Method paper

Catheterisation of the jugular vein of 12-day-old suckling piglets group-housed with littermates and the sow

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ABSTRACT

Collection of blood samples by venipuncture requires isolation, restraint and immobilisation, which is stressful for piglets and may result in unreliable readings. A jugular vein catheter allows frequent blood sampling with minimal stress to the piglets. Techniques for jugular vein catheter implantation in older pigs have been described, but less information is available for suckling piglets. This report describes the procedure of catheter implantation into an external jugular vein for longer-term frequent blood sampling in 48 12-day (**d**)-old suckling German Landrace piglets with a mean BW of 3.4 ± 0.7 kg. Catheterisation was performed either under general anaesthesia by injection of azaperone and ketamine (**a/k**) or by inhalation of isoflurane (**IsoF**). To determine the optimal incision site in the *sulcus jugularis*, the centre between the caudal edge of the *mandibula*, cranial shoulder and *sternum* was identified. After a small incision of the skin, the jugular vein was bluntly dissected and a catheter was inserted. The tip of the catheter was placed near the beginning of the right atrium. After wound closure, the surgical area was secured by disinfection and bandages. The piglets were returned to their littermates in the farrowing pen immediately after full recovery from anaesthesia, which was 5–7 h in a/k and 0.75 h in IsoF anaesthetised piglets after the onset of the surgery, respectively. The catheter was flushed daily with 0.9% NaCl-0.1% sodium citrate solution. To demonstrate the longer-term benefits of the catheter, on d 4 after surgery, a series of frequent blood sampling were performed after an oral xylose bolus. In total, 10 samples of 0.5 mL were taken before and every 30 min after the oral xylose bolus for 5 h; the blood volume was replaced with 0.9% NaCl solution. On d 4 after surgery, 41 of 48 implanted catheters were considered fully functional (90% of desired samples collected), three as partially functional, and four as non-functional, whereas two catheters were non-functional already from d 1 after surgery. The catheterised animals remained clinically healthy but showed less daily BW gain from age d 12 (day of surgery) to d 15 than non-catheterised control animals (0.12 ± 0.01 vs 0.23 ± 0.01 kg; n = 46; *P* < 0.05). In conclusion, the catheter implanted into an external jugular vein in 12-d-old suckling piglets remaining with the sow was patent for 4 d after surgery and allowed frequent blood sampling with minimal stress for the piglets.

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Implications

Collection of blood samples from pigs by venipuncture requires isolation, restraint, and immobilisation, which is stressful for the animals and may result in unreliable readings during scientific studies. Therefore, a permanent venous catheterisation procedure was developed specifically for suckling piglets group-housed with their litter and the sow for frequent blood sampling. A jugular catheter was used for up to 4 days after surgery and allowed frequent blood sampling with minimal stress to the animal. This technique promotes the 3R principle, especially in terms of limiting the number of animals (reduction) and their suffering (refinement).

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Specification table

Subject	Nutrition, Physiology and Functional Biology
Type of data	Table, Graph, Figure
How data were acquired	Data were obtained from suckling piglets that remained in the farrowing pen with their littermates and the sow during a study to collect frequent blood samples for metabolite analyses. Data on xylose concentrations in plasma were analysed by HPLC-Refractive Index Detector (Agilent 1200/1260 Infinity Series) and the OpenLab/ChemStation C.01.06 [61] software. BWs were measured by a Kern scale DE 150K20DL.
Data format	Raw and analysed data (Microsoft Excel,.docx, SigmaPlot,).
Parameters for data collection	BW data were recorded from day 10 of life to monitor daily growth. Plasma xylose concentration was measured as a function of time after ingestion of a xylose bolus. Jugular vein catheter functionality data were obtained.
Description of data collection	The data were collected from suckling piglets which were implanted with a permanent jugular vein catheter at 12 days of age. Piglets were weighed daily and given an oral bolus of xylose at 16 days of age after being separated from the dam for 1 h. Blood samples were collected every half h for 5 h.
Data source location	Research Institute for Farm Animal Biology, Institute of Nutritional Physiology, Dummerstorf, Germany
Data accessibility	Data and supplementary materials used for this paper can be obtained from the repository Repository name: Zenodo Metges, Cornelia, De Leonardis, Daria, & Vernunft, Andreas. (2023) . Data on piglet bodyweight_body temperature_catheter functionality_plasma xylose concentration_after jugular vein catheter surgery [Data set]. Zenodo. https://doi.org/10.5281/zenodo.10004876

Introduction

Taking a blood sample by vascular puncture from piglets requires isolation, fixation and immobilisation, which is stressful, may lead to unreliable results during scientific studies and precludes frequent sampling. Frequent blood sampling is required to analyse blood parameters over time, such as plasma concentration of various metabolites to observe the combined effects of feed intake, digestion, absorption and metabolism (Rasch et al., 2020). Repeated puncture of the jugular vein in pigs at short intervals (e.g. several times daily) would result in destruction of the vein, failure of samples and immense stress and defensive reactions in the animals. Implantation of a jugular vein catheter is a solution to sample blood frequently with minimal stress on the pigs and to improve the welfare of the experimental animals (Theil et al.,

2012). Published information on swine vascular catheterisation is mostly available in grower and adult pigs. Although catheterisation of weaned piglets is routinely performed in some facilities (Columbus et al., 2014, Pi et al., 2019), implantation of a venous catheter in suckling piglets is more challenging. Catheterisation in 6-day (d) -old and 21-d-old piglets has been previously reported (Gasthuys et al., 2017, Furbeyre and Labussiere, 2020). However, the catheter was only used for 24 h and the piglets were kept isolated.

To study functional macronutrient metabolism, e.g. in response to the ingestion of a meal or a specific nutrient, in this study a surgery procedure to implant a permanent external jugular catheter in suckling piglets with an average weight of less than 4 kg was established. During the study, the piglets were group-housed with their littermates and the sow. In addition to developing the procedure, the objectives were to determine the performance, durability, patency and functionality of the catheter, as well as the effects of surgery and anaesthesia on the piglets.

Material and methods

Animals and housing

A total of 48 suckling German Landrace male piglets born to sows of parity 2–9 at the pig facility of the Research Institute for Farm Animal Biology, Dummerstorf, Germany, were implanted a permanent jugular vein catheter at d 12 of age and a BW range of 2.08 to 4.8 kg (average BW of 3.4 ± 0.70 kg). The animals were used in an experiment within the EU "MonoGutHealth" project (Marie Skłodowska-Curie Innovative Training Networks, Grant agreement no. 955374). Once completely recovered from surgery, the piglets were immediately returned to the farrowing pen with their littermates and dam. In addition, 48 control (**CON**) piglets (average BW of 3.5 ± 0.75 kg) were studied under comparable conditions and used to determine normal daily BW gain (**BWG**) without the influence of catheter implantation and blood sampling.

Catheterisation

Anaesthesia, presurgical treatments and medication

Immediately before surgery, the piglets were taken individually from the farrowing pen into a 45 × 55 cm box lined with a blanket in the surgery room, weighed, and their body temperature measured. During winter time, a heating lamp was placed above the box.

They were injected intramuscularly (i.m.) with azaperone (2 mg/kg BW, Stresnil, Elanco Animal Health, Bad Homburg, Germany) and ketamine (25 mg/kg BW, Ursotamin Serumwerk Bernburg AG, Bernburg, Germany) (**a/k**) as recommended by the manufacturer (n = 18 piglets) or with isoflurane (**IsoF**) (Forane®, Baxter, Unterschleißheim, Germany) (n = 30 piglets). To assess the surgical tolerance, piglets were checked for the absence of a pedal reflex and the presence of eyeball torsion and mydriasis. In the case of an injection anaesthesia, an individualised supplemental dose of ketamine was administered into an ear vein (*vena auricularis*) to achieve full surgical tolerance or prolong anaesthesia, if necessary.

Piglets receiving IsoF anaesthesia were anaesthetised with a face mask (Eikemeyer, size 4, Tuttlingen, Germany) placed over the piglet's snout and delivering 4% IsoF in pure oxygen at a flow rate of 200 mL/min. After vigilance breakdown and assessed surgical tolerance, IsoF was reduced to 2% for the surgery and to 1% when the incisions were closed at the end of the procedure. A recommended dose of meloxicam (0.4 mg/kg BW Meloxicam 20 mg/mL; Melovem, Dopharma, Raamsdonksveer, Netherlands) was

administered i.m. before surgery. No concomitant antibiotic treatment was given.

Surgical procedure

The piglets were placed in dorsal recumbency with the four legs placed in caudal direction. Instruments used for the implantation of the jugular catheter are depicted in Supplementary Fig. S1. Commercially available catheter sets were used: Cavafix® Certo® with Splittocan® (B.Braun, Melsungen, Germany) or Leadercathexpert (Vygon, Aachen, Germany) (Supplementary Table S1). Surgical materials and drugs used are summarised in Supplementary Table S1.

The skin of the neck area was scrubbed three times with 70% isopropanol. The surgical area was covered with a surgical drape (Henry Schein GmbH, Berlin, Germany; Supplementary Fig. S2) and a window was cut in the surgical drape to expose the surgical site. The surgical field was treated with 7.5% Povidone-iodine (PVP-I; Braunol, B. Braun, Melsungen, Germany). To find the optimal incision site in the *sulcus jugularis*, the centre of a triangle between the caudal ramus of the *mandibula*, rostral end of shoulder and *manubrium* was determined as described (Flournoy and Mani, 2009). Then, a two- to three-cm incision (Fig. 1, a) at the caudal part of the *sulcus jugularis dexter* was made with a scalpel (Supplementary Fig. S1, no. 8). The subcutaneous tissue was blunt prepared (Fig. 1, b) with tweezers and a surgical curved clamp (Supplementary Fig. S1, no. 3 and 5). Separating *M. brachiocephalicus* and *M. sternocephalicus*, the V. *jugularis externa* was isolated from other tissues (Fig. 1, c). A schematic drawing for finding the optimal incision side and locating the jugular vein is provided in the supplementary material (Supplementary Fig. S3). The surgical clamp (Supplementary Fig. S1, no. 3) was used also to expose the

vein so that connective tissue could be carefully removed from the vein with surgical tweezers (Supplementary Fig. S1, no. 4). A permanent cranial ligature on the vein was made (Supplementary Fig. S4) using non-absorbable surgical suture (Polifile silk, 2/0 USP, Dermafil®, SMI, St. Vith Belgium) to avoid bleeding during or after the cannulation procedure. Care was taken not to place the ligature too far cranially, which could lead to possible occlusion of the *V. linguofacialis* and *V. maxillaris*. A hollow needle (Supplementary Fig. S1, no. 9) was used to prepare a subcutaneous channel between the vein and the back of the piglets to be used as catheter guide. The piglets were turned to the left side to facilitate the insertion of the hollow needle through the muscles and subcutis, to come out between the *scapulae* (Supplementary Fig. S5). The end of the catheter was passed through the hollow needle from the back, and the needle was removed ventrally. To allow multiple sampling from the catheter, the end of the catheter was equipped with a disposable stopcock (0.20 mL with SPIN-LOCK connector, B. Braun). Then, the posterior outer extension of the catheter was attached to the skin with the integrated catheter suture wing (Fig. 1, d).

The piglets were then placed in the original supine position, and special care was taken to maintain the sterility of the catheter by covering it with a surgical drape. Thereafter, the internal length of the catheter was determined individually for each piglet so that the end of the catheter would be near or in the right *atrium*. As reference point for shortening the catheter, the *olecranon* in extension position was used (Fig. 1, e). The catheter was shortened at a slight angle to facilitate insertion into the vein, avoiding sharp edges (Supplementary Fig. S1, no. 7). A syringe was then connected to the catheter and the catheter was filled with saline. The vein was exposed again with a surgical clamp (Fig. 1, f), and a small tweezer
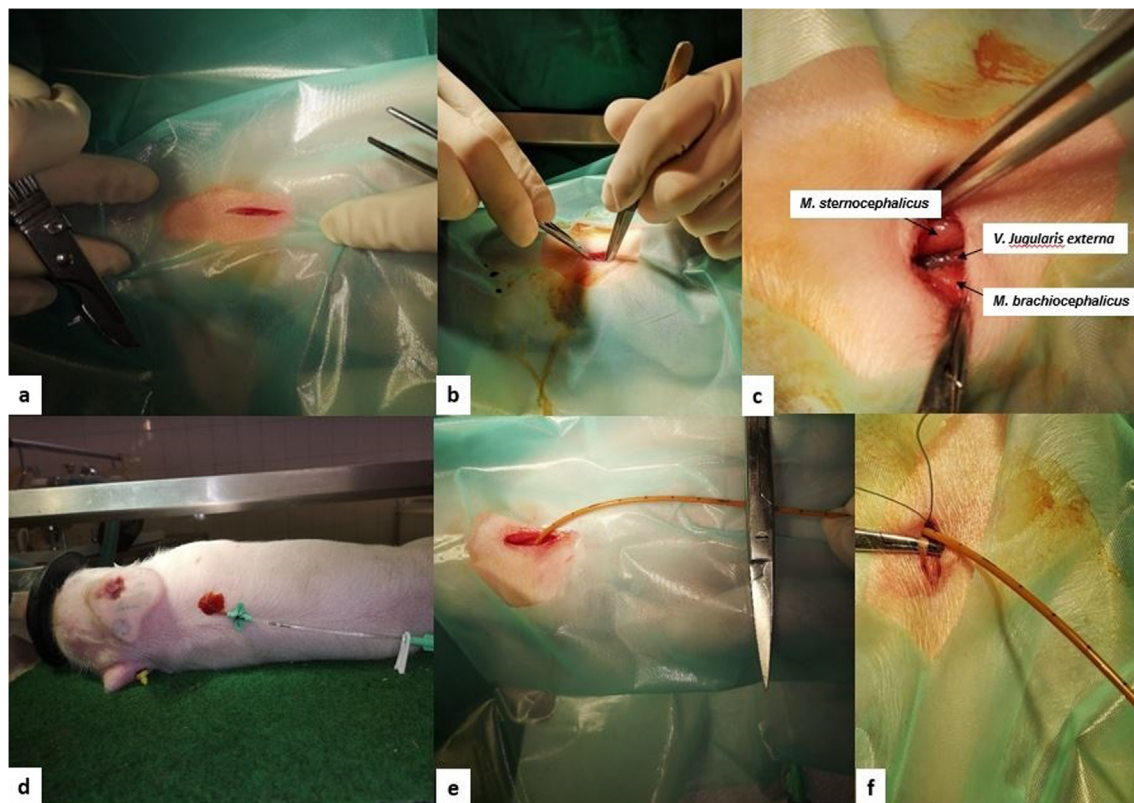

Fig. 1. Catheterisation procedure of the right jugular vein of suckling piglets at 12 day of age, (a) 2–3 cm incision at the right caudal part of *sulcus jugularis dexter* made with a scalpel, (b) subcutaneous tissue is blunt prepared, (c) isolation of the *V. jugularis externa* from other tissues, (d) the external extension of the catheter fixed with the integrated catheter suture wing (in red, Vet-Sept, ointment containing iodine), (e) determination of the inner length of the catheter, (f) vein exposed with clamp before opening and catheter insertion.

(Supplementary Fig. S1, no. 4) was used to stretch the vein caudally to the first ligature so that a small opening could be created through a cut (about one third in depth of the vein) with small, curved scissors (Supplementary Fig. S1, no. 6). Then the catheter could be inserted smoothly and, if necessary, with a slight twist, without damaging to the vein wall. Once the catheter has been inserted, its correct position and function were checked repeatedly via blood aspiration and flushing with 0.9% NaCl-0.1% sodium citrate solution (0.9% NaCl, Serumwerk Bernburg AG, Bernburg, Germany) during the next steps. Two additional ligatures (Polifile silk, 2/0 USP, Dermafil, SMI, St. Vith Belgium) were placed around the catheterised vein, cardiac from the catheter entry site to secure the catheter in the vein and prevent retrograde haemorrhage. The second ligature was additionally fixed to the adjacent tissue with a stitch. Then, the *V. jugularis* was positioned *in situ*.

The musculature and the subcutaneous tissue were adapted separately with a continuous suture using absorbable suture (Surgicryl PGA DS-24, reverse cutting 3/8 M circle 24 mm, SMI). The skin was closed with a monofilament non-absorbable suture material (Supramid black polyamide non-resorbable, SMI) by continuous suture according to Reverdin or a single U-stitch.

Postsurgery procedures

The surgery wound and the exit point of the catheter were treated with an ointment containing iodine (Vet-Sept100 mg/g PVP iodine; Livisto, Hamburg, Germany) and covered by a 5 × 5 cm piece of a soft self-adhesive plaster (Fixmull stretch, 5 cm × 10 m, Hypafix, Hamburg, Germany). A first layer of soft cotton bandage (10 cm × 3 m, Cellona, Rengsdorf, Germany) was draped crosswise around the chest. A cohesive bandage (Petflex cohesive bandage, 5 cm, Covetrus, Hamburg, Germany) was used as a second layer for final coverage to protect the catheter from contamination or bites from littermates (Supplementary Fig. S6).

The piglets were left in the transport box with a heating lamp under supervision until they were completely awake in quadrupedal position. After that, the piglets were brought back to their littermates in the farrowing pen. Signs of pain such as abnormal respiratory rate, posture, and vocalisation were assessed for each pig during the days postsurgery. The catheter was flushed twice daily with sodium citrate solution at 0900 and 1700 h to prevent blockage of the catheter and to check the status of sutures. In the first 4 d after surgery, all piglets were weighed daily at 0700 h and their BWG was calculated. In a subset of 25 animals, the body temperature was measured rectally on d1 and 4 after catheterisation. On the fourth day, the animal experiment was completed, and the animals were euthanised.

Blood sample collection and analyses

After a washout period of one d after surgery, frequent blood sampling was performed. For blood collection, the catheter was first drained of the blood mixed with the previous day's rinse by aspiration with a 2 mL syringe. Subsequently, the blood was collected with a clean syringe (Supplementary Fig. S7) and the catheter was rinsed again with 2 mL of physiological sodium citrate solution after each blood drawing to prevent clotting and to replace the volume.

For the assessment of the functionality of the catheter for this study, 10 blood samples taken on the fourth d (age d 16), were analysed. For this purpose, a xylose test was performed. Plasma concentrations of xylose were selected to demonstrate longitudinal blood sampling in suckling piglets. Piglets were given orally 400 mg/kg BW xylose (Roth, Karlsruhe, Germany) dissolved in 5 mL of tap water. Before (-15 min) and after the xylose bolus

dose, blood samples (0.5 mL) were taken (every 30 min) for a total period of 300 min. Between blood collections, the piglets were returned to their home pen. Blood samples were collected in EDTA tubes (Minicollect 0.5 mL K3EDTA, Greiner Bio-One GmbH, Frickenhausen, Germany), and plasma was prepared from blood samples via centrifugation at 1 573g for 20 min at 4 °C (Heraeus Multifuge 3 L-R; Kendro Laboratory Products, Osterode, Germany) and stored at -80 °C until analysis. Plasma was deproteinised by perchloric acid, and concentration of xylose was quantified by HPLC with a refractive index detector (1200/1260 Infinity Series, Agilent Technologies, Waldbronn, Germany) on a Rezex ROA-Organic Acid column (300 × 7.8 mm) column protected with a Carbo-H+ guard cartridge (4 × 3 mm) (Phenomenex, Aschaffenburg, Germany) as described (Junghans et al., 2010). Intra-assay and inter-assay CV were 1.4 and 2.0%, and the lower limit of quantification was at 1 μM.

Statistics

For data analysis, statistical tools of SigmaPlot for Windows Version 11.0 (Systat Software GmbH) were used. With the BWG data, a one-way analysis of variance (ANOVA) was performed and the significance of differences between groups was tested with Mann-Whitney Rank Sum Test when data were normally distributed or using a pairwise multiple comparison procedure (Dunńs method) as normality test failed. Differences between the repeated body temperature measurements were analysed with a paired t-test. Descriptive statistics and graphs were created with Microsoft® Office Excel 2013. All results are presented as means with their SEs. Differences between means were considered significant if the *P*-value was below 0.05.

Results

Anaesthesia

In a/k anaesthetised piglets, the time between i.m. administration and onset of the effect varied between 5 and 10 min. In 16 of 18 animals, the initially used i.m. dose (2 mg/kg BW azaperone and 25 mg/kg BW ketamine) caused deep sedation but did not achieve sufficient surgical tolerance. Therefore, a second or even third equivalent dose of ketamine had to be administered intravenously. In two cases, the surgical tolerance was not sufficient until the end of the surgery, so that another half dose had to be administered during the surgery. Piglets anaesthetised with a/k could stand secure and unassisted in quadrupedal position within 5–7 h postsurgery. Subsequently, they were returned to their litter, where some of them remained disoriented. This caused a further delay until the piglets could secure a teat to suckle.

The IsoF anaesthesia took effect 1–2 min after initiation of the narcotic gas flow, and the surgery could start earlier than in piglets anaesthetised with a/k. An initial IsoF concentration of 4% in pure oxygen proved sufficient for a rapid narcosis initiation. After vigilance breakdown and assessed surgical tolerance, IsoF could be reduced to 2% for the surgery procedures (after approximately 5 min). Some piglets showed respiratory depression, if the IsoF concentrations were not reduced in time. At the end of the surgery (wound closure), the IsoF concentration could be again reduced to 1%. After surgery, piglets recovered in approximately 15 min after termination of the gas flow and were then returned to their litter. From the start of the surgery, it took a total of 45 min until the isoF anaesthetised piglets could be returned to their pen and started to suckle.

Surgery

All 48 piglets could be successfully fitted with a catheter. The internal length of the catheter was determined individually for each piglet so that the end of the catheter was near to or in the right atrium. The reference point for shortening the catheter was the *olecranon* in extension position, resulting in an inner remaining catheter length of 20–25 cm from the integrated catheter suture wing (outer entrance to the skin). This prevents the catheter from retracting into smaller sections of the vessel and has turned out to be beneficial for the functionality of the catheter. In four piglets, insertion of the catheter into the vein during surgery was difficult, and took between 10 and 15 min longer. In two of the four piglets, another opening had to be made in the vein towards the heart because the first opening was lost or damaged while handling the vein during insertion. At the end of the procedure, these catheters were as functional as the others.

Once the piglets were sedated, the surgery from cutting the skin to the last suture took about 30 min, if no complication occurred. After the catheter functionality was established, 5 min were needed until the bandages were applied.

Catheter management

In this study, catheters were considered as functional on d 1, if blood could be drawn but was not collected during the daily flushing (Table 1). Functionality on d 4 was confirmed when 9 or 10 blood samples of the planned 10 samples were collected, partially functional if 8 or fewer samples were collected, and non-functional if no samples could be obtained during the 5-h collection period (Table 1). All animals were returned to their litters with a functioning catheter. On d 1 after surgery 2 of 48 catheters were not operational. In one animal without any obvious reason, the flushing was easily going in but no blood could be aspirated. One animal was found dead one day after surgery, with no external signs of bruises or wounds, and the catheter was still in. On d 4, in few cases, the catheter seemed not to work or to work only partially. However, by turning or repositioning the piglet's head, blood sampling was possible again. This position was very piglet specific. In other cases, catheter functionality was restored by flushing the catheter beforehand. If the catheter was classified as non-functional on d 4, this meant the above two recovery techniques were tried unsuccessfully on each of the 10 samplings. It happened that the catheter lost its functionality after the first few days of samples, but regained it the next day or during the next attempts of blood sampling. In case of non-functioning catheters (except in one case), an injection was still possible.

To allow optimal catheter function and to prevent damage or infection of the catheter, a two-layer bandage was applied. If the bandages were applied properly on the day of surgery, no serious problems occurred until d 4. During the daily check of catheter functionality, the bandages were checked and if the catheter was exposed to the outside, an additional layer was made with only the adhesive bandage material. However, a replacement of the bandages or a complete renewal was not necessary.

Effect of surgery on piglets

From d 10 to d 16 of age (d 4 after surgery), 46 piglets were weighed daily to follow their recovery after catheter surgery. From d 10 to d 12 of age (before surgery), the average BWG was 0.21 ± 0.01 kg (Fig. 2). From d 12 (day of surgery) to d 15, the mean BWG in the catheterised group decreased to 0.1 ± 0.01 kg ($P < 0.001$; Mann-Whitney Rank Sum Test), whereas animals in the CON group continued with a BWG of 0.22 ± 0.07 kg (n = 46). The piglets anaesthetised with a/k, had a BWG of 0.02 ± 0.04 kg from d 12 (day of surgery) to d 13 which was lower than in CON and those anaesthetised with IsoF at that day (CON 0.24 ± 0.02; IsoF 0.21 ± 0.02 kg, $P < 0.001$, Mann-Whitney Rank Sum Test). From d 13 to d 15, the BWG of a/k and IsoF anaesthetised animals were comparable (Dunn's Method). From the day of surgery, the BWG of the catheterised group were consistently lower compared to the CON group (Fig. 2, $P < 0.001$, Mann-Whitney Rank Sum Test). On the last day of the study (d 16), the BW of the catheterised animals was lower than that of the CON animals (3.8 ± 0.1 vs 4.4 ± 0.1 kg, $P < 0.01$; Mann-Whitney Rank Sum Test).

Mean body temperature before surgery was 39.1 ± 0.05 °C and was higher at 39.8 ± 0.15 °C ($P < 0.001$; paired t-test) on the last day of the study (d 16). However, it has to be mentioned that on d 16, the animals were observed sleeping under the heating lamp in the pen before measuring their temperature. After surgery, all animals were active, without signs of pain and suckling like their intact littermates, and only two of the catheterised animals had a body temperature of 41 °C.

Plasma sample analysis

In order to demonstrate catheter functionality, blood samples were collected frequently at 4 d after surgery, before and after the administration of a xylose bolus and plasma xylose concentrations were measured. For each sample, the piglets were taken from the pen and put back immediately thereafter. Plasma xylose concentrations increased from baseline and reached a maximum xylose concentration of approximately 1.2 mM 60 min after the bolus, then slowly decreased over the next 4 h (Fig. 3). From 41 of the 48 catheterised piglets, sufficient blood (0.5 mL) could be taken at all 10 or at least 9 of the 10 desired time points (total of 5 mL blood) to generate an informative data set.

Table 1

Functionality of jugular catheters recorded one or four days after insertion in 12-day-old suckling piglets (n = 48). Catheters were considered as functional on day 1 when blood could be aspirated during the daily flushing. Catheters were considered functional on day 4, if 9 or 10 blood samples of the desired 10 samples were collected, partially functional if 8 or less samples and not functional if no samples were obtained during a sampling series.

Days (d) after surgery	1 d Piglets		4 d Piglets	
	n	%	n	%
Catheters				
Functional	46	95	41	85
Partially functional	0	0	3	6
Not functional	2[*]	4	4	8

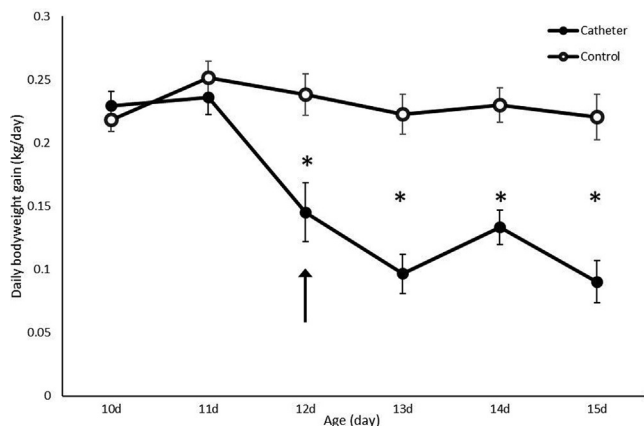[*] One animal was found dead the first day after surgery.

Fig. 2. Effect of jugular catheterisation of 12-d-old suckling piglets on BW gain (BWG). Shown is the BWG on days (d) 10 and 11 of age (before surgery), on the day of catheterisation (d 12) and the 3 days after surgery of the catheterised group (closed circles, n = 46) and the non-catheterised control group (open circles, n = 46). Values are means ± SEM. The arrow indicates the day of surgery; the asterisks indicate significant differences between groups; ($P < 0.001$, Mann-Whitney Rank Sum Test).
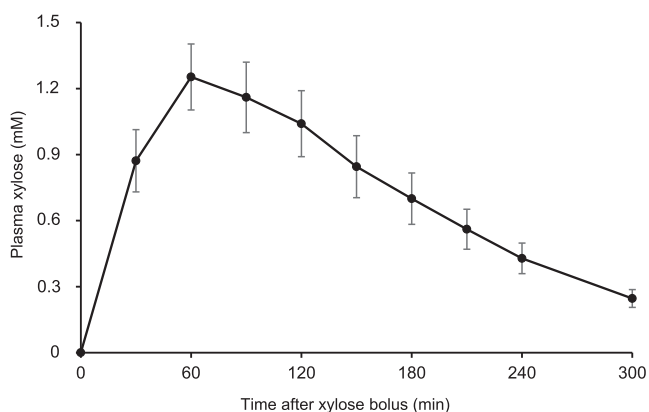

Fig. 3. Xylose concentration of blood plasma withdrawn from jugular vein catheters of suckling piglets aged 16 days with a BW of 3.85 ± 0.12 kg (n = 40). Values are means ± SEM.

Author's point of view

- The main outcomes of the study are that (1) even young piglets can be reliably catheterised with a relatively simple surgical technique while maintaining group housing with littermates and sow. (2) Anaesthesia with IsoF impairs piglet welfare less compared to a/k anaesthesia, because suckling piglets do not sleep as long and therefore, the fasting period was 5–6 h shorter, resulting in higher BWG in the first 24 h after surgery. (3) To protect the catheter in the days after surgery, double-layer bandages are recommended to avoid problems with contamination and littermate bites. (4) Antibiotic administration is not necessary. (5) We observed a surgery-related lower average BWG in catheterised piglets compared to intact control piglets, even if they appear healthy. (6) Catheterisation allows repeated collection of good quality blood samples over several days with minimal stress and pain to the piglets.
- In various study designs, frequent blood sampling is needed. For ethical and technical reasons, frequent blood collection by venipuncture is not possible because the pig needs to be caught and restrained, and blood sampling at intervals of a few min

increases the risk of vein damage, hematoma and swelling, causing stress, pain and suffering to the animal. In addition, blood collected by venipuncture is frequently haemolysed or influenced by stress hormones which could lead to unreliable metabolite concentrations (Theil et al., 2012) Therefore, permanent venous catheterisation can aid the implementation of the 3R principle.
- Our results confirm the reports of the principal usefulness of surgical central catheterisation of piglets (Gasthuys et al., 2017) but our experimental piglets were group-housed after catheterisation. We confirm that IsoF anaesthesia is much more animal-friendly (Daş et al., 2016) because the suckling piglets are active and suckling in less than one h after the surgery and the control of the depth of anaesthesia is easier with IsoF compared to an a/k anaesthesia. In addition, the higher dose required and the variability of the dose needed for young piglets are in accordance with current knowledge (Lahrmann, 2006). The double-layer, crosswise bandage turned out to be a good protection for the catheter, as it is elastic, difficult to bite through, and easy to change as observed by Furbeyre and Labussiere (2020). In a former experiment, we have tested various elastic jackets for covering the catheter and cannot recommend it because the jackets were chewed and destroyed by the littermate piglets. According to our results, adverse effects of catheter implantation on BWG of piglets should be expected. In contrast, the effects on BW were not seen by others if minimally invasive methods of catheter placement were used (Furbeyre and Labussiere, 2020).
- The outcomes of the study are limited in that we only tested the patency of catheters until the 4th day after surgery, as this was dictated by the study design of the underlying nutritional study. Because no autopsy was performed, we can only speculate as to the reasons for the <5% catheter failure. The causes for loss of functionality can lie in a displacement of the catheter tip to the vessel wall or into smaller vessel parts, but accumulation of blood clots on the catheter tip can also result in valve-like or total catheter obstruction (Furbeyre and Labussiere, 2020).
- During surgery, the following pitfalls were experienced: placing the skin incision in the correct location and quickly locating the vein, creating an opening and inserting the catheter in the vein (especially in very small piglets) without destroying it and placing catheter ligatures that are tight enough but do not choke the catheter. However, these pitfalls can be mastered by a skilled surgeon and repeated functionality checks during surgery. Sometimes, the catheter gets stuck in the vein after a few centimetres of insertion. This can be released by gently pulling back and rotating. In addition, care should be taken that the tip of the catheter is deeply inserted, at the start of the right atrium. Otherwise, a vein collapse could occur when blood is drawn. For this reason, the olecranon proved a suitable reference point for the right length of the catheter.
- Our results and observations benefit the planning and implementation of experiments in suckling piglets where blood samples are to be taken frequently. By accurately accounting for potential losses and the planning the number of required reserve animals, the number of experimental animals can be better determined and the effects of catheterisation can be considered in the experimental design. A further advantage of our surgical procedure is the avoidance of antibiotics when the investigation of the intestinal microbiome is considered. In addition, the experimental conditions can be refined by optimising anaesthesia and surgical techniques. In this way, the experimental designer can address the implementation of the 3Rs principle. Finally, our explanations can directly help those surgeons who want to learn and implement the surgical technique in practice.

- The data sets provided can be reused as comparative data in the evaluation and further development of different catheterisation techniques in pigs. In addition, they can be used to assess the results of other studies that have worked with catheterised pigs and want to assess the effect of catheterisation.

Conclusion

In conclusion, this study shows that permanent catheterisation of the jugular vein can be successfully used in young suckling piglets to obtain high-quality blood samples frequently and without stress, while maintaining group housing with littermates and sow. The presented technique can be implemented easily and practically with few technical resources and probable pitfalls should be possible to be mastered by a skilled and trained surgeon. The use of Isoflurane as anaesthetic can refine animal welfare and under good hygienic conditions, antibiotics can be avoided while sufficient pain prophylaxis and close monitoring of health are recommended. However, further growth development of the catheterised animals is to be expected and 4–8% of inserted catheters can lose their functionality a few days after the implementation. This must be taken into account when planning and interpreting experiments.

Ethics approval

The procedures were performed in accordance with the German Animal Protection Law and approved by the relevant authorities (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei, Mecklenburg-Vorpommern, Germany; permission no. 7221.3-1-078/20; 7221.3-1-022/21).

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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Author contributions

CCM conceived the study and acquired funding. AV and DDL performed the surgery and DDL collected blood. DDL wrote the first draft of the manuscript, which was edited and revised by CCM and AV. The final manuscript was read and agreed by all authors and AV had the final responsibility for the manuscript.

Declaration of interest

None.

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