4-30-2011

Evaluation of L-carnitine in ovo injection followed by L-carnitine feed supplementation on broiler hatching and growing characteristics

Michael Ray Dooley

EVALUATION OF *L-CARNITINE IN OVO* INJECTION FOLLOWED BY *L-CARNITINE* FEED SUPPLEMENTATION ON BROILER HATCHING AND GROWING CHARACTERISTICS

By

Michael Ray Dooley

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Poultry Science
in the Department of Poultry Science

Mississippi State, Mississippi

April 2011

EVALUATION OF *L-CARNITINE IN OVO* INJECTION FOLLOWED BY *L-CARNITINE* FEED SUPPLEMENTATION ON BROILER HATCHING AND GROWING CHARACTERISTICS

By

Michael Ray Dooley

Approved:

Alejandro Corzo Associate Professor of Poultry Science (Director of Thesis)	Aaron S. Kiess Assistant Professor of Poultry Science (Committee Member)
E. David Peebles Professor of Poultry Science (Committee Member)	Christopher D. McDaniel Professor of Poultry Science (Graduate Coordinator)

George Hopper
Interim Dean of the College of Agriculture and Life Sciences

Name: Michael Ray Dooley

Date of Degree: April 29, 2011

Institution: Mississippi State University

Major Field: Poultry Science

Major Professor: Dr. Alejandro Corzo

Title of Study: EVALUATION OF *L-CARNITINE IN OVO* INJECTION FOLLOWED BY *L-CARNITINE* FEED SUPPLEMENTATION ON BROILER HATCHING AND GROWING CHARACTERISTICS

Pages in Study: 42

Candidate for Degree of Master of Science

Ross × Ross 708 eggs were injected with commercial diluent containing supplemental *L-carnitine* at 8, 16, or 32 mg/100 µL concentrations using an automated multi-egg injector. After hatching, 1,080 male and female broiler chicks were distributed into 90 pens with chicks at each of the injected concentrations receiving feed that was or was not supplemented with 50 ppm of *L-carnitine*. Treatments did not affect incubation time or hatchability of fertilized eggs. Birds fed supplemental *L-carnitine* and injected *in ovo* with *L-carnitine* had lower body weight and ate less feed. The same birds exhibited a reduction in feed conversion compared to birds that did not receive supplemental dietary *L-carnitine*. Absolute breast weight was reduced in birds given *L-carnitine in ovo* and in the feed. Broiler diets containing 50 ppm *L-carnitine* appeared to be slightly toxic if provided with 8, 16, or 32 mg/100 µL of *L-carnitine* administered via *in ovo* injection.

Key words: *L-carnitine*, hatchability, *in ovo*

DEDICATION

I would like to dedicate this research to my beautiful wife, Mary Elizabeth Dooley, whose patience has guided me throughout this process. I would also like to dedicate this research to my parents, Dennis and Debbie Dooley, and my sister Jennifer Dooley, whose love has guided me throughout my whole educational process. I would also like to dedicate this paper to every other family member, friend, or important figure in my life for even the tiniest thing said or done to guide my life in the right direction. My family is the main reason that I was able to complete this process.

ACKNOWLEDGEMENTS

The author would like to express great gratitude and appreciation to the people that made sacrifices to ensure the completion of this thesis. First of all, my sincere thanks are due to Dr. Alex Corzo, my committee chairman, for his distinguished advice, his time and devotion, and his overall effort to help and assist me throughout my graduate process. I would also like to thank Dr. E. David Peebles and Dr. Aaron Kiess for their advice, comments, and hard work to make this process a more enjoyable experience. I would like to specially thank my fellow associates, Leonel Mejia, Bob Loar, and Keyla Lopez for their patience and help throughout the whole process. Special thanks to Leonel Mejia for the long afternoons, egg candling on the weekend, and helping with hatch monitoring. I would also like to extend my greatest gratitude to Sharon Womack and Wei Zhai for their guidance and help throughout the whole incubation, transfer, and hatching phases of the study. Other special thanks go to the farm workers for their work in weighing feed and birds throughout the study.

I would also like to thank my wife, Mary Elizabeth, for going back and forth to the farm at all hours of the day and night with me. I would like to thank her for being dedicated to me. I would have been lost in this whole process if not for her. I want to also thank my parents for their support, especially my mom for her guidance throughout my life.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

FIGURE Page

CHAPTER I

INTRODUCTION

During these tough economic times, poultry producing companies are searching for ways to cut costs. Nutritionally, companies do so by looking for cheaper ingredients, and researching feed ingredients that allow for better feed conversions. Feed accounts for up to 70 % of the total cost to raise a poultry broiler. As feed ingredient prices fluctuate, the survival of some companies could be in jeopardy. Hatcheries are also looking for new ways to increase livability and to increase hatch rate. Hatch rate is crucial and companies compete for better hatch rates, because a 1 – 2 % decrease in hatch rate could have negative consequences for most companies. Another major concern in the poultry industry is excessive fat within the animal. Excessive fat has caused leg problems and decreased mobility in flocks. Intense genetic selection has caused problems in flocks and those problems are now being noticed by the consumer.

The current study will explore the effects of *L-carnitine* supplementation via application of *in ovo* technology on commercial broiler hatching eggs and the further enhancement of subsequent chick performance through diet supplementation. Previous research has studied the effect of injecting *L-carnitine* into the embryo while other research has focused on the *L-carnitine* supplementation after hatch. However, in this study the metabolic ingredient *L-carnitine* will be administered via *in ovo* injection, along with the current vaccine regiment, alone or in combination with diet supplementation, to enhance the livability and overall performance of broiler chicks. No previous research

has studied these two effects of *L-carnitine* simultaneously. Effects of *in ovo* and dietary *L-carnitine* administrated on grow-out performance, and processing cuts were examined in detail.

L-carnitine was chosen because of the importance of *L-carnitine* in fat metabolism. Previous authors showed a decrease in fat deposition when *L-carnitine* was supplied in the feed (Kidd et. al 2005). Keralapurath et. al 2010 showed a decrease in hatch time with injections of .5 mg, 2 mg, and 8 mg of *L-carnitine* along with 100 µL commercial diluent and showed a significant decrease in hatch time. Therefore the levels of 8 mg, 16mg, and 32 mg of *L-carnitine* with 100 µL of commercial diluent were chosen to see if a significant effect could be observed for hatch time and hatchability. Supplementation of 50 ppm of *L-carnitine* was the standard for most research to test the effects of *L-carnitine* on grow-out and processing data.

CHAPTER II

REVIEW OF LITERATURE

Avian embryo

In contrast to mammals, avian embryo development is restricted by the nutrients within the egg, and embryo and chick weight are influenced by yolk nutrient status because of the chicks' yolk utilization post-hatch. The chick embryo develops outside the mother in a self-sufficient egg, is easy to manipulate, and is amenable to transplantation, explanation, and micro-dissection techniques. However, the disruption of the embryo is imminent when studying the development processes of the chick. To better study the process, El-Ghali et al. (2010) outlined modifications in the methods used in imaging the chicken embryo for a prolonged period of time *in ovo*. He also presented a new way of manipulating the embryonic environment as a way to look at the function of specific molecules during early development.

Chick embryos have limited ability to digest and absorb nutrients prior to hatch, as reflected by relatively low mRNA levels for sucrase-isomaltoase, choline, l-aminopeptidase, ATPase, and sodium glucose transporter (SGLT-1) in the small intestinal mucosa (Uni and Ferket, 2003). Carbohydrates are also critical during the final stage of chick embryo development, prior to emergence from the shell, and very little carbohydrate remains in the egg before hatch (Christensen et al., 1993).

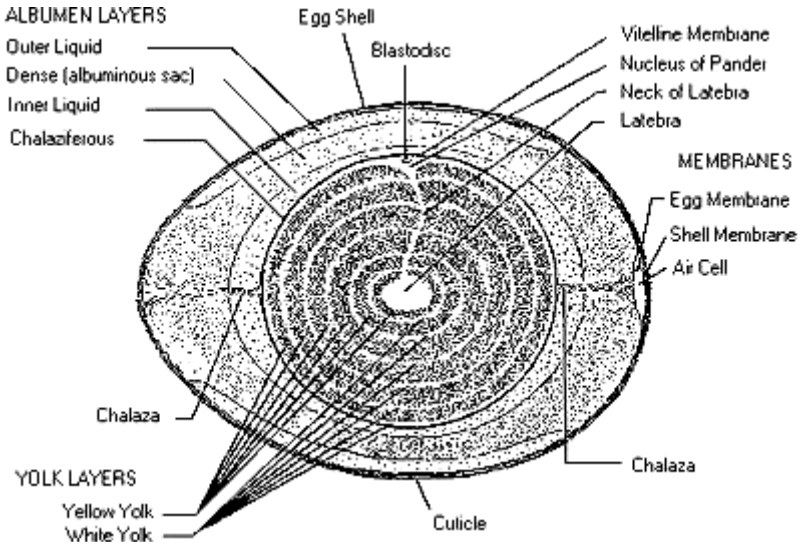

Figure 2.1 Components of a chicken egg viewed along its longitudinal axis (Smith, 2002).

The chicken egg starts as an egg yolk inside of a hen. An oocyte is produced by the hen's ovary in a process called ovulation. The yolk is released into the oviduct, where it can be fertilized internally by a sperm. The yolk continues down the oviduct (fertilized or unfertilized) and is covered with a membrane (vitelline membrane), structural fibers, and layers of albumin. This part of the oviduct is called the magnum. As the egg travels down through the oviduct, it is continually rotating within the spiraling tube. This movement twists the structural fibers (chalazae), which form rope-like strands that anchor the yolk in the thick egg white. There are two chalazae anchoring each yolk on opposite ends of the egg. The eggshell is deposited around the egg in the lower part of the oviduct of the hen just before it is laid. The shell is made of calcite, a crystalline form of calcium carbonate. This entire trip through the oviduct takes about one day. The fertilized blastodisc (blastoderm) grows and becomes the embryo. This table would explain why *L-carnitine* is limited within the egg. Waste products collect in a sack called the allantois. The exchange of oxygen and carbon dioxide gas occurs through the eggshell; the chorion

lines the inside surface of the egg and is connected to the blood vessels of the embryo. The embryo develops inside the egg for twenty one days (the incubation period), until a chick pecks its way out of its eggshell and is hatched.

The primary energy source of the embryo is the yolk, and as seen in the table below amino acids is a vital part of the egg. Since *L-carnitine* is synthesized from Methionine and Lysine which is only a small portion of the amino acids contained within the egg. Also since Methionine and Lysine are essential amino acids. Therefore carnitine is an essential nutrient in animals (Borum and Bennet 1986).

Table 2.1 Major ingredient list of an egg yolk (raw and fresh) USDA National Nutrient Database for Standard Reference, Release 23 (2010)

Nutritional value per 100 g (3.5 oz)	
Energy	1,325 kJ (317 kcal)
Carbohydrates	3.59 g
Fat	26.54 g
Protein	15.86 g
Tryptophan	0.177 g
Threonine	0.687 g
Isoleucine	0.866 g
Leucine	1.399 g
Lysine	1.217 g
Methionine	0.378 g
Cystine	0.264 g
Phenylalanine	0.681 g
Tyrosine	0.678 g
Valine	0.949 g
Arginine	1.099 g
Histidine	0.416 g

Alanine	0.836 g
Aspartic acid	1.550 g
Glutamic acid	1.970 g
Glycine	0.488 g
Proline	0.646 g
Serine	1.326 g
Water	52.31 g
Vitamin A equiv.	381 μg (42%)
Thiamine (Vit. B_1)	0.176 mg (14%)
Riboflavin (Vit. B_2)	0.528 mg (35%)
Pantothenic acid (B_5)	2.990 mg (60%)
Folate (Vit. B_9)	146 μg (37%)
Calcium	129 mg (13%)
Iron	2.73 mg (22%)
Magnesium	5 mg (1%)
Phosphorus	390 mg (56%)
Potassium	109 mg (2%)
Zinc	2.30 mg (23%)
Choline	682.3 mg
Cholesterol	1234 mg
One large egg contains 17 grams of yolk. Percentages are relative to US recommendations for adults.	

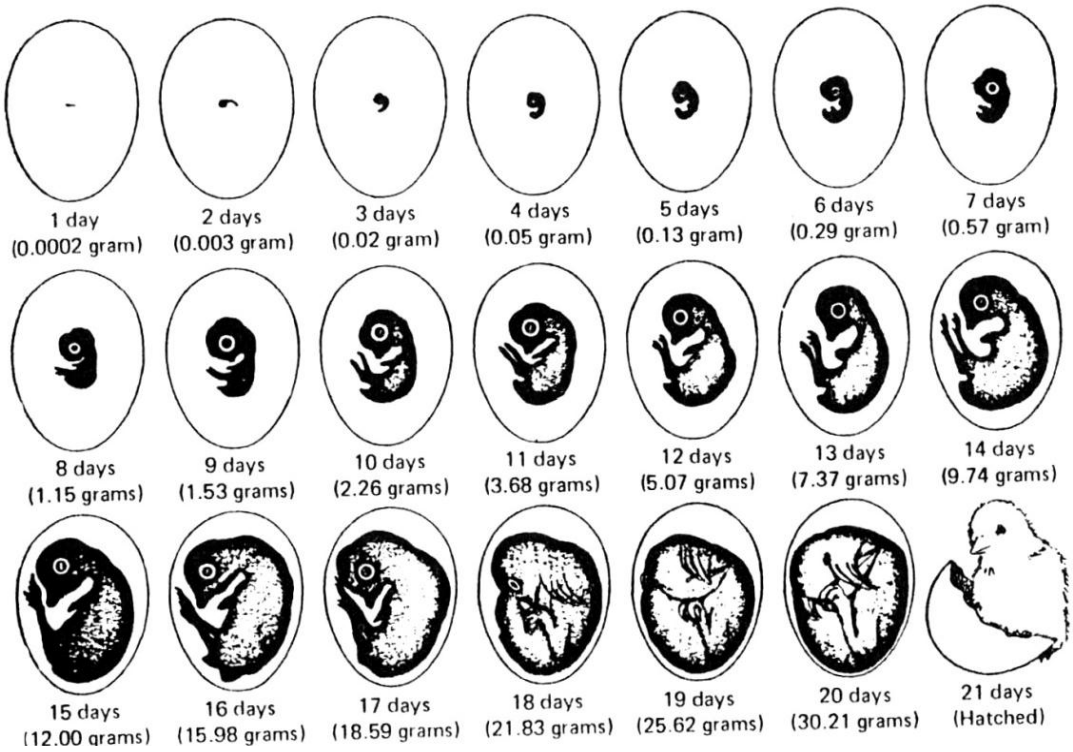

Figure 2.2 Daily body weight gain and changes in morphological appearance of a growing chick embryo (Parkhurst and Mountney, 1988)

The incubation process of a chicken embryo takes 21 days. The egg is placed in the incubator for the first 18 days and in the hatcher for the final 3 days. On Day 2 the heart, brain, and vascular system formation is occurring by the end of Day 3 of incubation the beak begins developing and limb buds appear. On Day 7 digits appear on the wings and feet. At this time the bird can be easily identified. At the beginning of Day 10, forelimbs appear to be wings. By Day 13 of incubation, the down and claws start to appear. Day 14, the chick moves into hatching position, and by Day 17, the chick's beak turns toward the air cell. On Day 19, the yolk sac begins to enter the body cavity, internal pipping starts, and the embryo begins external respiration. On Day 20, the yolk sac is completely drawn into the body cavity, and hatching of the chick begins on Day 21.

Since embryos are Poikiolotherms and cannot maintain their own body heat (Oppenheim and Levin, 1975), it is important to transfer eggs quickly from the incubator to the hatcher and to inject the embryo as quickly as possible. A decrease in the environmental temperature occurs during transfer of chicks and causes a decrease in embryonic body temperature followed by a decrease in embryonic metabolic rate, which subsequently delays the hatching process (Zakaria and al-Anezi, 1996). Suarez (1996) also reported that incubation time was delayed during the time that embryos experience cooling during transfer and injection. Many other factors affect time of hatch, such as the age of embryo, the temperature at which the embryo is exposed, and the genotype of the embryo.

Carnitine (*L-carnitine*)

Carnitine exists in two stereoisomers. The biologically active form is *L-carnitine*, whereas its enantiomer, D-carnitine, is biologically inactive. The precursors of *L-carnitine* are lysine and methionine, the vitamins B_6, B_{12}, C, folic acid and niacin, and the trace element iron. Carnitine was discovered in the early 1900s, but its importance in fatty acid metabolism was not established until fifty years later and, the biosynthesis of carnitine has been demonstrated within chick embryos (Bremer, 1983).

The kidneys produce about 25 % of the carnitine required by the body, and the other is acquired through the diet within red meat, poultry, fish, and dairy products. Therefore, 75% of the required amount must be obtained in the diet and for most healthy chickens it is met with ease; however, malnutrition and certain disorders can cause a deficiency. Stress, disease, and physical strain can also result in carnitine deficiency.

Symptoms of carnitine deficiency are a weakened enlarged heart, confusion, muscle weakness, and low blood sugar.

All precursors of *L-carnitine* are necessary as catalysis for the endogenous synthesis of *L-carnitine*, and its role as a transporter of long-chain fatty acids across the mitochondrial membrane and to facilitate β-oxidation of long chain fatty acids for energy production. β-oxidation occurs with the activation of fatty acids in the cytosol, and then those fatty acids are transported in the mitochondria. Once in the mitochondria, the fatty acids are broken down to generate Acetyl-CoA. This is very important in order for the chick to generate the required energy during the pipping process, and since short chain esterified carnitine is at the maximum level within the liver, brain, and heart on Day 18 of egg incubation (Rinaudo et al. 1991). This fact increases the importance of the time frame in which the *in ovo* injections of amino acids, sugars, and vaccines take place, further particularly since on Day 18 or 18.5 are times at which injections are given in the commercial industry. Also since piping begins on d 19 within the embryo, carnitine concentration in the late stage of embryonic growth becomes more important, because *L-carnitine* is limited in chicken embryos. Free carnitine levels also remain constant in all tissues during embryonic development. Rapid development, a high energy requirement, and a low level of *L-carnitine* synthesis in chicken embryos may make carnitine supplementation even more beneficial to chicken embryos (Zhai et al., 2008).

Carnitine also plays a significant role in energy metabolism as a carrier for the transport of the activated long chain fatty acids across the inner mitochondrial membrane, and causes free coenzyme-A to pool up. Once free coenzyme-A has pooled up, this stimulates the generation of metabolizable energy (Bremer, 1983). Also, carnitine influences fat metabolism by being integrally being involved in fatty acid oxidation

(Casillas and Newburgh, 1969; Nishida et al., 1989). Furthermore, carnitine has been shown to specifically facilitate the transfer of fatty acyl groups from the yolk into tissues of embryonic chicks via the yolk sac membrane (Casillas and Newburgh, 1969).

Carnitine is also a critically important nutrient in energy metabolism in most voluntary and cardiac muscles (Borum, and Bennet, 1986), and has been proven to be an important nutrient in both human and animal nutrition. Borum and Bennet (1986) stated "that an accurate assessment of the carnitine status of patients at risk from carnitine deficiency is fundamental to identification of those patients who require carnitine as the result of altered metabolism." This shows if carnitine deficiency goes unnoticed in humans or animals it could result in major problems with the nutrition of the organism.

L-carnitine has been reported to be found in cereal grains and their by-products (Borum, 1983). Because cereal grains are such an important and large part of poultry diets, it could be useful to incorporate this product in the diet (Rabie et al., 1997). The fact that the main sources of carnitine are found in the meat of animals, may pose a major problem since it is illegal to feed animal-based feed in some parts of the world. Numerous ways to manufacture plant based carnitine are becoming successful, but research field experiments set up to test the effects of carnitine on body weight gain, feed conversion, and feed intake are showing mixed results (Rabie et al., 1997; Kidd et al., 2009; Leibetseder, 1995; Lein and Horng, 2001). *L-carnitine* research has involved the testing of different levels and concentrations of ingredients, and has also focused on the effects of its supplementation of different levels, such as 0 to 300 mg/kg different kinds of effects have resulted from this research. *L-carnitine* supplementation in broilers resulted in decreased abdominal fat (Rabie et al., 1997; Kidd et al., 2009) but other studies (Leibetseder, 1995; Lein and Horng, 2001) with broilers have found no effects on

abdominal fat. Lien and Horng (2001) also showed no effects on body weight, feed consumption, abdominal fat pad, or liver weights in the response to the supplementation of *L-carnitine* in broilers. *L-carnitine* was fed to breeder hens at 21 weeks of age and onward, it decreased carcass fat and increased breast meat yield in progeny fed high-amino-acid-density diets (Kidd et al., 2005). However, de Beer and Coon (2009) showed that it had negative effects on body weight gain, reproductive traits, egg weight, fertility, and hatchability of broiler breeders. Leibetseder (1995) reported that supplementation with *L-carnitine* (500 mg) had no effect on egg production, feed intake, or body weight, but the content of yolks was significantly increased in the groups of commercial layers that were fed supplemental diets. Rabie et al. (1997) also observed that *L-carnitine* (50 mg) had positive effects on interior egg quality during the early egg stages of lay in commercial layers. *L-carnitine* has also been shown to improve yolk weight (Kita et al, 2005) and has had significant effects on relative albumen weight and albumen height when provided in the drinking water of laying hens (Çelik et. al., 2004). Although most of the research with layers is using the unfertilized edible egg, the importance of *L-carnitine* is still evident in the parts of the edible egg.

***In ovo* technology**

The standard procedure for hatchery-applied vaccines for Marek's disease virus and infectious bursal disease in the United States for broiler chicks is through the use of *in ovo* vaccination technology. Also, from a production standpoint, *in ovo* vaccination must maintain quality control and ease of application during daily operation to consistently achieve maximum vaccine efficacy, maintain chick quality, and support

subsequent bird health. The global expansion of this innovative technology has been further enhanced with the use of vectored vaccines for infectious bursal disease and Newcastle disease virus.

In ovo feeding

"*In ovo* feeding" is not a relatively new process. It has been around for almost a decade, and has been patented by Uni (2003). *In ovo* feeding refers to the administration of compounds into the embryonic amnion, which are subsequently consumed orally by the embryo and eventually come into contact with tissues of the digestive system (Uni et al., 2003). *In ovo* feeding can be explained furthermore as administration into the embryonic amnion a solution along with other natural compounds that modulate enteric development to improve hatchling's nutritional status during transition from embryonic nutrition to diet digestive competence. *In ovo* feeding ensures a readily available supply of nutrients and co-factors to increase the energy status of embryos (Shafey et. al, 2010) and help or even accelerate enteric development in the embryo, which might otherwise be limited by the availability of critical nutrients (Kadam et. al., 2008). The purpose of the *in ovo* feeding invention is to enhance enteric development of late term embryos (Uni et al., 2003). This is very critical in poultry because, as previously mentioned, a chick's entire nutrient supply is in the egg and if supplemental substances can enhance enteric development, then the chick is more likely to grow more efficiently once it has hatched.

In ovo feeding has been shown to increase in body weight, yolk sac weight, and pipping muscle size and eventually increase the size of eggs laid by breeders. *In ovo* feeding of late-term embryos has increased the body weights of Ross and Cobb strains of chicks by 5 to 6% when compared to the non-fed or non-injected chicks (Uni et al. 2005).

Tako et al. (2004) also noticed that *in ovo* feeding enhanced intestinal development by increasing the size of the villi and by increasing intestinal capacity to digest disaccharides, and hypothesized that *in ovo* feeding of β-Hydroxy β-methylbutyric acid could enhance intestinal development by enhancing the process of proliferation and differentiation of enterocytes or by lowering the rate of protein degradation. Further research has been conducted to examine more complex solutions and substances that can be injected into an egg in order to increase body weight in the live bird. *In ovo* feeding is important because all of a chick's nutrients are within the egg itself during the embryonic period and then in the yolk sac after hatch. This fact explains why experiments with all different kinds of substances are being introduced to eggs via *in ovo* injection like vaccines, antibiotics, vitamins, and competitive exclusion media.

Another important factor about *in ovo* feeding is its potential impact on liver glycogen levels in chicks. At the end of incubation, an embryo exhausts its energy reserves due to high demand of glucose to fuel all its hatching activities (Freeman, 1965; John et al., 1987, 1988; Christensen et al., 2001). Due to the high demands of a chick's nutrients supplemental solutions may aid the embryo in pipping out of the shell. *L-carnitine* level could possibly be a limiting factor for β-oxidation of fatty acids during emergence process. (Keralapurath et al., 2010).

Effects of *in ovo* injection of *L-carnitine* on egg hatchability

The injection of substances can greatly affect hatchability, because the injection site can provide a channel for bacteria invasion, and the needle can accidentally hit the embryo, killing it on contact. The poultry industry has employed *in ovo* injections for a long time now with realized economic benefits. *In ovo* injection is known to reduce

hatchability, however, the economic benefits outweigh the loss in hatch. Moore et al. (1994) have injected hormones into eggs with no significant treatment effects on hatchability. Zakaria and Al-Anezi (1996) injected vitamins into eggs and experienced an improvement in hatchability, an increase in body weight at hatch, and a decrease in cull chick percentage. Ohta et al. (1999) also showed that injecting amino acids into the embryo had no effect on the hatchability of the chick. Uni et al. (2005) reported using both the control and the Ross and Cobb strain of chicks showed similar hatch of fertilized eggs. This is important because this shows that injection of carnitine does not affect the hatchability of the embryo. Ohta et al. (1999) demonstrated that the *in ovo* amino acid injection into the yolk sac at Day 7 of incubation increased body weight of hatched chicks in comparison to water injection with no effect on hatchability. These experiments alone have shown that the injection of different compounds had no harsh effects on hatchability.

Many different solutions of *L-carnitine* have been injected with no detrimental effects on hatchability. Keralapurath et al. (2010) have further shown that *L-carnitine* up to 8 mg/100 μL of commercial diluent had no effects on hatch time or hatch of fertilized eggs. Keralapurath et al. (2010) also showed that *L-carnitine* increased pipping muscle size, which is important in the hatching process. A larger pipping muscle would be valuable in allowing the chick to break out of the shell. Shafey et al. (2010) also injected high concentrations of carnitine with no adverse effects on hatchability.

Supplementation

Dietary *L-carnitine* supplementation in poultry has been studied extensively (Lein and Horng, 2001; Kidd et al., 2005; Geng et al., 2007; Corduk et al., 2007; Peebles et al.,

2007). Dietary *L-carnitine* supplementation causes the generation of adenosine triphosphate (ATP) energy and improves energy utilization through promoting β-oxidation of fatty acids (Rinaudo et al., 1991). Therefore, supplementation of *L-carnitine* in the diet reduces the amount of long-chain fatty acids available for esterification to triacylglycerols and their subsequent storage in adipose tissue (Uni et al., 2005). Excess body fat in broilers is of concern, and *L-carnitine* fed to breeder hens that are 21 week of age or older has shown to decrease carcass fat and increase breast meat yield in progeny fed with high-amino-acid-density diets (Kidd et al., 2005).

Supplementary *L-carnitine* in broiler breeder diets

Research shows that in many species the female parent is primarily responsible for *L-carnitine* concentrations in the offspring (Kidd et al., 2005). Research has also shown that supplemental *L-carnitine* fed to breeder hens at 21 weeks of age and onward decreased carcass fat and increased breast meat yield in progeny fed high-amino-acid-density diets (Kidd et al., 2005). The addition of *L-carnitine* broiler breeder diets can therefore increase meat yield and decrease fat in broiler progeny.

Chicks hatched from young broiler breeders are smaller than chicks hatched from mature broiler breeder flocks. Because of this fact, chicks from younger breeder flocks exhibit poorer uniformity and exhibit more production-related problems than chicks from mature flocks. More research is being conducted to further study effects of the use of various supplemented products to increase body weight, improve feed conversion, and reduce feed intake. Because most producers employ some form of feed restriction in order to improve reproductive performance of their flock, more research is also conducted to observe differences in feed additives that could be added to the diet.

L-carnitine has not been shown to cause significant treatment main effects on percentage early, mid, late, or pipped embryonic mortalities, or for hatchability as a percentage of total or fertilized eggs and set (Peebles et al., 2007). However, there were significant breeder age main effects for percentage early embryonic mortality, and for hatchability as a percentage of total and fertilized eggs set (Peebles et al., 2007). Since all the nutrients for the baby chick are contained in the yolk of the egg, it is important to note that the nutrients are passed from the broiler breeder to the egg. Kidd et al. (2005) and Peebles et al. (2007) showed that *L-carnitine* can be passed from the mother to the progeny. Leibetseder (1995) found that the hatching rate was increased from 83% to 87% and from 82.4% to 85.3% in groups of broiler breeders supplemented with 50 and 100 mg *L-carnitine*, respectively. These drastic changes are critical since a pour hatching rate can bring financial loses to a company. Therefore, a company could benefit from the use of *L-carnitine* supplementation in broiler breeder diets.

Not all experiments have demonstrated positive results concerning *L-carnitine* supplementation in breeder diets. de Beer and Coon (2009) showed negative effects of *L-carnitine* on body weight gain, reproductive traits, egg weight, fertility, and hatchability. The results, however, showed that the increase in egg size, in response to *L-carnitine* was not significant. Results from de Beer and Coon (2009) are in contrast with those of Kidd et al. (2005) and Peebles et al. (2007) in not showing any effects on carcass fat or percent carcass fat. Since lysine and methionine are the first and second limiting amino acids in poultry, the contradictory results may be explained by an inefficient support of fatty acid transport by *L-carnitine*.

Supplementary *L-carnitine* in broiler diets

The use of multiple concentrations of supplemental *L-carnitine* in broiler diets has been studied extensively. *L-carnitine* has been shown to increase body weight, carcass fat, and feed conversion in broilers. However contradictory reports have gone as far as stating that *L-carnitine* decreases body weight and feed intake of broilers. Some researchers have used concentration as high as 300 mg/kg to explore the effects of *L-carnitine* on growth, carcass traits, and processing cuts (Rabie et al., 1997; Kidd et al., 2009; Leibetseder, 1995; Lein and Horng, 2001).

Rodehutscord (2002) used concentrations of 0 and 80 mg/kg of *L-carnitine* to test the effects of *L-carnitine* supplementation in association with various dietary fat levels. Geng (2007) used three different levels of *L-carnitine* (0, 75, 100 mg/kg) in broilers to test the effect on immune responses in ascites-susceptible broilers. Results from the Geng (2007) experiment showed no additional growth response in broilers provided supplemental *L-carnitine* but did show some positive effects on immune responses of broilers. Some researchers have found that adding *L-carnitine* to the diet results in decreased abdominal fat in broilers (Rabie et al., 1997; Kidd et al., 2009), while others (Leibetseder, 1995; Lein and Horng, 2001) have found no effects on abdominal fat. Xu et al. (2003) also showed no differences in weight gain, feed intake, or feed conversion with supplemented diets containing concentrations of 50 or 100 mg/kg of *L-carnitine*. Lien and Horng (2001) used 0 mg/kg and 160 mg/kg concentration of *L-carnitine* and showed no effects on body weight, feed consumption, or on abdominal fat pad, or liver weights. However serum triacylglycerol and nonesterified fatty acids were significantly higher in the carnitine fed group (Lien and Horng, 2001), which further supports the role of carnitine in fatty acid metabolism. However, Leibetseder (1995) showed that *L-*

carnitine supplementation increased *L-carnitine* concentrations in tissues of the liver, kidney, heart, and skeletal muscles. Excessive fat in these organs could possibly be decreased with *L-carnitine* supplementation.

Supplementary *L-carnitine* in commercial layer diets

L-carnitine supplementation in the diet of layers has been used to investigate the importance of *L-carnitine* to egg size, egg weight, yolk size, albumen contents, and egg shell strength. Layer fertility, hatch rate, and egg production factors to consider in *L-carnitine* research. However, results from laying hens are inconsistent just like broiler breeders and broilers. Leibetseder (1995) reported that diet supplementation with *L-carnitine* (500 mg) had no effects on egg production, feed intake, and body weight of commercial layers. However, percentage yolk percentage in eggs was significantly increased in the supplemented groups. Rabie et al (1997) also observed that *L-carnitine* (50 mg) had positive effects on interior egg quality during the early stages of lay in commercial layers. Late period in lay, *L-carnitine* exhibited beneficial effects on albumen quality and modified the components of the edible part of the egg (Rabie et al., 1997). Although egg weight was not affected by dietary *L-carnitine*, the percentage of egg white increased and egg yolk percentage decreased in the *L-carnitine* supplemented groups when compared with the controls (Rabie et al., 1997). The higher egg white percentage could be explained by the higher metabolic rate in the magnum and/or higher activity of the shell gland of treated birds when compared to the non-treated group (Rabie et al., 1997). The increase in *L-carnitine* content of eggs has been suggested to be a desirable effect for the food value of an egg, but it may also be beneficial to the development of the chick embryo. However, Rabie et al. (1997) demonstrated no effects on egg production

rate or daily feed intake. Adabi et al. (2006) also observed no effects on egg production except in the fifth and six weeks of the study. However, Adabi et al (2006) did show an *L-carnitine* effect on hatching rate and a 4% higher average fertility in response to *L-carnitine* supplementation.

L-carnitine research continuously alternates between positive and negative effects. *L-carnitine* has been reported to improve yolk weight (Kita et al, 2005 R). However, when provided in the drinking water of commerical laying hens, *L-carnitine* has also been reported to have significant effects on relative albumen weight and albumen height (Çelik et. al., 2004). Although Çelik provided supplemental *L-carnitine* in the drinking water, it is still important to note its contributions to the edible part of the egg.

References

Adabi, Sh. Golzar, Gh. Moghaddam, A. Taghizadeh, A. Nematollahi and T. Farahvash. 2006. Effect of *L-carnitine* and Vegetable Fat on Broiler Breeder Fertility, Hatchability, Egg Yolk and Serum Cholesterol and Triglyceride. Int. J. Poult. Sci. 5: 970-974.

de Beer, M. and C.N. Coon. 2009. The effect of different feed restriction programs and dietary *L-carnitine* supplementation on reproductive performance, efficiency, frame size and uniformity in broiler breeder hens. Int. J. Poult. Sci. 8: 409-425.

Borum, P.R. 1983. Carnitine. Annu. Rev. Nutr. 3:233–259

Borum PR and Bennett SG. 1986. Carnitine as an essential nutrient. J. Am. Coll Nutr. 5: 177-182.

Bremer, J. 1983. Carnitine metabolism and functions. Physiol. Rev. 63: 1420–1480.

Casillas, E.R. and R.W. Newburgh. 1969. Carnitine and derivatives in embryonic chick tissue. Biochim. Biophys. Acta. 184: 566–577.

Çelik, L.B., A. Tekeli and O. Öztürkcan. 2004. Effects of supplemental *L-carnitine* in drinking water on performance and egg quality of laying hens exposed to a high ambient temperature. J.Anim. Physiol. Anim. Nut. 88: 229-233

Christensen, V.L., M.J. Wineland, G.M. Fasenko, and W.E. Donaldson. 2001. Egg storage effects on plasma glucose and supply and demand tissue glycogen concentrations of broiler embryos. Poult. Sci. 80: 1729–1735.

Corduk, M., N. Ceylan, and F. Illdiz. 2007. Effects of dietary energy density and *L-carnitine* supplementation on growth performance, carcass traits and blood parameters of broiler chickens. S. Afr. J. Anim. Sci. 37: 65-72.

Cox, R.A. and C.L. Hoppel. 1973. Biosynthesis of carnitine and 4-N-trimethylamino-butyrate from 6-N-trimethyllysine. Biochem. J. 136: 1075

El-Ghali, Nissrine, M. Rabadi, A.M. Ezin, and M.E. de Bellard. 2010. New Methods for Chicken Embryo Manipulations. Micro. Res. Tech. 73: 58-66.

Freeman, B.M. 1965. The relationship between oxygen consumption, body temperature and surface area in the hatching and young chick. Br. Poult. Sci. 6:67–72.

Geng, A., Baoming Li, and Yuming Guo. 2007. Effects of dietary *L-carnitine* and coenzyme Q10 at different supplemental ages on growth performance and some immune responses in ascites-susceptible broilers. Arch. Anim. Nutr. 61: 50-60.
Kadam, M.M., S.K. Bhanja, A.B. Mandal, R. Thakur, P. Vasan, A. Bhattacharyya, and J.S. Tyagi. 2008. Effect of *in ovo* threonine supplementation on early growth, immunological responses and digestive enzyme activities in broiler chickens. Br. Poult. Sci. 49: 736-741.

Keralapurath, M.M, R.W. Keirs, A. Corzo, L.W. Bennett, R. Pulikanti, and E.D. Peebles. 2010. Effects of *in ovo* injection of *L-carnitine* on subsequent broiler chick tissue nutrient profiles. Poult. Sci. 89: 335-341.

Keralapurath, M.M, R.W. Keirs, A. Corzo, R. Pulikanti, W. Zhai, and E.D. Peebles. 2010. Effects of *in ovo* injection of *L-carnitine* on hatchability and subsequent broiler performance and slaughter yield. Poult. Sci. 89: 1497-1501.

Kidd, M.T., C.D. McDaniel, E.D. Peebles, S.J. Barber, A. Corzo, S.L. Branton, and J.C. Woodworth. 2005. Breeder hen dietary *L-carnitine* affects progeny carcase traits. Br. Poult. Sci. 46: 97–103.

Kidd, M. T., J. Gilbert, A. Corzo, W. S. Virden, and J. C. Woodworth. 2009. Dietary *L-carnitine* influences broiler thigh yield. Asi. Austr. J. Anim. Sci. 22: 681-685.

Kita, K., S.I. Nakajima, and J. Nakagawa. 2005. Dietary *L-carnitine* supplementation improves albumen quality of laying hens. Poult. Sci. 42: 79-83.

Leibetseder, J.1995. Studies on effects of *L-carnitine* in poultry. Arch. Anim. Nutr. 48: 97-108.

Lien, T-F. and Y-M. Horng. 2001. The effect of supplementary dietary *L-carnitine* on the growth performance, serum components, carcase traits and enzyme activities in relation to fatty acid β-oxidation of broiler chickens. Br. Poult. Sci. 42: 92-95.

Moore, Randle W., Carlton E. Dean, and Pamela S. Hargis. 1994. Effects of *in ovo* hormone administration at day eighteen of embryogenesis on posthatch growth of broilers. J. Appl. Poult. Res. 3: 31-39.

Nishida, N., T. Sugimoto, T. Takeughi, and Y. Kobayashi. 1989. Effect of *L-carnitine* on glycogen synthesis and ATP production in cultured hepatocytes of the newborn rat. J. Nutr. 119: 1705-1708.

Ohta, Y., N. Tsushima, K. Koide, M.T. Kidd, and T. Ishibashi. 1999. Effect of amino acid injection in Broiler Breeder Eggs on Embryonic Growth and Hatchability of Chicks. Poult. Sci. 78: 1493-1498.

Ohta, Y., M.T. Kidd, and T. Ishibashi. 2001. Embryo growth and amino acid concentrations profiles of broiler breeder eggs, embryos and chicks after *in ovo* administration of amino acids. Poult. Sci. 80: 1430-1436.
Oppenheim RW, and HL Levin. 1975. Short-term changes in incubation temperature: behavioral and physiological effects in the chick embryo from 6 to 20 days. Dev. Psychobiol. 8: 103-115.

Peebles, E. D., M.T. Kidd, C.D. McDaniel, J.P. Tanksley, H.M. Parker, A. Corzo, and J.C. Woodworth. 2007. Effects of breeder hen age and dietary *L-carnitine* on progeny embryogenesis. Br. Poult. Sci. 48: 299–307.

Rabie, M.H., M. Szilagyi, and T. Gippert. 1997. Effects of dietary *L-carnitine* on the performance and egg quality of laying hens from 65-73 weeks of age. Br. J. Nutr. 78: 615-623.

Rinaudo, M.T., M. Curto, R. Bruno, M. Piccinini, and C. Marino. 1991. Acid soluble, short chain esterified and free carnitine in the liver, heart, muscle and brain of pre and post hatched chicks. Int. J. Biochem. 23: 59-65

Rodehutscord, M., R. Timmler and A. Dieckmann. 2002. Effect of *L-carnitine* supplementation on utilization of energy and protein in broiler chicken fed different dietary fat levels. Arch. Anim. Nutr. 56: 431-441.

Shafey, T.M. H.A. Al-Batshan, A.N. Al-Pwaimer and K.A. Al-Samawei. 2010. Effects of *in ovo* administration of *L-carnitine* on hatchability performance, glycogen status and insulin-like growth factor-1 of broiler chickens. Br. Poult. Sci. 51: 122-131.

Suarez ME, HR Wilson, BN McPherson, FB Mather, and CJ Wilcox. 1996. Low temperature effects on embryonic development and hatch time. Poult Sci. 75: 924-932.

Tako, E., P.R. Ferket, and Z. Uni. 2004. Effects of *In ovo* Feeding of Carbonhydrates and β-Hydroxy-β-Methylbutyrate on the Development of Chicken Intestine. Poult. Sci. 83: 2023-2028.

Uni, Z. and P. Ferket. 2003. Enhancement of development of oviparous species by *in ovo* feeding, U. S. Patent No. 0035965.

Uni, Z., P.R. Ferket, E. Tako, and O. Kedar. 2005. *In ovo* feeding improves energy status of late-term chicken embryos. Poult. Sci. 84: 764–770.

Xu, Z.R., M.Q. Wang, H.X. Mao, X.A. Zhan, and C.H. Hu. 2003. Effects of *L-carnitine* on growth performance, carcass composition, and metabolism of lipids in male broilers. Poult. Sci. 82: 408-413

Zakaria AH, and M.A. al-Anezi.1996. Effect of ascorbic acid and cooling during egg incubation on hatchability, culling, mortality, and the body weights of broiler chickens. Poult. Sci. 75: 1204-1209.

Zhai, W., S. Neuman, M.A. Latour, and P.Y. Hester. 2008. The Effect of *in ovo* injection of *L-carnitine* on hatchability of White Leghorns. Poult. Sci. 87: 569–572.

CHAPTER III

EFFECTS OF *L-CARNITINE* VIA *IN OVO* INJECTION WITH- OR WITHOUT- *L-CARNITINE* FEED SUPPPLEMENTATION ON BROILER HATCHABILTITY AND POST-HATCH PERFORMANCE

Abstract

On d 18 of incubation, Ross × Ross 708 eggs were injected immediately before transfer with commercial diluent containing supplemental *L-carnitine* at concentrations of 8, 16, or 32 mg/100 µL using an automated multi-egg injector. Three control groups (non-injected and injected with or without diluent) were also included. After hatching, 1,080 male and female broiler chicks were distributed into 90 pens of an experimental broiler house. Chicks hatched from eggs that were injected with *L-carnitine* at each of the 3 dosages, received feed that was or was not supplemented with 50 ppm of *L-carnitine*, increasing the total number of treatments to 9 with 10 replicates pens per treatment during the grow-out phase of the experiment. None of the treatments had any effect on incubation time or hatchability of fertilized eggs. At the end of the grow-out phase, birds that were fed supplemental *L-carnitine* and had been injected with *L-carnitine in ovo* were lower in body weight and ate less feed. However, these same birds also exhibited a better feed conversion when compared to birds that did not receive supplemental dietary *L-carnitine*. Livability was not affected at hatch or at 21 and 45 d post-hatch by any of the experimental treatments. At processing, absolute and relative weights of the carcass, back-half or abdominal fat were found to be unaffected by any of the treatments. Breast

meat yield was also unaffected, but its absolute weight was reduced in birds given *L-carnitine in ovo* and in the feed. Based on the responses in feed consumption, final body weight and breast meat weight, supplementation of broiler diets with 50 ppm *L-carnitine* appeared to reach slightly toxic levels if provided with 8, 16, or 32 mg/100 µL of *L-carnitine* administered via *in ovo* injection.

Introduction

L-carnitine (β-hydroxy γ-trimethylaminobutyrate) is a water-soluble quaternary amine that occurs naturally in microorganisms, plants, and animals (Bremer, 1983), and can be biosynthesized endogenously from methionine and lysine (Cox and Hoppel, 1973) *L-carnitine* is also an essential nutrient in animals (Borum, 1983), and was first synthesized in chick embryos (Bremer, 1983). Amino acids administrated *in ovo* have been shown to stay within the egg and be used later in life (Bremer, 1983; de Beer and Coon, 2009). Since *L-carnitine* is an amino acid derivative, this could be valuable throughout the chick's life cycle.

The injection of nutrients into the embryonic amnion has become a topic of interest in poultry nutrition and is also a fairly new line of research. *In ovo* injection has proved to be a cost effective approach for vaccine administration to fertilized eggs in current commercial hatcheries. This has led to an interest in pursuing more research that explores the use of other nutrients that can be injected into eggs. *In ovo* feeding refers to the administration of compounds into the embryonic amnion that are orally consumed by the embryo which ultimately brings the internal nutrients into immediate contact with tissues of the digestive system (Uni and Ferket, 2003). *L-carnitine* has been recognized as a substance that facilitates the transfer of fatty acyl groups from the yolk into the tissues

of embryonic chicks via the yolk sac membrane. This in turn allows for the availability of more usable energy to the chick (Casillas and Newburgh, 1969). At the end of incubation, the embryo uses its energy reserves due to the high demand for glucose to fuel all of the hatching activities (Freeman, 1965; Christensen et al., 2001). Because of this, the chick requires extra energy for the pipping process. Keralapurath et al., 2010 showed that *L-carnitine* can increase the moisture of the pipping muscle which could in turn help aid the chick to pip from the egg. Rinaudo et al., 1991 also showed that ratio of chain esterified carnitine in the liver, brain, and heart are also at the maximum level at 18 d of incubation, which suggest the importance of fatty acid oxidation for energy production in embryos.

Dietary *L-carnitine* supplementation in poultry has been studied extensively (Lein and Horng, 2001; Kidd et al., 2005; Geng et al., 2007; Corduk et al., 2007; Peebles et al., 2007). Dietary *L-carnitine* supplementation causes the generation of adenosine triphosphate (ATP) energy and improves energy utilization through promoting β-oxidation of fatty acids (Rinaudo et al., 1991). Therefore, supplementation of *L-carnitine* in the diet reduces the amount of long-chain fatty acids available for esterification to triacylglycerols and their subsequent storage in adipose tissue (Uni et al., 2005). Excess body fat in broilers is of concern, and *L-carnitine* fed to breeder hens that are 21 week of age or older has shown to decrease carcass fat and increase breast meat yield in progeny fed with high-amino-acid-density diets (Kidd et al., 2005). This study will focus on the effects of *L-carnitine* provided via *in ovo* injection followed by an additional supply through dietary supplementation.

Materials and Methods

Incubation and treatments

Ross × Ross 708 broiler hatching eggs were obtained from a local commercial source. On the third day after collection, 1,440 eggs were individually weighed and all cracked, dirty, and misshapen eggs were discarded. Eggs were set on 10 tray levels of a Jamesway Model PS 500 single stage incubator. On each tray level, which corresponded to a replicate unit, all 9 treatment groups (*in ovo* injection and feed supplement combinations) were represented. Therefore, a total of 144 eggs were set on each tray level, with 16 eggs randomly assigned to each treatment group. Bulb temperatures and relative humidity were recorded using data loggers placed on levels 1, 5, and 10 of the incubator. Average dry bulb temperature was maintained between 37.0 and 37.5°C throughout incubation. On d 14 of incubation, eggs were candled and eggs that were unfertilized, contaminated, or contained dead embryos were discarded and recorded.

On d 18 of incubation, the embryonic amnion was injected according to the following treatment descriptions: 1) non-injected control, 2) injected without diluent (dry punch) control, 3) injection of 100 μL of diluent control, 4) injected with 100 μL of diluent containing 8 mg of supplemental *L-carnitine*, 5) injected with 100 μL of diluent containing 16 mg of supplemental of *L-carnitine*, 6) injected with 100 μL of diluent containing 32 mg of supplemental *L-carnitine*. After hatch, 3 treatment groups (treatments 7, 8, and 9) were added for the grow-out period. Then 3 additional treatments were created when chicks hatched from eggs that were injected with *L-carnitine* at each of the 3 dosages (treatments 4, 5, and 6) received feed that was supplemented with 50 ppm of *L-carnitine*. Control treatments (treatments 1, 2 and 3) were fed un-supplemented *L-carnitine* feed. The controls could not be fed supplemented *L-carnitine* because of the

limited space for the treatment in the incubator and within the grow-out facility. The compositions of the un-supplemented basal starter and grower diets are provided in Table 3.1.

Injection and Hatching

Eggs were injections immediately before transfer on d 18 of incubation. A modified Intellinject automated multi-egg injector was used to inject eggs. The air cells of the eggs were injected with a blunt tip injector needle [18.4-cm length and 1.27-mm bore width (o.d.)] to target the amnion. The needle provided an approximate 2.49 cm injection depth from the top of the large end of the egg (Keralapurath et al., 2010) with a standard error for injection volume of 0.1%. The injector was equipped with an automated cleaning cycle and was flushed and cleaned after each treatment group of eggs were injected to prevent cross contamination. The machine was primed using the appropriate treatment solution before the injection of the current treatment. A validation test using a water soluble dye confirmed that the material was being delivered into the amnion on d 18 of incubation. A 100 μL volume of treatment was injected into the large end of each egg. All eggs were left out of the incubator for 15 min including the industry egg during injection to maintain conditions for all treatments. Each treatment was placed into the hatching baskets corresponding to the same map as the incubator, in which one treatment at a time was placed to maintain conditions for all treatments.

Grow-out

On d 0 post hatch (21 d of incubation), 12 chicks belonging to the same treatment replicate group were randomly selected and transferred to one of 90 floor pens in a broiler grow-out house. On a daily basis, birds' condition and mortality were monitored,

and temperature and dead bird weights were recorded. Temperature was remained constant and adjusted according to industry standards and qualifications. Birds were maintained on 24 h of light for the first 5 d and then on 20 h of light per d thereafter. Chicks were provided ad libitum access to feed and water, and the use of 50 ppm supplemental dietary *L-carnitine* was used in accordance with the previously indicated treatment designations. All diets were formulated to meet or exceed NRC (1994) recommendations throughout the grow-out period.

Data collection

Hatch time for each treatment replication was monitored every 12 h from 19.5 d to 21.5 d. Hatchability was calculated and expressed as a percentage of fertilized eggs. After hatch, dead birds and their weights were recorded. Birds and feed were weighed and birds counted in each treatment replicate pen on d 21 and 45 of grow-out in order to calculate mean body weight gain and feed conversion ratio (FCR). On d 45 of post-hatch grow-out, 6 birds (3 males and 3 females) from each treatment replication were randomly selected, individually weighed, and processed. Weights of the carcass, abdominal fat pad, back-half, and breast meat were recorded

Statistical analysis

A randomized complete block experimental design was employed for the incubational and grow-out components of the study. Incubator tray levels and areas of the grow-out house represented specific replicate units and were considered as blocks, with all 9 treatments equally represented within each replicate unit. When moved to the grow-out house, chicks belonging to the same treatment replicate group were randomly assigned to an individual pen. The PROC REG option of SAS (SAS institute, 2003) was

used to regress the means for time of hatch and hatchability on *L-carnitine* concentration, the GLIMMIX procedure was used for the analysis of mortality data, and the MIXED procedure was used for the analysis of all other data. Fisher's protected least significant difference test was used to compare means. Comparisons between means were made when there were significant global effects, with all differences considered significant at $P \leq 0.05$.

Results and Discussion

Hatch time is best described as the amount of time that is required for a specific chick or group of chicks to hatch, whereas hatchability is calculated by dividing the number of chicks hatch by the number of fertile egg placed in the hatcher. Time of hatch and hatchability of fertilized eggs for each treatment group are provided in Table 3.2. Mean length of incubation (hatch time) and hatchability of fertilized eggs were not significantly different between individual treatments. In agreement with these results, Keralapurath et al. (2010) showed no significant effects on hatch time due to *in ovo* injected *L-carnitine* levels at 0.5, 2.0, and 8.0 mg/100 μL of commercial diluent. However overall, hatchability in the current study was low in comparison to other research (Xu et al., 2003; Uni et al., 2005; Zhai et al., 2008; Zakaria et al. 2009; Keralapurath et al., 2010). In the current study, all of the eggs were exposed to low temperature (27°C) for approximately 2 h when the eggs were transferred from the incubator to the hatcher after injection on d 18 of incubation. It took another hour for the eggs to warm up to normal hatching temperature after they were placed in the hatcher. Chicken embryos are not able to regulate their body temperature (Oppenheim and Levin, 1975), and a decrease in the environmental temperature can cause a decrease in

embryonic body temperature, followed by a decrease in embryonic metabolic rate, which subsequently delays the hatching process (Zakaria and al-Anezi, 1996). Suarez reported that incubation time was delayed as long as the time that embryos experience cooling (Suarez et al., 1996). Cooling embryos resulted in a longer incubation period. In the current study, when the embryos were moved out of the hatcher on d 21 of incubation, some of the embryos were still in the process of hatching. However, those embryos that hatched after d 21 of incubation were not counted in the calculation of hatchability. Nevertheless, in the current study, hatchability was not affected by the injection of *L-carnitine* at concentrations of 8, 16, or 32 mg/ μL as compared to the controls. This result is consistent with previous studies which have shown no detrimental effects on hatchability with the injection of *L-carnitine* (Xu et al., 2003; Uni et al., 2005; Zhai et al., 2008; Zakaria et al. 2009; Keralapurath et al., 2010). Keralapurath et al. (2010) showed that *L-carnitine* levels at 0.5, 2.0, and 8.0 mg/100 μL of commercial diluent were non-toxic to broiler embryos. Results from the current study also confirmed that 8 mg of *L-carnitine*/100 μL of commercial diluent are non-toxic to broiler embryos. However, if the concentrations are increase beyond 8 mg along with the supplementation of 50 ppm *L-carnitine*, slightly toxic levels may be noticed.

At 21 d of age, no affects were observed for the treatments involving the injection of *L-carnitine* compared to the 3 controls with no supplemental *L-carnitine*, but when comparing the injection of *L-carnitine* in combination with 50 ppm supplemental *L-carnitine* to the 3 controls there were significant differences in body weight ($P \leq 0.01$). However there were no significant differences in the injection of *L-carnitine* when compared to the injection of *L-carnitine* in combination with 50 ppm *L-carnitine* supplementation with respect to d 21 body weight. At d 21, there are no significant

differences in feed intake between the controls, the injection of *L-carnitine*, or the injection of *L-carnitine* in combination with 50 ppm *L-carnitine* supplementation ($P \leq 0.001$). However, feed intake begins to decrease beginning at the 32 mg injection of *L-carnitine* and continuing to decrease throughout all treatments containing the injection of *L-carnitine* combined with *L-carnitine* supplementation, and such reduction seemed to be less severe in those birds that received no *L-carnitine* or birds that received lower-*in ovo* *L-carnitine* doses (Table 3.3). Mortality and FCR were unaffected by the injection and the feed supplementation of *L-carnitine*. For the final body weight, the 3 controls do not exhibit significantly differ effects from the injections of *L-carnitine*. However, the 3 controls are significantly different from the *L-carnitine* injection with the supplemental *L-carnitine* and were observed to reduce final body weight ($P \leq 0.001$). Final feed consumption is somewhat similar to final body weight, in which there were no differences in the 3 controls and the injections of *L-carnitine*. However there is a significant difference observed when comparing the 3 controls and the injection of *L-carnitine* in combination with the supplemental *L-carnitine*, and once again beginning at the 32 mg injection of L-carnitine lower feed intake is observed when adding the supplemental *L-carnitine* ($P < 0.001$). The results observed at d 21 and d 45 of age are not in agreement with results from previous studies which showed that *L-carnitine* supplementation had no effect on body weight and feed intake (Moore et al., 1994; Lein and Horng, 2001). However, birds hatched from eggs that were injected with only 32 mg/100 μL of *L-carnitine* or that were injected with 8 or 16 mg/100 μL of *L-carnitine* in combination with a subsequent dietary supplement of 50 ppm *L-carnitine* exhibited an improvement in FCR ($P \leq 0.021$) when compared to birds in the three control treatments (Table 3.3) Results from several studies have shown that dietary *L-carnitine*

supplementation at 50, 75, 100, and 160 mg/kg with broilers was not toxic to broilers (Lein and Horng, 2001; Xu et al., 2003; Corduk et al., 2007; Beer and Coon, 2009). However, it appears from the current results that 50 ppm of supplemental dietary *L-carnitine* in combination with 8, 16, and 32 mg/100 µL of *L-carnitine* administrated via *in ovo* injection may be slightly toxic to broilers, as evidenced by the reductions in feed consumption, final body weight, and breast meat weight except for the 8 mg *L-carnitine* injection.

Although, the results for boneless-skinless breast meat weight ($P \leq 0.03$) are somewhat inconsistent and was shown to be lower in birds fed 50 ppm supplemental *L-carnitine* subsequent to the injection of 8 mg/100 µL of *L-carnitine* (Table 3.4). When comparing the 3 controls, the injections of *L-carnitine*, or the injections of *L-carnitine* in combination with 50 ppm supplemental *L-carnitine* there were no differences observed. However, in the 32 mg injection of *L-carnitine*, 8 mg injection of *L-carnitine* in combination with 50 ppm *L-carnitine*, and the 32 mg injection of *L-carnitine* in combination with the 50 ppm *L-carnitine* slightly exhibit lower breast meat weights. In contrast, breast meat weight was maximized in birds that were injected with only 8 mg/100 µL of *L-carnitine*. There were no responses to the treatments with regards to back-half or abdominal fat weights or yields, which is in agreement with previous findings (Moore et al., 1994; Lein and Horng, 2001). Conversely, previous studies have also shown opposite results, in that reductions in abdominal fat pad weight (Kidd et al., 2005; Geng et al., 2007) and increases in back-half yield (Kidd et al., 2009) where observed when supplemental *L-carnitine* was fed.

In ovo injection with *L-carnitine* at levels ranging from 0 to 16 mg of *L-carnitine*/100 µL of diluent did not have any beneficial or deleterious effects on hatching,

grow-out, or carcass trait production of broilers. Levels of *L-carnitine* at 32 mg of /100 µL of diluent, caused a decrease in final feed consumption, and the supplementation of *L-carnitine* via *in ovo* injection in combination with dietary supplementation resulted in a reduction of final body weight, feed consumption and total breast meat weight, but resulted in an improvement in FCR. These results suggest that providing *L-carnitine* to broilers both in the feed and at the embryonic level via *in ovo* injection may have deleterious consequences that warrant further investigation. The *in ovo* application of *L-carnitine* at dosages of 8 or 16 mg/100 µL of diluent did not negatively impact broiler production or carcass traits, but also failed to improve any of the parameters measured except for the increase noticed in breast meat weight in response to the 8 mg of *L-carnitine* injection.

Conclusions and applications

In conclusion, *L-carnitine* from 0 to 32 mg/100 µL of diluent did not have any effect on the hatching characteristics of injected eggs. When eggs were injected with 32 mg of *L-carnitine*/100 µL of diluent, there was a decrease in final feed consumption. The combination *L-carnitine* supplementation at 8, 16, or 32 mg/100 µL of diluent via *in ovo* injection with its supplementation in the feed at 50 ppm resulted in impairment in the growth, feed intake, and breast meat development, suggesting that toxic levels may have been reached.

References

de Beer, M. and C.N. Coon. 2009. The effect of different feed restriction programs and dietary *L-carnitine* supplementation on reproductive performance, efficiency, frame size and uniformity in broiler breeder hens. Int. J. Poult. Sci. 8: 409-425.

Borum, P.R. (1983). Carnitine. Annu. Rev. Nutr. 3: 233–259

Bremer, J. 1983. Carnitine metabolism and functions. Physiol. Rev. 63: 1420–1480.

Casillas, E.R. and R.W. Newburgh. 1969. Carnitine and derivatives in embryonic chick tissue. Biochim. Biophys. Acta. 184: 566–577.

Christensen, V.L., M.J. Wineland, G.M. Fasenko, and W.E. Donaldson. 2001. Egg storage effects on plasma glucose and supply and demand tissue glycogen concentrations of broiler embryos. Poult. Sci. 80: 1729–1735.

Corduk, M., N. Ceylan, and F. Illdiz. 2007. Effects of dietary energy density and *L-carnitine* supplementation on growth performance, carcass traits and blood parameters of broiler chickens. S. Afr. J. Anim. Sci. 37: 65-72.

Cox, R.A. and C.L. Hoppel. 1973. Biosynthesis of carnitine and 4-N-trimethylamino-butyrate from 6-N-trimethyllysine. Biochem. J. 136: 1075.

Freeman, B.M. (1965). The relationship between oxygen consumption, body temperature and surface area in the hatching and young chick. Br. Poult. Sci. 6: 67–72.

Geng, A., Baoming Li, and Yuming Guo. 2007. Effects of dietary *L-carnitine* and coenzyme Q10 at different supplemental ages on growth performance and some immune responses in ascites-susceptible broilers. Arch. Anim. Nutr. 61: 50-60.

Keralapurath, M.M, R.W. Keirs, A. Corzo, L.W. Bennett, R. Pulikanti, and E.D. Peebles. 2010. Effects of *in ovo* injection of *L-carnitine* on subsequent broiler chick tissue nutrient profiles. Poult. Sci. 89: 335-341.

Keralapurath, M.M, R.W. Keirs, A. Corzo, R. Pulikanti, W. Zhai, and E.D. Peebles. 2010. Effects of *in ovo* injection of *L-carnitine* on hatchability and subsequent broiler performance and slaughter yield. Poult. Sci. 89: 1497-1501.

Kidd, M.T., C.D. McDaniel, E.D. Peebles, S.J. Barber, A. Corzo, S.L. Branton, and J.C. Woodworth. 2005. Breeder hen dietary *L-carnitine* affects progeny carcase traits. Br. Poult. Sci. 46: 97–103.

Kidd, M. T., J. Gilbert, A. Corzo, W. S. Virden, and J. C. Woodworth. 2009. Dietary *L-carnitine* influences broiler thigh yield. Asi. Austr. J. Anim. Sci. 22: 681-685.
Lien, T-F. and Y-M. Horng. 2001. The effect of supplementary dietary *L-carnitine* on the growth performance, serum components, carcase traits and enzyme activities in relation to fatty acid β-oxidation of broiler chickens. Br. Poult. Sci. 42: 92-95.

Moore, Randle W., Carlton E. Dean, and Pamela S. Hargis. 1994. Effects of *in ovo* hormone administration at day eighteen of embryogenesis on posthatch growth of broilers. J. Appl. Poult. Res. 3: 31-39.

National Research Council, 1994: Nutrient Requirements of Poultry, 9th rev. edn. National Academy Press, Washignton DC, USA.

Oppenheim RW, and HL Levin.1975. Short-term changes in incubation temperature: behavioral and physiological effects in the chick embryo from 6 to 20 days. Dev Psychobiol. 8: 103-115.

Peebles, E. D., M.T. Kidd, C.D. McDaniel, J.P. Tanksley, H.M. Parker, A. Corzo, and J.C. Woodworth. 2007. Effects of breeder hen age and dietary *L-carnitine* on progeny embryogenesis. Br. Poult. Sci. 48: 299–307.

Rinaudo, M.T., M. Curto, R. Bruno, M. Piccinini, and C. Marino. 1991. Acid soluble, short chain esterified and free carnitine in the liver, heart, muscle and brain of pre and post hatched chicks. Int. J. Biochem. 23: 59-65.

SAS User' s Guide 2004. Version 9.1 ed. SAS Institute, Inc., Cary, NC.

Suarez ME, HR Wilson, BN McPherson, FB Mather, and CJ Wilcox.1996. Low temperature effects on embryonic development and hatch time. Poult Sci. 75: 924-932.

Uni, Z. and P. Ferket. 2003. Enhancement of development of oviparous species by *in ovo* feeding, U. S. Patent No. 0035965.

Uni, Z., P.R. Ferket, E. Tako, and O. Kedar. 2005. *In ovo* feeding improves energy status of late-term chicken embryos. Poult. Sci. 84: 764–770.

Xu, Z.R., M.Q. Wang, H.X. Mao, X.A. Zhan, and C.H. Hu. 2003. Effects of *L-carnitine* on growth performance, carcass composition, and metabolism of lipids in male broilers. Poult. Sci. 82: 408-413

Zakaria AH, and MA al-Anezi.1996. Effect of ascorbic acid and cooling during egg incubation on hatchability, culling, mortality, and the body weights of broiler chickens. Poult Sci. 75: 1204-1209.

Zakaria, A.H., P.W. Plumstead, H. Romero-Sanchez, N. Leksrisompong, and J. Brake. 2009. The effects of oviposition time on egg weight loss during storage and incubation, fertility, and hatchability of broiler hatching eggs. Poult. Sci. 88: 2712-2717

Zhai, W., S. Neuman, M.A. Latour, and P.Y. Hester. 2008. The Effect of *in ovo* injection of *L-carnitine* on hatchability of White Leghorns. Poult. Sci. 87: 569–572.

Table 3.1 Composition of experimental diets (%)

Ingredients	Starter (0-21 d)	Grower (21-45 d)
Corn	65.09	70.36
Soybean mill	26.35	21.02
ProPlus[1]	5.00	5.00
Poultry oil	0.79	1.17
Calcium carbonate	0.94	0.86
Dicalcium	0.87	0.69
Salt	0.32	0.33
L-Lysine-HCL	0.21	0.21
DL-Methionine	0.24	0.19
Coccidiostat[2]	0.05	0.05
L-Threonine	0.031	0.016
Vitamin/mineral premix[3]	0.05	0.05
Choline	0.047	0.063
Calculated composition		
ME (Kcal/kg)	3,075	3,150
Crude Protein (%)	21.1	19.0
Lysine (%)	1.30	1.15
TSAA (%)	0.94	0.83
Calcium	0.90	0.82
Available phosphorus (%)	0.45	0.41

[1] Animal protein blend with a CP value of 60%; HJ Baker & Bro Inc. (Little Rock, AR).
[2] Dietary inclusions of 60 g of salinomycin sodium per 907.2 kg of feed.
[3] Vitamin and mineral premix included the following per kilogram of diet: vitamin A (vitamin A acetate), 4,960 IU; cholecalciferol, 1,653 IU; vitamin E (source unspecified), 27 IU; menadione, 0.99 mg; vitamin B_{12}, 0.015 mg; folic acid, 0.8 mg; D-pantothenic acid, 15 mg; riboflavin, 5.4 mg; niacin, 45 mg; thiamin, 2.7 mg; D-biotin, 0.07 mg; pyridoxine, 5.3 mg; manganese, 90 mg; zinc, 83 mg; iron, 121 mg; copper, 12 mg; iodine, 0.5 mg; selenium, 0.3 mg.

Table 3.2 Hatch time and hatchability of fertilized Ross 708 broiler hatching eggs in in response to the *in ovo* injection of supplemental L-carnitine.

Treatment	Hatchability of Fertilized Eggs (%)	Hatch Time (hr)
Industry Egg	78.36	20.86
Dry Punch	77.32	20.93
Diluent	74.94	20.88
8 mg/100µL *L-carnitine*	76.00	20.88
16 mg/100µL *L-carnitine*	69.83	20.89
32 mg/100µL *L-carnitine*	76.06	20.88
SEM	4.322	0.021
P value	0.56	0.51

Table 3.3 Live performance at d 21 and d 45 of post-hatch of age Ross 708 broilers fed diets supplemented with L-carnitine subsequent to the *in ovo* injection of L-carnitine.

	Day 21				Day 45			
Treatment	BW (g)	Feed intake (g/bird)	FCR[1]	Mortality (%)	BW (kg)	Feed intake (kg/bird)	FCR[1]	Mortality (%)
Industry Egg	575[ab]	747[abcd]	1.337	2.1	2.431 [a]	4.247 [a]	1.747 [ab]	5.2
Dry Punch	582[a]	782[a]	1.342	2.5	2.437 [a]	4.220 [a]	1.732 [abc]	5.0
Diluent	583[a]	778[ab]	1.336	4.2	2.428 [a]	4.219 [a]	1.757 [a]	5.0
8 mg/100µL *L-carnitine*	566[abc]	753[abc]	1.332	1.7	2.400 [ab]	4.156 [ab]	1.732 [abc]	3.3
16 mg/100µL *L-carnitine*	571[ab]	772[ab]	1.338	0.8	2.435 [a]	4.229 [a]	1.745 [ab]	2.5
32 mg/100µL *L-carnitine*	550 [abcd]	734[bcde]	1.335	2.8	2.378 [abc]	4.052[bc]	1.716 [c]	2.8
8 mg/100µL *L-carnitine* w/50 ppm feed supplementation	526[cd]	721[cde]	1.315	0.9	2.322 [bcd]	3.968 [c]	1.717 [c]	2.8
16 mg/100µL *L-carnitine* w/50 ppm feed supplementation	541[bcd]	704[de]	1.303	5.6	2.272 [d]	3.892 [c]	1.713 [c]	4.2
32 mg/100µL *L-carnitine* w/50 ppm feed supplementation	522[d]	699[e]	1.344	5.8	2.286 [cd]	3.982 [c]	1.729 [bc]	7.5
SEM	14.6	17.0	0.0159	1.98	0.0346	0.0652	0.0096	2.22
P value	0.01	0.001	0.68	0.50	0.001	<.0001	0.021	0.81

[1]Values correspond to feed conversion adjusted for mortality weight.
[a-e]Values not sharing a common superscript within a column differ significantly

Table 3.4 Carcass traits of age Ross 708 broiler at 45 d of age in response to being fed diets supplemented with L-carnitine subsequent to the *in ovo* injection of L-carnitine.

In ovo Injection Type	Carcass		Breast		Back-half		Fat	
	Weight (kg)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)
Industry Egg	1.720	68.8	568 [ab]	22.4	709	28.5	24.9	0.99
Dry Punch	1.708	68.6	553 [abcd]	22.4	705	28.3	23.9	0.93
Diluent	1.713	68.3	552 [abcd]	21.8	709	28.3	27.4	1.09
8 mg/100µL *L-carnitine*	1.752	68.7	575 [a]	22.5	714	28.0	24.4	1.01
16 mg/100µL *L-carnitine*	1.720	68.6	563 [abc]	21.8	721	28.3	26.4	1.04
32 mg/100µL *L-carnitine*	1.687	68.2	544 [bcd]	21.7	709	28.3	26.8	1.08
8 mg/100µL *L-carnitine* w/50 ppm feed supplementation	1.667	68.2	527 [d]	21.7	688	28.6	24.8	1.03
16 mg/100µL *L-carnitine* w/50 ppm feed supplementation	1.692	68.9	551 [abcd]	22.3	694	28.2	24.0	0.99
32 mg/100µL *L-carnitine* w/50 ppm feed supplementation	1.666	68.2	537 [cd]	22.0	681	28.0	25.7	1.01
SEM	0.0249	0.243	10.4	0.253	11.2	0.16	1.56	0.059
P value	0.26	0.53	0.05	0.12	0.21	0.27	0.72	0.61

[a-d]Values not sharing a common superscript within a column differ significantly.

CHAPTER IV

SUMMARY

Many different products are continuously injected into eggs via the *in ovo* procedure. Commercial hatcheries vaccinate for Marek's disease and infectious bursal disease. With this study and previous studies, results have shown that these products can have harsh, good, or no effects at all. The studies have shown that the *in ovo* procedure does place a physical stress on the chick. Supplementation of the chick post-hatch as also been studied extensively. Various products have been studied throughout all three grow-out phases with many different kinds of affects. Supplementation of those various products post-hatch has shown increases in body weight, feed conversion, and processing cuts.

In the study, the process of *in ovo* injection of *L-carnitine* into the embryonic amnion of broiler breeder eggs was studied. The supplementation of *L-carnitine* (in the feed) post-hatch was also studied. *L-carnitine* did not affect hatchability or hatch time. However, *L-carnitine* did affect certain aspects of grow-out performance, such as feed conversion, body weight, feed intake, and breast wt. Also *L-carnitine* was found to be slightly toxic to broilers when injected *in ovo* combined w/50 ppm of the supplemented *L-carnitine*. Nevertheless, further research is needed to help explain the reasons for the different aspects of the grow-out performance.