined by an agar plate fluorescent antibody test (Baas and Jasper, 1972) that used direct labeling of colonies stained with anti-F-strain *M. gallisepticum* polyclonal antibodies produced in rabbits and labeled with fluorescein isothiocyanate (Kleven, 1981).

Pullets were placed on clean dry litter in a conventional house until 10 wk of age. A daily artificial lighting schedule followed a 13 L:11 D cycle. One 75-W incandescent light bulb was used to illuminate each 8.4 m^2 of floor space, providing a calculated intensity at bird level of 35.5 lx. Feed and water were provided for *ad libitum* consumption in each trial. At 10 wk of age, 11 pullets were randomly selected and placed in each of 16 negative pressure fiberglass biological isolation units (1.16 m^2). The units were housed in a previously described poultry disease isolation facility (Branton and Simmons, 1992). Hen numbers were reduced to 10 per unit at point-of-lay (22 wk of age) so that bird density was 0.116 m^2 / bird for the duration of each trial. At 18 wk of age, the length of the artificial lighting schedule was increased 15 min/d until a cycle of 16 h and 15 min of light per 7 h and 45 min of dark was achieved. Chickens were maintained on that schedule through the remainder of each of the trials. For the entirety of each trial, chickens had *ad libitum* access to feed and water. Pullet and layer diets were formulated to meet or exceed NRC (1994) recommendations. Ingredient percentages and calculated analyses of the diets were as described by Burnham et al., (2002). No medications were administered during either trial.

Treatments

Four experimental treatment groups were used. Each treatment group consisted of 4 isolation units containing 10 birds each for a total of 40 birds per treatment group. Treatment one (Controls) received no *M. gallisepticum* inoculation but were sham-inoculated via eye-drop in the right eye with sterile Frey's media. Treatment 2 contained birds that were eye-drop vaccinated in the right eye with ts11-strain *M. gallisepticum* at 10 wk of age (ts11/10). Birds belonging to treatment 3 received ts11-strain *M. gallisepticum* via eye-drop at 10 wk of age followed by a 22 wk overlay vaccination via eye-drop in the left eye with F-strain *M. gallisepticum* (ts11/10-F/22). Treatment 4 consisted of birds given ts11 strain *M. gallisepticum* at 10 wk of age via eye drop in the right eye followed by a 45 wk overlay vaccination of F-strain *M. gallisepticum* via eye drop in the left eye (ts11/10-F/45).

Data Collection

All data collected prior to wk 22 were designated as belonging to age interval I; all data collected from wk 22 - 44 was designated as belonging to age interval II; and all data collected from wk 45 - 57 were designated as belonging to interval III. Individual BW was recorded at 20 wk (interval I), at 24, 32, and 43 wk (interval II), and at 47 and 56 wk of age (interval III) in both trials 1 and 2. Egg production was recorded daily and analyzed weekly (weekly EP) from wk 23 (when Control treatment EP reached approximately 10%) through wk 44 (interval II) and from wk 45 through wk 55 (interval III) and was expressed as percent hen-day EP. Furthermore, the total number of eggs

produced per hen (total hen EP) from onset of lay through 55 wk (across all 3 intervals), and within intervals II (wk 22-44) and interval III (wk 45-55) separately, were likewise determined. In order to include all eggs that were laid prior to 10 % EP, the calculation of total hen EP across all 3 intervals was initiated when the first egg was laid (onset of lay). Age of onset of lay ranged between 18 and 21 wk. Total hen EP was calculated as total daily numbers of eggs produced as a percentage of total daily numbers of hens for each replicate group.

Beginning on wk 23 (when Control treatment EP reached approximately 10 %), eggs were collected 2 d per wk to determine egg weight (EW), eggshell breaking strength (ESS), and percentage incidences of eggshell pimpling (ESP), egg blood spots (EBS), and egg meat spots (EMS). Egg weight, ESS, ESP, EBS, and EMS determinations were recorded weekly in both trials from 23 to 57 wk of age. These determinations were made on all the same eggs that were produced within the 2 d time period. The ESS of each egg was determined by the technique described by Reece and Lott (1976), and ESP, EBS, and EMS incidences were determined using methods described by Branton et al., (1997). For determination of eggshell weight per unit of eggshell surface area (SWUSA), a total of 10 eggs per pen were weighed at 20, 24, 32, 43, and 46 wk of age in both trials. The eggs used for SWUSA measurements were different from the eggs used for determination of EW, ESS, ESP, EBS, and EMS. Determination of SWUSA was according to the procedure described by Peebles et al., (1994). All the above egg and eggshell determinations were recorded on the same day that the eggs were collected.

Statistical Analysis

A completely randomized experimental design, with trial as a block, was employed. Data prior to wk 22 (age interval I), from wk 22-44 (age interval II), and from wk 45-57 (age interval III) were analyzed separately. The data of both trials were pooled then analyzed together. Therefore, the results from both trials were not reported independently but were reported over both trials. Trial was considered as a random effect. All data within each age interval were subjected to a repeated measures analysis if parameters were examined at multiple age periods in an age interval. Otherwise, data (i.e. total hen EP) obtained within and across age interval was subjected to 1-way ANOVA.

In the first age interval, Controls and the ts11/10 treatment group were compared. In the second age interval, Controls and those having had ts11/10 and ts11/10-F/22 inoculations were compared. In the third age interval, Control, ts11/10, ts11/10-F/22, and ts11/10-F/45 groups were compared. Individual sample data within each of these replicate units were averaged prior to analysis. Least-squares means were compared in the event of significant global effects (Steel and Torrie, 1980). Global effects and differences among least-squares means were considered significant at $P \leq .05$. All data were analyzed using the MIXED procedure of SAS software (SAS Institute, 2003).

RESULTS AND DISCUSSION

All initial mycoplasmal cultures obtained from 5-wk old pullets, as well as SPA tests were negative for both *M. gallisepticum* and *M. synoviae*. As described by Zander (1984), *M. gallisepticum* is already established on many multi-age farms, and transmission from mature hens to replacement pullets ensures its existence. Therefore, in this study, birds were also later tested to ensure the absence of disease transmission between treatment groups. The aforementioned cultures and SPA tests were repeated at 27 and 51 wk. Throughout the 2 trials these sample tests on all 4 treatment groups showed that Control birds remained *Mycoplasma*–free (negative for both *M. gallisepticum* and *M. synoviae*), whereas the 3 vaccinated groups resulted in positive broth cultures, with FA and SPA results being positive for *M. gallisepticum* and negative for *M. synoviae*. The SPA and FA tests confirmed systemic infections in *M. gallisepticum*-inoculated birds, whether inoculated with the ts11- or F-strains of *M. gallisepticum*. Nevertheless, there was no significant difference in mortality between *M. gallisepticum*-free and *M. gallisepticum*-inoculated birds in either trial. More specifically, no significant age or treatment main effects or age by treatment interactions were found for bird mortality in any of the 3 age intervals examined. The few mortalities recorded had no apparent relationship to treatment application.

In large commercial multi-age layer facilities where depopulation followed by disinfection is impractical, vaccination programs are the best protection (Bermudez and Kalbac, 1988). Vaccination for diseases in animals is commonly practiced where the disease is endemic (Barile, 1985). Furthermore, early vaccination allows for the early

development of immunity to *Mycoplasmas* before EP begins. Therefore, in one of the treatment groups of this study, an early pre-lay vaccination was employed, and in 2 of the other treatments, the early vaccination was used in conjunction with later overlay vaccinations in order to confer continued protection during lay. Despite these treatment applications, only a main effect ($P \leq 0.04$) due to hen age for BW was observed in interval II; hen BW at wk 24, 32, and 43 were 1.34, 1.40, and 1.45 kg, respectively (pooled SEM = 0.022). Hen BW at wk 23 and 43 were significantly greater than that at wk 24, but BW at wk 32 and 43 were not significantly different. There was no treatment effect at wk 20 in interval I, and there was no significant treatment main effect or age by treatment interaction in interval II. In addition, there were no significant age or treatment main effects or an age by treatment interaction for BW in interval III. These BW data are consistent with those reported by Burnham et al., (2002), Peebles et al., (2007), and Viscione et al., (2008). Burnham et al., (2002) found no affect of a 12 wk F-strain *M. gallisepticum* inoculation on subsequent BW, Peebles et al., (2007) showed that BW was not affected by inoculation of F-strain *M. gallisepticum* at either 12 or 22 wk of age, and Viscione et al., (2008) noted no significant effects of F-strain *M. gallisepticum* inoculation at either 22 or 45 wk of age on layer hen BW. No previous research has reported the effects of pre-lay ts11-strain *M. gallisepticum* vaccinations on layer BW.

A hen age main effect was observed for weekly EP in interval III (wk 45-55; $P \leq 0.01$; data not shown); however, no significant treatment main effect or age by treatment interaction was observed for weekly EP in interval III. Also, no significant effects of any kind were observed for weekly EP in interval II. Furthermore, total hen EP across lay (intervals I - III) and total hen EP within interval II and within interval III were not

significantly affected by treatment. Although total hen EP within intervals II and III and across all 3 intervals was not affected by treatment, treatment means within interval II and interval III are provided in Table 1 for observation. In trials conducted by Branton et al., (1997), no negative effects were noted in EP or EW for birds that were challenged with F-strain *M. gallisepticum* prior to lay, when compared to control birds. The results of Branton et al., (1997) support other research previously conducted (Yoder 1978, 1991) and Mohammed et al., (1987) who reported that F-strain *M. gallisepticum* vaccination can reduce the negative impact of natural *M. gallisepticum* infections in layer flocks. However, in studies reported by Burnham et al., (2002), where egg-laying hens were inoculated at 12 wk of age with F-strain *M. gallisepticum*, it was reported that there was a decrease in total EP and that birds laid their first eggs approximately 1 wk later than controls that received only sham inoculations. Furthermore, Peebles et al., (2008) noted that F-strain *M. gallisepticum* inoculations given at either 12 or 22 wk of age decreased EP at the beginning of lay.

The lack of a pre-lay ts11-strain *M. gallisepticum* inoculation influence on EP in the current study suggests that the possible negative effect of a pre-lay F-strain *M. gallisepticum* inoculation on EP, as observed by Burnham et al., (2002) and Peebles et al., (2008), might be avoided by using a ts11/10 pre-lay vaccination program. This suggestion is supported by results from a study similar to the current one conducted by Branton et al., (2000), in which a pre-lay inoculation of ts11-strain *M. gallisepticum* alone was used. It was determined in that study that there were no significant differences in EP or other egg and eggshell quality parameters between the ts11-strain *M.*

gallisepticum vaccinated birds and controls, which indicated that a pre-lay ts11 vaccine had no negative impact on overall layer performance.

In a study reported by Branton et al., (1988), where layer hens were inoculated with F-strain *M. gallisepticum* at 45 wk of age, there was a significant decrease in EP compared to control birds that were not vaccinated. This was in agreement with earlier work published by Carpenter et al., (1981), who found that layers maintained free from *M. gallisepticum* infection laid an average of 8.7 eggs/ hen housed more than did flocks that were vaccinated with F-strain *M. gallisepticum* at 45 wk of age. The results of Branton et al., (1988), Carpenter et al., (1981), and Peebles et al., (2008) demonstrate further potential negative impacts of 22 and 45 wk F-strain *M. gallisepticum* inoculations on performance. Because EP was not affected by the usage of ts11/10-F/22 or ts11/10-F/45 vaccine treatments in the current 2 trials, it is also suggested that a pre-lay tsll/10 vaccination may help to prevent a significant drop in hen day EP when F-strain *M. gallisepticum* vaccination overlays are given at either 22 or 45 wk. A pre-lay tsll/10 inoculation might serve to reduce the negative impacts of F-strain *M. gallisepticum* inoculations given during lay.

An age by treatment interaction was observed for EW ($P \leq 0.04$) in interval II (Table 2.) At wk 27, EW was significantly larger in the ts11/10-F/22 treatment group compared to the Control and ts11/10 treatment groups. This was opposite to that at wk 38, where the Control and ts11/10 treatment birds had a greater EW than the ts11/10-F/22 birds. At wk 37, the Control and ts11/10-F/22 birds had a higher EW than the ts11/10 treatment group. There were no significant age or treatment main effects or age by treatment interactions in interval III. It has been noted that EW was unaffected by

inoculations with ts11-strain *M. gallisepticum* at 10 wk (Branton et al., 2000), F-strain *M. gallisepticum* at 12 wk (Burnham et al., 2002), F-strain *M. gallisepticum* at 12 and 22 wk (Peebles et al., 2008), and F-strain *M. gallisepticum* at 22 and 45 wk (Viscione et al., 2008). Branton et al., (1988) did find that F-strain *M. gallisepticum* inoculated at 45 wk increased EW over a 15-wk period, but this was noted in only 1 of 2 trials. Also, Burnham et al., (2002) found that the percentage of eggs belonging to the USDA undersized egg category decreased at 19 wk but later increased at 20 and 21 wk after a 12 wk F-strain *M. gallisepticum* inoculation. The inconsistent results of these aforementioned studies do not convincingly demonstrate that EW is susceptible to the various inoculation regimens described involving ts11- and F-strains of *M. gallisepticum*. However, despite the apparent innocuousness of a ts11/10 vaccination on EP, consideration should be given to possible subsequent effects that a ts11/10 vaccination might have on EW. This is suggested in this study because of the reduction in EW of the ts11/10 treatment group relative to the Control group at wk 37. Furthermore, these current data indicate that an overlay of F-strain *M. gallisepticum* at 22 wk may lead to respective increases and decreases in EW at 27 and 38 wk in birds that had been given ts11-strain *M. gallisepticum* vaccinations at 10 wk.

The effects of prelay and wk 22 F-strain *M. gallisepticum* inoculations on ESS, ESP, EBS, and EMS have not been previously investigated. Nevertheless, F-strain *M. gallisepticum* vaccinations at 45 wk (Branton et al., 1988) and ts11-strain *M. gallisepticum* vaccinations at 10 wk of age (Branton et al., 2000) have been reported to have no effect on these same parameters. However, in the current study, significant age-by- treatment interactions were observed for ESP ($P \leq 0.05$; Table 3) and EBS ($P \leq 0.05$;

Table 4) in interval III. At wk 53, ESP was significantly higher for the ts11/10-F45 treatment group than for the Control or ts11/10-F/22 treatment groups. The ESP of the ts11/10 treatment was not significantly different from the other 3 treatment groups. At wk 56, the ts11/10-F/45 treatment group had a significantly higher ESP than the Control, ts11/10, or ts11/10-F/22 treatment groups. Conversely, at wk 57, the ts11/10-F/45 treatment group was significantly lower than the Control or ts11/10 treatment group; however, the ts11/10-F/22 group was not significantly different from the other 3 groups. For EBS at wk 54, the incidence was higher for the ts/10-F/22 treatment when compared to the Control, ts11/10, and ts11/10-F/45 treatment groups. However, at wk 56, the ts11/10-F/45 treatment group had a significantly higher incidence of EBS than did the other 3 groups. There were no significant effects of any kind for ESP or EBS in interval II, for ESS or EMS in interval II or III, or for SWUSA in any of the 3 time intervals. The egg and eggshell quality parameters selected for these trials reflected the functionality of the ovary and specific segments of the oviduct as described by Branton et al., (2000). However, ESS, EMS, and SWUSA were not affected, and EW, ESP, and EBS were affected inconsistently by the combined vaccine treatments.

In conclusion, although possible effects of the combined vaccinations on EW, ESP, and EBS should be considered, they had no recurrent negative impact on egg and eggshell quality in the present study. Furthermore, the pre-lay ts11/10 vaccination appeared to prevent a decrease in EP when F-strain *M. gallisepticum* was given during lay. These results suggest that an overlay of F-strain *M. gallisepticum* on pre-lay ts11 vaccinated birds does not adversely impact layer performance. Each of the vaccine treatments examined in this study had specific strengths and weaknesses. As noted in

earlier work, the individual use of ts11- or F-strain *M. gallisepticum* vaccinations may respectively have an adverse effect on EP or may not confer continued protection against field strain *M. gallisepticum* infections. However, the ts11 and F-strain *M. gallisepticum* vaccine treatment combinations may overcome some of the weaknesses that pre-lay vaccines of ts11- or F- strains of *M. gallisepticum* may have if given alone. The pre-lay ts11- and lay F-strain *M. gallisepticum* vaccine combination shows promise as research continues to develop new and better vaccine protocols to eliminate the negative impacts of *Mycoplasma* vaccination. It is suggested that in order to better establish the protective benefits of a dual vaccination approach, this study should be followed by one using a virulent *M. gallisepticum* challenge during lay.

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TABLE 3.1. Total hen egg production (EP) of commercial layers in sham-inoculated control (Control), ts11 *M. gallisepticum* at 10 wk (ts11/10), ts11 *M. gallisepticum* at 10 wk with 22 wk F-strain *M. gallisepticum* overlay (ts11/10-F/22), and ts11 *M. gallisepticum* at 10 wk with 45 wk F-strain *M. gallisepticum* overlay (ts11/10-F/45) treatment groups within age intervals II (wk 22–44) and III (wk 45–55)[1]

	Age Interval	
Inoculation Treatment	II[2]	III[3]
	----------(%)----------	
Control	85.2	73.4
ts11/10	81.9	74.5
ts11/10-F/22	81.3	63.1
ts11/10-F/45	-----	70.2

[1]n = 4 replicate units for calculation of mean within each interval and inoculation treatment group.
[2]SEM based on pooled estimate of variance = 2.63.
[3]SEM based on pooled estimate of variance = 3.64.

TABLE 3.2. Egg weight (EW) of commercial layers in sham-inoculated control (Control), ts11 *M. gallisepticum* at 10 wk (ts11/10) and ts11 *M. gallisepticum* at 10 wk with 22 wk F-strain *M. gallisepticum* overlay (ts11/10-F/22) treatment groups at 27, 37, and 38 wk of age[1,2]

	Weeks of Age		
Inoculation Treatment	27	37	38
	----------(g)----------		
Control	50.7[b]	56.3[a]	56.8[a]
ts11/10	50.8[b]	53.9[b]	56.0[a]
ts11/10-F/22	53.1[a]	55.9[a]	52.9[b]

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]n = 4 replicate units for calculation of mean within each hen age and inoculation treatment group.

[2]SEM based on pooled estimate of variance = 0.75.

TABLE 3.3. Eggshell pimpling incidence (ESP) of commercial layers in sham-inoculated control (Control), ts11 *M. gallisepticum* at 10 wk (ts11/10), ts11 *M. gallisepticum* at 10 wk with 22 wk F-strain *M. gallisepticum* overlay (ts11/10-F/22), and ts11 *M. gallisepticum* at 10 wk with 45 wk F-strain *M. gallisepticum* overlay (ts11/10-F/45) treatment groups at 53, 56, and 57 wk of age[1,2]

	Weeks of Age		
Inoculation Treatment	53	56	57
	----------(%)----------		
Control	0.290[b]	0.223[b]	0.365[a]
ts11/10	0.389[ab]	0.273[b]	0.280[a]
ts11/10-F/22	0.301[b]	0.250[b]	0.268[ab]
ts11/10-F/45	0.502[a]	0.410[a]	0.143[b]

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]n = 4 replicate units for calculation of mean within each hen age and inoculation treatment group.

[2]SEM based on pooled estimate of variance = 0.0600.

TABLE 3.4. Egg blood spot incidence (EBS) of commercial layers in sham-inoculated control (Control), ts11 *M. gallisepticum* at 10 wk (ts11/10), ts11 *M. gallisepticum* at 10 wk with 22 wk F-strain *M. gallisepticum* overlay (ts11/10-F/22), and ts11 *M. gallisepticum* at 10 wk with 45 wk F-strain *M. gallisepticum* overlay (ts11/10-F/45) treatment groups at 54 and 56 wk of age[1, 2]

	Weeks of Age	
Inoculation Treatment	54	56
	----------(%)----------	
Control	0.000[b]	0.041[b]
ts11/10	0.016[b]	0.018[b]
ts11/10-F/22	0.183[a]	0.025[b]
ts11/10-F/45	0.018[b]	0.161[a]

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]n = 4 replicate units for calculation of mean within each hen age and inoculation treatment group.

[2]SEM based on pooled estimate of variance = 0.0300.

CHAPTER IV

EFFECTS OF TIME SPECIFIC F-STRAIN *MYCOPLASMA GALLISEPTICUM* INOCULATION OVERLAYS ON PRE-LAY TS11-STRAIN *MYCOPLASMA GALLISEPTICUM* INOCULATION ON BLOOD CHARACTERISTICS OF COMMERCIAL LAYING HENS

ABSTRACT

It has been established that *Mycoplasma gallisepticum* is a cause of great concern to commercial egg producers with multi-age layer facilities complexes. Vaccination programs have been implemented by many commercial egg producers to minimize egg production losses due to infection with wild strains of *M. gallisepticum*. When F-strain *M. gallisepticum* vaccine is given pre-lay, EP losses are decreased and the layer is protected from the more virulent strains of *M. gallisepticum.* The F-strain *M. gallisepticum* vaccine was the first live vaccine licensed for use in the United States; however, it is not completely apathogenic. Other vaccine strains (ts11 and 685) that are apathogenic have been licensed for use more recently. It has been proven that F-strain *M. gallisepticum* vaccine can displace more pathogenic strains of *M. gallisepticum.* Neither ts11 or 685 vaccine strains have been proven to displace wild type *M. gallisepticum* In the present study, two trials were conducted to determine the effects of two currently available live *Mycoplasma gallisepticum* vaccines (ts11 and F-strains) when used in

combination. The following 4 inoculation treatments were utilized: 1) sham inoculation at 10 wk of age, 2) ts11 at 10 wk, 3) ts11 at 10 wk overlaid by F at 22 wk, and 4) ts11 at 10 wk overlaid by F at 45 wk. Effects of vaccinations with the ts11 and F-strain *M. gallisepticum* on the blood characteristics of commercial Single Combed White Leghorn laying hens were investigated throughout lay. Variables measured in both trials were whole blood hematocrit, plasma protein, and serum cholesterol, triglycerides and calcium. No significant age or treatment main affects or age by treatment interactions were observed for any of the observed blood parameters except for serum calcium. Mean calcium levels were higher in the ts11/10 and ts11/-F/22 treatments in comparison to controls between 23 and 44 weeks of age. Overall it suggests that ts11 vaccination at 10 wk of age alone or combined with the F-strain vaccine overlays at either 22 or 45 wk did not adversely affect the blood of the treated birds.
(*Key words*: blood, hematology: commercial layer, F-strain *Mycoplasma gallisepticum*, ts11-strain *Mycoplasma gallisepticum*)

INTRODUCTION

Vaccine programs have been implemented by egg producers maintaining multi-age layer facilities to prevent losses from *Mycoplasma gallisepticum* (Carpenter et al., 1981; Mohammed et al., 1987; Kleven, 1998). Three live *M. gallisepticum* vaccines have been licensed for use to help prevent/control *M. gallisepticum* outbreaks (Evans et al., 1992; Ley et al., 1997). The F-strain *M. gallisepticum* vaccine strain has predominantly been used because of its proven ability not only to protect layers from field strains, but

also to displace more virulent field strains of *M. gallisepticum* and has a lower bird to bird transmission rate (Levisohn and Kleven, 1981; Kleven et al., 1990).

The F-strain *M. gallisepticum* vaccine, however, is not completely apathogenic (Lin and Kleven, 1982) and the spread to *M.gallisepticum*-free turkey and chicken farms has been reported (Ley et. al., 1993, Turner and Kleven, 1998). Apathogenic strains ts11 and 685 *M. gallisepticum* are considered to be safer than F-strain vaccines with little to no potential of spreading from bird to bird (Levisohn and Kleven, 1981; Kleven et al., 1990). The ts11 and 685-strains of vaccine have not proven to displace wild type *M. gallisepticum* (Kleven, 1998). Furthermore, these strains may not confer continued protection as does the F-strain throughout lay (Yoder, 1978, 1991; Mohammed et al., 1987).

Differential leukocyte counts of birds after exposure to *Mycoplasma* species have been described (Kerr and Olson, 1970; Branton et al., 1997); however, further characterization studies of the blood from birds infected with F-strain *M. gallisepticum* have just recently been initiated (Burnham et al., 2003) with no literature available for blood characteristics when birds are vaccinated with ts-11 strain *M. gallisepticum* vaccine. More testing is needed to determine if combinations of vaccines can lessen the impact of pre-lay F-strain *M. gallisepticum* vaccination on layer blood characteristics. Therefore, the objective of the current study was to determine the effects of pre-lay ts11-strain *M. gallisepticum* inoculations and time specific F-strain *M. gallisepticum* inoculation overlays administered during lay on blood characteristics of commercial laying hens.

MATERIALS AND METHODS

Pullet Housing and Management

Two trials were performed using Hy-Line W-36 pullets that were obtained at 1 d of age from a commercial source that was monitored and certified free of both *M. gallisepticum* and *M. synoviae* (USDA-APHIS-VS, 2003). Chickens were vaccinated at 10 d of age for infectious bursal disease via the drinking water. At 5 wk of age, 20 randomly selected chickens were tested for antibodies to both *M. gallisepticum* and *M. synoviae* using both the serum plate agglutination and the hemagglutination-inhibition tests (Yoder, 1975) and swabs were obtained from the choanal cleft (Branton et al., 1984) and placed into tubes containing Frey's broth medium (Frey et al.,1968) supplemented with an additional 0.15 thallium acetate and 10^6 IU penicillin-G/mL. Tubes were incubated at 37^o C for 30 d or until the phenol red indicator reaction occurred in the media. A sample from those that changed color was then inoculated onto Frey's-based agar and incubated at 37 C. Colonies with morphology suggestive of *Mycoplasma* species were examined by an agar plate fluorescent antibody test (Baas and Jasper, 1972) that used direct labeling of colonies stained with anti-F-strain *M. gallisepticum* polyclonal antibodies produced in rabbits and labeled with fluorescein isothiocyanate (Kleven, 1981).

Pullets were placed on clean dry litter in a conventional house until 10 wk of age. A daily artificial lighting schedule followed a 13 L:11 D cycle. One 75-W incandescent light bulb was used to illuminate each 8.4 m^2 of floor space, providing a calculated

intensity at bird level of 35.5 lx. Feed and water were provided for *ad libitum* consumption in each trial. At 10 wk of age, 11 pullets were randomly selected and placed in each of 16 negative pressure fiberglass biological isolation units (1.16 m^2). The units were housed in a previously described poultry disease isolation facility (Branton and Simmons, 1992). Hen numbers were reduced to 10 per unit at point-of-lay (22 wk of age) so that bird density was 0.116 m^2 / bird for the duration of each trial. At 18 wk of age, the length of the artificial lighting schedule was increased 15 min/d until a cycle of 16 h and 15 min of light per 7 h and 45 min of dark was achieved. Chickens were maintained on that schedule through the remainder of each of the trials. For the entirety of each trial, chickens had *ad libitum* access to feed and water. Pullet and layer diets were formulated to meet or exceed NRC (1994) recommendations. Ingredient percentages and calculated analyses of the diets were as described by Burnham et al., (2002). No medications were administered during either trial.

Treatments

Four experimental treatment groups were used. Each treatment group consisted of 4 isolation units containing 10 birds each for a total of 40 birds per treatment group. Treatment one **(Controls)** received no *M. gallisepticum* inoculation but were sham-inoculated via eye-drop in the right eye with sterile Frey's media. Treatment 2 contained birds that were eye-drop vaccinated in the right eye with ts11-strain *M. gallisepticum* at 10 wk of age **(ts11/10)**. Birds belonging to treatment 3 received ts11-strain *M. gallisepticum* via eye-drop at 10 wk of age followed by a 22 wk overlay vaccination via eye-drop in the left eye with F-strain *M. gallisepticum* **(ts11/10-F/22)**. Treatment 4

consisted of birds given ts11 strain *M. gallisepticum* at 10 wk of age via eye drop in the right eye followed by a 45 wk overlay vaccination of F-strain *M. gallisepticum* via eye drop in the left eye **(ts11/10-F/45)**.

Data Collection

Birds were bled from the left wing vein from two hens per isolation unit. In each trial, hen blood was drawn and harvested at the same time of day at 22, 24, 32, 43, and 55 wk of age. Results were recorded at 22, 24, 32, and 43 wk (interval I), and at and 55 wk of age (interval II) in both trials 1 and 2. Effects of vaccinations with the ts11 and F-strain *M. gallisepticum* on the blood characteristics of commercial Single Combed White Leghorn laying hens were investigated throughout lay. Variables measured in both trials were whole blood hematocrit **(HCT)**, plasma protein **(PP)**, and serum cholesterol **(SC)**, triglycerides **(ST)**, and calcium **(SCA)**. Hematocrit was expressed as percentage blood packed cell (primarily red blood cell) volume, was determined through use of capillary tubes that were centrifuged in a micro-HCT centrifuge and were then read with a microcapillary reader. Serum cholesterol and ST expressed in milligrams per deciliter and PP expressed in grams per deciliter were determined by placing 10 μL of serum or plasma for each test on test slides, which were analyzed on a Kodak Ektachem DT-60 analyzer[5] as described by Latour et al., (1996). Similarly, SCA concentrations expressed in milligrams per deciliter were determined by placing 10 μL of serum on a test slide which was analyzed on a Kodak Ektachem DTSC module analyzer5, according to procedures of Tietz (1986). Control analyses were performed to assure that each sample was in the appropriate test range for accurate analysis.

Statistical Analysis

A completely randomized experimental design, with trial as a block, was employed. Data from wk 22-44 (age interval I), and from wk 45-56 (age interval II) were analyzed separately. The data of both trials were pooled then analyzed together. Therefore, the results from both trials were not reported independently but were reported over both trials. Trial was considered as a random effect. All data within each age interval were subjected to a repeated measures analysis because parameters were examined at multiple age periods in each age interval

In the first age interval, Controls and those having had ts11/10 and ts11/10-F/22 inoculations were compared. In the second age interval, Control, ts11/10, ts11/10-F/22, and ts11/10-F/45 groups were compared. Individual sample data within each of these replicate units were averaged prior to analysis. Least-squares means were compared in the event of significant global effects (Steel and Torrie, 1980). Global effects and differences among least-squares means were considered significant at $P \leq 0.05$. All data were analyzed using the MIXED procedure of SAS software (SAS Institute, 2003).

RESULTS AND DISCUSSION

All initial mycoplasmal cultures obtained from 5-wk old pullets, as well as SPA tests were negative for both *M. gallisepticum* and *M. synoviae*. As described by Zander (1984), *M. gallisepticum* is already established on many multi-age farms, and transmission from mature hens to replacement pullets ensures its existence. Therefore, in this study, birds were also later tested to ensure the absence of disease transmission between treatment groups. Cultures and SPA test results on Control birds remained

negative throughout the 2 trials for both *M. gallisepticum* and *M. synoviae*. The aforementioned tests were repeated throughout the trial. These sample tests for all 4 treatment groups showed that Controls remained *Mycoplasma* - free, whereas the 3 vaccinated groups resulted in positive broth cultures with FA and SPA results being positive for *M. gallisepticum* and negative for *M. synoviae*. Throughout the study, SPA and FA tests confirmed systemic infections in *M. gallisepticum*-inoculated birds, whether the inoculation was with the ts11- or F-strains of *M. gallisepticum*

Previous studies have suggested that *M. gallisepticum* moves through the blood and has the ability to colonize the liver, as well as the reproductive organs in the bird. Estrogen release and the onset of egg production in laying hens drastically increases liver metabolism (Lorentz et al., 1938) and its production of neutral lipids (Heald and Badman, 1963), ST and phospholipids (Dashti et al., 1983). Eventually, these components are destined for yolk lipid deposition (Nimpf and Schneider, 1991; Walzem et al., 1999). Colonization of the liver by F-strain MG (F-strain *M. gallisepticum*) may negatively affect liver metabolism (Sahu and Olson, 1976). *M. gallisepticum* infections are often subclinical unless a secondary infection is present or outside stressors are present. Manifestations of *M. gallisepticum* infections usually occur in the respiratory system and lesions become extensive when complicated by other bacteria. The birds in this experiment were housed in biological isolation chambers and not exposed to other infective agents that could cause secondary infections or any outside stressors.

A treatment main affect ($P \leq 0.02$) was observed for SCA in response to the pre-lay ts/11-F/22 strain *M. gallisepticum* inoculation in interval II. Mean SCA for control, ts11/10, and ts11/10-F/22, treatments were 22.5, 26.7, and 28.4 mg/dL, respectively

(Pooled SEM = 1.50). Mean SCA levels were significantly higher in the ts11/10 and ts11/10-F/22 treatment groups in comparison to controls. Neither Peebles et al., (2007) nor Burnham et al., (2002a, 2003) reported changes in SCA; however, in this study, there was a significant increase in SCA in interval I for the treated birds. Nevertheless, the change of SCA at that time does not appear to be impacting the performance of the birds under these controlled conditions. It would be useful to repeat this experiment in a commercial facility where the layer hens were exposed to normal stressors and diseases. It is possible that the increase of SCA could contribute to weaker bones if excessive SCA is being pulled from the bones. According to Burnham et al., (2003), in trials where birds were vaccinated with F-strain vaccine at 12 wk of age and maintained under the same aforementioned conditions, HCT increased at 8 wk after challenge suggesting a compensatory polycythemic response the to the F-strain vaccination. Hematocrit levels returned to those of the controls, after this time, indicating that the birds adjusted through other physiological means to the F-strain vaccination stressor. In a previous report (Burnham et al., 2002a), these same birds also exhibited a subsequent delay in onset of lay and a decrease in total egg production in response to F-strain inoculation. In the present study where ts11 vaccine was given at 10 wk and F-strain vaccine was either given as an overlay at 22 wk or 45 wk, no significant treatment or age main affects or age by treatment interactions for HCT were observed when comparing each of the treatment groups with control birds. This in turn suggests that none of the treatment vaccinations increase stress on the layer hens.

When comparing the HCT results in this study to the aforementioned study (Burnham et al., 2002a), it appears that the 10 wk ts11 vaccination may have prevented

the stress response to either of the subsequent F-strain vaccinations. When a stressor such as F-strain *M. gallisepticum* is introduced, ST may become elevated in response to the infection. An elevation in ST is known to be a common response to the presence of infectious disease agents (Guyton and Hall, 1996). Burnham et al., (2003) under the same experimental conditions reported significant changes in each of the observed blood characteristics at some point when F-strain vaccine was given alone. In the present study, there were no significant age or treatment main effects or age by treatment interactions for ST or PP in either trial or for SCH in either trial.

An increase in PP and HCT which is often the result of dehydration has been reported by Burnham et al., (2003), to occur in response to pre-lay F strain *M. gallisepticum* ; however, this study did not observe an increase in either PP or HCT. This suggests that ts11/10 vaccination may prevent the dehydration that causes changes in PP and HCT (Boyd, 1981; Warriss et al., 1997). This further suggests that the 10 wk ts11 vaccination may prevent stress from the F-strain vaccinations given either at 22 or 45 wk of age. The results of the current study, when observing EP and blood characteristics suggests that the possible stress of a 12 wk F-strain *M. gallisepticum* inoculation on EP and blood characteristics, as observed by Burnham et al., (2002), might be avoided by using a ts11/10 pre-lay vaccination program.

In conclusion, although possible effects of the combined vaccinations on blood characteristics should be considered, they had no significant negative impacts on blood characteristics in the present study. Each of the vaccine treatments examined in this study had specific strengths and weaknesses; however, the ts11 and F-strain *M. gallisepticum* vaccine treatment combinations may overcome some of the weaknesses

that ts11 or F- strains of *M. gallisepticum* may have if given alone. This vaccine combination shows promise as research continues to develop new and better vaccine protocols to eliminate the negative impacts of *Mycoplasma* vaccination.

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CHAPTER V

EFFECTS OF TIME SPECIFIC F-STRAIN *MYCOPLASMA GALLISEPTICUM* INOCULATION OVERLAYS ON PRE-LAY TS11- STRAIN *MYCOPLASMA GALLISEPTICUM* INOCULATION ON DIGESTIVE AND REPRODUCTIVE ORGAN CHARACTERISTICS OF COMMERCIAL EGG LAYING HENS

ABSTRACT

Outbreaks of *Mycoplasma gallisepticum* infections can have devastating affects on commercial layer facilities. Three live vaccines are now available to control *M. gallisepticum* outbreaks. Experimental inoculation with the F-strain of *Mycoplasma gallisepticum* between 8 and 18 wk of age is known to affect reproductive performance in commercial layers. Two trials were conducted to observe the effects of 2 currently available live *Mycoplasma* vaccines (ts11- and F-strains) used in conjunction on digestive and reproductive organs. In the present study, the following 4 inoculation treatments were utilized: 1) sham inoculation at 10 wk of age, 2) ts11 at 10 wk, 3) ts11 at 10 wk overlaid by F at 22 wk, and 4) ts11 at 10 wk overlaid by F at 45 wk. Necropsy at the end of both trials using two birds per replication unit to observe the results on inoculated with liver weight, liver lipid and moisture contents, ovary weight, ovarian follicular hierarchy, and the weights, lengths, and histologies of the infundibulum, magnum, isthmus, uterus, and

vagina. In both trials, treatment groups showed no significant change in the average number of mature (diameter ≥ 12 mm) ovarian follicles in comparison to controls

(*Key words*: layer, liver, *Mycoplasma gallisepticum*, reproductive tract, small intestine)

INTRODUCTION

Mycoplasma gallisepticum is primarily a respiratory pathogen of meat-type chickens and turkeys, and a reproductive pathogen of table egg chickens. The impact of *M. gallisepticum* outbreaks, the ease of disease transmission and resulting systemic infection causes *M. gallisepticum* to be worrisome organism for egg layer facilities maintaining multiage layer facilities. Vaccination programs are presently being used to prevent egg production (**EP**) losses and control outbreaks of the more virulent strains. Three live vaccines are currently licensed for use in the United States. *M. gallisepticum* F-strain has been licensed for use the longest and has been proven to protect layers from wild strain *M. gallisepticum,* but is mildly pathogenic. More recently the two apathogenic strains are ts11-strain *M. gallisepticum* and 685-strain *M. gallisepticum* were licensed for use in the United States. These vaccines show virtually no bird to bird transmission, but have not been proven to displace wild type *M. gallisepticum* (Kleven et al., 1998) or confer continued protection throughout lay (Yoder, 1978, 1991; Mohammed et al., 1987).

Mycoplasma gallisepticum has the ability to not only colonize the respiratory tract of layers, but also, the liver and reproductive organs in the bird. It is often asymptomatic unless a secondary infection is involved or outside stressors such as ammonia, dust, crowding, cold/hot temperatures. Interactions with other viruses and bacteria that can occur under normal growing conditions can exacerbate and confuse the symptoms of *M.*

gallisepticum infections (Saif et al.,1970, Jordan; 1972, Springer et al, 1974;Kleven et al., 1998).

More testing is needed to determine if combinations of vaccines can lessen the impact of pre-lay F-strain *M. gallisepticum* vaccination. Therefore, the objective of the current study was to determine the effects of pre-lay ts11-strain *M. gallisepticum* inoculations and time specific F-strain *M. gallisepticum* inoculation overlays administered during lay on the performance characteristics of commercial laying hens.

MATERIALS AND METHODS

Pullet Housing and Management

Two trials were performed using Hy-Line W-36 pullets that were obtained at 1 d of age from a commercial source that was monitored and certified free of both *M. gallisepticum* and *M. synoviae* (USDA-APHIS-VS, 2003). Chickens were vaccinated at 10 d of age for infectious bursal disease via the drinking water. At 5 wk of age, 20 randomly selected chickens were tested for antibodies to both *M. gallisepticum* and *M. synoviae* using both the serum plate agglutination and the hemagglutination-inhibition tests (Yoder, 1975) and swabs were obtained from the choanal cleft (Branton et al., 1984) and placed into tubes containing Frey's broth medium (Frey et al., 1968) supplemented with an additional 0.15 thallium acetate and 10^6 IU penicillin-G/mL. Tubes were incubated at 37^o C for 30 d or until the phenol red indicator reaction occurred in the media. A sample from those that changed color was then inoculated onto Frey's-based agar and incubated at 37^o C. Colonies with morphology suggestive of *Mycoplasma*

species were examined by an agar plate fluorescent antibody test (Baas and Jasper, 1972) that used direct labeling of colonies stained with anti-F-strain *M. gallisepticum* polyclonal antibodies produced in rabbits and labeled with fluorescein isothiocyanate (Kleven, 1981).

Pullets were placed on clean dry litter in a conventional house until 10 wk of age. A daily artificial lighting schedule followed a 13 L:11 D cycle. One 75-W incandescent light bulb was used to illuminate each 8.4 m^2 of floor space, providing a calculated intensity at bird level of 35.5 lx. Feed and water were provided for *ad libitum* consumption in each trial. At 10 wk of age, 11 pullets were randomly selected and placed in each of 16 negative pressure fiberglass biological isolation units (1.16 m^2). The units were housed in a previously described poultry disease isolation facility (Branton and Simmons, 1992). Hen numbers were reduced to 10 per unit at point-of-lay (22 wk of age) so that bird density was 0.116 m^2 / bird for the duration of each trial. At 18 wk of age, the length of the artificial lighting schedule was increased 15 min/d until a cycle of 16 h and 15 min of light per 7 h and 45 min of dark was achieved. Chickens were maintained on that schedule through the remainder of each of the trials. For the entirety of each trial, chickens had *ad libitum* access to feed and water. Pullet and layer diets were formulated to meet or exceed NRC (1994) recommendations. Ingredient percentages and calculated analyses of the diets were as described by Burnham et al., (2002). No medications were administered during either trial.

Treatments

Four experimental treatment groups were used. Each treatment group consisted of 4 isolation units containing 10 birds each for a total of 40 birds per treatment group. Treatment one **(Controls)** received no *M. gallisepticum* inoculation but were sham-inoculated via eye-drop in the right eye with sterile Frey's media. Treatment 2 contained birds that were eye-drop vaccinated in the right eye with ts11-strain *M. gallisepticum* at 10 wk of age **(ts11/10)**. Birds belonging to treatment 3 received ts11-strain *M. gallisepticum* via eye-drop at 10 wk of age followed by a 22 wk overlay vaccination via eye-drop in the left eye with F-strain *M. gallisepticum* **(ts11/10-F/22)**. Treatment 4 consisted of birds given ts11 strain *M. gallisepticum* at 10 wk of age via eye drop in the right eye followed by a 45 wk overlay vaccination of F-strain *M. gallisepticum* via eye drop in the left eye **(ts11/10-F/45)**.

Data Collection

At the end of each trial (wk 56), 2 birds from each replicate unit were euthanized by cervical dislocation, their organs were removed, and the following parameters were determined: liver weight, color, and moisture and lipid content; ovary weight, mature follicle number (those that are ≥ 12 mm in diameter); total oviduct weight and length, total small intestine weight and length; oviduct segments (infundibulum, magnum, isthmus, and vagina) weights and lengths, and small intestine segments (duodenum, jejunum, and ileum), weights and lengths; total oviduct and small intestine weights were calculated as percentages of BW. Furthermore, oviduct and small intestine segment

weights were calculated as percentages of BW and total organ weight and oviduct and small intestine segment lengths were calculated as percentages of total organ length. The number of mature follicles in an ovary was assigned a category from zero to six, where zero indicates the absence of mature follicles, and where six is the number of maximum follicles recorded. The percentage of birds in each unit possessing zero, one, two, three, four, five, or six follicles was calculated. Livers were examined for color and for incidence of FLHS. Three categories were used for liver color and for incidence of FLHS. Three categories were used for liver color assignment: neutral, red, and yellow. The number of birds in each unit possessing a particular liver color was expressed as a percentage of total number of birds examined. Also, birds with normal livers or those exhibiting FLHS were calculated as percentages of the total number of birds in each unit.

Statistical Analysis

A completely randomized experimental design, with trial as a block, was employed. The data of both trials were pooled then analyzed together. Therefore, the results from both trials were not reported independently but were reported over both trials. Trial was considered as a random effect. All data was subjected to 1-way ANOVA. Individual sample data within each of these replicate units were averaged prior to analysis. Least-squares means were compared in the event of significant global effects (Steel and Torrie, 1980). Global effects and differences among least-squares means were considered significant at $P \leq .05$. All data were analyzed using the MIXED procedure of SAS software (SAS Institute, 2003).

RESULTS AND DISCUSSION

All initial mycoplasmal cultures obtained from 5-wk old pullets, as well as SPA tests, were negative for both *M. gallisepticum* and *M. synoviae*. Cultures and SPA test results on Control birds remained negative throughout the 2 trials for both *M. gallisepticum* and *M. synoviae*. The aforementioned tests were repeated at throughout the experiment. These sample tests for all 4 treatment groups showed that Controls remained *Mycoplasma* -free, whereas the 3 vaccinated groups resulted in positive broth cultures with FA and SPA results being positive for *M. gallisepticum* and negative for *M. synoviae*. Throughout the study, SPA and FA tests confirmed systemic infections in *M. gallisepticum*-inoculated birds, whether the inoculation was with the ts11- or F-strains of *M. gallisepticum*.

Vaccination for diseases in animals is commonly practiced where the disease is endemic (Barile, 1985). Furthermore, early vaccination allows for the early development of immunity to *Mycoplasmas* before egg production begins. No information is available in the literature for digestive and organ characteristics for birds inoculated with ts11-strain *M. gallisepticum*. Burnham et al., (2002b) reported that when F-strain *M. gallisepticum* vaccination was given at 12 wk of age, egg production and other egg characteristics beginning at onset of lay (18 wk of age) in birds inoculated with F -strain M. *gallisepticum* at 12 wk of age were different from un-inoculated controls. Burnham et al., (2002a) also reported that initiation of lay was delayed and that weekly egg production after 42 wk and overall average weekly egg production were reduced in layer hens inoculated with F -strain M. *gallisepticum* at 12 wk of age. Peebles et al., (2007) reported that lower magnum weight in birds vaccinated with F-strain vaccine at 12 wk

22. Percentage duodenal was also decreased in bird given the F-strain at 22 wk versus 12 wk.

M. gallisepticum can be transmitted both vertically (hen to eggs), (Glisson et al., 1984), and horizontally (bird to bird) *M. gallisepticum* can be cultured from many different organs in the layer hens. It has been cultured from the oviduct (Carlson and Howell, 1967; Domermuth et al., 1967; Hitchner et al., 1980), liver, spleen, uterus, and vagina (Sahu and Olson, 1976) and cloaca (Amin and Jordan, 1979; MacOwan et al., 1983) of chickens. Branton et al., (1997) suggest that certain egg and eggshell quality parameters can reflect the functioning of both ovary and special segments of oviduct. The ability of *M. gallisepticum* to colonize liver and reproductive organs or the bird suggests that possible quality parameters of egg production and quality could occur due to organ damage Hepatic lipidosis referred to as fatty liver syndrome, often precedes fatty liver hemorrhagic syndrome (FLHS), which has been associated with environmental heat stress in birds (Riddell,1997). However, there was no treatment main effect for all of parameters investigated at wk 56 except for the vagina as percent of the oviduct length. There was a significant ($P \leq 0.007$) main effect due to treatments for vagina length as a percent of the total oviduct length. Mean vagina length as a percentage of total oviduct length for the control, ts11/10, ts11/10-F/22, and ts11/-F/45, treatment groups were 5.49, 7.30, 5.64, and 5.36 percent respectively (Pooled SEM = 1.419). Mean vagina length as a percentage of total oviduct length was significantly higher in the ts11/10 treatment group in comparison to all the other 3 treatment groups.

In a study using ts11 vaccine, by Branton et al., (2000), egg production and egg quality parameters selected reflected the functionality of the ovary and specific segments

of the oviduct as described by Branton et al., (2000). No significant differences were observed for EP, blood/meat spots, egg size, mortality, or HU scores. This suggested that the ovary and various segments of the oviduct were not compromised by the ts11 vaccine. Furthermore, gross and histological changes were not observed upon necropsy. These findings appear to agree with the results of the present study.

However, Burnham et al., (2002b) reported that hen inoculated with F-strain *M. gallisepticum* at 12 wk had fewer mature ovarian follicles and a decreased magnal, isthmal and vaginal proportion of the reproductive tract at trial termination (60 wk) compared to F-strain *M. gallisepticum* free hens. Burnham suggested that the establishment of systemic F-strain *M.gallisepticum* infections and long term changes were the result of the disease rather than the F-strain *M.gallisepticum* vaccination.

The data in this trial differs from the above literature in that the significant treatment main effect was observed for interval II was isolated in the vagina as percent of the oviduct. The significance of the vagina to the overall performance is limited. Therefore, it is suggested that inoculation regime did not have an adverse effect on digestive or reproductive physiology.

Each of the vaccine treatments examined in this study had specific strengths and weaknesses. However, it did appear that the ts11 and F-strain *M. gallisepticum* vaccine treatment combinations overcame some of the weaknesses of pre-lay vaccines in which ts11- or F- strains of *M. gallisepticum* were given alone. In conclusion, the pre-lay ts11- and lay F-strain *M. gallisepticum* vaccine combination shows promise as research continues to develop new and better vaccine protocols to eliminate the negative impacts of *Mycoplasma* vaccination.

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CHAPTER VI

EFFECTS OF TIME SPECIFIC F-STRAIN *MYCOPLASMA GALLISEPTICUM* INOCULATION OVERLAYS ON PRE-LAY TS11-STRAIN *MYCOPLASMA GALLISEPTICUM* INOCULATION ON EGG YOLK COMPOSITIONS AND HAUGH UNIT SCORES OF COMMERCIAL LAYING HENS

ABSTRACT

Mycoplasma infections are virtually endemic in multiage layer chicken flocks with *M. gallisepticum* being the species of greatest concern to commercial egg producers. Live *M. gallisepticum* vaccines are presently being used to help control *M. gallisepticum* outbreaks. In the present study, two trials were conducted to compare the effects of two currently available live *Mycoplasma* vaccines (ts11- and F-strains) used in conjunction. The following 4 inoculation treatments were utilized: 1) sham inoculation at 10 wk of age, 2) ts11 at 10 wk, 3) ts11 at 10 wk overlaid by F at 22 wk, and 4) ts11 at 10 wk overlaid by F at 45 wk. In each trial at various ages between 23 and 57 wk of age, percent yolk moisture, percent yolk lipid, percent yolk weight, percent albumen weight, percent shell weight, and Haugh unit scores were assessed. At wk 32, percent yolk lipid was increased in eggs belonging to the ts11 at 10 wk and ts11 at 10 wk overlaid by F at 22 wk treatment groups. Treatment main effects were observed for percent egg albumen weight and Haugh unit scores. In comparison to controls, there was a significant

decrease in percent albumen in eggs due to the ts11 at 10 wk and F at 22 wk treatment, and there was a decrease in Haugh unit scores in the ts11 at 10 wk treatment. Percent yolk moisture, percent egg yolk weight, and percent egg shell weight in layers were not significantly affected by a 10 wk ts11 inoculation alone or in conjunction with subsequent overlay inoculations of F during lay. It is therefore suggested that the 10 wk inoculation of commercial layers with ts11 may reduce the negative impacts of a pre-lay F inoculation on performance while providing protection against subsequent field strain *M. gallisepticum* infections.

(Key words: commercial layer, egg characteristic, egg quality, F-strain *Mycoplasma gallisepticum*, ts11-strain *Mycoplasma gallisepticum*)

INTRODUCTION

Mycoplasma gallisepticum, the pathogen responsible for chronic respiratory disease in chickens, continues to be a costly problem for commercial table egg producers maintaining multi-age layer houses (Yoder, 1991). Vaccination programs using live whole cell vaccines are presently being used to control outbreaks and to help protect flocks from field strains of *M. gallisepticum*. Three live vaccines are commercially available for use in the United States. The most commonly used live vaccine is F-strain *M. gallisepticum* (Barbour et al., 2000). The F-strain *M. gallisepticum* vaccine strain is less virulent than many of the field strains and has a lower bird to bird transmission rate, yet is able to displace the more virulent stains of *M. gallisepticum* (Levison and Kleven, 1981; Kleven et al., 1990). The predominate means of vaccination with live *M. gallisepticum* vaccine strain in a commercial pullet setting is by coarse spray between 8

to 10 wk of (Burnham et al., 2003) allowing pullets to receive a mild infection and recover before coming into egg production (Yoder et al., 1984). Continuous use of F-strain *M. gallisepticum* vaccines for replacement flocks in multi-age commercial layer facilities has been proven to protect these flocks from field strains (Kleven et al., 1997).

The F-strain *M. gallisepticum* vaccine, however, is not totally apathogenic and has been reported to infect *M. gallisepticum* free birds and turkeys (Evans et al., 1992; Ley et. al., 1997). Once a bird is infected with *M. gallisepticum*, it is considered to be chronically infected for life (Brown et al., 1995). In a controlled study in biological isolation units, early vaccination with F-strain *M. gallisepticum* did not adversely affect egg production (**EP**) (Branton et al., 1997). It has also been established that producers that have used F-strain *M. gallisepticum* have also shown that no adverse effects on EP occur when F-strain *M. gallisepticum* vaccines are given pre-lay (Burnham et al., 2003). However, some field studies have determined that F-strain *M. gallisepticum* vaccination can reduce EP when compared to *Mycoplasma*-free birds (Carpenter et al.,1981; Mohammed et al., 1987; Branton et al., 1988). More recently, apathogenic whole cell live vaccines, including ts11-strain *M. gallisepticum* and 685-strain *M. gallisepticum*, have been licensed for use in layer chickens. These vaccines show virtually no bird to bird transmission, but have not been proven to displace wild type *M. gallisepticum* (Kleven et al., 1998). Furthermore, these strains may not confer continued protection throughout lay as does the F-strain (Yoder, 1978, 1991; Mohammed et al., 1987). Administration of the ts11 vaccine via eye drop, as per manufacturer instructions, is more time consuming and labor intensive than spray vaccination.

More testing is needed to determine if combinations of vaccines can lessen the impact of pre-lay F-strain *M. gallisepticum* vaccination. Therefore, the objective of the current study was to determine the effects of pre-lay ts11-strain *M. gallisepticum* inoculations and time specific F-strain *M. gallisepticum* inoculation overlays administered during lay on the egg quality of commercial laying hens.

MATERIALS AND METHODS

Pullet Housing and Management

Two trials were performed using Hy-Line W-36 pullets that were obtained at 1 d of age from a commercial source that was monitored and certified free of both *M. gallisepticum* and *M. synoviae* (USDA-APHIS-VS, 2003). Chickens were vaccinated at 10 d of age for infectious bursal disease via the drinking water. At 5 wk of age, 20 randomly selected chickens were tested for antibodies to both *M. gallisepticum* and *M. synoviae* using both the serum plate agglutination and the hemagglutination-inhibition tests (Yoder, 1975) and swabs were obtained from the choanal cleft (Branton et al., 1984) and placed into tubes containing Frey's broth medium (Frey et al., 1968) supplemented with an additional 0.15 thallium acetate and 10^6 IU penicillin-G/mL. Tubes were incubated at 37° C for 30 d or until the phenol red indicator reaction occurred in the media. A sample from those that changed color was then inoculated onto Frey's-based agar and incubated at 37° C. Colonies with morphology suggestive of *Mycoplasma* species were examined by an agar plate fluorescent antibody test (Baas and Jasper, 1972) that used direct labeling of colonies stained with anti-F-strain *M. gallisepticum* polyclonal

antibodies produced in rabbits and labeled with fluorescein isothiocyanate (Kleven, 1981).

Pullets were placed on clean dry litter in a conventional house until 10 wk of age. A daily artificial lighting schedule followed a 13 L:11 D cycle. One 75-W incandescent light bulb was used to illuminate each 8.4 m^2 of floor space, providing a calculated intensity at bird level of 35.5 lx. Feed and water were provided for *ad libitum* consumption in each trial. At 10 wk of age, 11 pullets were randomly selected and placed in each of 16 negative pressure fiberglass biological isolation units (1.16 m^2). The units were housed in a previously described poultry disease isolation facility (Branton and Simmons, 1992). Hen numbers were reduced to 10 per unit at point-of-lay (22 wk of age) so that bird density was 0.116 m^2 / bird for the duration of each trial. At 18 wk of age, the length of the artificial lighting schedule was increased 15 min/d until a cycle of 16 h and 15 min of light per 7 h and 45 min of dark was achieved. Chickens were maintained on that schedule through the remainder of each of the trials. For the entirety of each trial, chickens had *ad libitum* access to feed and water. Pullet and layer diets were formulated to meet or exceed NRC (1994) recommendations. Ingredient percentages and calculated analyses of the diets were as described by Burnham et al., (2002). No medications were administered during either trial.

Treatments

Four experimental treatment groups were used. Each treatment group consisted of 4 isolation units containing 10 birds each for a total of 40 birds per treatment group. Treatment one (**Controls**) received no *M. gallisepticum* inoculation but were sham-

inoculated via eye-drop in the right eye at 10 wk of age with sterile Frey's media. Treatment 2 contained birds that were eye-drop vaccinated in the right eye with ts11-strain *M. gallisepticum* at 10 wk of age (**ts11/10**). Birds belonging to treatment 3 received ts11-strain *M. gallisepticum* via eye-drop in the right eye at 10 wk of age followed by a 22 wk overlay vaccination via eye-drop in the left eye with F-strain *M. gallisepticum* (**ts11/10-F/22**). Treatment 4 consisted of birds given ts11 strain *M. gallisepticum* at 10 wk of age via eye drop in the right eye followed by a 45 wk overlay vaccination of F-strain *M. gallisepticum* via eye drop in the left eye (**ts11/10-F/45**).

The tsll vaccine titers for both trials were 1.0 X 10^{10} colony forming units/mL (**CFU/mL**). For trial 1, F-strain *M. gallisepticum* titers were 3.0 x $10^{10}$and 1.0 x 10^{8} CFU/mL at wk 22 and 45, respectively. For trial 2, F-strain *M. gallisepticum* titers were 7.5 x 10^{11} CFU/mL for both inoculation overlays (wk 22 and 45).

Data Collection and Egg Parameters Measured

All data collected from wk 23-44 was designated as belonging to age interval I; and all data collected from wk 45-57 were designated as belonging to interval II. Percent yolk lipid **(YL),** percent yolk moisture **(YM)**, percent egg yolk weight **(PY)**, percent egg albumen weight **(PA)**, and percent shell weight **(PSW)** were determined in eggs at 24, 32, and 43 wk of age (interval I), and at 47 and 56 wk of age (interval II) in both trials 1 and 2. Beginning on wk 23 (when Control group EP reached approximately 10 %) in both trials, eggs were collected weekly from 23 to 44 wk (interval I) and weekly from 45 to 57 wk (interval II) to determine egg Haugh unit scores **(HU)**. For determination of the above mentioned parameters, a total of 10 eggs were collected from each replicate unit

for an accurate estimate of each parameter (Buss, 1984). If less than 10 eggs were collected on a given d, the rest were collected the following d of the same wk. Egg processing and determinations of HU and of fresh yolk and albumen weights were made on the same day that eggs were collected. Eggshell (dried shell plus membranes) weight was determined according to the procedure described by Brake et al., (1984). Egg component (egg yolk, albumen, and shell) weights were expressed as percentages of total egg weight. Haugh unit scores were determined using a Model EQM egg quality management system (Technical Services and Supplies Limited, York, England) according to the procedure of Branton (1988).

Quantification of Yolk Moisture and Lipid Content

For YM determinations, yolk samples (2 g) were dried in a commercial oven (Chromalox Temperature Control, Edwin L. Wiegand Co. Pittsburgh, PA) according to the procedure of Peebles et al., (1999). After samples were removed from the oven, they were allowed 30 min to cool and the dry weights were recorded. Yolk moisture was calculated as the difference between the fresh and dry weight of the sample and was expressed as a percentage of fresh sample weight.

Yolk lipid was extracted according to the procedure described by Bligh and Dryer (1959), and as modified by Latour et al., (1998). A 3 g sample of fresh yolk was thoroughly mixed with 10 mL of methanol and 10 mL of chloroform in a 100 mL beaker. Mixing was done by hand until the mixture was a pasty homogenate. Water (5 mL) was added to the homogenate and the mixture was filtered through Whatman No. 1 filter paper in a Coors Buchner funnel. The filtered mixture was pulled under vacuum suction

into 50 mL centrifuge tubes. Additionally, the beaker that contained the original 3 g sample was rinsed with 3 mL of chloroform to extract any additional yolk, and that liquid was also poured through filter paper as described above. Centrifuge tubes were spun in a Beckman JB-6 centrifuge (Beckman Instruments, Inc., Fullerton, CA 93634) for 15 mins at 3000 rpm in order to separate the alcohol and lipid. The centrifuged samples contained two well-defined layers (lower chloroform-lipid layer and upper methanol-water layer). The upper layer was discarded and the lower chloroform-lipid layer was filtered through a column of anhydrous sodium sulfate into a glass tube. The chloroform containing lipid extract was placed into a V-Evap apparatus (Organomantion, Shrewsbury, MA 01545) and dried with a stream of air. Dried lipid samples were weighed and the expression of YL concentration was dry lipid sample weight as a percentage of total fresh yolk sample weight.

Statistical Analysis

A completely randomized experimental design, with trial as a block, was employed. Data from wk 23 - 44 (age interval I), and from wk 45 - 57 (age interval II) were analyzed separately. The data of both trials were pooled then analyzed together. Therefore, the results from both trials were not reported independently but were reported over both trials. Trial was considered as a random effect. All data within each age interval were subjected to a repeated measures analysis because parameters were examined at multiple age periods in each age interval. In the first age interval, Controls and those having had ts11/10 and ts11/10-F/22 inoculations were compared. In the second age interval, Control, ts11/10, ts11/10-F/22, and ts11/10-F/45 groups were

compared. Individual sample data within each of the replicate units were averaged prior to analysis. Least-squares means were compared in the event of significant global effects (Steel and Torrie, 1980). Global effects and differences among least-squares means were considered significant at $P \leq 0.05$. All data were analyzed using the MIXED procedure of SAS software (SAS Institute, 2003).

RESULTS AND DISCUSSION

All initial mycoplasmal cultures obtained from 5-wk old pullets, as well as SPA tests, were negative for both *M. gallisepticum* and *M. synoviae*. Cultures and SPA test results on Control birds remained negative throughout the 2 trials for both *M. gallisepticum* and *M. synoviae*. The aforementioned tests were repeated throughout the entire study. These sample tests for all 4 treatment groups showed that Controls remained *Mycoplasma* -free, whereas the 3 vaccinated groups resulted in positive broth cultures with FA and SPA results being positive for *M. gallisepticum* and negative for *M. synoviae*. There was no significant difference in mortality between *M. gallisepticum*- free and *M. gallisepticum* - inoculated birds in either trial. Throughout the study, SPA and FA tests confirmed systemic infections in *M. gallisepticum*-inoculated birds, whether the inoculation was with the ts11- or F-strains of *M. gallisepticum*.

No significant age or treatment main effects or age by treatment interactions were found for YM, PY, or PSW in either age interval examined. A hen age by treatment interaction ($P \leq 0.05$) was observed for YL (wk 24, 32, 43) in interval I (Table 1). In comparison to Controls, YL was significantly higher in the ts11/10 and ts11/10-F/22 vaccination regimens at wk 32. However, no significant age or treatment main effects or

age by treatment interaction was observed for YL in interval II. Also, no age or treatment main effects or age by treatment interaction was observed for PA in interval I. Furthermore, there was no significant age main effect or age by treatment interaction for PA in interval II; however, there was a treatment main effect ($P \leq 0.05$) for PA in interval II (across 47 and 56 wk). Mean egg PA for Control, ts11/10, ts11/10-F/22, and ts11/10-F/45 treatments in interval II were 59.5, 58.5, 57.2, and 60.1 %, respectively (pooled SEM = 0.86). Egg PA at wk 47 and 56 was significantly lower for ts11/10-F/22 treatment birds than for Control and ts11/10-F/45 treatments, with the ts11/10 treatment intermediate. For HU, there were no age or treatment main effects or age by treatment interactions in interval I, and there was no significant age main effect or age by treatment interaction in interval II. Nevertheless, a treatment main effect ($P \leq 0.05$) was observed for HU scores in interval II (45-57 wk). Mean HU for Control, ts11/10, ts11/10-F/22, and ts11/10-F/45 treatments were 93.6, 91.8, 93.9 and 93.3, respectively (pooled SEM = 1.84). In interval II, mean HU for the ts11/10 treatment group was significantly lower than that for the other 3 treatment groups. This result suggests that a prelay ts11-strain *M. gallisepticum* inoculation alone may lower albumen quality during postpeak lay.

In comparison to Controls, significant increases in YL were observed at 32 wk in birds administered either the ts11/10 or ts11/10-F/22 vaccination regimens. This result suggests that the inoculation of ts11 *M. gallisepticum* at 10 wk may increase YL in eggs up to 22 wk later. The basis for this effect is not clear, however, the synthesis, transport, and uptake of lipid in the liver, blood, and ovary, respectively, cannot be precluded as possible locations and means by which the ts11 *M. gallisepticum* organism may colonize and influence YL. Furthermore, these data imply that a subsequent inoculation of F-

strain *M. gallisepticum* at 22 wk does not modify the effect of the ts11/10 treatment on YL at 32 wk.

The distribution of fatty acids of various chain lengths is relatively constant in YL and is related to their synthesis in the hens' liver (Watkins and Kratzer, 1987; Watkins, 1995; Walzem, 1996; Speake and Thompson, 1999). Sturkie (1976) reported that approximately 32% of total egg weight is yolk, 11% of total egg weight is shell, and 57% of the total egg weight is albumen and that the egg weight is primarily the function of the four albumen layers. Albumen is secreted in the magnum in response to the pressure of the egg yolk. Sturkie (1976) also reported that the stimulation of albumen secretion is proportional to the size of the egg yolk. Therefore, the total egg size is directly related to ovarian follicular development consequently the smaller the egg yolk the smaller the total egg weight. HU scores reflect the inner quality of the egg, specifically albumen quality. The majority of the albumen formation occurs during the 2-3 hr the ovum remains in the magnum (Johnson, 1986; Branton et. al., 1988). *Mycoplasma gallisepticum* has been cultured from the liver (Sahu and Olson, 1976) and periovarian region (Fabricant and Levine, 1963) of chickens. The liver is considered to be the primary source of lipid production. The egg and eggshell quality parameters selected for these trials reflected the functionality of the ovary and specific segments of the oviduct as described by Branton et al., (2000). In a study using ts11 vaccine, by Branton et al., (2000), egg production and egg quality parameters were evaluated. No significant differences were observed for EP, blood/meat spots, egg size, mortality, or HU scores. This suggested that the ovary and various segments of the oviduct were not compromised by the ts11 vaccine. Furthermore, gross and histological changes were not observed upon necropsy.

Branton et al., (1997) reported that EP and other egg characteristic in birds inoculated with F-strain M. gallisepticum at 10 wk of age were not significantly different than controls. It has, however, been proven that F-strain *M. gallisepticum* given late in lay does reduce EP. These changes were the result of the disease rather than the vaccination. In a more recent report by Burnham et al., (2002), using birds inoculated at 12 wk of age, EP was reported to be significantly different in the F-strain vaccinated birds compared to the controls. Burnham et al., (2002) reported that onset of lay was delay and that a significant decrease in EP occurred. It was reported that inoculated hens had fewer mature ovarian follicles and a decreased magnal, isthmal and vaginal proportion of the reproductive tract at trial termination (60 wk) compared to F-strain *M. gallisepticum* free hens Burnham. Burnham suggested that the establishment of systemic F-strain *M. gallisepticum* infections and long term changes were the result of the disease rather than the F-strain *M. gallisepticum* vaccination. In further studies of yolk characteristics (Burnham et al., 2003), a decrease in YL was observed at 22 wk in the F-strain vaccinated birds suggesting that F-strain vaccination may inhibit YL disposition at this time. Increased percentages of YL post peak, however, may represent a compensatory increase in YL deposition in response to the earlier depression in YL.

Each of the vaccine treatments examined in this study had specific strengths and weaknesses. However, it did appear that the ts11 and F-strain *M. gallisepticum* vaccine treatment combinations overcame some of the weaknesses of pre-lay vaccines in which ts11- or F- strains of *M. gallisepticum* were given alone. The results of this study suggest that the combination of ts11 and F-strain *M. gallispticum* vaccinations may prevent a decrease in HU scores late in lay that were observed as a result of the individual ts11/10

treatment. Also, it appears that ts11 *M. gallispticum* vaccination at 10 wk prevented the drop in EP when the F-strain *M. gallispticum* vaccine was given late in lay. Therefore, the combination of ts11 and F-strain *M. gallisepticum* may result in a better outcome than vaccinations with either strain alone. In conclusion, the pre-lay ts11- and lay F-strain *M. gallisepticum* vaccine combination shows promise as research continues to develop new and better vaccine protocols to eliminate the negative impacts of *Mycoplasma* vaccination.

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TABLE 6.1. Percent yolk lipid concentration of eggs from commercial layers in sham-inoculated control (Control), ts11 *M. gallisepticum* at 10 wk (ts11/10), and ts11 *M. gallisepticum* at 10 wk with 22 wk F-strain *M. gallisepticum* overlay (ts11/10-F/22) treatment groups at 24, 32, and 43 wk of age (interval I)[1,2].

	Weeks of Age		
Inoculation Treatment	24	32	43
	----------(%)----------		
Control	22.1	20.8[b]	22.1
ts11/10	20.5	23.2[a]	22.1
ts11/10-F/22	21.9	23.4[a]	22.9

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]n = 4 replicate units for calculation of mean within each hen age and inoculation treatment group.

[2]SEM based on pooled estimate of variance = 0.95.

CHAPTER VII

SUMMARY

The primary goal of the present study was to investigate the effects of a ts11-strain M. *gallisepticum* inoculation alone, and in conjunction with F-strain *M. gallisepticum* overlays at 22 or 45 weeks of age during lay. F-strain *M. gallisepticum* has previously been shown to negatively affect laying hens' performances when given pre-lay or during post-peak lay, whereas ts11-strain M. *gallisepticum* inoculation administered pre-lay has previously been shown to not exert these negative effects.

In this study, it was shown that a pre-lay ts11 vaccination had a broad range effect on the physiology of the commercial layer without affecting overall performance. The effects on the bird's physiology included effects in the oviduct, blood, egg yolk, and egg albumen. More specifically, the pre-lay ts11/10 vaccination had unassociated effects on relative vaginal length, SCA, YL, and HU. Furthermore, the timing of an overlay vaccination using F-strain *M. gallisepticum* during lay had varied effects on some of the aforementioned physiological parameters without affecting overall performance. The effects of the timing of F-strain *M. gallisepticum* overlays included those on SCA, Yl, and HU. Both the early (wk 22) and late (wk 45) F-strain *M. gallisepticum* overlays ameliorated the adverse effects of the pre-lay ts11 vaccine on HU and the late overlay decreased YL and SCA in comparison to the early overlay.

These results confirm that pre-lay ts11-strain *M. gallisepticum* inoculations may be suitable for pre-lay F-strain *M. gallisepticum* inoculations, and the F-strain *M. gallisepticum* overlays during lay on pre-lay ts11-strain *M. gallisepticum* may also provide continual protection without eliciting any subsequent suppressive affects on performance. The data from this study indicates that using ts11-strain *M. gallisepticum* in conjunction with F-strain *M. gallisepticum* does not negatively affect laying hen performance. Furthermore, this inoculation combination offers benefits above the use of those achieved through either inoculation alone. Also, because F-strain *M. gallisepticum* is a more virulent strain, F-strain *M. gallisepticum* may offer stronger protection during lay than ts11-strain *M. gallisepticum*. The results of this study would be of particular interest in an industry setting in which flocks previously inoculated with ts11-strain *M. gallisepticum* are acquired by a company comfortable with a traditionally used F-strain *M. gallisepticum* inoculation regimen.