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# Ammonia and greenhouse gas emissions from an enriched cage laying hen facility

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## ABSTRACT

Ammonia, methane, nitrous oxide and carbon dioxide emissions were measured during a complete productive cycle in an enriched cage laying hen facility under typical oceanic conditions. Continuous monitoring of gas concentration, ventilation rate and environmental parameters were conducted from April 2012 to September 2013. The diurnal and seasonal pattern of gas emissions was analyzed.

Seasonality effect was found for $NH_3$ emission, showing an average emission of 144.9 mg $d^{-1}$ $hen^{-1}$ and 90.3 mg $d^{-1}$ $hen^{-1}$ in summer and winter respectively. An $NH_3$ emission factor of 7% of total N in manure was defined for this system. Greenhouse gases also showed a diurnal pattern differing in each case. For $CO_2$, even if averaged emission values (80.2 – 95.9 g $d^{-1}$ $hen^{-1}$) do not answer to seasonality, a distinct pattern was observed between winter and summer related to ventilation rate. On the contrary, in the case of both $CH_4$ and $N_2O$, averaged values varied between winter and summer, showing the opposite diurnal pattern among seasons for each gas. These results show that on-farm emission monitoring studies are necessary to identify gas emission patterns and define proper farm-scale mitigation measures.

**Keywords**: diurnal variation; emission factor; gas concentration; hen manure; seasonality; ventilation rate.

## 1. Introduction

Livestock intensification is associated to concerns on animal welfare and environmental issues such as air pollution. The need to improve the welfare and the productivity together with the mitigation of air pollution has led either governments or producers to the adoption of several international agreements. Regarding egg production sector in EU, intensified laying hen farms had to adopt Directive 1999/74/EC on animal welfare in 2012. According to this regulation, conventional cages (CC) are prohibited across EU since then. Despite alternative production systems have been implemented at varying levels in different EU countries, most CC farms have switched into enriched cage (EC) production system. In this sense, Spain, which is the 3rd egg producer country in EU (MAGRAMA, 2013), has currently more than 85% of its laying hen population producing through EC production system. From an environmental perspective, EU state members are obliged to reduce $NH_3$ and GHG losses by adopting Kyoto and Gothenburg protocols together with Directive 2001/81/EC concerning national emission ceilings (NEC). In order to reduce these emissions, EU created Directive 2010/75/EU, known as Industrial Emission Directive (IED). Laying hen farms with more than 40,000 hens are obliged to comply with IED Directive by implementing best available techniques to reduce gaseous losses.

Ammonia ($NH_3$) is one of the main pollutant gases associated with poultry operations, which also lead to poor indoor air quality that affects on the health of animals and workers (Portejoie, Martinez, Landmann, 2002). It also has an impact on vegetation, water and atmospheric environment (Henry & Aherne, 2014). It has been reported that $NH_3$ concentrations and emissions in poultry houses are usually higher than those from other livestock categories, e.g., dairy cattle and swine (Groot Koerkamp et al., 1998). In this sense, Nicholson, Chambers, and Walker (2004) concluded that strategies to reduce $NH_3$ emissions from poultry farming would be most effective if focused on housing and land spreading practices, where the greatest losses occur. On the contrary, methane ($CH_4$) and nitrous oxide ($N_2O$) emission from these facilities are lower if compared to other livestock productions, although according to IPCC (2013) both are greenhouse gases with a higher warming potential than carbon dioxide ($CO_2$).

The emission of $NH_3$ from poultry houses has been widely investigated although most of the studies on laying hen units have been carried out in Central and Northern European countries, and USA, where either the environmental conditions or production systems may differ with respect to South European countries. In contrast to $NH_3$, fewer data on the emissions of $CH_4$ and $N_2O$ from animal houses are available (Fournel, Pelletier, Godbout, Lagacé, Feddes, 2012a; Shepherd et al., 2015; Wathes, Holden, Sneath, White, Phillips, 1997 and Zhu, Dong, Zhou, Xin, Chen, 2011). Moreover, most of the research on air quality in laying hen houses in Europe has been conducted based on short-time measurements (Nimmermark, Lund, Gustafsson, Eduard, 2009), thus not covering seasonal variations, which have a known key effect on ventilation rates that directly affect pollutant concentrations in the barn. Long term and continuous monitoring is therefore needed to obtain deeper knowledge on gaseous emissions driving factors. This is a key element when proposing mitigation strategies that would better adapt to specific conditions.

The main objective of this paper was to report a sound baseline characterization of $NH_3$, $CH_4$, $CO_2$ and $N_2O$ concentrations and emissions from a commercial farm of laying hens under oceanic climatic conditions, located in the Basque Country. A second objective was to analyze the effect of factors such as ventilation, temperature, feeding or manure management on gaseous losses.

## 2. Material and methods

### *2.1.Animals and housing*

Approximately 52,000 Lohmann-Brown hens were housed in a commercial laying hen unit in a vertical tiered EC system adapted to Directive 1999/74/EC.

The house (Figure 1) was 17 m wide and 66 m long and enriched cages were arranged in 6 rows of 9 tier cages. The lighting period was 17:7 (light:dark) hours per day. The farm was selected to be representative of the current egg production facilities in the Basque Country in terms of management practices.

The hens were fed on a phase feeding system composed of three phases differing in crude protein content (Table 1).

Animal live weight (LW) was estimated from data provided by the supplier for a Lohmann Brown hen (Lohmann Tierzucht GMBH, 2013) according to animals' age. Bird mortality, laying rate, egg production, feed intake and feed conversion ratio was recorded on a daily basis by the producer and used to calculate bird inventory and productive parameters. Productive parameters of the laying hens during the experiment for different feeding phases are presented in Table 2.

The rate of lay reached 90% at week 23, remaining at 90.8% during the first feeding phase, and then, it decreased gradually until the end of the cycle (78.1 %). The feed conversion ratio in contrast, did not changed throughout the cycle and follows the ratios detailed by the animal supplier.

### *2.2.Environmental conditions*

Outside weather conditions of the location during the study were: average air temperature of 10.7ºC and 20.0ºC, air relative humidity of 76.0% and 86.3% and rainfall rate of 618 and 101mm (Euskalmet, 2014) for winter and summer respectively. These climate parameters are within the values recorded during the last 20 years for the Atlantic region, being representative of the oceanic conditions.

Five temperature and relative humidity (RH) sensors (Onset, HOBO U12-013, USA, precision ±0.35ºC and ±2.5%, respectively) were installed at the facility. One sensor was placed outside the house, two at the air inlets and the other two close to the fans. Temperature and RH were monitored and recorded every 15 min. An automated system (Tecno Poultry Equipment, Macronew 3, Italy) regulated inside temperature through windows opening, cooling system and the activation of 18 fans (EM50n, Munters, Sweden, air flow rate 42.125 $m^3 h^{-1}$ at differential pressure = 0 Pa) set up within a tunnel ventilation system (Figure 1).

Ventilation rate (VR) was measured under the usual rearing conditions of the farm following the methodology described by Calvet, Cambra-López, Blanes-Vidal, Estellés, and Torres (2010). The average percentage of operation of each fan was obtained every 5 minutes. An electronic data logger system (Binary Devices S.L., Datalogger 244, Spain) converted every second the electric signal from each fan into digital data on fan status. All on-line sensors were downloaded every 10 days.

The airflow rate of each fan was individually calibrated at different static pressures. Air was ducted 30 cm and the velocity measured by a hot wire anemometer (Testo 425, Germany, accuracy ± 0.03 m $s^{-1}$) at 25 locations in the section (ASHRAE, 2001). Static pressure was continuously measured and recorded every 5 min by a pressure drop meter (Veris, PX, USA, accuracy ±0.5Pa). The resulting average airflow rate of fans, associated to each pressure drop recorded in the building during calibration events, were used to create the corresponding linear relationship (Eq.1)

$$\begin{aligned} y &= -0.4405x + 43.909 \\ R^2 &= 0.7378 \end{aligned} \qquad \text{(Eq.1)}$$

where,

$y$ = *Airflow rate* ($10^3 m^3 h^{-1}$)

$x = Pressure\ Drop\ (Pa)$

Total ventilation rate for each hour was calculated by integrating the number of operating fans and the individual airflow rate as given in Eq.1 for each pressure drop recorded.

### *2.3.Manure characterization*

Manure was accumulated on belts under the cages from 2 to 4 days before being discharged. The producer recorded the time of each manure removal. A representative sample of manure from the belt (approximately 2 kg) was collected fortnightly and analyzed for dry matter (DM), total nitrogen (Ntot), ammonium nitrogen ($NH_4^+$-N), organic matter (OM) and pH. The amount of manure removed from the building was weighed at the farm after each manure removal.

### *2.4.Gas concentrations and emissions*

Gas concentrations ($NH_3$, $CH_4$, $CO_2$ and $N_2O$) were measured continuously over 18 months between April 2012 and September 2013 by using a factory calibrated INNOVA 1412 Photoacoustic multi gas analyzer (PAMGA) coupled with an INNOVA 1309 multipoint sampler (LumaSense, Denmark). According to the technical specification of the INNOVA 1412, the detection limit of the measurement is 0.2 ppm for $NH_3$, 0.4 ppm for $CH_4$, 0.03 ppm for $N_2O$ and 1.5 ppm for $CO_2$. A standard gas containing certified concentrations ($CO_2$: 15,000 ppm, $CH_4$:100 ppm, $N_2O$: 25 ppm) was used to verify the response of PAMGA over a set of automatically diluted reference concentrations. Besides, air from the barn was sampled in 10L Tedlar bags and measured by gas chromatography (Agilent, 7890A,USA) - and a second PAMGA (Bruel&Kjaer, 1302, Denmark). In this sense, when concentrations are confirmed with another measuring method, the uncertainty due to unexpected interferences can be neglected (Hassouna, Robin, Charpiot, Edouard, Meda, 2013).

Readings fell within 5 % RSD, thus it was not necessary to remove the analyser for recalibration.

Each gas sampling cycle was composed of 12 gas samples from different locations (4 at the air inlet opening and 8 sampling points next to the extraction fans). Each cycle interval lasted 20 minutes. The air was pumped from the sampling locations to the analyser through Teflon tubing (6 mm outside diameter, and 4 mm inside diameter) to avoid $NH_3$ adsorption to the sampling lines. Each tube was equipped with PFT-Filters (nSpire Health Ltd., Hertford, UK) to protect from dust. Sampling lines run completely inside the building. Thus, they were kept under a stable temperature, which prevented any in-line moisture condensation. Besides, all the air sampling and analysing equipment (PAMGA, multipoint sampler and external pump) were kept inside an air conditioned outdoor rack enclosure placed in a clean room to prevent them from excessive heat, dust and moist.

According to Equation 2, gas emissions were calculated in an hourly basis:

$$E = \left(C_{outlet} - C_{inlet}\right) \times V \qquad \text{(Eq.2)}$$

where E is the emission (mg $h^{-1}$), $C_{outlet}$ and $C_{inlet}$ is the outlet and inlet gas concentration, respectively (mg $m^{-3}$), and V is the VR in the building ($m^3$ $h^{-1}$).

Emission was also expressed both per hen (hens present in the hen house on the measurement day) in mg $d^{-1}hen^{-1}$ or per animal mass as g $h^{-1}$ 500 $kg^{-1}$ LW (considering the number of hens present in the building and the corresponding weight).

### *2.5.Data analysis*

Considering the high number of gas concentration and emission data collected during the experiment, an analysis of variance led to a high significance in the parameters considered. This kind of statistical analysis was not able to identify the effect of independent variables on gas emissions and concentrations. Therefore, average values and standard deviations are presented for results analysis.

The relationship among continuous variables (VR, temperature and relative humidity) and gas emissions was explored through a correlation analysis using PROC CORR of the statistical package SAS 9.3 (SAS, 2013). To analyze the effect of phase feeding in manure composition, one-way analysis of variance (PROC GLM) was performed using the same statistical package.

The diurnal variation of gas emissions was explored for the 2 extreme seasons (winter and summer). Daily average emissions were calculated to this aim. Data were modelled using the PROC NLIN of SAS, following a regression equation based on the Fourier Transform (Estellés, Calvet, Ogink, 2010):

$$X_h = T + \left[ A\cos\left( \frac{h2\pi}{24} - \frac{D2\pi}{24} \right) \right]$$

where, (Eq.3)

$X$ = Variable target

$T$ = Mean value

$A$ = Amplitude

$D$ = Time at which the maximum occurred

$h$ = target time

Significant differences are expressed at $P<0.05$.

## 3. Results and discussion

### *3.1. Environmental conditions and ventilation rates*

Daily average outside temperature during the measurement period ranged from 4.0ºC to 27.1ºC, reflecting typical weather conditions in the region. Inside the farm, temperature ranged from 18.0ºC to 25.4ºC with an average value of 22.4ºC. The daily mean indoor temperatures showed variations corresponding to seasonal variations of outdoor temperatures. Outside relative humidity presented a low variation (77.3 ± 11.8 %) during the measurement period (Table 3) due to the rainfall registered in the location along the year (1240 mm), which is characteristic of oceanic climate. Despite being slightly lower than those from outside, inside relative humidity remained more stable (66.1 ± 8.9) during the experimental period. In some occasions, when inside temperature was higher than 25 ºC, also the cooling refrigeration system was activated. This system operated 18 % of days, with an average activation of 12 h $d^{-1}$. The higher relative humidity values recorded at summer 2012 and 2013 may be related to the activation of the cooling system.

Daily average VR ranged from 1.1 $10^6$ $m^3$ $d^{-1}$ to 16.6 $10^6$ $m^3$ $d^{-1}$. As expected, VR were closely related to outdoor ambient temperatures (Figure 2), being higher in summer due to higher outdoor temperatures (Table 3, Figure 3). Despite the exponential relationship between temperature and VR, a saturation point would be expected for VR at higher temperatures.

### *3.2.Manure characteristics*

Manure composition and production are presented in Table 4. The mean DM content of manure was 27.2 %, which was slightly lower than values reported for manure belt systems by previous studies (Fabbri, Valli, Guarino, Costa, Mazzotta, 2007; Fournel et al., 2012a). Nonetheless, the observed DM content was within the range reported by EC (2003) for manure belt systems without manure drying tubes (25%-35%). No seasonality effects were observed on manure DM content during the experimental period ($P > 0.05$). The lack of a seasonality effect was attributed to the high RH values observed throughout the year (Table

3). As mentioned above, RH higher than 70% was typical for the humid oceanic weather conditions. Despite the higher VR measured in summer, the high RH of the incoming air would not have favoured water evaporation from manure as Kroodsma, Arkenbout, and Stoffers (1985) stated. In addition, the activation of the cooling system during the hottest days increased the air RH by 7% in comparison to summer RH values without cooling activation.

Total nitrogen content in the manure (5.3±0.8%) was influenced by the feeding phase. A significant decrease ($P<0.05$) on manure Ntot content was observed during the second and third phases compared to the first one (Table 4). Fournel et al. (2012a) and Fabbri et al. (2007) reported higher N content in the manure (6.5 and 7.1% of DM) in manure belt system than the values obtained in this study. On the contrary of Ntot, no relationship among feeding phase and $NH_4^+$-N content was observed.

The average value for manure pH was 7.6 (±0.5), which was in the range of the values reported by other authors. Fournel et al. (2012a) observed values of 6.7 while Fabbri et al. (2007) and Chepete, Xin, and Li (2011) recorded pH values of 8.3 and 8.6, respectively. A pH below 7 would have kept ammonia bound as $NH_4^+$-N, reducing $NH_3$ volatilization. Uric acid represents around 70% of the Ntot in poultry faeces. The pH recorded in this study, could have favoured around 60% uric acid degradation as described by Groot Koerkamp (1994).

## ***3.3.Gas Concentrations and Emissions***

### ***3.3.1. Ammonia***

Average inside $NH_3$ concentration measured in this study was 2.0 mg $m^{-3}$ (±1.4), which was within the range reported by Zhao, Shepherd, Li, and Xin (2015) for a similar EC facility. On the other hand, Ni et al. (2012) recorded higher mean $NH_3$ concentration (9 mg $m^{-3}$) in a CC facility with manure belt system. This difference was attributed to factors such as VR, flock density and manure management. Ventilation rate reported by Ni et al. was half of VR observed in the current study. Stocking rates reported by Ni et al. were 1.6 times higher (13.8 kg LW $m^{-3}$). Finally, despite manure removal frequency was similar between both studies, Ni et al. did not totally remove manure from the belts.

The maximum $NH_3$ concentration in the building was found during the first month of the flock (April 2012), which could be related to the combination of the low VR (Figure 3E) and the early laying stage period. Maximum $NH_3$ in our study was 12.1 mg $m^{-3}$, which is lower than the threshold of 17.4 mg $m^{-3}$ as aversive to laying hens reported in previous studies (Kristensen, Burgess, Demmers, Wathes, 2000). Variations in seasonal $NH_3$ concentrations were closely related to differences in outdoor temperatures and VR. Thus, at higher outdoor temperatures and VR, reduced $NH_3$ concentrations in the exhaust air were recorded (Table 3).

Average $NH_3$ emission in this study was 115.7±85.6 mg $d^{-1}$ $hen^{-1}$ (Table 3). Our results were slightly higher than those cited in EC (2003) for EC system with belt manure, being 95.9 mg $d^{-1}$ $hen^{-1}$. Other authors, such as Liang et al. (2005) and Fournel, Pelletier, Godbout, Lagacé, and Feddes (2012b), reported 94 mg $d^{-1}hen^{-1}$ and 87 mg $d^{-1}hen^{-1}$, respectively, for cage systems with belt manure removal system. This difference could be attributed to hen commercial strains, as when we referred emissions to 500 kg LW, values were similar, with 1.21 g $h^{-1}$ 500 $kg^{-1}$LW reported by Fournel et al. and 1.22 g $h^{-1}$ 500 $kg^{-1}$LW for our study. We attributed this difference to the commercial strain which weighed 1.5 kg LW $hen^{-1}$ in the case of Fournel et al. as VR (4.0 and 4.2 $m^3$ $h^{-1}$ $hen^{-1}$), manure removal frequency and pH were similar. Likewise, our results were consistent with those reported by Liang et al. (2005) with 1.28 g $h^{-1}$500 $kg^{-1}$LW. In this case, pH of the manure (7.4) and manure removal frequency (3-4 days) were similar among both studies.

However, emission rates may also range from 54.0 mg $d^{-1}$ $hen^{-1}$ to 169.9 mg $d^{-1}$ $hen^{-1}$ as Shepherd et al. (2015) and Fabbri et al. (2007) reported. These variations were mainly attributed to different VR of each experiment, which were 2.2 $m^3$ $hen^{-1}$ $h^{-1}$ and 6.2 $m^3$ $hen^{-1}$ $h^{-1}$, respectively. In this sense, $NH_3$ emission did not differ between our study and data reported by Fabbri et al. (2007) at similar VR in summer 2012 (Table 3). It is well known the influence of air velocity over the manure surface promoting $NH_3$ volatilization (Groot Koerkamp, 1994). Besides ventilation, other factors such as pH and manure DM content affecting $NH_3$ emissions (Elliot & Collins, 1982; Groot Koerkamp, 1994) were also more favourable in that study (pH = 8.3 and 37% DM, Fabbri et al., 2007).

The highest $NH_3$ emissions (11.9±3.3 mg $h^{-1}$ $hen^{-1}$) happened in summer conditions (weeks 28 and 83, Figure 3A). Several factors could explain these peak emissions, such as a 5-day manure accumulation inside the building in week 28 and a higher inside temperature (25ºC) and VR with 6.0 and 9.8 $m^3$ $h^{-1}$ $hen^{-1}$ in weeks 28 and 83 respectively. Other $NH_3$ emission peaks found in summer (weeks 34, 35, 39) or early autumn (week 44) could also be attributed to high temperature, ventilation and manure accumulation time inside the building. On the contrary, low emissions were found from end November 2012 to beginning of May 2013 (Figure 3A). During this period, average outside temperature was below 10ºC, which together with a low VR and an average of 3 day manure accumulation time could have contributed to lower emissions. Data collected during week 25 and week 28 suggested a potential effect of manure extraction frequency. For similar temperature, ventilation and RH conditions, lower emissions were registered in week 25 with a daily removal, whereas highest emissions occurred after 5 day accumulation period in week 28. The effect of manure removal frequency should therefore be further explored (Figure 3A).

Seasonal differences in $NH_3$ emission were observed in this study, in consistency with other authors (Da Borso & Chimenti, 1999; Nicholson et al., 2004). Mean $NH_3$ emission reached

90.3 mg $d^{-1}$ $hen^{-1}$ in winter 2013, 165.4 mg $d^{-1}$ $hen^{-1}$ in summer 2012 and 136.3 mg $d^{-1}$ $hen^{-1}$ in summer 2013, similar results to those obtained by Da Borso and Chiumenti  in summer in a similar production system in the north of Italy, with 134 mg $d^{-1}$ $hen^{-1}$. However, they obtained lower values in winter (27 mg $d^{-1}$ $hen^{-1}$), possibly due to lower temperatures in the area. This is the case of Nimmermark et al. (2009), who reported low $NH_3$ emissions in winter (75 mg $d^{-1}$ $hen^{-1}$) due to the low temperatures registered in Sweden (-6.1ºC), even with a 5-day manure removal frequency. In this sense, Groot Koerkamp, Keen, Van Niekerk, and Smit, (1995) found that less than 5% of uric acid is degraded under 15ºC. Nicholson et al. (2004) also reported higher $NH_3$ emission rates in summer than in winter (mean 3.2 $gh^{-1}$ 500 $kg^{-1}$LW and 1.4 g $h^{-1}$ 500 $kg^{-1}$LW respectively), in response to the different VR in summer (8.9 $m^3$ $s^{-1}$) and winter (1.4 $m^3$ $s^{-1}$). Shepherd et al. (2015) also found higher emission rates with ambient temperatures above 20ºC and greater velocities in the barn. This seasonal pattern was also observed by Wathes et al. (1997), who reported an $NH_3$ emission rate of 192 g $d^{-1}AU^{-1}$ in winter and 290 g $d^{-1}AU^{-1}$ in summer from four deep-pit layer houses in England (AU = 454kg LW equivalent).

Deep-pit houses, generally show higher emissions than cage systems, with values of 1,065 mg $d^{-1}$ $hen^{-1}$ (Fournel et al., 2012b), 870 mg $d^{-1}$ $hen^{-1}$ (Da Borso & Chiumenti, 1999) and 446 mg $d^{-1}$ $hen^{-1}$ (Fabbri et al., 2007) . The manure management in these installations, together with their building design, ventilation and temperature ranges would explain this variability. In aviary systems, emissions can be three times higher than in cage systems (Groot Koerkamp et al., 1995) and vary from 353 to 463 mg $d^{-1}$ $hen^{-1}$ (Dekker, Aarnink, de Boer, Groot Koerkamp, 2011), which suggests that a modification in the manure management system could reduce $NH_3$ emissions. Nevertheless, the highest $NH_3$ emissions were derived from floor production systems, with 2,100 mg $d^{-1}$ $hen^{-1}$ (Nimmermark et al., 2009) and free range housing systems, with 1,342 mg $d^{-1}$ $hen^{-1}$ (Dobeic & Pintarič, 2011).

Apart from housing characteristics, other factors such as feeding and manure management affect $NH_3$ emissions. During the period of study, feed crude protein ranged from 16.0 to 16.7%, resulting significantly different ($P<0.05$) Ntot content of manure (Table 2). Nevertheless this effect was not clearly observed on $NH_3$ emission, probably due to the existence of several interacting factors that explain in house $NH_3$ concentrations and emissions. In relation to manure management, both $NH_3$ concentration and emission showed short-lived peaks in an interval of 2-4 days, in coincidence with belt cleaning frequency. It has been previously described that belt cleaning operations lead to a decrease on $NH_3$ emissions (Dekker et al., 2011; Liang et al., 2005).

The parameters discussed in this paper related to density of hen population, housing type, ventilation regime and manure management had a significant impact on emissions. Thus, results from this study gave sound information to produce $NH_3$ emission factor (EF) for laying hen installations with belt cleaning systems. Ammonia EF for laying hen facilities are currently established by EMEP-Corinair guidebook (2013) based on excreted $NH_4^+$-N and uric acid. This guidebook considers that 41% of TAN is volatilized as $NH_3$, which would be equivalent to 29% of Ntot. Our results showed that 7% of Ntot was lost as $NH_3$ in EC system, in which manure was on average removed every 3 days. Ammonia EF given by EMEP-Corinair is based on a slurry whose TAN content accounts for 70% of Ntot. This guidebook assumes that all uric acid becomes $NH_4^+$-N. However, mean TAN content in our research represented about 30% of Ntot. This result suggests that not all the uric acid is converted into $NH_4^+$-N in belt system with frequent manure removals.

### *3.3.2. Methane*

Average $CH_4$ concentration was 3.4 mg $m^{-3}$ (±1.9). Methane concentration followed the similar pattern as $CH_4$ emissions, mainly from week 18 to 40 and 73 to 93 showing the opposite pattern to $CO_2$ (Figure 3B, 3D). Average $CH_4$ emission in this study was 90 mg $d^{-1}$

$hen^{-1}$. No clear effect of season was observed, similarly to Zhu et al. (2011). Nevertheless, $CH_4$ emissions increased in summer, when the highest water content in air was recorded. Thus, these data should be taken with caution as the combination of high air water content and $CH_4$ concentration lower to 20 ppm has been found to lead to $CH_4$ overestimation by PAMGA (Cortus, Jacobson, Hetchler, Heber, Bogan, 2015). Methane emission referred to animal mass (0.96 g $h^{-1}$ 500 $kg^{-1}$ LW) was in the same range than values reported by Fournel et al. (2012a), who found an average emission rate of 0.95 g $h^{-1}$ 500 $kg^{-1}$ LW. Fabbri et al. (2007) reported higher values (2.14 g $h^{-1}$ 500 $kg^{-1}$ LW).

The presence of manure on the belt did not affect emissions of $CH_4$ as described by Dekker et al. (2011) for aviary systems, where bedding exists as a mixture of faeces and bedding material. Theoretically, in this study, the absence of bedding, together with the frequent removal of manure would have favoured lower $CH_4$ emissions in comparison to aviary systems. However, Dekker et al. reported higher and lower $CH_4$ emission from three different types of aviary systems (1.70, 0.37, 0.64 g $h^{-1}$ 500 $kg^{-1}$ LW).

### ***3.3.3. Nitrous oxide***

Low concentrations were registered for $N_2O$, similarly to results reported by other authors for a variety of laying hen production systems (Fabbri et al., 2007; Jungbluth, Hartung, Brose, 2001). Further, denitrification is of minor importance in hen manure as it contains few nitrate or nitrite. Inside $N_2O$ concentrations were not always higher than outside concentrations, resulting, on some occasions, in negative emissions.

Average $N_2O$ emission was 4.5 mg $d^{-1}$ $hen^{-1}$ (Table 3) in our study, in the range of values registered by Fournel et al. (2012a) for cage systems, with 7.1 mg $d^{-1}$ $hen^{-1}$. The emission pattern of $N_2O$ was similar to that of $NH_3$. In contrast, the $N_2O$ emission rate was affected negatively by VR ($P<0.001$) as reported Dobeic and Pintarič (2011). This effect could be the reason that explains the lowest emission in summer ($P<0.05$).

### *3.3.4. Carbon dioxide*

Average indoor concentration was 1,984 mg $m^{-3}$±588. Carbon dioxide concentration was indicative of the barn VR, with lower $CO_2$ concentration corresponding to higher VR (Figure 3D). This relationship was also observed by Nimmermark et al. (2009) and Zhao et al. (2015), who found higher $CO_2$ concentrations with values up to 4,497 mg $m^{-3}$ and 3,985 mg $m^{-3}$, respectively, along with very low ventilation (0.9 and 2.2 $m^3$ $h^{-1}$ $hen^{-1}$). Dekker et al. (2011) reported an average $CO_2$ concentration of 2,734 mg $m^{-3}$ for aviary system, probably due to the lower VR and the contribution of manure from bedding.

The VR might also partly explain the seasonal $CO_2$ concentration pattern. In fact, the mean $CO_2$ concentration in winter was 2,511 mg $m^{-3}$. Lower outside temperatures and VR led to higher daily mean $CO_2$ concentration (Table 3).

Carbon dioxide in poultry houses is originated by hen exhalation and manure release, which in on site studies can not be partitioned. Both effects must be taken into account when estimating the VR with $CO_2$ balance method (Liang et al., 2005; Pedersen, Blanes-Vidal, Joergensen, Chwalibog, Haeussermann, 2008). Pedersen et al. estimated that 8.3% of $CO_2$ emissions measured in a laying hens house comes from manure. Also, Ni, Heber, Hanni, Lim, and Diehl (2011) suggested that manure is a significant source of $CO_2$ release in commercial layer barns. In our study it was observed a slight significant increase in $CO_2$ emission during the days between manure removals, is suggested due to manure accumulation.

### *3.4.Gas emission diurnal patterns*

A clear diurnal variation pattern was found for gas emissions, temperature, relative humidity and VR. These variations were expressed in hourly average terms for summer and winter separately (Figure 4). Regression parameters obtained for measured parameters were presented in Table 5. All models were statistically significant at least at $p<0.001$ (Table 5).

Relative humidity in bird houses is inversely related to temperature (Seedorf et al., 1998). Ventilation rate followed the same pattern as outdoor temperature, showing a higher difference between day and night in summer, with peak values between 12 and 17 h (Figure 4J). It was observed that maximum VR in winter were similar to the lowest VR in summer, with 3.1 $m^3$ $h^{-1}$ $hen^{-1}$ and 3.2 $m^3$ $h^{-1}$ $hen^{-1}$, respectively. Gas emission patterns differed among gases under the same ventilation regime for each season, suggesting other factors could be affecting emission processes.

In the case of $CO_2$, apart from the previously described effect of VR on those emissions, bird respiration affected $CO_2$ diurnal emission pattern (Figure 4C). Thus, the activation of light and consequent activity of laying hens caused a sudden increase at morning and quick drop at night (Figure 4C). In this sense, Von Wachenfelt, Pedersen, and Gustafsson, (2001) observed a large diurnal variation in $CO_2$ production, closely correlated with layer hen activity. Carbon dioxide emission pattern showed a higher difference between day and night in winter than in summer (Table 5), suggesting that low VR found in winter during night (1.3 $m^3$ $h^{-1}$ $hen^{-1}$) allowed for $CO_2$ accumulation in the building. This fact was not observed in summer, when $CO_2$ emissions were quite similar between day and night, not being affected by the twofold increase in VR in summer.

The opposite effect between season and diurnal emission patterns was found for $NH_3$, when the lowest $NH_3$ emission rate in summer was similar to the highest emission in winter, with 0.24 g $h^{-1}$ and 0.26 g $h^{-1}$ (Figure 4A). That is, $NH_3$ emissions were higher in response to VR in summer, but maintained the same difference between day and night as in winter. When tested how VR affected $NH_3$ emissions on an hourly basis, weak relationship was obtained, suggesting that the effect of ventilation was masked by other factors. There is little information on diurnal emission patterns in the literature for laying hen installations (Estellés, Calvet, Ogink, 2010). Other authors (Calvet, Cambra-López, Blanes-Vidal, Estellés, Torres, 2011) have also found opposite behaviours for $CO_2$ and $NH_3$ in broiler systems.

Methane emission values were similar in winter and summer around 15 h, decreasing steeply in winter after this time, while emissions persisted in summer. It was observed a higher difference between day and night in winter than in summer, indicating, like in the case of $CO_2$, that the low ventilation recorded in winter during night induced a certain accumulation of $CH_4$ that was emitted when ventilation reaches around 2 mg $m^{-3}$.

Although low $N_2O$ emissions are reported in the literature from cage systems, it was observed that emissions tended to be higher in winter than in summer. Nevertheless, as found in the other gases considered, the response along the day was higher in winter than in summer, supporting the hypothesis that a minimum VR was always used to avoid accumulation of gases in the building. The characteristics of this experiment, carried out in commercial running operations, made it difficult to establish strong relationships due to the confluence of different factors such as climatic conditions and management operations which were beyond the experimental control.

## 4. Conclusion

Gaseous concentrations and emissions were monitored in a laying hen facility during a complete laying cycle in relation to manure composition, management and environmental parameters. Ammonia EF was 7% of total N in manure in EC facility with belt cleaning system. Seasonal and diurnal variation was observed in $NH_3$ emissions, linked to VR and manure management.

Greenhouse gas emissions showed a diurnal pattern differing in each case. For $CO_2$, even if averaged emission values did not answer to seasonality, a distinct pattern was observed between winter and summer related to VR. On the contrary, in the case of both $CH_4$ and $N_2O$, averaged values varied between winter and summer, showing the opposite pattern. Nitrous oxide emissions were low throughout the study, making it difficult to detect differences in diurnal pattern between winter and summer. On-farm emission monitoring studies are necessary to identify gas emission patterns and define proper farm-scale mitigation measures.

## Acknowledgments

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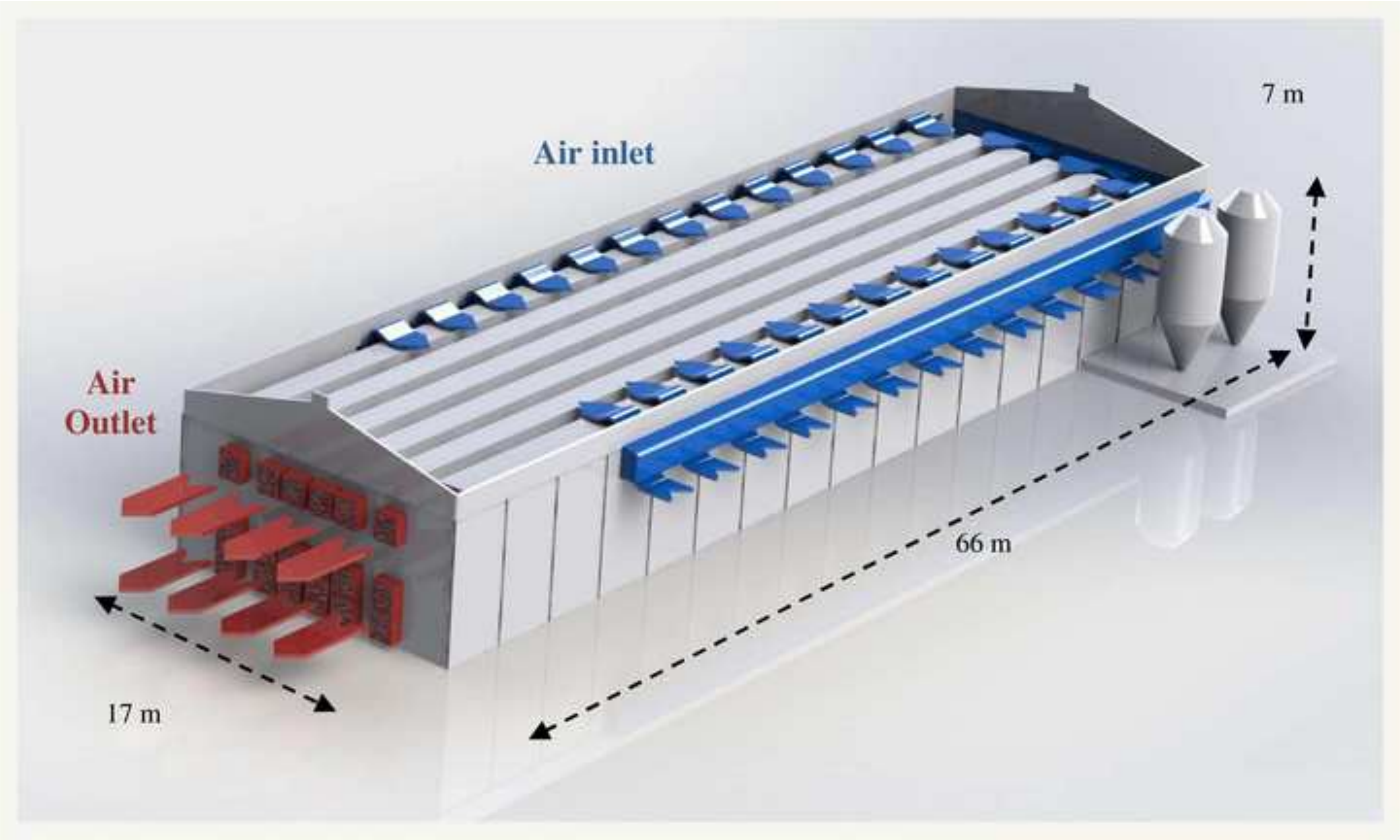
Figure1_Colour



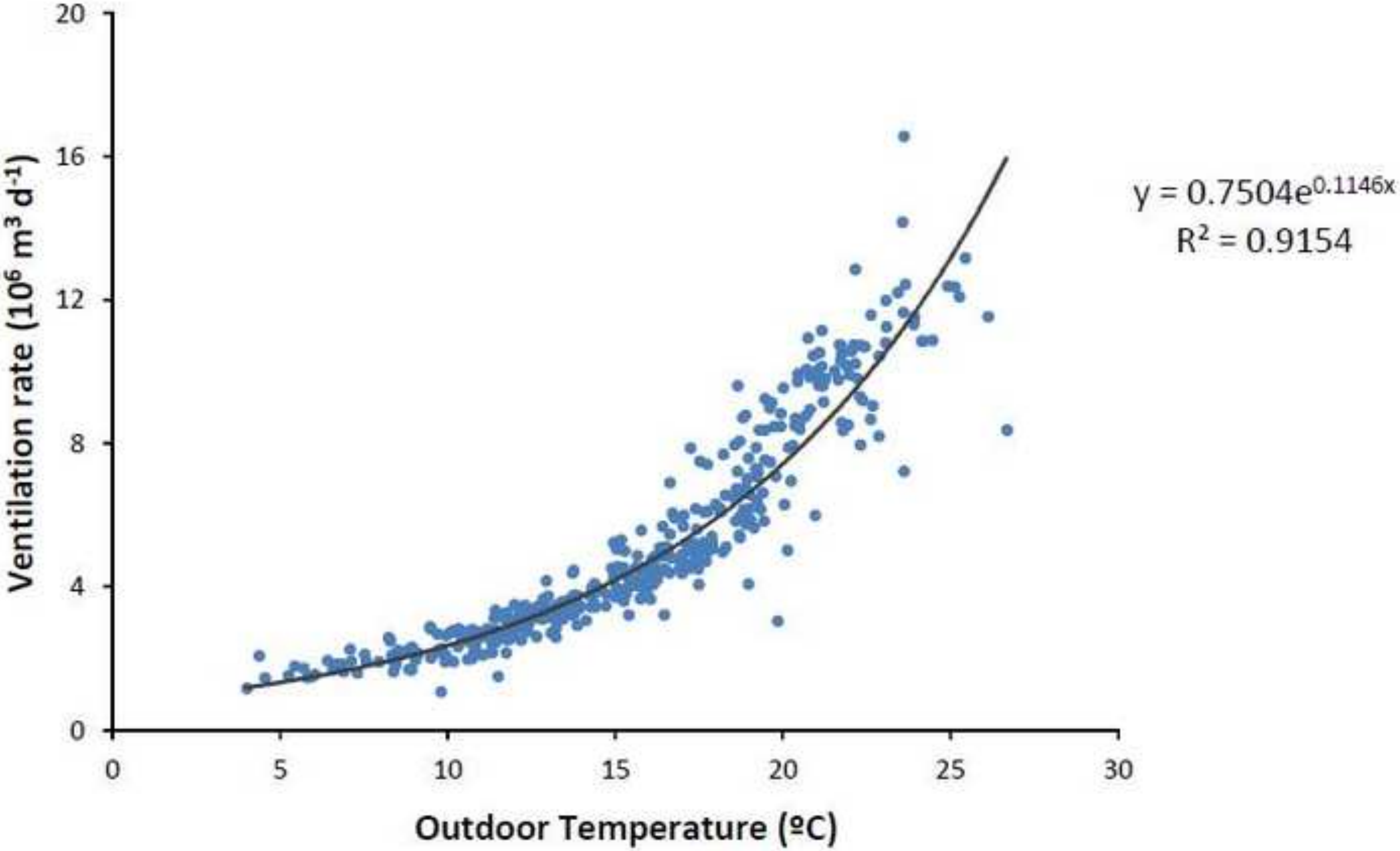
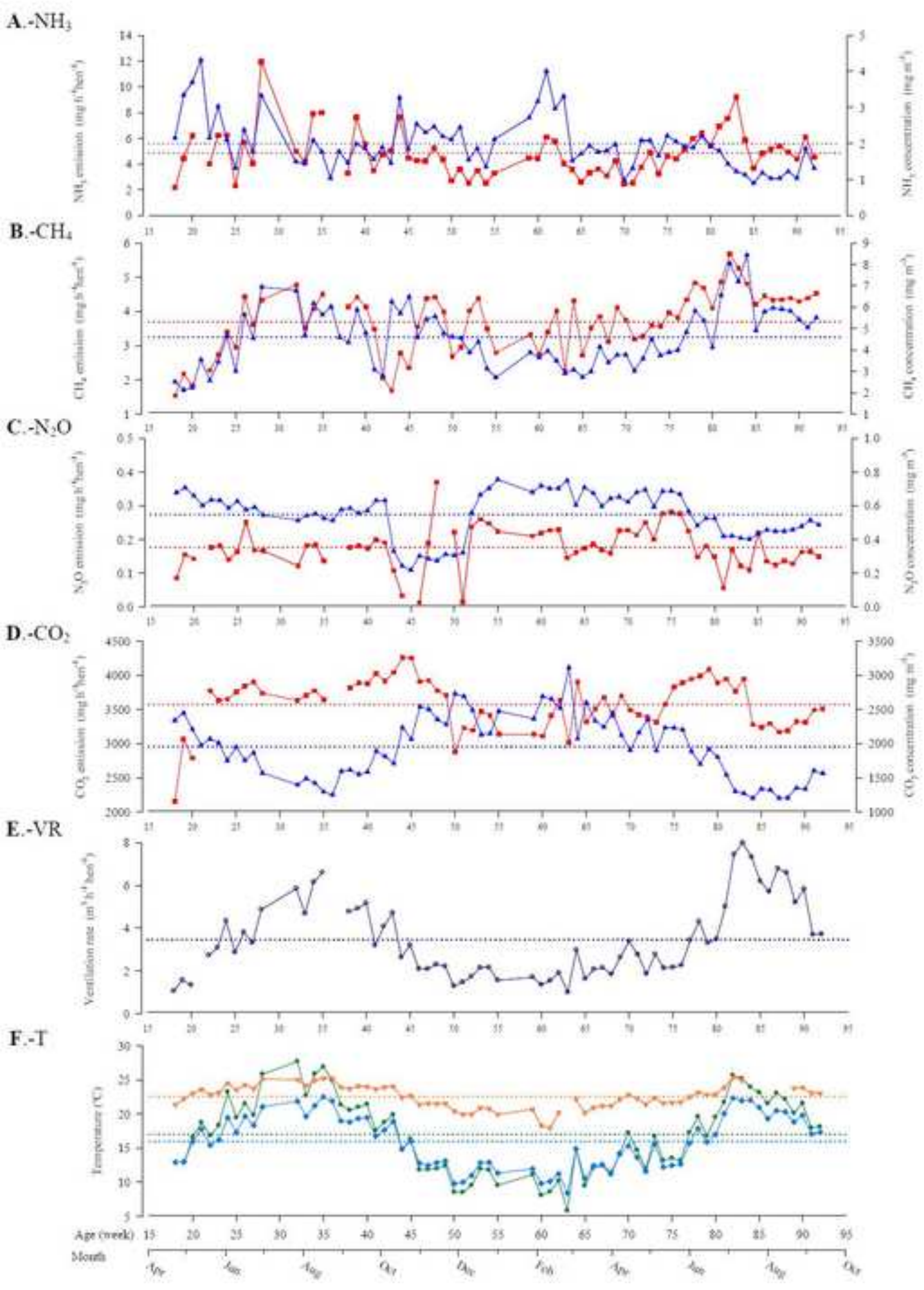

Ventilation rate ($10^6$ m$^3$ d$^{-1}$)
20
16
12
8
4
0
0
5
10
15
20
25
30
Outdoor Temperature (ºC)
y = 0.7504e$^{0.1146x}$
R$^2$ = 0.9154

Figure3_Colour



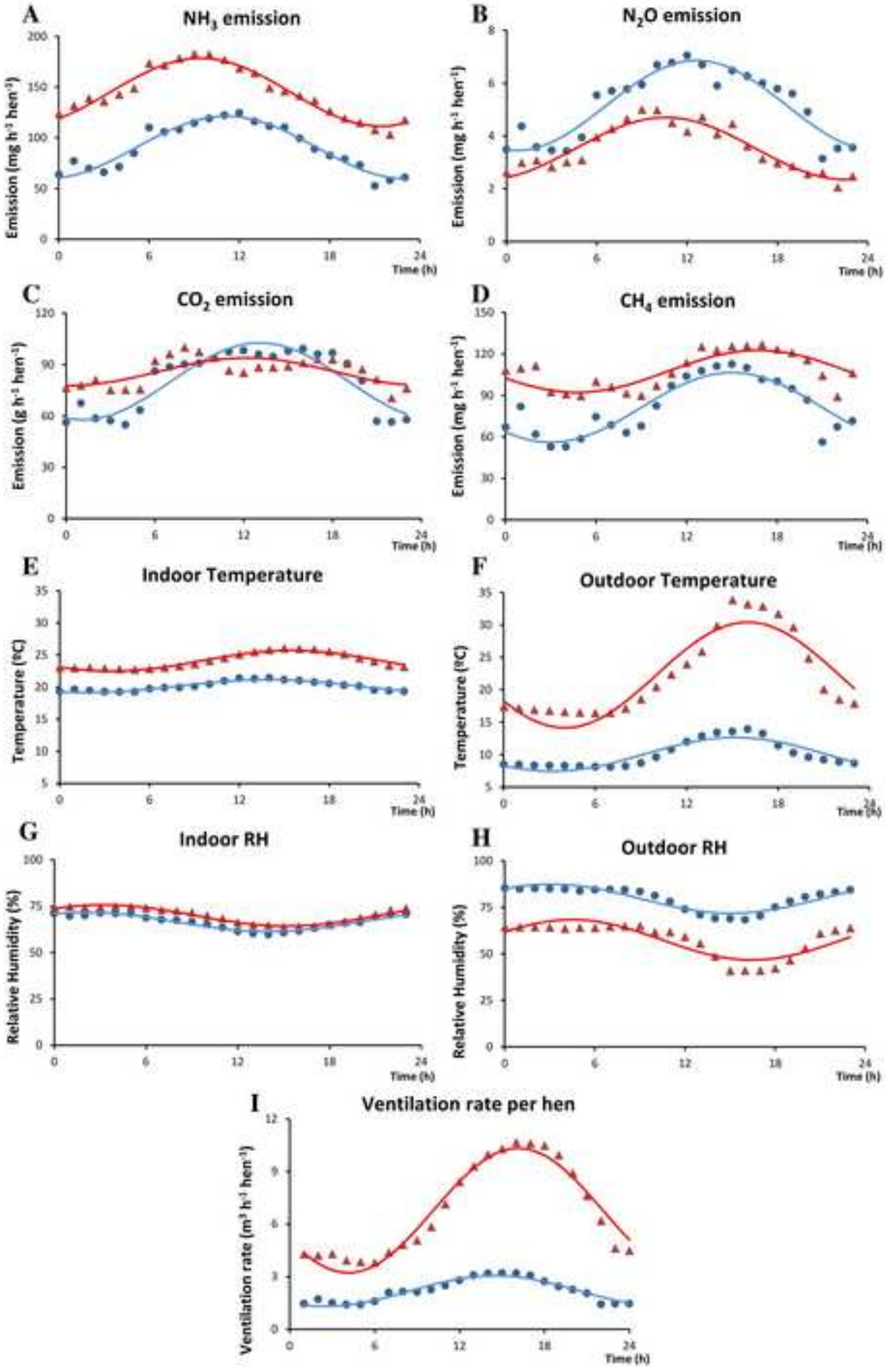
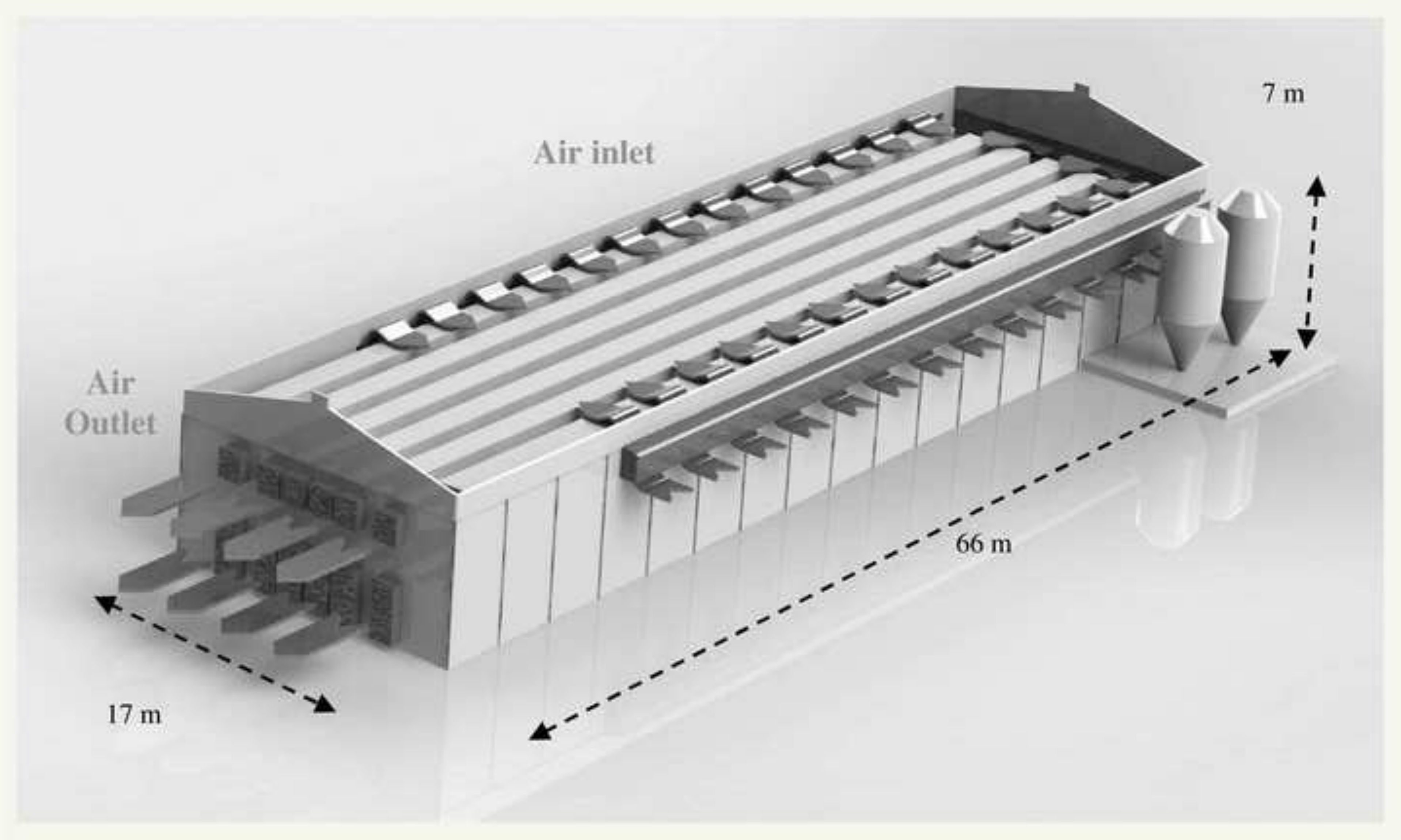

A
NH$_3$ emission
Emission (mg h$^{-1}$ hen$^{-1}$)
0
50
100
150
200
0
6
12
18
24
Time (h)
B
N$_2$O emission
Emission (mg h$^{-1}$ hen$^{-1}$)
0
2
4
6
8
0
6
12
18
24
Time (h)
C
CO$_2$ emission
Emission (g h$^{-1}$ hen$^{-1}$)
0
30
60
90
120
0
6
12
18
24
Time (h)
D
CH$_4$ emission
Emission (mg h$^{-1}$ hen$^{-1}$)
0
30
60
90
120
150
0
6
12
18
24
Time (h)
E
Indoor Temperature
Temperature (ºC)
5
10
15
20
25
30
35
0
6
12
18
24
Time (h)
F
Outdoor Temperature
Temperature (ºC)
5
10
15
20
25
30
35
0
6
12
18
24
Time (h)
G
Indoor RH
Relative Humidity (%)
0
25
50
75
100
0
6
12
18
24
Time (h)
H
Outdoor RH
Relative Humidity (%)
0
25
50
75
100
0
6
12
18
24
Time (h)
I
Ventilation rate per hen
Ventilation rate (m$^3$ h$^{-1}$ hen$^{-1}$)
0
3
6
9
12
0
6
12
18
24
Time (h)

Figure1_B&W



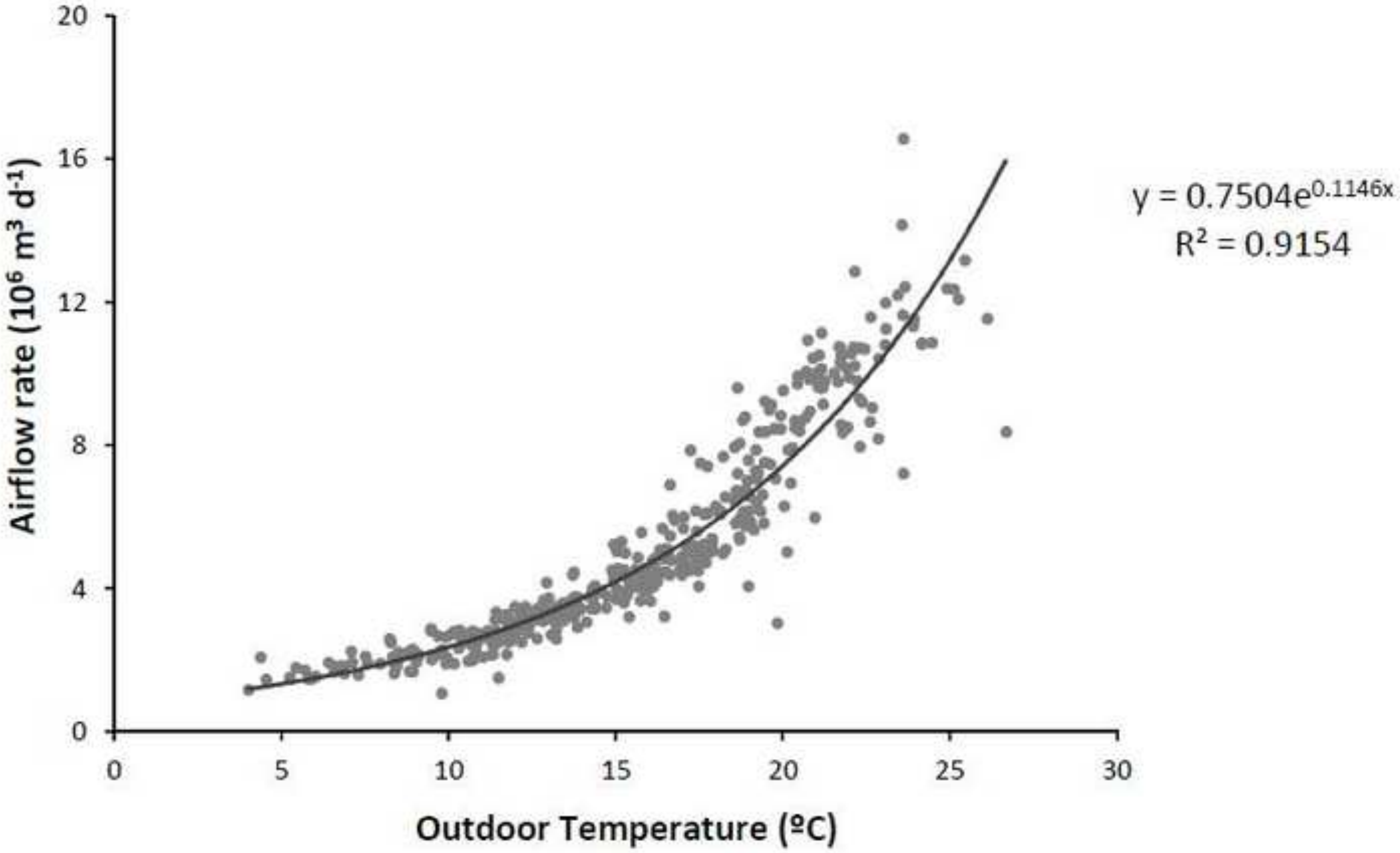
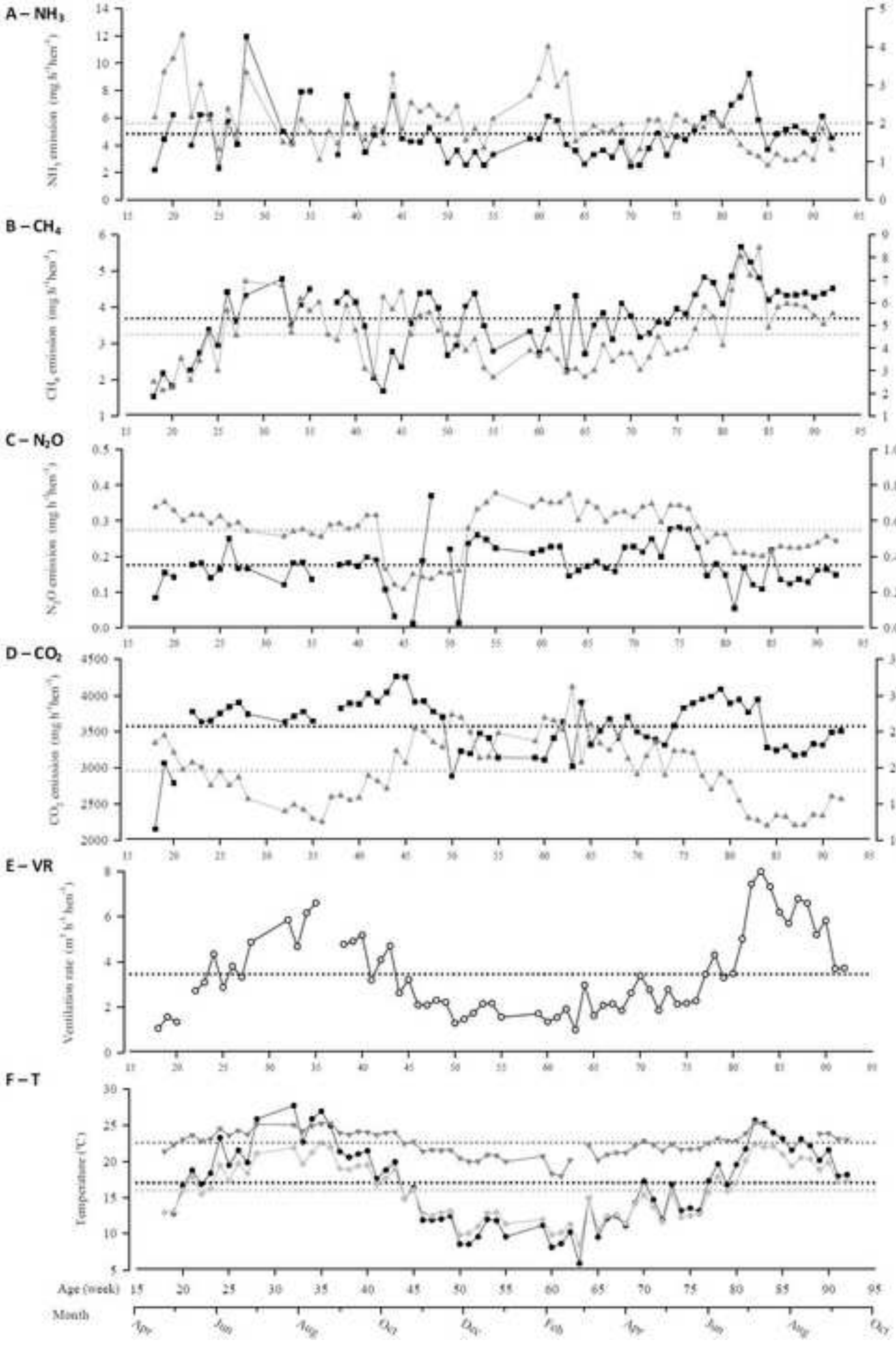
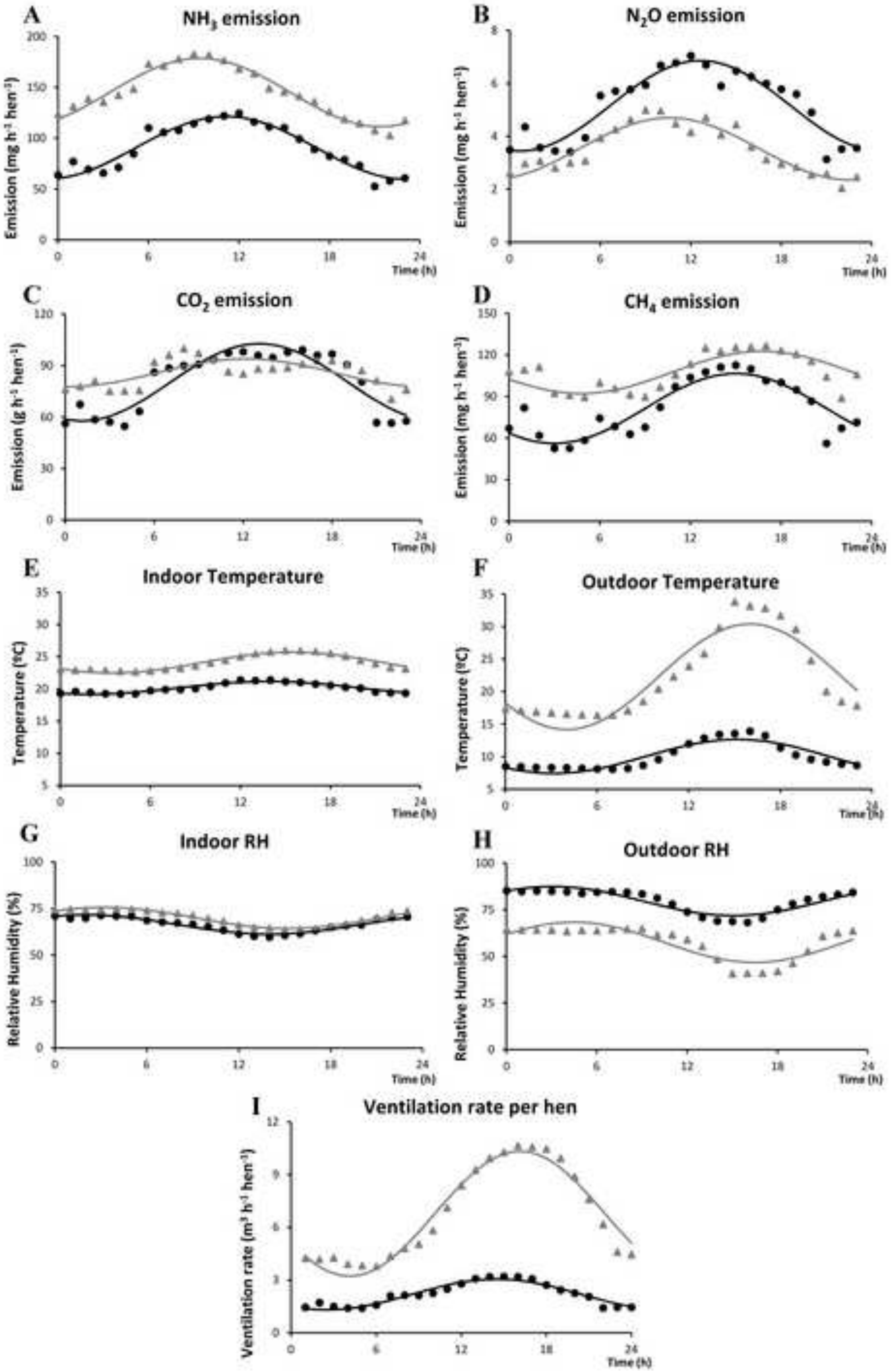

20
16
12
8
4
0
Airflow rate (10⁶ m³ d⁻¹)
0
5
10
15
20
25
30
Outdoor Temperature (ºC)
y = 0.7504e^0.1146x
R² = 0.9154

Figure3_B&W


A – $NH_3$
$NH_3$ emission (mg h⁻¹hen⁻¹)
$NH_3$ concentration (mg m⁻³)
0
2
4
6
8
10
12
14
0
1
2
3
4
5
B – $CH_4$
$CH_4$ emission (mg h⁻¹hen⁻¹)
$CH_4$ concentration (mg m⁻³)
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9
C – $N_2O$
$N_2O$ emission (mg h⁻¹hen⁻¹)
$N_2O$ concentration (mg m⁻³)
0.0
0.1
0.2
0.3
0.4
0.5
0.0
0.2
0.4
0.6
0.8
1.0
D – $CO_2$
$CO_2$ emission (mg h⁻¹hen⁻¹)
$CO_2$ concentration (mg m⁻³)
2000
2500
3000
3500
4000
4500
1000
1500
2000
2500
3000
3500
E – VR
Ventilation rate (m³ h⁻¹ hen⁻¹)
0
2
4
6
8
F – T
Temperature (°C)
5
10
15
20
25
30
Age (week)
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
Month
Apr
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Aug
Oct


A
NH$_3$ emission
Emission (mg h$^{-1}$ hen$^{-1}$)
0
50
100
150
200
0
6
12
18
24
Time (h)
B
N$_2$O emission
Emission (mg h$^{-1}$ hen$^{-1}$)
0
2
4
6
8
0
6
12
18
24
Time (h)
C
CO$_2$ emission
Emission (g h$^{-1}$ hen$^{-1}$)
0
30
60
90
120
0
6
12
18
24
Time (h)
D
CH$_4$ emission
Emission (mg h$^{-1}$ hen$^{-1}$)
0
30
60
90
120
150
0
6
12
18
24
Time (h)
E
Indoor Temperature
Temperature (ºC)
5
10
15
20
25
30
35
0
6
12
18
24
Time (h)
F
Outdoor Temperature
Temperature (ºC)
5
10
15
20
25
30
35
0
6
12
18
24
Time (h)
G
Indoor RH
Relative Humidity (%)
0
25
50
75
100
0
6
12
18
24
Time (h)
H
Outdoor RH
Relative Humidity (%)
0
25
50
75
100
0
6
12
18
24
Time (h)
I
Ventilation rate per hen
Ventilation rate (m$^3$ h$^{-1}$ hen$^{-1}$)
0
3
6
9
12
0
6
12
18
24
Time (h)

# Figure_Captions

**Figure 1.** Layout of the house and scheme of the tunnel ventilation system.

**Figure 2.** Relation of daily ventilation rate ($10^6$ $m^3d^{-1}$) and outdoor temperature (ºC).

**Figure 3.** Ammonia and GHG emission (■) and concentration (▲), and ventilation rate (o) per hen and; outlet (▼), inlet (♦) and outdoor (●) temperature during trial. Dotted lines represent average values.

**Figure 4.** Average hourly variations in $NH_3$, $CH_4$, $N_2O$ and $CO_2$ emissions, outdoor and indoor temperature and relative humidity, and ventilation rate for summer (▲) and winter (●). All modelled curves were significant at $P<0.001$.

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**Table**

Table 1. Composition of feed for the different feeding phases.

| | Phase 1 | Phase 2 | Phase 3 |
|---|---|---|---|
| | Weeks 18-54 | Weeks 55-74 | Weeks 74-93 |
| Feed Composition | Mean | Mean | Mean |
| Dry Matter (%) | 89.6 | 89.9 | 90.0 |
| Crude protein (% DM) | 16.7 | 16.2 | 16.0 |
| Crude fat (% DM) | 4.2 | 4.4 | 4.1 |
| Crude fiber (% DM) | 3.8 | 4.0 | 4.7 |
| Organic Matter (% DM) | 86.5 | 85.1 | 87.3 |

Table 2. Number of hens, body weight, mortality, percentage of laying, productivity, feed intake and conversion efficiency of laying hens monitored during different feeding phases.

| | Phase 1 | | Phase 2 | | Phase 3 | | Total | |
|---|---|---|---|---|---|---|---|---|
| | Weeks 18-55 | | Weeks 56-74 | | Weeks 75-93 | | | |
| Productive parameters | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Birds housed average | 52,144 | 353 | 50,950 | 232 | 49,952 | 353 | 51,294 | 969 |
| Bird weight (kg LW hen$^{-1}$)* | 1.9 | - | 2.0 | - | 2.1 | - | 2.0 | - |
| Mortality (% per phase)* | 2.4 | - | 1.8 | - | 2.3 | - | 6.6 | - |
| Laying rate (%) | 90.8 | 2.1 | 86.1 | 1.9 | 82.6 | 2.4 | 87.5 | 5.2 |
| Egg production (g place$^{-1}$) | 54.5 | 14.4 | 54.4 | 9.9 | 53.6 | 2.0 | 54.4 | 54.4 |
| Average daily feed intake (g hen$^{-1}$) | 113.2 | 23.5 | 124.3 | 17.7 | 124.0 | 25.9 | 118.9 | 23.2 |
| Feed Conversion Ratio | 2.2 | 0.4 | 2.1 | 0.0 | 2.1 | 0.0 | 2.1 | 0.3 |

* Average estimated value of the standard deviation

Table 3. Mean temperature (T) and relative humidity (RH) inside and outside the hen house, ventilation rate (VR), concentrations of GHG and ammonia ($NH_3$) outside and inside the hen house, emissions of GHG and $NH_3$ for each season. Data have been calculated on an hourly basis.

| | | 2012 | | | | | | 2013 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Spring | | Summer | | Autumn | | Winter | | Spring | | Summer | | Average | |
| | | Weeks 18-27 | | Weeks 28-40 | | Weeks 41-53 | | Weeks 54-66 | | Weeks 67-79 | | Weeks 80-93 | | | |
| Climatic conditions | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| T (ºC) | Outside | 16.5 | 4.0 | 20.3 | 3.2 | 15.1 | 3.8 | 11.2 | 2.9 | 13.9 | 3.8 | 19.7 | 3.3 | 15.7 | 4.8 |
| | Inside | 23.2 | 1.6 | 24.5 | 1.8 | 22.5 | 1.6 | 20.1 | 1.8 | 22.0 | 1.3 | 23.8 | 1.6 | 22.4 | 2.1 |
| RH (%) | Outside | 72.5 | 14.0 | 74.2 | 8.6 | 76.6 | 9.6 | 75.7 | 10.6 | 78.7 | 13.5 | 82.2 | 10.4 | 77.3 | 11.8 |
| | Inside | 62.7 | 7.9 | 68.1 | 7.6 | 64.5 | 7.3 | 66.5 | 11.7 | 64.5 | 7.8 | 71.5 | 6.8 | 66.1 | 8.9 |
| Ventilation rate ($m^3$ $hen^{-1}$ $h^{-1}$) | | 3.4 | 2.4 | 6.6 | 3.4 | 3.7 | 2.2 | 2.2 | 1.1 | 3.3 | 2.3 | 6.9 | 3.8 | 4.2 | 3.2 |
| Concentrations | | | | | | | | | | | | | | | |
| $NH_3$ ($mg/m^3$) | Inlet | 0.8 | 0.2 | 0.6 | 0.1 | 0.5 | 0.1 | 0.4 | 0.2 | 0.4 | 0.1 | 0.3 | 0.1 | 0.5 | 0.2 |
| | Outlet | 2.8 | 1.8 | 1.9 | 1.4 | 2.2 | 1.5 | 2.3 | 1.5 | 1.8 | 1.0 | 1.4 | 0.9 | 2.0 | 1.4 |
| $N_2O$ ($mg/m^3$) | Inlet | 0.6 | 0.0 | 0.5 | 0.0 | 0.4 | 0.1 | 0.6 | 0.1 | 0.5 | 0.1 | 0.4 | 0.0 | 0.5 | 0.1 |
| | Outlet | 0.6 | 0.1 | 0.6 | 0.1 | 0.4 | 0.2 | 0.6 | 0.2 | 0.6 | 0.1 | 0.5 | 0.1 | 0.5 | 0.2 |
| $CH_4$ ($mg/m^3$) | Inlet | 2.2 | 1.6 | 4.4 | 2.0 | 4.0 | 1.6 | 2.0 | 0.8 | 2.7 | 1.0 | 5.3 | 1.6 | 3.4 | 1.9 |
| | Outlet | 3.4 | 2.1 | 5.4 | 2.3 | 5.1 | 1.6 | 3.6 | 1.0 | 4.1 | 1.1 | 6.1 | 1.5 | 4.6 | 1.8 |
| $CO_2$ ($g/m^3$) | Inlet | 0.8 | 0.0 | 0.8 | 0.0 | 0.8 | 0.0 | 0.8 | 0.1 | 0.8 | 0.0 | 0.7 | 0.0 | 0.8 | 0.0 |
| | Outlet | 2.0 | 0.5 | 1.5 | 0.4 | 2.2 | 0.5 | 2.5 | 0.5 | 2.1 | 0.4 | 1.4 | 0.3 | 2.0 | 0.6 |
| Emissions | | | | | | | | | | | | | | | |
| $NH_3$ (mg $hen^{-1}$ $d^{-1}$) | | 118.1 | 85.6 | 165.4 | 129.8 | 119.5 | 77.9 | 90.3 | 68.1 | 98.8 | 68.5 | 136.3 | 86.4 | 115.7 | 85.6 |
| $N_2O$ (mg $hen^{-1}$ $d^{-1}$) | | 3.9 | 2.9 | 4.1 | 2.4 | 4.3 | 2.5 | 5.1 | 2.3 | 5.3 | 3.0 | 3.3 | 2.6 | 4.5 | 2.8 |
| $CH_4$ (mg $hen^{-1}$ $d^{-1}$) | | 66.4 | 36.9 | 100.0 | 32.4 | 78.0 | 38.5 | 81.2 | 39.1 | 92.1 | 34.3 | 110.1 | 28.3 | 90.0 | 37.5 |
| $CO_2$ (g $hen^{-1}$ $d^{-1}$) | | 82.3 | 23.0 | 90.5 | 13.5 | 95.9 | 20.6 | 80.2 | 21.9 | 88.1 | 23.2 | 83.9 | 15.4 | 86.4 | 20.9 |

Table 4. Composition and quantity of belt manure for the different feeding phases

| | | Phase 1<br>Weeks 18-55 | | Phase 2<br>Weeks 56-74 | | Phase 3<br>Weeks 75-93 | | Total | |
|---|---|---|---|---|---|---|---|---|---|
| Manure Composition | | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Dry Matter (%) | | 27.2 | 4.5 | 27.1 | 4.7 | 27.0 | 2.7 | 27.2 | 4.2 |
| pH | | 7.5 | 0.5 | 7.7 | 0.4 | 7.6 | 0.3 | 7.6 | 0.5 |
| Organic Matter (% of D.m.) | | 69.0 a | 5.1 | 70.7 ab | 2.8 | 72.4 b | 1.8 | 70.0 | 4.4 |
| Nitrogen (% of D.m.) | Ntot | 5.5 a | 0.7 | 4.9 b | 1.0 | 4.9 b | 0.4 | 5.3 | 0.8 |
| | $NH_4^+$-N | 1.6 | 1.0 | 1.5 | 0.6 | 1.8 | 0.6 | 1.6 | 0.8 |
| Manure quantity | | | | | | | | | |
| Faeces production | (g hen$^{-1}$ d$^{-1}$) | 95.5 | 14.2 | 96.3 | 16.0 | 98.3 | 5.4 | 97.2 | 14.5 |
| | (g DM hen$^{-1}$ d$^{-1}$) | 26.0 | 3.9 | 26.1 | 4.3 | 26.5 | 1.5 | 26.4 | 3.9 |

Different superscript letters in the same row indicate statistically significant differences at P<0.05

Table 5. Mean values, amplitudes and time at which the maximum occurred ($t_{max}$) in the modelization of diurnal patterns.

| | | Summer | | | Winter | | |
|---|---|---|---|---|---|---|---|
| Item | | Mean | Amplitude | $t_{max}$ | Mean | Amplitude | $t_{max}$ |
| $T_{out}$ | (ºC) | 22.3 | 8.1 | 16:09 | 10.0 | 2.6 | 15:11 |
| $T_{in}$ | (ºC) | 24.1 | 1.6 | 15:28 | 20.1 | 1.0 | 13:55 |
| $RH_{out}$ | (%) | 57.5 | 10.8 | 4:29 | 79.6 | 7.8 | 3:42 |
| $RH_{in}$ | (%) | 70.0 | 5.7 | 3:13 | 66.5 | 5.2 | 1:06 |
| VR | ($m^3h^{-1}hen^{-1}$) | 6.8 | 3.6 | 15:08 | 2.2 | 0.9 | 13:29 |
| $NH_3$ | (mg $d^{-1}hen^{-1}$) | 144.9 | 33.5 | 9:25 | 90.4 | 30.9 | 11:08 |
| $CH_4$ | (mg $d^{-1}hen^{-1}$) | 107.4 | 15.3 | 16:45 | 81.3 | 25.2 | 15:29 |
| $N_2O$ | (mg $d^{-1}hen^{-1}$) | 3.52 | 1.18 | 10:34 | 5.15 | 1.71 | 12:38 |
| $CO_2$ | (g $d^{-1}hen^{-1}$) | 85.8 | 8.1 | 12:12 | 80.2 | 22.5 | 13:51 |