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Multilevel sensor for monitoring external and internal environment of eggs

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ABSTRACT Although it is well known that incubation environment has a great influence on embryogenesis and post-hatching performance of birds, not much is known about how external thermal, sound and light stimuli are isolated by eggshells and perceived by embryos. In this context, this study aimed to develop, calibrate and evaluate a multilevel sensor for integrated monitoring of the external (incubator) and internal environment of eggs. The variables of interest for the external environment were air temperature and relative humidity. For the internal environment, shell temperature, internal temperature, luminosity and sound pressure level were considered. The sensor was developed with an ATmega328 microcontroller, in open-source prototyping, using electronic components which are compatible with the egg's physical structure. Calibrations were carried out in a controlled environment, comparing the multilevel sensor with commercial equipment, obtaining coefficients of determination of $R^2 > 0.90$ for all variables studied. The multilevel sensor was also validated, simulating a commercial incubation situation and comparing eggs with 2 shell colors (white and brown) and internal volume (intact and empty). Validation results showed that white-shelled eggs insulate less external light ($P < 0.001$) and full eggs presented higher internal temperatures, greater light and lower sound pressure levels compared to empty eggs ($P < 0.001$). The multilevel sensor developed here is an innovative proposal for monitoring, simultaneously and in real time, different variables of interest in the commercial incubation environment.

Key words: environmental stimuli, fertile egg, monitoring, egg shell, precision livestock farming

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INTRODUCTION

Embryogenesis comprises one-third of the total life of broiler chickens and is crucial to the success of the poultry industry. This process lasts around 21 d (or 504 h) and is influenced by environmental factors which affect embryonic development, incubation duration, hatchability and quality of newborn chicks (Bergoug et al., 2013; Mesquita et al., 2021).

In artificial incubation, environmental factors such as temperature and humidity of the internal environment of the incubators are key for production standardization and control. The temperature of the incubation environment is especially critical, affecting directly the most diverse aspects, ranging from the physical characteristics of the birds (visible) to other important factors such as immune and nutritional status, to the presence of infections or physiological disorders (Molenaar et al., 2011; Wijnen et al., 2020).

The ideal thermal zone for embryonic development is between 37 and 38°C (Bergoug et al., 2013; Wijnen et al., 2020), and variations, especially above this range, can compromise embryonic development and post-hatching chick performance. (Amjadian and Shahir, 2020; Tona et al., 2022). However, breaking a paradigm that only temperature and humidity are sufficient for successful incubation, recent literature has reported that other environmental stimuli can impact bird embryogenesis (Abdulateef et al., 2021; Hanafi et al., 2023).

Studies have shown the influence of light on embryonic development, even though commercial incubators traditionally provide a dark environment (Archer, 2017; Li et al., 2021; Tona et al., 2022). The explanation is that birds have advanced visual capacity and can detect light through the retina and pineal gland (Zhang et al., 2016), indicating the importance of photostimulation from the first days of incubation (El-Sabrout and Khalil, 2017; Wang et al., 2020; Tona et al., 2022).

Sound perception is another field of interest in embryogenesis in birds, supported by recent advances in

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the field of bioacoustics. The auditory system of birds is one of the first to develop, functioning since the 10th embryonic day (Tong et al., 2013). Considering this, studies have explored how different sounds influence cognition, learning, memory and neuronal development of chicks in the first days after hatching (Kesar, 2014; Donofre et al., 2020; Hanafi et al., 2023).

These stimuli can be natural sounds, the vocalization of the species, music and constant noise, the latter being the case in commercial incubators (Donofre et al., 2020). However, a scientific challenge lies in the lack of sensors to measure environmental variables inside eggs, which is crucial to understand the thermal, acoustic and light insulation provided by the shell and embryonic annexes (Donofre et al., 2018). This is mainly due to the fact that there are no commercially available sensors which measure environmental variables in very small spaces, such as the inside of an egg. This gap reveals the need for precision animal husbandry tools and robotics for more effective data collection.

In this context, electronic prototyping appears as a solution. Open-source sensors offer a low-cost, customizable and multifunctional alternative for measuring environmental properties in different contexts (Niranjan et al., 2021; Islam et al., 2022; Beyhan, 2023). Such devices can overcome the limitations of commercial sensors, especially in specific applications such as egg incubation.

This study aims to develop, calibrate and validate a monitoring system for the egg incubation process, referred to in this work as a multilevel sensor, with an emphasis on embryo perception. The ideal sensor must collect variables from the egg's external and internal microenvironment, have adequate size, being minimally invasive to the physical structure of the egg (shell), and allow data communication in real time.

MATERIAL AND METHODS

Development of a Multilevel Sensor

To meet the interests of this study, it was necessary to develop a unified monitoring system for the fertile egg incubation process. The term "multilevel" came from the premise that the incubation system has 2 distinct environments, the first being the environment of the artificial incubation machine, the incubators (external environment - macroclimate), and the second being the environment inside the egg, in direct contact with the embryo (internal environment - microclimate). The variables of interest for the external environment were air temperature (AT, °C) and relative humidity (RH, %). For the internal environment, shell temperature (ST, °C), internal temperature (IT, °C), internal luminosity (L, lux) and internal sound pressure level (SPL, dB) were considered. Figure 1 summarizes the processing diagram adopted for the project to monitor the internal and external environment of eggs.

Figure 2 presents the sensor assembly scheme, classified in this study as an minimum viable product (**MVP**) and the device under development, exposing the included sensors. As a prototyping tool, the open-source platform WeMos was tested, used in projects based on the Internet of Things with multiple applications, including for zootechnical purposes (Memon et al., 2019; Wahyuni et al., 2021; Rahmalisa et al., 2021; Niranjan et al., 2021). The device's microcontroller was the

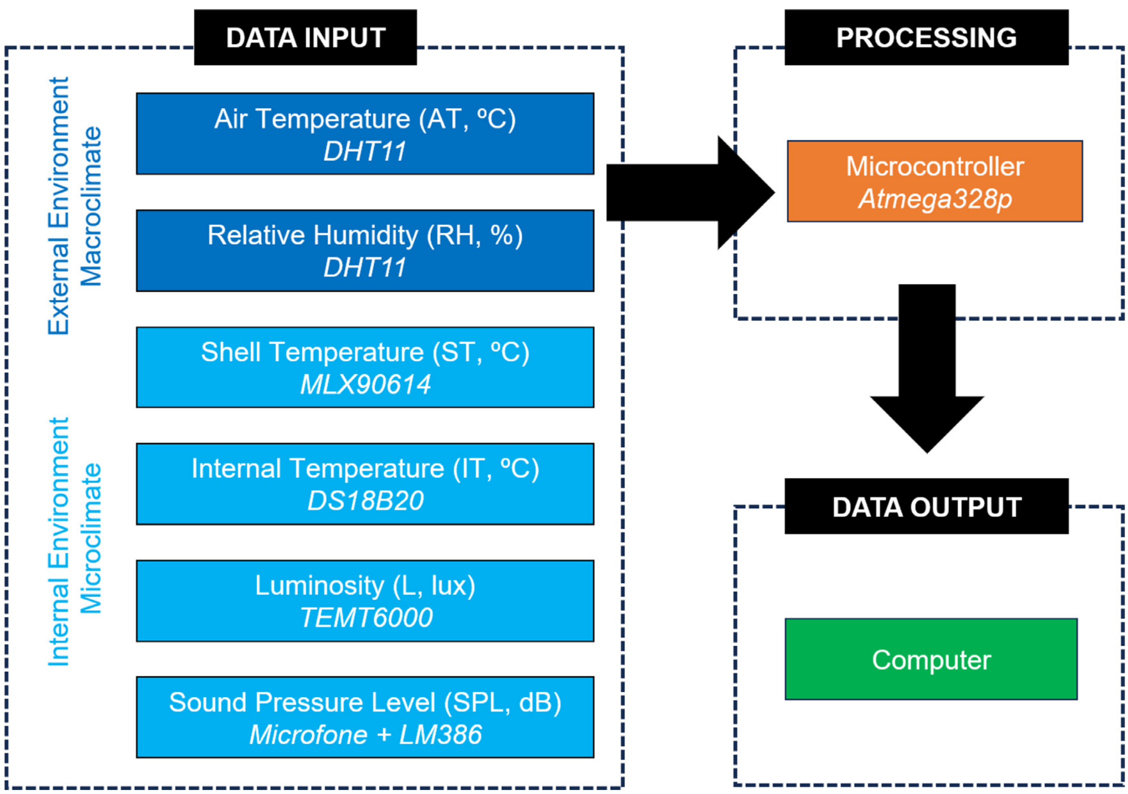
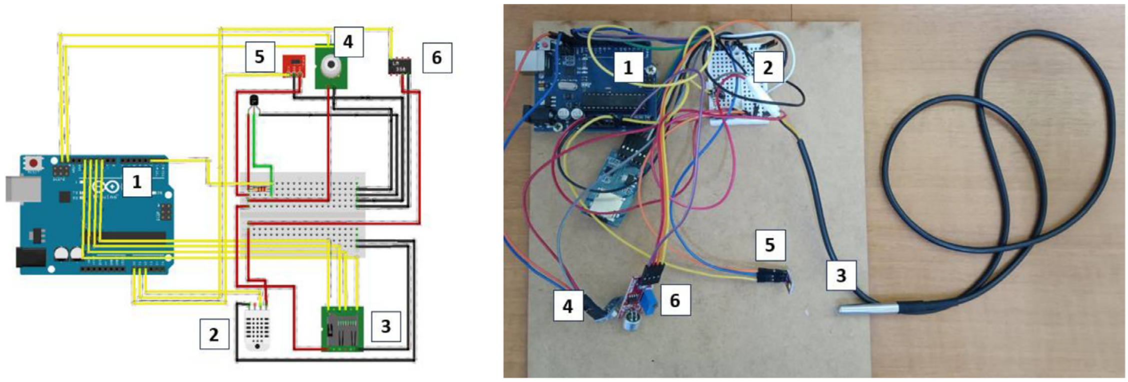


Figure 1. Multilevel sensor processing diagram.



(A) **(B)**

Figure 2. Schematic (A) and physical (B) prototype of the multilevel sensor. In the microcontroller (1), sensors were included to collect the temperature and relative humidity of the environment (2), Internal temperature (3), shell temperature (4), luminosity (5) and sound pressure level (6).

ATmega328, with a useful capacity of 14 digital ports and 6 analog ports and a micro-usb connector for power charging and programming.

The multilevel sensor included a set of specific sensor modules to collect the environmental variables of interest in this project: air temperature and relative humidity (DHT11), internal egg temperature (DS18B20), shell temperature (MLX90614), internal luminosity of the eggs (TEMT6000) and sound pressure level (Microphone + LM386). Furthermore, for the device to function, auxiliary modules were included, such as: breadboards, as the devices' structural material; memory card module, for data recording; and power modules, for energy supply and storage.

The Arduino IDE software was used to develop the programming logic in C++ language, compatible with the Atmega328p microcontroller used in prototyping. The choice of the C++ language followed the following criteria, as highlighted by Montironi et al. (2017): it is a language supported by the microcontroller, making the project technically viable; It can be implemented with the Arduino IDE and is compatible with libraries which facilitate handling of the hardware used.

Multilevel Sensor Calibration

After development, the device was calibrated, following the methodology adapted from Cavaliere et al., 2018; Donofre et al., (2018); Koestoer et al. (2019). In this way, the values of air temperature, relative humidity, internal temperature, surface temperature, sound pressure level and luminosity - all collected by the multilevel sensor - were calibrated by comparing them with readings obtained by commercial equipment.

The air temperature, relative humidity and internal temperature values offered by the multilevel sensor were compared with readings taken by a HOBO model U12-012 data logger (Onset, Piracicaba, Brazil). Eggshell temperature was compared with measurements carried out using an infrared thermometer model KR381 (Akrom, Piracicaba, Brazil). For the sound pressure level, obtained values were compared with a commercial decibel meter model DEC-490 (Instrutherm, Piracicaba, Brazil). The luminosity was calibrated using a commercial lux meter model KR832 from the Akrom, Piracicaba, Brazil, as a reference.

The comparative tests took place in a closed laboratory environment. The temperature and relative humidity tests were conducted in an oven, allowing controlled variation of these variables. In the comparative luminosity test, the lighting levels were obtained in a controlled environment with no access to the influence of external lighting and with the use of dimmable LED lamps. The evaluated sound pressure level values were obtained through the emission of a constant noise (white noise) performed by amplifier boxes. To guarantee acoustic insulation, the tests were conducted inside a Polystyrene (Styrofoam) structure.

As a descriptive method for evaluating the multilevel sensor in relation to reference equipment, regression analysis was used. For ambient temperature, relative humidity, shell temperature, internal temperature and luminosity, the simple linear regression model was applied. For sound pressure level, electrical signals from the multilevel sensor output needed to be converted to dB, after which comparison could be made with the commercial decibel meter. In this case, the analytical procedure followed the protocol carried out by Donofre et al. (2018) and Feitosa et al. (2014), and the descriptive model was adjusted using polynomial equations. From each of the models, the coefficient of determination (R^2) and its characteristic equation were extracted.

Validation and Practical Application of the Sensor

As an application, the multilevel sensor was validated, considering a commercial incubation situation. The insertion of the multilevel sensor in an incubation environment had 3 objectives. The first of these was to verify whether, in a practical situation, the developed sensor is applicable or not. Starting from the premise that the first objective is satisfied, the second objective was to understand whether or not the color of the eggshell influences the relationship between the external environment and internal environment, thus comparing brown-shelled and white-shelled eggs. The third objective was to evaluate thermal, acoustic and light insulation that the egg shell offers, thus comparing intact eggs and empty eggs (only the shell).

To simulate a standardized incubation process, eggs were inserted into a commercial incubator under conditions recommended for the species (Bergoug et al., 2013; Donofre et al., 2020): incubator temperature of 37.5 °C, relative humidity of 55% and automatic egg turning. The incubator used was a Chockmaster (Luna model, Piracicaba, Brazil) with automatic temperature and relative humidity control. Throughout the validation, temperature and humidity measurements in the incubator were collected by the multilevel sensor, in order to determine whether the environmental factors were in accordance with those recorded by the incubator control.

For the application of the sensor to measure environmental factors inside the eggs, 2 possible situations were considered: 1) empty eggs, only the shell and 2) intact eggs, with intact internal content. In order to empty the eggs, the internal contents (yolk/albumen) were removed through an opening made at the largest pole (air chamber) in the egg (Figure 3A). The procedure for removing the liquid, cleaning and drying the egg is detailed by Jones et al (2010). Two shell colors were also considered in the study, being 1) white-shelled eggs and 2) brown-shelled eggs (Figure 3B). The experimental design was therefore configured in a 2 × 2 factorial scheme, with 2 factors of internal content of the egg and 2 factors of shell color. Each condition tested used 24 units of eggs, which were considered experimental replicates.

For each of the egg samples, the following were measured: weight, shell temperature, internal temperature, internal luminosity and internal sound pressure level. All eggs tested came from the same marketing company, collected and processed on the same day and maintaining the same shelf life. Weight was measured using a semi-analytical scale (model BG2000, brand Gehaka, Piracicaba, Brazil). Shell temperature, internal temperature and luminosity were measured with the eggs inside the incubators, whereas the sound pressure level test was conducted in an acoustically isolated environment (inside a polystyrene structure), following the

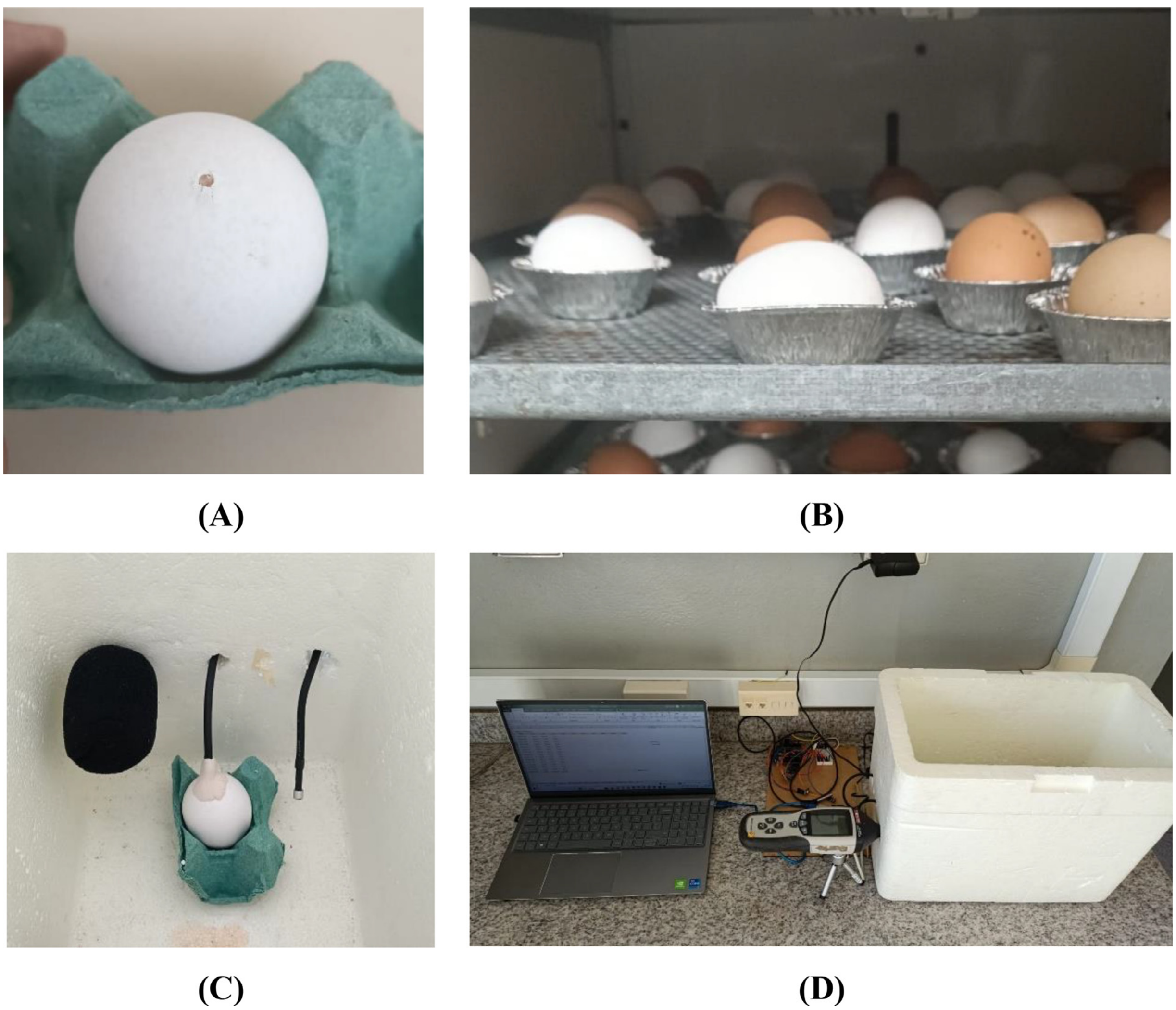


Figure 3. Multilevel sensor validation procedures: Opening the egg in the air chamber region in order to remove the internal liquid and insert sensors (A); set of samples of different shell colors in an incubation environment (B); and comparative testing of the sound pressure level inside (C) and outside (D) the test environment (Polystyrene box).

methodology used by Donofre et al., (2018) and presented in Figures 3C and 3D. In this case, the characteristic sound of a commercial incubator, 90 dB, was simulated, as described by Donofre et al. (2020).

In order to read the internal variables, the sensors were inserted through the opening made in the largest pole of the egg. The sensors were inserted into the air chamber, about 1 cm deep and near the liquid area of the egg. In empty eggs, a marking was made on the sensor wire so that their position was the same as that of intact eggs. The empty spaces between the sensor wire and the opening of the eggs were filled with synthetic putty in order to seal (Figure 3C).

In the analysis, the presence of outliers in the data was verified. Then, the normality of the residuals was assessed using the Shapiro-Wilk test ($P > 0.05$) (Shapiro and Wilk, 1965), while homogeneity was verified using the Levene test (Levene et al., 1960). Data were subjected to analysis of variance (**ANOVA**) to evaluate shell color, internal volume and interaction between internal volume and shell color. Means were compared using the Tukey test (with a probability of error of 5%). The following general linear model was used in the analysis:

$$Y_{ijk} = \mu + G_i + S_j + \left(G_i \times S_j\right) + e_{ijk}$$

Where: Y_{ijk} represents the responses of the set of dependent variables, μ is the general average, G_i is the i^{th} effect of the shell color ($i =$ brown or white), S_j is the j^{th} effect of the internal volume ($j =$ empty egg or whole egg), $G_i \times S_j$ is the effect of interactions between the internal volume and shell color, and e_{ijk} is the random error. Interactions were excluded from the initial model when they were not significant.

Furthermore, a canonical discriminant analysis (**CDA**) was carried out to discriminate the main variables that differentiate eggs according to internal volume and color, and which variables have discriminatory power. The general CDA model was:

$$Z_n = \propto + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n$$

Where: Z_n is the dependent variable (shell insulation), $\propto$ is the intercept, X_i is the explanatory variable and β_i is the discriminant coefficient for each explanatory variable. Discriminant power was assessed by % variance, Wilks' Lambda statistic and standardized coefficients. The Stepwise method was used to determine which environmental variables have the greatest influence on egg differentiation. All analyzes were performed in SPSS.

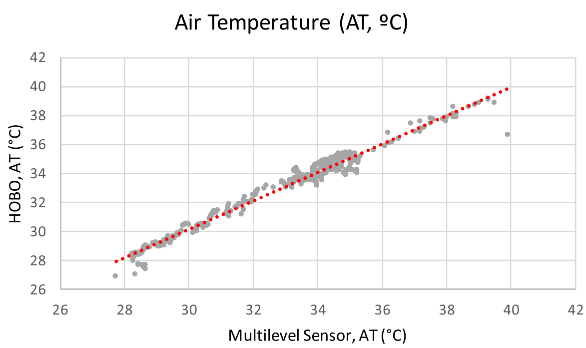
RESULTS AND DISCUSSION

Multilevel Sensor Calibration

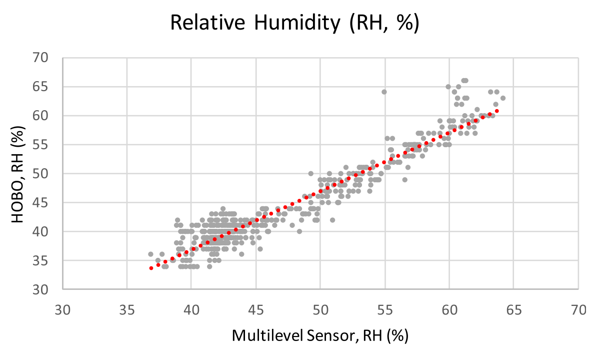
The first level of interest in this study is the incubator environment (external environment). As variables of interest at this level, temperature and relative humidity values collected by the developed sensor were compared with the reference sensor. The distribution of data, according to the regression graph, is presented in Figure 4. It is noted that, for both cases, the linear regression model (line in red) is the one that best represents the 2 sensors evaluated, which is characteristic in other temperature and relative humidity calibration processes (Santos et al., 2019, Koestoer et al., 2019; Pereira and Ramos, 2022). The ambient temperature reading range was between 27.7 and 39.9 °C for the commercial equipment and 26.9 and 39.2 °C for the multilevel sensor. For relative humidity, the range was between 36.9% and 64% for the commercial device and 34% and 66% for the proposed sensor. In this way, the calibration range is within the reference values which are possible on the commercial hatchery machine (Bergoug et al., 2013; Tona et al., 2022).

The second level of interest in this study is the interior of fertile eggs (internal environment). In this case, Figure 5 presents the regression graphs for internal temperature (5A), surface temperature (5B) and luminosity (5C). Just like in the internal environment monitoring, the adjustment curve used in all cases was the linear regression model, a behavior already expected for such physical quantities (Koestoer et al., 2019; Pereira and Ramos, 2022; Beyaz and Gül, 2022).

The reading range for shell temperature calibration was between 8.2 and 50.9 °C for the commercial equipment and 8.9 and 50.8 °C for the multilevel sensor. For surface temperature, the reading range was

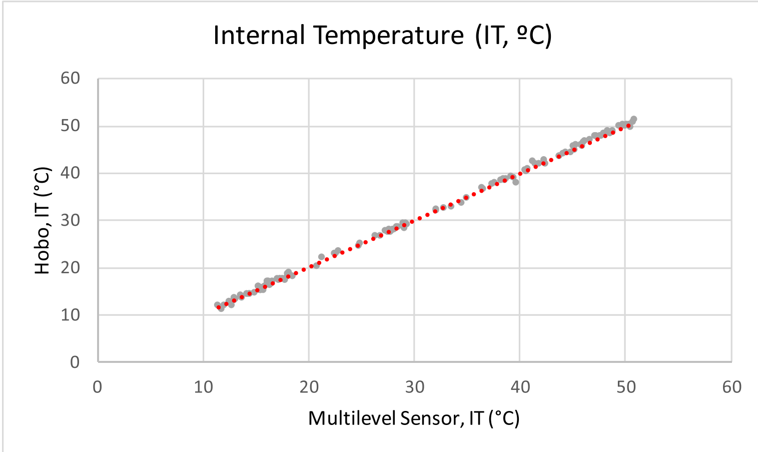


(A)

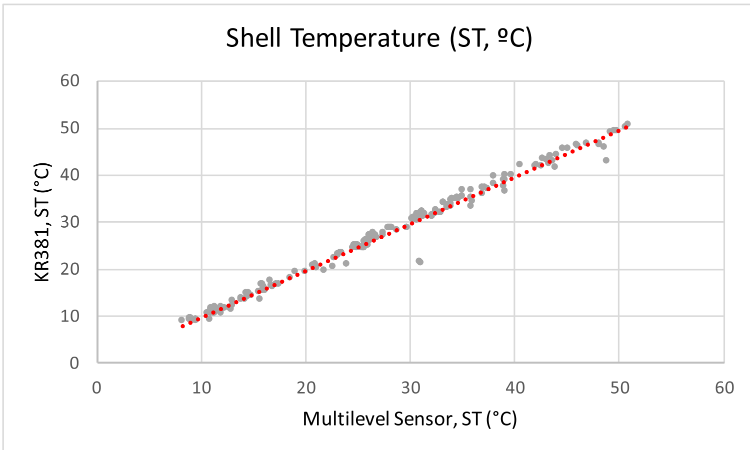


(B)

Figure 4. Regression graphs for variables collected from the external environment - ambient temperature (A) and relative humidity (B) - comparing the commercial device with the multilevel sensor.



(A)



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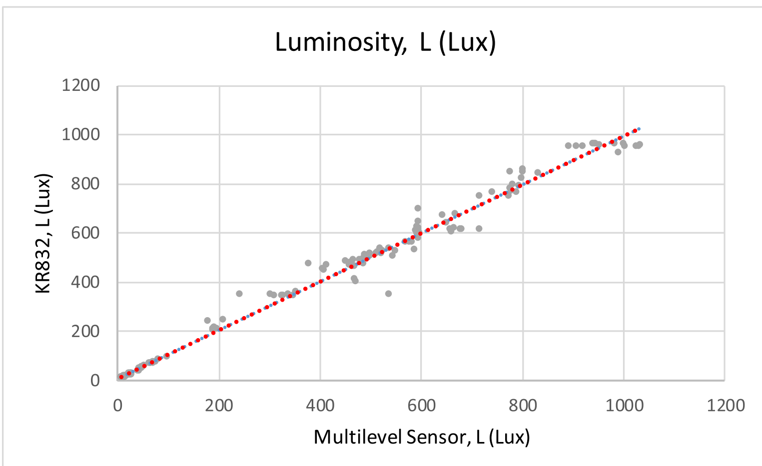

(C)

Figure 5. Regression graphs for variables collected from the internal environment - internal temperature (A), shell temperature (B), and luminosity (C) - comparing the commercial devices with the multilevel sensor.

between 11.5 and 50.8 °C for the reference equipment and 11.2 and 51.2 °C for the sensor developed in this project. As for luminosity, the collection range was between 6.2 and 1,033.0 lux for the commercial lux meter, while the multilevel sensor presented a range of 7.8 and 962.5 lux.

Sound pressure level values were also collected and compared with a commercial decibel meter. In this case,

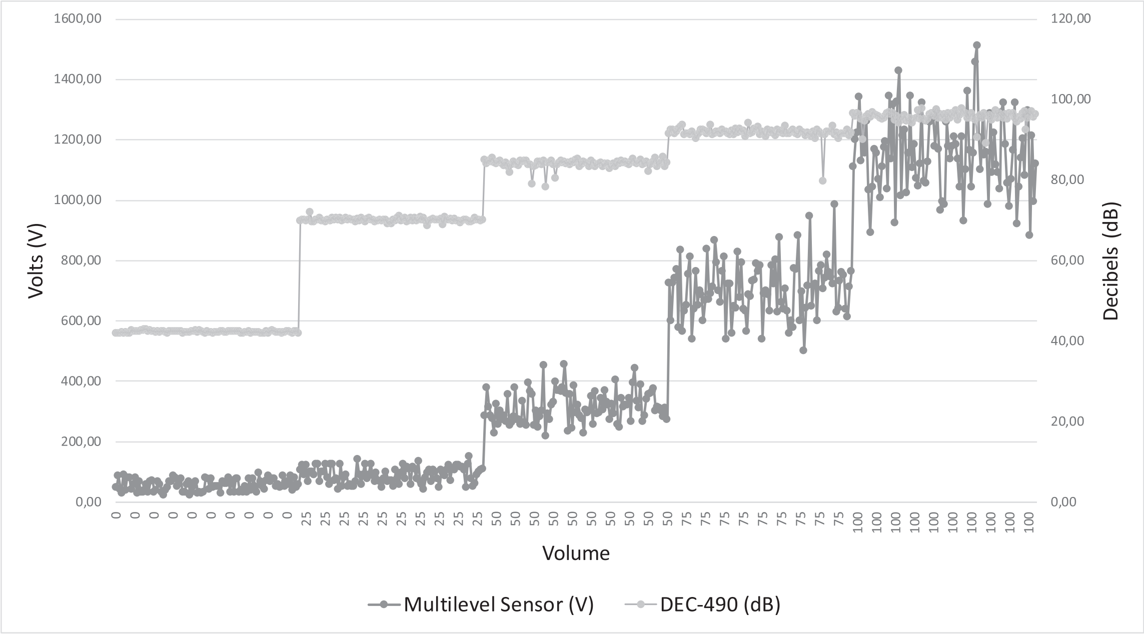

Figure 6. Reading variations of the commercial decibel meter (dB) and the multilevel sensor (V), according to the increase in tested volumes.

it is important to highlight that, while the commercial device offered the result directly in decibels (**dB**), the multilevel sensor offered the final result in volts (**V**), exhibiting a different data behavior than the previously evaluated variables. Figure 6 shows the distribution of the 2 databases, with 5 volume variations.

In the commercial decibel meter, the values ranged between an average of 42 dB and standard deviation of 0.21 dB, at minimum volume, and an average of 96 dB and standard deviation of 1.34 dB at maximum volume. As for the multilevel sensor, the values ranged from 57 V, standard deviation of 19.51 V at minimum volume and 1,157.00 V, with standard deviation of 125.91 V at maximum volume. The response pattern of the data corroborates Donofre et al. (2018), although the magnitudes are different considering that the volumes analyzed in the calibration were different and the physical characteristics of the Microphone + LM386 modules are different.

For calibration and conversion of values in V to dB, we adopted the analysis methodology presented and detailed by Donofre et al. (2018) and Feitosa et al. (2014), who had the same intention of converting an electrical quantity to a decibel scale. In this case, the regression model that best adapted to the sample set was the polynomial, in line with what is presented by reference methodologies. Robin and Plante (2022) explain this phenomenon by detailing that the sound pressure level is measured in decibel units by commercial decibel meters, which have a logarithmic scale as their

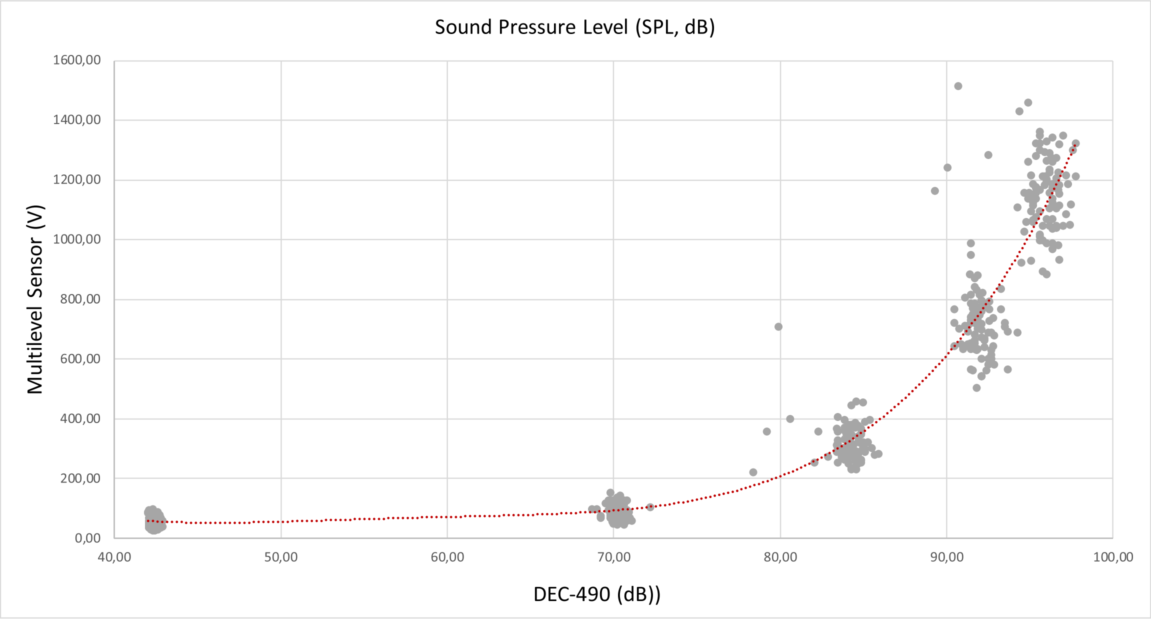

Figure 7. Regression graph of sound pressure level analysis, relating the commercial device with the multilevel sensor.

Table 1. Calibration equations and coefficient of determination of physical variables collected by the multilevel sensor (n = database size).

Variable	n	Calibration equation	Coefficient of determination (R^2)
		External environment	
Air temperature (AT, °C)	546	$y = 0.9988x + 0.81$	0.9755
Relative humidity (RH, %).	546	$y = 1.0115x - 3.5254$	0.9152
		Internal environment	
Surface temperature (ST, °C)	200	$y = 0.9936x - 0.1105$	0.9870
Internal temperature (IT, °C)	130	$y = 0.9986x + 0.0376$	0.9925
Luminosity (L, lux)	172	$y = 0.9879x + 6.1834$	0.9897
Sound pressure level (SPL, dB)	486	$y = 0.0005x^4 - 0.1051x^3 + 8.96x^2 - 336.29x + 4732.4$	0.9315

measurement base. The scatterplot for this variable is presented in Figure 7.

Table 1 summarizes the calibration equations and the coefficient of determination for all variables collected by the multilevel sensor. It is recalled that, except for sound pressure level, calibration equations represent a linear relationship, whereas the sound pressure level equation is a fourth-degree polynomial. Furthermore, all calibrated devices present coefficients of determination (R^2) above 0.90, with the lowest value of R^2 being recorded for the relative humidity of the air ($R^2 = 0.9152$) and the maximum value was for the internal temperature ($R^2 = 0.9925$).

Calibration is a fundamental part of the development and technical feasibility of monitoring equipment. Therefore, when evaluating new sensors, authors have incorporated the coefficient of determination (R^2) as an evaluation parameter. For the temperature variables, a high R^2 was expected, which is recurrent in other studies. Pereira and Ramos (2022), for example, obtained $R^2 = 1.000$ when comparing a DHT22 electronic module - also used in this study - with a commercial thermometer and Santos et al. (2019), similarly, obtained an $R^2 = 0.997$ when developing and calibrating an ambient air temperature sensor.

The literature presents varied R^2 when relative humidity sensors are calibrated. Koestoer et al. (2019) recorded an $R^2 = 0.907$ when comparing the DHT22 with a commercial hygrometer. On the other hand, Pereira and Ramos (2022) obtained R^2 between 0.883 and 0.998 for this same variable, when evaluating 4 different types of humidity sensors. Therefore, the R^2 presented in this study for relative humidity does not differ from other studies.

For luminosity, Beyaz and Gül (2022) obtained $R^2 = 0.992$ when testing the TEMT600 module, the same one used in prototyping the multilevel sensor, being close to the result obtained in this study. The aforementioned authors still achieved $R^2 = 1.000$ when using more sophisticated lux meters. Donofre et al. (2018), when developing a miniaturized decibel meter, obtained $R^2 = 0.984$ when compared with a commercial decibel meter, obtaining a higher correlation than that observed in this study.

Therefore, in general, the results of this study are in line with those presented in literature for all variables measured. Cunha and Martins (2004) argue that coefficients of determination greater than 0.90 are highly recommended when comparing sensors, ensuring high agreement between data collected by a newly developed device and reference (commercial) equipment. In this logic, all variables evaluated presented satisfactory R^2, above the minimum recommended value.

However, it is crucial to emphasize that each sensor is unique when it comes to the calibration process. As emphasized by Dias Neto et al. (2016), tuning curves should only be considered for the calibration of a specific sensor, as they can vary between sensors due to the unique electronic and microelectronic properties of the components of each equipment. Furthermore, deterioration and abrasion of sensors over time require periodic recalibrations, as reading errors tend to become recurrent as devices age. Therefore, calibration must be a continuous and customized process for each sensor, aiming to guarantee accuracy throughout the useful life of the measuring equipment.

Practical Application of the Multilevel Sensor in a Simulated Incubation

A descriptive analysis was made of the external variables collected by the multilevel sensor. For ambient temperature, the minimum value obtained was 35.11 °C, maximum value 37.93 °C, average 37.2 °C and standard deviation 0.52 °C. As for relative humidity, the minimum value recorded by the multilevel sensor was 32.03%, maximum value 58.69%, average 44.51% and standard deviation 8.37%. It is noteworthy that the *set-point temperature* was 37.5 °C and the relative humidity was 55%, as recommended by the incubator manufacturer and in accordance with what is necessary for the embryonic development of birds (Donofre et al., 2020). Therefore, during the experimental period, it can be stated that the average temperature value was 0.3 °C lower than the ideal, while humidity varied by 10.5% from the incubator reference value. The differences in relative humidity can be explained, mainly, by the constant opening of the incubator to handle the eggs and carry out other assessments.

Table 2 summarizes the results obtained for egg weight, in addition to measurements of internal egg

Table 2. Weight and internal variables of eggs, according to their shell color and internal volume.

Parameters	Egg color (**C**)		Internal volume (**IV**)		SEM	P - value		
	White	Brown	Empty	Full		C	IV	C*IV
Weight, g	30.84	31.18	6.81	55.22	24,620	0.661	< 0.0001	0.568
Surface temperature,°C	34.12	33.75	33.73	34.15	1,620	0.261	0.203	0.203
Internal temperature,°C	35.39	35.35	34.97	35.77	0.910	0.827	< 0.0001	0.560
Luminosity, Lux	26.52	15.69	19.50	22.70	6,720	<0.0001	< 0.0001	0.194
Sound Pressure Level, dB	76.20	76.68	79.21	73.67	8,500	0.772	0.001	0.316

Abbreviation: SEM, standard error mean.

variables made with the multilevel sensor. Comparing the samples by shell color (brown or white) and internal volume (empty or full), no interaction was observed between these 2 factors for any variable evaluated in this study ($P > 0.05$). Regarding shell colors, luminosity is the only quantity with statistical difference ($P < 0.0001$), since eggs with white shells accumulated greater intensity of internal light when compared to eggs with brown shells. When comparing eggs by their internal volume, it was observed that full eggs are heavier and accumulate a higher internal temperature and luminosity compared to empty eggs ($P < 0.0001$). On the other hand, empty eggs had a higher internal sound pressure level ($P < 0.001$).

When considering the average luminosity values obtained, it was observed that a white shell and a brown shell isolate 79.6% and 87.9% of the luminosity, respectively, when exposed to an environment with 130 lux. Moreover, empty eggs isolate 85.0% of external light, while full eggs isolate 82.54%. Results indicate, therefore, that external lighting influences the interior of eggs, as the composition of the eggshell does not represent a complete barrier against the passage of light. Therefore, although several studies have focused only on external lighting and the consequent impact on embryogenesis success (Tong et al. 2018; Li et al., 2021; Hanafi et al., 2023), recent studies already indicate that the composition and color of the shell also play a role in influencing the hatchability of eggs. Orellana et al. (2023), for example, observed that less translucent eggshells showed a higher percentage of hatchability (6.9% more) with chicks weighing 0.7 g heavier when compared to more translucent eggs. Furthermore, as the authors also mention, knowing the luminous insulation of the shell can also be an indirect indication of its thickness, another physical variable of great scientific and commercial interest.

When analyzing the average sound pressure level values, it was observed that full and empty eggs presented insulation of 18.8% and 11.9%, respectively, when exposed to an environment of 90 dB. Donofre et al. (2018), when exposing eggs to environments with 70 and 90 dB, also pointed out that, in addition to sound pressure levels varying according to the internal content of the egg, shell insulation also varies according to the external volume applied. The results of this study therefore reinforce that the eggshell is a barrier to sound, but the embryo is still exposed to a fraction of the sound present in the external environment, which can impact its development (Hanafi et al., 2023). However, more in-depth studies in bioacoustics are necessary to investigate the propagation of sound waves in a complex and closed structure, such as the egg.

Even though the temperature of the shell, in direct contact with the external environment of the incubator, did not present a statistical difference for any of the factors evaluated, the internal composition of the eggs is a factor to be considered in order to understand their thermal insulation. As it is vital for the incubation process, since the dependence on an external source of heat is fundamental for the embryogenesis of birds, understanding thermal exchanges and heat transfers is already a frequent study objective in incubation research (Turner et al., 1990; Van Brecht et al., 2005; Lourens et al., 2005; Bergoug et al., 2013). Based on this, the differences presented in this study for the intact egg and the empty egg suggest that in addition to the thermal insulation of the shell, there is isolation from the evaluated medium, air or liquid (albumen/yolk). Since air is a more efficient thermal insulation than liquid (Bergman et al., 2011), it is reasonable that empty eggs have a lower internal temperature than full eggs.

Finally, Figure 8 presents the graphic result of the CDA, thus discriminating which the main variables that differentiate eggs according to internal volume and shell color are, and which variables have discriminatory power. The first 2 canonical discriminant functions were significant ($P < 0.001$) and explained 100% of the data variation. A classificatory dynamic was observed with 92.7% of the eggs correctly classified into their group of origin, with the eggs being grouped more closely according to their internal volume, regardless of the color of the shell. According to the canonical discriminant analysis using the stepwise method for variables in studies (Table 1 − Supplementary Material) weight and luminosity are the main variables that presented discriminatory power.

The canonical discriminant analysis therefore indicates that egg weight has a great influence on the classification of treatments discussed in this study. The weight of the egg is directly related to the internal volume present in the egg, and this volume may be represented by the yolk, albumen, or the embryo itself, if the egg is fertile. Furthermore, throughout embryonic development, the weight of the egg is not constant, as the egg becomes heavier as the embryo grows (Donofre et al., 2020).

This fact therefore opens up new questions of scientific interest: What is the acoustic, light and thermal

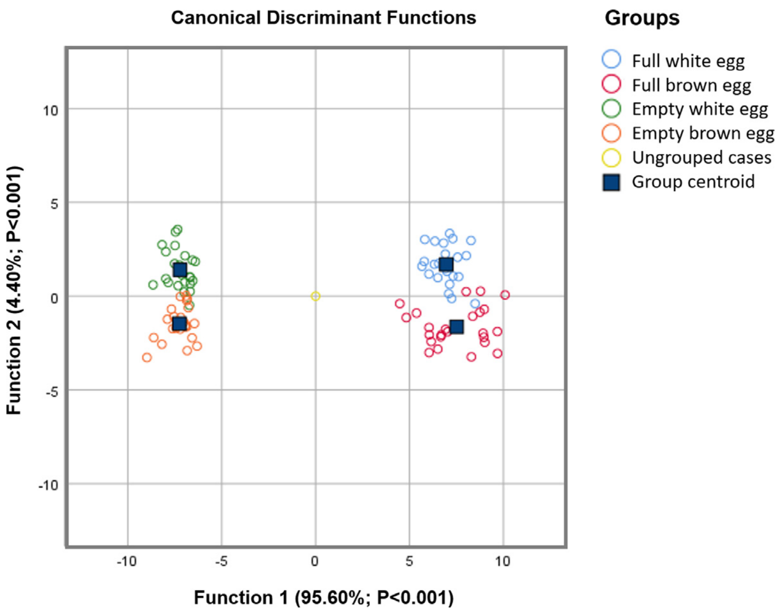

Figure 8. Biplot of the canonical discriminant analysis for weight and internal variables of the eggs, according to their shell color and internal volume.

behavior throughout an incubation process? Does this behavior change according to embryonic development and subsequent changes in the internal composition of the egg? Although answering these questions is not the interest of this study, in particular, the multilevel sensor developed in this work makes it possible to develop these new studies.

Technical Implications and Limitations

The multilevel sensor was also evaluated in this study considering its technical potential and limitations. In order to achieve this, it was considered that an efficient monitoring system must meet the following criteria: 1) Be capable of simultaneously collecting environmental variables from the incubation area and the microenvironment inside the eggs; 2) Have sensors of appropriate size for the object of study, something not always feasible with commercial devices; 3) And minimize changes made to the physical structure of the eggshell.

Meeting the first criterion, the multilevel sensor demonstrated efficiency by simultaneously recording variables from the external environment of the incubators and the interior of the eggs. Compared to commercial alternatives, the sensor has notable advantages. Using customizable prototyping tools, similarly to studies in other areas (Niranjan et al., 2021; Islam et al., 2022; Beyhan, 2023), this study presents unique equipment which integrates the collection of environmental variables of great interest for an incubation process, facilitating data collection and management. This eliminates the need for multiple commercial devices which operate in isolation and need to be spreadsheeted later in a single file. However, a limitation of this study is the presentation of a minimum viable product (**MVP**) of the sensor, which is less robust and has lower technical manufacturing rigor when compared to commercial devices (Kondaveeti et al., 2021). This trait therefore opens up opportunities for improvements in the encapsulation of the developed device and new tests when using more advanced sensor modules.

As for the second criterion, the multilevel sensor has cables and components appropriately sized for the use in the internal environment of the eggs. This work builds on the findings of Donofre et al. (2018) when developing a miniaturized sound level meter, adding new environmental variables of interest. With sensors such as a contact thermometer, infrared thermometer, lux meter and miniaturized decibel meter, the sensor enables measurements that cannot be performed with commercial equipment, expanding the frontiers of knowledge and opening up new research possibilities on thermal, acoustic and light conditions inside eggs.

The third criterion emphasizes the importance of minimizing changes made to the eggshell structure to carry out measurements. In order to insert the sensors, a hole of maximum 1 cm in diameter was made in the egg's air chamber (Figure 3A). This opening represents about 1.5% of the total surface of the egg, which has a surface area of approximately 70 cm^2 (Hughes, 1984). However, it is important to highlight that this intervention is an invasive practice and interrupts embryonic development, making it necessary to discard the egg after measurements. Another limitation, also mentioned by Donofre et al. (2018), is that the sound pressure and luminosity sensors cannot come into direct contact with the liquid medium, restricting their operation to the dry air chamber. These challenges therefore suggest the need for research focused on modeling for non-invasive estimates that consider the liquid medium. As a next step, understanding embryonic responses to variations in external stimuli, alongside with the geometric characteristics of the egg and physical properties of the shell, offers great potential for developing predictive models about the internal environment with no need to break the shell.

CONCLUSIONS

This study represents one of the first approaches to measure, simultaneously and in real time, relevant physical variables in the internal and external environment of eggs. These measurements offer a new perspective to understand thermal, acoustic and light perception to which avian embryos are exposed. The multilevel sensor demonstrated satisfactory reading quality compared to commercial devices, as evidenced by the high correlation between calibration records ($R^2 > 0.90$). It was also found that the color of the shell has a significant influence on the light insulation of the eggs, with brown shells providing greater resistance to the passage of light. Furthermore, intact eggs had higher internal temperatures, greater luminosity and lower sound pressure levels compared to empty eggs. As a future perspective, it is recommended to explore new sensor modules or predictive modeling that make the monitoring process noninvasive.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

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