



Correlations between Thermal Environment and Egg Quality of Two Layer Commercial Strains

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ABSTRACT

Heat stress limits the productivity of laying hens, as reflected by egg production and egg quality. The present study aimed at showing the correlations between egg quality parameters and environmental variables recorded on the day eggs were laid and on the previous days. Birds were housed in battery cages in a commercial poultry house. Main component analyses were used to verify associations between environmental and production variables, and Pearson's linear correlation tests were used to further investigate those associations. Analyses were carried out separately for two layer strains, Dekalb® White and Hy-Line® w36, and the variables egg weight (g), eggshell weight (g), specific gravity (g/cm³) and eggshell percentage (%) were compared with the environmental variables of the same day of the production, and one, two, three, and four days before egg production. Sound intensity measured inside the houses was positively associated with the quality parameters of eggs produced on the next day. Thermal environmental variables affected the egg quality differently in each strain, particularly air temperature, internal roof tile temperature, relative humidity, and air velocity. Ammonia concentration measured inside the houses was lower than 1ppm, and did not affect production performance.

INTRODUCTION

Heat stress limits layer performance, as the bird diverts feed metabolic energy to maintain its body temperature constant, resulting in lower egg production, and particularly in lower egg quality (Esmay, 1982; Hsu *et al.*, 1998; Teeter & Belay, 1993; Tinôco, 2001).

Under high environmental temperatures, layer respiratory rate increases from approximately 29 cycles per minute (mild environmental temperatures) to more than 100 cycles per minute (environmental temperatures above the thermoneutral zone). The resulting hyperventilation decreases carbon dioxide blood levels, which may decrease eggshell thickness in approximately 12% (Campos, 2000). Carbon dioxide is responsible for eggshell quality improvement, as it may promote acidosis, which is subsequently compensated by kidney uptake of bicarbonate. Therefore, heat stress causes losses in egg weight, eggshell percentage, eggshell weight, and egg specific gravity (Macari *et al.*, 1994; Nääs, 1992).

Hsu *et al.* (1998) found that temperature increase significantly decreases feed intake, egg production, mean egg weight, and live weight, and also influence some egg quality traits, such as eggshell thickness and egg specific gravity. Muiruri & Harison (1991) studied the performance of layers maintained under thermoneutral (25°C) or hot (35°C) temperatures, and concluded that environmental temperature did not influence egg weight or feed conversion ratio, but egg production



and feed intake significantly decreased

As to environmental ammonia concentration, Nääs *et al.* (2007) and Wathes *et al.* (2002) found that ammonia concentrations higher than 20ppm may cause respiratory disease and affect egg production.

Studying the effects of light intensity on egg quality, Renema *et al.* (2001) observed a 12% incidence of eggs below 55g when light intensity was 500 lux, whereas no differences in egg weight were detected when light intensity was below 50 lux.

Due to the importance of the environment on layer productivity, this study evaluated the correlations between egg quality parameters and environmental variables recorded at the time of lay of two layer genetic strains housed in battery cages in a commercial layer house.

MATERIALS AND METHODS

The experiment was carried out in two layer houses of a commercial farm located in Bastos, SP, Brazil. The local climate is Cwa according to Köppen's classification, characterized by hot temperate climate, with a well-defined dry season in winter.

Data were recorded for 63 days (between March and May, 2007) divided in three cycles of 21 days each. Eggs for quality analyses were collected during the last three days of each cycle.

Birds, cages, and houses

A total number of 184 34-week-old layers belonging to two genetic strains (92 Dekalb® White and 92 Hy-Line® w36) were housed in wire cages.

In this experiment, houses were built in the east-west direction, with the east and west walls closed with boards, and were not equipped with curtains. Roof material was different in each house. Both houses were equipped with wire battery cages, but with different dimensions. Chart 1 shows the main house

characteristics and cage dimensions. Differences between houses and among cages were not tested.

Both houses operated as usual and housed 34-week-old Hy-Line® w36 layers. For the experiment, Hy-Line® w36 birds were removed from 16 cages (eight per house), with an approximate distance of 20m from the west wall, which were used to house Dekalb® White layers of the same age. Other 16 cages (eight per house), at the same distance from the west wall, housed Hy-Line® w36 birds. All the experimental cages were monitored.

Cages closer to the center of the houses were monitored, with Dekalb® White birds housed closer to the north side of the barn and Hy-Line® w36 birds housed closer to the south side. Figure 1 shows the experimental layout of the cages inside the houses.

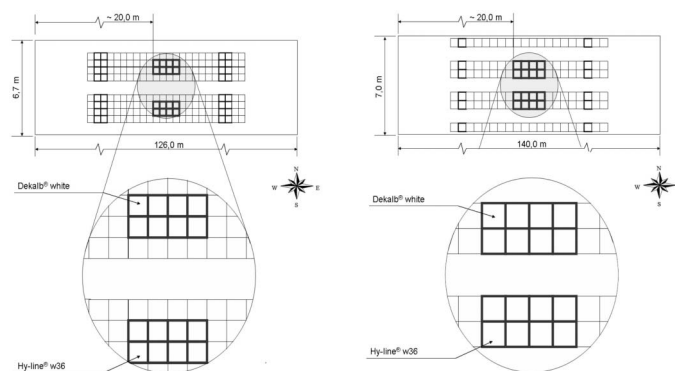


Figure 1 - Layout of the experimental setup in house 1 (a) and house 2 (b).

Feeding management and lighting program

A mixed artificial-natural lighting program was adopted. Lamps in both houses were lit at 04:00 am; during the day, natural lighting was used, and lights were turned on again at dusk and turned off at 09:00 pm, with a total period of light of 17h.

Chart 1 - Layer house characteristics.

Description	House 1	House 2
Latitude	21°57'30"	21°57'29"
Longitude	50°42'05"	50°42'03"
Altitude	444 m	456 m
Tiles	Asbestos with no external paint	Clay tiles with no external paint
Chimney	Yes	No
Roof extension	Yes	No
Roof slope	40 %	43 %
Height	2.56 m	2.32 m
Width	6.7 m	7.0 m
Length	126.0 m	140.0 m
Distance between house floor and ground (foot height)	2.0 m	1.5 m
Cage (width x depth x height)	50 cm x 45 cm x 45 cm	60 cm x 60 cm x 40 cm



Feed (17.8% protein and 2.750 kcal/kg ME) had the same formula during the entire experiment, and was fed *ad libitum*, according to the usual farm management. Daily feed intake was not monitored.

Environment monitoring

The following environmental variables were measured in a central point inside each house, in the corridor that separated the monitored cages, at bird height: air temperature, relative humidity, sound intensity, and light intensity (using a multi-function environmental analyzer, model THDL-400, manufactured by Instrutherm®); air velocity (using a digital anemometer, model AN-3010 manufactured by Icel®); internal roof tile temperature (infrared thermometer model Fluke66 manufactured by Fluke®); and ammonia concentration (gas detector model PAC7000 NH₃ manufactured by Dräger®).

Egg quality analyses

During the last three days of each production cycle, three eggs per cage were randomly collected. The following parameters were determined egg weight (EW, g), eggshell weight (ESW, g), specific gravity (SG, g/cm³), and eggshell percentage (ES%, %) at the Laboratory Unit of Avian Pathology of Bastos, SP.

Eggs from each experimental unit were weighed in a 0.01-g precision scale, and then used for specific gravity determination by immersing the eggs in recipients containing different saline solutions, and recording the density in which the eggs floated. Saline solutions were adjusted with the aid of a densimeter, in concentrations between 1.062 and 1.098 g/cm³, at 0.004 intervals.

Eggs were then broken for eggshell collection, which, after albumen and yolk removal, were dried in the air for 48 hours in the shade, and weighed. Eggshell percentage was calculated as the ratio between eggshell weight and egg weight.

Statistical analysis

Main component multivariate analysis was used to identify the associations between qualitative production parameters and house environmental variables.

The main component analysis is used to verify if there groups of associated variables and if typology evidences among variables can be detected (Johnson & Wichern, 1998). These relations among variables are evaluated with classic Euclidean distances in the case of comparison between individuals, and by correlation in the case of comparison between variables. In the

present experiment, the main component analysis was applied to verify if the observed experimental variables were associated. The association of two variables p and q , of n sample sizes, can be calculated by the expression:

$$\bar{X}_p = \sum_{i=1}^n p_i x_{ip} \quad ; \quad r_{(p,q)} = \sum_{i=1}^n m_i \left(\frac{x_{ip} - \bar{X}_p}{S_{xp}} \right) \left(\frac{x_{iq} - \bar{X}_q}{S_{xq}} \right) \quad (1)$$

Assuming that variables have the same importance ($m_i = 1/n$) in the present experiment, then:

$$r_{(p,q)} = \frac{1}{n} \sum_{i=1}^n \left(\frac{x_{ip} - \bar{X}_p}{S_{xp}} \right) \left(\frac{x_{iq} - \bar{X}_q}{S_{xq}} \right) \quad (2)$$

Assuming that the variables are represented by vectors in \mathfrak{R}^n , the set of vector extremities that represent the variables is the cloud of points N^k , where k is the number of studied variables. The main components are obtained by the orthogonal projection of the cloud of points N^k along each main direction u . The coordinate of the i th individual in the new axis system (origin in G , e u directions $\{u_\alpha, \alpha = 1, \dots, p\}$) is defined by the scale product $\langle w_i, u_\alpha \rangle$, where:

$$w_i = \left(\frac{x_{i1} - \bar{X}_1}{S_{x_1}} \right) e_1 + \left(\frac{x_{i2} - \bar{X}_2}{S_{x_2}} \right) e_2 + \dots + \left(\frac{x_{ik} - \bar{X}_k}{S_{x_k}} \right) e_k$$

and the base (e_1, e_2, \dots, e_k) is an *orthonormal base*, centered in G .

This cloud of points N^k is located in a hypersphere of radius 1, defined in \mathfrak{R}^n , as it is impossible to study the cloud shape in \mathfrak{R}^n ($n > k$) directly (Crivisqui, 1998). This means that the maximum number of components is defined by the number of studied variables.

When two main components are defined (u_1, u_2) for a study, the inertia relative to the origin of the projection of the cloud of points is maximized (Crivisqui, 1998). As the norm of the vectors that represent the variables is equal to 1, the projection coordinate of one variable over the other can be interpreted as the coefficient of correlation between the variables.

Therefore, for analyses of pairs of variables in the main component graphs, one must observe: 1) the magnitude of the vectors that summarize the variables in the plan, and which represents the contribution of these variables to the construction of the main



components, and 2) the angle established by two vectors, as the projection of one vector over the other corresponds to the correlation between these variables. Johnson & Wichern (1998) recommend that correlation tests are used together with main component analysis when data sets need to be further explored.

In the present study, five main component analyses were carried out for each genetic line with the objective of evaluating associations between egg quality parameters and environmental data obtained on the same day or on the days before the eggs were produced. These analyses are listed below.

- **Main component analysis 1:** egg qualitative parameters compared to environmental variables recorded on the same day eggs were produced;
- **Main component analysis 2:** egg qualitative parameters compared to environmental variables recorded the day before eggs were produced;
- **Main component analysis 3:** egg qualitative parameters compared to environmental variables recorded two days before eggs were produced;
- **Main component analysis 4:** egg qualitative parameters compared to environmental variables recorded three days before eggs were produced;
- **Main component analysis 5:** egg qualitative parameters compared to environmental variables recorded five days before eggs were produced.

Mean air temperature (T, °C), internal roof tile temperature (T_{Tile}, °C), air horizontal velocity at bird height (AirV, m/s), relative humidity (RH, %), sound intensity (dB, dB), and light intensity (Lux, lux), relative to daily measurement of environmental variables were calculated for main component analyses. In addition, maximum and minimum values of air temperature (T_{max}; T_{min}) and relative humidity (RH_{max}; RH_{min}) were determined.

All statistical analyses were carried out using Minitab® statistical software.

RESULTS AND DISCUSSION

Recorded ammonia concentration values were lower than 1ppm. According to Nääs et al. (2007) and Wathes et al. (2002), values above 20ppm may disturb bird behavior, and cause irritation of the respiratory

system and egg production decline. Therefore, the ammonia values measured in the present experiment did not pose any risk to production. Air ammonia levels were low in the measured houses because the north and the south sides were completely opened, favoring natural ventilation, and because of distance of the floor from the ground, allowing the contaminated air below the floor to be renewed before ammonia reached the birds.

Analysis of variance (ANOVA) results showed significant differences between genetic strains ($P < 0.05$) as to egg quality parameters, as found also by Franco-Jimenez et al. (2007). Therefore, analyses were carried out separately for each strain, which were submitted to five main component analyses to identify associations between qualitative production parameters and environmental variables.

Considering the main component analyses shown in Figures 2 and 3, a strong positive association of production parameters with internal roof tile temperature, air temperature, and light intensity in all analyses. Also, air relative humidity and sound intensity presented significant, but negative association. This association mimics what happens, for instance, when rain is about to fall, when heavy clouds appear in the sky, increasing air relative humidity at the same time that temperature and the intensity of the light that enters in the house decrease. Figure 1 shows the main component graphs for the environmental conditions on the same day of egg production of Dekalb® White (a) and Hy-Line® w36 (b) genetic strains.

Production parameters, despite presenting strong positive association among each other, did not present any association with environmental parameters measured on the same day of egg production (Figure 2), probably because the environmental variables measured on the same day eggs were produced had little or no influence on production. Table 1 shows the confidence interval for the mean of each environmental variable measured, determining the environmental conditions valid for the present study.

Table 1 - Confidence intervals and means of environmental variables.

	Lower limit	Mean	Upper limit
Sound intensity (dB)	64.80	66.17	67.36
Light intensity (lux)	174.2	179.6	184.0
Air velocity (m/s)	0.163	0.300	0.472
Air temperature (°C)	29.10	29.60	30.02
Relative humidity (%)	49.81	52.34	53.36
Internal roof tile temperature (°C)	38.52	39.82	43.29

The main component analyses of both strains showed the presence of moderate associations



between production and the environmental conditions measured four to one day before eggs were produced. This means that there were associations between production and average daily environment, but there was not a single day in which environment and production were strongly associated. Figure 3 shows the main component graphs of the association between production and environment on the day before eggs were laid for the strains Dekalb® White (a) and Hy-Line® w36 (b).

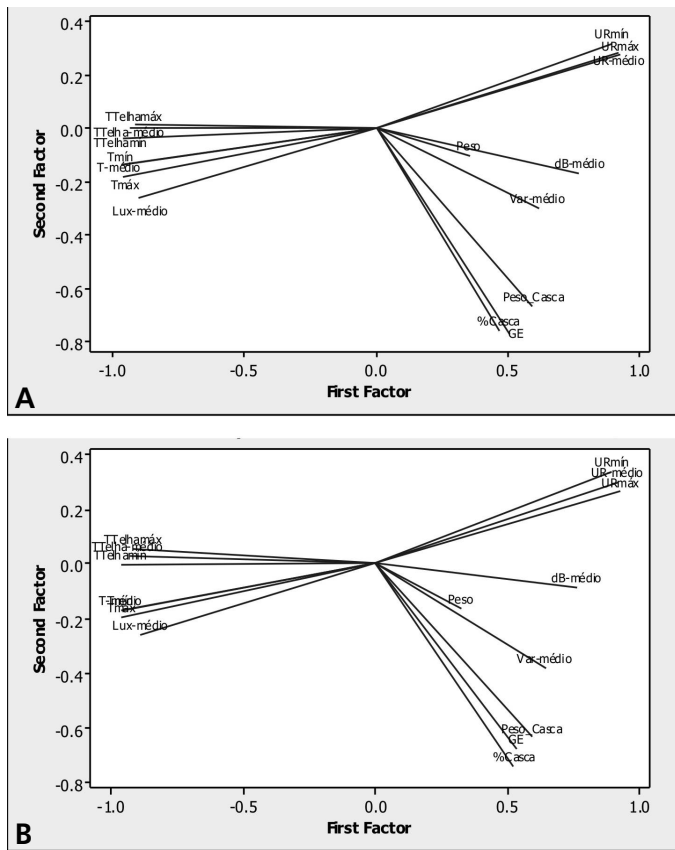


Figure 2 - Main component graphs of production and environmental parameters on the day eggs were laid of Dekalb® White (a) and Hy-Line® w36 (b) strains.

Legendas: dB-média=dB-mean; Lux-média =Lux-mean, Var-média=AirV-mean, Peso=Weight, Peso Casca = Eggshell weight, T-média= T-mean, Tmáx= Tmax, Tmín=Tmin, URmédia=RHmean, URmáx=RHmax, URmín=RHmin, TTeHamédia= TTile-mean, TTeHamáx= TTilemax, TTeHamín= TTilemin

Figure 3 é shows moderate negative association between egg quality parameters and environmental variables. Lin *et al.* (2006) asserted that high environmental temperatures are very stressful to poultry, and affect their productivity. This productivity reduction, according to Macari *et al.* (1994) and Nääs (1989), may be measured as quality loss of the

produced eggs. When Figure 2 is compared with Figure 3, it is observed that increases in air temperature and in internal roof tile temperature caused lower eggshell percentage, eggshell weight, and specific gravity values, resulting from the negative association between these two groups of variables. This association between qualitative production variables and air temperature was also observed by Hsu *et al.* (1998).

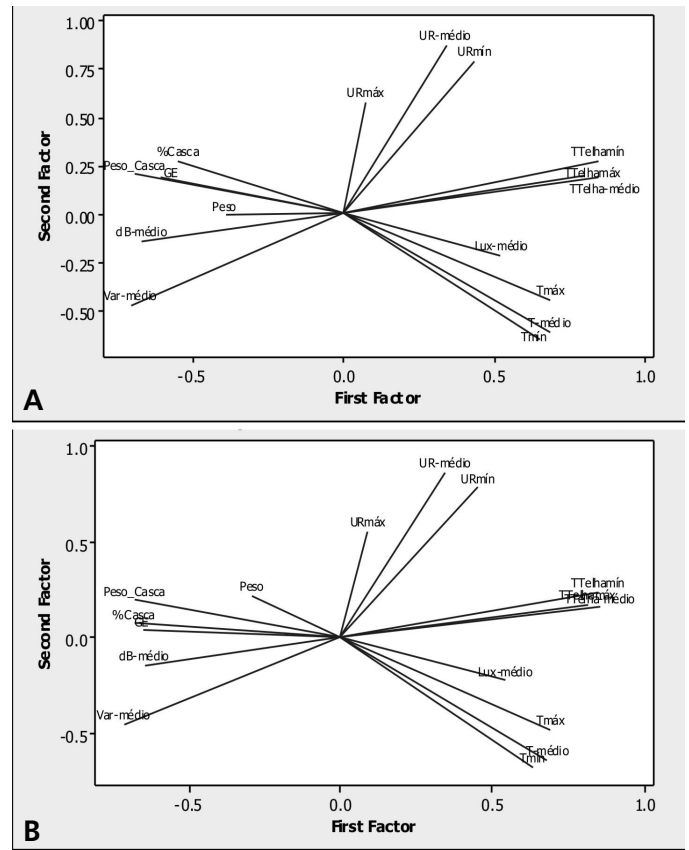


Figure 3 - Main component graphs of production and environmental parameters on the day before eggs were laid of Dekalb® White (a) and Hy-Line® w36 (b) strains.

Figure 3 also shows a positive association between sound intensity and production parameters. Sound intensity, as measured inside the house, included several components, such as external noise and machines working inside the house - background noise, and noises produced by the birds, among which vocalizations are highlighted. Although a more detailed analysis of the measured sound waves was not performed, it was observed that bird vocalization was the sound that most contributed for the variation of measured sound intensity. The analysis of Figure 3 showed that the increase of sound intensity inside the house was strongly associated with an improvement



of the quality of the eggs produced on the next day. This result could indicate that sound intensity could be used as a parameter to estimate the welfare of caged layers. However, further studies are required to evaluate the effects of this variable and of bird vocalization on layer welfare to validate this hypothesis.

The relation between noise produced by poultry and welfare was reported by Manteuffel *et al.* (2004), who related vocalization to certain emotional states. The authors said that, because vocalization is closely related to the physical-physiological status of the animal, it may be used as a non-invasive method to estimate welfare. Zimmerman *et al.* (2000) observed significant differences in the vocalization of layers submitted to different feeding regimes, with more frequent vocalization when hunger increased. Marx *et al.* (2001) related layer emotional state with different vocalization types during the process of gradual bird isolation, and concluded that this process resulted in an increase in vocalization related to stress.

For egg weight (EW), due to the low magnitude of its vector in the main component plan of all analyses, no evidences that confirm the existence (or not) of associations between this parameter and the other analyzed variables were found, which is consistent with the results of Muiruri & Harrison (1991). Mashaly *et al.* (2004) also tested the effect of three environmental conditions on the production performance of layers in the peak of lay (31 weeks of age). The following conditions were tested: thermoneutrality (constant temperature of 24°C); cyclic, simulating natural conditions of hot months (temperature varying between 23.9 and 35 °C); and heat stress (constant temperature of 35°C). The authors concluded that heat stress significantly influenced egg weight; however, no significant differences between thermoneutral and cyclic temperature on egg weight were observed after five weeks of continuous exposure.

Differences between strains were observed in the analyses of two, three, and four days before egg production. In order to further explore these data, Pearson's correlation tests (*r*) were carried out between variable pairs at a significance level of 1% ($P < 0.01$). Tables 2 and 3 show these results, and the significant results ($r > 0.5$) are highlighted.

The main component graphs shown in Figure 2 and Figure 3 (environment on the day eggs were produced and the day before, respectively) and the Pearson's correlations in Tables 2 and 3 indicate that light intensity did not affect egg quality, as shown by Renema *et al.* (2001).

Franco-Jimenez *et al.* (2007) and Vits *et al.* (2005) detected significant differences in eggshell quality between different layer strains submitted to different heat stress levels, suggesting that genetic strains have different acclimatization capacity and eggshell stabilization times. These differences can be observed in Tables 2 and 3, which show that all qualitative production parameters were influenced by the environment ($P < 0.01$).

Table 2 - Pearson's correlations between production variables egg weight (EW), specific gravity (SG), eggshell weight (ESW), and eggshell percentage (ES%) and environmental variables on the days before eggs were laid for Dekalb® White strain.

	EW	SG	ESW	ES%	
2 days before	dB-mean	-	0.292	-	
	Lux-mean	-	0.333	0.297	
	AirV-mean	0.324	0.430	0.528	0.408
	T-mean	-	-0.326	-0.375	-0.317
	Tmax	-	-0.258	-0.296	-0.265
	Tmin	-0.258	-0.348	-0.435	-0.330
	RH-mean	-0.313	-0.432	-0.490	-0.371
	RHmax	-0.264	-0.406	-0.420	-0.306
	RHmin	-0.314	-0.412	-0.516	-0.412
	TTile-mean	-	-0.292	-0.314	-
TTilemax	-	-0.293	-0.305	-	
TTilemin	-0.317	-0.267	-0.356	-	
3 days before	dB-mean	-	-	-	
	Lux-mean	-	-	-	
	AirV-mean	0.335	0.423	0.505	0.353
	T-mean	-0.287	-0.333	-0.430	-0.313
	Tmax	-0.291	-0.340	-0.417	-0.286
	Tmin	-0.224	-0.423	-0.488	-0.446
	RH-mean	-0.325	-0.424	-0.470	-0.328
	RHmax	-0.286	-0.341	-0.382	-0.254
	RHmin	-0.324	-0.531	-0.577	-0.470
	TTile-mean	-	-0.320	-0.343	-0.265
TTilemax	-	-0.290	-0.309	-	
TTilemin	-	-0.397	-0.404	-0.344	
4 days before	dB-mean	-	-	-	
	Lux-mean	-	-0.363	-0.323	-
	AirV-mean	0.294	0.367	0.450	0.338
	T-mean	-0.260	-0.349	-0.454	-0.388
	Tmax	-0.272	-0.274	-0.390	-0.298
	Tmin	-0.276	-0.408	-0.506	-0.432
	RH-mean	-0.315	-	-0.377	-
	RHmax	-	-	-	-
	RHmin	-0.351	-0.343	-0.499	-0.354
	TTile-mean	-	-0.499	-0.467	-0.400
TTilemax	-	-0.470	-0.432	-0.373	
TTilemin	-0.259	-0.487	-0.492	-0.394	

where: dB-mean = mean sound intensity on that day (dB); Lux-mean = mean light intensity on that day (lux); AirV-mean = mean horizontal air velocity on that day (m/s); T-mean = mean air temperature on that day (°C); Tmax = maximum air temperature on that day (°C); Tmin = minimum air temperature on that day (°C); RH-mean = mean air relative humidity on that day (%); RHmax = maximum air relative humidity on that day (%); RHmin = minimum air relative humidity on that day (%); TTile-mean = mean internal roof tile temperature on that day (°C); TTilemax = maximum internal roof tile temperature on that day (°C); TTilemin = minimum internal roof tile temperature on that day (°C).



Table 3 - Pearson's correlations between production variables egg weight (EW), specific gravity (SG), eggshell weight (ESW), and eggshell percentage (ES%) and environmental variables on the days before eggs were laid for Hy-Line® w36 strain.

	EW	SG	ESW	ES%	
2 days before	dB-mean	0.265	0.403	0.409	0.322
	Lux-mean	-	-	-	-
	AirV-mean	-	0.333	0.318	0.343
	T-mean	-0.263	-0.366	-0.433	-0.369
	Tmax	-	-0.312	-0.355	-0.314
	Tmin	-0.312	-0.381	-0.472	-0.365
	RH-mean	-	-	-	-
	RHmax	-	-	-	-
	RHmin	-	-0.344	-0.297	-0.259
	TTile-mean	-	-0.389	-0.403	-0.524
	TTilemax	-	-0.367	-0.379	-0.514
	TTilemin	-	-0.407	-0.451	-0.473
3 days before	dB-mean	-	-	-	-
	Lux-mean	-	-	-	-
	AirV-mean	-	0.539	0.549	0.572
	T-mean	-0.420	-0.366	-0.419	-
	Tmax	-0.378	-0.389	-0.442	-0.270
	Tmin	-0.413	-0.349	-0.429	-
	RH-mean	-0.299	-0.440	-0.474	-0.387
	RHmax	-0.264	-0.363	-0.393	-0.311
	RHmin	-0.326	-0.513	-0.564	-0.481
	TTile-mean	-	-0.348	-0.348	-0.490
	TTilemax	-	-0.328	-0.320	-0.465
	TTilemin	-	-0.390	-0.405	-0.529
4 days before	dB-mean	-	-	-	-
	Lux-mean	-	-0.300	-0.309	-0.319
	AirV-mean	-	0.427	0.472	0.478
	T-mean	-0.291	-0.341	-0.414	-0.296
	Tmax	-	-0.320	-0.364	-0.395
	Tmin	-0.380	-0.408	-0.479	-0.292
	RH-mean	-	-0.375	-0.396	-0.407
	RHmax	-	-	-	-0.337
	RHmin	-0.307	-0.490	-0.528	-0.450
	TTile-mean	-	-0.418	-0.464	-0.525
	TTilemax	-	-0.386	-0.433	-0.510
	TTilemin	-	-0.441	-0.491	-0.555

where: dB-mean = mean sound intensity on that day (dB); Lux-mean = mean light intensity on that day (lux); AirV-mean = mean horizontal air velocity on that day (m/s); T-mean = mean air temperature on that day (°C); Tmax = maximum air temperature on that day (°C); Tmin = minimum air temperature on that day (°C); RH-mean = mean air relative humidity on that day (%); RHmax = maximum air relative humidity on that day (%); RHmin = minimum air relative humidity on that day (%); TTile-mean = mean internal roof tile temperature on that day (°C); TTilemax = maximum internal roof tile temperature on that day (°C); TTilemin = minimum internal roof tile temperature on that day (°C).

No correlation between sound intensity measured three and four days before egg lay (Tables 2 and 3) with production parameters was detected, except for Dekalb® White, which eggshell weight showed a week correlation with this variable two days before egg was laid (Table 2). Tables 2 and 3 show that egg production had different correlations with environmental variables in both strains as a function of egg formation stage. Two and three days before production, air velocity and

relative humidity presented positive and negative correlations, respectively, with egg specific gravity, eggshell weight, and eggshell percentage, for both strains. Three days before production, for the strain Hy-Line® w36, roof tile temperature had a negative correlation with egg quality parameters (Table 3), and four days before production, production parameters were correlated with air temperature and internal roof tile production, particularly, for both strains. Production parameters of Hy-Line® w36 layers presented higher correlation values.

CONCLUSIONS

It was observed that air temperature, relative humidity, horizontal air velocity, and internal roof tile temperature affected egg quality at different intensities during egg formation and at different stages of egg formation in each genetic strain.

The low ammonia concentrations measured inside the poultry houses showed the efficiency of natural ventilation and air renewal in the monitored facilities, and did not affect egg quality.

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