

Validation of alternative behavioral observation methods in young broiler chickens

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ABSTRACT Continuous sampling provides the most complete data set for behavioral research; however, it often requires a prohibitive investment of time and labor. The objectives of this study were to validate behavioral observation methods of young broiler chickens using 1) 7 scan sampling intervals (0.5, 1, 3, 5, 10, 15, and 30 min) and 2) an automated tracking software program (EthoVision XT 14) compared to continuous behavioral observation, considered the gold standard for behavior observation. Ten 19-day-old Ross 708 broiler cockerels were included in this study. All behavior was video recorded over an 8-h period, and data were collected using a continuous sampling methodology. The same video files were utilized for analysis for scan sampling and automated tracking software analysis. For both analyses, the following criteria were used to identify which method accurately reflected the true duration and frequency for each behavior, as determined by continuous observation: $R^2 \geq 0.9$, slope was not differ-

ent from 1 ($P > 0.05$), and intercept was not different from 0 ($P > 0.05$). Active, eating, drinking, and maintenance behaviors were accurately estimated with 0.5-min scan sample intervals. Active, inactive, eating, and maintenance behaviors were accurately estimated with 1-min scan sample intervals. Inactive behavior was accurately estimated with 5-min scan sample intervals. The remainder of sampling intervals examined did not provide accurate estimates, and no scan sampling interval accurately estimated the number of behavior bouts. The automated tracking software was able to accurately detect true duration of inactive behavior but was unable to accurately detect activity. The results of this study suggest that high-frequency behaviors can be accurately observed with instantaneous scan sampling up to 1-min intervals. Automated tracking software can accurately identify inactivity in young broiler chickens, but further behavior identification will require refinement.

Key words: behavior, scan sampling, broiler, tracking software, validation

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INTRODUCTION

Ethology is an integral measurement utilized when assessing the welfare of livestock species. A comprehensive assessment of species-specific behaviors allows scientists to quantify the impact of housing and management practices by assessing deviations in these behaviors. This is particularly evident when comparing groups of animals with differing levels of welfare. Weeks et al. (2000) observed a significant deviation in feeding behavior between sound and lame broilers, indicating lameness has a negative effect on normal behavior. While ethology research in broiler chickens has historically focused on the quantification of ingestive and reproductive behavior (Noble et al., 1993; McGary et al., 2002; Bokkers and Koene, 2003; Bilcik et al., 2005; Skinner-Noble et al., 2005), recent research is utilizing behavior as an indirect measurement of animal prefer-

ence and affective states (Buckley et al., 2012; Mendes et al., 2013; Baxter et al., 2018; Raccoursier et al., 2019). This research, in turn, has influenced multiple aspects of poultry management and production.

Continuous sampling is considered to be the gold standard utilized across all species, as it offers the most complete assessment of an animal's behavior by providing a complete record of all behaviors and durations observed over a time period (Lehner, 1992). However, continuous sampling requires a substantial investment of time, labor, and resources on the scientist's behalf. This is especially evident when evaluating large animal populations or extended observation periods, and scan sampling may reduce the investment required to obtain usable data. Additionally, continuous sampling methodology may result in inaccurate data collection due to observer bias, fatigue, or skill level (Altmann, 1974; Oh et al., 2015); this can be mitigated by using automated data collection techniques. Therefore, validating alternative methodologies such as scan sampling or use of automated tracking software is critical.

Scan sampling, conducted as either instantaneous or one-zero sampling, may be a viable option to

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accurately assess behavior while minimizing time and labor investments by recording behavior at certain time intervals. Recent work has been conducted validating scan sampling methodologies in pastured lambs (Pullin et al., 2017), feedlot cattle (Mitlöhner et al., 2001), nursery piglets (Bowden et al., 2008), dairy calves (Miller-Cushon and DeVries, 2011), and laying hens (Daigle and Siegford, 2014). However, to the authors' knowledge, no work has been conducted validating scan sampling methodologies in young broiler chickens.

Manual behavior observation presents several obstacles to successful data collection, including but not limited to biased and poorly trained observers, insufficient ethogram development, and technological limitations of video and software equipment (Oh et al., 2015). The reproducibility of animal studies has become a concern in some scientific communities (Jilka, 2016), providing impetus to seek a more reliable observational method. Video tracking software has been used extensively in rodents (Pham et al., 2009; van Gorp et al., 2011; Aitken et al., 2017), zebrafish (Cachat et al., 2011; Mathur et al., 2011), and insects (Cullen et al., 2012; Rose et al., 2017) to objectively quantify activity levels. Video tracking software utilizes a pixel-by-pixel analysis to convert the center point of the subject to an x, y coordinate, allowing for subject tracking at a high sample rate. Additionally, the use of video tracking software allows for data to be collected that cannot be easily determined by a manual observer, such as velocity and total distance traveled (Noldus et al., 2001).

The objectives of this study were to validate alternative behavioral observation methods of young broiler chickens using 1) 7 different scan sampling intervals (0.5, 1, 3, 5, 10, 15, and 30 min) and 2) an automated tracking software program (EthoVision XT 14, Noldus, Netherlands) compared to the gold standard of continuous behavioral observation.

MATERIALS AND METHODS

The Ohio State University Institutional Animal Care and Use Committee in Columbus, Ohio, approved the protocol for this study June 7, 2018. These animals were cared for in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies, 2010).

Study Animals and Housing

This study was conducted at The Ohio State University Poultry Research Facility (Columbus, OH) in September 2018. Ten 19-day-old Ross 708 broiler cockerels were included in the study. Birds were individually housed in floor pens composed of wood and woven wire (76.2 cm × 76.2 cm × 76.2 cm), which were larger than the minimum space recommended by the National Chicken Council (6.5 lb/ft²; National Chicken Council, 2017). Birds were concurrently utilized for a feeding

trial, necessitating individual housing. Pens were facing each other with woven wire and an aisle in between, allowing for visual interaction. Pens were bedded with black mulch (Ohio Mulch, Columbus, OH), and birds had ad libitum access to feed and water. Feed was provided to each individual bird in a small plastic trough (7.6 cm × 10.1 cm). PVC nipple drinkers were custom made to span across 4 adjacent pens, offering 1 nipple to each individual bird (pen). Since birds were individually housed, each bird was provided a 10.1 cm length of chain which hung from the PVC pipe for enrichment.

Behavioral Measurements

Behavior was recorded with 1 of 10 color-hardwired IP video cameras per pen (Foscam, model FI19805P and model FI9900P, Houston, TX) recording at 960 pixels and 1,080 pixels, respectively. FI9805P cameras were used in pens 1 to 6 and FI9900P cameras were used in pens 7 to 10. To ensure camera type did not affect the results, t-tests were performed comparing each behavior for each method ($P > 0.41$). Each camera was positioned centrally over the pen at a height of 60.9 cm to ensure the bird was visualized at all times. Video was captured digitally using portable laptops with external USB hard drives and recorded continuously for 24 h. An 8-h subset of video data was utilized for observation, beginning with the onset of artificial daylight, coinciding with the expected highest activity levels (Schwean-Lardner et al., 2014), and continuing through the portion of the day with minimal external disruption.

Behavioral data (Table 1) were collected using a continuous sampling methodology, and the same video files were then utilized for analysis for scan sampling and automated tracking software analysis. Video was evaluated by 2 trained observers using The Observer XT 14 (Noldus, Netherlands). To ensure inter-observer reliability between the 2 observers, a 2-h subset of continuous video was selected at random, observed, and compared until 90% accuracy was achieved.

Statistical Analysis

Scan Sampling The individual chicken was the experimental unit. Following the methods described by Chen et al. (2016), continuous data were converted to 1-s interval samples (true values) using SAS software (version 9.4; SAS Institute Inc., Cary, NC). Seven instantaneous sample intervals (0.5, 1, 3, 5, 10, 15, and 30 min) were extrapolated from the 1-s intervals (Chen et al., 2016). Total duration and bout number were calculated over an 8-h period for each of the instantaneous sample intervals for each behavior. Linear regression (PROC REG) was used to conduct pairwise comparisons between the true values and the 7 sample intervals for each behavior (Chen et al., 2016). Sample intervals that met the following criteria were considered to accurately reflect the true duration and frequency for each behavior, based on Chen et al. (2016): 1) $R^2 \geq 0.9$, 2)

Table 1. Behavioral ethogram for manual observation.

Behavior	Description
Active	Behavior that involves movement and does not fall into the other categories. Primarily involves walking in pen.
Inactive	Sitting or standing with no forward motion or maintenance behaviors.
Eating	Head is over the feeder.
Drinking	Beak is touching the drinker nipple.
Maintenance	Scratching (using a foot to scratch at head), preening (grooming by using beak on body), wing stretch (extension of single wing without a flapping motion, often accompanied by a leg stretch), or dust bathing (interaction with bedding by tossing onto body or rolling in bedding).

Table 2. EthoVision detection settings.

Parameter	Setting
Subject detection	Dynamic subtraction
Automated setup	Yes
Brightness	21–27
Frame weight	1
Pixel smoothing	Low
Dropped frame correction	On
Track noise reduction	On
Subject contour	3 pixels
Subject erosion	3–5 pixels
Track filter	Minimal distance moved = 0.8 cm
Track smoothing	Lowess, half-window = 7

slope was not different from 1 ($P > 0.05$), and 3) intercept was not different from 0 ($P > 0.05$). Data were screened for linearity, normality, and independence using visual inspection of graphs; all assumptions were met.

Video Tracking Software Prior to analysis, EthoVision settings were optimized (Table 2) to prevent false detection of a subject and minimize “body wobble” (Noldus et al., 2001; Hen et al., 2004). This required using the “automatic detection” function first, then manually adjusting the brightness until only the bird was detected. Track smoothing settings were optimized as described in Cullen et al. (2012). Total duration for each behavior was obtained using PROC SUMMARY in SAS for each bird. Linear regression (PROC REG) was used to conduct pairwise comparisons between the total durations obtained from continuous observation and the total durations obtained from automated tracking software. Residuals were plotted and checked for assumptions, and data were screened for outliers. Cook’s distance was used to identify influential observations; individual birds were removed from the data set if the Cook’s distance was greater than $4/n$ (Dohoo et al., 2009).

RESULTS

Scan Sampling

Data for the 7 different instantaneous sample intervals were compared to 1-s sample intervals, representative of the continuous data. Results were analyzed as described in Chen et al. (2016) (Table 3). No behavior bouts were accurately estimated using any of the scan

sampling intervals (Table 4). Active, eating, and maintenance behavior duration was accurately estimated with 0.5 and 1-min scan sample intervals (Table 4). Inactive behavior duration was accurately estimated with 1 and 5-min scan sample intervals (Table 4). Drinking behavior duration was accurately estimated with only 0.5-min scan sample intervals (Table 4). Mean bout duration and number of bouts per hour were calculated for descriptive purposes (Table 5).

Automated Tracking Software

Video data of 10 birds were analyzed using EthoVision video tracking software and compared to continuous behavior observation. Automated tracking software was unable to consistently detect eating, drinking, and maintenance behaviors due to inconsistent nose and tail point identification; therefore, the final analysis included only active and inactive behaviors (Figures 1 and 2). One bird was excluded as an influential observation from both active and inactive behavior analysis, and one bird was excluded from the inactive analysis only. The automated tracking software was unable to accurately detect active behavior duration but able to accurately detect true duration of inactive behavior (Table 6).

DISCUSSION

Historically, broiler research has focused on the impact of housing and management practices on bird production, health, and carcass quality (Robinson, 1991; Kotula and Wang, 1994; Teeter and Belay, 1996; Ekstrand et al., 1997; Kannan et al., 1997). With an increase in consumer concern regarding the management and care of food animals (Vanhonacker et al., 2016; Erian and Phillips, 2017; Mulder and Zomer, 2017; Yang and Hong, 2019), research has shifted to address broiler welfare (Kittelsen et al., 2018; de Lima et al., 2019; Raccoursier et al., 2019). Behavior is an objective measurement that can be used to quantify welfare conditions of commercially reared broilers. However, work conducted to identify and validate accurate behavioral observation methodologies is limited. Therefore, the objectives of this study were to validate scan sampling and automated tracking software as alternative behavioral observation methodologies for young broiler chickens.

Table 3. R^2 (slope P -value, intercept P -value) for behaviors and scan sampling time intervals.¹

	0.5 min	1 min	3 min	5 min	10 min	15 min	30 min
Active							
Duration	0.98 (0.53, 0.69)*	0.95 (0.30, 0.17)*	0.73 (0.67, 0.71)	0.80 (0.61, 0.94)	0.57 (0.70, 0.91)	0.67 (0.06, 0.06)	0.57 (0.08, 0.09)
Bouts	0.51 (<0.01, <0.01)	0.36 (<0.01, <0.01)	0.19 (0.02, <0.01)	0.05 (<0.01, <0.01)	0.02 (0.06, <0.01)	0.06 (0.41, <0.01)	0.00 (0.3, <0.01)
Inactive							
Duration	0.99 (0.04, 0.16)	0.95 (0.3, 0.65)*	0.86 (0.39, 0.56)	0.93 (0.20, 0.08)*	0.79 (0.80, 0.78)	0.71 (0.38, 0.32)	0.61 (0.45, 0.30)
Bouts	0.65 (<0.01, <0.01)	0.27 (<0.01, <0.01)	<0.01 (<0.01, <0.01)	<0.01 (<0.01, <0.01)	0.00 (<0.01, <0.01)	0.03 (<0.01, <0.01)	0.14 (<0.01, <0.01)
Eating							
Duration	0.99 (0.08, 0.07)*	0.96 (0.11, 0.09)*	0.89 (0.76, 0.59)	0.84 (0.38, 0.18)	0.60 (0.74, 0.98)	0.85 (0.07, 0.04)	0.81 (0.06, 0.06)
Bouts	0.69 (<0.01, <0.01)	0.43 (<0.01, <0.01)	0.22 (<0.01, <0.01)	0.24 (<0.01, <0.01)	0.01 (<0.01, <0.01)	0.01 (<0.01, <0.01)	0.02 (<0.01, <0.01)
Drinking							
Duration	0.95 (0.66, 0.96)*	0.39 (0.05, 0.02)	0.52 (0.29, 0.21)	0.19 (0.88, 0.56)	0.28 (0.49, 0.79)	0.47 (0.12, 0.76)	0.3 (0.23, 0.64)
Bouts	0.59 (0.05, <0.01)	0.39 (<0.01, <0.01)	0.5 (0.04, <0.01)	0.01 (0.09, <0.01)	0.00 (0.28, <0.01)	0.05 (0.8, <0.01)	0.07 (0.9, <0.01)
Maintenance							
Duration	0.99 (0.15, 0.54)*	0.97 (0.24, 0.33)*	0.86 (0.43, 0.67)	0.86 (0.21, 0.40)	0.61 (0.50, 0.73)	0.64 (0.26, 0.22)	0.52 (0.26, 0.24)
Bouts	0.75 (0.34, <0.01)	0.66 (0.43, <0.01)	0.36 (0.19, <0.01)	0.34 (0.42, <0.01)	0.53 (0.97, <0.01)	0.04 (0.32, <0.01)	0.02 (0.66, <0.01)

¹The sampling intervals were considered accurate (*) if they met 3 criteria: $R^2 \geq 0.9$, slope was not different from 1 ($P > 0.05$), and intercept was not different from 0 ($P > 0.05$; Chen et al., 2016).

Scan sampling is an effective means to assess behavior in poultry when the behaviors of interest are frequent and long in duration (i.e., 80% of an individual's time budget; Martin and Bateson, 2012). Previous studies have demonstrated broilers spend >80% of their time budget active (2.5 to 4%, Weeks et al., 2000; 20%, Murphy and Preston, 1988), inactive (76 to 86%, Weeks et al., 2000; 64%, Murphy and Preston, 1988), or eating (5 to 6%, Weeks et al., 2000; 11.3%, Murphy and Preston, 1988). These 3 behaviors also comprised >80% of the time budget in this study, despite different proportions (17% active; 53% inactive; 21% eating). This variation may be attributed to multiple factors, including bird age, data collection method, and observation duration. Weeks et al. (2000) observed 39 to 49 D old broilers with 5-min instantaneous scan samples over a 1-h duration, whereas Murphy and Preston (1988) observed 19- to 50-day-old broilers with continuous observation for 1 h. In this study, 19-day-old broilers were observed with continuous observation for an 8-h time period. However, despite the variable proportion of time spent on each of these activities, the mean durations and frequencies (activity: 10 s, 62 bouts/h; inactivity: 22 s, 88 bouts/h; eating: 14 s, 57 bouts/h) were adequate to provide reliable estimates for total behavior duration at a 1-min scan sampling interval (Tables 4 and 5). Inactive behavior was not accurately estimated using a 0.5-min scan sampling interval. The high frequency of inactive behavior (88 bouts/h) resulted in an overestimation of total duration of inactivity with this short scan sample interval. Broilers have been observed to experience relatively long bouts of eating activity, 88 to 199 seconds per bout (Weeks et al., 2000). This study observed a shorter eating bout duration, but a high frequency allowed for accurate scan sampling at a 1-min interval. As described by Miller-Cushon and DeVries (2011), if the scan sampling interval is longer in duration than the behavior bout being observed, accurate estimation of true duration is difficult. Thus, drinking behavior was only accurately estimated at a 0.5-min interval, as the average duration and frequency were both low (5 s, 13 bouts/h). Additionally, drinking behavior represented only 2% of the overall time budget, making it a poor candidate for scan sampling. To accurately assess short duration or rare behavior patterns such as these, continuous sampling is ideal (Martin and Bateson, 2012). Similarly, maintenance behaviors, including preening, wing stretching, dust bathing, and grooming, were only 7% of the time budget. This was lower than expected, as Weeks et al. (2000) observed 7.5 to 10% of the time budget was spent on preening alone. However, while the average maintenance behavior duration was low (7 s), the frequency was relatively high (40 bouts/h), allowing for accurate estimation of true values using scan sampling up to 1-min intervals. These results are similar to previous work where behaviors composing <20% of the time budget were poorly estimated using instantaneous scan sampling intervals above 1 min (Pullin et al., 2017).

Table 4. Means (SD) for behavioral data obtained using instantaneous samples that were extrapolated from continuous observation of young broiler chickens.¹

	1 s ²	0.5 min	1 min	3 min	5 min	10 min	15 min	30 min
Active								
Total duration (h)	1.3 (0.3)	1.3 (0.3)*	1.2 (0.3)*	1.2 (0.4)	1.2 (0.4)	1.1 (0.4)	1.2 (0.9)	1.4 (1.0)
Total bouts	460.7 (157.1)	91.9 (17.8)	48.4 (7.1)	17.8 (4.4)	11.3 (2.3)	5.7 (2.0)	3.5 (2.2)	2.3 (1.3)
Inactive								
Total duration (h)	3.9 (0.8)	4.1 (0.7)	4.1 (0.8)*	4.1 (0.8)	4.2 (1.0)*	4.4 (0.9)	4.1 (1.2)	4.4 (1.4)
Total bouts	648.5 (289.3)	104.7 (23.2)	65.4 (13.9)	28.4 (6.2)	19.9 (4.3)	11.3 (2.2)	8.1 (1.6)	4.3 (1.1)
Eating								
Total duration (h)	1.5 (0.5)	1.6 (0.4)*	1.5 (0.4)*	1.5 (0.4)	1.7 (0.6)	1.7 (0.6)	1.7 (0.8)	1.5 (0.8)
Total bouts	439.8 (227.2)	79.5 (24.1)	45.3 (10.9)	17.8 (5.2)	13.1 (3.5)	7.3 (1.8)	5.1 (1.7)	2.6 (0.9)
Drinking								
Total duration (h)	0.1 (0.1)	0.2 (0.1)*	0.1 (0.0)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.2)
Total bouts	100.0 (64.6)	12.4 (5.7)	6.7 (1.7)	2.8 (1.2)	1.0 (0.9)	0.6 (0.8)	0.2 (0.4)	0.1 (0.3)
Maintenance								
Total duration (h)	0.6 (0.3)	0.6 (0.3)*	0.6 (0.3)*	0.5 (0.3)	0.5 (0.4)	0.5 (0.4)	0.6 (0.6)	0.6 (0.7)
Total bouts	293.2 (156.5)	42.1 (21.1)	23.3 (12.3)	8.2 (4.1)	5.4 (3.4)	2.7 (2.0)	2.1 (1.8)	0.7 (1.1)

¹The total duration and bouts generated by each sample interval were compared pairwise to the true values (represented by samples at 1-s intervals) using linear regression, and instantaneous samples were considered accurate (*) if they met 3 criteria: R² ≥ 0.9, slope was not different from 1 (P > 0.05), and intercept was not different from 0 (P > 0.05; Chen et al., 2016).

²The 1-s intervals represented the true values based on continuous observation to the nearest second.

Table 5. Mean bout duration in seconds, frequency, and proportion of total time budget for each behavior.

	Active	Inactive	Eating	Drinking	Maintenance
Mean bout duration (s)	10	22	13	5	7
Number of bouts per hour	62	88	59	13	40
% of total time budget	17	53	21	2	7

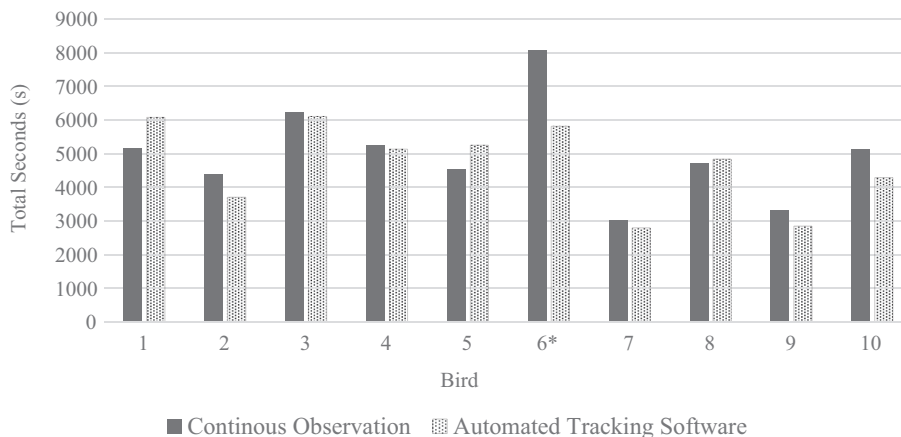


Figure 1. Total number of active seconds per bird, observed with continuous observation and automated tracking software. Birds were excluded from the final analysis (*) as influential observations if Cook’s distance was greater than 4/n.

In this study, scan sampling methodology was unable to accurately assess any behavior at a scan sampling interval above 5 min. This is likely due to the relative duration and frequency of these behaviors, as the mean duration for all behaviors was under 30 s. Inactive behavior exhibited the highest frequency (88 bouts/h), allowing for accurate duration estimates at the 5-min scan sampling interval. Additionally, behavior bouts were not accurately estimated for any be-

havior at any interval (Table 4). Scan sampling at all intervals resulted in underestimation of actual bout number for all behaviors. As continuous observation data contained behaviors that were less than 1 s in duration, many of these behavior bouts were excluded with scan sampling.

Automated tracking software programs quantify animal activity without requiring manual observation of the subject. This is an ideal tool for observing numerous

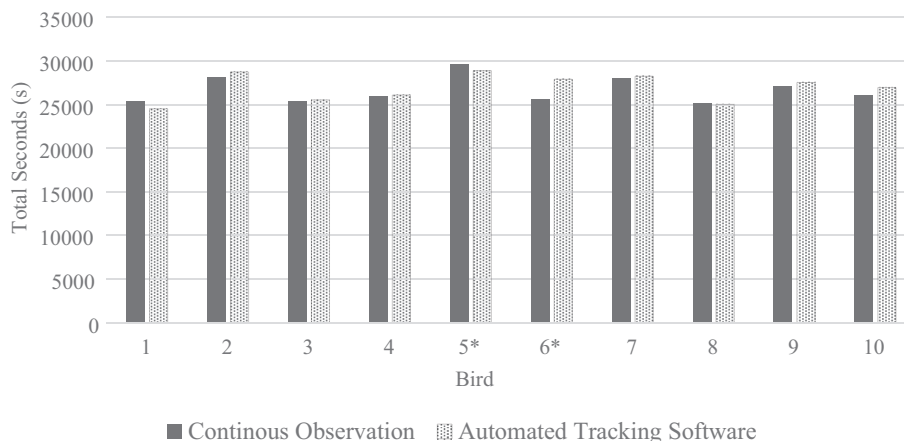


Figure 2. Total number of inactive seconds per bird, observed with continuous observation and automated tracking software. Birds were excluded from the final analysis (*) as influential observations if Cook's distance was greater than $4/n$.

Table 6. Linear regression comparing active and inactive behavior between continuous observation and EthoVision.

Behavior	N ¹	R ²	Slope P	Intercept P
Inactive (s)	8	0.91	0.19	0.20
Active (s)	9	0.78	0.63	0.59

¹Bird number after influential observations were removed (Cook's distance greater than $4/n$; Dohoo et al., 2009).

individual animals for long time periods, as was demonstrated in this study. While the application of this software is likely limited to individually housed animals, the ability to observe extensive time periods is a valuable tool for quantifying time budgets. Previous work has utilized automated tracking software programs to quantify bird behavior, but software validation is limited or non-existent (Agnvall et al., 2012); thus, this study provides preliminary data which may encourage further validation for use in group housing situations, as would be evident in industry applications. In our current study, the automated tracking software program was able to accurately determine the true duration of inactive behaviors in young broiler chickens but was unable to accurately detect active, eating, drinking, or maintenance behaviors. The program utilized in our study relies on detecting movement of the center point of a subject as a means to quantify activity. The potential functionality of this software includes 3-point subject identification, zone identification with movement into and out of zone, zone proximity, identification of changes in body shape (i.e., elongation from wing stretching behavior), and monitoring activity level via rate of pixel change. However, as this was the first investigation into application of this software to broiler behavior, many of these features did not perform as expected. The inability to consistently identify a nose or tail point did not allow for utilization of the eating and drinking zone parameters, and the maintenance behaviors involved pixel movement too subtle for accurate detection. This can be partially attributed to the limitations of the video technology utilized in this

study and may be improved with the use of more advanced recording equipment or advances in the automated tracking software program. Further work is needed to validate different broiler behaviors using this automated software program as well as assess the accuracy of the program's unique measurements including distance traveled and velocity.

CONCLUSION

The behavioral methodology of choice is dependent upon the types of behaviors the researcher seeks to observe, as well as the size of the subject pool and the amount of observation time required. Behaviors that are high frequency or long duration, as active, inactive, eating, and maintenance behavior were categorized in this study, can be accurately observed with instantaneous scan sampling up to 1-min intervals. The results of this study suggest that automated tracking software can accurately determine duration of inactivity based on movement of a center point of a subject.

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