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CHANGES IN THE MICROBIOTA OF AGED SQUAB MEAT DETERMINED BY CULTURE AND 16S rDNA ANALYSIS

Begoña Rubio*, Luisa Blanco[1], Verónica Casero[1], Ceferina Vieira[1], Patricia González-García[2], Jerson Garita-Cambronero[2]

[1] Estación Tecnológica de la Carne. Instituto Tecnológico Agrario de Castilla y León. C. Filiberto Villalobos, 5, 37770 Guijuelo, Salamanca.

[2] Laboratorio de Biología Molecular y Microbiología. Instituto Tecnológico Agrario de Castilla y León. Ctra. Burgos, Km 119. Finca Zamadueñas. 47071 Valladolid

*Corresponding author email: rubherbe@itacyl.es

I. INTRODUCTION

Squab meat is considered a delicacy, so there is a growing interest in this type of alternative meat [1]. Its consumption is mostly limited to specialty restaurants and a common culinary practice among chefs is to keep the squabs whole with their feathers and intestines intact and to hang them in a cooling chamber to age for some time. This treatment, called “faisandage”, is used to tenderize the meat for a period of up to 14-21 days. This practice could compromise meat safety due to bacterial growth, since the digestive tract of birds is the primary natural reservoir of both pathogenic and nonpathogenic bacteria [2, 3]. This study aims to evaluate the changes in amounts/proportions of meat microbiota in squab meat subjected to “faisandage” treatment by traditional microbiology testing and by amplicon sequencing of the 16S (rDNA) gene; thus, enhancing food quality assurance and consumer protection.

II. MATERIALS AND METHODS

Thirty whole squabs (*Columbia livia*) were slaughtered and transported under chilled conditions to the “Estación Tecnológica de la Carne” (Guijuelo, Salamanca, Spain). They were hung and stored in a cooling chamber at 0–4°C. Five squabs per day were selected for analysis at 0, 7, 12, 17, 21 and 27 days. At each sampling point, the squabs were dressed, with feathers removed, internal organs carefully extracted and the heads severed. Each carcass was then deboned using sterile forceps and scalpels, and a homogeneous sample was prepared from the meat.

Ten grams of the meat sample were homogenized in 90 mL of sterile tryptone water using a laboratory stomacher (dilution 10^{-1}). Serial decimal dilutions were made in sterile tryptone water and 1 ml of each dilution was pour-plated on 3 M Petrifilm Aerobic Count Plate and incubated at 30°C for 72 h to determine total viable count (TVC), on 3 M Petrifilm *Enterobacteriaceae* Count Plate and incubated at 37°C for 24 h to determine *Enterobacteriaceae* count, and on 3 M Petrifilm Selective *E. coli* Count Plates (3 M) and incubated at 42°C for 24 h to determine *E. coli* count. The results, expressed as log cfu/g, were statistically analyzed using one-way ANOVA. When statistical significances were found, the means were separated by Tukey's post hoc (significant difference test at 5% level).

For DNA extraction, 5 g of meat was mixed with 50 mL of Phosphate Buffered Saline and then, a 5 mL aliquot was collected and centrifuged at 13,000 rpm for 2 min at room temperature. After discarding the supernatant, total DNA was extracted from the pellet using the DNeasy PowerFood Microbial Kit (Qiagen) according to manufacturer’s recommendations. To assess bacterial diversity at each sampling point, amplicon sequencing of the V3-V4 region of the 16S rDNA gene was used. Sequencing libraries were prepared following the Illumina protocol and sequenced on a MiSeq system using the MiSeq Reagent Kit v3 (600 cycles, Illumina Inc.). Sterile molecular-grade water served as a negative control to assess background contamination in the laboratory environment, while a standardized microbial community sample (ZymoBIOMICS D6300) was used as a positive control. The resulting sequence reads were analyzed using an in-house bioinformatics pipeline. Read quality was assessed with MultiQC, and high-quality reads were processed using Qiime2 with the Dada2 algorithm to denoise sequences and remove chimeras. Laboratory contaminants were identified and filtered using Decontam. Taxonomic classification of amplicon sequence variants (ASVs) was performed using the SILVA v.138 database, retaining only bacterial ASVs for further analysis. Metadata and sequencing data were then imported into R for alpha and beta diversity analyses using the Phyloseq and MicrobiomeMarker packages. Finally, differentially abundant ASVs across sampling points were identified using ANCOM-BC.

III. RESULTS AND DISCUSSION

Results from the evaluation of bacterial indicators are shown in Table 1. Despite the differences ($p<0.05$) observed in the enterobacteria and *E. coli* counts, microbial stability was observed in squab meat over 27 days. Similar results were observed by Rubio et al. [4] in squab meat subjected to different aging periods (0-14 days), who pointed out that the origin of contamination was the evisceration stage, as the animal microbiota are mainly present in the digestive tract.

Table 1 –Mean (log CFU/g) ± SD of microbial counts in different aging times.

	Aging times					
	Day 0	Day 7	Day 12	Day 17	Day 21	Day 27
Total viable count	5.2±0.5	5.8±0.5	5.6±0.8	5.7±0.8	5.6±1.3	6.9±1.2
***Enterobacteriaceae* count**	3.3±0.4^{a}	4.3±0.6^{ab}	4.9±0.7^{bc}	3.7±1.1^{ab}	3.9±0.7^{ab}	5.3±1.2^{c}
***E. coli* count**	3.1±0.5^{ab}	4.1±0.6^{ab}	4.2±0.8^{b}	2.8±0.9^{ab}	2.4±0.8^{a}	2.6±1.3^{ab}

Different lowercase letters in the same row indicate significant differences between aging times by Tukey test (P < 0.05).

Regarding the metataxonomic analysis, from the 29 sequenced samples, 1,071,756 high-quality reads were classified into 1,103 ASVs, with a mean of 36,957 ASVs per sample. Taxonomic analysis placed the ASVs into 12 bacterial phyla, with Actinobacteriota, Firmicutes, and Proteobacteria being the ones that had a relative abundance of over 1% at all sampling points. The ASVs obtained were classified into 212 bacterial genera, 17 of which had a relative abundance of over 1% at all sampling points. The relative abundance of these genera varied throughout the experiment (Figure 1.A). Alpha diversity indices (Richness and Shannon index) showed no differences across sampling days (K-W; χ^2 = 7.8309; df = 5; p = 0.1658 and χ^2 = 1.1145; df = 5; p = 0.9528 for Richness and Shannon index, respectively). Pairwise PERMANOVA results revealed significant differences in microbial beta diversity between sampling points (Weighted Unifrac; pseudo-F = 1.585; R^2 = 0.25; p = 0.001). Statistical differences were observed in bacterial community composition at days 0, 7, and 12, whereas no significant differences were detected from day 12 to the end of the study (27 days). Finally, the ANCOM-BC analysis identified significant differential abundance in 18 taxa (p ≤ 0.001) across sampling points. Among them, *Actinomyces*, *Atopobium*, and *Gallibacterium* were significantly depleted after day 12, whereas all the remaining taxa (*Acinetobacter*, *Aeromonas*, *Bacillus*, *Enterococcus*, *Lysinibacillus*, *Pantoea*, *Paraclostridium*, *Pseudomonas*, *Serratia*, *Sphingobacterium*, and *Staphylococcus*) were enriched from day 12 onwards (Figure 1.B).

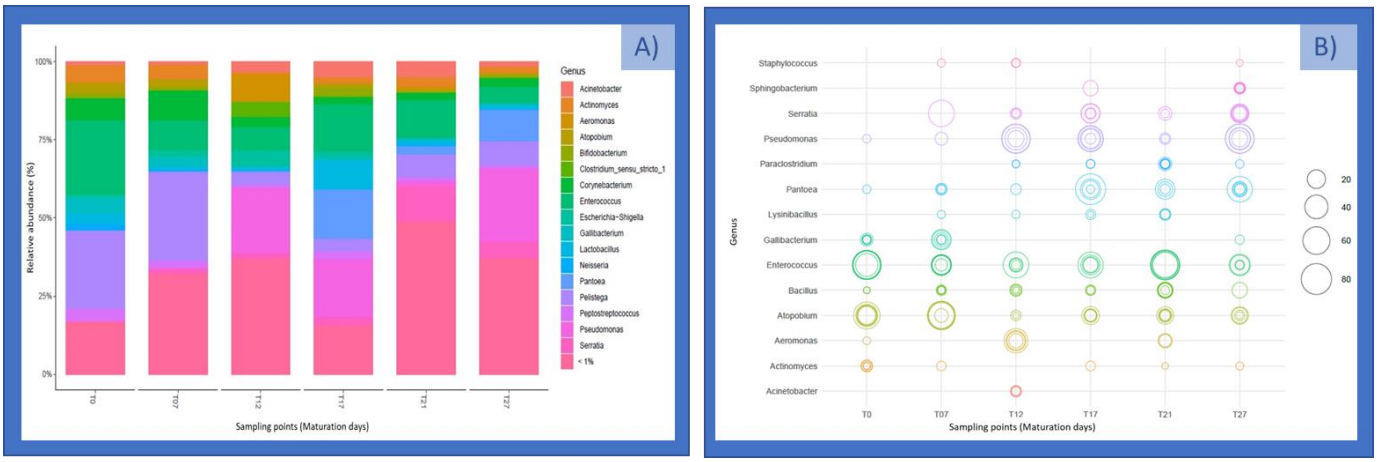


Figure 1. **A)** Bacterial genera with a relative abundance of over 1% and their population dynamics during the 27-day squab meat maturation process. **B)** Bacterial genera showing significant differential abundance during the 27-day squab meat maturation process. Different bubble sizes indicate the relative abundance of the taxa. The number of bubbles per sampling point represents the number of samples in which the genus was present (n = 5). *Samples were collected at days 0, 7, 12, 17, 21, and 27 (represented as T0 to T27 in the chart).

IV. CONCLUSION

Knowledge of the proportions of bacteria obtained by 16S rDNA could help determine the hygiene status of squabs meat for each ageing time, thus enhancing food quality assurance and consumer protection.

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REFERENCES

1. Prabakaran, R., Ezhil Valavan, S., & Thiruvenkadan, A. K. (2020). Diversified poultry production: An overview. *Journal of Entomology and Zoology Studies*, *8*(2), 211–217.
2. El-Rayes, T. K., Mohmed, E. A., & Ahmed, A. I. (2018). Assessment of dietary supplementation of turmeric (*curcuma longa*) as a phytobiotic on broiler performance and bacterial count. *Egyptian Journal of Nutrition and Feeds*, *21*(2), 519–528. doi:10.21608/ejnf.2018.75612.
3. Rubayet Bostami, A. B. M., Yang, C. J., Hossain, M. D., Islam, M. R., Ajoy, M. K. H., Rahmanand, M. M., & Sultana, A. (2021). Carcass traits, blood parameters and meat composition of quail and pigeon available in Bangladesh. *Meat Research*, *1*(1), Article 2. https://doi.org/10.55002/mr.1.1.
4. Rubio, B., Guzmán, J., Lera, L. A., & García, J. J. (2022). Effect of Aging Time of Squabs on Microbiological Characteristics and Quality Attributes of Their Meat. Journal of Culinary Science & Technology, 22(4), 667–679. https://doi.org/10.1080/15428052.2022.2073935.