The Effect of Dietary L-Carnitine on Semen Traits of White Leghorns[1]

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ABSTRACT A previous study conducted in our laboratory showed that feeding 500 ppm of dietary L-carnitine to young and aging White Leghorns for 5 wk improved sperm concentration and reduced sperm lipid peroxidation during the last half of supplementation. The current study examined the effect of feeding dosimetric as well as lower levels of L-carnitine for longer durations on semen traits of White Leghorns. In experiments 1 and 2, White Leghorns consumed diets supplemented with 0, 125, 250, or 500 mg of L-carnitine/kg of feed. For experiment 1, an 8-wk trial was conducted with 48 White Leghorns from 46 to 54 wk of age. For experiment 2, a 17-wk trial was conducted with 96 White Leghorn roosters from 46 to 63 wk of age. For experiment 3, 84 roosters were provided for ad libitum consumption a diet formulated to contain 0 or 125 ppm of L-carnitine beginning at hatch until 37 wk of age. Long-term consumption of 125 ppm of L-carnitine beginning at hatch was the only dietary treatment that sustained a persistent increase in sperm concentration. These results suggest that L-carnitine's antioxidant influence on sperm production begins before the onset of sexual maturity.

Key words: L-carnitine, semen trait, sperm concentration, dosimetry

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INTRODUCTION

L-Carnitine (β-hydroxy-γ-trimethylammonium butyrate) is a highly polar natural compound found in microorganisms, plants, and animals (Bremer, 1983). L-Carnitine plays a critical role in the maturation and motility of spermatozoa within the male reproductive tract (Menchini-Fabris et al., 1984; Jeulin et al., 1988; Ng et al., 2004). The mammalian epithelium secretes L-carnitine into the epididymal fluid, and it is subsequently transported into spermatozoa, where it accumulates as free L-carnitine as well as acetylated L-carnitine (Jeulin et al., 1994; Jeulin and Lewin, 1996; Enomoto et al., 2002). Two carnitine transporters in the basolateral and luminal membranes of the epididymal epithelium of the testes facilitate this transfer (Enomoto et al., 2002; Kobayashi et al., 2005). Epididymal fluid contains the highest levels of L-carnitine of all biological fluids with levels reaching as high as 100 m*M*, which is 2,000-fold greater than levels found in the blood (0.01 to 0.05 m*M*; Jeulin and Lewin, 1996). L-Carnitine content in seminal fluid is correlated positively to sperm concentration and motility (Menchini-Fabris et al., 1984; Matalliotakis et al., 2000; Lenzi et al., 2003). Within the epididymal lumen, sperm motility is initiated in parallel with increasing levels of L-carnitine (Jeulin and Lewin, 1996). Epididymal L-carnitine enhances spermatozoa's fertilizing capabilities (Hinton et al., 1979; Jeulin and Lewin, 1996; Enomoto et al., 2002). L-Carnitine accumulates in spermatozoa as they progress to the caudal region of the epididymis (Jeulin et al., 1994), whereas spermatozoa simultaneously gain motility and fertilizing capabilities (Kirby and Froman, 2000).

Fatty acid β-oxidation within the mitochondria requires L-carnitine (Bremer, 1983). Specifically, L-carnitine transports fatty acids into the sperm mitochondria where the oxidation of fatty acids is used as a main source of energy for epididymal spermatozoa. In addition to carnitine's role in energy metabolism, it also possesses antioxidant properties. Newly formed spermatozoa within the gonad are immobile and are therefore infertile, requiring modification in the epididymis to become fertile (Jeulin and Lewin, 1996). For example, total lipid content of spermatozoa decreases during maturation with changes in fatty acid composition. Saturated and monounsaturated fatty acids are reduced with a concomitant increase in the proportion of polyunsaturated fatty acids (**PUFA**), leading to a potential increase in the fluidity of the sperm membrane and perhaps increasing susceptibility to lipid peroxidation (Ladha, 1998). Because L-carnitine is involved in fatty acid transport for energy metabolism, it reduces lipid availability for peroxidation. Its antioxidant properties likely preserve other antioxidants (e.g., ascorbic acid), including antioxidant enzymes, against potential peroxidative damage (Kalaiselvi and Panneerselvam, 1998). L-Carnitine may serve as a scavenger of free radicals that cause lipid peroxidation. L-Carnitine reduces these free radicals or reactive oxygen species (**ROS**) and increases the forward moving ability of sperm

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as well as sperm viability in infertile men with prostate-vesiculo-epididymitis (Vicari and Calogero, 2001). The antioxidant status of aged rat brains was improved in a duration dependent manner through use of supplemental L-carnitine (Rani and Panneerselvam, 2001).

The ad libitum consumption of 500 ppm of dietary L-carnitine to young and aging White Leghorns for 5 wk improved sperm concentration during the last half of supplementation and reduced sperm lipid peroxidation. Multinucleated giant cells, composed mainly of aggregates of degenerated spermatocytes and spermatids, were reduced in the testes of roosters consuming L-carnitine as compared with control-fed birds. Collectively, these results suggest that dietary L-carnitine has antioxidant properties that may preserve sperm membranes in roosters, thereby extending the life span of sperm (Neuman et al., 2002). These investigators used only 1 dosage level of L-carnitine in their study, and the L-carnitine was fed to roosters for short duration; therefore, the objective of the present study was to investigate the effect of feeding dosimetric as well as lower levels of L-carnitine for longer durations on semen traits of White Leghorns.

MATERIALS AND METHODS

Experiment 1

An 8-wk trial was conducted with 48 White Leghorn roosters when they were 46 to 54 wk of age during cooler weather from September 26 to November 20. The line of chickens were maintained at Purdue University and were several generations removed from commercial strains. Roosters were housed singularly in a 30 cm × 36 cm deep cage resulting in 1,080 cm^2/bird. Feeder space was 30 cm. There were 2 nipple drinkers per cage. Birds were fed a corn-soybean based diet (Table 1). Feed was formulated to contain 0, 125, 250, or 500 ppm or mg of L-carnitine/kg of feed (Carniking, Lonza Inc., Allendale, NJ). Diets were fed ad libitum. There were 12 birds/treatment, and the semen of 2 roosters of adjacent cages from the same treatment was pooled to form 6 experimental units per treatment. Dietary treatments were assigned a colored microtracer in the feed so that investigators were blind to dietary treatments until termination of study.

Weekly semen traits and BW were determined, including a pretreatment (0 wk) sampling. Semen volume was determined gravimetrically by weighing each pooled sample to the nearest thousandth of a gram. The total weight was divided by the number of birds used to produce the ejaculate in each replicate and treatment (Bakst and Cecil, 1997). Sperm viability, expressed as percent dead sperm, was determined using a fluorometric technique based on the ethidium bromide exclusion procedure (Bakst and Cecil, 1997). Sperm concentration was determined using a hemocytometer procedure (Bakst and Cecil, 1997). Total sperm cells produced/rooster were calculated as sperm cells/mL × mL of semen volume. Feed consumption was also determined weekly except for pretreatment determination. The concentration of L-carnitine was measured via radio-enzymatic analysis (Metabolic Analysis Labs Inc., Madison, WI) on a pooled sample of feed and plasma obtained from the L-carnitine- and control-fed groups at the end of experiment. This experiment was conducted under guidelines approved by the Purdue University Animal Care and Use Committee.

Table 1. Composition of diets for White Leghorns in experiments 1 and 2

Item	Diet
Ingredient, %	
Ground yellow corn (6.12% CP)	86.80
Soybean meal (47.50% CP)	9.60
Dicalcium phosphate	1.50
Limestone, ground	0.70
Salt	0.40
Vitamin-mineral trace mix[1]	0.20
Mold inhibitor[2]	0.05
Microtracer[3] or L-carnitine[4]	0.75
Total	100.00
Calculated nutrient composition	
ME, kcal/kg	3,142.00
CP, %	9.87
Lys, %	0.51
Met, %	0.22
TSAA	0.45
Ca, %	0.64
Available P, %	0.36

[1]Provided per kilogram of diet: vitamin A, 5,320 IU; vitamin D_3, 2,172 IU; vitamin E, 27 IU; vitamin K, 0.93 mg; riboflavin, 3.6 mg; pantothenic acid, 5.3 mg; niacin, 17 mg; choline, 288 mg; vitamin B_{12}, 0.0044 mg; Mn, 6.5 mg; Zn, 49 mg; Fe, 26 mg; Cu, 3.1 mg; I, 0.89 mg; and Se, 0.207 mg.

[2]Myco curb Dry, Kemin Industries Inc., Des Moines, IA.

[3]Microtracers, San Francisco, CA.

[4]10% Carniking, Carniking 10, Lonza Inc., Allendale, NJ.

Data were analyzed using ANOVA with repeated measurements for all traits, except for plasma L-carnitine, using the mixed model procedure of SAS (2003). A 1-way ANOVA was used for plasma L-carnitine. The means of sperm and production traits of each supplemented diet was contrasted with the mean of the control diet through preplanned comparisons (Steel et al., 1997). For significant dietary treatment by age interactions or main effect for plasma L-carnitine, the Tukey-Kramer test was used to partition differences among means (Oehlert, 2000).

Experiment 2

A 17-wk trial was conducted with 96 White Leghorns when they were 46 to 63 wk of age during warmer weather from April 27 to August 24. The line of chickens was maintained at Purdue University and was several generations removed from commercial strains. Housing, diets, and approval for animal care and use were the same as experiment 1. There were 24 birds/treatment, and the semen of 2 roosters in adjacent cages from the same treatment was pooled, creating 12 experimental units per treatment.

Semen traits (semen volume, sperm viability, and sperm concentration as experiment 1) were determined weekly from 0 to 8 wk and then at 11, 12, and 17 wk following the initiation of the treatments. Feed consumption and BW were also determined weekly from 0 to 8 wk posttreatment. Lipid peroxidation of the semen was determined at 11, 12,

Table 2. Composition of diets for White Leghorns in experiment 3

Item	Diet		
	Starter 0 to 6 wk	Grower 6 to 8 wk	Breeder 8 to 37 wk
Ingredient, %			
Ground yellow corn (6.12% CP)	66.46	63.38	86.75
Soybean meal (47.50% CP)	29.34	21.82	9.60
Standard midds	—	11.00	—
Limestone, ground	1.20	1.20	0.70
Dicalcium phosphate	1.60	1.20	1.50
Salt	0.35	0.35	0.40
Breeder vitamin-mineral premix[1]	0.25	0.25	0.25
Mold inhibitor[2]	0.05	0.05	0.05
Microtracer[3] or L-carnitine[4]	0.75	0.75	0.75
Total	100.00	100.00	100.00
Calculated nutrient composition			
ME, kcal/kg	2,942.00	2,876.00	3,140.00
CP, %	18.00	16.00	9.87
Lys, %	1.04	0.89	0.51
Met, %	0.32	0.28	0.22
TSAA, %	0.65	0.59	0.45
Ca, %	0.90	0.80	0.64
Available P, %	0.41	0.35	0.36

[1]Provided per kilogram of diet: vitamin A, 11,013 IU; vitamin D_3, 3,525 IU; vitamin E, 33 IU; vitamin K, 2.75 mg; riboflavin, 7.7 mg; pantothenic acid, 17.6 mg; niacin, 55.1 mg; choline, 478 mg; vitamin B_{12}, 0.028 mg; pyridoxine, 5.0 mg; thiamine, 2.2 mg; folic acid, 1.1 mg; biotin, 0.22 mg; Mn, 64 mg; Zn, 75 mg; Fe, 40 mg; Cu, 10 mg; I, 1.85 mg; and Se, 0.3 mg.

[2]Myco curb Dry, Kemin Industries Inc., Des Moines, IA.

[3]Microtracers, San Francisco, CA.

[4]10% Carniking, Carniking 10, Lonza Inc., Allendale, NJ.

and 17 wk posttreatment by measuring malonaldehyde, which is the primary stable by-product of lipid peroxidation using a modified procedure (Neuman et al., 2002) of Cecil and Bakst (1993). The concentrations of L-carnitine for feed and plasma were measured as experiment 1.

For semen and production traits, data were analyzed using ANOVA with repeated measurements using the mixed model procedure of SAS (2003). In addition, a 1-way ANOVA was performed on semen traits each week and plasma L-carnitine concentrations using dietary treatment as the main effect. For the main effect of diet, the mean of each supplemented diet was contrasted with the mean of the control diet through preplanned comparisons for semen and production traits (Steel et al., 1997). Tukey-Kramer test was used to partition differences among plasma L-carnitine concentration means (Oehlert, 2000).

Experiment 3

Male hatchlings, derived from an experiment in which 18-d-old White Leghorn embryos were injected in ovo with sterilized saline, 1 μmol, or 2 μmol of L-carnitine, were used. Roosters from each of the 3 in ovo injection treatments were assigned randomly to 1 of 2 dietary treatments. Eighty-four Hy-Line W-36 White Leghorn roosters were provided for ad libitum consumption a corn-soybean based diet formulated to contain 0 or 125 ppm of L-carnitine (Carniking 10, Lonza Inc., Allendale, NJ) beginning at hatch until 37 wk of age from December 6 to August 24 (Table 2). A red or green microtracer (Microtracers, San Francisco, CA) with no nutritive value was assigned by the feed mill manager to either the control (0 ppm) or treated (125 ppm of L-carnitine) diet; its purpose was to serve as a marker to ensure that the correct feed was placed in the appropriate feed trough and to keep the investigators blind with respect to assignment of dietary treatments until termination of the experiment. Housing was the same as experiments 1 and 2.

The BW of individual roosters was determined when transferred to the breeder facility at 17 wk of age. Forty-two roosters were used for each of the 2 dietary treatments of 0 or 125 ppm of L-carnitine for a total of 84 roosters. Semen was collected artificially from each rooster at weekly intervals. The semen of 2 roosters in adjacent cages and on the same dietary treatment were pooled to compose an experimental unit, resulting in 21 semen samples per dietary treatment or a total of 42 samples. Weekly semen volume and hemocytometer-based sperm counts as described in experiment 1 were determined for each experimental unit from 22 to 37 wk of age. L-Carnitine concentrations in feed were measured as experiment 1. This experiment was conducted under guidelines approved by the Purdue University Animal Care and Use Committee.

Data were analyzed using ANOVA and the mixed model procedure of SAS (2003). Fixed effects included dietary L-carnitine, in ovo injection of L-carnitine, and age of the birds. Semen traits, but not BW, used repeated measurements. A *t*-test was conducted to determine if analyzed vs. calculated values of L-carnitine in the feed were different (Steel et al., 1997).

RESULTS

Experiment 1

Analyzed values for feed were higher (3, 167, 357, and 674 ppm) than formulated values (0, 125, 250, and 500 ppm, respectively). Feeding dietary L-carnitine for 8 wk did not affect feed consumption, BW, and semen viability, expressed as percent dead sperm (Table 3). Sperm concentration of roosters fed 125 ppm of L-carnitine was significantly higher than the controls ($P < 0.05$; Table 3).

Dietary effects on semen volume varied over time (dietary treatment × age interaction, $P < 0.003$; Figure 1). The Tukey-Kramer test used to partition differences among semen volume means showed no differences among diets within sampling time (wk posttreatment). The interaction was due to the unexplained decrease in semen volume from 2 to 3 wk posttreatment of roosters consuming 250 ppm of L-carnitine ($P < 0.01$) and also due to an increase in semen volume among roosters consuming 125 ppm at 6 wk as compared with 2 wk posttreatment ($P < 0.002$). In addition, roosters consuming 500 ppm of L-carnitine showed an increase in semen volume at 6 ($P < 0.0002$), 7 ($P < 0.02$), and 8 ($P < 0.007$) wk when compared with 2 wk posttreatment. However, total sperm cells (sperm cells/mL × mL of semen volume) produced by each rooster were not affected by the L-carnitine treatment ($P = 0.095$, Table 3).

Table 3. Effect of different levels of dietary L-carnitine on feed consumption, BW, and semen traits of White Leghorns in experiment 1

Dietary L-carnitine (mg/kg or ppm)	Feed consumption[1] (g/bird per d)	BW[2] (g)	Sperm concentration[2] (billion sperm/mL)	Semen volume[2] (mL)	Total sperm cells[2] ($\times 10^9$)	Dead sperm[2] (%)
0 (3)[3]	123	2,362	4.3	0.45	1.9	19
125 (167)	116	2,319	4.8*	0.49	2.4	19
250 (357)	117	2,355	4.3	0.54	2.3	19
500 (674)	119	2,355	4.5	0.57	2.6	17
SEM	3	53	0.2	0.05	0.3	1

[1]Values represent the least square means of 48 observations (6 samples/dietary treatment × 8 wk).

[2]Values represent the least square means of 54 observations per dietary treatment (6 samples/dietary treatment × 9 wk which includes pretreatment values).

[3]Numbers in parenthesis are analyzed values of L-carnitine in the diet.

*Roosters consuming L-carnitine at 125 mg/kg showed an increase in sperm concentration when compared with controls ($P < 0.05$).

Circulating levels of free and total L-carnitine were elevated in roosters supplemented with 500 ppm, but not 125 and 250 ppm, of L-carnitine as compared with controls ($P < 0.05$, Table 4).

Experiment 2

Feed was formulated to contain 0, 125, 250, and 500 ppm of L-carnitine with analyzed values of 0, 129, 267, and 611 ppm, respectively. Feeding dietary L-carnitine for 8 wk did not affect feed consumption and BW (data not shown), nor did it affect sperm concentration, semen volume, and sperm viability (Table 5). Sperm concentration of roosters fed 125 ppm of L-carnitine was higher than controls ($P < 0.007$) only at 11 wk posttreatment (Table 5). A trend for higher sperm concentration among roosters consuming 125 ppm was evident ($P < 0.07$) with reduced sperm oxidation ($P < 0.08$) from 11 to 17 wk posttreatment (Table 5).

Circulating levels of total L-carnitine measured at 8 wk following the initiation of the dietary treatments were elevated in roosters supplemented with L-carnitine as compared with controls. Free L-carnitine was also elevated in the blood of hens consuming 250 and 500 ppm, but not 125 ppm, as compared with controls ($P < 0.006$, Table 6).

Experiment 3

Chemical analysis of feed showed L-carnitine levels at 1 and 143 ppm for control (0) and supplemented diets (formulated at 125 ppm), respectively. The chemically analyzed L-carnitine values were not significantly different from the calculated values ($P = 0.32$, $P = 0.44$ for control and supplemented diets, respectively). The in ovo injection of L-carnitine did not affect sperm concentration, semen volume, and BW at 17 wk of age. Long-term consumption of 125 ppm of L-carnitine beginning at hatch resulted in a consistent increase in sperm concentration from 22 to 37 wk of age (Figure 2). Roosters consuming L-carnitine as compared with controls had increased sperm concentration (overall mean of 5.3 and 4.8×10^9 sperm/mL, respectively, SEM = 0.1, $P = 0.002$) with no effect on semen volume (0.49 and 0.50 mL, respectively, SEM = 0.02, $P = 0.87$). The 17-wk-old BW of cockerels consuming 125 ppm of L-carnitine was lower than controls (1,550 and 1,595 g, respectively, SEM = 17, $P < 0.006$).

DISCUSSION

Results from experiments 1 and 2 showed that consumption of L-carnitine for 8 wk by White Leghorns did not affect feed consumption and BW. These results are in agreement with those reported by other authors for broiler chickens (Barker and Sell, 1994; Xu et al., 2003), turkeys (Barker and Sell, 1994), quail (Sarica et al., 2005, 2007), and Leghorn roosters (Neuman et al., 2002). In these studies, Barker and Sell (1994) reported that supplementation of 0, 50, or 100 mg/kg of L-carnitine did not affect BW gain or feed efficiency of turkeys and broiler chickens. Xu et al. (2003) also reported that supplementation of 0, 25, 50, 75, or 100 mg/kg of L-carnitine had no effect on broiler daily gain or feed conversion. Sarica et al. (2005) showed that supplementation of 0, 30, 40, or 50 mg/kg of L-carnitine did not affect BW gain, cumulative feed intake, and feed conversion of immature Japanese quail. Mature quail consuming 250 and 500 ppm of L-carnitine from 5 to 20 wk of age also showed no effect on BW and feed intake (Sarica et al., 2007). Short-term feeding of 500 mg/kg of dietary L-carnitine for 4 to 5 wk did not affect feed consumption or BW of mature White Leghorn roosters (Neuman et al., 2002). However,

Table 4. Concentrations of L-carnitine in plasma of roosters (experiment 1)

Dietary L-carnitine (mg/kg or ppm)	Ester L-carnitine	Free L-carnitine	Total L-carnitine
	(μmol/L)		
0 (3)[1]	6[2]	11[b]	17[b]
125 (167)	8	17[ab]	26[ab]
250 (357)	7	17[ab]	24[b]
500 (674)	11	23[a]	33[a]
SEM	1	2	2

[a,b]Means within a column with no common letters are significantly different ($P < 0.05$).

[1]Numbers in parenthesis are analyzed values of L-carnitine in the diet.

[2]Values represent the mean of 2 pooled samples.

Table 5. Effect of different levels of dietary L-carnitine on semen traits of White Leghorns in experiment 2

Dietary L-carnitine (mg/kg or ppm)	Sperm concentration[1] (billion sperm/mL)	Semen volume[1] (mL)	Dead sperm[1] (%)	Sperm concentration[2] (billion sperm/mL)	Semen volume[2] (mL)	Sperm concentration[3] (billion sperm/mL)	Sperm lipid oxidation[3] (μg of malonaldehyde/ billion sperm)	Semen volume[3] (mL)
	0 to 8 wk posttreatment			11 wk posttreatment		11 to 17 wk posttreatment		
0 (0)[4]	3.9	0.53	9.9	3.7	0.50	4.0	37	0.50
125 (129)	3.8	0.49	9.0	4.7*	0.44	4.6†	30†	0.49
250 (267)	3.9	0.53	8.1	4.4	0.51	4.4	33	0.52
500 (611)	3.6	0.52	8.2	4.3	0.51	4.5	36	0.50
SEM	0.2	0.04	0.9	0.2	0.04	0.2	3	0.03

[1]Values represent the least square means of 108 observations per dietary treatment (12 samples/dietary treatment × 9 wk, which includes pretreatment values).

[2]Values represent the least square means of 12 observations per dietary treatment.

[3]Values represent the least square means of 36 observations (12 samples/dietary treatment × 3 time periods of 11, 12, and 17 wk posttreatment).

[4]Numbers in parenthesis are analyzed values of L-carnitine in the diet.

*Roosters consuming 125 ppm of L-carnitine showed an increase in sperm concentration when compared with controls ($P < 0.007$).

†Roosters consuming 125 ppm of L-carnitine showed an increase in sperm concentration ($P < 0.07$) and a decrease in sperm oxidation ($P < 0.08$) when compared with controls.

in experiment 3 of the current study, consumption of 125 ppm of L-carnitine lowered the 17-wk-old BW of cockerels as compared with controls, which could be due not only to the long-term consumption of L-carnitine, but also to the higher dosage (125 ppm) fed during growth and development than levels fed to immature broilers, turkeys, and quail (<100 ppm). The lowered 17-wk-old BW of L-carnitine-supplemented birds as compared with controls may have had beneficial effects on subsequent sperm production. Low growth lines of chickens have higher sperm concentration than high growth lines (Marini and Goodman, 1969).

Feeding 500 ppm of dietary L-carnitine as compared with the control diet increased plasma free and total L-carnitine concentration (Tables 4 and 6). Only in experiment 2, but not experiment 1 where variation was greater (SEM = 2), was circulating concentrations of total L-carnitine increased with all L-carnitine supplemented diets as compared with controls. L-Carnitine can be removed from blood and released into epididymal lumen by active transporters (Brooks, 1980; Enomoto et al., 2002; Kobayashi et al., 2005) and ultimately transported into spermatozoa (Jeulin and Lewin, 1996).

In experiment 1, dietary effects on semen volume (Figure 1) and sperm concentration (Table 3) suggest the possibility that roosters consuming L-carnitine are producing greater quantities of sperm cells as well as seminal fluid, thus diluting the sperm cells over a greater volume of semen. However, dilution of sperm did not occur as total sperms cells did not differ between roosters supplemented with L-carnitine vs. controls (Table 3).

Even though L-carnitine improved viability in other species with damaged sperm due to injury (Amendola et al., 1991; Agarwal and Said, 2004; Ng et al., 2004), in the present study, dietary L-carnitine supplementation did not affect sperm viability in healthy White Leghorn roosters. However, Japanese quail consuming 250 or 500 ppm of L-carnitine from 5 to 20 wk of age showed improvement in sperm viability compared with controls (Sarica et al., 2007).

The data of experiment 1 showed that, compared with controls, White Leghorns consuming 125 ppm of L-carnitine for 8 wk had increased sperm concentration. Dosimetric effects of L-carnitine supplementation in Leghorns were lacking, suggesting that future studies explore levels of 125 ppm or less. Prior experiments conducted in our lab showed that 500 ppm of dietary L-carnitine increased sperm concentration after 3 to 4 wk of supplementation (Neuman et al., 2002). In experiment 2, the lack of response in sperm concentration to consumption of 125 ppm of L-carnitine until 11 wk of supplementation is perplexing, and it can only be postulated that the summer heat may have been a factor (Joshi et al., 1980; McDaniel et al., 2004). Sperm concentration was down considerably in all treatment groups during wk 1 through 8 of supplementation (3.6 to 3.9 billion sperm/mL, Table 5), with a rebound near the end of the experiment when a break in the summer heat provided cooler in-house temperature (4.0 to 4.6 billion sperm/mL, Table 5). Though supplementation of L-carnitine in the diet increased circulating levels of total L-carnitine in experiment 2 (Table 6), plasma L-carnitine was measured only at 8 wk of age. This timing could have corresponded to the end of the heat, allowing birds 3 wk to

Table 6. Concentrations of L-carnitine in plasma after consuming diets for 8 wk in experiment 2

Dietary L-carnitine (mg/kg or ppm)	Ester L-carnitine[1]	Free L-carnitine[1]	Total L-carnitine[1]
		(μmol/L)	
0 (0)[2]	3	13[b]	16[c]
125 (129)	6	17[b]	24[b]
250 (267)	5	24[a]	29[b]
500 (611)	7	30[a]	36[a]
SEM	1	2	1

[a-c]Means within a column with no common superscript differ significantly ($P < 0.006$).

[1]Values represent the mean of 2 pooled plasma samples.

[2]Numbers in parentheses are analyzed values of L-carnitine in the diet.

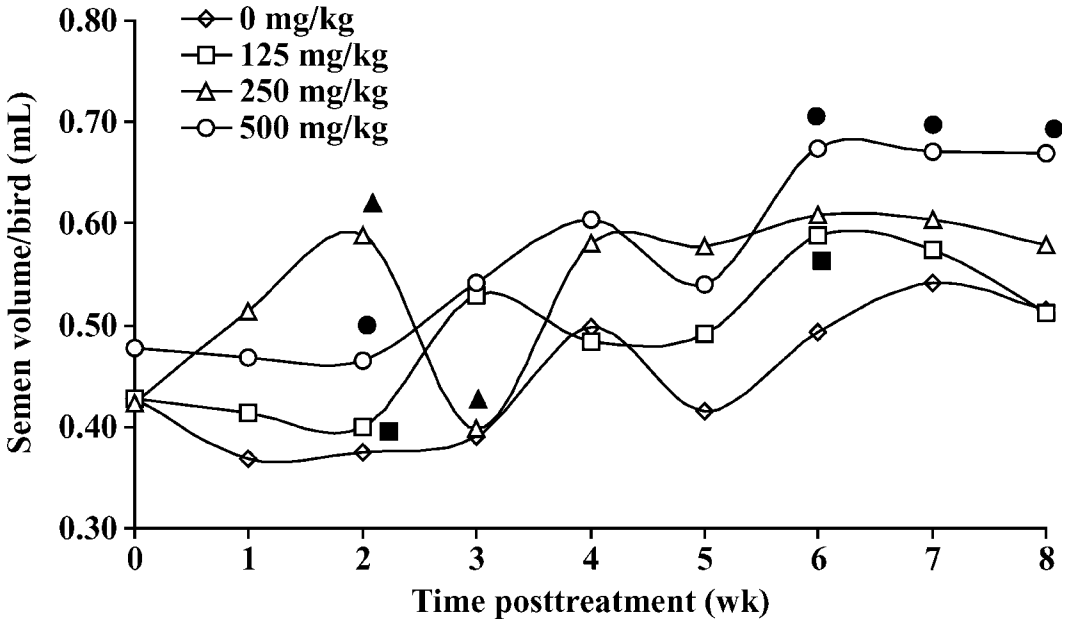

Figure 1. The effect of different levels of dietary L-carnitine on weekly semen volume of Hy-Line White Leghorns. Each value represents the least square mean of 6 observations per dietary treatment per time of sampling (SEM = 0.07). The symbol (▲) indicates the decline in semen volume from 2 to 3 wk posttreatment in roosters consuming 250 mg/kg of L-carnitine. The symbol (■) indicates an increase in semen volume of roosters consuming 125 mg/kg at 6 wk as compared with 2 wk posttreatment ($P < 0.002$). The symbol (●) indicates an increase in semen volume of roosters consuming 500 mg/kg of L-carnitine at 6 ($P < 0.0002$), 7 ($P < 0.02$), and 8 ($P < 0.007$) wk when compared with 2 wk posttreatment (experiment 1).

consume diets in cooler weather before experiencing enhanced spermatogenesis. Time required to complete a full cycle of spermatogenesis from spermatogonia to spermatozoa is about 12 to 13 d in birds (Lake, 1981; Lin and Jones, 1992). The majority of spermatozoa in the epididymis arrive at the lower ductus deferens in approximately 3 d (Etches, 1996).

Unlike experiments 1 and 2 in which L-carnitine was consumed for short durations (8 or 17 wk, respectively) with unsustained increases in sperm concentration, long-term consumption of 125 ppm of L-carnitine beginning at hatch in birds of experiment 3 resulted in a consistent increase in sperm concentration from 22 to 37 wk of age (Figure 2). The preparation of spermatogenesis in avians begins before the onset of sexual maturity (Lake, 1981; Etches, 1996). In young animals, L-carnitine synthesis could be minimized by the limited activity of γ-butyrobetaine hydroxylase, which catalyzes the synthesis of γ-butyrobetaine, an intermediate metabolite in the L-carnitine biosynthetic pathway (Hahn, 1981; Borum, 1983; Rebouche, 1992). Concentrations of total L-carnitine as well as levels of free and short chain esterified L-carnitine in the muscle, liver, and heart of embryonic and young chickens are lower than those found in adult tissues (Rinaudo et al., 1991). Due to the limited ability of young chicks to synthesize L-carnitine, supplementation of L-carnitine to the diet beginning at hatch may have prolonged beneficial effects to the chicks.

L-Carnitine acting as an antioxidant may be another explanation for its role in improving sperm concentration. The spermatozoon's middle-piece contains abundant mitochondria providing the enzymatic capability for energy metabolism needed for motility function of the spermatozoa (Etches, 1996). The ROS, which cause lipid peroxidation, can be the result of leakage of electrons from uncoupled oxidative phosphorylation within the mitochondria (Halliwell and Gutteridge, 1999; Surai, 2002). In order for sperm to become fertile, testicular spermatozoa must undergo postgonadal modification in the epididymis (Jeulin and Lewin, 1996). The proportion of PUFA in sperm membranes increases during maturation, potentially increasing susceptibility to lipid peroxidation (Ladha, 1998). Sperm is susceptible to peroxidation because of abundant mitochondria in the middle-piece and high concentrations of PUFA in the membrane (Phetteplace and Watkins, 1989; Surai et al., 1998). Whereas the proportion of PUFA and susceptibility to peroxidation may play a role in the survival of avian spermatozoa during in vitro manipulations such as storage (Cecil and Bakst, 1993), these factors may also affect spermatozoa's survival in vivo (Surai et al., 1998). L-Carnitine serves as a scavenger of ROS so as to minimize lipid peroxidation (Vicari and Calogero, 2001; Agarwal and Said, 2004; Agarwal et al., 2005). In our experiment 2, feeding 125 ppm of L-carnitine from 11 to 17 wk posttreatment increased sperm concentration ($P = 0.07$) and reduced sperm lipid peroxidation ($P = 0.08$, Table 5). These results agree with the experiments conducted by Neuman et al. (2002) in which they found that feeding 500 ppm of dietary L-carnitine to aging White Leghorn roosters (58 to 62 wk of age) for 5 wk not only improved sperm concentration during wk 3 and 4 of supplementation, but also reduced sperm lipid peroxidation during wk 3 through 5, and helped preserve testicular tissue. Similar results were obtained in a second experiment with younger roosters (32 to 37 wk of age) in which increases in sperm

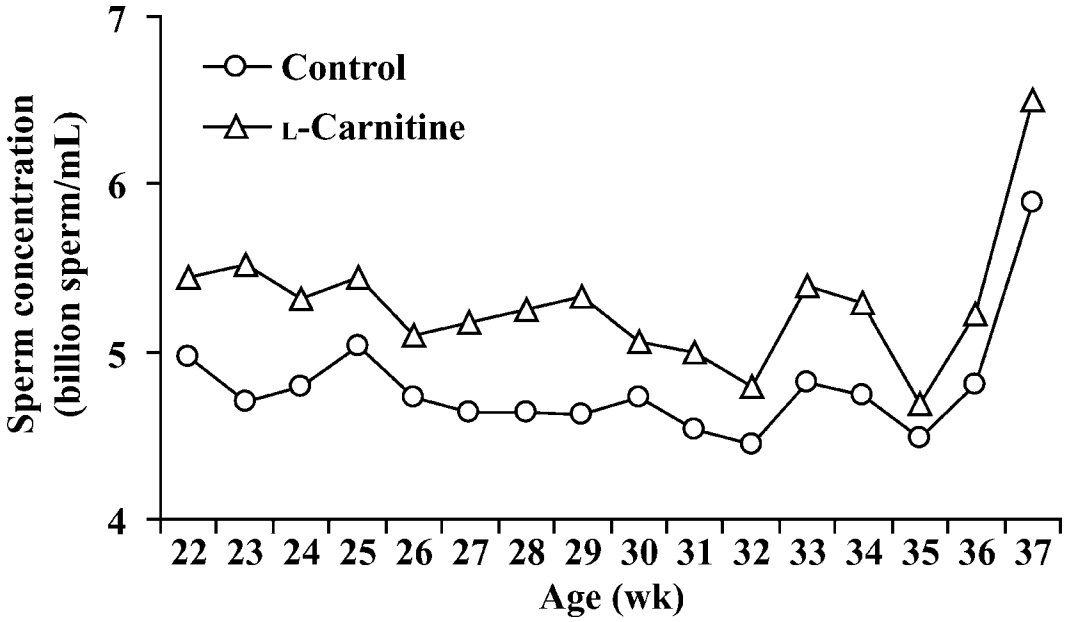

Figure 2. The consumption of L-carnitine increased weekly sperm concentration from 22 to 37 wk of age (P = 0.002) by 10%. Diets were supplemented with 125 ppm of L-carnitine beginning at 1 d of age. Each value represents the least square mean of 21 observations. The experimental unit was the pool of semen from 2 roosters housed in adjacent cages. SEM = 0.2 (experiment 3).

concentration and a decline in sperm lipid peroxidation were observed in the L-carnitine-fed birds during wk 4 and 5 of feeding the diets. These results suggest that L-carnitine provided to roosters in the feed has antioxidant properties, which may preserve sperm membranes, thereby extending the life span of sperm (Neuman et al., 2002).

Rooster's sperm can loose 97% of its cytoplasmic volume during spermiogenesis (Sprando and Russell, 1988), which can translate into a deficiency in cytoplasmic antioxidant enzymes, which metabolizes and neutralizes ROS (Strzezek et al., 2004). Aitken et al. (1993) postulated that sperm are susceptible to lipid peroxidation because of their poor defense mechanisms as well as the high content of PUFA in sperm membranes. The presence of antioxidants in the seminal plasma compensates for the deficiency in cytoplasmic enzymes within the spermatozoa (Strzezek et al., 2004). Lipid peroxidation can lead to cell degeneration. Tingari and Lake (1972) reported that some degenerating spermatozoa are reabsorbed by cells specifically lining the excurrent ducts of the reproductive tract. L-Carnitine is found in very high concentrations in the epididymal fluid (2,000-fold greater than blood levels, Jeulin and Lewin, 1996). The presence of high levels of L-carnitine in seminal plasma may act as an antioxidant to facilitate preservation of sperm membranes and reduce sperm reabsorption, thereby increasing sperm concentration.

In conclusion, long-term consumption of 125 ppm of L-carnitine beginning at hatch resulted in a sustained 10% increase in sperm concentration. L-Carnitine possesses antioxidant properties which increase the sperm concentration by preventing lipid peroxidation. Part of L-carnitine's effects in promoting spermatogenesis may begin before the onset of sexual maturity.

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