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An Attempt at Alleviating Heat Stress Infertility in Male Broiler Breeder Chickens with Dietary Ascorbic Acid

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Abstract: Previous research regarding the effect of heat stress on broiler breeders is very limited. The objective of the present study was to determine the amount of ascorbic acid in the broiler breeders' diet that will improve reproductive performance of males that are exposed to continuous heat stress. One hundred forty-four Ross males (18 weeks old) were divided equally among six temperature controlled rooms. Three rooms were used for heat treatment and three rooms served as controls (21°C). The temperature in the heat treatment rooms was increased in two four-week phases followed by a final three week recovery phase with the temperatures for each period being 29 (mild stress), 32 (severe stress), and 21°C, respectively. The roosters in each room were equally divided among four dietary treatments consisting of 0, 250, 500, and 1000 ppm of ascorbic acid. Heat stress significantl/y decreased sperm quality index, sperm viability, and fertility. The percentage of dead sperm rose significantly during both the mild and severe heat phases and dropped immediately upon initiation of the recovery phase. Sperm motility decreased linearly with increasing ambient temperature but rebounded upon the removal of heat stress. The rate of fertilization was decreased as a result of heat stress. Dietary ascorbic acid did not improve any of the semen characteristics of control or heat stressed birds. Administration of 500 and 1000 ppm of dietary ascorbic acid resulted in a depression of fertility over each day postinsemination when the males were heat stressed. In conclusion, dietary ascorbic acid at the levels used in the present study did not improve the reproductive performance of broiler breeder males under normal or heat stress conditions.

Key words: Broiler breeder, fertility, heat stress, ascorbic acid, sperm

Introduction

Heat stress decreases the reproductive ability of broiler breeder chickens (McDaniel *et al.*, 1995). Supplementation of dietary ascorbic acid can improve reproduction (Pardue and Thaxton, 1986), but it is questionable whether the detrimental reproductive effects of heat stress can be offset by an increase in dietary ascorbic acid.

It is well known that high ambient temperature coupled with high humidity has a detrimental effect on the poultry industry by decreasing fertility. Keirs (1982) noted that during summer months broiler breeder fertility can be decreased by as much as 15 %. This loss in fertility costs the poultry industry millions of dollars in revenue annually along with other heat related reductions in performance characteristics such as: feed intake, growth rate, egg production, egg quality, hatchability of fertile eggs, number of chicks per hen, and increased mortality (Van Kampen, 1981; Peebles and Brake, 1985; Muiruri and Harrison, 1991; McDaniel *et al*., 1995).

Research concerning the effects of heat stress on broiler breeder fertility and hatchability is limited. McDaniel *et al*. (1996) have shown that fertility of the broiler breeder male is greatly reduced during high ambient temperatures. McDaniel *et al*. (1996) discovered that when semen from broiler breeder males heat stressed at 27°C for 12 hours was used to inseminate hens, the number of sperm penetrating the egg was reduced by 48% as compared to results obtained when hens were inseminated with semen from males maintained at 21°C. Using artificial insemination, McDaniel *et al*. (1995) found that fertility declines 42% when the male broiler breeder is heat stressed at 32°C. McDaniel *et al*. (1995) also found that significant heat stress effects on male fertility are evident within only 12 hours at an average summer temperature of 29°C.

High ambient temperatures have been found to adversely affect semen characteristics of other breeds of poultry as well. Boone and Huston (1963) found that heat stressing White Plymouth Rock males for only two to four hours per day resulted in decreased sperm production. Parker and McSpadden (1943) stated that semen production is depressed during the summer months. However, Clark and Sarakoon (1967) reported that there was no change in Leghorn semen production with fluctuating temperatures of 21-38°C. Joshi *et al*. (1980) found that sperm concentration and semen volume as well as the number of live sperm and normal sperm decreased as a result of heat stress at 32°C for 40 days.

The physiological mechanisms that result in heat stress infertility are not completely understood, but most logically explained by increased body temperature. McDaniel *et al*. (1995) hypothesized that during the heat stress period, the increase in body temperature has a negative effect on gamete formation and the fertilization

process. Also, Karaca *et al.* (2002) found that changes in semen characteristics due to elevated body temperature alone contribute to heat stress infertility of broiler breeders. McDaniel *et al.* (1996) proved that fewer sperm from heat stressed birds are stored in the hen's oviduct and penetrate the ovum as compared to sperm from males who are maintained in a thermoneutral environment.

Physiological stressors, such as heat, disease, or overcrowding, may increase the chickens need for ascorbic acid (Nockels, 1984). Chickens require vitamin C (ascorbic acid) for amino acid and mineral metabolism as well as for the synthesis of hormones that are involved in the resistance to stressors. Ascorbic acid stimulates the activity of leukocytes and is involved in the production of antibodies. It is also involved in the synthesis of the sex hormones such as testosterone (McDowell, 1989). Testosterone is essential to the reproductive performance of males. Pardue and Thaxton (1986) reported that the testicular weights of young cockerels were increased by supplementing dietary ascorbic acid.

The chicken may not have the ability during stress to produce enough ascorbic acid to meet physiological demands (Pardue and Thaxton, 1986). The levels of ascorbic acid synthesized for physiological needs may only be sufficient when the environmental temperature does not cause any stress to the animal. Blood ascorbic acid levels are inversely proportional to environmental temperature within the range of 21 to 31°C (Thornton, 1961). Within this temperature range, as the environmental temperature increases, the blood ascorbic acid levels decrease. Jungck *et al.* (1947) reported that in humans, seminal fluid contains levels of ascorbic acid ten to fifteen times greater than that of blood, and a decline in seminal ascorbic acid has been associated with a decrease in fertility.

Under normal environmental conditions, dietary ascorbic acid has been shown to improve the fertility of poultry. Dobrescue (1987) found that supplementing ascorbic acid at the rate of 150 ppm increased sperm concentration, semen volume, and total number of sperm produced per turkey breeder male. He also found a significant decrease in sperm agglutination. Harris *et al.* (1974) documented that ascorbic acid supplementation reduces sperm agglutination and improves fertility. Pardue and Thaxton (1986) reported that feeding levels of 100 ppm increased testicular weights in eight week old broiler cockerels. Peebles and Brake (1985) found that feeding ascorbic acid to broiler breeders at the rate of 50 ppm improved fertility when compared to that of the controls.

The objective of the present study was to determine the amount of ascorbic acid in the broiler breeder's diet that would improve the reproductive performance of males exposed to heat stress. Testes weight, sperm viability, sperm concentration, sperm motility, and rate of fertilization were all used to measure reproductive performance.

Materials and Methods

Housing: One hundred forty-four Ross roosters (18 wk) were housed. The broiler breeder males were equally divided among six controlled temperature rooms (21°C), and individually caged, so that there were 24 cages per room. The males were given a 9 wk acclimation period to adjust to the cages and their environment. The roosters were exposed to 16 hours of light from 0500 to 2100h throughout the entire trial. They were fed a basal corn-soy ration (370 kcal ME per bird per day, 14% crude protein) formulated for broiler breeders at Mississippi State University, for the first 13 wk of the trial. In order to maintain body growth as recommended by the primary breeders schedule, the amount of feed was decreased from 120 g/bird/day to 115 g/bird/day (355 kcal of ME per bird per day, 14% crude protein) after the thirteenth week of the trial. Throughout the trial, room temperature and humidity were recorded daily in the rooster house.

On the same date as housing of the roosters, 360 Babcock White Leghorn (18 wk) hens were caged in a commercial layer house to determine the fertility of the roosters. The hens were individually caged in twenty-four groups, consisting of 15 hens each. Each group of hens corresponded with a group of roosters. The hens were given a 9 wk acclimation period to adjust to their cages and surrounding environment. They were fed *ad libitum* throughout the trial. Their ration was formulated at Mississippi State University to contain 2860 kcal of ME/kg, 14.5% crude protein, and 4% calcium. They were exposed to 17 h of light from 0500 to 2200h.

Treatment of roosters: Beginning 2 wk prior to heat stress, one fourth of the roosters in each room received one of four dietary treatments consisting of 0, 250, 500, or 1000 ppm of ascorbic acid. The trade name of the ascorbic acid was Rovimix Stay-C 35 (Hoffman-La Roche, Nutley, NJ, USA), and it was guaranteed to have 35% ascorbic acid equivalents. Ingredients included a mixture of mono, di, and tri phosphate esters of L-ascorbic acid and silicon dioxide to act as an anti-caking agent.

To evaluate the effects of heat stress on fertility, an 11 wk heat treatment period was conducted in two 4 wk heat stress phases followed by a final 3 wk recovery phase. Three rooms served as controls (21°C); the other 3 rooms served as heat treatment rooms. During the first or mild stress phase, the temperature in the 3 heat treated rooms was increased to 29°C. During the second or severe stress phase, the ambient temperature of these rooms was increased to 32°C. Finally, during the third or recovery phase, the temperature in these rooms was decreased to 21°C.

Temperatures were constant throughout the day. Humidity of the rooms was recorded daily and range from 40 to 80% relative humidity.

Feed consumption, body temperatures, and weight of roosters: Throughout the trial, feed consumption and male body temperature were recorded at the same time each week. Body temperature was measured for every bird by using a digital thermometer and a YSI thermistor probe 403 inserted 2 cm in the rectum. Body temperature was determined before feeding at 0800h. Also, at the same time every 2 wk, body weight was recorded for each male. At the end of the trial, all roosters were euthanized; body and testes weights were then recorded. Relative testes weights were determined by the following formula: relative testes weight = (testes weight / body weight * 100).

Semen analyses: Through out the trial, semen was collected from each male 3 d/wk using the abdominal massage method of Burrows and Quinn (1937). Beginning 3 wk prior to initiation of heat stress, semen was collected and analyzed for sperm concentration, viability, and motility every Monday. Sperm viability and concentration were measured (one replicate) by the fluorometric method of Biligili and Renden (1984). Two sperm quality index (SQI) readings, a measure of sperm motility, were recorded for each male's semen sample which was diluted with 0.85% NaCl (McDaniel *et al.*, 1998).

Semen collected every Wednesday during heat stress was pooled by dietary treatment group within a room and used for artificial insemination. Each pooled sample was used to inseminate the same group of hens (15) each week of the study. The original pooled semen was diluted with minimum essential media (Howarth, 1981) to a concentration of 1 x 10^9 sperm/ml for insemination. Each hen was inseminated with 50 x 10^6 sperm by giving them 50 µl of diluted semen from the corresponding group of roosters.

Fertility analyses: To obtain fertility for each treatment group, eggs collected on days 3 - 8 postinsemination were placed in an egg cooler and stored at 18°C for seven days. The eggs were then incubated in a Petersime incubator for seven days at a dry bulb temperature of 37.5°C and a wet bulb temperature of 29°C. The eggs were broken out seven days later to determine the occurrence of fertilization and early embryonic mortality.

Statistical analyses: An ANOVA with a split-split plot design was used to analyze the data. Whole plots represented the heat stress and control temperatures. The split plots represented the four different levels of ascorbic acid. The split-split plots represented each week of the study. The base experimental units were the rooms of the males. Fisher's protected LSD was used to separate the means. Also, linear and curvilinear regression was utilized to explain dietary and heat treatment effects over the weeks of the study (Steel and Torrie, 1980).

Results

Feed consumption, body temperature, and mortality: Heat stress treatment depressed feed consumption ($P < 0.01$, Table 1). The heat treated birds experienced a significant decrease in feed consumption, during the entire severe stress period and the second and third week of the recovery period (temperature by week interaction, $P < 0.03$, Fig. 1). Feed consumption for all treatments decreased after the first week of the severe stress period because the feed provided decreased from 120 g/bird/day to 115 g/bird/day. However, all dietary treatments had similar levels of feed consumption ($P > 0.7$, Table 2). In addition, there was no significant interaction among temperature, diet, and weeks of the study.

The heat treated males had a significantly higher body temperature when compared to the controls for the main effect of temperature ($P < 0.06$, Table l). The main effect of diet ($P < 0.03$, Table 2) revealed that body temperature increased by feeding 500 ppm of ascorbic acid. The body temperature of the heat treated males increased in a linear fashion from the third week of the mild stress period until the end of the severe stress period. In addition, this linear increase in body temperature resulted in significantly higher body temperatures for the treated males as compared to the controls during each week of the severe stress period. Immediately following the removal of heat stress, body temperatures of the heat treated males dropped to the control birds level (temperature by week interaction, $P < 0.0001$, Fig. 2). However, there were no significant interactions of temperature with diet or temperature with diet and week ($P > 0.2$, Fig. 3).

Heat stress increased mortality ($P < 0.08$, Table 1). However, mortality was unaffected by dietary treatments ($P > 0.2$, Table 2).

Body weight and testes weight: Neither temperature ($P>.3$, Table 1) nor diet ($P>.6$, Table 2) significantly altered body weight. Body weights increased over the entire study for both the control and heat stressed males in the same curvilinear fashion (data not shown).

Testes weights were increased in the heat stressed males as compared to the control males ($P<.03$, Table 1). Also, relative testes weight was increased as a result of heat treatment ($P<.03$, Table 1). Testes weights were unaffected by the main effect of diet ($P>.1$, Table 2). However, diet had a significant impact on relative testes weight ($P<.03$, Table 2). Birds fed the 1000 ppm

Table 1: Main effect of ambient temperature on feed consumption, body temperature, weekly mortality, body weight, testes weight, relative testes weight, semen characteristics, and fertility

Parameter	Temperature Treatment				
	Heat	Control	SEM	P	n
Feed Consumption (g)	112.0[b]	115.0[a]	0.46	0.01	144
Body Temperature (C)	41.71[a]	41.44[b]	0.074	0.06	144
Weekly Mortality (%)	2.5[a]	0.6[b]	0.58	0.08	144
Body Weight (kg)	4.33	4.27	0.035	0.30	84
Testes Weight (g)	34.4[a]	29.9[b]	0.99	0.03	12
Relative Testes Weight (%)	0.73[a]	0.65[b]	0.016	0.03	12
Dead Sperm (%)	10.6[a]	8.0[b]	0.74	0.06	156
Sperm Conc. Billions/ml	7.6	7.5	0.21	0.70	156
Sperm Quality Index	315[b]	352[a]	6.8	0.02	168
Fertility (%)	52[b]	71[a]	3.9	0.02	132

Table 2: Main effect of diet on feed consumption, body temperature, weekly mortality, body weight, testes weight, relative testes weight, semen characteristics, and fertility

Parameter	Ascorbic Acid (ppm)						
	0	250	500	1000	SEM	P	n
Feed Consumption (g)	113.2	112.5	114.0	113.6	0.96	0.7	72
Body Temperature (C)	41.51[bc]	41.65[ab]	41.67[a]	41.47[c]	0.048	0.03	72
Weekly Mortality (%)	0.9	1.3	1.0	3.1	0.79	0.2	72
Body Weight (kg)	4.5	4.6	4.7	4.6	0.10	0.66	42
Testes Weight (g)	30	32	30	34	1.2	0.12	6
Relative Testes Wt. (%)	0.68[ab]	0.71[ab]	0.65[b]	0.74[a]	0.019	0.03	6
Dead Sperm (%)	8	8	8	10	1.2	0.6	78
Sperm Conc. billions/mL	7.5	7.6	7.4	7.7	0.22	0.8	78
Sperm Quality Index	335	346	332	323	11	0.5	84
Fertility (%)	63	67	60	57	4.3	0.4	66

ascorbic acid diet had larger relative testes weights as compared to birds receiving the 500 ppm ascorbic acid diet.

Semen analyses: The percentage of dead sperm produced by heat stressed males was greater than that of control males (P<.06, Table 1). There was no main effect of diet found for sperm viability (P>.6, Table 2). The percentage of dead sperm of the control males exhibited a curvilinear decrease throughout the entire study. The percentage of dead sperm for the heat stressed males revealed a linear increase from week four of the mild heat stress period to the end of the severe heat stress period. Upon removal of stress, the percentage of dead sperm for the heat treated males immediately dropped and returned to a level similar to that of the control males later in the recovery period. In addition, the percentage of dead sperm produced by the heat treated males was significantly greater than that of the controls from the second week of the mild stress period until the first week of the recovery period (temperature by week interaction, P<.01, Fig. 4).

Neither temperature (P>0.7, Table 1) nor diet(P>0.8, Table 2) altered sperm concentration. Sperm concentration increased over the entire study for both the control and heat stressed males in the same curvilinear fashion (data not shown).

The heat stressed males showed a significant decrease in the SQI when compared to controls (P < 0.02, Table 1). However, the SQI was unaffected by dietary treatment (P>0.5, Table 2). The SQI for the heat stressed males decreased in a linear fashion from the first week of mild stress to the final week of severe stress. The SQI for the heat treated males during the last three weeks of the mild stress period, the severe stress period, and the first week of the recovery period was significantly less than that of the controls. Furthermore, upon removal of heat stress, the SQI of the stressed males immediately rebounded to a level near that of the controls (temperature by week interaction, P < .0001, Fig. 5). Also for the temperature treatment by week interaction, the SQI for the control groups increased in a linear fashion throughout the study.

Fertility analyses: Heat stress treatment decreased fertility when compared to that of the controls (P<.02,

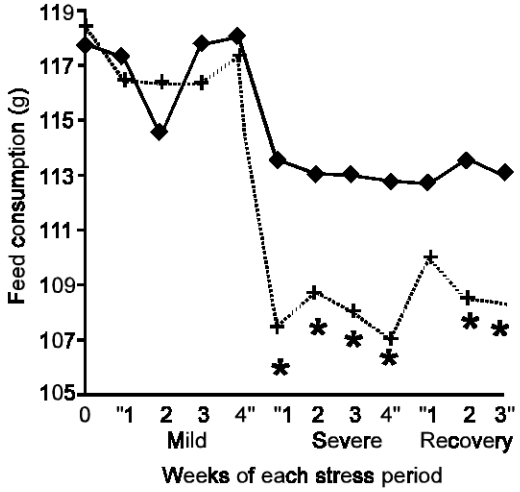

Fig. 1: The effect of heat stress on feed consumption during each week of the study. In the heat treatment rooms (+ dashed line), the birds were exposed to a mild stress, severe stress, and recovery period with temperatures of 29, 32 and 21°C respectively. The control treatment rooms (◆, Solid line) remained at 21°C throughout the trial. An asterisk denotes significant differences between the temperature treatments at any given week of the study (temperature by week interaction, P<0.03, SEM = 1.3). Each mean represents three replicates over four diets (n = 12)

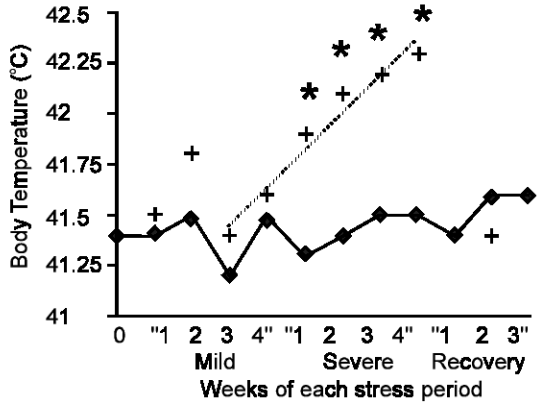

Fig. 2: The effect of heat stress on body temperature during each week of the study. In the heat treatment rooms (+ dashed line), the birds were exposed to a mild stress, severe stress, and recovery period with temperatures of 29, 32 and 21°C, respectively. The control treatment rooms (◆, solid line) remained at 21°C throughout the trial. Heat stressed birds showed a linear increase in body temperature from week three of the mild stress period to the final week of the severe stress period. An asterisk denotes significant differences between the temperature treatments at any given week of the study (temperature by week interaction, P<0.0001, SEM = 0.06). Each mean represents three replicates over four diets (n = 12).

Table 1). The main effect of diet was not significant (P>0.4, Table 2). A curvilinear relationship in fertility over each week of the study was shown for the control birds. The heat stressed male's fertility dropped drastically during the first two weeks of the severe stress period. However, fertility increased during the last two weeks of the severe stress period. Fertility once again decreased for the heat stressed males during the first week of the recovery period but began to level off in the last weeks of the recovery period. The stressed male's fertility was significantly less than that of the control males during the severe stress period and the first week of the recovery period (temperature by week interaction, P<0.05, Fig. 6). Fertility means were similar for the temperature by diet by week interaction (P>.5, Fig. 7).

When the males were heat stressed, 500 and 1000 ppm of dietary ascorbic acid proved to be detrimental to fertility over each day postinsemination (P<0.05, Fig. 8). All diets for the heat stressed males resulted in a similar rate (slope) of linear decline in fertility over days postinsemination. However, feeding the 500 and 1000 ppm ascorbic acid diets to the heat treated males resulted in significantly lower intercepts for these lines as compared to the control diet.

Discussion

The objective of this study was to determine the amount of ascorbic acid in the broiler breeder's diet that would improve the reproductive performance of males that are exposed to heat stress. It was shown that heat stress affected feed consumption, body temperature, mortality, semen characteristics and fertility. However, dietary ascorbic acid did not show evidence of being beneficial to the male broiler breeder's performance during heat stress.

Heat stress decreased feed consumption in the severe stress period and during the recovery period as compared to the controls. McDaniel *et al.* (1996) reported that feed consumption of broiler breeder males was decreased by 10g per bird per day when the temperature was increased to 32°C. Warren and Schnepel (1940) reported a 26% decrease in feed intake when ambient temperatures were raised from 16 to 35° C. Also, Payne (1966) found that feed intake was depressed by 31% when the ambient temperature was 35°C. Feed consumption may be reduced during heat stress to avoid excess heat production within the bird (Muiruri, 1989). However, in the present study feed consumption was also depressed in the last two weeks

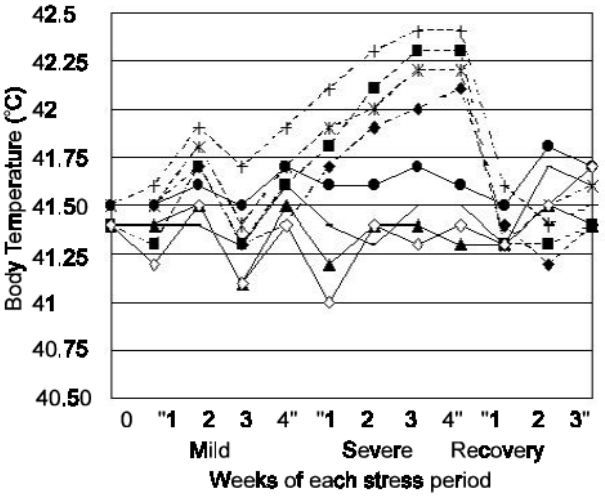

Fig. 3: Effect of heat stress and dietary ascorbic acid on body temperature during each week of the study. In the heat treatment rooms, the birds were exposed to a mild stress, severe stress, and recovery period with temperatures of 29, 32 and 21°C, respectively (dashed lines, 0-■, 250-+, 500-*, and 100 ppm-◆). The control treatment rooms remained at 21°C throughout the trial (solik lines, 0 - ▲, 250--, 500- ●, and 1000 ppm- ◇). There were no significant differences found (temperature by diet by week, P>0.2, SEM = 0.06). Each mean represents three replicates (n = 3).

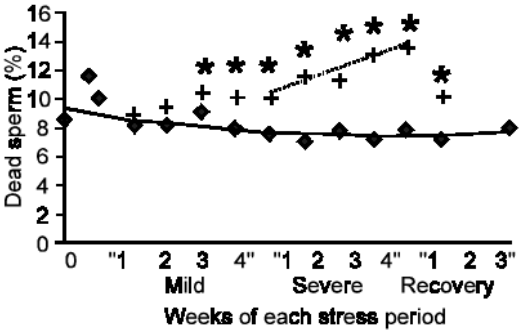

Fig. 4: The effect of heat stress on the percentage of dead sperm from individual males during each week of the study. In the heat treatment rooms the birds were exposed to a mild stress, severe stress, and recovery period with temperatures of 29, 32 and 21°C, respectively. The control treatment rooms (◆, solid line remained at 21°C throughout the trial. Heat stressed birds showed a linear increase in the percentage of dead sperm from the fourth week of the mild stress period to the fourth week of the severe stress period ($y = 0.098x + 10.1$, $r^2 = 0.95$, $P<0.0001$). A quadratic relationship for the percentage of dead sperm each week of the study was found for the control birds ($y = 0.0159x^2 + 0.34x + 9.29$, $r^2 = 0.58$, $P<0.01$). An asterisk denotes significant differences between the temperature treatments at any given week of the study (temperature by week interaction, P<0.01, SEM = 0.64). Each mean represents three replicates over four diets (n = 12).

of the recovery period.

May and Lott (1992) reported lower body weights for broilers during heat stress. Also, Teeter *et al.* (1992) reported that broilers exposed to high cyclic temperatures had significant decreases in body weight. However, in the present study, body weights were not affected by heat stress. One possible reason for this is that the males were fed a restricted diet unlike the broilers mentioned in the previous study. There was only a three gram difference found for feed consumption between the control and heat stressed groups (115 vs. 112g).

Heat treatment was shown to raise the body temperature of stressed males by 0.3°C as compared to the controls. As already proven in previous trials (Ahmad *et al.*, 1967; Harrison and Bieller, 1969), body temperature increases with an increase in ambient temperature. McDaniel *et al.* (1995) found that rectal temperatures were increased in heat stressed broiler breeders at ambient temperatures of 29.4°C. As observed by McDaniel *et al.* (1995), there was also a linear increase of body temperature during the severe stress period (32°C) in the present study. Apparently, when the ambient temperature reaches 32°C, the adult male broiler breeder is inefficient at regulating its core temperature resulting in a continual rise in rectal temperature.

Once the bird's body temperature exceeds the upper critical range, mortality usually follows quickly (Muiruri, 1989). Mortality was higher in the heat treatment rooms as compared to the control rooms. There was a trend toward higher mortality in the severe stress period, which indicates the birds had entered the zone of survival. McDaniel *et al.* (1996) found a 40 percent increase in weekly mortality within one week after the temperature was raised to 32°C. However, in the present study mortality for any treatment group never exceeded 20% in one week. The males of the present study were given four weeks to acclimate to 29°C prior to the severe stress; yet in the study by McDaniel *et al.* (1996) the birds were only given one week to acclimate to 27°C prior to the severe stress. This difference in the length and temperature of acclimation is most likely the reason for the greater mortality found by McDaniel *et al.* (1996).

The effects of heat stress on semen production and characteristics are debatable subjects among many

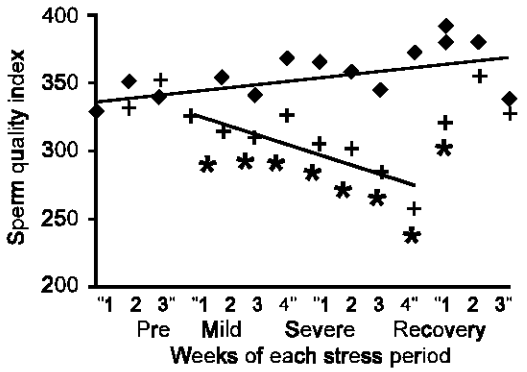

Fig. 5: The effect of heat stress on the sperm quality index (SQI) from individual males during each week of the study. In the heat treatment rooms the birds were exposed to a mild stress, severe stress, and recovery period with temperatures of 29, 32 and 21°C, respectively. The control treatment rooms(◆ , solid line) remained at 21°C throughout the trial. Heat stressed birds showed a linear decrease in the SQI from the first week of the mild stress period until the final week of the severe stress period ($y = -7.54x + 357.52$, $r^2 = 0.73$, $P<0.007$). A linear increase in the SQI is shown for the control birds throughout the study ($y = 2.75x + 330.86$ $r^2 = 0.32$, $P<0.04$). An asterisk denotes significant differences between the temperature treatments at any given week of the study (temperature by week interaction, $P<0.0001$, SEM = 11). Each mean represents three replicates over four diets (N = 12).

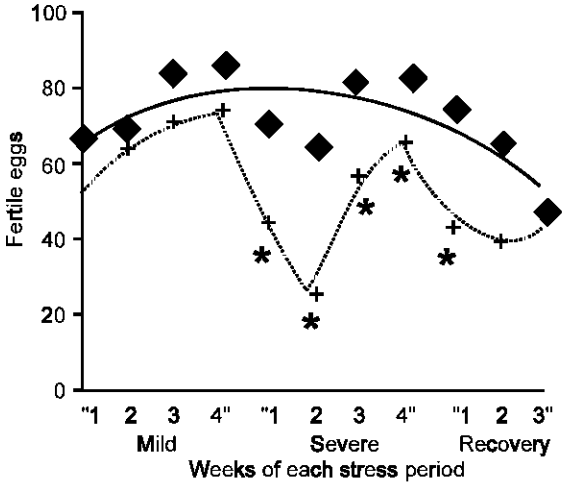

Fig. 6: The effect of heat stress on fertility during each week of the study. In the heat treatment rooms the birds were exposed to a mild stress, severe stress, and recovery period with temperatures of 29, 32 and 21°C, respectively. The control treatment rooms (◆ , solid line) remained at 21°C throughout the trial. A quadratic relationship for fertility with weeks of study was found for the control birds ($y = 8.04x^2 + 8.44x + 57.2$, $r^2 = 0.53$, $P<0.04$). An asterisk denotes significant differences between the temperature treatments at any given week of the study (temperature by week interaction, $P<0.05$, SEM = 5.2). Each mean represents three replicates over four diets (n=12).

researchers. Clark and Sarakoon (1967) found no deleterious effects of heat stress on semen characteristics of White Leghorn males when heat stressed under cyclic ambient temperatures, ranging from 21 to 38°C. However, Joshi *et al.* (1980) found that elevated ambient temperatures of 32°C decreased semen volume, sperm concentration, number of live sperm, and motility. These (Joshi *et al.*, 1980) findings support the results that were found in the present study, which showed that the percentage of dead sperm increased during the heat stress periods and then decreased immediately upon removal of heat stress. In addition, this is the first study to show that the sperm motility of modern broiler breeders is decreased as result of both mild and severe heat stress, but rebounds immediately once heat stress is removed. However, McDaniel *et al.* (1995) found that heat stress did not decrease semen quality of broiler breeders. On the other hand, McDaniel *et al.* (1996) found that heat stressing males at 32 C increased the percentage of dead sperm. The difference in the authors results may be due to the different lengths of, and temperatures during, the acclimation periods prior to heat stress. Along with sperm viability and sperm motility, fertility was also depressed by severe heat stress. Motility is an absolute requirement for sperm to reach the uterovaginal junction for storage prior to fertilization (Allen and Grigg, 1957). The sperm from heat stressed males may have been unable to cross the vagina and reach the sperm storage tubules in the uterovaginal junction due the lack of motility seen during the severe stress period. However, fertility of the stressed males decreased during the first part of the severe heat stress period, but increased during the later part of the severe heat stress period. There are several possible explanations for these results. During the severe stress period, some of the inferior males may have died leaving superior males that would improve fertility. Furthermore, the males may have acclimated to the ambient temperature. Acclimation may also explain why there was a decrease in fertility during the recovery period. The males may have acclimated to the 32°C environment, thus when the temperature was lowered to 21°C the birds were stressed resulting in a decrease in fertility. Another explanation for the post-heat stress decrease in fertility seen over weeks is an alteration in spermatozoa

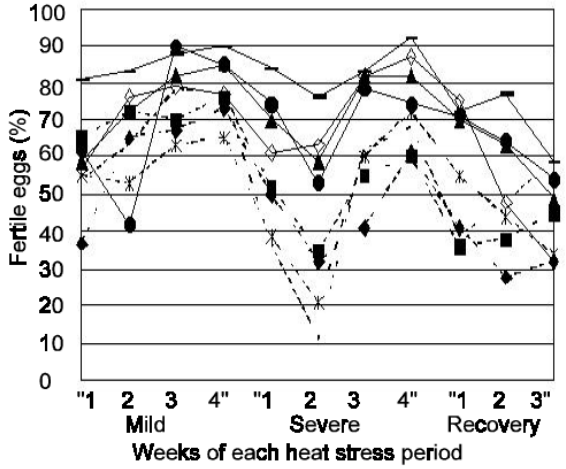

Fig. 7: Effect of heat stress and dietary ascorbic acid on fertility during each week of the study. In the heat treatment rooms the birds were exposed to a mild stress, sever stress, and recovery period with temperatures of 29, 32 and 21°C, respectively (dashed linse, 0- ■ , 250 -+, 500-* and 100 ppm- ◆). The control treatment rooms remained at 21°C throughout the trial (solid lines, 0- ▲ , 250 - -, 500 -● , and 1000 ppm -◇ . Each mean represents three replicates over six days postinsemination. There were no significant differences found (temperature by diet by week, P>0.5, SEM = 7.4).

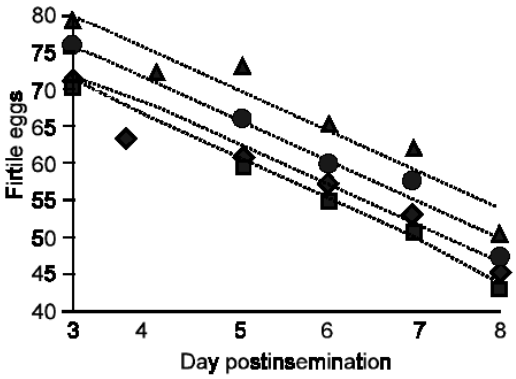

Fig. 8: Candling fertility during each day postinsemination when hens were inseminated with semen from heat stressed males in each dietary treatment group. When semen from males receiving 0 ppm of ascorbic acid was used to inseminate hens, a linear decline was seen over days postinsemination ($y = -5.5 x + 85.1$ $r^2 = 0.97$, $P<0.001$). A linear decrease was also seen in males receiving 250, 500 and 1000 ppm ascorbic acid ($y = -5.1x + 83$, $r^2 = 0.96$, $P<0.001$, $y = 4.86x + 77.71$, $r^2 = 0.99$, $P<0.001$ and $y = -5.57x + 78.5$, $r^2 = 0.98$, $P<0.001$, respectively) An asterisk denotes that the 500 and 1000 ppm treatment lines have significantly lower intercepts than the control diet line. All lines have the same slopes. Means represent three replicates over weeks (n= 66).

DNA. It is possible that in the testes of the males after heat stress, abnormal meiotic division may occur during spermatocytogenesis. This would result in abnormal sperm which may be able to penetrate the ovum but unable to complete the fertilization process.

In general, ascorbic acid was ineffective at improving broiler male performance under normal or heat stress conditions in the present study. McKee and Harrison (1995) found that by feeding 150 ppm of ascorbic acid, feed intake of heat stressed broilers was increased. Interestingly, the authors found that feeding 300 ppm of ascorbic acid to heat stressed broilers did not improve feed intake when compared to the controls. However, in the present study no level of ascorbic acid influenced feed consumption of the broiler breeder male. It is possible that because of the restricted diet, the effects of ascorbic acid on feed consumption could not be detected. In addition, ascorbic acid supplementation did not alter body weights in the present trial possibly due to the lack of an effect of ascorbic acid on feed consumption.

There was an increase in body temperature as a result of supplementation with 500 ppm of ascorbic acid. Interestingly, this increase in body temperature was not found for the males receiving 1000 ppm of ascorbic acid. Lyle and Moreng (1968) found no significant differences in the body temperature of heat stressed poultry breeds as a result of ascorbic acid supplementation. Thornton (1961) found that ascorbic acid lowered the body temperature of hens exposed to 37.8°C for 1.5 to 8.5 hours. Because the birds in the present study were continually exposed to heat stress, the ascorbic acid may not have been able to reduce body temperature.

Ascorbic acid has been reported in many studies to increase fertility and to increase semen quality. Dobrescu (1987) found that feeding 150 ppm of ascorbic acid to turkey toms increased semen volume and the number of sperm per ejaculate. Monsi and Onitchi (1991) found that feeding ascorbic acid levels of 125, 250, and 500 ppm also increased semen volume, sperm concentration, sperm per ejaculate, and number of motile sperm. Furthermore, Peebles and Brake (1985) found that feeding 50 ppm of dietary ascorbic acid to male and female broiler breeders improved fertility. However, these studies were performed under normal environmental conditions and not under the controlled heat stress conditions as in the present study. Research involving ascorbic acid supplementation is very inconsistent in its findings. This is more than likely due to the fact that ascorbic acid is an unstable

compound. Its biological activity can be greatly overestimated due to oxidative degradation, thus leading to false results (Mckee and Harrison, 1995). Peebles and Brake (1985) used an ascorbic acid that was coated with ethyl cellulose to retard oxidation. McKee and Harrison (1995) used a Rovimix STAY-C ascorbic acid that was shown to be 4 to 55 times more stable than L-ascorbic acid at 25°C. They reported that this product retained 93% of its biological activity after being stored at 25°C for 90 days in an airtight container at 25°C. In the present trial, a similar Rovimix STAY-C 35 ascorbic acid was used, which has a shelf-life of 18 months. This product was manufactured during November and was not used until the following September. It is possible that some oxidation could have occurred and lowered the biological activity of the ascorbic acid.

However, ascorbic acid did influence fertility in the present study. When the males were heat stressed, 500 and 1000 ppm of ascorbic acid had a detrimental effect on fertility over the 8 days postinsemination. Furthermore, Dobrescue (1987) found that supplementing toms with 150 ppm of ascorbic acid improved male characteristics. Peebles and Brake (1985) also found improved fertility by feeding 50 ppm of ascorbic acid, but no significant improvement by feeding 100 ppm of ascorbic acid. McKee and Harrison (1995) also found a biphasic response of stressed broilers to dietary ascorbic acid. They found that plasma corticosterone concentrations were significantly lower in broiler chicks supplemented with 150 ppm of ascorbic acid. Interestingly, stressed chicks supplemented with 300 ppm of ascorbic acid had the same plasma corticosterone levels as did the chicks that received no supplemental ascorbic acid. Apparently, only specific levels of ascorbic acid are beneficial to stressed poultry and the levels of ascorbic acid in the present study may not have been appropriate.

Lewis *et al.* (1997) suggests that ascorbic acid acts as a water soluble antioxidant in avian seminal plasma. Also in human seminal plasma, ascorbic acid is positively correlated with the percentage of spermatozoa that have normal morphology (Thiele *et al.*, 1995). However, Chen (1981) states that excessive amounts of ascorbic acid combined with only marginal amounts of vitamin E, significantly increases liver lipid peroxidation. It is possible that excess dietary ascorbic acid could cause oxidative damage to sperm. This pro-oxidative effect would explain why the males supplemented with 500 and 1000 ppm of ascorbic acid had inferior fertility over the 8 days postinsemination. Additional research should be conducted in which males are fed lower levels of ascorbic acid.

In summary, percentage dead sperm rose significantly with severe heat stress treatment, then dropped immediately upon the removal of heat stress. Furthermore, this was the first study to show that sperm motility of modern broiler breeders decreases with increasing ambient temperature and rebounds immediately upon the removal of heat stress. Also, fertility was significantly depressed in the heat treated males for the mild and severe stress period. However, ascorbic acid was ineffective at improving the broiler breeder male's performance under normal or heat stress conditions. In fact, 500 and 1000 ppm of ascorbic acid proved to be detrimental to fertility over the 8 days postinsemination when the males were heat stressed. In conclusion, ascorbic acid levels used in this study appeared to be inappropriate at reducing heat stress infertility. Additional research pertaining to heat stress infertility should be conducted using an array of lower dietary ascorbic acid levels.

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