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Method paper

Method: Standard operating procedure for the administration of swallowable devices to study pig's gut content in a non-invasive way

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ABSTRACT

Due to the evolution of welfare laws and the search for novel methods to study pig microbiota, the development of precise and non-invasive sampling methods is key to studying the microbial communities that inhabit the guts of pigs. Administering swallowable devices to pigs is always a challenge due to factors such as anatomy, the requirement for specific materials, and the need to restrain the animals. In this study, we describe a step-by-step protocol on how to administer Capsule for Sampling (**CapSa**), a biocompatible non-invasive device to study pig's microbiota without harming the animals. The validation of the protocol was done through two different studies. In Study 1, 92 Swiss Large White pigs (BW: 6.45–71.3 kg) were administered two capsules each and monitored for the following 3 days for capsule retrieval. On day 3, all pigs were euthanised to locate the missing capsules directly from their gastrointestinal tracts. In Study 2, 16 Swiss Large White pigs were selected at weaning and administered CapSas at five different timepoints (T1: 52 ± 3; T2: 70 ± 3; T3: 83 ± 3; T4: 110 ± 3; T5: 126 ± 3 days of age). To retrieve the capsules in the faeces, pigs were monitored 3 days postadministration. At T5, the pigs were slaughtered, and CapSas that were not found in the faeces, termed as missing CapSas, were retrieved from their gastrointestinal tracts. The protocol entails acclimation of the animals, housing modifications, administration of a prokinetic agent (prucalopride) to facilitate gastric emptying, and oesophageal intubations to overcome challenges related to administration, gastric blockage, and retrieval of the capsules. In Study 1, 46.74% of the administered CapSas were found in the faeces within 72 h postadministration, with 47.67% retrieved within the first 24 h, and 28.26% were located in the stomach. The CapSa retrieval was lowest in light pigs (<12 kg). In Study 2, 75.6% of CapSas were recovered in the faeces within 72 h postadministration, with 51.23% retrieved within the first 24 h. The CapSa retrieval rates varied depending on the administration time point being lowest at T1 and T3 and highest at T2 with intermediate values at T4 and T5. In both studies, the pH levels were affected by transit time ($P < 0.01$), resulting in a more acidic content when capsules were expelled after 36–40 h. To the contrary, the volume of the CapSa content was never affected by transit time ($P < 0.05$). In both studies, postmortem observations showed no health-related issues except one pig from Study 2 excluded due to respiratory distress. The present study describes a valid procedure for administering CapSa or any other swallowable devices in pigs. Moreover, this procedure is applicable to singular and repetitive administrations over the lifespan of pigs.
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Implications

The gut microbiota's impact on pig health and performance is of great importance for both research and the pig industry. Recognising the limitations of faecal microbiome studies due to variations in the small intestine's microbiome, we introduce CapSa. This biocompatible, non-invasive capsule, designed for oral administration, collects chyme from the small intestine, ensuring animal welfare. A unique standard operating procedure addresses pigs' anatomical challenges, facilitating CapSa's administration and faeces retrieval.

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This innovation advances accurate gut microbiota and chyme content research in swine, marking a significant step forward in the field.

Specification table

Subject	Physiology and Functional Biology
Type of data	Table, graph
How data were acquired	Data were gathered from two studies involving the administration of the Capsule for Sampling (CapSa). In Study 1, 92 pigs, with a BW ranging from 6.45 to 71.3 kg, each received two CapSas once. These pigs were monitored for 3 days and then slaughtered on the third day. Study 2 involved 16 pigs, each receiving two CapSas at five different ages (T1: 52 ± 3, T2: 70 ± 3, T3: 83 ± 3, T4: 110 ± 3, T5: 126 ± 3 days). In both studies, pigs were observed for 3 days following each administration, with slaughter occurring 3 days postadministration (Study 1) or at T5 (Study 2). The volume and pH of every retrieved capsule were measured.
Data format	Raw data, preprocessing data
Parameters for data collection	A total of 318 CapSas were orally administered. A total of 208 CapSas were retrieved in the faeces, 52 in the gastrointestinal tract, and 80 were not found. From the retrieved CapSas, 40–580 µl of chyme were collected. The pH ranged from 1 to 8.
Description of data collection	The number of CapSas administered and retrieved either in the faeces or postmortem in the gastrointestinal tract was determined. From the CapSas retrieved in the faeces, the chyme volume and pH were determined.
Data source location	Institution: Agroscope City/Town/Region: Posieux, Fribourg Canton Country: Switzerland Latitude and longitude (and GPS coordinates, if possible) for collected samples/data: 46°46′07.50″ N, 7°06′17.90″ E
Data accessibility	Data and Supplementary Materials used for this paper can be obtained from the repository. Access: https://zenodo.org/records/13132044
Related research article	García Viñado, I., Correa, F., Trevisi, P., Bee, G., Ollagnier, C., 2024. A non-invasive tool to collect small intestine content in post weaning pigs: validation study. Scientific Reports 14, 9964. https://doi.org/10.1038/s41598-024-59950-3.

Introduction

Microbiota, the diverse community of microorganisms residing in the gastrointestinal tract (**GIT**) of animals play a crucial role in host metabolism and in maintaining host health and overall well-being (Isaacson and Kim, 2012; Luo et al., 2022). Therefore, accurate and efficient microbiota sampling methods are essential for studying the composition and evolution of the microbial community within the porcine gut.

Traditional microbiota sampling methods in pigs have primarily relied on either invasive techniques, such as cannulation or post-mortem sampling, or non-invasive methods, such as collecting faecal samples and rectal swabs (Zhao et al., 2015). Although these methods provide valuable insights, they pose several challenges regarding accuracy and limitations in repeated sampling over time. Thus, there is a growing demand for non-invasive, repeatable, and less stressful sampling methods that can allow researchers to study intestinal microbiota (Amoako-Tuffour et al., 2014; Tang et al., 2020).

A novel approach to addressing these challenges is the development and implementation of swallowable devices designed to collect chyme samples at a determined location in the GIT and then transit the digestive tract. Some of these devices have already been used in humans (Rezaei Nejad et al., 2019; Folz et al., 2023; Shalon et al., 2023) and dogs (Menard et al., 2023). These devices offer a less invasive and more animal-friendly alternative for microbiota sampling. However, only one of these devices has been tested in pigs (Rezaei Nejad et al., 2019) demonstrating a lower rate of retrieval. This underscores the need for an administration procedure tailored specifically to pigs.

The capsule for sampling, referred to as **CapSa**, is a size 0 capsule that collects intestinal content directly from the pigs' guts. It is administered orally, and its sampling mechanism is based on the physicochemical properties of the environment. Its sampling mechanism has already been validated in vitro (García Viñado et al., 2022) and in vivo (García Viñado et al., 2024), specifically for collecting gut microbiota from the upper portion of the small intestine. However, the successful implementation of swallowable devices in pigs requires standardised administration protocols. Standardisation and refinement of these protocols are imperative to ensure consistent and reliable results. Factors such as administration procedure, materials and housing, and acclimation conditions must be optimised to overcome challenges due to the anatomy and physiology of the pig. In this study, a standardised administration protocol applicable to singular and repetitive administrations of CapSa in pigs was validated.

Materials and methods

Design and operating principle of the capsule for sampling

The CapSa measures 21.7 mm in length with a diameter of 7 mm, corresponding to a rigid size 0 capsule. Its movement along the digestive tract is purely passive, and its transit speed depends entirely on intestinal peristalsis. The capsule can collect a maximum of 400 µL of GIT content and is engineered to follow a specific sequence of actions: once ingested, it passes through the stomach to the small intestine, where it opens to collect a sample. Within 10 s after sampling, CapSa seals shut and continues its journey through the large intestine, ultimately being expelled with the faeces. The opening mechanism for sample collection is pH dependent (García Viñado et al., 2022).

Table 1
Numbers of pigs, their BW and sex distribution across the 10 runs in Study 1.

Run#	N	BW±SD, kg	Castrates, %	Females, %
1	3	17.9 ± 2.59	100.0	0.0
2	6	13.7 ± 1.40	50.0	50.0
3	6	13.45 ± 2.02	50.0	50.0
4	12	7.79 ± 0.98	50.0	50.0
5	9	13.6 ± 0.96	22.2	77.8
6	12	34.27 ± 1.62	8.3	91.7
7	12	41.45 ± 3.00	50.0	50.0
8	12	58.98 ± 2.51	50.0	50.0
9	12	61.15 ± 4.12	41.7	58.3
10	8	60.41 ± 7.27	50.0	50.0

Study design

Study 1: Validation of capsule administration protocol in pigs of various ages

A total of 92 Swiss Large White pigs from 6.45 to 71.3 kg BW were selected; 57% were females and 43% were castrated male pigs. (Table 1). The pigs originated from 10 different farrowing batches. All pigs were orally administered two capsules that were retrieved in the following 3 days from the faeces or directly from the animal after euthanasia on day 3.

Study 2: Tracking capsule retention and recovery across key developmental stages in Swiss Large White pigs

For this second study, a total of 16 Swiss Large White pigs were selected at weaning: 50% were females and 50% were castrated male pigs. The pigs came from the same farrowing batch, and from 4 litters (4 pigs per litter). All pigs were orally administered two capsules at five different time points: T1: 52 ± 3, T2: 70 ± 3, T3: 83 ± 3, T4: 110 ± 3, and T5: 126 ± 3 days of age (Table 2). For the 3 days following each administration, the pigs were monitored five times daily to ensure the collection of the capsules from their faeces. At 140 ± 5 days of age, all pigs were slaughtered, and we searched for any missing CapSas.

Preadministration preparation: housing, enrichment, and dietary adjustments for pigs prior to capsule administration

To administer CapSa, pigs from both studies were allocated in individual pens (total surface area of 4.47 m^2) 3 days prior to the administration. The straw was removed 2 days prior to the CapSa administration, and plastic toys, rope, and softwood were introduced as enrichment material. The pigs were accustomed to liquid meals 2 days before capsule administration. Pigs were provided with a half-liquid meal one day prior to CapSa administration (day −1) and had no access to food 12 h before capsule administration.

Table 2
BW and age of pigs at the different administration timepoints in Study 2.

Capsule administration	Age, d	BW±SD, kg
T1	52 ± 3	13.92 ± 1.79
T2	70 ± 3	21.48 ± 2.39
T3	83 ± 3	30.43 ± 3.13
T4	110 ± 3	59.16 ± 5.83
T5	126 ± 3	82.04 ± 8.47

Abbreviations: BW=Bodyweight; SD=Standard Deviation.

Prokinetic and capsule for sampling administration by oesophageal sondage

A prokinetic was administered 40 min before the administration of the CapSas. The administration of both the prokinetic agent and CapSas was facilitated via oesophageal sondages. For this procedure, pigs were placed into a pig sling adapted to their BW, and a trained individual conducted the oesophageal sondage using a sonde (see Supplementary Figure S1) and mouth gag (Mouth bite bar – small 20 mm, Ellegaard Goettingen minipigs, Dalmose, Denmark). For intubation, the sonde was gently inserted through the mouth gag and placed in the back of the throat. When the pig was inhaling, the sonde was gently pushed further, so that it was swallowed and reached the oesophagus. Afterwards, the sonde was pushed to the end of the oesophagus and then backed up a few centimetres. Attention was paid to aligning the pig's head with the rest of the body to ease the intubation, especially for the swallowing phase. When the sonde was in place, the CapSa or the prokinetic were delivered through the sonde. The procedure concluded with the careful removal of the sonde. For the administration of the prokinetic, prucalopride (Resolor ®, Takeda Pharma AG, Glattpark, Switzerland) at a dose of 0.15 mg/kg BW was dissolved in 10 ml of water and delivered through the sonde, which was subsequently rinsed with water to ensure full delivery of the dose of the prokinetic agent. For the CapSa administration, two CapSas were delivered via the sonde, followed by 10 ml of orange juice. Each CapSa was uniquely labelled with the pig ID and the capsule ID, that is, 1 or 2. All pigs were fed ad libitum 4 h after capsule administration with a standard diet formulated to meet the nutritional requirements of their production stage (Agroscope, 2005).

Capsule retrieval and collection of capsule's contents

Several modifications were made to the housing system to ensure the retrieval of the CapSas. In each box (4.47 m^2), the slatted floor area was reduced to 1.73 m^2. The openings of the slatted floor were narrower than CapSa's diameter. Furthermore, the faeces of the pigs were examined for the presence of the CapSas five times daily from day 1 until day 3 postadministration. On day 1 postadministration, a rectal lavage was conducted twice, utilising 50 mL of warm water combined with hand soap. Performing rectal lavages 24 h postadministration enables us to expedite the retrieval of CapSas from faeces, ensuring their safe retrieval.

Immediately after the retrieval of the CapSa, the outside of the capsule was cleaned with 70° alcohol to avoid contamination of the content. After the CapSa was opened, the content was extracted using a micropipette and sterile and DNA-free tips while measuring the volume. The content was then put in a sterile 0.5 ml Eppendorf (Eppendorf SE, Hamburg, Germany), snap frozen in liquid nitrogen, and stored at −80 °C until analysis. The pH of the sample was measured using litmus paper (Merck KGaA, Darmstadt, Germany) by immersing it on the inside of the empty capsule.

Postmortem observations

All pigs from Study 1 were euthanised 3 days after capsule administration. Meanwhile, pigs from Study 2 were sent to the slaughterhouse at 140 ± 5 days of age. In instances where the CapSas were not recovered from the faeces, the chyme of the pigs' GIT was examined. Additionally, all GITs were checked for macroscopic lesions potentially linked to the administration protocol of the CapSa (for example, gastric ulcers, intestinal perforations, etc).

Table 3
BW and sex distribution of pigs across the four BW categories in Study 1.

BW category	N	BW±SD, kg	Castrates, %	Females, %
XS (<12 kg)	14	8.31 ± 1.57	50.0	50.0
S (≥12 – 20 kg)	21	14.07 ± 1.41	66.7	33.3
M (≥20 – 40 kg)	17	34.34 ± 4.02	82.4	17.6
L (≥40 – 70 kg)	40	56.76 ± 7.99	52.5	47.5

Abbreviations: BW=Bodyweight; SD=Standard Deviation.

Calculations and statistical analysis

All statistical analyses were performed in R (v 4.3.1). For all statistical analyses, a difference was declared significant if the *P*-value < 0.05 and a trend was considered when $0.05 < P < 0.10$.

Study 1

For the pigs used in Study 1, four BW categories were defined: XS: < 12 kg BW; S: ≥ 12–20 kg BW; M: ≥ 20–40 kg BW; L: ≥ 40–70 kg BW (Table 3). The percentage of CapSas retrieved from the faeces, stomach, or not found was calculated based on the number of CapSas in each category divided by the number of CapSas administered. The capsule's transit time was calculated as the time between administration and retrieval from the faeces of the pigs. Percentages were analysed using linear regression with BW category, sex, and their interaction as fixed effects. An ANOVA was performed to check the effect of the BW category and sex on the outcome of the CapSas. The volume and pH of the CapSas were analysed using linear regression with BW category, sex, and the BW category × sex interaction as fixed effects and transit time as covariant. An ANOVA was performed to assess the effects of the BW category, sex, and time of retrieval on the pH and volume of the sample of the retrieved CapSas. Interactions were removed from the final model if not significant ($P > 0.05$). Type 3 ANOVA was used if the interaction was significant and type 2 ANOVA if not. Posthoc tests, such as least squares means, were performed when ANOVA detected an effect of BW category or sex on capsule result, transit time, volume and pH.

Study 2

The percentage of CapSas retrieved and transit time was calculated as described in Study 1. Percentages were analysed using linear regression with administration time, sex, and their interaction as fixed effects. An ANOVA was performed to check the effect of administration time and sex on the outcome of the CapSas. The pH and volume of the CapSas were analysed using linear regression with administration time, sex, transit time, and the administration time × sex interaction as fixed effects. An ANOVA was performed to assess the effects of administration time, sex, and time of retrieval on the pH and volume of the sample of the retrieved CapSas. Interactions were removed from the final model if not significant ($P > 0.05$). Type 3 ANOVA was used if the interaction was significant and type 2 ANOVA if not. Posthoc tests, such as least squares means, were performed when ANOVA detected an effect of administration time point or sex on capsule result, transit time, volume and pH.

Results

Study 1

Capsule retrieval and sample extraction

Of the 184 CapSas administered, 86 (46.74%) were found in the faeces within 72 h postadministration. Regarding the transit time of these 86 CapSas, 47.67% passed through the GIT within the first 24 h, an additional 48.84% were retrieved within the following 48 h postadministration, and the remaining 3.49% of CapSas were retrieved 72 h postadministration (Fig. 1). Furthermore, 28.26% of CapSas were found in the stomach, and 22.28% of CapSas were classified as "not found". Additionally, due to animal handling issues, five CapSas were not administered to three pigs: two with a low BW (6.91 and 7.2 kg) and one pig with a high BW of 41.8 kg. Independent of sex, the lowest percentage of retrieved CapSas was observed in the faeces of XS pigs, whereas the highest percentage was found in S pigs ($P < 0.05$). Intermediate values were observed for the M and L pigs. By contrast, the percentage of retrieved CapSas in the stomach was higher in the XS category compared to all the other BW categories ($P < 0.05$). Neither age nor sex had an impact on the percentage of capsules ($P \geq 0.30$). Out of the 86 capsules retrieved from faeces, 71 (82.5%) had a pH>5. The pH of the retrieved capsules was not affected by BW category ($P = 0.10$) or sex ($P = 0.38$). The mean values of pH ranged between 6.5 and 7 across all BW categories (Table 4). However, the pH of the samples was affected by the capsule transit time increase ($P < 0.01$), which could mean that the content becomes more acidic the longer the capsule takes to exit the stomach, possibly due to contamination with gastric chyme or bacterial fermentation within the capsule. The volume of the collected digesta samples was affected by the BW category ($P < 0.01$) and sex ($P = 0.02$) but not by the time of retrieval ($P > 0.05$) (Fig. 2). The highest sampled volumes were found in capsules from animals in BW category M and XS (246 and 242 µL, respectively), while lower volumes were found in S and L pigs (158 and 172 µL, respectively). In the case of sex, capsules from castrated males had higher volumes (226 versus 183 µL) (Table 4).

Postmortem observations

There were no abnormal health observations, and none of the pigs had to be euthanised for health-related issues. There was no tissue damage linked to the capsule administration and/or the capsule passage observed after euthanasia in any of the pigs. Every capsule retrieved on day 3 after administration was found in the stomach.

Study 2

Capsule retrieval and sample extraction

A total of 158 CapSas were administered, and within 72 h of administration, 121 (75.6%) were recovered in the faeces. However, 23.1% were classified as "not found", and 2 capsules could not be administered to one pig (1.2%) on T5. For the CapSas retrieved from faeces, 51.23% transited throughout the digestive tract within 24 h, and 38.02% in the following 48 h postadministration (Fig. 3). Only 10.74% were retrieved later in the following days after administration. Regardless of sex, the lowest CapSa retrieval rate was observed at T1 and T3, and the highest at T2, with intermediate values at T4 and T5 ($P < 0.05$) (Table 5). Only at T1 CapSas were retrieved after ≥ 312 h after administration. Age had an impact ($P < 0.05$) on the outcome of capsules (found in faeces, found late or not found) but not sex ($P > 0.05$) (Table 5). Out of the 121 capsules retrieved from faeces, 84.2% had a pH > 5.
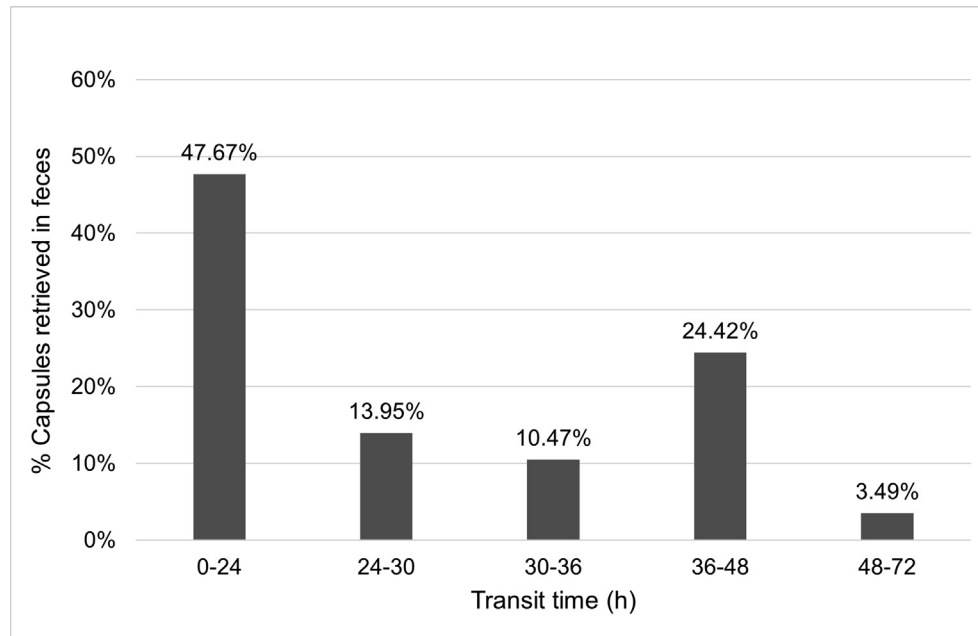
Fig. 1. Time (h) of transit of capsules found in faeces of pigs in Study 1. Time is calculated as the difference between the time of administration and the time of recovery.

Table 4
Summary of capsule retrieval rate, and characteristics (volume and pH) by BW category and sex of pigs in Study 1. Mean % calculated by linear models.

	BW category[1]				SEM	P[3]	Sex[2]		SEM	P[4]
	XS	S	M	L			C	F		
% Capsules[5]										
in faeces	2.2[a]	59.6[b]	62.1[ab]	57.4[ab]	16.10	0.03	50.7	40.0	9.89	0.42
in stomach	97.7[a]	21.1[b]	8.2[b]	10.8[b]	9.49	< 0.01	34.3	34.6	5.84	0.97
not found	0.00	19.3	29.7	31.8	14.04	0.30	15.0	25.4	8.64	0.37
Transit time, h[5]	70.3[a]	29.8[b]	34.6[b]	33.8[b]	11.67	< 0.01	43.8	40.5	3.77	0.24
Volume, μl[6]	242.0[ab]	158.0[a]	246.0[b]	172.0[a]	81.1	< 0.01	226.0[a]	183.0[b]	25.1	0.02
pH[6]	6.7	6.0	6.7	6.8	1.18	0.10	6.67	6.42	0.37	0.38

Abbreviations: BW=Bodyweight.
[ab]Values within a row with different superscripts differ ($P < 0.05$).
[1] XS: < 12 kg BW; S: ≥ 12–20 kg BW; M: ≥ 20–40 kg BW; L: ≥ 40–70 kg BW.
[2] C. castrate; F: female.
[3] P-value for the effect of BW category.
[4] P-value for the effect of sex.
[5] Percentages were analysed using linear regression with BW category, sex and their interaction as fixed effects. An ANOVA was performed to check the effect of BW category and sex on the outcome of the CapSas and transit time.
[6] Volume and pH were analysed using linear regression with BW category, sex and the BW category × sex interaction as fixed effects and transit time as covariant.

The pH of the retrieved capsules was not affected by the administration time point ($P = 0.10$), or by sex ($P = 0.12$). The mean values of pH ranged between 5.8 and 6.5 across all administration time points (Table 5). The pH of the CapSa samples was highly affected when the capsule transit time increased ($P < 0.01$, Table 5). In fact, capsules with a pH<5.5 are only retrieved after 36 h (Fig. 4). The volume of the collected digesta samples tended to be affected by the BW category ($P = 0.06$) but not by sex ($P = 0.21$) or the time of retrieval ($P = 0.97$) (Fig. 4). The highest sampled volumes were found in capsules from T2 (243 μL), followed by samples from T1 and T4 (219 and 217 μL, respectively), while lower volumes were found in T3 and T5 (184 and 192 μL, respectively) (Table 5).

Postmortem observations

None of the pigs had to be euthanised for health-related issues. Only one pig was excluded from capsule administration due to respiratory distress, which upon postmortem examination revealed upper oesophageal damage, probably due to an incorrect intubation procedure. As for the remaining pigs, there was no tissue dam-age linked to the capsule administration and/or the capsule passage observed after slaughter. There were no capsules found in the stomach after slaughter; probably, the capsules were expelled between administrations when the pig's faeces were not monitored.

Author's point of views

The present study showed a valid procedure for administering CapSa in pigs. To address gastric blockage in a pig's stomach and ensure successful CapSa administration and retrieval, several strategies were implemented. First, to facilitate the process, straw was removed from the pig's enclosure 2 days prior to the administration of the CapSas, and the pig's diet was switched from solid to liquid. This dietary modification and straw removal were aimed at shortening the digestive transit time and enhancing gastric emptying. According to the literature (Henze et al., 2021), gastric emptying is highly variable and can range from 20 to 233 h. By allowing the stomach to be emptier, we hoped for a faster and more efficient
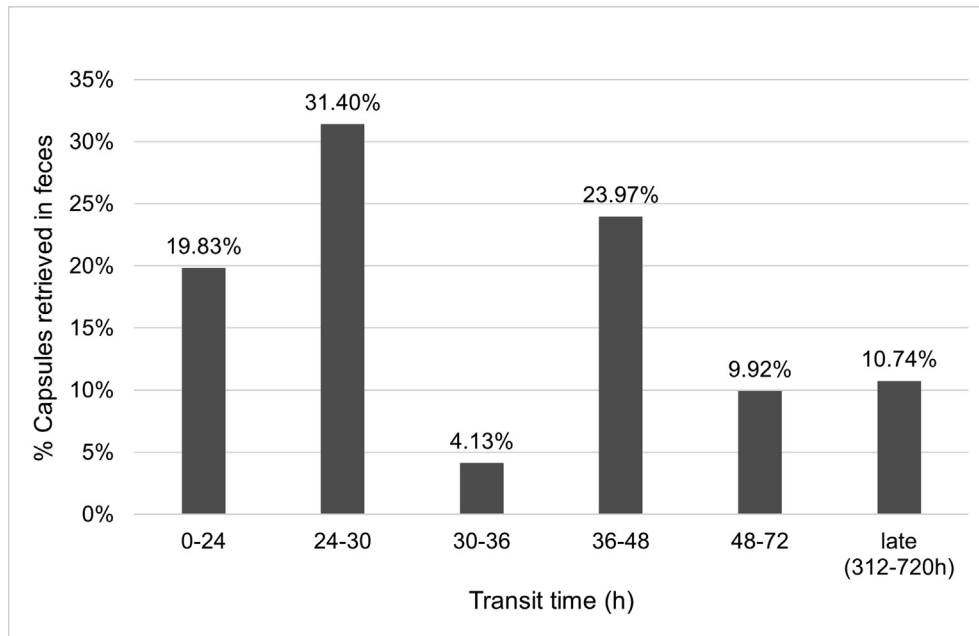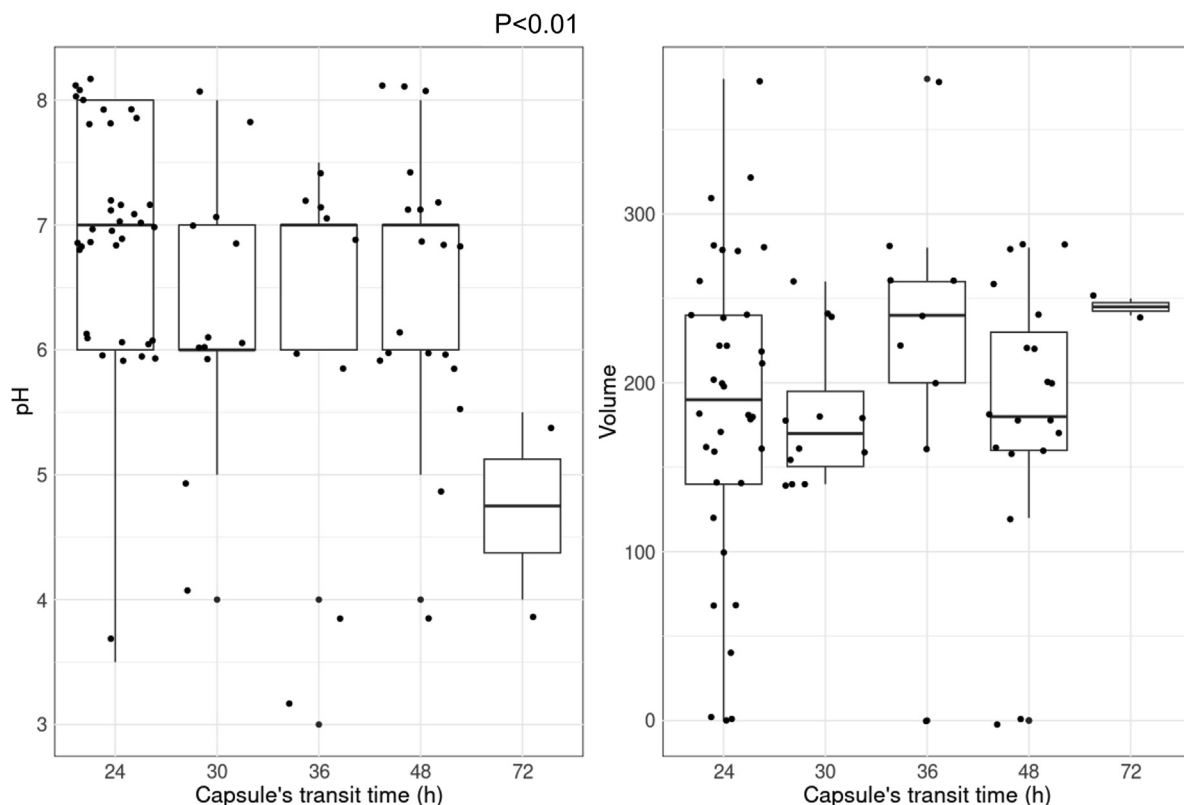
Fig. 2. pH and volume of capsule's content from pigs in Study 1 depending on capsule's transit time. The pH and volume of the capsule were analysed using a linear regression with transit time as fixed effect. Only *P*-values that are significant (<0.05) or tend to be significant (0.05 < *P* < 0.10) are shown.

Fig. 3. Time (h) of transit of capsules found in faeces of pigs in Study 2. Time is calculated as the difference between the time of administration and the time of recovery.

capsule passage through the GIT. Furthermore, on the day preceding CapSa administration, pigs were provided with a semi-liquid meal and fasted for at least 12 h before the capsule was given. Again, this allowed us to have an emptier GIT that would allow the capsule to pass through easier and faster. As has been proven in previous studies (Ochia, 1973), the rate of gastric emptying for

pigs is very rapid at first and later slows down after fasting. This sudden initial gush is due to the latent period between duodenal distension by gastric contents and its response in regulating gastric emptying.

Second, to assist the capsule in bypassing the gastric blockage, administration of a prokinetic agent was necessary to increase gas-

Table 5
Summary of capsule retrieval rate, and characteristics (volume and pH) by BW category and sex of pigs in Study 2. Mean % calculated by linear models.

	Time point of administration[1]							Sex[2]			
	T1	T2	T3	T4	T5	SEM	P	C	F	SEM	P
% Capsules[4]											
in faeces	56.2[a]	93.8[b]	59.4[a]	71.9 [ab]	62.5[ab]	4.74	0.02	67.5	70.0	3.00	0.58
in faeces "late"[3]	34.0[a]	0.0[b]	0.0[b]	0.0[b]	0.0[b]	1.40	< 0.01	7.5	6.3	0.88	0.37
not found	9.4[ab]	6.2[a]	40.6[b]	28.1[ab]	37.5[ab]	5.13	0.02	25.0	23.8	3.25	0.79
Transit time, h[4]	169.7[a]	39.4[b]	62.1[b]	30.7[b]	40.6[b]	27.7	< 0.01	64.4	72.6	15.7	0.70
Volume, µl[5]	219.0	243.0	184.0	217.0	192.0	17.8	0.06	220.0	202.0	10.25	0.21
pH[5]	5.8	6.4	6.4	6.4	6.5	0.195	0.10	6.4	6.2	0.11	0.12

[ab]Values within a row with different superscripts differ ($P < 0.05$).
[1] T1: 52 ± 3 d; T2: 70 ± 3 d; T3: 83 ± 3 d; T4; 110 ± 3 d; T5: 126 ± 3 d.
[2] C: castrate; F: female.
[3] In faeces "late": capsules retrieved more than 3 days after administration (e.g. capsules from T1 retrieved in faeces after administration in T2).
[4] Percentages were analysed using linear regression with time point of administration, sex and their interaction as fixed effects. An ANOVA was performed to check the effect of time point of administration and sex on the outcome of the CapSas and transit time.
[5] Volume and pH were analysed using linear regression with time point of administration, sex, transit time, and the time point of administration × sex interaction as fixed effects.
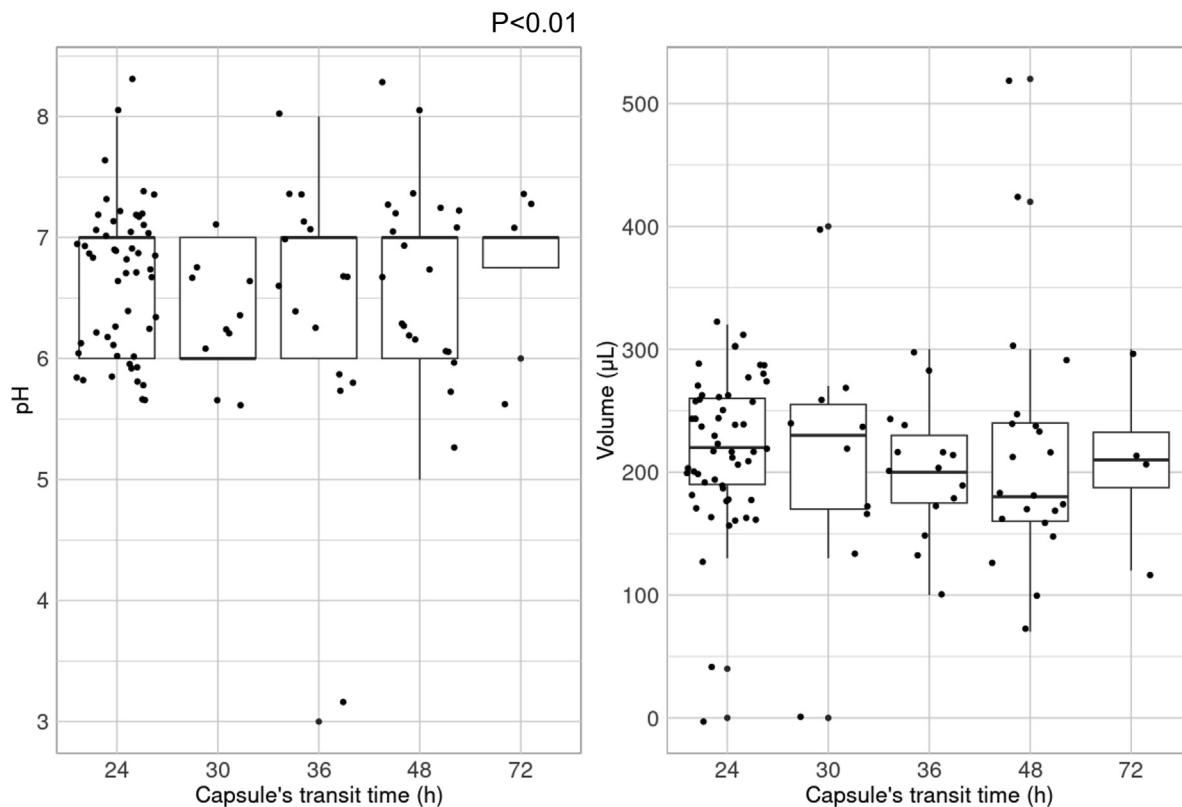
Fig. 4. pH and volume of capsule's content from pigs in Study 2 depending on capsule's transit time. The pH and volume of the capsule were analysed using a linear regression with transit time as fixed effect. Only *P*-values that are significant (<0.05) or tend to be significant (0.05 < *P* < 0.10) are shown.

tric contractions, facilitating passage through the pylorus and reducing the capsule's transit time through the GIT. The pig's stomach, characterised by its pronounced "C" shape and the close proximity of the gastric cardia to the pylorus, presents challenges for gastric emptying (Henze et al., 2021). The presence of a transverse pyloric fold, known as the "*torus pyloricus*" (Bal and Ghoshal, 1972; Kopácová et al., 2010), in the pyloric aperture further complicates the process. This anatomical feature, designed to prevent the passage of unprocessed solid gastric contents into the small intestine, contributes to slower gastric emptying and can result in particles larger than 1 cm being retained in the stomach for extended periods (Hossain et al., 1990; Davis et al., 2001). Among the prokinetic

agents evaluated, prucalopride, a "last generation" serotonergic 5-HT$_4$ agonist, has demonstrated efficacy in enhancing peristalsis within both the stomach and colon, thus promoting gastric emptying. Prucalopride's effectiveness, as noted in several studies (Priem et al., 2012; Camilleri and Atieh, 2021), distinguishes it from other prokinetics, particularly in maximising capsule retrieval rates. This attribute makes prucalopride a preferred choice for facilitating the passage of the capsule through the pig's GIT.

Third, to counteract the fasting-induced rise in gastric pH prior to administering the CapSa, orange juice was co-administered with the CapSas. The acidity of orange juice helps maintain an acidic environment around the capsule as it reaches the stomach, a

crucial step because the capsule's collection mechanism is triggered at a pH>6 (García Viñado et al., 2022), a condition typically found beyond the stomach, in the GIT.

Sondes were adapted to the size of the pig (see Supplementary Figure S1 for more detail). According to our measurements, the length of the oesophagus (L_o) of the pig is approximately 60% of the length of the pig from the groin to the base of the tail (L_{g-t}). The estimation of the size of the sonde was done using the following equation:

$$Lo = 0.6 \times Lg\text{-}t$$

To ensure at least the retrieval of one capsule per pig, all pigs were administered two CapSas. In the current study, the described protocol successfully enabled the recovery of 86 capsules (46.74%) in Study 1 and 121 capsules (75.6%) in Study 2. These findings are highly promising, indicating the efficacy of the administration protocol in both singular and repeated applications.

In Study 2, our results demonstrated that the efficacy of the administration protocol was maintained even with repeated application. Although there is a significant effect of administration time on the percentage of capsules recovered from faeces ($P = 0.02$), the retrieval rate consistently exceeded 50%, ranging from a minimum of 56.2% to a maximum of 93.8%. Notably, our findings indicate that the protocol's effectiveness is not compromised by repetition. We have also observed that the repeated administration of prucalopride does not appear to reduce the prokinetic effect of prucalopride in pigs; these findings are consistent with previous in vitro results in pigs (De Maeyer et al., 2009). Furthermore, regardless of sex, the protocol yielded consistent results, suggesting its applicability to both females and castrated males.

Transit time is strongly influenced ($P < 0.01$) by both BW category and time of administration (Tables 4 and 5). Indeed, the smallest pigs and the first time of administration had a longer transit time than the rest of the BW categories and the following time of administration, respectively. In Study 1, we observed that the longest transit time for the capsules occurred in XS pigs, averaging 70.3 h, while for other weight categories, it ranged between 29.8 and 34.6 h. This discrepancy may stem from a size disproportion between the capsule (size 0) and the GIT passage in small piglets. However, we cannot definitively confirm this hypothesis, since only one pig from the XS BW category expelled the capsule. In Study 2, the longest mean transit time was observed at the first administration time point (169.7 h), which significantly decreased in subsequent administration time points. This is attributed to the consistent effectiveness of the protocol in subsequent capsule administrations, which facilitated the expulsion of capsules from previous administrations.

This protocol could prove advantageous for other ingestible devices. A recent study utilising a non-invasive capsule to investigate microbiota was used in pigs (Rezaei Nejad et al., 2019) and encountered difficulties retrieving all capsules after administration. However, successful retrieval was achieved when administered to humans and macaques. The establishment of a standardised administration protocol could pave the way for utilising pigs as models for pharmacological and drug delivery studies.

Despite the overall promising results of the administration protocol, a notable limitation in our investigation was the inability to retrieve capsules from pigs with a light BW (category XS: < 12 kg). As shown in Study 1, 97.7% of CapSas administered to pigs belonging to the XS category were retrieved in the stomach postmortem. This block in the stomach is due to the presence of the *torus pyloricus*, which nearly entirely obstructs the pyloric exit for solid particles in small piglets.

Another limitation of this administration procedure is the precision of the required materials (sling, sondes, mouth gag, etc.) that have to be adapted to be used in pigs, as well as the training of the person executing the oesophageal intubation. Certain protocol specifications, such as the absence of straw in the box around the administration time or the liquid diet 2 days before administration, even though they are essential for the procedure to work, might have consequences for the gut microbiota and should be investigated in future studies.

In conclusion, this is a valid procedure for administering CapSa in pigs, overcoming the challenges of administration, gastric blockage, and retrieval. In the future, this standard operating procedure could serve to use CapSa to study the dynamic picture of the small intestine microbiota in pigs using the same pigs.

Peer Review Summary and Supplementary material

Peer Review Summary and Supplementary Material for this article (https://doi.org/10.1016/j.anopes.2024.100076) can be found at the foot of the online page, in Appendix A and B, respectively.

Ethics approval

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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IGV and CO validated the procedure and conducted the main statistical 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, PT, and GB supervised the study. IGV drafted the manuscript, and CO, PT, and GB critically reviewed the manuscript. All authors read and approved the final manuscript.

Declaration of interest

The authors declare that they have no conflict of interest relating to the content of this article.

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