Chapter

Review of Measures to Control Airborne Pollutants in Broiler Housing

José L.S. Pereira, Carla Garcia and Henrique Trindade

Abstract

Broiler housing is a significant source of airborne pollutants from animal production, which lead to degradation of indoor air quality and outdoor emissions, particularly ammonia, nitrous oxide, carbon dioxide, methane, hydrogen sulphide, odours and particulate matter. In this chapter, we first analyse the current state of the art on the consequences of these pollutants on broiler farming, farm workers, and the environment. This includes the factors affecting pollutants generation, quantification, and mitigation measures suppressing airborne pollutants. Next, we describe different best available techniques for environmental protection and sustainability of broiler production, namely feeds and feeding management, feed supplements, bedding management and treatment of exhaust air. Thus, broiler farms should select mitigation strategies based on several considerations, such as location, climate conditions, environmental policies and financial resources.

Keywords: ammonia, broiler, greenhouse gases, mitigation measure, odours, particulate matter

1. Introduction

Broiler meat is one of the main sources of protein for humans in the Mediterranean region and its consumption is expected to continue to increase until 2050. Globally, 337 million tons of meat were produced in 2019, 44% more than in 2000, with chicken meat representing more than half the increase [1]. In 2021, almost 121.5 million tons of broiler meat were produced in the World, from 74×10^9 broiler heads; in the same year, Europe produced ca. 19.5 million tons of broiler meat (11.3×10^9 heads) with Southern European countries being responsible by 17% of Europe production [2]. It has been estimated that around 10% of the carbon (C) footprint, land use and acidification potential caused by the European food basket is directly attributable to broiler meat consumption [3].

Broiler chickens have strict requirements in terms of thermal conditions and require high tech housing and management, equipped with automatic systems for feeding, drinking, heating, cooling, air conditioning and various sensors for data acquisition [4]. As can be observed in **Figure 1**, the broiler production includes farms of breeding hens for fertile eggs and of broilers for meat [5, 6]. From chicks to

Figure 1.
Commercial broiler houses of breeding hens for fertile eggs (above) and broilers for meat (below).

20 weeks of age, breeding hens are housed in buildings with a solid floor and litter material (pine shavings or rice hulls). Between 20 and 60 weeks of age, hens are moved to housing with deep litter/slatted floor and the manure is removed once per year. The broilers are housed in similar buildings with a rearing cycle between 30 and 42 days (1.4–2.4 kg liveweight) [4, 7].

The broiler production is a major pollution source of reactive nitrogen (N) losses, and the emissions intensity of chicken meat is 0.6 kg CO_2eq kg^{-1} [1]. This sector is a significant source of ammonia (NH_3), nitrous oxide (N_2O) and methane (CH_4) emissions [8].

This sector is linked to NH_3, N_2O and CH_4 emissions, and have an impact on global greenhouse gas (GHG) emissions, as well as bird and human health. Litter and manure can contain pesticide residues, microorganisms, pathogens, pharmaceuticals (antibiotics), hormones, heavy-metals, macronutrients (at improper ratios) and other pollutants which can lead to air, soil and water contamination as well as formation of antimicrobial/multidrug resistant strains of pathogens. Particulate matter (PM) emitted from intensive broiler production operations contain feather and skin fragments, faeces, feed particles, microorganisms and other pollutants, which can adversely impact poultry health as well as the health of farm workers and nearby inhabitants. Odours are another problem that can have an adverse impact on health and quality of life of workers and surrounding population [9].

Farms are required to comply with environmental legislation to be managed in a sustainable and environmentally friendly way, namely: (i) the Integrated Pollution Prevention and Control (IPPC) Directive (96/61/EC), which requires large livestock production facilities to implement best available techniques (BAT) for environmental protection; (ii) the National Emissions Ceiling (NEC) Directive (2016/2284/EU), which sets emission reduction commitments relative to 2005 levels for N oxides (NOx), non-methane volatile organic compounds (NMVOC), sulphur oxides (SOx), NH_3 and $PM_{2.5}$ by 2030; (iii) the Energy and Climate Action Directive (2018/1999/EU) for carbon neutrality, which targets reducing greenhouse gas (GHG) emissions by 45–55%, based on emissions recorded in 2005 EU Commission's Joint Research Centre reference document (BREF) was introduced in 2017 and provides the BAT guidelines for intensive poultry rearing operations [10].

The aim of this review was to provide a comprehensive overview of current knowledge about the impact of airborne pollutants (NH_3, N_2O, CO_2, CH_4, hydrogen sulphide (H_2S), odours and PM) from broiler houses, including analysis and discussion of best available techniques for environmental protection and sustainability of broiler production.

2. Gases

2.1 Ammonia

Broilers excrete most of the not metabolised N in the forms of uric acid (ca. 80%), NH_3 (ca. 10%) and urea (ca. 5%). The excretions are rich in uric acid ($C_5H_4N_4O_3$), being hydrolysed into urea ($CO(NH_2)_2$) through aerobic decomposition and followed by conversion to NH_4^+ by the urease enzyme found in manure Eq. (1). Then, NH_3 is prone to be released into the air as a gas that can affect both birds and farmworkers and escape to the atmosphere by building ventilation [11, 12].

$$C_5H_4N_4O_3 \underset{Uricase}{\Rightarrow} CO(NH_2)_2 \underset{Urease}{\Rightarrow} [CO_2] + NH_4^+ \underset{Volatilisation}{\Rightarrow} NH_3 \tag{1}$$

In terms of environmental impacts, the emission of NH_3 reacts with ammonium (NH_4^+) sulphate and nitrate (NO_3^-) and chloride particles, being transported over wide areas, adversely impacting air quality and visibility [13, 14]. Also, the wet deposition of NH_4^+ salt formed after NH_3 volatilisation led to toxicity of vegetation in the surrounding farm area (<1 km), whereas the wet deposition can induce soil acidity, severely damaged biodiversity and eutrophication of water bodies [13, 14].

Ammonia volatilisation is a result of complex biological, physical and chemical processes and depends on several factors (**Figure 2**) [11]. Thus, the factors that influence how NH_3 is formed and released into the broiler house environment are litter type, animal activity, bird age and density, manure handling, frequency of manure removal, ventilation rate and air velocity, while the factors that influence how manure bacteria and enzymes break down N to form NH_3 are N content, temperature, moisture/humidity and pH (**Figure 2**) [4, 15]. The litter's pH is a major factor regulating the volatilisation of NH_3 since it specifies the volatile NH_4^+/NH_3 ratio between their ionic and non-volatile forms (**Figure 2**) [16]. Among all factors affecting NH_3 volatilisation in broiler house litter, humidity is one of the most important influencing parameters [17]. Therefore, the increase of indoor air humidity and temperature will increase the moisture and temperature of the litter material and consequently will enhance the NH_3 volatilisation [4, 18]. The water trough should be placed carefully and checked regularly to prevent water leakage on the litter and the litter humidity in broiler houses should be 15–25% [16].

Ammonia, a colourless and highly water-soluble gas, is primarily an irritant and has been known to create health problems for animals in confinement building [19]. Ammonia concentrations in broiler houses should not exceed 25 ppm because birds' productivity is adversely affected above this limit (**Table 1**). Higher NH_3 levels also decrease the body weight gain, calorie conversion, general living conditions, carcass condemnation, and birds' immune system (**Table 1**) [12, 16, 21]. Furthermore, the NH_3 gas has a common pungent odour that humans can detect at concentrations of 20–30 ppm and irritates the conjunctiva, cornea, and mucous membranes of birds'

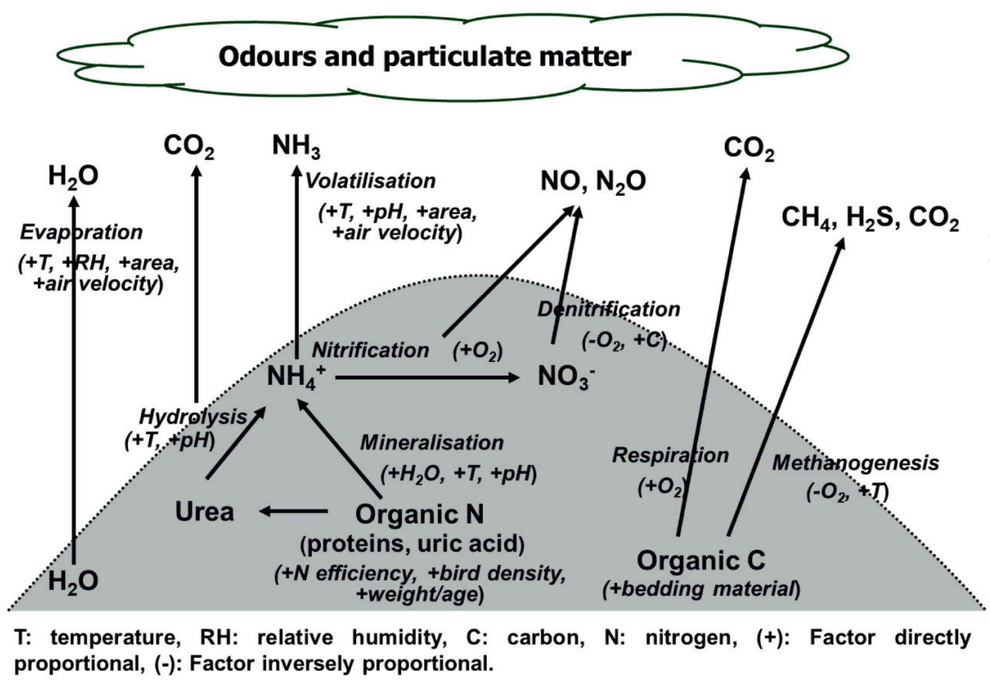

Figure 2.
Factors that influence the emission of airborne pollutants from the litter of broiler housing (from Méda et al. [15]).

Concentration	Effect
5 ppm	Lowest detectable level
6 ppm	Irritation of the eyes and the respiratory tract
11 ppm	Reduced animal performance
25 ppm	Maximum exposure level allowed for a period of one hour
35 ppm	Maximum exposure level allowed for a period of 10 minutes
40 ppm	Headache, nausea and loss of appetite in humans
50 ppm	Severe reduction in performance and animal health; increased possibility of pneumonia
100 ppm	Sneezing, salivation and irritation of mucus membranes in animals
≥300 ppm	An immediate threat to human life and health

Table 1.
Harmful effects of NH_3 concentrations in broilers and farm workers (from Birst et al. [20]).

respiratory tract at high concentrations (**Table 1**) [12, 16, 21]. High NH_3 levels can damage birds' respiratory systems' mucous membranes, increasing their susceptibility to respiratory infections, particularly to *Escherichia Coli* [12, 16, 21]. Also, high NH_3 levels increase susceptibility to Newcastle disease virus, increase the incidence of air sacculitis and keratoconjunctivitis and increase the prevalence of *Mycoplasma gallisepticum* [12, 16].

Previous studies [12, 18] recommend a limit of 10 ppm of NH_3 to maintain a good indoor air quality on broiler houses, but the threshold values of 20 ppm are recommended as limit for a short period exposure. Note that long-term NH_3 toxicity in the poultry house may increase the susceptibility of birds to the adverse effects of NH_3 even at 20 ppm (**Table 1**) [16]. However, the NH_3 gas significantly affects bird health and wellbeing, but the severity of damage depends on the concentration of NH_3 and duration of exposure (**Table 1**) [22]. Indoor NH_3 levels are also affected by housing and management factors, such as housing type, bird age and density, manure or litter conditions and handling schemes and building ventilation rate [15].

Season of the year, diurnal cycles, bird health and management practices can influence NH_3 volatilisation variability from broiler houses due to indoor and outdoor temperature and humidity differences associated to ventilation, bird activity and manure management conditions [20, 23]. Previous studies [4, 5, 7, 23, 24] observed that the lowest concentrations of NH_3 were recorded in summer period, although NH_3 emissions tended to be higher just in summer months because of a higher ventilation rate. The elevated levels of NH_3 in winter were attributed to the lower ventilation rate during cold weather. However, the average annual NH_3 emission rates from broiler houses using new litter material in each production cycle varied greatly among European countries, from 0.06 to 0.13 g day^{-1} $broiler^{-1}$ in Portugal [4, 5, 7], 0.17–0.19 g day^{-1} $broiler^{-1}$ in UK and France [25, 26] and 0.35–0.45 g day^{-1} $broiler^{-1}$ in Ireland and Spain [24, 27].

Previous studies [12, 16, 20, 28] compiled a comprehensive overview of the most promising NH_3 mitigation strategies from broiler housing such as oil and water spraying; aeration and ventilation system; filtration and biofiltration; acid scrubber; dietary manipulation; temperature and litter moisture control; immunisation; bedding materials; light intensity; manure managements; and solid additive and litter amendment. However, some of these mitigation technologies are expensive and/or needed more research to be implemented in commercial farms. Thus, **Table 2** resumes the best available techniques proposed to reduce NH_3 production in poultry houses. For Portugal, the Directive 2016/2284/EU sets a 20% NH_3 reduction from 2030 relative to 2005 levels.

2.2 Nitrous oxide

Nitrous oxide (and NO) can be released through nitrification and denitrification processes from litter Eq. (2). Nitrification is the bacterial oxidation from ammonium (NH_4^+) to NO_3^- under aerobic conditions, whereas denitrification is the reduction of NO_2^-/NO_3^- to N_2 under anaerobic conditions. The main influencing factors of nitrification and denitrification are oxygen pressure, presence off N-substrates, temperature and humidity. Consequently, litter type, stoking density and management affect the gas concentration and emission from broiler houses [15].

$$NH_4^+ \underset{Nitrification}{\Rightarrow} [N_2O] + [NO] + NO_3^- \underset{Denitrification}{\Rightarrow} [N_2O] + [NO] + N_2 \qquad (2)$$

Nitrous oxide is a colourless and non-flammable gas, with a slightly sweet odour. Known as "laughing gas" due to the euphoric effects of inhaling it, being anaesthetic and the maximum recommended indoor concentration is 3 ppm [19]. In terms of environmental impacts, N_2O in the atmosphere has a long life and contributes significantly to global warming and greenhouse effect [13, 14]. Also, contributes to the depletion of the ozone layer in the stratosphere through the photochemical decomposition of N_2O to NO [13, 14].

The rate of formation and emission of N_2O varies through time with changes in manure porosity, pH, temperature, moisture, amount of solids, N and protein content of the manure substrate (**Figure 2**) [32]. However, N_2O emission rates might be mostly related with the litter management (i.e., the interphase aerobic/anaerobic conditions of the litter), but litter temperature and protein content in the diet could enhance the N_2O loss (**Figure 2**) [4–6]. Moreover, previous studies reported low N_2O concentrations close to the detection limit levels and tended to be higher in winter than in summer, being negatively affected by the ventilation rate [4–6, 32]. The average annual N_2O emission rates from broiler houses with new litter material in each production cycle varied among European countries, ranging from 0 mg day^{-1} broiler^{-1} in France [25] to 2 to 6 mg day^{-1} broiler^{-1} in Portugal [4, 5] and 46 mg day^{-1} broiler^{-1} in Spain [5].

There are various options to reduce N_2O emissions, but the key approach is to improve overall N efficiency of broilers production [10, 32]. Therefore, improving animal feed conversion efficiency (dry matter, fibre, protein and mineral nutrition intake) becomes a major strategy for mitigating N_2O emissions from these animal species [10, 32]. The relationship between manure NH_3 volatilisation and N_2O emission is also complex because emissions of both may be reduced by diet manipulation or manure management, and if a mitigation technology reduces NH_3 losses, the preserved NH_4^+ may later increase storage N_2O emissions [13, 33]. On the other hand, gaseous losses of N will reduce the availability of N for nitrification and denitrification processes and, consequently, N_2O formation [34]. Nevertheless, it is crucial to consider potential pollution swapping when planning and implementing mitigation measures.

Mitigation measure	Technique	NH_3	Reference
Feeding	Multiphase feeding		[13]
	Low protein feeding, with or without supplementation of specific synthetic amino acids		[13]
	Increasing the non-starch polysaccharide content of the feed		[13]
	Supplementation of pH-lowering substances, such as benzoic acid		[13]
	Decrease of the protein content in the feed by 1% may decrease the NH_3 emissions by 10%	10%	[13]
	Clinoptilolite as feed additive	30%	[29]
	Yucca extract as feed additive	50%	[29]
Housing	New and largely rebuilt broiler housing	20–90%	[13]
	Naturally ventilated house or insulated fan-ventilated house with a fully littered floor and equipped with non-leaking drinking system	20–30%	[13]
	Litter with forced manure drying using internal air	40–60%	[13]
	Tiered floor and forced air drying	90%	[13]
	Tiered removable sides; forced air drying	90%	[13]
	Combideck system	40	[13]
	Magnesium sulfate as litter additive	45%	[5]
	Aluminium sulphate as litter additive	50–70%	[13, 30]
	Clinoptilolite as litter additive	28%	[6]
	Scrubbing of exhaust air	70–90%	[13]
	Biofiltration of exhaust air	50%	[31]

Table 2.
Best available techniques (relative to the reference technique: Deep litter; fan-ventilated house) proposed to reduce NH_3 losses in broiler houses.

2.3 Carbon dioxide

Carbon dioxide emission originates from the breathing of broilers, uric acid/urea hydrolysis of excretions and aerobic/anaerobic decomposition of litter material, the first source being the main source of this emission (**Figure 2**) [4–6, 15, 18]. Carbon dioxide production by broilers is proportional to their metabolic heat production, and thus to the metabolic body weight of the broilers, which in turn is affected by the temperature and broiler activity [21, 23].

Carbon dioxide is an odourless gas and the threshold limit is set to 3000 ppm, being asphyxiant at this level, increasing breathing, drowsiness, and headaches as concentration increases [19]. Under normal conditions, the concentration of CO_2 in broiler houses ranges from 500 to 3000 ppm, with a limit of 2500 ppm of CO_2 being recommended to maintain good indoor air quality [21]. There is no health risk for birds and humans at this level. Although CO_2 is rarely life threatening, some effects are reported for longer exposure to 3000–10,000 ppm, with negative effects on blood parameters (alkaline phosphatase) and immune system in broilers and respiratory and cardiovascular diseases in humans [21]. In terms of environmental impacts, CO_2 in the atmosphere contributes significantly to global warming, but does not contribute to the greenhouse effect because it is part of the so-called short C cycle, with CO_2 originated by animal production not being accounted in national inventories [14, 35].

The CO_2 concentrations observed in broiler houses are related with NH_3, with elevated levels in winter due the lower ventilation rate during cold weather [5]. Previous studies [4, 5, 7, 21, 24] reported an average CO_2 emission rate from 55.2 to 98.4 g day^{-1} broiler^{-1} in South of Europe for broiler using new litter material in each production cycle.

2.4 Methane

Methane is produced by the anaerobic microbial degradation of soluble lipids, carbohydrates, organic acids, proteins and other organic components Eq. (3). Methane emission originates from the anaerobic decomposition of litter material inside the building, depending on temperature and humidity, ventilation rate, excretion rate and litter management (**Figure 2**) [15, 24, 32].

$$Organic\ C \underset{Anaerobic\ decomposition}{\Rightarrow} CO_2 + CH_4 + H_2S \tag{3}$$

Methane is not considered to be toxic and the threshold limit is set to 5000 ppm, being odourless and concentrations from 5000 to 15,000 ppm are explosive, with several explosions have occurred due to ignition of methane rich air in poorly ventilated animal buildings [19, 21]. In terms of environmental impacts, CH_4 in the atmosphere has a strong radiative power and contributes significantly to global warming and greenhouse effect [13, 14].

Previous studies reported low CH_4 concentrations in broiler houses and the variability may be caused by differences in litter reactions within the broiler house during the growing period [24]. The annual average CH_4 emissions ranged from 0 to 18 mg day^{-1} broiler^{-1} in South of Europe [4, 5, 24, 25] for commercial broiler houses using new litter material in each production cycle, showing lower values than the IPCC emission factor (50 mg day^{-1} broiler^{-1}) [14].

2.5 Hydrogen sulphide

Hydrogen sulphide is formed by bacterial sulphate reduction and the decomposition of sulphur containing organic compounds in litter under anaerobic conditions (**Figure 2**) [36].

The H_2S as low odour thresholds (10 ppb) and when managed improperly, higher concentrations negatively affect humans, birds, and the environment. At low concentrations (<10 ppm), this gas is highly toxic, poisonous, deadly, odorous (odour of rotten eggs/low concentrations contributed significantly to odour), colourless, and heavier than air. The H_2S could cause dizziness, headaches, and irritation to the eyes and the respiratory tract. In addition to causing adverse effects to human and bird health, H_2S might be oxidised in the air forming sulphuric acid (H_2SO_4) resulting in acid rain that could cause ecological damage [19, 21].

Saksrithai and King [36] summarised a comprehensive overview of the most promising H_2S mitigation strategies from broiler, including feed supplementation (additives, prebiotics, and probiotics); manure manipulation (pH, moisture, and its microbial population); housing types; ventilation rates; and biofilters. In addition, the most promising singular methods to reduce 100% H_2S emissions are probiotic supplementation in feed, sawdust in manure, or a biofiltration system. Where cost and equipment availability may be prohibitive, combined methods (assuming additive effects) of fibrous by-products and manure moisture control via microorganisms or oil addition can reduce 100% emissions as well.

3. Odours

The odours are a significant source of gaseous pollution in broiler housing, derived from anaerobic and aerobic microbial activity during litter decomposition. The litter is considered the primary source of odour in broiler housing. The malodorous compounds are originated by organic particulate matter, volatile fatty acids, sulphurous (H_2S, mercaptans) and NH_3 (**Figure 2**) [37]. The main odorous compounds emitted on broiler farms are dimethyl sulphide, dimethyl disulphide, dimethyl trisulfide, nhexane, acetic acid, 2,3-butanedione, methanol, ethanol, 1-butanol, 2- butanol, 1-octen-3-ol, 3-methyl-1-butanol, 3-methyl-1-butanal, acetone, 2- butanone and 3-hydroxy-2-butanone [38].

Odour emissions from litter are complex due to the existence of multiple odorant sources within litter (i.e., fresh excreta, friable litter and cake), formation and emission of numerous odorants, and significant spatial and temporal variability of moisture content, porosity, pH, ventilation airflow, temperature, humidity and bird activity (**Figure 2**) [9, 38].

There has been limited studies of management strategies that reduce the formation and emission of odorants from broiler litter, mostly focussed on the perspective of reducing NH_3 emissions by air scrubbing, misting, filtering, ionising, oxidising and dispersing technologies [28, 38, 39]. In addition, strategies are focused on the perspective of reducing odours in the air through fogging technologies combined with the use of masking agents, counteractants, neutralizers and surface-enhanced absorption agents (**Table 3**) [28, 38, 39]. Dunlop et al. [40] selected management strategies with expected effectiveness to reduce odour emissions from broiler litter, being maintaining dry and friable litter; *in situ* aeration of litter; in shed windrowing/pasteurising of litter reuse; litter acidifying additives; litter adsorbent addition (activated carbon, silica gel or zeolite); and enzyme addition combined with heated incubation.

4. Particulate matter

In broiler housing, the PM consists of a complex mixture of solid and liquid materials such as litter materials, feathers, feeds, skin, excreta, dander and microorganism, whit about 90% organic content [41]. The classification of PM is based on particle size (aerodynamic diameter), considering $PM_{1.0}$ (≤1.0 μm), $PM_{2.5}$ (≤2.5 μm), $PM_{4.0}$ (≤4.0 μm), $PM_{10.0}$ (≤10.0 μm) and total suspended particle (TSP) (≤100.0 μm) [42]. The PM emissions from broiler housing are affected by various factors and change according to housing system or types, litter materials, diurnal and seasonal variation, ventilation system and velocity, temperature and relative humidity, birds age and type, activity and stocking density and manure management (**Figure 2**) [41].

The primary air emissions include PM with a high potential risk to air quality, public and bird health, and climate change. Cambra-López et al. [43] reported that the concentrations of TSP (inhalable PM) in broiler houses ranged from 1 to 14 mg m^{-3} in European countries. The World Health Organisation has recently (in 2021) amended the ambient air quality standards and proposed the maximum of $PM_{10.0}$ to be 15 μg m^{-3} for the annual average and 45 μg m^{-3} for the 24 h mean, while for $PM_{2.5}$ to be 5 μg m^{-3} for the annual average and 15 μg m^{-3} for the 24 h mean [41]. In Portugal, the Directive 2016/2284/EU establishes a 53% reduction of $PM_{2.5}$ from 2030 compared to 2005 levels. The levels of PM in broiler houses affect birds' health and welfare, including eye irritation, throat irritation, cough, phlegm, chest tightness, sneezing, headache, fever, nasal congestion, and wheezing, especially in cold periods when the house will have limited ventilation. Furthermore, long term exposure to PM in humans increases obstructive pulmonary disorder, chronic bronchitis, chronic obstructive pulmonary disease, pneumonia lesions, cardiovascular disease, asthma like symptoms, lung cancer, or even mortality. Similarly, a higher level of PM with endotoxin in birds causes impaired lung function, chronic bronchitis, pneumonia lesions, cardiovascular illness, and cardiotoxicity in chicken embryos and hatchling chickens and might increase the risk of mortality rates [21, 41, 43].

Principle	Technique	Pollutant
Microbiological	Aeration	Gases, odour
	Anaerobic digestion	Gases, odour
	Biofiltration	Gases, odour, PM
	Composting	Gases, odour
Biochemical	Enzyme additives	Gases, odour
Chemical	Acidification	NH_3
	Ozonation	Odour
Managerial	Best management practices	Gases, odour, PM
Physical	Absorption	Gases, odour
	Cooling	NH_3
	Covering	Gases, odour
	Drying	Gases, odour, PM
	Electrostatic precipitation	PM
	Fan plum deflector	Gases, odour, PM
	Filtering	PM
	Oil spraying	Gases, odour, PM
	Shelterbelt	Gases, odour, PM
	Solid separation	Gases, odour, PM
	Wet scrubbing	Gases, odour, PM
Physiological	Dietary manipulation	NH_3, odour
	Odour masking	Odour

Table 3.
Best available technologies for mitigation of airborne pollutants in broiler houses (from Ni [28]).

Table 3 show effective mitigation strategies to reduce PM in broiler houses using biochemical, chemical, managerial, physical, and physiological practices. The techniques to control PM will also reduce NH_3 and odours, which can be managing housing system and cleaning, light intensity, oil and water spraying, litter materials, electrostatic ionisation, filtration and biofiltration, acid scrubber, windbreaks and vegetative shelterbelts [28, 41, 43, 44].

5. Conclusions

In this review study, were summarised the primary reasons that affect the emission of NH_3, N_2O, CO_2, CH_4, H_2S, odours and PM in housing, then analysed the consequences of these pollutants on birds, workers and environment, and finally discussed different best available techniques for environmental protection and sustainability of broiler production, particularly feeds and feeding management, feed supplements, litter management and treatment of exhaust air. Thus, broiler farms should select mitigation strategies based on several considerations, such as location, climate conditions, environmental policies and financial resources.

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Conflict of interest

The authors declare no conflict of interest.

Author details

José L.S. Pereira[1,2*], Carla Garcia[1] and Henrique Trindade[2]

1 Agrarian School of Viseu, Polytechnic Institute of Viseu, Quinta da Alagoa, Viseu, Portugal

2 Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Inov4Agro, University of Trás-os-Montes and Alto Douro, Quinta de Prados, Vila Real, Portugal

*Address all correspondence to: jlpereira@esav.ipv.pt

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