ADAPTATION AND VALIDATION OF THE CO_2 BALANCE METHOD FOR VENTILATION RATE ESTIMATION IN LAYING HEN HOUSES

A. Katherín Carranza-Díaz[1,2,*], A. Dalila Larios-Martinez[2], Alexis Ruiz-González[1], Stéphane Godbout[2], Sébastien Fournel[1,**]

[1] Université Laval, Québec, Canada.
[2] Research and Development Institute for the Agri-Environment (IRDA), Québec, Canada.
Correspondence: *andrea-katherin.carranza-diaz.1@ulaval.ca, **sebastien.fournel@fsaa.ulaval.ca

HIGHLIGHTS

- Adapted CO_2 balance improves ventilation rate prediction in poultry housing systems.
- Respiration quotient set to 0.9 (day) and 0.85 (night) enhances model accuracy.
- Adjusting the CO_2 production rate (+18% day, +8% night) boosts model performance.
- Model accuracy declines with inlet-to-exhaust CO_2 differences below 150 ppm.

ABSTRACT. *Assessing the environmental impact of poultry farms requires accurately determining ventilation rates (VR) while minimizing resource use. Accurate VR estimates are crucial for precisely evaluating emissions, making VR a vital factor in enhancing the sustainability of poultry production. This study aims to adapt and validate the carbon dioxide (CO_2) balance method for VR prediction in laying hen houses. The adaptation was based on the CO_2 balance method from the International Commission of Agricultural and Biosystems Engineering (CIGR), which considers variables such as the CO_2 production rate of hens ($PRCO_2$), animal activity (AA), and the respiratory quotient of laying hens (RQ). Data collected from an experiment involving laying hens in conventional cages (CC) under laboratory conditions were used to adapt the VR prediction model. The adapted model was validated under both laboratory and commercial conditions in three housing systems: conventional cages (CC), enriched cages (EC), and cage-free systems (CF). At the laboratory level, validation was performed using data from two studies conducted in the same controlled laboratory conditions but with different housing systems (the first in CF and the second in the three systems simultaneously). At the commercial level, validation was performed using data collected from 30 commercial farms in Quebec, Canada. The resulting adaptations included setting AA to 1, RQ to 0.9 during the day and 0.85 at night, and $PRCO_2$ to 18% during the day and 8% at night. The adjusted model demonstrated an R^2 of 0.63 for VR prediction when the difference between exhaled and inlet CO_2 was less than 150 ppm. At the experimental level, all evaluated housing systems showed an R^2 greater than 0.63 and an average RMSE of 0.35 m^3 h^{-1} hen^{-1}. At the commercial level, housing systems exhibited an average predicted R^2 of 0.71 and an RMSE of 1.68 m^3 h^{-1} hen^{-1}. The adapted CO_2 balance method presented good predictive values across laboratory and commercial experiments.*

Keywords. *Airflow, Animal activity, Egg production, Gas production, Poultry building, Respiratory quotient.*

Adequate ventilation in poultry buildings is essential for optimizing animal health, enhancing productivity, and ensuring overall welfare. Proper airflow management helps regulate temperature, humidity, and air quality, which are critical factors in preventing respiratory diseases, reducing stress, and promoting efficient growth and reproduction (Wasti et al., 2020; Al-Abdullatif and Azzam, 2023; Apalowo et al., 2024). Next-generation livestock production systems are integrating new regulations focused on animal welfare considerations, allowing greater freedom of movement, which the public views as one of the most critical aspects of farm animal living conditions (Schuppli et al., 2014). However, transitioning to these systems has led to a significant increase in environmental impact, notably raising ammonia (NH_3) and greenhouse gas (GHG) emissions (Shepherd et al., 2015; Bist et al., 2023). From this perspective, accurately calculating gas emissions becomes crucial for developing effective reduction strategies that can be implemented in commercial livestock buildings.

The calculation of emissions is a product of concentration levels and ventilation rates (VR), thus accurate VR measurements are deemed essential for reliable emissions estimates. However, variability in VR is a primary source of uncertainty,

Submitted for review on 19 March 2025 as manuscript number PAFS 16383; approved for publication as a Research Article by Associate Editor Dr. John Linhoss and Community Editor Dr. Shafiqur Rahman of the Plant, Animal, & Facility Systems Community of ASABE on 25 July 2025.

Citation: Carranza Díaz, A. K., Larios-Martinez, A. D., Ruiz-Gonzalez, A., Godbout, S., & Fournel, S. (2025). Adaptation and validation of the CO_2 balance method for ventilation rate estimation in laying hen houses. *J. ASABE, 68*(5), 775-789. https://doi.org/10.13031/ja.16383

as precise measurements require high temporal resolution, overall accuracy, and precision (Ogink et al., 2013). Factors such as temperature variations, humidity, and the physical layout of the housing affect airflow patterns, complicating the precise measurement and control of VR. Several approaches are currently used for estimating VR, including direct measurements, tracer gas techniques, and heat and carbon dioxide (CO_2) balance methods. Direct measurement is one of the most common strategies for determining VR. It involves equipment to measure the air speed at the inlet and outlet directly. The VR is then determined by multiplying the air speed by the cross-sectional area of the fans. However, this method has limitations, such as measurement errors, issues related to the placement and location of the measurement devices, equipment calibration (Zhou et al., 2017; Park et al., 2018), facility size, and fan distribution (Gates et al., 2009; Calvet et al., 2013). Moreover, fan curves, representing the VR of fans at various static pressures, are typically used to assess the VR in poultry houses directly. Continuous static pressure monitoring allows calculating the VR for each fan and the total VR for the entire house (Gates et al., 2004; Shepherd et al., 2015). Robust data collection and analysis are essential, given the fluctuating external temperatures and dynamic ventilation requirements throughout the day (Park et al., 2018; Zhou et al., 2017). Unfortunately, results from available VR calculation methods are challenging to implement when comparing various production systems with specific characteristics (Calvet et al., 2013; Gates et al., 2009). The tracer gas technique, often using sulfur hexafluoride (SF_6), is commonly applied in livestock buildings to determine VR. It involves introducing a gas into the environment and measuring its concentration to estimate airflow (Phillips et al., 2001). However, the technique requires careful distribution of SF_6 to maintain uniform concentration, as its high density can lead to uneven mixing, which may affect accuracy (Hassouna et al., 2015). The heat balance method relates heat production from animal metabolism to temperature gradients inside the building to estimate VR (Hassouna et al., 2015). While this method is useful, its accuracy decreases over short periods, as animal activity and metabolic heat production can fluctuate throughout the day, leading to potential errors in VR estimation (Carranza-Diaz et al., 2024). The CO_2 balance method has shown promise due to its continuous and non-invasive measurement technique. This approach suggests that ventilation can elucidate the relationship between CO_2 production (mainly from animal metabolism and, if applicable, from litter and heating) and the CO_2 gradient between indoor and outdoor environments (Li et al., 2004; Liu et al., 2016; Rosa et al., 2019). By monitoring both indoor and outdoor CO_2 concentrations and understanding the CO_2 production rate of animals, it becomes feasible to calculate the VR required to maintain an acceptable CO_2 concentration.

Despite its potential, the CO_2 balance method has not been thoroughly validated or adapted for laying hen housing systems. Hens' CO_2 production rates and environmental influences need careful consideration. Numerous research projects (e.g., Carranza-Díaz et al., 2025) have explored the assessment of VR in laying hen facilities, employing the CO_2 balance method. Pedersen et al. (1998), Li et al. (2005), and Rosa et al. (2019) demonstrated a positive correlation between the direct and CO_2 balance methods for VR calculation. However, correlation analyses between CO_2 balance and direct methods have not always been convincing in egg production facilities, leading to poor estimates of VRs. Liu et al. (2016) indicated that the reliability of the CO_2 balance method depends on the precision of the animal metabolic rate and the presence of additional CO_2 sources not related to animal respiration (e.g., manure degradation, human activity, combustion devices), which may introduce uncertainty in the estimation of VR. These factors need additional research, improvement, and confirmation. The CO_2 method has advantages, including simplicity and low cost, but it also has some limitations and inconsistencies. For example, while it has been proven reliable for short-term spot evaluations lasting a few hours (Pedersen et al., 1998; Rosa et al., 2019; Carranza-Diaz et al., 2025), its application in real-time monitoring remains challenging due to fluctuations in animal activity and CO_2 production throughout the day. This highlights the need for additional research to enhance continuous assessment tools for improved monitoring of emissions and environmental control in livestock buildings.

The objective of this study was to: (1) adapt a model based on the CO_2 balance from the International Commission of Agricultural and Biosystems Engineering (CIGR) method (Pedersen et al., 2002) for VR estimation in laying hen housing systems; (2) validate the model under laboratory conditions; and (3) validate the model in three different commercial housing systems. The findings of this study could lead to more accurate VR estimations, allowing for more precise emissions assessment with minimal resources, ultimately supporting better environmental control in poultry houses and promoting the welfare and productivity of laying hens.

Materials and Methods

CO_2 Balance Method Adaptations

The CIGR equation for CO_2 balance (Pederson et al., 2002) calculates ventilation rate (VR) by relating the CO_2 produced within livestock buildings to the concentration difference between indoor and outdoor environments. For livestock buildings where CO_2 originates primarily from animals, this method provides the following equation to estimate VR on a 24-hour basis per heat production unit (hpu; 1 hpu = 1000 W of total heat at 20°C):

$$VR_{CO_2} = \frac{0.185}{\left([CO_2]_e - [CO_2]_i\right) \times 10^{-6}} \quad (1)$$

where

VR_{CO_2} is the ventilation rate per hpu ($m^3\ h^{-1}$)

0.185 ($m^3\ h^{-1}$) is the CO_2 production

$[CO_2]_e$ and $[CO_2]_i$ are the exhaust and inlet CO_2 concentrations (ppm), respectively.

Equation 1 was adapted to improve the accuracy of ventilation rate (VR) estimation in laying hen production. The adaptation process was conducted sequentially: (1) the effect of animal activity (AA) was evaluated; (2) if the inclusion of AA improved accuracy, the modification was retained; otherwise, the original equation was preserved; (3) the hen CO_2 production rate ($PRCO_2$) was assessed and adjusted accordingly; (4) the respiratory quotient (RQ), which influences $PRCO_2$, was analyzed and integrated. Figure 1 shows the scheme.

Equation 1 assumes a fixed animal activity factor (AA = 1). However, on an hourly basis, AA can vary significantly depending on the behavior of the hens. Therefore, based on the recommendations of Pederson et al. (2002), the first adaptation consisted of replacing the fixed value with dynamic AA values representative of hourly activity patterns (eq. 2):

$$AA = 1 - a \times \sin\left[\left(2 \times \frac{\pi}{24}\right) \times \left(h + 6 - h_{min}\right)\right] \quad (2)$$

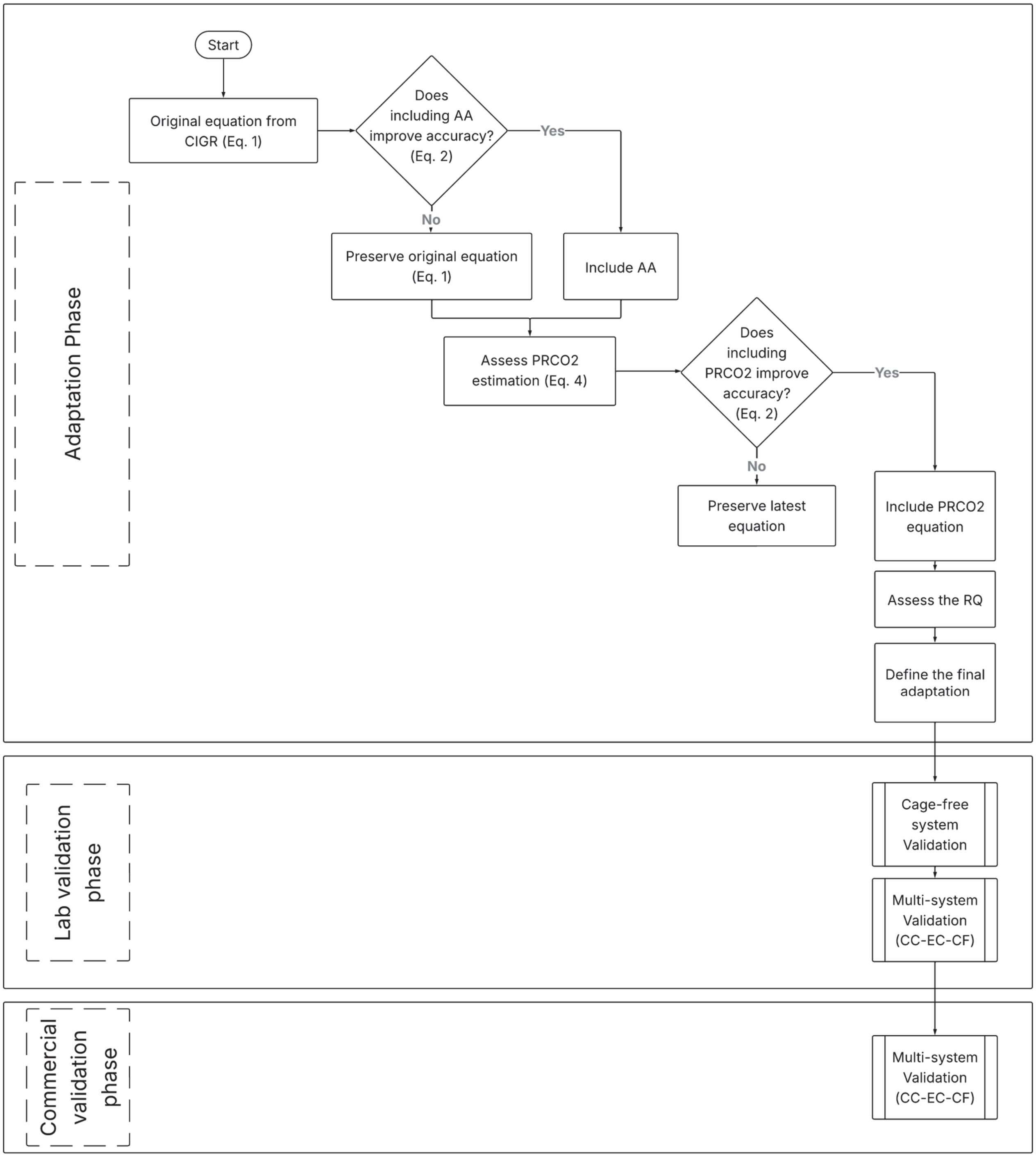

Figure 1. Schematic representation of the sequential adaptation and validation process. Abbreviations: AA = Animal activity, PRCO2 = CO_2 production rate, RQ = respiratory quotient, CC = conventional cage, EC = enriched cage, and CF = Cage-free.

where

AA = relative animal activity

a = constant expressing the amplitude and is equal to 0.61 for laying hens

h = hour

h_{min} = -0.1, which is the time of day with minimum activity for laying hens (which is at 23:55).

Although activity is closely linked to light and feeding schedules (Pedersen et al., 2002), for modeling purposes, a standardized 12-hour light/12-hour dark cycle was used to define the daily animal activity profile. This simplification was necessary to ensure consistency across trial periods, as the actual lighting schedule varied throughout the experimental weeks.

The adjustment can be made indirectly by multiplying animal activity by the VR estimated by the CO_2 balance (Pederson et al., 2002) and equation 1 becomes:

$$VR_{CO_2} = \frac{0.185}{\left([CO_2]_e - [CO_2]_i\right) \times 10^{-6}} * AA \quad (3)$$

Pederson et al. (2002) showed that the value of 0.185 m^3 h^{-1} in equation 1 corresponds to the CO_2 production rate of a standard laying hen over 24 hours and does not account for diurnal variations. Consequently, ventilation rate (VR) estimates based on this fixed value may diverge from actual ventilation, especially when animal activity varies throughout the day. To improve accuracy, the fixed term 0.185 m^3 h^{-1} in equation 1 was replaced with a dynamic estimation of CO_2 production rate ($PRCO_2$) calculated using equation 4. This equation, adapted from Hayes et al. (2013), estimates $PRCO_2$ (mL CO_2 s^{-1}) based on total heat production (THP; W hen^{-1}) and respiratory quotient (RQ) and includes CO_2 from manure:

$$PRCO_2 = \frac{THP}{\frac{16.18}{RQ} + 5.02} + CO_{2_{manure}} \quad (4)$$

where RQ is the volume of CO_2 released from the volume of oxygen utilized during the breathing activity of the hens (Brouwer, 1965). The constants 16.18 and 5.02 used in the equation represent the energy equivalents (in kJ) of 1 L of O_2 consumed and 1 L of CO_2 produced, respectively. These values are based on the principles of indirect calorimetry and allow for the estimation of respiratory CO_2 from total heat production (THP), adjusted by the RQ (Brouwer, 1965). $CO_{2_{manure}}$ is the CO_2 produced by manure (mL CO_2 s^{-1}).

The THP was estimated using the equations proposed by Pederson et al. (2002), specifically equation 5a for caged laying hens and equation 5b for hens housed on floors:

$$THP_{cages} = 6.28\ m^{0.75} + 25Y \quad (5a)$$

$$THP_{floor} = 6.80\ m^{0.75} + 25Y \quad (5b)$$

where m is the mass of the hen (kg) and Y is the egg production (kg d^{-1} hen^{-1}). In this study, "floor" refers to a littered floor system (i.e., solid floor covered with bedding material), which is the most common alternative housing system in our experimental conditions. These equations were developed at 20°C, so a temperature correction was necessary (Pederson et al., 2002). The correction equation (eq. 6) is based on the animal's heat dissipation needs.

$$THP_{corr} = 1000 + 20 \times (20 - T) \quad (6)$$

where THP_{corr} is the total heat production adjusted for the temperature (W hpu^{-1}) and T is the temperature (°C).

The proportion of CO_2 attributed to manure decomposition ($CO_{2manure}$) has been reported to vary seasonally, typically around 1% in winter and up to 5% in summer, mainly due to higher microbial activity at elevated temperatures (Hayes et al., 2013; Rosa et al., 2019). However, this proportion also depends on manure characteristics and the manure management system (e.g., type of collection, frequency of removal, and accumulation). This study used various manure collection systems during the adaptation and validation phases. Given this variability and the absence of direct measurements of manure CO_2, we adopted the seasonal correction factors (1% in cold and 5% in warm seasons) from the literature as reference values. Consequently, the metabolic CO_2 during the cold season was estimated to be 99%, while 95% of the total CO_2 was produced at the hen's barn during the warm season. For this study, the cold season was defined as October to April and the warm season as May to September, based on average temperature trends in southern Québec. The CO_2 produced by the heater was neglected because it was electric.

Finally, the RQ values found in the literature vary in a range between 0.80 and 0.99 (Ketelaars et al., 1985; Chepete and Xin, 2004; Liu et al., 2016; Rosa et al., 2019), depending on the age of the birds, metabolic rate, feed intake, and the individual status of the animal (Ouwerkerk and Pedersen, 1994). A range of RQ values reported in the literature for laying hens (0.85–0.99) was tested to assess the influence of different RQ values on model accuracy. To evaluate the impact of diurnal variation, daytime is defined as 6:00 AM to 5:59 PM and nighttime is defined as 6:00 PM to 5:59 AM, and distinct RQ values are applied for each period. Specifically, all possible combinations of RQ values for daytime (0.85 to 0.99) and nighttime (0.85 to 0.99) were generated, resulting in 225 combinations.

Adaptation of the Model Based on the CO_2 Balance Method

The adaptation was based on the data from Fournel et al. (2012) focusing on conventional cages (CC). The subsequent subsections summarize the experimental setup described in this article.

Experimental Rooms

The experiment took place at the Research and Development Institute for the Agri-Environment's livestock research facility called BABE ("Bilan agroenvironnemental des bâtiments d'élevage"), located at the Centre de recherche en sciences animales de Deschambault (CRSAD) in Quebec, Canada. Twelve rooms, each measuring 1.2 m in width, 2.4 m in length, and 2.4 m in height, were used for the experiment. Each room was designed to prevent air exchange with other rooms and was equipped with ventilation, electric heating, and lighting systems.

Animal Housing

Thirty white Lohmann LSL-Lite laying hens per room (n = 360), aged 19 weeks, were housed in conventional cages (485.5 mm wide x 507 mm deep x 540 mm high; Farmer Automatic, 2010) stacked in two columns of three cages. Each cage included five hens (492 cm^2 hen^{-1}). The study consisted of two consecutive eight-week trials from March to August 2010. The hens were fed daily with 100 g hen^{-1} of commercial laying hen feed throughout the experiment (35.4 g kg^{-1} total nitrogen, 904 g kg^{-1} dry matter content, 22% proteins, 6.55% fat, 4.88% acid detergent fiber; 2005-000, Aliments Breton, St-Bernard-de-Beauce, QC, Canada). The feed quantity was predetermined before the trials and distributed once daily at 09:00, following a consistent schedule across all experimental phases. Fresh water was provided ad libitum via a solenoid valve connected to a data logger, ensuring automatic water supply control. The lighting schedule varied throughout the trials: 13 hours per day during weeks 1–2, 13.5 hours per day during weeks 3–4, and 14 hours per day during weeks 4–8. The light period began daily at 06:00 and ended according to the designated photoperiod. Lights were switched on and off at fixed times. The lighting system was regulated to provide 40 lux at the hens' level (Light Meter, Lux/FC, 840020C, Sper Scientific Ltd.). To prevent interference from external light sources, cardboards were fixed to the windows of the doors separating the rooms from the working area. This lighting schedule was implemented to support animal adaptation and the initiation of rearing, in accordance with standard commercial practices.

Data Collection

Each experimental room was equipped with a 0.3 m diameter variable-speed exhaust fan (Model VEN812448, Ziehl Fan Série Z, IEL Technologie Agricole, Ste-Claire, QC, Canada). Ventilation operated continuously at a fixed rate to ensure air renewal. To maintain the desired room temperature (22–23°C), incoming air from outside was pre-conditioned (PCD) by a centralized system that combined an air conditioning unit and an electric resistance heater. This air was distributed to the 12 rooms via a duct network. In addition, each room had a secondary heater for fine temperature adjustment, regulated by an electronic controller (model RM-2V2S r-matic, IEL Technologie Agricole, Ste-Claire, QC, Canada). Temperature and relative humidity (RH) were measured every 15 min (sensor CS500, ±0.3°C, ±3% RH, Campbell Scientific Canada, Edmonton, AB, Canada). Ventilation rate was quantified using a 204 mm iris damper (model 200, ±5% accuracy, Continental Fan Manufacturing Inc., Buffalo, NY, USA), and pressure differentials were recorded every 15 min by averaging 10-second measurements.

Air CO2 Determination

Air from each room was pumped to a mobile laboratory using Teflon tubing for air sampling. In the mobile laboratory, CO_2 levels were measured by gas chromatography (GC; model 3600, accuracy ±30 ppmv, Varian, Walnut Creek, CA, USA) equipped with an electron capture detector. Samples from the experimental rooms were drawn through the GC injection loop every 15 min, with each room sampled sequentially. A complete set of CO_2 measurements for the 12 experimental rooms required a 3-hour sampling period, with one measurement taken for each room every 3 hours. Gas concentrations were synchronized with the ventilation rate data by aligning the timestamp of each CO_2 measurement with the corresponding 15-minute VR. For analyses requiring hourly values, we aggregated all available data within each hourly block (e.g., 00:00–00:59, 01:00–01:59), acknowledging that individual rooms were sampled at different times each day due to the rotating sequence. Calibration checks were conducted every three days between 12:00 PM and 1:00 PM using three standard gas concentrations to ensure measurement reliability.

Validation at the Laboratory Level

For model validation, data from two previously conducted studies that took place in the twelve laboratory-scale rooms of the BABE laboratory (Experimental Rooms) were used. The first laboratory study only tests the CF system, while the second laboratory study compares CC, EC, and CF housing approaches. Experimental conditions were fully described in the articles of Gonzalez-Mora et al. (2021) and Philippe et al. (2020) for the first and second studies, respectively. The subsequent subsections provide a detailed summary of the experimental setup in these articles.

Animal Housing

In the first study (Gonzalez-Mora et al., 2021), 144 white Lohmann LSL-Lite laying hens were used. Twelve hens were randomly housed in each experimental room from February to June 2019. Therefore, the bird age ranged from 19 to approximately 38 weeks during the study. The rooms were set up to comply with the housing requirements outlined for the CF system in the Code of Practice for the care and handling of pullets and laying hens, as established by the National Farm Animal Care Council of Canada (NFACC, 2016). Each room included a wire floor area (122 × 81 cm, 30 cm high) and a litter area (122 × 38 cm) covered with 5 cm of wood shavings. The wire floor featured two nest boxes, a feeder, two nipple drinkers, and two perches (5 and 45 cm high, 20 cm hen^{-1}). Hens were housed at a density of one per 1115 cm^2. Manure was collected below the wire floor, dried with forced air, and removed on a weekly basis. Lighting intensity in all rooms was maintained between 10 and 15 lux, with an immediate on/off cycle. A 13.5-hour light/10.5-hour dark photoperiod was implemented. The hens were fed with 100 g hen^{-1} d^{-1} of a commercial diet (Laying Hen 18% vegetable-based crude protein from Agri-Marché, St-Isidore, QC, Canada). Feeders were filled once in the morning, then verified three times a day and refilled by the end of the day. Feed and water were provided ad libitum. Water was supplied through a solenoid-activated valve connected to a data logger.

In the second study (Philippe et al., 2020), 360 Lohmann LSL-Lite laying hens were randomly allocated to one of three treatment groups (CC, EC, and CF). The CC system consisted of multi-deck battery cages, each accommodating five hens with 493 cm^2 per hen. These cages featured metallic mesh flooring, a linear feeder, and two nipple drinkers. The EC and

CF systems adhered to the European Directive 1999/74/CE. The EC cages housed ten hens each, providing 780 cm^2 per bird, excluding the pecking and scratching area (PSA). Each EC cage had two nests, a PSA, two perches, a linear feeder, and three nipple drinkers. The experimental phase spanned 70 days, from 23 to 34 weeks of age. The hens were fed a commercial corn-based diet (27910819, Aliments La Coop Fédérée, Joliette, QC, Canada) at a rate of 100 g hen^{-1} d^{-1}, distributed twice daily, with ad libitum access to water. The lighting system provided an intensity of 5 to 10 lux at the hens' level, with a 16-hour light and 8-hour dark photoperiod.

Data Collection and CO_2 Determination

In both studies, temperature, RH, and VR measurements were taken, as described in the Data collection section. Study 2 utilized the methodology described in the section dealing with air CO_2 determination. In study 1, CO_2 concentrations were measured using an infrared gas analyzer (FTIR, model DX4040, Gasmet, Helsinki, Finland). This analyzer, which employs a Fourier transform infrared spectrometer (FTIR) and an integrated sample gas pump, recorded concentration levels at 15-minute intervals.

VALIDATION AT THE COMMERCIAL LEVEL

The commercial-scale validation was based on data from (Carranza-Díaz et al., 2023). The subsections below summarize the research conditions.

Experimental Setup and Animal Housing

The study, conducted between 2020 and 2022, involved 30 commercial laying hen farms with mechanical ventilation systems in Southern Quebec, Canada (table 1). The laying hens, averaging 57 weeks old, were part of one of three production systems: CC (6 barns), EC (14 barns), and CF (10 barns). Each farm was visited once, during which two measurement sessions were performed on the same day: one in the morning (between 9:00 and 11:00 a.m.) and one in the afternoon (between 1:00 and 3:00 p.m.). The measurements included CO_2 concentrations, air velocity, temperature, and relative humidity (RH). They were carried out at multiple locations: near the ventilation fans, at the center of the barn, at the air intake, and outside the building to ensure representative data. The values obtained during the morning and afternoon sessions were averaged for further analysis.

The CO_2 concentrations were taken using an FTIR (model DX4040, Gasmet, Helsinki, Finland). Air velocity, humidity, and temperature were measured using a telescopic multifunction thermo-hygrometer-anemometer (VT210 + SFC300 probe, air velocity accuracy ± 3% of reading ± 0.1 m/s, temperature accuracy 3% of reading ± 0.25°C, KIMO, Chevry-Cossigny, France). The VR was estimated by multiplying the air velocity of each operating fan by its cross-sectional area and summing the individual flow rates to obtain the total VR for the facility.

The influence of breed on model accuracy is minimal, as only 4 out of the 30 farms (13.3%) raised a breed different from the one used for model adaptation. Given the small proportion of farms with an alternative breed, the impact of this factor on model variability is limited. As a result, any potential bias introduced by breed differences is considered low and unlikely to significantly affect the model's overall accuracy. The farms employed various manure management systems: 21 used manure belt drying systems, four used manure

Table 1. Summary of hen housing conditions and manure management system.

N°	Date dd-mm-yy	Housing System	Manure Management System	N° Hens	Average Hen Weight (kg)	Age (weeks)	Temperature (°C) Indoor	Temperature (°C) Outdoor	Relative Humidity (%) Indoor	Relative Humidity (%) Outdoor	Ventilation Rate (m^3 h^{-1} hen^{-1})
1	25-02-20	CC	Pit storage	20,000	1.6	64	20.93	7.70	49	48	1.04
2	10-03-20	EC	Manure belt drying system	22,800	1.8	56	20.09	4.47	55	72	0.76
3	31-07-20	CF	Manure drying on bedded floors	16,000	1.5	45	25.85	27.00	66	53	5.32
4	26-09-20	CF	Manure drying on bedded floors	15,000	1.7	55	25.46	25.00	59	49	0.80
5	27-10-20	EC	Pit storage	14,000	1.4	50	21.27	-0.40	63	71	0.56
6	03-11-20	CF	Manure conveyor and scraper system	18,000	1.9	50	22.07	0.80	67	46	1.08
7	17-11-20	EC	Pit storage	13,919	1.9	60	23.95	-0.70	55	57	0.53
8	24-11-20	CF	Manure belt drying system	19,500	1.8	69	18.54	-2.30	56	46	0.98
9	08-12-20	CC	Manure belt drying system	35,000	1.6	46	23.09	-2.00	57	58	0.78
10	15-12-20	EC	Manure belt drying system	17,000	1.8	57	22.24	-11.20	64	44	0.11
11	05-01-21	EC	Manure belt drying system	48,000	1.7	55	25.85	-2.40	66	61	0.58
12	07-01-21	EC	Manure belt drying system	14,484	1.7	65	22.62	1.00	57	46	0.61
13	27-01-21	EC	Manure belt drying system	19,997	1.8	57	23.73	0.64	54	44	0.63
14	29-01-21	EC	Manure belt drying system	9000	1.7	45	21.24	-6.70	57	37	0.86
15	09-02-21	EC	Manure belt drying system	92,000	1.7	50	22.96	-12.00	54	47	0.70
16	02-03-21	CF	Manure conveyor and scraper system	22,978	1.7	60	21.12	-6.24	66	34	0.97
17	13-03-21	EC	Manure belt drying system	12,000	1.8	55	20.51	-5.62	42	51	0.82
18	16-03-21	CF	Manure belt drying system	17,675	1.7	57	21.69	-1.80	52	34	0.16
19	16-04-21	CF	Manure belt drying system	12,935	1.7	55	24.21	5.80	56	74	0.52
20	02-12-21	CF	Manure conveyor and scraper system	33,417	1.6	43	23.16	5.75	71	71	2.07
21	23-11-21	CF	Manure conveyor and scraper system	13,694	1.6	58	21.08	-3.48	52	52	0.71
22	07-12-21	CF	Manure belt drying system	21,065	1.6	57	22.20	-3.09	58	44	0.46
23	18-01-22	CC	Manure belt drying system	16,510	1.7	51	17.14	-14.80	59	32	0.77
24	01-02-22	CC	Manure belt drying system	25,040	1.9	57	20.79	-11.43	62	47	0.45
25	22-02-22	CC	Manure belt drying system	44,028	1.8	68	22.01	-7.44	51	52	0.23
26	02-03-22	CC	Manure belt drying system	25,009	1.7	67	21.84	-5.76	57	50	0.55
27	29-07-22	EC	Manure belt drying system	22,500	1.8	73	26.9	25.6	52	53	3.33
28	01-08-22	EC	Manure belt drying system	10,518	1.8	64	26.69	28.5	63	56	11.45
29	02-09-22	EC	Manure belt drying system	33,600	1.7	59	23.46	30.01	51	38	2.32
30	12-09-22	EC	Manure belt drying system	9221	1.7	37	26.62	26.45	58	54	4.51

conveyor and scraper systems, two used manure drying on bedded floors, and the remaining three used pit storage systems to collect and store manure in underground pits or tanks beneath livestock housing areas.

Statistical Analysis

Two standard metrics were calculated to evaluate the performance between the direct method, the original CO_2 balance, and the adaptations of the latter approach: root mean squared error (RMSE) and the R-squared score (R^2). These metrics were calculated using Python modules, specifically the mean_squared_error and r2_score functions from sklearn (Pedregosa et al., 2011). According to Moore et al. (2013), an R^2 value below 0.30, between 0.30 and 0.50, between 0.50 and 0.70, and above 0.70, respectively, indicates a minimal, weak, moderate, and strong effect. These metrics are commonly used to evaluate a model's prediction (Kadiyala and Kumar, 2017; Srisa-An, 2021; Lin et al., 2023). Additionally, the mean absolute error (MAE) and mean relative error (MRE) were calculated to assess the accuracy of the adapted models in both absolute and relative terms. All statistical metrics were computed using non-aggregated data (data every 15 minutes). Data aggregation into hourly blocks, as explained before, was performed solely for visualization purposes in the hourly plots to represent average daily trends.

The determination of the VR propagation of uncertainties by the CO_2 balance adapted method is given by equation 7 (ASHRAE, 2005):

$$\frac{\partial VR}{VR} = \sqrt{\left(\frac{\partial VR}{\partial v_1}\ \varepsilon_1\right)^2 + \left(\frac{\partial VR}{\partial v_2}\ \varepsilon_2\right)^2 + \cdots + \left(\frac{\partial VR}{\partial v_n}\ \varepsilon_n\right)^2} \quad (7)$$

where v_n are the independent variables and ε_n are the uncertainties associated with each one.

Results and Discussion

Environmental Conditions and Laying Hen Performance

Table 2 presents data from the three phases of the project: adaptation, laboratory validations, and commercial validation. The table summarizes key parameters, including the number of hens, hen weight, indoor temperature and humidity, ventilation rate, and egg production, across different housing systems.

Model Adaptations

The VR determined by the original CIGR equation (eq. 1) produced an R^2 of 0.60 and an RMSE of 10.14 $m^3\ h^{-1}\ hen^{-1}$ (figs. 2A and 2B). This model exhibited significant errors, indicating poor predictive capability for VR, as it frequently overestimated direct measurements. The high variability observed suggests that assumptions of constant animal metabolism and steady-state conditions for CO_2 production and distribution might not always hold under rapidly changing conditions (Gates et al., 2009). The CIGR equation assumes a fixed CO_2 production rate per hen, which may not accurately reflect metabolic CO_2 production under varying housing conditions (Pederson et al., 2002). The metabolic CO_2 production refers to the carbon dioxide generated internally by the hens as a result of cellular respiration, which is influenced by their physiological state and activity levels.

The model using the animal activity (AA) calculation (eq. 3) led to a significant drop in performance, with an R^2 of 0.28 and an RMSE of 11.58 $m^3\ h^{-1}\ hen^{-1}$ (figs. 2C and 2D). This suggests that incorporating AA may not accurately capture the actual metabolic activity of hens in the evaluated conditions. Specifically, the ventilation rate in the research facility was primarily regulated by ambient temperature setpoints, rather than by animal activity, a practice similar to those observed in commercial barns. Additionally, laying hens are known for exhibiting relatively stable activity within light and dark periods due to their strong behavioral sensitivity to abrupt light transitions (Khalil et al., 2004; Mishra et al., 2005). While sharp activity shifts may occur at lights-on and lights-off, activity remains fairly constant during the rest of the period, which deviates from the smooth diurnal variation assumed by sinusoidal models commonly used in other species. This behavioral stability was reflected in our hourly AA calculations, which resulted in a daily average very close to 1 (0.98), supporting the use of a constant activity level (AA = 1) as a reasonable assumption for modeling purposes

Table 2. Hen performance and environmental parameters of sampled farms.

Parameter[a]	Adaptation – Fournel et al. (2012)	Lab Validation 1 – Gonzalez-Mora et al. (2021)	Lab Validation 2 – Philippe et al. (2020)	Commercial Validation – Carranza-Díaz et al. (2023)
System	CC[b]	CF[b]	CC–EC–CF[b]	CC–EC–CF[b]
Number of hens	30	12	30	~ 23,163
Quantity of experimental units	12 rooms	12 rooms	12 rooms	30 facilities
Hens' age at the beginning (weeks)	19[c]	19	23	56 ± 8.3[d]
Hens age at the end (weeks)	28[c]	38	34	
Hen weight (kg)	1.50 ± 0.06	1.73 ± 0.04	1.57 ± 0.04	1.72 ± 0.12
Indoor temperature (°C)	22.2 ± 0.8	21.8 ± 0.4	20.0 ± 1.55	22.6 ± 2.4
Indoor relative humidity (%)	40.0 ± 8.6	34.5 ± 8.4	28.5 ± 8.6	57.7 ± 6.3
Outdoor temperature (°C)	15.7 ± 8.3	4.2 ± 10.4	-1.2 ± 11.5	3.0 ±13.3
Outdoor relative humidity (%)	57.6 ± 19.4	61.6 ± 4.5	57.5 ± 22.3	50.7 ±11.0
Measured ventilation rate ($m^3\ h^{-1}\ hen^{-1}$)	3.84 ± 0.93	6.44 ± 1.45	4.08 ± 0.68	1.49 ± 2.24
Laying rate (%)	96.2[a]	97.7[a]	90.3[a]	~91.2
Egg production ($g\ d^{-1}\ hen^{-1}$)	54.12	60.00	59.23	61.70

[a] Average per experimental unit.
[b] CC = conventional cages, EC = enriched cages, and CF = cage-free system.
[c] Each trial.
[d] The average age ± sd of all the farms sampled is shown, since they were point measurements.

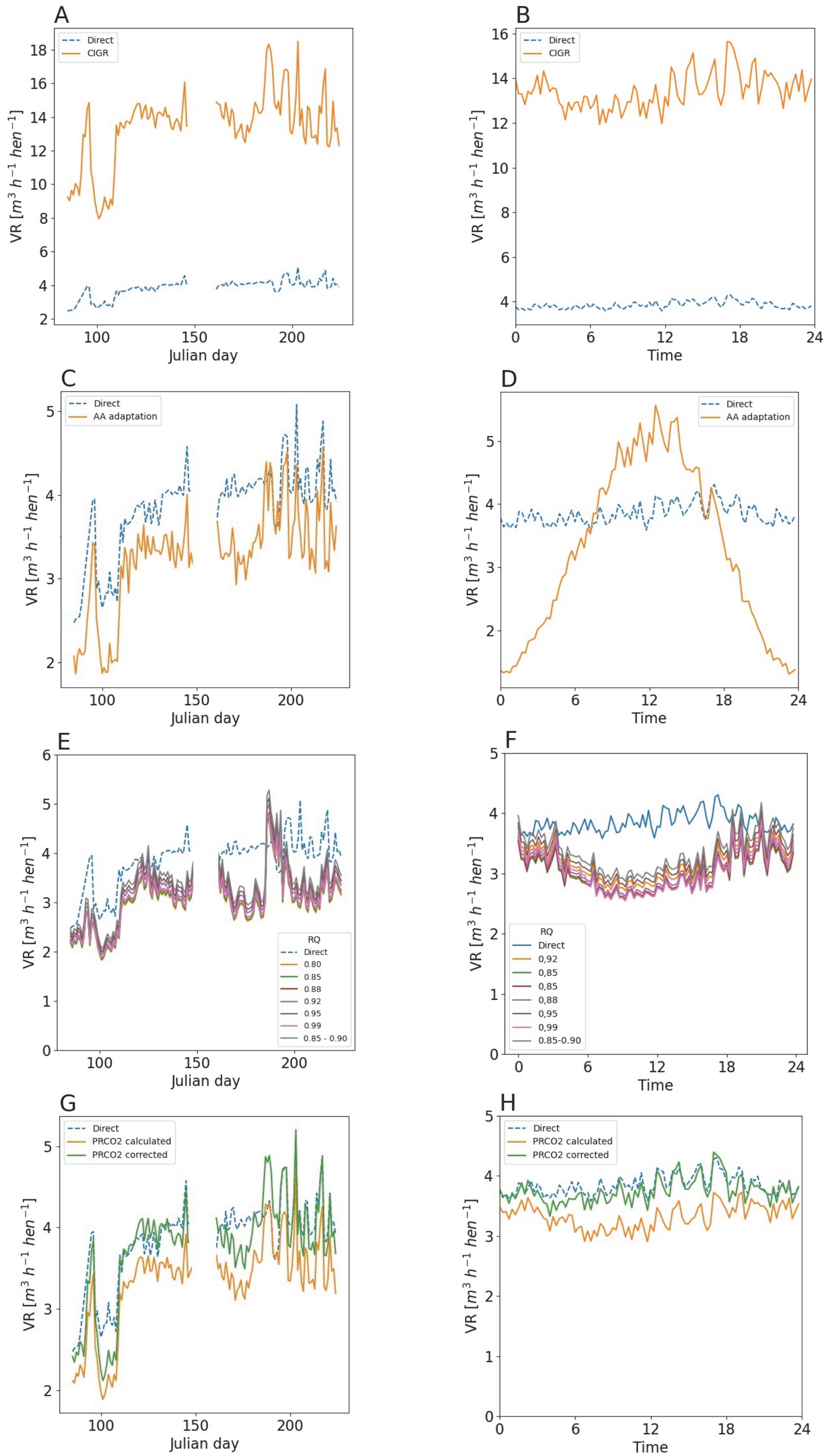

Figure 2. Comparison between VR calculated by the direct method and the CO_2 balance method adaptations: (A) CIGR equation by Julian day, (B) CIGR equation by hour of the day, (C) animal activity adaptation by Julian day, (D) animal activity adaptation by hour of the day, (E) respiration quotient adaptation by Julian day, (F) respiration quotient adaptation by hour of the day, (G) $PRCO_2$ calculation and correction adaptation by Julian day, and (H) $PRCO_2$ calculation and correction adaptation by Julian day. Note: hour of day data covers the interval from 00:00 to 23:45.

in laying hen facilities (Pedersen et al., 2002). Therefore, sinusoidal AA models may not be suitable for this species, and incorporating more physiologically or behaviorally realistic representations, possibly based on direct activity monitoring or refined light-period modeling, could improve future estimates.

The RMSE and R^2 values obtained for the different RQ inputs were similar, suggesting minor practical impact on the model's predictive performance (figs. 2E and 2F). However, the RQ values of 0.90 during the day and 0.85 at night showed the highest R^2 (0.61) and lowest RMSE (0.87 $m^3\ h^{-1}\ hen^{-1}$) (Supplemental Materials). These findings suggest that RQ values may provide a more accurate representation of the metabolic conditions of hens at different times of the day. Previous research has demonstrated that RQ values fluctuate with the age and metabolic state of hens, thereby influencing CO_2 production rates and the efficiency of ventilation systems (Liu et al., 2016). Several studies have directly measured O_2 consumption and CO_2 production to determine RQ values in laying hens. For instance, Hayes et al. (2013) conducted long-term monitoring of Hy-Line Brown hens housed in two commercial aviary houses, using a paramagnetic O_2 analyzer and a photoacoustic CO_2 analyzer over 19 months. Both houses operated under similar management practices, with the main difference being a 20-week age gap between the two flocks. The authors reported RQ values ranging from 0.60 to 1.23, with average daily values (mean ± SE) of 0.94 ± 0.01 and 0.95 ± 0.01 for houses 2 and 3, respectively. Similarly, Chepete and Xin (2004) used large-scale indirect calorimetry chambers designed to simulate commercial production conditions to measure RQ during daily light and dark periods at individual bird and room levels. Their study, which focused on W-36 pullets and layers housed in cages with ad libitum feeding and nipple drinkers for water access, reported RQ values ranging from 0.88 to 0.96, with an average of 0.91 for laying hens between 21 and 64 weeks of age. Additionally, Liu et al. (2016) found that RQ values differed by age, reporting an average of 0.88 for laying hens younger than 28 weeks and 0.82 for those older than 28 weeks. They also highlighted that uncertainties in VR modeling partly arise from uncertainties in the RQ used. Specifically, the RQ can theoretically vary between 0.76 and 1.17, and a 10% error in RQ can cause approximately a 7% error in CO_2 production estimates. Moreover, variability in CO_2 production not captured by RQ further contributes to modeling uncertainties. In our study, RQ variability did not significantly affect VR estimation, as the observed values remained within a narrow and predictable range. However, future research could benefit from direct measurements of O_2 and CO_2 to refine RQ estimations further and improve model accuracy.

The model with the $PRCO_2$ calculated by equation 4 showed slight improvement, with an R^2 of 0.61 and an RMSE of 0.83 $m^3\ h^{-1}\ hen^{-1}$ (figs. 2G and 2H). The calculated $PRCO_2$ likely provides a more direct measure of the hens' metabolic output, offering a more accurate basis for estimating VR (fig. 3). The study's light schedule, which varied from 13 to 14 hours daily, directly impacted the hens' behavior and metabolic activity. During periods of inactivity, such as when the hens are resting or sleeping, metabolic rates decrease, leading to lower CO_2 production. These periods typically align with the dark phase of the light schedule when the hens are less active. This variation must be accounted for in models estimating VR, as it affects the accuracy of CO_2-based calculations. An adjustment was applied to align the estimated VR with actual measurements better and to account for variations in metabolic activity and CO_2 production between day (6:00 AM to 5:59 PM) and night (6:00 PM to 5:59 AM) periods. As shown in figure 2, a consistent discrepancy was observed between the metabolic CO_2 emissions calculated using equation 4 and those measured in the rooms. The measured CO_2 emissions were determined by calculating the CO_2 flow in mL s^{-1} utilizing the concentration of CO_2 in the room air and the ventilation rate. On average, the calculated values underestimated measured emissions by 18% during the day and 8% at night. These percentages were therefore applied as correction factors. These values were established by analyzing the difference between the VR curves from direct and CO_2 balance methods. Although the values by the CO_2 balance method underestimated the actual measurements, the curves followed a similar trend (figs. 1G and 1H). The bias was calculated as the average difference between the two curves, leading to the adjustments that

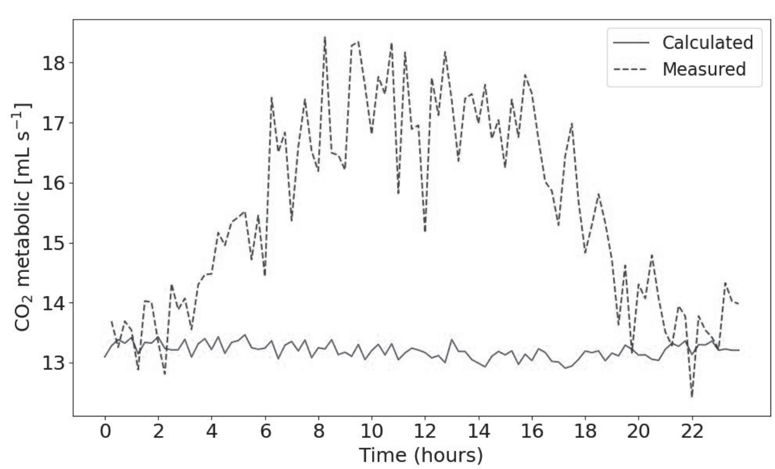

Figure 3. Calculated vs measured CO_2 metabolic rates.

improved the alignment between the predictions and actual measurements. This adjustment achieved an R^2 of 0.62 and an RMSE of 0.72 $m^3 h^{-1} hen^{-1}$.

The AA, RQ, and $PRCO_2$ are variables related to animal metabolism that depend on certain factors, including light exposure (England and Ruhnke, 2020), feeding schedules (Yan et al., 2023), and environmental conditions within the housing system (Rosario Díaz et al., 2018). As mentioned above, hens exhibit diurnal variations in their metabolic rates, which are closely linked to their exposure to light. Hens are more active during daylight hours, leading to increased feed intake and higher metabolic rates, which result in greater CO_2 production as their bodies process the nutrients from their feed (Blatchford et al., 2012; Olanrewaju et al., 2015). Feed intake is another critical factor affecting the metabolic rate of hens. When hens consume feed, their metabolic rate rises to process the nutrients, leading to increased CO_2 production (Barzegar et al., 2020). Heat production varies with the feeding schedule, peaking during daylight hours when animals are most active (Pedersen et al., 2015). Likewise, Ouwerkerk and Pedersen (1994) stated that RQ depends on the animal's metabolic rate, feed intake, and individual status. The commercial laying hen feed provided to the hens and the feeding schedule significantly influenced their metabolic activity and CO_2 output. Hens fed ad libitum exhibit higher metabolic activity and thus higher CO_2 production (Teofilo et al., 2022; Koutsoyiannis et al., 2023). Environmental factors such as temperature and humidity influence metabolic rates and CO_2 production. According to Koutsoyiannis et al. (2023), elevated temperatures can lead to increased CO_2 production because of heightened respiratory activity for thermoregulation (e.g., panting). However, equation 6 accounts for heat dissipation requirements by adjusting the THP: at higher ambient temperatures, hens release less sensible heat, resulting in lower corrected THP values, even when CO_2 production increases. This study also established that lower temperatures could reduce metabolic rates and CO_2 output. At night, lower outdoor temperatures reduce heat transmission and losses, leading to lower VRs and higher indoor humidity than expected (Pedersen et al., 2015; Chauhan et al., 2023). Similarly, humidity levels can impact the respiratory efficiency of hens, affecting their metabolic CO_2 production (Wasti et al., 2020). By not accounting for these environmental variations, the CIGR equation's assumption of uniform conditions contributed to the overestimation of VRs.

The final adaptation, which incorporates the percentage of adjustments to the calculated $PRCO_2$, sets AA to 1, and specifies RQ values for day and night, yields the best model performance. This comprehensive approach effectively integrated the most accurate predictors of VR, resulting in a model that provides precise and reliable estimates. The final equation used to estimate VR with the CO_2 balance adapted method is given by:

$$VR_{CO_2} = \frac{PRCO_2}{\left([CO_2]_e - [CO_2]_i\right)} * AA * 3600 \quad (8)$$

where

VR_{CO_2} = ventilation rate estimated by the CO_2 balance adapted method ($m^3 h^{-1}$)

$[CO_2]_e$ = exhaust CO_2 concentration (ppm)

$[CO_2]_i$ = inlet CO_2 concentration (ppm)

AA = animal activity factor, for laying hens = 1 (dimensionless)

3600 = conversion factor from seconds to hours.

$PRCO_2$ = total CO_2 production ($mL\ s^{-1}$), as calculated by equation 9:

$$PRCO_2 = \left[\frac{THP}{\frac{16.18}{RQ} + 5.02} * (1 + c)\right] + CO_{2\,manure} \quad (9)$$

where

THP = total heat production, corrected for ambient temperature ($W\ hen^{-1}$), from equations 5a and 5b

RQ = respiratory quotient, (0.85 during daytime (06:00–17:59) and 0.90 during nighttime (18:00–05:59) (dimensionless))

$CO_{2\,manure}$ = CO_2 emissions from manure ($mL\ s^{-1}$)

c = correction for daytime and nighttime metabolic CO_2 variations, i.e. 0.18 during daytime and 0.08 at nighttime

The total propagated uncertainty in VR was estimated to be ±6.2%.

Model Validations

First Laboratory Experiment

The adapted CO_2 balance model was validated in CF conditions of Gonzalez-Mora et al. (2021), obtaining an R^2 of 0.09 and an RMSE of 4.51 (table 3). This is likely due to the intrinsic characteristics of each housing system, such as increased mobility and space, which introduce greater variability in the model variables. Additionally, the validation revealed another factor that diminishes the predictive potential of the adapted CO_2 balance model. The model can be error-prone when the threshold difference between the exhaust and inlet CO_2 concentration is not sufficiently high (fig. 4). This may be attributed to the experimental conditions in the first study (Gonzalez-Mora et al., 2021), where only 12 hens were housed per room. In such small-scale setups, the ventilation rate per hen (6.44 ± 1.45 $m^3 h^{-1} hen^{-1}$) was substantially higher than under commercial conditions, likely diluting the metabolic CO_2 and thereby reducing the concentration gradient. The threshold difference observation aligns with Liu et al. (2016), who summarized baseline data on CO_2 production from laying hens and identified uncertainties in the CO_2 balance approach for estimating VR. They found that model accuracy decreased when including data where the differences between exhaust and inlet CO_2 concentrations did not exceed 50 ppm.

The difference in CO_2 concentration between indoor and outdoor air (ΔCO_2) falls below a certain threshold, the reliability of VR estimates decreases significantly, particularly due to the influence of sensor accuracy and measurement noise. However, establishing a minimum ΔCO_2 threshold based on sensor resolution and uncertainty is a critical step

Table 3. Comparison of VR by direct method and adapted CO_2 balance considering the threshold of differences between the exhaust and inlet CO_2 concentrations.[a]

CO_2 Concentrations Delta (ppm)	Data Points	Direct VR (m^3 h^{-1} hen^{-1})[b]	Adapted CO_2 Balance VR (m^3 h^{-1} hen^{-1})[b]	MAE	MSE	R^2	RMSE (m^3 h^{-1} hen^{-1})
>0	4748	6.40 ± 1.45	7.01 ± 4.69	1.53	20.35	0.09	4.51
>50	4743	6.40 ± 1.45	6.93 ± 3.47	1.45	10.45	0.15	3.23
>100	4646	6.39 ± 1.45	6.54 ± 2.10	1.08	2.79	0.38	1.67
>150	4576	6.35 ± 1.34	6.38 ± 1.64	0.98	1.36	0.51	1.17
>200	4475	6.24 ± 1.10	6.27 ± 1.48	0.97	1.31	0.41	1.15

[a] MAE = mean absolute error, MSE = mean squared error, R^2 = determination coefficient, and RMSE = root mean squared error.

[b] Values are means ± standard deviations.

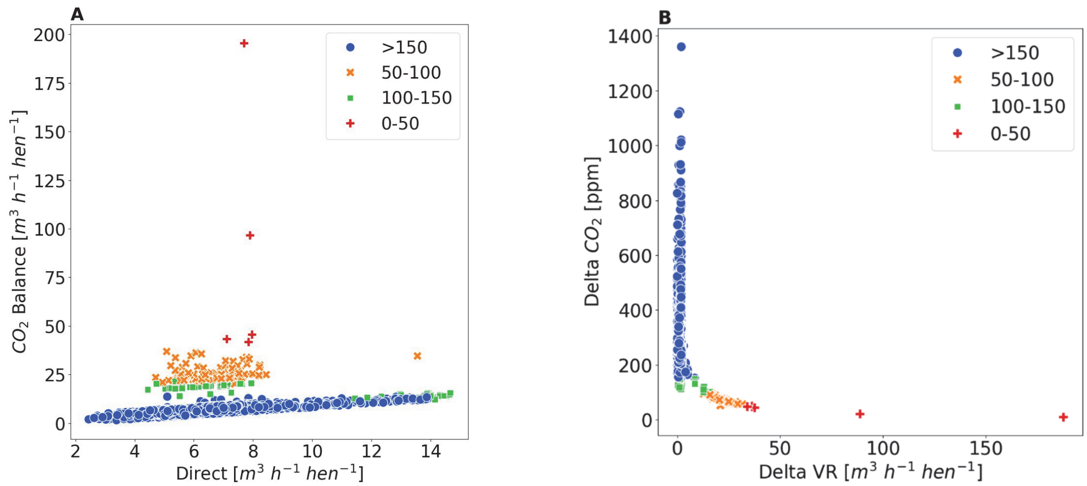

Figure 4. (A) Comparison of VR by direct method and adapted CO_2 balance considering the threshold of differences between exhaust and inlet CO_2 concentrations. (B) Differences between exhaust and inlet CO_2 concentrations (Delta CO_2) vs differences between VR by direct method and adapted CO_2 balance (Delta VR).

for improving the robustness of CO_2 balance methods. Discarding or flagging data below this threshold could help minimize error propagation and improve overall model accuracy, especially under conditions of low ventilation or low bird activity. In our model, the highest correlation was observed when the difference exceeded 150 ppm. However, accuracy decreased beyond 200 ppm due to missing data.

Second Laboratory Experiment

The results (fig. 5) from the adapted CO_2 balance model, validated in the three laying hen housing systems (Philippe et al., 2020), revealed varying performance metrics in VR using both the direct method and the adapted CO_2 balance. Values for MAE and MSE were similar for the three systems, but CF achieves the highest R^2, suggesting the best overall fit to the data, followed by CC and EC (table 4). In this case, the difference between inlet and exhaust CO_2 concentrations was greater than 200 ppm for all data points. Although CF housing systems are generally associated with increased variability due to greater hen mobility and environmental heterogeneity, the better model performance in the second experiment can be attributed to other experimental conditions, such as higher CO_2 differentials and a larger number of animals.

Commercial Validation

For commercial validation, all three systems correlate well with the model, with an R^2 greater than 0.60 at the experimental level (table 5). The EC system exhibits a strong

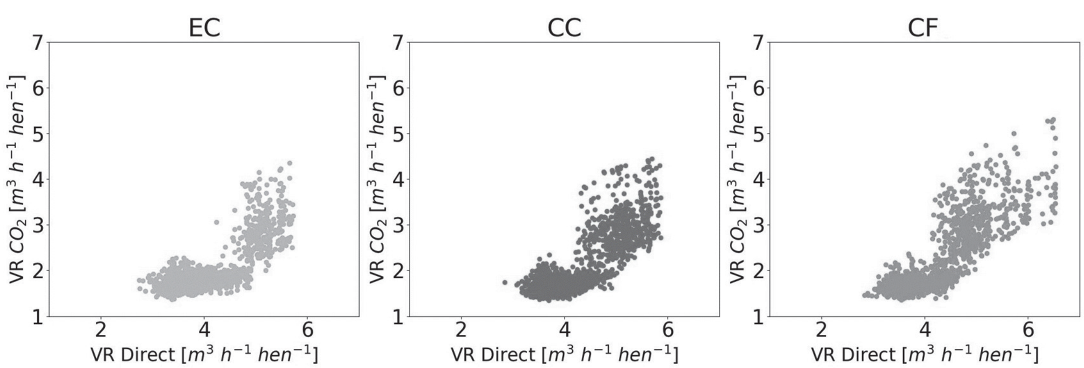

Figure 5. Comparison of VR by direct method and adapted CO_2 balance for the enriched cage (EC) system, conventional cage (CC) system, and cage-free system (CF) under laboratory conditions.

Table 4. Comparison of VR by direct method and adapted CO_2 balance for the validation with commercial housing systems.[a]

System	Data Points	VR Calculated by Direct Method (m^3 h^{-1} hen^{-1})[b]	VR Calculated by Adapted CO_2 Balance Model (m^3 h^{-1} hen^{-1})[b]	MAE	MSE	R^2	RMSE (m^3 h^{-1} hen^{-1})
CC	1951	4.16 ± 0.65	1.99 ± 0.61	2.17	4.85	0.67	2.20
EC	1959	3.97 ± 0.65	1.93 ± 0.50	2.04	4.30	0.63	2.07
CF	1954	4.10 ± 0.71	2.06 ± 0.72	2.03	4.28	0.72	2.07

[b] Values are means ± standard deviations.

[a] MAE = mean absolute error, MSE = mean squared error, R^2 = determination coefficient, RMSE = root mean squared error, CC = conventional cage, EC = enriched cage, and CF = cage-free system.

Table 5. Comparison of VR by direct method and adapted CO_2 balance concerning the system in commercial validations.

System	Data Points	VR Calculated by Direct Method (m^3 h^{-1} hen^{-1})*	VR Calculated by Adapted CO_2 Balance Model (m^3 h^{-1} hen^{-1})*	MAE	MSE	R^2	RMSE (m^3 h^{-1} hen^{-1})
CC	12	0.64 ± 0.29	0.76 ± 0.21	0.16	0.05	0.62	0.21
EC	28	1.98 ± 2.95	2.56 ± 3.46	0.63	1.35	0.92	1.16
CF	20	1.31 ± 1.46	2.38 ± 3.54	1.43	6.60	0.74	2.57

[a] MAE = mean absolute error, MSE = mean squared error, R^2 = determination coefficient, RMSE = root mean squared error, CC = conventional cage, EC = enriched cage, and CF = cage-free system.

[b] Values are means ± standard deviations.

correlation with moderate error metrics and a higher R^2. This strong correlation is likely influenced not only by the system's moderate variability in CO_2 production but also by the larger number of data points available for this system. Conversely, the CF system shows the highest error metrics (MAE, MSE, and RMSE), indicating larger deviations between the predicted and actual VR values despite having a good R^2. This suggests that the variability in CO_2 production due to the dynamic and varied activities of hens in cage-free systems results in higher prediction errors. At the same time, the model accurately captures the overall trend. The CC system presents a lower correlation. However, this could be attributed to the difference in data size compared to the other two systems.

Impact of Housing System on the Validation Performance

The level of environmental control in each system has a significant influence on the model's performance. In cage systems (CC and EC), hens are confined to controlled spaces that could produce more uniform CO_2 production rates. The restricted space limits movement, leading to stable metabolic rates and predictable CO_2 output (Tactacan et al., 2009; Zhao et al., 2014; Liu et al., 2020). The limited space and consistent diet also contribute to predictable metabolic processes, resulting in less variation in RQ (Chepete et al., 2004; Barzegar et al., 2020). This stability could simplify the integration of $PRCO_2$ and RQ into the CO_2 balance model, facilitating easier prediction of VR. In CF systems, hens have more freedom to move, perch, and engage in natural behaviors, leading to higher and more variable $PRCO_2$ due to increased activity levels and changes in metabolic processes. This variability in activity and diet influences RQ, affecting the accuracy of models used to estimate VR (Hayes et al., 2012; Barzegar et al., 2020). Consequently, the model requires flexibility to adjust $PRCO_2$ and RQ values based on real-time metabolic states and behavior patterns.

Several studies have assessed VR for laying hens by the CO_2 balance method (Li et al., 2004; Liu et al., 2016; Pedersen et al., 1998; Rosa et al., 2019). However, none of these studies were conducted on systems without cages. For instance, the experimental design of Pedersen et al. (1998) included a CF system. Still, due to inherent differences between the housing systems (in nine buildings), the hens were kept in cages in six buildings and aviary systems in the remaining three. Consequently, only the largest group with cages was considered for the regression analysis. In addition, these authors used time interval integration to analyze their data into intervals, thereby improving the predictive ability of their models. Pedersen et al. (1998) studied VR in livestock buildings for laying hens and found that the CO_2 balance method provided good results with an R^2 of 0.90 on a 24-hour basis. Li et al. (2004) compared the VR obtained by direct measurement with the CO_2 balance in a commercial laying hen house at different time intervals (each hour, every two hours, and daily). The results showed strong correlations (R^2 above 0.90). As Carranza-Díaz et al. (2025) observed, utilizing time interval integration positively reduces the noise and variability in the data.

With the adaptations employed in this study, the model can be applied to CC, EC, and CF systems, enabling more continuous VR calculations. Under an experimental scale scenario, the model demonstrates consistent performance across various systems, but fine-tuning is particularly critical in CF systems. The significant variability in hen behavior and environmental factors in these systems leads to greater fluctuations in CO_2 production, introducing larger errors and more accurately reflecting the complexity of real-world conditions. On a commercial scale, although the model's application has been limited to a small number of farms and a single climate region, the robust results suggest its potential for broader use. These promising outcomes indicate that, with further validation and refinement, the adapted CO_2 balance model could become a valuable tool for accurately predicting VR across diverse poultry farming systems and regions, ultimately enhancing management practices and animal welfare. Future research should further investigate several key factors influencing CO_2 production. For example, light exposure and feeding schedules affect birds' metabolic activity and behavior, impacting CO_2 output or updated physiological parameters for modern genotypes. Moreover, manure management, including removal frequency, bedding substrate, and accumulation patterns, plays a dual role: directly contributing to CO_2 emissions through microbial decomposition and indirectly affecting animal behavior, especially in CF systems where birds interact more with the litter, increasing exploratory activity and metabolic demand. Integrating these parameters could help improve model accuracy and adaptability across a broader range of commercial settings.

This study adapted and validated the CO_2 balance method to predict VR in laying hen housing systems. The key findings highlight the model's ability to improve prediction accuracy through $PRCO_2$, and RQ adjustments, particularly in controlled environments. Although the model initially exhibited variability, subsequent refinements led to significant improvements, making it a valuable tool for predicting VR. This research provides a reliable method for estimating VR, which could enable more precise airflow control when integrated into long-term monitoring systems. By accurately determining VR, this approach allows for accurate measurement of actual emissions based on concentration levels. Improved VR estimates contribute to more precise emission factor calculations, helping the industry better understand its environmental impact.

CONCLUSION

This study adapted and validated the CO_2 balance method for calculating VR in laying hen housing systems. The results highlight the potential of the adapted model to provide accurate VR estimates, even under varying conditions. The original CO_2 balance model, derived from the CIGR equation, exhibited limitations in capturing the full range of factors affecting VR, resulting in high prediction errors. However, incorporating specific RQ values for day and night, and adjusting the $PRCO_2$, significantly improved the model's accuracy, yielding the best performance. This comprehensive adaptation demonstrates the model's potential for more precise VR estimation.

The adapted model was validated across three housing systems (CC, EC, and CF) at both the experimental and commercial scales. Despite the inherent differences in hen activity and environmental conditions among these systems, the model consistently achieved high R^2, indicating good predictive capability. The commercial validation of the adapted CO_2 balance model demonstrates a good correlation, despite the limited number of farms and the model's application in only one climate region.

Unlike direct measurement techniques, the adapted method is less resource-intensive and more feasible for on-farm application, offering a practical and scalable tool for estimating VR in commercial poultry production. This enables more accurate emission factor calculations and supports real-time monitoring efforts for environmental management.

Beyond its practical application, this study addresses knowledge gaps by critically evaluating core modeling assumptions, such as the appropriateness of constant versus variable activity profiles and the impacts of ambient temperature, housing design, and CO_2 gradients on model reliability. Although some initial prediction variability was observed, targeted refinements reduced error and confirmed the model's robustness across multiple systems. Additionally, this work contributes to the field by improving the accessibility and accuracy of VR estimation methods, encouraging their integration into routine environmental monitoring in poultry operations, and laying a foundation for future studies focused on refining physiological parameters, breed-specific CO_2 production, and manure emission dynamics.

SUPPLEMENTAL MATERIAL

The supplemental materials mentioned in this article are available for download from the ASABE Figshare repository at: https://doi.org/10.13031/29839631

ACKNOWLEDGMENTS

This work was supported by the "Partenariat pour l'innovation en agroalimentaire" program (PPIA04) under the Canadian Agricultural Partnership between the "Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec" (MAPAQ) and the federal government. We also gratefully acknowledge the financial and technical support of Université Laval and the Research and Development Institute for the Agri-Environment (IRDA).

REFERENCES

Al-Abdullatif, A., & Azzam, M. M. (2023). Effects of hot arid environments on the production performance, carcass traits, and fatty acids composition of breast meat in broiler chickens. *Life, 13*(6), 1239. https://doi.org/10.3390/life13061239

Apalowo, O. O., Ekunseitan, D. A., & Fasina, Y. O. (2024). Impact of heat stress on broiler chicken production. *Poultry, 3*(2), 107-128. https://doi.org/10.3390/poultry3020010

ASHRAE. (2005). Engineering analysis of experimental data. Atlanta, GA: ASHRAE. Retrieved from https://webstore.ansi.org/previewpages/ASHRAE/preview_ASHRAE+Guideline+2-2005.pdf

Barzegar, S., Wu, S.-B., Choct, M., & Swick, R. A. (2020). Factors affecting energy metabolism and evaluating net energy of poultry feed. *Poultr. Sci., 99*(1), 487-498. https://doi.org/10.3382/ps/pez554

Bist, R. B., Subedi, S., Chai, L., & Yang, X. (2023). Ammonia emissions, impacts, and mitigation strategies for poultry production: A critical review. *J. Environ. Manag., 328*, 116919. https://doi.org/10.1016/j.jenvman.2022.116919

Blatchford, R. A., Archer, G. S., & Mench, J. A. (2012). Contrast in light intensity, rather than day length, influences the behavior and health of broiler chickens. *Poultr. Sci., 91*(8), 1768-1774. https://doi.org/10.3382/ps.2011-02051

Brouwer, E. (1965). Report of Sub-Committee on Constants and Factors. *Proc. 3rd Symp. on Energy Metabolism* (pp. 441-443). Academic Press.

Calvet, S., Gates, R. S., Zhang, G., Estellés, F., Ogink, N. W., Pedersen, S., & Berckmans, D. (2013). Measuring gas emissions from livestock buildings: A review on uncertainty analysis and error sources. *Biosyst. Eng., 116*(3), 221-231. https://doi.org/10.1016/j.biosystemseng.2012.11.004

Carranza-Díaz, A. K., Larios-Martinez, A. D., Ruiz-González, A., Duchaine, C., Boulianne, M., Godbout, S., & Fournel, S. (2023).

Assessing greenhouse gas and ammonia emissions: A comparison of three egg production systems in Québec, Canada. *Can. Biosyst. Eng., 65*(1), 5.01-5.12. https://doi.org/10.7451/CBE.2023.65.5.1

Carranza-Díaz, A. K., Larios-Martinez, A. D., Ruiz-González, A., Godbout, S., & Fournel, S. (2025). Assessing ventilation rate using indirect measurement methods in experimental bench-scale houses for laying hens. *J. ASABE, 68*(2), 147-159. https://doi.org/10.13031/ja.16115

Chauhan, S. S., Zhang, M., Osei-Amponsah, R., Clarke, I., Sejian, V., Warner, R., & Dunshea, F. R. (2023). Impact of heat stress on ruminant livestock production and meat quality, and strategies for amelioration. *Anim. Front., 13*(5), 60-68. https://doi.org/10.1093/af/vfad046

Chepete, H. J., & Xin, H. (2004). Ventilation rates of a laying hen house based on new vs. old heat and moisture production data. *Appl. Eng. Agric., 20*(6), 835-842. https://doi.org/10.13031/2013.17722

Chepete, H. J., Xin, H., Puma, M., & Gates, R. (2004). Heat and moisture production of poultry and their housing systems: Pullets and layers. *ASHRAE Trans., 110*(2).

England, A., & Ruhnke, I. (2020). The influence of light of different wavelengths on laying hen production and egg quality. *World's Poult. Sci. J., 76*(3), 443-458. https://doi.org/10.1080/00439339.2020.1789023

Farmer Automatic. (2010). Multi-Deck system layer cage. [Online] https://farmerautomatic.com/brochure/MultiDeckSystem.pdf (page visited on May 16, 2024)

Fournel, S., Pelletier, F., Godbout, S., Lagacé, R., & Feddes, J. (2012). Greenhouse gas emissions from three cage layer housing systems. *Animals, 2*(1), 1-15. https://doi.org/10.3390/ani2010001

Gates, R. S., Casey, K. D., Xin, H., & Burns, R. T. (2009). Building emissions uncertainty estimates. *Trans. ASABE, 52*(4), 1345-1351. https://doi.org/10.13031/2013.27784

Gates, R. S., Casey, K. D., Xin, H., Wheeler, E. F., & Simmons, J. D. (2004). Fan assessment numeration system (fans) design and calibration specifications. *Trans. ASAE, 47*(5), 1709-1715. https://doi.org/10.13031/2013.17613

Gonzalez-Mora, A. F., Larios, A. D., Rousseau, A. N., Godbout, S., Morin, C., Palacios, J. H.,... Fournel, S. (2021). Assessing environmental control strategies in cage-free egg production systems: Effect on spatial occupancy and natural behaviors. *Animals, 11*(1), 17. https://doi.org/10.3390/ani11010017

Hassouna, M., Eglin, T., Cellier, P., Colomb, V., Cohan, J.-P., Décuq, C.,... Rochette, Y. (2015). Measuring emissions from livestock farming: Greenhouse gases, ammonia and nitrogen oxides. Ademe and INRA.

Hayes, M. D., Xin, H., Li, H., Shepherd, T. A., Zhao, Y., & Stinn, J. P. (2013). Heat and moisture production of Hy-Line Brown hens in aviary houses in the Midwestern U.S. *Trans. ASABE, 56*(2), 753-761. https://doi.org/10.13031/2013.42663

Hayes, M. D., Xin, H., Li, H., Shepherd, T., Zhao, Y., & Stinn, J. P. (2012). Bioenergetics of Hy-Line Brown hens in aviary houses. *Proc. 2012 IX Int. Livestock Environment Symp. (ILES IX).* St. Joseph, MI: ASABE. https://doi.org/10.13031/2013.41576

Kadiyala, A., & Kumar, A. (2017). Applications of Python to evaluate environmental data science problems. *Environ. Prog. Sustain. Energy, 36*(6), 1580-1586. https://doi.org/10.1002/ep.12786

Ketelaars, E. H., Arets, A., Van der Hel, W., Wilbrink, A. J., & Verstegen, M. W. (1985). Effect of housing systems on the energy balance of laying hens. *Neth. J. Agric. Sci., 33*(1), 35-43. https://doi.org/10.18174/njas.v33i1.16869

Khalil, A. M., Matsui, K., & Takeda, K.-I. (2004). Diurnal and oviposition-related changes in heart rate, body temperature and locomotor activity of laying hens. *Anim. Sci. J., 75*(2), 169-174. https://doi.org/10.1111/j.1740-0929.2004.00172.x

Koutsoyiannis, D., Onof, C., Kundzewicz, Z. W., & Christofides, A. (2023). On hens, eggs, temperatures and CO_2: Causal links in earth's atmosphere. *Sci, 5*(3), 35. https://doi.org/10.3390/sci5030035

Li, H., Xin, H., Liang, Y., Gates, R. S., Wheeler, E. F., & Heber, A. J. (2004). Comparison of direct vs. Indirect ventilation rate determination for manure belt laying hen houses. Paper No. 044177. *Proc. 2004 ASAE/CSAE Annual Int. Meeting.* ASABE. https://doi.org/10.13031/2013.16892

Li, H., Xin, H., Liang, Y., Gates, R. S., Wheeler, E. F., & Heber, A. J. (2005). Comparison of direct vs. Indirect ventilation rate determinations in layer barns using manure belts. *Trans. ASAE, 48*(1), 367-372. https://doi.org/10.13031/2013.17950

Lin, Z., Huang, X., & Chen, J. (2023). Prediction model of sailing ship price based on random forest regression. *Highlights Bus. Econ. Manag., 16*, 29–33. https://doi.org/10.54097/hbem.v16i.10530

Liu, Y., Zhang, G., Rong, L., Li, Z., Wang, S., & Wang, C. (2020). Concentrations and emissions of ammonia from different laying hen production systems of conventional cage, aviary and natural mating colony cage in North China Plain. *Appl. Sci., 10*(19), 6820. https://doi.org/10.3390/app10196820

Liu, Z., Powers, W., & Harmon, J. (2016). Estimating ventilation rates of animal houses through CO_2 balance. *Trans. ASABE, 59*(1), 321-328. https://doi.org/10.13031/trans.59.10235

Mishra, A., Koene, P., Schouten, W., Spruijt, B., van Beek, P., & Metz, J. H. (2005). Temporal and sequential structure of behavior and facility usage of laying hens in an enriched environment. *Poultr. Sci., 84*(7), 979-991. https://doi.org/10.1093/ps/84.7.979

Moore, D. S., Notz, W., & Fligner, M. A. (2013). *The basic practice of statistics* (6th ed.). Freeman.

NFACC. (2016). *Code of practice for the care and handling of pullets and laying hens.* Retrieved from https://www.nfacc.ca/codes-of-practice/pullets-and-laying-hens

Ogink, N. W., Mosquera, J., Calvet, S., & Zhang, G. (2013). Methods for measuring gas emissions from naturally ventilated livestock buildings: Developments over the last decade and perspectives for improvement. *Biosyst. Eng., 116*(3), 297-308. https://doi.org/10.1016/j.biosystemseng.2012.10.005

Olanrewaju, H. A., Miller, W. W., Maslin, W. R., Collier, S. D., Purswell, J. L., & Branton, S. L. (2015). Influence of photoperiod, light intensity and their interaction on health indices of modern broilers grown to heavy weights. *Int. J. Poult. Sci., 14*(4), 183–190. https://doi.org/10.3923/ijps.2015.183.190

Ouwerkerk, E. N., & Pedersen, S. (1994). Application of the carbon dioxide mass balance method to evaluate ventilation rates in livestock buildings. *Proc. XII CIGR World Congress on Agricultural Engineering.*

Park, G., Lee, I.-b., Yeo, U.-h., Ha, T., Kim, R.-w., & Lee, S.-y. (2018). Ventilation rate formula for mechanically ventilated broiler houses considering aerodynamics and ventilation operating conditions. *Biosyst. Eng., 175*, 82-95. https://doi.org/10.1016/j.biosystemseng.2018.09.002

Pedersen, S., Jørgensen, H., & Theil, P. K. (2015). The influence of diurnal variation in animal activity and digestion on animal heat production. Agric. Eng. Int.: CIGR J., Special Issue: 18th World Congress of CIGR. Engineering analysis of experimental data. Atlanta, GA: ASHRAE. Retrieved from https://webstore.ansi.org/previewpages/ASHRAE/preview_ASHRAE+Guideline+2-2005.pdf

Pedersen, S., Takai, H., Johnsen, J. O., Metz, J. H., Groot Koerkamp, P. W., Uenk, G. H.,... Wathes, C. M. (1998). A comparison of three balance methods for calculating ventilation

rates in livestock buildings. *J. Agric. Eng. Res., 70*(1), 25-37. https://doi.org/10.1006/jaer.1997.0276
Pederson, S., & Sällvik, K. (2002). International Commission of Agricultural Engineering. Section II - 4th report of working group on climatization of animal houses : Heat and moisture production at animal and house levels. Research Centre Bygholm, Danish Institute of Agricultural Sciences. Retrieved from https://www.cigr.org/sites/default/files/documets/CIGR_4TH_WORK_GR.pdf
Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O.,... Duchesnay, E. (2011). Scikit-learn: Machine Learning in Python. *J. Mach. Learn. Res., 12*(2011), 2825-2830.
Philippe, F. X., Mahmoudi, Y., Cinq-Mars, D., Lefrançois, M., Moula, N., Palacios, J.,... Godbout, S. (2020). Comparison of egg production, quality and composition in three production systems for laying hens. *Livest. Sci., 232*, 103917. https://doi.org/10.1016/j.livsci.2020.103917
Phillips, V. R., Lee, D. S., Scholtens, R., Garland, J. A., & Sneath, R. W. (2001). SE — Structures and Environment: A review of methods for measuring emission rates of ammonia from livestock buildings and slurry or manure stores, Part 2: Monitoring flux rates, concentrations and airflow rates. *J. Agric. Eng. Res., 78*(1), 1-14. https://doi.org/10.1006/jaer.2000.0618
Rosa, E., Arriaga, H., Calvet, S., & Merino, P. (2019). Assessing ventilation rate measurements in a mechanically ventilated laying hen facility. *Poultr. Sci., 98*(3), 1211-1221. https://doi.org/10.3382/ps/pey524
Rosario Díaz, L. Á., Alavéz Ramírez, R., Caballero Caballero, M., Chiñas Castillo, F., Montes Bernabé, J. L., & Silva Rivera, M. E. (2018). Análisis de condiciones higrotérmicas para las gallinas de postura en el estado de Oaxaca. *Revista Mexicana de Ciencias Agrícolas, 9*(SPE21), 4317-4327. https://doi.org/10.29312/remexca.v0i21.1533
Schuppli, C. A., von Keyserlingk, M. A., & Weary, D. M. (2014). Access to pasture for dairy cows: Responses from an online engagement. *J. Anim. Sci., 92*(11), 5185-5192. https://doi.org/10.2527/jas.2014-7725
Shepherd, T. A., Zhao, Y., Li, H., Stinn, J. P., Hayes, M. D., & Xin, H. (2015). Environmental assessment of three egg production systems — Part II. Ammonia, greenhouse gas, and particulate matter emissions. *Poultr. Sci., 94*(3), 534-543. https://doi.org/10.3382/ps/peu075
Srisa-An, C. (2021). Guideline of collinearity - Avoidable regression models on time-series analysis. *Proc. 2021 2nd Int. Conf. on Big Data Analytics and Practices (IBDAP)*, (pp. 28-32). https://doi.org/10.1109/IBDAP52511.2021.9552165
Tactacan, G. B., Guenter, W., Lewis, N. J., Rodriguez-Lecompte, J. C., & House, J. D. (2009). Performance and welfare of laying hens in conventional and enriched cages. *Poultr. Sci., 88*(4), 698-707. https://doi.org/10.3382/ps.2008-00369
Teofilo, G. F., Lizana, R. R., de Souza Camargos, R., Leme, B. B., Morillo, F. A., Silva, R. L.,... Sakomura, N. K. (2021). Effect of feed restriction on the maintenance energy requirement of broiler breeders. *Anim. Biosci., 35*(5), 690. https://doi.org/10.5713/ab.21.0183
Wasti, S., Sah, N., & Mishra, B. (2020). Impact of heat stress on poultry health and performances, and potential mitigation strategies. *Animals, 10*(8), 1266. https://doi.org/10.3390/ani10081266
Yan, J., Wang, J., Chen, J., Shi, H., Liao, X., Pan, C.,... Yang, X. (2023). Adjusting phosphate feeding regimen according to daily rhythm increases eggshell quality via enhancing medullary bone remodeling in laying hens. *J. Anim. Sci. Biotechnol., 14*(1), 17. https://doi.org/10.1186/s40104-023-00829-0
Zhao, Y., Xin, H., Shepherd, T. A., & Li, H. (2014). Concentrations of ammonia, greenhouse gases and particulate matters in conventional cage, aviary, and enriched colony laying-hen houses. Paper No. 14190402. *Proc. 2014 ASABE and CSBE/SCGAB Annual Int. Meeting.* St. Joseph, MI: ASABE. https://doi.org/10.13031/aim.20141904027
Zhou, Z., Qin, Z., Li, H., Sun, Q., Xia, L., & Yu, G. (2017). Balance model to calculate the ventilation rate of a mechanically ventilated finishing pig house in east china. *Proc. 2017 Int. Conf. on Material Science, Energy and Environmental Engineering (MSEEE 2017)* (pp. 200-206). Atlantis Press. https://doi.org/10.2991/mseee-17.2017.37

NOMENCLATURE

AA = Animal activity
CC = Conventional cage
CF = Cage-free
CIGR = International Commission of Agricultural and Biosystems Engineering
EC = Enriched cage
GHG = Greenhouse gas
HPU = Heat production unit
MAE = Mean absolute error
MSE = Mean squared error
PCD = Pre-conditioned duct
$PRCO_2$ = CO_2 production rate (mL CO_2 s^{-1})
RQ = Respiration quotient
THP = Total heat production (J h^{-1})
VR = Ventilation rate (m^3 h^{-1})