The Effects of In Ovo Feeding of Fatty Acids and Antioxidants on Broiler Chicken Hatchability and Chick Tissue Lipids

by

Travis P. Schaal

A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Animal Sciences (Honors Scholar)

Presented May 29, 2008
Commencement June 2008

AN ABSTRACT OF THE THESIS OF

Travis P. Schaal for the degree of Honors Baccalaureate of Science in Animal Sciences presented on May 29, 2008 Title: The Effects of In Ovo Feeding of Fatty Acids and Antioxidants on Broiler Chicken Hatchability and Chick Tissue Lipids.

Abstract approved: _____

Gita Cherian

Hatchability of broiler eggs has not increased over the last twenty years. Increasing hatchability with in ovo technology may be possible through feeding metabolic modulators to the developing embryo. Exogenous fatty acids and antioxidants provided during incubation may enhance polyunsaturated fatty acid, lipid, and antioxidant status of the chicken embryo. This hypothesis was evaluated by conducting three experiments: first, in ovo feeding of polyunsaturated fatty acids (conjugated linoleic acid (CLA), alpha-linolenic acid (LNA), and second, injection of palmitate/carnitine (PC) or control. In experiment three, in ovo injection of 10 IU or 20 IU of vitamin E (VE) was performed. Hatchability of chicks receiving CLA and LNA was reduced compared saline injection and chick livers showed no difference in total lipid ($p>0.05$). Hatchability was reduced in PC and control groups compared to the non-injected treatment. Lipid content of liver and heart tissue was increased in the PC injected chicks compared to control ($p<0.05$). Hatchability of fertile eggs was 88% for both VE injected treatments. Chicks injected with VE had increased brain weight as percent of body weight compared to non-injected chicks ($p<0.05$). Brain total lipid and VE content were increased in all three injected chicks compared with non-injected ($p<0.05$). In ovo feeding of VE may enhance brain tissue lipids and the antioxidant status of hatched chicks.

Key Words: hatchability, in ovo feeding, palmitate, carnitine, vitamin E, polyunsaturated fatty acid

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APPROVED:

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Mentor, representing Animal Sciences

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Dean, University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

_____

Travis P. Schaal, Author

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1. INTRODUCTION

1.1 Background:

The highly integrated and efficient modern poultry industry produces a wholesome protein source for a growing world population. The breeding, hatching, and rearing of meat type chickens (broilers) and turkeys operate at small tolerances for error. The profit in the industry is surprisingly low, especially with rising feed costs of feed. Corn and soybean meal comprise the bulk of poultry rations. These commodities face rising values as a major portion of the production is funneled into ethanol production (Skrzycki, 2008). Streamlined processes and automation have made the expansion of the poultry industry possible and are now necessary its survival. Intensive genetic selection for high producing birds and nutritional research for optimal rations have provided broilers that can reach market weight in excess of six pounds in only 42 days. The 21 day incubation period for a chicken egg makes up one third of a bird's entire life cycle and contributes a major role in the production of an animal protein source that nearly all cultures of the world consume.

Commercial hatcheries incubate and hatch fertile eggs derived from primary breeder flocks. Grow-out farms must receive quality chicks from hatcheries to avoid set-backs in growth that may arise from weak chicks due to exposure to pathogens or other stressors. Along with providing healthy chicks for grow-out, the percentage of fertile eggs incubated that hatch (hatchability) is an important factor to the industry. As genetic

selection, nutrition, and management of poultry flocks has improved over the last twenty years, hatchability of broiler or turkey eggs set in commercial hatcheries has not increased (Schaal and Cherian, 2007). Nearly twenty percent of the broiler and turkey eggs incubated do not hatch, equating to a loss of nearly 500 million dollars to the United States poultry industry in the year 2005 alone (Schaal and Cherian, 2007). The industry relies on the ability of birds to meet their genetic potential (in terms of weight gain, feed conversion, etc.) through proper management to generate profits. The potential exists to improve hatchability of viable embryos to further increase profits. Each year, more eggs are set to hatch a greater number of broilers (Schaal and Cherian, 2007). An increased hatchability would allow for fewer eggs to be set and fewer dollars expended on energy to maintain incubation. In the same way problems such as late hatches, unthrifty chicks, pathogen exposure, feed withdrawal, etc. are addressed as causes for declines in profit, hatchability should be addressed as a major loss to the industry.

1.2 In Ovo Technology:

The world consumption of poultry products is rising and the broiler industry requires healthy, fast growing, and well muscled birds to meet the consumers' demands. Technological advances have streamlined incubation and broiler management resulting in monetary savings for producers, through efficient production of uniform products. Embrex Corporation has revolutionized broiler incubation through their development of INOVOJECT® products that provide in ovo vaccinations during incubation (Embrex, 2007). As incubated eggs are transferred from setters to hatchers at day 18 of incubation, they are processed through automated injection machines (Johnston, et. al, 1997). A

needle is inserted through the egg shell and vaccines and other biological are introduced into the amnion, air cell, or the chick itself (Embrex, 2007; Johnston, et. al, 1997). Current vaccinations administered in ovo include Marek's vaccine, infectious bursal virus, avian influenza virus, and vaccines to prevent coccidiosis (Embrex, 2007; Johnston et. al. 1997). Chick vaccinations were traditionally reserved for the day of hatch and added to an already stressful first day of life. After hatching, chicks are graded, sorted, counted, boxed and transported (usually by truck) to grow-out facilities that may be up to two days drive from the hatchery.

1.3 In Ovo Feeding:

Advancing the use of in ovo technology has become a "hot spot" in research today. Beyond vaccines, any number of nutrients or compounds can be provided to the developing embryo via this route of administration. In ovo injections have created new opportunities to improve the health and development of broiler chickens. Employing the same technology as with vaccination, it is possible to provide developing embryos with exogenous nutrients in a process developed by Uni and Ferket (2003) known as "in ovo feeding". Supplying embryos with exogenous nutrients in ovo may improve hatchability, increase hatched chick weight, and/or the final body weight of broilers through modulating embryo gut morphology (Uni and Ferket, 2003). The ability to improve any of these economic traits or improve livability and performance of birds may lead to increased returns and efficiency for the industry. Any discovery leading to improved animal performance could be readily adopted into the industry through the in ovo infrastructure already in place in broiler hatcheries.

The site of in ovo injection and stage of incubation when injections are administered have also been explored with varying objectives in research that has centered about in ovo feeding. Ohata and Kidd (2001) determined the optimum placement site of in ovo injection of amino acids to be the yolk or extra embryonic coelom on day 7 of incubation. Previous work by those researchers concluded that amino acid injection increased the body weight of hatched chicks (Ohata et. al., 1999). Specific compounds that may prove beneficial in the formation and development of the embryonic gut and digestive tract's ability to utilize carbohydrates upon hatching have also been explored (Tako, et. al., 2004, Uni et. al., 2005). The embryo's diet differs from that of the hatched chick placed on the farm in terms of composition of nutrients. The nutritional requirements of intestinal villi and digestive enzymes also are different between developing embryo and hatched chick (Uni et. al, 2003). The fat and protein based diet of the embryo is replaced by a ration high in carbohydrates (predominately corn and soybean meal) on the farm. Decreasing the time for the gut to transition to a carbohydrate diet decreases the chick's need to produce glucose via gluconeogenesis (Uni et. al, 2005). Uni et. al. (2003) have also shown that the morphological changes associated with gut development in the embryo prior to hatching prepare the small intestine for a diet high in carbohydrates through the production of specific brush border enzymes as well as increased villi length and number.

The egg is a "closed nutrition" system once it is laid by the hen. All the nutrients the developing embryo will utilize are packaged inside the shell, meaning the availability and form of the nutrients in the egg are essential to the development of a viable chick. Cherian et al. (1996) and Latour et. al. (1997) showed that modulating the antioxidant

(e.g. vitamin E) and fatty acid (e.g. omega-3) profile of the hen's diet has an effect on subsequent egg antioxidant and fatty acid profiles. Hens fed high levels of antioxidants produced eggs with higher concentrations of antioxidants and chicks hatched from those eggs had higher concentrations of antioxidants in their tissues (Surai et. al., 1999; Cherian et. al. 1997). Supplementing the hen's diet with oils containing beneficial omega-three fatty acids and/or high levels of antioxidants can be costly. In contrast, one can more easily, and cost effectively, administer a set amount of nutrient directly to individual viable embryos with in ovo technology. The ability to directly supply growing embryos with specific compounds may decrease the need for long term formulation of enriched rations for maternal diets to achieve similar effect. In ovo injections may also provide a more accurate dose at the specific time for peak absorption of specific nutrients, cofactors, or metabolic modulators by the embryo.

Nearly 80% of the 5-6g of lipid found in the yolk of the egg is absorbed by the embryo for energy production and structural membrane synthesis (Noble and Cocchi, 1990). As a result, fatty acid oxidation provides over 90 percent of the energy requirements for the chick embryo (Romanoff, 1960). The common use of low quality (low antioxidant, low essential fatty acids, highly oxidized) fats in breeder hen diets provides the growing embryos with a low quality fat source during development (Cherian et. al.,1996; van Kempen and McComas, 2002). Low quality fat may predispose the embryo to increased metabolic disorders resulting from lipid peroxidation and damage to cellular membranes. During the third week of incubation, the embryo increases the uptake of fatty acids which may increase the potential for oxidative stress to occur (Noble and Cocchi, 1990; Latour et al., 2000). Another time of potential increased oxidative

stress occurs as the chick begins pipping when it must switch to active pulmonary respiration of oxygen as opposed to respiring via gas exchange from the temporary embryonic structures (Garcia et al., 1986; Moran 2007). With such important roles in energy production for the embryo, fat sources are essential to fuel the heart and for deposition of polyunsaturated fatty acids (PUFA) in tissues such as the brain (Cherian and Sim 1992). As such, in ovo administration of high quality fatty acids may prove beneficial for improving energy production during embryogenesis and hatching.

The use of antioxidants, especially vitamin E (VE) has been proven to reduce harmful peroxidation of lipids and cholesterols in animal models (Singh, et. al., 2005). Surai et. al. (1997, 1999) report a positive correlation between high levels of dietary VE in the maternal hen's diet and increased VE levels in eggs and subsequent chick tissues. Gore and Qureshi (1997) have examined the role of VE administered in-ovo on immune system and immunoglobulin levels in turkey poults and broiler chicks. Exogenous vitamin E increased the IgM levels of poults and the IgG levels of chicks when measured 7 and 14 days, respectively, after embryonic exposure to VE (Gore and Qureshi, 1997).

Besides the health benefits of an increased immune response, exogenous VE administered in ovo at the time of increased fatty acid oxidation may prove beneficial in reducing the production of free radical oxygen species that can cause serious damage to the highly polyunsaturated fatty acids of cellular membranes (Cherian and Sim, 1992; Cherian and Sim, 1997; Surai et al., 1999). Zahi, et. al. (2008) explored in ovo injection of carnitine and noted no increase in hatchability of layer type embryos receiving the amino acid. However, the role of carnitine in transport of fatty acids across the mitochondrial membrane, as well as its ability to act as an antioxidant may allow for

increased oxidation of fatty acids for energy and protection from free radicals (Zahi, et. al, 2008). Low quality fats added to diets of breeder hens as an inexpensive energy source may be counteracted with the in ovo administration of antioxidants to protect the lipids of the yolk as well as the plasma membranes of the chicks' cells from damage.

If beneficial effects of in ovo feeding are determined, they may be readily adapted into the industry through existing hatchery infrastructure. Improving the energy availability for the developing embryo and protecting the cellular membranes and fatty acid reserves from peroxidation may improve the embryo's ability to hatch and to perform to its genetic potential. Thus, the present study includes the three following main objectives:

1.3.1 Objective One: To conduct a survey on the hatchability observed in commercial broiler and turkey hatcheries in United States from the year 1985 to 2005.

1.3.2 Objective Two: To develop a method to deliver fatty acids to incubating embryos in order to determine the effect of exogenous fatty acids on chick hatchability**.**

It is hypothesized that the embryos receiving an exogenous supply of fatty acids will produce more energy during the stressful process of hatching and will have a higher hatchability rate.

1.3.3 Objective Three: To determine the effect of an exogenous supply of antioxidants on chick embryo health and hatchability.

It is hypothesized that the embryos receiving an exogenous supply of antioxidants will have improved hatchability and hatched chicks will reflect higher levels of antioxidants and total lipids in their tissues.

2. OBJECTIVE ONE: A SURVEY ON THE HATCHABILITY OF BROILER ANDTURKEY EGGS IN THE UNITED STATES FROM 1985 TO 2005[1]

2.1 Introduction:

Commercial poultry production is one of the fastest growing capital-intensive animal industries in North America. The value of the US broiler industry has grown from $5.68 billion in 1985 to $20.9 billion in 2005 (Crop Reporting Board, 1986; National Agricultural Statistics Service, 2006a). Turkey production has witnessed a 77% increase in value since 1985, totaling $3.23 billion in 2005 (Crop Reporting Board, 1986; National Agricultural Statistics Service, 2006b). Following this trend, each year an increasing number of broiler and turkey eggs have been set in commercial hatcheries in the United States. In 2005, over 11 billion broiler eggs and 343 million turkey eggs were set in US hatcheries, compared with 5.6 billion broiler eggs and 259 million turkey eggs in 1985 (Crop Reporting Board, 1986; National Agricultural Statistics Service, 2006b).

Considering the increase in broiler and turkey production in the past 20-year period, we were interested in determining if hatchability had changed. We hypothesized that advances in nutrition, genetic selection, and management would have increased hatchability of broiler and turkey eggs. The objectives of this study were to examine published data on broiler and turkey eggs set in US commercial hatcheries between 1985 and 2005, calculate the hatchability of broiler and turkey eggs in terms of chicks and

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[1]A version of this chapter has been published previously (Schaal, T., and G. Cherian. 2007. Poult. Sci. 86:598-600).

poults placed for grow out, and to also estimate the economic impact associated with changes in hatchability during this time period.

2.2 Materials and Methods:

Data regarding US commercial broiler and turkey hatching eggs between 1985 and 2005 were obtained from the Economic Research Service, USDA Poultry Yearbook (89007; 2004), National Agricultural Statistics Service, USDA Hatchery Production Annual Summaries, USDA Poultry Production and Value Summaries, and the USDA Economic Research Service web sites. Annual hatchability was calculated from these data using the following equation:

(total chicks or poults placed for grow-out) / (total eggs set) x 100%

The annual economic impact associated with hatchability was calculated from the following equation:

(total eggs set – total chicks or poults placed) x (annual average price paid by farmers per chick or poult)

Hatchability loss calculated includes eggs that did not hatch, non-fertile eggs, embryo mortality, non-salable chicks and poults, or excess production of chicks and poults.

2.3 Results and Discussion:

The number of broiler and turkey eggs set in US commercial hatcheries as illustrated in Figures 1 and 2, increased substantially each year from 1985 to 2005. Between 1985 and 2005, the number of broiler eggs set increased by 98%; 5.6 billion broiler eggs were set in 1985 compared with 11 billion eggs in 2005 (Crop Reporting Board, 1986; National Agricultural Statistics Service, 2006b). Turkey hatcheries experienced a peak, with the highest number of eggs set in 1995. The number of eggs set in 2005 represented an increase of 33% compared with 1985 figures. The trend of setting more eggs during this period can be associated with the increased consumer demand for poultry, and increased exports of poultry from the United States. Between 1987 and 1998, the average US per capita consumption of chicken and turkey increased by 37.3 and 21.1%, respectively (Ollinger et al., 2000). United States exports of poultry (chicken and turkey combined) during this time increased by a substantial 502% (Ollinger et al., 2000). In order to meet the rising demand for poultry, the industry has set an increased number of broiler or turkey eggs.

Figure 1. Total broiler eggs set and hatchability percentage in US hatcheries from 1985 through 2005.

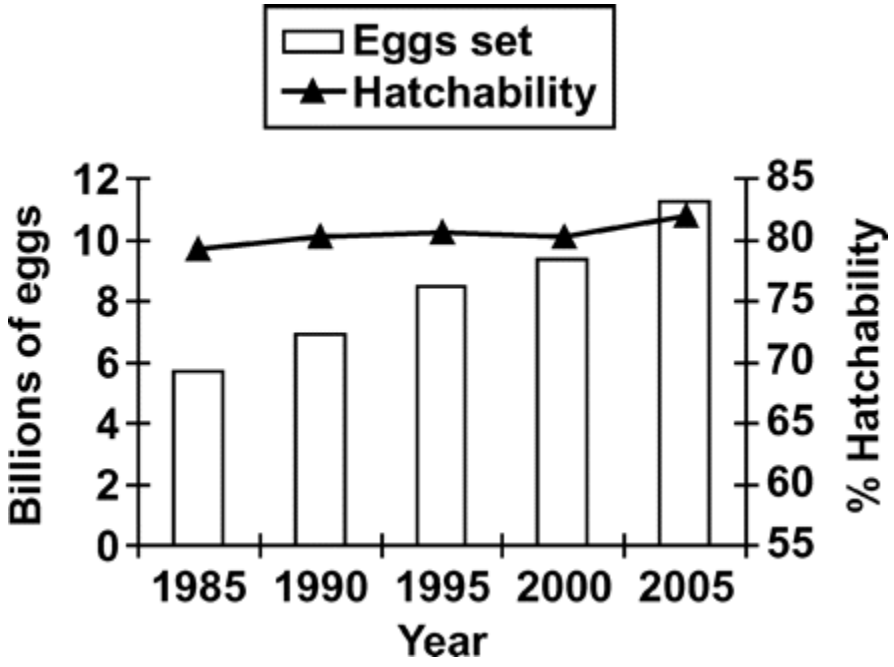
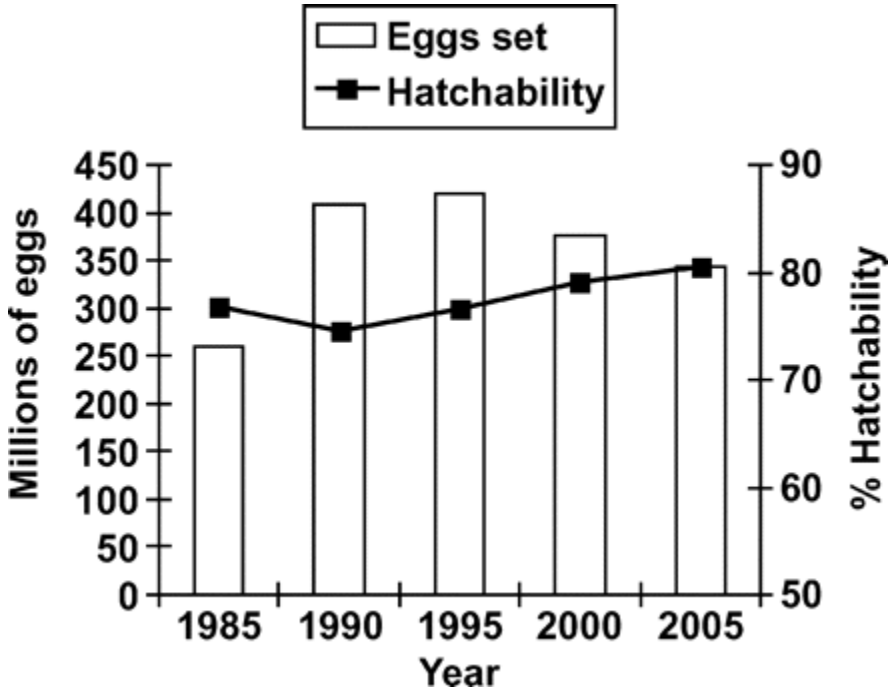


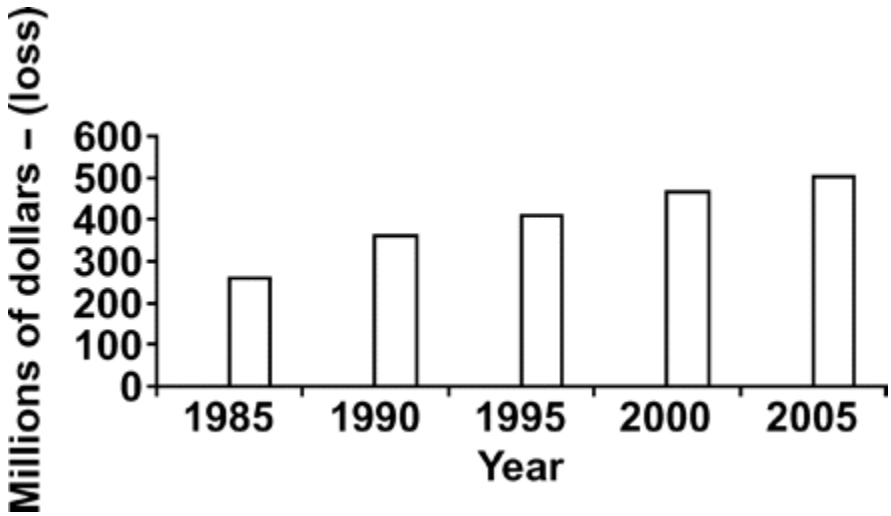
Figure 2. Total turkey eggs set and hatchability percentage in US hatcheries from 1985 through 2005.



With large increases in the numbers of eggs set and chicks and poults placed for grow out, hatchability did not increase. Broiler hatchability ranged from 79 to 82%, whereas turkey egg hatchability ranged from 76 to 80% (Figures 1 and 2). The staggering 20% of incubated broiler and turkey eggs that did not hatch had a large economic impact on the industry. To correlate a monetary value with hatchability, we used the annual average prices paid by farmers for day-old chicks and poults, as recorded by the National Agricultural Statistics Service (2004a,b). Even within such an integrated industry, each chick and poult hatched carries a value because of the costs of rearing breeder birds as well as the expense of incubation. Although farmers may not actually pay at the farm gate per chick, to estimate a monetary cost with hatchability, we had to use the average annual prices farmers paid for chicks and poults for our calculations of the value of day-old chicks and poults. The average value per broiler chick in 1985 was $0.172, whereas the value per poult was $0.96, while in 2005 the prices were $0.21 per broiler chick and $1.15 per poult, respectively (National Agricultural Statistics Service, 2004a,b).

The total calculated economic impact associated with hatchability for broilers and turkeys in 1985 was a loss to the industry of $260 million. This loss has increased each year since 1985 (Figure 3), resulting in a $500 million loss associated with broiler and turkey hatchability in 2005. If the industry were able to improve the average hatchability of turkey and broiler eggs by 1%, the increased returns would total more than $25 million. In addition, an improvement in hatchability would allow more birds to be placed for grow out, further increasing returns to the industry.

Figure 3. Total economic impact associated with the hatchability of broiler and turkey eggs from 1985 through 2005



In the past 20 years, advancements have been made through genetic selection, nutrition, and management of broiler and turkey breeder flocks resulting in increased bird growth, increased carcass yield, and decreased number of days to market. Hatchability, however, has remained unchanged over the last 20 years. Hocking and Robertson (2005) reported that genetic selection for increased bird size has resulted in low-quality eggs from breeder flocks. Current management of breeder stocks does not place adequate emphasis on hatchability, creating a need to explore avenues that will increase hatchability while maintaining the highly selected economic traits of the birds. Further research on improving hatchability should be a priority will lead to increased economic returns to the poultry industry.

3. OBJECTIVE TWO: THE EFFECT OF IN OVO FEEDING OF FATTY ACIDS ON HATCHABILITY AND CHICK TISSUE LIPID CONTENT

3.1 Introduction:

The lack of improved hatchability of broiler eggs over the last twenty years may be associated with various factors including diet of the breeder hen. The yolk fat of an egg is crucial to the development of the chicken embryo in terms of energy production (Noble and Cocchi, 1990). The quality of fat available in the yolk is directly linked to the diet of the breeder hen (Cherian et al. 1997). If the hen is fed low quality fats (high level saturated fats, highly oxidized, low levels of essential fatty acids), her eggs will reflect the same lipid profile, forcing subsequent embryos to utilize poor quality fats for energy production and structural membrane synthesis (van Kempen and McComas, 2002; Cherian et al.,1996). Providing embryos with exogenous fatty acids may allow for increased energy production, as well as allow for more polyunsaturated fatty acid accretion in vital tissues during the stressful process of hatching.

Conjugated linoleic acid (CLA), a polyunsaturated fatty acid, has been shown to alter the lipid metabolism in chicken embryos derived from hens fed high levels of CLA (Latour et al., 2000). CLA is implicated in the immune function as an anti-inflammatory compound that decreases blood platelet aggregation. Another polyunsaturated fatty acid of interest is alpha-linolenic acid (LNA). An essential omega-3 fatty acid, LNA has been associated with increasing the n-3:n-6 ratio of poly unsaturated fatty acids (PUFA) levels in chicks through the maternal feeding of diets rich in LNA (Cherian and Sim, 2001). High levels of LNA affect the activity of liver desaturases which ultimately affects the

PUFA content of cellular membranes increasing the fluidity of cellular plasma membranes (Cherian and Sim, 2001).

Palmitate is a medium chain fatty acid that can be readily utilized by the body without the need for restructuring by chylomicrons in the lymphatic system. By decreasing rate of hydrolysis for energy production, palmitate may allow for more rapid energy production during hatching. Carnitine, an amino acid which aids in the transport of fatty acids across the mitochondrial membrane for oxidation, may further increase the embryo's ability to utilize exogenous palmitate (Zahi et al., 2008). In ovo feeding of a palmitate and carnitine mixture (PC) may increase the rate of fatty acid absorption, hydrolysis, and oxidation to produce more energy during hatching.

The objectives of the present experiments were to develop a method for introducing exogenous fatty acids to the developing chicken embryo, and to determine the effects of in ovo feeding of CLA, LNA (Experiment one), and PC (experiment two) on hatchability, and chick total lipid content of tissues. It is hypothesized that embryos having an exogenous supply of fatty acids (CLA, LNA, PC) will have a higher hatchability.

3.2. Materials and Methods:

Two experiments were conducted to determine the effects of in ovo injection of exogenous fatty acids on the hatchability of broiler eggs.

3.2.1 Egg Collection: In experiment one, a total of 79 Cobb broiler eggs were collected from ongoing research at the Poultry Research Facility at Oregon State University. For experiment two, 91 eggs were collected from Cobb broiler breeders from

ongoing research at the Poultry Research Facility at Oregon State University (source 1). In addition, 45 Cobb broiler eggs were purchased from a commercial hatchery (source 2) (Tangent, OR). The two sources of eggs were used to reduce variation that may arise due to diet and management of the breeder flock. All eggs were stored in a holding room at 18.3C for one week prior to setting for incubation.

3.2.2 Incubation Conditions: Eggs were removed from the holding room and allowed to warm to room temperature (Jamesway Model 252). All eggs for each experiment were set in the same tray in the same incubator. Throughout incubation, a dry bulb temperature of 37.5C and wet bulb temperature of 28.3C were maintained from day 0-18 of incubation. On day 18 of incubation, all eggs were sorted by treatment, transferred to hatching trays and the dry bulb temperature was reduced to 36.3C and the wet bulb temperature was increased to 30.2C. At the time of in ovo injection the eggs were first candled and clear eggs (non-fertile) were removed.

3.2.3 In Ovo Injection: In ovo injection of compounds in both experiments was facilitated through a modified Noor et al. (1995) method. All eggs were removed from the incubation tray and placed in paper egg flats. Eggs were randomly assigned to a treatment and marked with permanent marker for identification. All eggs were cleaned with a Kim-wipe and 70% ethanol to disinfect the shell surface. A sharp dissection probe was dipped in ethanol to sterilize and used to make a small puncture in eggshells of injected treatments. A 23 gauge, 1.25 inch needle and 1mL syringe was used to administer all injections. Prior to each injection (between eggs) the needle was immersed in 70% ethanol and replaced between treatments. The needle was fully inserted through the hole created by the dissection probe and the contents of the syringe were injected into

the amnion of the egg. Paraffin wax was used to seal the puncture hole and prevent bacterial infection. Upon completion of all injections, all eggs were returned to the incubator tray and returned to the incubator.

3.2.4 Experiment One: Eggs were removed from the incubator simultaneously on day 14 of incubation to facilitate in ovo injections. Eggs were randomly sorted into three treatments. The first treatment (n=27) received 0.2gm CLA diluted in 0.2mL 70% ethanol, the second treatment (n=25) received 0.1gm LNA + 0.1gm CLA (total 0.2gm lipid) dissolved in 0.2 mL 70% ethanol, and the third treatment (n=20) received 0.2mL injection of sterile 8.8% saline solution (control).

3.2.5 Experiment Two: Eggs were removed from the incubator on day 15 of incubation to administer in ovo injections. Eggs were randomly assigned to one of following three treatments: control received 0.2 mL carrier (70% ethanol) (n=30 source 1; n=15 source 2), 0.2 mL PC injection (4mg palmitate, 1 μM carnitine, 70% ethanol) (n=14 source 2; n=30 source 1), and no injection (n=29 source 1; n=13 source 2).

3.2.6 Sample Collection: On the day of hatch, all chicks from each treatment were counted and non-hatched eggs examined to determine embryo status (early dead, mid dead, late dead, or pipping). Hatchability was recorded as percent of fertile eggs that hatched in each treatment. In experiment one, liver tissue samples were collected from hatched chicks from each treatment (n=6) and subjected to total lipid analysis. In experiment two, tissue samples collected from hatched chicks (n=6) were analyzed. Lipid analysis of blood plasma, heart, liver, yolk sac, and brain were conducted. Weights of all tissues in experiment two were also recorded. Tissues in both experiments were stored at -30 ^{o}C until analyzed.

3.2.7 Lipid Analyses: Total lipids were extracted from collected tissues (experiment one = liver; experiment two = blood plasma, liver, heart, brain, and yolk sac) by the method of Folch et al. (1957) and modified by Cherian et al. (2005). The whole tissue samples (0.5 mL of blood plasma) were weighed into a screw-capped test tubes each with 18 mL of chloroform and methanol solution (2:1, vol/vol), and homogenized with a polytron (Type PT10/35; Brinkman Instruments, Westbury, NY) for 30 sec at high speed. After an overnight incubation at 4C, 4 mL of 0.88% sodium chloride solution was added and mixed. The phases were separated by centrifugation, and the lower chloroform layer was collected for lipid analysis. Total lipids were determined gravimetrically.

***3.2.8 Statistical Analysis*:** A one way ANOVA was used to compare treatment means (SAS Institute, 2001). Means were compared by Duncan multiple range comparison test with level of significance $p<0.05$ (Steel and Torrie, 1980).

3.3 Results:

3.3.1 Experiment One: Hatchability is summarized in Figure 4. The saline group had a value of 80%, the CLA, 30% and the LNA and 55%. Total lipid content of hatched chick liver tissue (percent) from each treatment is summarized in Table 1. Total lipid content of liver tissue did not differ among the three treatment groups ($p>0.05$).

Figure 4. Effect of in ovo feeding of conjugated linoleic acid and alpha-linolenic acid on the hatchability of fertile eggs.

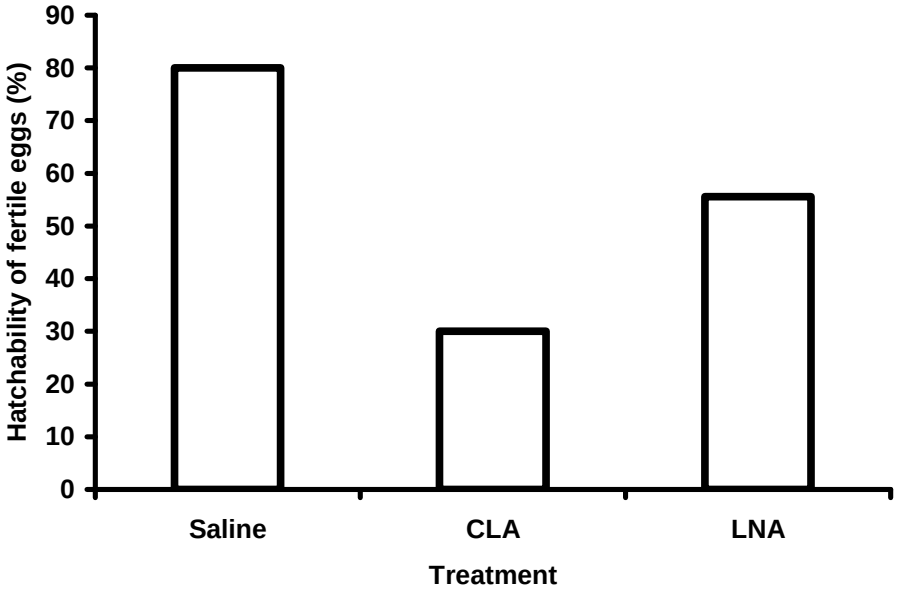


Table 1. Effect of in ovo feeding of conjugated linoleic acid, alpha-linolenic acid, or saline on hatched chick liver total lipid content (as percent of total tissue weight).

Treatment	Liver Total Lipid %
Saline	16.83
CLA	16.57
LNA	17.06

3.3.2 Experiment Two: Hatchability of eggs from the two sources that were subjected to the three experimental treatments are summarized in Figure 5. The treatment receiving in ovo feeding of PC and the control injection group had reduced hatchability compared to the non-injected control. Nearly 90% of fertile eggs in the non non-injected treatment hatched compared to 40% of control injection and roughly 60% hatchability of PC injected eggs. Differences in hatchability were similar among treatments consisting of commercial (source 2) and University (source 1) derived eggs. A slight drop is noticed

between source 2 and source 1 eggs receiving PC with hatchability over 10% higher in the Commercial eggs.

Figure 5. Effect of in ovo feeding of palmitate/carnitine on the hatchability of Source 1 and Source 2 fertile eggs.

Source 1 and Source 2 represent university and commercial, respectively.
Control and Palmitate represent eggs in ovo injected with control ethanol carrier and palmitate/carnitine, respectively.

Table 2 summarizes the average hatched chick weight and organ weight for source 2 eggs. Non-injected chicks had a higher average liver weight than chicks receiving control or PC injection ($p<0.05$). Average organ weight as percent of total chick body weight for all collected tissues of source 2 eggs are outlined in Table 3. Non-injected chicks had a higher liver weight as percent of total body weight than chicks receiving PC injection ($p< 0.05$), but no difference was observed between PC and control injection treatments. Heart weight as a percent of total body weight was higher in non-injected chicks compared to the PC injection treatment ($p<0.05$). No difference was

observed in the heart weight as percent of total body weight between control injection and PC injected treatments. There were no differences between the body weights, brain weights, yolk sac weights and the respective organ weights per unit body weight did not differ among treatments ($p>0.05$).

Table 2. The effect of in ovo feeding of palmitate/carnitine on hatched chick body weight and tissue weights (Source 2).

	---------- Chick and organ weight (g) ----------				
Treatment	**Chick**	**Heart**	**Liver**	**Yolk Sac**	**Brain**
Non-injected	48.41	0.34	1.07[a]	7.62	0.74
Control	47.19	0.32	0.94[b]	7.07	0.74
Palmitate/Carnitine	46.29	0.30	0.93[b]	6.24	0.68

[a,b]Means within a column not sharing a common superscript differ significantly ($p < 0.05$).
Control and Palmitate/Carnitine represent eggs in ovo injected with control ethanol carrier and palmitate/carnitine, respectively (n=6).

Table 3. The effect of in ovo feeding of palmitate/carnitine on hatched chick tissue weights as a percent of chick body weight (Source 2)

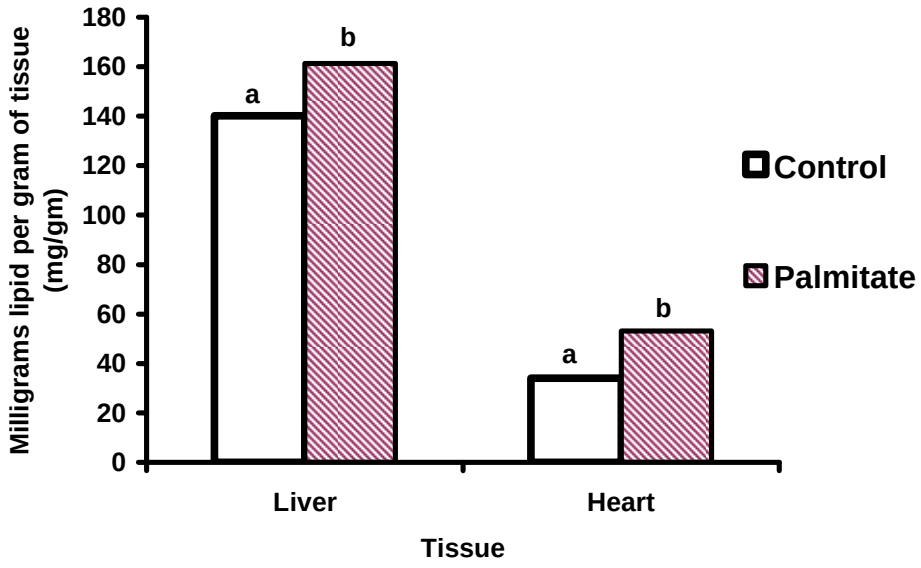
	---------- Organ weight as percent chick body weight (%) ----------			
Treatment	**Heart**	**Liver**	**Yolk Sac**	**Brain**
Non-injected	0.72[a]	2.28[a]	13.18	1.59
Control	0.69[a,b]	2.03[a,b]	14.64	1.51
Palmitate/Carnitine	0.63[b]	1.91[b]	15.81	1.34

[a,b]Means within a column not sharing a common superscript differ significantly ($p < 0.05$).
Control and Palmitate/Carnitine represent eggs in ovo injected with control ethanol carrier and palmitate/carnitine, respectively (n=6).

Total lipid analysis revealed that source 2 chicks receiving PC through in ovo injection had higher levels of lipids in their liver tissue than the control injected group ($p<0.05$) (Figure 6). Figure 6 also shows a similar observation, in that PC injection increased the total lipid content of heart tissue in hatched chicks compared to the control injection group ($p<0.01$). No differences were observed in the tissue lipid content of brain,

yolk sac or blood plasma between the PC injected and control injected treatments ($p>0.05$) (Table 4).

Figure 6. Effect of in ovo feeding of palmitate/carnitine on total lipid content of hatched chick tissues (Source 2).



[a,b]Columns not sharing common letters within each tissue group indicates significant difference ($p< 0.05$). Control and Palmitate represent eggs in ovo injected with control ethanol carrier and palmitate/carnitine, respectively (n=6).

Table 4. Tissue lipid concentration of hatched chicks (Source 2).

Treatment	Brain	Yolk Sac	Blood Plasma*
	mg lipid/gm tissue		
Control	54.18	234.21	4.49
Palmitate/Carnitine	57.27	242.08	6.00

*Plasma value mg lipid/mL blood plasma
Control and Palmitate/Carnitine represent eggs in ovo injected with ethanol carrier and palmitate/carnitine, respectively (n=6).

3.4 Discussion:

The goal of optimizing in-ovo injection technique was accomplished in experiment one. As evident by the hatchability of the saline injected treatment, there were

no adverse affects on hatchability as a result of the procedure or the benign compound provided. Hatchability among the experimental treatments that received injection of exogenous fatty acids (both CLA and LNA) had reduced hatchability compared to the saline injection group. The hatchability of the LNA treatment was higher than the CLA treatment. Both CLA and LNA injections reduced hatchability but did not affect liver tissue lipid content. As a result, the hypothesis that in ovo feeding of exogenous CLA and LNA would increase hatchability cannot be supported. However, the use of ethanol as a carrier to dilute the fatty acids and provide an aqueous injection is most likely the cause for the reduced hatchability observed in both experimental treatments. CLA has been implicated in reduced hatchability of fertile eggs when supplemented to breeder hen diets (Muma et al., 2006).

The use of ethanol as a carrier may have contributed to the drastic drop in hatchability among experimental treatments. The PC blend may have provided some benefit in counteracting the harmful action of the ethanol as an increase in hatchability over the control (ethanol carrier) treatment was observed. In ovo feeding of PC increased the lipid content of liver tissue in hatched chicks compared to control injection among commercially derived eggs. Increased lipids in the liver may have allowed for improved energy production at the time of hatch and could explain the difference between respective hatchability of the control and PC injected treatments.

The hypothesis for experiment two cannot be supported as hatchability was not increased through the in ovo feeding of PC. Surprising results were observed in regards to the action of PC in reversing the negative affects of ethanol injected in ovo. Future studies should include the use of water soluble fatty acid derivatives to prevent any

deleterious effects of ethanol on the developing embryo. Also, an increase in sample size (more eggs per treatment) and replication of experiments may provide more significant results in regards to hatchability.

4. OBJECTIVE THREE: THE EFFECT OF IN OVO FEEDING OF VITAMIN E ON HATCHABILITY, CHICK PLASMA AND TISSUE VITAMIN E AND POLYUNSATURATED FATTY ACID CONCENTRATIONS

4.1 Introduction:

The yolk of the egg provides fat that is oxidized by the embryo to produce energy for growth (Noble and Cocci, 1990; Romanoff, 1960). Yolk fat composition is dependant on breeder hen diet and the common use of low quality fats (low in antioxidant levels, highly oxidized etc.) in breeder hen diets do not produce an environment hospitable to embryo development that relies on fats (Cherian et. al.,1996; van Kempen and McComas, 2002). About day 14 of incubation, the embryo increases the uptake of fatty acids which may further increase the potential for lipid peroxidation to occur (Noble and Cocchi, 1990; Latour et al., 2000). As the chick begins pipping, the use of the chorioallantoic membrane for respiration begins to subside as pulmonary respiration of oxygen occurs, adding to the possible presence of radical oxygen species (Garcia et al., 1986; Moran 2007). Fats are also essential fuel for the heart and for deposition (of polyunsaturated fatty acids (PUFA)) in the brain (Cherian and Sim 1992).

The use of antioxidants, to protect lipids and cholesterols from peroxidation has been proven in animal models (Singh, et. al., 2005). The antioxidant Vitamin E (VE) has also been reported to have improved the immune response in turkeys and broilers as measured by the IgM levels of poults and the IgG levels of chicks (Gore and Qureshi, 1997). In addition to an increased immune response, exogenous VE administered around day 14 of incubation (the time of intense fatty acid oxidation) may reduce the production of radical oxygen species that can cause serious damage to the highly polyunsaturated

fatty acids of cellular membranes (Cherian and Sim, 1992; Cherian and Sim, 1997; Surai et al., 1999).

Low quality fats added to diets of breeder hens as an inexpensive energy source may be counteracted with the in ovo administration of antioxidants to protect the lipids of the yolk as well as the plasma membranes of chicks' cells from damage. Increased incidence of peroxidation of membrane lipids caused by free radical species may cause harm to the health of the developing embryo. In ovo feeding of VE may protect lipid membranes from the harmful effects of radical oxygen species and allow for increased lipid utilization for energy production to improve hatchability.

In the present experiment we wanted to observe the effects of in ovo feeding exogenous VE on the hatchability of broiler eggs and the fatty acid profile, total lipid content, and VE concentration of hatched chick tissues.

4.2 Materials and Methods:

4.2.1 Egg collection: A total of 100 Cobb broiler eggs were purchased from a commercial hatchery (Tangent, OR). All eggs were stored in a holding room at 18.3C for one week prior to incubation.

4.2.2 Incubation Conditions: Eggs were removed from the holding room and allowed to warm to room temperature. Care of all hatching eggs prior to and during hatching was constant among all treatments. Eggs in all treatments were set in the same tray in the same Jamesway Model 252 incubator with a dry bulb temperature of 37.5C and wet bulb temperature of 28.3C maintained from day 0-18 of incubation. On day 18 of incubation, all eggs were sorted by treatment, transferred to hatching trays, and the dry

bulb temperature was reduced to 36.3C while the wet bulb temperature was increased to 30.2C.

4.2.3 In ovo injection: On day 14 of incubation all eggs were removed from the incubator to facilitate in ovo injections (same protocol for injection as outlined in section 3.2.3). All eggs were candled and infertile eggs were removed. Viable eggs were randomly assorted into four treatments: injection of 10 IU VE (n=25), 20 IU VE (n=25), 100µL control vegetable oil (positive control) (n=25), and no injection (negative control) (n=19). For VE treatments, water dispersible VE capsules (0.4g = 400 IU VE) were diluted with vegetable oil at the following ratios: 0.3 g / 3 mL oil = 10 IU VE, and 0.6 g / 3 mL oil = 20 IU VE. Each VE injection consisted of 100µL. After completion of injections all eggs were returned to the same incubation tray and placed back in the incubator.

4.2.4 Sample collection: On the day of hatch all hatched chicks were counted and non-hatched eggs were opened to determine embryo status (pipping, late dead, mid dead, early dead). Hatched chicks from each treatment were weighed and dispatched to collect the following tissues: brain, liver, heart, yolk sac, bursa, and blood plasma. Weights of all tissues were recorded.

4.2.5 Lipid Analyses: Total lipids were extracted from collected tissues (blood plasma, liver, heart, brain, and yolk sac) by the method of Folch et al. (1957) and modified by Cherian et al. (2005). Approximately half of each collected tissue (0.5 mL of plasma) was weighed into a screw-capped test tube with 18 mL of chloroform and methanol solution (2:1, vol/vol), and homogenized with a polytron (Type PT10/35; Brinkman Instruments, Westbury, NY) for 30 sec at high speed. After an overnight

incubation at 4C, 4 mL of 0.88% sodium chloride solution was added and mixed. The phases were separated by centrifugation, and the lower chloroform layer was collected for lipid analysis. Total lipids were determined gravimetrically.

Lipids from all collected tissue extracts (2 mL) were placed into 16-mL screw-capped glass tubes and dried in a block heater at 39C under a gentle stream of nitrogen. The dried lipids were re-solubilized in 2 mL of boron trifluoride/methanol (10% w/w) and were heated in a 95–100C water bath for 60 min and fatty acid methyl esters (FAME) were prepared. The FAME were separated and quantified by gas chromatography. Analysis of FA composition was performed with an HP 6890 gas chromatograph (Hewlett-Packard Co., Wilmington, DE) equipped with an autosampler, FID, and fused-silica capillary column, 100 m × 0.25 mm × 0.2 μm film thickness (SP-2560; Supelco, Bellefonte, PA). Sample (2 μL) was injected with helium as a carrier gas onto the column programmed for ramped oven temperatures (initial temperature was 110C, held for 0.5 min, then ramped at 20C/min to 200C and held for 50 min, then ramped at 10C/min to 230C and held for 5.0 min). Inlet and detector temperatures were both 250C. Peak areas and percentages were calculated using Hewlett-Packard ChemStation software. FAME were identified by comparison with retention times of authentic standards (Matreya, Pleasant Gap, PA). FA values and total lipids are expressed as weight percentages.

4.2.6 Tocopherol Analysis: A modification of the methods by Podda et al. (1996), and Nierenberg and Nann (1992) were used for analysis of tissue α-tocopherols,. A known weight (0.1-0.2gm) of tissue sample was with homogenized with 2 mL PBS utilizing a Precision Scientific Co. 50/60 cycle 1/100hp homogenizer with Teflon homogenizing bit at low speed. A known amount of homogenate (0.4-0.5gm) was

transferred to into a 12mL screw capped tube. Distilled water (0.5mL) was added to each tube. A set of three standards were prepared utilizing 0.025mL, 0.05mL, and 0.1mL working vitamin E standard (DL-alpha-tocopherol molar concentration 0.000642). All tubes received 0.1mL of internal standard (0.125mL stock solution/5mL ethanol; stock solution: 10mg rac-5-,7-dimethyltocol, Matreya LLC, Pleasant Gap, PA, dissolved per mL ethanol).

For blood plasma samples, 0.2mL plasma was added to a 12mL screw-capped tube, 0.8mL distilled water was added and the mixture was vortexed. Three standards were created with extra plasma and 0.1mL, 0.2mL, and 0.05mL working vitamin E standard. All tubes received 0.1mL of internal standard.

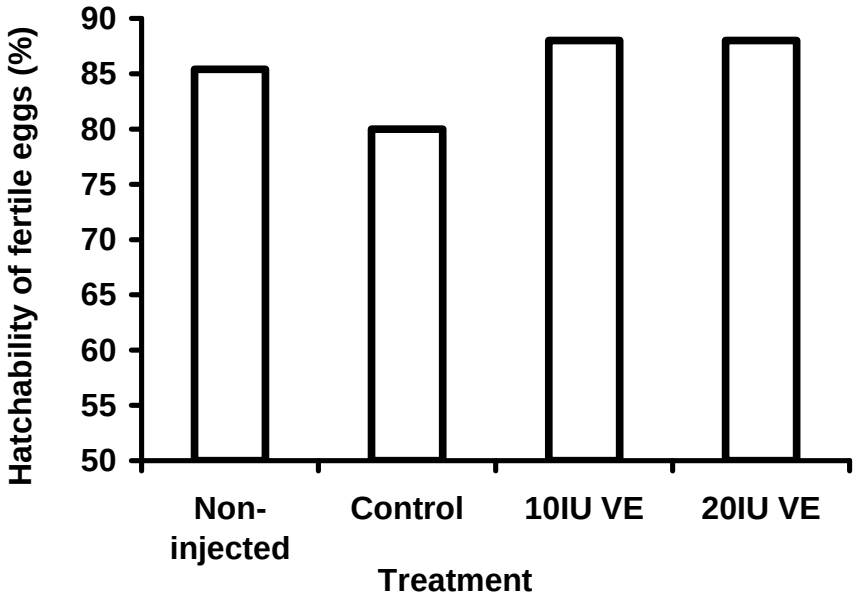
Plasma and tissue samples were saponified with alcoholic KOH, extracted with hexane, dried under nitrogen, re-suspended in ethanol, then auto-injected into a high performance liquid chromatography (HPLC) system. The HPLC system consisted of a Shimadzu LC-2010A HT controller and auto injector (Shimazdu Scientific Instruments). The column used was a Supelcosil LC-18, 250 × 4.6 mm, 5.0 μm particle size, and pore size of 120 angstroms. An isocratic mobile phase delivery system (97.5% methnaol:2.5% distilled water (v:v)) was used, with a flow rate of 1.0mL/min and total run time of 16 min. The Ultraviolet detector was set at a wavelength of 295nm. Peak areas were integrated using a Shimadzu EZStart V7.1.1 Chromatography software package.

4.2.7 Statistical Analysis: A one way ANOVA was used to compare treatment means (SAS Institute, 2001). Means were compared by Duncan multiple comparison test with level of significance $p<0.05$ (Steel and Torrie, 1980).

4.3 Results:

Figure 7 summarizes the hatchability for the treatments. Hatchability of control injected chicks was numerically reduced compared to the experimental treatments and the non-injected control group. Chicks that received VE in ovo hatched at a slightly higher rate (88% for both 10 IU and 20 IU treatments) than the non injected control (85.4%), however no statistical analysis was performed.

Figure 7. Effect of in ovo feeding of vitamin E on the hatchability of fertile eggs.



Non-injected and Control represent eggs receiving no in ovo injection or injection of vegetable oil, respectively.

The average hatched chick weight and tissue weights are contained in Table 5. Mean brain weights of the control injected and both VE injected treatments were significantly greater than the non injected chicks ($p<0.05$). No difference was observed in

the body weight of chicks or the weights of liver, heart, yolk sac, or bursa between treatments ($p>0.05$).

Table 5. Effect of in ovo feeding of vitamin E on hatched chick body weight and tissue weights.

	Chick and organ weight (g)					
Treatment	**Chick**	**Liver**	**Heart**	**Brain**	**Yolk Sac**	**Bursa**
Non-injected	48.97	1.038	0.368	0.775[a]	5.018	0.078
Control	47.23	1.133	0.376	0.971[b]	5.375	0.057
10 IU VE	47.80	1.073	0.351	0.917[b]	5.432	0.046
20 IU VE	45.14	1.007	0.346	0.964[b]	4.183	0.060

[a,b]Means within a column not sharing a common superscript differ significantly ($p<0.05$).
Non-injected and Control represent eggs receiving no in ovo injection or injection of vegetable oil, respectively (n=6).

Table 6 lists the relative average organ weights as percent of chick body weight in all treatments. Brain weight as a percent of total chick weight was increased in all the injected treatments (control, 10 IU and 20 IU VE) compared the non-injected control ($p<0.05$). No difference was observed between treatments for the remaining tissue weights as percent of total chick weights ($p>0.05$).

Table 6. Effect of in ovo feeding of vitamin E on hatched chick tissue weights as a percent of chick body weight.

	Organ weight as percent chick body weight (%)				
Treatment	**Liver**	**Heart**	**Brain**	**Yolk Sac**	**Bursa**
Non-injected	2.129	0.752	1.591[a]	10.207	0.161
Control	2.401	0.795	2.055[b]	11.420	0.119
10 IU VE	2.244	0.734	1.920[b]	11.324	0.096
20 IU VE	2.254	0.766	2.122[b]	9.176	0.134

[a,b]Means within a column not sharing a common superscript differ significantly ($p<0.05$).
Non-injected and Control represent eggs receiving no in ovo injection or injection of vegetable oil, respectively (n=6).

Total lipid analysis of brain tissue in all three injected treatments (control, 10 IU and 20 IU VE) compared to non-injected chicks were also increased ($p<0.05$) (Table 7).

Non injected chicks had the lowest level of brain total lipids at 4.29%. Total lipid content of blood plasma was not increased in the control group compared to the 20 IU VE injected treatment or non injected chicks ($p<0.05$). No difference was observed between the 10 IU VE group and all other treatments.

Table 7. Effect of in ovo feeding of vitamin E on total lipid content of tissues from hatched chicks.

Treatment	Liver	Heart	Brain	Yolk Sac	Plasma
	percent lipid of total tissue wt.				
Non-injected	17.13	3.21	4.26[c]	10.62	2.63[a]
Control	18.72	3.10	4.69[a]	11.09	2.25[b]
10 IU VE	17.10	3.10	4.53[a,b]	9.41	2.49[a,b]
20 IU VE	17.29	2.77	4.47[b]	11.14	2.59[a]

[a-c]Means within a column not sharing a common superscript differ significantly ($p<0.05$).
Non-injected and Control represent eggs receiving no in ovo injection or injection of vegetable oil, respectively (n=6).

Polyunsaturated fatty acid concentrations of tissues collected from hatch chicks are summarized in Table 8. Chicks in the injection of control group had higher levels of PUFA in brain tissue than the non injected treatment ($p<0.05$).

Table 8. Effect of in ovo feeding of vitamin E on the polyunsaturated fatty acid (PUFA) content of tissues from hatched chicks.

Treatment	Liver	Brain	Yolk Sac	Plasma
	percent PUFA			
Non-injected	21.77	23.36[b]	15.39	32.59
Control	20.59	24.38[a]	16.06	33.32
10 IU VE	21.29	23.89[a,b]	14.96	32.07
20 IU VE	20.80	23.99[a,b]	14.69	33.13

[a,b]Means within a column not sharing a common superscript differ significantly ($p<0.05$).
Non-injected and Control represent eggs receiving no in ovo injection or injection of vegetable oil, respectively (n=6).

The concentration of n-6 fatty acids in tissues from hatched chicks are summarized in Table 9. Yolk sac tissue of chicks in the control group had increased percent n-6 FA than non injected chicks ($p<0.05$).

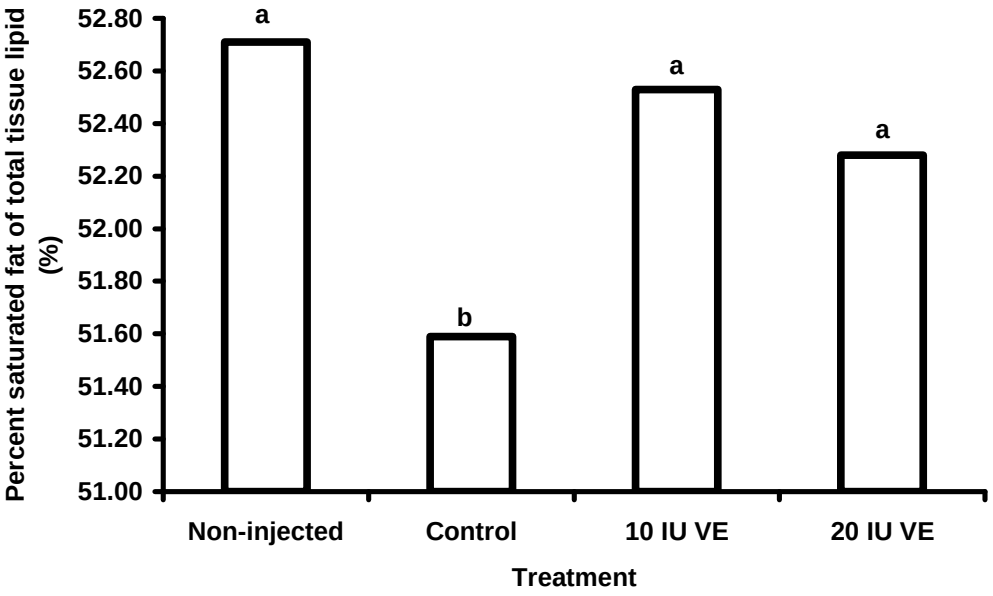
Table 9. Effect of in ovo feeding of vitamin E on the n-6 fatty acid content of tissues from hatched chicks.

Treatment	Brain	Liver	Yolk Sac	Plasma
	---------- percent n-6 fatty acids ----------			
Non-injected	12.38	8.65	1.43[b]	11.66
Control	12.79	7.61	1.89[a]	12.03
10 IU VE	16.61	7.95	1.59[a,b]	11.55
20 IU VE	12.28	7.98	1.43[a,b]	12.09

[a,b]Means within a column not sharing a common superscript differ significantly ($p<0.05$).
Non-injected and Control represent eggs receiving no in ovo injection or injection of vegetable oil, respectively (n=6).

The concentration of saturated fat (as percent of total tissue lipid) in hatched chick brain tissue was increased in non injected, 10 IU VE, and 20 IU VE treatments respectively, compared with the control injected group (Figure 8).

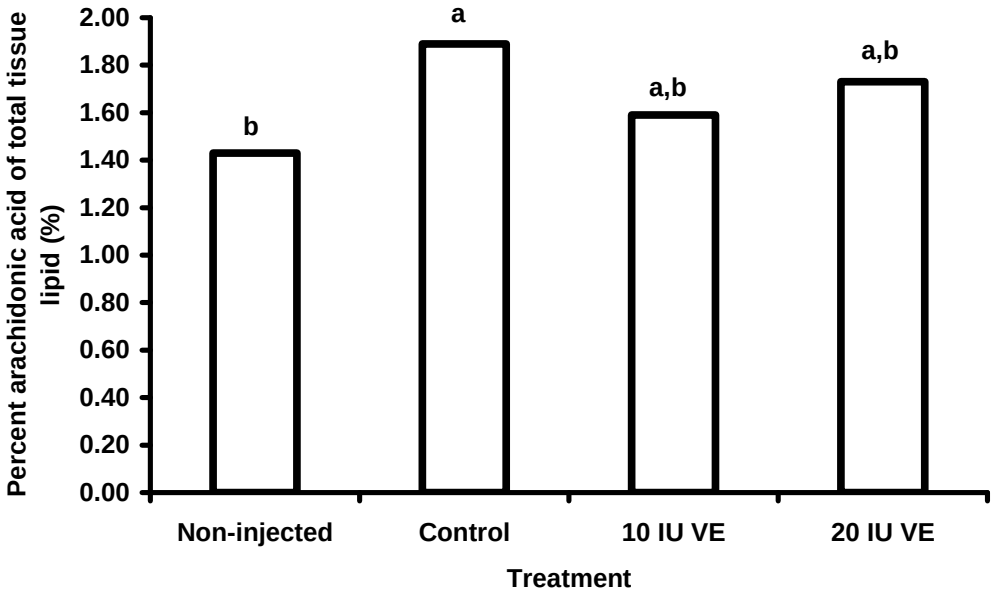
Figure 8. Effect of in ovo feeding of vitamin E on the saturated fat concentration of brain tissue from hatched chicks.



[a,b]Columns not sharing common letters differ significantly ($p < 0.05$).
Non-injected and Control represent

Figure 9 summarizes the concentration of Arachidonic acid (AA) in hatched chick yolk sac tissue. Control injected chicks' yolk sac had increased AA than chicks in the non injected treatment ($p<0.05$).

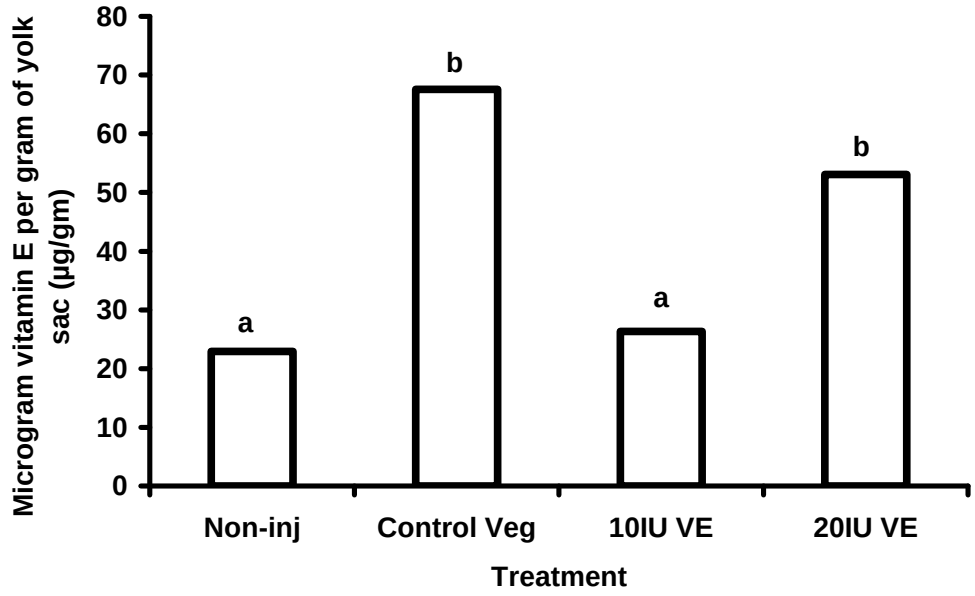
Figure 9. Effect of ino ovo feeding of vitamin E on the arachidonic acid concentration of yolk sac tissue from hatched chicks.



[a,b]Columns not sharing common letters differ significantly ($p < 0.05$).
Non-injected and Control represent eggs receiving no in ovo injection or injection of vegetable oil, respectively (n=6).

VE levels of yolk sac in control injected and 20 IU VE injected chicks were increased compared with levels in non-injected and 10 IU VE injected chicks ($p<0.05$) (Figure 10). Non-injected chicks had 22.9µg VE per gm of yolk sac while control injected chicks had 67.5µg VE per gm of yolk sac. Chicks that received 20 IU of VE in ovo had 53.0µg VE per gm of yolk sac while the 10 IU VE treatment had only 26.3µg VE per gm of yolk sac.

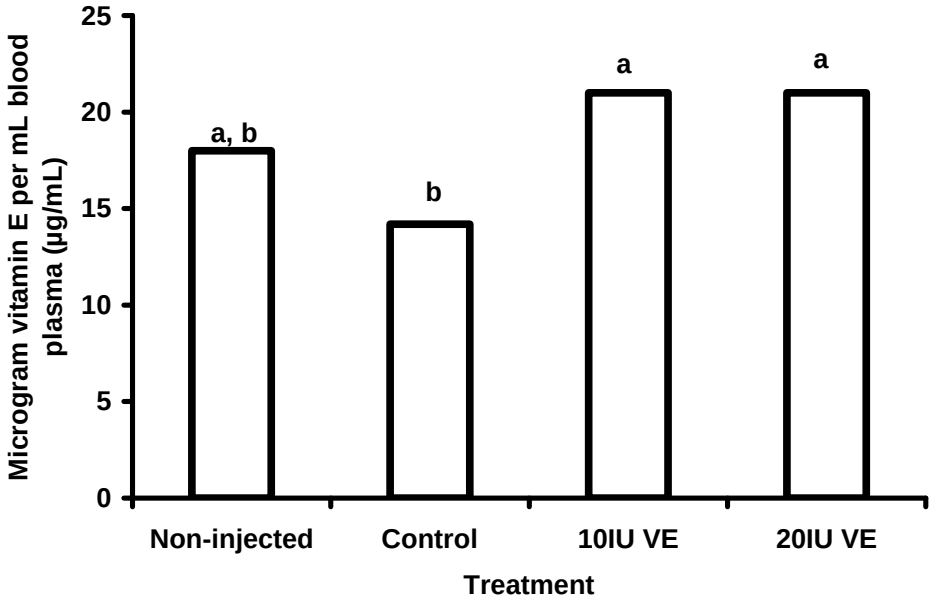
Figure 10. Effect of in ovo feeding of vitamin E on hatched chick yolk sac vitamin E concentration.



[a,b]Columns not sharing common letters differ significantly ($p < 0.05$).
Non-injected and Control represent eggs receiving no in ovo injection or injection of vegetable oil, respectively (n=6).

Blood plasma VE levels in both VE injected treatments (10 IU and 20 IU VE) were higher than in control injected chicks ($p<0.05$), but not different from the non injected group (Figure 11). Both 10 IU and 20 IU VE treatments had VE levels of 21.0µg VE per mL of plasma, while control injected chicks had only 14.2µg VE per mL of plasma and Non-injected chicks had 18.0µg VE per mL of plasma.

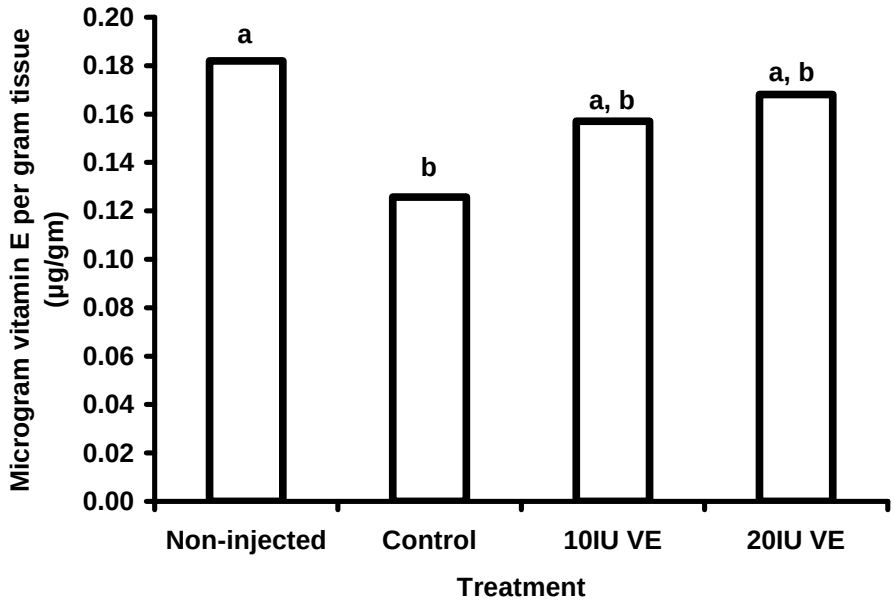
Figure 11. Effect of in ovo feeding vitamin E on hatched chick blood plasma vitamin E concentration.



[a,b]Columns not sharing common letters differ significantly ($p < 0.05$).
Non-injected and Control represent eggs receiving no in ovo injection or injection of vegetable oil, respectively (n=6).

Concentration of vitamin E in heart tissue (Figure 12) was higher ($p<0.05$) in non-injected chicks compared with the control injection. Neither VE injected treatment differed from the non injected group.

Figure 12. Effect of in ovo feeding of vitamin E on hatched chick heart vitamin E concentration.

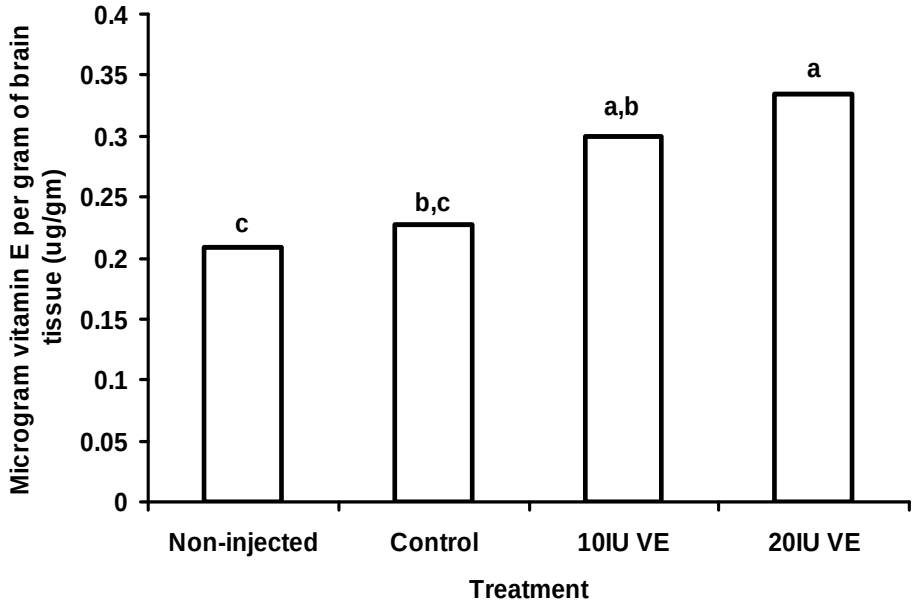


[a,b]Columns not sharing common letters differ significantly ($p<0.05$).
Non-injected and Control represent eggs receiving no in ovo injection or injection of vegetable oil, respectively (n=6).

Brain tissues VE levels for each treatment are shown in Figure 13. VE levels in the VE injected treatments were significantly higher than in non-injected chicks ($p<0.02$). The 20 IU VE treatment had the highest level VE of 0.33µg VE per gm of brain tissue. Non injected chick had an average of 0.21µg VE per gm of brain tissue.

No differences were observed among the VE content of liver tissues of all treatments ($p>0.05$).

Figure 13. Effect of in ovo feeding of vitamin E on hatched chick brain vitamin E concentration.



[a-c]Columns not sharing common letters differ significantly ($p<0.05$).
Non-injected and Control represent eggs receiving no in ovo injection or injection of vegetable oil, respectively (n=6).

4.4 Discussion:

Overall, hatchability appeared to have increased in the experimental treatments but replications will be necessary to perform statistical analysis. As seen in Figure 7, both experimental treatments of VE injection may have increased hatchability (2.6% without replication) over the control treatments. Both VE injected treatments had improved hatchability compared with non injected and control treatments possibly suggesting the in ovo feeding of vitamin E could enhance the ability of the chick to produce energy for hatching.

Total lipid content of the brain tissue was increased in all three injected treatments. The VE level of brain tissue was also increased among the injected treatments compared

to non-injected control and may suggest the in ovo feeding of antioxidants acted to protect the PUFA of brain tissue, although relative levels of PUFA in the brain was increased in the control injected group compared to the non injected treatment. The AA concentration was also increased in the yolk sac of control injected chicks compared with the non injected group, while the concentration of saturated fat was lower in the yolk sac of the control group compared with the non injected, 10 IU VE, and 20 IU VE treatments. The vegetable oil carrier could have played a role in improving the fatty acid availability for the growing chick and may have provided exogenous fatty acids for energy production.

The administration of the injection through the large end of the egg may have benefited the brain of the chick if the needle were directed towards the brain at time of injection. This may explain the increased lipid content of brain tissue as well as increased VE concentration of brain tissue in injected treatments. Localization of injection may also explain the reduced VE level of heart tissue in the control group compared with the non-injected treatment.

Total lipids of blood plasma were highest in the non-injected and 20IU VE injected treatments although the blood plasma levels of VE were highest in both the VE injected treatments. Levels of VE in blood plasma were also increased in the experimental treatments, with the highest levels occurring in the 20 IU injected group (figure 2). Vitamin E concentrations in both the liver and brain also appear higher in experimental treatments compared to the controls. In both tissues the 20 IU group had the highest concentration of vitamin E per gram. Chick weight was unaffected throughout the treatments

The in ovo feeding of VE may enhance the antioxidant status of hatched chick tissues and protect lipid membranes from radical oxygen species. The use of a vegetable oil carrier proved effective as a carrier for the VE and also altered the FA content of select hatched chick tissues compared to control. Determining the constituent fats of the vegetable oil may prove beneficial in future work as well as the antioxidant concentration (if any) of the oil.

5. CONCLUSIONS

5.1 Final Thoughts:

In ovo technology provides an intriguing method for potentially increasing the hatchability of broiler eggs. The injection of beneficial compounds into embryos to affect chick health or modulate metabolism can be seen as a way to effectively improve production with minimal labor, costs, or harm to the embryo.

Experiment one proved beneficial in developing the methods for providing compounds in ovo to developing embryos. The use of ethanol as a carrier for fatty acids may have contributed to the viability of embryos and reduced the effectiveness of the in ovo feeding of fatty acids compared to a saline solution. The study was useful as key principles were developed such as protocol for injection, and the refinement of assays that will allow for the expansion of future studies.

Antioxidants provided through in ovo feeding may affect hatchability and/or the tissue lipid content of hatched chicks. In ovo feeding of VE may have improved the hatchability of embryos receiving VE in ovo in experiment three. The effects of in ovo feeding of 10 IU and 20 IU of VE appeared to have a significant influence on the brain tissue and blood plasma VE levels of hatched chicks. Vegetable oil provided in ovo may also increase the n-6, AA, and PUFA levels of tissues in hatched chicks compared to non injected treatments.

Overall, the success of in ovo feeding will rely on capitalizing on the peak utilization of nutrients by the embryo in the proper amount and with an inert carrier that will not affect embryo livability.

5.2 Future Work:

In order to prove the effects of antioxidant on increasing hatchability, the study size (number treatments and controls) need to be increased and replicated. The long-term effects of in ovo feeding on chick health should also be explored with grow outs of hatched chicks to market weight while tracking production characteristics (growth rate, feed conversion, mortality), and immune responses (IgG, IgY levels) following exposure to antigen. Other antioxidant compounds such as Vitamin A or Se could be utilized in future studies to determine effects on hatchability and tissue lipid content.

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