

## THE TURKEY INTESTINAL MICROBIOME: A NEXT GENERATION SEQUENCING APPROACH

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### Summary

The diversity and functions of microbes in the gastrointestinal tract is a field of ongoing research, aiming to establish the correlation between them and different genetic changes, diseases, relationship with therapy and the influence on metabolism or immune system of the host (Moura-Alvarez et al. 2014, Danzeisen et al. 2013). The distribution of bacterial population depends on the environment, the different zones of the organism in which microbes grow, the age and the diet of the host.

For our study, samples from four parts of the intestine of turkeys (duodenum, jejunum, ileum and cecum) were collected to obtain a characterisation of intestinal microbiome. Four abundant phyla were found: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. For these phyla analysis at family level were performed, and results shown that Lactobacillaceae family has the higher percentage in samples, while at genus level *Lactobacillus* (phylum Firmicutes), is the most abundant and homogeneously distributed bacterial genus presented in the gut of turkeys, except in cecum zone.

### INTRODUCTION

The word “microbiome” defines at genetic level the presence of different microorganisms in the host, without distinguish among beneficial, neutral or damaging. Considering bacteria species, they are distributed with different density in the organism of the host, depending on the area in which they are located. The chemical and physiological conditions of each part of the organism can determine the growth of bacterial populations.

The gastrointestinal microflora is composed by fungi, protozoa and bacteria, the last one being the predominant microorganisms. Each tract develops different populations of bacteria, that continue to change during growth of birds as correlated to age, diet, breed, and geographic location (Danzeisen et al. 2013, Choi et al. 2014, Scupham et al. 2008). Intestine bacterial populations contribute to the production of vitamins and stimulate the immune system in avian species, but also can cause localized or systemic infections. Independently from health status and life condition (domestic or wild animals), avian microbiome is composed at phylum level by Firmicutes, Bacteroidetes and Proteobacteria, while *Lactobacillus* and *Enterococcus* (belonging to Firmicutes phylum) are the most abundant genera (Oakley et al. 2014).

The aim of this study was to inspect the bacterial distribution, analysing four tracts of the intestine of turkeys: duodenum, jejunum, ileum and cecum.

## **MATERIALS AND METHODS**

Samples were collected from both mucosa and content of the intestine of turkeys:

- 4 samples from duodenum tract
- 8 samples from jejunum tract
- 4 samples from ileum tract
- 4 samples from cecum tract

Bacterial DNA was extracted and then quantified by nanodrop.

To obtain informations about bacterial populations, the hypervariable regions V1-V2 of 16S rRNA gene were amplified by PCR, and then Ion torrent - Next Generation Sequencing (Life Tecnology) analysis were performed. With this tecnology we are able to have a lot of information from samples in few time as compared to other techniques.

## **RESULTS**

The sequences of intestinal turkey microbiome were analysed at phylum, family, genus and species level. Distribution of bacteria in duodenum, jejunum and ileum part is homogeneous at various taxonomic levels, while the composition of cecum one is different.

### **Phylum level.**

The most abundant phyla found are: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria.

Our data show that Firmicutes is the most abundant phylum (range percentage: 45% - 99%) in three intestinal tracts of duodenum, jejunum and ileum, except in the cecum (range percentage: 29% - 52%).

Bacteroidetes phylum (range percentage: 37% - 57%) and Proteobacteria phylum (percentage range: 7% - 12%) are homogeneous and abundant only in cecum zone. Actinobacteria is presents in only few samples, with a percentage up to 22%.

**Family level.** The most abundant family is Lactobacillaceae (phylum Firmicutes), presents in three intestinal tracts (range from 32% to 99%), except in cecum one (range from 1% to 2,28%).

In ileum zone, samples belong mainly to two families: Lactobacillaceae and Pepto-streptococcaceae (both belong to Firmicutes phylum).

In cecum zone, the distribution of bacteria is completely different from the other samples. The most abundant families detected are: Bacteroidaceae (range from 7% to 30%), Order Bacteroidales (range from 10% to 30%) and Lachnospiraceae (range from 12% to 29%).

**Genus and species level.** The major part of sequences found were classified up to family level. The few ones that can be classified at genus level belong to *Lactobacillus*. In this genus the most abundant species is *L. crispatus*: in duodenum, jejunum and ileum zone the range is from 1% to 87%, and in cecum zone is from 0.08% to 0.27%.

## DISCUSSION AND CONCLUSION

This is the first study in which intestinale microbiome in turkeys is characterised. Using Ion Torrent – Next Generation Sequencing, we have obtained information about different bacterial populations that live in the intestine of turkeys in four zones of it.

Predominant bacteria phyla found in our samples were: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. In particular the percentage of Firmicutes is higher respect the other phyla, and is almost homogeneous in duodenum, jejunum and ileum zone, but not in cecum one. The most abundant family found in our samples is Lactobacillaceae, belonging to phylum Firmicutes, and of this family the most abundant genus is *Lactobacillus*.

The cecum zone shows a different distribution of bacteria, at phylum level we obtained a high percentage of Bacteroidetes and Firmicutes, while Proteobacteria is homogeneously ditributed but with a lower percentage respect the other phyla. At family level Bacteroidaceae, belonging to Bacteroidetes phylum, was the most abundant family found togheter with Lachnospiraceae and Veillonellaceae families, both belonging to Firmicutes phylum.

Our data were in agreement with the ones of previous studies:

- Firmicutes is the most abundant phylum in the intestine except for cecum zone.
- Cecum zone has a different bacterial distribution, respect the other intastinal zones.
- Lactobacillaceae is the most abundant bacterial family in the intestine of avian species.
- *Lactobacillus* is the most abundant genus found in chickens and turkeys.

*Lactobacillus* genus was analysed at species level. In all the intestinal areas, except the cecum, *Lactobacillus crispatus* was the most abundant species found, with a percentage until 85%.

Previous studies describe the genus *Lactobacillus* like the most abundant in the upper part of the intestine of animals, togheter with *Enterococcus* and *Bifidobacterium* (Oakley et al. 2014, Wei et al. 2013). They also report the most abundant species in the intestine belonging to *Lactobacillus* genus: *L. johnsonii* and *L. reuteri*. Those results are not in agreement whit what reported in the present finding because we did not found high percentages of *Enterococcus* and *Bifidobacterium* . On the contrary, the most abundant species found was *L. crispatus*.

## REFERENCES

1. Danzeisen JL, Calvert AJ, Noll SL, McComb B, Sherwood JS, Logue CM, Johnson TJ. Succession of the turkey gastrointestinal bacterial microbiome related to weight gain. PeerJ. 2013 Dec23;1:e237. doi: 10.7717/peerj.237. eCollection 2013.

2. Oakley BB, Lillehoj HS, Kogut MH, Kim WK, Maurer JJ, Pedroso A, Lee MD, Collett SR, Johnson TJ, Cox NA. The chicken gastrointestinal microbiome. *FEMS Microbiol Lett.* 2014 Nov;360(2):100-12. doi: 10.1111/1574-6968.12608. Epub 2014 Oct 13.
3. Moura-Alvarez J, Nuñez LF, Astolfi-Ferreira CS, Knöbl T, Chacón JL, Moreno AM, Jones RC, Ferreira AJ. Detection of enteric pathogens in Turkey flocks affected with severe enteritis, in Brazil. *Trop Anim Health Prod.* 2014 Aug;46(6):1051-8. doi: 10.1007/s11250-014-0612-7. Epub 2014 May 12.
4. Scupham AJ, Patton TG, Bent E, Bayles DO. Comparison of the cecal microbiota of domestic and wild turkeys. *Microb Ecol.* 2008 Aug;56(2):322-31. doi: 10.1007/s00248-007-9349-4. Epub 2008 Jan 8.
5. Choi J.H., Kim G.B., and Cha C.J.: Spatial heterogeneity and stability of bacterial community in the gastrointestinal tracts of boiler chickens. *Poultry Science* 93 :1942–1950, 2014.
6. Wei S., Morrison M., and Yu Z.: Bacterial census of poultry intestinal microbiome. *Poultry Science* 92 :671–683, 2013.