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NON-TECHNICAL SUMMARY

Maternal conditioning effect: Transfer of volatile from different sow gestation and lactation diets to amniotic fluid, milk, faeces and carpal glands

Project duration

2 years 0 months

Project purpose

- (a) Basic research

Key words

Maternal diet, Vertical transfer, Volatiles, Feed intake, Feeding behaviour

Animal types	Life stages
Pigs	adult, pregnant, neonate, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this research is to determine whether volatiles from different maternal feed ingredients have different transfer efficiencies into maternal fluids and subsequent effect on feeding behaviour of piglets.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

When piglets are weaned from their sow, they can often have a prolonged period of time where they are reluctant to consume food as it is unfamiliar to them, which is detrimental to piglet health. Poor feed intake immediately after weaning results in gut morphology alterations, inflammation, reduced nutrient absorption and digestion resulting in an increase in pathogenic bacteria (Moeser et al., 2017). Therefore a key aim in pig production is to maximise feed intake immediately after weaning. By boosting the levels of key aromatic compounds in maternal feed using supplemental volatiles, it is hypothesised that there will be an increased transfer into maternal amniotic fluid and milk, creating feed preferences in piglets that can overcome the reluctance to eat after weaning.

What outputs do you think you will see at the end of this project?

The results of this research could be beneficial in determining whether certain feed ingredients increase volatile transfer to maternal fluids and improve piglet feeding behaviours after weaning. The weaning process can be stressful for pigs and result in prolonged periods of time where they do not eat, this can lead to inflammation in the gut, which can have detrimental effects on the pigs health and weight. By reducing time to first feed and stress associated with unfamiliar feed formats, the health and welfare of pigs could be improved. If these improvements are observed, modification of pig diets to include higher levels of the specific volatiles identified could reduce neophobia in newly weaned piglets and positively alter their feeding behaviour. By understanding this transfer, it could lead to modifications of the maternal diet that would benefit the pig industry worldwide.

Who or what will benefit from these outputs, and how?

The benefit of the outputs will not be realised until all laboratory analyses are conducted after the trial has been complete. Once outputs have been assessed, data and information will be published. Results obtained from this trial will be beneficial for future researchers to understand which feed ingredients have a higher transfer into maternal fluids and whether different maternal fluids (e.g. amniotic fluid or milk) have different transfer efficiencies. By reducing neophobia in piglets and

increasing their feed intake after weaning, it is expected that the health of the piglets will improve compared to piglets who take several hours to eat after weaning. Overall, in the long-term these results could benefit the pig industry worldwide as reduced feed intake after weaning and the subsequent effects on piglet health can be an area of significant economic loss.

How will you look to maximise the outputs of this work?

The results obtained from this study will be published regardless of whether they are the expected outcome. This work is in collaboration with researchers from Australia and therefore outputs will be shared across the pig industry worldwide.

Species and numbers of animals expected to be used

- Pigs: 188

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The species chosen allows for research to be applicable to commercially farmed pigs. The transfer of volatiles specifically happens between sows and their piglets and therefore it is essential to use this stage of pig production for the research.

Typically, what will be done to an animal used in your project?

Sows: 60 sows will be moved into commercial, indoor freedom farrowing crates 5-7 days prior to the expected farrowing date (as part of standard farm practice) and will be fed one of four experimental dies (L1: Low sensory diet; L1+: L1 with volatile booster; L2: high sensory diet; L2+: L2 with volatile booster). Upon entry to the farrowing house, all 60 sows will have blood samples taken from their mammary vein, a swab taken from their carpal gland, a urine sample and a faecal sample will be obtained by inserting two fingers into the rectum to stimulate defecation.

Sows will be induced to increase their likelihood of farrowing during working hours for subsequent sample collection. At farrowing, a colostrum sample will be collected from the first functional anterior teat of 32 sows in total (8 per treatment), between the birth of the 5th and 8th piglet. At day 5 and 12 after birth, from the sows that had samples successfully collected at farrowing, a blood sample will be taken from their mammary vein, a swab taken from their carpal gland, a urine sample and a faecal sample will be obtained by inserting two fingers into the rectum to stimulate defecation. A milk sample will also be collected by separating piglets for one hour prior to the morning feed (sows will still be able to see and hear piglets), the reintroduction of piglets is hoped to stimulate the mammary gland to enable a milk sample to be collected.

Piglets: At farrowing, amniotic fluid will be collected from 32 litters in total, using a collecting tray. From these 32 litters, the umbilical cord from 3 piglets per litter (96 in total) will have a hemostatic clip placed on the loose end and two further clips placed 2-5 cm from the proximal end, the cord will be cut between these clips and the cord attached to the pig will be disinfected. Blood will be collected from the umbilical vein/artery using a needle. From the same piglets, a faecal swab will be taken by inserting a sterile cotton swab into the rectum and rotating, this will be considered the meconium sample.

From the same 3 piglets that were sampled at farrowing, a faecal swab will be collected using a sterile cotton swab inserted into the rectum at 2, 12 and 21 days after birth.

Post-weaning: Approximately 500 pigs from the original 60 sows will be used for the 14 day production trial. During the 14 day trial, the 96 piglets sampled during the pre-weaning stage will be balanced across treatment pens after weaning. The same pigs, with an additional 32 pigs, equalling 128 pigs in total (2 pigs per pen from 64 pens in total) will have a rectal swab taken, a blood sample collected from the jugular vein and a saliva sample collected by exposing pigs to a sponge for them to chew on, this will be exposed to the pigs prior to sample collection to prevent the novel aspect influencing data obtained. Then 10 days after weaning, a saliva sample will be collected again using a sponge. Finally, at day 14 after weaning, rectal swabs will be taken. At day 14, 6 pigs per treatment (24 in total) will be selected for euthanasia under Schedule 1 of the Animals (Scientific Procedures) Act 1986 and dissection of tissue samples. For the remaining pigs on trial (296), they will be inspected and returned to the commercial herd, this will be the end of the trial.

What are the expected impacts and/or adverse effects for the animals during your project?

Blood sampling: Possible adverse effects include short-term discomfort whilst being restrained (if applicable) as well as at the injection site during and immediately after collection of blood. However, fully trained technicians will complete the sampling and therefore will ensure the animals comfort and minimise stress. Samples will be collected using aseptic techniques. Prior to blood sampling from the sows, an anaesthetic topic cream will be applied to the injection site to minimise discomfort and pain to the sow. All pigs will be monitored to ensure infection of the injection site does not occur, by carrying out daily health checks.

Rectal swabs (sow and piglets): Possible adverse effects include short term discomfort whilst being restrained as well as during the insertion of two fingers (sows) or a sterile cotton swab (piglets/post-wean pigs). However, this reduces the need for a technician to be present in the pen for long periods of time, which can cause stress to the pigs within the pen. Swabs will also be taken by a fully trained technician to minimise stress and discomfort.

Umbilical cord: Possible adverse effects include short term discomfort as the piglet is held while the umbilical cord is clipped and removed. There is the potential for infection to occur at the site of removal, however this will be disinfected upon removal to minimise risk. Blood will only be collected from the umbilical cord once it has been removed. This will be conducted by fully trained technicians and all piglets will be monitored daily to ensure infection of the removal site on the piglet does not occur.

Milk collection: There may be mild levels of stress for sows and their piglets during the separation period prior to milk collection. Piglets will be provided sufficient space and stay within the farrowing pen to ensure the sow can still see and hear her piglets. Sows and her piglets will be closely monitored throughout the separation period to ensure sows do not become higher than mild level of distress, at which point the separation would cease.

Saliva samples- potential stress due to a novel item in the pen, although this will be gradually introduced to reduce that impact for sample collection. Pigs will be able to freely chew on the sponge.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild severity for all sows (60) and piglets/pigs (128) within the trial.

What will happen to animals at the end of this project?

- Kept alive
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The program of work aims to identify the transfer efficiencies of volatiles from sows to piglets and therefore it is not feasible to conduct this form of work in any other animal. Furthermore, given the experimental diets could be beneficial to commercial farming practices worldwide, it is essential to determine their effects on commercially bred and housed pigs.

Which non-animal alternatives did you consider for use in this project?

None

Why were they not suitable?

Given the potential benefit of the sow diets to commercially housed piglets, they are the only appropriate species option and will allow results to be applicable to the industry.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

A sample size has been determined using information gathered from previous published research and similar studies conducted by the researchers involved.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The number of animals used is the least amount of animals per treatment, as determined by a power calculation, while also allowing for some animals to be removed from the trial if required, without compromising the statistical validity of results. This experiment aims to detect a significant difference ($P<0.05$) between volatile profile in the amniotic fluid, milk, colostrum, blood, carpal fluid and faeces from sows receiving diets with different volatile profiles. Based on previous research as presented by Val-Laillet et al., 2018 and the use of an online power-sample-size calculator (https://www.gigacalculator.com/calculators/power-sample-sizecalculator.php), with a standardised difference of 0.85, a significance of 0.05 (95% confidence) and 80% power, the minimum sample size required is 8 sows per treatment to find statistically relevant results. Sampling 60 sows at the start of the trial will ensure we can sample from and follow eight sows per treatment (32 in total across both production batches) throughout the farrowing period.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Given the potential difficulty to obtain amniotic and colostrum samples from sows at farrowing, to ensure that litters that prove successful in collection of these samples, all sows will be sampled from at the first time point (entry into the farrowing house). This means that there will not be misalignment in full sample sets over-time. After collection of samples at farrowing, those that were a success, will be the sows/piglets that are sampled from repeatedly, to reduce the total number of animals sampled and to enable accurate result to be determined over time. This includes into the post-weaning stage.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

To determine different transfer efficiencies of volatiles from sow feed to sows and their piglets, analysis (such as gas chromatography and mass spectrometry) of samples from a multitude of fluids will need to be carried out. This will also enable identification of varying transfer efficiencies through different maternal fluids. Volatiles will be measured in sow blood, carpal gland fluid, amniotic fluid, umbilical cord blood, colostrum/milk, urine and faeces. Upon entry to the farrowing house, sow blood, carpal gland fluid, urine and faecal samples will be collected for analysis to determine volatile levels. Sow faeces will also be used to asses faecal microbiome and compare to that of her piglets. Prior to blood sampling from the sows, an anaesthetic topic cream will be applied to the injection site to minimise discomfort and pain to the sow. Amniotic fluid will be collected from 32 litters using a collection tray. Piglet umbilical blood and cord will be collected by placing a hemostatic clip on the loose end of the umbilical cord and two further clips at the proximal end, where the cord will be cut between. The remaining cord attached to the piglet will be disinfected and from the cut cord, blood will be extracted using a needle. This will prevent extraction while still attached to the piglet. From the same piglets, a sterile cotton swab will be inserted into the rectum and immediately removed to collect the meconium/faecal samples required.

The same sows and piglets will be sampled from at each time point to reduce the total number of animals sampled. Sampling over time is important to determine whether volatile transfer from feed to sows and her piglets changes over time.

After weaning, the same 96 pigs, with an additional 32, to total 128 pigs (2 pigs per pen) will have a faecal swab, blood sample and saliva sample to determine volatile levels after weaning. The saliva sample will also be used to measure stress levels, to determine whether familiarity with volatiles in the feed reduces stress. Saliva samples will be taken again at day 10 to determine differences in stress response over time and at day 14, faecal swabs will be repeated to determine differences in bacterial composition over time.

Why can’t you use animals that are less sentient?

Given there are direct interactions expected between the sow and her piglets during the gestating and farrowing period, and that the expected results could be beneficial for commercially managed pigs, it would not be appropriate to use any other species than commercially bred and reared pigs.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The regulated procedures involved, will not cause pain, suffering or lasting harm more than mild severity as these procedures will be carried out by fully trained staff that possess a Home Office Personal Licence and up-to-date training. During the entire research trial, all sows and her piglets will be health checked daily to ensure the health of the pigs are maintained. Any piglets showing signs of ill-health, as determined by trained research technicians and/or the veterinarian, will be treated with relevant medication, removed from the trial (if deemed necessary) or euthanized appropriately. A dedicated pig veterinary specialist will be available in these situations. Prior to blood sampling from the sows, an anaesthetic topic cream will be applied to the injection site to minimise discomfort and pain to the sow.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

For blood sampling of the mammary vein, the procedures documented in Scollo et al., 2019 will be adhered too: Scollo, A., Bresciani, C., Romano, G., Tagliaferri, L., Righi, F., Parmigiani, E. and Mazzoni, C., 2019. A novel blood-sampling technique in lactating sows: the mammary vein route. The Veterinary Journal, 254, p.105397. As well as the advice regarding volumes presented by Swindle MM (2010). Blood collection in swine.

For blood sampling of pigs during the post-weaning stage of production, the NC3Rs guidance on blood sampling pigs from the external jugular vein (non-surgical) will be used Available at: https://www.nc3rs.org.uk/3rs-resources/blood-sampling/blood-sampling-pig#anchor_4.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Regularly check the NC3Rs website and read the regular emails received as part of the licensee email list from NC3Rs as well as actively looking for advances in the area that could effectively advance the research.