Quantitative analysis of the intestinal bacterial communities in broiler chickens using qPCR and metagenomic analysis

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The 16S rDNA sequence analysis has been one of the most used approaches for microbiota taxonomic profiling. Among the available techniques, real-time quantitative PCR (qPCR) and next generation sequencing (NGS) and metagenomic analysis has been widely used by researchers, because in addition to qualitative, it also provide quantitative data. Thus, the aim of our study was to compare the effectiveness of these 2 methods for quantification of 3 groups of bacteria of the intestinal microbiota of broilers fed on a standard corn-soybean based diet. The absolute quantification by qPCR was performed on the equipment 7500 Real-Time PCR System (Applied Biosystems®) using specific primers to accurately discriminate organisms belonging to the family Enterobacteriaceae and Lactobacillus and Enterococcus genera. The sequencing of the 160bp amplicons, covering the hypervariable V3 region of the 16S rDNA was performed in HiScanSQ apparatus (Illumina®). Sequences were checked for low quality reads, chimeric reads and sequencing errors using the software Mothur. In addition, Mothur was also used to align sequences; calculate distances; clustering sequences into operational taxonomic units (OTU); constructs rarefaction curves and assign taxonomic for each sequence using the Silva Database. Using the genus Lactobacillus as a reference, once its qPCR amplicon encompasses the complete V3 region of 16S rRNA gene, qPCR results revealed a lower 16S rDNA copy number for Enterobacteriaceae family and Enterococcus genus (0.02 and 0.14, respectively), while NGS and metagenomic analysis showed relative amounts of 0.51 and 0.22 in the total number of reads, and 0.40 and 0.10 in the number of identified OTUs for Enterobacteriaceae family and Enterococcus genus, respectively. Although both methods were efficient for detecting similar trends in population size, the numerical magnitude of this characteristic showed a large discrepancy between qPCR and NGS analysis, indicating that 16S rRNA hypervariable region targets for family and genus-specific primers can influence the qPCR results, misestimating the size of microbial population under study. Our results strongly suggest that due to its low discriminating power, this family Enterobacteriaceae primer pair should not be used in studies involving the estimation of species richness and evenness of this group of bacteria. Supported by: FAPESP, Embrapa

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