The Effect of Dietary Ascorbic Acid on Semen Traits and Testis Histology of Male Turkey Breeders


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ABSTRACT

A 9-mo field trial was conducted to evaluate the effects of dietary L-ascorbic acid (AA) on semen traits of 144 male turkey breeders. Dietary AA treatments were initiated when birds were 30 wk of age. Semen and blood collection began at 32 wk of age. Three treatments with four pens per treatment and 12 birds per pen were fed 0, 75, and 150 mg/kg AA during the first 4 mo of their reproductive cycle. Levels of AA were doubled in the supplemented diets to 150 and 300 mg/kg during Months 5 to 9. Semen traits and blood AA were unaffected by dietary AA. When birds were 65 wk of age, testes were removed from 12 birds per treatment for histological analysis. Multinucleated giant cells (MGC), indicative of degeneration, were observed in the testes of 7 of the 12 control birds but were absent from AA-supplemented birds (P < 0.02). The antioxidant properties of AA may delay formation of these degenerative cells. In conclusion, dietary AA levels employed in the current study did not affect semen traits or testis weight but were associated with reduced formation of MGC in the testes of 65 wk-old breeder toms.

(Key words: L-ascorbic acid, multinucleated giant cells, semen traits, male turkey breeder, vitamin C)

INTRODUCTION

Unlike primates and the guinea pig, domestic fowl has the ability to synthesize ascorbic acid (AA); thus, AA has traditionally been excluded from poultry diets. However, under conditions of heat stress (Perek and Kendler, 1962, 1963; Njoku, 1986; Cheng et al., 1990) and disease (Pardue et al., 1985a; Pardue and Thaxton, 1986; Gross, 1988; 1992), birds may be incapable of synthesizing adequate amounts of AA to meet metabolic demands. Consequently, supplemental AA was found to be beneficial. Inconsistencies on the effects of supplementary AA in birds (Sifri et al., 1977; Kafri and Cherry, 1984; Pardue et al., 1985b) may be due to this particular vitamin being easily oxidized. For example, the shelf life of pharmaceutical grade AA is only about 30 d. A new form of AA, Rovimix Stay-C 35®/H23041, containing a mixture of mono-, di-, and triphosphate esters of L-ascorbic acid, is currently available on the market. The esterification of AA at Position 2 protects it from oxidation. After opening the container, the shelf life of Rovimix® is about 90 to 120 d and is stable after pelleting of feed.

The reproductive effects of AA have been researched extensively in mammals. Men who consume supplemental ascorbate showed improved sperm quality (Dawson et al., 1990). Fraga et al. (1991) and Luck et al. (1995) concluded that the antioxidant properties of AA are essential to maintain membranes and the genetic integrity of sperm cells by preventing oxidative damage to sperm DNA. To date, little research has been conducted on the reproductive effects of AA in the avian male. White Rock roosters fed 100 mg of AA/kg of feed showed improved semen volume and sperm concentration (Perek and Snapir, 1963). Eight-week-old male chickens supplemented with 100 mg/kg AA had significantly greater testicular weights than control birds (Pardue and Thaxton, 1986). Despite previous work with chickens, research concerning the reproductive efficiency of AA in the turkey breeder male is lacking. Thus, the primary objective of our study was to evaluate the effects of dietary AA on semen traits and testis histology of male turkey breeders.

MATERIALS AND METHODS

A 9-mo field trial was conducted using 144 turkey breeder toms (British United Turkeys of America) of the
same hatch housed at a single industry turkey breeder facility from July through March. The same personnel managed and cared for the birds throughout the trial. Birds were allotted 13 h of light (2.5 lx) at 30 wk of age with 15-min increases in light per month until a maximum light duration of 15 h and 15 min was obtained. Ascorbic acid diets were initiated when birds were 30 wk of age; semen and blood collection began when birds were 32 wk of age. Three dietary treatments were assigned randomly to four pens per treatment with 12 birds per pen. Dietary treatments consisted of 0, 75, and 150 mg of AA/kg of feed during Months 1 through 4 of semen production. Levels of AA were doubled in the supplemented diets to 150 and 300 mg/kg of feed during Months 5 through 9. A freshly prepared basal diet was mixed each month, split into three batches, mixed with appropriate amounts of Rovimix® Stay-C 35® and/or Microtracer®©, and pelleted. Color-coded Microtracer® were added to the appropriate dietary treatment to ensure that each pen received the correct diet. To prevent obesity, tom breeders were feed-restricted, providing a daily allotment of 0.45 kg of feed per bird, resulting in a daily calculated ingestion of 33.75 and 67.50 mg of AA/bird when fed 75 and 150 mg of AA/kg of feed, respectively. Water was provided for 4 h/d at the time of feeding.

Semen traits and AA concentrations were measured monthly throughout the reproductive cycle of tom breeders until 65 wk of age. Monthly collections of semen and blood and semen trait analysis were done in the morning at approximately the same hours of the day by the same personnel throughout the study. Semen was pooled per pen prior to analyses. Each pool of semen per pen was weighed on a gram scale to determine semen volume per bird. Sperm concentration was determined using a hemocytometer (Bakst and Cecil, 1997). Sperm viability, expressed as percentage dead sperm, was determined using a fluorometric ethidium bromide exclusion procedure (Bilgili and Renden, 1984) modified by Bakst and Cecil (1997).

Three milliliters of blood was collected into heparinized vacutainer tubes from the brachial vein of three randomly selected birds per pen. Blood was pooled per pen and centrifuged at 275 × g for 10 min. Plasma was snap frozen in dry ice until L-ascorbic acid analysis could be performed the following day. Five hundred microliters of pooled plasma sample per pen was measured for AA by using spectrophotometric analysis;6 samples were read at 578 nm. Diets were analyzed for AA content monthly using HPLC (Wang et al., 1988).

At 65 wk of age, 12 randomly selected birds per treatment (three birds per pen) were weighed and processed at the Purdue University Abattoir. Testes were excised and weighed. Testis weights were expressed relative to body weight. 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). Three preparations of each left testis of each bird were examined microscopically.

Semen traits and AA concentrations were analyzed by one-way ANOVA with a split-plot in time or age of the bird. Dietary treatment and time were considered fixed effects, and pens were considered random. Pen replicates within dietary treatment served as the error term for the main effect of dietary treatment. The experimental unit was the pen. The presence or absence of multinucleated giant cells (MGC) in testicular tissue was analyzed by chi-squared (Steel et al., 1997).

**RESULTS AND DISCUSSION**

Dietary AA did not affect semen volume, sperm concentration, or percentage dead spermatozoa (Table 1), and there were no significant treatment × age interactions. Significant age-related declines were observed in semen traits (data not presented).

Plasma concentrations of AA did not differ among birds fed the three dietary treatments (Table 1). Despite that blood collection was purposely delayed for 30 min following the initiation of feeding during the last 5 mo of the trial, to allow for gastrointestinal absorption of nutrients, an increase in AA concentrations in the blood of birds fed supplemental AA was not observed. This lack of an increase in plasma AA following supplementa-

tion has been reported previously (Pardue et al., 1984) and could be due to a number of factors including poor dietary absorption, rapid tissue utilization, or renal excretion.

Continuous administration of AA in the drinking water (5, 25, 50, and 100 ppm) for 4 wk to broiler cockerels did not affect plasma levels of AA when compared to unsupplemented controls (0 ppm). However, broiler cockerels supplemented with 250, 500, 1,000, or 2,000 ppm of AA in the drinking water for 4 wk showed significant increases in plasma AA. The larger body weight and blood volume of an adult breeder tom as compared to an immature cockerel could partially explain why 250 ppm of AA in the water resulted in an increase in plasma AA in broilers but not in turkeys fed 300 mg of AA/kg in the feed. In addition, the feed-restriction program implemented with turkey breeder toms of the current study to prevent obesity might not have allowed for sustained increases in circulating levels of AA; the immature breeders cockerels were provided drinking water ad libitum under a continuous photoperiod (Pardue et al., 1984).

When extremely high levels of AA were fed (1,200 or 3,300 ppm) to chickens, an increase in plasma levels of AA was observed (Herrick and Nockels, 1969; Dorr and Nockels, 1971). Acute administration with a subcutaneous injection of 50 mg of AA caused a momentary increase in plasma AA within 25 min, but plasma levels then subsequently declined to pretreatment levels within 2 h postinjection (Satterfield et al., 1945), suggesting rapid renal clearance or tissue utilization. Thus, it appears that

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5 Microtracers®, San Francisco, CA 94124.
6 R-Biopharm, Marshall, MI 49068.
TABLE 1. The effect of dietary ascorbic acid (AA) on semen traits, plasma AA concentrations, and testes weight of turkey breeders

<table>
<thead>
<tr>
<th>Dietary AA (mg/kg)</th>
<th>Semen volume/bird (mL)</th>
<th>Sperm concentration ($\times 10^9$ cells/mL)</th>
<th>Dead sperm (%)</th>
<th>Plasma AA concentration ($\mu$g/mL)</th>
<th>Total testes weight (g)</th>
<th>Relative testes weight (g/kg × 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.53$^1$</td>
<td>8.3$^1$</td>
<td>11.4$^1$</td>
<td>7.5$^1$</td>
<td>58$^2$</td>
<td>211$^2$</td>
</tr>
<tr>
<td>75/150</td>
<td>0.49</td>
<td>8.0</td>
<td>12.4</td>
<td>6.9</td>
<td>53</td>
<td>187</td>
</tr>
<tr>
<td>150/300</td>
<td>0.55</td>
<td>8.1</td>
<td>11.5</td>
<td>6.9</td>
<td>63</td>
<td>230</td>
</tr>
<tr>
<td>SEM</td>
<td>0.02</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>5</td>
<td>21</td>
</tr>
</tbody>
</table>

$^1$Values represent the mean averaged across age (9 mo × 4 replicates or n = 36 observations).
$^2$Values represent the mean of 12 observations.

high supplemental levels of AA are needed to cause a concomitant increase in plasma AA. Analysis of feed samples from the three diets showed no AA in the unsupplemented diets and an average of 88% and 94% of claim in the lower and higher levels of AA-supplemented diets, respectively (data not shown).

Body weight of tom breeders at 65 wk of age was also unaffected by dietary treatments (mean = 28 ± 1 kg/bird on all diets). Unlike the results of Pardue and Thaxton (1986), who observed a significant increase in testes weights of 8-wk-old broiler cockerels supplemented with 100 mg/kg AA as compared to controls, dietary AA in the present study had no effect on absolute or relative testes weight (Table 1). However, differences among dietary treatments were noted in the histological examination of testicular tissue (Figure 1). Seven out of 12 control testes contained several countable MGC (Figure 1a). These MGC were absent from testes of birds fed supplemental dietary AA (Figure 1b). Testicular MGC are composed mainly 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).

The presence of MGC in the testes of control birds and their absence in the testes of birds supplemented with AA was significant ($P < 0.02$). The antioxidant properties of AA might have delayed the formation of these degenerative cells in birds receiving supplemental dietary AA. The effect of dietary AA in reducing testicular MGC has not been previously reported; however, age-related MGC, along with other developmental and regressive changes in the testes, have been indicated in Himalayan rabbits (Tsunenari and Kast, 1992), in autopsied men ranging in ages from 23 to 97 yr (Coyne and Dervan, 1997), and in middle-aged cats (Elcock and Schoning, 1984). Premature onset of testicular MGC has been observed in young boars after being exposed to a viral outbreak (Sur et al., 1997), in rats subjected to cobalt toxicity (Corrier et al., 1985), and in chickens fed Neem seed kernel meal (Mohan et al., 1997).

In conclusion, dietary AA levels in the current study did not affect semen traits or testis weight but was associated with a reduced formation of MGC in the testes of 65-wk-old breeder toms. The doubling of dietary AA levels from 75 and 150 mg/kg to 150 and 300 mg/kg, respectively, during Months 5 to 9 of semen production was ineffective in improving semen traits of breeder toms. Higher levels of AA, than utilized in the current study, may be needed to improve semen traits.

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FIGURE 1. Left testes of 65-wk-old breeder toms stained with hematoxylin and eosin. Bar scale = 20 µm. A) Testis from a control-fed turkey breeder tom. Magnification is 100×. Arrows indicate multinucleated giant cells (MGC). B) Testis from a turkey breeder fed 300 mg/kg ascorbic acid. Note the absence of MGC. Magnification is 100×.
REFERENCES


