

## SUPPLEMENTARY INFORMATION

### Microbial characterization of inocula

The primers 341F (5'- CCTACGGGNGGCWGCAG) and 785R (5'- GACTACHVGGGTATCTAATCC) that target the V3-V4 region of the 16S rRNA gene (Klindworth et al., 2013), with an extra wobble position in the reverse primer to make it more universal, were used to target total bacteria. The DNA extracts had a DNA concentration of 20 ng  $\mu\text{L}^{-1}$ , and were free of RNA, which was validated with agarose gel electrophoresis. The PCR mix included 1  $\mu\text{L}$  of DNA extract, 15 pmol of both the forward primer 341F 5'- NNNNNNNNNTCCTACGGGNGGCWGCAG and reverse primer 785R 5'- NNNNNNNNNNTGACTACHVGGGTATCTAAKCC (Klindworth et al., 2013), 20  $\mu\text{L}$  volume of MyTaq buffer containing 1.5 units MyTaq DNA polymerase (Bioline) and 2  $\mu\text{L}$  of BioStabII PCR Enhancer (Sigma). For each sample, the forward and reverse primers had the same unique 10-nt barcode sequence. The PCR contained an initial predenaturation step for 2 min at 96 °C, followed by 20 cycles using the following parameters: 96°C for 15 s, 50°C for 30 s, 70°C for 90 s. The PCR products were purified using Ampure XP beads, according to the manufacturer's instructions, to remove primer dimer and other small mispriming products, followed by an additional purification on MinElute columns (Qiagen). Finally, about 100 ng of each purified amplicon pool DNA was used to construct Illumina libraries by means of adaptor ligation using the Ovation Rapid DR Multiplex System 1-96 (NuGEN). Illumina libraries were pooled and size selected by preparative gel electrophoresis.

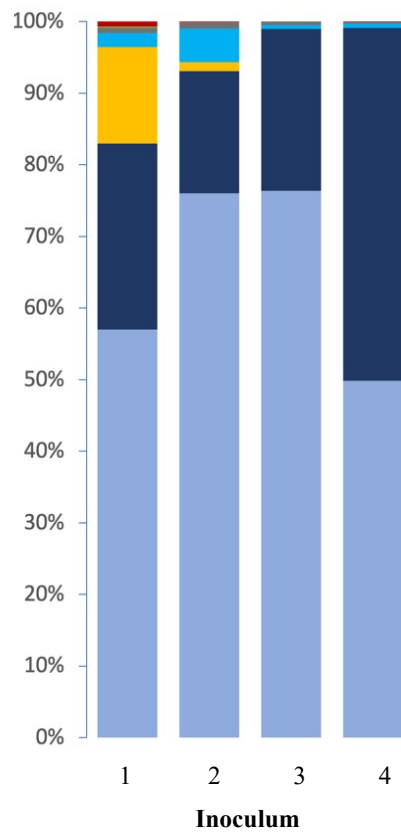
The Mothur software package (v.1.44.3), and guidelines developed by Schloss et al. (2009) were used to process the raw Illumina data using R version 4.1.0 on a x86\_64-pc-linux-gnu (64-bit) system. The forward and reverse reads were assembled into contigs by a heuristic approach, taking the Phred quality scores into account. Ambiguous contigs or with unsatisfying overlap were removed, and the remaining sequences were aligned to the mothur formatted silva

seed v138 database. Those sequences not aligning within the region targeted by the primer set or sequences with homopolymer stretches with a length  $> 12$  bp were removed. The sequences were pre-clustered, allowing mismatch for every 100 bp of sequence. Chimeric sequences were removed with VSEARCH (v2.13.3) (Rognes et al., 2016). Classification of the sequences was carried out by a naïve Bayesian classifier (Wang et al., 2007), using the RDP 16S rRNA gene training set, release 16, with an 85% cut-off for the pseudobootstrap confidence score. Taxa that were annotated as Chloroplast, Mitochondria, unknown, Archaea or Eukarya at the kingdom level were excluded. Sequences were clustered into OTUs with an average linkage, and at a 97% sequence identity, using the OptiClust method (Westcott and Schloss, 2017). If sequences could not be classified at the (super) Kingdom level, they were removed. Representative sequences were picked for each OTU as the most abundant sequence within that OTU.

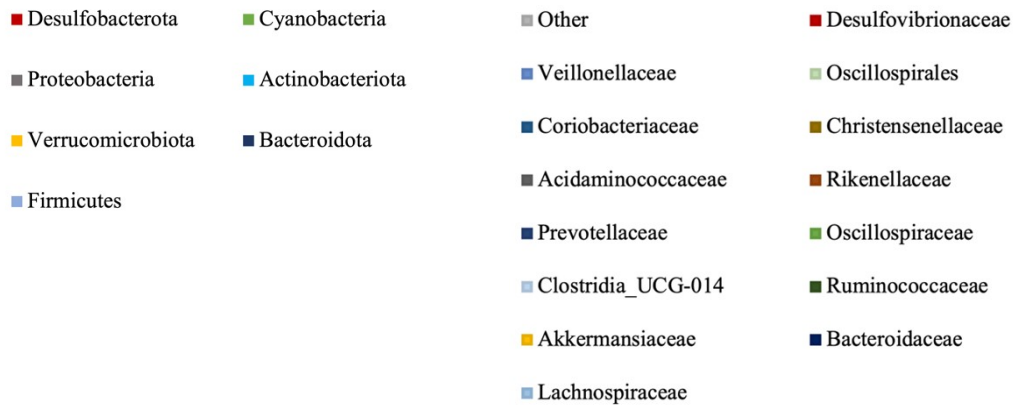
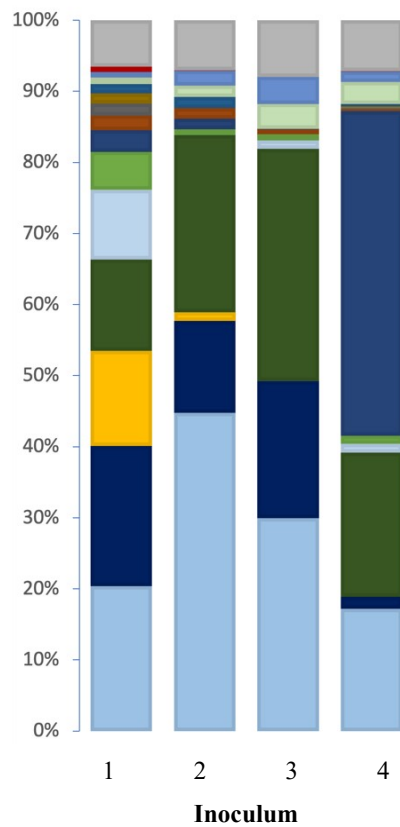
**Supplementary Table 1.** Composition of the simulated digestive fluids.

MOUTH		STOMACH		SMALL INTESTINE			
Saliva		Gastric Juice		Duodenal Juice		Bile Juice	
0.900 g	KCl	2.750 g	NaCl	7.010 g	NaCl	5.260 g	NaCl
0.200 g	KSCN	0.270 g	NaH <sub>2</sub> PO <sub>4</sub>	3.390 g	NaHCO <sub>3</sub>	5.790 g	NaHCO <sub>3</sub>
0.900 g	NaH <sub>2</sub> PO <sub>4</sub>	0.820 g	KCl	0.080 g	KH <sub>2</sub> PO <sub>4</sub>	0.380 g	KCl
0.570 g	Na <sub>2</sub> SO <sub>4</sub>	0.400 g	CaCl <sub>2</sub> · 2H <sub>2</sub> O	0.560 g	KCl	0.150 ml	HCL (37%)
0.300 g	NaCl	0.037 g	NH <sub>4</sub> Cl	0.050 g	MgCl <sub>2</sub>		
1.690 g	NaHCO <sub>3</sub>	7.500 ml	HCl (37%)	0.180 ml	HCl (37%)		
0.007 g	NaNO <sub>2</sub>	6.000 ml	Urea	4.000 ml	Urea	10.00 ml	Urea
8.000 ml	Urea	0.020 g	Glucuronic acid	1.000 g	BSA	1.800 g	BSA
0.012 g	Uric acid	0.650 g	Glucose	9.000 g	Pancreatin	30.00 g	Bile
0.290 g	Amylase	0.330 g	Glucosamine -HCl	1.500 g	Lipase	0.222 g	CaCl <sub>2</sub>
		0.018 g	Ascorbic acid	0.200 g	CaCl <sub>2</sub>		
		1.000 g	BSA				
		2.500 g	Pepsin				
		3.000 g	Mucin				
		0.011 g	FeSO <sub>4</sub> · 7H <sub>2</sub> O				

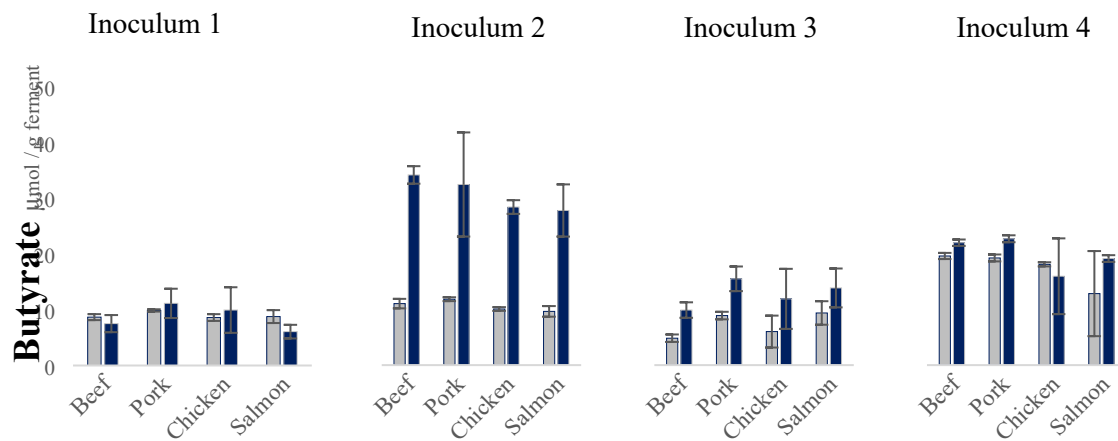
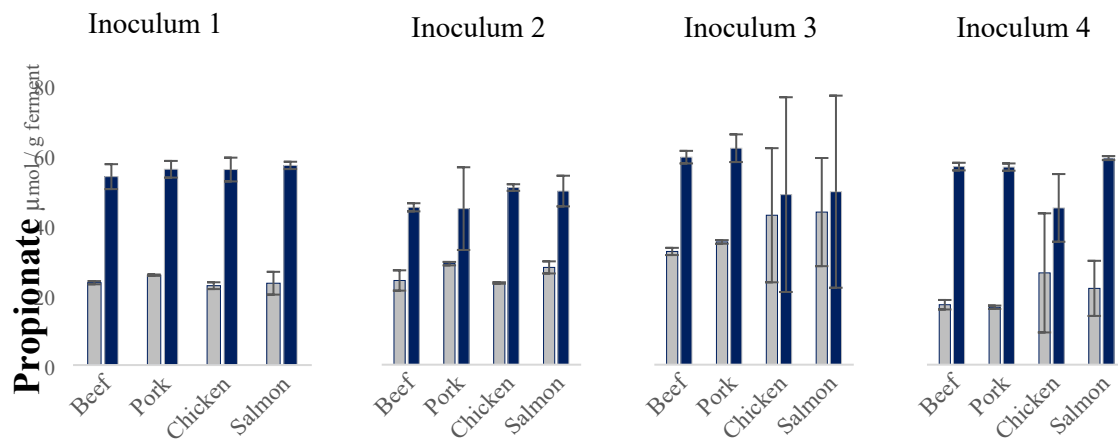
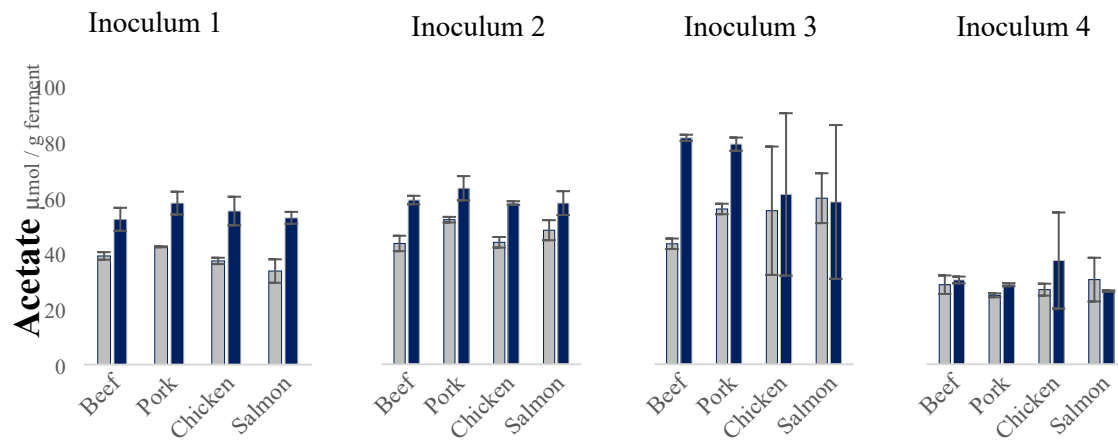
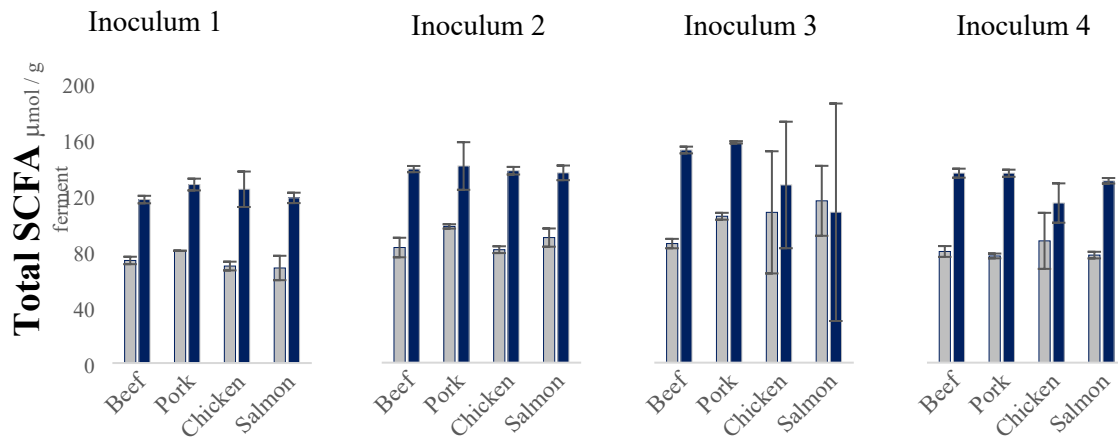
(A)

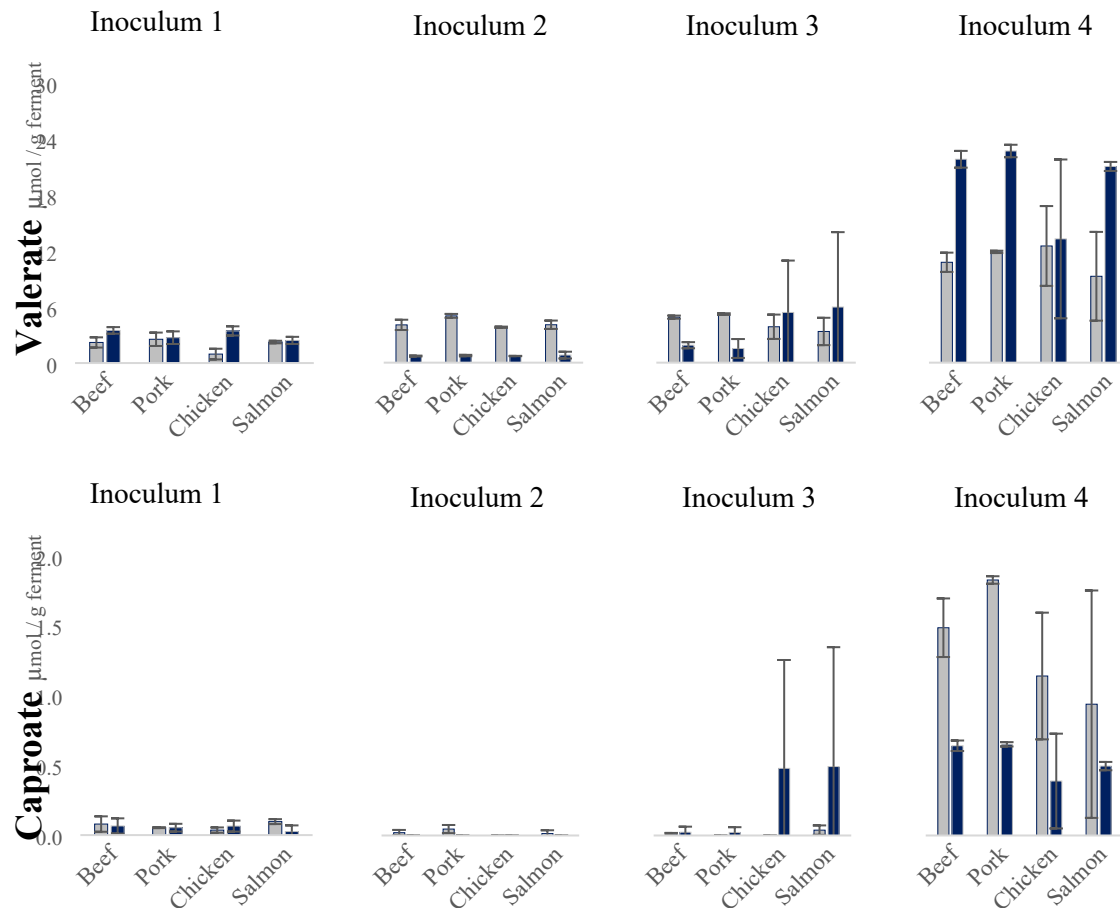


(B)

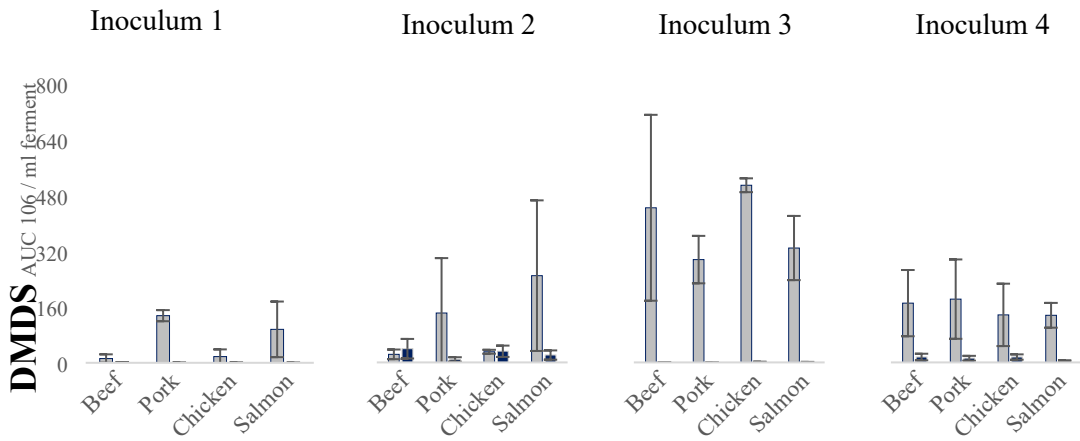
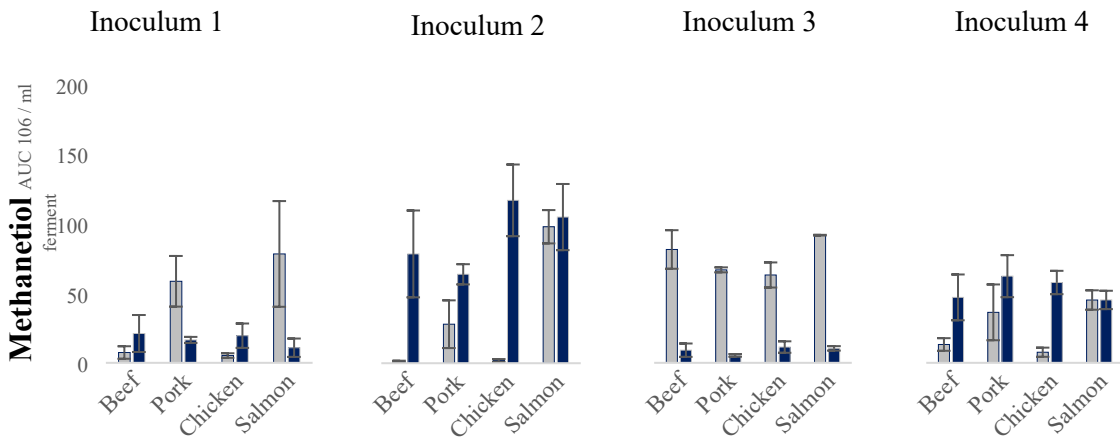
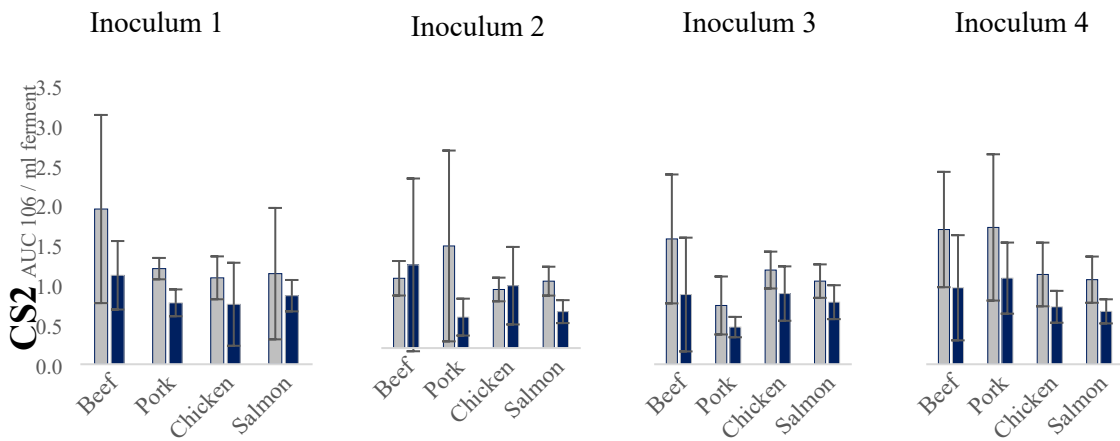
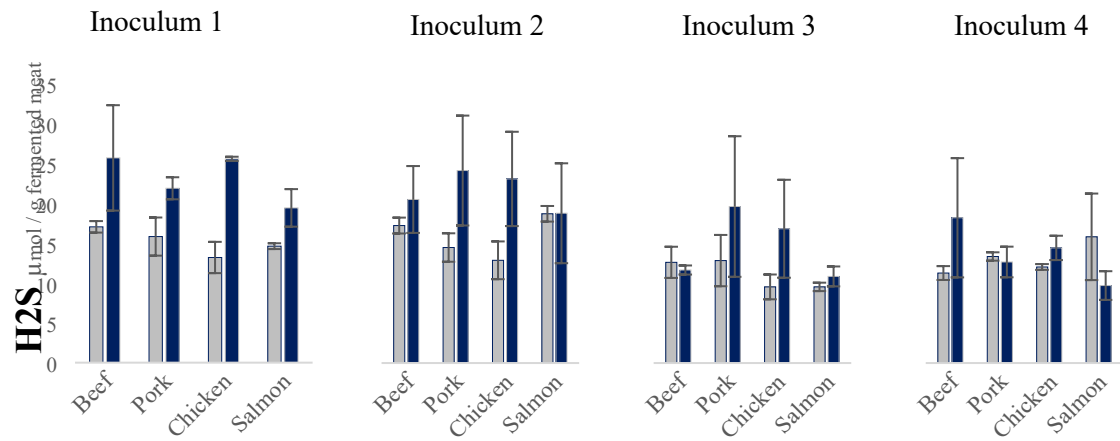


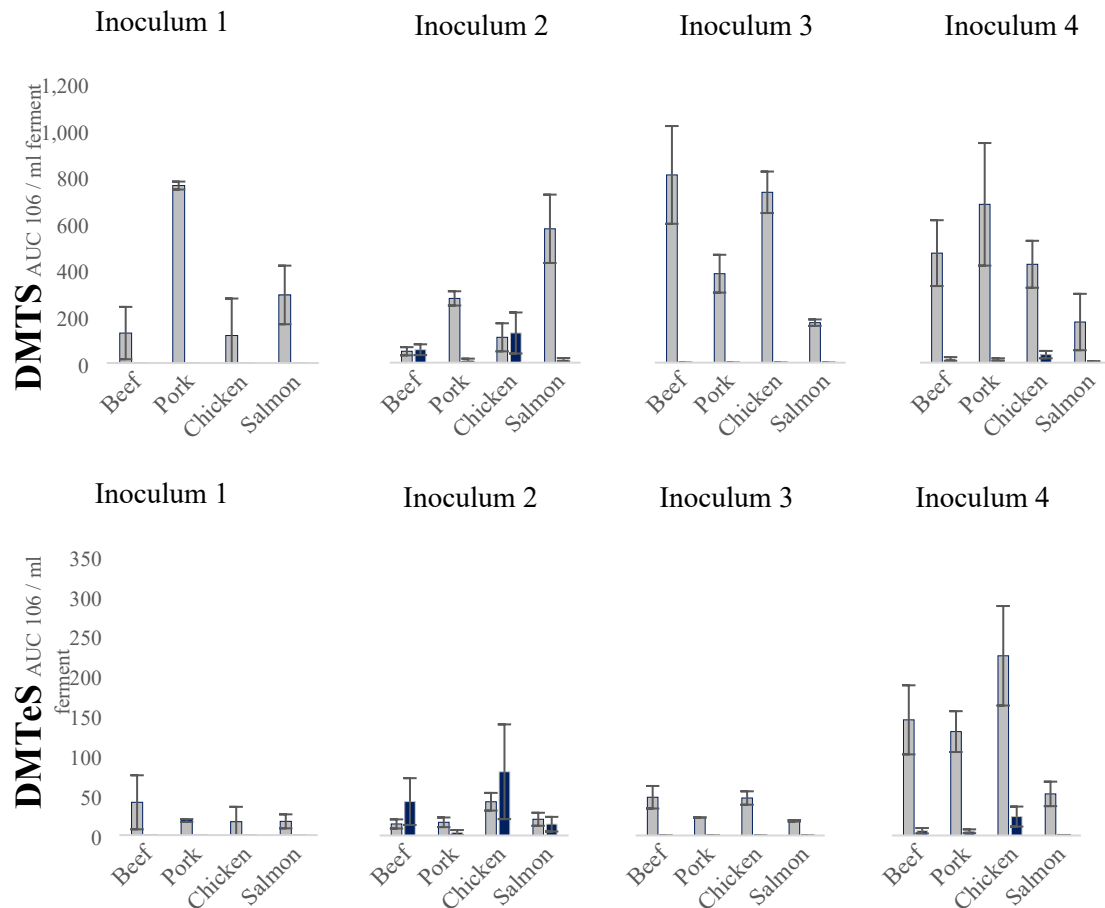
**Supplementary Figure 1.** Microbial community characterization of inocula at the phylum (A) and family (B) level.





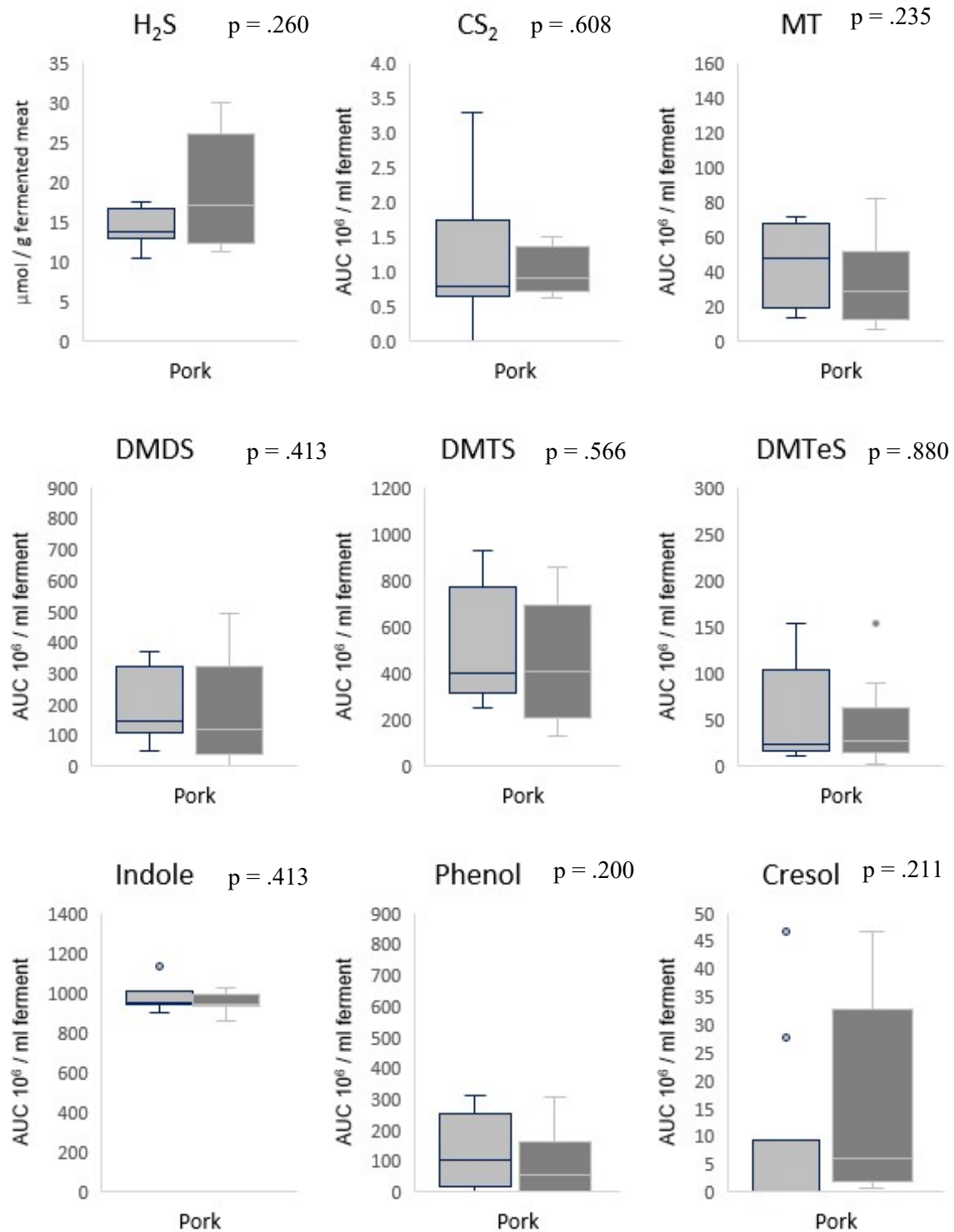
**Supplementary Figure 2.** Levels of SCFA in ferments of muscle sources with (■) or without FOS (□) for the different inocula.



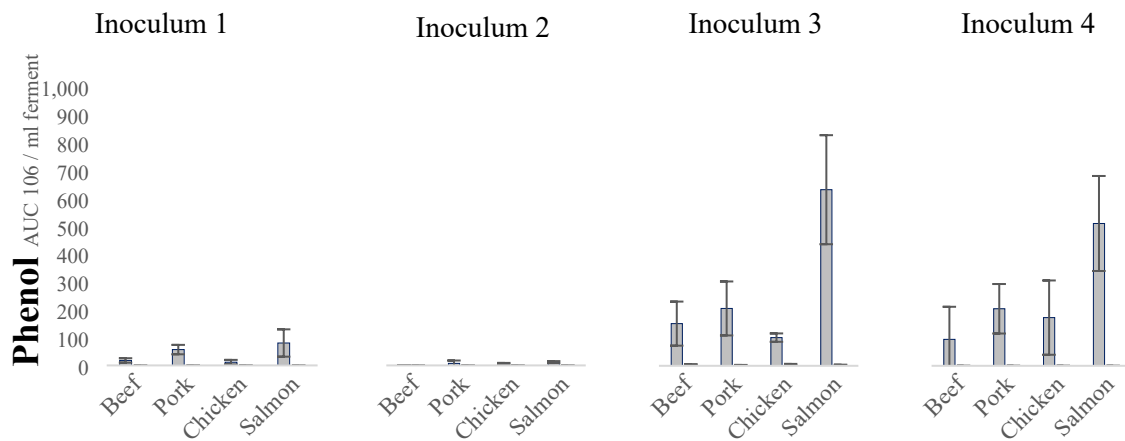
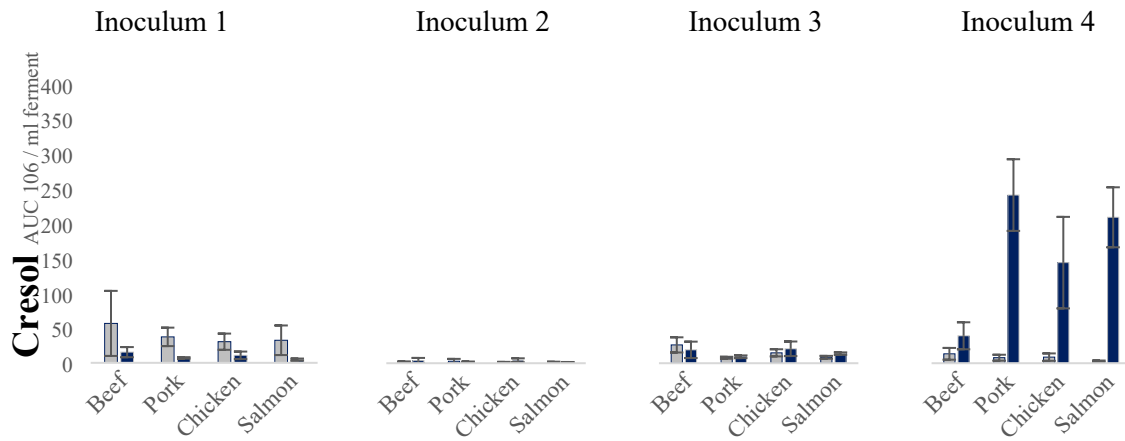
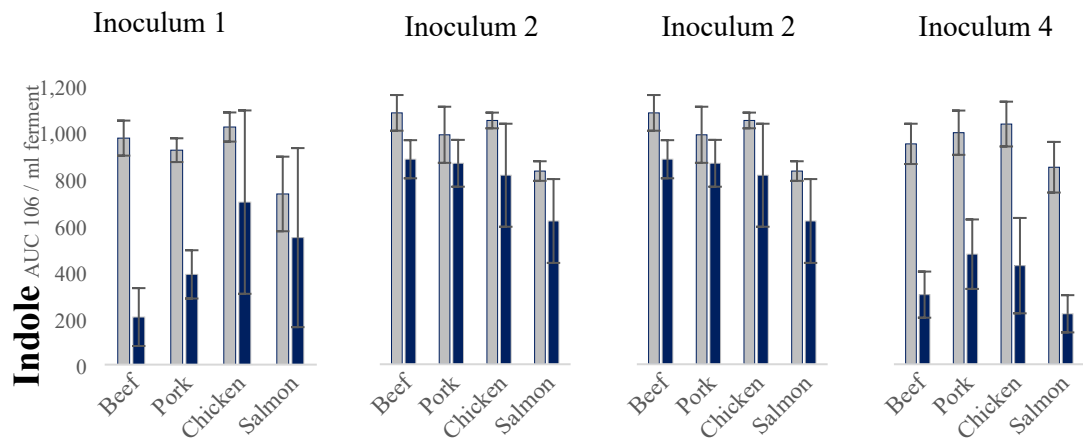
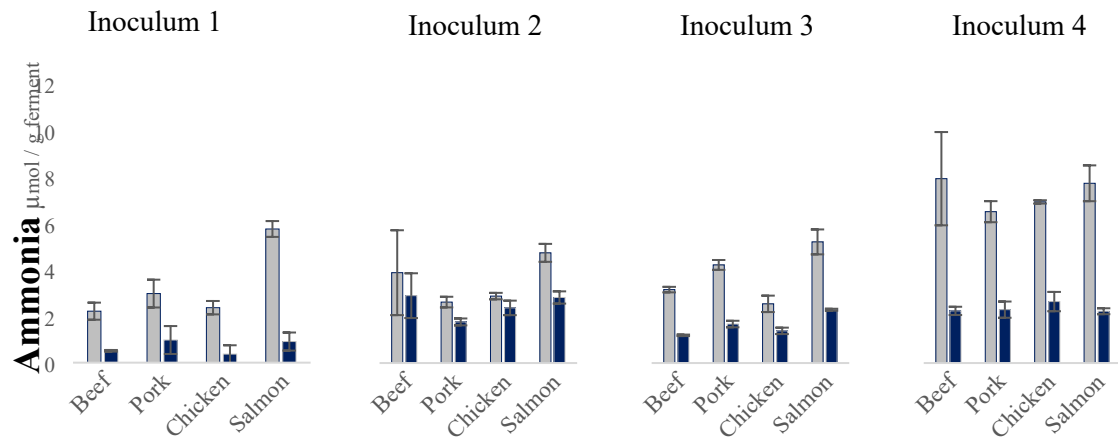


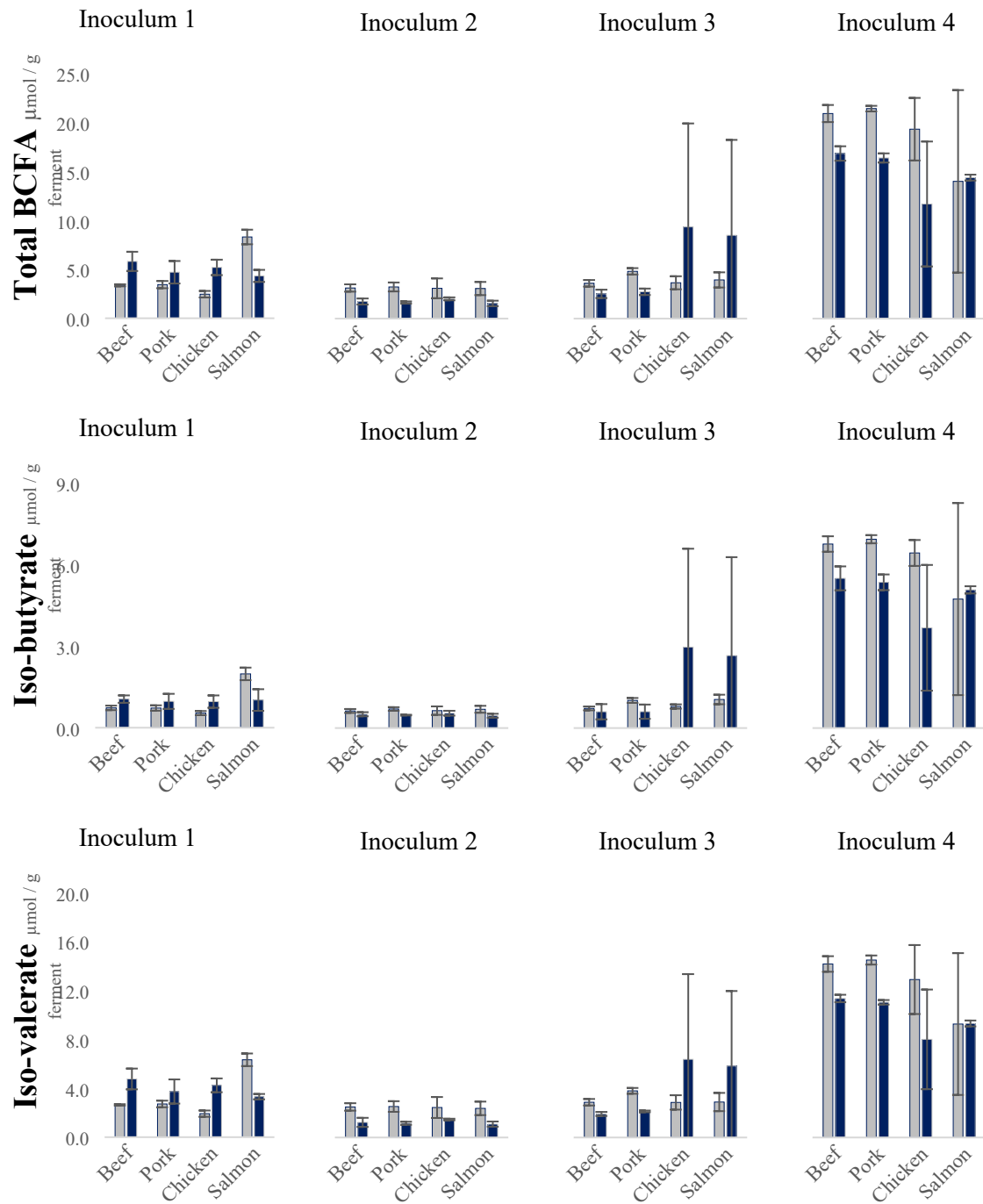
**Supplementary Figure 3.** Levels of sulfur metabolites in ferments (24h) of muscle sources with (■) or without FOS (■) for the different inocula.





**Supplementary Figure 4.** Levels of sulfur and protein metabolites in ferments of muscle sources with (□) or without (■) mucin.





**Supplementary Figure 5.** Levels of non-sulfur protein fermentation metabolites in ferments of muscle sources with (■) or without FOS (■) for the different inocula.

## REFERENCES SUPPORTING INFORMATION

- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic acids research*, 41(1), e1. <https://doi.org/10.1093/nar/gks808>
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- Westcott, S. L., & Schloss, P. D. (2017). OptiClust, an Improved Method for Assigning Amplicon-Based Sequence Data to Operational Taxonomic Units. *mSphere*, 2(2), e00073-17. <https://doi.org/10.1128/mSphereDirect.00073-17>