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

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Abstract

Nowadays, the preventive use of antibiotics in intensive farming system is common and this management practice involves spreading of drugs in the environment, contributing to the phenomenon of antibiotic resistance. For this reason, different professional figures work on the development of drugs reduction strategies. Due to the high priority of this issue, it is of great importance the early detection of any health problem in intensive farming. 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. A patent pending device, able to give information about air composition, was tested on air from an experimental poultry farm, in order to observe if air quality data were related to the presence of Coccidiosis, one of the most detrimental enteric pathology in poultry. Air samples were collected once a week in Nalophan® bags and transported to the laboratory for the instrumental analysis. The device was able to discriminate between infected and not infected flocks at a very early stage, when only 250 oocysts per gram of faeces were present. These results were also confirmed analysing air samples from a commercial poultry farm, because all samples were correctly classified by the device in infected or not infected class. 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.

Keywords

Precision Livestock Farming; poultry; coccidiosis detection; air quality; Volatile Organic Compounds

Symbols and abbreviations

VOCs	Volatile Organic Compounds
opg	oocysts per gram
PCA	Principal Component Analysis
LDA	Linear Discriminant Analysis
DPs	Discrimination Powers

35	KNN	K Nearest Neighbors
36	PLF	Precision Livestock Farming

1. Introduction

Enteric disorders represent a major health issue in intensive broiler farming system. One of the most common and detrimental enteric disease in poultry farming is Coccidiosis, which is caused by protozoa of the family Eimeridae. Coccidia parasites are present in almost every poultry farm and most species belong to the genus Eimeria that infects different poultry intestine tracts. Seven species of Eimeria infect the chicken with absolute host 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, including faeces and litter (Blake & Tomley, 2014), and the infection spreads quickly among the animals, due to the environmental and hygienic 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 the damage and inflammation and could include loss of appetite and diarrhoea with consequent drop in the 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 higher 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 the public's 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 (Speksnijder, Mevius, Bruschke, & Wagenaar, 2015). Antimicrobials resistance is the global health issue integrating human, animal, and environmental health that makes public bodies as UN or the WHO setting a global agenda to contrast the crisis (Laxminarayan, Sridhar, Blaser, Wang, & Woolhouse, 2016). Even if substantial funds have been committed in the United States and Europe to tackle antimicrobials resistance, there is the need to incentivize 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 of beginning 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 in counting the oocysts present in poultry 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 promptly detecting the onset of the infection through the monitoring of chemical species present in the air of poultry houses. Indeed the odour from birds in the house is influenced by their health status and, in particular, enteric problems are characterised by peculiar odour properties (Sohn et al., 2008). Moreover the use of the tested device might allow the reduction of antibiotics in livestock, since they are considered important "tools" in many parts of the industrialized world and the intensive use of these drugs could result into high levels of antibiotic resistance in commensal and pathogenic bacteria (Alali et al., 2009; Berge, Atwill, & Sischo, 2005).

Several studies have explored the possibility to diagnose pathologies in livestock and in humans via identification of Volatile Organic Compounds (VOCs) produced by pathogens, host-pathogen interactions and biochemical pathways (Ellis et al., 2014). 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 were 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.

The goals of this pilot study were to observe if differences in air quality inside pens hosting broilers infected or not by coccidia exist and if 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 only analysing the air inside it.

2. Material and methods

2.1 Animal housing, samples collection and analysis

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

One hundred and twenty Ross 308 one day old chicks were split in two separate pens (called A and B) with standardised temperature, and rearing conditions. The room was 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^3h^{-1} per kg of live weight respectively, as requested by animals of that size. The temperature and air velocity were the same of the two pen (A and B). Pens measured 2x3 meters (**Figure 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/CE for the protection of chickens kept for meat production.

Both groups were fed with the same diet, but the coccidiostatic Robenidine was added in the feed of group A in the concentration of 66 ppm.

Weekly, fresh faeces and air samples were collected in four points (circles in **Figure 1**) within the pens A and B. Faeces were sampled to count the oocysts, according to the Mc Master method (Holdsworth et al., 2004) and the number of oocysts, expressed as oocysts per gram (opg), was employed as Gold Standard. The four faeces samples were mixed to obtain a single sample in which oocysts were counted and calculated per gram. Air sampling was carried out following the recommendations described in the European Standard EN 13725 (CEN, 2003). Air was drawn into disposable Nalophan® bags, using a special sampler (**Figure 2**) 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 (Dincer, Odabasi, & Muezzinoglu, 2006). Air samples were transported to the laboratory and analysed within 1 hour after the sampling. The analysis were performed with a patent pending technology, developed to respond to the volatile organic compounds present in poultry intensive farming.

2.2 Tests in an intensive poultry farm

Fresh faeces and air samples were also collected inside an intensive poultry farm, located in Ospitaletto (Brescia, BS), in order to test if the patent pending technology worked correctly also in a real case situation. The barn measured 50x12 meters and hosted about ten thousands Ross 308 broilers. Ventilation was transitional tunnel, the litter was made of rice hull and the bird maximum stocking density was 33 kg m^{-2}. Animals were fed with the same diet of group A of the previous trial (with addition of coccidiostatic), in order to prevent coccidiosis and the relative economic loss for the farmer. Faeces and air were sampled in the first, fourth and fifth week of the production cycle. The choice of these weeks was determined by an unexpected avian influenza outbreak that limited the access to the farm.

Air was drawn in two points located in the middle of the building into Nalophan® bags and then the samples were transported to the laboratory for the instrumental analysis. At the same time, faeces were collected to count the oocysts.

2.3 Statistical analysis

The data obtained with the new technology on air samples collected in the experimental facilities were processed using multivariate statistical techniques, specifically Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA). PCA is a statistical method useful for exploring large datasets and displaying differences in different samples (Bro & Smilde, 2014). Discrimination powers (DPs), which are an inverse measure of the overlapping of two groups of measurement points, between group A and group B were also calculated along the PCA, according to the description given in the manual of Winmuster Airsense Analytics (Schwerin, Germany) that provides a number of analyzing precedures. The farther a group lies from one another, the less they overlap and the better they can be distinguished. LDA is a statistical technique that allows creating classes of samples, taking care about the distribution within classes and the distances between each class.

Then, a correlation classifier statistical procedure was performed on data obtained from air samples of the commercial broiler farm in order to verify the validity of the classification system. The correlation classifier technique investigates the space angles, which indicate the location of the pattern of classes with respect to the new measurements (**Figure 3**). The samples were classified according to the K Nearest Neighbors (KNN), with K equal to 3.

3. Results and Discussion

3.1 Oocysts count

Fresh faeces were sampled in group A and group B during the experimental trial to count the oocysts, used as Gold Standard in this experiment. As shown in **Figure 4**, only the group B developed an infection of coccidiosis, associated with clinical signs (diarrhoea). In particular, the oocysts per gram (opg) were 250 at week 1, until the infection reached the highest value of 37,300 opg at week 4. The following sampling (week 5) resulted in 1,800 opg and the same drop was observed at week 6, when 1,100 opg were counted. The complete trend is reported in **Figure 4**.

At the beginning of the trial, the number of oocysts increased and then dropped after the fourth week; this drop can be explained by the immunity provoked by the infection from coccidia (Rose, 1976).

3.2 Principal Component Analysis and Linear Discriminant Analysis of data from air samples

Data obtained from air analysis with the patent pending technology were processed using the Principal Component Analysis (PCA) in order to find possible differences between the two poultry groups in pens A and B.

According to the presence or absence of oocysts in the faeces, PCA showed good ability in the discrimination between air samples from group A and group B (**Figure 5**), in particular along the second principal component (14% of explained variance), which distinguish samples collected in the same day. Conversely, the first principal component (72% of explained variance) seems to explain air changes during the production cycle. The changes in air features in group A during the weeks are probably related to chicks growing, while in group B the presence of coccidia in the gut probably modifies the normal trend.

Discrimination powers (DPs) along the PCA were calculated between the two groups for each week and reported in **Table 1**. The value was quite high (0.95) even in the first week (250 opg, as shown in **Figure 4**), when the infection was at a very early stage, and this could set the basis for the development of a new device able to rapidly detect the onset of coccidiosis in poultry farms.

Week	Discrimination Power
1	0.95
2	0.92
3	0.95

4	0.93
5	0.90
6	0.93

Table 1. Discrimination powers calculated along the PCA between Group A and Group B during each week of the production cycle.

Since PCA has shown differences between air samples from infected and not infected animals, classes were modelled using Linear Discriminant Analysis (**Figure 6**), which showed that the "between-groups" difference was relevant starting from the first week, confirming PCA findings. Here, the information about the presence of the infection is captured by the first component, which explains 48.5% of the total variance.

3.3 Tests on samples from a commercial poultry farm

In order to evaluate whether the patent pending device was able to predict the animal health status in an intensive poultry farm, the K Nearest Neighbors (KNN) classification technique was carried out on air samples coming from the commercial farm located in Ospitaletto, using data collected in the experimental trial as reference dataset.

LDA was performed on the reference data, grouping them in infected (group B) and not infected samples (group A), as shown in **Figure 7**. Then, the new samples were classified inside these groups (**Figure 7**). During the production cycle, two Nalophan® bags were filled with the air from two points in the middle of the building in the first, fourth and fifth week.

Air sampled in the first week was classified by the device in group A. The classification resulted correct with respect to the oocysts count, which was zero for faeces collected in the same day. On the other hand, air samples collected in week four and five were classified in group B. These results were confirmed by the oocysts count that was equal to 50,000 and 10,000 in week four and five, respectively. Although the feed was added with coccidiostatic, parasites were present due to hygienic conditions and overcrowding of animals in a typical intensive broiler farm.

4. Conclusions

This pilot study has shown that the patent pending technology is able to discriminate between animals infected or not by coccidiosis and its hypothetical application in livestock farming could be extremely beneficial in the future. Air analysis revealed that the discrimination was effective even when the infection was at a very early stage (250 opg). The patent pending instrument 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 (Vranken & Berckmans, 2017).

Many studies have explored the possibility to diagnose pathologies analysing the generated VOCs pattern, which acts as a "fingerprint" for each specific disease (Wilson & Baietto, 2011). In other words, the complex biochemical modifications that occur during a disease state produce a typical and characteristic VOC pattern that can be related to the presence of the same disease (Pavlou & Turner, 2000). The physiological reason of the change in air composition inside broiler houses during coccidiosis infection was not investigated, thus it gives many opportunities for future studies in the identification of specific biomarkers and in the development of more reliable and precise technologies, avoiding possible interferences. In addition, more data from different commercial poultry farms will allow to verify to which extent the outcomes of this technology are affected by diverse management practices.

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Figure captions

Figure 1. Schemes of pens A and B and locations of sampling points 1 to 4.

Figure 2. System employed to sample air in Nalophan® bags.

Figure 3.Classification of new measurement (indicated by X) using k-nearest neighbors algorithm (k-NN)). The new object is classified for one class having the pattern with the smallest angles to new the pattern. . (source: elaboration on Airsense protocol manual).

Figure 4. Oocysts count in groups A and B during the production cycle.

Figure 5. PCA plot for PC1 and PC2 components in group A and B. The first two PCs value are more than 85% of the total variances (PC1 is 72.02 % and PC2 is 13.96%).

Figure 6. LDA plot: air samples projection onto the first 2 linear discriminats (LD1 and LD2) that explains 69.26% of the total variance (LD1 is 48.50% and LD2 is 20.76%).

Figure 7. KNN classification (K = 3) of air samples collected in the intensive poultry farm with respect to the classes obtained with LDA on the reference dataset (experimental trial). Red crosses represent air samples collected from Ospitaletto poultry farm in the first week, blue crosses in the fourth week and blue triangles in the fifth week, respectively.

Fig. 1

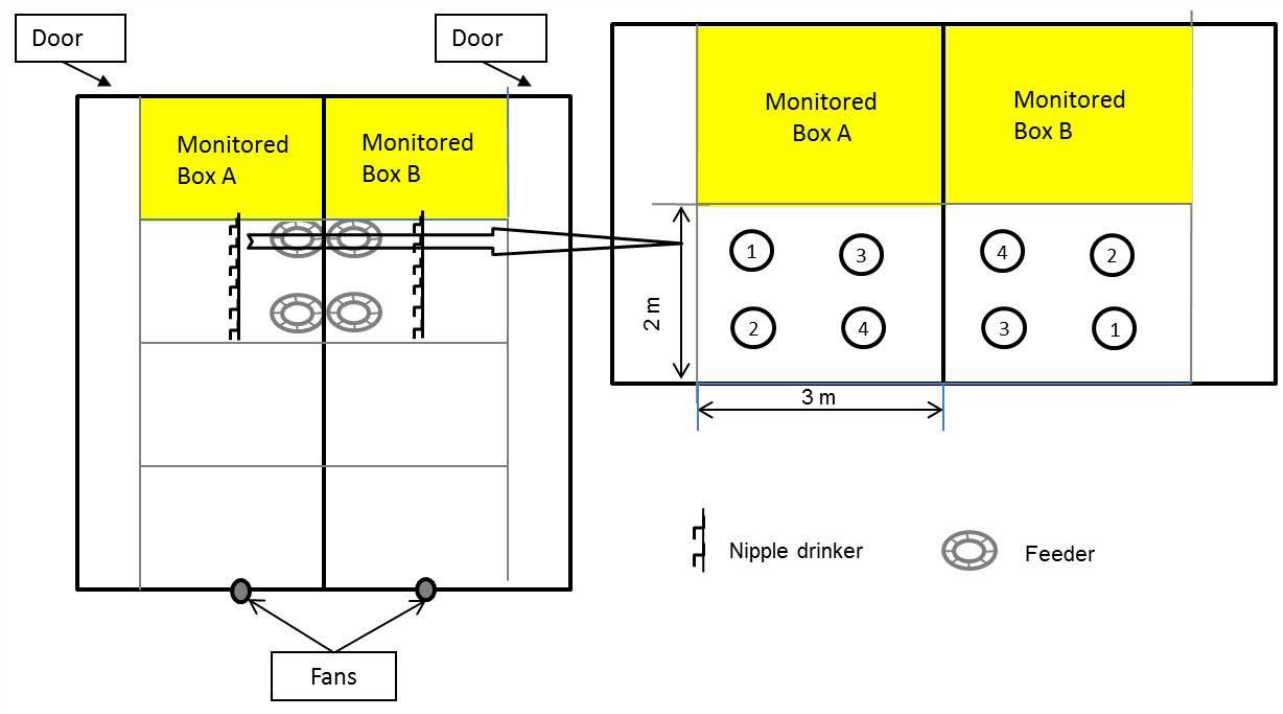


Fig. 2

Fig. 3

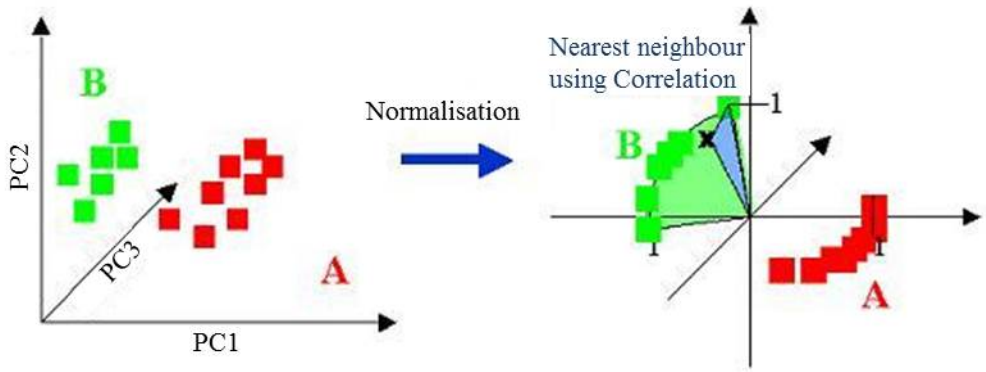
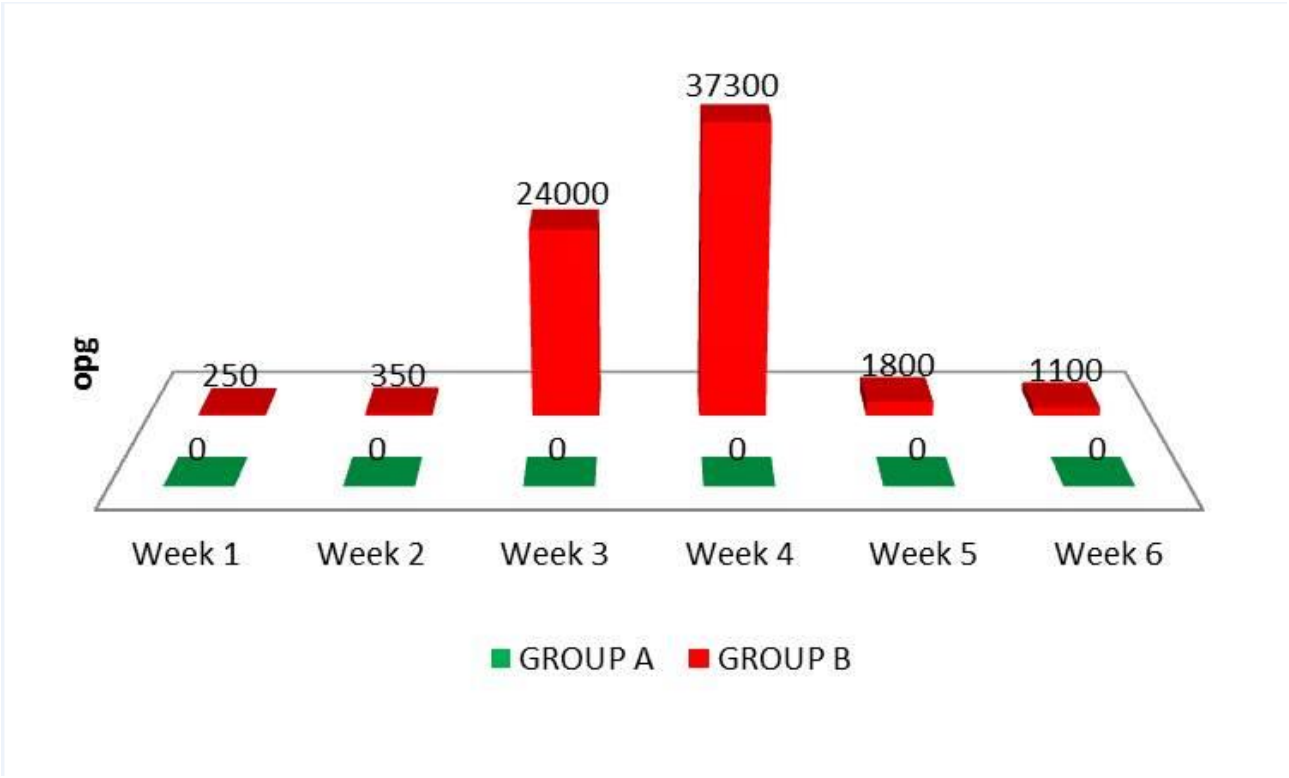

B
PC2
PC3
A
PC1
Normalisation
Nearest neighbour
using Correlation
1
B
A

Fig. 4



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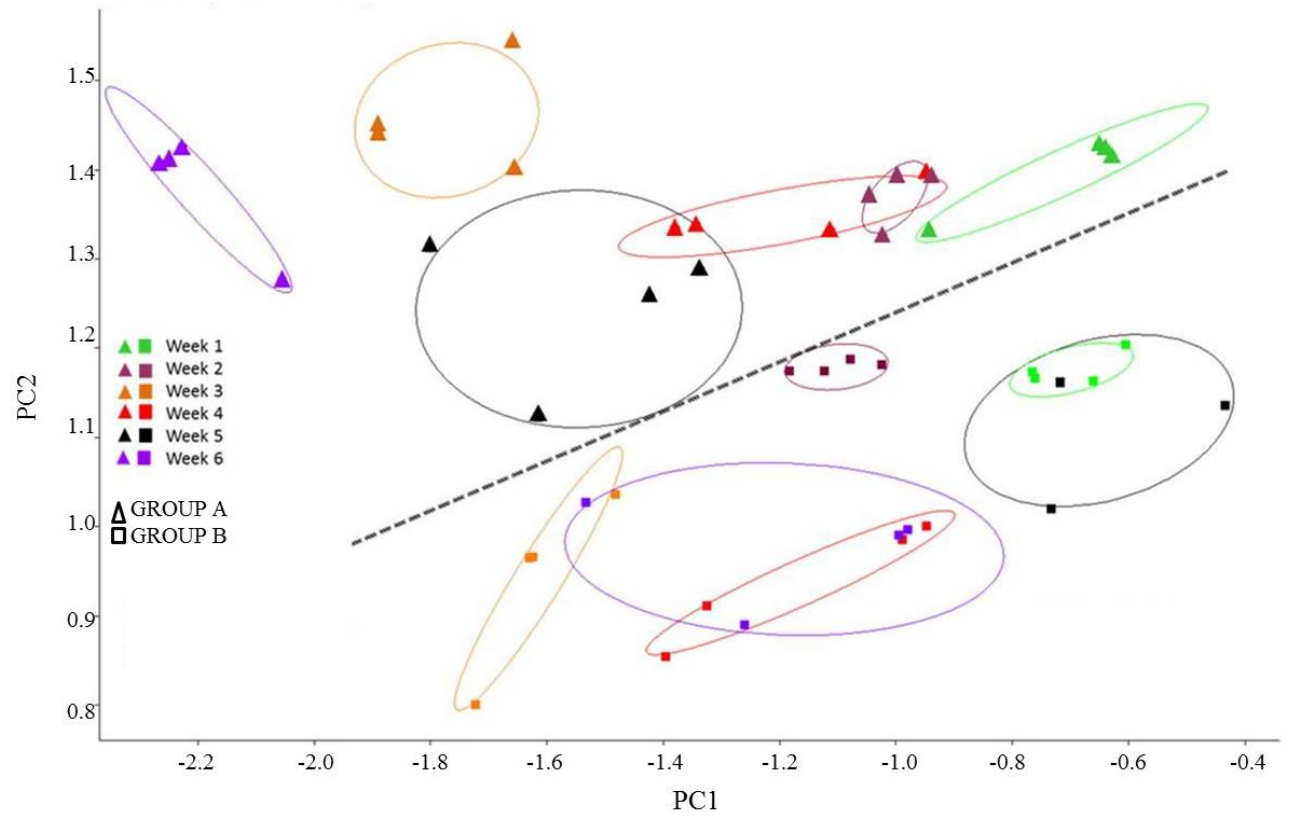
332 **Fig. 5**



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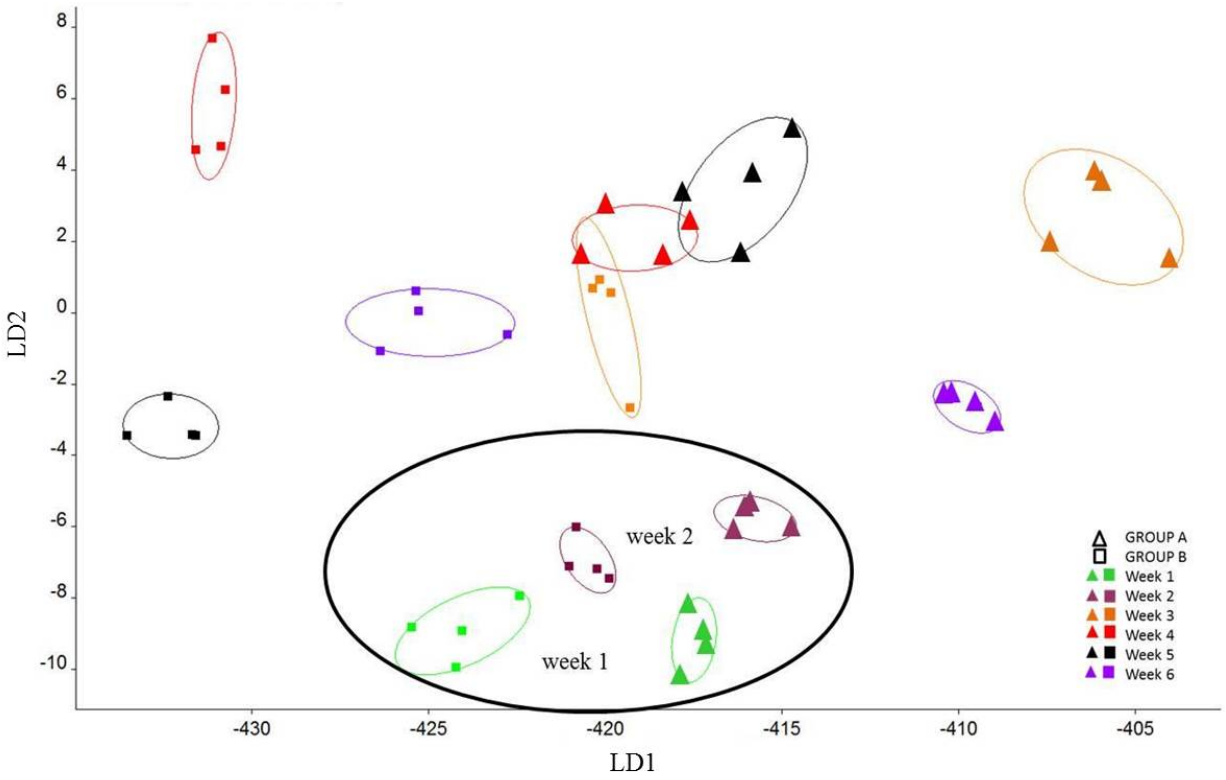

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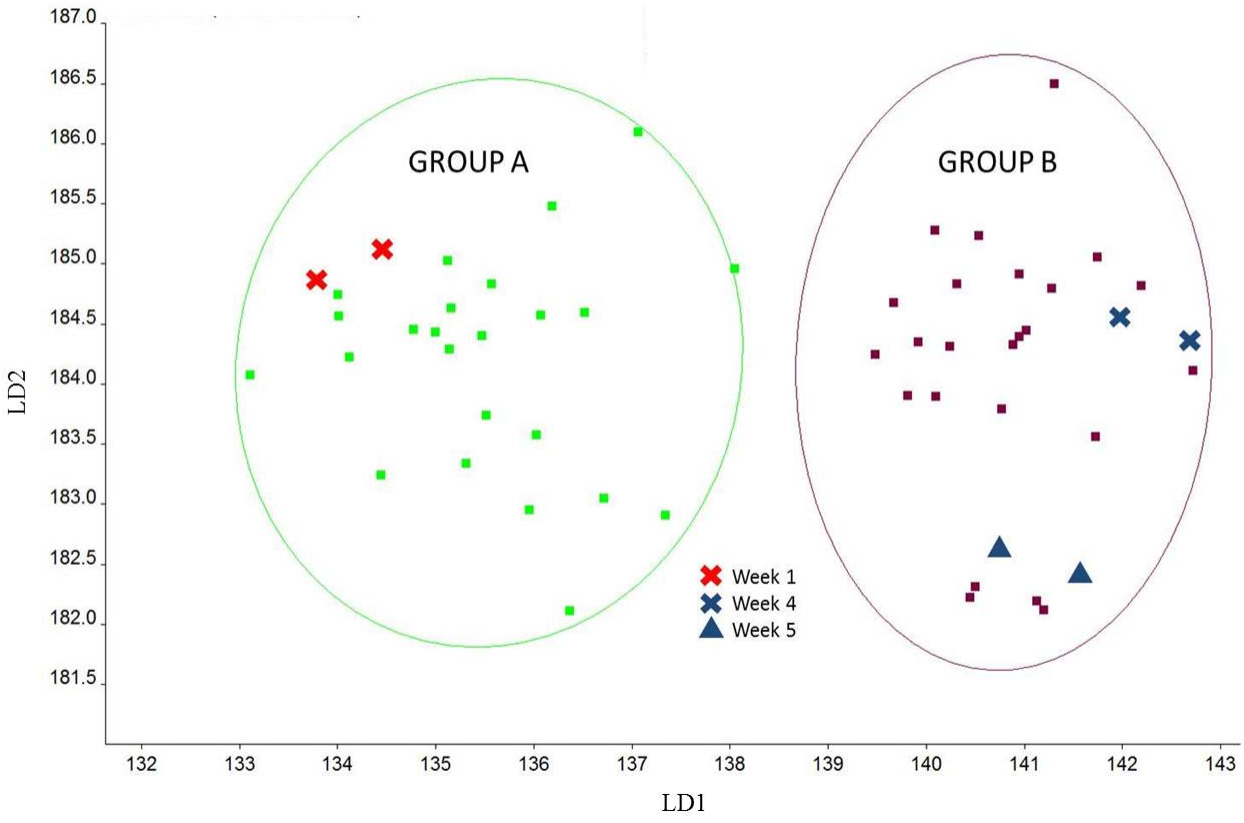
336 **Fig. 7**



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