Intermittent Biotelemetric Monitoring of Electrocardiograms and Temperature in Male Broilers at Risk for Sudden Death Syndrome

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ABSTRACT Biotelemetry was used to acquire electrocardiograms (ECG) and temperature measurements in a study of male broilers at risk for sudden death syndrome (SDS), a fatal condition that may have underlying cardiovascular mechanisms. Day-old (Day 1) Arbor Acres × Arbor Acres male chicks were randomly assigned to two different diets: control (Diet A) and one that contained elements that contribute to SDS (Diet B). The heaviest birds in each group on Day 13 underwent surgery on Day 15 to have transmitters with temperature sensors and ECG electrodes implanted. After surgery, three controls and three implanted birds from each diet group were kept in individual cages and exposed to 23 h of light and 1 h of darkness during each 24-h cycle. Implantation did not affect weight gain between Days 13 and 22 (P = 0.396). Temperature measurements and 1-min ECG were taken every 15 min. Heart rate and heart rate variability were measured from three 2-s segments in two dark and two light period samples during Days 17 to 19. Diet B decreased weight gain (P = 0.045), lowered heart rate (P < 0.0001), and increased internal temperature (P < 0.0001). Heart rate variability was lower during dark versus light periods (P = 0.004), which indicates that the birds rested during the dark periods, but was not affected by diet (P = 0.651). Thus, biotelemetry provided a useful method for intermittent physiological monitoring of poultry on different diets and under changing environmental conditions.

(Key words: sudden death syndrome, broiler, biotelemetry, electrocardiogram, temperature)

INTRODUCTION Sudden death syndrome (SDS) affects many types of animals and can lead to the deaths of 2 to 4% of male broilers in a flock (Buckley et al., 1987; Julian, 1990). The underlying physiological mechanisms that result in SDS are unknown but are thought to be diet-related metabolic disturbances leading to cardiac dysfunction (Chung et al., 1993; Squires and Summers, 1993; Olkowski and Classen, 1995; Julian, 1998). Increased cardiac irritability in rapidly growing broilers has been reported (Greenlee et al., 1989). Birds begin dying from SDS within 3 d of hatching with the total number of deaths peaking around 3 wk and continuing up to 12 wk of age (Gardiner et al., 1988).

Olkowski and Classen (1997) used needle electrodes planted subcutaneously to record electrocardiogram (ECG) from six birds dying of SDS (18 to 28 d old) and from matched controls that died from cervical dislocation. Although death in SDS birds was due to ventricular fibrillation, the underlying causes of cardiac arrhythmogenesis could not be determined because recordings were not initiated until symptoms, which presumably occurred in response to the initiation of ventricular fibrillation, were observed. Unbalanced control of cardiac activity by the central nervous system, metabolic disorders, and cardiomyopathy are all possible causes of this lethal arrhythmia.

Low heart rate variability has been found in human hypertensive patients free of coronary artery disease (Chakko et al., 1993) and is a predictor for arrhythmia-related complications in patients who have survived myocardial infarction (Kleiger et al., 1987). One study in humans reported lower heart rate variability in young babies (<30 d old) who died of sudden infant death syndrome (SIDS) than in controls (Schectman et al., 1988), but another study found no difference (Antila et al., 1990). The peak ages for birds dying from SDS are analogous to those for

©2002 Poultry Science Association, Inc.
Received for publication September 20, 2001.
Accepted for publication February 21, 2002.
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Abbreviation Key: bpm = beats per minute; ECG = electrocardiogram; NCSU = North Carolina State University; RMSM = root mean square measure of R-R intervals; R-R interval = time between heartbeats; SDS = sudden death syndrome; SIDS = sudden infant death syndrome.
human victims of SIDS. Thus, lower heart rate variability could also be a predictor of risk for SDS in young broilers.

Few recent studies have attempted to use biotelemetry for long-term monitoring of physiological variables in chickens. Hamrita et al. (1997) used implanted sensors in six white mature layer hens to measure deep body temperature responses to ambient temperature. They found diurnal variations in deep body temperature during constant temperature conditions and noticeable changes in deep body temperature in response to increases in ambient temperature, even when the environmental changes did not cause any noticeable stress symptoms such as panting. They (Lacey et al., 2000) concluded that biotelemetric measurements of deep body temperature could be used as early indicators of stress and have used this system for subsequent studies. Aerts et al. (1998) recorded ECG and subcutaneous body temperature from a single Ross female broiler to develop a model that could be used for climate control. Samples were taken every 4.5 min during eight experiments, each of which lasted 24 h. They developed a model that could predict the response of heart rate to changes in air temperature and to light-dark alterations. They plan to use this model to develop a feedback-control system that would use physiological measurements to control the environment of production animals.

The purpose of this study was to determine whether biotelemetry could provide useful physiological information about broilers at risk for SDS. Intermittent acquisition of physiological measurements over extended periods could give new insights into the underlying mechanisms of SDS.

MATERIALS AND METHODS

One-day-old (Day 1) Arbor Acres × Arbor Acres male chicks (n = 110) were divided into 10 groups of 10 chicks and weighed as a group. An individually weighed eleventh chick was then added to each group. Five groups were assigned to Diet A (control), and five groups were assigned to Diet B, which contained tallow and other elements that have been associated with an increased risk for SDS (Table 1; Blair et al., 1990, 1993). The nutritional variables that have been hypothesized to be related to SDS include the cation-anion balance, levels of sodium, available phosphorus, fatty acid composition, use of ionophore coccidiostats, and total energy level. All chicks were vaccinated for Marek’s disease and received wing bands. Each group was placed in a different cage in a Petersime Brooder in the Poultry Science Department at North Carolina State University (NCSU) and was fed ad libitum. Birds were weighed individually on Days 8 and Day 13. One bird from the Diet A group died from undetermined causes during the night between Days 5 and 6.

The three heaviest birds from each group based on Day-13 weights were selected for surgery on Day 15 to have transmitters (Model TL11M2-C50-PXT) implanted subcutaneously at the base of the right side of the neck with ECG leads placed over the right shoulder and left groin areas. Each cylindrical transmitter was 30 mm long, had a 15-mm diameter, weighed approximately 11 g, and contained a temperature transducer and leads for recording an ECG. The next three heaviest birds in each group were kept as controls.

Originally, we had also planned to implant a pressure transducer in the femoral artery in addition to the other procedures, but pilot studies indicated that it would not be possible to do this procedure in the young, 15-d-old chicks. During surgery, all birds were anesthetized with 3% isoflurane via mask inhalation, followed by intubation with an endotracheal tube. The birds were placed on a circulating hot water blanket in sternal recumbency, and the feathers over the right lateral neck, right shoulder, and left groin areas were plucked. The skin was aseptically prepared with 1% povidone iodine solution, and the bird was draped with a sterile surgical drape. A 3-cm skin incision was made over the right lateral proximal neck, and the subcutaneous tissue was gently undermined to facilitate placement of the transmitter under the skin. A 1-cm skin incision was made over the lateral right shoulder in the region of the proximal humerus. A stainless steel curved catheter was introduced into the shoulder skin incision and passed to the neck incision to facilitate transfer of one ECG lead to the right shoulder area. The insulation on the distal 1 cm of the lead was stripped, and the bare wire was sutured in two places to the proximal deltoid muscle using 4-0 polyglatin 910 suture. A third 1-cm skin incision was made over the left proximal groin area. The catheter was introduced at this skin incision and passed under the skin over the bird’s back to the right neck incision. The second ECG lead was passed through this catheter to the left groin area. The bare wire was sutured to the abdominal oblique muscle of the body wall medial to the proximal femur, using the same technique as described above. All skin incisions were closed with 4-0 polyglatin 910 suture using a simple continuous pattern. Surgical procedures were completed in less than 30 min on all birds, and there were no complications due to anesthesia or surgery with these birds. All procedures were approved by the NCSU Institutional Animal Care and Use Committee.

After surgery, the implanted birds and controls were housed in individual cages in the same room in the laboratory animal facilities at the North Carolina State College of Veterinary Medicine. The cages for implanted birds each contained four water-resistant receivers (Model RLA2000) that were connected to a receiver multiplexer (Model RMS10). All of the birds were able to move about freely within their cages. Room temperature was maintained between 20 ± 1 C and 22 ± 1 C during the study. Lights were kept on in the room for 23 h each day and were turned off for 1 h between 0500 and 0600 h, a regimen that has been shown to increase the rate of SDS (Blair et al., 1993). Each bird was fed the same diet, ad libitum, that it had received for the previous 15 d, i.e., Diet A or B. All birds

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2Data Sciences International, St. Paul, MN.
3Betadine, Purdue Frederick Co, Norwalk, CT.
4Vicryl, Ethicon, Inc., Somerville, NJ.
TABLE 1. Composition of two different diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Diet A (%)</th>
<th>Diet B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>57.2</td>
<td>30.7</td>
</tr>
<tr>
<td>Soy</td>
<td>26.0</td>
<td>35.4</td>
</tr>
<tr>
<td>Wheat</td>
<td>...</td>
<td>26.0</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.1</td>
<td>1.54</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>1.2</td>
<td>1.54</td>
</tr>
<tr>
<td>Corn oil</td>
<td>3.6</td>
<td>...</td>
</tr>
<tr>
<td>Tallow</td>
<td>...</td>
<td>4.0</td>
</tr>
<tr>
<td>Poultry meal</td>
<td>10.0</td>
<td>...</td>
</tr>
<tr>
<td>Ethoxyquin (67%)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.2</td>
<td>0.07</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.2</td>
<td>0.34</td>
</tr>
<tr>
<td>Choline chloride (60%)</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>Minerals1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamins2</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Salinomycin</td>
<td>...</td>
<td>0.1</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>0.25</td>
<td>...</td>
</tr>
<tr>
<td>0.02% Selenium</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Metabolizable energy (Mcal/kg)</td>
<td>3.15</td>
<td>2.95</td>
</tr>
<tr>
<td>% Crude protein</td>
<td>23.2</td>
<td>23.8</td>
</tr>
<tr>
<td>% Calcium</td>
<td>1.07</td>
<td>1.04</td>
</tr>
<tr>
<td>% Phosphorus</td>
<td>0.48</td>
<td>0.42</td>
</tr>
<tr>
<td>% Sodium</td>
<td>0.21</td>
<td>0.17</td>
</tr>
</tbody>
</table>

1Provided per kilogram of diet: Zn (as ZnSO4·7H2O), 120 mg; Mn (as MnSO4·H2O), 120 mg; Fe (as FeSO4·7H2O), 80 mg; Cu (as CuSO4·5H2O), 10 mg; I (as NaI·2H2O), 2.5 mg; Co (as CoSO4·H2O), 1 mg.

2Provided per kilogram of diet: vitamin A, 13,200 IU; cholecalciferol, 4,000 IU; vitamin E, 66 IU; vitamin B12, 0.0396 mg; riboflavin, 3.2 mg; niacin, 110 mg; d-pantothenate, 0.22 mg; menadione, 0.4 mg; folic acid, 2.2 mg; thiamine, 4.0 mg; pyridoxine, 7.9 mg; d-biotin, 0.253 mg; Se, 0.3 mg; ethoxyquin, 100 mg.

were weighed on Day 22, which was 1 wk after surgery for the implanted birds.

Every 15 min, a computer system2 acquired signals for 1 min from the body temperature sensor and the bipolar ECG electrode (1,000 samples/s) from each bird. Continuous sampling was not possible because the system was designed to sample from one animal at a time, i.e., sequentially rather than simultaneously. It would not have been possible to store the volume of data that would have been generated had simultaneous sampling for 24 h/d been feasible. The temperature and ECG signals were received by the computer system, digitized, converted to degrees or voltage based on the calibration values for the individual sensors, and stored directly on disk. Temperature data and ECG were evaluated at 0530 and 0545 h (during the hour of darkness) and at 1730 and 1745 h (during the light period) for 3 d beginning 2 d after surgery (Days 17 to 19).

Three 2-s intervals during each 1-min ECG were analyzed to provide information on heart rate and heart rate variability. A 5-point first derivative (Blanchard et al., 1989) was used to find the maximum negative deflection in the R wave of each heartbeat in the analyzed segments. The time of the maximum negative derivative, relative to the beginning of each 2-s analysis segment, was used as the fiducial point for determining the time period between consecutive R waves, i.e., the R-R interval. Graphs of the ECG were then inspected to confirm that the identified peaks were actually R waves. The intervals between successive R waves (RR) were determined, and an average R-R interval, R-Ravg, was calculated for the three 2-s intervals together. The standard deviation of the mean (RMSM) of the R-R intervals was obtained and used as a measure of heart rate variability (Antila et al., 1990) as follows:

\[
RMSM = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (RR_i - RR_{avg})^2} \quad [1]
\]

This study used a repeated measures nested design in which the main focuses of the study were diet and lighting conditions, and their interactions were subjected to regression analysis using the general linear models procedure of SAS software (SAS Institute, 1986). The statistical model used was

\[
Y_{ijklm} = A_{ik} + B_j + X_k + \delta_l + \tau_{k(j)} + e_{ijklm} \quad [2]
\]

where \(Y_{ijklm}\) is the individual observation (RR-interval, heart rate variability, or temperature), \(A_{ik}\) is the random effect with variance \(\sigma_A^2\) due to the diet within bird, \(B\) is the condition (light or dark), \(X_k\) is the diet (A or B), \(\delta_l\) is the date (Day 17, 18, or 19), \(\tau_{k(j)}\) is condition within time (0530-dark, 0545-dark, 1730-light, or 1745-light), and \(e_{ijklm}\) is an error with variance \(\sigma^2\). Values of \(P < 0.05\) were considered to be significant.

RESULTS AND DISCUSSION

Weight

The implanted chicks, irrespective of diet, weighed more than the non-implanted controls on Day 13 \((P = 0.003)\) because the heaviest chicks were selected for implantation (Table 2). Weight differences between the implanted and non-implanted groups disappeared by Day 22. There was no difference in the amount of weight gained by the implanted and non-implanted birds between Days 13 and 22.
Thus, implantation of the transmitters did not adversely affect weight gain.

On Days 8 and 13, there was no difference in the mean weights of the birds on Diet A and Diet B that were selected for the study (Table 2). By Day 22, the Diet A birds had gained an average of 40 g more than the Diet B birds since Day 13, a difference that just achieved significance ($P = 0.0001$) for the Diet B group over the 3-d analysis period. These rates were higher than the mean rate of 265 bpm reported by Mukai et al. (1996) in their study of 5- to 24-mo-old birds, most of which were female, but are consistent with other results that have shown that heart rates are lower in male birds than in females. Ringer et al. (1957) reported mean rates of 474 bpm for 1-wk-old birds with rates increasing until 2 to 3 wk of age and declining thereafter. They found that sex differences occurred by the 12th week with mean rates of 346 bpm for males and 385 bpm for females.

### Internal Temperature

There was no difference in internal temperature for birds under dark versus light conditions (Table 3). However, differences in internal temperature between the birds on the two diets cannot be attributed to environmental differences because the birds were housed in the same room and exposed to the same variations in room temperature. Thus, the higher internal temperature and lower overall weight gain (Table 2) of the Diet B group may indicate the presence of metabolic processes that were not contributing to growth.

### Heart Rate

Heart rate was measured in terms of intervals between successive R waves. There was no difference in R-R intervals for birds under dark versus light conditions (Table 3).

### TABLE 2. Effect of diet and implantation on weight (mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>Day 8 (g)</th>
<th>Day 13 (g)</th>
<th>Day 22 (g)</th>
<th>Weight gain (g) (from Days 13 to 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet A (n = 6)</td>
<td>176 ± 2</td>
<td>398 ± 4</td>
<td>928 ± 14</td>
<td>530 ± 14</td>
</tr>
<tr>
<td>Diet B (n = 6)</td>
<td>184 ± 3</td>
<td>402 ± 6</td>
<td>891 ± 6</td>
<td>490 ± 10</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.054</td>
<td>0.628</td>
<td>0.037</td>
<td>0.045</td>
</tr>
<tr>
<td>Implanted (n = 6)</td>
<td>182 ± 2</td>
<td>409 ± 4</td>
<td>909 ± 12</td>
<td>500 ± 15</td>
</tr>
<tr>
<td>Not implanted (n = 6)</td>
<td>179 ± 4</td>
<td>391 ± 1</td>
<td>910 ± 15</td>
<td>519 ± 15</td>
</tr>
<tr>
<td>$P^2$</td>
<td>0.506</td>
<td>0.003</td>
<td>0.959</td>
<td>0.396</td>
</tr>
</tbody>
</table>

$^1$Corrected for transmitter weight.

$^2$Two-sample $t$-test assuming equal variances, two-tail.

The mean R-R interval was longer for the Diet B group than the Diet A group. These R-R intervals corresponded to mean heart rates of 400 beats per minute (bpm) for the Diet A group and 377 bpm for the Diet B group. These rates were higher than the mean rate of 265 bpm reported by Mukai et al. (1996) in their study of 5- to 24-mo-old birds, most of which were female, but are consistent with other results that have shown that heart rates are lower in male birds than in females. Ringer et al. (1957) reported mean rates of 474 bpm for 1-wk-old birds with rates increasing until 2 to 3 wk of age and declining thereafter. They found that sex differences occurred by the 12th week with mean rates of 346 bpm for males and 385 bpm for females.

### Heart Rate Variability

Heart rate variability, as measured by RMSM, was lower during dark periods than during light ones (Table 3). Higher heart rate variability indicated that the heart rate was changing more often. The lower heart rate variability during darkness might simply have indicated that the birds were resting. During the light periods, the birds might have been responding to changes in the environment, which could include the activities of the other birds and human intrusions into the room. Human survivors of cardiac arrest have been found to have lower 24-h heart rate variability than controls and to also have their lowest heart rate variability immediately after awakening in the morning, the period that corresponds to the highest incidence of sudden cardiac death (Huikuri et al., 1992).

Although there were significant diet-dependent differences in heart rate, the current study found no diet-dependent differences in heart rate variability, which indicates...
these particular diets did not affect the ability of chicken hearts to respond to internal and external environmental changes.

**Biotelemetry**

Biotelemetry has been shown to be a valuable method for providing temperature measurements in experimental animals (Gallaher et al., 1985; Varosi et al., 1990; Hamrita et al., 1997; Lacey et al., 2000). However, temperature is a physiological variable that changes relatively slowly over time as compared to ECG. ECG must be sampled at a much faster rate because the signal changes rapidly. Arrhythmia analysis is even more complicated because it requires continuous signal acquisition. The data acquisition schedule that was used for this study, 1-min ECG every 15 min from each of six birds, was necessary to stay within the throughput and data storage limitations of the computer system. Such limitations made it virtually impossible to identify birds at risk for SDS and to continuously acquire ECG from those birds. Recent increases in computing speed and storage capacities make this type of data acquisition feasible, but it is still not possible to identify individual birds that will die from SDS with any certainty. However, this study has demonstrated that intermittent physiological monitoring of chickens with biotelemetry is a useful method for studying responses to diet and to environmental changes, e.g., dark versus light conditions.

**ACKNOWLEDGMENTS**

The authors thank Julie R. Coats, Thomas H. Dodd, Tagbo Ekwuemi-Okoli, Javier Gayo, Timothy R. Seaboch, Ho Yong Shin, Debbie Whitt Smith, and Jason A. Osborne for technical assistance. Funding for this project was provided by the North Carolina Agricultural Research Service, a Howard Hughes Medical Institute Grant, a grant from the Poultry Foundation, and NSF grant BIR-9634022.

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