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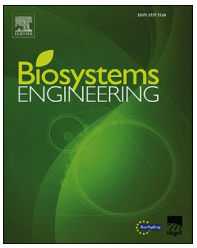

Special Issue: Engineering Advances in Precision Livestock Farming

Research Paper

A pilot study to detect coccidiosis in poultry farms at early stage from air analysis

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Nowadays, the preventive use of antibiotics in intensive farming system is common and this management practice lead to the spreading of drugs in the environment, contributing to the phenomena of antibiotic resistance. For this reason, different professional figures work on the development of drug reduction strategies. Due to the high priority of this issue, early detection of any health problem is of great importance in intensive farming. Precision Livestock Farming (PLF), through the combination of cheap technologies and specific algorithms, can provide valuable and rapid information for farmers starting from the huge amount of data that can be collected in real time at farm level. A prototype, able to give information about air fingerprint, was developed and tested in an experimental poultry farm in order to observe if air quality data were related to the presence of coccidiosis. Air samples were collected once a week in Nalophan® bags and transported to the laboratory for instrumental analysis. The prototype was able to discriminate between infected and not infected pens at a very early stage, when only 250 oocysts g^{-1} [faeces] (opg) were present in one pen. These results were also confirmed by analysing air samples in a commercial poultry farm, since all samples were correctly classified by the prototype in infected or not infected pen. This pilot study has shown that this technology could be installed in farms to continuously monitor health status of broilers, supporting farmers in the sustainable management of their activities.

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1. Introduction

Enteric disorders represent a major health issue in intensive broiler farming. One of the most common and detrimental enteric diseases in poultry farming is coccidiosis, which is caused by protozoa of the family Eimeridae. Coccidia are present in almost every poultry farm and most species belong to the genus *Eimeria* that infects intestinal tracts of poultry. Seven species of *Eimeria* infect the chicken with absolute host

Abbreviations: VOCs, Volatile Organic Compounds; opg, oocysts g^{-1} [faeces]; PCA, Principal Component Analysis; LDA, Linear Discriminant Analysis; DPs, Discrimination Powers; KNN, K Nearest Neighbours; PLF, Precision Livestock Farming.

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specificity, causing haemorrhagic diarrhoea (*Eimeria brunetti*, *Eimeria necatrix*, and *Eimeria tenella*) or malabsorption (*Eimeria acervulina*, *Eimeria maxima*, *Eimeria mitis*, and *Eimeria praecox*). These parasites persist for long periods in poultry houses (Blake & Tomley, 2014), and the infection spreads quickly among the animals, due to the environmental and hygiene conditions and the high number of animals reared (McDougald & Fitz-Coy, 2013; Peek & Landman, 2011).

Clinical disease occurs only after the ingestion of a relatively large number of sporulated oocysts (the infectious form of coccidia) by susceptible birds and the infectious process may last up to 4–7 days in host cells with extensive damage to the intestinal mucosa (Chapman, 2014).

The infection hits the digestive tract and younger animals are affected the most. Symptoms of coccidiosis depend on the degree of damage and inflammation and could include loss of appetite and diarrhoea with consequent drop in productive performances (Dalloul & Lillehoj, 2006). Also subclinical infection has consequences on poultry performance, with serious economic losses, poor product quality and increase in carcass condemnation at slaughter (Williams, 1999).

The global economic impact of coccidiosis has been estimated to be greater than $3 billion USD per year due to production losses combined with costs of prevention and treatment (Dalloul & Lillehoj, 2006). The broiler industry relies on in-feed prophylaxis with application of anticoccidial drugs (Haug, Gjevre, Thebo, Mattsson, & Kaldhusdal, 2008), but this clashes with public concern regarding the use of drugs in intensive farming. Use of antimicrobials and anticoccidial drugs in animals poses a potential risk for public health since it contributes to the selection and spread of resistant microbes in the environment (Alali et al., 2009; Berge, Atwill, & Sischo, 2005; Speksnijder, Mevius, Bruschke, & Wagenaar, 2015). Antimicrobial resistance is a global health issue integrating human, animal, and environmental health that has led public bodies such as UN or the WHO to set a global agenda to address the crisis (Laxminarayan, Sridhar, Blaser, Wang, & Woolhouse, 2016). Even if substantial funds have been committed in the United States and Europe to tackle antimicrobial resistance, there is a need to incentivise the development of new vaccines, diagnostics, novel therapies, and stewardship methods (Laxminarayan et al., 2016).

In particular, diagnostic techniques must be rapid and sufficiently inexpensive if they aim to prevent the decision to begin antibiotic treatment. Indeed, the application of specific diagnostics is important for carrying out rational and effective control measurements (McDougald & Fitz-Coy, 2013).

Nowadays, the available techniques to diagnose coccidiosis consist of counting the oocysts present in faeces and in evaluating the lesions provoked by coccidia in different intestinal tracts of dead or culled animals (Johnson & Reid, 1970). However, these methods are time consuming and only few laboratories are equipped to perform them. In addition, the evaluation of intestinal lesions in culled animals involves ethical issues.

The development of an alternative diagnostic tool might allow the prompt detection of the onset of infections. Several studies have explored the possibility of diagnosing pathologies in livestock and in humans via identification of Volatile Organic Compounds (VOCs) produced by pathogens, host–pathogen interactions and biochemical pathways (Guffanti, Pifferi, Falciola, & Ferrante, 2018). VOCs are present in blood, breath, stool, sweat, skin, urine and vaginal fluids of humans and animals and their qualitative and quantitative composition is influenced by pathophysiological responses to infections, toxins or endogenous metabolic pathway perturbations (Ellis et al., 2014). For instance, VOCs analysis has been explored as a method to diagnose bovine respiratory disease, brucellosis and bovine tuberculosis in cattle (Ellis et al., 2014; Peled et al., 2012; Purkhart et al., 2011). In poultry, VOCs have been analysed to evaluate air quality in sheds (Chang & Chen, 2003; Sohn et al., 2008; Trabue et al., 2010), but they have never been monitored to determine if birds were affected by enteric pathologies. Odours in the barn are influenced by poultry health status and, in particular, enteric problems are characterised by peculiar odour properties (Sohn et al., 2008).

The goals of this pilot study were to observe whether differences in air quality inside pens hosting broilers infected or not by coccidia exist and whether the obtained dataset was able to give information about the presence or the absence of the infection in animals reared in a commercial poultry farm by only analysing the air inside it.

2. Material and methods

2.1. Experimental trial

The trial was carried out in the experimental facilities of Università degli Studi di Milano (Lodi, Italy) and lasted for 45 days.

One hundred and twenty Ross 308 one day old chicks were split in two separate rooms with standardised temperature, and rearing conditions. The rooms were equipped to monitor the ventilation rate and internal temperature. Over the experimental period, the internal temperature and the ventilation rate ranged from 20 to 30 °C and from 0.074 to 1.091 $m^3\ h^{-1}\ kg^{-1}$ [live mass] respectively, as required by animals of that size. The temperature and air velocity were the same in the two rooms. In each room the animals were reared in 2 m × 3 m pen identified as pen A and B (Fig. 1). The floor was covered with wood shavings and the bird stocking density was 30 kg m^{-2}, according to the Council Directive 2007/43/EC for the protection of chickens kept for meat production.

Both groups were fed the same diet, but the coccidiostatic Robenidine ($C_{15}H_{14}Cl_3N_5$) was added to the feed of pen A at a concentration of 66 ppm.

Faeces and air samples were weekly collected in four points in each pen (A and B, circles in Fig. 1) to evaluate the possible presence of coccidiosis.

Faeces were collected in vials to perform the oocysts count in the laboratory of avian pathology, according to the Mc Master method (Holdsworth et al., 2004). The number of oocysts, expressed as oocysts g^{-1} [faeces] (opg), was used as a Gold Standard (Tullo et al., 2017) to indicate the health status of broiler (infected/not infected) and was used as a reference compared to the VOCs analysis performed on air samples.

To observe differences in air quality between pens, air sampling was carried out following the recommendations described in the European Standard EN 13725 (CEN, 2003). In each room, air was drawn into disposable Nalophan® bags, using a special sampler (Fig. 2) that works according to the

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