



Article

The Sequential Behavior Pattern Analysis of Broiler Chickens Exposed to Heat Stress

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Abstract: Broiler productivity is dependent on a range of variables; among them, the rearing environment is a significant factor for proper well-being and productivity. Behavior indicates the bird's initial response to an adverse environment and is capable of providing an indicator of well-being in real-time. The present study aims to identify and characterize the sequential pattern of broilers' behavior when exposed to thermoneutral conditions (TNZ) and thermal stress (HS) by constant heat. The research was carried out in a climatic chamber with 18 broilers under thermoneutral conditions and heat stress for three consecutive days (at three different ages). The behavior database was first analyzed using one-way ANOVA, Tukey test by age, and Boxplot graphs, and then the sequence of the behaviors was evaluated using the generalized sequential pattern (GSP) algorithm. We were able to predict behavioral patterns at the different temperatures assessed from the behavioral sequences. Birds in HS were prostrate, identified by the shorter behavioral sequence, such as the {Lying down, Eating} pattern, unlike TNZ ({Lying down, Walking, Drinking, Walking, Lying down}), which indicates a tendency to increase behaviors (feeding and locomotor activities) that guarantee the better welfare of the birds. The sequence of behaviors 'Lying down' followed by 'Lying laterally' occurred only in HS, which represents a stressful thermal environment for the bird. Using the pattern mining sequences approach, we were able to identify temporal relationships between thermal stress and broiler behavior, confirming the need for further studies on the use of temporal behavior sequences in environmental controllers.

Keywords: animal welfare; animal behavior; behavioral pattern detection; sequential pattern mining



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1. Introduction

Extreme weather events are responsible for considerable losses in agriculture worldwide. In countries located in tropical and intertropical zones such as Brazil, climate change is increasingly visible. With forecasts of high average air temperatures, climate changes, and heatwaves capable of causing substantial losses in broiler production [1,2], the management of the internal environment in poultry broiler chicken is a critical factor in maintaining the bird's well-being and productivity, as it cannot move in search of better rearing environmental conditions.

Research that improves the environment's quality for raising broilers is essential [3,4]. Previous studies show that monitoring the behavior of broilers might provide real-time information on thermal comfort in response to exposure to different thermal environments [1,5–8], since the animal is used as a biosensor [9,10], becoming a valuable non-invasive tool for environmental analysis [11]. Behavioral changes can occur quickly and

at a lower metabolic cost than physiological adjustments [12]. Nevertheless, the matters relating to the rearing environment and animal welfare are multifactorial; therefore, it is important to measure a wide range of indicators [3,13,14].

New research has been using precision livestock farming concepts, combining the automation and optimization of productive resources [15,16] to make broiler production even more competitive and reducing interference from the outside environment inside the houses. In general, merging the technology of sensors and actuators, a database of animal behavior, mathematical models, and the discovery of knowledge in the data applied together and interconnected can maximize animal production's potential concerning the environment and welfare [4,17,18]. Data mining becomes an ally for decision-making in precision livestock farming [12,19,20].

The generalized sequential pattern algorithm (GSP) is a mining method that can detect recurrent sequences that exceed a user-specified support threshold. The method was first presented by Agrawal and Srikant [21] and later applied to analyze tourist behavior [22], the learning behavior of university students after exposure to educational games [23], and the behavior of book lending transactions [24]. The mining of sequential patterns in poultry has been used to assess growing chicks' behavior under heat and cold stress [8]. However, there is no evidence of any current literature study focusing on broilers' sequential behavior close to slaughter.

This research theorizes that broilers have sequential behavior patterns, specifically under environmental rearing conditions of thermoneutrality and heat stress. We believe that identifying the behavioral sequence might help to develop a smart environmental control process based on visual flock analysis. Thus, the present study's objective is to identify and characterize the sequential behavior pattern of broilers in a thermoneutral rearing environment and under thermal stress due to constant heat exposure.

2. Materials and Methods

2.1. Housing, Animals, and Management

The experiment consisted of raising 18 male Hubbard[®] broilers in a controlled environment house. Birds in the fourth (aged 28 to 30 days old), fifth (aged 35 to 37 days old), and sixth (aged 42 to 44 days old) week of growth were exposed to continuous heat stress for 72 h (in total). The experiment was part of extensive research approved by the UFSM ethics committee (087/2012).

Initially, 600 birds were reared in a conventional experimental house (east–west orientation; 29°43'26' S; 53°43'07' W, and 113 m altitude), receiving water and mash commercial feed ad libitum (Table 1), and all rearing conditions were according to the breeders' recommendations [25]. When the birds reached the observation age, 18 broilers were moved into an environmental chamber beside the experimental broiler house. The birds were selected according to the homogeneity ($\pm 2.5\%$) of the initial flock.

Table 1. Nutritional levels * of diets according to bird age.

Nutritional Levels	Age (Week)		
	4th	5th	6th
Metabolizable energy (kcal/kg)	3153	3198	3247
Crude protein (%)	19.87	19.03	18.16
Calcium (%)	0.75	0.66	0.61
Digestible phosphorus (%)	0.29	0.28	0.26
Sodium (%)	0.20	0.20	0.19
Digestible lysine (%)	1.10	1.05	1.00
Digestible methionine (%)	0.57	0.56	0.53
Digestible methionine + cystine (%)	0.80	0.77	0.73

* As suggested by Rostagno et al. [26] and Hubbard [25].

Two days before starting exposure to thermal stress, the birds were taken to the chamber, remaining under thermoneutral conditions before thermal stress. This period was considered the adaptation of the birds to the new environmental conditions. At the end of the five days (two days of adaptation and three days of heat treatment), the birds returned to the experimental broiler house; in the following week, new birds were selected so that there was no thermal conditioning of the new birds. The birds were marked on the back with non-toxic ink to provide individual identification.

2.2. Experimental Set-Up

The chickens were distributed inside the chamber in two experimental rooms, each with nine birds. The rooms were 0.8 m wide \times 1.1 m long \times 1.1 m high (Figure 1), insulated with 0.12 m styrofoam between two plywood walls. The chamber's environmental control was carried out with commercial air conditioning (air renewal of 12 m³ h⁻¹) monitored with digital thermometers with visualization outside the room. The recommended lighting was 25 lux (white fluorescent lamp; intrutherm LDR-225 luminometer) with 16 h of light according to the breeder's manual [25]. Each pen contained a video camera positioned on the upper side; the footage was taken 24 h a day, generating videos with a resolution of 704 \times 480 pixels and 30 frames per second. Inside the chamber, a tubular feeder and nipple drinker were used in the experimental pens, and rice husk was used as litter.

The thermal stress consisted of a temperature 8 °C above the thermoneutral temperature for the birds' age (Table 2). This value was chosen because it is close to the approximate values found during Brazil's broilers' lairage. The birds were kept under constant thermal stress for three days, featuring a heatwave, described as a period with uncomfortable temperatures for at least two days above 32 °C.

Table 2. The values of temperature (°C) and relative air humidity (%) that were used to stress the broilers at the fourth, fifth, and sixth weeks of growth.

Age (Week)	Thermoneutral Temperature * (°C)	Air Relative Humidity (%)	Heat Stress (°C)	Air Relative Humidity (%)
4th	20.00 \pm 1.3	73.35 \pm 2.1	28.00 \pm 1.0	61.01 \pm 2.5
5th	19.00 \pm 0.9	69.45 \pm 2.5	27.00 \pm 1.1	57.41 \pm 2.3
6th	18.00 \pm 0.7	74.21 \pm 2.3	26.00 \pm 0.9	63.61 \pm 2.0

* Values based on Hubbard [25].

Continuous video recording of the birds was performed to assess their behavior. The dark period (8 h) [25] was not analyzed since no individual identification of the bird was possible. Thus, the total hours analyzed for each day were 16 h, totaling 48 h of video recording each week, for the heat stress and thermoneutral exposure.

The final data set was composed of 1 behavioral attribute that can assume 12 different values (Table 3) corresponding to the elaborated ethogram consisting of basic behaviors, based on previous studies related to the behavior and welfare of broilers [12,27,28].

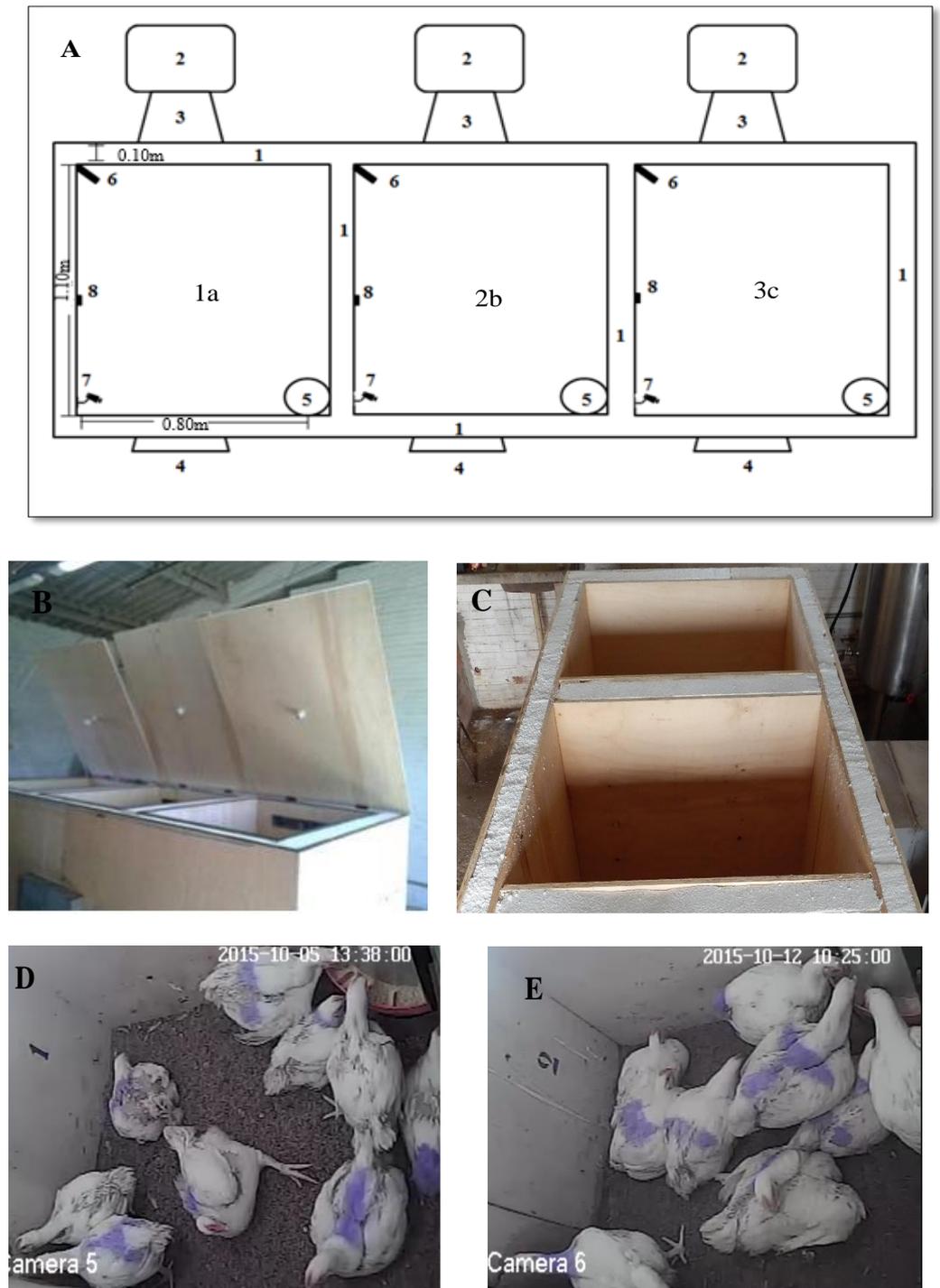


Figure 1. (A) Scheme of climate chambers. (1a: Chamber with high temperature; 2b: Chamber with comfort temperature; 3c: Chamber without experiment; 1: Internal polystyrene insulation; 2: Air conditioning; 3: Air intake; 4: Air outlet; 5: Feeder; 6: Drinker; 7: Video camera; 8: Datalogger and thermometer sensor). (B) External view of the climatic chamber and (C) inner part (under construction) showing the seal. (D) Internal view of the cameras with the birds at 35 days old and exposed to heat stress. (E) Internal view of the cameras with the birds at 42 days old exposed to thermoneutral temperature.

Table 3. Descriptive ethogram of the 12 observed broiler behaviors for the composition of the data set.

Ethogram of Observed Behaviors	
Eating	The bird is in front of the feeder and ingests feed
Drinking	The bird is in front of the drinker and ingests water
Foraging	The bird stands in an upright position and uses both feet to peck at or move litter material in search of food
Lying down	The bird lies in the litter while the head is resting on the ground or is erect
Walking	The bird moves at a slow pace
Running	The bird moves at a fast pace (at least three steps quicker than normal * walking)
Preening	The bird cleans and aligns the feathers using the beak
Litter pecking	The bird pecks the litter with the beak
Wing flap	Flaps wings while standing on the ground
Dust bathing	Bathing in the dust with the use of wings, head, neck, and legs
Stretching	The bird stretches one wing and one leg of the same body hemisphere
Lying laterally	The bird lies laterally with a stretched leg

* Nääs et al. [29].

After the video recording and storage, the images were analyzed using the methodology proposed by Schiassi et al. [6], equivalent to 10 continuous minutes every hour. For every 60 min, the intermediate 10 min were used for analysis continuously. The behaviors were recorded in a continuous sequence for later analysis. For example, if a bird (Bird_x) was eating after walking and drinking water and then lying down, the database sequence was described as Bird_x = <Eating, Walking, Drinking, Lying down>. A different behavior was considered when the bird presented at least 10 s performing a particular behavior and changing to a new one [30,31].

2.3. Data Analysis

The frequency data of the behaviors (lying down, eating, walking, preening, lying laterally, drinking, and dust bathing) were used for a one-way analysis of variance (ANOVA) and Tukey test (95% confidence) comparing the environmental condition factor—thermoneutral (TNZ) and thermal stress (HS)—with 16 repetitions per day/age (28, 29, and 30 days of age; 35, 36, and 37 days of age; and 42, 43, and 44 days of age) and in each ambient condition, totaling 48 N for the thermoneutral condition and 48 N for the thermal stress condition.

Then, we used the generalized sequential pattern (GSP) algorithm proposed by Agrawal and Srikant [21] and Srikant and Agrawal [32] to describe the birds’ behavioral sequence. The GSP algorithm was designed for mining sparse and generalized sequential patterns that are repeated over time. A description of the GSP algorithm’s operation can be found in Bureva et al. [33] and Branco et al. [8].

The sequence pattern mining approach finds repeated strings that exceed the minimum support limit declared by the user. The support of a sequence is the percentage of a finite ordered list of elements in the database that contain the sequence.

The support value of any given sequence reveals how frequent this sequence is. To calculate the support of a sequence, we use Equation (1).

$$\text{support}(s) = \frac{|\text{Number of occurrences } S|}{|\text{Total of sequence in the data set}|} \rightarrow [0; 1] \tag{1}$$

A sequence S consists of a list of temporally ordered behaviors. For example, a sequence of behaviors is described as S = <i₁, i₂, . . . , i_n>, where item i₁ occurs before item i₂, which occurs before item i₃, and so forth. The number of elements in a sequence is the length of that sequence. In this study, the example sequence, S = <Walking, Eating, Stretching, Lying down> has a size of 4 and means that it is normal for the bird to show behaviors in that order. For this study, the user’s support value was 40%, based on the database’s initial analysis, and the value shows the most relevant and non-repeated strings (as opposed to cases with a support value below 40%).

Weka software [34] was used to perform the sequential pattern mining task (GSP), designed to aggregate algorithms from different approaches/paradigms in the sub-area of artificial intelligence dedicated to the study of machine learning.

3. Results

The current study presents the frequency of the broilers' main behaviors during the final growth period (4th, 5th, and 6th weeks; Table 4) and the most relevant behavior sequences (Table 5), taking into account the chronological occurrence order.

Table 4. Average frequencies of observed behaviors under HS and TNZ compared by the Tukey test during the 4th, 5th, and 6th week of growth.

Age (Wk)	Ambient Condition	Observed Behavior						
		Lying Down	Eating	Walking	Preening	Lying Laterally	Drinking	Dust Bathing
4th	HS	18.17 ± 6.10 a	9.31 ± 3.22 a	18.00 ± 8.88 a	4.65 ± 2.49 a	1.65 ± 1.51 a	4.00 ± 2.63 a	0.167 ± 0.78 a
	TNZ	17.35 ± 4.69 a	10.56 ± 3.75 a	15.63 ± 9.49 a	2.92 ± 2.14 b	0.40 ± 0.74 b	3.90 ± 3.23 a	0.104 ± 0.31 a
	<i>p</i> -value	0.466	0.083	0.233	0.0001	0.0001	0.863	0.607
5th	HS	18.06 ± 5.66 a	7.77 ± 3.02 a	11.54 ± 6.86 a	3.85 ± 1.87 a	2.31 ± 1.79 a	4.08 ± 2.44 a	0.19 ± 0.70 a
	TNZ	16.15 ± 4.98 a	6.63 ± 2.90 a	11.88 ± 8.06 a	3.73 ± 2.29 a	0.38 ± 0.98 b	2.79 ± 2.29 b	0.04 ± 0.20 a
	<i>p</i> -value	0.082	0.061	0.828	0.770	0.0001	0.009	0.171
6th	HS	18.86 ± 5.23 a	7.00 ± 2.82 a	11.02 ± 6.34 a	4.33 ± 2.11 a	1.54 ± 1.25 a	4.46 ± 2.70 a	0.21 ± 0.92 a
	TNZ	18.98 ± 5.61 a	8.02 ± 2.88 a	10.71 ± 7.56 a	3.48 ± 1.81 b	0.71 ± 0.94 a	3.50 ± 2.53 a	0.06 ± 0.32 a
	<i>p</i> -value	0.925	0.082	0.827	0.036	0.0001	0.076	0.303

Table 5. Main patterns of sequential behavior for thermoneutral conditions and thermal stress (sequence size ranging from 2 to 7).

Age (Week)	The Pattern of Sequential Behaviors	
	Thermoneutral Temperature	Heat Stress
4th	<{Eating, Lying down, Eating}> (n = 6)	<{Lying down, Preening, Walking, Eating}> (n = 5)
	<{Lying down, Walking, Drinking, Walking, Lying down }> (n = 5)	<{Lying down, Preening, Lying laterally}> (n = 4)
	<{Lying down, Eating }> (n = 7)	<{Lying down, Eating }> (n = 4)
	<{Eating, Walking, Lying down }> (n = 4)	<{Eating, Lying down }> (n = 4)
	<{Eating, Walking, Lying down, Walking, Eating}> (4)	<{Lying down, Lying laterally}> (n = 5)
	<{Lying down, Walking, Eating}> (n = 5)	<{Lying down, Preening}> (n = 5)
5th	<{Eating, Walking, Lying down, Walking, Eating, Walking, Lying down}> (n = 4)	<{Eating, Lying down}> (n = 7)
	<{Lying down, Preening}> (n = 6)	<{Lying down, Eating, Lying down}>(n = 6)
	<{Lying down, Eating }> (n = 4)	<{Lying down, Preening}> (n = 4)
	<{Lying down, Walking, Drinking}> (n = 6)	<{Lying down, Eating, Walking, Lying down}> (n = 4)
	<{Lying down, Walking, Eating}> (n = 4)	
	<{Eating, Lying down}> (n = 6)	
6th	<{Eating, Walking, Lying down, Walking, Eating}> (n = 4)	<{Lying down, Preening, Lying laterally}> n = (4)
	<{Lying down, Preening}> (n = 9)	<{Lying down, Walking, Eating, Lying down}> (n = 4)
	<{Lying down, Eating, Lying down}> (n = 8)	<{Lying down, Preening}> (n = 7)
	<{Eating, Walking, Lying down}> (n = 4)	<{Lying down, Lying laterally}> (n = 4)
	<{Lying down, Eating}> (n = 5)	<{Lying down, Eating, Lying down}> (n = 5)
	<{Eating, Lying down}> (n = 7)	<{Eating, Lying down }> (n = 7)

n = number of broilers performing the described behavior for the support of 40%.

3.1. The Behavioral Frequency Approach

The 'Preening' behavior showed significant frequency during the 4th week of growth (4.65 ± 2.49 , $p = 0.0001$) and during the 6th week (4.33 ± 2.11 , $p = 0.036$), indicating higher values in the tested condition HS, when compared to TNZ. The 'Lying laterally' behavior was significant during all studied ages (1.65 ± 1.51 , $p = 0.0001$; 2.31 ± 1.79 , $p = 0.0001$; 1.54 ± 1.25 , $p = 0.0001$), suggesting high incidence in the HS condition compared to TNZ. The 'Drinking' behavior was also significant in the 5th week of growth when broilers were exposed to HS compared to those inside a TNZ area (4.08 ± 2.44 , $p = 0.009$) (Table 4).

Means that do not share the same letter (a, b) differ by Tukey test (95% confidence).

When exposed to heat stress, broilers showed a higher frequency of some behaviors concerning TNZ, such as increased water cooler use, bed bath, and lying sideways under the bed, regardless of age. Birds experiencing thermal comfort usually feed regularly since the feeding behavior is the first indicator of bird welfare. In the present study, broilers have increased water consumption in HS and higher feed consumption in TNZ. We found that, during heat stress, broilers were lying on their side at all ages ($p < 0.001$), and this is a behavior related to thermal stress.

3.2. The GSP Algorithm's Approach

Unique behavior patterns were found, which corresponds to the total number of birds remaining in that behavior during the observation period, such as {Eating} and {Lying down}, comprising a unique sequence. These behaviors were observed at both ages and temperatures assessed, ensuring a natural broiler pattern. Some behaviors were not seen because they had less than 40% support, among them 'Foraging' and 'Running' [8].

The most relevant behavior sequences obtained using the GSP algorithm are shown in Table 5. The GSP algorithm's approach indicates that a sequential pattern is characterized by events that occur in a temporal order and appear with significant frequency in a database. The support chosen was 40% for presenting the percentage of a finite ordered list of elements in the database containing the sequence and representing the most relevant sequential patterns for the proposal. The support value of any sequence reveals how frequent that sequence is.

We were able to differentiate and characterize sequences of behaviors for both temperature limits studied. Activity and feeding behaviors were more characteristic in conditions of thermal comfort, such as the behavioral sequences {Lying down, Walking, Eating}, {Lying down, Walking, Drinking, Walking, Lying down}, and {Lying down, Walking, Drinking}, with a frequency above four birds (support 40%). Unlike HS, the birds were more prostrated, shown in the shorter behavioral sequence, such as the pattern {Lying down, Eating}. This scenario indicates that the birds decrease their locomotor activities when under heat stress.

The 'Preening' behavior is considered typical behavior of broilers, as it appears in both ages and treatments, with a primary sequence of {Lying down, Preening}. However, during HS, the behavior sequence {Lying down, Preening, Lying laterally} was more frequent. On the other hand, the sequential behavior {Lying down, Lying laterally} can be characterized to differentiate an apparent situation from thermal discomfort, appearing only at the ages of 28 and 42 days in the situation of 8 °C above the thermoneutrality range.

Behaviors that did not differ between treatments can be explained by the bird not exceeding homeothermy's critical limits and managing to guarantee the desired performance.

4. Discussion

Broilers outside the thermoneutral zone tend to change their behavior, negatively influencing their performance. Because of this, the quick and early diagnosis of problems in the flock is critical [15], and this may be done through the monitoring of the activities and behavior of the broilers. As the assessment of behavioral responses is a non-invasive measure, it can be combined with technologies allowing automated assessment in an

efficient and real-time manner [16]. Therefore, data mining is becoming more popular in the poultry industry to analyze the constant and vast data sets generated by the increasing use of sensors in the current poultry industry [18,19,35–38]. Data mining also supports the development of an early warning system for precision livestock systems [11,39,40].

We recommend, in this study, the use of sequential pattern mining to characterize events that happen over time and appear with significant frequency in a database since these patterns can predict a future event based on the previous ones. Based on this assumption, we were able to identify sequences of characteristic behaviors of broilers under thermal stress or not. Ingestive behavior is the first suggestive behavior that the bird demonstrates when it is in an unsuitable environment. Although feeding behaviors were seen at both temperatures (Tables 4 and 5), a difference in the sequential pattern of behavior was observed. Food intake behavior can be an adaptive mechanism in the face of thermal stress, reducing when the ambient temperature increases [41–44]. Drinking behavior was found to be the opposite, with birds increasing their water consumption during exposure to high temperatures [41] in homeostatic response to water loss through evaporation. The sequence patterns indicate that they remained lying down and only rose in order to drink and eat: {Eating, Lying down} and {Lying down, Eating, Walking, Lying down} (Table 5).

Nevertheless, broilers exposed to thermal stress tended to decrease their locomotor activities in an attempt to reduce the heat generated by movement [42,45]. Shorter sequences of behavior performed at lower frequency occurred under HS. On the contrary, the activity and feeding behaviors were more characteristics under conditions of thermal comfort, with a frequency above four birds: ({Lying down, Walking, Eating}, {Lying down, Walking, Drinking, Walking, Lying down}, and {Lying down, Walking, Drinking} (Table 5).

Previous studies show that broilers spend 60 to 80% of their time resting [27,29,40]. However, aging birds decrease their activities, especially their locomotor and floor-scratching activities [30,46] and their rapid growth rate [28]. Nevertheless, both low and high rates of activity behaviors can be detrimental to the welfare of birds [15,47]. Thus, it is vital to ensure that chickens have appropriate activity levels at any age [15,48]. Activity indexes are easily visualized through automated monitoring employing video cameras, demonstrating the importance of work in this area [15,35,37].

The sequential pattern {Lying down, Lying laterally}, which appeared only under thermal stress by heat, proves that, under conditions of thermal stress, the bird uses such behavior to favor the thermal exchange of heat by conduction [49]. Although the 'preening' behavior is considered a comfort behavior [16,38], this behavior showed great frequency during HS. However, it can also be performed in stressful situations, serving as a mechanism to relieve stress [50], such as the sequence observed during HS <{Lying down, Preening, Lying laterally}>, preceding the characteristic behavior of stress that was lying on the side.

Animals modify their behavior in response to changes in the environment, indicating the animal's well-being. Although they can adapt their behavior to variations in the thermal environment, it is not recommended that there be a considerable variation in temperature [51]. Observation of birds' behavior is an indicator of bird welfare, and the greater the repertoire of behaviors performed by birds, the greater the indication that they are in better conditions [48]. Research that optimizes the set of variables is necessary to find solutions that provide adequate welfare levels for broilers [52] to achieve the flock's productive and economic efficiency.

Nowadays, most environmental control in broiler houses relies mainly on temperature monitoring, although there is the possibility of using integrated temperature, relative humidity, and gas sensors for environmental control in broiler housing. The study of broiler behavior sequential patterns might help in environmental control decision-making by integrating behavioral feedback into an environmental control process. Such a combination of knowledge might help to optimize broiler housing environmental control.

Providing faster and more accurate information is essential for current poultry to detect problems early [15,36,38]. Since a broiler's lifetime is limited to 6 weeks on average,

ensuring an adequate flock thermal environment and welfare during their lifespan is a critical task. The present study's thermal stress lasted for three days, making it difficult for the bird to adapt in the short term, with no significant changes in the bird's behavior. Future studies can assess thermal stress exposure's intensity levels to identify meat production losses since countries with tropical and subtropical climates indicate increased heatwave occurrence. Another possibility is that the sequential behavior algorithm might be the basis of the smart environmental control of livestock houses, as Morota et al. [20] suggested when implementing automation in farm animal production. Such an application might be further developed using a visual aid process.

5. Conclusions

Sequential pattern mining can identify temporal relationships between constant thermal stress and broiler behavior close to slaughter. Ingestive and locomotive behaviors are different in terms of comfort or thermal discomfort. The sequential patterns of {Lying down, Walking, Drinking} and {Lying down, Walking, Drinking, Walking, Lying down} are seen in thermoneutral conditions but not HS, in which birds become more prostrated, identified by the length of the {Eating, Lying down} sequences of behavior. Another sequential pattern characteristic of HS is {Lying down, Lying laterally}. Longer sequences are seen in TNZ, indicating that broilers' well-being is optimized.

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