Relationship of Late Embryo Loss and Anomalies in Broiler Offspring

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Primary Audience: Broiler Integrators, Hatchery Managers, Live Production Personnel, Veterinarians

SUMMARY

A previous study of Mississippi broiler complexes indicated a significant increase (0.640 to 1.77%) of embryonic total gross anatomical anomalies (TA) in the hatch residue. A separate field study in a single broiler complex of 8 breeder flocks, each hatching in 2 machines on the same day, illustrated a positive correlation between TA and total late stage embryo loss (TLEL; 16 to 21 d of incubation). The current study examined egg hatch residue from 92 selected breeder flocks that were hatched in each of 2 machines on the same date, with each respective machine being identified during egg residue analysis as having either low or high TLEL. Five makes of multistage incubational units were utilized within 10 different hatcheries in 7 states. The eggs were from breeders that comprised 32 weekly age groups (wk 27 to 61) and 5 separate strain crosses. When the egg residue results from the 92 flocks (hatching in 184 machines) were placed into 1 of 2 groups according to level (low and high) of TLEL, the high TLEL group showed a significantly lower estimated hatching efficiency (EHF) and significantly higher levels of TA and embryo head misorientations toward the small end of the egg than did the low TLEL group. The percentage change in EHF was 1.28% between eggs belonging to low (93.8%) and high (92.6%) TLEL groups. Also, TLEL in both low and high groups was significantly ($P \leq 0.0001$) correlated with EHF, TA, total cracked or crushed eggs, and with eggs having embryo heads misoriented toward the small end. These results strongly suggest that TA plays a minor role, if at all, as a cause of lower EHF and that TA incidence may serve as a biomarker for hatching efficiency.

Key words: anomaly, broiler, embryo, total late stage embryo loss, mortality


DESCRIPTION OF PROBLEM

Embryonic total gross anatomical anomalies (TA) within avian species due to various causes have been reported over many years. Examples of such TA and their causes that have been reported include alcohol- and ether-induced eye abnormalities [1]; eye, beak, and appendage monstrosities produced from Se salt injections [2]; dwarfism and shortened down resulting from riboflavin deficiencies [3]; congenital skeletal deformities caused by a biotin deficiency [4]; and spinal chord deformities induced by the application of a low-intensity electromagnetic field. Use of trade names in this publication does not imply endorsement by the Mississippi Agricultural and Forestry Experiment Station of these products nor similar ones not mentioned.

1Journal article number J-10956 from the Mississippi Agricultural and Forestry Experiment Station.

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Table 1. Hatch residue analyses from years 2003 to 2004

<table>
<thead>
<tr>
<th>TLEL</th>
<th>EHT</th>
<th>FZ</th>
<th>TEL</th>
<th>TLEL</th>
<th>LEL16</th>
<th>LEL18</th>
<th>LEL19</th>
<th>LEL21T</th>
<th>LEL21D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low²</td>
<td>86.3</td>
<td>91.8</td>
<td>5.63</td>
<td>2.96</td>
<td>0.140</td>
<td>0.870</td>
<td>1.35</td>
<td>0.620</td>
<td>0.110</td>
</tr>
<tr>
<td>High³</td>
<td>85.5</td>
<td>92.2</td>
<td>6.79</td>
<td>4.00</td>
<td>0.190</td>
<td>1.15</td>
<td>1.87</td>
<td>0.810</td>
<td>0.200</td>
</tr>
<tr>
<td>Difference (%)</td>
<td>0.800</td>
<td>0.400</td>
<td>1.16</td>
<td>1.04</td>
<td>0.050</td>
<td>0.280</td>
<td>0.520</td>
<td>0.190</td>
<td>0.090</td>
</tr>
<tr>
<td>Percentage change (high-low TLEL)</td>
<td>−0.927</td>
<td>0.436</td>
<td>20.6</td>
<td>35.1</td>
<td>35.7</td>
<td>32.2</td>
<td>38.5</td>
<td>30.6</td>
<td>81.8</td>
</tr>
</tbody>
</table>

¹EHT = total estimated hatchability; FZ = fertilization; TEL = total embryo loss (0 to 21 of incubation); TLEL = total late stage embryo loss (past d 12 of incubation); LEL16 = late stage embryo loss at d 16 of incubation; LEL18 = late stage embryo loss at d 18 of incubation; LEL19 = late stage embryo loss at d 19 of incubation; LEL21T = late stage embryo loss at d 21 of incubation (both live and dead); and LEL21D = late stage embryo loss at d 21 of incubation (dead only); n = 92 observations (flocks) for calculation of mean within each machine (low or high) category. Differences between low and high TLEL group means for each parameter were not statistically compared.

²In the low TLEL group, hatch residues of 1,123,876 broiler hatching eggs were observed.

³In the high TLEL group, hatch residues of 1,120,108 broiler hatching eggs were observed.

and its subsequent inhibition of ornithine decarboxylase activity [5].

The incidence of TA cited in this study is one of several hatching parameters routinely documented while auditing the efficiency of a hatchery and its hatching process by utilizing the hatching efficiency analysis system (HEAS). The HEAS program examines and catalogs the number and characteristics of all hatching egg residue from 4 selected trays from a single flock in 1 specific machine. This becomes a single HEAS machine-flock record. It is a computer database file accumulating all data in real numbers. The HEAS computer database file was composed of over 5,000 machine-flock records before 1998. Recall of the data can be by hatch date, company, complex, flock, flock age, machine, or by any combination of the aforementioned categories. It vividly highlights a variety of management errors resulting from flock or machine problems. This can include flock differences between houses, positional differences within a machine, or an error in inventory accountability.

Chronological trends and the incidence of embryonic TA were addressed in a field study [6], utilizing the HEAS that documented the increase in broiler chick TA in the state of Mississippi from an average of 0.640% from July to November 1998 to 1.77% from April to October 1999. A TA incidence of 0.390% before 1998 for 24 broiler complexes in 9 states within the United States was noted in that same study. At that time, the relationship of TA to other hatch residue parameters including percentage total late stage embryo loss (TLEL), which encompasses dead and live embryos past d 12 of incubation (16 to 21 d), was unknown.

Most broiler complexes in the United States now have breeder flocks maintained in multiple houses on the same premise. These breeder flocks are scheduled for a biweekly hatching egg pickup, with each pickup frequently supplying enough eggs (often exceeding 30,000) from a single flock to set 2 or more setters on the same date. This transition in broiler breeder egg supply often results in eggs from the same flock hatching in 2 or more machines on the same hatching day that are available to be studied using the HEAS.

A recent field study was conducted that consisted of 8 different breeder flocks, each hatching in 2 separate machines, with all 16 machines hatching in the same hatchery on the same hatch date [7]. Each respective breeder flock was separated by their machine difference in TLEL. An 8-flock HEAS composite of the breeder hens laying eggs exhibiting low TLEL incidence (3.31%) was compared with the respective HEAS flock composite of the same breeder hens laying eggs of high TLEL incidence (5.21%). The incidence of TA for the low TLEL group was 2.03% and that for the high TLEL group was 3.43%. Overall, there was a very highly significant ($P \leq 0.0001$) positive correlation (0.928) between TA and TLEL. The percentage of embryos (16 d of incubation and older) belonging to the lower TLEL group that had heads which were misoriented toward the small end of the egg (SEUP) was 0.330%, and the percentage of similarly aged SEUP embryos belonging to the high TLEL group was 0.710%.
Other questions remain to be answered regarding the incidence of TA in broiler embryos. These questions would include the prevalence and significance of TA increases in other US broiler hatcheries in various states, the level of involvement for commonly used multistaged incubational equipment, the incidence of TA related to major broiler breeder strains and crosses, and the correlation of TA with TLEL, SEUP, and other hatching parameters. The objective of this study was to address these and other related questions.

**MATERIALS AND METHODS**

**General**

To provide a chronological comparison, an HEAS database was utilized that had been generated over an 18-yr period before 1998 using eggs collected from 5,179 breeder flocks that were set in 5,179 respective incubator machines (a composite of 5,179 machine-flock records) [8].

A standard form of the HEAS was used to facilitate tabulation, and each flock’s hatching egg residue breakout was completed during the hatching process and immediately following its respective hatch pull. This HEAS database included 55,581,571 broiler hatching eggs set with information tabulated on the residues of over 480,000 of those eggs. Hatch residue observations were categorized under the following parameters (in units of percentage): total estimated hatchability; fertilization; estimated hatchability of fertilized eggs (or estimated hatching efficiency (EHF)); total embryo loss (d 0 to 21 of incubation); TLEL; SEUP; total cracked or crushed eggs (d 0 to 21 of incubation); TA; and brain anomalies. The observations in each category of the pre-1998 HEAS database were summarized but were not later subjected to statistical analysis. Mean total estimated hatchability is preferred for each respective flock-machine HEAS record rather than the actual hatch for each individual flock in their respective machine. The same flock is often simultaneously set and hatched in multiple incubational machines on the same dates, with the resulting actual hatch listed being provided as an
Table 3. Correlations of hatch residue percentage TEEL in low and high percentage TLEL groups with EHF, TLEL, SEUP, CNC, TA, and BA percentages and correlations of percentage TLEL in low and high percentage TLEL groups with EHF, TEEL, SEUP, CNC, TA, and BA percentages from years 2003 to 2004

<table>
<thead>
<tr>
<th>Item</th>
<th>EHF</th>
<th>TEEL</th>
<th>TLEL</th>
<th>SEUP</th>
<th>CNC</th>
<th>TA</th>
<th>BA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEEL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low TLEL</td>
<td>−0.6172</td>
<td>—</td>
<td>0.191</td>
<td>0.088</td>
<td>0.180</td>
<td>0.245</td>
<td>−0.014</td>
</tr>
<tr>
<td></td>
<td>0.00013</td>
<td>0.068</td>
<td>0.404</td>
<td>0.086</td>
<td>0.019</td>
<td>0.897</td>
<td></td>
</tr>
<tr>
<td>High TLEL</td>
<td>−0.600</td>
<td>—</td>
<td>0.174</td>
<td>0.128</td>
<td>0.179</td>
<td>0.183</td>
<td>−0.152</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>0.098</td>
<td>0.225</td>
<td>0.088</td>
<td>0.080</td>
<td>0.147</td>
<td></td>
</tr>
<tr>
<td>TLEL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low TLEL</td>
<td>−0.874</td>
<td>0.191</td>
<td>—</td>
<td>0.558</td>
<td>0.464</td>
<td>0.886</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>0.068</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.297</td>
<td></td>
</tr>
<tr>
<td>High TLEL</td>
<td>−0.873</td>
<td>0.174</td>
<td>—</td>
<td>0.562</td>
<td>0.408</td>
<td>0.832</td>
<td>−0.145</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>0.098</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.167</td>
<td></td>
</tr>
</tbody>
</table>

1TEEL = total early stage embryo loss (through d 12 of incubation); TLEL = total late stage embryo loss (past d 12 of incubation); EHF = estimated hatchability of fertilized eggs (or estimated hatching efficiency); SEUP = eggs with embryo heads oriented toward the small end; CNC = total cracked or crushed eggs (d 0 to 21 of incubation); TA = total anomalies; and BA = total brain anomalies; n = 92 observations (flocks) for correlated parameters within each machine (low or high) category.

2Correlation coefficient.

3\( P > | r | \).

average for that flock in all machines. Total estimated hatchability reflects the difference in TLEL for the same flock in different machines and likewise accounts for any fertilization differences found in the same flock placed in multiple housing on the same breeder farm. The cracked and crushed eggs occur primarily outside the machines during the transfer of eggs from the setter to hatcher on d 18 to 19 of embryonation.

Using information for 2003 and 2004 of the HEAS computer database, 92 breeder flocks were selected from which eggs were collected, set, and hatched in 184 incubational units. Only those breeder flocks with eggs set in and hatched from 2 separate incubational units at the same hatchery and on the same date were selected. The flocks were categorized as having either low or high TLEL based on their respective machine results at hatch. The selected breeder flocks included 3 major US broiler strains that were presented as 5 strain crosses, with each strain cross represented by a unique numerical code.

According to the time when their respective eggs were laid, the breeder flocks were classified into 32 age groups ranging from 27 through 61 wk of age. Hatching eggs were set in 5 different types of incubational units that were derived from 2 basic multistage hatching systems. Usually, 2 consecutive hatch dates, at each of 10 different hatcheries across 7 states, were represented. This included a total of 16 individual hatch dates from January 7, 2003 through August 13, 2004. Collectively, both low and high TLEL groups represented a total of 2,243,984 hatching eggs set. Preference was given to machine-flock units set full (at hatch, the hatcher

Table 4. Correlation of hatch residue percentage total late stage embryo loss (past d 12 of incubation) groups with total anomalies within incubational machine and within strain cross from years 2003 to 2004

<table>
<thead>
<tr>
<th>Item</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Machine</td>
<td>0.8231</td>
<td>0.692</td>
<td>0.809</td>
<td>0.926</td>
<td>0.821</td>
</tr>
<tr>
<td></td>
<td>0.00012</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>203</td>
<td>58</td>
<td>24</td>
<td>20</td>
<td>62</td>
</tr>
<tr>
<td>Strain cross</td>
<td>0.806</td>
<td>0.926</td>
<td>0.748</td>
<td>0.629</td>
<td>0.780</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.016</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>18</td>
<td>38</td>
<td>14</td>
<td>96</td>
</tr>
</tbody>
</table>

1Correlation coefficient.

2\( P > | r | \).

3n for each correlated parameter.
unit contained a full compliment of eggs from a single flock). Flocks were rejected when total cracked or crushed eggs exceeded one-third of all TLEL. All flocks were subjected to in ovo injection at transfer, which required the individual egg to be presented large end up. This resulted in a needle hole being left at the point of injection. During the residue breakout, all eggs had their large end oriented to the left. These actions allowed close monitoring of the eggs’ large end orientation when they were delivered to the hatchery and set and an accurate determination of those embryos that were truly misoriented during incubation.

As designated by the HEAS program, all hatch residues remaining on each of 4 respective hatcher trays for every flock-machine unit selected were tabulated. Starting at the top, the 4 trays of each unit selected were tray numbers 1, 5, and 10 and the bottom tray in the first vertical row of trays on the left side facing the hatcher. Every tray was tagged and labeled according to tray number, flock identity, and hatcher number. For analytical purposes, the 2 HEAS records for each of the 92 breeder flocks were listed according to their respective machine’s degree of TLEL (low or high). Also, their respective strain cross code, machine code, age in weeks, and other related data including percentages of total estimated hatchability, fertilization, total embryo loss, TLEL, and late stage embryo losses specifically at d 16, 18, 19, and 21 (both live and dead and dead only), total cracked or crushed eggs, TA, and brain anomalies were obtained. Mean EHF, total early stage embryo loss, SEUP, total cracked or crushed eggs, TA, and brain anomalies were obtained. Mean EHF, total early stage embryo loss, SEUP, total cracked or crushed eggs, TA, and brain anomalies (key hatching parameters) in the low TLEL group were statistically compared with those belonging to the high TLEL group [9]. Furthermore, within low and high TLEL categories, correlations between total early stage embryo loss and EHF, TLEL, SEUP, total cracked or crushed eggs, TA, and brain anomalies and between TLEL and EHF, SEUP, total cracked or crushed eggs, TA, and brain anomalies were determined [9]. Composite means of the other supplementary parameters (total estimated hatchability, fertilization, total embryo loss, TLEL, late stage embryo losses specifically at d 16, 18, 19, and 21 (both live and dead and dead only) in low and high TLEL categories are provided but were not statistically compared.

RESULTS AND DISCUSSION

Before 1998, mean percentages of total estimated hatchability, fertilization, total embryo loss, TLEL, and late stage embryo losses specifically at d 16, 18, 19, and 21 (both live and dead and dead only) of incubation were determined to be 85.5, 92.5, 6.86, 3.80, 0.210, 0.640, 1.86, 1.10, and 0.110%, respectively [8]. Also, before 1998, mean percentages of EHF, total early stage embryo loss, SEUP, total cracked or crushed eggs, TA, and brain anomalies were determined to be 92.5, 3.06, 0.380, 0.560, 0.390, and 0.180%, respectively [8].

In the 2003 and 2004 study, mean TLEL in the low and high categories was 2.96 and 4.00%, respectively. This corresponded to a 1.04% difference or a 35.1% increase in the level of TLEL from the low to the high group (Table 1). Also, in 2003 and 2004, mean total estimated hatchability and fertilization of the low TLEL group were 86.3 and 91.8%, respectively. Conversely, the high TLEL group had a mean total estimated hatchability of 85.5% and a 92.2% level of fertilization (Table 1).

Total estimated hatchability and fertilization in the 2003 and 2004 low and high TLEL groups resulted in respective EHF values of 93.8 and 92.6%. These EHF values in the low and high groups from 2003 and 2004 were significantly different (P ≤ 0.0001; Table 2). An increase of 1.41% for EHF in the 2003 and 2004 low TLEL group (93.8%; Table 2) was observed when compared with the pre-1998 HEAS database (92.5%). Also, a decrease of 22.1% for TLEL in the 2003 and 2004 low TLEL group (2.96%; Table 1) was observed when compared with the pre-1998 HEAS database (3.80%). When compared with the pre-1998 HEAS database, the high TLEL group had similar composite averages for fertilization and EHF. Furthermore, this study indicates that total estimated hatchability of the high TLEL group was the same as that of the pre-1998 HEAS database (85.5%). This occurred despite a 1.02% decrease in total embryo loss and a 485% increase in TA in the high TLEL group from that in the pre-1998 HEAS database.

In this study, increases in embryonic losses at d 16, 18, and 19 of incubation in the high TLEL
Figure 1. (Panel A) Scatter plot of total late stage embryo loss (TLEL; past d 12 of incubation) vs. total anomalies (TA) within the low TLEL category (TLEL = 0.78855 + 1.36944 × TA; R² = 0.7851; P ≤ 0.0001). (Panel B) Scatter plot of TLEL (past d 12 of incubation) vs. TA within the high TLEL category (TLEL = 0.65012 + 1.46633 × TA; R² = 0.6926; P ≤ 0.0001).

The incidence of total cracked or crushed eggs was 0.076% higher (14.6% increase) in the high TLEL group (0.595%) compared with that in the low TLEL group (0.519%; Table 2). Cracked or crushed eggs resulted almost exclusively from physical trauma outside the confines of the incubational units, with the majority occurring at transfer (68% in the HEAS database and 88% in this study). This confounding variable was dealt with through flock selections noted in the methodology. There was a significant gain of 0.700% in TA from the low to high TLEL group (a 44.3% increase; Table 2). When compared with the pre-1998 HEAS database, the TA of the 2003 and 2004 low TLEL group was 307% higher, whereas the TA of the high TLEL group was 485% higher. If TA was actually a cause of TLEL, then TLEL would be expected to be higher during 2003 and 2004 compared with the pre-1998 HEAS database, as was TA. Furthermore, an increase in EHF would not be expected. This is based on the finding that TA is positively correlated with TLEL in both low and high TLEL groups, whereas TLEL and EHF are negatively correlated. However, despite a drastic 307% increase in TA of the low TLEL group from the pre-1998 to the 2003 and 2004 period, there was a simultaneous 22.1% decrease in TLEL and a 1.41% increase in EHF of the low TLEL group. Therefore, it is suggested that TA plays a minor role, if any, as a cause of TLEL or of subsequent changes in EHF and that the observed increase in TA may be facilitated by other factors with little or no effect on TLEL and EHF. The combined average TA for the low and high TLEL groups was 1.93%, which closely approximated the TA of 1.77% observed in Mississippi in 1999 [6]. There was a 14.8% increase in the incidence of brain anomalies of the high TLEL group (0.310%) when compared with the low TLEL group (0.270%; Table 2), and the occurrence of brain anomalies in the pre-1998 HEAS database was 0.180%.

Correlations of total early stage embryo loss in low and high TLEL groups with EHF, TLEL, SEUP, total cracked or crushed eggs, TA, and brain anomalies and of TLEL in low and high TLEL groups with EHF, SEUP, total cracked or crushed eggs, TA, and brain anomalies from years 2003 to 2004 are provided in Table 3. Total early stage embryo loss in the low TLEL group was
significantly correlated with EHF \((P \leq 0.0001)\) and TA \((P \leq 0.019)\), and TLEL in the low and high TLEL groups was very highly significantly correlated \((P \leq 0.0001)\) with EHF, SEUP, total cracked or crushed eggs, and TA. Scatter plots of TLEL vs. TA within low and high TLEL categories shows that there is a linear relationship between the parameters within both low \((\text{TA} = 0.78855 + 1.36944 \times \text{TA}; R^2 = 0.7851; P \leq 0.0001)\) and high \((\text{TA} = 0.65012 + 1.46633 \times \text{TA}; R^2 = 0.6926; P \leq 0.0001)\) TLEL categories (Figure 1).

Correlation analyses of breeder flock age with total early stage embryo loss, TLEL, SEUP, total cracked or crushed eggs, TA, and brain anomalies showed that breeder age was only significantly correlated with number of total cracked or crushed eggs. The coefficient for the age and total cracked or crushed eggs correlation was 0.265 (not in a table; \(P \leq 0.0003\)). Overall (across incubation machine and strain cross), there was also a significant correlation between TLEL and TA. The coefficient for the TLEL and TA correlation was 0.823 (not in a table; \(P \leq 0.0001\)). Correlations of TLEL and TA within each incubational machine and strain cross examined are provided in Table 4. Positive correlations between these 2 parameters were significant within all machines and strain crosses. Correlations within all incubational machines and strain crosses examined were significant \((P \leq 0.0001)\) except for strain cross 4, which was significant at \(P \leq 0.016\). This exception is most likely because strain cross 4 was examined in only 1 hatchery that had a severely compromised hatching environment.

In the Mississippi field study [6], a significant increase in TA was documented for the state of Mississippi, but this did not include the relationship of TA to other hatching parameters such as TLEL. The current study conclusively documented the relationship of TA to TLEL within 10 hatcheries in 7 broiler production states. It is suggested that this relationship provides a basis for the use of TA incidence as an associated embryological index or biomarker for EHF.

### CONCLUSIONS AND APPLICATIONS

1. The increase in TA incidence seems to have involved most major US broiler breeding stock, their breed strain crosses, all multistage incubator equipment in which their hatching eggs were set, and numerous broiler hatcheries in many broiler production states within the United States.

2. A 307% increase in TA in the low TLEL group from the pre-1998 to 2003 to 2004 period, which involved 92 flocks, did not appear to negatively affect hatching performance, because there was an associated percentage increase in EHF (1.41%), and fertilization was essentially the same.

3. Results of the 2003 and 2004 low TLEL group showing an increase of 1.30% in EHF over the pre-1998 HEAS database, with its respective TA increasing by 307%, suggests that TA plays a minor role, if any, as a cause of TLEL or of subsequent changes in EHF.

4. Currently, EHF, TA, and SEUP are significantly correlated with TLEL during the hatching process of broiler eggs. The value of this relationship allows TA incidence to be considered as a biomarker for EHF.

5. Possible environmental variation between different hatching machines should also be considered, because a decrease (1.28%) in EHF between low and high TLEL groups was observed, although eggs from the same 92 flocks were hatched on the same day and in the same hatchery.

6. The HEAS documentation of the hatchery residue of eggs from the same flock in 1 of 2 machines categorized by TLEL level on the same date increases the pragmatic use of EHF and highlights its relationship to the conditions of the incubational environment.

7. The HEAS system using 1 flock in 2 machines also pragmatically documents EHF loss for a specific hatching date and for those incubational units involved.

8. Level of total cracked or crushed eggs was significantly correlated to TLEL, but number of total cracked or crushed eggs is a factor that must be accounted for in an HEAS study.
REFERENCES AND NOTES


9. Means were generated from egg residue results of 92 flocks in 184 machines. Each machine was categorized as having either low or high TLEL, and each replicate observation (184) represents a particular flock-machine (low or high TLEL) combination. Means for EHF, total early stage embryo loss, SEUP, total cracked or crushed eggs, TA, and brain anomalies belonging to TLEL low and high groups were compared by paired (eggs from each flock were divided into low and high TLEL incubation machine categories) t-test. Correlations involving those parameters, including TLEL, were determined by Pearson’s correlation, and TLEL and TA relationships within low and high TLEL categories were further analyzed by linear regression [10]. The TTEST, CORR, and REG procedures of SAS software, Version 6.12 [11], were used, respectively, for the t-test, correlation, and regression analyses. Statements of significance were based on P ≤ 0.05, unless otherwise indicated.


Acknowledgments

This work was funded through Hatch project MISV-2993 and funds from the College of Veterinary Medicine and the Mississippi Agricultural and Forestry Experiment Station at Mississippi State University.