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An innovative approach for analysing and evaluating enteric diseases in poultry farm

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ABSTRACT
Volatile organic compounds (VOCs) produced by pathogens, host-pathogen interactions and biochemical pathways are present everywhere such as in blood, breath, faeces, sweat, skin, urine, and vaginal fluids. Their qualitative and quantitative composition is influenced by pathophysiological responses to infections, toxins, or endogenous metabolic pathway perturbations. In poultry, VOCs analysis has been explored to evaluate air quality in sheds, but they have never been monitored to determine if birds were affected by enteric diseases. The enteric disorders represent one of the most important groups of diseases that affect poultry and cause illness, mortality, and economic losses. For this reason, monitoring the health status of broilers and the early detection of any health problem is of great importance in intensive farming, especially nowadays that antibiotics are banned. Precision Livestock Farming, through the combination of cheap technologies and specific algorithms, can provide valuable information for farmers starting from the huge amount of data collected in real time at farm level.
This study was aimed to the application of a PLF diagnostic tool, sensible to the variation of volatile organic compounds, to promptly recognize enteric problems recognize enteric problems (as coccidiosis and necrotic enteritis) in intensive farming, supporting veterinarians and enabling specific treatments in case of disease.

Section: RESEARCH PAPER

Keywords: diseases; poultry farm; VOCs; coccidiosis; necrotic enteritis

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1. INTRODUCTION

During the past 73 years, the world's poultry industry has grown and consequently the new needs, that were came, changed in housing, equipment, and farm management of poultry [1]. With the increased demand for high-quality food, the global poultry industry has undergone numerous changes. One of them was genetic selection, which resulted in animals with faster growth, better feed conversion, higher meat yields and lower mortality rates [1]-[2]. The change in house structure and equipment have led to an increase in the stocking density per farm [1], that can affect the health condition of the flock.

Broilers and meat turkeys are animals that have good performance and food efficiency. Any slight change from the optimal condition can cause a disruption of the growth process and all performance. The key is good intestinal health [3]-[4].

Enteric disorders represent a major health issue in intensive broiler farming indeed various types of these pathologies affect poultry causing illness, increase of mortality rates, decrease weight gain, increase medication costs, increase feed conversion rate. Consequently, the enteric disease in poultry have high economic impact on producers [5]. For this reason, in poultry farming, disease prevention plays a fundamental role in the business economy [1].

Different pathogens (bacteria, viruses, and parasites) can cause enteric disorders either on its own, in synergy with other microorganisms or with non-infectious causes such as feed and/or factors related to management and environmental factors. However, it is difficult to determine whether the cause of any enteric disorders is of infectious or non-infectious origin [3]-[6]. Besides in modern intensive poultry system the environmental condition and the high number of animal reared might promote these enteric diseases development. Enteric disease in poultry can be caused by a wide range of infectious agents, and the most important is Clostridia and the infections with this bacterium can cause different clinical signs. The

Clostridium perfringens, that is spores are ubiquitous in poultry farm litter, is the cause of the necrotic enteritis that is the most common clostridial enteric disorder in poultry [1],[7]-[9].

The enteric diseases infection affects the digestive tract and younger animals are influenced. The most clinical symptoms include inappetence, tendency to huddle, marked depression and diarrhea. Also, subclinical infection has serious consequences on poultry performance [10].

Coccidiosis, which is caused by protozoa of the family Eimeridae also is common and could cause detrimental enteric diseases in poultry farming. Coccidia are present in almost every poultry farm and most species belong to the genus Eimeria that infects intestinal tracts of poultry. These parasites persist for long periods in poultry houses [11] and the infection spreads quickly among the animals, due to the environmental and hygiene conditions and the high number of animals reared [12]-[13]. 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 [14]. The outbreaks of necrotic enteritis in broiler farms cause a global economic loss estimated to be over $2 billion annually [15]-[17].

Coccidiosis is well known that is a predisposing factor of necrotic enteritis (NE). Necrotic enteritis can develop when clostridium perfringens multiplies exponentially in the intestinal tract producing a wide range of toxins that lead to necrosis [9]. The clinical symptoms of NE are characterized by a sudden increase in animal mortality often with no preceding warning signs. In some cases, an early indicator of this pathologies is a wet litter. Instead, the subclinical form is characterized by a decrease in feed-conversion ratio with the chronic damage to the intestinal mucosa and an economic loss in the poultry production industry.

The addition of antibiotic growth-promoters (AGPs) to the poultry feed improves the performance of the animals. In particular, these products are able to reduce intestinal disorders acting on the intestinal flora and improving digestion, nutrient absorption and balance in the microbial population. By contrast, the use of AGPs can promote the development of antibiotic resistance trough the increase of the prevalence of drug-resistant bacteria. The European Union (EU), based on the "precautionary principle" and on the experience of other countries such as Sweden, Denmark, Germany, and the Netherlands, has decided to ban the use of AGPs from January 2006 [3]. Whereas therefore the preventive use of antimicrobials (penicillin, amoxicillin, ampicillin, erythromycin, dihydro-streptomycin and tetracyclin) is forbidden, in the last years several vaccines were tested and showed promising results [1].

How do you recognize this enteric problem?

Nowadays, the available techniques of diagnosis are based upon clinical signs, lesions on post-mortem examination. A presumptive diagnosis can be done from the case history, clinical signs, lesions and staining fresh smears of upper part of the intestinal tract with Gram stain showing an abundant number of clostridia organisms. The confirmation of the diagnosis is based on isolation of C. colinum from liver, intestine, and/or spleen [7]. To detected coccidiosis the available techniques, 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 [18]. For NE the isolation of C. perfringens from the intestinal tract can be useful for its diagnosis [1]

To prevent the problems of enteric disorders, the development of an alternative control system, that can promptly detect the onset of the infestation, is needed. The 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 [12].

Volatile Organic Compounds (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 [19]. For instance, VOCs analysis has been explored as a method to diagnose pathologies in livestock and in humans [20]. In poultry, VOCs have been analysed to evaluate air quality in sheds [21]-[23], 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 characterized by peculiar odour properties [22].

The goals of this pilot study were to evaluate if there was a difference in the air quality inside pens hosting broilers infected or not by enteric diseases like Coccidiosis or Necrotic Enteritis. The system was able to give information about the presence or the absence of an intestinal alterations in animals reared in different commercial poultry farms by only analysing the air inside it. The system, based on sensors, was developed for an early and non-invasive detection of any enteric disorders of poultry. The use of this innovative approach might provide fast and reliable results. This research aims for the development of a new detection method for a rapid and a relatively cheap detection of a dismicrobism in the poultries farm.

2. MATERIAL AND METHODS

In the first part of the experiment, where the objective was early identification of coccidiosis, the trial was carried out in four different intensive poultry farms located in Northern Italy. In all of the farms, Ross 308 males were reared and, based on the EU Council Directive on Broiler Welfare [24], the stocking density was 33 kg/m^2. All farms were equipped with forced ventilation by negative-pressure systems, automatic systems to monitor the environmental parameters, and litters of wood shavings. In order to classify the dust level in the broiler shed, the dust sheet test was performed [25]. While in the second part of the experiment, where the objective was to test the capacity of the same device to also detect the Necrotic Enteritis, the trial was performed in two other intensive poultry farms always located in Northern Italy. The farming conditions were the same as in the previous four farms.

2.1. Innovative approach

The detect system was developed to draw and to analyse the air samples, giving a response on VOCs present in the air. The system is a device which, using Metal Oxide Semiconductor sensors (MOS), is able to identifies and creates an odour print of the air mixture analysed. MOS are able to alter their electrical characteristics in presence of different VOCs, thereby providing a diversified electrical signal in response to their concentration in the air mixture.

In this way the system is able to recognize the environmental changes of VOCs due to the presence of gut dismicrobism in the poultries. These changes in the intestinal flora or/and in the enzymatic activity can refer to digestive disorders. The system is therefore able to analyse the air in the flock and detect intestinal disorders in poultry farms at early stage, when the pathology is not yet evident. Deeper understanding of these variations should be facilitating the veterinarian in the development of effective disease-control measures to treat. The enteric disorders, outlined by the system and associated with VOCs, could be both of a pathology (like viral agents, bacterial agents, and parasites) and metabolic (as feed, management, and environmental factors) origin. The system is based on a patent technology (International Publication Number: WO2017/212437).

2.2. Application field

The system has been validated with the Coccidiosis, since the oocyst counts, expressed as oocysts g-1 (OPG), obtained from the examination of faeces was used as a Gold Standard. The oocyst counts are able to indicate the health status of broiler (infected/not infected) and it was used as a reference compared to the VOCs analysis performed on air samples.

In fact, 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 [18]. The next step was to test the system for others enteric infectious disease in poultry like necrotic enteritis (NE) and for non-infectious enteric disorders in poultry. A diagnosis of NE should be based on different criteria and the confirm of the diagnosis is possible only postmortem examination.

2.3. Coccidiosis

To detect coccidiosis the device was installed in the middle of the building of the four farms and was tested online 24/7. The device based on a metal oxide semiconductor (MOS) did not directly measure the presence of pathogens but rather the presence of VOCs, which are well correlated with animal health [26]. The air was continuously drawn into the device throughout the production cycle by means of a polytetrafluoroethylene tube (Teflon). The air was sampled at 40 cm above the floor and drawn into a small chamber consisting of an electronic non-specific sensor array that is sensitive to a wide range of VOCs. Changes in the electrical characteristics of the sensors are due to the superficial reaction resulting from gas absorption. The electrical signals produced from the sensors were recorded every 10 s for further analysis.

2.4. Necrotic Enteritis

To evaluate if the device is also able to detect the presence or absence of Necrotic Enteritis it was decided to not install it directly in the farm but use it in the laboratory analysing some bags of air collected in the poultry farms. In particular, five air bags were collected in each of the two sheds present in each of the two poultry farms monitored. For a total of twenty samples analysed. Air sampling was carried out following the recommendations described in the European Standard EN 13725 (CEN, 2003). The air was drawn into disposable Nalophan® bags using a special sampler that works according to the lung principle. The sampler drew the air directly into the bag by evacuating the tightly closed atmospheric pressure vessel in which the bag was placed [27]. The bags containing air from the poultry facility were transported to the laboratory and analysed within 1 h after sampling with the device developed for the coccidiosis.

2.5. Statistical analysis

To focus the analysis on early warning for coccidiosis, we analysed the results (prediction plot) obtained from the algorithm. The sensors' data of each cycle, that was collected in the broiler farms, were so processed in real time by the algorithm and the prediction was computed automatically.

For the Necrotic Enteritis, to discriminate between healthy and sick animals, the data obtained with the device on air samples were processed using multivariate statistical techniques, specifically Cluster analysis and Principal Component Analysis (PCA).

3. RESULTS

3.1. Coccidiosis

The algorithm system was able to alert with an early warning one week before outbreak of the coccidiosis disease as shown in Figure 1. On this topic two studies were published [26],[28].

3.2. Necrotic Enteritis

Cluster Analysis showed a good ability to discriminate between air samples from healthy and sick animals (Figure 2). In this figure cluster analysis, carried out without a priori knowledge of the animals' disease status, is shown: the analysis was able to divide the two populations of healthy and sick sharply.

Principal Component Analysis (PCA) performed on healthy and sick animals seems to have identified the sensors responsible for this discrimination (Figure 3). These results should be confirmed by on field data collection 24/7.

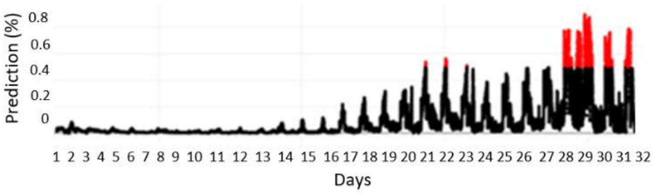

Figure 1. Prediction plot. The first red peaks represent the early warning instead the following red peaks represent the outbreak of the coccidiosis.

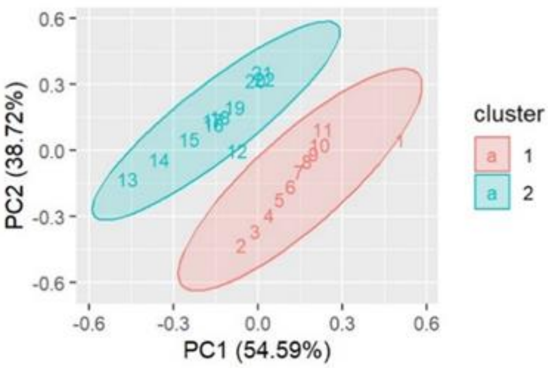

Figure 2. Cluster Analysis.

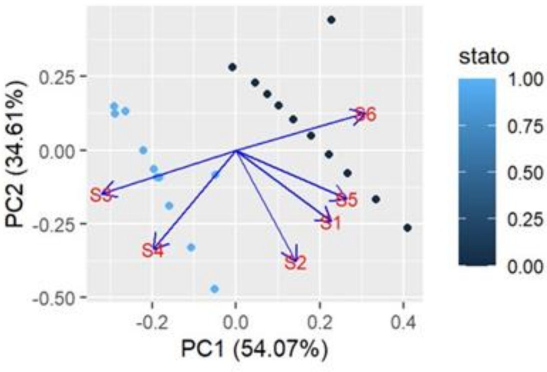

Figure 3. Principal Component Analysis.

4. CONCLUSIONS

For coccidiosis, the system used perfectly suits the methodologies and the goals of Precision Livestock Farming (PLF), which consists of non-invasive automated technologies that can support farmers with early warning systems for the identification of production, health, and welfare problems on farms.

For Necrotic Enteritis the study instead was only a pilot, and it has shown encouraging results. Indeed, from these results it is possible to report that also for this pathology the system seems to be able to discriminate between VOCs emitted by healthy animals and those sick sharply.

As for coccidiosis also for Necrotic Enteritis the application of this system in livestock farming could be extremely beneficial. For this reason, from these results there are many opportunities for future studies.

Currently this system has also been installed in turkey farms since the necrotic enteritis affects a variety of bird species (chickens, turkeys, ostriches, quail etc.) and its outbreak can be an important disease, in terms of mortality and welfare.

The development of this innovative approach for the early and non-invasive identification of any enteric diseases in poultry was able to promptly identify the onset of coccidiosis infestation and showed encouraging results for necrotic enteritis.

Enteric disorders represent a serious health problem in intensive broiler farming and in a future research VOCs analysis will be implemented to identify other intestinal pathologies.

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