monogut health

Optimal gut function in monogastric livestock

VALIDATION OF A NON-INVASIVE TOOL TO COLLECT SMALL INTESTINAL CONTENT IN PIGS

-CapSa-

Inés García Viñado (ESR2)

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 955374.

Abstract

IN-VIVO VALIDATION OF A NON-INVASIVE TOOL TO COLLECT INTESTINAL CONTENT IN PIGS -CAPSA-

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Pig microbiome has become a focus of intense research in recent years, because its modulation can greatly impact pigs' health. So far, stool samples have been the only non-invasive mean to study the gut microbiome. However, microbiome in the gut differs from the one in the stool. Capsule for Sampling (CapSa) is a swallowable device that transits through the natural digestive pathway, collects small intestine content, and is excreted in feces. In this validation study, 93 pigs (ranging from 6 to 67 kg bodyweight (BW)) were administrated 2 CapSas each and monitored for 3 days until recovery of the CapSas in the feces. Upon retrieval from feces, CapSa contents were extracted, their pH were measured, and they were stored at -80°C until microbiota analysis. Three days after administration, pigs were euthanized and content from small and large intestine, and feces were collected for microbiome analysis.
In total, 38 CapSas were retrieved from 28 pigs (n=9; 12±3.4kg BW fed a starter diet; n=19; 50.5±12.3kg BW fed a standard grower diet). Bacterial composition of CapSas were compared with the microbiota extracted from 3 segments of the small and large intestine, and the feces of the same pig. Pairwise Adonis contrast demonstrated that CapSa sample composition was different ($P<0.001$) from the large intestine and feces, while it was more similar to the composition of the segment 1 and 2 of the small intestine for lighter pigs fed with a starter diet (P adj=0.32 and R2=0.10; P adj=0.06 and R2=0.12) but not (P adj=0.001 and R2=0.12; P adj=0.001 and R2=0.14) for heavier pigs, fed with a grower diet. The CapSa samples were consistently characterized by the abundance of certain bacteria like Terrisporobacter (LDA=4.42, P=0.002 and LDA=4.61 and $P<0.01$; in lighter and heavier pigs respectively). CapSa has been validated for repeated collections of the gut microbiota in postweaning pigs, but needs to be confirmed in grower pigs with more precise post-mortem samplings.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N°955374.

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Continue with EAAP presentation…….

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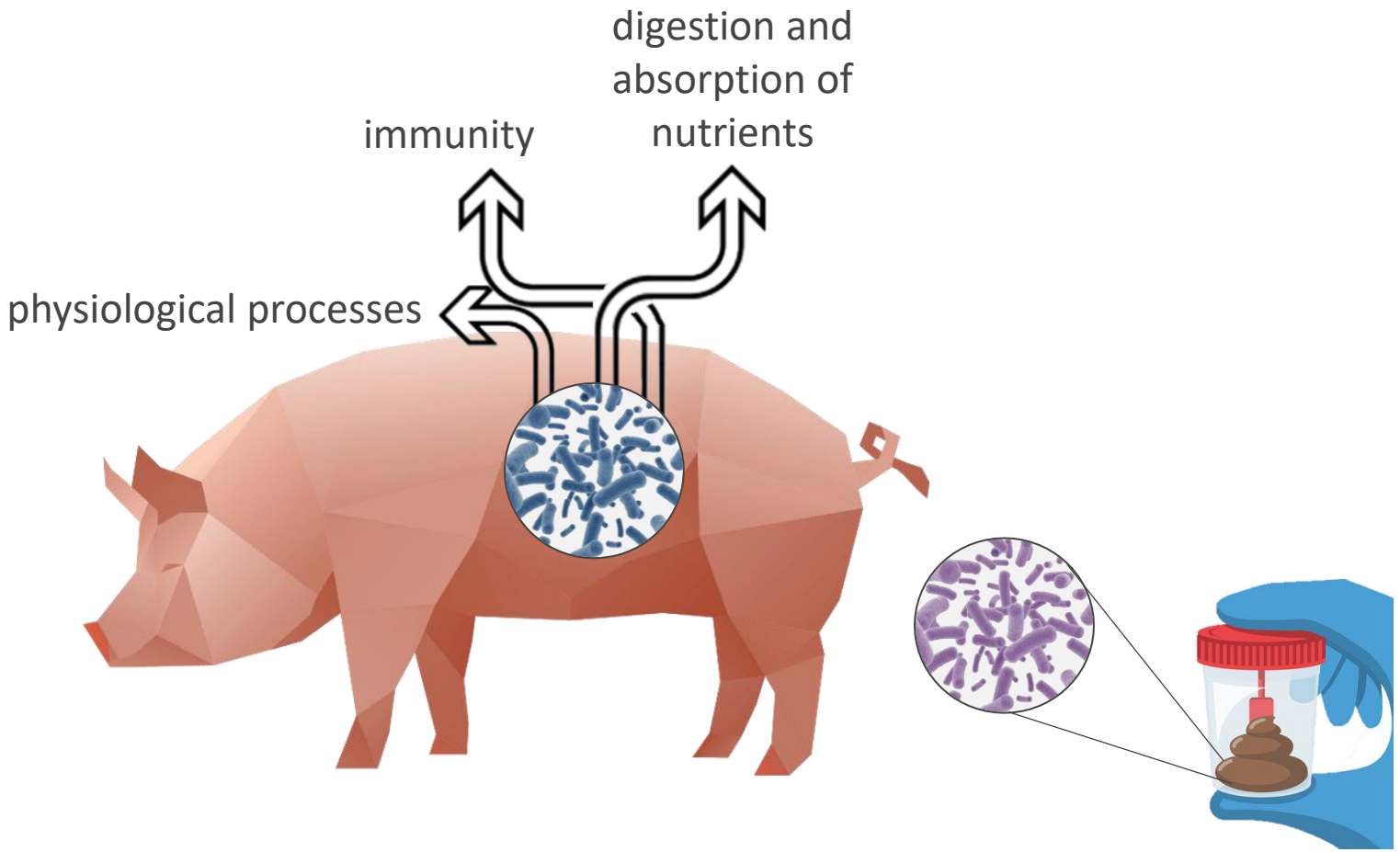
Introduction



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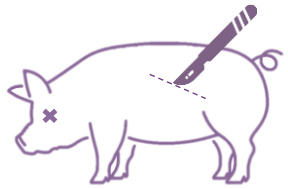
Methods to sample microbiota:

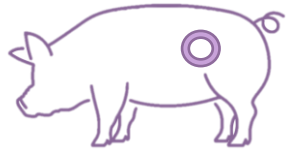


Post-mortem sampling

Feces sampling

Endoscopy

Biopsies



Cannulated animals

Ethical and practical reasons

Accuracy?

Frontiers in Cellular and Infection Microbiology, Review, 2020

Current Sampling Methods for Gut Microbiota: A Call for More Precise Devices

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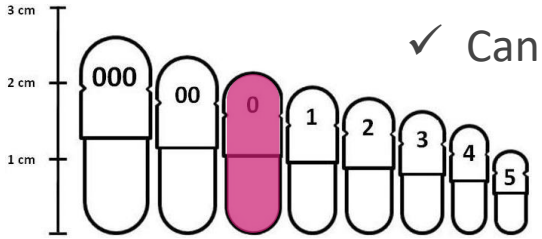
Objectives

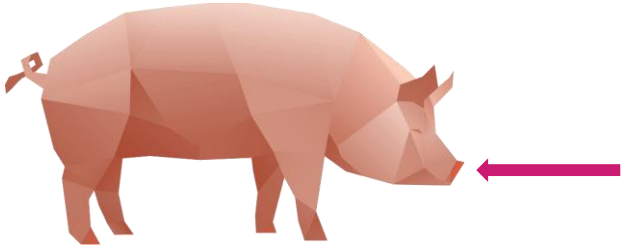
⇨ **Validate a new sampling capsule to perform non-invasive collection of the intestinal microbiota.**



✓ Can collect up to 400µL of liquid.

✓ 3D printed and biocompatible

✓ Retrieval in the feces



✓ Oral administration

✓ Opening based on physicochemical properties of the environment

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Study design

BW category	n=	BW ± SD (kg)	% males	% females
Size XS (>12kg)	14	8.31 ± 1.60	50	50
Size S (≥ 12 - 20kg)	21	14.06 ± 1.40	66.7	33.3
Size M (≥20-40kg)	17	34.34 ± 4.01	82.35	17.65
Size L (≥40-70kg)	32	55.85 ± 8.00	53.1	46.9
TOTAL	84	33.12 ± 20.51	69.1	38.1

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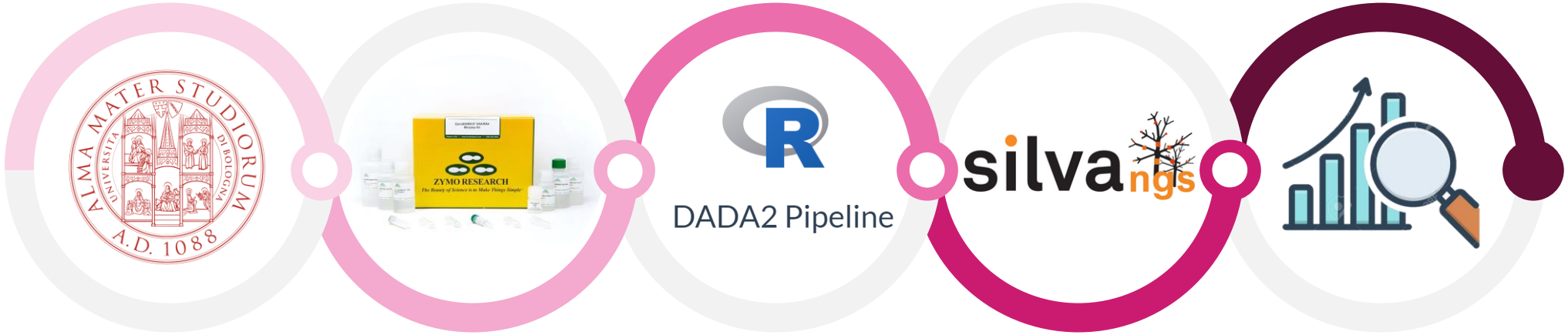
Microbiota analysis

DNA extraction

Taxonomy assignments



Shipping of Samples to Unibo

Microbiota analysis

Beta diversity analysis and Pairwise Adonis test

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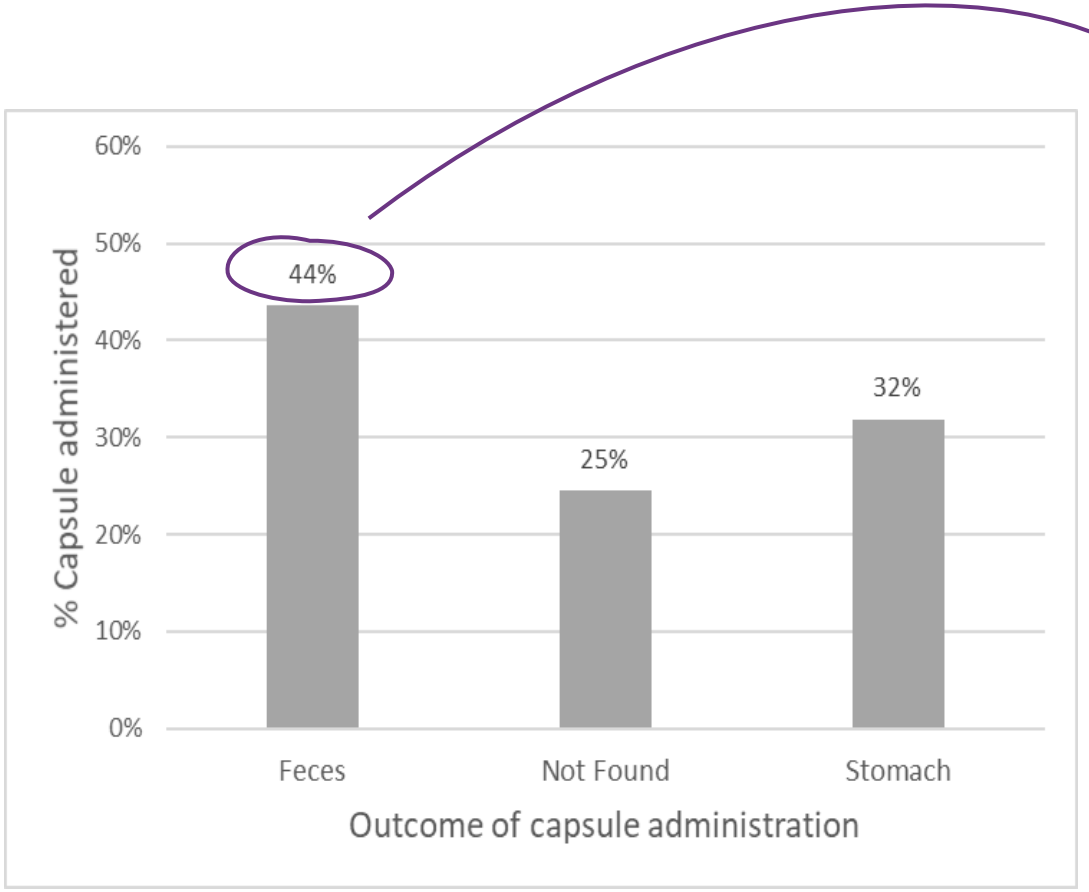
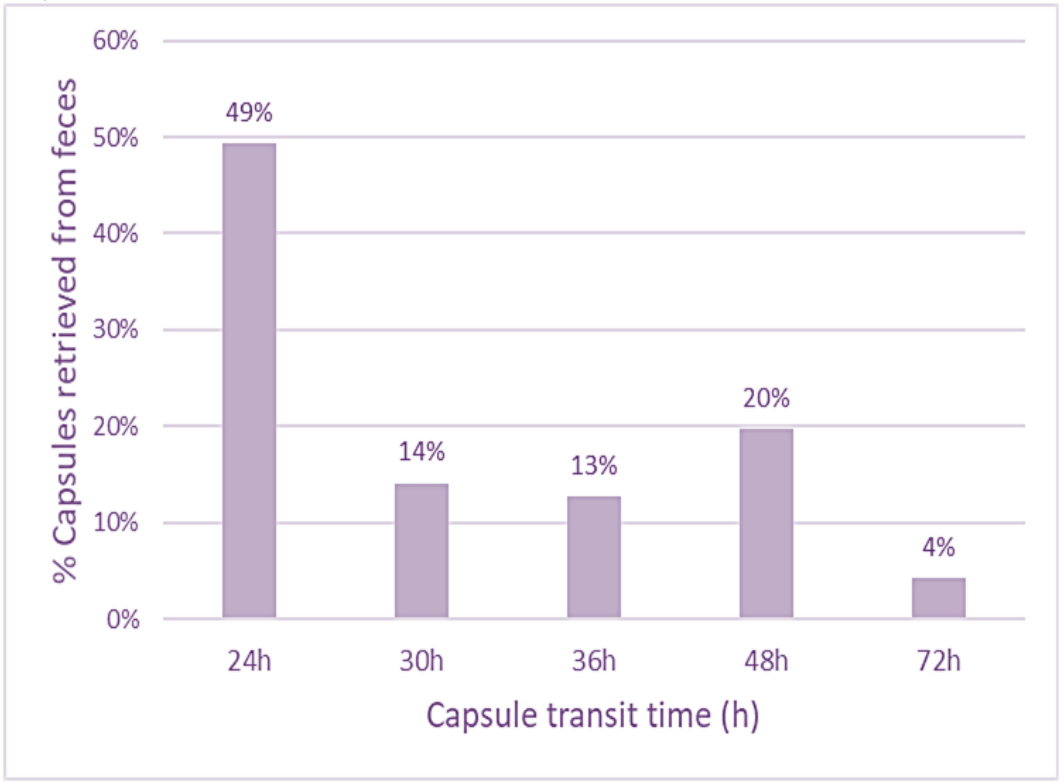
Capsule Retrieval





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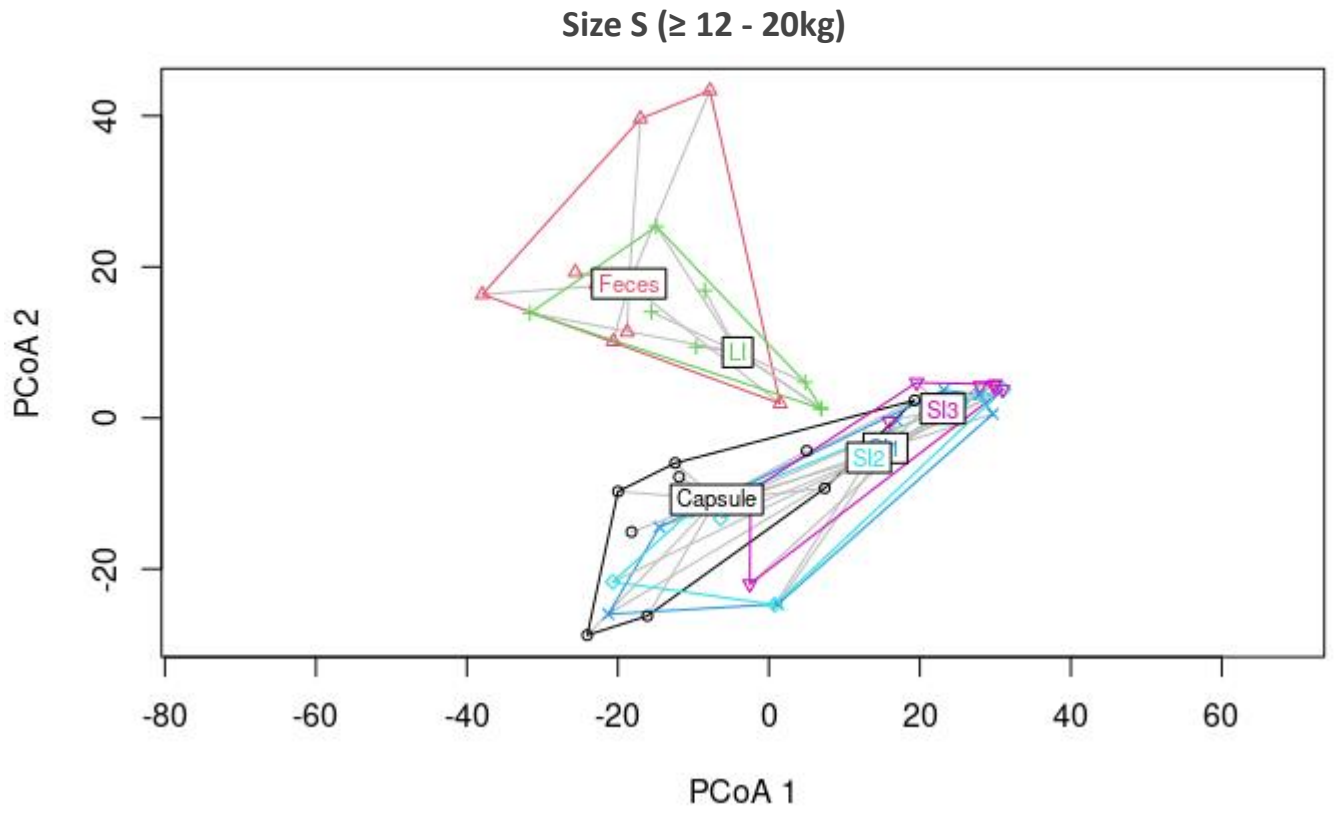
Microbiota analysis in postweaning pigs

PCoA plot generated using a Euclidean distance matrix based on clr transformed data



n= 8 pigs
10 capsules

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Microbiota analysis in postweaning pigs

Comparisons	SumsOfSqs	F.Model	r^2	p	p adj
Capsule vs Large intestine	5007.93	2.56	0.11	0.00	0.00
Capsule vs Feces	7921.83	3.33	0.14	0.00	0.00
Capsule vs Seg. 3	6514.44	4.51	0.19	0.00	0.01
Capsule vs Seg. 1	3623.75	2.06	0.10	0.02	0.32
Capsule vs Seg. 2	4464.66	2.65	0.12	0.00	0.06
Large intestine vs Faeces	3026.24	1.15	0.06	0.23	1.00
Large intestine vs Seg. 3	4902.91	3.23	0.16	0.00	0.01
Large intestine vs Seg. 1	3982.20	2.09	0.12	0.00	0.14
Large intestine vs Seg. 2	4257.28	2.36	0.12	0.00	0.08
Faeces vs Seg. 3	9863.60	4.88	0.23	0.00	0.00
Faeces vs Seg. 1	8160.77	3.35	0.18	0.00	0.00
Faeces vs Seg. 2	8615.03	3.73	0.18	0.00	0.00
Seg. 3 vs Seg. 1	1280.95	1.02	0.06	0.32	1.00
Seg. 3 vs Seg. 2	1490.14	1.24	0.07	0.18	1.00
Seg. 1 vs Seg. 2	972.36	0.62	0.03	0.82	1.00

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Microbiota analysis in fattening pigs

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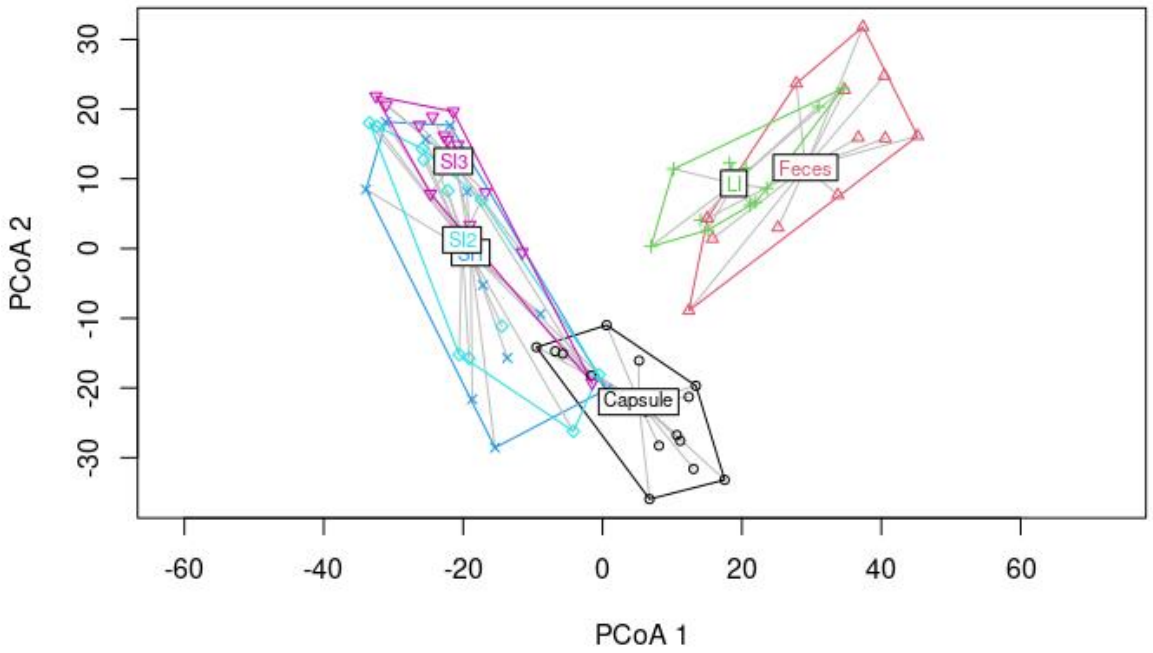
PCoA plot generated using a Euclidean distance matrix based on clr transformed data

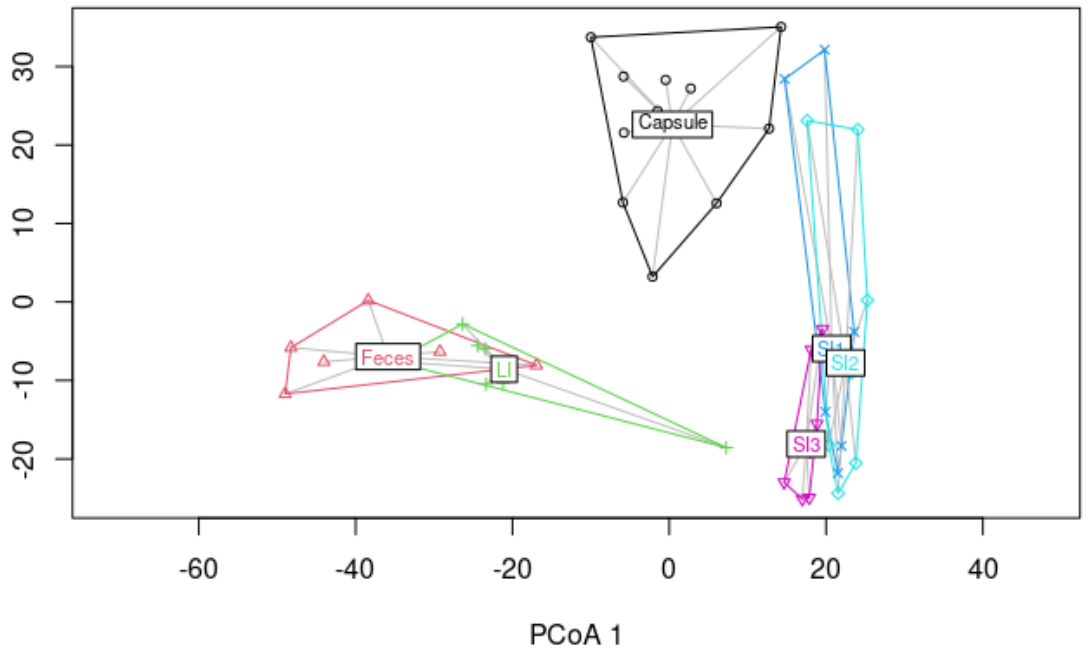
n=9 pigs
12 capsules

Size M (≥20-40kg)



n=11 pigs
15 capsules

Size L (≥40-70kg)



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Microbiota analysis in fattening pigs

Comparisons	SumsOfSqs	F.Model	r^2	p	p adj
Capsule vs Large intestine	19711.91	8.02	0.16	0.00	0.00
Capsule vs Feces	25825.26	8.41	0.16	0.00	0.00
Capsule vs Seg. 3	20762.67	11.19	0.20	0.00	0.00
Capsule vs Seg. 1	13818.34	5.79	0.12	0.00	0.00
Capsule vs Seg. 2	15532.08	7.15	0.14	0.00	0.00
Large intestine vs Faeces	10850.50	3.26	0.08	0.00	0.00
Large intestine vs Seg. 3	19499.73	10.66	0.23	0.00	0.00
Large intestine vs Seg. 1	19530.96	7.85	0.19	0.00	0.00
Large intestine vs Seg. 2	19869.27	8.94	0.21	0.00	0.00
Faeces vs Seg. 3	28579.00	11.14	0.24	0.00	0.00
Faeces vs Seg. 1	25792.52	7.83	0.19	0.00	0.00
Faeces vs Seg. 2	27073.75	9.02	0.21	0.00	0.00
Seg. 3 vs Seg. 1	6167.91	3.62	0.09	0.00	0.00
Seg. 3 vs Seg. 2	4484.882	3.05	0.08	0.00	0.01
Seg. 1 vs Seg. 2	1576.26	0.74	0.02	0.76	1

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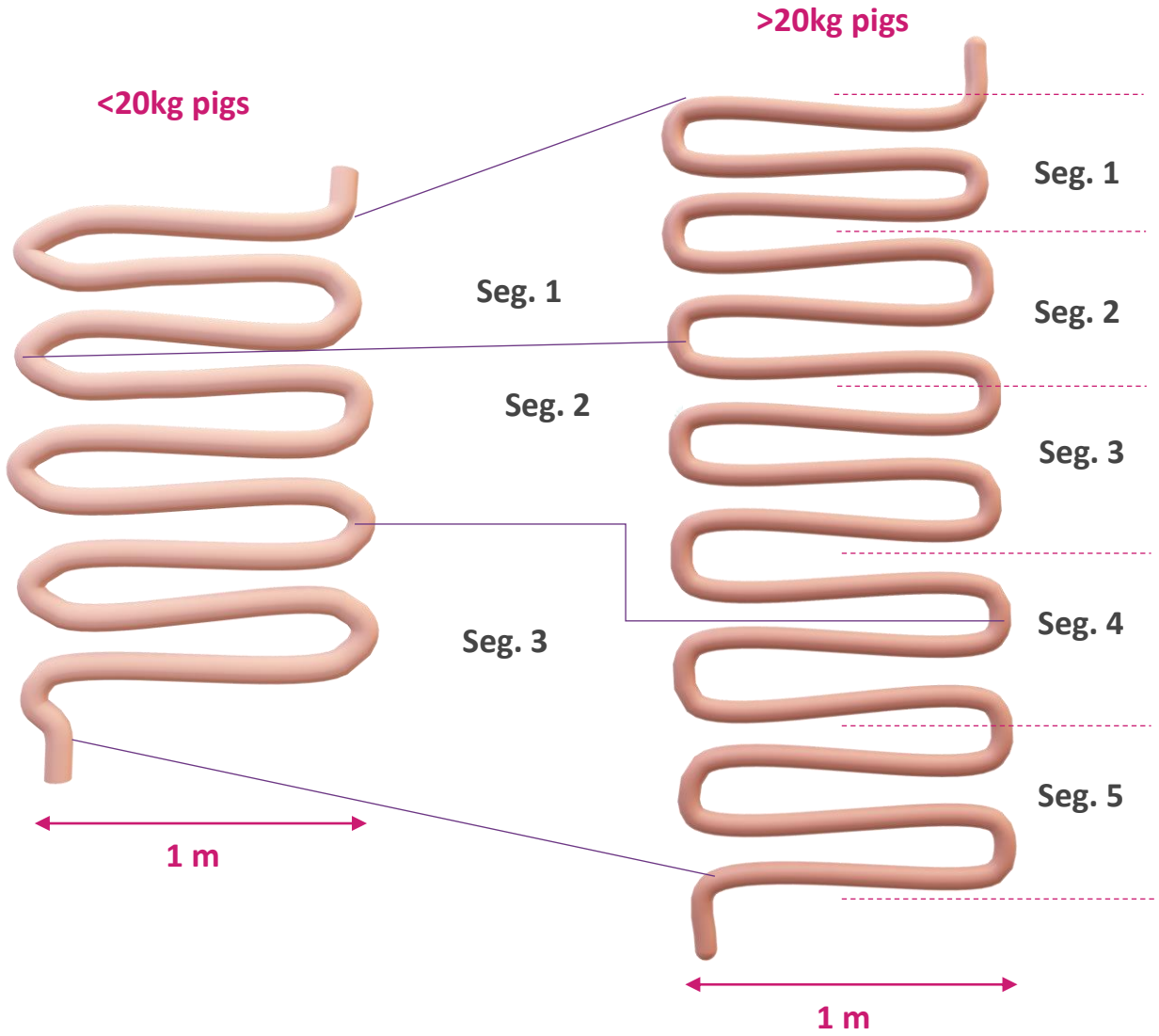
Why is the microbial composition of the capsule content similar to Seg 1 & 2 on pigs below 20kg, but not after?



Take home message

- First validation of a non-invasive device to collect and analyzed small intestine microbiota in postweaning pigs
- First time that a protocol has been standardized to administrate swallowable devices in pigs

Overall this opens the door to new ways to study the small intestine microbiota in pigs.

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THANK YOU

Do you have any questions?

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