www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Effect of L-carnitine on fertility, hatchability and sex hormones in duck breeder

Hazim J. Al-Daraji and Anmar O. Tahir

University of Baghdad, College of Agriculture, Department of Animal Production, Baghdad, Iraq

Abstract

In this experiment, 30 weeks-old, eighty four Iraqi drakes and sixty ducks were randomly divided into 4 equal groups and fed dietary L-carnitine for 12 weeks (0 mg/kg diet; control group (T_1), 50 mg/kg diet (T_2), 100 mg/kg diet (T_3) and 150 mg/kg diet (T_4). The traits examined were egg fertility, hatchability and mortality of embryos and some blood sex hormones. L-carnitine significantly increased ($P \leq 0.01$) the fertility, hatchability of fertile eggs and hatchability of total eggs and significantly decreased ($P \leq 0.01$) embryonic mortality during all three hatching cycles. However, the supplementation of dietary L-carnitine at levels of 50, 100 or 150 mg/kg to a basal diet significantly increased ($P \leq 0.01$) blood testosterone in drakes during all three months of experiment. Moreover, supplemental dietary L-carnitine at level of 50, 100 or 150 mg/kg to a basal diet significantly increased ($P \leq 0.01$) blood oestrogen and progesterone in mature ducks during all three months of experiment. We concluded positive effect of L-carnitine on the reproductive performance of drakes and ducks.

Keywords: Carnitine; fertility; hatchability; sex hormones; ducks

To cite this article: Al-Daraji HJ and AO Tahir, 2014. Effect of L-carnitine on duck breeder fertility, hatchability and sex hormones. Res. Opin. Anim. Vet. Sci., 4(11): 608-613.

Introduction

L-carnitine has a close resemblance with amino acid. This amino acid like compound is formed inside the body of animals. The precursors for L-carnitine are lysine and methionine. In addition, vitamins B_6, vitamin B_{12}, vitamin C, folic acid and niacin and iron are also necessary as catalytic agents for the endogenous synthesis of L-carnitine. The highest synthesizing capacity is found in the liver. L-carinitine is a natural, vitamin like substance that acts in the cells as a receptor molecule for activated fatty acids. A shortage of this substance results primarily in impaired energy metabolism and membrane function (Harmeyer, 2002). The effect of L-carnitine on reproductive parameters has been assessed in human and boars. Infertile men have significantly lower seminal carnitine concentration than fertile men. When utilized as an epididymal marker, L-carnitine level was elevated in fertile compared to infertile White Leghorn birds (Neuman et al., 2002). The major metabolic role of L-carnitine appears to be the transport of long-chain fatty acids into the mitochondria for B-oxidation (Coulter, 1995). Thus dietary L-carnitine supplementation could improve fatty acid and energy utilization to meet endogenous requirements (Gropp et al., 1994). With laying hens, supplemental dietary L-carnitine resulted in an improvement in the albumen quality of eggs such as albumen height and Haugh unit score, during the early and late stages of laying period (Rabie et al., 1997a&b). Leibetseder (1995) reported that egg hatchability increased from 83 to 87% and 82.4 to 85.3% when broiler breeder were fed on diets supplemented with L-carnitine at levels of 50 and 100 mg/kg respectively. Various studies (Adel et al., 2009; Moradi et al., 2010) have demonstrated the effectiveness of L-carnitine in treating male infertility by increasing sperm count, motility and semen volume. L-carnitine also has antioxidant properties that protect sperm membrane against toxic reactive oxygen species. Pignatelli et al. (2003) demonstrated that L-carnitine reduces oxidative stress via interference with arachidonic acid

***Corresponding author:** Prof. Dr. Hazim J. Al-Daraji, University of Baghdad, College of Agriculture, Department of Animal Production, Baghdad, Iraq; E-mail: hazimaldaraji@coagri.uobaghdad.edu.iq

incorporation into phospholipids and protein kinase C mediated NADPH oxidase system. It is also proposed that L-carnitine exerts antioxidant properties as a result of repairing mechanism by which elevated intracellular toxic acetyl-CoA is removed and fatty acids in membrane phospholipids are replaced (Vicari and Calogero, 2001). Al-Daraji and Tahir (2013) found that dietary supplementation with L-carnitine at level of 50, 100 or 150 mg/kg of diet improved semen quality of Iraqi drakes. L-Carnitine is also known to fulfil important role in sperm maturation and metabolism when spermatozoa passes through the epididymis (Yakushiji et al., 2006). A study reported that L-carnitine can increase the number of viable spermatozoa and the number of doses for artificial insemination (Yeste et al., 2010). L-carnitine plays a key role in sperm metabolism by providing readily available energy for use by spermatozoa, which positively affects sperm motility, maturation and the spermatogenic process. This beneficial effect is mediated by the transport of long chain fatty acids across the inner membrane of the mitochondria for utilization in metabolism through β-oxidation (Matalliotakis et al., 2000). Various feeding studies have indicated that supplementary dietary L-carnitine has helped birds to achieve the targeted egg weight earlier at the beginning of the egg production cycle (Leibetseder, 1995). It was hypothesized that breeder birds fed diets supplemented with L-carnitine showed an improvement in semen traits and fertility parameters by preventing lipid peroxidation of sperm membrane (Sarica et al., 2007). The objective of the present study was to investigate the different levels of L-carnitine on fertility, hatchability and sex hormones in Iraqi drakes and ducks.

Materials and Methods

In this experiment, we kept 30 weeks old, eighty four Iraqi drakes and sixty Iraqi ducks of the same age in individual cages with size 0.6×0.8×0.6 m. The birds were bred at the Poultry farm of the Department of Animal Production, College of Agriculture, University of Baghdad, Iraq. Both male and female groups were randomly divided into 4 equal groups (each group contained 12 drakes and 15 ducks) according to the dietary L-carnitine (L-Carnitine Xtreme 60 ct, Dymatize Nutrition Company, USA) supplementation for 12 weeks (0 mg/kg diet; control group (T_1), 50 mg/kg diet (T_2), 100 mg/kg diet (T_3) and 150 mg/kg diet (T_4). Each male and female group was further divided into three replicates. A photoperiod of 24 h was maintained throughout the experimental period. Feed and water were provided *ad libitum* and the diet was presented in mash form. It was formulated to be isocaloric and isonitrogenous and their composition was determined according to the NRC (1994) as shown in Table 1. The experiment was started when the age of drakes and ducks reached 32 weeks. Semen samples were collected from all drakes by placing the female (teaser method) in the cage of the drake using an artificial vagina (Gerzilov, 2000). In our study, we used ejaculates with the following quality. Females were inseminated at 3 periods of their reproductive cycle (32–35, 36–39 and 40–43 wks). Semen samples of all drakes in each treatment group were inseminated into females from the similar treatment group. The collection vial was sealed to minimize possible contamination and evaporation. A one ml tuberculin syringe was used for inseminations directly into the oviduct of ducks. To prevent injury, insemination was not attempted until each hen laid at least one egg (Al-Daraji, 2007). Unlike the domestic chickens and turkeys, it was impossible to avert the oviduct. The syringe containing 0.05 ml of semen ($175\pm25\times10^6$ spermatozoa per dose) was gently inserted on the left side of the cloaca and somewhat dorsally until the oviduct was discovered. The syringe was then gently inserted one or two centimetres and the semen was deposited (Al-Daraji et al., 2012). Eggs were collected from the 2nd to the 22nd day after insemination, stored for 1 week at 16°C and then incubated under standard conditions (Sellier et al., 2005). In order to assess their apparent status (unfertile, dead or live embryo), eggs were candled with a UV lamp on Day 6 and with an incandescent lamp 3 days prior to hatching. Embryo mortality was classified according to the period of incubation at which it was observed (early, 0–6 days; medium, 6–25 days; late, >25 days). The number of both hatched and unhatched eggs was evaluated after the incubation and egg hatching completed. In the case of unhatched eggs, the number of unfertile eggs and eggs containing dead embryos were recorded. And then each of fertility and hatchability rates and embryonic mortality were calculated.

Blood samples were collected randomly from three male and female birds in each month of experiment. Blood was sampled by puncturing the brachial vein with a small (25 G) needle and collecting blood in heparinized micro-hematocrit tubes. Pressure was applied to the wound if bleeding continued after sampling. Both wings were bled during the sampling from each bird in each month of experiment. Samples were kept on ice in the field, and transported to camp later in the day. Samples were immediately centrifuged. Plasma was drawn off with a micro syringe and placed in labelled plastic tubes, then stored and transported in liquid nitrogen. At the laboratory of College of Agriculture, University of Baghdad, samples were frozen at -20°C until analyzed. Plasma testosterone and oestrogen and progesterone concentration were determined using the commercially available kits (Diagnostic Products Corporation, CA, USA).

Table 1: Composition of feed composition and calculated chemical analysis of experimental basal diet

Ingredients	(%)
Yellow corn	39
Wheat	33.7
Soya bean meal (44 %)	13
Protein*	5
Limestone	6
Vegetable oil	2
Dicalcium Phosphate	1
NaCl	0.3
Total	100
Calculated Chemical composition**	
Crud Protein (%)	15.2
Energy (kcal/kg)	2927
Lysine (%)	0.7
Methionine (%)	0.3
Cysteine, %	0.25
Calcium (%)	2.7
Available Phosphorus (%)	0.3

*Concentration protein (BROCON – 5 SPECIAL W) each 1kg of vit. and min. premix contained: 3.25% Crud protein; 3.5% Crud fat; 1% Crud fiber; 6% Calcium; 3% Available phosphorus; 2.2% sodium; 3.5% methionine; 3.90% methionine + cysteine; 3.25% Lysine; 2100 kcal/kg metabolizable energy; 200000 IU Vit A; 40000 IU Vit.D_3; 500 mg Vit. E; 30 mg Vit. K_3; 15 mg Vit. B_1, B_2; 150 mg Vit. B_3; 20 mg Vit. B_6; 300 mg Vit. B_{12}; 10 mg Folic acid; 50 mg Biotin; 800 mg Zinc; 100 mg Copper; 15 mg Iodine ; 1 mg Iron; 2 mg Selenium; 1.2 mg Manganese; 6 mg Cobalt; and antioxidant 90 mg; **Calculated Chemical composition (NRC, 1994).

The data were analyzed using an analysis of variance (ANOVA) with the general linear model procedure of the SAS program (SAS Institute, 2004). The means of variables were compared using Duncan's multiple- range test (Duncan, 1955).

Results and Discussion

L-carnitine increased significantly (P≤0.01) the fertility percentage and hatchability of total eggs during the experimental period. It was observed that T_4 recorded the highest fertility and hatchability of total eggs in comparison with other treatments (T_1, T_2 and T_3) as shown in Table 2. Hatchability of fertile egg increased significantly (P≤0.01) in T_2 and T_3. It increased significantly (P≤0.01) in T_3 and T_4 during second month and all the treated groups in third month compared to the control. Percentage of embryonic mortality was significantly lower in the experimental groups (T_2, T_3 and T_4) as compared with control group (T_1) during the whole experimental period. The fertility and hatching rates increased significantly, which is also supported by the findings reported by Thiemel and Jelinek (2004). The better results may be due to the beneficial results of L-carnitine. Our results were also confirmed by the findings of Neuman et al. (2002) who reported that L-carnitine increased sperm cells in breeding roosters. Our previous studies (Al-Daraji and Tahir, 2013&2014) revealed increased activity of semen quality in breeding roosters in response to L-carnitine supplementation. Most feed ingredients in the commercial diets of domesticated hens are from plants that are low in L-carnitine. For this reason, freshly laid eggs have low concentrations of free L-carnitine and

Table 2: Effect of dietay L-carnitine supplementation on fertility and hatchability and embryonic mortality (%) of eggs

Treatments	Periods			Overall mean
	First month	Second month	Third month	
Fertility (%)				
T1	77.24±1.11^{c}	75.27±1.55^{c}	78.13±0.74^{d}	76.88±1.13^{d}
T2	83.65±1.35^{b}	82.38±1.35^{b}	83.66±0.37^{c}	83.23±1.02^{c}
T3	86.00±0.89^{b}	85.06±0.39^{b}	85.78±0.45^{b}	85.61±0.57^{b}
T4	90.67±0.45^{a}	91.70±1.39^{a}	91.14±0.58^{a}	91.17±0.80^{a}
Hatchability of total eggs (%)				
T1	69.34±1.67^{c}	74.37±0.55^{c}	74.34±0.19^{c}	72.68±0.80^{c}
T2	75.83±0.89^{b}	76.48±0.81^{b}	76.72±0.42^{b}	76.34±0.70^{b}
T3	78.84±1.09^{b}	78.13±0.29^{b}	78.68±0.65^{b}	78.55±0.67^{b}
T4	85.19±1.97^{a}	85.74±0.76^{a}	84.73±1.20^{a}	85.22±1.31^{a}
Hatchability of fertile eggs (%)				
T1	82.11±0.46^{c}	82.12±1.22^{b}	88.04±2.20^{b}	84.09±1.29^{c}
T2	90.07±1.32^{a}	84.22±0.91^{b}	94.62±1.44^{a}	89.64±1.22^{b}
T3	91.90±0.28^{a}	93.05±0.61^{a}	94.8±2.01^{a}	93.25±0.96^{a}
T4	85.41±1.56^{b}	93.92±0.58^{a}	94.90±1.90^{a}	91.41±1.34^{ab}
Embryonic mortality (%)				
T1	17.89±0.46^{a}	17.88±1.22^{a}	11.96±2.20^{a}	15.91±1.29^{a}
T2	9.93±1.32^{c}	15.78±0.91^{b}	5.38±1.44^{b}	10.36±1.22^{b}
T3	8.10±0.28^{c}	6.95±0.61^{c}	5.20±2.01^{b}	6.75±0.96^{c}
T4	14.59±1.56^{b}	6.08±0.58^{c}	5.10±1.90^{b}	8.59±1.34^{bc}

T_1: Control, T_2: 50 mg L-carnitine /kg of diet, T_3: 100 mg L-carnitine /kg of diet, T_4: 150 mg L-carnitine /kg of diet.
Means in same column with different superscript were significantly different (P≤0.01).

acetyl L-carnitine (Zhai et al., 2008a&b). It was previously reported that supplementation of hen's diets with L-carnitine increased yolk L-carnitine concentrations (Moran, 2007; Peebles et al., 2007). Moreover, the chick embryo may have limited capability to synthesize L-carnitine during incubation (Sato et al., 2006). Gamma-butyrobetaine is required for biosynthesis of L-carnitine. This compound is limited in embryos and young animals due to the low activity of γ-butyrobetaine hydroxylase (Agarwal et al., 2005). Low levels of L-carnitine in the embryo may be fulfilled by supplementation of L-carnitine. Hatchability of eggs increased from broiler breeder hens consuming diets supplemented with 50 or 100 mg of L-carnitine for 3 weeks (Leibetseder, 1995). Even though de novo synthesis of L-carnitine occurs in birds, exogenous L-carnitine may be beneficial, especially in embryos and hatchling (Kidd et al., 2005). The conversion of fatty acids into esterified L-carnitine is an essential step for fatty acid oxidation (Salmanzadeh et al., 2012). Embryos and young chickens have much lower level of free and total L-carnitine as well as short chain esterified L-carnitine in muscles, liver and heart as compared with adult tissues (Koksal et al., 2011). The ratio of esterified short chain L-carnitine to free L-carnitine reaches the highest level on d 18 of incubation in all tissues. This ratio is even higher than in growing chicks, reflecting the high demand for fatty acid utilization for energy production in embryos (Kucukersan et al., 2011). L-Carnitine could reduce the incidence of late dead embryos, in particularly those chicks that die during the piping process leading to improved hatchability. Uni et al. (2005) suggested that less glucose may be used as an energy source due to more conversion of fatty acids into energy during *in ovo* L-carnitine injection.

Figure 1 showed that compared within the first, second, third months or overall means, there was a significant increase ($P \leq 0.01$) in the level of testosterone in all treated groups (T_2, T_3 or T_4) in comparison with control group (T_1). In addition, the results shown in Figure 2 indicated that the level of oestrogen increased significantly ($P \leq 0.01$) in all experimental groups relative to the control group during all periods of experiment. However, there was significant increase ($P<0.01$) in the level of progesterone in all treated groups (T_2, T_3 and T_4) compared with the control group (T_1) during all stages of experiment (Fig. 3).

In our study, the levels of testosterone, oestrogen and progesterone increased in the L-carnitine-treated group as shown in Figures 1, 2 and 3. This result is consistent with reports by Tanis et al. (2010), Mohamed and Farghaly (2009) in which testosterone, oestrogen or progesterone levels increased after L-carnitine administration. The administration of a dietary supplement containing acetyl-L-carnitine has been

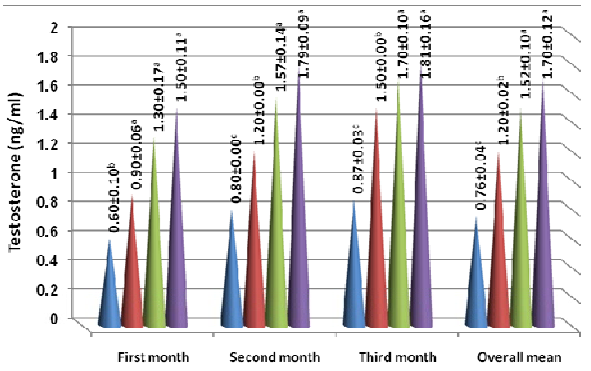

Fig. 1: Effect of dietay L-carnitine on serum testosterone (ng/ml) of drakes

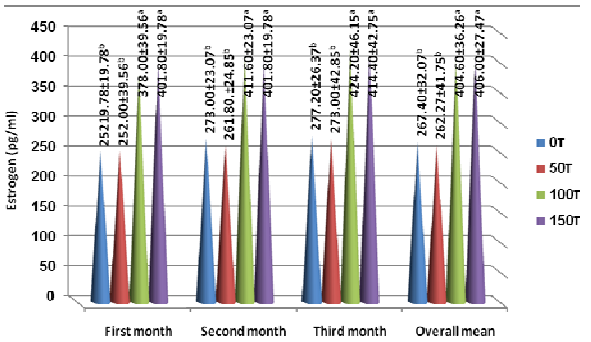

Fig. 2: Effect of dietay L-carnitine on serum oestrogen (pg/ml) of ducks

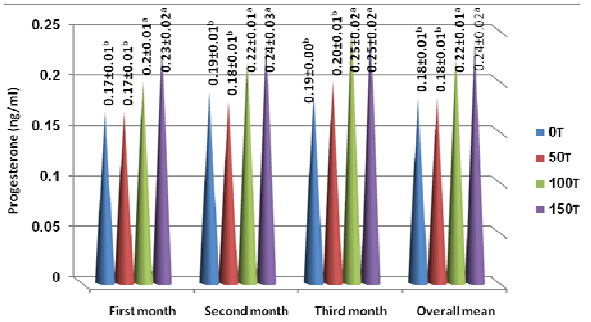

Fig. 3: Effect of dietay L-carnitine on serum progesterone (ng/ml) of ducks

shown to cause an increase in testosterone levels by increasing nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) levels via enhanced acetylcholine levels. NO activates the release of luteinising hormone-releasing hormone (LHRH), which reaches the pituitary and activates the release of luteinising hormone (LH) via the activation of neural NO synthase (NOs) in the pituitary gland. In addition, LH works with receptors located on the surface of Leydig cells to control the production and secretion of testosterone. The subsequent binding of LH to its receptor allows signalling through the cAMP pathway

via guanosine triphosphate binding proteins. Signal transduction occurs through the protein kinase A pathway, which ultimately causes the release of testosterone after 30-60 minutes of LH stimulation (Manna et al., 2009). Al-Rubiey (2012) found that histological examination shows new findings of significant improvement in the number of Leydig cells in L-carnitine treated male albino Wister rats compared to placebo. Agarwal and Said (2004) concluded that L-carnitine enhances the oxidation of fatty acids which stimulates oestrogen and progesterone biosynthesis by increasing the regeneration of the reducing equivalents necessary for the cholesterol side-chain cleavage reaction.

The results of the present study showed that oral administration of L-carnitine improved reproductive performance in Iraqi ducks and drakes.

References

Adel, R.A., Abd-Allah, A.R.A., Helal, G.K., Al-Yahya, A.A., Aleisa, A.M. and Al-Rejaie, S.S. 2009. Pro-inflammatory and oxidative stress pathways which compromise sperm motility and survival may be altered by L-carnitine. *Oxidative Medicine and Cellular Longevity*, 2: 73-81.

Agarwal, A. and Said, T.M. 2004. Carnitine and male infertility. *Reproductive Bio Medicine Online*, 8: 376-384.

Agarwal, A., Prabakaran S.A. and Said, T.M. 2005. Prevention of oxidative stress injury to sperm. *Journal of Andrology*, 26: 654-660.

Al-Daraji, H.J. 2007. Artificial insemination in domestic birds. Ministry of Higher Education and Scientific Research, University of Baghdad, College of Agriculture, Baghdad, Iraq.

Al-Daraji, H.J. and Tahir, A.O. 2014. Effect of L-carnitine supplementation on drake semen quality. *South African Journal of Animal Science*, 44: 18-25.

Al-Daraji, H.J. and Tahir, A.O. 2013. Effect of L-carnitine supplementation on seminal plasma quality of Iraqi drakes. *Indian Journal of Applied Research*, 3: 10-14.

Al-Daraji, H.J., Al-Mashadani, H.A., Al Hayani, W.K. and Merza, H.A. 2012. The first trial for semen collection and artificial insemination in duck and geese birds in Iraq by using new techniques. Patent issued from C.O.S.Q.C. No. 3367 on 5 / 1 / 2012.

Al-Rubiey, F.K. 2012. Effect of L-carnitine and meloxicam treatment on testicular Leydig cell numbers of varicocelized rats. *Middle East Fertility Society Journal*, 17: 47-53.

Coulter, D.L. 1995. Carnitine deficiency in epilepsy: risk factors and treatment. *Journal of Child Neurology*, 10 (Supp.2): 2S32-2S39.

Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics*, 11: 1-42.

Gerzilov, V. 2000. A method for obtaining semen from the species Muscovy duck (*Cairina moschata*). *Journal of Animal Science*, 4: 56-63.

Gropp, J.M., Schumacher, A. and Schweigert, F.J. 1994. Recent research in vitamin nutrition with special emphasis to vitamin A, carotene and L-carnitine. In: *proceedings of the meeting of the Arkansas Nutrition Conference, Fayetteville, Arkansas Poultry Federation*, Pp: 124-134.

Harmeyer, J. 2002. The physiological role of L-carnitine. *Lohman Information*, 27: 15-21.

Kidd, M.T, McDaniel, C.D., Peebles, E.D., Barber, S.J., Corzo, A., Branton, S.L. and Woodworth, J.C. 2005. Breeder hen dietary L-carnitine affects progeny carcase traits. *British Poultry Science*, 46: 91-103.

Koksal, B.H., Kucukersan, M.K. and Cakin, K. 2011. Effects of L-carnitine and/or inulin supplementation in energy depressed diets on growth performance, carcass traits, visceral organs and some blood biochemical parameters in broilers. *Revue de Médecine Vétérinaire*, 162: 519-525.

Kucukersan, M.K., Koksal, B.H. and Cakin, K. 2011. Effects of dietary L-carnitine and/or inulin supplementation on growth performance, carcass traits, visceral organs and some blood biochemical parameters in broilers. *Revue de Médecine Vétérinaire*, 162: 552-557.

Leibetseder, J. 1995. Studies on effects of L- carnitine in poultry. *Archives of Animal Nutrition*, 48: 97-108.

Manna, P.R., Dyson, M.T. and Stocco, D.M. 2009. Regulation of the steroidogenic acute regulatory protein gene expression: present and future perspectives. *Molecular Human Reproduction*, 15: 321-333.

Matalliotakis, I., Koumantakis, Y., Evageliou, A., Matalliotakis, G., Goumenou, A. and Koumantakis, E. 2000. L-Carnitine levels in the seminal plasma of fertile and infertile men: Correlation with sperm quality. *International Journal of Fertility*, 45: 236-240.

Mohamed, N.E. and Farghaly, A.A. 2009. Evaluation of the protective effect of L-carnitine on radiation induced free oxygen radicals and genotoxicity in male mice. *Researcher*, 1: 7-15.

Moradi, M., Moradi, A., Alemi, M., Ahmadnia, H., Abdi, H. and Ahmadi, A. 2010. Safety and efficacy of clomiphene citrate and L-carnitine in idiopathic male infertility. *Urology Journal*, 7: 188-193.

Moran, E.T., Jr. 2007. Nutrition of the developing embryo and hatchling. *Poultry Science*, 86: 1043-1049.

National Research Council. 1994. Nutrient Requirements of Poultry, 9th revised ed. Washington, D.C. National Academy Press.

Neuman, S.L., Lin, T. and Hester, P.Y. 2002. The effect of dietary carnitine on semen traits of white leghorn roosters. *Poultry Science*, 81: 495-50.

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

Pignatelli, P., Lenti, L., Sanguigni, V., Frati, G., Simeoni, I. and Gazzaniga, P.P. 2003. Carnitine inhibits arachidonic acid turnover, platelet function, and oxidative stress. *The American Journal of Physiology: Heart and Circulatory Physiology*, 284: 41-48.

Rabie, M.H., Szilagyi, M. and Gippert, T. 1997a. Effect of dietary L- carnitine on the performance and egg quality of laying hens from 65-73 weeks of age. *British Journal of Nutrition*, 78: 615-623.

Rabie, M.H., Szilagyi, M. and Gippert, T. 1997b. Influence of supplemental dietary L- carnitine on performance and egg quality of pullets during the early laying period. *Allattenyesztes es Takarmanyozas*, 46: 457- 468.

Salmanzadeh, M., Ebrahimnezhad, Y. and Shahryar, H.A. 2012. Effects of in ovo administration of L-carnitine on hatchability, subsequent performance, carcass traits and blood cholesterol of turkey poults. *Revue De Medecine Veterinaire*, 163: 448-453.

Sarica, S., Corduk, M., Suicmez, M., Cedden, F., Yildirim, M. and Kilinc, K. 2007. The effects of dietary L-carnitine supplementation on semen traits, reproductive parameters, and testicular histology of Japanese quail breeders. *Journal of Applied Poultry Research*, 16:178-186.

SAS Institute, 2004. SAS/STAT User's Guide. Relase Version 7.00. SAS Institute. Cary. North Carolina.

Sato, M., Tachibana, T. and Furuse, M. 2006. Heat production and lipid metabolism in broiler and layer chickens during embryonic development. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 143: 382-388.

Sellier, N., Brun, J.M., Richard, M.M., Batellier, F., Dupuy, V. and Brillard, J.P. 2005. Comparison of fertility and embryo mortality following artificial insemination of common duck females (*Anas Platyrhynchos*) with semen from common or Muscovy (*Cairina Moschata*) drakes. *Theriogenology*, 64: 429-39.

Tanis, D., Orrell, D. and Long, D.W. 2010. The effects of a dietary supplement, free test™, on serum free and total testosterone levels in weight-trained male subjects. Applied Nutriceuticals® In-House Pilot Work, April 2010.

Thiemel, J. and Jelinek, P. 2004. The effect of carnitine on hatching rate and metabolic profile of blood in breeding layers. *Czech Journal of Animal Science*, 49: 517-523.

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

Vicari, E. and A.E. Calogero. 2001. Effects of treatment with carnitines in infertile patients with prostato-vesico-epididymitis. *Human Reproduction*, 16: 2338-2342.

Yakushiji, K., Kai, S., Yamauchi, M., Kuwajima, M., Osada, Y. and Toshimori, K. 2006. Expression and distribution of OCTN2 in mouse epididymis and its association with obstructive azoospermia in juvenile visceral steatosis mice. *International Journal of Urology*, 13: 420-426.

Yeste, M., Sancho, S., Briz, M., Pinart, E., Bussalleu, E. and Bonet, S. 2010. A diet supplemented with L-carnitine improves the sperm quality of Piétrain but not of Duroc and Large White boars when photoperiod and temperature increase. *Theriogenology*, 73: 577-586.

Zhai, W., Neuman, S.L., Latour, M.A. and Hester, P.Y. 2008a. The effect of in ovo injection of L-carnitine on hatchability of White Leghorns. *Poultry Science,* 87: 569-572.

Zhai, W., Neuman, S.L., Latour, M.A. and Hester, P.Y. 2008b. The effect of male and female supplementation of L-carnitine on reproductive traits of white Leghorns. *Poultry Science*, 87: 1171-1181.