

# Microbial equilibrium of *Meleagris gallopavo* Turkey intestine and its modulation by non-antibiotic feed supplementations

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Turkey is the second produced poultry in Europe after broiler chicken. As animal health and development is dependent on gastrointestinal (GI) microflora, antibiotics were used as growth-promoters until the recent European authority ban. Thus, alternative substitutes are welcome.

In present study we evaluate effects of non-antibiotic feed supplementations on modulation of microbial equilibrium in GI tract of *Meleagris gallopavo*. Five different diets were used: (1) Fructooligosaccharides (sc FOS); (2) essential oil compounds (EO); (3) essential oil extracts (EOX); (4) association of natural plants and EOX; (5) CCPA diet (used as a reference). Ten birds in each diet group were sacrificed at age of 4-wk- and 7-wk- and their intestinal and caecal contents were analyzed. Microbial community was analyzed by using DGGE method with universal eubacterial and *Lactobacillus* genus-specific 16S rDNA primers.

The majority of identified bacteria belong to four genera: *Bacteroides*, *Clostridium*, *Lactobacillus*, and *Ruminococcus*. We observed modulation in *Lactobacillus*, *Bacteroides* and *Clostridium* microflora depending on age, GI compartment and used diet. These results are relevant and provide insights in understanding of the mechanism of action of the different non-antibiotic feed supplementations on the gut microbiota of turkey.

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**Keywords:** turkey hen; microbial equilibrium; gastrointestinal tract; non-antibiotic feed supplementation; DGGE.

## Introduction

An important rise in bacterial resistance to antibiotics has appeared over the last two decades presenting a significant danger for human and animal health. This resistance is mostly due to the abusive use of antibiotics in animal industry field. To limit its expansion, several measures were engaged and, for example, the systematic use of antibiotics as growth promoters in animal feed was banned by the European Union in January the 1<sup>st</sup> 2006. Since this date, poultry production particularly in turkey knows economical difficulties due to a lower production and to increasing digestive disorders. Therefore, search for alternative feed strategies to antibiotics became a priority for the animal industry.

This work presents the investigations undertaken to determine the effect of several dietary supplementations on microbial equilibrium in GI tract of *Meleagris gallopavo*. The dietary supplementations used included essential oils, natural plants and/or fructooligosaccharides (sc FOS) prebiotic. Microbial equilibrium in the turkey GI tract was studied by using phylogenetic analysis with amplification of the eubacterial 16S rDNA genes (Handelsman, 2004) and their separation in DGGE (Denaturant Gradient Gel Electrophoresis) (Muyzer and Smalla, 1998).

## Material and Methods

*Animals, treatments and sampling.* Turkey were housed in the same farmhouse in five different building in Brittany, France.

In each building, the same number of 1-day-old turkeys (BUT 9) (equally number of males and females) were randomly allocated in each different building. The birds were raised on floor at a stocking density of 7 turkeys per square meter of edifice. Feeds were offered *ad libitum* and constant access to water was provided via one bell type drinker per pen. The feeding program for all treatments was divided in seven regimes from 0 to slaughter age (112 days). The starter diet was fed from 0 to 14 days, the three grower diets from 15 to 56 days, and the three finisher diets up to 112 days. The diets used were based on wheat, maize and soybean meal. The starter and first grower diets were offered as a crumb and all other diets were fed as pellet. All starter and grower diets contained coccidiostat, Elancoban (70 ppm). No antibiotics were used until the GI samples were taken from the animals.

Treatments were: (1) Fructooligosaccharides (sc FOS); (2) essential oil compounds (EO) at equal doses; (3) essential oils extracts (EOX) - at decreasing doses; (4) association of natural plants and EOX and (5) CCPA reference diet.

All of the additives were premixed in calcium carbonate and wheat before being added at 0.2 % to the respective diet.

Ten birds in each diet group were killed by cervical dislocation at age of 4-weeks and 7-weeks respectively. The small intestine was rapidly excised at Meckel's diverticulum to the ileocecal junction (ileum). The ileal content was collected and pooled for the turkeys within each five different diets at each sampling time. The two caeca were also removed from each bird and the contents pooled for the turkeys within each diet at each sampling time in the same way that it was done for the small intestine.

Samples were collected in sterile tubes, immediately frozen and stored at  $-20^{\circ}\text{C}$  until analysed for the microflora profile.

*Eubacterial DNA extraction.* 25 mg of the twenty different pool samples are subjected to a DNA extraction with QIAamp DNA mini kit (Qiagen, California, USA). Extraction efficiency is thus controlled by electrophoresis on a 1 % agarose gel and DNA extracts are quantified by spectrophotometry on a Victor plate reader (Perkin-Elmer, Massachusetts, USA).

*DNA amplification and DGGE.* A nested PCR on 16S rDNA genes is carried out on each of the twenty DNA extracts. First amplification is realized with PU1-PU2 eubacterial universal primer set (Weisburg *et al.*, 1990) and the second with HDA1-HDA2 eubacterial universal primer set (Walter *et al.*, 2000) or with LAC1-LAC2 Lactic Acid Bacteria-specific primer set (Walter *et al.*, 2001). HDA1-HDA2 and LAC1-LAC2 fragments are separated electrophoretically in DGGE which denaturant gradient lies between 30 to 55 % and between 22.5 to 55 % respectively. Migration is realized at 130 V until xylene cyanol reach the bottom of the gel. The revelation of the gels is assured by an ethidium bromure bath at 1  $\mu\text{g}/\text{mL}$  and their reading under an UV transilluminator. Bands of interest are cut with sterile scalpel blades.

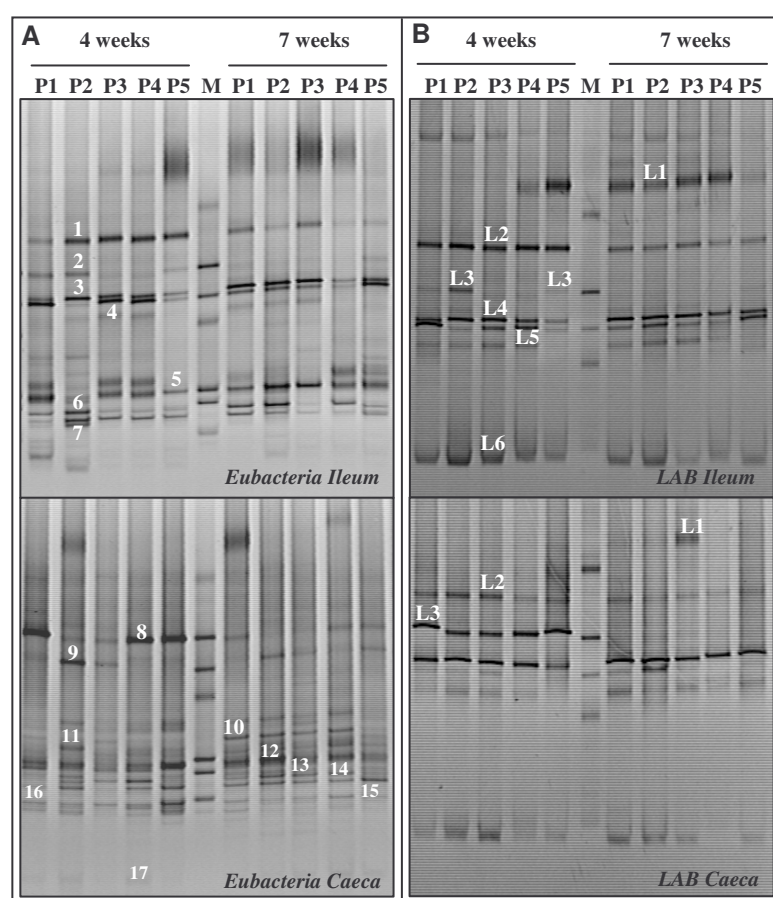
*Sequencing and bacterial identification.* After reamplification, bands of interest are sequenced (Genome Express, France) and the sequence results are compared with the RDPII 16S rDNA database (Cole *et al.*, 2007). The bacterial identification is thus deduced from the best similarity percentage and best S<sub>ab</sub> score.

## Results and discussion

Figure 1 represents the DGGE gel analyses of intestinal segments sampled at 4 and 7 weeks by using different primer sets. Seventeen bacterial species were identified by using the eubacteria specific primer set (Figure 1A). Six bacterial spp. were identified by using the Lactic Acid Bacteria (LAB)-specific primer sets (Figure 1B). Principal genus that has been modulated by used diet treatments were: *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Escherichia*, *Lactobacillus* and *Ruminococcus* (Table 1). The profile of bacteria belonging to the *Clostridium* and *Lactobacillus* genus was highly depend on the type of used diet. Variability of the bacterial profiles was also depending on the bird age and intestinal compartment. The highest eubacterial variability depending on the age and diet type was observed in the caecal compartment (CC). Minor modulation of the eubacterial composition in the

intestinal compartment (IC) was also observed. Importantly, analysis of the DGGE gel by using eubacterial primer set demonstrated that bacteria from the *Lactobacillus* genus follow the same variations observed in the LAB-specific gels. Bacteria from the *Clostridium* genus demonstrated a high variability between treatments particularly in CC. The appearance of the *Bacteroides* spp in CC was associated with the *Ruminococcus* microflora decrease in P1, P4 and P5 treatments at the both ages. Some variability in bifidobacteria profile depending on the used diet was detected in CC in 4 wk old birds. Effectively, this genus is more represented in CCPA reference diet. In LAB-specific gels, the variation of LAB was observed in the IC. In the treatment P2, band L3 appears while band L5 disappears. Moreover, a reversion in the presence/absence of L1 was observed between control and treatments at the both ages.

In summary, P2 treatment leads to the highest modulation of the analyzed microflora when compared to reference treatment P5. For example, the identified *Ruminococcus* and *Lactobacillus* L3 are highly associable with the treatment P2 in CC and IC respectively at the 4 wk old birds. These results prove the potential of certain non-antibiotic feed supplementations to modulate the microflora equilibrium.



**Figure 1.** DGGE gels of 16S rDNA gene eubacterial (A) or Lactic Acid Bacteria-specific (B) amplifications. (P1 to P4: treatments 1 to 4; P5: CCPA reference treatment; M: Markers)

**Table 1.** Match results of the excised band sequences (Specie information remains confidential).

DGGE bands	Identified genus
1 to 4 and L1 to L6	<i>Lactobacillus</i>
5	<i>Escherichia</i>
6, 7 and 10 to 15	<i>Clostridium</i>
8	<i>Bacteroides</i>
9	<i>Ruminococcus</i>
16, 17	<i>Bifidobacterium</i>

As little information about the turkey gut microflora is available in the literature, data comparison and correlation are almost difficult. On the contrary, broiler chicken microflora is well documented and information on the microflora of this poultry provided by Gong *et al.* (2006) or Lu *et al.* (2003) is related with the data given in this study for the turkey gut microflora. For example, *Lactobacillus* in ileum and *Clostridium* and *Bacteroides* in caeca are the majority genus in turkey as well as in broiler chicken.

## Conclusion

DGGE technique is efficient to monitor the principal modulations of the eubacterial intestinal microflora of the turkey. In present work we proved that feed supplementations as sc FOS, essential oil and natural plants have a potential as digestive flora regulators. Future works, conducted by CCPA, will determine the relation between microflora modulation of these non-antibiotic feed supplementations and the zootechnical performances of the animals.

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