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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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The Effects of Dietary Acetylsalicylic Acid on Heat Stress Infertility of Broiler Breeder Males

C.D. McDaniel and H.M. Parker
Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762, USA
E-mail: cmcdaniel@poultry.msstate.edu

Abstract: An attempt was made at improving fertility of male broiler breeders exposed to elevated ambient temperatures. Acetylsalicylic acid (ASA, aspirin) is a potent antipyretic drug that has been shown to lower the body temperature of heat stressed chickens. Because deviation in body temperature above normal is negatively correlated with fertility, the objective of the present study was to determine if ASA would lower rectal temperature of heat stressed male broiler breeders and improve fertility. Thirty six Arbor Acres roosters were divided equally among three controlled temperature rooms and caged individually. Half of the males in each room were fed .15% ASA, while the other half of the birds received the control (C) diet. Males were fed the C and ASA diets beginning 1 wk prior to heat stress treatment. After this pretreatment period, the temperature in all three controlled temperature rooms was increased to 29°C. Following one wk at 29°C, room temperature was increased further to 32°C for 3 wk. Once every wk of the experiment, 120 hens were inseminated with 50 million sperm from either ASA or C fed males. Dietary ASA did not lower the body temperature of the heat stressed roosters. Males fed ASA consumed less feed than males fed the C diet. In general, semen characteristics, such as semen volume, sperm concentration, and percentage of dead sperm produced, were unaffected by dietary treatment. However, addition of ASA to the heat stressed male's diet resulted in a linear decrease in fertility over Weeks 2 through 4 of the experiment and a greater reduction in fertility over days postinsemination than that obtained for males receiving the C diet. *In vivo* sperm-egg penetration was similar whether hens were inseminated with semen from C or ASA fed males. In conclusion, .15% ASA in the male's diet does not decrease body temperature when roosters are exposed to elevated ambient temperatures. In addition, 0.15% dietary ASA appears to be detrimental to fertility of heat stressed broiler breeder males.

Key words: Acetylsalicylic acid, heat stress, fertility, broiler breeder, body temperature

Introduction

Body temperature of mature broiler breeder males increases approximately one degree C during exposure to an ambient temperature of 32°C. Concurrent with this increase in male rectal body temperature is a decrease of 42 and 52% in candling fertility and *in vivo* sperm-egg penetration, respectively. Candling fertility and *in vivo* sperm-egg penetration have been shown to be highly correlated with male rectal body temperature (McDaniel *et al*., 1995b). These correlations indicate that it may be possible to alleviate heat stress infertility of male broiler breeders by lowering body temperature during elevated environmental temperatures.

Acetylsalicylic acid (ASA, aspirin) is a well known antipyretic drug (Weissmann, 1991). Aspirin inhibits prostaglandin synthesis and "resets the hypothalamic thermostat". It has been demonstrated that feeding ASA to chickens during heat stress lowers body temperature. Adams and Rogler (1968) found that feeding 0.05% ASA to broilers decreased body temperature by as much as 0.3 degrees C when birds were exposed to 29°C as compared to results obtained for birds fed a control (C) diet. In 1963, Glick determined that feeding 0.15, 0.30 and 0.60% ASA decreased body temperature 0.4, 1.2, and 0.6 degrees C respectively, when 5 and 56 d old New Hampshire chicks were heat stressed at 40.6°C. Hutchins *et al*. (1962) found that feeding 0.25 and 0.5% sodium salicylate, a less potent antipyretic analog of ASA (Weissmann, 1991), decreased the body temperature of chicks exposed to 40.6°C by approximately .3 degrees C. The correct dietary level of ASA may also decrease the body temperature of heat stressed broiler breeders. In addition, it appears that feeding ASA to hens during heat stress conditions improves egg quality and egg production (Oluyemi and Adebanjo, 1979; Reid *et al*., 1964). Because egg mass has been shown to be correlated with packed sperm volume (Marks, 1978), it is possible that during heat stress conditions the ASA mechanism responsible for increasing egg quality and egg production may also increase sperm quality and sperm production.

Therefore, the objectives of the present study were to determine if feeding ASA to heat stressed broiler breeder roosters would lower body temperature and increase overall fertility.

Materials and Methods

Housing and Environment: Thirty six Arbor Acres

roosters were caged individually in three controlled temperature rooms (12 males/room). Maximum and minimum temperature in each room was recorded daily with a high-low thermometer. The birds were maintained at 21°C (Maximum = 21±.6°C; Minimum = 18±.6°C) for 18 wk prior to the initiation of treatments, at which time the animals were 38 wk of age. Also, 120 Arbor Acres hens of the same age were caged individually in a closed-sided house with conventional environmental controls. The hens were divided equally among 12 groups of cages. The roosters were given 120 g of feed per bird per day of a standard breeder diet (2915 kcal ME/kg, 15% CP, and 3% Ca). The hens were fed the same breeder diet to maintain the recommended weight. Both hens and roosters were exposed to a photoperiod of 16 h of light per d.

The diet of six of the males in each room was supplemented with 0.15% ASA, while the remaining six males in each room received the C diet. The experimental diets were initiated 1 wk prior to heat stress treatment. After this prestress wk, a 4 wk heat stress period was conducted. During the first wk of the heat stress period, the ambient temperature in each room was increased to 29°C (Maximum = 30±.6°C; Minimum = 28±1°C). For the remaining 3 wk of the study, the temperature in each room was further elevated to 32°C (Maximum = 33±1°C; Minimum = 31±1°C). Relative humidity was recorded daily with a VWR digital hygrometer model 1022486. Relative humidity averaged 24±3% during the 4 wk of the experiment.

Body temperature: Body temperature was determined with a Cole-Parmer thermistor thermometer Model 8402 and a YSI thermistor probe 403 inserted 6 cm into the rectum. Rectal body temperature of each male was measured during the second through the eighth d of the prestress wk at 0800 hr before feeding. During the heat stress period, rectal body temperature was measured during the first d of each wk. Because body temperature changes after feeding (Wilson *et al.*, 1989) and because the level of aspirin in the blood would theoretically be the highest after feeding, measurements were made at 1 h before feeding and at 2 h after feeding during the heat stress wk. In addition, at the end of every wk of the study, feed consumption and mortality were calculated for each replicate of males.

Individual male semen characteristics: On the third and sixth d of each wk of the study, semen characteristics were analyzed for each individual male. The semen characteristics measured included the following: semen volume, packed sperm volume, and sperm viability. Packed sperm volume was determined using the technique of Maeza and Buss (1976). Sperm viability was analyzed by obtaining the percentage of dead sperm with the fluorometric method of Bilgili and Renden (1984).

Pooled semen characteristics and artificial insemination: On the first d of each wk of the heat stress period, semen was collected from each male and pooled by treatment group within a room. Sperm motility, concentration, and viability were determined for each pool of semen. Motility was scored from 1 (least motile) to 5 (most motile) using the swirl method (Cherms, 1968; Graham *et al.*, 1982). Sperm concentration was estimated by using the packed sperm volume method of Maeza and Buss (1976), while sperm viability was determined with the fluorometric method of Bilgili and Renden (1984). Semen was diluted with minimum essential medium (Howarth, 1981) to a concentration of 50 million sperm/50 µL and inseminated into hens. At 1400 h, six of the 12 groups of hens were inseminated with semen from the three ASA replicates, while the other six groups of hens were inseminated with semen from the three C replicates (2 groups per replicate per treatment).

Fertilization Characteristics: During the first wk of the heat stress period, *in vivo* sperm-egg penetration was determined in each egg laid postinsemination. The method used for determining sperm-egg penetration was that of Bramwell *et al.* (1995). The perivitelline layer from oviposited eggs was removed, fixed with 20% formalin, and stained with Schiff's reagent. The number of sperm penetration holes were counted in a 1.35 mm^2 area surrounding the germinal disc.

During the remaining three wk of the heat stress period, daily sperm-egg penetration was analyzed for three of the groups of hens inseminated with semen from each of the ASA replicates and for three of the groups of hens inseminated with semen from each of the C replicates. For determination of candling fertility and hatchability, eggs from the remaining six groups of hens were incubated. Candling fertility of each egg laid postinsemination was measured at 10 d of incubation. All unhatched eggs were opened to determine true fertility. Prior to incubation, eggs were stored in a cooler at 13.3°C and set once every wk. Hen-day egg production averaged 70% throughout the experiment. Therefore, approximately seven eggs were analyzed for either sperm-egg penetration or candling fertility for each replicate during each d postinsemination.

Statistical analysis: Randomized complete block designs with either split-plots or split-split plots in time (time of d, d, or wk) were used to analyze the data. The controlled temperature rooms served as blocks. Dietary treatment groups, which were crossed with rooms, were the experimental units. Linear and curvilinear regression analyses were also utilized to determine the relationship among treatment means over time (d or wk). Percentage

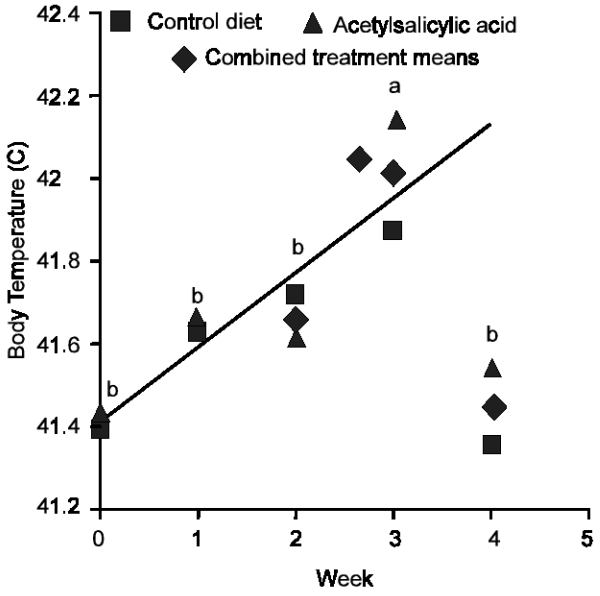

Fig. 1: Rectal body temperature of males fed a control diet or a diet containing 0.15% acetylsalicylic acid during each wk of the experiment (treatment by wk interaction, P>0.5, SEM = 0.11, n = 3). The combined treatment means are also displayed. Birds were exposed to 21°C during week 0. During week 1, the ambient temperature was elevated to 29°C. For weeks 2 through 4, the ambient temperature was increased further to 32°C. A significant linear increase in body temperature across weeks 0 through 3 of the experiment was noted for the combined treatment means ($y = 0.18x + 41.4$, $r^2 = 0.90$, P<0.05). Letter superscripts indicate a significant wk effect (P<0.01). Combined treatment means with different superscripts are significantly different [(P<0.05, SEM = 0.088, n = 6 (3 replicates time 2 diets)].

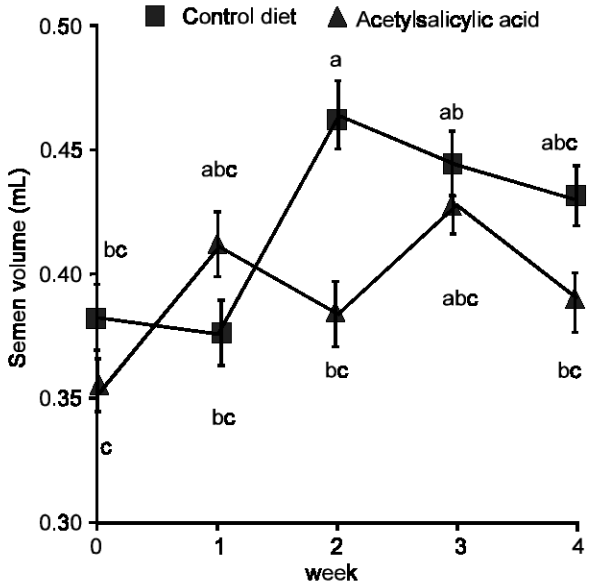

Fig. 2: Semen volume produced by males fed a control diet or a diet containing 0.15% acetylsalicylic acid during each wk of the experiment (treatment by wk interaction, P<0.028). Birds were exposed to 21°C during week 0. During week 1, the ambient temperature was elevated to 29°C. For weeks 2 through 4, the ambient temperature was increased further to 32°C. Values are displayed as the mean ±SEM. Means with different superscripts are significantly different (P<0.05). Values represent the replicate means over the 3rd and 6th d of the wk for 3 replicates of 6 males each (n = 6).

data were transformed using arcsin square root transformation. However, statistical patterns were similar between transformed and untransformed data, therefore only untransformed data will be presented. Student Newman Keul's sequential range test was used to separate interaction means (Steel and Torrie, 1980).

Results

Body temperature: Rectal body temperature, as measured before feeding, was similar between the C and ASA fed males during the prestress wk (Fig. 1). The means across treatment groups increased linearly from the prestress wk until the third wk of heat stress and then decreased. Additionally, body temperature increased approximately 0.7°C within 2 h of feeding (Table 1). However, the addition of ASA to the diet did not suppress this increase in body temperature associated with feeding. Supplementation of 0.15% ASA in the male broiler breeder's diet decreased feed consumption by approximately 4 g/bird/d as compared to feed consumption of roosters fed the C diet (Table 2). Mortality appeared to be unaffected by dietary treatment.

Individual male semen characteristics: As indicated in Fig. 2, a significant treatment group by wk of treatment interaction was obtained for semen volume. Semen volume was elevated in the C fed birds but not in the ASA fed birds during the second wk of heat stress. Packed sperm volume appeared to be unaffected by dietary treatment (Fig. 3), although a quadratic decline was noted among weekly means with increasing exposure to heat stress. A significant treatment group by wk of treatment interaction was also noted for sperm viability (Fig. 4). During the fourth wk of heat stress, the percentage of dead sperm produced by the ASA fed males was significantly greater than that of the C fed males. In addition, the percentage of dead sperm produced during the third d of the wk was less than that

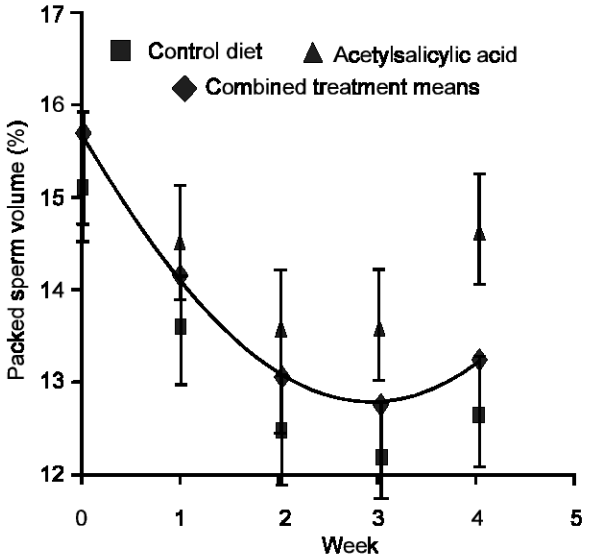

Fig. 3: Packed sperm volume of semen produced by males fed a control diet or a diet containing 0.15% acetylsalicylic acid during each wk of the experiment (treatment by wk interaction, P<0.11). The combined treatment means are also displayed. Birds were exposed to 21°C during week 0. During week 1, the ambient temperature was elevated to 29°C. For weeks 2 through 4, the ambient temperature was increased further to 32°C. A significant quadratic relationship between packed sperm volume and wk of the experiment was detected for the combined treatment means ($y = -1.5x + 0.33x^2 + 15.4$, $r^2 = 0.92$, P<0.08). Individual treatment means are displayed as mean±SEM. Individual treatment means represent the replicate means over the 3rd and 6th day of the wk for 3 replicates of 6 males each (n=6).

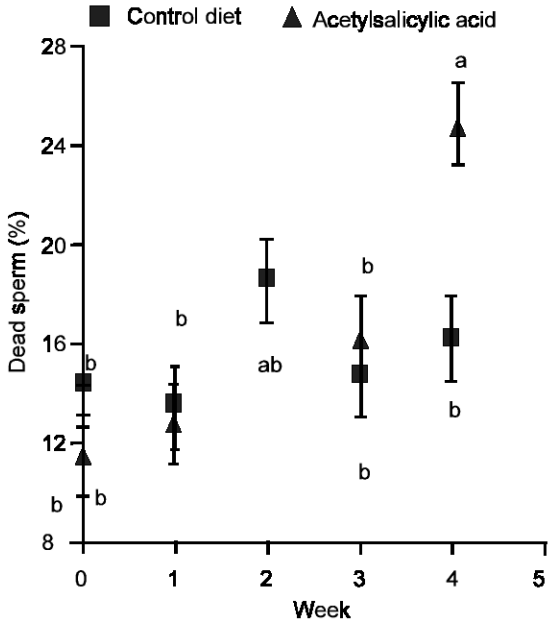

Fig. 4: The percentage of dead sperm in semen produced by males fed a control diet or a diet containing 0.15% acetylsalicylic acid during each wk of the experiment (treatment by wk interaction, P<0.056). Birds were exposed to 21°C during week 0. During week 1, the ambient temperature was elevated to 29°C. For weeks 2 through 4, the ambient temperature was increased further to 32°C values are displayed as the mean ±SEM. Means with different superscripts are significantly different (P<0.05). Values represent the replicate means over the 3rd and 6th d of the wk for 3 replicates of 6 males each (n = 6). A significant d effect was also obtained [Day 3 = 14.4%, Day 6 = 17.9%, P<0.007, SEM = 0.21, N = 30 (3 replicates times 5 wk times 2 treatments)].

produced during the sixth d of the wk (14.4 *vs* 17.9, SEM = .21, P < .007).

Pooled semen characteristics: All of the pooled semen characteristics were similar between the C and ASA fed males (Table 2). The treatment group by wk of treatment interactions for the pooled semen characteristics were not statistically significant.

Fertilization characteristics: As shown in Fig. 5, the main effect means for *in vivo* sperm-egg penetration, candling fertility, and hatchability of fertile eggs were not significantly different between the C and ASA treatment groups. In addition, no interaction between treatment and length of exposure to heat stress was seen for any of the fertilization characteristics. However, over the four wk of the heat stress period, sperm-egg penetration of sperm from the C males decreased linearly (Fig. 6).

While sperm-egg penetration by sperm from the ASA fed males appeared to decrease over weeks of the experiment, no linear component was detected. Similar quadratic declines in sperm-egg penetration over each d postinsemination were seen when hens were inseminated with semen from either C or ASA fed birds (Fig. 7).

Fertility, as a result of inseminating hens with semen from ASA fed males, decreased linearly from the second to the fourth wk of heat stress treatment (Fig. 8). No weekly linear decline in fertility was noted when hens were inseminated with semen from C males. However, means over each 7 d postinsemination period revealed a quadratic descent in fertility of the C males. A much sharper linear decline over d postinsemination was noted for fertility of the ASA fed males (Fig. 9). In addition, the line for the ASA treatment group was found to have a

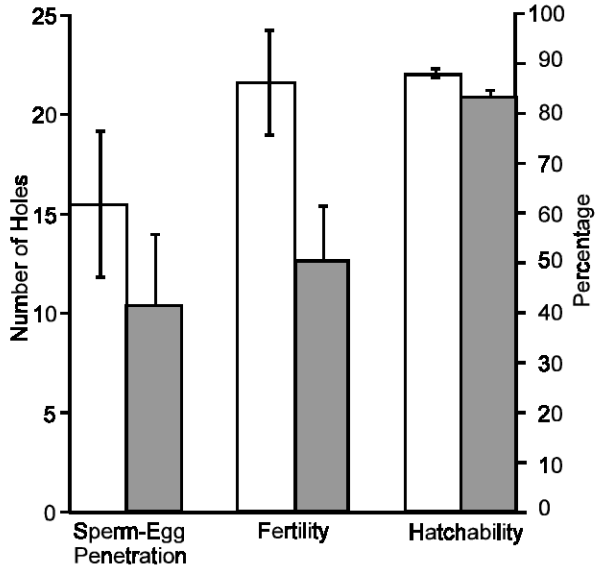

Fig. 5: *In vivo* sperm egg penetration, candling fertility, and the hatchability of fertile eggs when hens were inseminated with semen from males fed a control diet (open bars) or a diet containing 0.15% acetylsalicylic acid (hatched bars). Values are displayed as the mean ± SEM. For sperm egg penetration (P>0.42), values represent the main effect means over 4 wk and the 2nd through the 8th d postinsemination for three replicates (n = 84). For fertility (P>0.14) and hatchability (P>0.12), values represent the main effect means over 3 wk and the 2nd through the 8th d postinsemination for three replicates (n = 63).

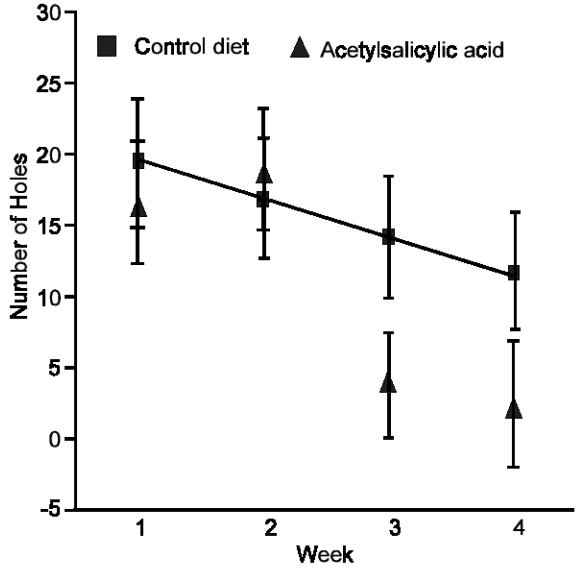

Fig. 6: *In vivo* sperm egg penetration when hens were inseminated with semen from males fed a control diet or a diet containing 0.15% acetylsalicylic acid during each wk of the experiment (treatment by wk interaction, P>0.50). Birds were exposed to 21°C during week 0. During week 1, the ambient temperature was elevated to 29°C. For weeks 2 through 4, the ambient temperature was increased further to 32°C. A significant linear decline in sperm egg penetration over wk of the experiment was noted for the control treatment group (y = -2.7x + 22.2, r^2 = 0.92, P<0.04). Values are displayed as mean ± SEM. Values represent the replicate means over the 2nd through the 8th d postinsemination for 3 replicates (n = 21).

significantly greater slope and smaller intercept than the C treatment group line. The hatchability of fertile eggs was similar between the C and ASA treatment groups during each wk of the experiment (Fig. 10).

Discussion

Previous research has shown that the body temperature of broiler breeders will increase following exposure to temperatures as low as 29 and 32°C, as was observed in the present study (McDaniel *et al.*, 1995a and 1995b). Although earlier accounts indicate that ASA in the poultry diet will decrease body temperature during heat stress conditions (Adams and Rogler, 1968; Glick, 1963), no such effect was seen in the present study. Even after feeding, when body temperature (Wilson *et al.*, 1989) and plasma ASA are most likely the highest, ASA failed to decrease the body temperature of heat stressed roosters. Wilson *et al.* (1973) noted an apparent increase in body temperature of heat stressed White Leghorn chicks given 3, 30, or 300 mg ASA as compared to birds receiving no ASA. Possible explanations, for the opposing results obtained in the current study as compared to those of Adams and Rogler (1968) and Glick (1963), may involve the age of the birds and feeding methods used. The present research used sexually mature birds that were feed restricted and not chicks that were given feed *ad libitum* as in this previous research.

Even though roosters were feed restricted, .15% dietary ASA was detrimental to feed consumption during the heat stress period. McDaniel *et al.* (1993a) has shown that feeding White Leghorn breeders 0.1 and 0.2% ASA *ad libitum* did not alter feed consumption, however, 0.4% ASA did significantly decrease feed consumption as compared to birds on a C diet. It is possible that in the present study a suboptimal level of ASA was used for maximizing its antipyretic properties while preventing any harmful side-effects (Weissmann, 1991).

Previous research has shown that fertility is negatively

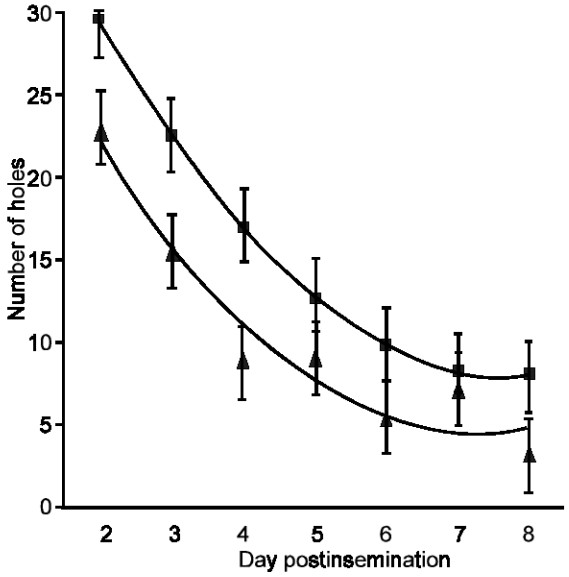

Fig. 7: *In vivo* sperm egg penetration during each d postinsemination when hens were inseminated with semen from males fed a control diet or a diet containing 0.15% acetylsalicylic acid (treatment by d postinsemination interaction, P>0.66). Significant quadratic declines in sperm egg penetration ver d postinsemination were seen for the control treatment group ($y = -10.1x + 0.66x^2 + 47.1$, $r^2 = 0.98$, P <0.0003) as well as the acetylsalicylic acid treatment group ($y = -9.1x + 0.63x^2 + 37.7$, $r^2 = 0.93$, P<0.0047). Both lines had similar slopes and intercepts (P>0.17). Values are displayed as mean ± SEM. Values represent the replicate means over 4 wk for 3 replicates (n = 12).

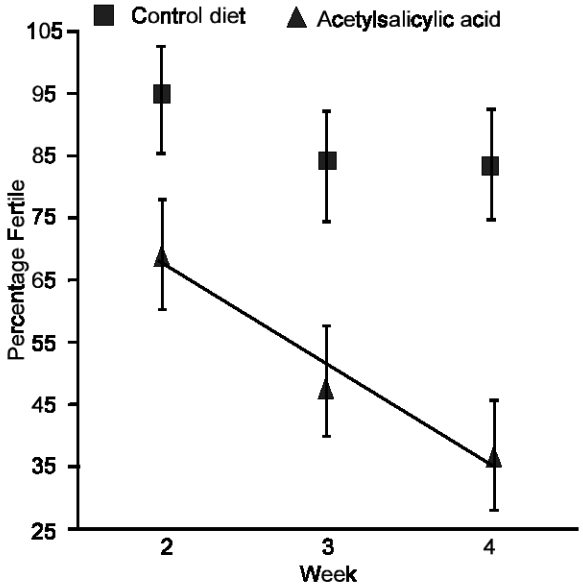

Fig. 8: Candling fertility when hens were inseminated with semen from males fed a control diet or a diet containing 0.15% acetylsalicylic acid during each wk of the experiment (treatment by wk interaction, P<0.5). Birds were exposed to 21°C during week 0. During week 1, the ambient temperature was elevated to 29° C. For weeks 2 through 4, the ambient temperature was increased further to 32°C. A significant linear decline in fertility over wk of the experiment was noted for the acetylsalicylic acid treatment group ($y = -16x + 83.5$, $r^2 = 0.97$, P<0.10). Values are displayed as mean ± SEM. Values represent the replicate means over the 2nd through the 8th d postinsemination for 3 replicates (n = 21).

correlated with male rectal body temperature when body temperature is increased as a result of heat stress (McDaniel *et al.*, 1995b). Possibly because dietary ASA did not lower the body temperature of heat stressed rooster in the present research, fertility and sperm-egg penetration were not improved in comparison to feeding males the C diet. In fact, when analyzed with linear and curvilinear regression, fertility over each d postinsemination was lower for birds receiving the ASA diet than for birds given the C diet, even though semen characteristic were similar between the two treatment groups. Additionally, fertility of ASA fed birds and not C birds decreased linearly with each wk of study. It is possible that the level of dietary ASA used in this study was too extreme. McDaniel *et al.* (1993b) found that feeding layer breeder hens 0.2% ASA did not alter fertility whereas .4% decreased fertility. The decrease in feed consumption, observed in the present study when birds were fed ASA, may partially account for the reduction in fertility for the ASA treatment group. Several studies have demonstrated that male reproductive performance is depressed during conditions of excessive feed restriction (Parker and McSpadden, 1943; Parker and Arscott, 1964; Sharlin *et al.*, 1981; Robinson *et al.*, 1993). The percentage of fertile eggs obtained in the present study, when semen from heat stressed males fed the C diet was used to inseminate hens, was high as compared to previous research when broiler breeder males were heat stressed at a similar temperature. McDaniel *et al.* (1995a) found that candling fertility of heat stressed males was only 55% after exposure to 32 C for 3 wk, whereas in the present study fertility of C fed males was 85% during the 3 wk of treatment. Possibly the lower relative humidity (24%) maintained in the present study, as compared to that (40%) maintained by McDaniel *et al.* (1995a), may not have been high enough to greatly depress fertility. It is well documented that an increase in environmental water vapor decreases

Table 1: Effects of feeding acetylsalicylic acid (ASA) to heat stressed males on body temperature (C) before and after feeding[1]

Time	Control	ASA	Mean	SEM	P <
Before feeding	41.64	41.74	41.69	0.042	0.007
After feeding	42.32	42.45	42.38		
Mean	41.98	42.10			
SEM	0.069				
P <	0.35				

[1]Values represent the replicate means over 4 wk of 3 replicates with 6 birds each (n = 12).

Table 2: Effects of feeding acetylsalicylic acid (ASA) to heat stressed males on mortality, feed consumption, and pooled semen characteristics[1]

Treatment	Mortality -(%)-	Feed Consumption -(g/bird/d)-	Sperm Motility	Packed Sperm Volume -(%)-	Dead Sperm -(%)-
Control	4	116.4	4.3	12.4	10
ASA	9	112.7	4.0	13.8	15
SEM	2.0	0.80	0.18	0.82	1.9
P <	0.22	0.08	0.43	0.37	0.19

[1]Values represent the replicate means over 4 wk for 3 replicates of 6 birds each (n = 12).

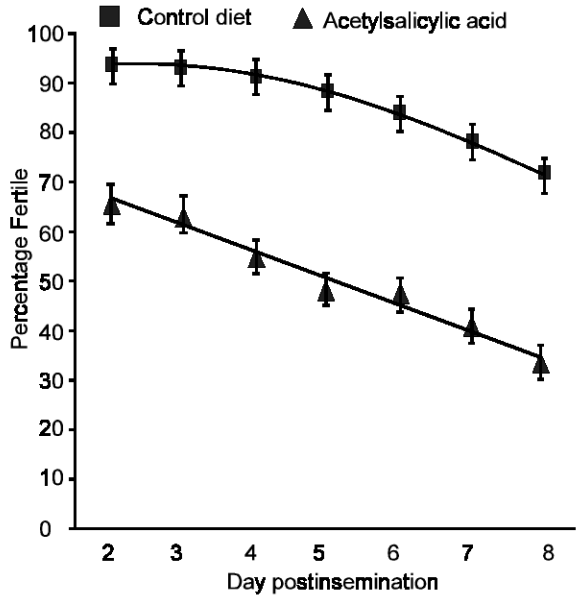

Fig. 9: Candling fertility during each d postinsemination when hens were inseminated with semen from males fed a control diet or a diet containing 0.15% acetylsalicylic acid (treatment by d postinsemination interaction, P>0.48). A significant quadratic decline in sperm egg penetration over d postinsemination was seen for the control treatment group ($y = 3x - 0.66x^2 + 90.9$, $r^2 = 0.99$, P<0.0001). For the acetylsalicylic acid treatment group a much sharper linear decline over d postinsemination was noted ($y = -5.2x + 77.5$, $r^2 = 0.98$, P <0.0001). Each line has different slopes and intercepts (P<0.05). Values are displayed as mean ± SEM. Values represent the replicate means over 3 wk for 3 replicates (n = 9).

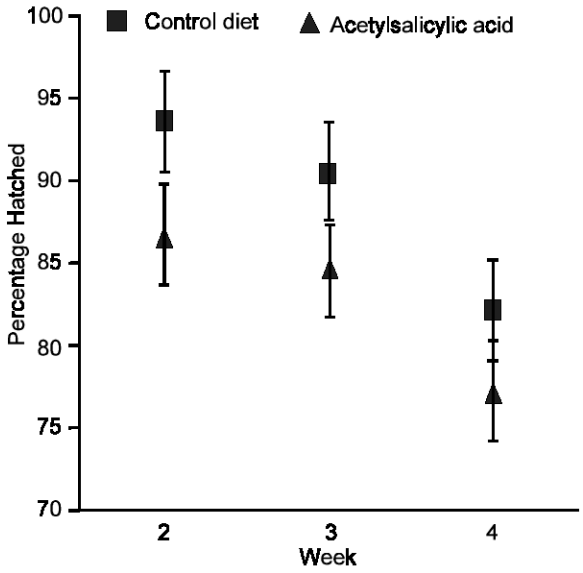

Fig. 10: The hatchability of fertile eggs when hens were inseminated with semen from males fed a control diet or a diet containing 0.15% acetylsalicylic acid during each wk of the experiment (treatment by wk interaction, P>0.5). Birds were exposed to 21°C during week 0. During week 1, the ambient temperature was elevated to 29°C. For weeks 2 through 4, the ambient temperature was increased further to 32°C. Values are displayed as mean ± SEM. Values represent the replicate means over the 2nd through the 8th d postinsemination for 3 replicates (n = 21).

performance of poultry exposed to elevated temperatures (Adams and Rogler, 1968; Smith and

Oliver, 1971). This depressing effect is most likely due to a loss in evaporative cooling efficiency at high relative humidities (Sturkie, 1986). However, as was mentioned previously, body temperature was significantly increased in the present study when males were heat stressed, indicating a response during exposure to increased environmental temperatures even at 24% relative humidity.

In conclusion, 0.15% ASA in the heat stressed male's diet did not decrease body temperature. In addition, 0.15% dietary ASA appears to be detrimental to fertility of heat stressed broiler breeder males.

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