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Sampling intestinal microbiota in growing pigs: evaluation of CapSa, an ingestible capsule

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ABSTRACT

This study aims to investigate Capsa, an ingestible capsule designed to collect the contents of the small intestine as it passes through the gastrointestinal tract. Eight Swiss Large White pigs weighing between 52.5 and 71.3 kg were administered two capsules each and monitored for three days before euthanasia for post-mortem sampling. Samples were collected from six equally divided segments of the small intestine, along with separate sampling of the solid and liquid contents of each segment when feasible. Samples were also obtained from the large intestine and faeces to determine CapSa's sampling location. Fifteen capsules were retrieved from faecal samples (93.75%), with 87.5% recovered on the first day post-administration. Only one capsule was not recovered. Comparative analysis of the bacterial composition of the capsules and post-mortem samples was conducted using a Permutational Multivariate Analysis of Variance (PERMANOVA) model (Adonis), with sample type as a factor. The results revealed significant differences in bacterial composition between capsules and samples from the large intestine and faeces ($p < 0.01$). However, no significant difference was observed between capsule content and the liquid and solid parts of the fourth segment of the small intestine ($p > 0.05$). This study provides evidence that CapSa can effectively sample the intestinal microbiota of the middle part of the small intestine in growing pigs.

HIGHLIGHTS

- CapSa effectively collects the contents of the small intestine as it passes through the gastrointestinal tract.
- Comparative analysis shows significant differences in bacterial composition between CapSa capsules and large intestine/faeces samples.
- CapSa exhibits the capability to sample the intestinal microbiota of the median part of the small intestine in growing pigs.

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Intestinal microbiota; small intestine sampling; ingestible capsule

Introduction

In recent years, significant research has highlighted the intricate relationship between gut microbiota and pig health (Schokker et al. 2015; Jang et al. 2020). Microbiota play crucial roles in diverse physiological processes, such as immunity and nutrient digestion (Fouhse et al. 2016; Luo et al. 2022), indicating their indirect impact on animal growth (Mahmud et al. 2023).

Despite a steady increase in pigs' feed intake and body weight, the composition of faecal microbiota during the growing period remains very stable under normal physiological conditions (Luo et al. 2022). This stability reduces susceptibility to infectious enteric diseases and optimises the animals' growth potential (Luo et al. 2022). Research has identified a relationship between enterotypes and feed intake in growing-finishing pigs (Yang et al. 2018), and different gut microbiota compositions have been linked to feed efficiency (McCormack et al. 2017; Tan et al. 2017). For instance, an Operational Taxonomic Unit (OTU)-based analysis of faecal samples revealed 31 OTUs associated with dietary polysaccharide metabolism that could potentially affect feed efficiency in 140-day-old finisher pigs (Yang et al. 2017), suggesting that modulating microbial composition could improve feed efficiency. However, faecal microbiota analysis is

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imperfect as a proxy for studying gut microbiota, as its profile differs significantly from that of the small intestine (Zhao et al. 2015; Gresse et al. 2019).

Alternative approaches to faecal sampling, such as animal cannulation or the collection of chyme samples subsequent to slaughter, face ethical concerns (Castillo and Hernández 2021) and sampling limitations of a single collection per animal (Choudhury et al. 2019). A new generation of non-invasive devices for sampling gut microbiota has emerged in human studies (Waimin et al. 2020; Park et al. 2022; Shalon et al. 2023) and in studies with dogs (Menard et al. 2023). However, there are currently no devices specifically tailored for studying pig gut microbiota. Only two devices, which were originally designed for human use (Rezaei Nejad et al. 2019; Nejati et al. 2022), have been tested in pigs. However, in one of the studies (Rezaei Nejad et al. 2019), the capsules were not retrieved after administration, while the second study tested the devices in only two pigs, with the objective of collecting samples in the colon.

The aim of this study is to test and validate a capsule prototype (CapSa) for non-invasive sampling of the intestinal microbiota of large pigs in the grower finisher stage. Capsa has already been successfully used to sample gut microbiota in small pigs in the post-weaning stage (García Viñado et al. 2024).

Materials and methods

Animals and rearing conditions

For the study, eight Swiss Large White pigs with bodyweights (BWs) ranging from 52.5 to 71.3 kg were used. Pigs were housed in groups of four. All pigs had *ad libitum* access to a standard starter diet formulated to meet the nutritional requirements of fattening pigs (Agroscope 2005) (Supplementary Table 1). Water was available *ad libitum* and distributed *via* nipple drinkers. As previously described by García Viñado et al. (2024), pens with a total surface area of 4.47 m^2 were specifically designed to collect the capsules by minimising the slatted area and reducing the openings of the slatted area to a size smaller than the capsule diameter of 7 mm.

Description of the capsule (CapSa)

The capsule studied is 21.7 mm long and 7 mm in diameter, corresponding to a size 0 hard capsule. Capsa passively moves along the gastrointestinal tract (GIT) at a speed that depends entirely on intestinal peristalsis. CapSa can collect a maximum of 400 µL (García Viñado et al. 2024). *In vitro* results show that CapSa can withstand two hours in an acidic-aqueous medium (pH $<$ 3), and then samples within 1 h after transfer to an aqueous medium at pH 7 (García Viñado et al. 2022). Moreover, it has been validated in post-weaning pigs (García Viñado et al. 2024).

Preparation of the animals and administration of the capsule

Three measures were taken prior to capsule administration to reduce intestinal load and shorten transit time. First, two days before the CapSa (-2 d) was administered, pigs were fed the finisher diet in liquid form (ration of 1 kg of finisher diet mixed with 2 L of water), and straw was removed from the pens. Second, one day before the administration of the CapSa (-1 d), the pigs had access to only half of their feed ration, and the feed was removed 12 h before CapSa administration. Third, to increase gastric emptying and thus facilitate CapSa transit through the stomach, 0.16 ± 0.001 mg/kg BW of prucalopride (Resolor®, Takeda Pharma AG, Glattpark, Switzerland) was administered orally *via* an oesophageal probe 40 min prior to administration. Prucalopride is a 5-HT_4 serotonin agonist that stimulates GIT peristalsis and increases gastric emptying (Briejer et al. 2001; Camilleri and Atieh 2021).

On the day of administration (0 d), each pig received two CapSas. The capsules were administered by oesophageal sondage, while the pigs were kept in a sling adapted to their BW. A 10 mL bolus of orange juice was then administered to flush the capsule in the stomach. Every CapSa was assigned a unique number, linking it to the pig ID.

Capsule recovery and sample processing

From 0 d to three days after administration (3 d), pens were inspected five times a day to look for CapSas expelled in the faeces. The specifically designed slatted area of the pens allowed searching for the capsule by sieving faeces with water over the slatted surface. Capsules retrieved from the faeces were directly transported to the laboratory. The outside of the capsule was cleaned with 70% alcohol to avoid contamination after opening. The identification of the capsule was recorded, its content was extracted, and the volume of the content was determined. The content was transferred to a 0.5 mL Eppendorf tube (Eppendorf SE, Hamburg, Germany), flash frozen by immersion in liquid nitrogen, and stored at −80 °C until analysis. The pH of the content was measured using Litmus

paper (Merck KGaA, Darmstadt, Germany) by cleaning the inside of the capsule after extraction.

Post-mortem sampling

Three days after capsule administration, all pigs were euthanised by electronarcosis. The GIT was extracted and samples of the colon and faeces were collected. Immediately after euthanasia, the abdominal cavity was opened, and the viscera were collected and placed on a table. The gastrointestinal tract was carefully unfolded, beginning just after the stomach. The small intestine was divided into six equal segments (Segments 1, 2, 3, 4, 5 and 6) and each segment was immediately sampled. After delineating a 3-meter segment of the intestine, a sample was taken by concentrating the contents of the small intestine in the central part of the segment. This process was repeated until all six segments were sampled. Additionally, prior to storage in sterile 2 mL Eppendorf tubes, the segment contents were also separated into the solid and liquid phases by sedimentation for 5 min in 50 mL tubes (Ratiolab®, Germany) cooled on ice. Subsequently, all tubes were submerged in liquid nitrogen and stored at −80 °C until further analysis. The GIT was carefully examined at the end of the sampling to ensure that all capsules had been retrieved.

Evaluation of capsule innocuity

To assess the innocuity of CapSa administration, passage, and retrieval, various parameters were considered to ensure the pigs' well-being. Post-mortem macroscopic observations were conducted to assess the presence of any tissue damage related to CapSa administration and/or passage (e.g. gastric ulcers and intestinal perforations). The faecal score was determined using a 4-level scoring scale, as follows: 1 = normal (firm but not hard), 2 = soft (does not hold form, piles but spreads slightly), 3 = runny (spreads readily), and 4 = watery (liquid consistency, splatters). Throughout the study duration, the overall health of the pigs was monitored continuously.

Microbiota analysis

Only capsule samples with a pH $>$ 5.5 and recovered within 48 h of administration were used for microbiota analysis. Bacterial DNA was extracted using the HostZERO™ Microbial DNA Kit (Zymo Research, California, USA) following the manufacturer's instructions. The DNA concentration (ng/μL) and purity (absorbance ratio 260/280 and 260/230, respectively) were verified spectrophotometrically on NanoDrop™ (Fisher Scientific, 13 Schwerte, Germany). The V3-V4 region of the 16S rRNA gene (∼ 460 bp) was amplified by PCR using Platinum™ Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific, Italy) and the universal primers Pro341F: 5′-TCGTCGGCAGCGTCAGATGTGTA TAAGAGACAGCCTACG GGNBGCASCAG-3′ and Pro805R:5′-GTCTCGTGGGCTCGGA GATGTGTATAAGAGACAGGACTA CNVGGGTATCTAATCC-3′ (Takahashi et al. 2014). The PCR reaction conditions for amplification of DNA were as follows: initial denaturation at 94 °C for 1 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension 65 °C for 45 s, ending with 1 cycle at 68 °C for 7 min (Takahashi et al. 2014). Amplicons were then sequenced by Illumina MiSeq 300 × 2 bp with the MiSeq® V3-V4 reagent kit on the MiSeq-Illumina® platform. Microbiota analysis was performed using the DADA2 pipeline (Callahan et al. 2016) according to the Silva database taxonomy, version 138 (Quast et al. 2013). For the DADA2 pipeline, primers were removed from the raw sequences, and based on the average quality score, forward and reverse reads were trimmed at positions 280 and 260. All other DADA2 parameters were maintained at their default settings.

Statistical analysis

All statistical analyses were performed in R (v 4.3.1). The percentage of CapSas recovered in the faeces and stomach or those not found was calculated based on the total number of CapSas administered. Capsule transit time was calculated as the time between capsule administration and recovery in the faeces. Capsule pH and volume were analysed using linear regression with transit time as fixed effects (function 'lm' in package 'lm4') (Bates et al. 2015). An ANOVA was performed to check the effect of time of retrieval on the pH and volume of the sample of the retrieved capsules (function 'Anova' in package 'car') (Weisberg and Fox 2011). Post hoc tests, such as least squares means, were performed when ANOVA detected the effect of time of retrieval on pH and volume.

Statistical analysis of alpha and beta diversity, as well as taxonomic analysis, was performed using 'phyloseq' (McMurdie and Holmes 2013) v1.38, 'vegan' v2.6 (Dixon 2003) and 'microbiomeutilities' v1.0. For the alpha diversity analysis, data were rarefied to the lowest sample depth to avoid bias linked to different sampling efforts. The Wilcoxon signed-rank test (procedure in R) was used to test for differences in the alpha diversity indices (Chao1, Shannon and Simpson diversity) of the CapSas microbiota content and those obtained from the six segments of the small intestine,

large intestine, and faeces. For beta diversity, a dissimilarity matrix using Euclidean distances from the centred log-transformed (clr) data was constructed, and the results were represented using a principal coordinate analysis (PCoA) plot. Differences were tested using a PERMANOVA model (Adonis) with 9999 permutations, including sample type (CapSa, six segments, large intestine, faeces) as the main factor. Pairwise contrasts between capsules and post-mortem samples were performed using the pairwiseAdonis function included in the 'PairwiseAdonis' package (Martinez Arbizu 2020). Bonferroni correction was then applied to adjust the p values for multiple comparisons. Differences in the taxonomic composition between samples were tested using linear discriminant analysis (LDA) effect size (LEfSe), aggregating the data at the genus level. The LDA score cut-off of 3 was used to discriminate bacterial taxa. For all statistical analyses, a difference was considered significant if $p < 0.05$ and a trend if $0.05 < p < 0.10$.

Results

CapSa innocuity

All pigs remained healthy throughout the study. No macroscopic tissue damage was observed following euthanasia related to CapSa administration and/or passage. The administration of CapSa had no impact on the faecal scores or the occurrence of diarrhoea, with none of the pigs exhibiting a faecal score higher than 1.

CapSa administration, recovery, and the volume and pH of the content

All CapSas were successfully administered to all pigs. A total of 15 capsules were recovered in faeces (93.75%), one of which was found to be empty. Overall, 87.5% of the CapSas were recovered on the first day post administration and 6.25% on the second day. Only one capsule was not found in the faeces or inside the pig after euthanasia. In this study, we recovered at least one capsule from each pig (Figure 1). Of the 15 CapSas recovered within 48 h, 14 had a pH $>$ 5.5 and were therefore included in the microbiota analysis. The pH of the retrieved content was 6.60 ± 0.51 (mean + standard deviation, STD), and the sampled volume was 172.67 ± 72.26 µL. Neither the volume nor the pH of the CapSa content was affected by transit time ($p > 0.05$) (Figure 2).

Post-mortem sampling

We successfully collected faecal and large intestine samples from all pigs as well as from Segments 1 to 6 of the small intestine. However, due to consistency differences, we could only obtain samples of both the solid and liquid phases from Segments 1 to 4 of the small intestine. In the upper part of the small intestine, particularly in Segments 1 to 4, the content tends to be more liquid in nature. In these segments, sedimentation was fast and efficient in separating the solid phase from the liquid phase. However, in Segments 5 and 6, and occasionally in Segment 4, the liquid phase was limited, making collection impossible.

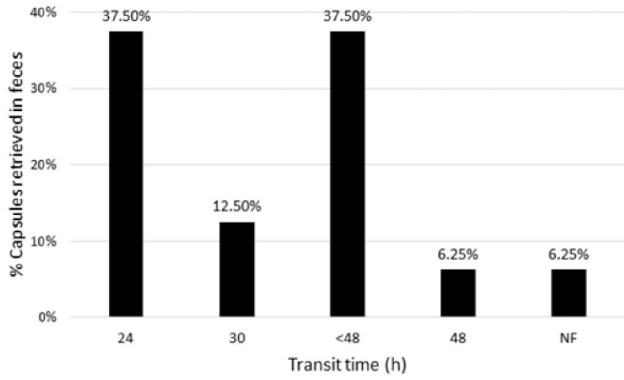

Figure 1. Time (h) of transit of capsules found in faeces. Time was calculated as the difference between the time of administration and the time of recovery. Transit time strongly influenced the number of capsules recovered in faeces, and the majority of the capsules were found within 24 h after administration. NF = Not found.

Microbiota analysis

Except for one CapSa sample, bacterial DNA was successfully extracted, amplified, and sequenced. The PCoA plot showed a clustering of the samples based on sample type, with large intestine and faecal samples tending to cluster together and CapSa and intestinal segment samples from two distinct clusters showing no overlap between them (Figure 3). The Adonis test proved that bacterial composition was affected by sample type ($r^2 = 0.33$, $p = 0.001$). The pairwise Adonis test shows that except for the solid and liquid phases of Segment 4 ($r^2 = 0.26$, $p_{adj} = 0.36$ and $r^2 = 0.26$, $p_{adj} = 0.31$, respectively), the microbial composition of the CapSa content was significantly different from all other sample types (Table 1).

Overall alpha diversity (InvSimpson) was lower ($p \leq 0.03$) in the CapSa content compared to the total, liquid, or solid fractions of Segments 2, 3 and 4 (except solid fraction of Segment 4) and solid fraction of Segment 1. No differences in alpha diversity were observed between CapSa and the rest of the samples (Figure 4A). The Chao1 index was higher ($p \leq 0.02$) in CapSa samples compared to the total fractions of

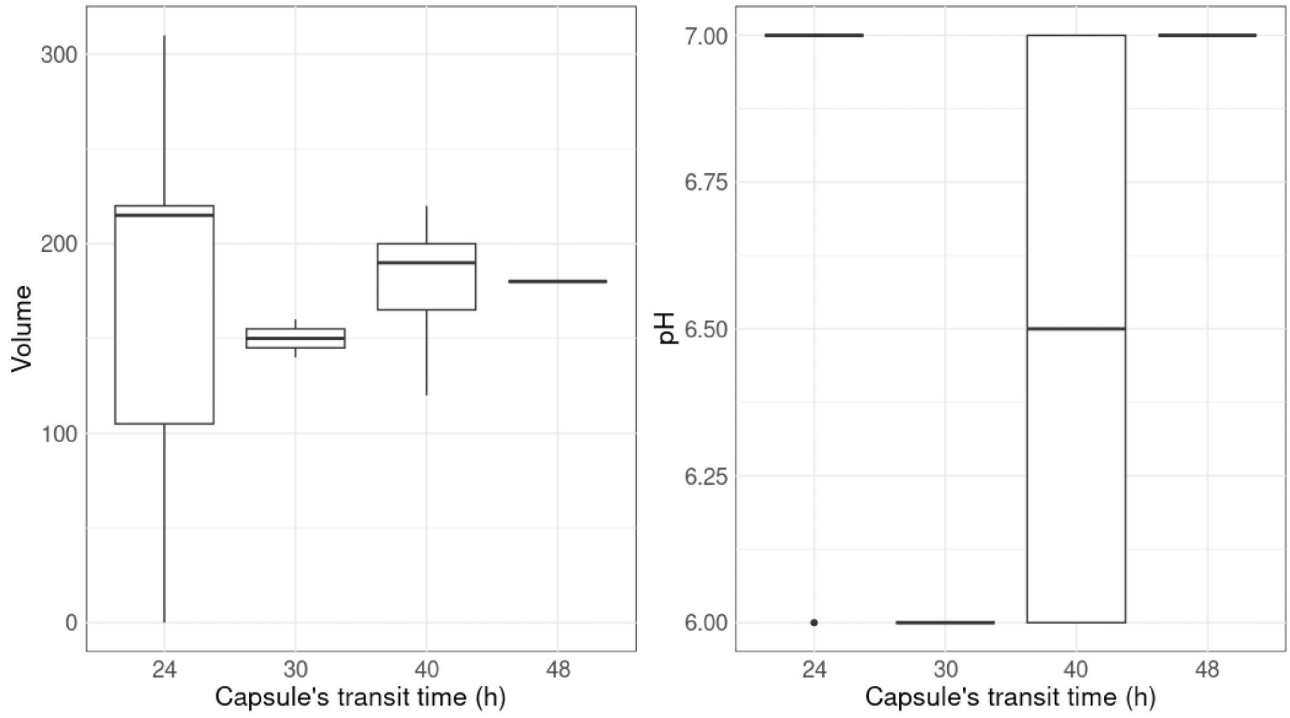

Figure 2. Volume (μL) and pH of capsule contents as a function of transit time (h).

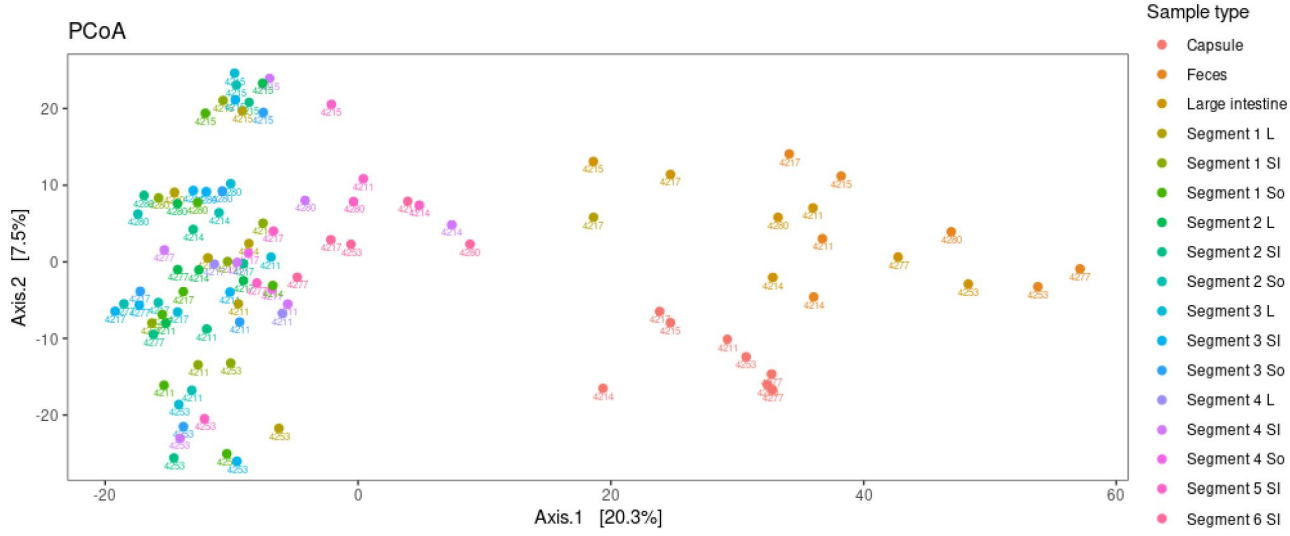

Figure 3. Principal coordinate analysis (PCoA) of Euclidean distance matrices of clr-transformed data. Samples are coloured based on sample type and labelled according to the subject. SI: small intestine sample; L: liquid phase sample; so: solid phase sample.

Segments 2, 3 and 5, the solid fractions of Segments 2 and 3, the liquid fractions of Segments 3 and 4, large intestine, and faeces. However, it was lower in the CapSa samples compared to the total fraction of Segment 1 ($p = 0.014$) (Figure 4B). The Shannon diversity index was lower ($p \leq 0.04$) in the CapSa samples compared to the total fraction of Segment 1, the liquid fractions of Segments 2 and 3, and the solid fraction of Segment 1 (Figure 4C). By contrast, microbiota distribution was similar between the CapSa samples and the rest of the samples.

LEfSe analysis revealed different biomarkers characterising each sample type (Figure 5). Capsules had four major biomarkers: *Clostridium sensu stricto 1* (LDA score = 5.06, $p < 0.01$), *Terrisporobacter* (LDA score = 4.54, $p < 0.01$), *Turicibacter* (LDA score = 3.92, $p < 0.01$), and *Methanosphaera* (LDA score = 3.00, $p < 0.01$). Faeces had one biomarker: *Methanobrevibacter* (LDA score = 3.37, $p < 0.01$). The large intestine had four biomarkers: *Blautia* (LDA score = 3.84, $p < 0.01$), *Subdoligranulum* (LDA score = 3.72, $p < 0.01$), *Solobacterium* (LDA score = 3.28, $p < 0.01$), and an unidentified genera of the

Table 1. Adonis pairwise comparisons of microbiota structure between capsules and gastrointestinal content samples, calculated using Euclidian distances.

Pairwise comparisons	SumsOfSqs	F. Model	r^2	p	p_{adj}
Capsule vs. Segment 1 SI	9525.21	6.77	0.34	<0.01	0.04
Capsule vs. Segment 2 SI	9772.48	6.66	0.33	<0.01	<0.01
Capsule vs. Segment 3 SI	10132.53	6.92	0.34	<0.01	<0.01
Capsule vs. Segment 4 SI	8290.68	5.81	0.30	<0.01	<0.01
Capsule vs. Segment 5 SI	8000.25	6.24	0.32	<0.01	<0.01
Capsule vs. Segment 6 SI	5929.84	4.61	0.29	<0.01	0.01
Capsule vs. Large intestine	7872.01	3.70	0.22	<0.01	<0.01
Capsule vs. Faeces	8154.14	3.18	0.19	<0.01	<0.01
Capsule vs. Segment 1 So	9642.59	6.56	0.33	<0.01	<0.01
Capsule vs. Segment 1 L	8974.71	5.11	0.28	<0.01	<0.01
Capsule vs. Segment 2 So	9677.64	6.77	0.36	<0.01	<0.01
Capsule vs. Segment 2 L	9199.97	6.71	0.35	<0.01	<0.01
Capsule vs. Segment 3 So	8342.67	5.81	0.34	<0.01	<0.01
Capsule vs. Segment 3 L	8101.19	5.61	0.33	<0.01	<0.01
Capsule vs. Segment 4 So	4519.61	2.93	0.26	0.02	0.36
Capsule vs. Segment 4 L	4452.47	2.90	0.26	0.01	0.31

SI = small intestine (without separation in solid and liquid phase); L = liquid phase; So = solid phase; SumsOfSqs = Sum of square reflecting total variance; F. Model = F-test value; r^2 = r-square value, reflects grouping differences; the higher the value, the higher the grouping differences; $p = p$ value; p_{adj} = p values adjusted for multiple comparison using the Bonferroni correction.

Erysipelotrichaceae family (LDA score = 3.22, $p < 0.01$). The liquid phase of Segment 1 had one main biomarker: unidentified genera of the *Lachnospiraceae* family (LDA score = 3.15, $p < 0.01$). The liquid phase of Segment 2 had one biomarker: *Bifidobacterium* (LDA score = 4.47, $p < 0.01$), similar to the solid phase of that same segment, which had *Limosilactobacillus* (LDA score = 5.25, $p < 0.01$). The liquid phase of Segment 3 had 3 biomarkers: *Ligilactobacillus* (LDA score = 4.33, $p < 0.01$). Segment 4 had one main biomarker: *HT002* (from *Lactobacillaceae* family, LDA score = 5.12, $p < 0.01$). Segment 5 also had one main biomarker: Lactobacillus (LDA score = 5.26, $p < 0.01$).

Discussion

A standardised protocol was previously designed by García Viñado et al. (2024) for the administration of CapSa to post-weaning pigs. However, the faecal CapSa recovery rate in heavier pigs was higher in the present study, at 93.75%, demonstrating that the protocol, combined with a high level of expertise in handling the pigs, is highly effective as a non-invasive sampling method for GIT content. The majority of the CapSas were recovered within the first day after administration, indicating efficient passage through the GIT. The shorter transit time and the higher recovery rate observed in this study compared to the previous study (García Viñado et al. 2024) can be attributed to the higher BW, as it seems easier for CapSa to exit the stomach in larger pigs. Indeed, none of the capsules were found in the stomach after euthanasia, contrary to findings in other studies (García Viñado et al. 2024). The CapSa contained a digesta with a pH > 5.5. In agreement with the results obtained with post-weaning pigs (García Viñado et al. 2024), our findings confirm that CapSas sampled the contents of segments beyond the stomach, as the fasting stomach typically does not exceed a pH > 5.5 (Reynaud et al. 2020).

Using PCoA and the Adonis test, we observed notable differences in microbial composition between the CapSa samples and samples from the large intestine and faeces, underscoring the unique microbial ecosystems across the GIT (Crespo-Piazuelo et al. 2018). Although certain phyla, such as Firmicutes, consistently inhabit the entire length of the intestine, variations in factors such as pH and oxygen concentration can cause other phyla to differ along the GIT (Crespo-Piazuelo et al. 2018). Our findings corroborate previous studies (Zhao et al. 2015) demonstrating differences in microbial composition between faecal and small intestine samples (Table 1). The similarity between the microbial content of the CapSa and the liquid and solid fractions of Segment 4 ($r^2 = 0.26$, $p_{adj} = 0.31$ and $r^2 = 0.26$, $p_{adj} = 0.36$, respectively; Table 1) indicates that the CapSas sampled in the midsection of the small intestine. However, as indicated by the results of the pairwise Adonis test (Table 1), the CapSa content differed significantly from that of Segment 4 when compared as a whole rather than separated into liquid and solid fractions. Our first hypothesis is that the limited volume of the Capsa sample (approximately 170 µL) hampered the capturing the diversity and richness of Segment 4 when both liquid and solid phases were mixed together. Separating the two phases into liquid and solid reduced the richness and diversity of each subsample, which became similar to those found in the Capsa sample.

A previous study demonstrated that CapSa sampled gut content from the first segment of the small intestine in postweaning piglets (García Viñado et al. 2024). However, in this study, the CapSa content differed from the bacterial composition of the first segment, sampling further along the small intestine, specifically in the jejunum (Segment 4), as shown in the pairwise Adonis analysis. It is noteworthy that the age of the pigs not only influences organ size but also transit time. Snoeck et al. (2004) observed that transit time was significantly prolonged immediately after weaning and returned to normal 3 weeks after weaning. The increase in transit time appeared to be related to retention in the stomach and colon (Snoeck et al. 2004). Considering this, we hypothesised that in our

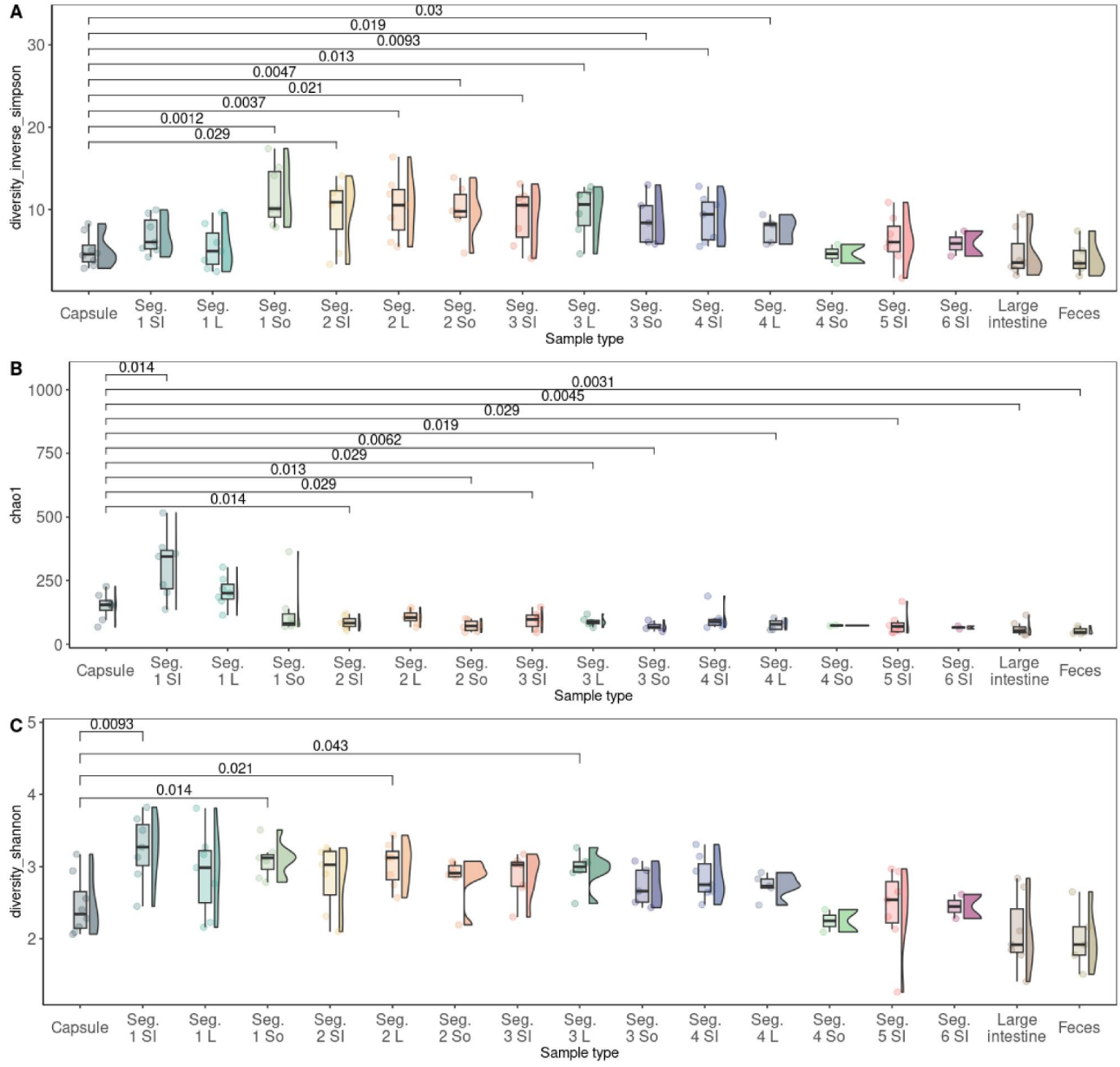

Figure 4. Box plots showing alpha diversity values for inverse Simpson (A), Chao1 (B) and Shannon index (C) for each sample. Only *p*-values <0.10 are shown. SI: small intestine sample; L: liquid phase sample; so: solid phase sample.

study, the capsule opened and closed further along the intestine due to faster transit. We speculate that in young pigs, the smaller size of the stomach and the space occupied by the *torus pyloricus* might result in a less forceful expulsion of intestinal content compared to larger growing pigs with a bigger pylorus and larger small intestine diameter. Moreover, in both post-weaning and growing pig studies, feeding occurred four hours after CapSa administration. Larger pigs may better handle stress, adapting more effectively to handling and intubation, thereby exhibiting a quicker return to feeding post-manipulation. This, coupled with the known peak in transit time immediately after feeding (Krawielitzki et al. 1990), suggests that the sooner they are fed, the faster the capsule advances. Taking into account these factors and previous findings, in post-weaning pigs, CapSa is observed to open in the first segment of the small intestine, whereas in growing pigs, it is expected to open in a more distal position.

To assess whether CapSa provided a representative sample of Segment 4, we also evaluated various alpha diversity indices. No differences in species richness (Chao 1) were observed between the Capsa samples and the total and solid fractions of Segment 4. The liquid fraction had a lower richness. Indices that simultaneously compared the number of species present (richness) and the relative abundance of each species

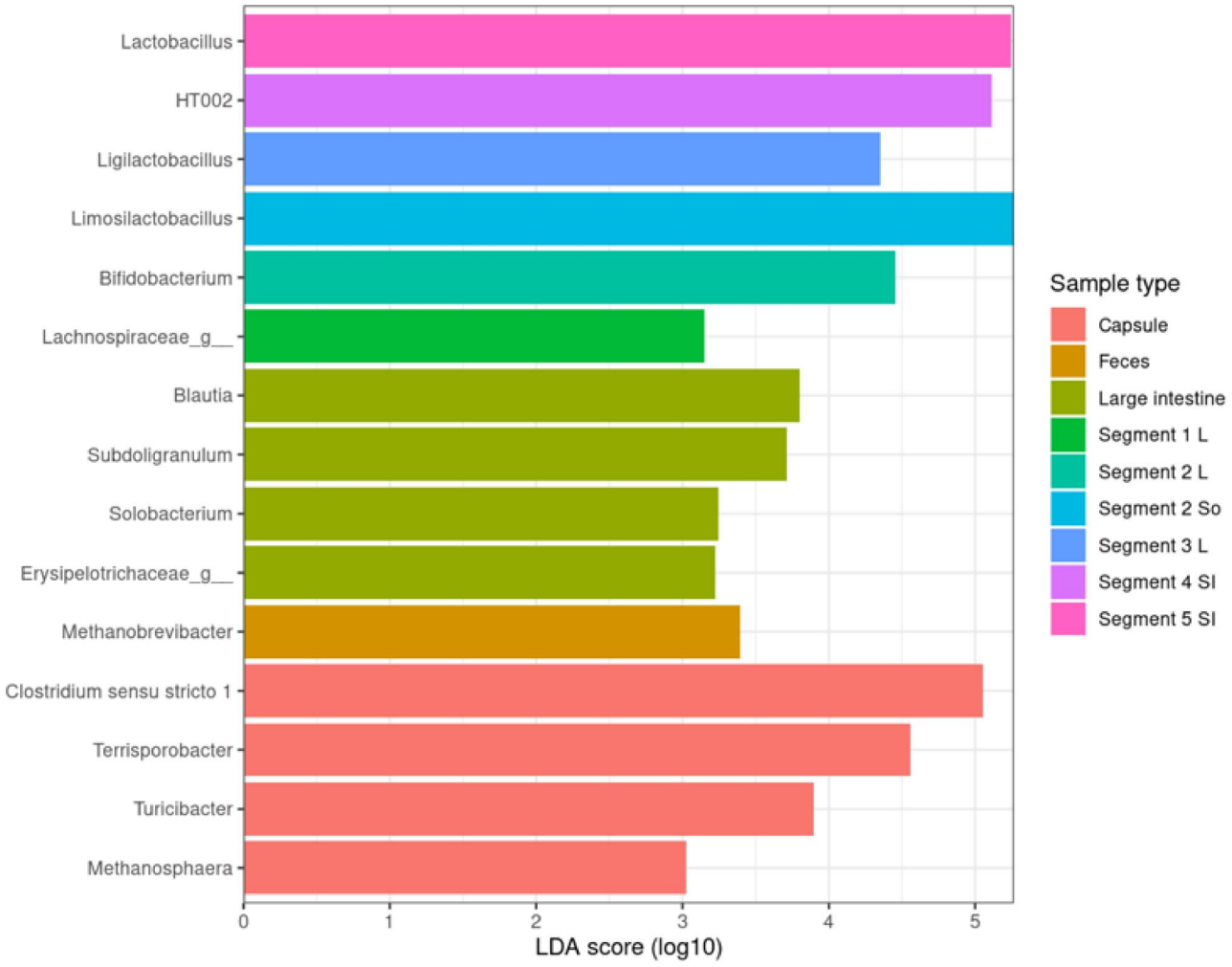

Figure 5. Bar plot of linear discriminant analysis (LDA) effect size (LEfSe). SI: small intestine sample; L: liquid phase sample; so: solid phase sample.

(evenness) yielded conflicting results. Indeed, the Shannon index showed no difference between CapSa and any of the sample fractions from Segment 4. By contrast, the Capsa samples had lower richness and evenness compared to the total and liquid fractions of Segment 4, while no difference was found compared to the solid fraction. In summary, the assessment of alpha diversity indices suggests that CapSa effectively reflects alpha diversity in Segment 4 of the small intestine.

Additionally, the presence of characteristic genera, such as *Clostridium sensu stricto 1* and *Terrisporobacter*, in capsule samples underscores CapSa's ability to capture representative microbiota from the small intestine. These genera have been proven to be the most prevalent in the jejunum of growing pigs in other studies (Wu et al. 2021). Further, the abundance of *Terrisporobacter* and *Clostridium sensu stricto 1* in the GIT has been positively correlated with adult pig weight gain (Kim et al. 2016; Yu et al. 2024).

Conclusion

The capsule sample more closely resembles the composition found in the solid and liquid phases of the fourth segment of the small intestine. However, although the composition of the capsule did not mirror that of the Segment 4 sample as a whole, it aligned with this segment when its liquid and solid phases were examined separately. The present results provide valuable insights on the exact sampling location of the capsule, though they should be considered within the context of the limited sample size ($n = 8$).

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Ethics approval

All experimental procedures were in compliance with Swiss animal welfare guidelines and were approved (No. 2021-39-

FR) by the Cantonal Veterinary Office of Fribourg (Switzerland). All methods are reported in accordance with the ARRIVE guidelines.

Authors' contributions

IGV, CO, and FC validated the procedure and conducted the main statistical and bioinformatics analyses. CO conceived the study, and CO and GB secured substantial funding. IGV and CO performed the animal experiments, recorded the data, and collected and processed the capsules' samples. CO, GB, and PT supervised the study. IGV drafted the manuscript, and CO, GB, FC, and PT critically reviewed the manuscript. All authors read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. Raw sequences are available at the NCBI sequence read archive (SRA under accession number PRJNA1107150).

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