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# Broiler behavior: Influence of thermal stress, age, and period of the day

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**ABSTRACT** - This study evaluated the behavior of broiler chickens subjected to thermal stress during different periods of the day and age of birds. A total of 180 one-day-old male broiler chicks were allocated to three chambers. Each chamber contained five boxes, with 12 chicks/box. Birds were assigned to a completely randomized design. The birds were filmed, and the images recorded every minute during the two periods: morning (from 08:00 to 09:00 h) and afternoon (from 17:00 to 18:00 h), at 7, 14, and 35 d of age. The frequency of the behavioral category (water intake, feed intake, resting, exploration, and comfort) was determined. Data were subjected to variance analysis for mixed models with rearing temperatures (cold, usual, and hot) and age of birds (7, 14, and 35 d) as subdivided plot, and periods of the day (morning and afternoon) as sub-subdivided plot. Birds at cold and usual temperatures showed higher feed intake than those at hot temperature in the morning. Birds exposed to cold and usual temperatures explored the environment less frequently at 35 d when compared with 14 d. However, chickens at hot temperature showed decreased exploration according to the increase of age. Birds spent more time feeding during the morning, regardless of age and rearing temperature. Also, during this period of the day, chickens spent most of the time exploring the environment. The frequency of feed intake and exploration decreases with the increase of age. The similarity of behavior between chickens reared at cold and usual temperatures may be due to a change in the thermal comfort zone of the birds. It suggests that the real thermal comfort temperature is between the two temperature ranges studied (cold and usual).

**Keywords:** animal behavior, birds, heat stress, rearing temperature, welfare

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## 1. Introduction

The environmental conditions in which poultry are reared may affect productivity and are an important study area of the poultry industry. It is known that heat stress affects profits, especially in tropical areas (Piestun et al., 2011). Furthermore, broilers have a thermoregulatory system, which is more adapted for heat retention, rather than dissipation, thereby acerbating heat stress and limiting productivity.

To facilitate heat loss, birds show behavioral changes such as panting, depression, holding wings away from the body (to expose the highly vascularized ventral region), and ruffling feathers (to promote peripheral vasodilation and intensify blood flow to the exposed surface areas) (Tan et al., 2010; Wang et al., 2018). In addition, under heat-stress conditions, birds express peripheral blood flow and decreased activity rhythm for longer periods (Sevegnani et al., 2005; Barbosa Filho et al., 2007), increased water intake, and decreased feed intake (Attia et al., 2019; Mutibvu et al., 2018).

On the other hand, in cold environments, birds exhibit cutaneous vasoconstriction, skeletal muscle tremors, and behavioral mechanisms, such as altered body posture, shrinkage, and grouping behavior (Bícego and Mortola, 2017).

Age influences some broiler behaviors. With increasing age, feeding and movement decreases, whereas sitting and lying behaviors increase (Bergmann et al., 2017). For example, Weeks et al. (2000) found that to maintain body temperature, seven-week-old birds spent an average of 76% of their time in a lying position. Broilers also adjust their behavior during the hottest period of day to maximize the dissipation of body heat and avoid overheating (Egbuniwe et al., 2018; Santos et al., 2019; Tickle and Codd, 2019).

One method to assess animal welfare is behavioral observations, analyzing critical points and discovering areas for improvement. However, there is a lack of literature correlating a bird's behavior according to the variables of rearing temperature, period of the day, and age. The combination of these variables could provide innovative information for an accurate behavioral profile for birds under ideal and stressed conditions.

Temperature bands optimal for broiler growth have been set according to temperate climates (Cassuce et al., 2013). However, these values fluctuate due to genetic evolution, growth means and management, housing density, and thermal stress severity, allowing adaptation and acclimatization of species to environmental specific conditions of different climatic regions of the world (Cassuce et al., 2013). Based on that, the temperatures currently considered as comfortable may be outdated, and research that investigates temperature ranges for broilers acclimated is essential (Cassuce et al., 2013).

For greater accuracy of rearing temperatures, the present study evaluated the behavior of broiler chickens subjected to cold, usual, and hot temperatures; the behavior was recorded in the morning and afternoon at 7, 14, and 35 d of age.

## 2. Material and Methods

This study was conducted in accordance with the ethical principles for animal experimentation, adopted by the Brazilian College of Animal Experimentation (COBEA), and the experimental procedures were approved by the local Committee for Ethical Animal Use (CEUA) with protocol n. 7377/2010, in Jaboticabal, SP, Brazil (21°14'05" South latitude, 48°17'09" West longitude, and average altitude of 615.01 m).

Two thousand (2,000) fertile eggs from 47-week-old broiler breeders (Cobb 500®) obtained from a commercial hatchery (Globoaves, Itirapina, SP, Brazil) were individually weighed and homogeneously distributed by weight (67±2 g) in ten incubators (250 eggs each) (Premium Ecológica, Belo Horizonte, MG, Brazil), all equipped with automatic temperature control and egg turning (one turn every 2 h until day 18 of incubation). Temperature and relative humidity were maintained, respectively, at 37.5 °C and 60% until day 18 of incubation, and at 36.5 °C and 70% from day 18 until hatching.

At hatching, five hundred and forty (540) male chicks were distributed homogeneously by body weight in three rearing temperature treatments: usual (recommended by the Cobb Broiler Management Guide, 2013), cold, and hot. Broilers were housed in three climatic chambers with automatic temperature control and dark/light regime (2h:22h D:L for all rearing treatments) each containing 15 boxes.

The climatic chambers had automatic exhaust fans, heating lamps, and cooling blocks, programed to the desired temperature treatment (Figure 1). Three thermohygrometers (Instrutemp, ITHT 2250, SP, Brazil), installed at bird height in the center of the chambers, were used to measure the temperatures. The maximum and minimum temperatures were measured at 8:00 and 17:00 h daily.

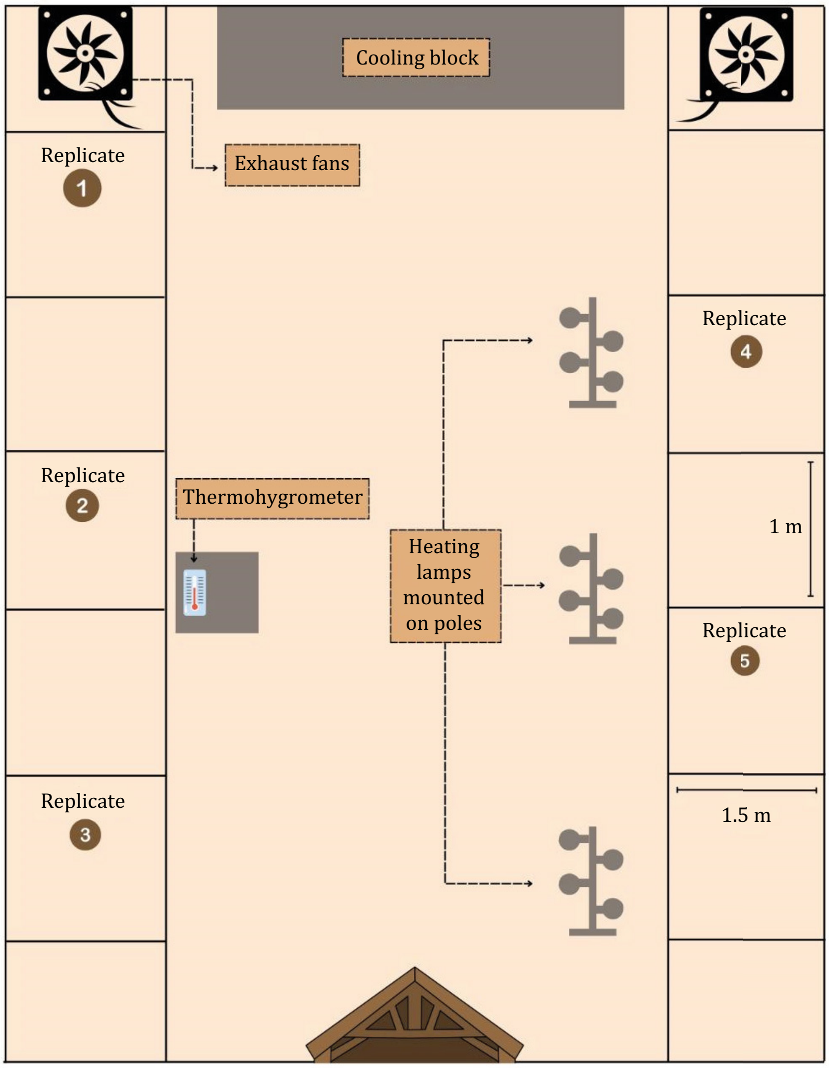



**Figure 1 -** Climatic chamber used with the necessary equipment to control and measure temperature.

Five replicates (boxes) with 12 broilers per incubation temperature were housed in each climatic chamber, totalizing 15 boxes (1.5 × 1 m) with the floor covered with wood shavings.

One climatic chamber was used for each temperature treatment (Sgavioli et al., 2019). The weekly average temperatures inside the chambers (cold, usual, and hot), up to the sixth week of age, are shown in Figure 2.

Broilers were raised up to 42 days of age receiving water and diet *ad libitum* and were fed two diets formulated on corn and soybean meal, adjusted for two phases: starter diet (1-21 days: 2,883 kcal/kg diet metabolizable energy (ME), 21.27% crude protein (CP), 0.88% digestible methionine + cysteine,

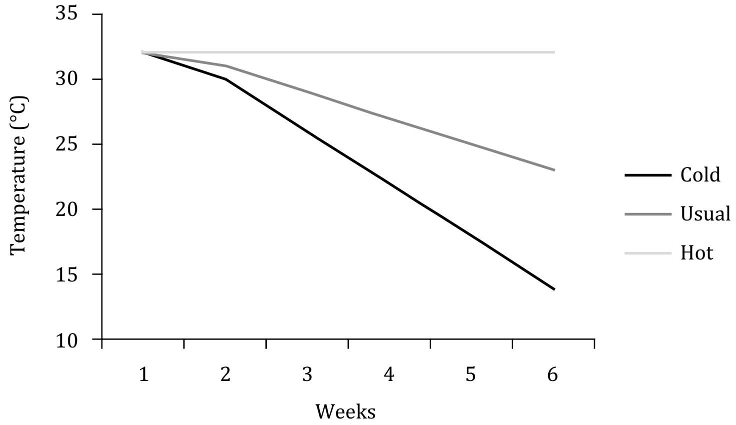



**Figure 2 -** Weekly average temperatures inside the chambers during the experimental period for the treatments.

0.56% digestible methionine, 1.22% digestible lysine, 0.85% Ca, 0.19% Na, 0.42% available P) and grower diet (22-42 days old: 3,121 kcal/kg diet ME, 18.86% CP, 0.77% digestible methionine + cysteine, 0.49% digestible methionine, 1.05% digestible lysine, 0.69% Ca, 0.20% Na, 0.32% available P), following the nutritional requirements established by Rostagno et al. (2011).

Chicks were vaccinated against Marek's Disease according to the recommended immunization schedule. The following vaccination program was completed during the experimental period: infectious bursal disease (IBD) (mild strain) on day 7 via eye drops; and Newcastle disease and IBD (hot strain) via drinking water using powdered milk as vehicle (2 g $L^{-1}$) on day 14.

Broiler behavior was recorded using a video camera (Intelbras, 420tvl 25 m infrared, Santa Catarina, Brazil) in each chamber. The cameras were installed over five boxes per chamber, where the cameras were able to record the entire box. The cameras recorded in continuous real-time mode directly to a computer system and data were stored in a standalone DVR (Luxvisio, 16 channels, 480 × 480, Rio Grande do Sul, Brazil) for 1 h twice a day (08:00 to 09:00 h in the morning and 17:00 to 18:00 h in the afternoon) at 7, 14, and 35 d old.

For each climatic chamber, the images from each one-hour period were analyzed every minute. The video frame was stopped at 60-second intervals, and the behavior of each bird was recorded (adapt of Tenório et al., 2017). A total of 120 records per day (morning plus afternoon) per bird for each of the three ages were registered. The minute-to-minute analysis was used based on a previous analysis showing the absence of a significant difference in the behavior frequency between continuous and minute-to-minute image analysis (adapted from Tenório et al., 2017).

The frequency of the following behavioral category was determined: drinking (water intake) and eating (feed intake or pecking food), resting (lying or sitting, not performing any other behaviors, birds may or may not have been sleeping), exploration (walking and/or exploring their surroundings by scratching the ground with their feet and beak), and comfort (dust-bathing; for preening behavior, birds contort to reach their uropygial glands and groom their feathers with their beaks; birds stretch out a wing and a leg of the same side of the body for stretching behavior; birds bristles their plumage and move their body laterally for bristling behavior, and birds flap and spread their wings wide open [Picoli, 2004; Pereira et al., 2013]). The data were expressed as a percent.

The data were subjected to variance analysis using the MIXED function of SAS® (Statistical Analysis System, 2002), for mixed models with three rearing temperatures (cold, usual, and hot) and age of birds (7, 14, and 35 d) as a subdivided plot and period of the day (morning and afternoon) as a sub-subdivided plot, and analyzed for the presence of outliers (Box-and-Whisker plot), normal distribution of studentized errors (Cramer-Von-Mises test), and homogeneity of variances (Brown-Forsythe).

All studied parameters were analyzed statistically using the model described below:

$Y_{ijklm}$ = μ + (rearing temperatures)$_i$ + (age of birds)$_j$ + (period of the day)$_k$ + (rearing temperatures × age of birds)$_{ij}$ + (age of birds × period of the day)$_{jk}$ + (rearing temperatures × period of the day)$_{ik}$ + (rearing temperatures × age of birds × period of the day)$_{ijk}$ + $e_{ijklm}$ (Eq. 1)

in which Y is the dependent variables, μ is the general mean value, and $e_{ijklm}$ is the error term associate to sub-experimental unit.

Among the 15 different covariance structures tested for the analyses, the one that best fitted the statistical model was chosen based on the lower value of the corrected Akaike information criterion (AICC) (Wang and Goonewardene, 2004). The model included fixed effects of rearing temperature, age of birds, periods of the day, as well as effects of the interactions between factors. Significant differences between means (P<0.05) were compared by Tukey's test.

For broiler behavior variables, statistical analyses were carried out considering each box as experimental unit (five boxes or replicates per treatment).

## 3. Results

There was interaction between rearing temperature and age of birds (P = 0.0018) and between age of birds and period of the day (P<0.0001) for water intake behavior (Table 1). Birds at 35 d increased water intake when raised under hot temperature, compared with usual temperature (P<0.05). Regarding the cold temperature, the frequency with which the birds consumed water was higher at 7 d, when compared with 35 d (P<0.05) (Figure 3A). The frequency of water intake in the afternoon was higher at 7 d than at 14 and 35 d (P<0.05). In addition, higher water intake occurred in the afternoon at 7 d (Figure 3B).

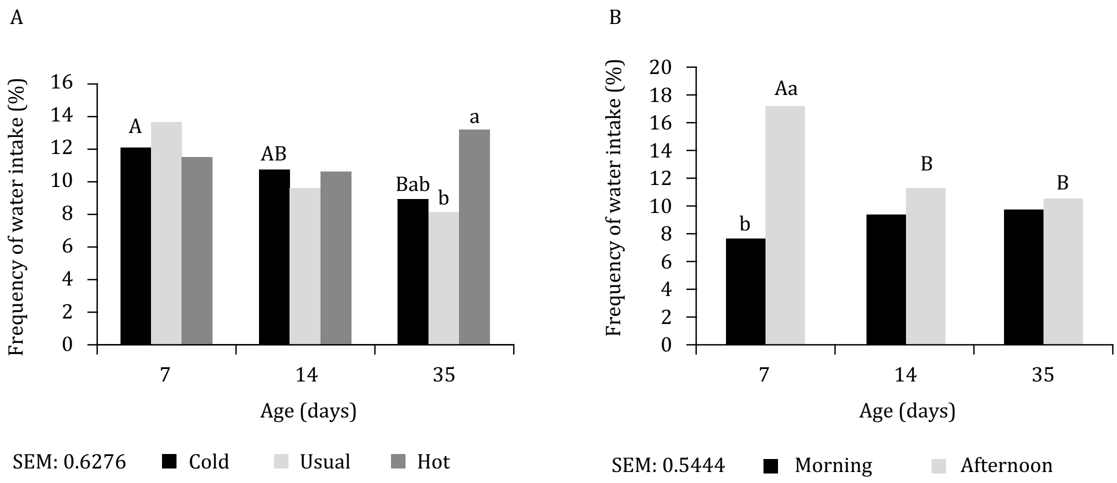
**Table 1 -** Effect of rearing temperature (RT), age, and period of the day (PD) on the behavior of broilers

| Variable | Behavior | | | | |
|---|---|---|---|---|---|
| | WIB | FEB | REB | EXP | COM |
| RT | 0.0224 | 0.0307 | 0.2138 | 0.0344 | 0.4164 |
| Age | 0.0043 | <0.0001 | <0.0001 | <0.0001 | 0.0338 |
| PD | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0744 |
| RT × age | 0.0018 | 0.0482 | 0.0014 | <0.0001 | 0.9311 |
| RT × PD | 0.9377 | 0.0217 | 0.2677 | 0.0060 | <0.0001 |
| Age × PD | <0.0001 | <0.0001 | 0.0180 | <0.0001 | <0.0001 |
| RT × age × PD | 0.0560 | 0.0821 | 0.3494 | 0.0715 | 0.2317 |
| SEM | 0.011 | 0.573 | 1.196 | 0.768 | 0.224 |

WIB - water intake behavior; FEB - feeding behavior; REB - resting behavior; EXP - exploratory behavior; COM - comfort behavior, (P<0.05); SEM - standard error of the mean.

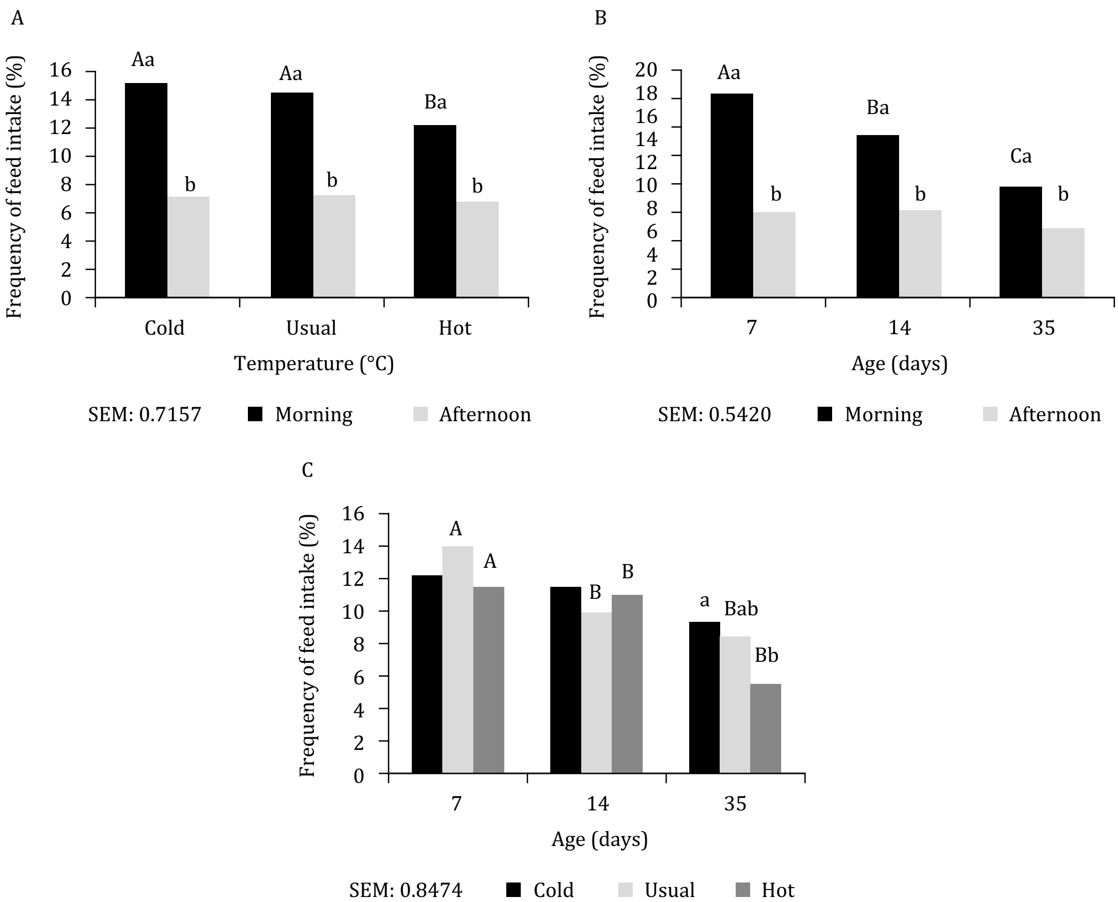
The behavior of feed intake was influenced by the interaction between rearing temperature and age of birds (P = 0.0482), rearing temperature and period of the day (P = 0.0217), and age of birds and period of the day (P<0.0001) (Table 1). In all rearing temperatures and age of birds, feed intake was higher (P<0.05) in the morning than in the afternoon (Figures 4A and 4B).

Birds at cold and usual temperatures had higher feed intake (P<0.05) than those at hot temperature, in the morning (Figure 4A). Frequency of feed intake during the morning decreased with the increase of age (P<0.05) (Figure 4B). Birds subjected to usual and hot temperatures had higher feed intake at 7 d when compared with 14 and 35 d (P<0.05). At 35 d, higher feed intake occurred for birds raised under cold temperature (P<0.05) when compared with hot temperature (Figure 4C).




A-B; a-b - Distinct upper- and lowercase letters indicate significant difference between age and rearing temperature, respectively (P<0.05).
A-B; a-b - Distinct upper- and lowercase letters indicate significant difference between age and period of the day, respectively (P<0.05).

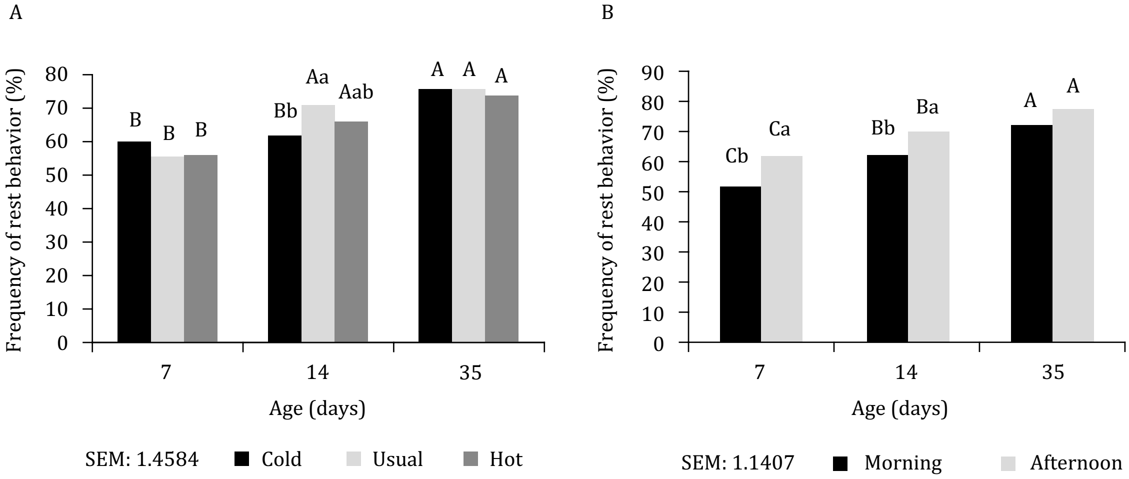
**Figure 3 -** Frequency (%) of water intake of broilers according to (A) rearing temperature (cold, usual, and hot) at 7, 14, and 35 d and (B) period of the day (morning and afternoon) at 7, 14, and 35 d.




A-B; a-b - Distinct upper- and lowercase letters indicate significant difference between period of the day and rearing temperature, respectively (P<0.05).
A-B; a-b - Distinct upper- and lowercase letters indicate significant difference between age and period of the day, respectively (P<0.05).
A-B; a-b - distinct upper- and lowercase letters indicate significant difference between age and rearing temperature, respectively (P<0.05).

**Figure 4 -** Frequency (%) of feed intake of broilers according to (A) rearing temperature (cold, usual, and hot) and period of the day (morning and afternoon), (B) period of the day (morning and afternoon) at 7, 14, and 35 d, and (C) rearing temperature (cold, usual, and hot) at 7, 14, and 35 d.

There was interaction between rearing temperature and age of birds (P = 0.0014) and between age of birds and period of the day (P = 0.0180) for resting behavior (Table 1). Birds reared at usual and hot temperatures remained at rest in higher frequency at 14 and 35 d (P<0.05), when compared with 7 d, while birds reared at cold temperature remained at rest in higher frequency just at 35 d (P<0.05), when compared with 14 and 7 d. At 14 d, birds reared at cold temperature remained less at rest compared with those at usual temperature (P<0.05) (Figure 5A). In both periods of the day (morning and afternoon), the frequency with which the birds were at rest is greater with the increase of age (P<0.05). At 7 and 14 d, birds showed higher frequency of resting during the afternoon than in the morning (P<0.05) (Figure 5B).



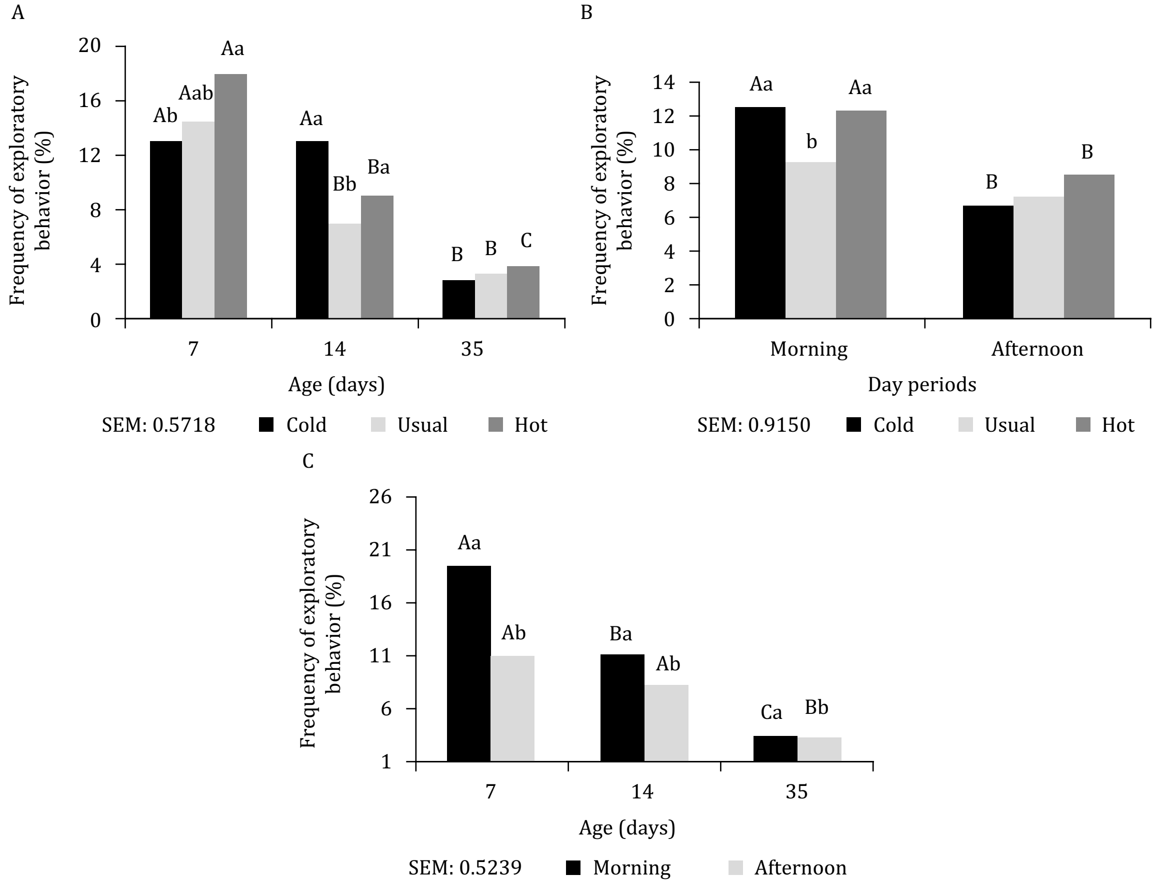

A-B; a-b - Distinct upper- and lowercase letters indicate significant difference between age and rearing temperature, respectively (P<0.05).
A-B; a-b - Distinct upper- and lowercase letters indicate significant difference between period of the day and age, respectively (P<0.05).

**Figure 5** - Frequency (%) of resting behavior of broilers according to (A) rearing temperature (cold, usual, and hot) at 7, 14, and 35 d and (B) period of the day (morning and afternoon) at 7, 14, and 35 d.

For exploratory behavior, there was an interaction between rearing temperature and age of birds (P<0.0001), rearing temperature and period of the day (P = 0.0060), and between age of birds and period of the day (P<0.0001) (Table 1).

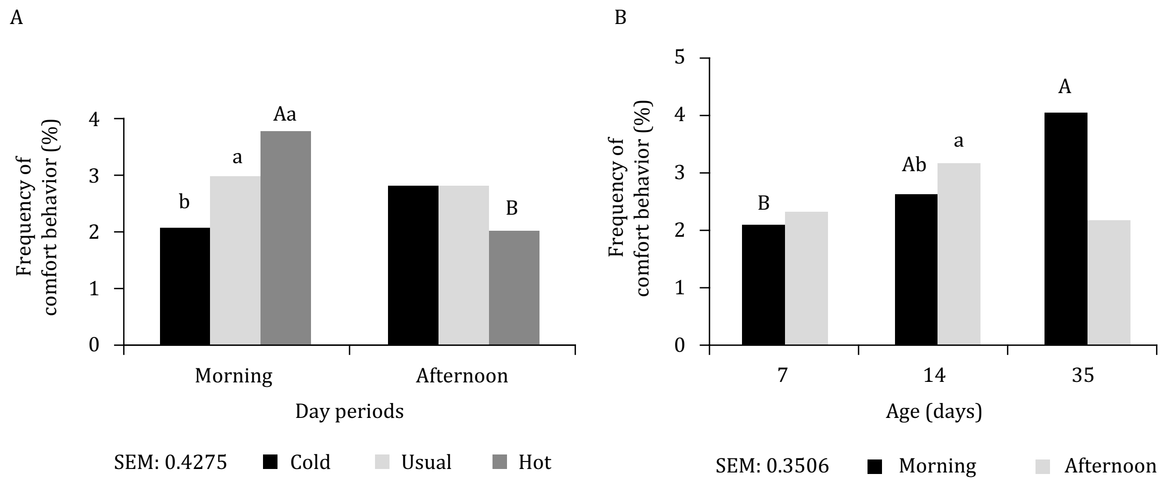
Birds exposed to cold and usual temperatures explored the environment less frequently at 35 d compared with 14 d (P<0.05). However, chickens at hot temperature showed decreased exploration according to the increase of age (P<0.05). At 14 d, the frequency of that behavior was higher for birds exposed to hot and cold when compared with usual temperature (P<0.05) (Figure 6A). Birds subjected to cold and hot temperatures explored the environment especially during the morning (P<0.05) (Figure 6B). At all ages (7, 14 and 35 d), birds explored the environment more during the morning than in the afternoon (P<0.05) (Figure 6C). In the afternoon, the birds explored the environment more at 7 and 14 d (P<0.05) when compared with 35 d (Figure 6C).

For comfort behavior (Table 1), there was an interaction between rearing temperature and period of the day (P<0.0001) and between age of birds and period of the day (P<0.0001). During the morning, birds subjected to hot and usual temperatures remained in comfort when compared with birds at cold temperature (P<0.05). Birds under hot temperature showed higher frequency of comfort behavior during the morning when compared with the afternoon (P<0.05) (Figure 7A) and, according to the age, this behavior was higher at 7 d (P<0.05). However, there was higher frequency of comfort behavior in the afternoon at 14 d (P<0.05) (Figure 7B).




A-B; a-b - Distinct upper- and lowercase letters indicate significant difference between age and rearing temperature, respectively (P<0.05).
A-B; a-b - Distinct upper- and lowercase letters indicate significant difference between rearing temperature and day period, respectively (P<0.05).
A-B; a-b - Distinct upper- and lowercase letters indicate significant difference between age and period of the day, respectively (P<0.05).

**Figure 6 -** Frequency (%) of exploratory behavior of broilers according to (A) rearing temperature (cold, usual, and hot) at 7, 14, and 35 d, (B) rearing temperature (cold, usual, and hot) and period of the day (morning and afternoon), and (C) age (7, 14, and 35 d) and period of the day (morning and afternoon).




A-B; a-b - Distinct upper- and lowercase letters indicate significant difference between rearing temperature and day period, respectively (P<0.05).
A-B; a-b - Distinct upper- and lowercase letters indicate significant difference between age and period of the day, respectively (P<0.05).

**Figure 7 -** Frequency (%) of comfort behavior of broilers according to (A) rearing temperature (cold, usual, and hot) and period of the day (morning and afternoon) and (B) period of the day (morning and afternoon) at 7, 14, and 35 d.

## 4. Discussion

This study evaluated the effects of rearing temperature, age, and period of the day on the behavior of broiler chickens. Significant effects of the analyzed factors on the evaluated characteristics (drinking, eating, resting, exploration, and comfort) were observed. Observing behavioral changes can help both producers and genetic suppliers because, in addition to being non-invasive, it can be combined with technology for automated, real-time evaluation (Fraess et al., 2016).

In this study, hot temperature increased water intake frequency in birds at 35 d, when compared with the usual temperature. In heat stress conditions, higher water intake is directly related to the increased demand for evaporative heat loss (Branco et al., 2021) and is fundamental for the maintenance of hydroelectrolytic balance (Macari and Maiorka, 2017).

Our results show that birds subjected to cold and usual temperatures presented higher frequency of feed intake during the morning and also at 35 d. On the other hand, reducing feed intake is an adaptive mechanism of birds in heat stress (Santos et al., 2019; Branco et al., 2021; Vandana et al., 2021) and is one of the main effects (Macari et al., 2002) for reducing heat from the metabolism of nutrients (Macari and Maiorka, 2017). Based on our results, we can infer that if birds are subjected to hot temperatures, feed intake is compromised. The hypothesis could be confirmed by the performance results (Ferreira et al., 2015), in which feed intake was affected by the rearing temperature, with minor feed intake for birds under hot temperature.

Birds at 14 d, raised at usual temperature, remain longer at rest than birds kept at cold temperature. This may be correlated with a higher need for heat loss due to heat transfer between the broilers and the floor litter (Li et al., 2019; Branco et al., 2021).

Regardless of the period of the day, with the increase of age there was a higher frequency of rest. Also, chickens at hot temperature showed decreased exploration according to the increase of age, while birds exposed to cold and usual temperatures explored the environment less frequently only at 35 d. These results confirm the hypothesis that resting behavior is directly related to the birds' need to keep their body temperature (Khalifa and El Iraqi, 2014). The behavioral thermoregulatory mechanism, considered the oldest one on the phylogenetic scale, is related to the contact with hot or cold surfaces (Bícego and Mortola, 2017).

Previous studies have shown that broilers spend 60 to 80% of their time resting (Nääs et al., 2018; Guo et al., 2020), and it increases as birds age, mainly due to their accelerated growth rate (Mack et al., 2013). As observed in the study, both temperatures above and below the usual (at 35 days of age) can be harmful to the welfare of birds (Rushen et al., 2011). Therefore, it is important to ensure that they have normal activity levels at every rearing stage (Zhao et al., 2014).

Birds spend more time feeding and exploring the environment during the morning, regardless of age and rearing temperature, as was observed by Barbosa Filho (2007). However, these results differ from Kristensen et al. (2006), who observed lower movement in the morning (10:00 h) and peaks in the afternoon (15:30 h).

Broilers reared under hot and usual temperature exhibit comfort behavior more frequently in the morning. There was also greater comfort behavior for birds at 14 d in the afternoon. However, it is uncertain whether the greater comfort of the birds is due to a possible influence of the circadian rhythm of the birds; therefore, more studies are needed for clarification. Furthermore, despite the preening behavior being considered a comfort behavior (Fraess et al., 2016), in the study by Branco et al. (2021), it showed high frequency during heat stress as a mechanism to relieve stress (Henson et al., 2012).

In this study, in general, it seems that birds reared in the cold temperature did not present a behavioral repertoire representative of cold stress, but rather of environmental comfort, mainly because they showed behavior similar to the birds raised under usual temperature. Additional studies on the ambient temperature recommended by the current management guides are needed. This is especially true in the final rearing stage.

Due to the wide scope and complexity of the present study, it was possible to obtain a complete overview of the behavioral pattern of broilers. Such results could serve as a basis to assist in the management of birds, because they demonstrate the peaks of feeding and exploration behaviors.

## 5. Conclusions

The behavioral similarity between broilers reared at cold and usual temperatures may be due to a change of the thermal comfort zone of the birds. It suggests that the real thermal comfort temperature is between the two temperature ranges studied (cold and usual).

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: S. Sgavioli and I.C. Boleli. Data curation: S. Sgavioli, E.T. Santos, C.H.F. Domingues, D.M.C. Castiblanco and P.H.M. Rodrigues. Formal analysis: S. Sgavioli, E.T. Santos, C.H.F. Domingues and D.M.C. Castiblanco. Funding acquisition: I.C. Boleli. Investigation: S. Sgavioli, E.T. Santos, C.H.F. Domingues, D.M.C. Castiblanco and A.R. Almeida. Methodology: S. Sgavioli, P.H.M. Rodrigues and A.R. Almeida. Project administration: S. Sgavioli and I.C. Boleli. Resources: P.H.M. Rodrigues. Supervision: I.C. Boleli. Writing – original draft: S. Sgavioli, C.P. Zeferino, A.R. Almeida and I.C. Boleli. Writing – review & editing: S. Sgavioli, C.P. Zeferino, A.R. Almeida and I.C. Boleli.

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