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Automatic analysis of high, medium, and low activities of broilers with heat stress operations via image processing and machine learning

Oluwadamilola Moyin Oso [a], Nicolas Mejia-Abaunza [a], Venkat Umesh Chandra Bodempudi [a,b], Xixi Chen [c], Chongxiao Chen [a], Samuel E Aggrey [a], Guoming Li [a,b,*]

[a] Department of Poultry Science, University of Georgia, GA 30602, USA
[b] Institute for Artificial Intelligence, The University of Georgia, Athens, GA 30602, USA
[c] Nutribins LLC, Covina, CA, 91723 USA

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ABSTRACT

Heat stress is a major welfare problem in the poultry industry, altering broilers' activity levels. Advancements in image processing and machine learning provide opportunities to automatically quantify and analyze broiler activity. This study aimed to evaluate the effects of moderate heat exposure on broiler behavioral activity via image processing and machine learning. 132 Cobb 500 broilers were raised in 2 nutritional treatment groups, each with 3 replicates. The control groups were fed a basal diet, while the variation groups were fed a diet with 0.05 % 25-hydroxyvitamin D_3. All birds were raised under standard environmental conditions for 27 days before exposure to cyclic heat of 29.56 $\pm$ 1.34 °C and humidity of 76.97 % $\pm$ 5.98 % from 8:00–18:00 and thermoneutral conditions of 26.67 $\pm$ 1.76 °C and 80.23 % $\pm$ 3.05 % from 18:00–8:00. Birds were continuously video recorded, and the bird activity index (BAI) was analyzed by subtracting consecutive frames and summing up pixel differences. The treatment effect was analyzed using two-way ANOVA with a P-value < 0.05. K-means clustering was used to determine BAI as high, medium, and low levels. The result showed a significantly higher ($P < 0.01$) activity index in the variation group in contrast to the control. Absolute values of high and medium BAI were significantly lower with cyclical heating operations than those without heating operations. The BAI was also higher at the onset and end of the heating operations and moderately correlated to flock age ($|r| =$ 0.35–0.45). The high, medium, and low BAI performed differently with different nutritional treatments, temperature ranges, and relative humidity ranges. It is concluded that the BAI is a useful tool for predicting broiler heat stress, but the prediction effectiveness could be influenced by bird age, diets, temperature, humidity, and behavior metrics.

Introduction

The United States (US) poultry industry produced over 9 billion broilers in 2023, valued at over 42 billion dollars (USDA, 2024). Despite continuing to produce affordable meat proteins for humans, the industry (especially the US broiler Southeastern regions) faces challenges such as heat stress (HS) that can impact production efficiency and animal health. Heat stress, caused by high ambient temperatures results from inefficiency in dissipating excess heat of broilers and can negatively impact broiler feed intake, weight gain, health, and consequently significant economic loss (Sakomura et al., 2013; Habashy et al., 2017a; Beckford et al., 2020). According to St-Pierre et al. (2003), the annual average loss associated with heat stress in the US poultry industry is estimated to be around $128 million to $165 million, and these numbers may increase with global warming and economic inflation in recent years.

Chickens experience HS when they are exposed to ambient temperatures above the upper limit of the thermoneutral zone (McNab, 2002), and the degree of impact of heat stress depends on relative humidity, frequency and duration of the heat exposure The thermoneutral zone of broiler chickens were 28–34, 25–31, 22–28, 20–25, 18–24, and 18–24 °C for each of the first six weeks of age (Cassuce et al., 2013), and any temperature beyond 25 °C predisposed chickens to heat stress, especially in the later phases of production (Donkoh, 1989). To mimic

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* Corresponding author. Guoming Li, Department of Poultry Science, Institute for Artificial Intelligence, The University of Georgia, Athens, GA 30602, USA.
E-mail address: gmli@uga.edu (G. Li).

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natural temperature patterns for heat stress studies, researchers have exposed broilers to cyclical heating of varying temperatures ranging from moderate to high. Sakomura et al. (2013) subjected broilers to heat-stressing temperatures of 25–31 °C that suppressed feed intake. Branco et al., (2021) found that temperature above 32 °C altered the behavioral sequence of birds, while Beckford et al., (2020) reported that 34–36 °C temperature decreased feed intake and body weight and increased mortality. These effects of heat stress are due to significant physiological, neuroendocrinological, and behavioral alterations accompanying exposure to temperatures beyond thermotolerance zone (Quinteiro-Filho et al., 2010; Beckford et al., 2020).

Many environmental, management, and nutritional strategies have been adopted to ameliorate the heat stress effects, including but not limited to reducing stocking density, increasing ventilation, providing cool water and electrolyte supplementation, feeding birds in early morning and late evening, supplementing dietary vitamins (Deepika et al., 2020), and supplementing with glucose (Ariyo et al., 2023; Kwakye et al., 2023). Vitamin D_3 and its active metabolite 25-hydroxyvitamin D_3 have been proven to support immune function, reduce inflammation (Abascal-Ponciano et al., 2022), and facilitate adaptation (Xu et al., 2021), thus enhancing overall welfare. The appropriate timing windows for the vitamin inclusion is of importance to decrease heat stress impacts while maintaining economic profits. To seek homeostatic under heat stress, chickens behave adaptively by panting, wing flapping, staying stationary, and altering consummatory behaviors (Mack et al. 2013; Li et al., 2015), which may provide direct indicators for the timing windows selection. However, gold standard methods like manually observing these behavior changes could be time-consuming.

Various tools have proven efficiency and accuracy for automatic behavior monitoring that provides insights into timely intervention strategies. Some of the existing sensor-based time series analysis tools include the use of radio frequency identification (RFID) tags, accelerometers, audio sensors, and thermography sensors. The RFID tags and accelerometers are a wearable sensor that could capture small movement but could pose discomfort to birds. It is impractical and expensive to attach sensors onto thousands of individual animals in large-scale studies (Casey-Trott and Widowski 2018; Adler et al., 2023). The audio sensor is non-invasive, but sensitive to background noise (e.g., fans) and may not be able to directly capture in-depth information about activities. Thermography sensors are a non-invasive tool that is less prone to background noise captures, but it is cost-prohibited to monitor large-scale flocks. Using normal cameras and image processing algorithms to extract bird activity index (BAI) is more promising as it effectively mitigate the abovementioned challenges. Computer vision features non-invasiveness and cost effectiveness in animal behavior measurements, especially in large-scale studies or field applications (Li et al., 2021). Previous studies have demonstrated potential of using computer vision algorithms in estimating poultry thermal comfort (Pereira et al., 2013; 2020). BAI is one of the effective computer vision algorithms for identifying animal stress in group level and defined as pixel changes caused by moving animals to the total bird-representative pixels between consecutive frames (Aydin et al., 2010; Silvera et al., 2017; Yang et al., 2020). The mechanism of BAI is that animal movement can cause intensity (pixel values) changes spatially and consequently overall pixel alternations in an entire frame. Li et al., (2019) applied the BAI to analyze diurnal rhythms of group-housed layer pullets with free choices between light and dim environments and observed that pullets behaved more actively under the light than under the dim environment. Kozak et al., (2016) classified BAI of laying hens into low, moderate and high BAI intensities and found that age and bird strain affect time budget of the BAI levels, and accelerometers with machine learning models are useful tools in identifying BAI levels. However, whether the BAI is effective in evaluating broiler heat stress especially under various nutrition treatments (e.g., with or without vitamin D_3) remains unclear. Classifying the BAI into different levels (i.e., low, medium, and high) with machine learning (Kozak et al., 2016) will further aid in distinguishing variations among different behavioral activities and patterns and further provide insights into precision management. These behavioral changes can be stress indicators and could be effective in heat stress prediction. Based on observation, behaviors requiring energetic body movements such as wing flapping, running, jumping, aggression and shaking phase of dust bathing might result in high BAI. Medium BAI could be associated with walking, stretching, gentle pecking, preening, foraging and feeding. While passive behaviors without any significant body movement such as sitting, lying and sleeping may possibly result in low BAI (Kozak et al., 2016). Critical questions, such as efficiency of image processing and machine learning and hourly and daily behavioral changes, are unsolved for BAI evaluation under heat stress operations. Investigating this will not only contribute to existing knowledge of broilers' behavioral response to heat stress but could also serve as a baseline for categorizing BAI. Practically, this study provides a non- invasive animal-based measure for evaluating broiler's response to heat exposure, forming the basis for developing real-time monitoring systems. The objective of this study was to evaluate the effects of moderate heat exposure on BAI in high, medium, and low levels via image processing and machine learning.

Materials and methods

The experiment was conducted in an environmentally controlled room of the Poultry Science Research Center at the University of Georgia, between July and August 2023. Experimental protocol and procedures were approved by the University of Georgia's Institutional Animal Care and Use Committee (IACUC number: A2022 12-012-Y1-A0).

Animal management, and experiment

One hundred and thirty-two Cobb 500 male birds were raised in six pens and two groups (control versus variation groups) with each pen accommodating 22 birds, resulting in three replicates per group. Each pen was measured at 1.22 m wide ×1.52 m long (Fig. 1). The birds in the control group were fed with commercial standard diets: starter (22.96 % Crude protein (CP) and 2,893 kcal/kg; 0 to 14-day of age), grower (20.99 % CP, 2,945 kcal/kg, 15 to 28-day of age) and finisher (19.54 % CP, 3,043 kcal/kg; 29 to 42-day of age), while those in the variation group were fed with the diet with additional 69 μg/kg of 25-hydroxyvitamin D_3 (Smart D, Nutribins). Feed and water were supplied *ad libitum*, and the birds were reared under standard environmental conditions following the Cobb management guideline (Cobb, 2021) before they were subjected to cyclical heat from day 28. From 28 to 42-day of age, birds were exposed daily to an average temperature of 29.56 ± 1.34 °C and relative humidity of 76.97 % ± 5.98 % from 8:00–18:00 (heat stress operation hours). During the non-heat stress operation hours (00:00–8:00 and 18:00–24:00), the average temperature of 26.67 ± 1.76 °C and relative humidity of 80.23 % ± 3.05 % were recorded, as shown in Fig. 2. The birds were raised (post-brooding) under 20L:4D photoperiod (ON at 6:00 and OFF at 2:00) with a light intensity of 5 lux.

Data collection

A security camera (NHD-887MSB, Swann Security, Santa Fe Springs, CA) was installed on the ceiling of each pen with distance of 3.05 meters from the ground. Broiler birds were continuously recorded with a 16-channel video recorder (SRDVR-85680H-US, Swann Security, Sacta Fe Springs, CA). The video recording was set at a resolution of 1024 × 768 pixels at a sample rate of 15 frames per second (fps), and 24 video episodes per hour per pen were recorded on daily basis. The videos were stored as .MP4 files on a 20-terabyte external hard disk. Recordings made on days 28, 30, 32, 34, 36, 38 and 40 of age were processed and analyzed.

Fig. 1. Photos of the experimental pens and broiler chickens during the experiment.

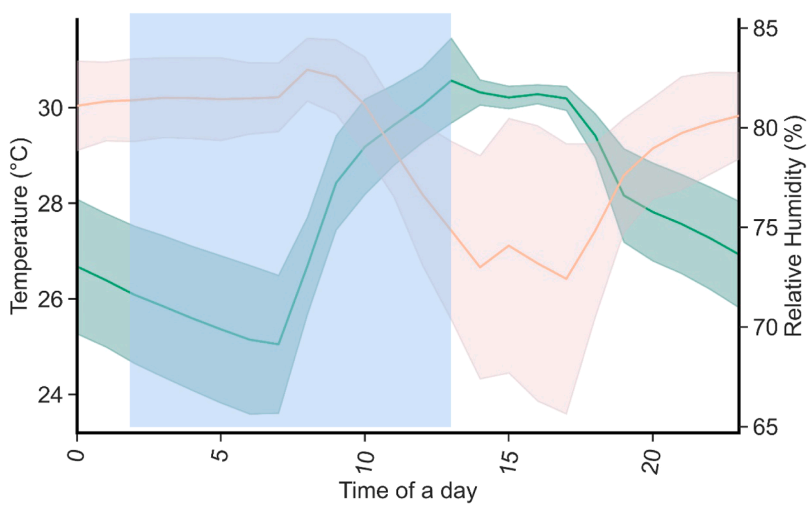

Fig. 2. The cyclical heating temperature (red) and relative humidity chart (green). The blue shading area indicates heat stress operation hours from 8:00 to 18:00. As temperature rises, there is a drop in the relative humidity and vice versa.

Image processing

Each video file was converted to RGB frames with the rate of 15 fps via a software (Free Video to JPG converter, version 2.1.2). In each extracted frame, the pen area or region of interest was cropped with predefined spatial coordinates, for reducing inferences from other unnecessary areas. As indicated in Fig. 3a, bird area was determined by creating a polygon around the bird and summing up all broiler-representing pixels from binarized images (Eq. 1). The bird area was used to normalize the BAI to reduce the effects of body size variations across ages.

$$I_a(x,\ y,\ t)\ = \begin{cases} 1, \text{ if } [\mathrm{I(x,\ y,\ t)}\ -\ \mathrm{I(x,\ y,\ t-1)}] > \tau \\ 0, \ \textit{otherwise} \end{cases} \tag{1}$$

where $I(x,\ y,\ t)$ is the intensity value at the location of (x,y) for the current frame; $I(x,\ y,\ t-1)$ is the intensity value at the location of (x,y) for the previous frame; $I_a(x,\ y,\ t)$ is the intensity value (0 or 1) at the location of (x,y) after binarization; and τ is the threshold for binarization which was set at 30 pixels in this study.

The pixel intensity ($I_N(x,\ y,\ t)$) at time t was normalized by bird area ($A(t)$) and indicated in Eq. 2.

$$I_N(x,\ y,\ t)\ = \frac{I_a(x,\ y,\ t)}{A(t)} \tag{2}$$

Then the BAI can be obtained by summing up the normalized pixels into hourly or daily basis (T) and suggested in Eq. 3.

$$BAI\ = \sum_T I_N(x,\ y,\ t) \tag{3}$$

Fig. 3b shows the working flow for computing BAI with Python. For each pen, the number of frames extracted was 54,000 in an hour and 1,296,000 in a day. Animal caretakers visited the pens daily, and their appearance can stimulate bird activity, leading to interference for calculating the BAI. The human visiting periods (average 2 min) for each pen were removed from the calculation.

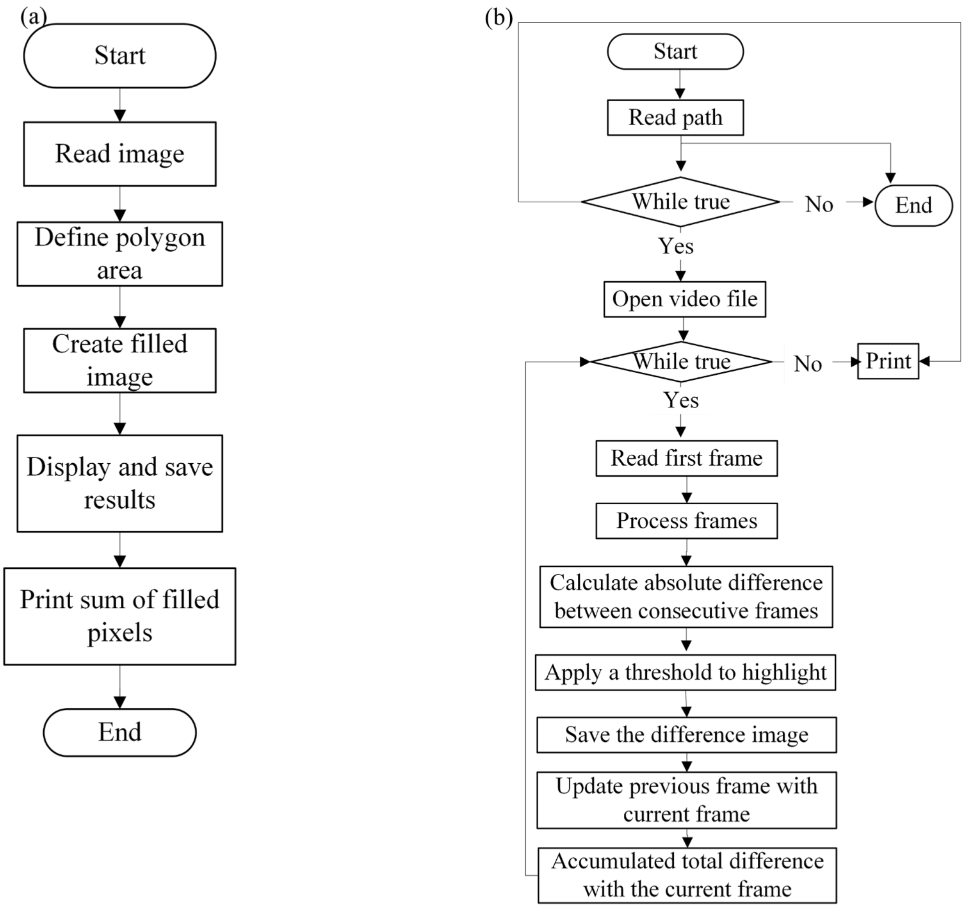

Fig. 3. Flowchart for image processing to determine total pixels for one bird (a) and bird activity index (b).

Data analysis

K-means clustering is an unsupervised machine learning algorithm that groups dataset into k distinct clusters and was used to cluster BAI into high, medium, and low in this study. K-means algorithm partitions data into clusters by minimizing variance within clusters and iterates to determine the optimal centroids. Inertia measures the compactness of clusters by calculating the sum of squared Euclidean distances from each point to its assigned cluster centroid (Eq. 4). Centroid is the means of the coordinates of all the points within a cluster. K-means++ method in the Python-based library, Scikit-learn, was used to initialize the centroids to improve the convergence of the algorithm. Using the elbow method, the optimal number of centroids were determined when the inertia measures reached a state of litter or no change following a progressing number of clusters. BAI feature was selected for clustering, and a fixed random state of zero was used to ensure reproducibility of result.

$$Inertia = \sum_{i=1}^{n} \min_{\mu_j \in C}\left(\|x_i - \mu_j\|^2 \right) \tag{4}$$

where C is a set of all centroids; μ_j is the centroid of cluster j ($j = 3$ in this case, indicating high, medium, and low BAI); x_i is the ith data point of BAI; $min_{\mu_j \in C}\left(\|x_i - \mu_j\|^2 \right)$ is the minimal squared Euclidean distance between data point x_i and centroid μ_j.

The BAI for each extracted frame was clustered into high, medium, and low, and the time budget (second/hour) in respective levels of BAI was summarized from each extracted frame and averaged into hourly basis. The percentage of respective levels of BAI was obtained by dividing the time budget of high, medium, and low BAIs with 3600 seconds in an hour. The high, medium, and low BAIs were analyzed with different time of a day, treatments (control and variation), temperature, and relative humidity to gain more insights into the BAI applications.

Correlations between high BAI, medium BAI, low BAI, flock age, temperature, and relative humidity were assessed. The strength and direction of linear relationships between those variables was determined by computing the Pearson correlation coefficient (r, Eq. 5). According to the definition of Akoglu (2018), the correlation is perfect with $r = \pm 1.0$, strong with r between ± 0.7 and ± 1.0, moderate with r between ± 0.4 and ± 0.7, weak with r between ± 0.1 and ± 0.4, and zero with $r = 0$.

$$r = \frac{\sum (X_i - \overline{X})(Y_i - \overline{Y})}{\sqrt{\sum (X_i - \overline{X})^2 \sum (Y_i - \overline{Y})^2}} \tag{5}$$

where X_i and Y_i are sample points for comparative variables; and $\overline{X}$ and $\overline{Y}$ are the means of respective sample sets.

Statistical Analysis

The effects of treatment (control and variation) and heating operations (with and without cyclical heating) on the BAI metrics were analyzed using two-way analysis of variance (ANOVA) (Eq. 6). The BAI metrics include time budget (seconds/hour) for high, medium, and low BAIs, percentage (%) of the time budget for high, medium, and low BAIs, and the absolute value per bird basis for high, medium, and low BAIs.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \tag{6}$$

where Y_{ijk} is the BAI metrics; μ is the least square mean of the BAI metrics; α_i is the treatment, i = control and variation; β_j is the heating operations, j = with and without cyclical heating; $(\alpha\beta)_{ij}$ is the interaction effect of treatment and heating operations; and ε_{ijk} is the random error.

The experimental unit was pen, and the repeated measurements were conducted from days 28 to 40. The total number of observations of the BAI metrics for statistical analysis is listed on Table 1. The separation of means for the BAI metrics was implemented using Tukey test in SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). The effect was considered significant with P-value less than 0.05.

Results

The elbow plot (Fig. 4a) shows two notable elbow points when the number of clusters was set to 2 (point k1) and 3 (point k2). The k1 value indicates a significant drop in inertia, thus capturing distinct data variations for unsupervised clustering, while k2 with finer granularity captured detailed sub-structure after k1. Inertial decline after point k2 was insignificant. K-means clustering graph (Fig. 4b) highlights the clusters with their centroids for high, medium, and low BAI. The cluster centroids were 1115.96 for high BAI, 740.47 for medium BAI, and 413.39 for low BAI.

No significant differences ($P \geq 0.22$) were recorded in the time budget and percentage time budget of chickens with and without heating operations for high, medium, and low BAI (Table 2). However, absolute values of high and medium BAI per bird with heating operations were significantly lower than those without heating operations ($P < 0.01$).

The time budget and percentage of the time budget for high and medium BAI were significantly lower in the control nutritional treatment groups than those in the variation nutritional treatment groups ($P < 0.01$), while the scenario was opposite for the low BAI ($P < 0.01$). The absolute values of high and medium BAI were not significantly different between the two nutritional treatment groups ($P \geq 0.30$), while those of low BAI were significantly higher in variation groups ($P = 0.04$).

There was no interactive effect of heating operations and nutritional treatments on time budget, percentage of time budget, and absolute values for high, medium, and low BAI ($P \geq 0.54$).

Fig. 5 shows the hourly trend of BAI across different time of a day and flock ages under the two nutritional treatments. The hourly BAI increased at the beginning of heating operations (after 6:00) and decline at the end of a day (after 17:00) (Fig. 5a). The hourly BAI gradually declined as bird aged from days 28 to day 40 for both nutritional treatments (Fig. 5b). High BAI became more prevalent at 8:00 and 17:00, while low BAI occurrences diminished at these two time slots (Fig. 5c). When considering flock age (Fig. 5d), a smaller frequency of medium BAI was observed on days 28 and 30. However from days 32, the medium BAI occurrences increased with increase in age while high BAI diminished. Low BAI occurrences gradually increased as birds aged.

The number of occurrences of low BAI was notably more in the control nutritional treatment groups in comparison to variation ones (Fig. 6a), while medium and high BAI was lower in control groups.

Fig. 6b shows the relationship between temperature ranging from 23–32 °C with 2 °C intervals and the three levels of BAI. The number of occurrences for high, medium, and low BAIs tended to increase with temperature from 23–27 °C. Then high BAI occurrences decreased with temperature increasing from 28 to 29 °C and stayed stable afterward. The medium BAI occurrences increased with temperature increasing from 28 to 29 °C but decreased with temperature moving from 30 to 31 °C. The low BAI occurrences remained stable in between 27 to 30 °C but decreased with temperature elevating over 30 °C.

Fig. 6c shows the relationship between relative humidity ranging from 61 % to 86 % with 1 % intervals and the frequency of high, medium, and low BAI. Overall, the high BAI occurrences increased from 61 % to 66 %, decreased from 67 % to 70 %, increased again from 71 % to 79 %, remained stable between 80 % to 84 %, and dropped over 84 %. The medium BAI occurrences were not obvious before 68 %, increased from 69 % to 80 %, stayed stable in between 81 % to 84 %, and drop dramatically over 84 %. The low BAI occurrences slightly fluctuated in between 63 % to 72 %, increased from 73 % to 82 %, and dropped afterward.

The correlations between variables are presented in Fig. 6d. High and medium BAI showed negative moderate correlation with flock age ($r = -0.39$ or -0.45), while low BAI was positively, moderately correlated with flock age ($r = 0.42$). High and medium BAI had negative weak correlation with relative humidity ($r = -0.16$ or -0.14), and low BAI was positively weakly correlated with relative humidity. The three levels of BAI seemly had no correlation with temperature with the absolute r values less than 0.09. Interestingly, the three levels of BAI had strong correlation with each other with absolute r values greater than 0.8.

Three-point clustering (k2) captured detailed sub-structure after k1 and was optimal for separating BAI into high, medium, and low. Heating operation reduced the absolute values of high and medium BAI per bird while nutritional treatment alters percentages of high, medium and low BAI. Sudden increase or decrease in temperature influenced BAI in relation to time of the day and flock age regardless of the nutritional treatment. Flock age mainly impacted medium and high BAI. Nutritional treatments, temperature and relative humidity affected the number of occurrences of low, medium or high BAI. Both temperature and relative humidity slightly increased as the number of occurrences of medium and high BAI decreased and vice vera while low BAI had an inverse relationship with temperature and relative humidity.

Discussion

Heat exposure influences broiler behavioral activities, and these activities can be classified as high, medium, and low levels via unsupervised machine learning models. The onset and end of heating operations relatively increased high BAI, and temperature of 27–28 °C increased all levels of BAI, but above 30 °C, all behavioral activity declined and could be indicative of heat stress. The elbow plot showed 2 points for data clustering. k1 gave a broad classification based on the major data clustering structure. However, k2 provided a more detailed clustering of BAI data. In addition, k2 was useful in classifying the BAI of the birds as high, medium, and low, thus ensuring the clusters provide more meaningful insights. Based on the total number of transitions among three tiers of an aviary system captured by radio frequency identification systems (Lorentz et al., 2024) and accelerometers (Kozak et al., 2016), the authors categorized laying hens (from weeks 34 to 42, and weeks 10–37 respectively) into low, medium, and high groups. Thus, three is the optimum number of clusters for classifying the BAI levels from technical and biological standpoints. The existence of three clusters implied that there were three distinct behavioral activity levels that could be influenced by moderate heat exposure. According to Kozak et al. (2016), behaviors involving small postural movement were associated with low-level BAI. Behaviors such as litter pecking, litter scratching, feeding, drinking, preening, and stretching resulted in medium-level BAI. Behaviors such as running, jumping, wing flapping, and body shaking generated high-level BAI. The compactness of values within the high BAI cluster suggests less variability, and consistency in behavioral activities within this group, unlike the low, and medium with

Table 1
Number of observations of the BAI metrics for statistical analysis.

Nutritional Treatment	Heating operations	Total BAI	Time budget and percentage of time budget for BAI levels		
			High	Medium	Low
Control	With	210	29	45	136
	Without	294	40	47	207
Variation	With	210	61	82	67
	Without	294	88	101	105

Note: BAI= Bird activity index.

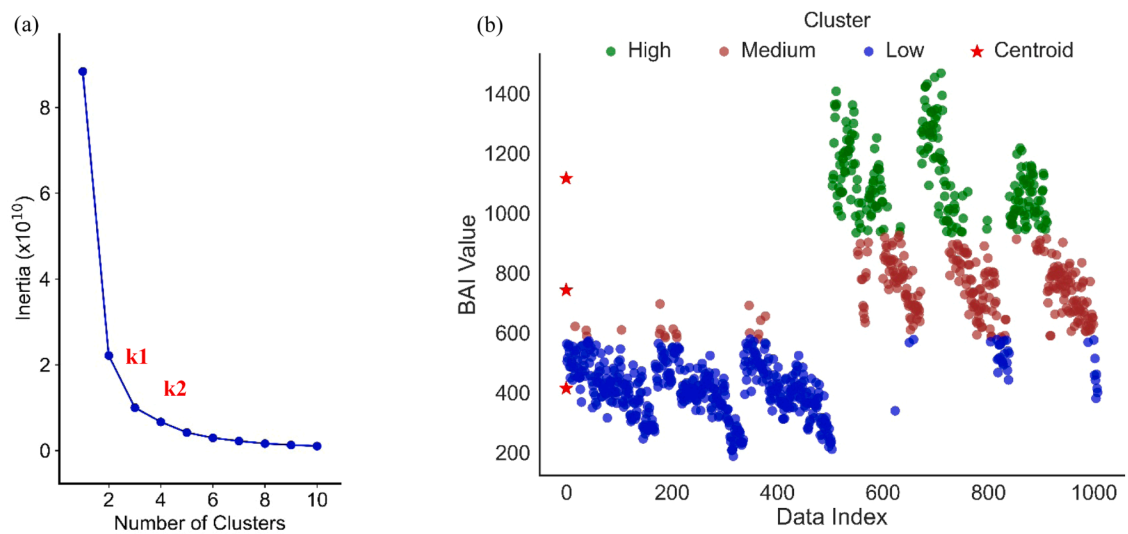

Fig. 4. K-means clustering for separating high, medium, and low bird activity index (BAI): (a) elbow plot to determine the optimal number of clusters, which was three in this case; (b) visualization of distribution and cluster centroid for high, medium, and low BAI.

Table 2
Effect of heating operations and nutritional treatments on BAI metrics of broiler chickens.

		Time budget (seconds/hour)			Percentage of the time budget (%)			Absolute value (per bird)		
	Parameter	High BAI	Medium BAI	Low BAI	High BAI	Medium BAI	Low BAI	High BAI	Medium BAI	Low BAI
Heating operations	With	203.54	1127.02	2269.45	5.65	31.30	63.04	114.60 [b]	266.32 [b]	197.29
	Without	186.49	1105.45	2303.67	5.19	30.77	64.04	151.01[a]	384.77 [a]	197.68
	SEM	10.56	23.68	37.23	0.29	0.78	1.03	7.12	7.83	1.89
	P-Value	0.23	0.56	0.48	0.22	0.60	0.46	<0.01	<0.01	0.88
Nutritional treatment	Control	167.64 [b]	919.65 [b]	2512.39 [a]	4.66[b]	25.55[b]	69.80[a]	138.04	330.74	194.69 [b]
	Variation	222.39 [a]	1312.82 [a]	2060.72 [b]	6.18[a]	36.53[a]	57.29[b]	127.57	320.35	200.29 [a]
	SEM	9.78	25.95	34.47	0.27	0.720	0.950	7.69	8.46	2.03
	P-Value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.30	0.35	0.04
Interaction (Heating operations-Nutritional Treatment)	With-Control	176.44	936.64	2486.92	4.90	26.02	69.08	120.76	272.53	193.67
	Without-Control	158.84	902.66	2537.87	4.41	25.08	70.51	108.43	260.11	200.92
	With-Variation	230.64	1317.39	2051.97	6.41	36.59	56.70	155.32	388.95	195.70
	Without-Variation	214.15	1308.25	2069.46	5.96	36.46	57.58	146.7	380.58	199.66
	SEM	14.94	39.63	52.65	0.41	1.10	1.46	10.88	11.96	2.88
	P-Value	0.97	0.73	0.73	0.96	0.69	0.75	0.85	0.85	0.54

[a,b] Means having the different superscript within each column indicate significant differences ($P < 0.05$). BAI is bird activity index, and SEM is standard error.

some dispersed data points.

The heating operation decreased the absolute values of high and medium BAI levels without interrupting the proportion of time (time budget) spent on each of the three BAI levels. This suggests that heating operations can decrease the intensity of behaviors (quality) regardless of time budget. Relative to low BAI levels, more energy might be utilized to attain both high and medium BAI levels in broiler chickens. Heat stress reduces feed intake and, subsequently, the energy intake of broiler chickens (Attia and Hassan., 2017; Habashy et al., 2017b; Branco et al., 2021). Hence, heating operation reduces the energy intake (Habashy et al., 2017b) of the birds, which could have forced them to conserve energy by reducing activities such as physical movement. Increased activity increases heat production (Nielsen et al., 2012). Hence, there is a reduction in the absolute values of high and medium BAI levels as observed under heating operation in the current study. Identifying and tracking medium and high activities associated with specific behaviors and analyzing movement rate or energy expenditure when performing these behaviors with or without heating operations may provide more insight into broiler management under heat stress.

Based on the number of occurrences of each level of BAI on hourly basis, the BAI increased at the onset as well as the end of heating operations (Fig. 5a). The spike in BAI at these periods of the day may indicate short bursts of activity to seek thermoregulation. These spikes correspond to increased high BAI and reduced low BAI. The sudden shift in thermal environment may cause birds to move more frequently or vigorously during the first hour of heating or the end of heating operation, leading to intense spike in activity that normalize as the birds acclimatize to the new temperature. The putative behavioral thermoregulation process may involve increased movement towards the drinker, feeding, lying, panting, wing spreading, dust bathing, and changes in spatial distribution (Santos et al., 2019). This is in resonance with the findings of Nielsen et al. (2012) who highlighted that fast-growing broilers adapt to environmental temperature using behavioral changes. In summary, BAI could be a tool to monitor thermoregulatory behaviors especially at the onset and end of heating operations in broiler chickens.

Age influenced all levels of BAI. The decline in BAI as bird aged was expected due to decline in muscle strength, bone health and increase in

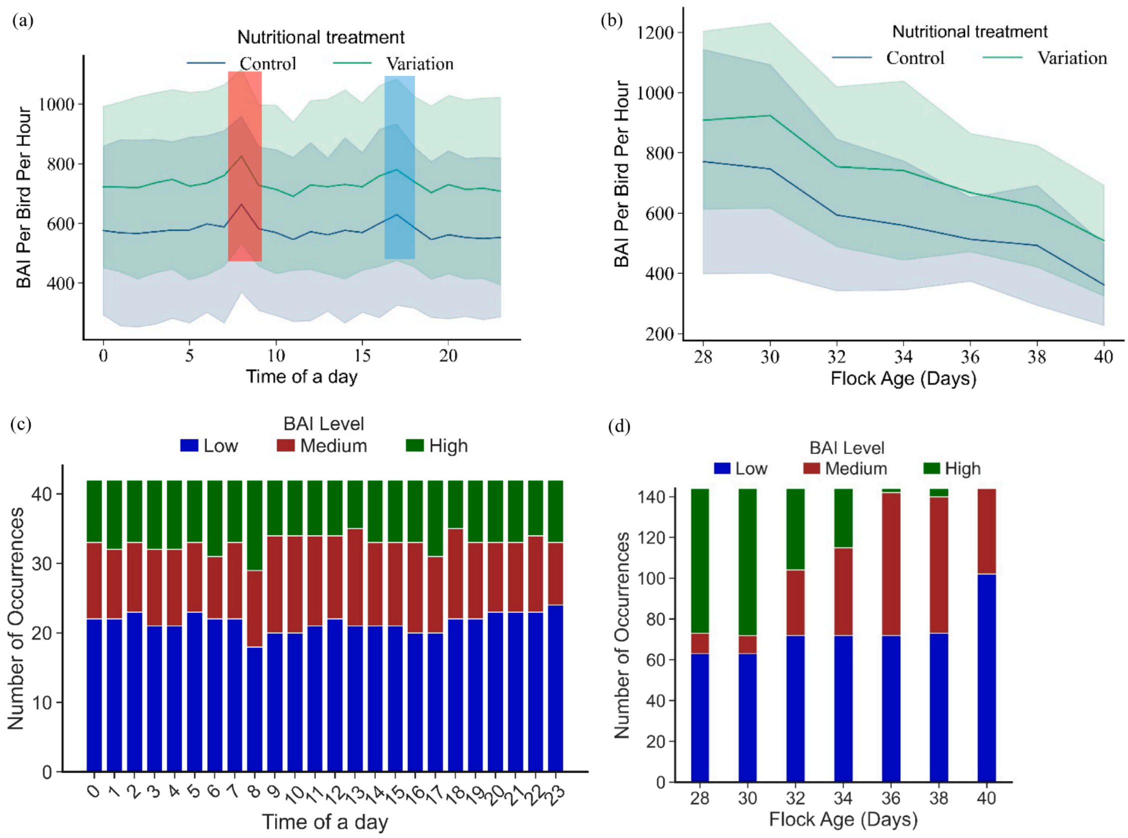

Fig. 5. Hourly bird activity index (BAI) across different time of a day (a) and flock age (b) with two nutritional treatments. Red bar indicates the onset of heating operations, and blue bar indicates the end of heating operations. Number of occurrences of high, medium, and low bird activity index (BAI) based on time of a day (c) and flock age (d).

body mass leading to a decrease in the free floor spaces within the pen. This may restrict birds' activities such as frequency of feeding (McLean et al., 2002). Existing research supports these findings, stating that broiler activity level declined with ages (Yang et al., 2020), and most active behaviors were less observed with increasing age (Jacobs et al., 2021). The observed trend in our study suggests correlation exist between flock age and BAI, confirmed by the correlation matrix.

The 25-hydroxyvitamin D3 is a great source of vitamin D3 in broiler diets owing to its high absorption rate and great metabolic activity as demonstrated by the research of Bar et al., (1980). Vitamin D3 is also known to alleviate the negative effect of oxidative stress in broiler (Nong et al., 2023) and enable them to cope better with high temperatures without compromising welfare. The significantly lower time budget and percentage of the time budget for high and medium BAI in the control nutritional treatment groups relative to the variation nutritional treatment groups and the inverse relationship observed in the low BAI implies that birds in the variation nutritional treatment group spent more time on high and medium activities rather than on low activity. Although the absolute values of high and medium BAI were similar between the two nutritional treatment groups, those of low BAI were significantly higher in variation groups. Based on flock age, absolute values of BAI in control groups were lower than that in variation groups. This could be explained by the higher occurrences of low BAI in the control groups (as seen in Fig. 6a), while the variation group had balance occurrences for low, medium, and high BAI, indicating a better welfare in the birds raised in variation groups. Low BAI is typically associated with sitting or other stationary behaviors (Kozak et al., 2016), which might increase the chances of bird lameness (Silvera et al., 2017; Van Hertem et al., 2018).

Understanding the pattern of activity (based on BAI level) relative to temperature is crucial to environmental management. As shown in Fig. 6b, BAI increased with temperature until 30 °C and declined afterwards. This implies that the BAI of birds was greatly impaired when birds were exposed to temperature beyond 30 °C. All levels of BAI increased with temperatures until 27–28 °C. Although the total number of BAI occurrences increased with 29–30 °C temperature, however, the occurrences of high BAI diminished at this point. Temperature of 27–28 °C appeared optimum for promoting activity at all levels. This suggests temperature around 27–28 °C as the most favorable thermal range for all levels of activity involved in behavioral expression especially when exposed to cyclical heating. Since optimum behavioral expression is linked to thermal comfort, this finding may challenge the existing knowledge that thermal comfort zone in older birds lies between 18–24 °C as reported by Cassuce et al. (2013) and Cahaner et al. (2008). It could be inferred that heating operation could have conditioned the birds such that there was a shift in thermotolerance threshold in the process of acclimation thus affecting the optimum range for activity levels, therefore future studies may need to verify this. Temperatures above 30 °C depressed behavioral activity levels and could be indicative of heat stress. This is aligned with the findings of Branco et al. (2021) who demonstrated that exposure to heat stress of 32 °C altered the behavioral sequence of birds.

The relative humidity recorded during the cyclical heating (76.97 % ± 5.98 %) and non-cyclical heating (80.23 % ± 3.05 %) fell within the range of 69.0–93.0 %, recorded by Egbuniwe et al. (2018) during a heat stress study. Similarly, Queiroz et al. (2015) reported relative humidity of 78–87 % during their study. Ajakaiye et al. (2010) studied the effect of vitamin supplementation on heat stressed layers and reported humidity of 84.6 % inside the experimental pen and 81.5 % outside the experimental pen. High relative humidity is known to affect thermal comfort by reducing evaporative loss (Oliveira et al., 2006). In summary, the recorded relative humidity in the two different periods aligned with

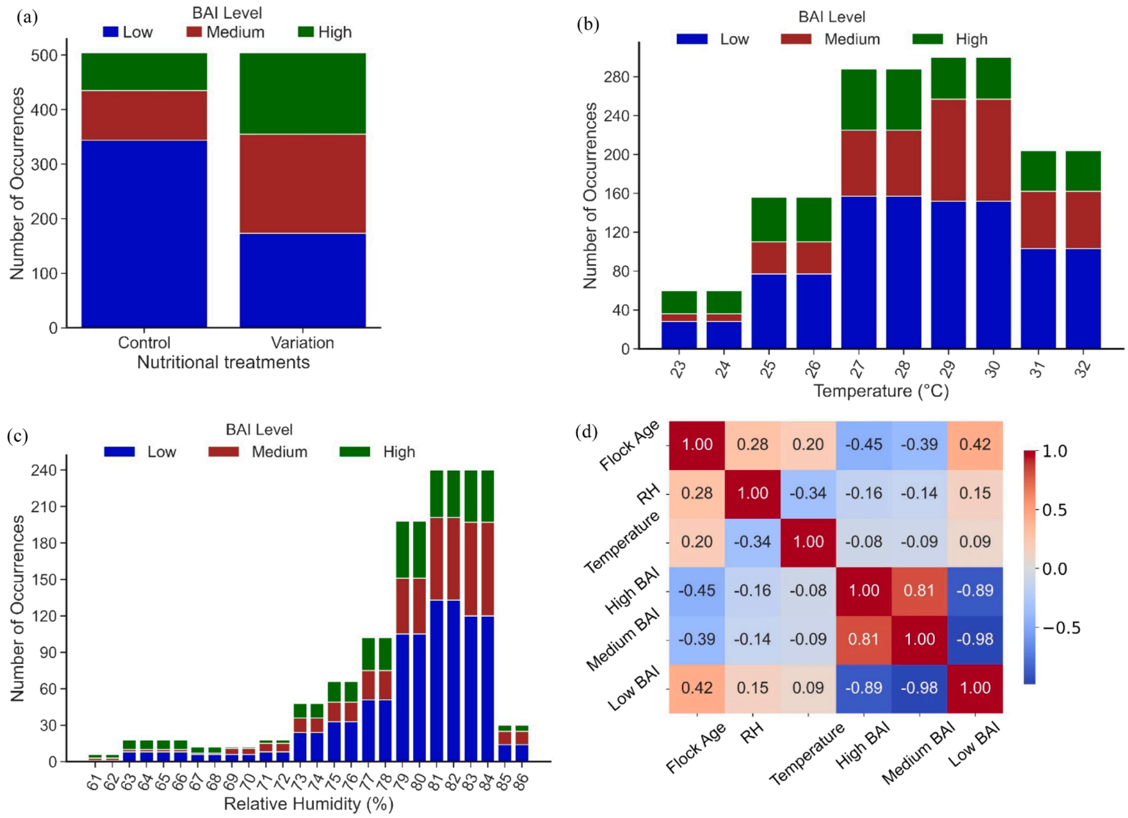

Fig. 6. Frequency of high, medium, and low bird activity index (BAI) in relation to nutritional treatments (a) temperature (b) relative humidity (c). Correlation between bird activity index (BAI), environmental variables, and flock age (d).

previous studies should be reasonable. Relative humidity also impacted all levels of BAI (Fig. 6c). The evaporative cooling process such as panting that accompanies increase in relative humidity could have resulted to the increase in low, medium and high BAI from 73 % to 82 %, 69 % to 80 % and 71 % to 79 % respectively. The stable BAI reported beyond this point suggested acclimation, and relative humidity of 84 % and over might have impaired broiler's ability to dissipate heat, leading to a drop in all levels of BAI which could be indicative of heat stress. This finding is similar to the result of Pereira and Naas (2008) who found out that the relative slightly above 85 % with a corresponding temperature of 22.5–30 °C lies outside the real time thermoneutral zone of broiler breeders. The weak negative correlation of high and medium BAI with relative humidity showed that as humidity increased, broilers may engage less in behaviors that could result in high or medium BAI (although this effect was weak). The weak correlation of low BAI and humidity may indicate that relative humidity could have interfered with other factors such as temperature. Although the temperature and relative humidity were investigated separately for the BAI, they jointly impact animal feelings on ambient environments, such as Temperature Humidity Index integrating both temperature and humidity (Pereira and Naas 2008; Moraes et al., 2008; Ying et al., 2019). Future directions also include independent and interactive impacts of temperature and humidity on BAI.

The negative correlation between high, medium BAI and flock age suggests that as broiler grew older, the probability of carrying out high or medium level activities decreased. Similarly, Kozak et al., 2016) found that high intensity activity decreased in laying flock as the birds increased in age. Broilers tended to opt for low level activity by resting more towards the end of the production cycle. The moderate positive correlation of low BAI and flock age confirmed this. The shift in distribution of BAI levels across flock age suggest an interdependency and this is confirmed by the strong correlations between the three levels of BAI. The strong correlation between the three levels of BAI indicates synchronized changes in behavioral activity, and this could be very useful in predictive behavioral modeling.

In large-scale studies, the behavioral interactions of broilers may differ based on stocking density. Broilers were less active under higher stocking density as walking decreased due to more interference between conspecifics (Simitzis et al., 2012). Furthermore, birds' social interactions could be different between small pens and large-scale pens (Campbell and Horton 2023). Temperature, humidity, and ammonia can be distributed unevenly in different local regions, resulting in different patterns of behavior. For instance, ammonia concentration of 15 ppm and above could increase head shaking behavior as demonstrated by Liu et al. (2021). Thus, BAI thresholds should be validated with various stocking densities and environmental conditions when the technique is applied to large-scale studies.

BAI can be integrated into IoT-based automated systems or smart environmental controllers for early detection of heat stress in broiler houses. This includes camera sensors (e.g., EyeNamic (World Food Innovations, 2017)) and ceiling robot (e.g., ChickenBoy (Farm Automation Today, 2021)) that are connected to base station with data storage, BAI analysis algorithms and alarming systems. The implementation of this will reduce over $128 million economic losses associated with heat stress in the United States (St-Pierre et al. (2003)), although the technology would require initial cost for camera devices ($ 17 to over $ 100 per camera depending on the brands) and data storage ($ 0.020 to 0.023 for 1 gigabyte per camera per day depending on the region). The software could be developed and integrated into alarm systems. The total cost may vary depending on the size of the pen, the type and number of required vision systems or cameras, and the volume of data generated. For instance, the cost of data storage may depend on the number of cameras. The constraint with this is the initial cost of installation but the long-term benefits outweigh this. These benefits include improved management system, real time monitoring and alert system for early detection of irregular behaviors. This will further ensure early intervention to minimize the cost of production and enhance profitability.

This study has certain limitations that should be acknowledged. The environmental variability caused by small lighting variation (Aydin et al., 2010), equipment interference and dust level (Guo et al., 2020) may impact image processing accuracy, by introducing noises in the background. This is a challenge for the existing technique of BAI as highlighted by Failla et al. (2021) and Massari et al. (2022). Background image subtraction may be a potential solution to this. Apart from this, Yang et al. (2020) demonstrated that adopting shorter sampling time (0.04 seconds) of BAI increased accuracy. Another limiting factor was the temperature. Although the broilers reacted to the temperature changes in current study, more research may be necessary to expose broilers to higher temperature in order to capture more in-depth behavioral responses especially at the acute phase of heat stress.

Conclusion

Broilers were exposed in moderate heating stress (average temperature of 29.56 $\pm$ 1.34 °C and relative humidity of 76.97 % $\pm$ 5.98 %) and fed with two different diets, and bird activity index (BAI) was analyzed from days 28 to 40 and clustered into high, medium, and low via unsupervised machine learning models. Absolute values of high and medium BAI were significantly lower with heating operations than those without heating operations ($P < 0.01$). The BAI was also higher at the onset and end of heating operations and moderately, negatively correlated with flock ages. It is concluded that the BAI is a viable tool for predicting heat stress, but the prediction efficiency and accuracy could be influenced with different nutrition treatments and behavior metrics.

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Author contribution

Conceptualization: G.L.; Data curation: O.M.O and G.L.; Formal analysis: O.M.O and G.L.; Funding acquisition: C.C and G.L.; Investigation: O.M.O, C.C and G.L.; Methodology: G.L.; Project administration: G. L.; Resources: C.C and G.L.; Software: G.L, O.M.O and V.U.C.B.; Supervision: G.L.; Validation: O.M.O and G.L.; Visualization: O.M.O and G.L.; Roles/Writing - original draft: O.M.O and G.L.; Writing - review & editing: O.M.O, N.M., V.U.C.B, X.C, C.C, S.A and G.L.

Declaration of competing interest

The authors declare no conflict of interest.

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