Home Office

NON-TECHNICAL SUMMARY

Effect of wheat bran and probiotic intake on the oxidative status in the colon and blood of finisher pigs

Project duration

2 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Pigs, Wheat-bran, Probiotics, Pig health, Gut health

Animal types	Life stages
Pigs	adult

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 understand the benefits of feeding wheat-bran alongside a probiotic on markers of health such as oxidative stress and inflammation within the gastrointestinal tract.

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?

Wheat-bran contains Ferulic acid that has previously been shown to have beneficial effects on pig and human gastrointestinal tract health. However, its bio-accessibility within cereals is limited, reducing the capacity for beneficial effects. By combining wheat-bran with a probiotic, such as Pediococcus acidilactici or Lactobacillus plantarum, it is hypothesised that ferulic acid could become more accessible to the pig. Such probiotics result in the degradation of cell wall polysaccharides which may release bound ferulic acid, and therefore promote benefits such as reduced oxidative stress and inflammation.

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 the inclusion of a probiotic alongside wheat-bran could increase the bioavailability of ferulic acid and promote gastrointestinal tract health in finisher pigs. If significant improvements are observed, modification of pig diets to include wheat bran and a probiotic could improve colonic health through promoting the growth of probiotic bacteria strains within the colon, increasing short chain-fatty acid concentration, reducing the number of pathogenic E.coli species present as well as reducing inflammation through an increased release of ferulic acid. Furthermore, the results could lead to further work to increase the use of wheat-bran instead of wheat in pig diets as means to also reduce feed costs. By gaining a better understanding of the availability of ferulic acid with a probiotic, this could support collaboration with human nutrition and health researchers to improve human gastrointestinal tract health, given the similarity between the pig and human gut. This could be beneficial for understanding how to reduce human diseases associated with oxidative stress, such as diabetes, cancer and cardiovascular disease.

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 in the area to show whether

beneficial effects are observed. This could lead to subsequent performance trials to determine its validity for use in pig feed, which could lead to reduced feed costs for farmers. In terms of human application, this work could provide human food and health researchers with an avenue for further investigation of wheat bran and probiotic consumption for potential application to reduce oxidative stress related diseases specifically in the colon.

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 will be in collaboration with animal and food scientists and therefore outputs will be shared across the pig industry as well as across researchers in animal and human health and nutrition.

Species and numbers of animals expected to be used

- Pigs: 36 pigs

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 results to be applicable to commercially farmed pigs alongside humans. Finisher pigs have been selected because they have a more stable gut microbiome and are less immunologically challenged compared to younger pigs, such as those at weaning. Determining the effect of these diets in a stable gut microbiome can enable more accurate results from the gastrointestinal tract microbiome and can increase consistency of the results compared to the use of younger pigs, whose gut microbiome can be highly varied.

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

Thirty-six pigs will be selected to go onto the trial at approximately 16 weeks old, they will be fed one of three diets (Control diet; control diet + 20% wheat bran; control diet + 20% wheat bran + probiotic) until approximately 22 weeks of age. During this time frame, all pigs will have blood samples taken on day 0 (16 weeks old) as well as days 14, 24, 34 and 42 of the trial. Ten pigs per treatment will be randomly selected to be humanely euthanized under Schedule 1 of the Animals (Scientific Procedures) Act 1986, the remaining six pigs will return to the commercial herd for commercial slaughter. Ten pigs per treatment will allow suitable power for significant differences to be observed with regards to gastrointestinal tract analysis.

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 as well as at the injection site during and immediately after collection of blood. However, home office trained technicians will complete the sampling and therefore will ensure the animals comfort and minimise stress. Samples will be collected using aseptic techniques. All pigs will be monitored to ensure infection of the injection site does not occur, by carrying out daily health checks.

Euthanasia: At week 22 ten pigs per treatment will be randomly selected to be humanely euthanized, the remaining six pigs will return to the commercial herd for commercial slaughter. Animals will be killed humanely and appropriately based on methods described in the Animals (Scientific Procedures) Act 1986, Schedule 1. Based on this a captive bolt will be used, followed by exsanguination to confirm death and prevent prolonged pain and/or distress.

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 pigs (36) within the trial.

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

- Killed
- Kept alive

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 whether the use of a probiotic in conjunction with a diet containing 20% wheat-bran enables more ferulic acid to be available within the gastrointestinal tract of pigs and humans. It is not feasible to conduct this form of work in humans given the requirement for collection of colonic samples at the end of the trial to determine effects on the microbiome and therefore pigs are the most appropriate animal to use. Furthermore, given the benefit of these experimental diets could be applicable to commercial farming, it is essential to determine their effects on commercial housed and reared pigs.

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

In vitro work was considered to complete this work.

Why were they not suitable?

As there can be a dynamic interaction between the host, and their gastrointestinal tract microbiome, in particular the colon in the case of ferulic acid, it is not feasible to replicate this work in vitro.

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 from similar studies that have found differences in markers of inflammation and oxidative stress in response to probiotics in pig diets (Yin et al., 2004; Molist et al., 2010).

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

A power analysis has been conducted in R (v. 4.1.3) to determine the sample size, using a One Way Anova. This calculation included an effect size of 0.6, a significance level of 0.05 and an 80% probability of results not being due to change. This resulted in a sample size of 9.9906. To allow for production losses, 12 pigs per treatment will be used to ensure significant differences could still be obtained if pigs are removed from the trial or cannot be sampled.

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

The same animals will be used for sampling at each time point to reduce the total number of animals sampled and to enable accurate results to be determined over time. Ten pigs per treatment will then be humanely euthanized at the end of the trial to collect further samples for identification of microbial differences within the GIT, minimising the likelihood of further investigation.

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.

The trial will run under commercial pig farming conditions. To measure oxidative stress and inflammatory markers over time in pigs, blood samples are the most appropriate method to use. The use of pigs allow potential beneficial effects to be applicable for commercial pigs as well as humans. Blood sampling will be conducted by fully trained and licenced personnel and therefore pain and suffering will be minimised.

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

Given there can be interactions between the gastrointestinal tract microbiota and the host, and that the products used in this research could be beneficial for commercially raised pigs, it would not be appropriate to use any other species than commercially 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. If blood cannot be taken after 2 attempts the animal will be returned to the pen without a sample collection. During the entire research trial, all pigs will be health checked daily to ensure the health of the pigs is maintained. Any pigs showing signs of ill-health, as determined by trained research technicians and/or the veterinarian, will be treated with relevant medication or euthanized appropriately. A dedicated pig veterinary specialist will be available in these situations.

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

The NC3Rs guidance on blood sampling pigs from the external jugular vein (non-surgical). 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 look for advances in the area that could effectively advance the research.