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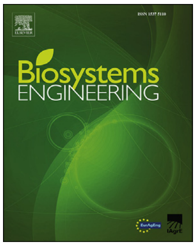

Research Paper

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

Oier Alberdi [a,*], *Haritz Arriaga* [a], *Salvador Calvet* [b], *Fernando Estellés* [b], *Pilar Merino* [a]

[a] *NEIKER-Tecnalia, Consevation of Natural Resources, Bizkaia Technology Park, P. 812, 48160, Derio, Bizkaia, Spain*
[b] *Universitat Politécnica de Valencia, Institute of Animal Science and Technology, 46022, Valencia, Spain*

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

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. On the contrary, diurnal pattern of NH_3 emission did not differ between these seasons. For CO_2, mean emission values did not show seasonality, although the diurnal pattern differed between winter and summer. Results obtained for CH_4 and N_2O emissions did not provide sufficient evidence to determine either seasonality or diurnal effect on these gases.

An NH_3 emission factor of 7% of total N in manure was defined for this system. These losses increased at higher ventilation rates and lower belt cleaning frequencies. Thus, NH_3 mitigation strategies at housing level should consider both parameters. Further studies would be necessary to determine how these factors regulate NH_3 emission at laying hen houses.

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. Alternative production systems have been implemented at varying levels in different EU countries, and most CC farms have switched to enriched cages (EC). In this sense, Spain, which is the 4th egg producer country in EU (MAGRAMA, 2015), has currently more than 85% of its laying hen population producing through EC production system. From an environmental perspective, EU

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* *Corresponding author.*
E-mail address: oalberdi@neiker.eus (O. Alberdi).

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state members are obliged to reduce NH_3 and GHG losses by adopting Gothenburg and Kyoto 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 leads to poor indoor air quality that affects 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 (Groot Koerkamp, 1994) and USA (Zhao, Shepherd, Li, & Xin, 2015), 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; Zhu, Dong, Zhou, Xin, & Chen, 2011). Moreover, most of the research on air quality in laying hen houses in Europe has been based on short-time measurements (Nimmermark, Lund, Gustafsson, & Eduard, 2009), thus not covering seasonal variations. 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 (northern Spain). A second objective was to analyse 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 (Fig. 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 (CP) 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 hen age. Bird mortality, laying rate, egg production, feed intake and feed conversion ratio was daily recorded by the producer. Productive parameters of the laying hens during the experiment for different feeding phases are presented in Table 2.

Maximum laying rate (93%) was reached at week 23 and decreased gradually until the end of the cycle (78%). Feed conversion averaged 2.1 throughout the cycle in accordance with the technical datasheet for Lohmann Brown hens.

2.2. *Environmental conditions*

Outside weather conditions of the location during the study were: average air temperature of 10.7 °C and 20.0 °C, air relative humidity (RH) of 76.0% and 86.3% and rainfall rate of 618 and 101 mm (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 climate conditions.

Five temperature and RH sensors (Onset, HOBO U12-013, USA, precision ±0.35 °C 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 (Fig. 1).

Ventilation rate (VR) was measured under the usual rearing conditions at the facility according to the methodology described by Calvet, Cambra-López, Blanes-Vidal, Estellés, and Torres (2010). An electronic data logger (Binary Devices S.L., Datalogger 244, Spain) converted every second the electric signal from each fan into digital data on fan status. The average percentage of operation of each fan was obtained every 5 min.

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 metre (Veris, PX, USA, accuracy ±0.5 Pa). 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))

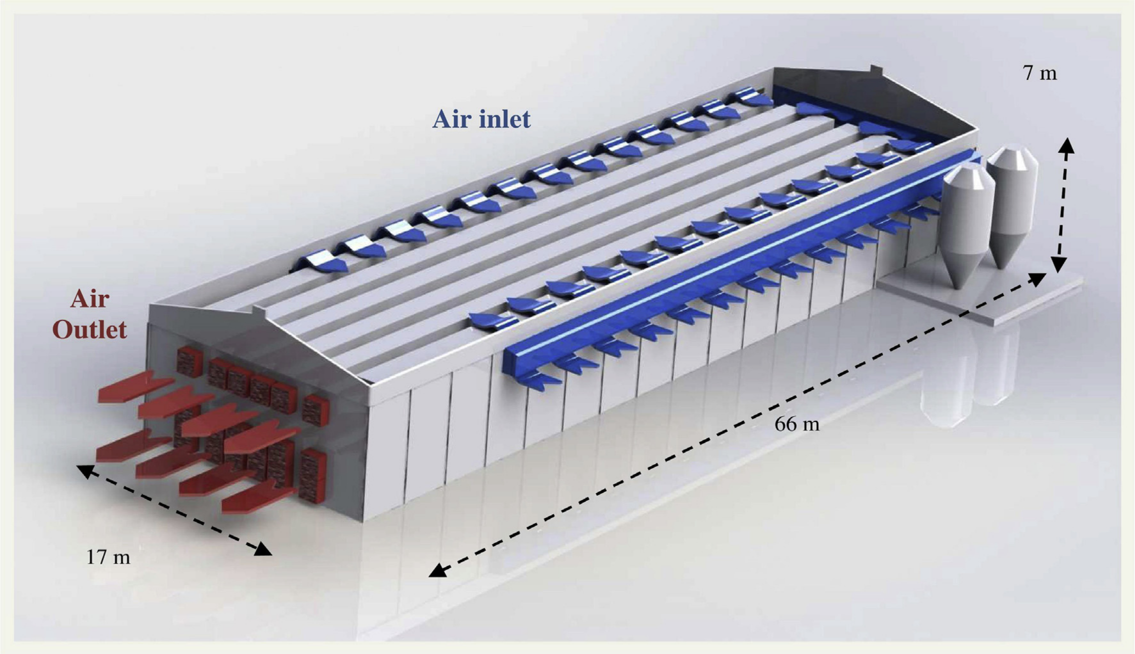

Fig. 1 – Layout of the house and scheme of the tunnel ventilation system.

$$y = -0.4405x + 43.909$$
$$R^2 = 0.7378 \quad (1)$$

where,

y = Airflow rate (10^3 m^3 h^{-1})
x = Pressure Drop (Pa)

Total VR 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.

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

Feed composition	Phase 1	Phase 2	Phase 3
	Weeks 18–54	Weeks 55–74	Weeks 74–93
	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 fibre (% DM)	3.8	4.0	4.7
Organic matter (% DM)	86.5	85.1	87.3

2.3. *Manure characterization*

Despite manure used to be accumulated on belts from 1 to 5 days, the most frequent interval between removals was 3 days. The producer recorded the time of each manure removal. A representative sample of manure from belts located at different corridors (approximately 2 kg) was collected fortnightly and analysed for dry matter (DM), total nitrogen (Ntot), ammonium nitrogen (NH_4^+–N), organic matter (OM) and pH. After each removal, the amount of manure removed from the building was weighed at the farm. Afterwards, manure was exported out of the farm.

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.

Productive parameters	Phase 1		Phase 2		Phase 3		Total	
	Weeks 18–55		Weeks 56–74		Weeks 75–93			
	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})[a]	1.9	–	2.0	–	2.1	–	2.0	–
Mortality (% per phase)[a]	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
Feed intake (g d^{-1} 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

[a] Average estimated value of the standard deviation.

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 an INNOVA 1412 Photoacoustic multi gas analyser (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. PAMGA was calibrated before the start of the trial. Two further calibrations were performed during the experimental period. In addition, 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 10 L Tedlar bags and NH_3 was tested by a recently calibrated second PAMGA (Bruel & Kjaer, 1302, Denmark). Ammonia concentration values biased less than 5% between both analysers. 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).

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 min. 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 stable temperature, which prevented any in-line moisture condensation. All the air sampling and analysing equipment (PAMGA, multipoint sampler and external pump) were placed in a clean room and kept inside an air conditioned rack to protect them from excessive heat, dust and moist.

According to Equation (2), gas emissions were calculated on an hourly basis:

$$E = (C_{outlet} - C_{inlet}) \times V \tag{2}$$

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

Emissions were also expressed either per hen unit (number of hens at the facility on the measurement day) as mg d^{-1} hen^{-1} or per animal mass unit as g h^{-1} 500 kg^{-1} LW (considering the number of hens in the building and their 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 such as VR, temperature and RH, and gas emissions was studied through a correlation analysis using PROC CORR of the statistical package SAS 9.3 (SAS, 2013). One-way analysis of variance (PROC GLM) was performed to analyse the effect of phase feeding in manure composition.

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

$$Xh = T + \left[A\cos\left(\frac{h2\pi}{24} - \frac{D2\pi}{24}\right)\right] \tag{3}$$

where,

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 4.0 °C in winter to 27.1 °C in summer, reflecting typical weather conditions in the region. Temperature amplitude was lower inside the facility and ranged from 18.0 °C to 25.4 °C (mean value, 22.4 °C). Daily mean indoor temperatures were related to seasonal temperature variations outdoors. Outside RH 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. Inside RH was slightly lower and remained stable (66.1 ± 8.9) during the experimental period. Additionally, when inside temperature was higher than 25 °C, the cooling refrigeration system was activated. This system operated 18% of days, with an average activation of 12 h d^{-1}. Cooling system would have contributed to increase RH conditions in summer conditions.

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 (Fig. 2), being higher in summer due to higher outdoor temperatures (Table 3, Fig. 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

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						Average	
		Spring		Summer		Autumn		Winter		Spring		Summer			
		Weeks 18–27		Weeks 28–40		Weeks 41–53		Weeks 54–66		Weeks 67–79		Weeks 80–93			
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Climatic conditions															
T (°C)	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
VR ($m^3\ h^{-1}\ hen^{-1}$)		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
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
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
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 ($mg\ d^{-1}\ hen^{-1}$)		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
CH_4 ($mg\ d^{-1}\ hen^{-1}$)		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
N_2O ($mg\ d^{-1}\ hen^{-1}$)		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
CO_2 ($g\ d^{-1}\ hen^{-1}$)		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

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 ($P > 0.05$). It was attributed to the high RH values observed throughout the year (Table 3). 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.

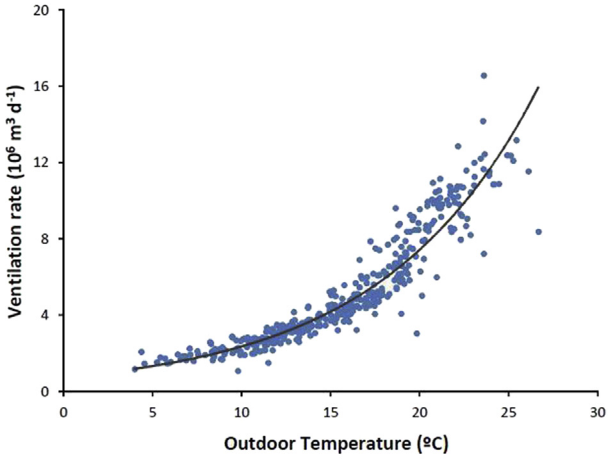

Fig. 2 – Relation of daily ventilation rate ($10^6\ m^3\ d^{-1}$) and outdoor temperature (°C). Fitted equation: $y = 0.7504e^{0.1146x}$, $R^2 = 0.9154$.

Total N content of manure, which averaged 5.3% DM (±0.8%) during the study, was significantly influenced by the feeding phase ($P < 0.05$). As Table 4 shows, manure Ntot content decreased during the second and third phases. 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. In contrast to Ntot, no relationship between 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 NH_3 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}, which was within the range reported by Zhao et al. (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, although manure removal frequency

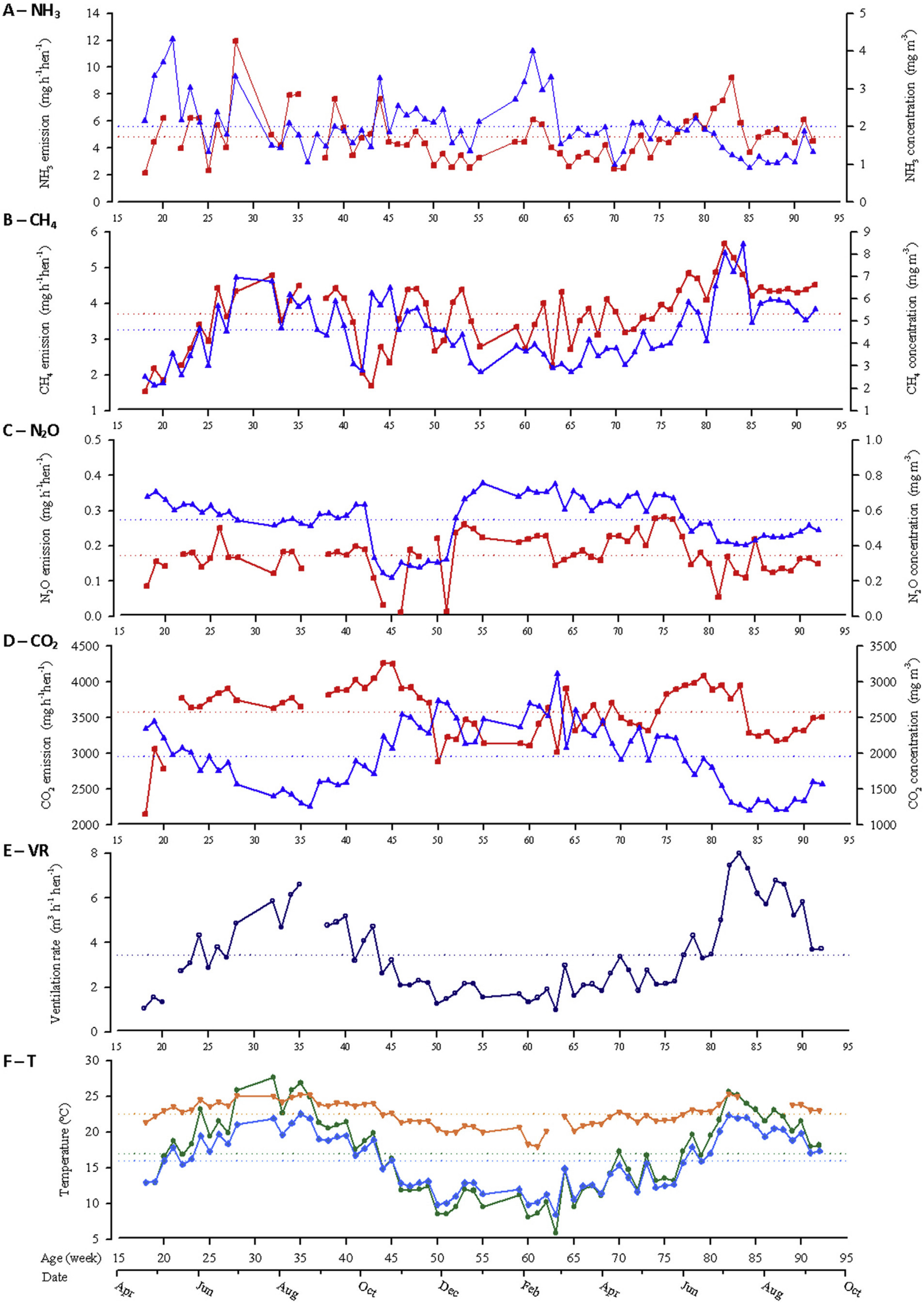

Fig. 3 – Ammonia and GHG emission (■) and concentration (▲), and ventilation rate (o) per hen and; outlet (▼), inlet (♦) and outdoor (●) temperature during trial. Dotted lines represent average values.

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

	Phase 1		Phase 2		Phase 3		Total	
	Weeks 18–55		Weeks 56–74		Weeks 75–93			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Manure composition								
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 (% DM)	69.0 a	5.1	70.7 ab	2.8	72.4 b	1.8	70.0	4.4
Total nitrogen (% DM)	5.5 a	0.7	4.9 b	1.0	4.9 b	0.4	5.3	0.8
Ammonium nitrogen (% DM)	1.6	1.0	1.5	0.6	1.8	0.6	1.6	0.8
Manure quantity								
Faeces production (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 $P < 0.05$.

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 (Fig. 3A), which would be related to the combination of the low VR (Fig. 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 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 was 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).

The highest NH_3 emissions (11.9 mg h^{-1} hen^{-1}) happened in summer conditions (weeks 28 and 83, Fig. 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 (25 °C) 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 (Fig. 3A). During this period, average outside temperature was below 10 °C, 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 (Fig. 3A).

Seasonal differences in NH_3 emission were observed in this study, consistent with other authors (Da Borso & Chiumenti, 1999; Nicholson et al., 2004). Mean NH_3 emission reached 90.3 mg d^{-1} hen^{-1} in winter and 144.9 mg d^{-1} hen^{-1} in summer. Similar results (134 mg d^{-1} hen^{-1}) were obtained by Da Borso and Chiumenti in summer in a cage system under similar climate conditions in Italy. 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 inside temperatures (14.5 °C) registered in Scandinavian region, 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 15 °C. Nicholson et al. (2004) also reported higher NH_3 emission rates in summer than in winter (mean 3.2 g h^{-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 20 °C and greater velocities in the barn. This seasonal pattern was also observed by Wathes et al. (1997), who reported that NH_3 emission rate increased by 51% from winter to summer season at deep-pit layer houses in England.

Deep-pit houses, generally show higher emissions than cage systems, with values of 1065 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 2100 mg d^{-1} hen^{-1} (Nimmermark et al., 2009) and free range housing systems, with 1342 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 CP ranged from 16.0 to 16.7%, resulting significantly different ($P < 0.05$) Ntot content of manure (Table 4). 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 coincidence with belt cleaning frequency. It has been previously described that belt cleaning operations lead to a decrease on NH_3 emissions at house level (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 housing with belt cleaning systems. Ammonia EF for laying hen facilities are currently established by EMEP-EEA 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-EEA 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}. Methane concentration followed the similar pattern as CH_4 emissions, mainly from week 18–40 and 73–93 (Fig. 3B). 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 RH was recorded (Table 3). 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. (2011) 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 in negligible emissions.

Average N_2O emission was 4.5 mg d^{-1} hen^{-1} (Table 3), which was within the range reported by Fournel et al. (2012a) for cage systems (7.1 mg d^{-1} hen^{-1}). Nitrous oxide emission rate was negatively affected by VR ($P < 0.001$) as reported Dobeic and Pintarič (2011). This effect should be the reason that explains the lowest emission in summer.

3.3.4. *Carbon dioxide*

Average indoor concentration was 1984 mg m^{-3}. Carbon dioxide concentration was indicative of the barn VR, with lower CO_2 concentration corresponding to higher VR (Fig. 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 4497 mg m^{-3} and 3985 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 2734 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 2511 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 hen house comes from manure. Ni, Heber, Hanni, Lim, and Diehl (2011) also suggested that manure is a source of CO_2 release in commercial layer barns. In our study it was observed a slight increase in CO_2 emission during the days between manure removals. That could be due to manure accumulation.

3.4. *Gas emission diurnal patterns*

A clear diurnal variation pattern was found for gas emissions, temperature, RH and VR. These variations were expressed in hourly average terms for summer and winter separately (Fig. 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).

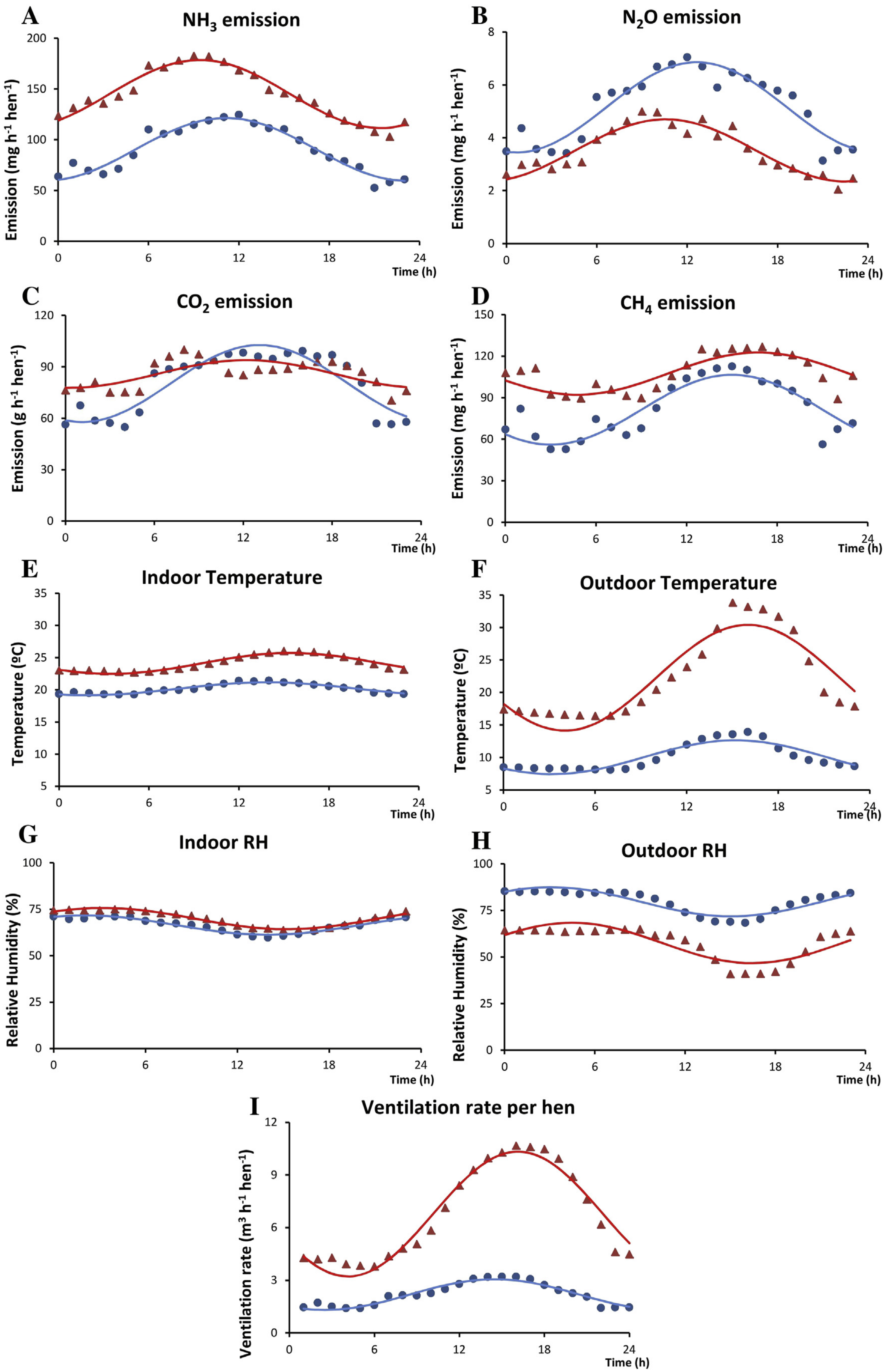

Fig. 4 – Average hourly variations in NH_3, CH_4, N_2O and CO_2 emissions, outdoor and indoor temperature and relative humidity, and ventilation rate for summer (▲) and winter (●). All modelled curves were significant at $P < 0.001$.

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

Item		Summer			Winter		
		Mean	Amplitude	t_{max}	Mean	Amplitude	t_{max}
T_{out}	(°C)	22.3	8.1	16:09	10.0	2.6	15:11
T_{in}	(°C)	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	($m^3\ h^{-1}\ hen^{-1}$)	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	($mg\ d^{-1}\ hen^{-1}$)	3.5	1.2	10:34	5.2	1.7	12:38
CO_2	($g\ d^{-1}\ hen^{-1}$)	85.8	8.1	12:12	80.2	22.5	13:51

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 (Fig. 4I). 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 (Fig. 4C). Thus, the activation of light and consequent activity of laying hens caused a sudden increase at morning and quick drop at night (Fig. 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 $g\ h^{-1}$ (Fig. 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 et al., 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 $m^{-3}\ h^{-1}\ hen^{-1}$.

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, as 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 (NH_3, CH_4, N_2O and CO_2) were monitored in a laying hen EC facility during a complete cycle under Oceanic climate conditions. A different seasonality effect on both total gas amount and diurnal emission pattern was found for each gas. In this sense, NH_3 showed quantitatively higher emission in summer than in winter, while diurnal pattern was similar. On the contrary, CO_2 presented a distinct diurnal pattern among the seasons while differences in CO_2 emission were low. Consequently, the identification of seasonal and diurnal patterns should be used to optimize sampling strategies for similar types of facilities to produce reference emission values at regional scale.

Ammonia EF at housing level was 7% of total N in manure. These losses were increased at higher VR and lower belt cleaning frequencies. Thus, NH_3 mitigation strategies at housing level should consider both parameters. Further studies would be necessary to determine how these factors regulate NH_3 emission at laying hen houses.

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