POULTRYNSECT

D3.6 Report on poultry gut microbiota

Deliverable 3.6

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Abbreviations	
ASV	Amplicon Sequence Variant
BSF	Black Soldier Fly
BSFL	Black Soldier Fly Larvae
OTU	Operational Taxonomic Unit
QIIME	Quantitative Insights Into Microbial Ecology
RT PCR	Real Time Polymerase Chain Reaction
WP	Work Package

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Introduction

Introduction

The POULTRYNSECT Work Package 3 ***"Laboratory and Sensorial Analyses"*** aims to evaluate the impact of Black Soldier Fly (BSF) live larvae inclusion as feed ingredient in chicken diet on chicken health and meat quality. This Deliverable (3.6) reports the microbiota composition of cecal gut samples obtained from the first and second *in vivo* feeding trials performed with Label Naked Neck and Bionda di Saluzzo chickens, respectively.

1. Material and Methods

In the first trial, a total of 240 Label naked neck birds were distributed in four experimental groups according to gender and treatment (10 chickens/pen, 60 birds/treatment):

1. males fed basal organic feed;
2. males fed basal organic feed +10% BSF supplementation;
3. females fed basal organic feed;
4. females fed basal organic feed +10% BSF supplementation.

The birds were fed with the experimental diets from day 20 to day 82 (time of slaughter) as described in D2.2.

In the second trial, a total of 144 Bianca di Saluzzo male chickens were hatched and reared until 39 days at the Avian Conservation Centre of Local Genetic Resources of the University of Turin (north-west of Italy) and then selected for the experiment on the basis of the average body weight. The trial was carried out from the end of May 2022 until the middle of October 2022. The initial weight of the birds was around 300 g.

The birds were allotted into 18 pens, after being selected and distributed in three experimental groups, according to the diet and live BSF larvae supplementation (8 chicken/pen, 48 birds/treatment):

1. birds fed commercial feed;
2. birds fed sustainable feed;
3. birds fed sustainable feed +15% live BSF larvae supplementation.

Feed and water were provided ad libitum (18 % crude protein, 4.1% crude fat for the commercial feed and 18.2% crude protein, 4% crude fat for the sustainable one) (Mangimi Monge di Monge Antonio e C. Snc). The feed composition of the commercial diet and the alternative one were respectively, as it follows, mainly composed by:

1. commercial diet: corn meal, soybean meal, soybean oil
2. sustainable diet: corn meal, corn gluten, field bean, pea protein, sunflower flour, barley flour.

1.1 Microbiota composition

At slaughter, samples of caecal content were collected from 18 birds/treatment (3 birds/replicate) and stored at −80 °C prior to DNA extraction and sequencing.
Total DNA was extracted from each sample using the RNeasy Power Microbiome KIT (Qiagen. Milan. Italy) following the manufacturer's instructions. One microliter of RNase (Illumina Inc. San Diego. CA) was added to digest RNA in the DNA samples with an incubation of 1 h at 37 °C. DNA was quantified using Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., Wiington, DE, USA) and standardized at 5 ng/μL.
The extracted DNA was used to assess the microbiota by the amplification of the V3-V4 region of the 16S rRNA gene (F: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGA CAGCCTACGGGNGGCWGCAG-3′; R: 5′-GTCTCGTGGGCTCGGAGATGTGTATAA GAGACAGGACTACHVGGGTATCTAATCC-3′) [**Klindworth et al. 2013**]. The PCR products were purified according to the Illumina metagenomic standard procedure (Illumina Inc., San Diego, CA, USA). Sequencing was performed with a MiSeq Illumina instrument with V3

chemistry and generated 250 bp paired-end reads in accordance with the manufacturer's instructions.

Regarding microbiota, paired-end reads were first merged using FLASH software with default parameters [**Magoc and Salzberg, 2011**]. Joint reads were further quality filtered (at Phred < Q20) using QIIME 1.9.0 software through a multiple_split_libraries_fastq.py script [**Caporaso, et al. 2010**] and the pipeline recently described in [**Biasato et al. 2018**]. Operational Taxonomic Unit (OTU) clustering was obtained at 97% of similarity by the pick_otus.py script and taxonomy assignment was assessed by Greengenes 16S rRNA gene database v. 2013 using the RDP Classifier, with a minimum confidence score of 0.80. The OTU table was rarefied at the lowest number of sequence (12.337 reads) and display the higher taxonomy resolution. The vegan package of R was used to calculate the alpha diversity [**Dixon, 2003**]. The diversity indices were further analysed using the Wilcoxon rank sum test to assess differences between the dietary treatments. Weighted UniFrac distance matrices and OTU table generated through QIIME were used to perform Adonis and Anosim statistical tests in R environment. A Generalized Linear Model was used in order to test the importance of insect administration on the relative abundance of OTU.

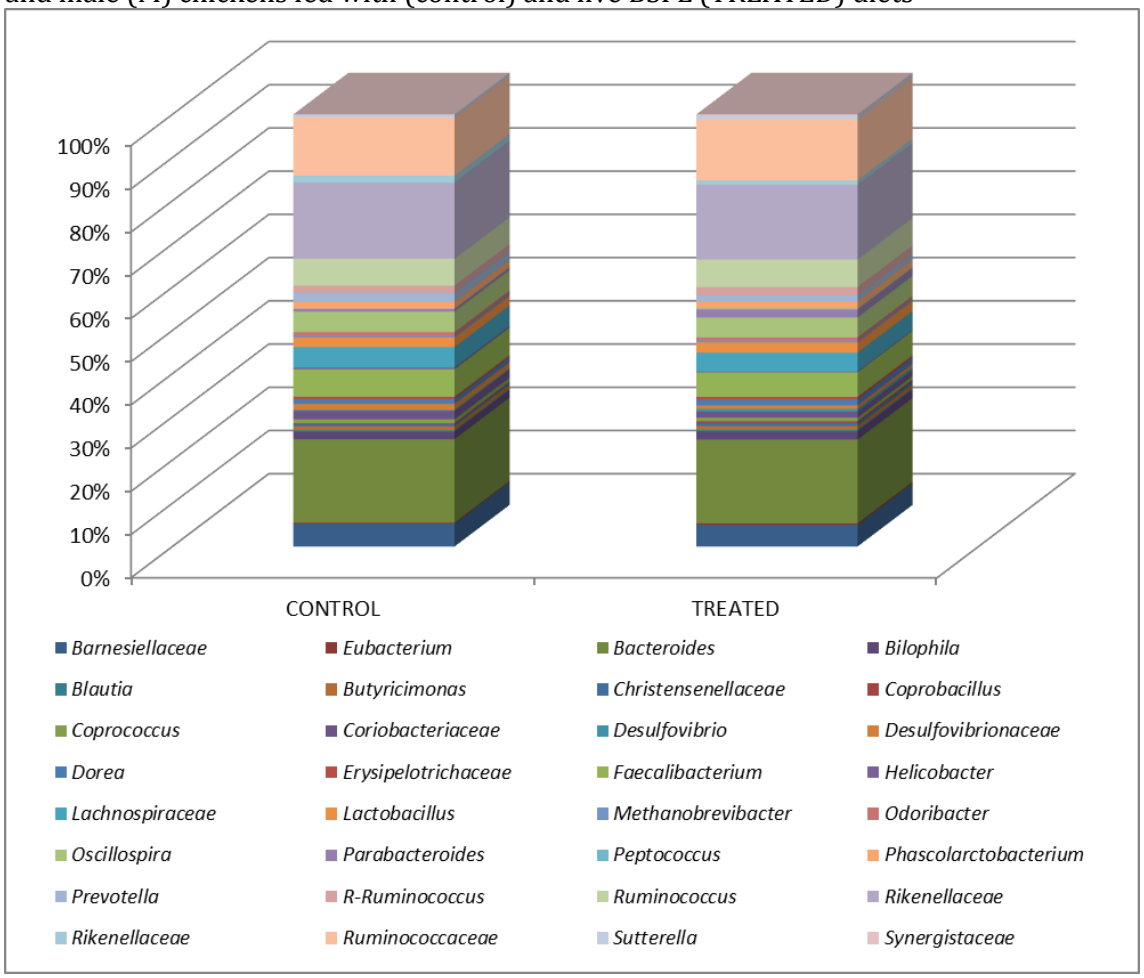
2. Preliminary results and discussion

2.1 Caecal microbiota characterization

As far as the first trial (2021) is concerned, after sequencing and quality filtering, a total of 320038 reads were used with an average value of 5334 reads/sample and a sample coverage of 99%.

The microbiota of birds in the two diets was characterized by the presence of Bacteroides (18 and 17% of the relative frequency on average in control and treated birds respectively), Rikenellaceae (17 and 15%), Ruminococcaceae (12 and 13%), Faecalibacterium (6 and 5 %), Ruminococcus (6 %), Barnesiellaceae (5 and 4 %), Oscillospira (4 %), Lachnospiraceae (2 %), Lactobacillus (2 %) and Prevotella (2 and 1 %) (Fig. 1) . However, it was possible to observe that the minor fraction of ASVs (relative frequency < 1%) was influenced by nutrition (fdr < 0.05). In detail, the treated (L) samples were characterized by the predominance of *Christensenellaceae*, *Coprobacillus* and *Synergistaceae*. Therefore, no differences were observed between male (M) and female (F) broilers between the two (control *vs* BSF Larvae) dietary treatments.

Figure 1: Relative abundance of the main bacterial genera in cecal samples of female (F) and male (M) chickens fed with (control) and live BSFL (TREATED) diets



As far as the second trial (2022) is concerned, though no significant difference in alpha diversity parameters was observed (data not shown), the analysed samples were separated based on their microbiota composition. In particular, Control samples were separate from samples belong to different diets (Figure 2, $P<0.05$). Observing the microbiota in detail the differences were due to five different genera: *Faecalibacterium, Odoribacter* and *Negativibacillus* which showed the lowest relative frequency in the Control samples; while *Lactobacillus* and *Oscillospiraceae* were present in high relative frequency in the Control samples.

Samples of the three different diets showed the same metataxonomic composition although with some variations according to the diets (Figure 3). *Bacteroides, Rikenellaceae, Clostrdia, Prevotellaceae* and *Lactobacillus* were the most abundant genera in the dataset (Figure 3). Subdominant ASVs were present in all the samples between the diets but without any significant differences in their relative frequency.

Figure 2: Bacterial community composition (weighted UniFrac beta diversity, PCA plots) in cecal samples of Bionda di Saluzzo chickens slaughtered at 137 days fed with control (C), sustainable and sustainable live BSF larvae diets.

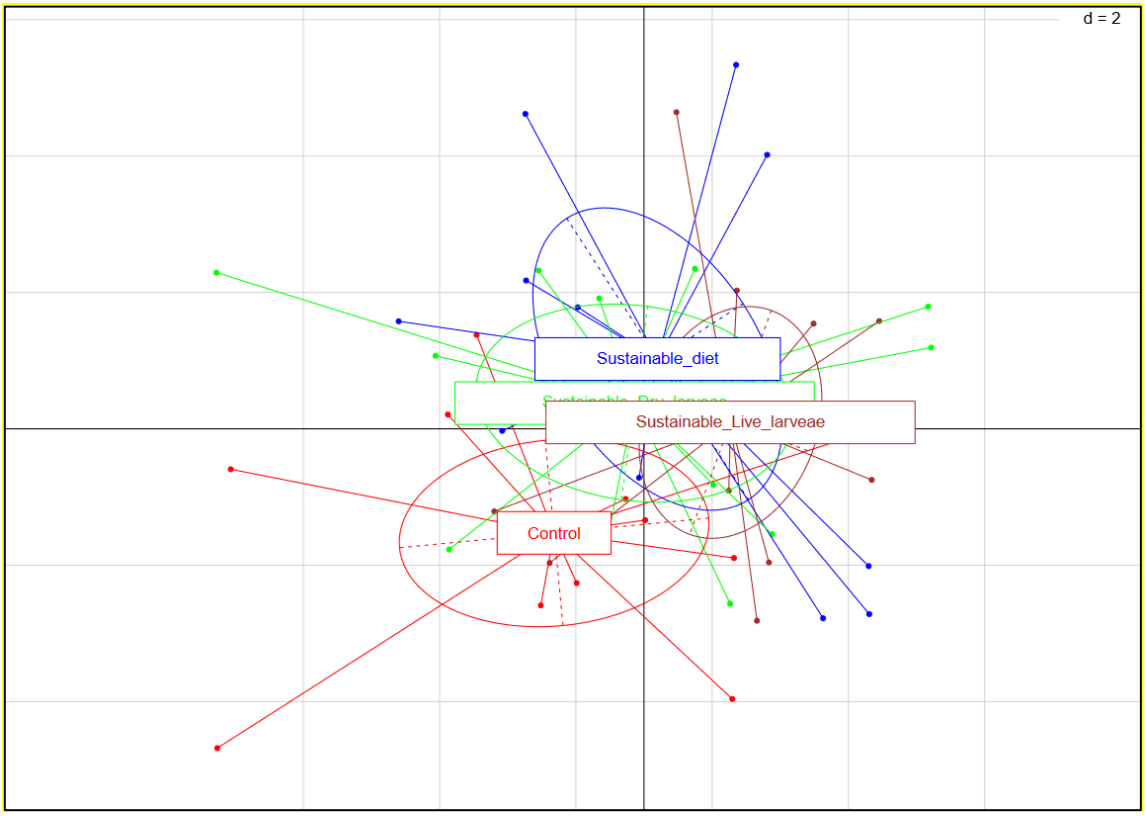
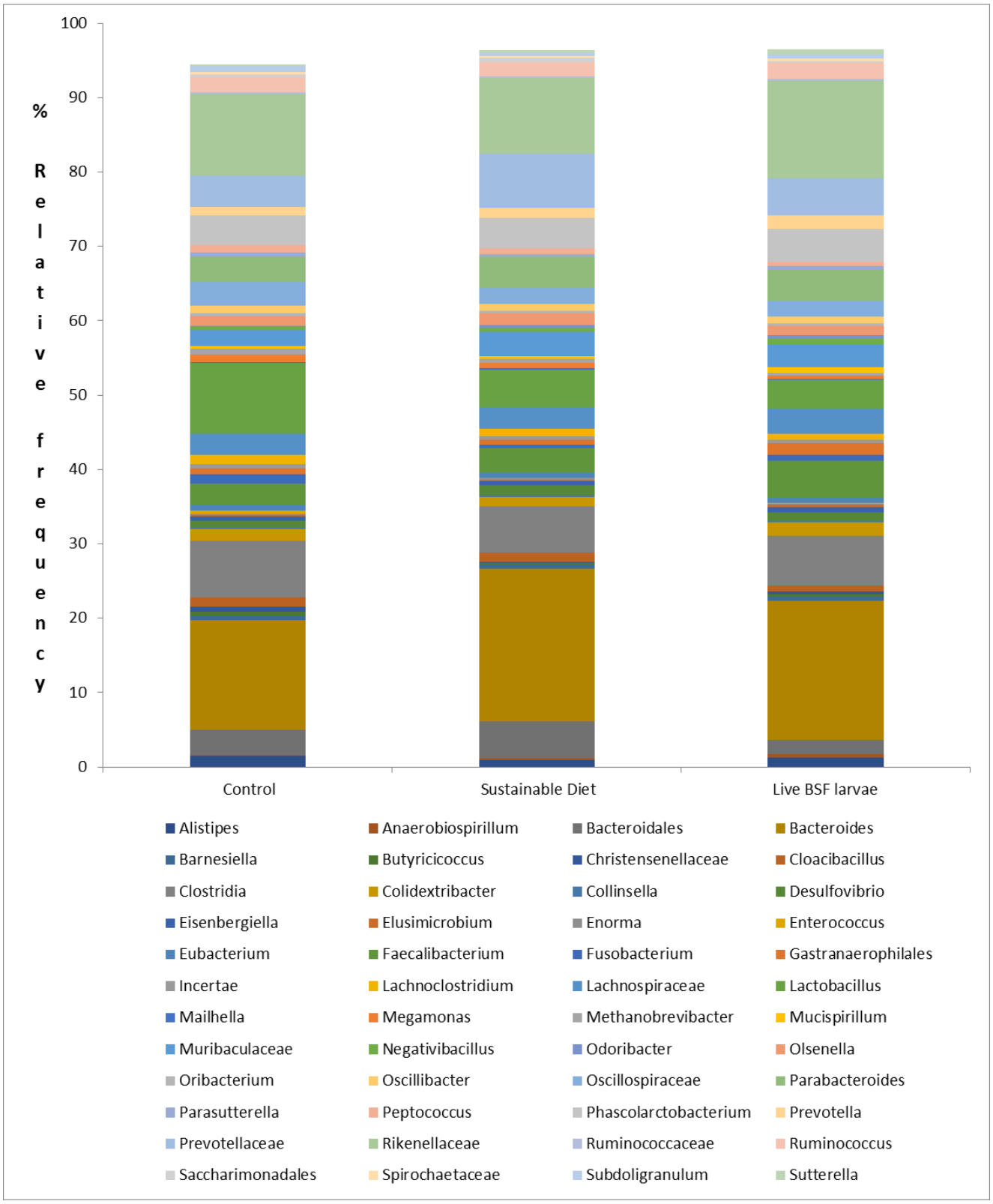


Figure 3: Relative abundance of the main bacterial genera in caecal samples of Bionda di Saluzzo chickens slaughtered at 137 days fed with control, sustainable and live BSF larvae diets



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