The Effect of Dietary Carnitine on Semen Traits of White Leghorn Roosters

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ABSTRACT Carnitine has antioxidant properties that protect sperm membranes against toxic reactive oxygen species. Carnitine also functions to reduce the availability of lipids for peroxidation by transporting fatty acids into the mitochondria for β-oxidation to generate adenosine triphosphate (ATP) energy. Because the effects of this supplemental amino acid on the reproductive performance of the avian breeder male are unknown, the objective of the current study was to evaluate the antioxidant role of dietary L-carnitine on semen traits and testicular histology in Leghorn breeder roosters. Two experiments were conducted in which birds were fed a control diet or one supplemented with 500 mg of carnitine/kg of diet. For Experiment 1, dietary treatments were fed to older birds (n = 12 birds/treatment) when they were 58 to 62 wk of age. For Experiment 2, younger birds were fed dietary treatments between 32 to 37 wk of age (n = 14 experimental units/treatment with three roosters composing an experimental unit for a total of 84 roosters). Semen traits and lipid peroxidation of sperm, determined by measuring malonaldehyde, were examined weekly. Feeding dietary carnitine to young and aging White Leghorn roosters ad libitum for 5 wk not only improved sperm concentration during the last half of supplementation but also reduced sperm lipid peroxidation. Testicular tissue of birds fed dietary carnitine for 5 wk was preserved as indicated by a reduction in multinucleated giant cells. These results suggest that dietary carnitine has antioxidant properties that may preserve sperm membranes in roosters, thereby extending the life span of sperm.

(Key words: carnitine, roosters, semen traits, sperm concentration, antioxidant)

INTRODUCTION

The effects of carnitine on reproductive parameters have been assessed in humans and boars. Infertile men have significantly lower seminal carnitine concentrations than fertile men (Soufir et al., 1984). When utilized as an epididymal marker and correlated with sperm concentration, L-carnitine levels are elevated in fertile vs. infertile men (Jouannet et al., 1981). Boars supplemented with 720 mg/d of dietary carnitine had a significant increase in semen volume vs. control-fed boars (Baumgartner, 1998). Although the effects of dietary carnitine on carcass composition (Barker and Sell, 1994), egg production, and hatchability (Leibetseder, 1995) have been assessed in poultry, the effects of this supplemental amino acid on the reproductive performance of the avian breeder male are unknown.

Free radicals or reactive oxygen species (ROS) are deleterious to cell membranes. Exposure of cell membranes to ROS induces lipid peroxidation causing membrane breakdown and loss of function (Halliwell and Gutteridge, 1984). Lipid peroxidation results when intracellular production of ROS overcomes the antioxidant defense mechanisms utilized by cells including sperm (Alvarez et al., 1987; Alvarez and Storey, 1989), and an immediate accumulation of lipid peroxides occurs in the plasma membrane. Unlike somatic cells, which depend on cytoplasmic enzymes such as superoxide dismutase (SOD) and glutathione peroxidase to defend against peroxidation, spermatozoa release most of their cytoplasm immediately prior to spermiation, and as a result, lose their enzymatic protection (Wang et al., 1997). Avian sperm cell membranes have a much greater concentration of polyunsaturated fatty acids than mammalian sperm cells (Cerolini et al., 1997) and are therefore more susceptible to lipid peroxidation during in vitro handling and storage of sperm, which is the primary cause of fertility dysfunction (Cecil and Bakst, 1993). Thus, determining the level of

Abbreviation Key: ROS = reactive oxygen species; SOD = superoxide dismutase; AA = ascorbic acid; SQA = sperm quality analyzer; SQI = sperm quality index; MAL = malonaldehyde; MGC = multinucleated giant cells.
sperm lipid peroxidation can be utilized as a biochemical index of semen quality (Jones et al., 1978; Alvarez et al., 1987).

Carnitine has antioxidant properties, which may protect sperm membranes from toxic oxygen metabolites. It also functions to reduce the availability of lipids for peroxidation by transporting fatty acids into the mitochondria for β-oxidation to generate adenosine triphosphate (ATP) energy. This transport of fatty acids into the mitochondria for catabolism reduces the amount of lipid available for peroxidation (Kalaiselvi and Panneerselvam, 1998). Vitamin C or ascorbic acid (AA) is a cofactor at the two hydroxylation steps in the carnitine biosynthetic pathway (Hulse et al., 1978). Both hydroxylation processes involving AA are catalyzed by 2-oxoglutarate dioxygenases. Ferrous iron is needed to activate the oxygen; however, a ferric iron-oxygen complex forms and inactivates the dioxygenase enzyme. Ascorbate is needed to restore enzymatic activity by reducing the ferric ion of the inactivated enzyme (Stryer, 1995) so that carnitine biosynthesis may proceed.

The antioxidant effects of L-carnitine have been assessed in the plasma, liver, and kidney of young and aged rats. Aged rats administered carnitine show significant increases in concentrations of AA and Vitamin E, as well as an increase in the activity of antioxidant enzymes such as SOD and glutathione peroxidase, whereas lipid peroxides decline in both age groups, as compared to control-fed rats. Because AA levels are elevated in rats administered carnitine, exogenous supplemental carnitine may spare AA, thereby enhancing its tissue levels (Kalaiselvi and Panneerselvam, 1998).

Superoxide dismutase is an antioxidant enzyme found in avian and mammalian semen (Froman and Thurston, 1981; Kalaiselvi and Panneerselvam, 1998; Surai et al., 1998a). Its purpose is to scavenge for biological oxidants like ROS and protect cells from peroxidative damage (Froman and Thurston, 1981). Carnitine, through its antioxidant properties, has been shown to increase the activity and levels of antioxidant enzymes, like SOD in aging rats (Kalaiselvi and Panneerselvam, 1998). As with vitamins C and E, carnitine may also work together with SOD to preserve the lipid membrane surrounding sperm, thus reducing lipid peroxidation. If carnitine increases the activity of sperm SOD, this increase would further substantiate that L-carnitine is in fact acting as an antioxidant.

Because AA increased and lipid peroxidases declined when aged rats were supplemented with L-carnitine, it was proposed that these same effects might occur in breeder birds supplemented with dietary L-carnitine. In addition, it was hypothesized that roosters fed carnitine would show an improvement in semen traits by preventing oxidation of sperm membranes. Thus, the objectives of the current study were to evaluate the effects of dietary L-carnitine on sperm traits, plasma AA concentration, and testicular histology and to determine if carnitine served as an antioxidant in layer breeder roosters.

MATERIALS AND METHODS

Experiment 1

A 6-wk trial was conducted with 24 White Leghorn layer breeder roosters. The 57-wk old birds were housed in individual wire cages with 1,084 cm² of floor space/bird. Birds were fed a corn-soy diet, given ad libitum access to feed and water, and randomly assigned to one of two dietary treatments. Each group of 12 birds received a diet supplemented with 500 mg of L-carnitine/kg of diet or a control diet containing red microtracer at 58 wk of age. The microtracer had no nutritive value; its purpose was to serve as a marker to ensure that the correct feed was placed in the appropriate feed trough. The 10% CP basal diet contained 3,170 kcal ME/kg of feed. The composition of the vitamin and mineral mix per kilogram of basal diet included 5,320 IU vitamin A, 2,172 IU vitamin D₃, 27 IU vitamin E, 0.93 mg vitamin K, 3.6 mg riboflavin, 5.3 mg pantothenic acid, 17 mg niacin, 288 mg choline, 4.4 µg vitamin B₁₂, 6.5 mg manganese, 49 mg zinc, 26 mg iron, 3.1 mg copper, 0.89 mg iodine, and 207 µg selenium. The experimental diets were fed for 5 wk.

Weekly semen and blood analyses were conducted, including a pretreatment (0 wk) sampling. Semen volume was determined gravimetrically by using a balance. Sperm viability, expressed as percentage of dead sperm, was analyzed using the ethidium bromide exclusion procedure (Bilgili and Renden, 1984) as modified by Bakst and Cecil (1997). Semen concentration was determined with a hemocytometer (Bakst and Cecil, 1997).

Feed consumption and BW were determined weekly. Individual plastic feed troughs were utilized to measure feed consumption per bird. The concentration of carnitine was measured via radio-enzymatic analysis on a pooled sample of feed and plasma obtained from the carnitine- and control-fed groups.

At 63 wk of age, birds were euthanized and testes were excised for histological and morphological analyses. The right and left testes of each bird were weighed. Testes weights were expressed relative to BW. The left testis of each bird was cut into serial cross sections 5 mm in thickness and fixed in 10% neutral buffered formalin. Fixed samples were processed and stained with hematoxylin and eosin (Prophet et al., 1994). Histological examination of five preparations of the left testis of each bird was conducted blindly.

Lipid peroxidation of the semen was determined by measuring malonaldehyde (MAL), which is the primary stable by-product of lipid peroxidation using a modified procedure of Cecil and Bakst (1993). Fifty-microliter aliquots of semen from each bird were pipetted into separate 20-mL capacity glass vials, along with 125 µL of 1-mM sodium L-ascorbate and 0.2 mM ferrous sulfate. Sodium L-ascorbate and ferrous sulfate were added to

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4Carniking 10, Lonza, Inc., Fair Lawn, NJ.
5Microtracers, San Francisco, CA.
6Metabolic Analysis Labs, Inc., Madison, WI.
each vial sample to promote peroxidation (Wang et al., 1997). Each vial was loosely capped to prevent evaporation but also to further circulate oxygen. Samples were incubated at 37 °C and placed on a shaker at 150 rpm for 4 h. After incubation, samples were immediately placed on ice, and 150 µL of 40% trichloroacetic acid; 150 µL of PBS, pH 7.0; and 500 µL of 1% 1,1,3,3 thiobarbituric acid, diluted in PBS, were added to each sample. Samples were mixed, capped, and incubated at 80 °C for 20 min. Following the second incubation, 1 mL of deionized water was added to each vial. Samples were transferred to 2.0 mL microcentrifuge tubes and centrifuged at 1,500 × g for 15 min at 25 °C. The resulting supernatant was measured with a spectrophotometer at 520 nm to determine MAL production.

For AA analysis, 6 mL of blood was collected from the brachial vein of each bird using heparinized needles and syringes. Blood was spun at 275 × g for 10 min, and plasma was frozen at −20 °C until AA analysis could be performed the following day. Five hundred microliters of plasma per bird was analyzed individually for AA by using spectrophotometry, samples were read at 578 nm.

**Experiment 2**

A 7-wk trial was conducted with 84 White Leghorn layer breeder roosters. The 32-wk-old birds were housed individually in wire cages with 1,084 cm² of floor space/bird. Birds were fed a corn-soy diet as described in Experiment 1, given access to feed and water ad libitum, and were randomly assigned to one of two dietary treatments. Each group of 42 birds received a diet supplemented with 500 mg of L-carnitine/kg of diet and colored microtracer (5 g carnitine + 2.5 g microtracer/kg feed) or a control diet containing microtracer (7.5 g microtracer/kg feed). Dietary treatments were color-coded; the investigators were blind as to which diet was assigned which color code. The code was broken after all data were collected and statistically analyzed.

Each dietary treatment was administered to 14 experimental units. Semen and blood were pooled from three roosters to form each experimental unit to reduce the probability of missing data from urate contamination or lack of an ejaculate. Any semen sample that was contaminated was not incorporated into the experimental unit.

Semen volume and sperm viability were determined as described previously for Experiment 1. In addition, sperm quantity and quality were measured during Weeks 1 through 5 of the experiment using the OptiBreed Sperm Quality Analyzer (SQA) which is indicative of motility, viability, and concentration through a sperm quality index (SQI; McDaniel et al., 1998). Semen was diluted 70-fold with 0.85% saline, mixed thoroughly, and drawn into a capillary tube; the outer capillary tube was washed with a tissue prior to placing it into the SQA. Duplicate readings were performed in 20 s at room temperature. Pretreatment data for the SQA were excluded due to inadequate dilution of semen for SQI readings. Sperm concentration was determined with a hemocytometer (Bakst and Cecil, 1997).

Lipid peroxidation of the semen was determined by measuring the stable by-product MAL as described in Experiment 1 (Cecil and Bakst, 1993). Sperm concentration of each experimental unit of semen was adjusted in vitro for equal sperm concentration by diluting with the appropriate amount of PBS.

For AA analysis, 3 mL of blood was collected from the brachial vein of each bird by using heparinized needles and syringes. Blood was pooled within each experimental unit, centrifuged, and processed. Plasma AA was measured weekly as described in Experiment 1 except for the last week of the experiment in which blood samples were not collected.

For determination of SOD during Weeks 0 through 4, seminal plasma was separated from the sperm pellet of each sample pool by centrifuging at 500 × g for 10 min at 20 °C, three times. Two hundred microliters of seminal plasma of each sample was pipetted into clean microcentrifuge tubes, diluted with 400 µL of 0.01 M phosphate buffer, free-thawed twice, and sonicated at 4 °C for 10 s. After centrifugation, the resulting supernatant was used in a colorimetric assay (Surai et al., 1998a).

Weekly data collections on feed consumption, BW, semen traits, plasma AA, SOD, and MAL production were conducted including a pretreatment (0-wk) sampling. Semen volume, viability, AA, and SOD were determined during the pretreatment period prior to initiation of the dietary treatments. Although the MAL assay and sperm concentration were measured during Week 0, experimental diets were initiated 3 d prior to their analysis. At termination of the trial, testes were collected and examined microscopically as previously described in Experiment 1. Carnitine concentrations were measured via radio-enzymatic analysis on two feed samples and two plasma pools, one from each dietary treatment.

**Statistical Analysis**

Semen traits, feed consumption, BW, AA concentrations, MAL production, and SOD activity were analyzed by one-way ANOVA within each week. A one-way ANOVA with a split-plot in time was employed for determination of dietary treatment × time interactions. For any significant treatment × time interaction, means were partitioned using Student-Newman Keuls' multiple-range test. The quantitative detection of multinucleated giant cells and testes weights were analyzed using a one-way ANOVA. For Experiment 1, the individual bird was deemed the experimental unit with 12 experimental units per dietary treatment. For Experiment 2, three roosters formed each experimental unit with 14 experimental units per treatment (Steel et al., 1997). The general linear models

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7 New Brunswick Scientific Co., Inc., Edison, NJ.
8 R-Biopharm, Marshall, MI.
9 OptiBreed, Alpharma, Inc., Fort Lee, NJ.
10 Randox Labs, Ltd., Antrim, UK.
procedure of the SAS system was utilized (SAS Institute, 1988). An analysis of covariance was conducted to adjust for pretreatment BW in Experiment 2 (Steel et al., 1997).

RESULTS AND DISCUSSION

Experiment 1

Feeding dietary carnitine (500 mg/kg) did not affect feed consumption or BW of mature roosters (Table 1). Similar findings were obtained by Barker and Sell (1994) in which supplemental carnitine (50 or 100 mg/kg) did not affect BW gain, feed utilization, or carcass composition of immature turkey poult and broilers. Feed analysis verified that the supplemented diets contained the appropriate amount of carnitine. Circulating levels of esterified and free carnitine were elevated in birds supplemented with 500 mg of carnitine/kg of diet as compared to control-fed birds (Table 2).

Supplemental dietary carnitine did not significantly affect testes weight, semen volume, or sperm viability (% dead sperm, Table 1). Sperm concentration of birds fed dietary carnitine did not differ from controls during pretreatment (0 wk), 1 or 2 wk; however, by Weeks 3 and 4, roosters fed carnitine had significantly greater sperm concentrations than control-fed birds ($P < 0.02$; Figure 1). Differences in sperm concentration were not observed during Week 5 of feeding the diets (Figure 1). Spermatogenesis, which occurs within the seminiferous tubules of the testis, is the sequence of events that transforms spermatogonia into mature spermatozoa. The length of spermatogenesis is approximately 13 to 15 d in birds (Lake, 1981), which may explain why a significant increase in sperm concentration did not occur until 21 d (Week 3) of feeding the diets.

A possible explanation for the increase in sperm concentration of carnitine-fed birds was that carnitine facilitated the preservation of the sperm lipid membranes, thereby extending sperm longevity. When the same volume of semen was added to each assay tube, and expressed as micrograms of MAL per milliliter of semen, similar levels of sperm peroxidation were observed with no differences between dietary treatments (Figure 2). However, because the carnitine-fed birds had more sperm than the control-fed birds, the data were expressed as micrograms of MAL per billion sperm cells, which resulted in a significant reduction in lipid peroxidation for the carnitine-fed birds as compared to the controls during Weeks 3 ($P < 0.04$) and 4 ($P < 0.01$, Figure 3) of feeding the diets. During Week 5 of the study, the MAL assay was repeated adjusted for sperm concentration. Individual semen samples were diluted with the appropriate amount of PBS so that each assay tube had equal sperm numbers. The results indicate that carnitine-fed birds still produced significantly lower amounts of MAL as compared to control-fed birds ($P < 0.001$, Figure 4), further substantiating that carnitine was in fact acting as an antioxidant. Differences in MAL production were not evident between dietary treatments during Week 5 when the same volume of semen was used in each assay tube or without prior adjustment for sperm concentration (Figures 2 and 3, respectively).

### Table 1. Effect of dietary carnitine on feed consumption, body weight, testis weights, and semen traits (Experiment 1)

<table>
<thead>
<tr>
<th>Dietary treatment (mg/kg)</th>
<th>Feed consumption (g/bird/day)</th>
<th>Live body weight (kg)</th>
<th>Absolute testes weight (g)</th>
<th>Relative testes weight (g/kg)</th>
<th>Semen volume (mL)</th>
<th>Dead sperm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>80$^1$</td>
<td>2.39$^1$</td>
<td>20$^1$</td>
<td>8.2$^1$</td>
<td>0.53$^1$</td>
<td>10.3$^4$</td>
</tr>
<tr>
<td>Carnitine (500)</td>
<td>3</td>
<td>2.38</td>
<td>21</td>
<td>8.8</td>
<td>0.48</td>
<td>10.4</td>
</tr>
<tr>
<td>SEM</td>
<td>3</td>
<td>0.07</td>
<td>1</td>
<td>0.5</td>
<td>0.05</td>
<td>0.5</td>
</tr>
</tbody>
</table>

$^1$Values represent the least square means of 60 observations (12 birds/dietary treatment $\times$ 5 wk, includes pretreatment values).

$^2$Values represent the least square means of 12 birds per dietary treatment. Testes were collected after birds had been on supplemental carnitine for 5 wk.

$^3$Values represent the least square means of 56 to 57 observations (10 to 12 birds/dietary treatment $\times$ 5 wk, which includes pretreatment values).

$^4$Values represent the least square means of 20 to 24 observations of wk 3 and 4 of feeding the diets (10 to 12 birds/dietary treatment $\times$ 2 wk).

### Table 2. Concentrations of L-carnitine in feed and plasma

<table>
<thead>
<tr>
<th>Sample$^1$</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Carnitine</td>
</tr>
<tr>
<td>Feed (mg/kg)</td>
<td>0</td>
<td>500.7</td>
</tr>
<tr>
<td>Plasma concentrations (µmoles/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ester carnitine</td>
<td>0.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Free carnitine</td>
<td>17.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Total carnitine</td>
<td>17.3</td>
<td>29.6</td>
</tr>
</tbody>
</table>

$^1$A pooled sample was measured in triplicate.
Plasma AA concentration was also measured as AA is a cofactor at the two hydroxylation steps in the carnitine biosynthetic pathway and also because AA has antioxidant properties (Stryer, 1995). Plasma AA did not differ between dietary treatments until the last week of the study when carnitine-fed birds had significantly higher concentrations than controls ($P < 0.03$, Figure 5). Similar results were observed by Kalaiselvi and Panneerselvam (1998) who determined that rats administered carnitine also had elevated endogenous levels of AA. These data suggest that carnitine may spare AA after prolonged supplementation.

Carnitine-fed birds had significantly fewer multinucleated giant cells per testes (MGC, $\bar{x} = 1$) than control-fed birds ($\bar{x} = 4$ MGC; $P < 0.05$, SEM = 1, Figure 6). Testicular MGC are described as a degenerative syndrome resulting presumably from the inability of tetraploid primary spermatocytes to complete meiotic division; thus, maturation arrests at the spermatid stage of development (Bloom and Fawcett, 1975; Corrier et al., 1985). Multinucleated giant cells are composed primarily of aggregates of degenerated spermatocytes and spermatids and are often sloughed into the lumen of seminiferous tubules (Corrier et al., 1985; Sur et al., 1997).

**Experiment 2**

Because birds assigned to the carnitine-supplemented diet had greater BW than birds assigned to the control diet at Week 0 (pretreatment period), an analysis of covariance, which adjusted for pretreatment BW, was performed. Although birds gained weight during the experimental trial, the control birds gained more weight than the carnitine-fed birds, resulting in a significant dietary treatment by age interaction ($P < 0.02$, Figure 7). Apparently, younger breeder birds (32 to 37 wk of age) compared to the older birds of Experiment 1 (58 to 62 wk of age) were more responsive to the metabolic effects of 500 mg of carnitine/kg of diet on fat metabolism. This apparent slowing of weight gain by the carnitine-fed birds...
The effect of feeding 500 mg of dietary carnitine/kg of diet on plasma ascorbic acid levels (Experiment 1). Within a week, least square means ± SEM with no common letters differ significantly ($P < 0.03$).

**FIGURE 5.** The effect of feeding 500 mg of dietary carnitine/kg of diet on body weight (Experiment 2). Within a week, least square means ± SEM with no common letter differ significantly ($P < 0.04$).

**FIGURE 6.** Left testes of 63-wk-old White Leghorn roosters stained with hematoxylin and eosin. Bar scale = 20 µm. A) Testis from a control-fed rooster. Magnification is 50×. Arrows indicate multinucleated giant cells (MGC). B) Testis from a rooster fed 500 mg of carnitine/kg of diet. Magnification is 50×. Note the absence of MGC (Experiment 1).

is in contrast to the lack of an effect on BW in immature poults and broiler fed up to 100 mg/kg of carnitine (Barker and Sell, 1994), but the differences in response could be attributed to age and dose differences.

Supplemental carnitine in the diet did not affect feed consumption (Table 3). Feed analysis confirmed that the supplemented diets contained the appropriate amount of carnitine (Table 2). Circulating levels of esterified and free carnitine were elevated in birds supplemented with 500 mg of carnitine/kg of diet as compared to control-fed birds (Table 2).

Testes weight, sperm quality as measured with a SQA,9 semen volume, plasma AA, and SOD activity were unaffected by dietary carnitine (Table 3). Dietary effects on sperm concentration varied over time (significant dietary treatment × time interaction, $P < 0.005$). Roosters fed carnitine had sperm concentrations similar to controls during pretreatment (0 wk), 1 wk, and 2 wk of feeding the diets; however, during Week 3, control-fed birds had an unexplainable increase in sperm concentration as compared to carnitine-fed birds ($P < 0.004$). On the contrary, carnitine-fed birds had higher sperm concentration than control-fed birds at Week 4 ($P < 0.03$) with a similar trend observed at Week 5, in which birds supplemented with dietary carnitine still maintained higher levels of sperm concentration ($P < 0.07$) than control-fed birds (Figure 8). Although the SQI is indicative of sperm concentration as well as sperm motility and viability (McDaniel et al., 1998), the SQA may not have been sensitive enough to detect these small but significant differences in sperm concentration between treatments as determined through hemocytometry.

For each week of the trial, sperm concentration was adjusted in vitro so that each MAL assay tube had equal sperm numbers. When the level of lipid peroxidation was expressed as micrograms MAL per billion sperm, carnitine-fed birds as compared to controls produced significantly less MAL at Week 4 ($P < 0.05$) with a similar trend occurring at Week 5 ($P < 0.07$; Figure 9). When MAL production was reported as micrograms MAL per milliliter of semen, no differences between treatments were observed (data not presented).

Not only were sperm concentration and lipid peroxidation affected by the diets, but a decline in percentage of
TABLE 3. Effect of dietary carnitine on feed consumption, testes weights, semen traits, ascorbic acid and superoxide dismutase activity (Experiment 2)

<table>
<thead>
<tr>
<th>Dietary treatment (mg/kg)</th>
<th>Feed consumption (g/bird/day)</th>
<th>Absolute testes weight (g)</th>
<th>Relative testes weight (g/kg)</th>
<th>Sperm quality index</th>
<th>Semen volume (mL)</th>
<th>Ascorbic acid (µg/mL)</th>
<th>Superoxide dismutase activity (units/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>71²</td>
<td>24.3²</td>
<td>13.0²</td>
<td>245³</td>
<td>0.46³</td>
<td>3.24</td>
<td>0.74³</td>
</tr>
<tr>
<td>Carnitine (500)</td>
<td>72</td>
<td>23.4</td>
<td>12.2</td>
<td>221</td>
<td>0.47</td>
<td>3.0</td>
<td>0.87</td>
</tr>
<tr>
<td>SEM</td>
<td>1</td>
<td>0.6</td>
<td>0.3</td>
<td>13</td>
<td>0.02</td>
<td>0.2</td>
<td>0.09</td>
</tr>
</tbody>
</table>

1Values represent the least square means of 84 observations (14 experimental units/dietary treatment with three birds in each experimental unit × 6 wk, including pretreatment values).
2Values represent the least square means of 14 observations (14 experimental units/dietary treatment with three birds in each experimental unit). Testes were collected after birds had been on supplemental carnitine for 6 wk.
3Values represent the least square means of 70 observations (14 experimental units/dietary treatment with three birds in each experimental unit × 5 wk, does not include pretreatment values).
4Values represent the least square means of 70 observations (14 experimental units/dietary treatment with three birds in each experimental unit × 5 wk, includes pretreatment values).

dead sperm was observed in the carnitine-supplemented birds during Week 4 of feeding the diets as compared to controls (P < 0.02, Figure 10). Histological evaluation of the testes showed that carnitine-fed birds had significantly fewer multinucleated giant cells per testes (MGC, x = 0.6) than control-fed birds (x = 1.6 MGC; SEM = 0.3, P < 0.007).

Generation of ROS, such as the superoxide anion, the hydroxyl radical, and hydrogen peroxide, can cause oxidative damage to liver, kidney, brain, lung and are particularly responsible for sperm dysfunction (Hsu et al., 1998) in humans (Aitken et al., 1989; Kessopoulou et al., 1992; Aitken et al., 1993), cattle (Beconi et al., 1991; O’Flaherty et al., 1997), rats (Shang et al., 1999) and chickens and turkeys (Froman and Thurston, 1981; Surai et al., 1998a,b). Intracellular production of ROS results in the immediate accumulation of lipid peroxides in the plasma membrane and cytosol of cells (Aitken et al., 1993). This accretion has also been correlated with the impaired ability of sperm to partake in the acrosome reaction and sperm-oocyte fusion and may be responsible for a reduction in motility (Aitken and Clarkson, 1987a,b; Aitken et al., 1993).

To reduce the incidence of peroxidation, organisms have evolved antioxidants and antioxidant enzymes that prevent this chain reaction by interfering with the process of peroxidation by scavenging for ROS (Halliwell and Gutteridge, 1984). Catalase, SOD, and glutathione peroxidase are three types of antioxidant enzymes, the latter two identified in birds, that scavenge for biological oxidants and protect cells from peroxidative damage (Froman and Thurston, 1981). These enzymes, however, have limited repair ability against ROS due to their intracellular location in the mitochondria and cytosol of cells (Halliwell, 1994). Similarly, AA, vitamin E, and L-carnitine are antioxidants that are found intra- and extracellularly, and, like sperm antioxidant enzymes, intracellular antioxidants are confined to the midpiece region of sperm and offer little protection to membrane lipids of the acrosome and tailpiece (Aitken et al., 1993) to effectively combat peroxidative damage.

Circulating free iron is able to catalyze ROS leading to lipid membrane degradation. Carnitine has iron-chelating
properties, which may allow carnitine and acetylcarnitine to partially prevent the generation of ROS by binding with free iron. Because carnitine also functions as an antioxidant and participates in fatty acid transport for energy metabolism, it likely preserves other antioxidants, such as AA, and antioxidant enzymes against potential peroxidative damage, thereby reducing the availability of lipids for peroxidation (Kalaiselvi and Panneerselvam, 1998). However, in the current study, supplemental carnitine had little effect on plasma AA levels (exception at Week 4, Experiment 1) and did not affect the activity of SOD in seminal plasma.

In Experiment 1, feeding 500 mg of carnitine/kg of diet to aging White Leghorn roosters (58 to 62 wk of age) for 5 wk not only improved sperm concentration during the third and fourth weeks of supplementation, but also reduced sperm lipid peroxidation during Weeks 3 through 5, and helped to preserve testicular tissue. Similar results were obtained in Experiment 2 with younger roosters (32 to 37 wk of age) in which increases in sperm concentration were obtained in Experiment 2 with younger roosters (32 wk of age) and a decline in sperm lipid peroxidation were observed to 37 wk of age) in which increases in sperm concentration were obtained in Experiment 2 with younger roosters (32 wk of age) and a decline in sperm lipid peroxidation were observed to 37 wk of age) in which increases in sperm concentration were obtained in Experiment 2 with younger roosters (32 wk of age) and a decline in sperm lipid peroxidation were observed to 37 wk of age) in which increases in sperm concentration were obtained in Experiment 2 with younger roosters (32 wk of age) and a decline in sperm lipid peroxidation were observed to 37 wk of age) in which increases in sperm concentration were obtained (P<0.02).

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