Centre Eau Terre Environnement

CONTRIBUTION D’UN MODÈLE STATISTIQUE (RANDOM FOREST) À L’ÉLEVAGE DE PRÉCISION DE SYSTÈMES DE POULES PONDEUSES EN VOLIÈRE COMME OUTIL DE PRÉDICTION DE LA QUALITÉ DE L’AIR ET DE LA PERFORMANCE DU POULAILLER

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RÉSUMÉ

L’industrie des œufs de consommation s’est développée de manière fulgurante depuis les années 70s. Aujourd’hui, l’élevage de poules pondeuses se fait avec des systèmes de production de forte intensité, tant en densité de confinement qu’en taux de rendement. Cela dit, une forte intensité implique, malheureusement, une empreinte environnementale importante. Dans ce cas précis, on fait référence à la production d’ammoniac et de gaz à effet de serre (GES) qui dégradent la qualité de l’air ainsi que le bien-être tant des animaux que des travailleurs. De plus, le bien-être animal et l’empreinte environnementale sont devenus des sujets d’intérêt chez les consommateurs. Ainsi, l’élevage de poules pondeuses doit avoir recours à une production plus contrôlée et automatisée afin de mieux gérer ce bien-être et cette empreinte. C’est pourquoi cette étude a porté sur l’évaluation des effets de trois Techniques de Mitigation Environnementales (TME) sur le bien-être et la performance d’un système avicole en volière, ainsi que sur l’applicabilité d’un modèle statistique (*Random Forest*) pour prédire la dynamique des fluctuations journalières du taux de ponte (TP), et les émissions d’ammoniac issue du stockage de fientes. Les résultats ont illustré que les TME n’ont pas eu un effet sur les comportements naturels des animaux, ni sur le TP, mais qu’une diminution de l’espace litière pouvait induire des différences dans la distribution spatiale du poulailler au bâtiment. Par ailleurs, le modèle *Random Forest* a présenté une capacité prédictive satisfaisante sur les deux variables d’intérêt. Les prédicteurs ont également été validés par une analyse multivariée des conditions environnementales et hygrothermiques à l’intérieure du site d’élevage. L’étude représente une contribution originale à l’élevage de précision.

Mots-clés : poules pondeuses, systèmes d’élevage, empreinte environnementale, qualité de l’air, bien-être, Random Forest, modélisation, élevage de précision

ABSTRACT

Since the 1970, worldwide egg production has undergone substantial development with, laying hen houses becoming intensive systems characterized by high animal density and yields. These intensive systems have an important contribution to the environmental footprint, linked to ammonia and greenhouse gases (GHG) emissions; negatively affecting air quality and welfare of both animals and caretakers. Nowadays, animal welfare and environmental footprint are subjects of interests to consumers. Thus, to deal with these concerns, laying hen housing systems must be adapted with automated control systems. Hence, this study focused on the evaluation of three Environmental Control Strategies (ECS) in terms of animal welfare and egg yield of a cage-free system; as well as on applying a statistical model known as Random Forest to predict the dynamics of egg-yield daily fluctuations and ammonia emissions in controlled manure storage. Results showed that neither the natural animal behaviors nor the egg-yield were affected by the applied ECSs. However, the reduction of litter surface produced significant differences in the animal spatial distribution. Further, the Random Forest model predicted satisfactorily two variables of interest and the model predictors were also identified using a multivariate analysis of indoor environmental and hygrothermal conditions. This research represents an original contribution to Precision Livestock Farming.

Keywords : Laying hen, livestock systems, environemental footprint, air quality, welfare, Random Forest, modelling, precision livestock farming

TABLE DES MATIÈRES

REMERCIEMENTS ..... **III**

RÉSUMÉ ..... **V**

ABSTRACT ..... **VII**

TABLE DES MATIÈRES ..... **IX**

LISTE DES FIGURES ..... **XIII**

LISTE DES TABLEAUX ..... **XV**

1 INTRODUCTION **1**

1.1 LES SYSTEMES D'ELEVAGE DE POULES PONDEUSES 1

1.1.1 Les systèmes en cages conventionnelles 1

1.1.2 Les systèmes en cages aménagées 2

1.1.3 Les systèmes en volière et en plein air 2

1.2 POLITIQUES D'ADAPTATIONS DES SYSTEMES D'ELEVAGES DES POULES PONDEUSES 3

1.3 LES SYSTÈMES EN VOLIÈRE ET LE BIEN-ÊTRE ANIMAL 4

1.4 ENJEUX ENVIRONNEMENTAUX À LA FERME : SYSTÈMES EN VOLIÈRE 4

1.5 PLF : MISE EN ŒUVRE DE L'IMPORTANCE DE LA MODÉLISATION 5

1.5.1 Complexité des biosystèmes 6

1.6 OUTILS DE MODÉLISATION 6

1.6.1 Modèles empiriques et mécanistes 6

1.6.2 Modèles statistiques de dernière génération : « Machine Learning » 7

1.7 INTÉRÊT DE L'ÉTUDE 7

1.8 BIBLIOGRAPHIE 8

2 PREMIER ARTICLE **13**

2.1 ABSTRACT 15

2.2 INTRODUCTION 15

2.3 MATERIALS AND METHODS 17

2.3.1 Experimental bench-scale rooms 17

2.3.2 Animal and housing 18

2.3.3 Environmental control strategies (ECSs) 19

2.3.4 Spatial occupancy and laying hen ethology analysis 20

2.3.5 Video recordings and observation analysis 22

2.3.6 Statistical Analysis 23

2.4 RESULTS 24

2.4.1 Spatial occupancy (SO) 24

2.4.3 *Laying hen behavior (LHB)* ... *26*
2.5 DISCUSSION ... 28
2.5.1 *Space occupancy (SO) preference* ... *28*
2.5.2 *Patterns in animal behaviors* ... *28*
2.6 CONCLUSION ... 29
2.7 ACKNOWLEDGMENT ... 30
2.8 BIBLIOGRAPHIE 1ER ARTICLE ... 30

3 DEUXIÈME ARTICLE ... 35
3.1 ABSTRACT ... 37
3.2 INTRODUCTION ... 37
3.3 MATERIALS AND METHODS ... 41
3.3.1 *Animal housing* ... *41*
3.3.2 *Indoor environmental and hygrothermal monitoring* ... *41*
3.3.3 *Egg handling* ... *42*
3.3.4 *Environmental control strategies (ECS)* ... *42*
3.3.5 *Egg yield modeling* ... *44*
3.3.6 *Data analysis* ... *45*
3.3.7 *Input data* ... *46*
3.3.8 *Variable importance* ... *47*
3.3.9 *Model evaluation* ... *47*
3.3.10 *Scenario analysis* ... *47*
3.4 RESULTS AND DISCUSSION ... 48
3.4.1 *Hygrothermal, atmospheric pressure and ventilation conditions* ... *48*
3.4.2 *Indoor environmental conditions* ... *50*
3.4.3 *HDEP and EGC time series* ... *52*
3.4.4 *Random Forest for HDEP predictions* ... *56*
3.5 CONCLUSION ... 62
3.6 ACKNOWLEDGMENT ... 63
3.7 BIBLIOGRAPHIE 2E ARTICLE ... 63

4 TROISIÈME ARTICLE ... 67
4.1 ABSTRACT ... 69
4.2 INTRODUCTION ... 69
4.3 MATERIALS AND METHODOLOGY ... 72
4.3.1 *Source of laying hen manure samples* ... *72*
4.3.2 *Laying hen manure characteristics* ... *72*
4.3.3 *Set-up for measuring ammonia emissions* ... *74*
4.3.4 *Mechanistic modelling* ... *74*

4.3.5 Statistical modelling *77*
4.3.6 Model evaluation *80*
4.4 RESULTS AND DISCUSSION 81
4.4.1 Litter samples *81*
4.4.2 Temperature and relative humidity *81*
4.4.3 Observed ammonia concentrations *82*
4.4.4 Mechanistic modelling of NH_3-N concentrations *83*
4.4.5 Random Forest algorithm for NH_3-N predictions *86*
4.5 CONCLUSIONS 90
4.6 ACKNOWLEDGEMENTS 91
4.7 BIBLIOGRAPHIE 3E ARTICLE 91
5 DISCUSSION GÉNÉRALE ET CONCLUSION 97
5.1 ANALYSE DES TECHNIQUES DE MITIGATION ENVIRONNEMENTALE (TME) 97
5.2 MODELISATION DE LA PRODUCTION DES ŒUFS DE CONSOMMATION 98
5.3 MODELISATION DES CONCENTRATIONS D'AMMONIAC 98
5.4 SYSTEMES D'ELEVAGE DES POULES PONDEUSES PLUS AUTONOMES 99
5.5 DEFIS ET OPPORTUNITES DE LA MODELISATION A L'AIDE DE MODELES STATISTIQUES DANS LES SYSTEMES D'ELEVAGE DE POULES PONDEUSES. 100
6 BIBLIOGRAPHIE 101
7 ANNEXE I 105
8 ANNEXE II 107

LISTE DES FIGURES

FIGURE 1. 3D LAYOUT OF AN EXPERIMENTAL ROOM 18

FIGURE 2. VIDEO RECORDING AT AN EXPERIMENTAL ROOM 22

FIGURE 3. RELATIVE FREQUENCIES OF SO WITH STANDARD DEVIATIONS FROM EXPERIMENTAL CFS. A) BATCH 1. B) BATCH 2. 25

FIGURE 4. AVERAGE COUNTS OF LAYING HENS DISPLAYING THE FIVE NATURAL AND SPECIES-SPECIFIC BEHAVIORS WITH STANDARD DEVIATIONS. A) BATCH1. B) BATCH 2. 27

FIGURE 5. BOXPLOT OF HYGROTHERMAL, ATMOSPHERIC PRESSURE AND VENTILATION CONDITIONS OF THREE ECSS AND THE CONTROL FOR BOTH BATCHES 1 AND 2: (A) TEMPERATURE, (B) RELATIVE HUMIDITY, (C) ATMOSPHERIC PRESSURE, AND (D) OUTLET AIRFLOW RATE. 50

FIGURE 6. BOXPLOT OF GAS EMISSIONS INSIDE THE THREE ECSS AND THE CONTROL FOR BOTH BATCHES 1 AND 2: (A) CO_2, (B) CH_4, (C) N_2O, AND (D) NH_3. 52

FIGURE 7. HEN-DAY EGG PRODUCTION INDEX (HDEP) AND EGG CLEANLINESS INDEX (EGC) TIME SERIES. BLACK BOLD DASHED LINES (--): MEAN VALUES. BLACK DASHED LINES (--): STANDARD DEVIATIONS. 54

FIGURE 8. EGG PRODUCTION CURVES FOR THE CONTROL TREATMENT WITH SEVERAL AVERAGING WINDOW SIZES, EXPRESSED IN DAYS. RED DASHED LINES (--): STANDARD DEVIATION INTERVALS. 55

FIGURE 9. LINEAR DISCRIMINANT ANALYSIS (LDA). LEFT: SCORE PLOT. RIGHT: BIPLOT OF LOADINGS. ADAPTED FROM: PARENT (2020) 56

FIGURE 10. OPTIMAL PARAMETERS OF RANDOM FOREST (RF) MODELS BY SLIDING WINDOW SIZE: (A) RMSE AND (B) R^2....................57

FIGURE 11. OBSERVED AND SIMULATED HDEP FLUCTUATIONS USING A RANDOM FOREST MODEL WITH A 59

FIGURE 12. SIMULATED (•, RED POINTS) AND OBSERVED (−, BLACK LINE) HDEP TIME SERIES. 59

FIGURE 13. PREDICTIVE VARIABLE IMPORTANCE BY THE INCREMENT OF THE MSE (%)....................60

FIGURE 14. SCENARIO ANALYSIS BY INCREASING TEMPERATURE 5% 62

FIGURE 15. ILLUSTRATION OF THE NH_3 VOLATILIZATION PROCESS IN LIQUID MANURE. FROM NI *ET AL.* (2009). USED WITH PERMISSION. 75

FIGURE 16. TIME SERIES OF OUTDOOR TEMPERATURE AND RELATIVE HUMIDITY OVER THE CONTROLLED STORAGE PERIOD OF LAYING HEN LITTER SAMPLES AT IRSTEA LABORATORY....................82

FIGURE 17. TIME SERIES OF NH_3-N CONCENTRATIONS FROM THE CONTROLLED STORAGE OF THE TWO SAMPLES OF LAYING HEN MANURE 83

FIGURE 18. DATA DISTRIBUTION FROM THE CONTROLLED STORAGE OF THE TWO SAMPLES OF LAYING HEN MANURE: LHM1 (LEFT) AND LHM2 (RIGHT). 83

FIGURE 19. LEFT: $C_{G,0}$ PREDICTIONS FROM LITTER A (TEMP = 12.9 TO 27.2 °C, $\boldsymbol{Kf}$ = 1.87 L KG^{-1}). RIGHT: $C_{G,0}$ PREDICTIONS FROM LITTER B (TEMP = 19.2 TO 30.0 °C, $\boldsymbol{Kf}$= 2.15 L KG^{-1}). 84

Figure 20. Observed and simulated NH_3-N concentrations using the mechanistic model. 85
Figure 21. Time series NH_3-N over a controlled experimental laying hen manure storage. Simulated values of the mechanistic model (red points) and observed values (black points). 85
Figure 22. Evolution of normalized RMSE values with *mtry* and *ntree* variations for a RF model using a 10-fold cross-validation re-sampling method with repetitions (n = 5). 86
Figure 23. Observed and simulated NH3-N concentrations using the statistical model (RF) 87
Figure 24. Simulated (+) and observed (•) NH_3-N concentration time series for the testing process in RF using LHM1 data 88
Figure 25. Simulated (+) and observed (•) NH_3-N concentration time series using the LHM2 data 88
Figure 26. Predictive variable importance by increment of the MSE (%) 89
Figure 27. ACP analysis by projecting Euclidian distances in 2D-space from a set of variables selected to predict NH_3-N concentrations over manure storage. 90
Figure 28. Weekly data distribution of temperature by treatment 107
Figure 29. Weekly data distribution of relative humidity by treatment 108
Figure 30. Weekly data distribution of atmospheric pressure by treatment 109
Figure 31. Weekly data distribution of airflow rate by treatment 110
Figure 32. Weekly data distribution of CO_2 concentrations by treatment 111
Figure 33. Weekly data distribution of CH_4 concentrations by treatment 112
Figure 34. Weekly data distribution of N_2O concentrations by treatment 113
Figure 35. Weekly data distribution of NH_3 concentrations by treatment 114

LISTE DES TABLEAUX

TABLE 1. ECSS APPLIED AT THE EXPERIMENTAL CFS 20
TABLE 2. LAYING HEN BEHAVIORS OBSERVED INSIDE ALL EXPERIMENTAL CFS...............................21
TABLE 3. F TEST FOR TREATMENT EFFECT ON BEHAVIOR COUNTS IN LAYING HENS 26
TABLE 4. LIST OF TREATMENTS APPLIED WITHIN THE CFRS. 43
TABLE 5. AVERAGES OF HDEP AND EGC BY TREATMENT. 53
TABLE 6. OPTIMAL RF MODEL PARAMETERS AS A FUNCTION OF AVERAGING WINDOW SIZES.............57
TABLE 7. INITIAL CHARACTERISTICS OF LAYING HEN MANURE FROM TWO MANURE SAMPLES (LHM1 AND LHM2). 81
TABLE 8. RELATIVE FREQUENCIES OF SPATIAL OCCUPANCY (SO, %) OF LAYING HENS AS A FUNCTION OF TREATMENT 105
TABLE 9. AVERAGE ± SD OF NUMBER OF LAYING HENS' BEHAVIORS FOR EACH EXPERIMENTAL SETUP. 106

1 INTRODUCTION

Depuis les 50 dernières années, l'industrie des œufs de consommation a continuellement augmentée sa productivité. Entre les années 2008 et 2013, la production globale des œufs a eu une croissance annuelle moyenne de 1.8% (Bertechini, 2017). De plus, selon FAOSTAT (2019), cette croissance connait une augmentation annuelle de 2.6% depuis l'année 2015. En faisant l'hypothèse d'une croissance constante, le taux de croissance de la production d'œufs deviendra au cours des prochaines années supérieure à celui de la viande bovine (FAO, 2010). Selon Agriculture and Agri-Food Canada (2020) au cours des cinq dernières années, la production des œufs au Canada a augmenté de 2.5% par année, ainsi la capacité des fermes peut maintenant varier entre 100 000 et 400 000 poules pondeuses. La poule Leghorn blanche est certes la race la plus souvent rencontrée dans ces fermes, car elle peut produire environ 340 œufs par année. En 2019, 1 172 fermes ovocoles étaient enregistrées au Canada. Le Québec pour sa part possédait 20.3% des contingents de la production nationale. En France, selon le *Comité National pour la Promotion de l'œufs* − CNPO (2020) le taux de croissance annuel de la production des œufs de consommation depuis 2013 est d'environ 2% avec une production annuelle moyenne d'environ 270 œufs par poule. En 2020, en France environ 2 800 élevages des poules pondeuses produisaient 15 milliards d'œufs par an, dont environ 36% de ses élevages se trouvaient dans la région de la Bretagne (Agreste, 2019)

1.1 Les systèmes d'élevage de poules pondeuses

Les bâtiments d'élevage de poules pondeuses peuvent être réparties en deux grands groupes, à savoir : les systèmes conventionnels (élevage en cages ou en batterie) et les systèmes alternatifs (élevage en cages aménagées, en volière ou en plein air).

1.1.1 Les systèmes en cages conventionnelles

Les systèmes en cages ou « en batterie » sont des bâtiments constitués de cages grillagées où les poules sont élevées avec une densité de plancher souvent inférieure à 500 cm^2/poule. Les batteries sont mises en place dans une structure en « A » permettant d'accumuler le fumier en dessous des batteries empilées (Mahmoudi, 2016; Pelletier & Godbout, 2016). Ces systèmes sont reconnus pour leur efficacité de contrôle, tant sur les poules, que les conditions sanitaires et environnementales. Cependant, le bien-être animal est limité par l'espace disponible par animal imposant intrinsèquement des contraintes sur les comportements naturels liées à l'espèce (p. ex. le bain de poussière, picorer, nidifier) (Appleby, 2003).

1.1.2 Les systèmes en cages aménagées

Les systèmes en cages aménagées ou « enrichis » sont des bâtiments ou logements alternatifs composés de cages grillagées, mais avec une surface minimale de 2000 cm^2 où les poules sont élevées avec une densité d'environ 750 cm^2/poule. Les cages, dites aménagées, offrent des nids, perchoirs, et parfois de la litière, garantissant à la poule la possibilité d'exprimer quelques comportements naturels (Misslin, 2017). Cependant, ces systèmes peuvent accentuer des problèmes des déviation du bréchet des poules selon la conception des perchoirs à l'intérieur des cages; ainsi que des comportements négatifs, p. ex., le picotage ou le cannibalisme, et la circulation des animaux dépendamment de la densité des animaux par cage enrichi (Mahmoudi, 2016).

1.1.3 Les systèmes en volière et en plein air

Les systèmes en volière sont des logements alternatifs avec une espace libre, parfois à plusieurs niveaux, disponible aux poules permettant un élevage plus dynamique. Ces systèmes ont souvent une surface disponible de 929 à 1 115 cm^2 par poule. Ils sont composés de nids, des perchoirs, passerelles, grattoirs et d'une espace litière (NFACC, 2017; Philippe *et al.*, 2020).

D'ailleurs, les systèmes en plein air ont des caractéristiques identiques aux systèmes en volières, mais ils ont une espace à l'extérieur du bâtiment qui permet à la poule de sortir pendant la journée en fonction de l'état du poulailler et des conditions météorologiques. Les systèmes en plein air utilisent des ouvertures sur les murs du bâtiment qui permettent la libre circulation des animaux (Misslin, 2017).

Ces deux systèmes offrent par ailleurs moins de moyens de contrôle sur les conditions environnementales (p. ex., émissions de gaz et de poussière), sur les conditions de santé du poulailler, ainsi que sur la production d'œufs, étant donné la dynamique du système animal-environnement-météo-production. Néanmoins, ces types de logements permettent : (i) une adaptation à l'environnement faite par les poules tout au long de la période de production, (ii) des meilleures conditions de bien-être directement liées à la libre circulation des oiseaux, ainsi que (iii) la possibilité d'effectuer leur comportements naturels (Mahmoudi, 2016; Philippe *et al.*, 2020; Zhao *et al.*, 2015).

Le bien-être animal peut comprendre divers aspects chez les poules. Il peut se mesurer à partir des changements dans la physionomie animale, dans le métabolisme de la poule, ainsi que dans les comportements naturels (Mahmoudi, 2016). Les systèmes avicoles en volière peuvent accentuer des changements dans la physionomie, p.ex. une déformation du bréchet découlant de plusieurs causes, à savoir : la fréquence de l'endroit de repos, le déplacement à l'intérieur du bâtiment à l'aide des vols courts ou des sauts, ainsi que les chutes générés par un déplacement vertical (Rentsch *et al.*, 2019; Stratmann *et al.*, 2019). Néanmoins, l'accès à la litière offert par ces systèmes permet l'expression des différents comportements, p. ex. le bain de poussières ou le lissage de plumes, offrant une ambiance plus naturelle et moins contraignante (Mahmoudi, 2016).

1.2 Politiques d'adaptations des systèmes d'élevages des poules pondeuses

Le constant taux de croissance de cette filière de production agricole a encouragé les pays comme le Canada et la France à mettre en place des politiques sur les exploitations des poules pondeuses. Depuis 2000, l'organisation mondiale de la santé animale (OIE) a contribué à l'élaboration des normes intergouvernementales du bien-être animal à l'échelle internationale. Ceci vient répondre aux intérêts de ces 182 pays membres sur diverses thématiques de soins et santé animale, ainsi que de la sécurité alimentaire (OIE, 2019). Ces normes s'appuient sur le document précurseur des normes relatives au conception des logements et de la protection des poules pondeuses produit par le conseil de l'Union Européenne (1999). Celles-ci ont permis de rédiger une politique sur la transition des systèmes conventionnels vers des systèmes alternatifs, c-à-d., cage aménagées, systèmes en volière ou systèmes en plein air. Au Canada, les systèmes alternatifs ont été introduits par le *Code de pratiques pour le soin et la manipulation de poulettes et pondeuses* (2017) dans le cadre d'une stratégie pour faciliter le passage des systèmes actuels d'élevages des poules pondeuses vers les systèmes alternatifs sur une période de 8 ans (Pelletier *et al.*, 2018).

En 2018, 80% des exploitation des poules pondeuses était dotées de cages conventionnelles au Canada, le 20% restant avait des cages aménagées ou en volière (Pelletier *et al.*, 2018). Au Québec, les systèmes conventionnelles sont interdits depuis janvier 2015 pour les nouveaux entrepreneurs d'œufs de consommation (Philippe *et al.*, 2020). En France, les systèmes conventionnels ont été supprimés en 2012, ouvrant la place à l'installation de cages aménagées et d'autres types de systèmes. De nos jours, l'élevage en cage aménagées représente 47% de la production française, l'autre 53% se fait élevages en volière ou en plein air (CNPO, 2020).

1.3 Les systèmes en volière et le bien-être animal

Le bien-être animal s'est insurgé dans la modulation de la demande d'œufs de consommation sur le marché. Selon une enquête menée par le *Comité National pour la Promotion de l'Oeuf* - CNPO (2019) en France, 95% des répondants était en accord avec l'initiative facilitant la transition vers les systèmes alternatifs des poules pondeuses. Cette tendance a été aussi observée dans les marchés au Canada et aux États-Unis. Les demandes des consommateurs sont liées à la production d'œufs d'une qualité nutritionnel, tout en respectant des conditions du bien-être animal et de la protection environnementales (Pelletier *et al.*, 2018). De là, les systèmes alternatifs, plus spécifiquement, les systèmes en volière ont commencé à s'instaurer de plus en plus dans la filière de production d'œufs de consommation, puisqu'ils peuvent offrir un bien-être aux animaux leur permettant d'exprimer des comportements naturels.

1.4 Enjeux environnementaux à la ferme : systèmes en volière

Bien entendu, les systèmes en volières ont une dynamique associée à la libre circulation des poules pondeuses tout au cours de la période de production. Ce dynamisme entraîne des enjeux environnementaux sur les différents composants du système de production : bâtiment, stockage de fientes, et l'épandage des engrais organiques.

Dans les systèmes d'élevage de poules pondeuses, la densité des animaux, ainsi que la gestion des fumier jouent un rôle très importante au sein des émissions de gaz et de poussières vers l'air ambient (David *et al.*, 2015a; David *et al.*, 2015b). Ce type de production animal peut contribuer à l'émission de gaz à effet serre (GES) telles que le dioxyde de carbone (CO_2), la vapeur d'eau (H_2O), le méthane (CH_4) et le protoxyde d'azote (N_2O), à partir de la respiration des poules, ainsi que des processus biochimiques associés à la dégradation et la gestion du fumier (Xin *et al.*, 2011).

Le dégagement d'ammoniac est aussi un sous-produit qui peut avoir lieu, soit au bâtiment, soit au stockage, avec la dégradation des déjections des animales. L'ammoniac, sous forme gazeuse, émis à l'air ambient peut réagir avec les molécules d'azote ou de souffre disponibles dans l'atmosphère produisant des sels (Finlayson-Pitts & Pitts Jr, 1999). Ces sels peuvent parcourir de longues distances et ainsi contribuer à des enjeux environnementaux tels que l'eutrophisation des cours d'eau, l'augmentation des polluants atmosphériques, la contamination de l'eau souterraines, ainsi que la modification des espèces végétales (Cape *et al.*, 2009; Larios *et al.*, 2018; Sutton *et al.*, 2009). De plus, un taux de concentration d'ammoniac élevé à l'intérieur du bâtiment peut entraîner des conditions de santé non favorables tant pour le poules que pour les travailleurs (Oloyo, 2018).

Par ailleurs, la production de poussières est aussi une source de pollution atmosphérique engendrée par ce type de systèmes alternatifs. La libre circulation des animaux à l'intérieur des bâtiments, l'accès à une espace litière, les conditions environnementales, ainsi que la gestion du fumier, engendrent la production des particules PM_{10} (< 10 µm) et $PM_{2.5}$ (< 2.5 µm) nocives pour les animaux et les fermiers. Celles-ci sont transportées vers l'atmosphère à l'aide de la ventilation in-situ de la production (David *et al.*, 2015b).

1.5 PLF : mise en œuvre de l'importance de la modélisation

L'intérêt des organismes et des consommateurs pour améliorer le bien-être animal, ainsi que celui des travailleurs, ont entrainé de fortes modifications dans la production animal donnant lieu à de nouveaux domaines de recherches comme l'élevage de précision ou « Precision Livestock Farming » (Berckmans, 2006; Fournel *et al.*, 2017)

Divers chercheurs ont discuté la nécessité de mettre en place l'élevage de précision. Celle-ci s'intéresse aux différents processus liés à la production animal, à savoir : le contrôle de l'alimentation, le taux de croissance, la gestion de maladies, ainsi que le contrôle environnemental et le bien-être animal (Berckmans, 2006; Fournel *et al.*, 2017; Frost *et al.*, 1997). De technologie assez développée existent pour réaliser un contrôle, et une prise de décision en temps réel, dans une filière en croissance comme celui de l'agriculture et les activités d'élevage (Berckmans, 2006). Quelques critères sont nécessaires pour assurer le bon fonctionnement de ces types de systèmes, à savoir : (i) des mesures en continue des variables liées aux différent processus, (ii) des algorithmes robustes et performants, (iii) des données permettant de prédire les comportements animaux induits par des changements de leur environnement, (iv) un système de contrôle et d'actionneurs qui vont réagir à ces comportements, et (v) un retour de l'information afin de compléter un cycle auto performant (Berckmans, 2006; Frost *et al.*, 1997; Wathes *et al.*, 2008). Alors, le PLF est devenu un sujet de recherche basé sur l'utilisation de modèles numériques dans le but de contribuer à cette tendance de recherche. Toutefois, ce domaine est encore peu documenté dans la littérature quant aux systèmes de production agricoles, surtout pour les sites de production animale.

1.5.1 Complexité des biosystèmes

Les systèmes d'élevage de poules pondeuses, sont avant tout des biosystèmes ayant des interactions complexes entre ses occupants et ses différentes composantes. Le système animal-environnement-météo-production inclut différents agents qui peuvent avoir des interactions tout au long d'une période de production, à savoir : les processus métaboliques des animaux, les flux de chaleurs sensible et latente d'un poulailler avec leur environnement, les défis des changements climatiques, les processus physico-chimiques dans les fientes déposées au sol, l'activité microbienne aux différents endroits (p.ex., dans les animaux, la litière ou dans l'air ambient). S'ajoute à ces agents les développements technologiques et la sensibilisation des consommateurs au bien-être animal et les enjeux environnementaux.

1.6 Outils de modélisation

La modélisation numérique correspond à un ensemble d'outils, mathématiques, statistiques et computationnelles, qui servent à anticiper une variable ou un évènement avec une précision déterminée (Kuhn & Johnson, 2013). La modélisation a été appliquée à différents domaines bien entendu dans la filière agricole. Trois branches principales de modélisation ont été développées au fil du temps, à savoir : les modèles empiriques, les modèles mécanistes, et les modèles statistiques incluent la dernière génération de ces derniers modèles le « Machine Learning ».

1.6.1 Modèles empiriques et mécanistes

Les modèles empiriques sont générés à partir de la réalisation des expériences qui mettent en évidence le comportement d'une variable de réponse sans chercher les causes qui décrivent le phénomène, souvent ils sont de modèles linéaires ou non-linéaires. Ces modèles sont caractérisées pour avoir une meilleure performance en autant qu'il existe une quantité substantielle d'observations (Grömping, 2009; Narinc *et al.*, 2014). Le modèle empirique devient mécaniste avec l'application des concepts théoriques de base, dans ce cas les sciences appliquées, tout au cours du développement du modèle. Ainsi la modélisation mécaniste s'appuie sur la représentation mathématique des phénomènes physiques, chimiques ou biologiques qui peuvent décrire le cas en étude (Narinc *et al.*, 2014).

Divers modèles empiriques et mécanistes ont été appliqués dans les systèmes d'élevage. Fournel *et al.* (2017) ont présenté une revue des modèles bioénergétiques basés sur des bilans de masses et d'énergie pour décrire le confort thermique de l'animal. Ni (1999) et Montes *et al.* (2009) ont regroupé les modèles mécanistes développés pour simuler l'émission d'ammoniac du stockage des fientes liquides dans les sites de production animal. De même, Bjerg *et al.* (2013) ont décrit des travaux de modélisation réalisés pour prédire les émissions d'ammoniac, mais à l'intérieur des sites d'élevages ventilés naturellement.

Dans la production d'œuf de consommation, la modélisation empirique a été utilisé pour prédire aussi le comportement du taux de ponte dans un poulailler à l'aide de la régression non-linéaire, c'est le cas du modèle Gamma, du modèle McNally, du modèle McMillan, du modèle Adams-Bell, entre autres (Gorgulu & Akilli, 2018; Narinc *et al.*, 2014).

1.6.2 Modèles statistiques de dernière génération : « Machine Learning »

Les modèles statistiques sont développés en utilisant : (i) un ensemble des données obtenues du cas en étude et (ii) les corrélations entre les variables prédictives et la variable de sortie obtenues à partir de fonctions mathématiques, ainsi que statistiques (Afroz *et al.*, 2018). C'est le cas des modèles comme les réseaux des neurones (ANN), le *Support Vector Machines* (SVM) ou le *Random Forest* (RF), pour en citer quelques-uns. Le *Machine Learning* (ML) a été largement utilisé dans les systèmes de Chauffage, Ventilation et Climatisation (CVC) pour améliorer le confort thermique humain. Toutefois, ce type de modèle commence à prendre une certaine place dans la filière agricole, bien entendu dans les sites d'élevage, étant donné la complexité intrinsèque de ces biosystèmes.

Dans les sites d'élevage, l'application de ces modèles a été dirigé vers différents branches, à savoir : le confort thermique des animaux (Lopes, 2009; Queiroz *et al.*, 2005; Xie *et al.*, 2014), la consommation de la nourriture (Cross *et al.*, 2018; Demmers *et al.*, 2018), le suivi des conditions environnementales du bâtiment (Shen *et al.*, 2018; Xie *et al.*, 2017), le comportement animal (Jensen *et al.*, 2020), la gestion des fumiers (Chen *et al.*, 2008), la gestion énergétique à la ferme (Ribeiro *et al.*, 2018; Sefeedpari *et al.*, 2016), ainsi que le suivi de la production du site d'élevage, dans ce cas dans les systèmes de production de poules pondeuses (Ahmad, 2011; Gorgulu & Akilli, 2018; Ramírez-Morales *et al.*, 2017).

1.7 Intérêt de l'étude

Les systèmes d'élevage de poules pondeuses englobent différents aspects tout au cours d'une période de production, p. ex., les aspects économiques, techniques, environnementaux, liés au bien-être. De plus, la filière de production d'œufs de consommation est composée de différentes composantes, dont plusieurs impliquent directement le bâtiment, le stockage et l'épandage. Cette étude a mis l'intérêt sur deux axes principaux : la production au bâtiment, ainsi que le stockage du fumier; et plus spécifiquement sur les besoins : (i) d'étudier l'empreinte environnementale des systèmes en volière de poules pondeuses, en tenant compte du bien-être animal; ainsi que (ii) de contribuer vers les avancements de l'élevage de précision.

Certes, l'implantation des systèmes plus autonomes dans ces sites d'élevage demande l'observation et l'apprentissage du système animal-environnement-météo-production et ses interactions; ainsi que le développement d'algorithmes robustes et performantes. C'est pourquoi, la première étude de trois réalisées dans le cadre de ce mémoire s'est focalisée sur la compréhension du bien-être des poules pondeuses dans un système d'élevage en volière, à l'échelle expérimentale, à partir de l'observation des patrons des comportements de celles-ci suite à la mise en place de trois techniques de mitigation (TME) visant à améliorer les conditions environnementales. La deuxième étude a porté sur l'analyse des effets de ces TME sur le taux de ponte (TP) et la propreté des œufs, ainsi que sur la prévision des anomalies journalières du TP en utilisant les conditions environnementales et météorologiques comme variables d'entrée dans l'entrainement d'un modèle statistique. Enfin, la troisième étude s'est penchée sur la qualité de l'air, plus spécifiquement, sur l'émission d'ammoniac dans le stockage contrôlé de fientes de poules pondeuses, ainsi que la prévision des concentrations d'ammoniac en utilisant et comparant des approches de modélisation mécaniste et statistique. Les résultats de ces trois études sont rapportés dans les trois articles qui constituent ce mémoire de maîtrise.

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2 PREMIER ARTICLE

Assessing environmental control strategies in cage-free egg production systems: Effect on spatial occupancy and natural behaviors

Évaluation de techniques de contrôle environnementales dans les systèmes de production d’œufs en volière : Effet sur l’occupation de l’espace et sur les comportements naturels

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Sébastien Fournel a participé à la révision générale de l'article et a fait une retroaction pour l'analyse, la présentation et la discussion des résultats.

2.1 Abstract

Animal welfare concerns have been a challenging issue for producers and international marketing. In laying hen production, cage-free systems (CFS) have been identified as an alternative to ensure the laying hens' well-being. Nevertheless, in CFS, important environmental issues have been reported, decreasing indoor air quality. Environmental control strategies (ECS) have been designed to enhance indoor air quality in CFSs. However, little information exists about the effect of these ECSs on natural animal behaviors. Four strategies and one control were tested in an experimental CFS, previously designed to track behavioral variables using video recordings over seven time-lapses of 1 hour per day. Spatial occupancy (SO) and laying hen behaviors (LHB) were registered. One statistical analysis was applied to evaluate the effect of ECS on SO and LHB using a multinomial response model. Results show lower chances to use litter area within the reduction of litter allowance treatment (T17) ($p < 0.05$). Neither the four ECSs nor the control implemented in this experiment affected the natural behaviors of the hens. However, stress patterns and high activity were reported in the T17 treatment. This study shows that it is possible to use these ECSs without disrupting laying hens' natural behaviors.

Keywords: animal welfare, cage-free systems, laying hen behaviors, spatial occupancy, air quality, litter allowance.

2.2 Introduction

In the last fifty years, different laying hen production systems have been designed to improve animal welfare and respond to international market demands. Directives focusing on housing design and alternative methods to rearing laying hens were provided by the European Commission (1999) to promote the egg industry's minimum standards. Modifications included restricting the use of conventional cages, fostering experimental research, and encouraging political discussions on implementing enriched cages and cage-free systems (CFS) (Appleby, 2003; NFACC, 2017; Shields et al., 2017)

CFS have been considered the best housing alternative for laying hens regarding animal welfare; allowing them to display a broader range of natural behaviors (Hartcher & Jones, 2017). These systems provide litter areas, nest boxes, multi-level perches, feeders and drinkers, ramps, and novel objects, such as long sticks with color bands. Access to a litter surface allows hens to carry out natural and species-specific behaviors such as dust bathing, foraging, scratching, and pecking activities (Colson, 2006). However, these activities can lead to environmental issues such as an increase in ammonia (NH_3) emissions, airborne dust production, and undesirable indoor air quality for hens and workers (Oliveira et al., 2018; Pelletier & Godbout, 2016).

High NH_3 concentrations (> 25 ppm) in CFS can induce respiratory disease to bird subjects to aerial viruses (Ritz et al., 2004), and decrease in feed intake (Beker et al., 2004; Oloyo, 2018), as well as irritation of the trachea, ocular damage by corneal lessons (Olanrewaju et al., 2007), and increase in hen mortality (David et al., 2015a). Furthermore, hen behavior and spatial occupancy can be affected by NH_3 concentrations where animals can reduce preening and foraging activities while in all likelihood preferring areas with low NH_3 concentrations (Kristensen et al., 2000).

Airborne dust or particulate matter (PM) from litter disturbance can be transported as bio-aerosols carrying biological and organic substances (viruses, bacteria, fungal spores, gaseous pollutants, manure, and food residues), affecting the health of workers, hens, and people living close to confined laying hens housing systems (Costa & Guarino, 2009; Farokhi et al., 2018; Yang et al., 2017). Human and animal respiratory and heart illnesses have been linked to particle exposures less than 10 µm (< PM_{10}). These particles can be inhaled, reaching the lower respiratory tract of humans (important residence time in the human body, might get into the bloodstream through the alveoli walls and create chronic diseases) (Löndahl et al., 2007; WHO, 1999). Likewise, health concerns due to high dust levels in CFSs have been reported (Michel & Huonnic, 2003). Hypotheses regarding the discomfort linked to the presence of airborne dust induced by laying-hen behavior have been made; however, there exists an evident lack of research activities to compare with other birds' housing systems (David et al., 2015b).

Factors favoring NH_3 and PM emissions include litter properties; environmental conditions such as air humidity, temperature, and ventilation rate, free animal movements; as well as manure management systems (Godbout et al., 2011; Lin et al., 2017; Oliveira et al., 2018). To improve indoor air quality reducing dust and NH_3, directly or indirectly, several environmental control strategies (ECS) have been suggested, namely: (i) incorporation of inert materials in aviary diet, *e.g.* biochar, zeolite or bentonite, to improve N retention in poultry manure (Prasai et al., 2018; Romero et al., 2012); (ii) litter amendment by adding natural zeolites, chemical compounds, *i.e.*, aluminum sulfate or ferric compounds (Qasim et al., 2017; Schneider et al., 2016; Zhang et al., 2016) or bedding materials such as white wood shavings or silage maize. (van Harn et al., 2012); (iii) sprinkling of neutral electrolyzed water (Chai et al., 2018); (iv) negative and positive air-bulk ionization; (v) electrostatic precipitation; and (vi) oil spraying systems (Aarnink et al., 2011; Griffin & Vardaman, 1970; Winkel et al., 2016). These ECSs have shown the potential to affect several aspects linked with dust and NH_3 volatilization, namely: N retention in manure storage, poultry manure amendment, and particulate matter abatement. Nevertheless, to our knowledge, animal behavior assessment, more specifically on laying hen behaviors, has been the subject of few studies, and the focus has been on animal physiognomy alterations. Thus, this study aims to evaluate the effect of four strategies on spatial occupancy and laying hen behavior. The outcomes of this study are expected to contribute to the implementation of animal-friendly ECSs without negatively impacting their natural behavior and thereby the welfare in laying hen CFS.

2.3 Materials and methods

The study was conducted in an experimental laboratory on animal production of the Research and Development Institute for the Agri-Environment (IRDA), located on the site of the *Centre de recherche en sciences animales de Deschambault* (CRSAD, Deschambault Animal Sciences Research Center), Quebec, Canada. The *Comité de protection des animaux* of the research center (CPA-CRSAD) approved the use and treatment of hens in this study (authorization number 19AVCPA01).

2.3.1 Experimental bench-scale rooms

The study took place in twelve independent bench-scale rooms (122 cm length, 119 cm wide). Each room was equipped with a variable-speed exhaust fan. The incoming air, drawn from outside, was preconditioned with an air conditioning unit and a heated electrical resistance unit to maintain an optimal temperature between 22°C and 23°C inside the rooms throughout the experimental period. The pre-conditioned air was distributed to all the rooms by using a series of ducts.

The rooms were laid out to meet the CFS housing requirements stipulated in the Code of practice for the care and handling of pullets and laying hens of the National Farm Animal Care Council of Canada (NFACC, 2017). Hens had free access to a wire floor space (122 cm long, 81 cm wide and 30 cm high) and a litter space at floor level (122 cm length and 38 cm wide) (Figure 1). At the beginning of each experiment, the litter space was covered with a 5 cm thick wood shaving bedding. The wire floor was made with a square mesh wire cloth, and it was equipped with two nest boxes (30 cm x 33 cm) delimited by black plastic curtains and lined with a washable plastic mesh. The animal density was one hen per 1115 cm^2. Two nipple drinkers and a linear feeder were installed in the wire floor space. The bird droppings collected beneath the wire floor were dried with forced air and removed once a week. A perforated 7.5 cm diameter duct blew air from a 10 cm blower (VTX-400, Atmosphere, Terrebonne, Quebec, Canada) fixed beneath the air inlet.

Several holes (diameter: 5 mm, 160 mm apart), were positioned at a 45° angle. Then, based on airflow of 0.7 m3 hr^{-1} hen^{-1}, the blower was adjusted to obtain an air velocity of 2 m s^{-1}. Two PVC-pipes (round profile) were installed over the wire floor area of each room (5 cm and 45 cm high), resulting in a linear space of 20 cm per hen.

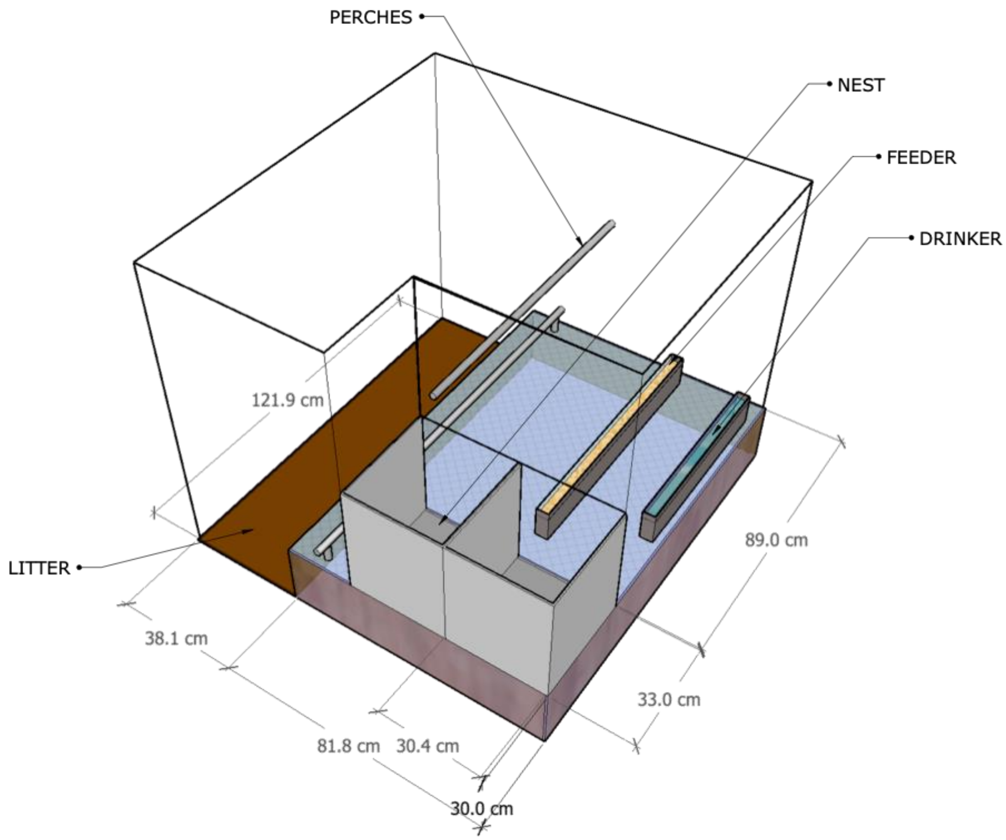

Figure 1. 3D layout of an experimental room

2.3.2 Animal and housing

Lohmann LSL-Lite laying-hens arrived at 19 weeks of age and were housed in the experimental rooms from February to June 2019 (n = 144, 12 hens per room). Trimming of the beak (at one-day-old) and vaccination requirements were done before their arrival at the experimental facility. Hens were individually weighted, placing the animals in a plastic recipient (8 L) with non-adjustable cover (to reduce stress) put on a digital platform scale previously tared. Then, the 144 hens were randomly selected to distribute them into the twelve experimental rooms. Four hens were randomly selected from each room and identified by painting a blue spot on their back. Further information about blue spot identification would be mentioned in section 2.3.5.

An adaptation period of two weeks was provided at the beginning of the experiment. The two-week period allowed (i) the hens recognize their environment and (ii) the caretakers to carry out flash hen behavior visual observations, ensuring well-being conditions and animal adaptability.

Hens were fed with a commercial diet (Laying hen 18% VG, Agri-Marché, St-Isidore, Quebec, Canada). Feeders were filled once in the morning. Then, they were verified three times per day (by the caretakers) and refilled by the end of the day. Water was provided by a solenoid-activated valve connected to a data logger. Access to feed and water was ad libitum, and the amounts consumed were daily quantified for each room. The lighting intensity was 10 to 15 lux with equal lighting periods for all the rooms (Light Meter, Lux/FC, 840020C, Sper Scientific Ltd., Scottsdale, AZ, USA). An immediate on/off light cycle was set for each room.

2.3.3 Environmental control strategies (ECSs)

Treatments included two combined ECSs previously selected by a panel of expert based on available literature. One treatment was proposed by the *Fédération de Producteurs d'Oeufs du Québec* (FPOQ, Quebec's Federation of Egg Producers) to evaluate the possibility to decrease litter surface. Then, optimal parameters of the combined ECSs were selected following an experimental pre-test procedure focusing on economic and technical aspects (Gonzalez-Mora *et al.*, 2020a).

The four ECSs studied included: decrease of litter surface area (T17); use of heated floor (HFOS), and litter amendment with biochar material (AOS) both combined with established oil sprinkling periods; as well as one single oil sprinkling treatment (OS). The experiment was divided into two consecutive experimental batches of eight weeks each (batch 1 and 2). A one-day time interval was used between batch 1 and 2 where the rooms were completely cleaned, ensuring any interference from the applied treatments in batch 1. The same laying-hens from batch 1 were housed for batch 2.

Table 1 provides a summary of the four treatments. One control (Ctrl) was settled during the experimentation with three repetitions (n = 3) for both batches 1 and 2, respectively. The control was a traditional aviary system with 33% of available litter surface. All treatments were applied to the litter space placed at floor level. It should be noted that AOS treatment was assessed in batch 1, while the OS treatment was evaluated only in batch 2. This experimental framework was selected to (i) evaluate the combination of applying acid adsorbent and sprinkling vegetable-oil, and (ii) the sole effect of sprinkled vegetable-oil, considering the number of experimental rooms and the replicate per treatment. The litter surface in all treatments was 33% of the total available floor area following the NFACC recommendations, except for treatment T17 where a reduced litter surface area was evaluated (17%). The wire floor area, nest boxes, perches, drinkers, and feeders followed the same specifications and dimensions for all the treatments as described in section 2.3.1.

Table 1. ECSs applied at the experimental CFS

Batch	Abb.	Treatment	Description*
1 - 2	T17	Decrease of litter surface area	17% of litter area, reduction of litter surface from 33% to 17%. n = 3 (rooms 1, 5 and 11).
1 – 2	HFOS	Heated floor + oil sprinkling	33% of litter area, installation of a heated floor fixed to 27ºC. Spraying an oily emulsion over litter (1.17 L/m^2/week). n = 3 (rooms 2, 6 and 10).
1	AOS	Litter adsorbent + oil sprinkling	33% of litter area, addition of 10%-litter of acid adsorbent (Active biochar). Spraying an oily emulsion over litter (1.17 L/m^2/week). n = 3 (rooms 3, 7 and 9).
2	OS	Oil sprinkling	33% of litter area. Spraying an oily emulsion over litter (1.17 L/m^2/week). n = 2 (rooms 7 and 9).

***Room design was based in a traditional aviary system, NFACC (2017).**

***Abb. = abbreviations**

The HFOS treatment included the sprinkling of an acid emulsion made with vegetal oil and an organic acid solution. The application was carried out twice a week at a dosage of 585 ml m^{-2} of litter area. Sprinkling was carried out only on the litter surface. In this treatment, the underlying floor temperature was kept at 27°C using an electrically heated floor system (EHF). The AOS treatment included the addition of an activated spruce-fir based biochar (Airex Energy Company, BiocharFX, Laval, Quebec, Canada), equivalent to 10% of the litter mass. The biochar particle size was ~1 mm, which was activated by acid protonation, after drying at 100°C. The activation was made by immersion in a concentrated solution of sulfuric and nitric acid (Fan et al., 2018). The OS treatment included the spraying of an acid emulsion twice a week, similar to the HFOS treatment, at a dosage of 585 ml m^{-2}. In this case, the litter area was kept at room temperature. The mass of litter placed in each room was 900 g for T17 and 1800 g for the other treatments.

2.3.4 Spatial occupancy and laying hen ethology analysis

Spatial occupancy and laying hen behavior were monitored for both 8-week experiments (batches 1 and 2). A video recording system (POE Security Camera System 4CH) was set up inside the experimental rooms to record hens throughout the day. Four cameras were used for this study (POE Cameras) connected to a Network Video Recorder (NRV), saving 4TB HDD (Hard disk drivers) of data; that is, recording simultaneously four experimental rooms.

To observe spatial occupancy (SO), each bench-scale room was divided into six main areas: Nest (N), Litter (L), Perches (P), Feeder (F), Drinker (D) and Nest-feeder (NF). The N-region was obtained by subtracting the number of observed hens in the other areas from the total number of hens since it was not possible to count the number of birds inside the nest at each observation. The NF-region was established as the free space located between the feeders and the nest according to the room design (Figure 1).

Ten laying hen behaviors (LHB) were observed according to Blokhuis (1984), Kristensen *et al.* (2000), and Weeks and Nicol (2006). Observed behaviors are described in Table 2. One additional behavior was added in the second batch to document any non-reported behavior (nBr) at the time of observation.

Table 2. Laying hen behaviors observed inside all experimental CFS

Behaviors	**Description**
Scratching (S)	Bird scratching itself or scratching the litter with its feet. (Bracke & Hopster, 2006)
Kneeling (K)	Includes events when the hen lies down over the litter, wire floor, or even on perches in a relaxing position.
Ruffling Feathers (RF)	The hen ruffles the feather without shaking its body. This activity could be observed in a standing or sitting position.
Body Shaking (BS)	Similar to RF but with an instant shaking movement.
Preening (PR)	The beak is in contact with the feathers. (Blokhuis, 1984)
Dustbathing (DB)	Sitting position and arbitrary movements where the body, the feathers, the legs, and the beak could be in contact with the litter. (Shields & Duncan, 2009)
Perching (P)	A hen is stand up or sitting on a perch more than 2s.
Feeding (F)	Head in the feeder trough ingesting food. (Webster & Hurnik, 1990)
Drinking (D)	Beak within the plane of the drinker. (Webster & Hurnik, 1990)
Supplementary behaviors	
Other (O)	Other behaviors such as fluttering (wing flapping), sleeping (head through the feathers above the wing base), pecking or foraging, and stretching feathers.
Non-reported behavior (nBr)	A hen does not show any of the other behaviors. Stationary or motionless at the time of observation.

2.3.5 Video recordings and observation analysis

Two and three random days were selected for the video recording of each treatment for batch 1 and 2, respectively. One day of observations included seven 1-hour recordings covering most of the day, that is: 6h–7h/ 8h–9h/ 11h–12h/ 14h–15h/ 17h–18h/ 20h–21h/ 21h–22h. Each observation was made over a 1-min video at the half-way time of each 1-hour recording by two trained observers. A total of 154 and 252 videos were analyzed for batches 1 and 2, respectively. The quantity of videos was not equal for both batches due to a lag time between the beginning of the batch 1 and the video recording system installation. Both SO and LHB were analyzed using the video recordings (Figure 2). For SO assessment, all the flock was considered for the score, while for LHB only four (4) laying hens, identified with the blue spot on the back, were observed.

Figure 2. Video recording at an experimental room

The SO were assessed using the relative frequency of an event as proposed by Kozak *et al.* (2016) where the number of hens per section over one day was divided by the total number of hens observed on the same day and multiplying by 100 to get a percentage (Equation 1).

$$\boldsymbol{SO}|_j\,(\%) = \frac{\boldsymbol{NoH_i}}{\boldsymbol{TNH}}\,\boldsymbol{x\,100} \qquad \textbf{Equation 1}$$

Where NoH_i is the number of hens observed in the i-th section over one day, TNH is the total number of hens observed on the same day and $SO\big|_j$ is the relative frequency of spatial occupancy in the j-th day in the i-th section. Furthermore, LHB analyses were carry out by summarizing the daily number of hens per treatment expressing a specific natural behavior. Then, the counts were summarized and divided by the number of rooms undergoing the same treatment.

2.3.6 Statistical Analysis

The response variable of the SO analysis for each treatment was assumed to have a multinomial distribution. Therefore, a multinomial response model was applied using the PROC GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA, 2016) and the general logic link function. Treatment effect was tested with the F-test ($p < 0.05$). The response category having the highest relative frequency, "Perches", was used as the baseline in the model. The odds that an animal under treatment A occupied space i rather than reference space, *i.e.*, perches, is the probability ratio Odds (Treat A) $Odds\ (Treat\ A) = \Pi_{i,Treat\ A}/\Pi_{p,Treat\ A}$, where $\Pi_{i,Treat\ A}$ and $\Pi_{p,Treat\ A}$ are the relative frequencies of SO in the i-space and perches area under treatment A. Treatment comparisons over spatial occupancy were performed using estimations of odds ratios (Equation 2),

$$\boldsymbol{OR}(\boldsymbol{A},\boldsymbol{B}) = \frac{\boldsymbol{Odds}(\boldsymbol{Treat\ A})}{\boldsymbol{Odds}(\boldsymbol{Treat\ B})} \qquad \textbf{Equation 2}$$

For the LHB, observed counts of each behavior were analyzed using a generalized linear model. The procedure PROC GLIMMIX of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA, 2016) with logic link function was used to accommodate the assumption that counts have a Poisson distribution. The F-test for fixed effect was applied to assess the significance of the treatment effect on mean counts ($p < 0.05$). Mean counts per treatment for each behavior were estimated and its standard deviations (±) are presented, respectively

2.4 Results

2.4.1 Spatial occupancy (SO)

The relative frequencies (%) of SO for the first and second batches are shown in Figure 3. In the case of the first batch, values from the HFOS and AOS treatments have similar frequencies for all the six regions when compared to the control, except for nest area where larger values of 9.6% and 8.4% were calculated. As mentioned before, it is noteworthy that frequency values in nest area were calculated by approximative subtraction since there was not any camera available inside the nest boxes. Low use of litter was observed in the T17 treatment, with a relative frequency of 8.9 ± 0.8%, compared to 26-29% observed in the other treatments and the control. The odds of animal presence in the litter area were 3.45 times higher in control than that of the T17 treatment ($p = 0.0001$). It was observed that occupancy in feeders and nest-feeders area for the T17 treatment showed higher relative frequencies, with percentages of 27.8 ± 9.0 and 20.8 ± 2.0%, compare to 16-22% and 9-11% observed in the other treatments and the control, respectively. In fact, hens preferred the feeder area 1.69 times more in T17 when compared to the HFOS treatment ($p = 0.04$). Furthermore, the odds of occupancy in nest-feeders were 1.74 and 2.24 times higher in T17 treatment than that in the HFOS treatment ($p = 0.05$) and control ($p = 0.0078$).

For the second batch, similar relative frequencies were observed for the perches, feeders, and drinkers area for the three treatments and the control with average frequencies of 24.8 ± 2.2%, 18.8 ± 1.0%, and 5.8 ± 1.0%, respectively (Figure 3b). Moreover, a low-frequency value was found in litter area for the T17 treatment, with a percentage of 11.6 ± 2.6%, compared to 25-31% observed in the other treatments and the control. The odds of animal frequency in litter area were 2.06 and 2.61 times higher for the HFOS ($p = 0.0051$) and OS ($p = 0.0002$) treatments than that of the T17 treatment. There was not any observed significant treatment effect in SO regarding random odds ratios between the HFOS and OS treatments, and the control.

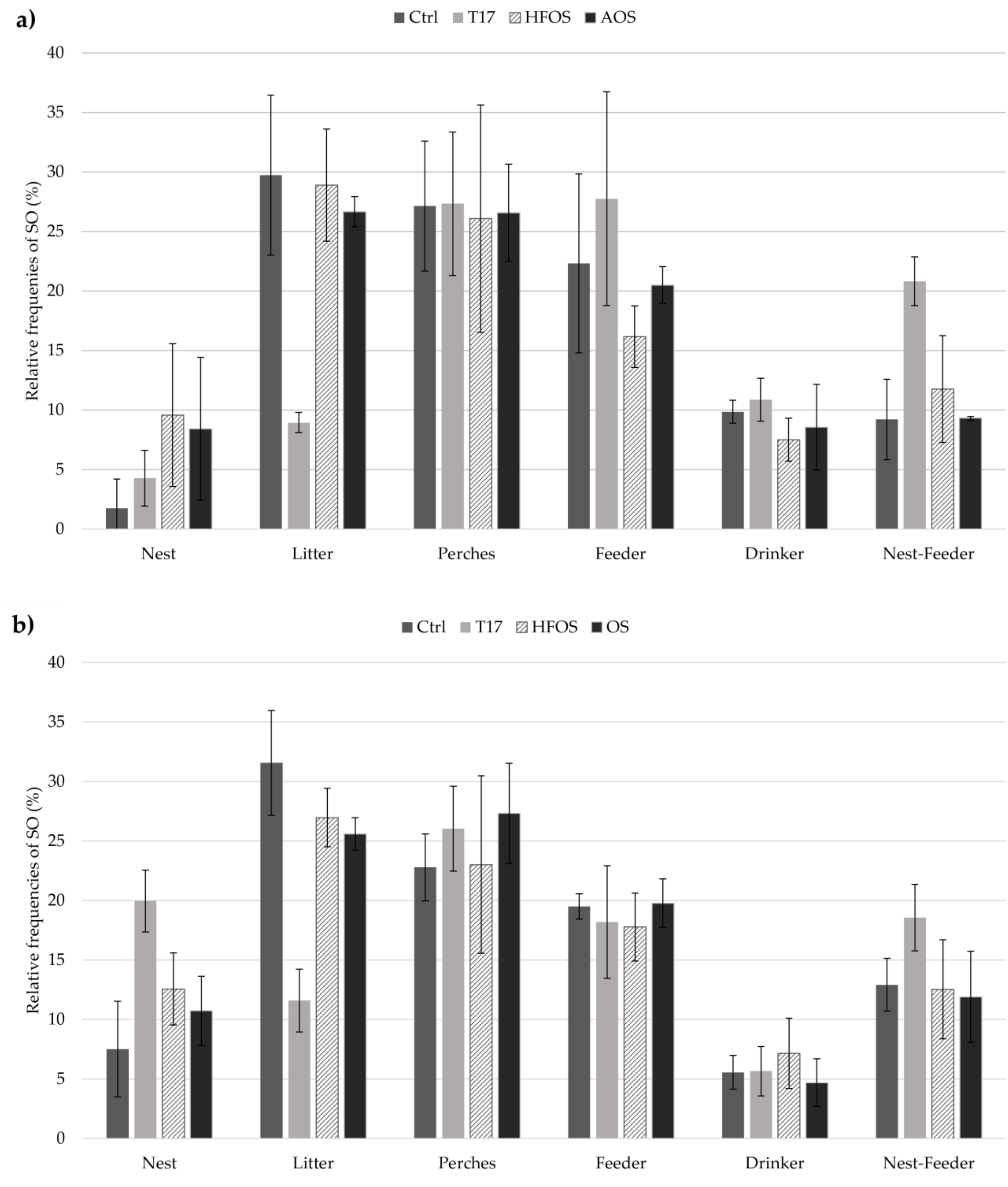

Figure 3. Relative frequencies of SO with standard deviations from experimental CFS. a) batch 1. B) batch 2.

2.4.3 Laying hen behavior (LHB)

The average counts of laying hens performing a natural or species-specific behavior per day for batches 1 and 2 are shown in Figure 4 and data is presented in Appendix I. Preening, perching, and feeding seem to be the most frequently observed behaviors reported in the experiment. Average values with standard deviations of 15 ± 2, 13 ± 2, and 13 ± 1 hens were observed doing these main activities for the three treatments and the control in batch 1. However, these behaviors had higher values in batch 2 (preening = 21 ± 1 hens, perching = 18 ± 3 hens, and feeding = 20 ± 1 hens). The difference between batches could be due to the number of observed days per room for the LHB analysis (Two random days for batch 1 vs. three random days for batch 2).

Species-specific behaviors such as dust bathing (DB) were reported for all three treatments and the control in both batches 1 and 2. These values were similar over the three treatments with average values and standard deviation of 3 ± 1 and 2 ± 1 hens observed for batches 1 and 2. Other animal behaviors, *i.e.*, scratching, kneeling, ruffling feathers, body shaking, and drinking, did not show a particular trend. Furthermore, no significant effect of treatment was detected on any other behaviors following statistical analysis (Table 3).

Table 3. F test for treatment effect on behavior counts in laying hens

Behavior	Batch 1		Batch 2	
	F-value	Pr < F	F-value	Pr < F
Scratching	0.36	0.78	1.75	0.23
Kneeling	0.06	0.98	1.33	0.33
Ruffling Feathers	1.65	0.26	0.63	0.62
Body Shaking	0.94	0.47	0.20	0.89
Preening*	0.35	0.79	0.28	0.84
Dustbathing*	0.77	0.55	1.38	0.32
Perching*	0.97	0.46	1.35	0.33
Feeding*	0.30	0.82	0.17	0.92
Drinking*	0.65	0.61	0.23	0.87

***Behavior selected for depth analysis.**

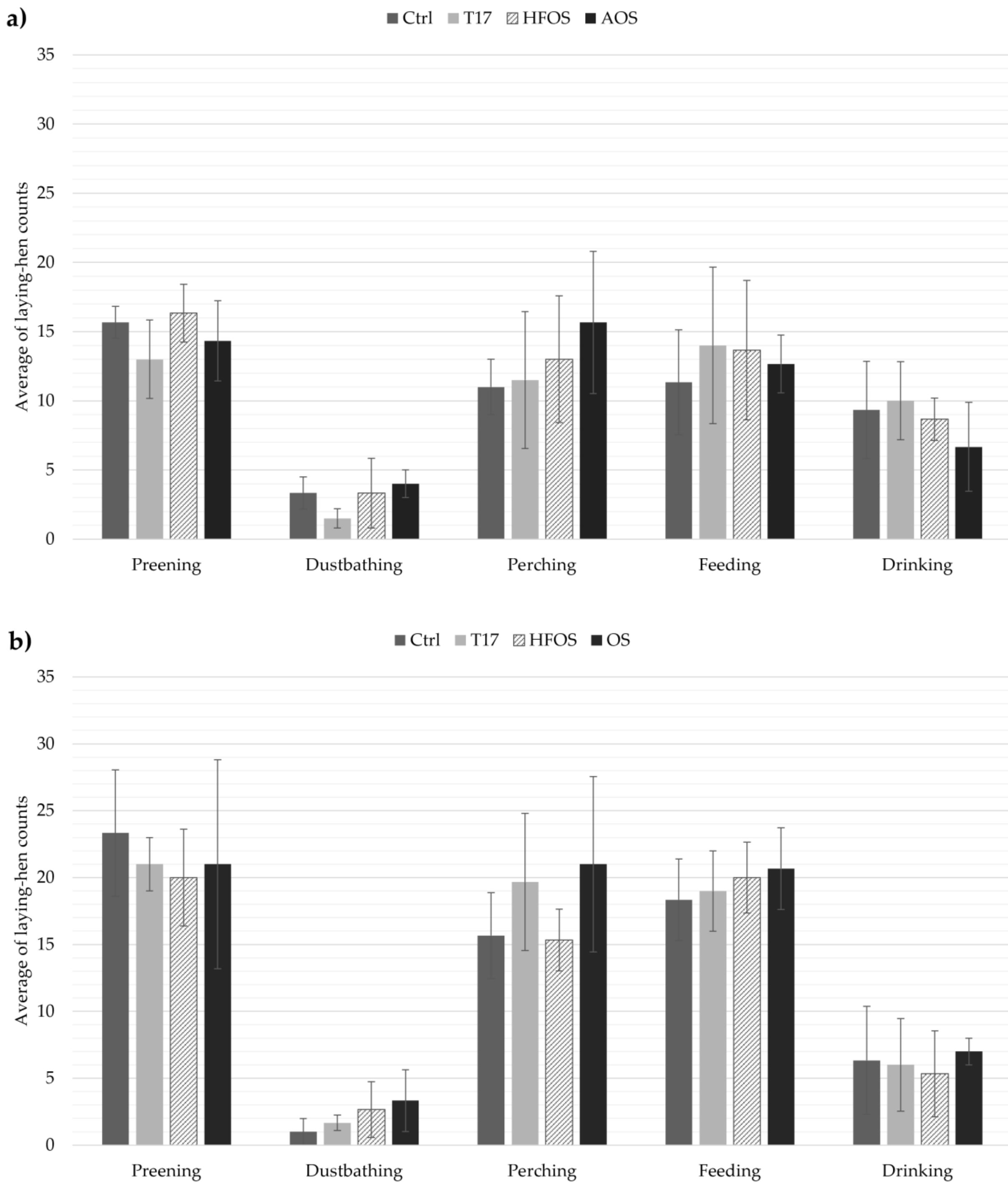

Figure 4. Average counts of laying hens displaying the five natural and species-specific behaviors with standard deviations. a) Batch1. b) Batch 2.

2.5 Discussion

2.5.1 Space occupancy (SO) preference

Our results demonstrate that litter, perch, and feeder areas are the main locations where hens spend most of their time (Figure 3a and 3b). Litter and perch areas seem to be suitable to express the main natural behaviors that are preening, perching, and foraging (Shields & Duncan, 2009). Hens spent most of their time performing these behaviors.

The use of litter area was the lowest for the T17 treatment when compared to all other treatments and the control. There were 2 to 3 times more odds that hens would prefer litter in the other treatments. This means that reducing the litter surface area could lead to an unbalanced distribution of the flock within CFS. Furthermore, abnormal repetitive behaviors were identified while analyzing the video recordings for the T17 treatment, stress patterns as defined by Garner (2005) were recorded. Thus, hens could be conditioned by the litter space available, promoting competition, and stress behavior inside the flock.

Displacement of animal behaviors could be observed if there were a motivational conflict. Taylor (2010) defined this as normal behaviors taking place in an inappropriate situation, *e.g.*, excess of feeding or preening. High activity of the flock and higher odds of feeder and nest-feeder area preference, observed in the T17 treatment, seems to be the result of motivational conflicts in the flock. However, it is recommended to assess more evidence to identify displacement behaviors for a litter reduction treatment (T17). It should be mentioned that anomalies within spatial distribution between nest and perch areas were observed as the light went out. In some cases, hens preferred rest in nest boxes than perched during the night. These distributions were attributed due to the shutdown lighting program which was configurated with immediate shutdowns.

2.5.2 Patterns in animal behaviors

Nesting activity in private places seems to be one of the preferred activities for laying hens, several studies have shown the preference for hens towards private spaces to lay eggs as a behavioral need (Hartcher & Jones, 2017; Shields & Duncan, 2009; Weeks & Nicol, 2006). Use of the nesting area was observed in this experiment with relative frequencies between 5% to 20%. In average, the use of nest area was similar compared to those relative proportions reported by Kristensen et al. (2000). However, relative frequency of the nesting activity should be treated with prudence because these frequencies could be influenced by two aspects: (i) the short time lag used for observations, and (ii) the bird counts methodology in nesting area using an approximation by subtracting the number of observed hens in other areas from the total number of hens.

Preening, perching, and feeding were the most frequent behaviors observed in this experiment (Figure 4). These results are in agreement with Kristensen et al. (2000) who reported that about 14% of the time budget of laying hens is dedicated to preening. Also, Cordiner and Savory (2001) showed an elevated proportion of time to perform perching activity during the day. Furthermore, perching behaviors are also related to preening or resting activities (Blokhuis, 1984), which explains also high-frequency values for these behaviors.

In this study, natural and species-specific behaviors were observed for all treatments. There was not any significant effect linked to the four ECSs in any of the nine laying hen behaviors considered (Table 3). This was also reported by Engel et al. (2018) who did not find any effect of litter allowance and nest-box availability on laying hen behaviors such as preening, scratching, etc., within modified cages. Other authors suggested a minimal effect on feeding, drinking, and resting behavior when space allowance is modified in furnished cages (Albentosa et al., 2007). These results mean that to use ECSs for improving indoor air quality could be possible without affecting animal welfare, more specifically, with their natural animal behaviors.

2.6 Conclusion

Nowadays, animal behavior remains an important factor when assessing the quality of laying hen housing systems in light of animal welfare demands from markets and consumer's needs. According to the literature, the application of ECSs within these systems have demonstrated the potential to improve air quality conditions for animals and workers in terms of airborne dust and gas concentrations. However, studies of the effect on laying hen behaviors are deemed necessary to better understand animal well-being.

This study provided an animal welfare analysis based on spatial occupancy and natural animal behaviors after the application of different ECSs. This paper indicates that the natural behavior of laying hens in a cage-free experimental system using different ECSs was not affected. However, the reduction of litter surface produced significant differences in the spatial distribution of the flock. Abnormal behaviors, as well as high activity in the flock have also indicated a possibly stressful environment for hens where litter surface was limited. Moreover, it is noteworthy that reduced litter allowance could be the limiting factor to trigger some behaviors performed in the litter area. Though there was not any significant effect observed over the laying hen behavior analysis, reduction of litter area is not recommended as animals could undergo stress patterns and unbalance spatial distribution. Besides, video recordings covering private places, i.e., nest boxes, could be useful to improve the analysis of spatial occupancy and animal behaviors. Also, gradual on/off lighting programs must be applied to reduce anomalies within spatial distribution in the flock while resting during the night. Additional (i.e., validation) research work should be conducted at an on-farm production scale with an online and integrated monitoring system to keep up with Precision Livestock Farming trends (Fournel et al., 2017).

2.7 Acknowledgment

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3 DEUXIÈME ARTICLE

Assessing environmental control strategies in cage-free egg production systems: Egg production analysis and statistical modelling applications using Random Forest algorithms

Évaluation de techniques de contrôle environnementales dans les systèmes de production d'œufs en volière : analyse de la production d'œufs et la modélisation statistique en utilisant l'algorithme Random Forest

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Andres F. Gonzalez Mora a contribué à l'organisation, l'observation et le traitement des données. M. Gonzalez a réalisé les activités de modélisation et d'analyse des résultats. Il a rédigé au complet l'article ci-nommé.

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3.1 Abstract

Since 1990, worldwide egg production has had an average increase of about 2.8% per year, reaching 76.8 million metric tons in 2018. This increase has drawn the attention of animal welfare advocates. In Canada, new challenges have emerged in light of animal welfare, production rates and farm's capacity. Also, monitoring egg production has been one primary activity to understand interactions and variability in laying hen housing systems. Therefore, assessing available environmental control strategies (ECS) and developing early warning systems based on on-line monitoring systems in egg production have become an important field of research. This study uses two statistical approaches to identify the key independent variables to explain the effect of three ECSs applied to an experimental laying hen cage-free system (CFS), namely: Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA). Then, a machine learning method known as Random Forest (RF) was developed to predict daily fluctuations of the hen-day egg production index (HDEP) using observed indoor environmental and hygrothermal conditions as input variables. A variable importance analysis further confirmed the governing variables of the egg production time series. An inquiry-driven scenario analysis was performed to identify potential changes in egg production when varying the input of the RF model. Among the predictors, the temperature was the first governing variable, followed by hen's age and relative humidity since gas concentrations were under recommended thresholds. The scenario analysis revealed that an increase in temperature of 5% can negatively affect egg yield. Although the ECSs did not disrupt the egg production in the experimental aviary systems, they had an effect on egg cleanliness, induced mainly by the available litter space. The proposed ECSs have the potential to enhance animal welfare in CFSs.

Keywords: egg yield, aviary systems, statistical modeling, air quality, gas emissions, laying hen, machine learning, early warning system, scenario analysis.

3.2 Introduction

Since 1990,worldwide egg production has had an average increase of about 2.8% per year; reaching 76.8 million metric tons in 2018 (FAOSTAT, 2019). In Canada, the demand for egg products is also growing, as well as the egg production rate. In 2019, food demand for eggs increased about 1.25% from 2018, and the annual increment in egg production was about 4.7% per year since 2015. Thus, new challenges have emerged in light of production rates and farm's capacity (Agriculture and Agri-Food Canada, 2019a; FAOSTAT, 2019). To date, the capacity of

Canadian egg farms ranges from 100 000 up to 400 000 laying hens; each producing on average 340 eggs per year with the Province of Quebec having a 20.1% of country's quota allocation (Agriculture and Agri-Food Canada, 2019b).

This impressive growth of the egg industry has drawn the attention of animal welfare advocates. Thus, egg production systems have adapted over the last twenty years; starting with the EU Council Directive 99/74 whereby conventional laying hen systems have shifted from batteries of cages to alternative systems such as furnished cages or cage-free systems (CFS) (Shields *et al.*, 2017). In Canada, these alternative systems were introduced with the 2017 Code of Practice for laying hens. The Code set forth best in-farm practices based on the Animal Care Program (ACP), along with a transition strategy to move from conventional systems to CFSs over a projected period of 8 years (NFACC, 2017; Pelletier *et al.*, 2018). In Quebec, conventional cages have been prohibited since 2015 for all new egg producers (Philippe *et al.*, 2020).

Egg production and international market demand combined with technological developments, environmental changes, consumer awareness, have produced complex interactions. Moreover, due to the increase in food production, sustainable laying hen housing systems must meet the demand while minimizing the environmental footprint (Pelletier *et al.*, 2018). For these reasons, deployment of several strategies to reduce the environmental impact, as well as an understanding of the complex interactions to ensure high egg production performances, have become highly relevant research subjects.

In laying hen housing systems, manure management and animal density play key roles in gas and dust emissions (David *et al.*, 2015a; David *et al.*, 2015b). Ammonia represents one of these harmful gases produced by the degradation of uric acid from stored feces. High levels of ammonia (> 25 ppm) inside laying hen houses can reduce feed intake and animal growth. Furthermore, high ammonia concentrations over large periods of time can lead to the development of respiratory illnesses and increase susceptibility to viral diseases, affecting egg quality and egg production (Oloyo, 2018; Xin *et al.*, 2011; Zhao *et al.*, 2015). Laying hen houses can also contribute to greenhouse gases (GHG) such as CO_2, H_2O, CH_4, and N_2O. These GHGs are the outcomes of the bird's respiratory cycles and the biochemical processes associated with manure management (Xin *et al.*, 2011). Production of particle matter (PM), such as PM_{10} (< 10 µm) and $PM_{2.5}$ (< 2.5 µm) results from the interaction between animal activity levels, access to litter, and environmental conditions. PMs can be harmful, because these can be inhaled and cause serious health problems for animals and caretakers (David *et al.*, 2015b; Xin *et al.*, 2011).

Comparison between conventional and alternative laying hen housing systems have shown that conventional cages lead to low levels of environmental impact and high egg production performance when compared to CFSs. Nevertheless, animal well-being has not been quite well assessed for these systems (Pelletier *et al.*, 2018). Thus, sustainable strategies to improve air quality in CFSs have become focal points in light of increased animal welfare and workers' health (Abín *et al.*, 2018). Therefore, to curb aggravating indoor conditions, several environmental control strategies (ECSs) have been proposed such as: (i) amendments of chemical and natural compounds to the litter (Qasim *et al.*, 2017; Schneider *et al.*, 2016; Zhang *et al.*, 2016), (ii) different bedding materials (van Harn *et al.*, 2012), (iii) incorporation of inert material in hen feed (Prasai *et al.*, 2018), (iv) application of sprinkling neutral electrolyzed water to the litter (Chai *et al.*, 2018), (v) air-bulk ionization and (vi) use of vegetable oil spraying systems on the litter (Aarnink *et al.*, 2011; Winkel *et al.*, 2016)

On the other hand, monitoring egg production has been one primary activity to understand interactions and variability in laying hen housing systems (Godbout *et al.*, 2020). Egg yield is usually defined as the ratio of the total number of laid eggs and the total number of producing laying hens housed over different time intervals (days, weeks, years) (Misslin, 2017). Indeed, egg production time series could serve as a decision-making tool to egg producers, since it could supply information about flock performance, illness patterns, or egg quality and on-farm economic profits. Tracking egg production provides a good indicator of nutrient intake balances, improvement of on-farm practices plans, and the effect of environmental conditions (Gorgulu & Akilli, 2018; Misslin, 2017). Moreover, it has been observed that variability in egg production curves could be triggered by several factors, namely: animal genetic, feed intake, water consumption, lighting program, and the ambient temperature (Ahmad, 2011). The shifting towards new alternative systems has led to new egg cleanliness challenges which also affect production efficiency and economic gains. In Canada, an egg not intended for human consumption is defined as an egg with a broken or unbroken shell with adhering dirt; that is blood, yolk, or manure spots (Egg Farmers of Canada, 2018). A high percentage of dirty eggs have been reported in CFSs when compared to conventional and furnished cages (Holt *et al.*, 2011). These aspects have encouraged the use of modeling techniques to predict egg yield given the increasing bird populations within CFSs and need to increase net farm income, along with economic projections and interaction analyses with environmental conditions, animal health, and farming activities (Ramírez-Morales *et al.*, 2017).

Several non-linear regression models have been proposed to identify time-dependent fluctuations of egg production, namely: the Gamma model, the McNally model, the McMillan model, the Adams-Bell model, the Compartmental model, to name a few. (Gorgulu & Akilli, 2018; Narinc *et al.*, 2014). These non-linear mathematical expressions have specific constants related to initial production, rate of increase, and decrease of egg production, the initial day of egg-laying, or biological aspects. Also, data-driven models have emerged in egg production modeling, such as Artificial Neural Networks (Ahmad, 2011; Ramírez-Morales *et al.*, 2017) or Least-square Support Vector Machines (Gorgulu & Akilli, 2018), using feed intake, mortality, counts of live animals, as well as historical registered egg production, as input variables. However, there is no evidence that environmental data, such as, gas concentrations, have been used as input of data-driven models to predict egg production. The available literature on modeling egg yield using environmental conditions is still scarce and additional research is recommended to establish their interaction with egg yield (Gorgulu & Akilli, 2018).

The objective of this paper is to present the results of a project focusing on the effect of selected ECSs on egg production in a laying hen aviary system. In passing, our study contributed to a larger project that also investigated the effect of these ECSs on air quality and animal welfare. The ECSs were selected by a panel of experts following an extensive review of available literature and pre-testing performed in a laboratory of the Research and Development Institute for the Agri-Environment (IRDA), Québec (Canada). Regarding air quality, the results which are introduced in a companion paper by Gonzalez-Mora *et al.* (2020a) showed there was not any significant difference in NH_3 concentrations with respect to the selected ECSs, but there were major differences in airborne dust emissions. Likewise, Gonzalez-Mora *et al.* (2020b) observed that selected ECSs did not have any effect on natural hen behaviors and spatial occupancy inside the experimental CFS, except for the reducing litter space strategy. Thus, three specific sub-objectives were proposed for this study, namely: (i) evaluation of the effect of the selected ECSs on egg production and egg cleanliness in an experimental aviary system; (ii) prediction of daily fluctuations in egg production with a statistical model, known as Random Forest, using observed indoor environmental and hygrothermal conditions as input variables; and (iii) assessment of the effect of varying the explanatory variables on the egg production curve.

3.3 Materials and methods

3.3.1 Animal housing

Twelve experimental cage-free livestock rooms (CFR) were used to shelter Lohmann LSL-Lite laying hens (12 hens per CFR) from February to June 2019. The experiment was divided into two consecutive experimental batches of eight weeks each (batch 1 and 2). CFRs were located at the livestock building agri-environmental assessment laboratory of the *Research and Development Institute for the Agri-Environment* (IRDA), in Quebec (Canada). Animals arrived at 19 weeks of age following vaccination requirements. Then, hens were individually weighted and randomly placed in the CFRs.

The bench-scale CFRs (122 cm length, 119 cm wide) were equipped with a variable-speed exhaust fan. CFRs consisted of ground and an upper floor conception. Ground-level was a litter space initially conditioned with 5 cm thick wood shaving bedding. The upper floor was a square mesh wire cloth furnished with two nest boxes, one linear feeder, two aviary nipple drinkers, and two perch PVC-round pipes. CFR design and livestock units (*e.g.*, linear feeder) met the requirements of the Code of practice for the care and handling of laying hens of the National farm animal care council of Canada (NFACC, 2017). A detailed description of the CFR design and conception is available in Gonzalez-Mora *et al.* (2020b).

The incoming air was pre-conditioned with a conditioning unit and a heated electrical resistance. This way the incoming air was could meet the target temperature inside CFRs throughout the experimental housing period (from 22°C to 23°C).

3.3.2 Indoor environmental and hygrothermal monitoring

Hygrothermal conditions (*i.e.*, temperature, relative humidity (RH)); gas emissions (*i.e.*, carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4), and ammonia (NH_3)); atmospheric pressure and ventilation rates were monitored before application of any ECSs (2 weeks before) and throughout both batches 1 and 2. Temperature and RH were measured with a T&RH probe (Model CS500, Campbell Scientific, Inc., Canada corp.), gas emissions were measured using an infrared gas analyzer (FTIR, model DX4040, Gasmet) and ventilation rates were calculated by the pressure difference (ΔP) downstream from a 204-mm iris orifice damper (Model 200, Continental Fan Manufacturing Inc., Buffalo, NY, USA; accuracy ± 5%) installed in the exhaust duct of each CFR. A data logger recorded hygrothermal data, gas emissions, and ΔP. The ensuing data were uploaded to a computer every 15 minutes.

3.3.3 Egg handling

Laid eggs were collected, counted, and classified (clean/dirty) daily for each CFR. Total laid eggs, clean and dirty ones, were manually registered in an experimental book and daily reports digitized and stored in a digital database.

3.3.3.1 Hen-day egg production index

Equation 3 introduces the mathematical expression used to calculate hen-day egg production index (HDEP) (Misslin, 2017). This equation was used to generate egg production evolution (*i.e.*, time series). HDEP helps farmers to observe critical lower thresholds of egg production induced by several factors such as diseases, age, weather, or environmental conditions. (Misslin, 2017):

$$\boldsymbol{HDEP}\ (\%) = \frac{\boldsymbol{TLEc}}{\boldsymbol{THc}} \boldsymbol{x\ 100} \qquad \textbf{Equation 3}$$

Where *TLEc* is the total laid eggs counts, and *THc* is the total number of laying hens.

3.3.3.2 Daily egg cleanliness index

An egg cleanliness index (EGC) was defined as the daily ratio of clean eggs (CEc) and the total number of eggs (TEc) counted (Equation 4). Clean eggs were collected from the nest egg collector following the quality requirements of Egg Farmers of Canada (2018). Otherwise, eggs were classified as dirty. It should be noted that eggs could be laid either in the litter space or wire floor area, however, these eggs were classified as dirty.

$$\boldsymbol{EGC} = \frac{\boldsymbol{CEc}}{\boldsymbol{TEc}} \qquad \textbf{Equation 4}$$

3.3.4 Environmental control strategies (ECS)

A treatment proposed by the *Fédération des Producteurs d'Oeufs du Québec* (FPOQ, Quebec's Federation of Egg Producers) (T1), two combined ECSs (T2 and T3), and a control (Ctrl) were applied and equally distributed over the 12 CFRs to evaluate indoor air quality conditions, animal welfare, and egg production (see Table 4). The control was a traditional aviary system with 33%

of available litter surface following the NFACC recommendations (n = 3). A review of different ECSs by a panel of experts led to the selection and implementation of combined ECSs. Then, pre-testing was carried out in the agri-environmental assessment laboratory at IRDA to establish the best conditions among the selected strategies seeking reduction of ammonia concentration from hen litter box-samples (Godbout *et al.*, 2020). T1 was proposed by the FPOQ to evaluate the possibility to decrease the litter surface. More information is available in Gonzalez-Mora *et al.* (2020a) and Gonzalez-Mora *et al.* (2020b).

Table 4. List of treatments applied within the CFRs.

Abb.	ECSs*	CFRs* (Room number)	Description**
T1	Reduced litter surface area	1 - 5 - 11	17% of litter area, reduction of litter surface from 33% to 17% (n = 3).
T2	Heated floor + oil sprinkling	2 - 6 - 10	33% of litter area, installation of a heated floor set to 27ºC. Spraying an oily emulsion over litter (1.17 L/m^2/week) (n = 3)
T3	Litter absorbent + oil sprinkling	3 - 7 - 9	33% of litter area, addition of 10%-litter of acid adsorbent (Active biochar). Spraying an oily emulsion over litter (1.17 L/m^2/week) (n = 3)

*CFRs: experimental cage-free rooms, and ECSs: Environmental control strategies.

**CFRs design was based on a traditional aviary system (NFACC, 2017)

3.3.4.1 Feature and data classification analysis

Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) were applied to observe interactions of selected ECSs and treatments with the HDEP and EGC observations. PCA is a non-supervised method which uses Euclidian distances to assess feature classification. On the other hand, LDA is a technique largely used for data classification where data is reduced from a high dimensional to a low dimensional space in light of obtained projections which can be maximized between-class distances (Balakrishnama & Ganapathiraju, 1998; Parent, 2020; Ye *et al.*, 2005). The R programming language (R Core Team, 2020) was used to perform the analysis using the *ade4* package (Dray & Dufour, 2007) and following the methodology proposed by Parent (2020).

3.3.5 Egg yield modeling

The application of non-linear models was limited due to the availability of initial parameters for the models. In this study, hens were housed for 15 weeks, instead of an entire egg production period. This experimental framework does not allow to have parameters such as: the initial production, the initial day of egg-laying or the rate of increase. Thus, the use of a data-driven model known as Random Forest, along with moving averages, was proposed to assess egg yield modeling.

3.3.5.1 Moving averages

Moving averages were used to estimate the egg production trend using several window sizes. Sliding windows allowed to observe egg yield trends over time by assuming a locally linear regression from a specific window size (span). These simple mathematical transformations could also reduce noise and follow a similar approach from local weighted regression functions (LOESS). Five intervals were proposed, namely, 3, 5, 7, 10, and 14 days. We assumed that the trend had a linear behavior within the interval considered. Then, Equation 5 describes the mathematical expression used to determine the moving average as proposed by Brandt (2014).

$$\boldsymbol{u_i = \frac{1}{k+1}\sum_{j=i-k}^{i} y_j} \qquad \textbf{Equation 5}$$

Where k is the window size, y_j is the observed value and u_i is the unweighted mean of the measurements for the times $t_{i-k}, t_{i-k+1}, t_{i-1}, t_i$

3.3.5.2 Random Forest

Random Forest model (RF) was selected to predict daily fluctuations in egg yield using indoor environmental and thermal data obtained from the traditional experimental aviary system (*i.e.*, Ctrl). Then, RF was used to establish the correlation between either environmental variables or hygrothermal variables and egg production. RF is a machine learning algorithm proposed by Breiman (2001). The decision trees and the bagging re-sampling techniques are the theoretical basis of this data-driven model. Decisions trees can be constructed by the classification and regression trees (CART) (Breiman *et al.*, 1984). CART algorithm draws trees expanding first a very large quantity of nodes followed by a pruning step; that is removal of branches which reflect non-improvement in the prediction performance, using a pruning criterion. Nevertheless, RF based on CART use unpruned trees and they grow until reaching a terminal node (Grömping, 2009).

Regression trees are well-defined if all the data have only numerical values, thus a numerical output will be obtained once the model is trained. On the other hand, bagging (or "bootstrap aggregating") is a re-sampling technique that creates several training subsets (or "bags") by randomly selecting the data using a with-replacement sampling; that is, a value could be taken more than once inside a bag. This method aims to increase the diversity in the input data used for the training process. Moreover, decisions trees are relatively unstable models in light of reproduced diversity in the response for small perturbations of data. In this case, no learning is performed at each training subset generated. Once the bag is created, the training process starts. Several decisions trees are generated for each training subset. For each decision tree, about 1/3 of the training subset is left out which has no participation in the model development (known as the Out-of-Bag: OOB). Thus, model-predictive responses are averaged to provide an ensemble prediction of the target variable. An estimate error rate (Equation 6) associated with this final prediction is calculated using the OOB on the training set to estimate the accuracy of the RF model (Breiman, 2001)

$$\boldsymbol{OOB-MSE} = \frac{\mathbf{1}}{\boldsymbol{nN}} \sum_{j=1}^{N} \sum_{i=1}^{n} \left(\boldsymbol{y}_{i,j} - \hat{y}_{i,j}\right)^2 \qquad \textbf{Equation 6}$$

Where $y_{i,j}$ is the observation i at the j regression tree, $\hat{y}_{i,j}$ is the prediction from observation i at the same regression tree, n is the number of observations in the OOB and N is the total number of trees.

RF is a user-friendly algorithm because only two parameters must be optimized, namely, the number of predictors to split at each node (*mtry*) and the number of trees to develop in the forest (*ntree*) (Liaw & Wiener, 2002).

3.3.6 Data analysis

Time series of hygrothermal and gas emissions recordings were analyzed using measures of central tendency and dispersion. A Kruskal-Wallis test with a pairwise Wilcoxon test (KW/PWt) were applied to observe data comparisons. Also, Shapiro-Wilk test (SWt) was used to test normal distribution behavior within the data. A p-value of less than 0.05 was established to accept null hypothesis (H_o) within tests. This analysis allowed to: (i) perform statistical comparisons between the treatments and the control, and (ii) establish the mean or median daily values as the principal predictors for modeling.

3.3.7 Input data

RF model was built in the R programming language version 4.0.0 (R Core Team, 2020) using the *caret* package (Kuhn, 2020). The *randomForest* package (Liaw & Wiener, 2002) was also used to perform the model framework. The following variables were selected as input variables or predictors, namely: CO_2, CH_4, N_2O and NH_3 concentrations, indoor temperature (Temp), relative humidity (RH), atmospheric pressure (Pressure), outlet airflow (AirFlow) and hen's age expressed in days (DoA). A variable with a random number (Random) from 0 to 1 was also added to the input dataset.

3.3.7.1 Data splitting

Data needed to be divided into training and testing datasets to build and validate the RF modeling. Therefore, the original dataset was randomly split into training (70%) and testing (30%) subsets. In all cases, the training dataset was re-sampled into several training subsets by 10-fold cross-validation with 5-repetitions to increase the diversity within predictions and to get better performance in model estimation as proposed by Polikar (2006) and Kuhn and Johnson (2013).

3.3.7.2 Model parametrization

RF model parametrization was achieved by using the train function from the *caret* package (Kuhn, 2020). The number of predictors at each split (mtry) and number of trees (ntree) were varied from 1 to 10 predictors, and from 1 000 to 3 500 trees with increments of 1 predictor and 500 trees. Optimal values of *mtry* and *ntree* parameters were selected using the lowest RMSE. Ten predictors were used for modelling; that is, four indoor environmental variables, two hygrothermal variables, ventilation rate, atmospheric pressure, hen age, and one random variable. An iterative algorithm was used to generate different RF models of different window sizes. One RF model, with the lowest RMSE, was selected for each window. Then, the RMSE evolution was observed to select the best RF model.

3.3.8 Variable importance

A variable importance analysis was used to rank the predictors by evaluating changes in the prediction after training was completed. This analysis provided information about the driving forces involved in the process and highlighted the key variables useful for monitoring activities in livestock farming. Variable importance was assessed for each predictor as follows: (i) for the OOB approach, the values were randomly permuted (OOBp), (ii) a decision tree was grown using the new data and new predictions calculated, (iii) a mean square error (MSE) was estimated from new predictions. Thus, the importance of a predictor was defined by an increase in the difference of the estimated MSE from OOBp and the MSE using the original OOB, expressed in percentage (%IncMSE) (Breiman, 2001; Liaw & Wiener, 2002).

3.3.9 Model evaluation

Root mean square error (RMSE) and the coefficient of determination (R^2) were calculated to evaluate model performance (Equation 7 and Equation 8):

$$\boldsymbol{RMSE} = \sqrt{\frac{\sum_{i=1}^{N}(\boldsymbol{y_i} - \boldsymbol{\hat{y}_i})^2}{\boldsymbol{N}}} \qquad \textbf{Equation 7}$$

$$\boldsymbol{R^2} = \mathbf{1} - \frac{\sum_{i=1}^{N}(\boldsymbol{y_i} - \boldsymbol{\hat{y}_i})^2}{\sum_{i=1}^{N}(\boldsymbol{y_i} - \boldsymbol{\bar{y}})^2} \qquad \textbf{Equation 8}$$

Where, y_i and $\hat{y}_i$ are observed and predicted values, $\bar{y}$ is the mean value of observed data and N is the quantity of data.

3.3.10 Scenario analysis

An inquiry-driven scenario analysis was performed to identify potential changes in egg production, as well as model response when varying the input of the selected RF model. The following procedure was adapted from Alcamo (2008):

1. Select the RF model with the best performance characteristics to predict time-dependent egg yield fluctuations and use it in a predictive mode.
2. Identify a "driving force" or the variable with more importance observed after applying the variable importance analysis.

3. Apply an increase of 5% to the driving force and add new values to the original dataset

4. Run the selected predictive model and analyze results.

3.4 Results and discussion

3.4.1 Hygrothermal, atmospheric pressure and ventilation conditions

The hygrothermal conditions, atmospheric pressure and ventilation of each treatment and one control for batches 1 and 2 are presented in Figure 5 (see Appendix II for distribution at the weekly scale). A total of about 2050 measures were analyzed for each treatment, except for treatment T3 where the number of data analyzed was of about 1300 measures. Statistical differences were observed in temperatures from T1 and T3 comparing to the control in the batch 1, where average values were 22.06 ± 0.03°C, 22.05 ± 0.02°C and 21.68 ± 0.02°C, respectively (±, 95% C.I., KW/PWt: $p < 0.05$). Also, significant differences were observed for T1, T2, and T3 with respect to the control in batch 2, where average values were 21.99 ± 0.04°C, 21.51 ± 0.03°C, 22.02 ± 0.03°C, and 21.63 ± 0.03°C, respectively (±, 95% C.I., KW/PWt: $p < 0.05$). Thus, temperatures in T1 and T3 were slightly higher than those measured in the control, while treatment T2 reported similar temperature values when compared to the control most of the time. There were any statistical differences in relative humidity (RH) between treatments and control in batches 1 and 2. However, RH for batch 2 was higher than that reported for batch 1 for all treatments. The average values of RH for T1, T2, T3 and control for batch 1 were 20.6 ± 1.8%, 19.0 ± 1.7%, 19.8 ± 1.6%, and 20.0 ± 1.8% (±, 95% C.I.), respectively; while the average of RH from batch 2 were 34.6± 1.8%, 34.0 ± 1.8%, 34.1 ± 1.8%, and 35.1 ± 1.9% (±, 95% C.I.), respectively.

Atmospheric pressure was similar for all treatments throughout the entire experiment with a marginal reduction in the average values for batch 2 in contrast with batch 1. Moreover, higher airflow rates were observed from T1, T2 and T3 from batch 1 in contrast to the control (KW/PWt: $p < 0.05$), with average values of 2.120± 0.007 l/min, 2.106± 0.006 l/min, 2.126± 0.007 l/min and, 2.065± 0.009 l/min (±, 95% C.I.), respectively. Airflow rates were similar between T1, T2 and control in batch 2 with average values of 2.012± 0.019 l/min, 1.998 ± 0.020 l/min, and 2.020± 0.020 l/min (±, 95% C.I.), respectively. However, lower values of airflow rates were observed for T3 compare to control (KW/PWt: $p < 0.05$) where the average was around 1.946 ± 0.02 l/min (±, 95% C.I.). These could be attributed to the airflow network design, and measurement process. Confidence intervals were established using the Central Limit Theorem method as recommended by Pek *et al.* (2017); since temperature, RH, atmospheric pressure, and airflow rate did not draw from a normal distribution (SWt: $p < 0.05$), and the number of data was higher than 40 samples ($n > 40$).

Ventilation rates were lower for batch 2 compare to batch 1 producing an increase in the RH as observed in the Figure 5. Furthermore, higher temperatures in T1 and T3 could be associated with several aspects, namely: (i) high activity observed in hens because of the litter reduction treatment (Gonzalez-Mora *et al.*, 2020b), probably, increasing natural animal heat production in T1, (ii) litter accumulation under the metallic wire floor because of less presence of hens in litter space increasing some gas emissions and thereby temperature (see section 3.4.2), and (iii) increase in litter thickness from agglomerations, generated by the interaction between the biochar and the excreta in the case of treatment T3, producing also an increase in gas emission and temperature. Nevertheless, temperature and RH conditions were considered appropriate for hen housing over all treatments and the control according to Oloyo (2018).

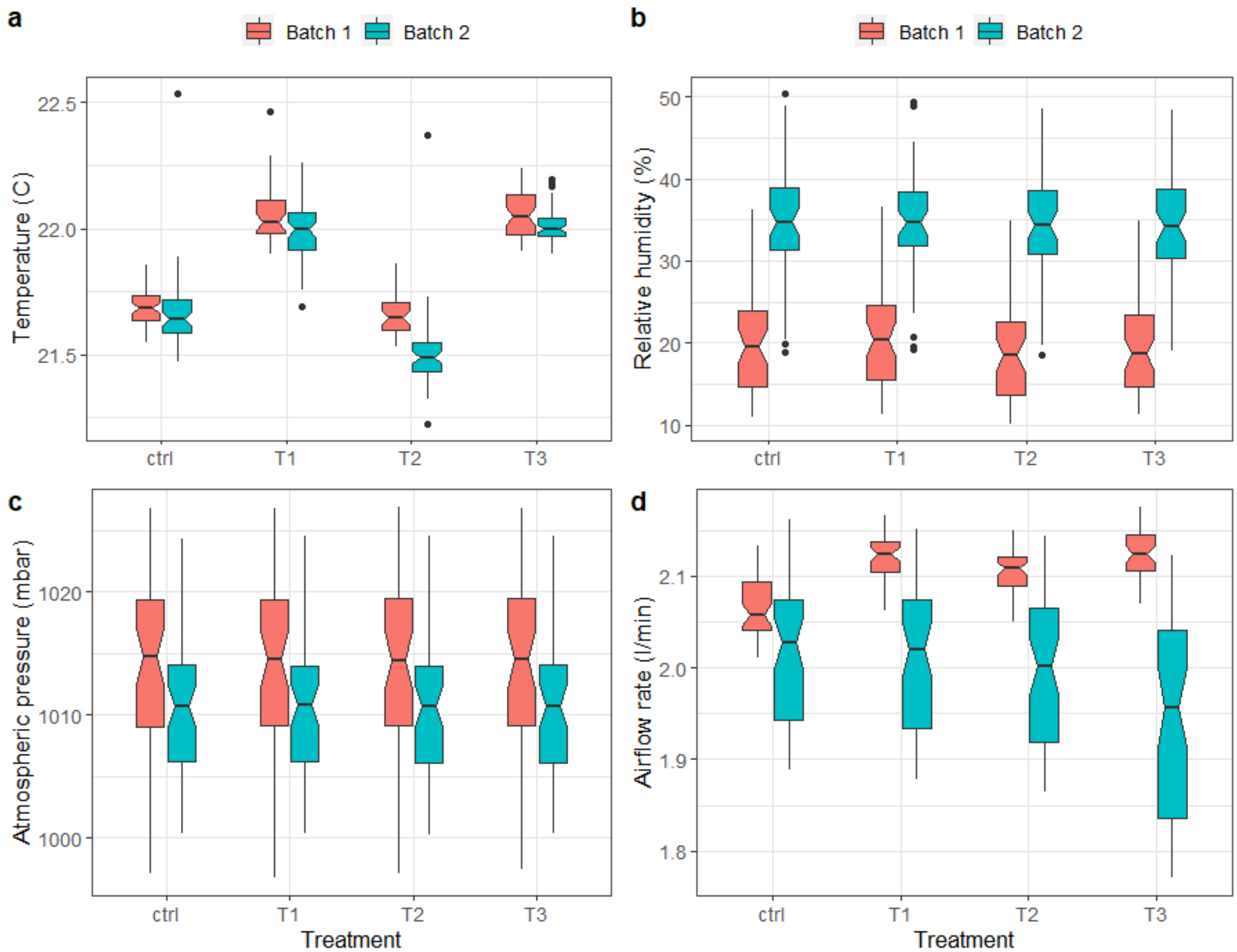

Figure 5. Boxplot of hygrothermal, atmospheric pressure and ventilation conditions of three ECSs and the control for both batches 1 and 2: (a) temperature, (b) relative humidity, (c) atmospheric pressure, and (d) outlet airflow rate.

3.4.2 Indoor environmental conditions

Indoor environmental conditions of treatments T1, T2, T3 and the control for batches 1 and 2 are summarized in Figure 6. Detailed weekly data distributions by treatment and by gas concentration are presented in Appendix II and only the overall analysis is mentioned in this section. Significant differences were found in CO_2 emissions in T2, with an average value of 26.39 ± 0.72 kg/yr/hen, compared to the control in batch 1, where the average of CO_2 emissions was 28.59 ± 0.93 kg/yr/hen (±, 95% C.I., KW/PWt: $p < 0.05$). The average values of CO_2 emissions in batches 1 and 2 ranged from 26.29 ± 0.74 to 28.59 ± 0.93 kg/yr/hen and from 24.77 ± 0.14 to 26.28 ± 0.16 kg/yr/hen (±, 95% C.I.), respectively. On the other hand, there were any statistical differences observed in CH_4 emissions between treatments and the control. The average values of CH_4 emissions in batches 1 and 2 ranged from 31.2 ± 5.88 to 35.1 ± 6.21 g/yr/hen and from

30.3 ± 3.57 to 35.4 ± 4.49 g/yr/hen (±, 95% C.I.), respectively, showing similarities in methane emissions between batches. N_2O emissions from T1, T2 and T3 were significantly higher than emissions for the control in batch 1 (KW/PWt: $p < 0.05$) with average values of 1.75 ± 0.10 g/yr/hen, 1.78 ± 0.09 g/yr/hen, 1.73 ± 0.20 g/yr/hen and 1.69 ± 0.10 g/yr/hen (±, 95% C.I.), respectively. However, no statistical differences of N_2O emissions were observed between treatments and control in batch 2 where average values ranged from 1.95 ± 0.19 to 2.13 ± 0.23 g/yr/hen (±, 95% C.I.). Also, no significant differences in ammonia emissions were observed between treatments T1, T2 and T3 and the control for batches 1 and 2. Average values of NH_3 emissions ranged from 7.99 ± 0.95 to 10.46 ± 1.66 g/yr/hen and from 21.03 ± 2.89 to 34.56 ± 5.91 g/yr/hen (±, 95% C.I.), highlighting an increase in emissions for batch 2 in contrast to batch 1.

CO_2 emissions in this experiment are in line with those cited by Shepherd *et al.* (2015) and Hayes *et al.* (2013) for aviary housing systems (cage-free systems), being 27.01 kg/yr/hen and 28.36 kg/yr/hen. Also, Fournel *et al.* (2012) observed similar CO_2 emissions in an experimental conventional system set up with a manure belt system and forced air drying. The authors reported emissions of about 28.7 kg/yr/hen. Average of CH_4 emissions were marginally higher than those reported by Shepherd *et al.* (2015), Hayes *et al.* (2013) and Fournel *et al.* (2012), where emissions were about 25.5 g/yr/hen, 26.0 g/yr/hen and 27.7 g/yr/hen, respectively. However, these emissions were similar to those cited by Alberdi *et al.* (2016) for enriched cages, and slightly lower than emissions reported by Zhu *et al.* (2011) for naturally ventilated cage layer housing, being 32.8 g/yr/hen and 40.8 g/yr/hen, respectively.

On the other hand, N_2O emissions were lower than those cited by Zhu *et al.* (2011) in cage layer houses, being 3.43 g/yr/hen. However, emissions rates in N_2O for this experiment should be treated with caution, since measured N_2O concentrations (ANNEXE II) were close to the atmospheric concentration threshold (0.331 ppm, WMO (2020)), limiting the detection of this gas inside the gas analyzer. This behavior was also observed by Hayes *et al.* (2013) in aviary systems, where N_2O concentrations were excluded because of the low concentrations observed.

For ammonia, the average of NH_3 emissions were lower than those reported by Shepherd *et al.* (2015) and Hayes *et al.* (2013), being 40.8 g/yr/hen and 55.0 g/yr/hen, respectively. It is noteworthy that increase in NH_3 emissions for batch 2 could be influenced by the decrease in the airflow rate observed within the same batch. Nevertheless, ammonia emissions were lower than the mean value for aviary houses reported by Hayes *et al.* (2013), being 54.7 g/yr/hen. Lower values of ammonia emissions should be treated also with prudence, because they were influenced by the number of housed animals, the ventilation rate applied, as well as the RH and temperature conditions used at laboratory scale. Hygrothermal conditions, gas emissions, as well as airborne dust emissions are discussed in more details in the companion paper of Gonzalez-Mora *et al.* (2020a).

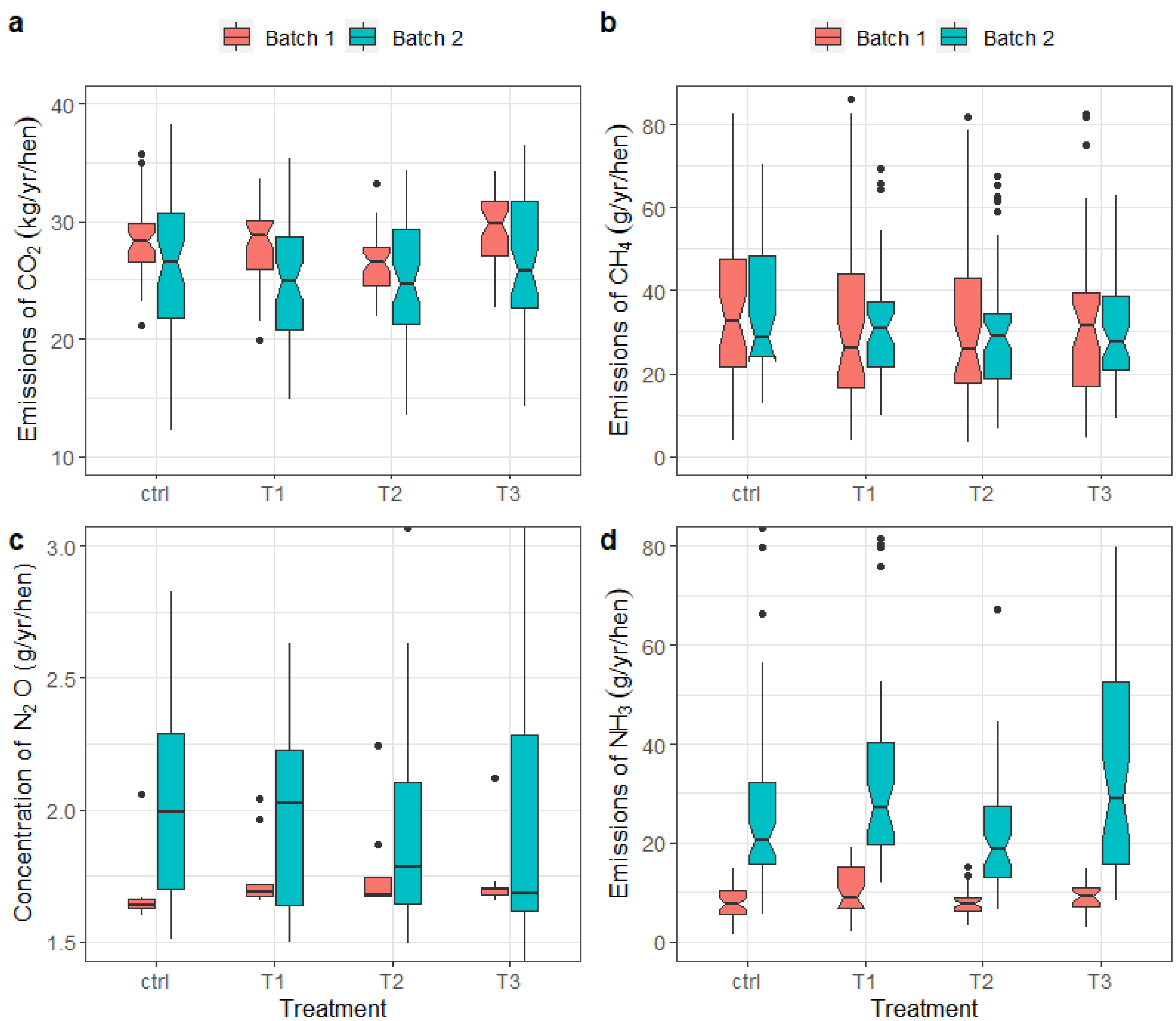

Figure 6. Boxplot of gas emissions inside the three ECSs and the control for both batches 1 and 2: (a) CO_2, (b) CH_4, (c) N_2O, and (d) NH_3.

3.4.3 HDEP and EGC time series

Egg yield and egg cleanliness time series of each treatment and the control are presented in Figure 7. Daily fluctuations can be seen over the entire selected housing period. HDEP and EGC trends seem to have a marginally constant behavior from 158 to 261 days of age (See section 3.4.3.1). The overall averages ± SD (standard deviation) of HDEP and EGC were 97.6 ± 4.2%

and 87.0 ± 6.9%, respectively. The min., max., mean and SD of HDEP and EGC, as well as the p-values of the Shapiro-Wilk test, calculated for each treatment, are summarized in Table 5. Results show that T1 had a slightly superior HDEP mean (98%) compared to the other treatments. However, the lowest min. HDEP value was found for treatments T1 and T3 (83.3%), respectively. Also, it is noteworthy T3 had the lowest HDEP mean and highest SD. This was also noticed for the EGC observations. Though this treatment had the highest max. HDEP value when compared to all treatments, and the same max. EGC values T1 and T2. Furthermore, T2 was the treatment with the highest mean EGC percentage (89.9%), followed by the control and T1. Daily fluctuations of HDEP and EGC were not drawn from a normal distribution (SWt: $p < 0.05$), except for treatment T1. Similar HDEP values were observed throughout treatments. However, significant differences were found in EGC from T2 and T3 compared to control (KW/PWt: $p < 0.05$). Higher fluctuations for T3 could be associated with the increase in litter thickness produced by the application of the active adsorbent material (biochar). Thus, laying hens were prompted to lay eggs in the litter space. These laid eggs are more susceptible to break or get dirty and they have a direct impact on the index values. Also, T3 was not replicated beyond the 29 weeks of age because of an interest in testing the last treatment with only oil sprinkling routines (Gonzalez-Mora *et al.*, 2020a). Also, the differences detected in EGC from T2 and T3 set forth the relative negative impact observed in aviary systems in terms of egg quality and cleanliness index (Philippe *et al.*, 2020).

Table 5. Averages of HDEP and EGC by treatment.

Treat.	HDEP (%)					EGC (%)				
	Min.	**Max.**	**Mean**	**SD**	**p-value***	**Min.**	**Max.**	**Mean**	**SD**	**p-value***
CTRL	86.1	108.0	97.7	3.4	< 0.05	65.7	97.3	87.9	6.3	< 0.05
T1	83.3	114.0	98.0	3.6	< 0.05	72.2	100	87.3	5.3	0.3
T2	86.1	108.0	97.4	4.4	< 0.05	72.2	100	89.9	5.5	< 0.05
T3	83.3	117.0	97.1	5.2	< 0.05	61.5	100	82.9	8.4	< 0.05

*p-value obtained from Shapiro-Wilk test

** Min., Max., Mean. and SD are the minimum, maximum and mean values of the HDEP and the EGC throughout the whole experiment for each treatment (T1, T2, T3) and control.

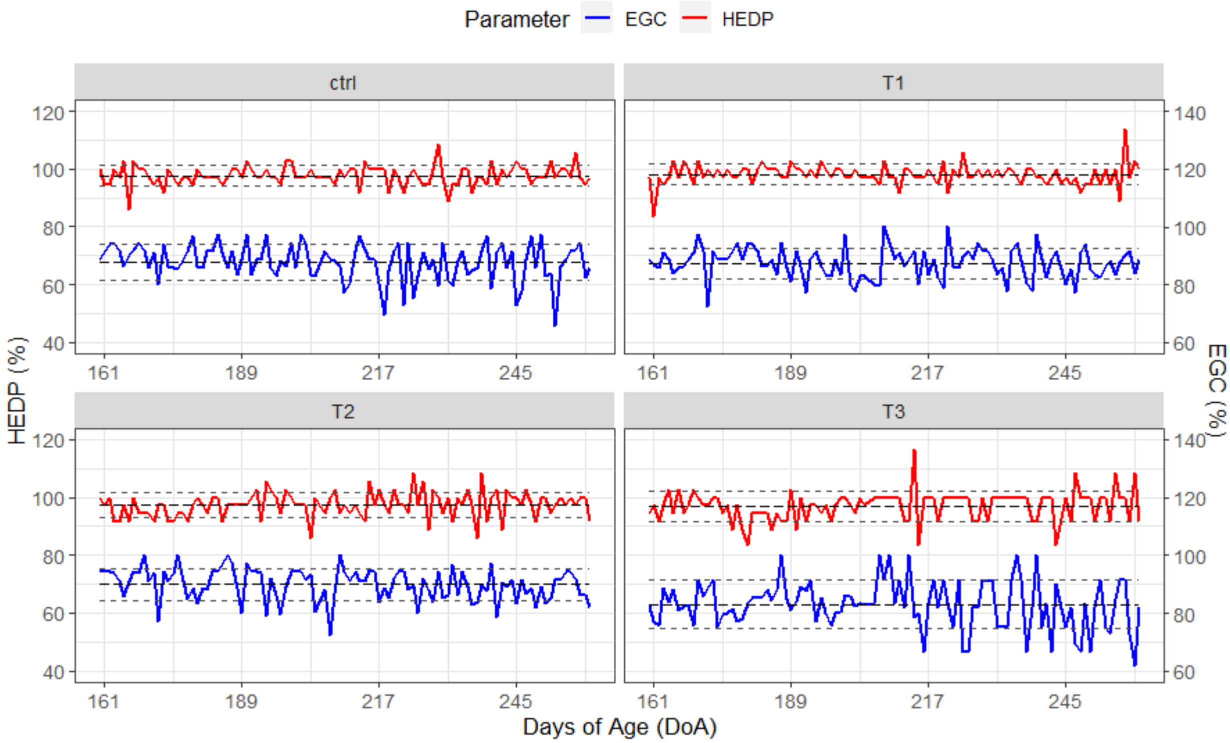

Figure 7. Hen-day egg production index (HDEP) and Egg cleanliness index (EGC) time series. Black bold dashed lines (--): mean values. Black dashed lines (--): standard deviations.

3.4.3.1 HDEP trend

HDEP values displayed a constant trend from week 22 to week 37, ranging mostly from 95% to 100% (Figure 8). A clear reduction in noise was observed when using a window size larger than 7 days, whereas marginal reductions could be appreciated between 7, 10, and 14 days. This could be noticed by the narrowing patron of the standard deviation intervals (red long dashed lines) throughout the windows in Figure 8. The constant trend over the production period was also observed by Ahmad (2011) for brown and white shelled commercial laying hens. Also, Ramírez-Morales *et al.* (2017) observed this constant behavior for daily egg production per bird in a selected flock (No. 8).

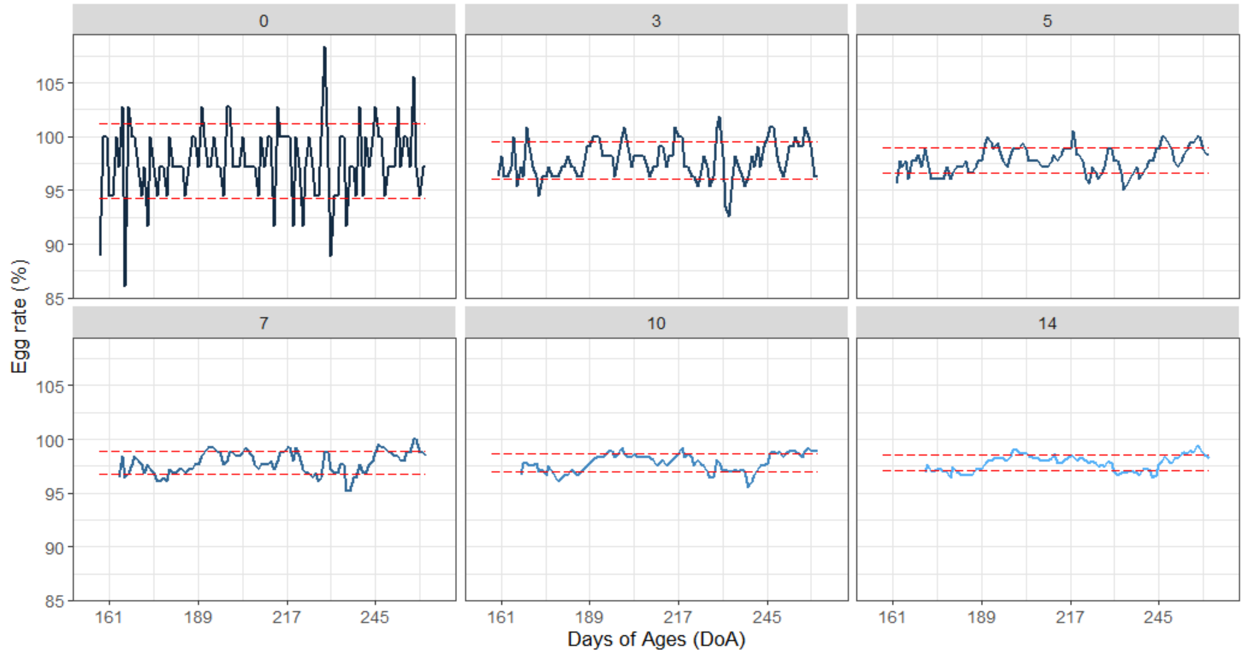

Figure 8. Egg production curves for the control treatment with several averaging window sizes, expressed in days. Red dashed lines (--): Standard deviation intervals.

3.4.3.2 Effect of treatments over HDEP and EGC

LDA was performed with three continuous variables: days of age (*doa*), egg yield (*hdep*) and egg cleanliness (*egc*), and one categorical variable (*i.e.*, *treatments*) (Figure 9). The first axis (LD1) was mostly associated with the *egc* and reproduced 42.7% of the data variability. The second axis (LD2) was composed mainly by the *hdep* and explained 30.3% of the variability. Hence, 73.1% of the variability in data was explained by the first two axes. Hen's age did not influence data variability since the data seemed to follow uniform trends along the days of age (see section 3.4.3.1). There were not any significant differences observed in HDEP between treatments and control with a confidence interval of 95%. However, the confidence regions of the mean discriminant scores (white ellipses) for T2 and T3 seemed to have a marginal distancing compared to the control of the LD1 axis. This distancing confirms the significant differences in T2 and T3 obtained from the Kruskal-Wallis test in terms of EGC. Thus, outcomes from the statistical test showed that the ECSs did not disrupt the laying hen performance in the experimental aviary systems. However, there is still an impact in egg cleanliness induced mainly by the litter space available for hens.

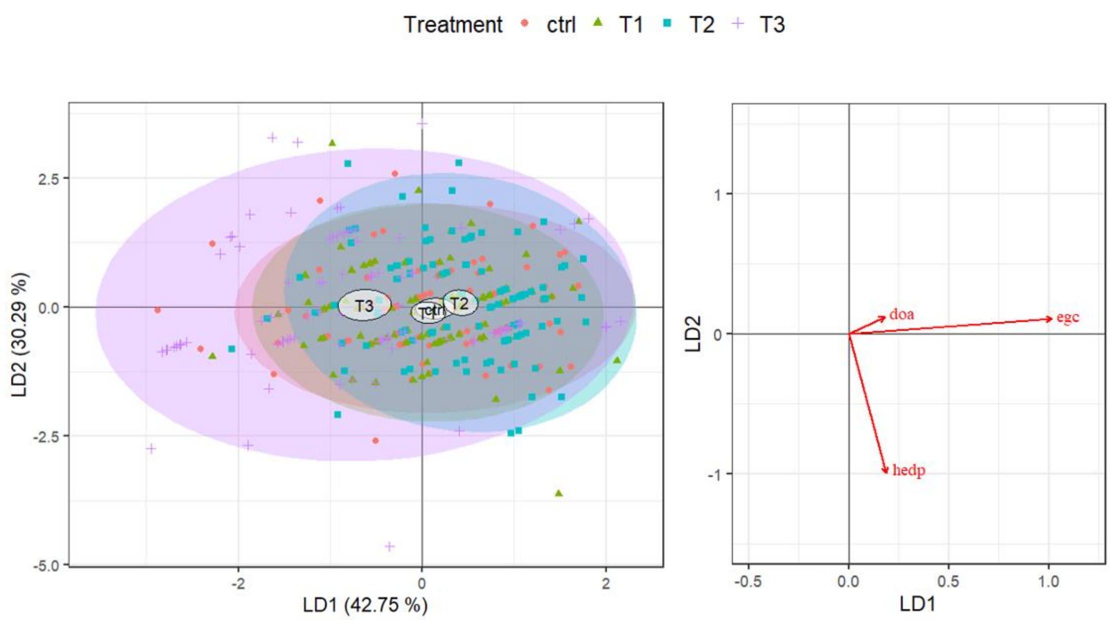

Figure 9. Linear discriminant analysis (LDA). Left: Score plot. Right: Biplot of loadings. Adapted from: Parent (2020)

3.4.4 Random Forest for HDEP predictions

3.4.4.1 Optimal model parameters

Optimal parameters of RF models using different averaging window sizes (Figure 10) revealed that the best model performance was achieved with a window size of 14 days (RMSE = 0.31%, R^2 = 0.81). It is noteworthy there was an increase in model accuracy as the window size increased. However, optimal parameters, *i.e.*, *mtry* and *ntree*, were different depending on the training procedure of each RF model and the window size (Table 6). There were two RF models with equal optimal parameters, but different accuracies explained by different window sizes, namely: RF models with window sizes of 5 days and 14 days with *mtry* and *ntree* values of 2 predictors and 2500 trees.

The use of averaging (*i.e.*, sliding) windows were also adopted by Ramírez-Morales *et al.* (2017) to predict egg yield fluctuations using artificial neural networks and features related to egg production as inputs. In our study, moving averages were used with RF algorithms to predict also HDEP daily fluctuations using environmental and hygrothermal characteristics. However, the selection of a window size depends on two main factors, namely: model accuracy and on-farm practices and management. Indeed, egg producers should prefer short periods to determine whether or not there are anomalies in a commercial egg production facility. Thus, large windows could be risky in light of taking decisions when facing sudden drops in the egg production curve, even more so when used to anticipate them. Nevertheless, a window size of either 10 or 14 days seems to be suitable and reliable. For this work, a 14-day RF model, with *mtry* and *ntree* parameter values of 2 predictors and 2500 trees, was selected to predict HDEP daily fluctuations.

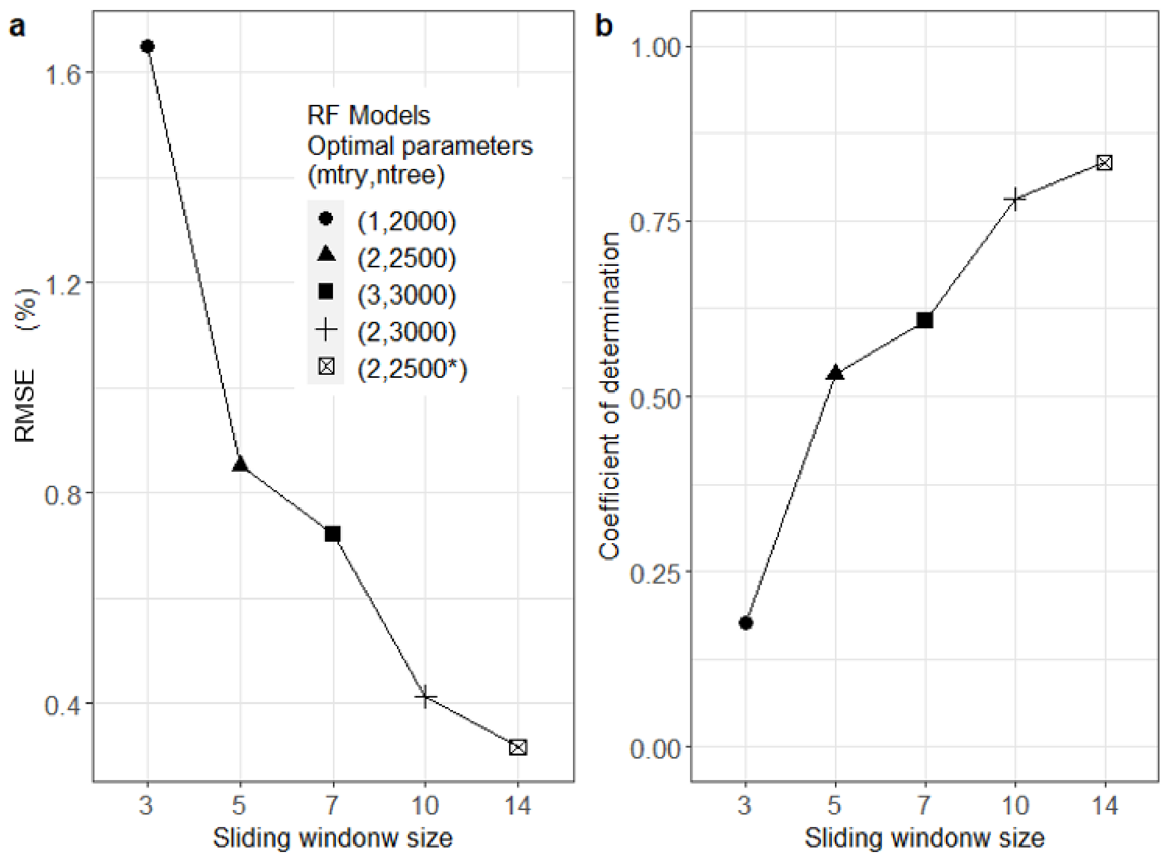

Figure 10. Optimal parameters of Random Forest (RF) models by sliding window size: (a) RMSE and (b) R^2

Table 6. Optimal RF model parameters as a function of averaging window sizes

Averaging window	mtry	ntree	RMSE (%)	R^2
3	1	2000	1.640	0.17
5	2	2500	0.846	0.54
7	3	3000	0.712	0.63
10	2	3000	0.396	0.81
14	2	2500	0.312	0.84

* *mtry:* number of predictors at each split node
*** ntree: number of trees in the forest.*

3.4.4.2 Simulation of HDEP

Simulated fluctuations contrasted well with HDEP observations (Figure 11). A RF model with a window size of 14 days was able to reproduce 84.7% and 77.6% of data variability over the training and testing datasets, respectively. The intercept (%) and slope (-), as well as the standard deviation from linear regressions were 0.61 ± 0.06 and 37.93 ± 6.63. Also, the RMSE was lower for the training (0.153%) than the testing (0.376%), where mean HDEP fluctuations were 97.77% and 97.78%. Several statistical models have been used to predict egg production curves from laying houses. Ahmad (2011) applied a general regression neural network-predicted model to simulate egg production from a US commercial strain throughout an intermediate production period (from weeks 22 to 36) using feed consumption as an input variable. Artificial neural networks (ANN) were also applied by Ramírez-Morales *et al.* (2017) using a multilayer perceptron (MLP) along with sliding windows as optimization methodology to determine anomalies in egg production. In their study, numbers of eggs, mortality, live animal, and cracked eggs were the relevant features used for modeling. As a result of their work, a warning classifier using a window size of 18 days was successfully developed with high accuracy. Akilli and Gorgulu (2020) aimed to compare multivariate, nonlinear, fuzzy regression techniques with a classical nonlinear regression to simulate egg yield curves, namely: ANN and least squares support vector machines (LSSVM). Akilli and Gorgulu (2020) successfully applied these two statistical models; getting high performance to estimate the values of daily and weekly egg production, showing suitable methods for early warning and egg production curve analysis. Also, other works have been proposed to fit egg production curves using support vector machines (Gorgulu & Akilli, 2018; Morales *et al.*, 2016). Observed and simulated HDEP time series (Figure 12) showed that RF modeling with a window size of 14 days was able to fit well daily fluctuations of egg production using data not used for training.

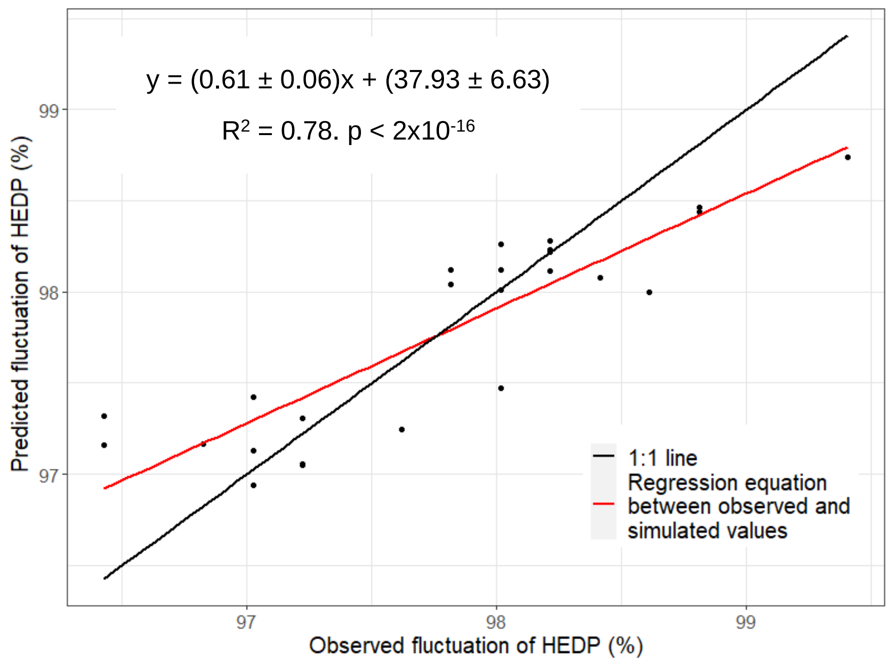

Figure 11. Observed and simulated HEDP fluctuations using a Random Forest model with a window size of 14 days.

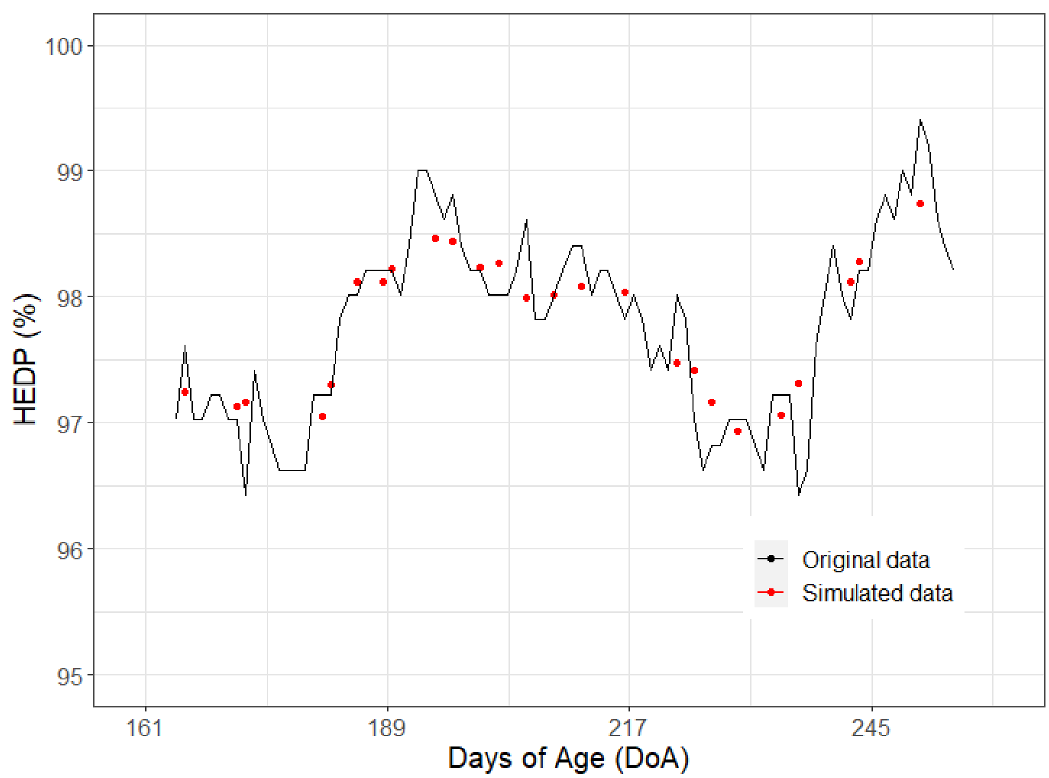

Figure 12. Simulated (•, red points) and observed (−, black line) HEDP time series.

3.4.4.3 Variable importance

Temperature was found to be the most important variable (*i.e.*, main governing factor) along with hen's age and relative humidity in light of predicting egg production daily fluctuations among hygrothermal and gas concentrations variables (Figure 13). Although the temperature range in our experiment was under the threshold recommended for aviary housing (< 35°C) (Oloyo, 2018), the model was more sensitive to change in this variable when gas emissions where under the threshold values recommended in the literature (see section 3.4.2). Indeed, the temperature is one of the major factors in livestock production. Livestock design and hygrothermal control systems have been subject of interest to ensure proper indoor environmental conditions for animals. Indoor temperature also has an important influence over animal heat transfer and energy consumption. Then, confined livestock units are mainly designed to manage temperature and relative humidity since these variables govern heat stress and livestock production (Fournel *et al.*, 2017). Further, it has been observed that abrupt changes in temperature could enhance negative effects in the health of bird population, mortality, feed intake, water consumption, body weight gain, or egg production (Oloyo, 2018). These parameters along with naturally produced sensible and latent heat fluxes by laying hens in large flocks of commercial scale in Canada (*i.e.*, up to 400 000 hens) singled out indoor temperature as a key variable to monitor. Importance analysis was validated by the random variable which did not have any importance among all predictors.

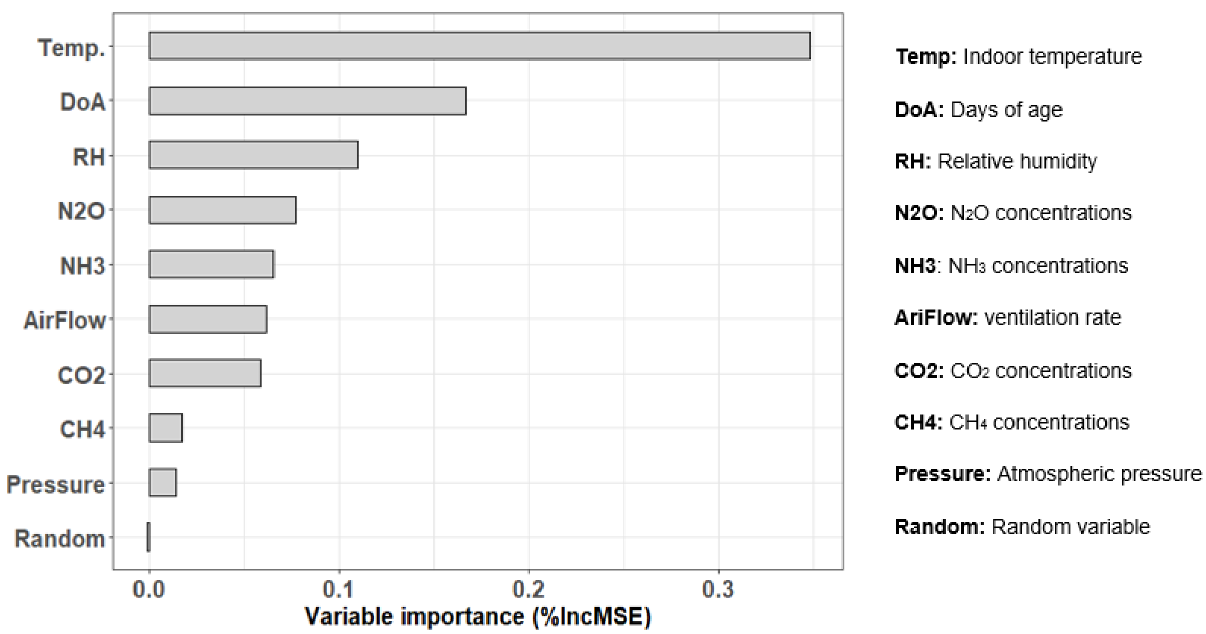

Figure 13. Predictive variable importance by the increment of the MSE (%)

3.4.4.4 Scenario analysis

Figure 14 displays egg production curves from measured and simulated data after applying an increment of 5% to temperature (Scenario: Inc. Temp. 5%). The increment of 5% was proposed to observe the effect in laying performance within little changes in temperature, as well as the model response to these changes. Average values of each egg production curve are also presented. It can be observed that HDEP undergoes a decrease mostly at peak of productions (from days 180 to 217 and from 245 until to the end). Besides, when observed and simulated HDEP values are below the average, they remain quite similar. However, overall egg production decreases when the temperature increases by 5% when compared to measured data. This could be observed by highlighting the average values of both curves, showing a negative effect on egg yield as a result of an increase in temperature.

Thus, the indoor temperature continues to be a relevant variable in climate control (combined with indoor environmental and hygrothermal monitoring), considering that other variables, such as, RH, CO_2, NH_3, CH_4, or NO_2, are under the recommended thresholds for laying hen housing. Oloyo (2018) has also mentioned drops in egg production under high temperatures, highlighting the importance to control temperature in laying hen houses. Furthermore, temperatures higher than 35°C can influence heat stress events for animals; leading to a decrease in feed intake, egg quality, and quantity, as well as, health and well-being in hens (Xin *et al.*, 2011). Nevertheless, it is highly recommended to continue the training process of the RF model with large data sets. This could increase our confidence in model accuracy when predicting outputs from inquiry-driven scenario analysis.

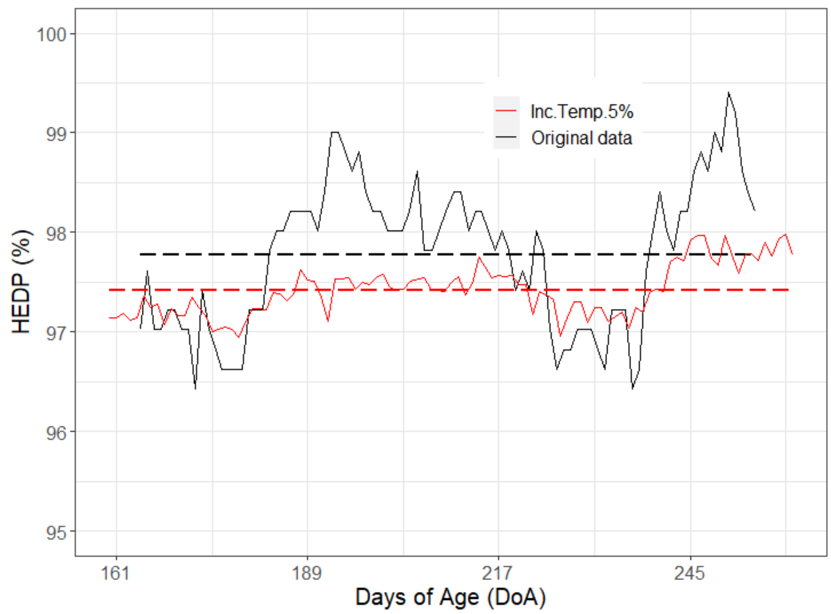

Figure 14. Scenario analysis by increasing temperature 5%

3.5 Conclusion

Environmental control strategies (ECS) are alternatives to reduce environmental impact from enriched and cage-free aviary housing systems. Evaluating outcomes from the application of these strategies have become a subject of interest in light of identifying suitable laying hen production systems with low negative environmental effects and high profitability. In this study, an integral egg production analysis along with a statistical modeling application was performed to evaluate the effect of different ECSs on egg yield curves and predict HDEP daily fluctuations, highlighting possible correlations within indoor environmental and hygrothermal conditions. The proposed combined ECSs have potential applications in CFSs from a point of view of productivity since they have not shown negative effects on egg production and egg cleanliness measured for experimental CFS. Nevertheless, additional research is needed to evaluate the implementation of ECSs at the commercial scale. Meanwhile, moving averages can be useful to exacerbate HDEP trend, though, sliding window sizes must be selected with caution because of the risk of dismissing sudden drops in egg production curves. These should be also selected depending on on-farm practices and egg producer needs.

Random Forest (RF) modeling was used in this study as another suitable approach among statistical models to generate early warning systems in egg production systems. Goodness of fit of the RF model showed a satisfactory performance to predict daily fluctuations in egg yield using indoor environmental and climate variables. This highlights the potential of machine learning approaches to deal with biosystems and their complexities. The contribution of this machine learning application has the potential to be useful for egg production modeling and monitoring. Likewise, it is highly recommended to apply critical thinking to validate the outcomes of statistical models with physical, chemical, or biological phenomena related to egg production systems. Besides, scenario analysis with RF represents a good framework to evaluate new potential applications. Nevertheless, the use of large data sets is paramount to enhance the training process illustrating all possible events recorded in the past. This study contributes to the recent research filed of monitoring systems and Precision Livestock Farming applied for laying hen production systems.

3.6 Acknowledgment

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4 TROISIÈME ARTICLE

Prediction of ammonia concentrations from a controlled storage of laying hen manure using two modelling frameworks: mechanistic and machine learning

Prévision des concentrations d'ammoniac émises lors du stockage de fientes de poules pondeuses en conditions controlées à l'aide de deux techniques de modélisation : mécaniste et machine learning

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Fabrice Guiziou a collaboré à la collecte des données. Il a participé à la révision au complet du document scientifique, apportant des suggestions et commentaires dans les différentes sections du document.

4.1 Abstract

Ammonia emissions (AE) are likely linked to a wide range of environmental and human health issues. Poultry production is one activity that contributes to AE (about 4.4% of global AE), wherein emissions can come from in-situ production and manure storage. Thus, manure management practices have become subject of interest focusing on the reduction of N contributions. However, tracking and control AE from commercial laying hen facilities are time and resource intensive, and can lead to negative consequences for farm economics and workers' welfare. Simulating AE from laying hen manure (LHM) can provide a powerful framework to formulate and analyze control strategies within laying hen housing systems. Emissions from LHM storage tanks under laboratory conditions were recorded in an experimental set up at IRSTEA laboratories (France). Volatilization tanks were designed to store LHM from commercial facility for seven weeks using a Photoacoustic Gas Monitor tracking system. Two modelling techniques were employed to simulate ammonia concentrations: a mechanistic model based on a set of physicochemical equations (rMM) and a machine learning method known as Random Forest (RF). Statistical parameters such as root mean squared error (RMSE) and the coefficient of determination (R^2) were applied to measure model performance. The rMM used in this study did not prove to be a convincing modelling approach with a RMSE and R^2 values of 80.8 mg N m^{-3} and 0.50, respectively. Nevertheless, RF model had a good performance for predicting ammonia concentrations with RMSE and R^2 values of 10.9 mg N m^{-3} and 0.93, respectively, highlighting the potential of using machine learning (ML) tools for environmental tracking in egg production facilities.

Keywords: ammonia emissions, laying hen, poultry manure, air quality, manure storage, Random Forest, mechanistic model, machine learning.

4.2 Introduction

Ammonia emissions (AE) are likely linked to a wide range of environmental and human health issues. Indeed, high stocks of ammonia (NH_3) in natural environment can lead to eutrophication of water bodies or increase of atmospheric pollutants (ADEME, 2018; Sutton *et al.*, 2009). NH_3 can react with nitrogen oxides or sulfuric molecules by nucleation process to produce salts such as, ammonium sulfates ($(NH_4)_2SO_4$) or ammonium nitrates (NH_4NO_3), with small particle sizes (< 2.5 µm) (CITEPA, 2019; Finlayson-Pitts & Pitts Jr, 1999). These NH_3 salts have low deposition rates and, thus, long atmospheric residence times and, under specific conditions, travel long distances (up to 2500 km); negatively affecting air quality or even changes of vegetable species (Irwin & Williams, 1988; Krupa, 2003; Sutton *et al.*, 2009).

NH_3 and ammonium (NH_4^+) deposition, by wet (*e.g*., rainout) or dry (*e.g*., diffusion, Brownian motion, sedimentation, or impaction) processes, can increase nitrogen in the soil matrix; disrupting the N-cycle and potentially leading to increasing outputs of nitrogen compounds in the atmosphere, namely: nitrogen gas (N_2), nitrous oxides (N_2O), nitric oxides (NO), even loads of carbon dioxide (CO_2) due to microbial activity. Furthermore, excess of soil nitrates can enhance groundwater contamination (Cape *et al.*, 2009).

From a global perspective, Van Der Molen *et al.* (1990) and recently the United Nations Economic Commission for Europe (UNECE) (2019) estimated that 80% of the AE came from agricultural activities; livestock farming contributing 50%. In France, agriculture is a major contributor of local AE (94%), wherein 41% are credited to livestock manure (CITEPA, 2019). Not surprisingly, manure management practices have been scrutinized and the subject of studies focusing on the reduction of N contributions from anthropogenic activities.

Ritz *et al.* (2004) have estimated that poultry production contributes to 4.4% (3.6 million of tons) of the global NH_3 emission. These emissions come from different hotspots within the production chain, namely: feed transport, in-situ production, poultry manure storage and land application for fertilization (Martin *et al.*, 2014). Often, AE contributions from in-situ production can be considered as manure storage due to the management practices of manure belt systems. In France, this activity results in the production of 140 000 tons of N excreted from poultry farms (Itavi, 2013; Loyon, 2018). N excretion can be linked to the amino acid ratio and high proteins content in the feed. In fact, 2/3 of the ingested N is rejected as uric acid leading to NH_3 volatilization (Ritz et al., 2004). Therefore, high levels of NH_3 can be reached without good control and management practices, conducive to negative environmental consequences, sub-optimal poultry performance or poor health conditions for the animals and workers (Ritz *et al.*, 2004).

Modelling can be viewed as an ensemble of mathematical, statistical, and computational tools to predict a target variable with a certainty degree of accuracy, *e.g.*, NH_3 volatilization phenomena (Kuhn & Johnson, 2013). Simulating AE can be considered as a relevant framework to formulate and analyze control strategies within animal housing systems. Moreover, knowledge derived from predictive models can be used to design and complement measurement methods which can be time and resource intensive (Arogo *et al.*, 2006). Computational methods were also recommended by the National Research Council to: (i) study the interactions between system components; that is gas emissions from animal housing systems, manure storage systems and organic fertilizer applications as proposed by Mansell *et al.* (2005), and (ii) develop an alternative approach instead of relying on emissions factor (EF) (Ni *et al.*, 2011). Modelling ammonia volatilization from manure storage systems remains challenging when considering the complexity of the governing processes and uncertainty associated with field measurements (Roman & Olaru, 2018). Notably, chemical reactions, biological activity and physical phenomena are not easy processes to model. Hence, different modelling methodologies have been proposed such as process-based modelling (*e.g.*, empiric or mechanistic models) or black-box modelling (i.e., statistic or data-driven models).

Several models have been developed to illustrate the AE for different livestock housing systems (cattle, pigs, poultry) using: (i) empirical models (McQuilling & Adams, 2015; Pinder *et al.*, 2004), (ii) mechanistic models (Berthiaume *et al.*, 2005; Bjerg *et al.*, 2013; Monteny *et al.*, 1998; Montes *et al.*, 2009; Ni, 1999; Sommer & Olesen, 2000), or (iii) statistical models (Aneja *et al.*, 2001; Harper *et al.*, 2004; Huijsmans *et al.*, 2018; Lim *et al.*, 2007; Shen *et al.*, 2018; Xie *et al.*, 2017). Moreover, empirical and mechanistic models have often been combined with the same goal in mind as introduced by Liu *et al.* (2009) or most recently by Tong *et al.* (2020).

Random Forest (RF) is known as a fast, accurate and simple statistical model also referred to as a machine leaning (ML) based model. RF has the potential to be a powerful method to predict AE in livestock housing systems because of its capacity to: (i) deal with missing data, (ii) perform well with small datasets, (iii) order data by variable importance analysis and (iv) be computationally fast with large dataset (Breiman, 2001; Philibert *et al.*, 2013). RFs have been used in various agricultural applications, for example in: (i) livestock farming to classify animal behavior in fattening pigs using data from video recordings (Jensen *et al.*, 2020); (ii) air quality and atmospheric pollution to predict N_2O emissions from a compilation of global studies (Philibert *et al.*, 2013), and (iii) water management to forecast water table depth in cranberry fields (Brédy *et al.*, 2020; Gumiere *et al.*, 2020). However, to our knowledge, there is no evidence of an application of RF to describe NH_3 volatilization from laying hen manure storage facilities.

The objective of this study was to evaluate the effect of environmental and meteorological conditions on NH_3 emission within a controlled storage tank filled with laying hen manure using a mechanistic model, based on a set of physicochemical equations (rMM), and a machine learning method, based on a RF model (RF).

4.3 Materials and methodology

4.3.1 Source of laying hen manure samples

Two laying hen manure samples were obtained from an egg production farm located in the north of the Brittany region in France (22400, Côtes d’Armor, France). The farm included two two-levels laying hen houses sheltering around 120 000 laying hens in a cage-free system (four livestock rooms). Manure is taken out periodically to a storage building using a conveyor belt system, where the manure is dried with an on-farm dryer. These conveyors are automatically controlled at different hours throughout the day. Both samples came from the upper (LHM1) and ground (LHM2) levels of one two-level hen house. LHM1 and LHM2 were sampled directly from the conveyor belt before entry to the drying section and placed in plastic closed containers for transportation to the laboratory. Transport and storage laboratory conditions were the same for both samples.

4.3.2 Laying hen manure characteristics

4.3.2.1 Dry and organic matter

Dry matter (DM) and organic matter (OM) were calculated using the protocols recommended by the *Comité Européen de Normalisation*, EN 12880 (Characterization of sludge – Determination of dry residue and water content), EN 12879 (Characterization of sludge – Determination of the loss on ignition of dry mass), EN 15935 (Sludge, treated biowaste, soil and waste - Determination of loss on ignition), and the *Norme Français*, NF U44-171. Laying hen manure (LHM) samples were manually homogenized and placed in ceramic containers previously weighted (M_0). Samples were weighted (M_1) with a Sartorius balance (accuracy ± 0.001 g) and placed in an oven at a temperature of 105°C for 48 hours. Then, first-stage dried weights (M_2) were obtained. Samples were placed in the oven again at a temperature of 250 ± 25°C for 2 hours, then dried at temperatures of 550 ± 25°C for another 2 hours. Second-stage dried weights (M_3) were also recorded. Equation 9 and Equation 10 were used to determine DM and OM as follows.

$$DM(\%) = \frac{M_2 - M_0}{M_1 - M_0}\, x\, 100 \qquad \textbf{Equation 9}$$

$$OM(\%) = \frac{M_2 - M_3}{M_1 - M_0} x\ 100 \qquad \textbf{Equation 10}$$

4.3.2.2 Total Kjeldahl Nitrogen (TKN)

Total nitrogen, that is organic and ammoniacal nitrogen, was determined using protocol NF EN 13342 (Characterization of sludge - Determination of Kjeldahl nitrogen). VAPODEST® steam distillation was used to perform the analysis. Five homogenized manure samples (3.0 ± 0.2 g) were placed into glass graduated cylinders. Samples were previously mineralized using a heating process and an acid solution (H_2SO_4) with Kjeldahl catalyzers (2 capsules: Kjeltabs) over three phases: 30 min at 180°C, 30 min at 250°C, and 2 hours at 420°C. Mineralization allowed for the transformation all nitrogen forms into ammonium (NH_4^+).

The analysis was divided into two stages: (i) one distillation to capture nitrogen compounds using a solution of Boric acid (H_3BO_4 – 4%), and (ii) one titration using a sulfuric acid solution (H_2SO_4 – 0.2 N) to reach the target pH value. The total nitrogen concentration was calculated by acidification of a basic solution (boric acid + ammonium).

$$[N - NH_4{}^+] = \frac{2.8\ (V_e - V_b)}{m_p} \qquad \textbf{Equation 11}$$

Where V_e was the volume of acid solution added for acidification of manure samples. V_b was the acid solution volume added to the control sample; that is distilled water. m_p was the mass of manure sample (g). Ammonium concentrations were expressed in kg N ton^{-1} as reported in other studies.

4.3.2.3 Ammonium (NH_4^+) and pH

Ammonium analysis was performed by Büchi K360 steam distillation. This procedure is similar to the TKN analysis; however, mineralization was not applied to analyze only the NH_4^+ concentration of the manure samples. Equation 11 was used to determine NH_4^+. pH was assessed following protocol NF EN 13037 (Soil improvers and growing media - Determination of pH). Manure samples (87.5 ± 0.2 g) were mechanically homogenized (360 ° agitation over 1 hour) with demineralized water to obtain a 20/80 solid-liquid solution. Then, a pH meter, previously calibrated (accuracy: ± 0.01 pH unit), was used for the measurement of manure solutions.

4.3.3 Set-up for measuring ammonia emissions

An experimental set-up was used to measure ammonia, as well as other gas emissions (CO_2, CH_4, H_2O). The set-up, described in Hassouna *et al.* (2016), had three principal sections: (i) a metallic board with twelve hermetic-glass containers (volume 5 L) where manure samples had three repetitions (n = 3), each container equipped with an in-out airflow system; (ii) a module to measure ammonia concentration by acid trapping, and (iii) an automatic gas tracking module, an INNOVA 1412i photo-acoustic gas analyzer (LumaSense Technologies), a fast sampling loop module was implemented to vary gas inputs to the gas analyzer to measure different gas concentrations.

4.3.4 Mechanistic modelling

4.3.4.1 Equilibrium gas phase ammonia concentrations

Enzymatic, microbial, and chemical reactions all participate to the transformation and mass transfer of ammoniac in animal excreta. Uric acid, through catabolism processes of purine bases, contributes to the production of NH_4^+ in laying hen manure. Once, urines and feces are excreted by the animal, uric acid degradation starts with the mineralization of enzymatic compounds such as allantoin, allantoic acid, ureidoglycolate, glyoxylate and urease. This last compound reacts with water to produce ammoniac (NH_3) and carbon dioxide (CO_2) (Carlile, 1984; Mowrer *et al.*, 2014). Therefore, a constant interaction between NH_3 and H^+ protons allows to produce ammonium (NH_4^+) throughout an acid-base reaction in the manure. This continuous conversion is considered a reversible reaction where dissociation (K_d) or association (K_a) constants regulate the equilibrium state (Ni, 1999; Zhang *et al.*, 1994). A diffusion mass transfer process, induced by concentration gradients, drives the transfer of NH_3 in the manure. Moreover, Henry's law regulates the apportionment of NH_3 in liquid and gas phases. Then, once the gas phase has reached the manure-atmosphere interface, NH_3 is released by convective mass transfer (Monteny *et al.*, 1998; Ni *et al.*, 2009; Ni, 1999) (Figure 15).

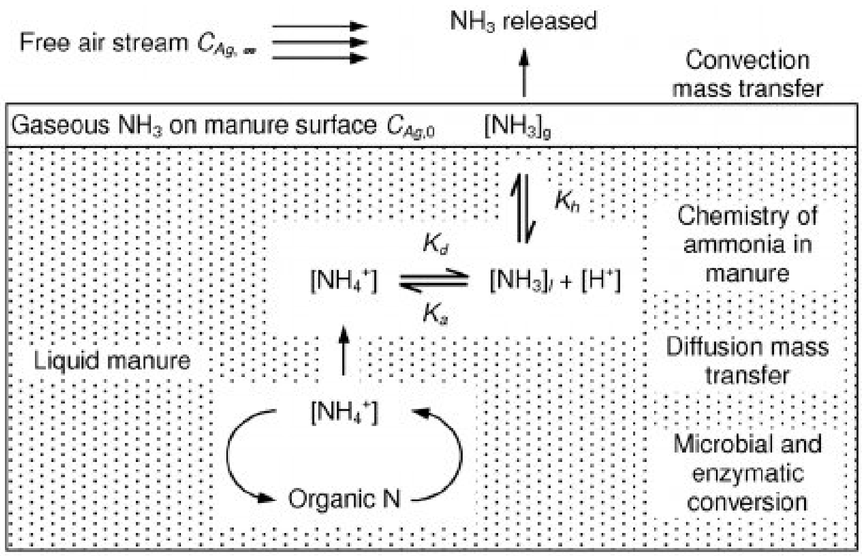

Figure 15. Illustration of the NH_3 volatilization process in liquid manure. From Ni *et al.* (2009). Used with permission.

Microbial activity also participates to the NH_3 transformation in manure. NH_4^+ ions are converted into organic nitrogen (Norg.) by assimilation or ammonification (Sánchez-Monedero *et al.*, 2001; Witter & Lopez-Real, 1987). In addition, the animal waste contains absorbed and dissolved NH_4^+ due to liquid-solid composition of these materials (Liu *et al.*, 2009)

4.3.4.2 The Core model

The core of the mechanistic model, that of Liu *et al.* (2009), is built using fundamental laws and empirical equations to predict free gas ammonia concentrations ($C_{g,0}$) above the laying hen manure surface. In Equation 12, $C_{g,0}$ (mg N m^{-3}) is calculated by multiplying the total available nitrogen (TKN, µg g^{-1}) by a correction factor (f_c) divided by Henry's constant (H). f_c is a dimensionless parameter to obtain the free ammonia nitrogen over the TKN where the dissociation constant (k_d) and the pH of the manure are the independent variables (Equation 13). The manure temperature (T_f) in Kelvin, is used to determine Henry's constant which represents the equilibrium between the gas and liquid phases of free ammonia.

$$C_{g,0} = [NH_3 - N]_{gas,bound} = \frac{17}{14} . 1000. \frac{TKN . f_c}{H} \qquad \textbf{Equation 12}$$

$$f_c = \frac{1}{1 + \frac{10^{-pH}}{k_d}}$$ **Equation 13**

$$log\, H = -1{,}69 + \frac{1477}{T_f}$$ **Equation 14**

k_d in manure (Equation 15) is calculated by multiplying the dissociation constant solution (k_{d0}) in (Equation 16) and α (Equation 17) whose independent variables include water density (ρ_{H_2O}), moisture content (MC) in decimal dry basis (d.b.), and the Freundlich partition coefficient (K_f) in L kg^{-1}. k_d accounts for the effects of the solids and ions in the mass of manure.

$$k_d = \alpha \,.\, k_{d0}$$ **Equation 15**

$$k_{d0} = 10^{-0{,}0918 - \frac{2729{,}92}{T_f}}$$ **Equation 16**

$$\alpha = \left(1 + K_f \frac{\rho_{H_2O}}{MC}\right)^{-1}$$ **Equation 17**

The Freundlich coefficient participates to the absorbed/dissolved N-NH_4^+ equilibrium in the solid-liquid phase of the manure matrix. However, K_f values have yet to be reported in the literature. Thus, Liu *et al.* (2009) proposed the following Equation :

$$K_f = 0{,}00672\, (10^{-pH})^{-0{,}412}\, {T_f}^{-0{,}759}$$ **Equation 18**

4.3.4.3 Model calibration and validation

The mechanistic model (rMM) was programmed using VBA MS-Excel. Model calibration was performed by varying initial parameters (TKN, pH and MC) to corroborate the dynamics of $C_{g,0}$ at several temperatures. Validation was achieved in two phases by: (i) plotting time-series of $C_{g,0}$ in mg m^{-3} from two poultry litter samples (A and B) over a range of temperatures, from 12.8 to 27.2°C and 19.2 to 30.0°C, as reported by Liu *et al.* (2009), and (ii) comparing coefficients of regression equations of observed and simulated values of $C_{g,0}$. Initial litter properties, as well as, original regression equation of observed $C_{g,0}$ was obtained from the results and discussion section of the aforementioned publication.

4.3.4.4 Input data

For modelling purposes, TKN, MC and pH were first set, while T_f was varied according to the experimental measurements. It should be noted that manure and *in-situ* outdoor temperatures were assumed to be equal. In other words, the thermal characteristics of the mass of stored manure throughout the experimentation were considered to be homogeneous, thereby assuming that there was not a significant gradient of temperature between the core and boundaries of the manure volume. Temperatures were recorded over the 2019 spring season.

4.3.5 Statistical modelling

4.3.5.1 Ensemble-based models & Decision trees

The concept of Ensembled based models (EBMs) were probably introduced by Dasarathy and Sheela (1979) where a composite classifier system design, *i.e.*, a linear/nearest neighbor classifier, was presented for optimal partitioning problems. These EBMs are mostly inspired by the decision-making process of humans. Indeed, decisions often require a second or more opinions before they are approved whatever the topic of discussion. Thus, ensemble systems are related to daily human life experiences by using several experts (predictors or classifiers) to obtain a particular answer in data analysis (Polikar, 2006). The strategy of these EBMs is to combine different models instead of using only one to improve performance and accuracy when modelling. Each model must be characterized by an error or weaknesses; thus, EBMs use combination rules throughout several model-outputs to correct the error from individual base models and obtain a good generalization performance (Zhang & Haghani, 2015). Diversity is the piecewise characteristic to successfully apply EBMs in any system or biosystem. Hence, diversity from the base models could be achieved by using different training data and unstable predictors; that is classifiers that are very sensitive to a small perturbation. And these are used during the model development stage to obtain differences in the decision boundaries from each model. The training dataset structure could be obtained by applying resampling techniques with and without replacement, namely: bagging, boosting or k-fold data split methods (Polikar, 2006).

Decision-trees are one of the powerful machine learning (ML) methods with high predictive accuracies and sensitive responses in the training dataset once small perturbations are implemented. Besides, fast, and easy algorithms, as well as, variance reduction could be allocated to this sort of classification and regression methods (Ogutu *et al.*, 2011; Zhang & Haghani, 2015).

Classification and regression trees (CART) are algorithms developed for building search-trees proposed by Breiman *et al.* (1984). CART algorithm draws trees expanding first a very large quantity of nodes followed by a prune step; that is using a pruning criterion removal of branches reflecting non-improvement in the prediction performance. Pruning degree usually is defined by the resampling method used (*e.g.* cross-validation). Thus, resampling techniques could participate in three ways over the model building procedure, namely: pruning, diversity and even in tuning parameters in specific cases.

4.3.5.2 Random Forest

Bagging (or bootstrapped aggregating) is one technique applied to obtain the diversity within decision trees and represents the fundamental theory of Random Forest algorithm (RF). This re-sampling technique proposed by Breiman (1996) builds sequences of training data subsets; choosing random values with replacement, a value could be repeated or not, from the original learning dataset. Bagging method has an effect of instability which reduces the mean squared error (MSE) averaged of the entire training dataset. However, this improvement in accuracy depends also on training data typology, in other words small changes in the entire training dataset could generate large changes in the predictions. Bagging method is related with the training set required to start the building tree process, moreover, the building process is important as well. Thus, the Bagging and the CART concepts were considered to introduce the RF concept which is in fact a successor of bagging trees methods.

Random Forest is defined by Breiman (2001) as a classifier/regressor made of a large number of un-pruned decision trees that once generated, they vote for the most performant class/value. Randomization in RF can be achieved in two ways: (i) search-trees based on random training subsets selection and (ii) at each split, by choosing the best predictor (*i.e.* input variables) among a group of predictors randomly selected (Grömping, 2009). RF was considered in our study because of its user-friendly parametrization and simplicity between the available tree-based models (TBMs). Two parameters must be established in this algorithm which determine the final model accuracy: the random sampling of inputs considered in the split during the building process (mtry), and the quantity of trees in the forest (ntree) (Liaw & Wiener, 2002).

4.3.5.3 Pre-processing and input data

RF algorithm was built in the R programming language version 4.0.0 (R Core Team, 2020). The *randomForest* (Liaw & Wiener, 2002) and the *caret* (Kuhn, 2020) packages were used to perform model parametrization and training. Data was obtained from the controlled experimental storage of laying hen manure of the 2019 spring season. Gas concentrations, (carbon dioxide (CO_2), methane (CH_4), water vapor (H_2O)) and meteorological variables (atmospheric pressure (Pressure), relative humidity (RH) and outdoor temperature (OutTemp)), were selected for model development. Input variables were scaled from zero to one reduce the differences in orders of magnitude among all the variables. In addition, an external random variable (random numbers from 0 to 1) was added to the data to validate the variable importance analysis within RF.

4.3.5.4 Data splitting

A dataset including 368 observations of each variable (CO_2, CH_4, H_2O, Pressure, RH and OutTemp) was used for RF. The default datalogging interval was 3 hours. Entire dataset was randomly split into a training (70%) and testing (30%) datasets using the *caTools* package (Tuszynski, 2020). The RF learning process was performed by selecting randomly several training subsets by applying 10-fold cross validation with 5 repetitions as re-sampling method based on recommendations from the literature (Kuhn & Johnson, 2013).

4.3.5.5 Model parametrization

Optimal model parameters, *mtry* and *ntree*, were selected following the lowest RMSE value obtained for training. For this purpose, the *train* function from the caret package was applied. Seven predictors (six input variables + one additional vector with random numbers), and a range of numbers of trees from 1000 to 3500 trees, with increments of 1 predictor and 500 trees at each step, were evaluated to identify the best performances of RF. The *ntree* range was based on recommendations taken from the literature and the default characteristic of the function. Testing different numbers of trees allowed to observe the statistical response and behavior of the model to reduce computational time without disrupting model performance.

4.3.5.6 Variable importance analysis

Once the learning process is done, RF can estimate the importance of predictors to rank the effect of an input variable over the final prediction. It should be noted that RF does an additional internal split at each re-sample training set leaving out about 1/3 of data (OOB: Out-Of-Bag). Variable importance is executed for each OOB predictor (OOB-*x*) as follows: (i) all values in OOB-x are randomly permuted, (ii) a new search-tree is grown calculating new predictions, and (iii) an internal prediction error (IPEp) is estimated. Then, the importance is defined on how much the IPEp exceeds the prediction error from the original OOB (IPE) (Breiman, 2001; Liaw & Wiener, 2002). This analysis is also known as permutation based MSE reduction, since increase in mean square error parameter (%IncMSE) is use to determinate importance (Breiman & Wald Lecture, 2002).

4.3.6 Model evaluation

Two statistical measures were applied to evaluate the performance of both models: The root mean squared error (RMSE) and the coefficient of determination (R^2) (Equation 19 and Equation 20)

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{N}} \qquad \textbf{Equation 19}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{N}(y_i - \bar{y})^2} \qquad \textbf{Equation 20}$$

Where, y_i and $\hat{y}_i$ are the observed and predicted $C_{g,0}$, $\bar{y}$ is the mean value of observed data and N is the quantity of data. RMSE should be close to zero and R^2 close to one for a good prediction. RMSE was selected as critical parameter to evaluate the model predictive capacity whereas R^2 was applied to determine the reproduction of data variability from observed data. Intercepts and slope from R^2 regressions are presented to compare with the target regression line where simulated values are equal to observed data (line 1:1, y = x). Thus, intercepts should be close to zero and slopes close to one.

4.4 Results and discussion

4.4.1 Litter samples

The initial characteristics of two laying hen litter samples are presented in Table 7. Dry matter (DM) values of 34.2 and 39.3% d.b. were found for laying hen manure samples 1 and 2, respectively (LHM1 & LHM2); values also reported by German *et al.* (2017), Ghaly and Alhattab (2013), SATEGE (2013) and Dekker *et al.* (2011). Available nitrogen of 20.1 and 22.2 kg N ton^{-1} were also consistent with studies given by Ghaly and Alhattab (2013) and Koerkamp (1994). However, higher total nitrogen values have been reported in poultry manure by Murakami *et al.* (2011). Moreover, pH and ammonia concentration ([NH_4^+]) were similar to those reported in the literature (Bertrand *et al.*, 1994; Pote *et al.*, 2003; SATEGE, 2013). Lower characteristic values observed in LHM1 compared to LHM2 could be due to two aspects: (i) the uncertainty related to the laboratory test and equipment, and (ii) the sample's source. Both manure samples came from different livestock rooms placed in the same two-level laying hen house. In this case, LHM1 was sampled from the upper level, which was probably affected by ground level hygrothermal conditions. However, the experimental framework in this study was limited to assess these phenomena. In general, manure characteristics were similar to those reported in the available literature.

Table 7. Initial characteristics of laying hen manure from two manure samples (LHM1 and LHM2).

ID	LHM1	LHM2
Dry matter (%)	34.2 ± 1.1 *(3.3)*	39.3 ± 0.4 *(1.1)*
Organic matter (%)	25.6 ± 0.6 *(2.5)*	29.3 ± 0.4 *(1.4)*
TKN (kg N ton^{-1})	20.1 ± 0,9 *(4.5)*	22.2 ± 1.7 *(7.8)*
NH_4^+ (kg N ton^{-1})	2.7 ± 0,1 *(3.7)*	4.6 ± 0.2 *(4.4)*
pH	8.5	8.4

( ) Coefficients of variation in %

4.4.2 Temperature and relative humidity

Diurnal temperatures were observed from 12th of March to 29th of April 2019 with values fluctuating between 12.9 to 31.2°C. The average and standard deviation (SD) of temperature for the storage period was 20.9 ± 3.0°C. Relative humidity fluctuated between 16.2 to 74.5% with an average and SD of 36.3 ± 8.1%. Temperature and relative humidity time series are presented in Figure 16.

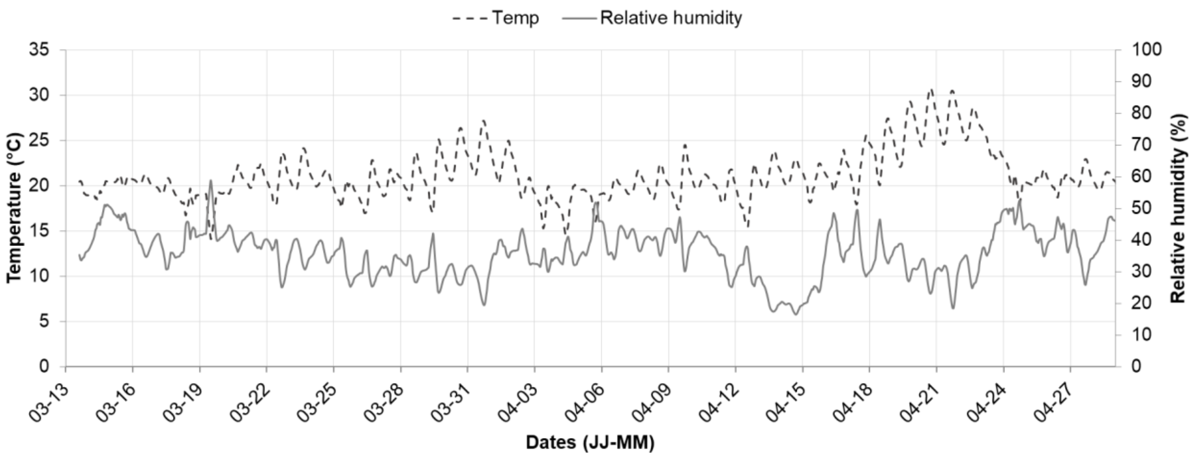

Figure 16. Time series of outdoor temperature and relative humidity over the controlled storage period of laying hen litter samples at IRSTEA laboratory

4.4.3 Observed ammonia concentrations

Time series of NH_3-N concentrations from LHM1 and LHM2 are presented in the Figure 17. Similar concentrations can be seen for LHM1 and LHM2 belonging to the same laying hen house with values ranging from 137.5 to 365.7 mg N m^{-3}. Higher values were observed from March 31st to April 02nd and April 21st to 24th. It should be noted that peaks of outdoor temperatures were also reported for the same dates. Average and SD of 210.5 ± 44.5 mg N m^{-3} and 206.2 ± 46.2 mg N m^{-3} were observed for samples LHM1 and LHM2. Also, diurnal fluctuations of NH_3-N were also observed for the two manure samples during the controlled storage period. This behavior was also observed for the other variables measured within the controlled manure storage, validating the repeatability of the dynamics of the gas and meteorological variables (Figure 18).

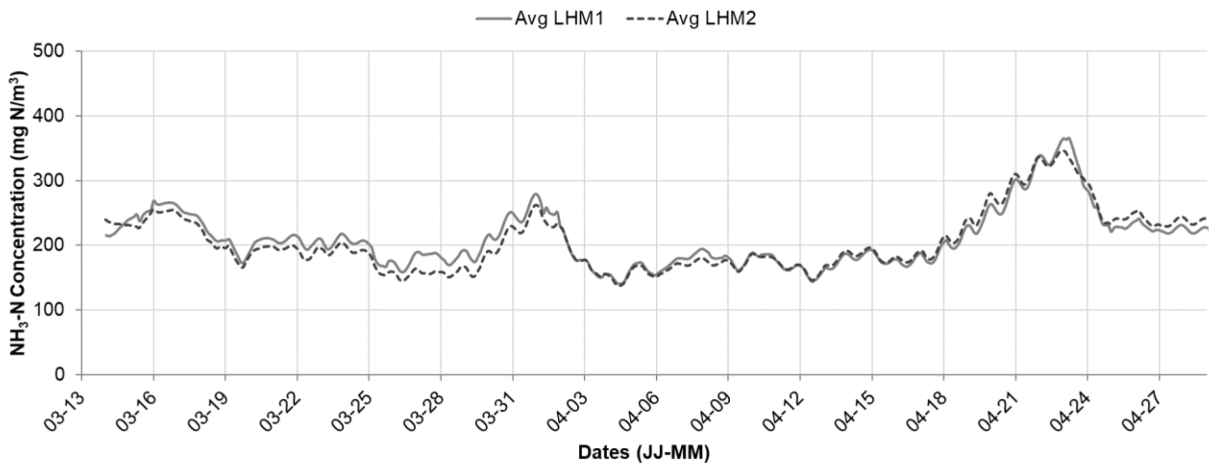

Figure 17. Time series of NH_3-N concentrations from the controlled storage of the two samples of laying hen manure

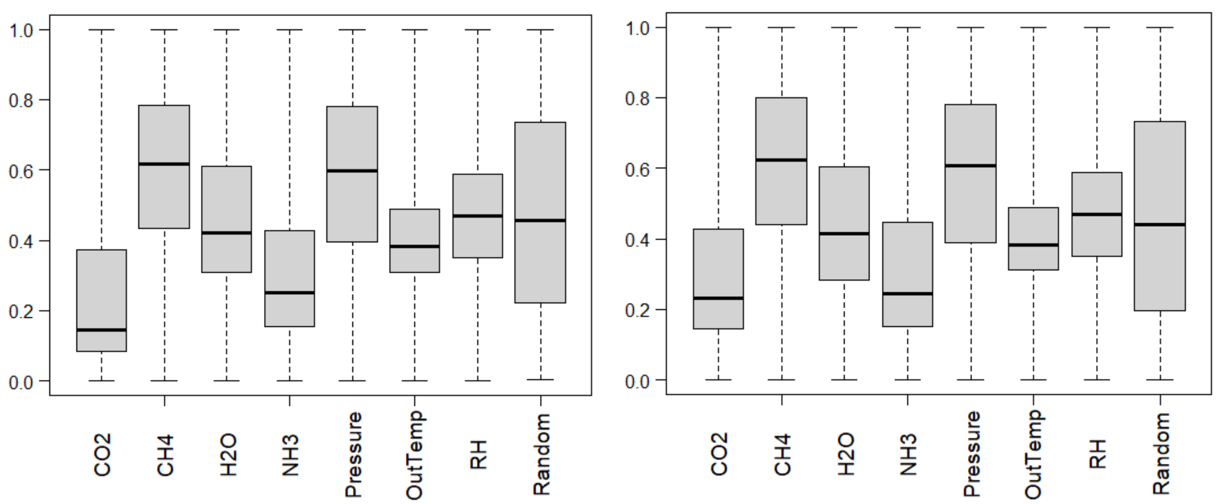

Figure 18. Data distribution from the controlled storage of the two samples of laying hen manure: LHM1 (left) and LHM2 (right).

4.4.4 Mechanistic modelling of NH_3-N concentrations

4.4.4.1 Model validation

Before applying the Liu *et al.* (2009) model to our study, we first validated the results obtained with our computer program (rMM). As shown in Figure 19, values of $C_{g,0}$ at various temperatures of both models have similar shapes. It is noteworthy there is a slight underestimation of $C_{g,0}$ values at higher temperatures for the *rMM* for both litter samples (Litter A and B) when compared to values published by Liu *et al.* (2009). In the case of litter A, a tiny difference was observed in the values of the exponential coefficients and the exponents from both regressions. Besides, the exponential coefficient in litter B from *rMM* duplicated the coefficient given by the exponential regression of the observed values, but the exponents were nearly similar. This was also reported in Liu *et al.* (2009) under similar temperature conditions and a K_f = 2.15 L kg^{-1} (K_f : the Freundlich coefficient). In addition, an adjusted pH value (pH = 8.59) was used as an initial parameter value for litter sample A, instead of using a pH of 8.49 as indicated in the original paper. This change was deemed necessary to have a visually better fit when compared to the original regression. Since $C_{g,0}$ predictions at several temperatures were visually repeatable, we assumed that our *rMM* was validated and could be used with our data set (Figure 19).

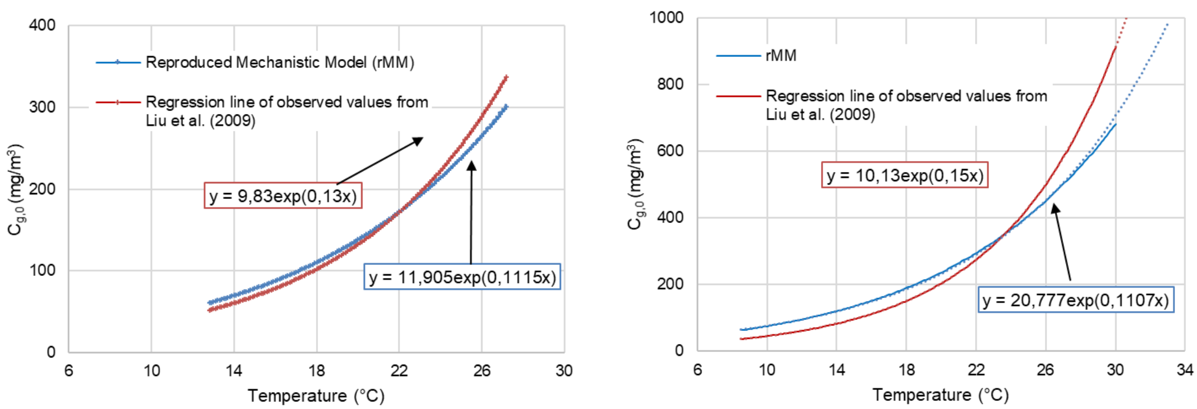

Figure 19. Left: $C_{g,0}$ predictions from litter A (Temp = 12.9 to 27.2 °C, K_f = 1.87 L kg^{-1}). Right: $C_{g,0}$ predictions from litter B (Temp = 19.2 to 30.0 °C, K_f= 2.15 L kg^{-1}).

4.4.4.2 Simulations of NH_3-N concentration

Comparison between observed and simulated NH_3-N concentrations of LHM throughout the controlled storage period are presented in Figure 20. An average of 313.4 mg N m^{-3} of simulated NH_3-N concentrations was obtained. Concentrations from both LHMs were averaged thanks to the repeatability observed concentration time series for both samples (see section 4.4.3). The results show that the mechanistic model was able to explain 51.25% of the variability with a RSME of 61.51 mg N m^{-3}. The intercept (mg N m^{-3}) and slope (-) of the regression equation with their SD were – 38.64 ± 14.69 and 1.40 ± 0.06. This performance is not as good as that achieved by Liu *et al.* (2009) (R^2 = 80%). It should be noted that manure heterogeneity could have affected the assessment of the initial parameter values and final results. Indeed, the composition of LHM could have excreta, hen feathers, rocks, and eggshells. Moreover, within the same range of NH_3-N concentrations, the dispersion of data obtained in our experiments was larger than that reported in Liu *et al.* (2009). This could explain the lower coefficient of determination achieved with the *rMM*. In addition, overestimation was observed throughout almost all the experimental storage and even more apparent for high concentrations. This is well observed in the time series of NH_3-N concentrations (Figure 21).

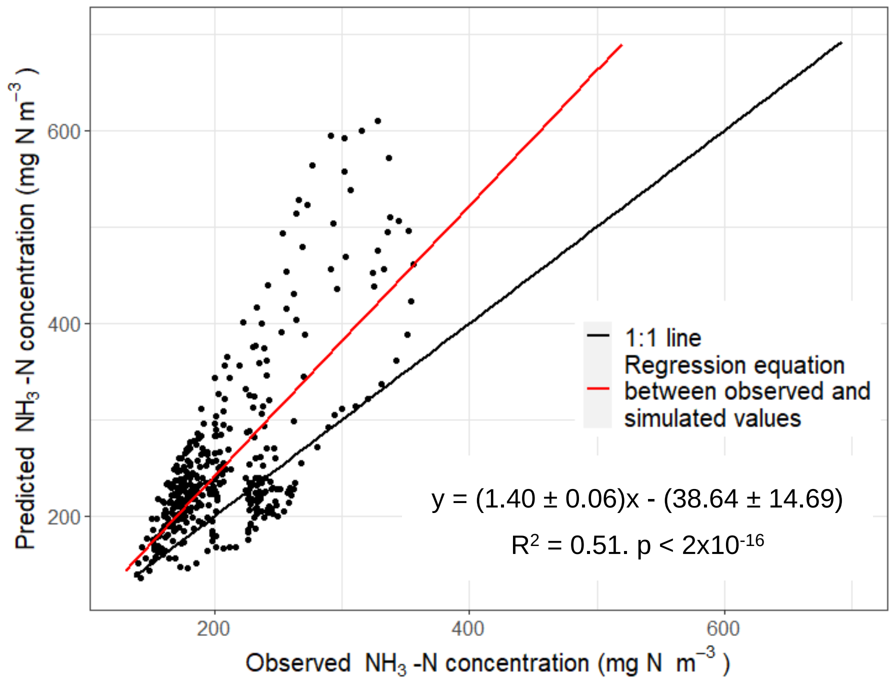

Figure 20. Observed and simulated NH_3-N concentrations using the mechanistic model.

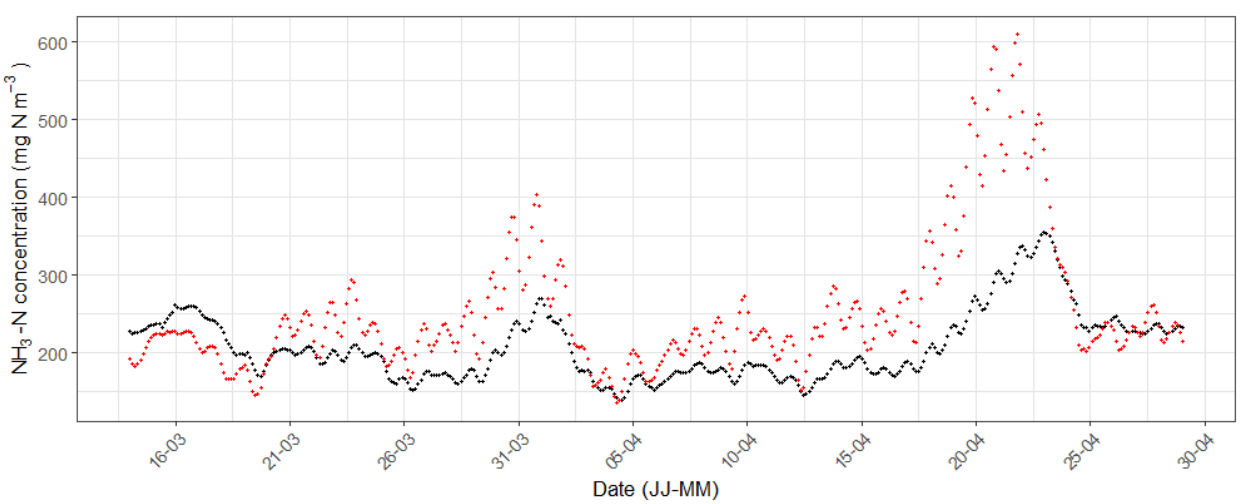

Figure 21. Time series NH_3-N over a controlled experimental laying hen manure storage. Simulated values of the mechanistic model (red points) and observed values (black points).

4.4.5 Random Forest algorithm for NH_3-N predictions

4.4.5.1 Optimal model parameters

The smallest normalized RMSE value (0.0634) was observed when using four randomly sampled predictors (*mtry* = 4) and 2000 trees for training (Figure 22). There were also small differences among all the *ntree* sets regarding the average of RMSE (avgRMSE) at each *mtry* value (< 0,7%). Decreases on avgRMSE were large at the beginning between *mtry* values of 1 and 2 (slope ≈ - 0,03), moderate for *mtry* sets of 2 to 4 predictors (slope ≈ - 0,01), and slightly decrease for *mtry* sets of 4 to 6 predictors (slope ≈ - 0,001), except for the last value (*mtry* = 7) where the avgRMSE value had a minor increase. In general, the pattern of RMSE values showed a considerable degradation when increasing the number of predictors at each split (*mtry*). In relation to the number of trees (*ntree*), these results showed that using a quantity of 1000 and 2500 trees provided the small model errors (black and blue lines) whereas 3000 trees gave a better one (green line) considering all the *ntree* sets selected for the entire RMSE analysis.

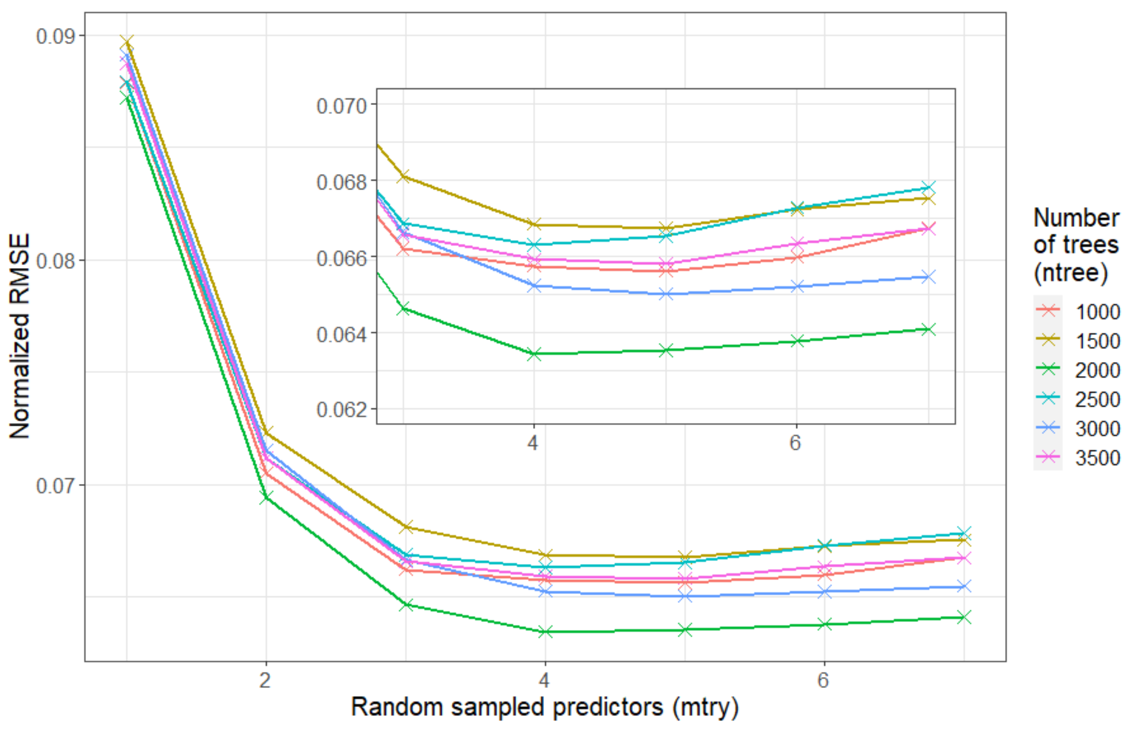

Figure 22. Evolution of normalized RMSE values with *mtry* and *ntree* variations for a RF model using a 10-fold cross-validation re-sampling method with repetitions (n = 5).

4.4.5.2 Simulations of NH_3-N concentrations with RF-algorithm

Simulated data compared well with observed NH_3-N concentrations (see Figure 23). The statistical model was able to explain 98.7% and 93.7% of the variability of the data observed over the training and testing procedures. The intercept (mg N m^{-3}) and slope (-) of the regression with their standard deviation (SD) were 30.51 ± 4.66 and 0.85 ± 0.02. Values of RMSE were lower during training (4.8 mg N m^{-3}) than testing (10.9 mg N m^{-3}), where mean NH_3-N concentrations were 207.9 and 212.5 mg N m^{-3}, respectively.

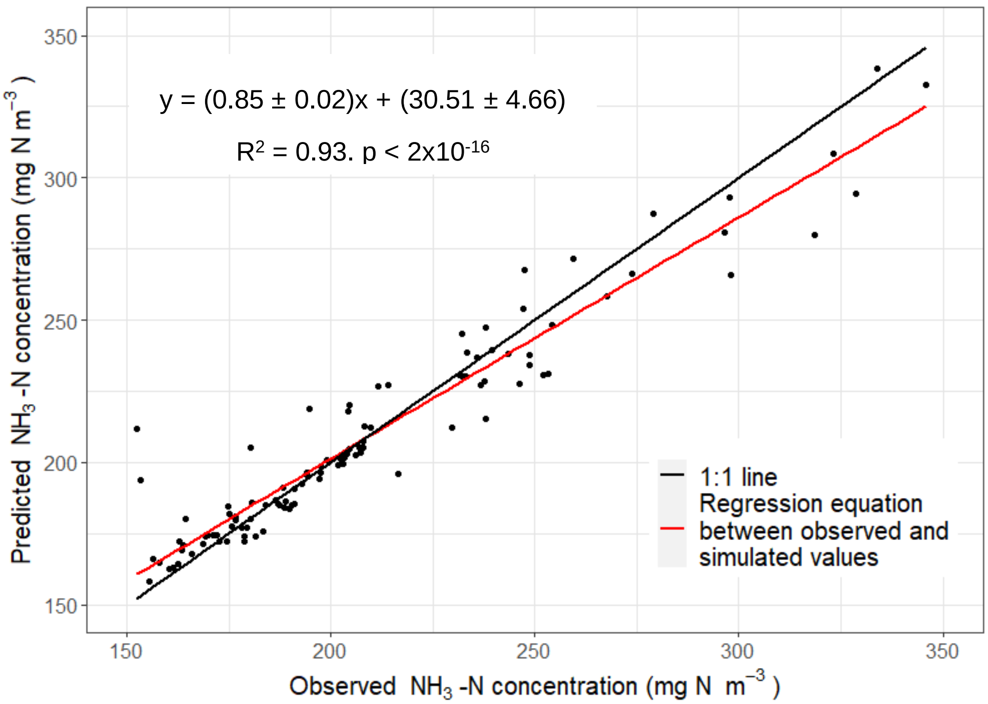

Figure 23. Observed and simulated NH3-N concentrations using the statistical model (RF)

Measured and simulated NH_3-N concentration time series are presented in Figure 24. RF was able to fit extremely well the daily fluctuations of ammonia when compared to the mechanistic model. Moreover, the simulated concentrations from the LHM2 sample also showed a good fit for 361 observations which were not used for the training process (mean: 201.6 mg N m^{-3}, RMSE = 10.8 mg N m^{-3}, R^2 = 0.91). These results are presented in Figure 25.

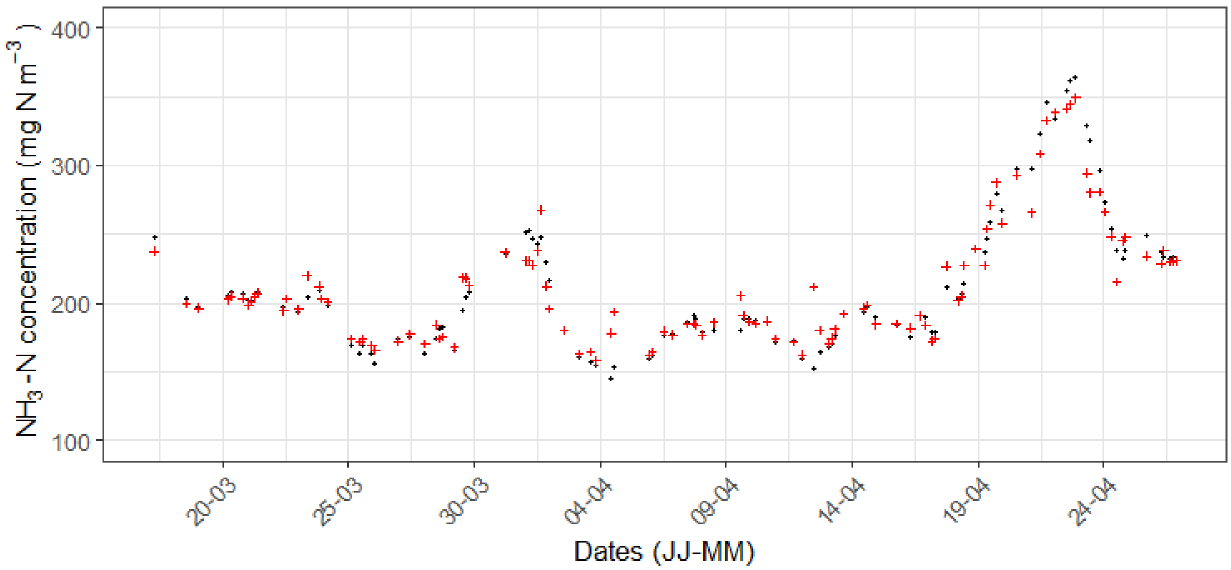

Figure 24. Simulated (+) and observed (•) NH_3-N concentration time series for the testing process in RF using LHM1 data

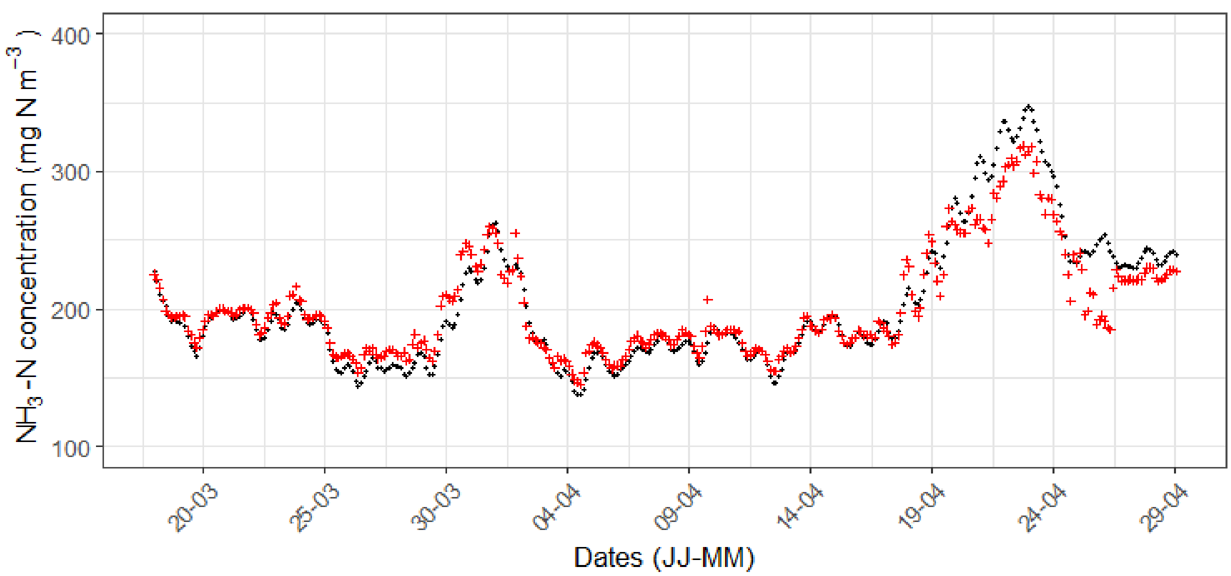

Figure 25. Simulated (+) and observed (•) NH_3-N concentration time series using the LHM2 data

4.4.5.3 Variable importance

The relative importance of selected predictors for the RF model is presented in Figure 26. Water content of the laying hen manure (H20) and outdoor temperature (OutTemp) were the most important predictors, followed by the CO_2 concentrations (CO2) and the atmospheric pressure (Pressure). The random number variable did not have any importance in the modelling process; validating the consistency of the variable importance analysis performed by the RF model.

Water content in laying hen manure increased the MSE (~ +0.03%) of simulated NH_3-N concentrations. Indeed, water molecules participate in different chemical reactions during the decomposition of uric acid, where NH_3 and CO_2 are the main outputs of either volatilization or mineralization in the manure matrix (Mowrer *et al.*, 2014). Moreover, temperatures (~ +0.025% in MSE) may affect the convective mass transfer at the manure - free air interface (Ni, 1999).

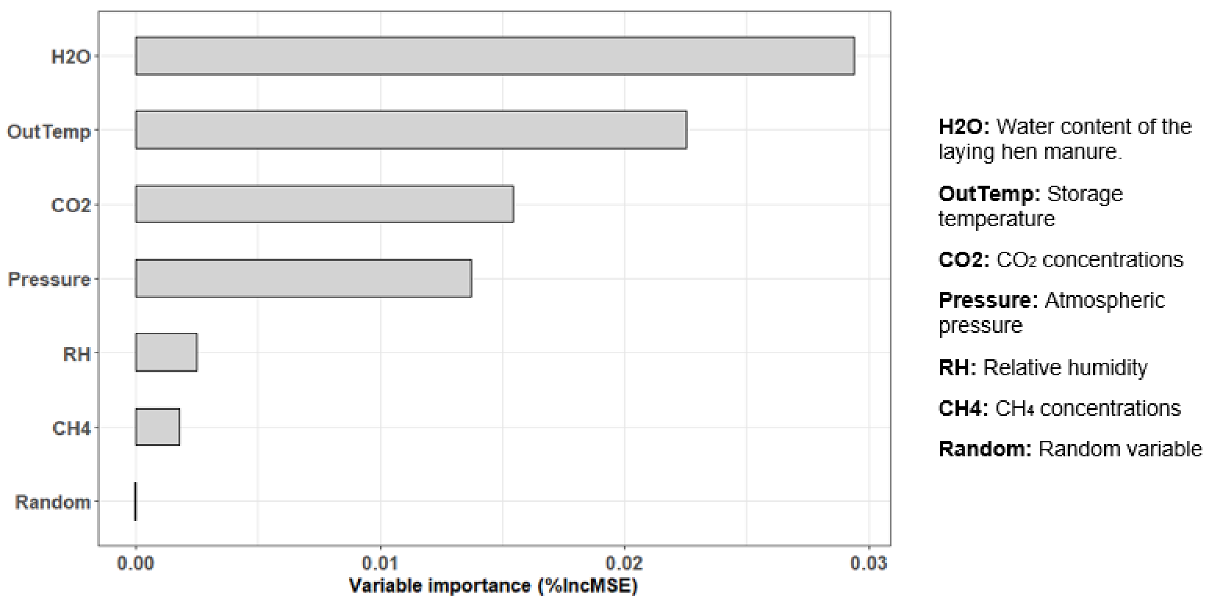

Figure 26. Predictive variable importance by increment of the MSE (%)

An ACP analysis was carried out with the seven predictors considered for RF modelling. The first axis was composed of water content in manure samples (H20), outdoor temperature (OutTemp) and relative humidity (RH) variables, reproducing 36.9% of the data variability. Further, CH_4 concentration (CH4), atmospheric pressure (Pressure) and CO_2 concentration (CO2) constituted the second axis which explained 31.2% of the entire data variability. A cumulative proportion of 68.1% of data variability was explained with the two first axes (Figure 27). These results confirmed the variable importance analysis of the RF model which had a similar order of variable importance for modelling with the H20 and OutTemp variables having important effect in data variability.

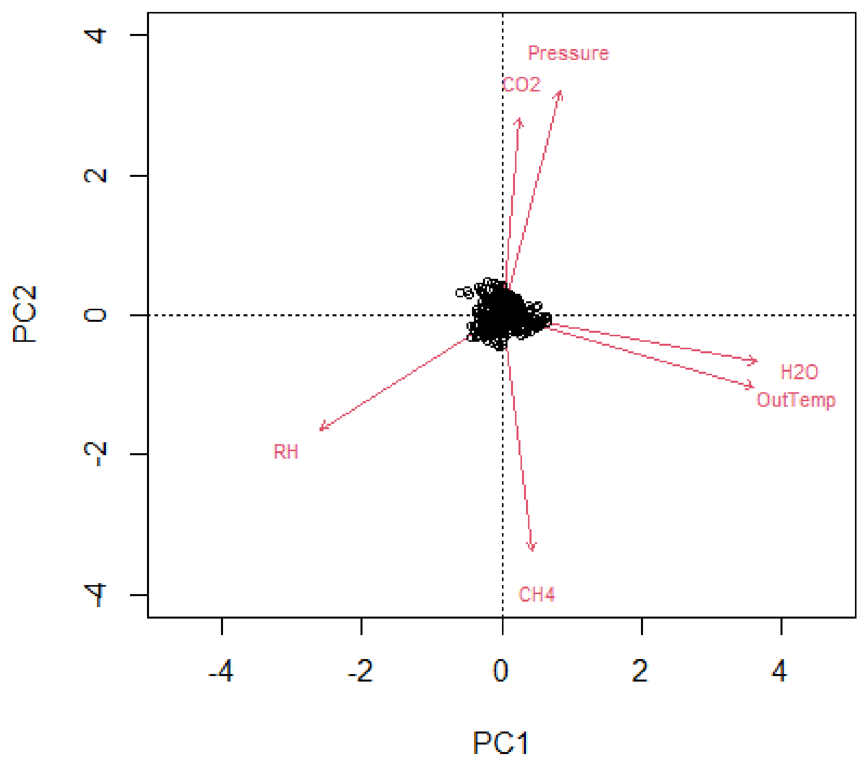

Figure 27. ACP analysis by projecting Euclidian distances in 2D-space from a set of variables selected to predict NH_3-N concentrations over manure storage.

4.5 Conclusions

Two modeling techniques were applied to predict ammonia concentrations in laying hen manure storage tanks under laboratory conditions (rMM and RF). *rMM* did not prove to be a satisfactory approach for simulating NH_3-N concentrations (RMSE of 61.5 mg N m^{-3}), but RF showed a good level of accuracy (RMSE of 10.9 mg N m^{-3}); highlighting the potential of using machine learning (ML) tools for environmental tracking within manure storage units. Process-based models are meant to be sensitive to parameters known to govern system changes. For example, some parameters, such as the Freundlich coefficient (K_f), are empirically derived under controlled conditions. Thus, there is a high likelihood of inducing a systematic bias in *rMM* results since these conditions are rarely perfectly matched under actual conditions. On the other end, a statistical approach such as RF can provide a satisfactory performance even with a small number of observations. Nevertheless, prediction accuracy could be affected if the input data were out of the range of data used during the training process. The use of large datasets over long periods of time is always recommended in *ML* approaches.

In this study, an analysis of variable importance used for the development of the RF model was successfully performed for prediction of ammonia concentration from experimental storage tanks. Results were consistent with known physicochemical phenomena governing the gas emission process and an ACP analysis. The water content of manure and the air temperature were deemed the most relevant variables to predict ammonia concentrations in the experimental laying hen manure storage tanks.

In summary, this study highlighted the usefulness of a statistical modeling approach to simulate a complex biosystem. This suggests that *ML* could provide a highly suitable approach to describe ammonia emission in livestock farms. That being mentioned, it is highly recommended to use critical and logical thinking to validate the statistical model in terms of the physical, biological, or chemical phenomena governing the subject of interest. Additional studies are also recommended to assess the modeling performance of ammonia emissions in commercial poultry farms using these *ML* techniques. The outcome of this study could prove to be useful for guiding future research based on the implementation of *ML* techniques for environmental control, mitigation scenarios of gas emissions and efficiency purposes in commercial poultry farms.

4.6 Acknowledgements

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5 DISCUSSION GÉNÉRALE ET CONCLUSION

5.1 Analyse des techniques de mitigation environnementale (TME)

La mise en place de trois TME a été bien accomplie. Celles-ci incluaient : la diminution de l'espace litière offert aux poules, l'utilisation d'une plaque chauffante au plancher, ainsi que l'application de biochar, ces deux dernières étant combinées avec l'aspersion d'une émulsion comestible à base d'huile végétale. L'évaluation des effets de ces TME sur le bien-être des animaux a mis en évidence : (i) un impact négatif sur la distribution spatiale des poules avec la réduction de l'espace litière favorisant ainsi des comportements agonistiques, et un éventuel stress animal avec des comportements anormales récurrents, et (ii) la faisabilité d'utiliser les trois stratégies sans avoir un effet sur les comportements naturels des poules, même si la distribution spatiale a été perturbée par la réduction de la litière. Ceci a été aussi observée par Albentosa *et al.* (2007) et Engel *et al.* (2018).

Par ailleurs, l'analyse sur le taux ponte et la propreté des œufs a montré l'applicabilité de ces TME offrant une meilleure qualité de l'air sans avoir eu un effet sur la performance d'un poulailler en termes de quantité d'œufs pondus par poule. En revanche, il a été remarqué que les TME n'ont pas eu une influence sur la propreté des œufs, car les mêmes impacts négatifs ont été observés dans les salles expérimentales en volière atteignant les mêmes conclusions au sujet d'une quantité plus élevée d'œufs sales rapportés. Autrement dit, les TME proposés n'ont pas aidé à réduire cet aspect négatif caractérisant les systèmes en volière (Philippe *et al.*, 2020). Il est important de noter que les TME ont été établis, en principe, pour améliorer la qualité de l'air dans le poulailler.

Bien que des différences significatives sur les conditions ambiantes à l'intérieur du bâtiment et les émissions des gaz entre traitements et le contrôle aient été observées, celles-ci étaient inférieures aux seuils recommandés dans la littérature. La température a varié entre 21°C et 23°C, des températures optimales pour les poules selon Holik (2015) et Oloyo (2018). L'humidité relative a varié entre 20% et 40%, des valeurs inférieures à 85%, seuil de la prolifération des microorganismes produisant des problèmes de santé pour les animaux (Oloyo, 2018). De plus, les concentrations de NH_3 et CO_2 étaient inférieures aux concentrations recommandées de 25 ppm et 2500 ppm selon Oloyo (2018). Ainsi, les émissions d'ammoniac et des gaz à effet serre étaient similaires aux émissions rapportées dans la littérature dans les cas de systèmes avicoles. Ces conditions ont permis de mettre en place une ambiance d'élevage optimale tant pour les poules que pour l'environnement tout au long de la période de production étudiée. La comparaison des conditions météorologiques, ainsi que des émissions des gaz est discuté plus en détails par Gonzalez-Mora *et al.* (2020a).

5.2 Modélisation de la production des œufs de consommation

L'utilisation d'un modèle statistique de type *Random Forest* (RF), en tant qu'outil de prédiction de la courbe du taux de ponte d'un poulailler, a mis en évidence le potentiel de cet algorithme d'apprentissage autonome dans ce type biosystème complexe avec une performance satisfaisante (R^2 : 0.77 et RMSE : 0.3%). Ceci a également été observé par divers auteurs qui ont utilisé le *Machine Learning* (ML) pour obtenir des systèmes d'alerte dans les systèmes de poules pondeuses à l'aide de réseaux de neurones (Ahmad, 2011; Ramírez-Morales *et al.*, 2017) ou *Support Vector Machines* (SVM) (Akilli & Gorgulu, 2020; Gorgulu & Akilli, 2018).

Le modèle RF se caractérise par sa simplicité et sa versatilité sur différents aspects, à savoir : (i) une haute capacité de travailler avec une quantité limitée de données, (ii) une bonne performance en utilisant des prédicteurs de différentes origines, et (iii) une quantité minimale de paramètres à régler pour la modélisation (Liaw & Wiener, 2002; Polikar, 2006). L'étude a mise en œuvre la conformité des caractéristiques du RF ci-mentionnées. Dans ce cas précis, la combinaison du RF avec une technique d'analyse de séries de données ordonnées, c-à-d., la moyenne mobile, a permis de développer un modèle capable d'anticiper les anomalies journalières du taux de ponte dans un élevage expérimental, en utilisant des variables météorologiques et environnementales à l'intérieur du bâtiment, ainsi que les conditions de ventilation du site. De plus, l'étude de l'importance des variables a illustré le besoin de bien contrôler la température et l'humidité relative dans les systèmes en volière, si les conditions des concentrations des gaz correspondent aux seuils optimaux recommandés.

5.3 Modélisation des concentrations d'ammoniac

La modélisation des concentrations d'ammoniac dans le stockage de fientes de poules pondeuses a été réalisée en utilisant deux approches, à savoir : un modèle mécaniste et un modèle statistique de type *Random Forest* (RF). Le modèle mécaniste proposé par Liu *et al.* (2009) et Tong *et al.* (2020) a été reproduit avec succès, néanmoins, celui-ci n'a pas été satisfaisant en ce qui a trait à la prédiction des concentrations d'ammoniac en comparaison avec les concentrations mesurées dans les salles expérimentales. Cela ne veut pas dire que ces types de modèles ne peuvent pas avoir une bonne performance. Au contraire, plusieurs auteurs ont démontré l'applicabilité de la modélisation mécaniste pour caractériser les émissions d'ammoniac dans les systèmes d'élevage (Berthiaume *et al.*, 2005; Bjerg *et al.*, 2013; Liu *et al.*, 2009; Monteny *et al.*, 1998; Montes *et al.*, 2009; Ni, 1999; Sommer & Olesen, 2000; Tong *et al.*, 2020). Cependant, la performance de ces modèles peut être altérée par différents aspects, à savoir : (i) la complexité inhérente dans les émissions d'ammoniac induite par différents processus physiques, chimiques ou biologiques, (ii) le recours à une quantité élevée de paramètres, parfois, difficiles à mesurer in-situ et même différentes de ce qui peut être observé en laboratoire, et (iii) la sensibilité associée à ces paramètres (Ni *et al.*, 2011).

Par ailleurs, le modèle statique RF a obtenu une haute performance dans la prédiction des concentrations d'ammoniac émises durant le stockage des fientes en conditions contrôlées (R^2 : 0.93 et RMSE : 10.9 mg N m^{-3}). À date, l'application du RF dans le domaine de la gestion des fientes est peu documentée dans la littérature. Néanmoins, Chen *et al.* (2008) ont proposé une méthode pour déterminer les caractéristiques chimiques des fientes de poules pondeuses à l'aide d'un réseau de neurones (ou Artificial Neural Networks : ANN) en utilisant des caractéristiques physicochimiques, à savoir : le pH, la conductivité électrique et la densité relative. Chen *et al.* (2008) ont également obtenu des résultats performants avec des ANN en comparaison avec des modèles linéaires. Ni *et al.* (2011) ont par ailleurs proposé l'application de *Data Mining* et ANN afin de générer un lien entre les avancements en termes de la modélisation et les inventaires globales des émissions d'ammoniac crées au fil des années. Certes, ceci met en évidence le potentiel de recherche et l'applicabilité des algorithmes de type *Machine Learning* dans la filière de gestions des fumiers des sites d'élevage de poules pondeuses afin d'avancer les connaissances sur l'empreinte environnemental inhérente à ce domaine.

5.4 Systèmes d'élevage des poules pondeuses plus autonomes

Les systèmes d'élevage de poules pondeuses sont les lieux de diverses interactions complexes. Premièrement, les interactions biologiques découlant de la manipulation d'animaux vivants. Ces interactions sont tributaires de la santé des volailles, des processus de respiration, du métabolisme et de la digestion de l'animal, du taux de croissance ; mais aussi elles sont aussi les fruits des processus microbiologiques dans la dégradation des composées azotées ou carboniques à l'intérieur de la tract digestive de l'animal, dans la litière générée par les animaux ou dans l'air ambient du bâtiment (Fournel *et al.*, 2017; Sánchez-Monedero *et al.*, 2001; Wrest Park History, 2009). Deuxièmement, il y a les interactions chimiques issues des réactions chimiques à l'intérieur de la poule suivant sa physionomie, de même que dans son entourage (*e.g.*, dégradation de l'acide urique des excretas, réaction acido-basique constante entre l'ammonium et l'ammoniac dans les fientes) (Mowrer *et al.*, 2014; Ni *et al.*, 2009). Troisièmement, il y se produit également des interactions physiques, à savoir : les flux de chaleur entre les animaux et leur environnement, les interactions entre l'air de l'extérieur et celle à l'intérieur du site d'élevage, le transfert de masse des concentrations de gaz entre la litière, soit dans le site d'élevage soit dans le stockage, avec l'air ambiante ; ou le changement climatique (Fournel *et al.*, 2017; Ni, 1999). En parallèle, les avancements technologiques, les demandes des consommateurs, ainsi que l'empreinte environnementale, apportent aussi de la complexité à ces biosystèmes (Pelletier *et al.*, 2018).

L'équilibre entre ces interactions est le sujet d'intérêt de l'élevage de précision (Precision Livestok Farming). Par exemple, un équilibre entre le régime alimentaire, la consommation de la nourriture, et le dégagement d'ammoniac dans le site d'élevage, peut aider à avoir de meilleures conditions environnementales, ainsi que des économies à la ferme. En effet, une régime alimentaire, avec des quantités contrôlées de protéines, peut conduire à une diminution du taux d'azote excrété par la poule dont les deux tiers sont sous forme d'acide urique (Ritz *et al.*, 2004). Ce qui peut diminuer aussi les quantités d'ammoniac dégagées dans la litière au cours d'une période de production (Wrest Park History, 2009). Plusieurs exemples peuvent être décrits pour d'autres interactions. C'est pourquoi, les systèmes d'élevage des poules pondeuses sont en train de se diriger vers des systèmes plus autonomes où il existe un contrôle des différents paramètres, ainsi qu'une automatisation à l'aide de différents équipements des mesures. Cependant, la modélisation a un rôle très important dans ce domaine, établissant la connexion entre ce qui peut être mesuré et ce qui peut être contrôlé.

5.5 Défis et opportunités de la modélisation à l'aide de modèles statistiques dans les systèmes d'élevage de poules pondeuses.

La filière de production des œufs de consommation a plusieurs composantes et chacune peut avoir des impacts négatifs sur l'environnement, sur l'entourage de la production, sur l'économie de la ferme ou sur une ville quelconque. Ces conséquences peuvent affecter aussi les animaux, ainsi que les travailleurs. Les résultats de cette étude ont permis de faire l'observation et l'analyse de ces conséquences pour deux composantes, à savoir : la production à la ferme et le stockage des fientes de poules pondeuses, en l'occurrence les conditions environnementales, ainsi que le bien-être des animaux. D'ailleurs, l'étude s'est penchée sur l'analyse de la mise en œuvre de trois stratégies de contrôle environnemental (TME) pour réduire l'empreinte environnementale dans les systèmes en volière de poules pondeuses. Cette étude a également contribué avec l'application d'un modèle statistique de type *Random Forest* à la possibilité de mettre en place de systèmes plus autonomes dans les sites de production animale.

L'application du modèle *Random Forest* a permis d'illustrer toute l'autonomie liée à aux processus statistiques menés à l'intérieur de ces algorithmes afin de prédire une variable. Parfois, cette autonomie peut produire des modèles qui ne permettent pas d'avancer notre compréhension. C'est pourquoi, il est bien recommandé de toujours confronter la modélisation en utilisant ces algorithmes de *Machine Learning* avec la pensée critique et ce afin de valider les résultats produits par ce type de modèles.

De plus, la compilation d'une grande quantité des données est aussi un aspect important de la modélisation avec des outils statistiques. Puisque, dans le cas de *Random Forest*, une quantité élevée des données permettra un apprentissage plus performant du modèle, menant ainsi à des résultats plus fiables.

D'ailleurs, bien que la performance du *Random Forest* ait été mise en œuvre dans cette étude, il est recommandé aussi d'évaluer l'applicabilité du modèle à l'échelle commercial où, probablement, l'historique de données enregistrées au cours des années pourra aider au développement de modèles statistiques encore plus performantes.

Enfin, il est important aussi de promouvoir le développement des systèmes de contrôles autonomes avec la participation de senseurs, d'outils informatiques, ainsi que d'actionneurs. Ceci dans le but de construire des sites d'élevage ayant une production plus contrôlée et automatisée, respectueuse du bien-être des animaux et de l'environnement en général.

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7 ANNEXE I

Table 8. Relative frequencies of spatial occupancy (SO, %) of laying hens as a function of treatment

REGION	BATCH 1				BATCH 2			
	T17	HFOS	AOS	Ctrl	T17	HFOS	OS	Ctrl
Nest	4.3 ± 2.3	9.6 ± 6.0	8.4 ± 6.0	1.7 ± 2.5	20.0 ± 2.6	12.6 ± 3.0	10.7 ± 2.9	7.5 ± 4.0
Litter	8.9 ± 0.8	28.9 ± 4.7	26.7 ± 1.3	29.7 ± 6.7	11.6 ± 2.6	27.0 ± 2.5	25.6 ± 1.4	31.6 ± 4.4
Perches	27.3 ± 6.0	26.1 ± 9.5	26.6 ± 4.1	27.1 ± 5.5	26.0 ± 3.6	23.0 ± 7.5	27.3 ± 4.2	22.8 ± 2.8
Feeder	27.8 ± 9.0	16.2 ± 2.6	20.5 ± 1.5	22.3 ± 7.5	18.2 ± 4.7	17.8 ± 2.9	19.8 ± 2.0	19.5 ± 1.1
Drinker	10.9 ± 1.8	7.5 ± 1.8	8.6 ± 3.6	9.9 ± 1.0	5.6 ± 2.1	7.1 ± 3.0	4.7 ± 2.0	5.6 ± 1.4
Nest-Feeder	20.8 ± 2.0	11.8 ± 4.5	9.3 ± 0.2	9.2 ± 3.4	18.6 ± 2.8	12.5 ± 4.2	11.9 ± 3.8	12.9 ± 2.2

Table 9. Average ± SD of number of laying hens' behaviors for each experimental setup.

BEHAVIOUR	BATCH 1				BATCH 2			
	T17	HFOS	AOS	Ctrl	T17	HFOS	OS	Ctrl
Scratch	1 ± 0	1 ± 1	1 ± 1	0 ± 0	3 ± 2	7 ± 3	6 ± 7	4 ± 3
Kneel Down	9 ± 1	5 ± 3	4 ± 4	5 ± 1	9 ± 4	7 ± 3	10 ± 5	9 ± 5
Ruffle Feathers	2 ± 1	3 ± 1	3 ± 1	4 ± 2	4 ± 2	5 ± 1	2 ± 2	4 ± 2
Body Shaking	3 ± 4	4 ± 2	5 ± 3	2 ± 1	3 ± 2	4 ± 1	3 ± 1	3 ± 2
Preening*	13 ± 3	16 ± 2	14 ± 3	16 ± 1	21 ± 2	20 ± 4	21 ± 8	23 ± 5
Dustbathing*	2 ± 1	3 ± 3	4 ± 1	3 ± 1	2 ± 1	3 ± 2	3 ± 2	1 ± 1
Perching*	12 ± 5	13 ± 5	16 ± 5	11 ± 2	20 ± 5	15 ± 2	21 ± 7	16 ± 3
Feeding*	14 ± 6	14 ± 5	13 ± 2	11 ± 4	19 ± 3	20 ± 3	21 ± 3	18 ± 3
Drinking*	10 ± 3	9 ± 2	7 ± 3	9 ± 4	6 ± 3	5 ± 3	7 ± 1	6 ± 4
Other	-	-	-	-	3 ± 1	5 ± 1	7 ± 2	3 ± 1
nBr	-	-	-	-	8 ± 3	6 ± 6	8 ± 1	5 ± 3

8 ANNEXE II

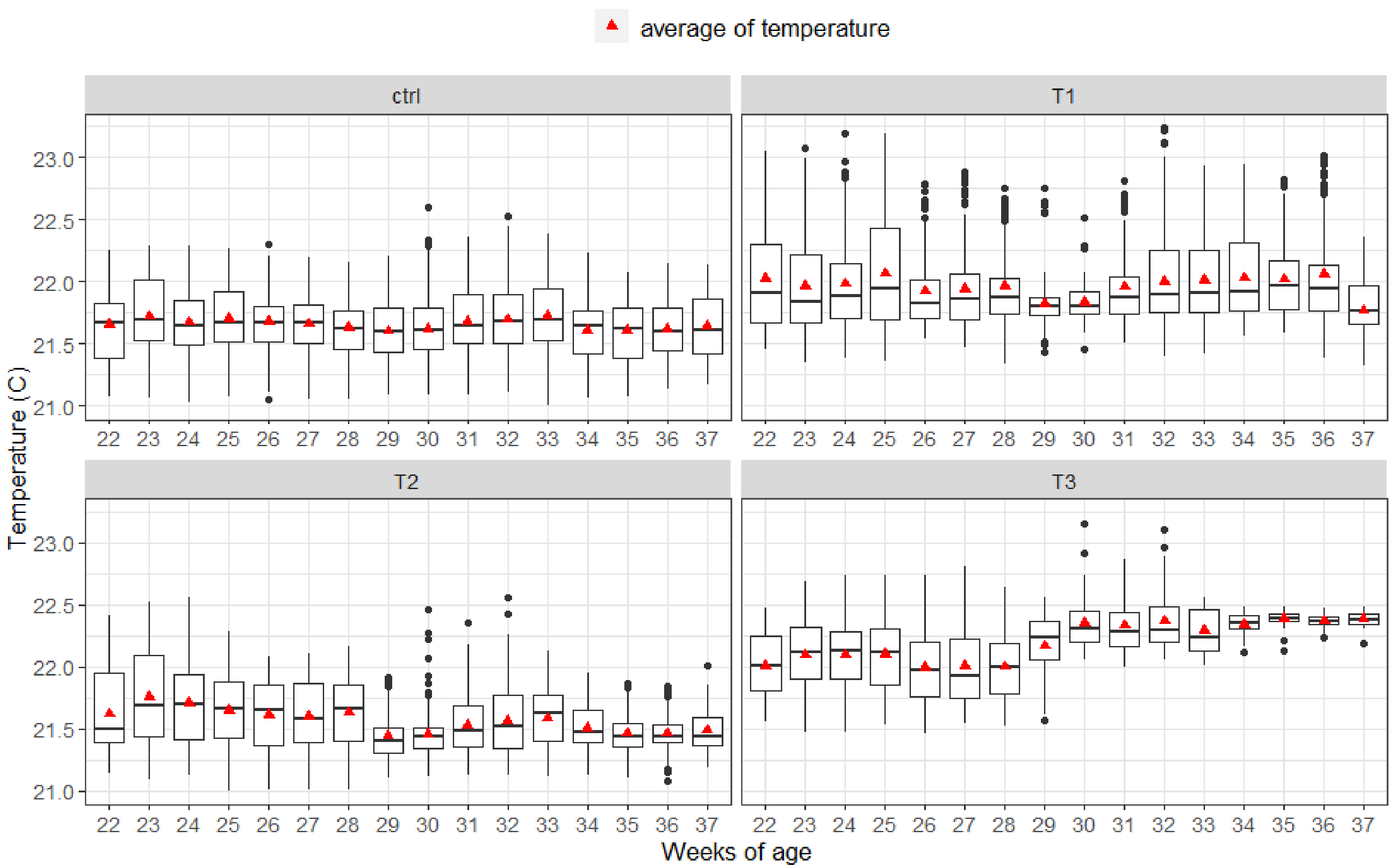

Figure 28. Weekly data distribution of temperature by treatment

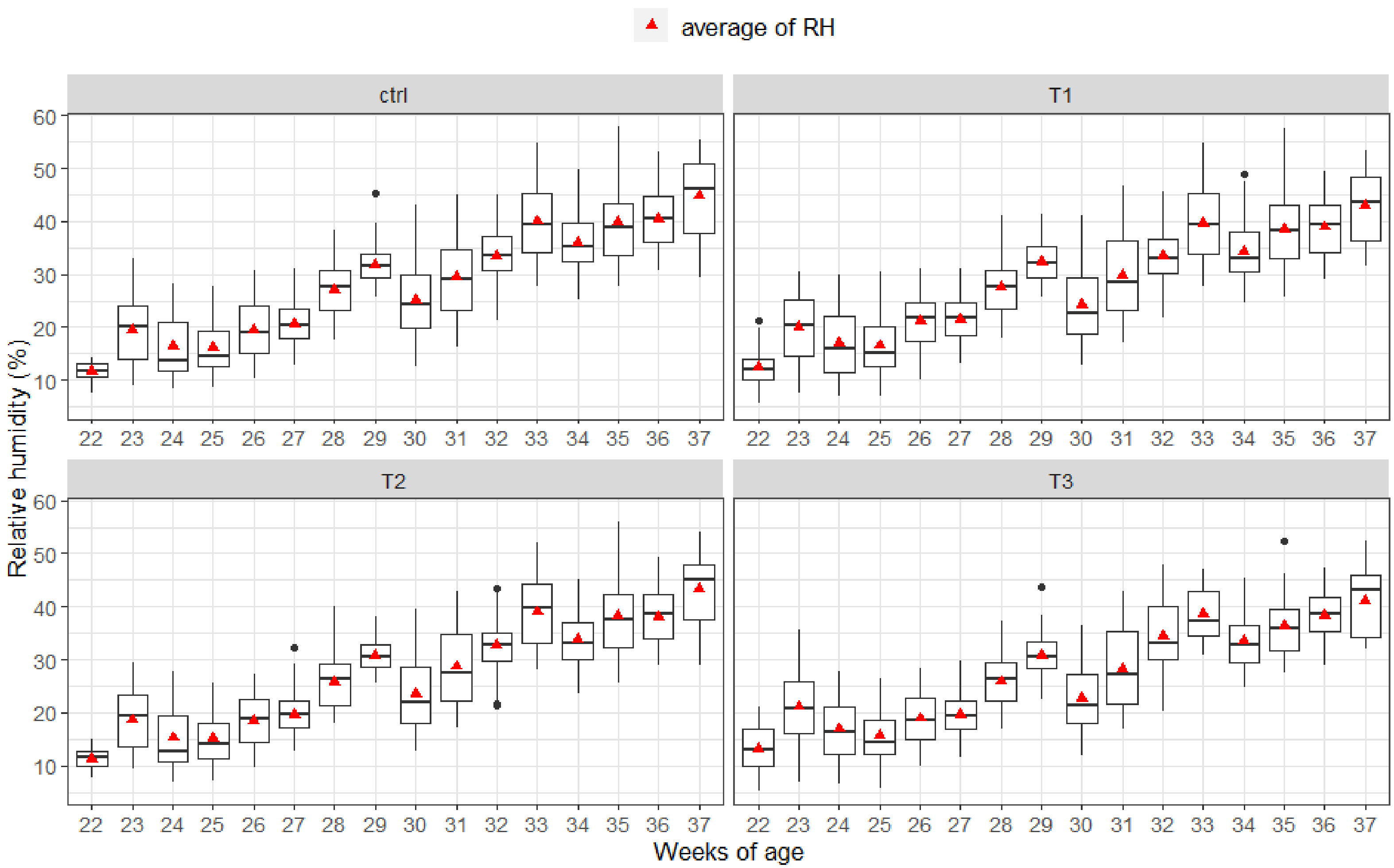

Figure 29. Weekly data distribution of relative humidity by treatment

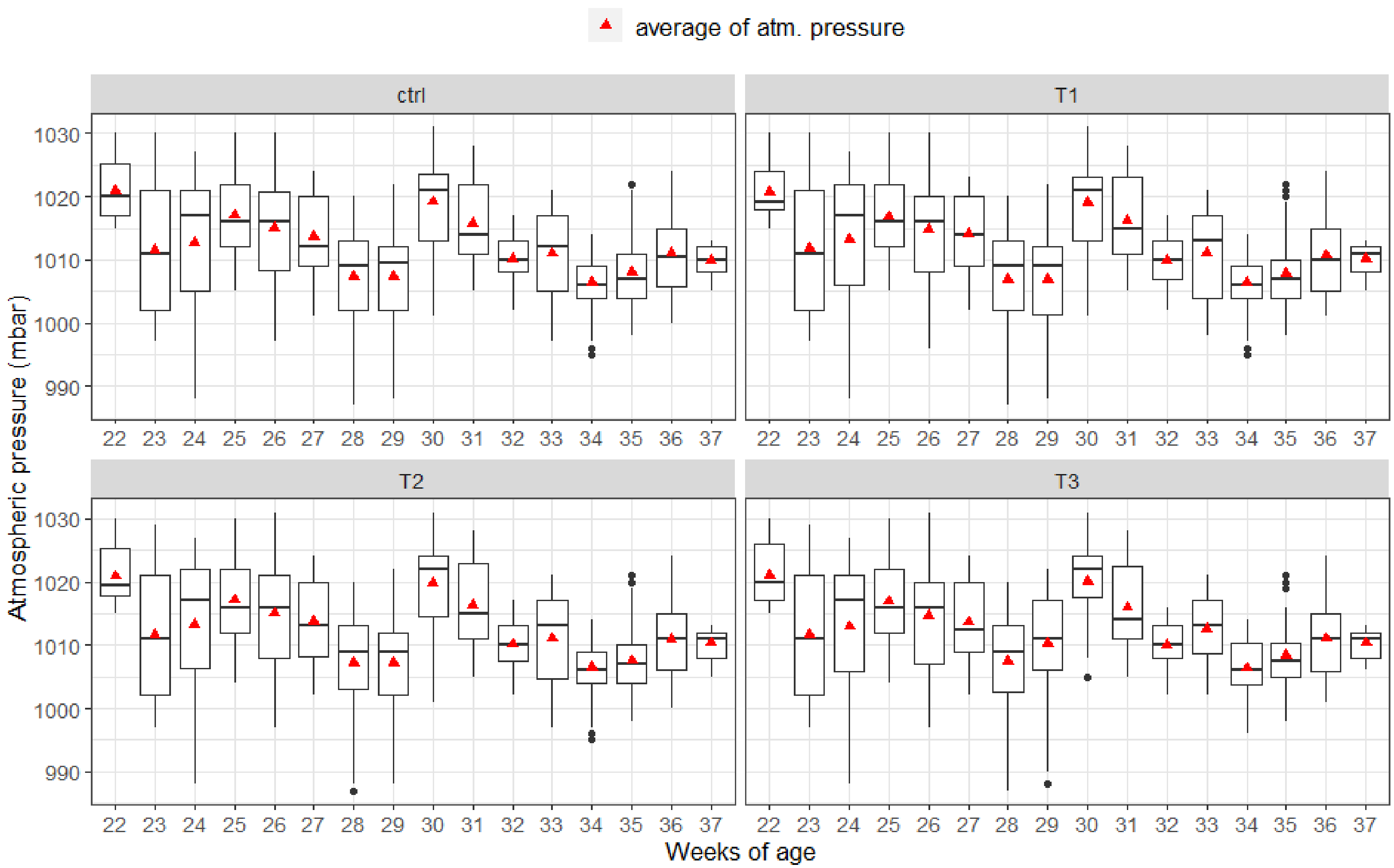

Figure 30. Weekly data distribution of atmospheric pressure by treatment

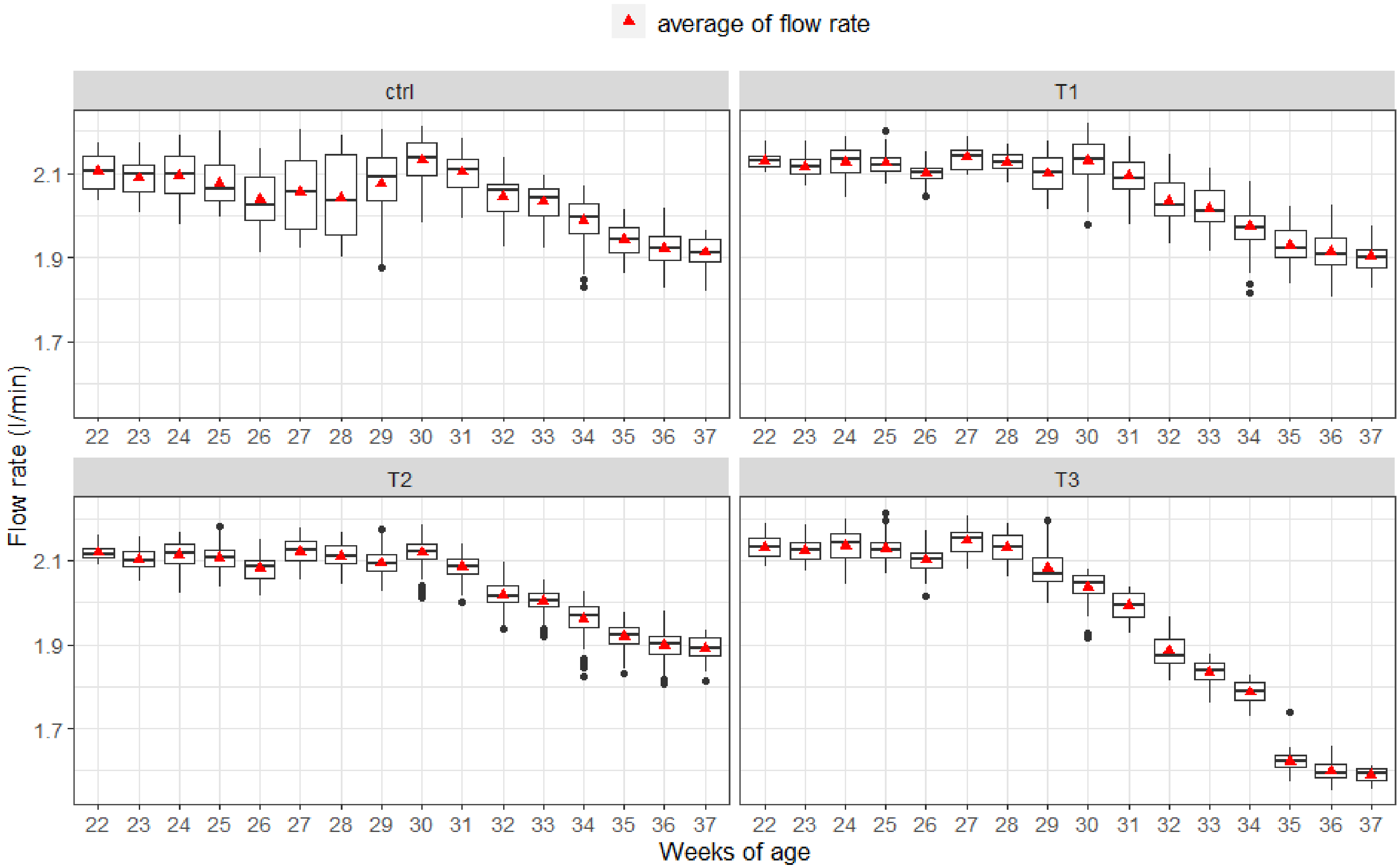

Figure 31. Weekly data distribution of airflow rate by treatment

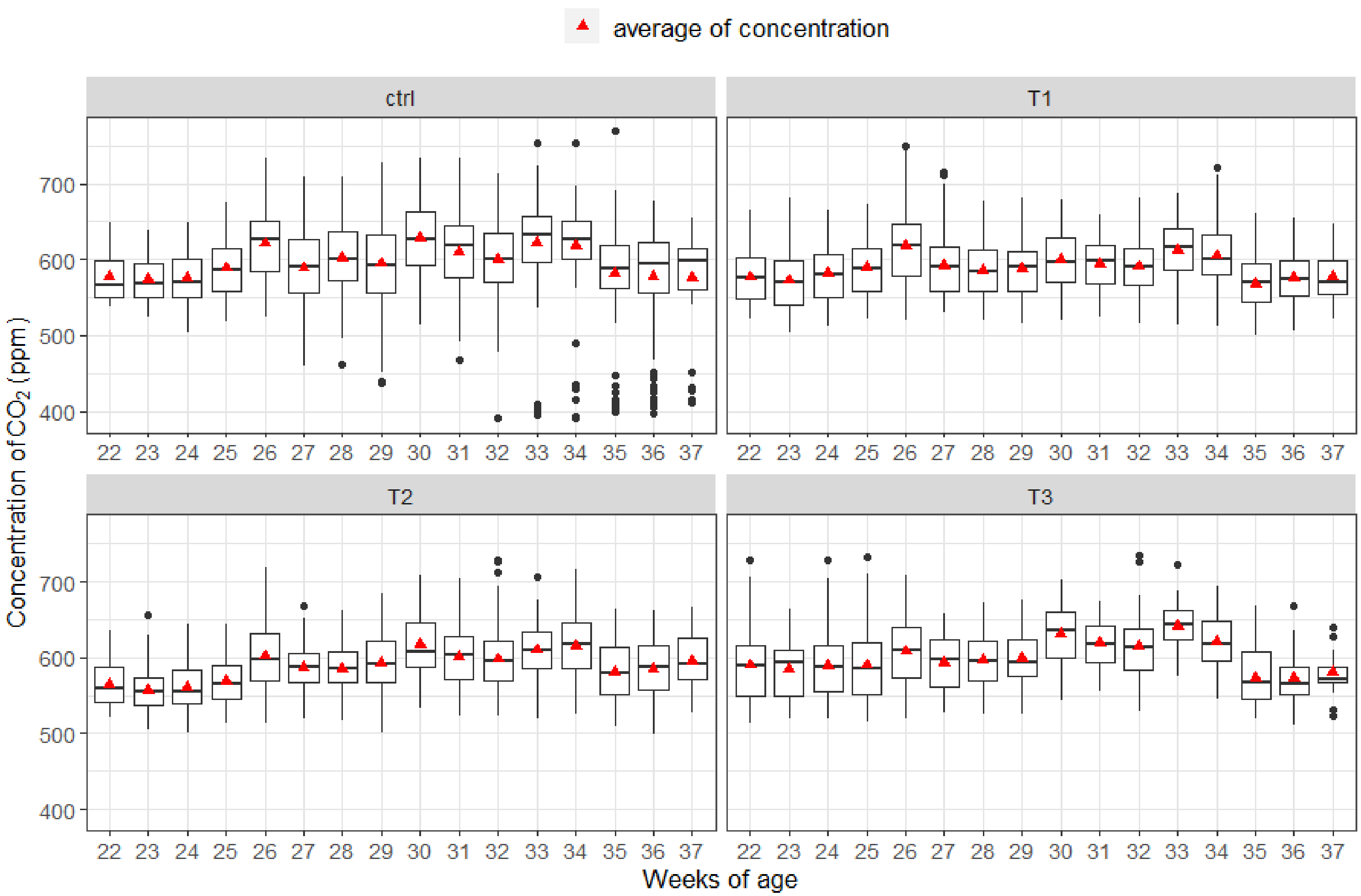

Figure 32. Weekly data distribution of CO_2 concentrations by treatment

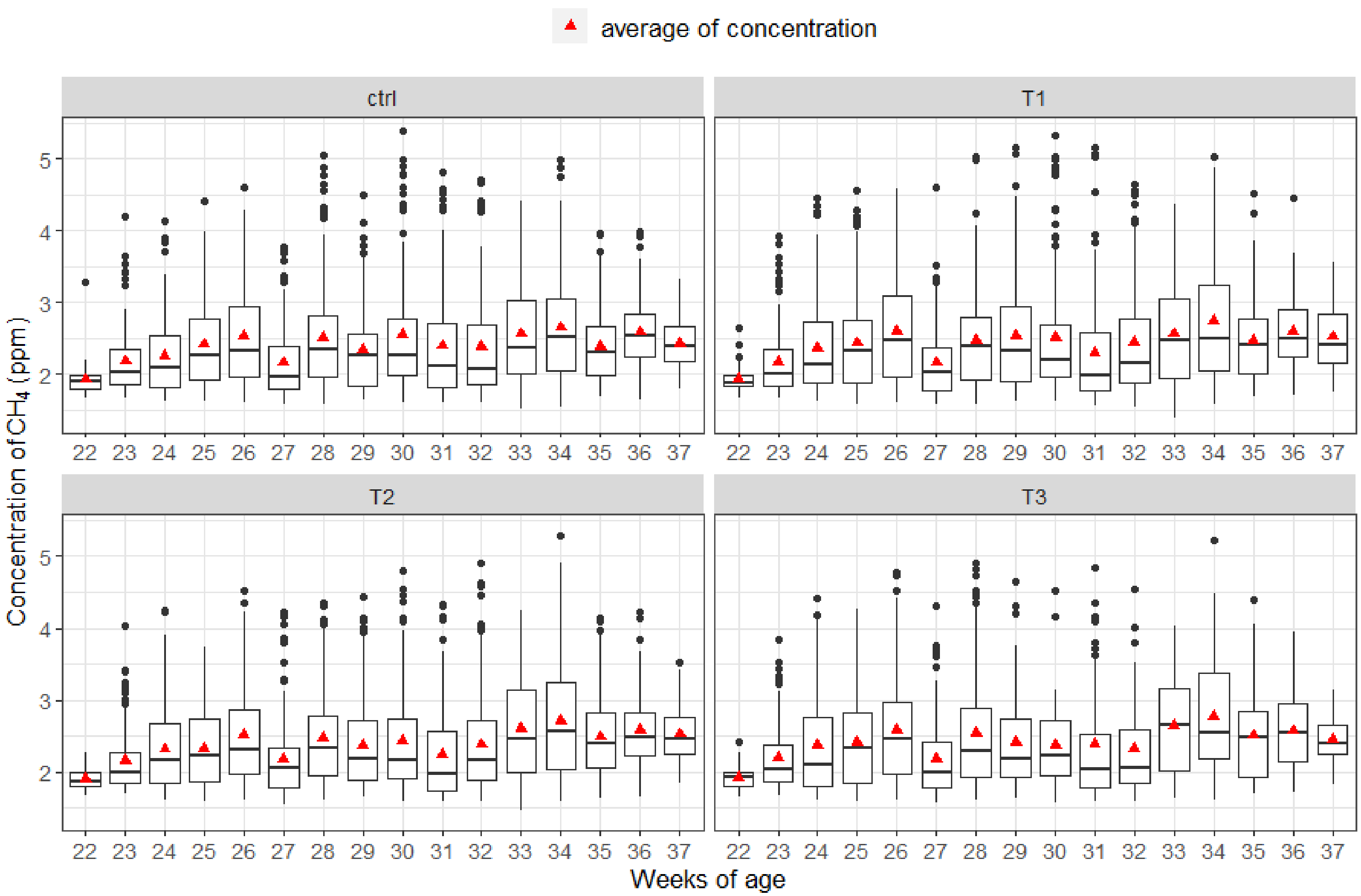

Figure 33. Weekly data distribution of CH_4 concentrations by treatment

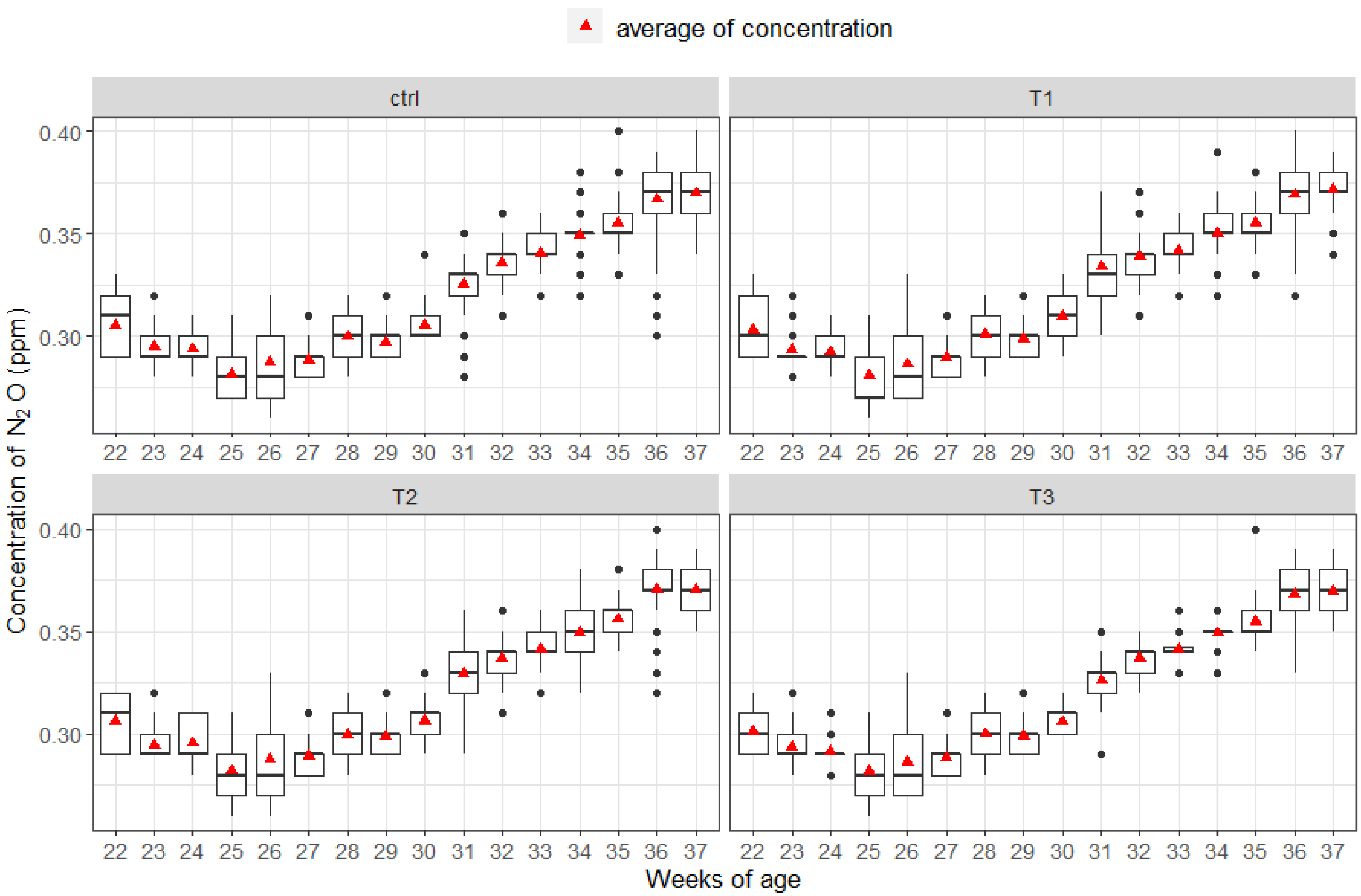

Figure 34. Weekly data distribution of N_2O concentrations by treatment

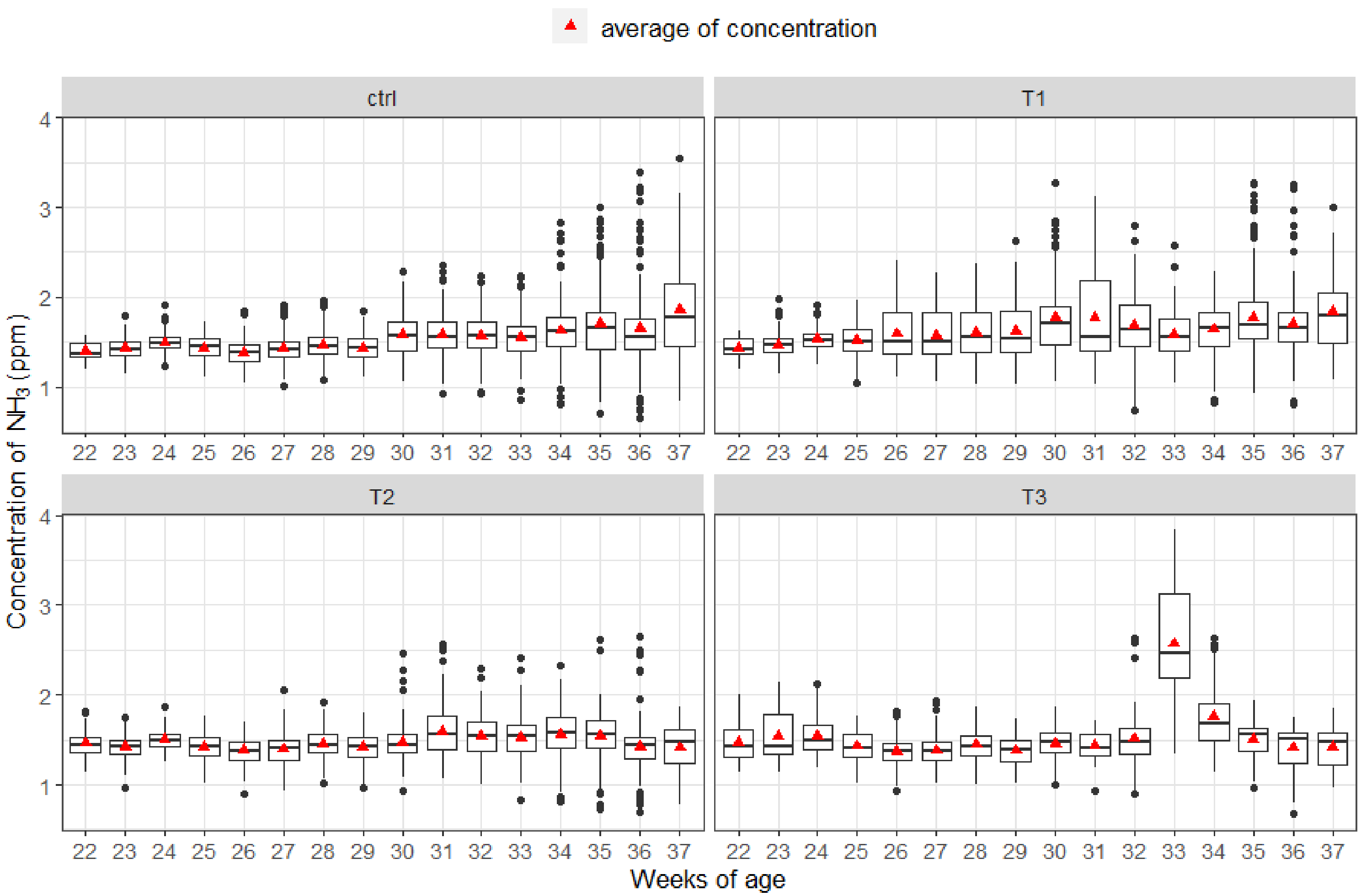

Figure 35. Weekly data distribution of NH_3 concentrations by treatment