PRESLAUGHTER FEED WITHDRAWAL TIME AND ITS EFFECT ON RABBIT'S CAECUM AND FAECES MICROBIOTA

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I. INTRODUCTION

Preslaughter feed withdrawal (FWT) is commonly used in the livestock industry to reduce transport-related sickness that is associated with stress, downers, animal death and microbial contamination (vomiting, diarrhoea). A smaller and emptier gastrointestinal tract (GIT) that results from feed withdrawal also decrease the risk of viscera puncture during evisceration [1]. In hogs, longer FWTs have been shown to increase faecal shedding of *Enterobacteriaceae* and *Escherichia coli* creating a contamination risk for the carcasses and threatening product safety [2]. Our objective is to study the effect of FWT on rabbit's gastrointestinal microbiota without the stress caused by crating and transport.

II. MATERIALS AND METHODS

A total of 72 Grimaud rabbits (35-days old) were allocated by weight into 12 cages, each with six animals (three males and three females) per cages to reflect commercial practices. Rabbits were fed *ad libitum* with a commercial diet until they reached 2.5 kg. Animals from one cage was sacrificed every 2-3h in a separated room by excision of the jugular vein after proper stunning using a non-penetrating captive bolt pistol. Caecum content and faeces were harvested directly from the colon and immediately frozen at -80°C. Total genomic DNA was extracted (DNeasy PowerLyzer PowerSoil Kit**;** Qiagen, Hilden, Germany) from 250 mg of faecal or caecum samples. DNA (750 ng/rabbit) was pooled to obtain two sample composites (male and female) per FWT. Sequences from 16S rRNA gene amplicon (Illumina MiSeq) were categorised based on sample type (faeces or caecum), sex (female or male) and FWT (0 to 24 h). Beta diversity (between samples) was calculated using the Bray-Curtis dissimilarities [3] with the phyloseq R package (version 1.36.0). Principal coordinate analysis (PCoA) was used to visualize the distances using the ampvis2 R package (version 2.7.10). Correlation with physiological variables (blood lactate, cortisol, glucose and urea concentrations, GIT weight, stomach content weight, stomach and caecum pH and stomach DM) was added to the graphs as red arrows, using the envfit function of the vegan R package (version 2.5-7) [4]. To improve detection of *Enterobacteriaceae*, specific PCR analyses were performed (forward primer: 5'-GACCTCGCGAGAGCA-3'; reverse primer: 5'-CCTACTTCTTTTGCAACCCA-3').

III. RESULTS AND DISCUSSION

For the β-diversity analysis, three FWT groups were created for the principal coordinate analysis (PCoA). Group 1 includes FWTs between 0 h and 6 h, Group 2 between 8 h and 14 h (caecotrophy 8–12 h) and Group 3 between 16 h and 24 h (Fig. 1). These PCoA results reveal changes in the microbial community structures as the FWT increased (all P value < 0.002; PERMANOVA). For both caecum and faeces, FWT led to more microbiota variations between samples compared to sex. PCoA based on Unweighted and Weighted UniFrac distances gave similar results (data not shown). The vectors shown overlapping the PCoA analyses (Fig. 1) indicate that blood urea concentration and caecum pH increased with longer FWTs. However, blood glucose concentrations, stomach pH, stomach DM, stomach content weight and

GIT weight were higher with shorter FWTs (all P < 0.001; ANOVA GLM procedure). *Enterobacteriaceae* were in low abundance in our study, as indicated by the lack of identification by 16S amplicon analysis. Using specific primers for PCR analysis, *Enterobacteriaceae* were only found in male faeces after 18 h and 22 h of FWT (data not shown).

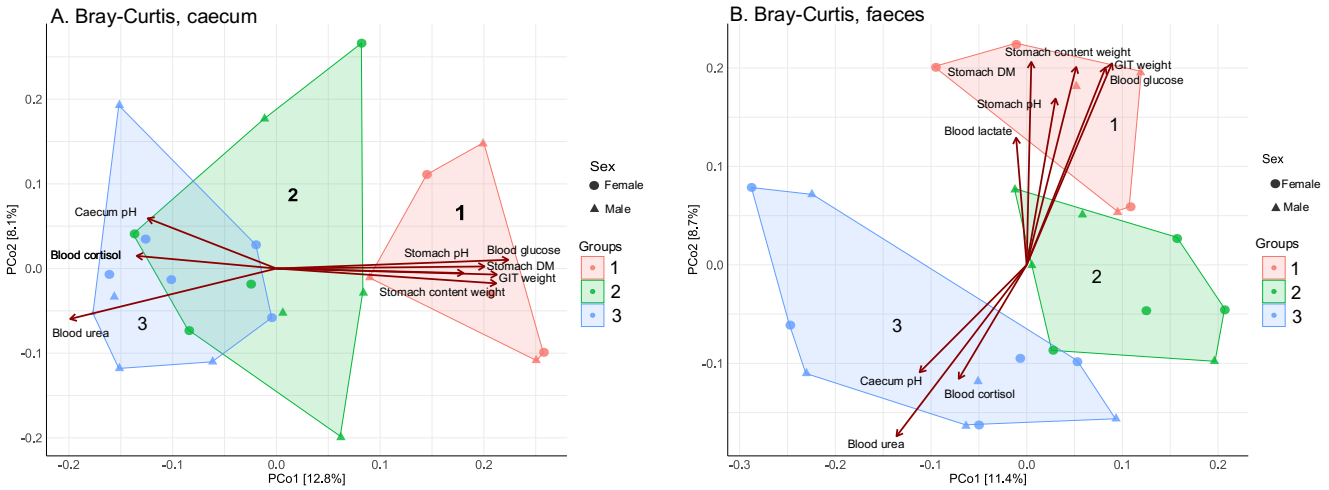

Figure 1. Principal coordinate analysis illustrating β-diversity based on the sequencing of the 16S gene amplicons using Bray-Curtis distance matrix according to sample type (caecum, A or faeces, B) and feed withdrawal time (FWT) from rabbits slaughtered onsite. Group 1 = 0–6 h, group 2 = 8–14 h (caecotrophy 8–12 h) and group 3 = 16–24 h of FWT. Correlation vectors (in red) of the top 10 contributive physiological parameters for the sample distribution are also represented. Arrows are displayed at a scale of 25%. Stomach DM: Stomach dry matter. GIT weight: Gastrointestinal tract weight.

IV. CONCLUSION

Beta diversity indicated a difference in microflora composition between the different FWT groups. A FWT longer than 18 h is not recommended as *Enterobacteriaceae* were detected in the faeces of male rabbits after 18 h and 22 h of FWT. Also, the limited numbers detected by metataxonomic and PCR analyses suggest a lower risk of faecal shedding for rabbits compared to other animal species such as pigs and poultry. Nonetheless, longer FWTs could still present the risk of faecal shedding of those undesirable organisms and contaminate the carcass during slaughter.

ACKNOWLEDGEMENTS

This study was carried out with financial support from the *Programme de soutien à l'innovation en agroalimentaire*, a program derived from the Growing Forward agreement between the *Ministère de l'agriculture des pêcheries et de l'alimentation du Québec* (MAPAQ) and Agriculture and Agri-Food Canada.

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