Continuous heart rate variability monitoring of freely moving chicken through a wearable electrocardiography recording system

P. Ahmmed ,* J. Reynolds ,* A. Bozkurt ,* and P. Regmi[†,1]

*Department of Electrical and Computer Engineering, North Carolina State University, Raleigh, NC 27695, USA; and †Department of Poultry Science, University of Georgia, Athens, GA 30602, USA

ABSTRACT Identification and quantification of stress and stress inducing factors are important components of animal welfare assessment and essential parts of poultry management. Measurement of the autonomic nervous system's influence on cardiac function using heart rate and heart rate variability (**HR/HRV**) indices can provide a non-invasive assessment of the welfare status of an animal. This paper presents a preliminary study showing the feasibility of continuous long-term measurement of HR/HRV indices in freely moving chicken. We developed and evaluated an electrocardiography (**ECG**) based HR/HRV recording system that can be used as a poultry wearable backpack for research studies. The backpack system was first validated against a commercial ECG amplifier, and the corresponding estimations of HR values matched well with each other. Then, an in vivo proof-of-concept experiment was conducted on floor-reared chickens to collect ECG data for 2 weeks. The extracted HR/HRV values show strong alignment with circadian patterns and well-defined sleep cycles. Wearable devices, like the backpack ECG system used in this study, may be best suited for application in freely moving poultry to get an insight into circadian abnormalities and sleep quality for stress and welfare management.

Key words: electrocardiography, heart rate variability, chicken, animal welfare, wearables

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INTRODUCTION

In recent years, the livestock industry in the United States has been undergoing a massive transformation driven by consumer concerns for welfare-friendly husbandry systems. For instance, statistics from the United States Department of Agriculture (**USDA**) estimates nearly 30% of the 330 million table-egg-laying hens in the United States occupied new "cage-free" housing in 2021, and this number is expected to rise rapidly in the coming years. These noncage housing systems not only require more complex designs with enrichment for birds but can also act as a source of psychosocial and physical stresses that are associated with different health and welfare challenges. Major welfare challenges of the modern poultry management systems include aggressive pecking and cannibalism, high prevalence of keel bone fractures, and footpad disorders (Lay et al., 2011). Similarly, health challenges include pathogenic infections of different viral, bacterial, and protozoal origin.

The assessment of welfare status at individual bird level relies on accurate quantification of the stress response. The sympathetic arm of the autonomic nervous system (**ANS**) is the primary orchestrator of the sympatho-adrenal (flight or fight) stress response. On the other hand, the parasympathetic arm represents the vagal response involved in regulating visceral functions when an animal is challenged with external or internal stimuli. As a result, autonomic function has been researched extensively in human and other animal models to quantify stress and to determine the physiological and psychological plasticity of an organism to cope with stress (as reviewed by Gaidica and Dantzer, 2020). Branches of ANS also interact rhythmically to regulate cardiac activity resulting in the variation of the interbeat intervals, commonly known as heart rate variability (**HRV**). Examining HRV metrics has since emerged as a non-invasive measure of autonomic nervous function as it relates to stress, affective states, behavior, and diseases in animals (as reviewed by von Borell et al., 2007). In general, only a limited number of studies with HRV have been conducted in chickens and these include assessment of acute stress in divergent genetic lines of laying hens selected for feather pecking (Korte et al., 1998) and development of cardiovascular rhythm during embryonic development and at post-hatch (Aubert et al., 2004). The majority of the studies in

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[1]Corresponding author: pregmi@uga.edu, pr01814@uga.edu (PR)

livestock and birds attempt to draw inferences from HRV indices through electrocardiography (**ECG**) recordings that only span a few minutes before, during, or after the stress exposure. Like other continuous physiological parameters, heart rate (**HR**) and HRV have been demonstrated to follow circadian pattern (Li et al., 2011) and even vary with the sleep stages (da Estrela et al., 2021). Shorter ECG recordings may fail to accommodate circadian changes in cardiac activity and confound the results of the experiment. Furthermore, shorter recordings might be unable to account for cardiac changes that can occur in anticipatory manner prior to the exhibition of altered behavior or the changes that may persist even after recovery from the stress challenge (Mohammadi et al., 2019). For the measurements to be useful in monitoring stress and welfare in poultry, a system capable of monitoring HR/HRV in a freely moving bird for a longer duration is required. Long-term monitoring can add considerable precision to the inferences drawn from the measurements depending on the time of the day when they were collected. Specifically, continuous HR/HRV monitoring can be a good alternative to electroencephalogram in measuring sleep quality as an effect of stress (da Estrela et al., 2021).

We developed a wearable bioelectric recording system capable of monitoring heart rate and its variability through ECG signals. Such an ECG based measurement usually consumes very low power and hence is better suited for long-term continuous data acquisition. This paper describes the design and validation of the wearable system through a preliminary proof-of-concept experiment for collecting long-term biopotential measurements in poultry birds.

MATERIALS AND METHODS

System Overview

The proposed "backpack" system is developed upon a wireless microcontroller (CC2642, Texas Instruments, Dallas, TX) equipped with Bluetooth Low Energy (**BLE**) radio communication. The on-board analog front-end (AFE) for recording ECG (MAX30003, Maxim Integrated, San Jose, CA) is connected to two standard needle electrodes (see Figure 1a) through a network of passive components working as a filter and providing electrical protection for the animal in compliance with AAMI (Association for the Advancement of Medical Instrumentation) standards for safe current levels. The AFE amplifies and filters the biopotential signal and sends the digitized data to the microcontroller through a Serial Peripheral Interface (**SPI**) bus.

The system is primarily designed for standalone operation in the farmhouse. Hence, offline data storage is implemented with an on-board micro-Secure Digital (**SD**) memory card. However, for real-time debugging purposes, the BLE functionality is also enabled. A 30×30 mm^2 printed circuit board (**PCB**) contains all the electronics and the contact pads for connecting the battery and the ECG electrodes. The microcontroller needs a 3.3 V direct current (**DC**) supply to comply with the recommended range of micro-SD cards. However, the analog operation of the ECG AFE needs a 1.8 V DC supply. Hence, two voltage regulators of 3.3 V and 1.8 V (both from the XCL210 series, Torex Semiconductor Ltd., Chuo City, Tokyo, Japan) are used in the system. The backpack system can be powered from either a lithium-ion polymer (LiPo) battery or a micro-USB power supply. Charging circuitry (MCP73831, Microchip Technology, Inc., Chandler, AZ) to recharge the LiPo battery from the micro-USB power supply is also included.

Low power consumption of the overall system is important for long-term monitoring applications. To estimate the power consumption of the backpack system, the current drawn from a 3.7 V DC supply (nominal voltage of LiPo batteries) was measured using a source meter (Model 2450, Keithley Instruments, Cleveland, OH). During this measurement, the backpack was recording ECG with a sampling rate of 128 Hz and performing one write operation (the smallest writable sector of the SD card is 512-bytes long) every 1.25 s. A standard SD card with a memory of 32 GB can store 2 y of ECG data and a smaller memory size can be used for cost efficiency as needed.

As the ECG AFE consumes very little power and the microcontroller is programmed to utilize its low-power sleep mode as much as possible, the system's power consumption was dominated mostly by the SD card's read and write cycles. We measured an average current of 0.8 mA (less than 3 mW power). This would allow a standard 480 mAh LiPo battery to last for approximately 25 d. Depending on the experimental duration lighter or heavier batteries can be used within the constraints of animal comfort. The weight of the overall system was 15.4 g (5.9 g for the board and 9.5 g for the battery).

In Vivo Experiments

A few in vivo experiments were performed on 20-wk-old White Leghorn pullets (*Gallus gallus domesticus*). All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of NC State University, Raleigh, NC, USA.

First, a short in vivo validation experiment was performed on a chicken to measure the accuracy of the backpack system in estimating the HR/HRV in comparison to a commercial ECG amplifier as a gold standard. After validation, a long-term study was performed with continuous ECG data collected for approximately two weeks. For these experiments, the devices were attached to the chickens using a fabric vest with a pocket acting as a backpack (see Figure 1b). Chickens were reared in floor pens and the focal birds selected for the experiment were acclimated to the fabric vest one week prior to the sensor attachment and data acquisition.

System Validation For validating the backpack system, simultaneous ECG data were collected using a

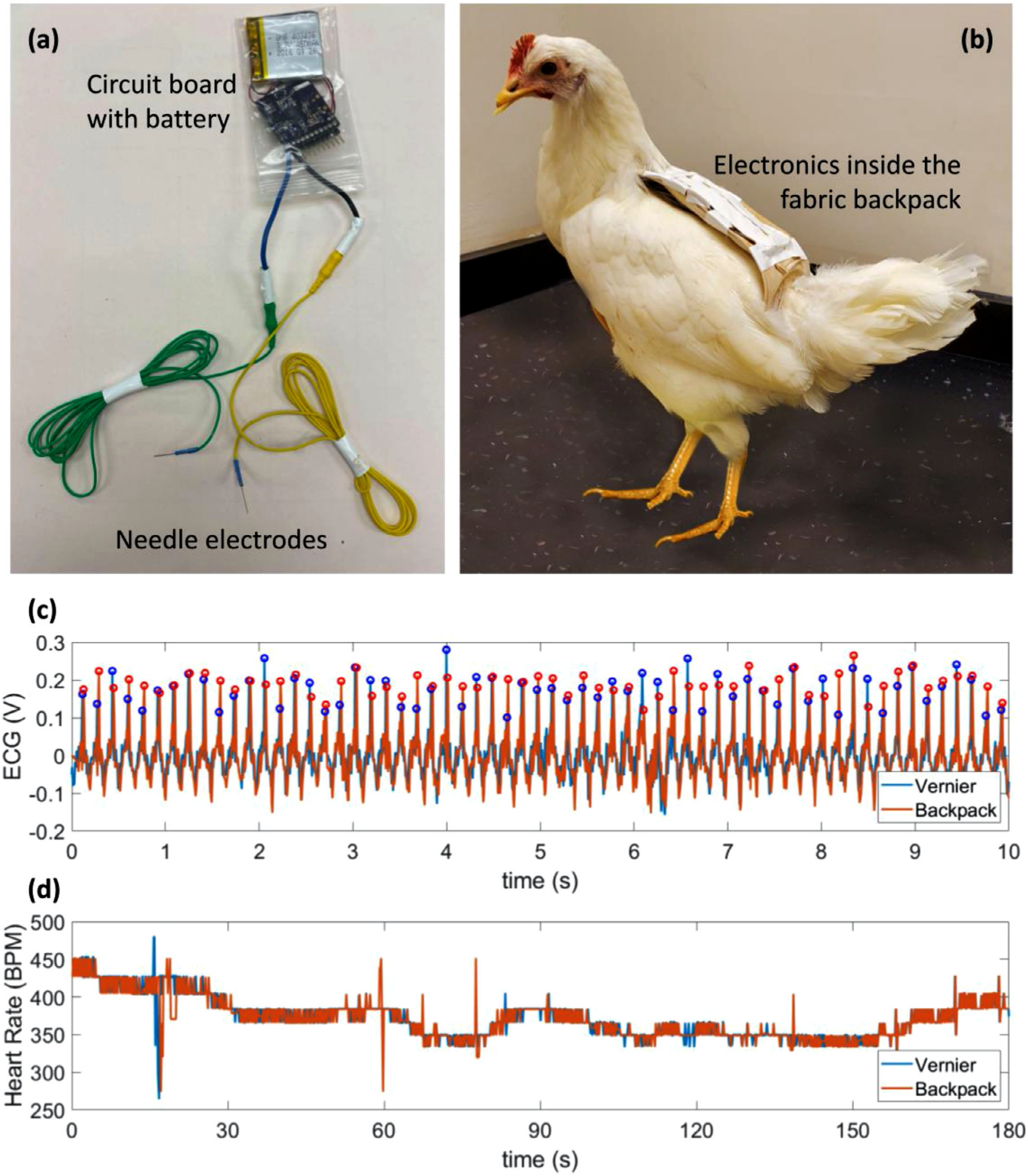

Figure 1. (a) Picture of the sensor package with electronic components and the electrodes; (b) Picture of a chicken wearing a backpack with the electronic sensor package; (c) Example plot of ECG waveforms recorded simultaneously using two devices during the validation experiment; (d) Transient plot of HR values extracted from the two devices.

commercial device (Go Direct EKG Sensor, Vernier Software & Technology, LLC, Beaverton, OR). Both the systems used 27-gauge uncoated stainless steel needle electrodes (TSS-1315, Technomed USA, Inc., White Bear Township, MN). Two pairs of electrodes were inserted subdermally on the dorsal side of the anesthetized chicken for the two systems and a driven right leg (**DRL**) electrode was inserted on the right thigh for the Go Direct device. While the backpack device used the micro-SD card for data storage, the data from the Go Direct device was collected directly to a laptop through a micro-USB connection. The experiment ended after 3 min of simultaneous data collection.

A 25-point moving average filter extracted the baseline artifacts in the ECG data recorded from both the devices with the same sampling frequency of 128 Hz. The clean ECG signals for each system (see Figure 1c) were obtained by subtracting the baselines from the raw data where the obtained graphs clearly coincided and overlapped with each other. Then, the peaks in the filtered ECG signals were detected using a library function *findpeaks* in MATLAB (The MathWorks, Inc., Natick, MA) to obtain the R-R intervals (**RRI**) of the ECG signal. The HR values in beats per minute (**BPM**) were calculated from these RRI values (see Figure 1d). Visual observations of these recordings between the developed backpack system and commercial reference system demonstrate close to 100% matching.

The mean ± SD of the HR values extracted from the backpack device and the reference device are 373.59 ± 27.70 BPM and 373.78 ± 27.68 B.M. respectively, which are almost equal. The accuracy of the individual HR values is estimated by the mean absolute error which is only 7.11 BPM (1.9%) and Pearson's correlation coefficient of 0.85 with respect to the reference system. Although the chicken exhibited higher than usual HR due to the stress from experimental handling, this study demonstrates the ability of the backpack system to provide accurate HR/HRV information about the animal subjects.

Long-term Study During the experimental setup, first, a few feathers were removed from the dorsal side of the chicken to prepare the site of implantation. Two sterilized needle electrodes were inserted subdermally along the mid-dorsal line facing away from each other. Then, we established a temporary data connection between the backpack and a remote laptop computer to visualize the data and confirm that the electrode placement was correct. After this initial verification, the device, along with the battery, was secured with a small plastic zipper bag and inserted into a pocket on the fabric backpack. Finally, the electrodes were secured on the skin, the extra wires were tucked under the backpack with medical adhesive tapes, and the backpack was put on the chicken.

After the sensor attachment, 2 chickens were left with the rest of the flock in their home pens. They were monitored daily to check the sensor attachment and observe their behavior in the flock.

We should note a major failure point with other backpacks, beyond these two, we deployed but discarded the data due to discontinuity of the experiment. The standard commercial needle electrodes are designed to connect to external equipment and hence come with longer wires. If these wires of the needle electrodes are not shortened or securely stored inside the backpack, the chicken itself or its conspecifics demonstrate a behavioral pattern of pecking and pulling off the electrodes. This is a known limitation of any wearable system and can be handled with a careful ergonomic design to secure the electronics and wiring better.

Once the raw data was extracted from the microSD cards, the ECG baseline was calculated using a 25-point moving average filter. The baseline was subtracted from the raw signal to remove major motion artifacts. Cleaner portions of the data were first segmented using an amplitude threshold. Peak detection using the library function *findpeaks* in MATLAB was applied on each 5-s bin of the segmented data. Finally, the segments that have a mean RRI less than 500 ms (corresponding lower limit on HR is 120 B.M., the ratio between maximum and minimum HR less than 2.2, and average correlation coefficient greater than 0.66 with the template (Orphanidou et al., 2015) were classified as the 'usable' segments.

RESULTS AND DISCUSSION

This study investigated the feasibility and application of long-term HRV measurement using a wearable sensor in laying hens. After plotting the average HR values in each of the usable segments from the 2 chickens throughout the whole experiment, prominent circadian patterns of the HR values were observed which inspired us to condense the plots into 24-h periods. Figure 2 shows the daily plots of averages of RRI values and 3 HRV indices extracted from all the usable segments in each 30-min window (only the days with at least 20% usable segments are shown). The HRV indices include standard deviation of the RRI values (**SDNN**), standard deviation of absolute differences between successive RRI values (**SDSD**), and the ratio of power from the low frequency (**LF**) and high frequency (**HF**) regions of the power spectral density plots (Shaffer and Ginsberg, 2017). Although the LF and HF zones are well-defined in humans (0.04−0.15 Hz and 0.15−0.4 Hz, respectively) based on their relationship with the autonomic functions, such standards are yet to be set in case of poultry birds. Moreover, we failed to find any circadian pattern in the LF/HF values using the frequency ranges defined for humans, mostly because of the difference of HR values in humans and chickens. As the HR of chickens is about 4 times higher than that of humans, the frequency range for calculating LF and HF power was chosen to be 0.16−0.6 Hz and 0.6−1.6 Hz, respectively. A similar LF (0.195−0.74) and HF (0.78−2.5) zone was previously reported in HRV study in chicken embryos at the end of the incubation period where the mean HR was reported to be 246 beats/min (Aubert et al., 2004). A validation of the exact frequency ranges in chickens is subject to further investigation.

Although the functional implications of the data require data collection from a significant number of animals, it is obvious from the plots in Figure 2 that both chickens showed well-defined sleep cycles synchronized with the lighting schedule (5:00 am to 3:00 pm). Both chickens had distinctively higher mean RRI (lower HR), lower SDSD, and higher LF/HF values during sleep compared to the light hours. The lower HR obtained during the dark hours is most likely due to the absence of confounding influences of metabolic and physical activity on HRV (Li et al., 2011). Similar to the results of this study, decrease in HR during the night phase has also been observed in human subjects. However, contrary to the results in humans, the SDNN and SDSD values decreased and the LF/HF values increased in chickens during sleep (Li et al., 2011). Various factors including the sleep stages, metabolism, core body temperature and the blood pressure regulation mechanism (baroreflex activity and the renin-angiotensin system) can influence circadian HRV changes. A reduced SDSD

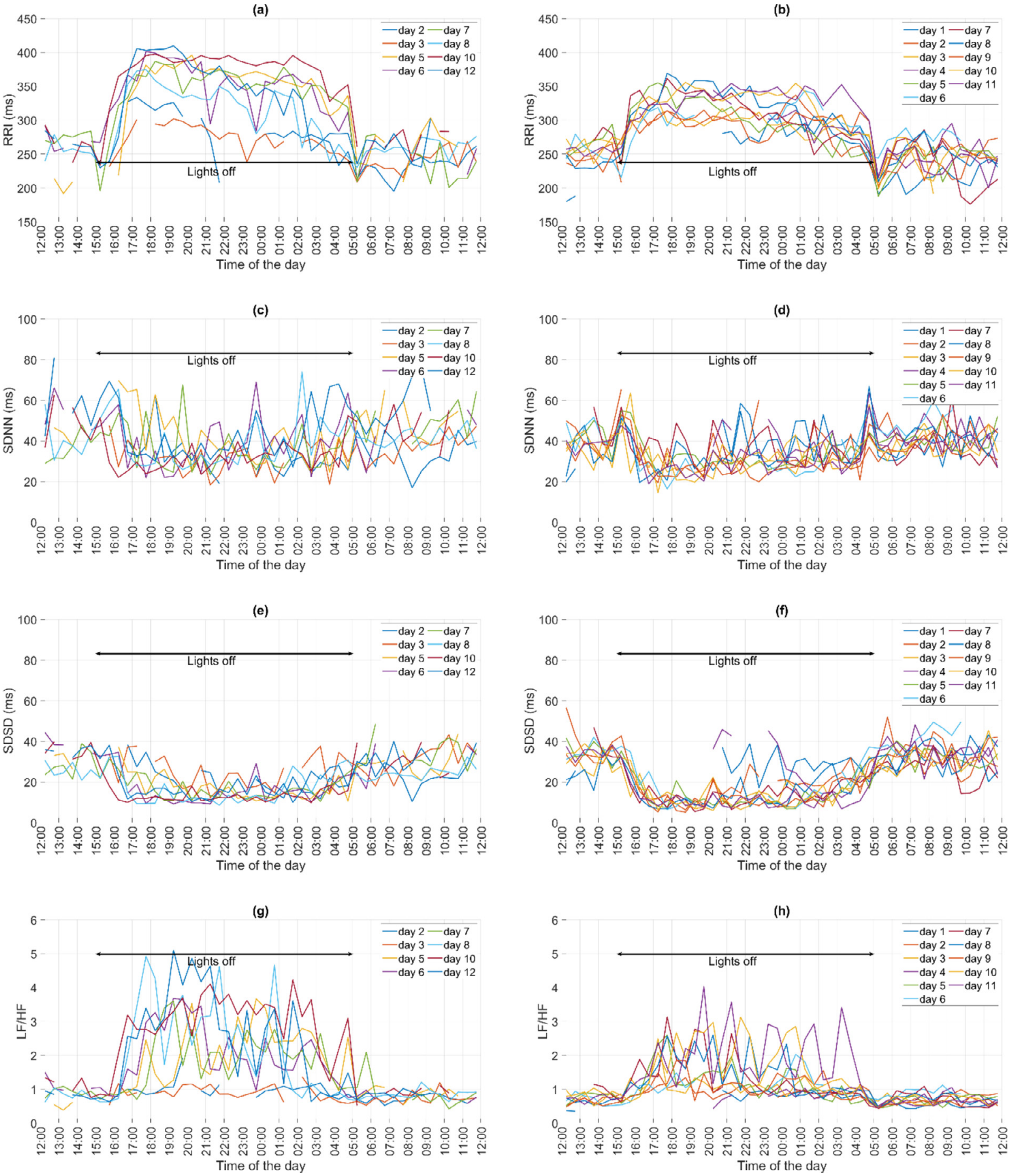

Figure 2. Daily plots of heart rate variability indices from two chickens exhibiting circadian trends of (a-b) the R-to-R interval (RRI); (c-d) the standard deviation of RRI; (e-f) the standard deviation of the absolute differences between adjacent RRI values, (g-h) the ratio of power in LF and HF range of the spectral density.

together with an increased LF/HF ratio during the lights-off period in our study could indicate a reduced HF band activity. These results point toward sympathetic dominance on resting HR in chicken. Sympathetic domination on resting HR was previously observed in chicken during short-term pharmacological blockade of ANS (Matsui and Sugano, 1987). On the other hand, a circadian pattern of increased blood pressure at night than during the day has been observed in chickens (Savory et al., 2006). An increase in the LF/HF ratio at night could also be due to the increased LF band power because of greater baroreflex activity in chickens at night. Anyway, one must be cautious about relying entirely on HRV indices as a proxy to sympatho-vagal balance particularly when pharmacological studies involving HRV indices during partial or total ANS blockade in chickens are lacking. We are also unaware of other studies in chickens monitoring HRV over longer periods. Broader experiments with more animals along with structured pharmacological studies could reveal the correlation of the estimated sleep quality with respect to the induced stress in commercial poultry.

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DISCLOSURES

The authors of the manuscript entitled "Continuous heart rate variability monitoring of freely moving chicken through a wearable electrocardiography recording system" declare no conflict of interest during the preparation and publication of the manuscript.

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