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Scotland's Rural College

AW0521 - Determining potential impacts of Precision Breeding on Animal Welfare FINAL REPORT

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Print publication: 18/11/2023

Document Version
Publisher's PDF, also known as Version of record

Link to publication

Citation for pulished version (APA):
Haskell, MJ., Li, B., Prentice, PMP., & Dwyer, CM. (2023). *AW0521 - Determining potential impacts of Precision Breeding on Animal Welfare FINAL REPORT*. DEFRA.
https://sciencesearch.defra.gov.uk/ProjectDetails?ProjectId=21137

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Evidence Project Final Report

Department for Environment Food & Rural Affairs

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1. Defra Project code: AW0521
2. Project title: **Determining potential impacts of Precision Breeding on Animal Welfare**
3. Contractor organisation(s): SRUC
4. Total Defra project costs (agreed fixed price): £ 49,885
5. Project: start date 24/10/2022
   end date 01/05/2023

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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

1. Introduction of the Genetic Technology (Precision Breeding) Act in 2023 paved the way for the use of precision breeding technologies (e.g., genome editing) in livestock in England. However, while recognising that there may be major benefits inferred by increased disease resistance and other traits, concern has been raised about the possible wider effects of the use of the technology on animal welfare. This project aimed to **understand the current situation with respect to level of use** and development of precision-bred animals and to **consider what welfare indicators should be used to assess welfare** in general, and for specific types of edits.
2. **A mapping and scoping phase indicated that few companies have initiated data collection** or development of precision-bred animals that might be present in/be imported into England. The **pig and fish sectors were the only sectors to indicate that development has commenced**. Mapping suggested that the first animals are bred in research/university environments where animals are kept under the auspices of ASPA. Apart from the fish and pig sectors,the chicken, cattle and sheep sectors also expressed some interest in developing precision-bred animals in the near future (I.e.., in the next 5 years). For the equine sector, only a few stakeholders expressed some interest in using precison-breeding technologies to improve specific traits such as disease resistance or resilience to environmental stress, but there is **no intention to use PB in equine breeding practice in the immediate future.**
3. **Expert consultation and a review of the literature indicated that the Five Domains Model** was the most appropriate of current animal welfare models to use to build indicator lists. This model includes nutritional state, health, environmental responses, behavioural interactions and mental state. While most animal welfare assessment protocols assess the effects of housing and management on welfare outcomes for animals, it is **biological functioning** that is the most important aspect to assess in the precision breeding context.
4. **Welfare assessment indicator lists** were drawn up for the three main species that are in the most advanced stage of use of precision breeding. These were **pigs, poultry and salmon.** Indicator lists were constructed that drew on industry handbooks, current animal welfare assessment schemes and relevant literature. These indicator lists aimed to facilitate a **holistic assessment of overall animal welfare** to detect changes in functioning across the Five Domains. The indicator lists contain **welfare indicators that assess the animal across its**

lifetime, compared with a **control group** of the same breed and same age and sex ratio. **Three levels of assessmen**t were considered: basic, enhanced and enhanced plus. The basic level of assessment does not fully cover the five domains in all three species, so **SRUC strongly recommends that the enhanced level of assessment is adopted**.

5. In addition to the overall welfare assessment indicator lists, **three cases studies** were considered to determine how and when to add **additional welfare indicators** to these lists. The aim was to cover welfare-related traits and production-relate traits. To this end, the specific traits considered were PRRS virus, avian influenza and the hypothetical case of myostatin in fish. As animals carrying these edits are not available for inspection, a risk assessment was limited to 'consequence characterisation': ie., identifying possible consequences of gene editing on welfare. These case studies showed that a wider consideration of the edit and the pathways involved needs to be investigated. In addition to the overall holistic assessment using the Basic, Enhanced or Enhanced Plus levels, assessment using additional welfare indicators that are relevant to the specific edit may be required.
6. **Three webinars/workshops** were held to present results to stakeholders. In addition, a meeting was held with equine stakeholders and numerous **discussions were held with individual stakeholders** to gain information on aspects of precision breeding.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Exchange).

1. Objectives

The aim of the project is to understand how precision breeding might be used by breeding companies operating within England and to create some lists of welfare indicators to allow welfare benefits and harms to be assessed in key livestock species.

Objectives (as outlined in the Tender proposal):
Objective 1: Scoping phase and mapping of the steps. This Objective aims to understand the status of precision-bred animals in the UK and globally for key species of interest including pigs, chickens, fish, cattle, sheep, and horses. Mapping/scoping of the main steps involved in developing precision-bred genetic lines will be drafted for key species of interest (including pigs, chickens, fish, cattle, sheep), with more focus on pigs, chickens, and fish (specifically farmed salmon) considering the current development of precision-bred animals across breeding sectors. The map will address assessment of (i) data which are already collected (or to be collected) for developing new precision-bred genetic lines; (ii) data which are already collected from conventional breeding processes which link to animal welfare; (ii) opportunities for collecting new welfare data; (iii) participants and resources involved in the main steps of developing new precision-bred genetic lines.
Objective 2: Establishing a framework to organise welfare criteria. We will review the uses of each of the known different welfare frameworks in terms of the ease of use, applicability to the species and ability to detect possible harms and benefits of precision breeding at the developmental points identified in Objective 1, and over the lifetime of an animal produced through precision breeding. The outcome of this Objective will be an unbiased assessment of the relative utility of each framework, and a recommendation for the best approach to be used in this context.
Objective 3: Options for mandatory species-specific assessments. Using the framework identified as most appropriate under Objective 2, and the development maps designed in Objective 1, we will draw up a list of species-specific welfare assessment criteria. These will focus on the most relevant welfare outcome measures for each developmental timepoint and will use animal-based assessment measures as these are widely considered to be the most indicative of animal experience. The outcome of this objective will be three options for the assessment of the welfare of the species undergoing precision breeding.
Objective 4: Create trait-specific assessments including case studies. A set of 3 case studies will be developed focusing on current industry directions or desired directions, one for each species, which will consider possible welfare-related manipulations (such as opportunities to develop sex-specific eggs from layer lines, opportunities to minimize the presence of specific diseases) as well as production-focused manipulations (to increase growth rate, food conversion efficiency or lean tissue content).
Objective 5: Stakeholder engagement. We plan 3 workshops to be conducted at the start of the project, at the end of Objective 4 and at the end of the project.

2. Extent to which the objectives have been met

The Objectives described in the Tender document were fully addressed and completed apart from the exceptions noted below.
Objective 1 aimed to assess the resources required for each step of breeding and assessment process. It was not possible to fully assess the resources required for each step of the breeding and assessment process as these costs are not published.
Objective 3 aimed to assess the costs of implementation of the assessment. The resources, time and the skills required were detailed in the preamble for each indicator list, but exact costs will depend on the skills of the personnel present in each organisation and the equipment available to them.
The initial call document from DEFRA proposed that in **Objective 4**, a risk assessment would be carried out to assess welfare impacts of specific gene edits. We followed the EFSA Panel (AHAW) (2012) definition of risk assessment which includes (i) exposure assessment, (ii) consequence characterisation and (iii) risk characterisation. However, only the ‘consequence characterisation’ process was possible. As there has been no research on production-related traits, a hypothetical edit that would increase fillet size in fish was considered. This was a myostatin gene edit that has been used in other fish (and terrestrial) species to increase muscularity. It is possible that edits to other genes may be used. Please see Section 3 below for further explanation.

3. Details of methods, results, and discussion of results

3.1 Objective 1: Scoping phase - Mapping the steps involved in developing new precision bred genetic lines

3.1.1 Background

Precision breeding (PB) technologies, such as genome editing (GE), may have the potential to improve animal health, welfare and productivity in farm animals. As precision breeding is generally at the early stage of development for most animal breeding sectors, the Objective 1 of this project aimed to understand the status and recent developments of precision-bred animals in the UK for key species of interest including pigs, chickens, fish, cattle, sheep, and horses. Mapping/scoping of the main steps involved in developing precision-bred genetic lines took place for key species of interest (including pigs, chickens, fish, cattle, sheep), with more focus on pigs, chickens, and fish (specifically farmed salmon) considering the current development of precision-bred animals across breeding sectors.

3.1.2 Methods

3.1.2.1 Survey

Two anonymised surveys were set up and sent to relevant industry stakeholders to understand their breeding status and initial ideas on utilizing and managing precision breeding technologies (e.g., genome-editing). The first survey (Survey 1) was designed and sent to industry stakeholders in the breeding sectors for pigs, chickens, cattle, sheep, and fish, including 75 email contacts covering all major breeding companies/organisations in the UK and leading breeding industries worldwide for these animal species. These contact people were known to Defra staff from previous projects, were breeding company staff known to SRUC or were identified as relevant contacts from breeding company websites. The second survey (Survey 2) was designed for the equine sector considering the potential difference in breeding practices between the livestock sector and the equine sector (e.g., horses are partly considered as companion animals). The equine survey (i.e., Survey 2) was sent to 23 relevant UK stakeholders in the equine sector (including equine industries, welfare council and welfare organisations, equine associations/societies/foundations, Stud Book Committees worldwide, and academic researchers in related areas). The two surveys were designed as two independent questionnaires and were analyzed separately.

For Survey 1, the questionnaire was to collect information for pigs, chickens, fish, cattle, and sheep. The relevant industry stakeholders were asked to specify the animal species they work on, as the first question in the questionnaire. The rest of the questionnaire included questions on: (1) data which are already collected for precision-bred lines in the organisation/company; (2) data which will be collected in the next 5 years for precision-bred lines in the organisation/company; (3) data which are already collected from conventional breeding processes which link to animal welfare in the organisation/company; (4) data which will be collected as new welfare data in the organisation/company; (5) any other information related to developing precision-bred animals and animal welfare.

For Survey 2, the questionnaire aimed to collect information for the equine sector. The equine questionnaire was designed based on Defra's stakeholder meeting with equine stakeholders on "The potential use of precision breeding technologies in the equine sector" on April 27th, 2023. This meeting identified the fact that the equine industry was further away from using gene editing than the livestock industries. Therefore, the survey included questions on the use of precision breeding and other breeding methodology and sent to a wider range of stakeholders. The equine questionnaire included questions on: (1) traditional breeding practices or technologies currently used in the organisation/company for equines; (2) precision breeding techniques used in the organisation/company with respect to equines; (3) future plans for using precision breeding techniques with respect to equines; (4) barriers to using precision breeding techniques within the organisation or sector; (5) interest in importing precision bred equines or germinal products into England; (6) data which are already collected from conventional breeding processes which link to equine welfare; (7) data to collect as new welfare data; (5) any other information related to precision breeding technologies and welfare in the equine sector.

The questionnaire of each survey was designed by the SRUC researcher team and was approved by Defra with ethics checks before survey release. The stakeholder lists involved in the survey were suggested by both the Defra team and the SRUC team, and the stakeholder lists were approved by Defra before survey release. The surveys were officially released for responses on April 17th, 2023 for Survey 1, and on June 07th, 2023 for Survey 2. Both surveys were open for 2 weeks for responses with reminder emails sent during the 2 weeks. The JISC Online Survey was used for both surveys as a safe and well-established tool for Academic Research, Education and Public Sector organisations.

Both surveys were designed as anonymous questionnaires. Survey recipients were asked about their company/organisation's name as an optional question. The Privacy Notice was addressed and attached to each survey. In the Privacy Notice, it was stated that all survey raw data will be kept confidential and maintained by the SRUC research team of this project and Defra within the period of April 1st, 2023 – March 31st, 2024 for Survey 1, and within the period of June 1st, 2023 – May 31st, 2024 for Survey 2. All survey raw data will be deleted when the periods end to maintain the timeliness and confidentiality of these data. The general findings and statistics from the surveys will be shared with the Welsh Government and Scottish Government and may be used as part of a wider evidence base to inform future policy direction. The Northern Ireland Administration was not contacted in this project as not companies registered there were identified.

3.1.2.2 Interviews
Based on the literature review and survey responses, the industry stakeholders in the pig sector and fish sector gave feedback on their existing data collection practices for precision-bred animals. To further understand the status of the pig and fish sectors where data collection for precision-bred animals have already been initiated, interviews were set up with key industry stakeholders and academic researchers in the pig and fish sectors to further discuss current data collection and future plans for breeding precision-bred animals, techniques and resources involved, and any animal welfare data they have collected or will collect that can be used to ensure animal welfare of the precision-bred animals.

3.1.2.3 Literature Review
Literature (e.g., scientific journals, reports) were studied to understand the research status of developing precision-bred animals (e.g., genome editing for farm animals). Discussions and meetings were set up with related academic researchers in animal behavior, welfare, animal genetics including genome editing techniques applied to breeding.

3.1.2.4 Mapping/Scoping
The mapping/scoping was evidence-based, integrating stakeholders' responses from Survey 1, interviews and workshops to address (i) data which are already collected (or to be collected in the next 5 years) for developing new precision-bred genetic lines; (ii) data which are already collected from conventional breeding processes which link to animal welfare; (ii) opportunities for collecting new welfare data; (iii) participants and resources involved in the main steps of developing new precision-bred genetic lines.

The mapping process of this project aimed to provide one general map covering multiple farm animal species of interest (including pigs, chickens, fish, cattle, sheep), recognizing potential differences between sectors. For some species, the development of precision-bred animals has been initiated in the breeding sector. For other species, the breeding processes for developing precision-bred animals are not clear so far. Considering the fact that precision breeding is at the early stage of development for most animal breeding sectors, mapping/scoping developed from this project may need updating when situations change.

3.1.3 Results

3.1.3.1 Survey Response Rates

For Survey 1 (survey for pigs, chickens, fish, cattle, and sheep sectors), a total of 31 individual responses were received from 75 contacts in major breeding companies/organisations in the UK and leading breeding industries worldwide from pigs, chickens, fish, cattle, and sheep sectors. The overall response rate of Survey 1 was 41% (i.e., 31 out of 75). Specifically, the survey response rates for each species were 45%, 33%, 44%, 38%, 54% for pigs, chickens, fish, cattle, and sheep sector, respectively (**Table 1**). It should be noted that the actual response rate may be higher as a few breeding industries informed us that one representative in the sector would respond.

For Survey 2 (equine survey), a total of 8 individual responses were received out of the 23 contacts in the UK equine sector (including equine industries, welfare council and welfare organisations, equine associations/societies/ foundations, Stud Book Committees worldwide, and academic researchers in related area). The survey response rate for the equine sector was 35% (i.e., 8 out of 23). It should be noted that the actual response rate of this survey may be higher as one representative per organisation or group responded.

Table 1. The number of survey questionnaires sent, the number of responses received, and the response rate for pigs, chickens, fish, cattle, sheep sector (i.e., Survey 1), and for the equine sector (i.e., Survey 2)

Sector	No. of survey sent	No. of responses	Response rate[1]
Pig	11	5	45%
Chicken	18	6	33%
Fish	9	4	44%
Cattle	24	9	38%
Sheep	13	7	54%
Horse	23	8	35%

[1]It should be noted that the actual response rate for each species may be higher than the response rate reported in this table, as groups of breeding industries had informed us that just one representative would respond.

3.1.3.2 Data that have been collected for developing new precision-bred genetic lines

From Survey 1, among all 31 survey responses from the pig, chicken, fish, cattle, and sheep sectors, the majority of the responses (80.6%) indicated that no data have been collected from precision bred genetic lines from their sectors/companies. A total of 4 responses (12.9% of the responses) indicated that their breeding sector/company have already collected data from precision bred genetic lines (e.g., from genome-edited animals) in the past 10 years (**Figure 1**). These companies that have collected data from precision bred genetic lines are from companies based outside of the UK. These 4 responses were from the fish sector (2 responses from 2 companies/organisations) and the pig sector (2 responses from the same company/organisation). For the pig sector, the responses notified that they have collected data for the precision-bred lines on traits related to health and disease resistance, production, reproduction, feed intake, animal behavior, genetic information, and disease information. They have assessed direct and indirect responses from a genetic edit for resistance to Porcine Reproductive and Respiratory Syndrome (PRRS) through the entire life-cycle of the pig. For the fish sector, the data collected for precision-bred lines were for resistance to viral infection, production and reproduction data including sterility, sex determination, growth, and fillet yield.

Figure 1. The descriptive statistics of the stakeholders' responses to the question on if the breeding sector/company have already collected data from precision bred genetic lines (e.g., from genome-edited animals) in the past 10 years: 4 responded "Yes", 25 responded "No", 2 responded "Not sure", and none for "Prefer not to say". The proportions for each answer (Yes/No/Not sure/Prefer not to say) are in brackets.

3.1.3.3 Data to be collected for developing new precision-bred genetic lines

When it comes to the future plans (e.g., in the next five years), the majority (51.6%) answered "not sure" about collecting data from precision genetic lines in the next five years (**Figure 2**). About one third of the 31 responses (i.e., 11 responses; 35.5%) plan to collect new or more data from precision genetic lines (Figure 2). The data to be collected were related to health and disease resistance, production, reproduction, feed intake, behavior, animal welfare and ethics, genetic information, and methane production (**Figure 3**). About 12.9% of the stakeholders (i.e., 4 responses) have no plans to collect data from precision genetic lines (Figure 2). The ability to collect new data suggests that new genetic lines carrying precision-bred edits are likely to produced.

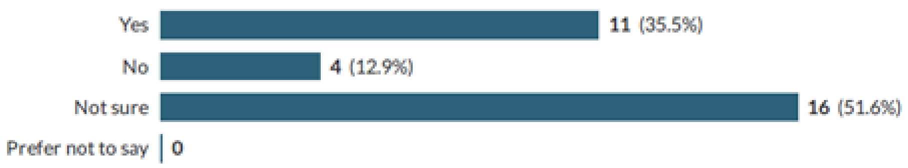

Figure 2. The descriptive statistics of the stakeholders' responses to the question on if their breeding sector/company plan to collect new or more data from precision bred genetic lines (e.g., in the next 5 years): 11 responded "Yes", 4 responded "No", 16 responded "Not sure", and none for "Prefer not to say". The proportions for each answer (Yes/No/Not sure/Prefer not to say) are in brackets.

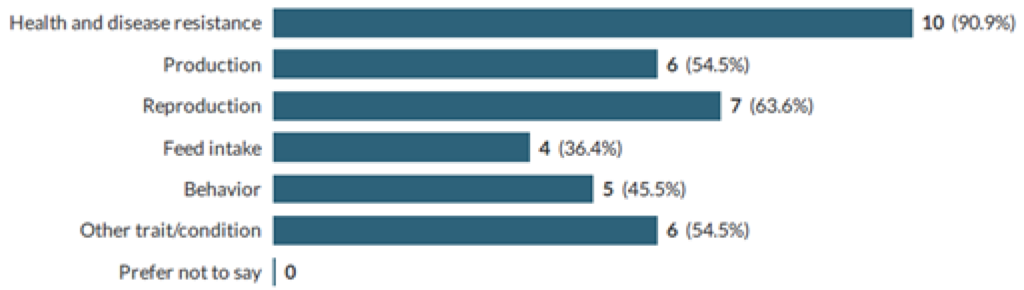

Figure 3. Description of the data that the industry stakeholders plan to collect from precision bred genetic lines in the future (e.g., in the next 5 years). In the "Other trait/condition" category, stakeholders mentioned data for: Animal welfare and ethics, genetic information, and methane production.

More specifically, different breeding sectors tend to have different plans for future data collection from precision bred genetic lines (**Table 2**).:

- In the pig sector, 3 responses from 2 companies/organisations plan to collect new or more data from precision genetic lines, mainly data related to health and disease resistance, reproduction, behavior, feed intake, animal welfare and ethics, and genetic information.
- In the chicken sector, 2 responses (from the same company/organisation) plan to collect new or more data from precision genetic lines on data related to in ovo sexing.
- In the fish sector, all 4 responses (from 4 companies/organisations) have plans to collect new or more data from precision genetic lines. The data of interest to collect from precision genetic lines are for health and disease resistance, reproduction including sterility, sex determination, feed intake, behavior, growth, fillet yield, egg, smolt, growth-out and harvest (quality type trait), general welfare condition of fish in all life stages.
- In the cattle sector, most stakeholders (i.e., 8 out of 9) answered "not sure" of future plans to collect data from precision genetic lines. One stakeholder answered "yes" and plans to collect data from precision genetic lines on health and disease resistance, production, reproduction, feed intake, behavior, and methane production.
- In the sheep sector, most stakeholders are either "not sure" of their plans or have no plans to collect data from precision genetic lines. One stakeholder has plans to collect data from precision genetic lines on health/disease resistance and production.

Table 2. The number of responses answering Yes/No/Not Sure to the question on if the breeding sector/company plan to collect new or more data from precision bred genetic lines in the future (e.g., in the next 5 years) from each sector.

Sector	Number of "Yes"	Number of "No"	Number of "Not sure"	Total
Pig	3	1	1	5
Chicken	2	1	3	6
Fish	4	0	0	4
Cattle	1	0	8	9
Sheep	1	2	4	7

3.1.3.4 Data collected from conventional breeding processes for animal welfare

Among all 31 survey responses from the pig, chicken, fish, cattle, and sheep sectors, the majority (87.1%) indicated that animal welfare-related data have already been collected from current breeding practices in their sector/company (**Figure 4; Figure 5**). The collected welfare data covers animal health, living environment, nutrition status, behavior, welfare of young animals, and other types of welfare data (Figure 5). Specifically for each sector:

- In the pig sector, welfare-related data have been collected for animal health (e.g., disease, injuries), animal living environment, animal nutrition status (e.g., water intake, feed intake), welfare for young animals, animal behavior linked to welfare (e.g., sleep, stress, social activities)
- In the chicken sector, welfare-related data have been collected for animal health (e.g., disease, injuries), animal living environment, animal nutrition status (e.g., water intake, feed intake), animal

behavior linked to welfare (e.g., sleep, stress, social activities), welfare for young animals, and in ovo sexing.

- In the fish sector, welfare-related data have been collected for animal health (e.g., operational welfare indicators – ordinal score data for different types of injuries in fish, wounds and fin damage, snout damages, disease-related stressors), animal living environment, animal nutrition status (e.g., feed consumption, loss of appetite days), animal behavior linked to welfare (e.g., sleep, stress, social activities), welfare for young animals.
- In the cattle sector, welfare-related data have been collected for animal health (e.g., lameness, mastitis, digital dermatitis, ketosis, bovine TB), animal nutrition status (e.g., water intake, feed intake), animal living environment, animal behavior linked to welfare (e.g., 3D camera based surveillance), welfare for young animals, calving traits, longevity, polled status, survival events, body condition score, body weight, fertility.
- In the sheep sector, welfare-related data have been collected for animal health (e.g., parasite resistance, Scrapie resistance), welfare for young animals, faecal egg count, lamb and ewe survival, ewe longevity, body condition score in adults, liveweight, lambing ease, lamb mortality, longevity, animal behavior linked to welfare (e.g., sleep, stress, social activities), animal response to AI and ET.

Figure 4. The descriptive statistics of the stakeholders' responses on if welfare-related data have already been collected from the current breeding practice in the sector/company: 27 responded "Yes", 2 responded "No", 2 responded "Not sure", and none for "Prefer not to say". The proportions for each answer (Yes/No/Not sure/Prefer not to say) are in brackets.

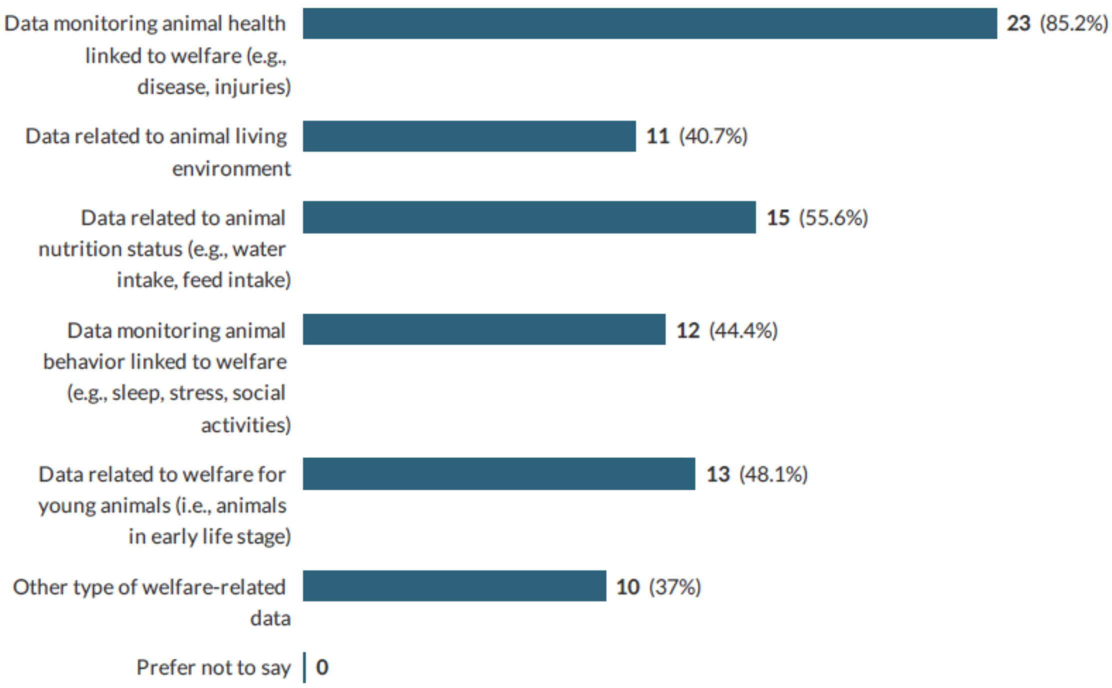

Figure 5. Description of the welfare-related data that the industry stakeholders have collected from current breeding practice. In the "Other type of welfare-related data" category, stakeholders mentioned data for calving traits (cattle), lambing ease (sheep), longevity (cattle, sheep), polled status (cattle), faecal egg count (sheep), lamb and ewe survival (sheep), body condition score and/or liveweight (multiple species), parasite resistance (sheep), animal response to AI and ET (sheep), in ovo sexing (chicken), and Code EFABAR adopted in the pig sector.

3.1.3.5 Opportunities for collecting new welfare data

The majority (64.5%) plan to collect new or more welfare data in the next 5 years (**Figure 6**). About one third (i.e., 29%) answered "not sure" about collecting new or more welfare data, and 6.5% had no plans to collect more welfare data (Figure 6). For the stakeholders who plan to collect more welfare data, 90% will collect data that monitor animal health linked to welfare (**Figure 7**), followed by data that monitor animal behavior (65%), data related to animal nutrition status (60%), data related to welfare for young animals (50%), data related to animal living environment (40%), and other type of data (15%) including image data on udder conformation and other functional conformation traits, phenotypes from videos and other techniques to achieve automated measurements that go beyond what are measured today.

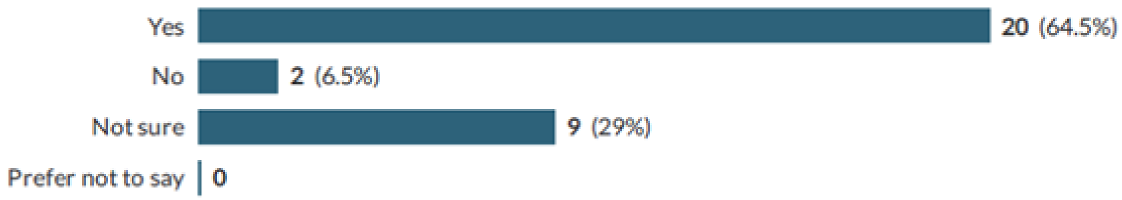

Figure 6. The descriptive statistics of the stakeholders' responses on if they plan to collect new or more welfare-related data in the next 5 years: 20 responded "Yes", 2 responded "No", 9 responded "Not sure", and none for "Prefer not to say". The proportions for each answer (Yes/No/Not sure/Prefer not to say) are in brackets.

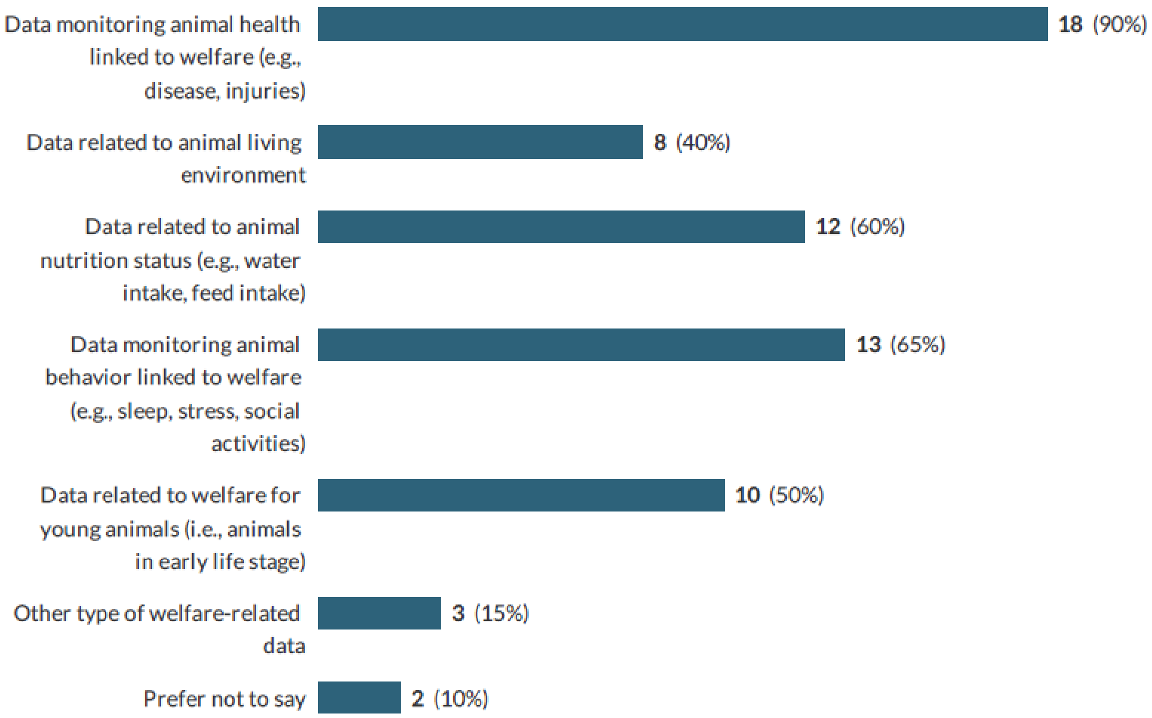

Figure 7. Description of the welfare-related data that the industry stakeholders plan to collect in the next 5 years. In the "Other type of welfare-related data" category, stakeholders mentioned image data on udder conformation and other functional conformation traits, phenotypes from videos and other techniques to achieve automated measurements that go beyond what are measured today.

3.1.3.6 Participants and resources involved in the main steps of developing new precision bred (PB) genetic lines

Based on current findings from this project, the development of new precision-bred genetic lines is mostly initiated from collaboration between research institutions and breeding industry (**Figure 8**). The research focusses on understanding the genetic basis of the targeted traits, precision breeding techniques (e.g., genome editing techniques), and correlated responses to other traits/performances. The research outcome builds the basis for setting up a breeding stock of precision-bred animals (e.g., in a nucleus herd for PB animals) in the breeding industry. After the breeding stock for PB animals is established, we recommend that health and welfare assessments are conducted for the PB animals in the breeding stock as well as the genetic information of the edited animals and their offspring. These assessments may need participants from industry stakeholders, researchers, and the welfare advisory body that will be established under the Act, under guidance from Defra. When the assessment for PB animals is completed, the ongoing maintenance of the breeding stock may need to be evaluated on a certain regular basis for the PB animals' genetic information and performances under guidance from Defra.

Please note that the current mapping/scoping developed in this project is targeted for the key species of interest (including pigs, chickens, fish, cattle, sheep), with more focus on pigs, chickens, and fish considering their status of developing precision-bred genetic lines. The mapping/scoping developed in this project is not appliable to the equine sector.

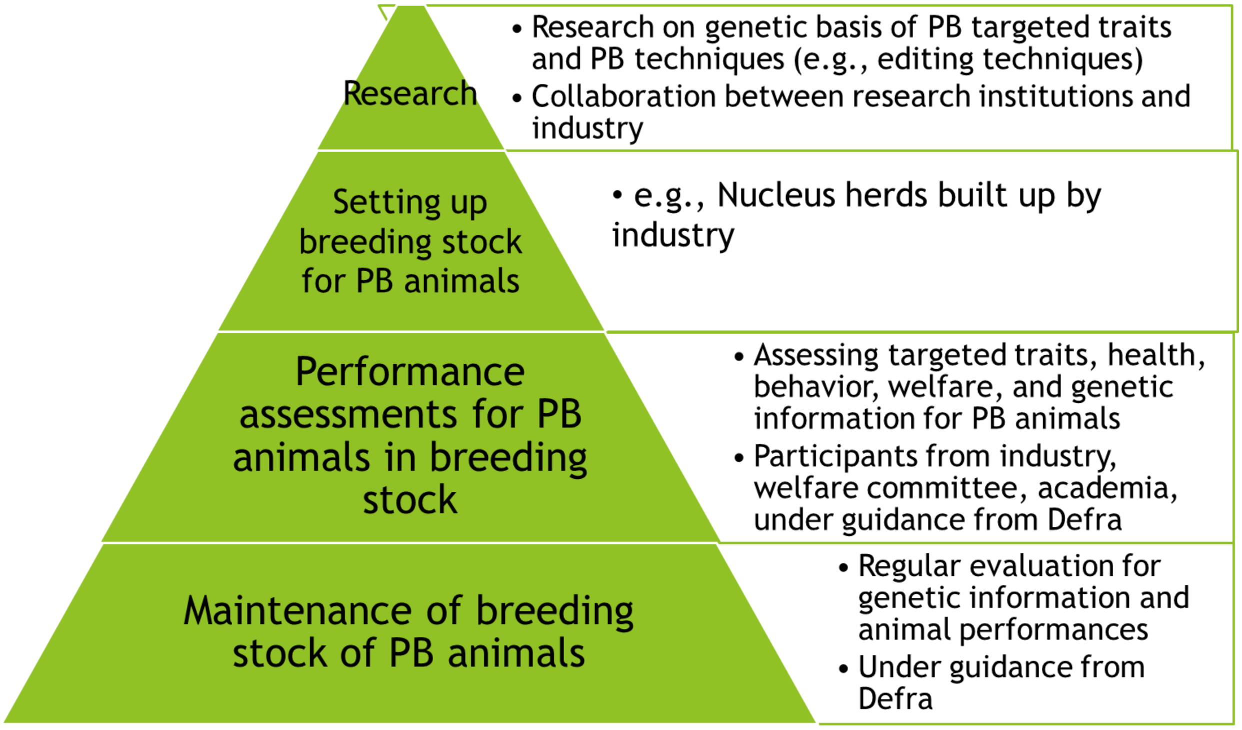

Figure 8. Participants and resources involved in the main steps of developing new precision bred (PB) genetic lines.

3.1.3.7 Survey findings from the equine sector

Based on the equine survey results, traditional breeding practices/technologies currently used in the equine sector include natural covering, artificial insemination using chilled and/or frozen semen, embryo transfer, OPU-ICSI, frozen embryo transfer. The development of precision-bred lines is not yet initiated in the breeding practice in the equine sector and there is no clear intention to use PB in the equine breeding practice in immediate future. Few stakeholders express their interest in using precision breeding techniques to improve specific disease resistance in the future, however, most stakeholders are worried that the current regulations in the UK and EU, the cost and expertise required, the mandatory use of natural cover in some cases, and the data/resource limitations will be the barriers to using precision breeding techniques within equine sector. In addition, equine stakeholders from academia and World Horse Welfare mentioned certain research development of embryos created through ICSI in private storage and a research publication on "Generation of myostatin edited horse embryos using CRISPR/Cas9 technology and somatic cell nuclear transfer" (Moro et al., 2020, https://doi.org/10.1038/s41598-020-72040-4) from academia.

About 63% of the equine stakeholders indicated that animal welfare-related data have been collected from their current breeding practices, covering data related to animal health (e.g., veterinary records, body condition and injuries), living environment, nutrition status, behavior, welfare of young animals, and other welfare-related data including linear scores capturing limbs, development, joints, foot balance and care, dental, weight, horse welfare and care concerns, conformation as pertaining to a horse's ability to live a fulfilled and pain free life, and welfare related to the herd of retired and rescued equines. In the future (i.e., in the next 5 years), 62.5% of the responded stakeholders (5 out of 8) plan to collect new data related to horse welfare, including data related to equine health, nutrition status, welfare of young animals, behavior, living environment, and other type of data such as use of the whip in racing, welfare status of racehorses, linear scoring, and data from Horselife longitudinal study.

3.1.4 Conclusions

The mapping/scoping phase (objective 1) of the project integrated a wide range of stakeholder engagement events to understand the status and recent development of precision-bred animals in the UK for key species of interest including pigs, chickens, fish, cattle, sheep, and horses. Precision breeding is generally at an early stage of development for most animal breeding sectors in the UK. Some data have been collected from PB genetic lines from the pig and fish sector (mainly from companies based outside UK and partly from universities), and there may be more data to be collected from PB genetic lines in the next five years (mainly in the fish, pig, chicken sectors). Mapping/scoping of the main steps involved in developing precision-bred genetic lines were drafted for key species of interest (pigs, chickens, fish, cattle, sheep), with more focus on pigs, chickens, and fish (specifically farmed salmon) considering the current development of precision-bred animals across breeding sectors. Considering the fact that precision breeding is at the early stage of development for most animal breeding sectors, mapping/scoping developed from this project may need updating when situations change.

3.2 Objective 2: Establishing a framework

3.2.1 Background

The purpose of this part of the project was to provide a suitable framework on which the work on specific aspects of the Welfare Declaration (as outlined in Objectives 3 and 4) can be built.

The oldest and best-known animal welfare framework is the Five Freedoms (FAWC 1979), which has subsequently been operationalized in the EU FP6 Welfare Quality project in 2005 (and used by EU FP7 AWIN project, 2011) into 4 Principles and 12 welfare criteria. Parallel developments of the Five Domains model (Mellor & Reid, 1994) have also considered methods for assessing animal welfare and, through various iterations (most recently Mellor et al., 2020), has been expanded to include positive as well as negative welfare traits, and the interactions that animals will have with their keepers and other animals. This approach has been gaining wide acceptance and has been used to assess the welfare of, for example, laboratory animals, equids, wildlife species considered as pests and zoo animals as well as farmed species. An important component of these assessments is a focus on measurements made at the animal level (Animal Based Measures, ABMs) as a better method to evaluate welfare rather than assessment of provision of resources. To a lesser extent the Animal Welfare Assessment Grid (Wolfensohn et al., 2015) has also been used, mainly with animals used in research and zoo species, to consider lifetime impacts on animal welfare. Other approaches, such as that advocated by Dawkins (2004), to assess whether animals are healthy and have what they need, assessments of Quality of Life (QoL) (Scott et al., 2007; Broom, 2007) and the Sharp and Saunders model (2011) which assesses the relative humaneness of different options, have also been proposed. This latter model has only been applied to pest control thus far, but does contain some relevant features, such as assessing the severity and duration of a welfare harm, that could be useful in this context. Each of these approaches may have value in determining the most appropriate method to assess welfare, and therefore they were all considered as part of the achievement of this objective.

3.2.2 Evaluation of welfare assessment frameworks

The initial phase of this work involved a workshop with the Animal Behaviour and Welfare Research Group at SRUC to discuss the most useful approach. In our initial discussions and scoping, it was decided that a framework suitable for assessment of welfare in precision-bred animals must have the following features: (i) cover the full range of domains of animal welfare; (ii) represent the current scientific thinking regarding the range of animal experience (i.e., reflect current thought in sentience and positive and negative welfare); (iii) contain indicators that assess the animals function and emotional experience, rather than the effect of the management or environment on the animal and (iv) allow indices to be scored on individual animals.

It was concluded that no single framework would be entirely suitable for use as they have all been designed to assess how well the animal was coping within the environment in which it was managed, rather than an evaluation of the capacity of the animal to have good welfare, which is required for the welfare declaration. However, some aspects or frameworks for welfare were preferred over others, and an evaluation of the merits and issues with each framework is given in Table 1 below.

Table 1. *Overview of the main benefits and issues with the selected Welfare Assessment frameworks*.

Framework	***Positive benefits***	***Issues***

Five Freedoms	Useful checklist of different aspects of welfare that should be considered in an evaluation; some of these are framed in terms of the animal's feelings/emotions	Focus on negative aspects of welfare predominantly
Welfare Quality approach	List of measurable welfare indicators, many of which have been validated; some indicators do attempt to assess positive welfare	Purpose is to evaluate welfare of farmed animals in their environments; thus many indicators are at the group level and consider environmental features although mostly focused on animal-based indicators
Five Domains	Considered the most progressive and advanced conceptualisation of animal welfare, including both positive and negative welfare states and focuses on animal welfare as primarily being the mental state of the animal	May require modification to assess the functional abilities of the animal; derived on the basis of evaluating the environment or other impacts on the animal rather than the functionality of the animal
Animal Welfare Assessment Grid	Considers the impacts of management or experimental procedures on the individual animal and takes a 'whole life' perspective	Not all indicators are validated; also includes impacts of external factors predominantly on animal responses. Most indicators are assessed through expert/owner opinion
Quality of Life assessments	Focus on the individual animal; does contain elements of functionality	Tends to be related to end of life care; mostly focused on negative aspects of welfare
Sharp & Saunders model	Allows comparison between different levels or approaches for management; related to Five Domains model (in how it has been used); considers severity and duration of welfare harms	Focus on negative aspects of welfare but could possibly be modified to include positive

Overall, the *Five Domains model* was preferred because of its focus on animal feelings and capacity to consider both positive and negative aspects of welfare. It will, however, require some modification to be fit for purpose. Mostly this involves removing any indicators that are resource-based or relate to environmental issues and replacing/re-evaluating these in terms of the animal's capacities. In this regard aspects of *QoL assessment* can be useful, as are laboratory animal assessments for genetically modified rodents (e.g. Wells et al., 2006). This paper reports on the recommendations of a Working Group to review the potential welfare issues for genetically altered (GA) mice (acknowledging that many changes may have no adverse effects) and offers some recommendations for timing and types of functional assessments that may be adapted to livestock scenarios. They do not, however, consider any opportunity for positive welfare to be elicited by the modifications (largely as, for mice models, these modifications are almost exclusively for human benefit and biomedical reasons). Some possible indicators that are suggested for these assessments include tests of functional and ability to perform key behavioural activities (such as 'integrating-to-the-nest' in mice; Rock et al., 2014), which could inform possible livestock welfare assessments.

The work in laboratory mice suggests several key time points when health and welfare of GA animals could be assessed. These include the neonatal period, weaning, puberty and adulthood. These may be useful time periods to consider for mammalian livestock (as informed by Objective 1 mapping activities) but will need to be modified for poultry (e.g. hatch, early development, puberty, adult) and salmon (e.g, hatch (alevin), fry, parr, smolt, adult.

3.2.3 Conclusions

Therefore, some of the main conclusions of this discussion are:

1. The Five Domains model of welfare is the best framework to use in this work.
2. Some indicators will need to be reconsidered or formulated as functional rather than environmentally influenced ABMs.
3. The timing of assessments should follow major changes in an animal's stage of life/life history, as welfare impacts may be manifested when there are changes in behaviour or physiology due to development or life history events (e.g, parturition). Mapping from Objective 1 will also inform the timing of assessments.

4. The duration of welfare impacts (positive and negative should be assessed by repeated testing and severity should be assessed by using scoring systems that cover the full range of possibilities.

3.3 Objective 3: Options for mandatory species-specific assessment

3.3.1 Aims

The aim of this objective was to create lists of welfare indicators for the three livestock species that the mapping exercise had identified as being the most likely to be using, or intending to use, precision breeding technologies. These species were: pigs, poultry (laying hens and broiler chickens) and farmed salmon.

The welfare indicator lists are attached as Appendices I, II, and III.

3.3.2 Assessments of animal biological function

There are many welfare assessment protocols that contain lists of welfare indicators. Well-known protocols for livestock such as Welfare Quality®, AWIN and AssureWel have been widely used in commercial practice. These protocols are designed to determine whether the management, physical and social environment of the animals is adequately providing for a good standard of welfare. Additionally, the breeding companies often include assessments of welfare in their breeding programmes. However, the approach required to assess the effects of precision breeding on welfare is different. In this instance, the welfare concerns are principally around the precision-bred animal's biological functioning. In this case we define 'biological functioning' as meaning the capacity of the animal to achieve normal physiological and behavioural functioning and species-appropriate cognitive and emotional processing. Therefore, essentially the aim of the indicator list and the protocol that will be formed from it is to ask 'Is the animal healthy? Is it growing normally? Is it showing normal behaviour?'. In this case, the growth, behaviour and healthy of the parent stock or a non-precision bred population should be used as a benchmark to define the normal range for that particular breed or strain of animal. A function-based approach requires that health, growth and behavioural assessments are central to the approach and that animal-based indicators are primarily used. Farm records (a management-based indicator) are used to gather information across time for traits such as disease occurrence.

3.3.3 Methods used to create indicator lists

The list of indicators allows assessment of whether the welfare of animals produced from precision-breeding methodology has been positively or negatively affected by the presence of the edited gene that they carry. The work is based on the scientific evidence on how best to assess animal welfare. The lists are based around the Five Domains Model (Mellor et al., 2020) as outlined in Objective 2 as it covers a wide range of animal functioning, including mental state, and is the framework currently favoured by animal welfare scientists. The Five Domains model is increasingly being used as the basis for welfare assessment protocols across a number of species (e.g. Beef Cattle: Meat and Livestock Australia (MLA, 2021); Redwings Horse Sanctuary (www.redwings.co.uk)).

To create the lists, workshops with welfare experts from each species were held. The experts were presented with the project aims and asked about their knowledge of welfare indicators used in the commercial sector, in welfare assessment protocols and welfare assessment methods used by animal welfare scientists in experimental contexts.

A literature review was done, assessing indicators across a range of sources. Wherever possible, the indicators were taken from sources detailing current industry practice in assessing health and welfare (e.g., industry management handbooks, AHDB) or current welfare assessment protocols (e.g., AssureWel, Welfare Quality®). The review of the literature also yielded welfare indicators that have been used in experimental settings within animal welfare science. It was important proviso in the selection of the indicators, that they had been validated against other welfare indicators (i.e., had construct or face validity), and had been used in on-farm settings (ie., had not solely been used in experimental settings). This limited the extent to which all aspects of the different 'sub-categories' of welfare detailed in the Five Domains model could be assessed.

3.3.4 Welfare assessment levels

A key objective was to create indicator lists at different levels. Thus, three levels were created. The welfare indicators were divided into 'basic', 'enhanced' and 'enhanced plus'. The breeding industry manuals and basic health checks and routine monitoring checks by farm staff or veterinarinans form the basis of the 'basic' level of welfare assessment.

However, the basic level indicators do not cover all of the Five Domains and thus domains of animal welfare considered as important by the scientific community are not assessed. Specifically, two out of the five domains ('behaviour' and 'mental state') are not typically assessed if the basic level of assessment is used.

The 'enhanced' level includes indicators that assess welfare across the Five Domains. A number of behavioural indicators, primarily involving behavioural observations, are included in the enhanced level.

Some of the 'enhanced plus' level are behavioural tests that give a more in-depth analysis of the animal's cognitive and emotional functioning (e.g., for poultry and pigs), and some are laboratory tests. As such, these tests typically require specialised equipment or testing expertise, which may not be currently available in the breeding companies. The 'enhanced plus' level also includes some indicators that can be used to give a full picture of animal functioning but that need further development for use in an on-farm welfare assessment setting.

Based on the current scientific understanding of animal welfare, SRUC recommends that indicators covering all five domains should form part of a holistic assessment of health and welfare in precision- bred animals to help identify positive outcomes and/or unintended consequences that may result from the precision-breeding methods. We therefore recommend that the 'enhanced' level of assessment should be mandatory.

The 'enhanced plus' level should also be considered as it allows a more comprehensive assessment of welfare. However, research in needed to develop some of the test for on-farm use, and expertise will need to be gained or outsourced by the breeding companies or welfare assessment tasks outsourced to groups with the appropriate skills and resources.

Consideration of an optional 'lifetime' assessment was also included in the project proposal. However, it was considered that assessing a precision-bred animal across its lifespan is necessary to allow a full assessment of the effects of any gene edit. For production animals, this should be to the natural slaughter age. For animals to be kept for breeding, this would be through the first parturition and the subsequent lactation for females.

3.3.5 Assessment setting and control animals

In order to assess any positive or negative effects of the gene edit on animal welfare, the welfare of the precision-bred animal must be compared to a 'baseline' or 'control' group. As there are known differences between different breeds and strains, and also differences in behaviour shown in different housing facilities, and feeding and management regimes, the best controls will be a group of animals of the genotype from which the precision-bred line was created, of the same age and sex ratio, and housed and managed in the same way (ideally by the same people in the same facility). Consideration should be given to what environment (intensive or extensive) the progeny of the animals will experience.

The exact number of animals required has not been covered in this project but should involve input from industry on likely numbers of animals produced for the first population reared on breeding company facilities (i.e., not under the auspices of ASPA (1986). See the 'Future Work' section for a discussion on this.

3.3.6 Key life-stage assessments

As the effects of the edited gene may manifest themselves at different stages of life, it is important to assess animals across the key life stages. For each species, the key life stages have been identified in the indicator lists, and are typically the key developmental stages, such as birth, nutritional independence (weaning), appropriate growth stages/commercial production stages and reproductive stages.

3.3.7 Gathering data, skills and resources

The welfare indicators show what data need to be collected. However, the method used to gather the data is not specified to allow the industry flexibility in the methods used. As discussed above, many of the basic level indicators (such as health checks and growth rates) may already be gathered as part of normal animal husbandry and health monitoring routines, and only require the data to be inputted. Behavioural time budget data (for activity, feeding, resting etc) can be extracted from video recordings or recorded through technological approaches that monitor activity and feeding (accelerometers for activity, automated feeders etc.). For example, measures of feeding behaviour may be collected by automated means, or could be provided from assessments of video recordings, or from check sheets taken from manual observations of animals.

In terms of additional staff training, the indicators that are routinely collected will clearly require no extra training or resources. For indicators that are not routinely assessed, training of staff will be required. The purchase of equipment such as video-recording equipment, or sensors may be required. Alternatively, the behavioural analysis could be outsourced to welfare specialists.

3.3.8 Conclusions

Three welfare indicator lists have been created for farmed salmon, pigs and poultry (laying hens and broiler chickens). The lists contain welfare indicators at three levels: basic, enhanced and enhanced plus. The sets of indicators span the lifetime of the animal concerned to allow welfare effects that manifest themselves at different ages to be assessed. The data may be collected manually or via the use of technology. To allow the effects of the edit to be quantified, a group of control or baseline animals must be assessed alongside the group of gene edited animals, ideally in the same facility and of the same age and sex. While breeding companies currently include a number of assessments of health and physical fitness in their breeding programmes and monitoring is carried out during routine checks, the domains of behavioural interactions and mental state are not currently covered sufficiently. SRUC recommends that the suite of indicators at the 'enhanced' level are adopted.

3.4 Objective 4: Trait-specific risk assessments including case studies

3.4.1 Introduction

The aim of this objective was to develop a set of 3 case studies focusing on current industry directions or desired directions for PB in that species, one for each species. The purpose of the case studies was to conduct an analysis of the specific possible welfare risks and opportunities from the edits, considering the pathways in which the proteins resulting from an edited gene may be involved. A further set of relevant welfare indicators, are suggested for each case study.

The EFSA (2012a,b) risk assessment framework was followed as far as possible. This definition includes (i) exposure assessment (duration), (ii) consequence characterisation (effects) and (iii) risk characterisation (probabilities/magnitude). However, only the 'consequence characterisation' process was possible. Please see Section below for further explanation.

The three case studies are attached as Appendices IV, V and VI.

3.4.2 Selection of Case Studies

Based on stakeholder engagements and discussion with industry representatives and the Defra Steering Group, three case studies were selected for this project. From our discussions it was clear that, currently, the terrestrial livestock industry were focused primarily on edits associated with disease resistance, with less focus on production traits. For pigs, the case study was selected based on the known existence of pigs that have been edited for Porcine Reproduction and Respiratory Syndrome Virus (PRRSV) resistance (Burkard et al., 2017), where several generations of gene-edited pigs have been created. For poultry, although there is no published data yet on gene-edited chickens with resistance to disease traits, we were aware from stakeholder discussions that research into edits for resistance to Avian Influenza Virus (AIV) was underway, and in vitro studies provided evidence of the likely gene target for conferring resistance to AIV (Long et al., 2019).
For salmon, unlike other species, the results of the survey (see Sections 3.1.3.2 and 3.1.3.3) and discussion with representatives of the salmon industry indicated that there was more interest in edits that may enhance productivity including edits for fillet yield and sterility (although the later edit may also have other benefits). Although no published data were yet available for salmon, increased fillet yield in other fish species (such as carp (Zhong et al., 2016), seabream (Ohama et al., 2020) and catfish (Khalil et al., 2017)) have been achieved through gene-edits of the myostatin muscle-suppressor gene (MSTN). Therefore, this gene was selected as the target gene for the case study.

3.4.3 Risk Assessments

EFSA (2012a) defines a risk assessment framework as including (i) exposure assessment (duration), (ii) consequence characterisation (effects) and (iii) risk characterisation (probabilities/magnitude). For a population of precision-bred animals all containing the same edit, the exposure will be the same, and is likely across the lifetime of the animal. Risk characterisation is difficult where there are no animals 'on the ground' or data to analyse to assess the magnitude of effects. Therefore, the risk assessment carried out in this project explored the 'consequence characterisation' or assessment of the nature of the effects on animal welfare in each scenario.

For each case study, the site of the edit was identified if known or could be inferred from the literature (CD163 in PRRSV resistant pigs, ANP32A in AIV resistant poultry). Exploration of the pathways in which these genes and proteins were involved, using databases of gene pathways (https://www.ncbi.nlm.nih.gov/gene/8125#pathways, UniProt), were conducted to identify the known possible biological processes in which the gene may be involved.
For salmon, the candidate MSTN gene is currently unidentified, as it appears that myostatin is expressed in more tissues and may have a more diverse role in fish than in mammalian species. Gene-edits in salmon have also not been conducted, to the best of our knowledge. However, searches in https://www.ensembl.org/index.html suggested that some myostatin-related target genes for Atlantic salmon were known (e.g. mstnb, gdf8, mstn, mstn-1) and were considered potential targets for future approaches to boost muscle yield in salmon. In addition, MSTN edits from other fish species were used as a basis for conducting the risk assessment for potential welfare impacts in salmon of a future similar edit.
It is acknowledged that these risk assessments consider *theoretical* rather than actual risks to welfare. Currently only one case study is based on actual production of live gene-edited animals (PRSSV), where only information on a small number of animals kept under research conditions are available, for a very limited number of traits (for example, evidence is presented that the gene-edit is not lethal and produces animals that are qualitatively normal in terms of growth and reproduction; Burkard et al., 2018).

The opportunities for good or improved welfare from the gene edits were also assessed. Where the edits lead, or are likely to lead, to improved disease resistance to common endemic threats, then evaluation of the welfare benefits that accrue from the gene-edits form a relevant part of the welfare case study. In both the disease case studies selected (PRRSV and AIV), the edits target the virus's ability to invade the host cells and replicate, thus providing disease resistance and suppressing the virus's ability to spread in the population. It is also relevant, therefore, to consider that there may be indirect benefits to non-edited animals from proximity to the gene-edited population.

The potential for increase muscle yield in fish using a myostatin gene edit should be considered with care, as there is documented evidence for immune function and developmental issues in other fish species (e.g., Zhong et al., 2016). However, increased monitoring of welfare indicators assessing growth, development and disease incidence would likely detect any issues.

3.4.4 Additional case-study specific welfare assessments

As described in Objective 3, SRUC recommends that welfare assessment of gene-edited animals covers the Five Domains of welfare as described by Mellor et al (2020), and that a broad approach to welfare assessment is adopted. This is considered advantageous also since it means a similar approach can be adopted for each new edit, without requiring a new set of metrics, focusing on bespoke welfare indicators to be collected for each welfare declaration for a new edit. Where the involvement of the gene product in other pathways is not well understood, or where the edit may potentially result in widespread impacts in many biological systems, then the wider approach suggested is also considered optimal to allow welfare impacts and benefits to be identified.

Where there are potential additional consequences of the edit, and Defra wish to be reassured that specific detrimental impacts have not been realized through the edit, then a small number of additional welfare indicators (or implementation of the indicators covered in Objective 3 at higher frequency or specific timings) have been suggested. The welfare advisory body may require for these trait-specific data to be presented in addition to the general welfare assessment data.

3.4.5 Conclusions

Three case studies, involving two disease resistant edits and one production edit, were conducted. Currently, there is limited information in the scientific literature of actual edited animals and outcomes, thus the case studies are necessarily based on potential or theoretical edits and possible risks to, or opportunities to positively influence, the welfare state of the edited animal. It is likely that these would require modification or revision in future years once more edited animals are available and further generations of animals could be assessed.

3.5 Objective 5: Stakeholder engagement

3.5.1 Introduction

The aim of this Objective was to engage widely with stakeholders to explain the approaches taken, to learn from industry and others the current state of developments, and to understand the range of opportunities and concerns that all stakeholders may have about the use of gene-edited animals in general, as well as their views on the welfare indicator lists produced.

3.5.2 Stakeholder Workshops

To achieve these engagement aims, we held 3 stakeholder workshops (workshop 2 was conducted twice with two different groups of stakeholders). Each workshop was held online, as this was considered most time efficient for all participants. Workshops 1 and 3 were held in a webinar format, where presentations were made to participants and participants had the opportunity to post questions to the project team. These were answered verbally in the meeting, via written responses in the chat box during the meeting and a written response was circulated to all participants following the webinar. Workshop 2 was more interactive and consisted of a short presentation, focused discussions in break out rooms (6 in total) and a feedback session. Throughout the project approximately 90 stakeholders have been engaged in the various activities. The specific focus and activities of each workshop were:

Workshop 1 (91 registered participants), 06/12/2022: An overview of the project was presented, focusing on Defra's requirements in tendering for the work and SRUC's planned approaches in meeting these requirements. Information on the planned survey for Objective 1 was given to prepare industry participants for future contacts. Responses to questions on approach and inputs to planned work received.

Workshop 2 (a: industry focused: 35 participants; b: NGO/other focused: 28 participants), 26/04/2023: An update on project progress was shared with participants and an introduction to the approach taken for Objective 3. Breakout groups to discuss the approach taken and the types of welfare indicators developed, using pigs as an example species. Feedback on the approach collated and used to inform further developments.

Workshop 3 (~45 registered participants), 26/07/2023: The draft welfare assessment lists of indicators were shared with potential participants approximately 2 weeks prior to the meeting, and feedback collated. This final workshop presented the work done and the final outcomes and conclusions from the study. The feedback from stakeholders on the indicator lists showed that respondents were concerned about the number of animals to be sampled and issues around accounting for natural variation between animals and how thresholds would be set for between-population effects. The number of generations to record, and how many reproductive cycles should be assessed for breeding animals were issues that were also raised. All of these issues were also identified by the project team as requiring further study (see Section 5 'Future Work' below). In addition, it was suggested that physiological indicators could also be assessed. The use of non-invasive sampling methods (such as the sampling of excreta, hair or saliva) need to be used for this purpose. This idea definitely has merit, but there are no physiological tests being used routinely used in welfare assessment protocols on farms. Methodologies could be developed for this purpose and added to the indicator lists.

3.5.3 Other engagement with stakeholders

Stakeholder workshops and the surveys of Objective 1 were supplemented with one-to-one meetings or interviews with representative groups:

1. Representatives of breeding companies: PIC (pigs), Aviagen (broiler chickens), Hendrix (laying hens), BenchMark Genetics (salmon), AquaTech (salmon), FrontFoot (general).
2. Representatives from research organisations with expertise in gene-editing (Roslin, PIC, BenchMark Genetics)
3. Representatives from animal welfare NGOs (Compassion in World Farming, RSPCA).

These meetings allowed detailed questions to be asked of industry, and focused on understanding the approaches to developing gene-edited animals in more detail, as well as an overview of the current methods for assessing welfare and practical issues of the feasibility of making additional welfare assessment measures.

Steering Group meetings

Five meetings were held with the wider steering group for the project. These allowed greater discussion of the work and outcomes with representatives of relevant Government departments and bodies (including Defra, the Home Office and the Animals in Science Regulation Unit), the Animal Welfare Committee, Animal and Plant Health Agency and the project team.

3.5.4 Horses

A specific workshop was held with equine stakeholders to address and discuss the likely use of gene-editing in horse breeding. The main areas of possible future interest involved using precision breeding in very small populations of rare equine breeds to avoid or remove deleterious mutations and some interests in breeding horses for specific performance traits. It was concluded, however, that these developments were likely to be many years away from being implemented.

3.6 Overall Conclusions

There were a number of conclusions. Firstly, there are very few livestock species where research and development has reached the point where precision-bred animals are in existence, and mostly these animals appear to exist in small numbers in research facilities. However, discussions with industtry suggest that there is great interest in the potential of using precision breeding to resolve disease issues and work with traits difficult to address with current genetic or genomic selection tools. To safeguard the welfare of these precision-bred animals, welfare indicators must be used to ensure that welfare is not adversely affected by the presence of any edited gene. These indicators must assess the physical, immunological and mental functioning of animals in comparison with the most closely related genetics strains available. Indicator lists that cover the Five Domains of animal welfare have been drawn up, but additional checks or increasing frequency of monitoring will be necessary when edits to specific genes within regulatory pathways are made. Stakeholders with a welfare interest suggest that these indicators should sit within a monitoring framework that safeguards animals in all settings from research facilities to on-farm use.

4. Main implications of the findings

There were a number of implications of these findings across Objectives 1 to 5. The mapping exercise suggested that a number of major breeding companies have current or future plans to produce precision-bred animals, but particularly in the pig, poultry and fish sectors. There is the opportunity to collect some information on the health and welfare of these animals as breeding companies have implemented a number of health and welfare assessments to date. The Five Domains Model was considered the best model to use as a framework to build a welfare assessment indicator list as it includes mental state. Construction of the welfare indicator lists for three key species for which precision breeding will likely be used in the near future (pigs, poultry, salmon) was completed. There were three levels of assessment: basic, enhanced, and enhanced plus. The basic levels were based on the breeding company management handbooks and basic health and growth checks. However, the basic level of assessment does not cover all of the domains for all of the species. Behavioural interactions and mental state are not covered for poultry and pigs. It is recommended that the ‘enhanced’ level is adopted, but assessing the animals requires conducting analysis of behaviour, while ‘enhanced plus’ levels of assessment requires conduction of specific behavioural tests that require specialised equipment. Analysis of the case studies suggests that the first step will be to determine what pathways the edited gene is involved in, and then to determine whether the alteration of the gene will affect the functioning of these pathways. In addition to the overall holistic assessment using the Basic, Enhanced or Enhanced Plus levels, assessment using additional welfare indicators that are relevant to the specific edit may be required.

5. Possible future work

In conducting this work, it became apparent that previous approaches to animal welfare assessment (such as Welfare Quality, AWIN or AssureWel), which essentially consider the impact of the environment on the individual or group of animals, were not suitable for the specific needs of this project. We did, therefore, need to consider this in a different way, that is, what might be the impact of a change in the gene expression profile of an animal on its ability to live with a good standard of welfare? The focus was thus on the biological and mental functioning of the animal, in any environment in which it might be kept, and what might be the key aspects of its welfare that could be compromised or enhanced. This meant that some approaches, such as systematic reviews or other methods of data collation, were less useful since this approach to welfare has been much less a focus for scientific study. There are some parallels with work on laboratory rodents, where knock-out mice and other manipulations have been used widely to create models for human disease or as a means to facilitate research into specific pathways. Working groups for genetically altered (GA) mice (which include precision-bred mice) have been established and these have tried to solve the related issue of how to ensure the welfare of GA animals and established some guidelines (Wells et al., 2006). However, although the principles are useful, and aspects such as the need to assess animals at key points in development were used in this work, the report does identify that further work is needed to establish objective welfare metrics for GA animals. Similar approaches may also be required for gene-edited non-laboratory animals to understand the impact on welfare state of alterations to genetic expression.

In addition to the broader question of how the animal’s biological function with respect to animal welfare should best be assessed, a number of specific additional research or evidence needs were identified that relate more specifically to the implementation of a welfare declaration associated with an edited animal:

How to conduct on-going monitoring: The initial cohort of animals on which welfare data would be required are those gene-edited animals that are held within the facilities of the breeding company wishing to market the edited animal. However, these animals may be kept in relatively small numbers, and in higher quality facilities, presumably with optimal nutritional, health and environmental management. On commercial farms animals may be kept at higher stocking density, with less consistent nutrition and health management and potentially exposed to variable impacts of disease agents, environmental temperature, relative humidity, air quality etc. To be reassured that the gene edit does not interact with these variable environments in a negative way for welfare, it is recommended that further monitoring of a representative sample of animals in a range of commercial environments should be carried out to understand how the edited animals function under commercial husbandry conditions. How this might be achieved, and which metrics might be involved requires further work, including possible modelling of the expected prevalence of issues and the numbers of animals likely to be required to achieve appropriate monitoring of different traits. The frequency of any monitoring, the target traits and the numbers of animals required are currently not clear. Greater welfare benefits may accrue in some environments, thus it could be beneficial for the industry to also understand how the edited animals perform in a variety of conditions.

During the project, it became clear that once animals (as commerical stock) are sold by the breeding companies to commercial farms, they do not retain any rights to obtain data on the health, growth or welfare of these animals. Nor are they able to implement any additional assessments on these commercial farms. This information is likely be what is needed to monitor the welfare and performance of PB animals in commercial contexts. How this monitoring data is collected from commercial farms (if this is the approach taken to understanding the performance of PB in commercial contexts) needs to be considered.

How many generations to assess: Some beneficial or deleterious aspects of the gene edit may not become apparent until the 2nd or 3rd generation when homozygosity (or heterozygosity) is achieved. For example, if an edit impacts on the development of gametes in an *in utero* mammalian foetus this may not become evident until the offspring of the edited animal themselves produce offspring.

Deciding on what differences in welfare outcomes are acceptable: We have advocated that the welfare outcomes of precision-bred animals is compared with the results of the same assessments on 'control' animals of the same breed/strain. It is quite possible that there will be differences between the two groups in the welfare outcomes. Where these differences reveal detrimental effects on the welfare of the precision-bred animals, decisions need to be made on what level of negative impact is acceptable. Is there a threshold beyond which the impact is unacceptable? Or should effects be weighed against possible positive impacts of the edit? Expert elicitation studies or ethical frameworks may need to be used to aid decision-making. Trade-offs of this nature are discussed below.

Relative weighting of welfare issues: In all welfare assessments there may be a need to weight positive aspects of welfare against negative aspects. This may be something that is particularly critical in welfare assessments of edited animals. For example, where edits confer disease resistance to a severe and widespread disease which causes significant welfare impacts (such as PRRSV in pigs) should that welfare benefit of disease resistance be sufficient to mitigate other potential welfare harms (such as impaired wound healing), and how should these welfare costs and benefits be balanced? Where the edit (such as myostatin in salmon) may be entirely production-related, should the burden of proof of welfare impacts be greater than where there may be implicit welfare benefits in creating the edited animal? This is an important issue requiring ethical consideration and analysis. Empirical scientific evidence from welfare data will also help to quantify the impacts and refine the questions.

Extending welfare assessment protocols: indicators that were validated and feasible for on-farm use were not available for all sub-categories of the Five Domains model. As animal welfare science creates these methodologies, they should be considered for inclusion in the indicator list. Likewise, technological solutions to remotely and automatically assess welfare are likely to be developed in near future, and the application of these methods should also be considered.

6. Resulting actions

As the outcomes of the project have been presented to the stakeholders and the steering group, no further knowledge exchange activities have been carried out. However, as there is increasing interest in the regulation of precision breeding technologies within the UK (e.g., Scotland) and across the world (e.g., US, NZ) there is potential to publish an account of the methodologies and major outcomes of the project, but taking care to protect sensitive information imparted in the surveys.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

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APPENDIX I

List of welfare assessment indicators to assess effects of precision breeding on welfare

Pigs

July 11, 2023

Aim and scope of this document

This indicator list was compiled by SRUC as part of a Defra-funded project that aimed to assess the impacts of precision breeding on animal welfare (AW0521).

This list outlines welfare indicators recommended for use in assessing animal welfare within one production cycle for animals that would normally reach slaughter age in a commercial company and indicators to be used with breeding animals. The list is designed to assess the welfare of the animal carrying the gene edit, and does not assess any effects that the edited animal might have on non-edited animals (e.g., in terms of increased/decreased propensity for disease transmission or aggression). The number of generations to assess and how/when to monitor animals in commercial contexts (including responses to transport and slaughter) will be considered in future work.

The animals used as parents or germ-line material such as egg/sperm donors or embryo recipients are not included for consideration using this indicator list. Currently, these animals are located in universities or research institutes, and therefore the animals are created and cared for under the auspices of the Animals (Scientific Procedures) Act 1986. If this situation changes, and breeding companies start to house and use donor or recipient animals within their own facilities, this exclusion from welfare assessment will need to be reviewed.

Introduction

This list of indicators allows assessment of whether the welfare of animals produced from precision-breeding methodology has been positively or negatively affected by the presence of the edited gene that they carry. The work is based on the scientific evidence on how best to assess animal welfare. Existing welfare assessment indicators used in the commercial sector and welfare assessment methods used by animal welfare scientists in experimental contexts were used as the evidence base to create this list of indicators.

Welfare assessment protocols designed for livestock such as Welfare Quality®, AWIN and AssureWel are designed to determine whether the management, physical and social environment of the animals is adequately providing for a good standard of welfare. The aim of the present indicator list is different: here the aim is to determine whether the precision-bred animal's biological functioning is similar to their non-edited counterparts. Essentially the assessment asks 'Is the animal healthy? Is it growing normally? Is it showing normal behaviour?' A function-based approach requires that health, growth and behavioural assessments are central to the approach and that animal-based indicators are primarily used. Farm records (a management-based indicator) are used to gather information across time for traits such as disease occurrence.

Assessment setting and control animals

To determine what is typical for the strain and age of animals being assessed, a control group of animals must also be assessed to act as a 'baseline' to allow for a comparison of the data between the two groups. As there are known differences between different breeds and strains, and also differences in behaviour shown in different housing facilities, and feeding and management regimes, the best controls will be a group of animals of the genotype from which the precision-bred line was created, of the same age and sex ratio, and housed and managed in the same way (ideally by the same people in the same facility). Consideration should be given to what environment (intensive or extensive) the progeny of the animals will experience.

Assessment Framework

The Five Domains Model (Mellor et al., 2020; Table 1) has been used as the basis of this protocol to ensure that all aspects of welfare are covered. The five domains are: nutrition, environment, health, behavioural interactions and mental state. This model is used as it includes mental state as well as domains which cover all aspects of biological function. It is the welfare framework that is currently the most widely accepted by animal welfare scientists and has been used as the basis of a number of welfare assessment protocols across a number of species (e.g. Beef Cattle: Meat and Livestock Australia (MLA, 2021); Redwings Horse Sanctuary (www.redwings.co.uk)). These components of welfare underlie welfare standards used by groups such as Global Animal Partnership (USA) that cover the major livestock species.

Welfare indicators and levels of welfare assessment

Welfare indicators were extracted from a number of sources to match against the Five Domains. Wherever possible, the indicators were taken from sources detailing current industry practice in assessing health and welfare (e.g., industry management handbooks, AHDB) or current welfare assessment protocols (e.g., AssureWel, Welfare Quality®). We also included welfare indicators that have been used in experimental settings within animal welfare science and had been validated against other welfare indicators (I.e., had construct or face validity).

The welfare indicators are divided into 'basic', 'enhanced' and 'enhanced plus'. For the 'basic' level, Defra asked us to explore with the breeding industry the types of indicators currently assessed, including basic health and welfare checks likely to be done by farm staff or veterinarians as part of routine monitoring linked to breeding programmes.

However, the basic level indicators do not cover all of the Five Domains and thus domains of animal welfare considered as important by the scientific community are not assessed. Specifically, two out of the five domains ('behaviour' and 'mental state') are not typically assessed if the basic level of assessment is used.

The 'enhanced' level includes indicators that assess welfare across the five domains. A number of behavioural indicators, primarily involving behavioural observations, are included in the enhanced level. The 'enhanced plus' level includes behavioural tests as indicators that give a more in-depth analysis of the animal's cognitive and emotional functioning. The 'enhanced plus' level also includes some indicators that can be used to give a full picture of animal functioning but that need development for use in a welfare assessment setting.

Based on the current scientific understanding of animal welfare, SRUC recommends that indicators covering all five domains should form part of a holistic assessment of health and welfare in precision-bred animals to help identify positive outcomes and/or unintended consequences that may result

from the precision-breeding methods. We therefore recommend that the 'enhanced' level of assessment should be mandatory.

The 'Enhanced plus' level should also be considered as it allows an assessment of cognition and an assessment of whether an animal can live a life worth living or a good life (as described by FAWC (2009)).

Consideration of an optional 'lifetime' assessment was also included in the project proposal to Defra. However, it was considered that assessing a precision-bred animal across its lifespan is necessary to allow a full assessment of the effects of any gene edit. For production animals, this should be to the natural slaughter age. For animals to be kept for breeding, this would be through the first parturition and the subsequent lactation for females.

Key life-stage assessments

As the effects of the edited gene may manifest themselves at different stages of life, it is important to assess animals across the key life stages. For each species, the key life stages have been identified, and are typically the key developmental stages, such as birth, nutritional independence (weaning), appropriate growth stages/commercial production stages and reproductive stages (e.g., parturition and lactation) (Tables 2 and 3).

Gathering data, skills and resources

Throughout, the list for the welfare declaration focuses on the welfare indicators that are needed, but the method used to gather the data is open to allow industry flexibility. Data on some of these indicators may already be gathered as part of normal animal husbandry and health monitoring routines. Data can be recorded manually, using a checklist for the health checks for instance and measures of weight and growth. Behavioural time budget data (for activity, feeding, resting etc) can be extracted from video recordings or recorded through technological approaches that monitor activity and feeding (accelerometers for activity, automated feeders etc.). For example, measures of feeding behaviour may be collected by automated means, or could be provided from assessments of video recordings, or from check sheets taken from manual observations of animals.

Indicators that are routinely collected will clearly require no extra training or resources. For indicators that are not routinely assessed, training of staff will be required. The purchase of equipment such as video-recording equipment, or sensors may be required. Alternatively, the behavioural analysis could be outsourced.

Control group of animals

It is envisaged that the assessments would be carried in a matched group of animals from the 'base' genotype of the same age and sex, and in the same housing and management conditions. The exact number of animals required has not been covered in this project but should involve input from industry on likely numbers of animals produced for the first population reared on breeding company facilities (i.e., not under the auspices of ASPA (1986).

Indicators by welfare domain

Table 1. Table showing definitions of the Five Domains and example indicators

Domain	Definition and example indicators
Nutrition	The nutrition domain refers to the ability to ingest sufficient feed and water for good body functioning
	Example indicators: weight at key stages, body condition, water intake, feeding behaviour
Environment	This domain encompasses responses to environmental stimuli such as light, heat, daylight and alarming stimuli
	Example indicators: thermal competence, appropriate circadian responses, resting postures
Health	This refers to injury, functional impairment and physical fitness
	Example indicators: results of health checks, disease records, recordings of general injuries and species-specific injuries
Behavioural interactions	This domain refers to the capability of the animal to interact with the environment, with other animals and with humans
	Example indicators: vitality, behavioural flexibility, body care (grooming, scratching), social behaviour and maternal behaviour
Mental state	This refers to the quality of the emotional state of the animal
	Example indicators: Qualitative Behavioural Assessment (QBA), presence of play behaviours

Species specific welfare assessment indicators

Table 2. Key life stages for pigs

Stage	**Description**
Immediate post-natal period	Piglet – less than 12h
Post-natal period	Piglet – 12- 72h
Weaner	Weaned from sow and reared in nursery/weaner facility (~28-70 days (7-30kg))
Grower	70-112 days (30-60kg)
Finisher	112-150 days (60-110kg)
Reproductive adult	Gilts: on entry to the breeding herd (~140-180 days (70-90kg) or at first breeding, and through first gestation. Boars: on entry to the breeding herd (~90-180 days) or age at first breeding.
Lactating adult (females)	Gilts and sows (through first lactating period)

Table 3. Schedule of assessments showing what indicators are suggested for each life stage and welfare levels for the indicators.

Details of each indicator are shown in the text below. Tests in italics exist in the experimental literature but have not been adapted for use in on-farm welfare assessments. Basic, enhanced (E) and enhanced plus (E+) levels are shown. Asterisks indicate tests which are not yet fully developed for use in a welfare assessment protocol.

Age/ tests	Immediate post-natal	Post-natal	Weaner	Grower/Finisher	Adult
Basic	-Birthweight -Birth abnormalities -Head morphology	-Mortality/ interventions -Health checks	-Basic feed and water intake -Growth from -Physical abnormalities -Mortality -Disease records -Health checks -General injuries	-Growth -Mortality -Disease records -General injuries -QBA -Physical abnormalities	-Body condition -Mobility (gait) -Mortality -Disease records -Health checks -Dystocia -Physical abnormalities -Litter size
E	-Vitality test		-Water intake patterns -Feed intake patterns -Activity/resting -Species-specific *i*njuries -Social interactions, grooming -QBA	-Water intake -Feed intake -Activity/resting -Social interactions grooming -Species-specific injuries -Social synchronisation*--QBA	-QBA
E+			-Thermal competence* -Startle test -Behavioural flexibility* -Social synchronisation* -Play*	-Thermal competence* -Startle test -Behavioural flexibility* -Play*	

Table 4. Table showing indicators by domain and welfare level (basic, enhanced and enhanced plus).

	Nutrition	Environment	Health	Behaviour	Mental state
Basic	-Birthweight -Growth -Body condition -Basic feed and water intake		-Birth abnormalities -Mortality/ interventions -Head morphology -Health checks -Physical abnormalities -Disease records -Mobility -General injuries -Dystocia		
Enhanced	-Water intake patterns -Feed intake patterns	-Activity/ Resting	-Species-specific injuries	-Piglet vitality -Social interactions -Grooming	-QBA
Enhanced plus		-Thermal competence -Startle test		-Behavioural flexibility -Social synchrony	-Play

Indicator list

The assessments are presented by domain and by life stage. The welfare level (basic, enhanced or enhanced plus) is also indicated.

Nutrition

The nutrition domain refers to the ability to ingest sufficient feed and water for good body functioning

Birth weight - basic

Life stage	Piglet in the immediate neonatal period (within 12 h of birth)
Aim of indicator	Piglet is weighed within 12 hr of birth on a calibrated scale. Birth weight is a well-known survival and vigour/vitality indicator
Scoring	Weight recorded
Source	Baxter et al. 2008 ; Tuchsherer et al. 2000; Edwards et al. 2002

Growth - basic

Life stage	Birth to weaning, weaning to grower stage, grower to finisher/slaughter weight (or across relevant management periods appropriate to the farm e.g. grower to slaughter age in 'grow out' systems)
Aim of indicator	To assess development of the animal
Scoring	Measure bodyweight using a calibrated scale at each time point and use the previous measure to calculate growth across each period.
Source	AHDB Pork

Body condition - basic

Life stage	At weaning, and grower to finisher stage, and at slaughter weight, and monthly during reproductive period and lactating period
Aim of indicator	To assess the physical condition of the animal as an assessment of its ability to convert feed to body tissue
Scoring	Method: • Assess sows by considering several locations, such as shoulders, ribs, backbone and hips • Score the sows by touch, using the palm of the hand; and by sight where this is not possible. Remember you cannot condition score with your eyes alone • Score the sows on a scale of 1 to 5; half scores may be used for mid-ranges Scoring: 1. Emaciated: Shoulders, individual ribs, hips and backbone are visually apparent 2. Thin: Shoulders, ribs, hips and backbone are quite easily felt when pressure is applied with the palm of the hand 3. Acceptable/optimal: Shoulders, ribs, hips and backbone can only be felt when pressure is applied 4. Fat: Shoulders, ribs, hips and backbone cannot be felt even when pressure is applied 5. Grossly fat: Fat deposits are clearly visible
Source	AHDB Pork

Feed and water check - basic

Life stage	During weaning period, grower and/or finisher period, during reproductive/lactating period
Aim of indicator	The aim is to provide a basic check to determine whether the animal is ingesting feed and water
Scoring	Observe each group of pigs in the post-feeding period (up to 3h) and record (yes/no) whether each pig has ingested feed and water.
Source	Farm monitoring check

Feed intake - enhanced

Life stage	During weaning period, grower and/or finisher period, during reproductive/lactating period
Aim of indicator	The aim is to determine whether the pig is showing appropriate feeding behaviour. Automated methods of feed intake, live observations or video-recordings of feeding behaviour can be used.

Scoring	Record the number of feeding bouts and the length of the feeding bouts of each individual animal. Frequency of bouts and means (minimum and maximum) length of bout can be reported. *Suggested schedule: It is suggested that 2x 3h periods (a active period (after fresh feed delivery or lights on) and a non-active period (typically mid-afternoon) are recorded for 2 days at each life stage. If individual automated feed or water intake data is available, then full days should be presented.* *It is estimated that analysis of this type of video footage using a simple ethogram would take 10-12 days for a group of ten animals at each life stage.*
Source	Standard behavioural time budget assessment

Water intake - enhanced

Life stage	During weaning period, grower and/or finisher period, during reproductive/lactating period
Aim of indicator	The aim is to determine whether the pig is drinking appropriately. Automated methods of assessing water intakes, live observations or video-recordings can be used.
Scoring	Record the number of drinking bouts and the length of the drinking bouts per day. Frequency of bouts and means (minimum and maximum) length of bout can be reported. *Suggested schedule: record with feeding as above.*
Source	Standard behavioural time budget assessment

Environment

This domain encompasses responses to environmental stimuli such as light, heat, circadian rhythms and alarming stimuli

Activity/resting/circadian patterns (time budget analysis) - enhanced

Life stage	During weaning period, grower and/or finisher period, during reproductive/lactating period
Aim of indicator	The aim is to determine whether the pigs show appropriate levels of activity and lying, resting and appropriate resting postures. Are they capable of showing both sternal and ventral recumbency? Is the ratio between standing and lying typical for that strain of pig? Does the pig rest at night?
Scoring	Over a 2 x 24h periods within each life-stage period, assess the amount of time spent standing and lying (and lying postures). This can be done using sensor technology or via video-recording. *Suggested schedule: Every 30 mins across 2 x 24hr periods for each life stage, record whether each animal is standing or lying (including lying posture). Analysing this video footage is estimated to take 1-2 days for one group of ten animals per life stage.*
Source	Standard behavioural time budget assessment

Unexpected stimuli/startle test – enhanced plus

Life stage	At weaning, and during grower/finisher phase
Aim of indicator	The aim of this test is to determine whether the pig shows appropriate responses to unexpected stimuli. The appropriate behaviours are to show a startle or freeze response. The protocol from Statham et al. 2020 should be followed (See Appendix). Pigs should be tested in small groups. In the Statham et al (2020) study, a balloon was burst and the response recorded.
Scoring	0 – no startle reaction 1 - No jump, but reaction (side step, head up, head turn, muscle ripple, ear prick) 2 – Jump on spot or spin around to face stimulus 3 – Large jump with movement, typically away from the stimulus 4 - Flee
Source	Statham et al 2020

Additional tests that require further development for use in a Welfare Assessment setting (see Appendix 1 for further details)

Thermal competence – enhanced plus

Health

This domain contains indicators that assess the health and injury status of the animals

Birth abnormalities - basic

Life stage	Immediate neonatal period
Aim of indicator	The piglet should be checked for birth abnormalities within 24h of birth.
Scoring	Record the presence of any abnormalities
	Farm monitoring check

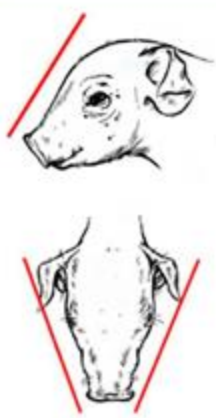
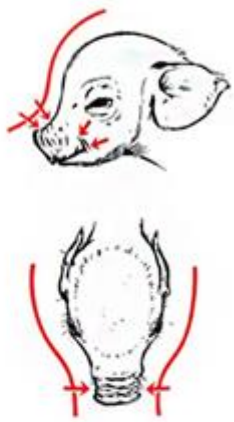
N.B. Stillbirths will be scored as part of the vitality score and typical production recordings

Records of piglet mortality/intervention required - basic

Life stage	Post-natal period (up to 72h)
Aim of indicator	Death of piglets in the cohort of PB or control animals and those that required human intervention (e.g., by farm staff or vets) within the first 72h of life should be recorded. Litter size should also be recorded.
Scoring	Record the number of deaths and cause of death (including savaging) within this period, and number of animals requiring human intervention
	Farm monitoring check

Head morphology - basic

<table>
<tr><td>Life stage</td><td>Piglet in the immediate neonatal period (within 12 h of birth)</td></tr>
<tr><td>Aim of indicator</td><td>Head morphology is a good indicator of piglet vitality. It is often used as an indicator of intrauterine growth restriction (IUGR) and is therefore can also be used as an indicator of reproductive success in reproductive stage females.</td></tr>
<tr><td>Scoring</td><td>Score piglets based on IUGR status as either 1 = Normal, 2 = Moderate, 3 = Severe

This is based on how many of the following characteristics piglets present with:
1. steep, dolphin-like forehead; (2) bulging eyes; and (3) wrinkles perpendicular to the mouth.
Using these criteria, the following three-point piglet head-morphology score was used:
1 = normal head shape, no criteria met
2 = moderate IUGR head morphology, one or two criteria met; and
3 = severe IUGR head morphology, all criteria met.
E.g.
<table>
<tr><th>1 = Normal</th><th>2 = Moderate</th><th>3 = Severe</th></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td>NORMAL PIGLET
</td><td></td><td>IUGR PIGLET
</td></tr>
</table>
</td></tr>
<tr><td>Source</td><td>Hales et al. 2013; Matheson et al. 2018; Huting et al., 2018</td></tr>
</table>

Health checks - basic

Life stage	Post-natal period, at weaning, at transfer to grower pens, at slaughter age and monthly during pregnancy and lactating period
Aim of indicator	This list of health checks represents a basic clinical examination of the animal.
Scoring	Record the value for each indicator: • Respiratory rate • Coat/skin condition: normal/abnormal • Temperature • Presence of diarrhoae
Source	Basic clinical examination

Physical abnormalities -basic

Life stage	At weaning, and transition from grower to finisher and at slaughter weight, during reproductive/lactating period
Aim of indicator	This indicator should be used to assess the presence of any physical abnormalities including hernias, swollen joints or abnormal feet, presence of supernumerary teats (e.g., overgrown hooves)
Scoring	Record the presence of any abnormalities
	Farm monitoring check

Disease records -basic

Life stage	Birth to weaning, weaning to grower period, finisher to slaughter age grower to finisher (or weaning to slaughter weight if this system is used), during reproductive/lactating period
Aim of indicator	The aim of this indicator is to determine whether animals suffer the same levels of disease
Scoring	Record any incidence of disease for each animal in the PB and control group. Identify the disease agent.
	Farm monitoring check

Mobility - basic

Life stage	Adult reproductive stage animal – monthly during reproductive/lactating period
Aim of indicator	The aim is to assess the mobility and gait of the adult animal. Move animals out of accommodation and walk them at least 20m (e.g. walk to weigher or to new accommodation).
Scoring	0. Normal. Even strides, rear end sways slightly while walking, pig is able to accelerate and change direction rapidly. Stands normally. 1. Stiff. Abnormal stride length, movements no longer fluent, pig appears stiff. Pig still able to accelerate and change direction. Stands normally. 2. Slight lameness. Shortened stride, lameness detected, swagger of rear end while walking, no hindrance in pig's agility. Uneven posture while standing. 3. Pig slow to get up (may dog-sit), Shortened stride, Minimum weight-bearing on affected limb (standing on toes), Swagger of rear end while walking. May still trot and gallop. 4. Limping. Pig reluctant to get up, holds limb off floor while standing, avoids placing affected limb on the floor while moving. 5. Downer. Pig unresponsive- does not move and struggles to stand when encouraged to do so.
Source	The scoring system, here used for sows, is based closely on one that has been developed for use in growing and finishing pigs by Main et al (2000), using the standing and walking gait parts of their scale. The assessment starts from the point of getting them up and out of their dry sow pens. Main et al., 2000; D'Eath 2012

General injuries - basic

Life stage	Birth to weaning, weaning to grower period, grower to slaughter age, during reproductive/lactating period
Aim of indicator	The aim of this indicator is to assess the number of general injuries
Scoring	• **0** No Lesion - Pigs without any of the below body marks • **1** Mild - Pigs with mild body marks. Linear lesion longer than 10cm or if there are 3 or more 3cm lesions or if there is a circular area larger than 1cm diameter • **2** Severe - Pigs with severe body marks. Lesion is larger than 5x5cm diameter, or lesion extends into deeper layers of skin, or lesions cover a large percentage of skin (>25%)
Source	D'Eath et al 2021

Species-specific injuries: ear-biting - enhanced

Life stage	Birth to weaning, weaning to grower period, grower to slaughter age
Aim of indicator	The aim of this indicator is to determine whether the pig has suffered from ear-biting
Scoring	**Severity** 0: No Damage 1: Red/Minor Scratches 2: Damaged skin 3: Wound – raw flesh **Freshness** 0: No Wound 1: Intact scab or healed old wound 2: Not intact scab – older blood, red tissue 3: Fresh bite/scratch or wound, not bleeding or weeping 4: Fresh bite/scratch or wound – weeping or bleeding (bloodied) **Size / % of ear affected** 0: No damage 1: Top or bottom of ear damaged 2: Top & Bottom of ear damaged 3: Large % of circumference damaged **Swelling/Fluid Filled** 0: No swelling 1: Swelling 2: Unrelated fluid filled ear
Source	D'Eath et al 2021

Species-specific injuries: tail-biting - enhanced

Life stage	Birth to weaning, weaning to grower period, grower to slaughter age
Aim of indicator	The aim of this indicator is to determine whether the pig has suffered from tail-biting
Scoring	**Severity** 0: No tail damage 1: Flattened - tail is not round, feels flattened like it has been sucked or chewed 2: Red – general redness or red marks, no broken skin 3: Bite marks or scratches – not just red, can see the puncture wounds 4: Wound – Raw flesh, chewed, damaged tail **Wound Freshness** 0: No Wound 1: Intact scab or healed old wound. 2: Not intact scab – older blood, red tissue 3: Fresh bite/scratch or wound, not bleeding or weeping 4: Fresh bite/scratch or wound – weeping but not bleeding (can include bloodied, blood stuck to tail hair) 5: Fresh bite/scratch or wound – bleeding – dripping with blood & splattering own bottom or other pigs.
Source	D'Eath et al 2021

Species specific injuries: flank biting - enhanced

Life stage	Birth to weaning, weaning to grower period, grower to slaughter age
Aim of indicator	The aim of this indicator is to determine whether the pig has suffered from flank-biting
Scoring	Severity 0: No Damage 1: Red/Minor Scratches 2: Damaged skin 3: Wound – raw flesh **Freshness** 0: No Wound 1: Intact scab or healed old wound 2: Not intact scab – older blood, red tissue 3: Fresh bite/scratch or wound, not bleeding or weeping 4: Fresh bite/scratch or wound – weeping or bleeding (bloodied) **Size** <2cm >2cm
Source	D'Eath et al 2021

Dystocia – basic

Life stage	At point of parturition for gilts and sows in the reproductive period
Aim of indicator	The aim of this indicator is to record the difficulty of the parturition process.
Scoring	Ease of farrowing (EFS) can be scored based on the protocol by Mainau et al. (2010) which included different factors 1. Rectal temperature was measured in the sow 90 min after farrowing was completed. 2. A visual assessment (VA) of farrowing was done by farmer for each sow using a 4 categorical subjective scale (1= high difficulty at farrowing; 2 = middle difficulty at farrowing; 3 = low difficulty at farrowing; 4 = spontaneous or easy farrowing). 3. The total duration of farrowing, the birth interval, and the litter size were also recorded by direct observation. 4. Meconium Aspiration Syndrome (MAS) can be scored on the piglets as an indicator of a difficult birth 5. Record whether or not a manual examination and extraction of piglets was done 6. Record if oxytocin or another uteronic has been used 
Source	Farrowing ease: Mainau et al. 2010 MAS: Mota-Rojas et al. 2012 Baxter et al., 2008

N.B. The piglet vitality test and the neonatal survival results will also form part of the assessment of dystocia

Behavioural interactions

This domain refers to the ability of the animal to interact appropriately to the environment and to other animals.

Activity and resting, and the startle test will also form part of the assessment of behaviour.

Piglet vitality test (bucket test) - enhanced

Life stage	Neonatal piglet (~3h after the end of farrowing) – 'Bucket test'
Aim of indicator	Score the piglets on both indicators when the piglets are processed after farrowing.
Scoring	Stimulation of the udder (U) 0: the piglet does NOT show head movements emulating the stimulation of the udder or the search behaviour during a period of 30 s. 1: the piglet shows head movements emulating the stimulation of the udder or the search behaviour during a period of 30 s. Mobility of the piglet (M) 0: the piglet is NOT able to rotate 360° on its axis or to do around in circles following the perimeter of a bucket during a period of 30 s. 1: the piglet is able to rotate 360°
Source	Muns et al. 2013

Social interactions and body maintenance - enhanced

Life stage	At weaning, and at transition from grower to finishing period and at slaughter age, during reproductive period
Aim of indicator	The aim of this assessment is to determine whether pigs are capable of positive and negative species typical social interactions and show awareness of the condition of their skin/hair. Pigs should be observed during high activity periods of the day for up to 3h. Occurrence of positive affiliative behaviours and negative (aggressive) and self-scratching/rubbing behaviours should be recorded.
Scoring	Counts of positive and negative interactions Counts of scratching/rubbing
	Standard behavioural time budget assessment

Additional tests that require further development for use in a Welfare Assessment setting (see Appendix Ia for further details):

Behavioural flexibility test - enhanced plus

Social synchronisation - enhanced plus

Mental state

This domain refers to the quality of the emotional state of the animal and its ability to experience positive and negative emotional states. Several of the indicators in the 'Behavioural interactions' section also give an indication of mental state (e.g. quality of social interactions).

Qualitative Behavioural Analysis (QBA) - enhanced

Life stage	At weaning, and at transition from grower to finishing period and at slaughter age, during reproductive/lactating period
Aim of indicator	QBA is used to assess the mental experience of animals. Each animal is observed for 10-1 seconds and scored according to the Welfare Quality protocol
Scoring	Use terms from Welfare Quality Terms applied to growing pigs: Active, relaxed, fearful, agitated, calm, content, happy, tense, enjoying, frustrated, sociable, bored, playful, distressed, positively occupied, listless, lively, indifferent, irritable, aimless.
Source	Welfare Quality Assessment Protocol for Pigs (see Appendix 1)

Play – enhanced plus

Life stage	At weaning, and at transition from grower to finishing period and at slaughter age
Aim of indicator	The aim of this indicator is to determine whether the pigs are capable of experiencing the positive emotional states associated with play. Suggested method: Enrichment stimulus put into pen and latency to respond and amount of play within 30 mins recorded.
Scoring	Occurrence of play over a day should be recorded – via video or using pedometers.
Source	Standard behavioural time budget assessment

Further work

Further work is required to determine how many animals to assess in both the precision-bred and control line groups. It is possible that differences in scores will be detected between the precision-bred and control groups. Consideration is needed to determine the limit at which any negative effects on the precision-bred population is acceptable, and how positive effects (e.g., reductions in disease) might be traded off against any potential negative effects (e.g., improved disease resistance but reduced activity).

The Precision Breeding Act states that 'An application under this section must include a declaration that the notifier does not expect the health or welfare of the relevant animal or its qualifying progeny to be adversely affected...by any precision bred trait ("an animal welfare declaration"). How many generations of animals that should be assessed, and how and where they are assessed needs to be addressed but is outside the scope of this project.

The longer-term monitoring of welfare and performance of animals in commercial settings where the environment and management maybe different to nucleus herds or research facilities, and the impact of the crossing the gene into different genetic backgrounds also needs to be considered but is beyond the scope of this project.

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Appendix Ia

Indicators that have been used in experimental studies and published, but need some development to allow them to be used in an on-farm welfare context (e.g., development of a user-friendly scoring system.).

Environment domain

Thermal competence – enhanced plus

Life stage	Neonatal phase, weaner phase
Aim of indicator	The aim is to determine whether the pig is responding appropriately to the microclimate in the pen i.e., seeking out heat in cold microclimates or cooler areas.
Scoring	There is currently no validated method to assess thermal competency. Rectal temperature can be used as a indicator of whether the pig is effectively regulating its core body temperature. However, this would need to be assessed during a period during which the temperature was outside the thermal-comfort zone of the piglets. Behavioural test: Suggest that identities of any piglet not seeking heat lamp or contact with the sow in cold conditions, or seeking cool areas in hot conditions. Alternatively, infra-red thermography could be used to assess core temperature.
Source	Pearce et al., 2013; Review by Guevara et al., 2022

Behavioural interactions

Behavioural flexibility test - enhanced plus

Life stage	At weaning, and in grower/finisher stage
Aim of indicator	The aim of this test is to assess the learning and memory capabilities of the animals. Currently, no test exists that has been validated for use in an on-farm setting, as multiple training sessions are typically required. However, it is possible that modified version of a reversal learning test in which 2 feeders are put into the pen, initially with feed in both, then with feed in just one for a period and then the food is moved to the other. The ability of pigs to learn this reversal is then measured (after Bolhuis et al. 2004). Alternatively, an object recognition test could be used. In this test, pigs are repeatedly given access to a single novel object. It is expected that they habituate to it. This object is then replaced with another object, and the level of interest is assessed. A social recognition test could also be used.
Scoring	TBC

Social synchronisation - enhanced plus

Life stage	At weaning, and at transition from grower to finishing period and at slaughter age
Aim of indicator	The aim of this assessment is to determine whether the pig is following social cues provided by other pigs. Pigs should be observed during active and resting periods. Whether the individual is performing the behaviour that the other animals are should be recorded. There is currently no validated method for assessing social interactions.
Scoring	

APPENDIX II

Welfare assessment protocol to assess effects of precision breeding on welfare

Poultry – broiler chickens and laying hens

Aim and scope of this document

This indicator list was compiled by SRUC as part of a Defra-funded project that aimed to assess the impacts of precision breeding on animal welfare (AW0521).

This list is designed to assess welfare in broiler chickens and laying hens, as these sectors are where precision-breeding is currently most likely to be used. The list outlines welfare indicators recommended for use in assessing animal welfare within one production cycle for animals that would normally reach slaughter age in a commercial company and indicators to be used with breeding animals. The description of each indicator shows which type of bird it is applicable to. The list is designed to assess the welfare of the animal carrying the gene edit, and does not assess any effects that the edited animal might have on non-edited animals (e.g., in terms of increased/decreased propensity for disease transmission or aggression). The number of generations to assess and how/when to monitor animals in commercial contexts (including responses to catching, transport and slaughter) will be considered in future work.

The animals used as parents or germ-line material such as egg/sperm donors are not included for consideration using this indicator list. Currently, these animals are located in universities or research institutes, and therefore the animals are created and cared for under the auspices of the Animals (Scientific Procedures) Act 1986. If this situation changes, and breeding companies start to house and use donor or recipient animals within their own facilities, this exclusion from welfare assessment will need to be reviewed.

Introduction

This list of indicators allows assessment of whether the welfare of animals produced from precision-breeding methodology has been positively or negatively affected by the presence of the edited gene that they carry. The work is based on the scientific evidence on how best to assess animal welfare. Existing welfare assessment indicators used in the commercial sector and welfare assessment methods used by animal welfare scientists in experimental contexts were used as the evidence base to create this list of indicators.

Welfare assessment protocols designed for livestock such as Welfare Quality®, AWIN and AssureWel are designed to determine whether the management, physical and social environment of the animals is adequately providing for a good standard of welfare. The aim of the present indicator list is different: here the aim is to determine whether the precision-bred animal's biological functioning is similar to their non-edited counterparts. Essentially the assessment asks 'Is the animal healthy? Is it growing normally? Is it showing normal behaviour?' A function-based approach requires that health, growth and behavioural assessments are central to the approach and that animal-based indicators are primarily used. Farm records (a management-based indicator) are used to gather information across time for traits such as disease occurrence.

Assessment setting and control animals

To determine what is typical for the strain and age of animals being assessed, a control group of animals must also be assessed to act as a 'baseline' to allow for a comparison of the data between the two groups. As there are known differences between different breeds and strains, and also differences in behaviour shown in different housing facilities, and feeding and management regimes, the best controls will be a group of animals of the genotype from which the precision-bred line was created, of the same age and sex ratio, and housed and managed in the same way (ideally by the same people in the same facility).

Assessment Framework

The Five Domains Model (Mellor et al., 2020; Table 1) has been used as the basis of this protocol to ensure that all aspects of welfare are covered. The five domains are: nutrition, environment, health, behavioural interactions and mental state. This model is used as it includes mental state as well as domains which cover all aspects of biological function. It is the welfare framework that is currently the most widely accepted by animal welfare scientists and has been used as the basis of a number of welfare assessment protocols across a number of species (e.g. Beef Cattle: Meat and Livestock Australia (MLA, 2021); Redwings Horse Sanctuary (www.redwings.co.uk)). These components of welfare underlie welfare standards used by groups such as Global Animal Partnership (USA) that cover the major livestock species including broiler chickens and laying hens.

Welfare indicators and levels of welfare assessment

Welfare indicators were extracted from a number of sources to match against the Five Domains. Wherever possible, the indicators were taken from sources detailing current industry practice in assessing health and welfare (e.g., industry management handbooks) or current welfare assessment protocols (e.g., AssureWel, Welfare Quality®). We also included welfare indicators that have been used in experimental settings within animal welfare science and had been validated against other welfare indicators (I.e., had construct or face validity).

The welfare indicators are divided into 'basic', 'enhanced' and 'enhanced plus'. For the 'basic' level, Defra asked us to explore with the breeding industry the types of indicators currently assessed, including basic health and welfare checks likely to be done by farm staff or veterinarians as part of routine monitoring linked to breeding programmes.

However, the basic level indicators do not cover all of the Five Domains and thus domains of animal welfare considered as important by the scientific community are not assessed. Specifically, two out of the five domains ('behaviour' and 'mental state') are not typically assessed if the basic level of assessment is used.

The 'enhanced' level includes indicators that assess welfare across the five domains. A number of behavioural indicators, primarily involving behavioural observations, are included in the enhanced level. The 'enhanced plus' level includes behavioural tests as indicators that give a more in-depth analysis of the animal's cognitive and emotional functioning. The 'enhanced plus' level also includes some indicators that can be used to give a full picture of animal functioning but that need development for use in a welfare assessment setting.

Based on the current scientific understanding of animal welfare, SRUC recommends that indicators covering all five domains should form part of a holistic assessment of health and welfare in precision-bred animals to help identify positive outcomes and/or unintended consequences that may result from the precision-breeding methods. We therefore recommend that the 'enhanced' level of assessment should be mandatory.

The 'Enhanced plus' level should also be considered as it allows an assessment of cognition and an assessment of whether an animal can live a life worth living or a good life (as described by FAWC (2009)).

Consideration of an optional 'lifetime' assessment was also included in the project proposal to Defra. However, it was considered that assessing a precision-bred animal across its lifespan is necessary to allow a full assessment of the effects of any gene edit. For production animals, this should be to the natural slaughter age. For animals to be kept for breeding, this would be through the first laying period.

Key life-stage assessments

As the effects of the edited gene may manifest themselves at different stages of life, it is important to assess animals across the key life stages. For each species, the key life stages have been identified, and are typically the key developmental stages, such as hatching, nutritional independence, appropriate growth stages/commercial production stages and reproductive stages (e.g., point of lay) (Tables 2 and 3).

Gathering data, skills and resources

Throughout, the list for the welfare declaration focuses on the welfare indicators that are needed, but the method used to gather the data is open to allow industry flexibility. Data on some of these indicators may already be gathered as part of normal animal husbandry and health monitoring routines. Data can be recorded manually, using a checklist for the health checks for instance and measures of weight and growth. Behavioural time budget data (for activity, feeding, resting etc) can be extracted from video recordings or recorded through technological approaches that monitor activity and feeding (accelerometers for activity, automated feeders etc.). For example, measures of feeding behaviour may be collected by automated means, or could be provided from assessments of video recordings, or from check sheets taken from manual observations of animals.

Indicators that are routinely collected will clearly require no extra training or resources. For indicators that are not routinely assessed, training of staff will be required. The purchase of equipment such as video-recording equipment, or sensors may be required. Alternatively, the behavioural analysis could be outsourced.

Control group of animals

It is envisaged that the assessments would be carried in a matched group of animals from the 'base' genotype of the same age and sex, and in the same housing and management conditions. The exact number of animals required has not been covered in this project but should involve input from industry on likely numbers of animals produced for the first population reared on breeding company facilities (i.e., not under the auspices of Animals (Scientific Procedures) Act (1986).

Table 1. Table showing definitions of the Five Domains and example indicators

Domain	Definition and example indicators
Nutrition	The nutrition domain refers to the ability to ingest sufficient feed and water for good body functioning
	Example indicators: weight at key stages, body condition, water intake, feeding behaviour
Environment	This domain encompasses responses to environmental stimuli such as light, heat, daylight and alarming stimuli
	Example indicators: thermal competence, appropriate circadian responses, resting postures
Health	This refers to injury, functional impairment and physical fitness
	Example indicators: results of health checks, disease records, recordings of general injuries and species-specific injuries
Behavioural interactions	This domain refers to the capability of the animal to interact with the environment, with other animals and with humans
	Example indicators: vitality, behavioural flexibility, body care (grooming, scratching), social behaviour and maternal behaviour
Mental state	This refers to the quality of the emotional state of the animal
	Example indicators: Qualitative Behavioural Assessment (QBA), presence of play behaviours

Table 2a. Key life stages for broilers

Stage	**Description**
Day old	Day 0 of life
48h	Within 48h of hatch
Post hatch period	Day 2 to day 10
Growing period	Day 10 to slaughter age for broilers
Reproductive adult	Animals that are reared as breeding stock

Table 2b. Key life stages for laying hens

Stage	**Description**
Day old	Day 0 of life
48h	Within 48h of hatch
Post hatch period	Day 2 to day 10

Rearing period	Day 10 to point of lay (approximately 24 weeks)
Productive period	Animal in the laying period
Reproductive adult	Animals that are reared as breeding stock

Table 3. Schedule of assessments showing what indicators are suggested for each life stage and welfare levels for the indicators. Details of each indicator are shown in the text below. Tests in italics exist in the experimental literature but have not been adapted for use in on-farm welfare assessments. Basic, enhanced (E) and enhanced plus (E+) levels are shown. Asterisks indicate tests which are not yet fully developed for use in a welfare assessment protocol.

Age	Day old	48h	Day 2-10	Growing/ rearing	Reproductive adult (laying hens)
Welfare level Basic	Hatchability Crop fill Viability Body temperature Abnormalities	Crop fill Viability	Body temperature Growth Abnormalities Mortality/ interventions	Growth Abnormalities Mortality/ Interventions General Injuries Species-specific injuries Gait Disease records	Growth Mortality/ interventions General Injuries Species-specific injuries Gait Disease records
Enhanced			Feed intake Water intake Activity	Feed intake Water intake Activity Perching Avoidance Social interactions QBA	Feed intake Water intake Activity Perching
Enhanced plus		Thermal competence*		Startle test Novel object test Behavioural flexibility* Social synchrony*	

Table 4. Table showing indicators by domain and welfare level (basic, enhanced and enhanced plus). Asterisks indicate tests which are not yet fully developed for use in a welfare assessment protocol.

	Nutrition	Environment	Health	Behaviour	Mental state
Basic	Hatchability Crop fill Viability Growth	Body temperature	Abnormalities Mortality/ Interventions Disease records General injury Species-specific injuries Feather quality		
Enhanced	Feed intake Water intake	Activity	Gait	Perching Avoidance Social interactions	QBA
Enhanced+		Startle test Novel object test Thermal competence*		Behavioural flexibility* Social synchrony*	

Indicator list

The assessments are presented by domain and by life stage. The welfare level (basic, enhanced or enhanced plus) is also indicated.

Nutrition

The nutrition domain refers to the ability to ingest sufficient feed and water for good body functioning

Hatchability - basic

Life stage	Day old
Description of indicator	The aim of this indicator is to assess the hatchability of the neonatal chick as a measure

	of its nutritional status, and also the status of the parent stock.
Scoring	Record the percentage of live chicks that hatch from eggs (and correspondingly, number of dead-in-shell animals)
Source	Farm monitoring check

Crop fill - basic

Life stage	Day old, 48h
Description of indicator	The aim of this indicator is to determine whether the chick has been able to find food and water
Scoring	Feel the crop of each chick. 0=Crop is empty (indicating the bird has not found food or water) 1=Crop is full but hard, or feed texture apparent (indicates that the bird has found food but not water) 2=Crop is full but soft and rounded (indicates that the bird has found both food and water)
Source	Ross (2018) Management Handbook – Parent Stock

Viability - basic

Life stage	Day old, 48h
Description of indicator	The aim of this indicator is to assess the viability of the neonatal chick as a measure of its nutritional status, and also the status of the dam.
Scoring	Assess movement in the chick. Does each chick move when approached by a human (Y/N)?
Source	Farm monitoring check

Growth - basic

Life stage	Broilers: Weigh at Day old and then weekly until slaughter age is reached Laying hens: Weigh at Day Old and then weekly until 16 weeks of age and then monthly (as per parent stock management)

Description of indicator	Growth rate is a good indicator of nutritional status and general health and welfare. Growth is most important during the first 6 weeks of life.
Scoring	Weigh using a calibrated weigh-scale that is accurate to */-20g. Calculate growth for each week, weight range and coefficient of variation. i
Source	Broilers: Ross (2018) Management Handbook – Parent Stock Hendrix Genetics (2022) Parent Stock Management Guide

Feed and water check - basic

Life stage	Broilers: Day 10 and in week before intended age of slaughter Laying hens: Day 10, point of lay, middle and end of laying period
Description of indicator	The aim is to provide a basic check to determine whether the bird is ingesting feed and water
Scoring	Observe each group of animals in the post-feeding period (up to 3h) and record (yes/no) whether each animal has ingested feed and water.
Source	Farm monitoring check

Feed intake - enhanced

Life stage	Broilers: Day 10 and in week before intended age of slaughter Laying hens: Day 10, point of lay, middle and end of laying period
Description of indicator	The aim is to determine whether the bird is showing appropriate feeding behaviour. Automated methods of assessing intake, live observations or video-recordings of feeding behaviour can be used.
Scoring	Record the number of feeding bouts and the length of the feeding bouts. If individual feed intake is available, this should be recorded as well. Frequency of bouts and means (minimum and maximum) length of bout can be reported. *Suggested schedule: It is suggested that 2x 3h periods: an active period (after fresh feed*

	delivery or lights on) and a non-active period (typically mid-afternoon) are recorded for 2 days at each life stage. If individual automated feed intake data is available, then full days can be presented. *It is estimated that analysis of this type of video footage using a simple ethogram would take 10-12 days for a group of ten animals at each life stage.*
Source	Standard behavioural time budget assessment

Water intake - enhanced

Life stage	Broilers: Day 10 and in week before intended age of slaughter Laying hens: Day 10, point of lay, middle and end of first laying cycle
Description of indicator	The aim is to determine whether the bird is drinking appropriately. Automated methods of assessing intake, live observations or video-recordings of feeding behaviour can be used.
Scoring	Record the number of drinking bouts and the length of the drinking bouts per day. Frequency of bouts and means (minimum and maximum) length of bout can be reported. *Suggested schedule: record with feeding as above.*
Source	Standard behavioural time budget assessment

Environment

This domain encompasses responses to environmental stimuli such as light, heat, circadian rhythms and alarming stimuli.

Body temperature - basic

Life stage	Day old and day 10
Description of indicator	The aim is to determine whether the bird is maintaining body temperature correctly.
Scoring	Using an appropriate thermometer, take the cloacal temperature of the bird.
Source	Ross (2018) Management Handbook – Parent Stock Hendrix Genetics (2022)

Activity/resting/circadian patterns - enhanced

Life stage	Broilers: Day 10, and in week before intended age of slaughter Laying hens: Day 10, point of lay, end of first laying cycle
Description of indicator	The aim is to determine whether the birds show appropriate levels of activity and resting. Do the birds show resting behaviours at night? Are they active during the normal feeding periods?
Scoring	Over a 2 x 24h periods within each life-stage period, assess the amount of time spent standing and sitting. This can be done using sensor technology or via video-recording. *Suggested schedule: Every 30 mins across 2 x 24hr periods for each life stage, record whether each animal is standing or sitting/resting (including use of perches for resting). Analysing this video footage is estimated to take 1-2 days for one group of ten animals per life stage.*
Source	Standard behavioural time budget assessment

Startle test – enhanced plus

Life stage	Broilers: when growing and functionally capable (Week 3) Laying hens: at point of lay
Description of indicator	The aim of this test is to determine whether the bird shows appropriate responses to unexpected stimuli. The appropriate behaviours are to show a startle or freeze response. A mild stressor (e.g., banging a metal object against the feeder) can be used to elicit a startle response.

Scoring	The time taken to resume previous behaviour (resting, feeding or movement) is recorded.
Source	Elston et al., 2000

Novel object test – enhanced plus

Life stage	Laying hens: at point of lay Broilers: when growing and functionally capable (Week 3)
Description of indicator	The aim of this test is to determine whether the bird shows appropriate responses to a novel environmental stimuli.
Scoring	The test involves placing brightly coloured object into the pen. After waiting for 5 mins to allow the birds to settle after entering the house, place the novel object (NO) in the litter and step back 1.5 m. Starting immediately, count every 10 seconds (for a total of 2 minutes) the number of hens at a distance of less than 1 birds length of the NO. For each individual, the number of recordings within 1 bird's length of the NO is tallied and presented
Source	Welfare Quality® (2009)

For additional environmental tests for the Enhanced Plus level that require further development for use in a welfare assessment setting see Appendix IIa for further details.

Thermal competence – enhanced plus

Health

This domain contains indicators that assess the health and injury status of the animals

Early life abnormalities - basic

Life stage	Day Old
Description of indicator	The chicks should be checked for hatching abnormalities within 24h of birth, such as unhealed navels, twisted neck, leg/foot deformities
Scoring	Record the presence of any abnormalities
Source	Farm monitoring check

N.B. Number of ‘dead-in-shells' will be scored as part of the hatchability score

Physical abnormalities –basic

Life stage	Broilers: Day 10, and at slaughter weight Laying hens: Day 10, point of lay, middle and end of laying period
Description of indicator	The aim of this indicator is to determine whether any physical abnormalities are present in the birds. Record deformed toes, feet and beak abnormalities (in untrimmed birds). Also prolapse, keel bone deviations and factures for laying hens.
Scoring	Record the presence of any abnormalities for each bird
Source	Farm monitoring check

Mortality, culling and interventions - basic

Life stage	Broilers: Day 10; Weekly until slaughter age Laying hens: Day 10; weekly to week 16, then monthly to end of first laying cycle
Description of indicator	The aim of this indicator is to assess the mortality and the number of farm staff interventions (extra feeding, add heat source etc.) across the different life stages
Scoring	Calculate % mortality, % of birds culled (with reasons for culling) and % of animals which received interventions
Source	Farm monitoring check

Disease records - basic

Life stage	Broilers: Day old, post-hatch period, from hatch to slaughter age Laying hens: Day old, post-hatch period, from hatch to point of lay, and point of lay to middle and end of first laying cycle
Description of indicator	The aim of this indicator is to determine whether animals suffer the same levels of disease
Scoring	Record any incidence of disease for each animal in the PB and control group. Identify the disease agent where possible.
Source	Farm monitoring check

Gait - enhanced

Life stage	Broilers: Week before intended slaughter age (suggest Week 4) Laying hens: Week 4, week 30 and end of lay
Description of indicator	The aim of this indicator is to assess foot and leg health
Scoring	0. Normal, dextrous and agile 1. Slight abnormality, but difficult to define 2. Definite and identifiable abnormality 3. Obvious abnormality, affects ability to move 4. Severe abnormality, only takes a few steps 5. Incapable of walking
Source	Welfare Quality® (2009)

General injuries – basic

Life stage	Broilers: Week before intended slaughter age (suggest Week 4) Laying hens: Week 4, week 30 and end of lay
Description of indicator	The aim of this indicators is to assess the number of general injuries of the bird
Scoring	Count the number of injuries to comb, legs, body etc (excluding hock burn, FPD and cloacal pecking)
Source	Farm monitoring check

Species specific injuries - Hock burn -basic

Life stage	Broilers: Week before intended slaughter age (suggest Week 4) Laying hens: Week 4, week 30 and end of lay
Description of indicator	The aim of this indicators is to assess the level of hock burn suffered by the birds. This is an indicator of skin quality and also of activity.
Scoring	Individual level: a – No evidence of hock burn (score '0') b – Minimal evidence of hock burn (score '1' and '2') c – Evidence of hock burn (score '3' and '4') (see Welfare Quality® (2009) manual for details and illustrations of the scores)
Source	Welfare Quality® (2009)

Species specific injuries - Foot pad dermatitis and bumblefoot – basic

Life stage	Broilers: Week before intended slaughter age (suggest Week 4) Laying hens: Week 4, week 30 and end of lay
Description of indicator	The aim of this indicator is to assess the level of footpad dermatitis suffered by the birds. This is an indicator of skin quality.
Scoring	Individual level: 0 – Feet intact, no or minimal proliferation of epithelium 1 – Necrosis or proliferation of epithelium or chronic bumble foot with no or moderate swelling 2 – Swollen (dorsally visible) (see Welfare Quality® (2009) manual for details and illustrations of the scores)
Source	Welfare Quality® (2009)

Species specific injuries – Breast blisters – basic

Life stage	Broilers: Week before intended slaughter age (suggest Week 4)
Description of indicator	The aim of this indicator is to determine whether the birds are suffering from breast blisters which might indicate an alteration in skin quality.

Scoring	Individual level: 0 – No breast blister 1 – Breast blister present (see Welfare Quality® (2009) manual for details and illustrations of the scores)
Source	Welfare Quality® (2009)

Species specific injuries - feather-pecking; cloacal pecking - basic

Life stage	Broilers: post-hatch period, from hatch to slaughter age Laying hens: from hatch to point of lay, and point of lay to mid- and end of first laying period
Description of indicator	The aim of this indicator is to determine whether the birds are suffering from feather-pecking or cloacal pecking.
Scoring	Visually assess the head/neck area and back/vent area of the bird and score both these areas according the scoring system below: 0 = No/Minimal feather loss. No bare skin visible, no or slight wear, only single feathers missing 1 = Slight feather loss. Moderate wear, damaged feathers or 2 or more adjacent feathers missing up to bare skin visible < 5cm maximum dimension 2 = Moderate/Severe feather loss. Bare skin visible ≥ 5cm maximum dimension
Source	AssureWel; RSPCA; www.featherwel.org

Behavioural interactions

This domain refers to the ability of the animal to interact appropriately to the environment and to other animals.

Perching - advanced

Life stage	Laying hens: during rearing period (before point of lay), during laying period
Description of indicator	The aim is to determine whether the birds are capable of showing appropriate use of perches
Scoring	Assess time on perch and whether the birds show appropriate perching and resting behaviour. Birds should be observed during a dark period in the presence of a perch. Using instantaneous scan-sampling method, record whether the bird is using the perch every 30 mins through the dark period.
Source	Standard behavioural time budget assessment

Avoidance distance test – enhanced

Life stage	Broilers – when growing and functionally capable (Week 3) Laying hens: during rearing period (before point of lay), during laying period
Description of indicator	The aim is to determine whether the birds are capable of showing appropriate response to a potentially startling object – an approaching human
Scoring	Walk slowly parallel to the slatted floor through the litter area at a distance of 1.5 meter from the edge of the slatted area. The hand is held in a fixed position in front of the abdomen of the assessor, directly above and in line with the bird's feet. When a hen is sitting on the edge of the slatted area, turn 90 degrees and stand facing the hen. Then walk with a pace of one step per second towards the hen, looking at its toes. When the hen turns away or retreats (both feet step aside or away), the distance is measured from the hand of the assessor to the earlier position of the feet of the hen.

	Record the closest distance to which the bird can be approached
Source	Welfare Quality® (2009)

Social interactions – negative interactions - enhanced

Life stage	Broilers: when growing and functionally capable (Week 3) Laying hens: at point of lay
Description of indicator	The aim of this assessment is to determine whether the birds are performing a high level of negative social interactions. Birds should be observed during activity periods of the day for 1-2 h (include with observations of feeding and drinking.
Scoring	Over a 1h period during the most activity period of the day, score the number of: Aggressive behaviour: fighting, aggressive pecking at or chasing other birds. A social behaviour to establish pecking order. Injurious feather pecking - includes pulling out feathers, pecking at wounds or vent. Believed to be redirected foraging behaviour. Both are often signalled by a loud squawk or vocalisation.
Source	AssureWel (2013)

For additional tests for the Enhanced Plus level that require further development for use in a welfare assessment setting see Appendix 1 for further details.

Behavioural flexibility test - enhanced plus

Social synchrony - enhanced plus

Mental state

This domain refers to the quality of the emotional state of the animal and its ability to experience positive and negative emotional states. Several of the indicators in the 'Behavioural interactions' section also give an indication of mental state (e.g. quality of social interactions).

Qualitative Behavioural Analysis (QBA) - enhanced

Life stage	Broilers: when growing and functionally capable (e.g., Week 3) Laying hens: at point of lay
Description of indicator	QBA is used to assess the mental experience of animals. Each animal is observed for up to 1 minute and scored according to the Welfare Quality protocol
Scoring	The terms used for the QBA broiler assessment are: Active, Calm, Friendly, Relaxed, Content, Positively occupied, Helpless, Tense, Scared, Comfortable, Inquisitive, Drowsy, Fearful, Unsure, Playful, Agitated, Energetic, Nervous, Confident, Frustrated, Distressed, Depressed, Bored
Source	Welfare Quality® (2009)

Further work

Further work is required to determine how many animals to assess in both the precision-bred and control line groups. It is possible that differences in scores will be detected between the precision-bred and control groups. Consideration is needed to determine the limit at which any negative effects on the precision-bred population is acceptable, and how positive effects (e.g., reductions in disease) might be traded off against any potential negative effects (e.g., improved disease resistance but reduced activity).

The Precision Breeding Act states that 'An application under this section must include a declaration that the notifier does not expect the health or welfare of the relevant animal or its qualifying progeny to be adversely affected...by any precision bred trait ("an animal welfare declaration"). How many generations of animals that should be assessed, and how and where they are assessed needs to be addressed but is outside the scope of this project.

The longer-term monitoring of welfare and performance of animals in commercial settings where the environment and management maybe different to nucleus herds or research facilities, and the impact of the crossing the gene into different genetic backgrounds also needs to be considered but is beyond the scope of this project.

References

AssureWel (2013) Laying Hen Assessment. http://www.assurewel.org/layinghens.html

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Ross (2018). Management Handbook – Parent Stock

RSPCA Assured (www.rspcaassured.org.uk/farm-animal-welfare/

Welfare Quality® (2009). Welfare Quality® assessment protocol for poultry (broilers, laying hens). Welfare Quality® Consortium, Lelystad, Netherlands.

Appendix IIa

Indicators that have been used in experimental studies and published, but need some development to allow them to be used in an on-farm welfare context (e.g., development of a user-friendly scoring system).

Environment domain

Thermal competence - enhanced plus

Life stage	48h
Description of indicator	The aim of this indicator is to determine whether the bird is making the appropriate response to a deviation from normal temperatures. It is standard practice in commercial rearing facilities to determine whether the temperature of the house is appropriate by assessing the spatial distribution of the birds (e.g., Ross Breeders – Management handbook (2018)). If they are evenly distributed, the ambient temperature is correct; if they are huddled together, it is too cold or it they are evenly spaced and avoid any heat source, they are too hot. It is suggested that the ability of the chick to respond to temperature deviations could be tested by reducing or raising the temperature of

	the house and observing whether chicks respond appropriately
Scoring	Record whether each bird does not move away from the heat source in hot conditions or does not huddle with others in cool conditions. Infra-red thermography could also be used.
	TO BE DEVELOPED

Behavioural interactions domain

Social synchrony - enhanced plus

Life stage	Laying hens (at point of lay) Broilers – when growing and functionally capable (Week 3)
Description of indicator	The aim of this assessment is to determine whether the bird is following social cues provided by other birds. Birds should be observed during active and resting periods. Whether the individual is performing the behaviour that the other animals are should be recorded.
Scoring	

Behavioural flexibility test - enhanced plus

Life stage	Laying hens (at point of lay) Broilers – when growing and functionally capable (Week 3)
Description of indicator	The aim of this test is to assess the learning and memory capabilities of the animals. Currently, no test exists that has been validated for use in an on-farm setting, as multiple training sessions are typically required. However, it is possible that modified version of a reversal learning test in which 2 feeders are put into the pen, initially with feed in both, then with feed in just one for a period and then the food is moved to the other.
Scoring	TBC

APPENDIX III

Welfare assessment protocol to assess effects of precision breeding on welfare

Atlantic Salmon (*Salmo salar)*

11th July, 2023

Aim and scope of this document

This indicator list was compiled by SRUC as part of a Defra-funded project that aimed to assess the impacts of precision breeding on animal welfare (AW0521).

This list outlines welfare indicators recommended for use in assessing animal welfare within one production cycle for animals that would normally reach slaughter age in a commercial company and indicators to be used with breeding animals. The list is designed to assess the welfare of the animal carrying the gene edit, and does not assess any effects that the edited animal might have on non-edited animals (e.g., in terms of increased/decreased propensity for disease transmission or aggression, or as escapees). The number of generations to assess and how/when to monitor animals in commercial contexts (including responses to catching, transport and slaughter) will be considered in future work.

The animals used as parents or germ-line material such as egg/sperm donors are not included for consideration using this indicator list. Currently, these animals are located in universities or research institutes, and therefore the animals are created and cared for under the auspices of the Animals (Scientific Procedures) Act 1986. If this situation changes, and breeding companies start to keep and use donor or recipient animals within their own facilities, this exclusion from welfare assessment will need to be reviewed.

Introduction

This list of indicators allows assessment of whether the welfare of animals produced from precision-breeding methodology has been positively or negatively affected by the presence of the edited gene that they carry. The work is based on the scientific evidence on how best to assess animal welfare. Existing welfare assessment indicators used in the commercial sector and welfare assessment methods used by animal welfare scientists in experimental contexts were used as the evidence base to create this list of indicators.

The lists of indicators in welfare assessment protocols designed for terrestrial livestock such as Welfare Quality®, AWIN and AssureWel and SWIM1.0 and SWIM2.0 for salmon (Stein et al., 2013; Pettersen et al.,2014) are designed to determine whether the management, physical and social environment of the animals is adequately providing for a good standard of welfare. The aim of the present indicator list is different: here the aim is to determine whether the precision-bred animal's biological functioning is similar to their non-edited counterparts. Essentially the assessment asks 'Is the animal healthy? Is it growing normally? Is it showing normal behaviour?' A function-based approach requires that health, growth and behavioural assessments are central to the approach and that animal-based indicators are primarily used. Farm records (a management-based indicator) are used to gather information across time for traits such as disease occurrence.

Assessment setting and control animals

To determine what is typical for the strain and age of animals being assessed, a control group of animals must also be assessed to act as a 'baseline' to allow for a comparison of the data between the two groups. As there are known differences between different breeds and strains, and also differences in behaviour shown in different physical facilities, and feeding and management regimes, the best controls will be a group of animals of the genotype from which the precision-bred line was created, of the same age and sex ratio, and kept and managed in the same way (ideally by the same people in the same facility).

Assessment Framework

The Five Domains Model (Mellor et al., 2020; Table 1) has been used as the basis of this protocol to ensure that all aspects of welfare are covered. The five domains are: nutrition, environment, health, behavioural interactions and mental state. This model is used as it includes mental state as well as domains which cover all aspects of biological function. It is the welfare framework that is currently the most widely accepted by animal welfare scientists and has been used as the basis of a number of welfare assessment protocols across a number of species (e.g. Beef Cattle: Meat and Livestock Australia (MLA, 2021); Redwings Horse Sanctuary (www.redwings.co.uk)). These components of welfare underlie welfare standards used by groups such as Global Animal Partnership (USA) that cover the major livestock species including farmed salmon. While mental state is not currently included in assessment protocols for farmed salmon, the sentience of fish has been recognised and development of indicators for this domain into assessment protocols for salmon is underway.

Welfare indicators and levels of welfare assessment

Welfare indicators were extracted from a number of sources to match against the Five Domains. Wherever possible, the measures were taken from sources detailing current industry practice in assessing health and welfare (e.g., Ashley, 2007; Martins et al., 2012; Noble et al., 2018; Rey et al., 2019) or current welfare assessment protocols (e.g., Global Aquaculture Alliance's (GAA) 'Best Aquaculture Practices' (BAP) certification, RSPCA Welfare Standards for Farmed Atlantic Salmon). We also included welfare indicators that have been used in experimental settings within animal welfare science and had been validated against other welfare indicators (I.e., had construct or face validity).

The welfare indicators are divided into 'basic', 'enhanced' and 'enhanced plus'. For the 'basic' level, Defra asked us to explore with the breeding industry the types of indicators currently assessed, including basic health and welfare checks likely to be done by farm staff or veterinarians as part of routine monitoring linked to breeding programmes.

However, the basic level indicators do not cover all of the Five Domains and thus domains of animal welfare considered as important by the scientific community are not assessed. Specifically, two out of the five domains ('behaviour' and 'mental state') are not typically assessed if the basic level of assessment is used.

The 'enhanced' level includes indicators that assess welfare across the five domains. A number of behavioural indicators, primarily involving behavioural observations, are included in the enhanced level. The 'enhanced plus' level includes behavioural tests as indicators that give a more in-depth analysis of the animal's cognitive and emotional functioning. The 'enhanced plus' level also includes some indicators that can be used to give a full picture of animal functioning but that need development for use in a welfare assessment setting.

Based on the current scientific understanding of animal welfare, SRUC recommends that indicators covering all five domains should form part of a holistic assessment of health and welfare in precision-

bred animals to help identify positive outcomes and/or unintended consequences that may result from the precision-breeding methods. We therefore recommend that the 'enhanced' level of assessment should be mandatory.

The 'Enhanced plus' level should also be considered as it allows an assessment of cognition and an assessment of whether an animal can live a life worth living or a good life (as described by FAWC (2009)).

Consideration of an optional 'lifetime' assessment was also included in the project proposal to Defra. However, it was considered that assessing a precision-bred animal across its lifespan is necessary to allow a full assessment of the effects of any gene edit. For production animals, this should be to the natural slaughter age. For animals to be kept for breeding, this would be through the first laying period.

Key life-stage assessments

As the effects of the edited gene may manifest themselves at different stages of life, it is important to assess animals across the key life stages. For each species, the key life stages have been identified. For salmon the key developmental stages are: egg, first feeding, parr, smolt, or where management changes occur in commercial production (transition from fresh to sea water). (Tables 2 and 3).

Gathering data, skills and resources

Throughout, the list for the welfare declaration focuses on the welfare indicators that are needed, but the method used to gather the data is open to allow industry flexibility. Data on some of these indicators may already be gathered as part of normal animal husbandry and health monitoring routines. Data can be recorded manually, using a checklist for the health checks for instance, or and measures of feed intake and growth. Video recordings can be used or technological approaches that monitor activity or feeding. For example, measures of feeding behaviour may be collected by automated means, or could be provided from assessments of video recordings, or from check sheets taken from manual observations of animals.

Indicators that are routinely collected will clearly require no extra training or resources. For indicators that are not routinely assessed, training of staff will be required. The purchase of equipment such as video-recording equipment, or sensors may be required. Alternatively, the behavioural analysis could be outsourced.

Control group of animals

It is envisaged that the assessments would be carried in a matched group of animals from the 'base' genotype of the same age and sex, and in the same tanks/pens and management conditions. The exact number of animals required has not been covered in this project but should involve input from industry on likely numbers of animals produced for the first population reared on breeding company facilities (i.e., not under the auspices of Animals (Scientific Procedures) Act (1986).

Indicators by welfare domain

Table 1. Table showing definitions of the Five Domains and example indicators for salmon

Domain	Definition and example measures

Nutrition	The nutrition domain refers to the ability to ingest sufficient feed for growth and development
	Measures: Growth, Feed Conversion Rate
Environment	This domain encompasses responses to environmental stimuli such as temperature, light, and alarming stimuli
	Example measures: thermoregulation, swimming performance, smoltification
Health	This refers to injury, functional impairment and physical fitness
	Example measures: morphological health checks, disease records, pathogen challenge test
Behavioural interactions	This domain refers to the capability of the animal to interact with the environment, with other animals and with humans
	Example measures: aggression (fin biting), foraging behaviour
Mental state	This refers to the quality of the emotional state of the animal
	Example measures: Qualitative Behavioural Assessment

Species specific welfare assessment measures for salmon

Table 2. Key life stages for salmon

Stage	**Description**
Egg	Eggs are incubated in controlled hatcheries until they hatch
Alevin	Yolk still attached 0.1g – 0.3g
Fry	First feeding. First sorted for size ('graded') at around 5g and moved to first feeding tanks.
Parr	Development of skin colouration (vertical bars) for camouflage along their sides.
Smolt	The stage of adaption to salt water (~75g-400g, depending on when smolting is induced)
Adult	After smoltification, smolts are transferred to saltwater sea cages or net pens for on-growing. Matured after one year at sea (3-4kg), or matured after 18-24 months (5-10kg).

Suggested schedule of assessments

Table 3. Schedule of assessments showing what indicators are suggested for each life stage and welfare levels for the indicators.

Details of each indicator are shown in the text below. Tests in italics exist in the experimental literature but have not been adapted for use in on-farm welfare assessments. Basic, enhanced (E) and enhanced plus (E+) levels are shown. Asterisks indicate tests which are not yet fully developed for use in a welfare assessment protocol.

Age	Egg	Alevin	Fry	Parr	Smolt	Adult
Basic	– Mortality – Egg Quality	– Mortality	– Mortalities – Standard Growth Rate (SGR) – Feed Intake – Swimming Performance: Behavioural assessment [b] – Health measures (morphological scores) – Aggression/fin damage	Same as fry	Same as fry with the addition of: – Smoltification: Smolt Test – Smoltification: Condition Factor	Same as fry with the addition of: – Disease: Sea Lice Score
E	–	–		– *Startle test* [a] – Swimming Performance: Ucrit test – *Disease: Challenge Test* – *QBA*	– *QBA* – *Disease: Challenge Test* – *Smoltification: RT qPCR*	– *QBA* – *Disease: Challenge Test*
E+	–	–	Foraging behaviour Food anticipatory behaviour	– *Behavioural fever test* [b]		

[a] Also applies to the Health domain; [b] Also applies to the Behavioural Interactions domain

Table 4. Table showing indicators by domain and welfare level (basic, enhanced and enhanced plus).

Domain	Nutrition	Environment	Health	Behavioural Interactions	Mental State
Tests Basic	– Growth (SGR) – Feed Intake (FCR)	– Swimming Performance: Behavioural assessment [b] – Smoltification: Smolt Index – Smoltification: Condition Factor	– Egg Quality – Mortality – Body and Spine Condition – Skin Condition – Eye Condition – Mouth/ Jaw Deformities – Opercular Damage – Disease: Sea Lice Score	– Aggression: Dorsal Fin Damage	
Enhanced	–	– *Smoltification: RT qPCR* – *Swimming Performance: Ucrit test* – *Startle test* [a]	– *Disease: Challenge test*	–	– *QBA*
Enhanced+	–	– *Behavioural fever test* [b]	–	– *Foraging Behaviour* – *Food Anticipatory Behaviour*	–

[a] Also applies to the Health domain

[b] Also applies to the Behavioural I**nteractions domain**

Assessments

The assessments are presented by domain and by life stage.

Nutrition

The nutrition domain refers to the ability to ingest sufficient feed for good body functioning.

Growth - Basic

Life stage	First feeding onwards
Aim of indicator	In a commercial setting this is practiced in the grow out phase, but this could be a viable test between control and PB groups at any life stage
Scoring	Specific Growth Rate (SGR), calculated by measuring the change in weight (g) over a specific period (days). The formula is: SGR = ((ln(final weight) - ln(initial weight)) / time) * 100 SGR should be compared between PB and control groups, and any deviation in SGR from the control (positive or negative) should be reported.
Source	(Noble et al., 2018)

Feed Intake - Basic

Life stage	First feeding onwards.
Aim of indicator	In a commercial setting this is practiced in the grow out phase, but this could be viable test between control and PB groups
Scoring	Feed Conversion Ratio (FCR), calculated as: FCR = Feed Consumed (in weight) / Weight Gain For example, if 1000 kg of feed is consumed by the salmon, and the weight gain of the population is 500 kg, the FCR would be calculated as: FCR = 1000 kg / 500 kg = 2 An FCR value of 2 indicates that it took 2 kg of feed to produce 1 kg of weight gain in the salmon.

	FCR should be compared between PB and control groups, and any deviation from the control (positive or negative) should be reported.
Source	BAP Certification

Environment

This domain encompasses responses to environmental stimuli such as light, heat, circadian rhythms and alarming stimuli. Many of these measures will cross over with the Behaviour and Health domain.

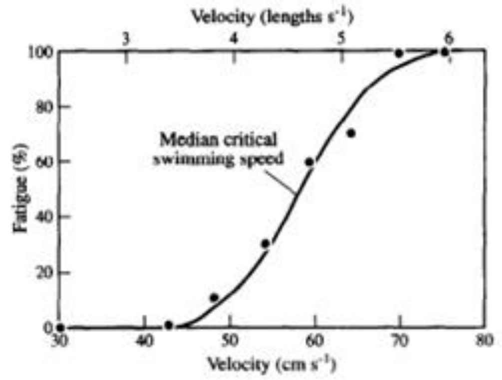
Swimming Performance – Basic

Life stage	Fry, parr, smolt, adult
Aim of indicator	The aim is to assess whether fish are swimming normally at each life stage. Altered swimming performance may occur due to variety of factors (e.g., suboptimal velocity, water quality or other stress factors). This assessment method directly observes the behaviour of fish at the individual and group level to assess swimming performance, and as such there is cross over between the Environmental and the Behavioural domain. Behavioural observations are made directly from tanks, analysis of video recordings, or live surveillance of video cameras. At both the individual and group level, and at each stage of life. Fish should comfortably hold and adjust position in the water column (flow rate should be adjusted to accommodate fish at each life stage)
Scoring	Individual level o Observe the behaviour of fish in both PB and control groups, looking for any signs of stress or abnormal behaviour. Is there any presence of: – Laboured and/ or fatigued swimming? Yes/No

	– Erratic swimming (increase in manoeuvre complexity)? Yes/ No – Gulping at the surface? Yes/ No – Fish cease schooling and become inactive, staying close to the surface or net wall (sea cage only)? Yes/ No Group Level o Observe the behaviour of fish in both PB and control groups, looking for any signs of stress or abnormal behaviour. Is there any deviation from the following normal group level behaviour: o Polarised group cohesion, with fish swimming comfortably, holding and adjusting position in the water column with ease? Yes/No o Tank/ cage distribution reflect natural behaviour (e.g., fry position at the bottom of tanks; at dawn or warmer temps, fish descend to the bottom of the water column (adults in sea cages only)? Yes/No o Presence of: – Laboured and/ or fatigued swimming? Yes/ No – Gulping at the surface? Yes/ No – Fish cease schooling and become inactive? Yes/ No
Source	(Martins et al., 2012)

UCrit: Swimming performance -Critical Swimming Speed – Enhanced

Life stage	Parr, smolt, adult
Aim of indicator	The aim is to assess whether there is a significant difference in Critical Swimming Speed (a measure of swimming performance) between fish from PB and control groups Critical Swimming Speed (Ucrit) o Defined as the maximum incoming velocity against which a fish can successfully swim and maintain position. o Measured using a water tunnel or swim chamber. o Water flow velocity is gradually increased until the fish reaches its

	maximum sustainable swimming speed. Measured in body length/ sec (BL/sec) o Can be measured individually or in small groups, e.g., when 50% of fish have fatigued, the mean maximum sustainable speed is reached (Fig 1) o *Lab based measure, requiring equipment and standardised conditions*  Fig. 1. Percentage of young sockeye salmon fatigued within 300 min at 15°C when forced to swim at the velocity indicated, expressed in lengths/sec. Mean length of the sample of 104 fish was 13.6 cm. (From Brett J. R. (1967) Swimming performance of sockeye salmon (*Oncorhynchus nerka*) in relation to fatigue time and temperature. *J. Fish. Res. Bd. Can.* **24,** 1731–1741. Reproduced by permission.) (taken from Hammer, 1995)
Scoring	Critical swimming speed (Ucrit) of PB population is compared to that of control groups, check for any significant deviations in swimming speed from base line/ control groups
Source	(Brett, 1964; Hammer, 1995; Thorstad et al., 2000; Wolter & Arlinghaus, 2004)

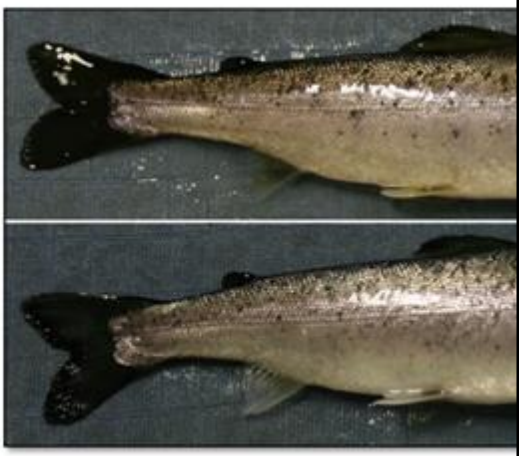
Unexpected stimuli/startle test - Enhanced

Life stage	
Aim of indicator	The aim of this test is to determine whether PB fish shows appropriate responses to unexpected stimuli. The startle response in salmon is a sequence of evasive behaviours

	triggered by the displacement of water by an approaching predator (McIntyre et al., 2012) . This simple protocol from (Raby et al., 2012) involves the tail grab response (as 1 of 5 reflex action mortality predictors (RAMP) to check for the presence or absence of natural reflexes to stressors), which is assessed by a handler attempting to grab the tail of the fish while the fish is submerged in water (in a fish bag or holding trough); a positive response is characterized by the fish attempting to burst-swim immediately upon contact.
Scoring	0. Unimpaired response 1. Impaired response Scoring is categorical and conservative, so that if the handler is in any doubt as to whether the reflex was present, it is recorded as impaired. Compare scores between a sample of fish from PB and control groups.
Source	(McIntyre et al., 2012; Raby et al., 2012)

Smoltification: Smolt Index - Basic

Life stage	Pre-smolt/ smolt
Aim of indicator	The aim of this test is to determine whether fish are showing appropriate development in terms of smoltification, using non-invasive methods currently used in industry. Smoltification rates between PB and Control groups can be compared. Using the Smolt Index – a non-invasive commercial index used to evaluate the physical characteristics of fish undergoing smoltification, to help determine the readiness of salmon to transition from freshwater to seawater environments. Changes in body morphology and skin reflectance are monitored and recorded using this scoring system. Visual checks and observations must be made for several weeks during the period- prior to smolting (silvering, swim pattern, shape).

	Comparison between PB and Control groups should be conducted to check smoltification is occurring at the same rate.
Scoring	As a guide, the RSPCA recommends the use of the smol of C. Findlay, of the Fish Vet Group Inverness. **Smolt score** **Score** **Appearance** 1 Parr marks clear, light coloured back, flanks g 2 Parr marks fading, back and fins light, flanks 3 Parr marks faint, back and fins darkening, fla 4 Parr marks very faint, dark back, yellow only flanks silver. 5 Parr marks gone, back dark, dark margin to fi silvering colour dominant.  *Fig. 3.2.8-1. Upper picture: A salmon that is not completely yellowish colour on the operculum and the area around the p smoltified salmon. Photos: Jonatan Nilsson* Picture courtesy of (Noble et al., 2018)
Source	(RSPCA 2018, Noble et al., 2018)

Smoltification: Condition Factor - Basic

Life stage	Pre-smolt/ smolt
Aim of indicator	The aim of this test is to determine whether fish are showing appropriate development in terms of condition factors associated with smoltification. Condition factors of PB and Control groups should be compared to check no significant deviations from normal are present. Condition factor is (K) is a well-accepted tool for assessing the nutritional status of fish (Bolger &

	Conolly, 1989; Nash et al., 2006). The higher the K value, the rounder the fish. Condition factor commonly decreases in smolting salmonids relative to non-smolts (Folmar and Dickhoff 1980; Hoar 1988). $K = 100 \times Weight(g) \times Length(cm)^{-3}$
Scoring	Condition factors of a sample from PB and Control groups should be compared to check no significant deviations are present.
Source	(Folmar & Dickhoff, 1980; Hoar, 1976; Noble et al., 2018)

Smoltification: RT qPCR tests – Enhanced

Life stage	Pre-smolt/ Smolt
Aim of indicator	This test uses Real-Time qPCR analysis to assess when salmon enter smoltification. RT-qPCR expression of mRNA encoding for two protein isoforms of the NKA alpha catalytic subunit are analysed, the first is upregulated in freshwater (FW) and the second is upregulated in saltwater (SW). The test looks at the ratio of the relative expression of the SW over FW (SW/FW), which is the primary quantity used in practice to determine whether fish are ready for seawater transfer. This test is generally used alongside the Smolt Index and the K factor (described above) to measure development of smoltification in each population This invasive test requires sending a gill biopsy to a commercial lab for analysis (ANALYTIQ (pharmaq.com))
Scoring	Gene expression is compared between PB and control groups, looking for significant deviations in development of smoltification between groups.
Source	(Handeland et al., 2014; Khaw et al., 2021)

Additional environmental tests that require further development for use in a Welfare Assessment setting (see Appendix IIIa for further details):

Behavioural fever

Health

This domain contains indicators that assess the health and injury status of the animals

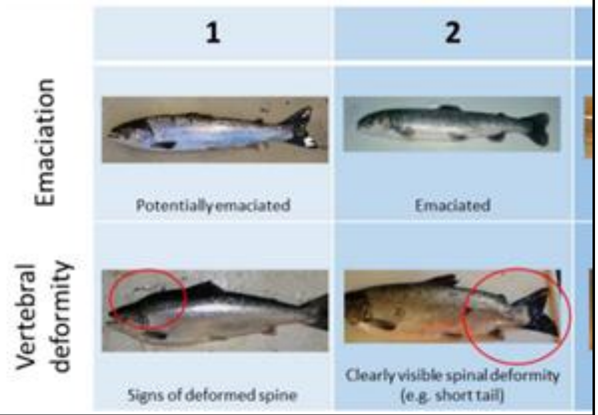
Egg quality -basic

Life stage	Egg
Aim of indicator	The eggs should be checked for development and viability. RSPCA mortality thresholds (shown below) are relevant to assessment of impacts of PB. RSPCA mortality threshold: Eggs to 1st feed (~10 weeks): not to exceed 6% mortality weekly
Scoring	Egg quality between PB and control groups should be compared. Any significant deviations should be recorded.
Source	RSPCA 2018

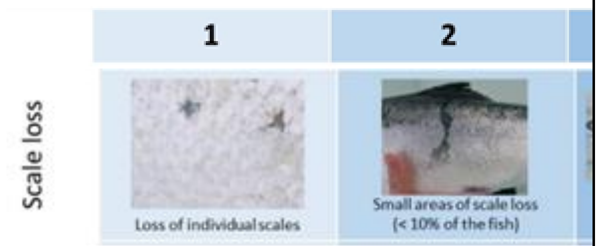
Mortality - basic

<table>
<tr><td>Life stage</td><td>Fry, parr, smolt, adult</td></tr>
<tr><td>Aim of indicator</td><td>Mortalities should be checked daily. RSPCA mortality thresholds (shown below) are relevant to assessment of impacts of PB.
RSPCA Thresholds:
Fry: not to exceed 3% weekly
Parr: not to exceed 1.5% weekly
Smolt: not to exceed 1.5% weekly
Adult:
<table>
<tr><td>Site average weight (g)</td><td>Max. Weekly mortality (%)</td><td>Ma</td></tr>
<tr><td>Under 750</td><td>1.5</td><td></td></tr>
<tr><td>750+</td><td>1.0</td><td></td></tr>
</table></td></tr>
<tr><td>Scoring</td><td>Mortality between PB and control groups should be compared. Any significant deviations should be recorded.</td></tr>
<tr><td>Source</td><td>RSPCA 2018</td></tr>
</table>

Body condition and spine - basic

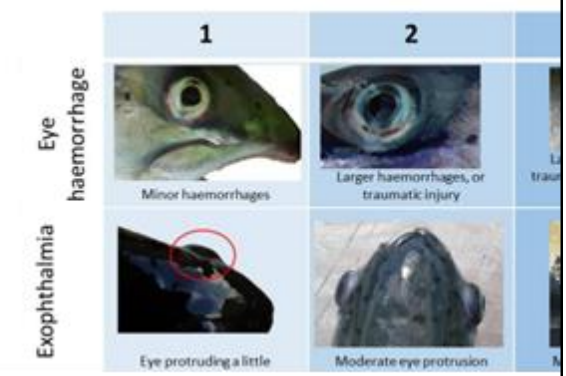
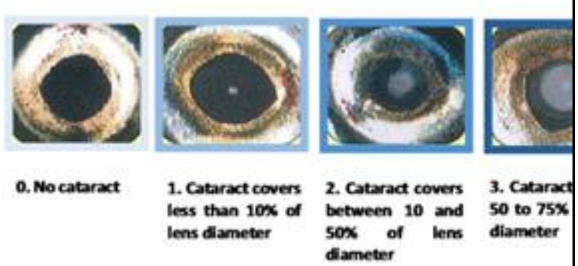
Life stage	Parr, smolt, adult
Aim of indicator	This measure should be used to assess the body condition and presence of any physical abnormalities or deformities, including indication of malnourishment. **Severity** 1: Signs of deformed spine, potentially emaciated 2: Deformed spine, malnourished 3: Extremely deformed spine, emaciated 
Scoring	Body condition and spine scores between PB and control groups should be compared. Any significant deviations should be recorded.
Source	(Noble et al., 2018)

Scale loss/ skin damage - basic

Life stage	Parr, smolt, adult
Aim of indicator	This measure should be used to assess skin condition, including scale loss. The presence, severity and frequency of scale loss and epidermal damage and wounds should be regularly monitored, especially as the fish approach smolt transfer. **Severity** 1: Loss of individual scales 2: Small area of scale loss (<10%) 3: Large areas of scale loss (≥10%) 

Scoring	Skin condition scores between PB and control groups should be compared. Any significant deviations should be recorded.
Source	(Noble et al., 2018) RSPCA 2018

Eye condition - basic

Life stage	Parr, smolt, adult
Aim of indicator	This measure should be used to assess eye status. Eyes are very vulnerable to mechanical trauma, leading to haemorrhages or desiccation during handling. Exophthalmus (“pop eye”) is often a non-specific sign of disease while cataract or loss of transparency of the eye lens can be caused by number of factors and is more frequent in later life stages, such as smolts and post-smolts. **Severity** 1: No damage 2: Loss of one eye 3: Loss of two eyes  **Cataracts** 
Scoring	Eye condition scores between PB and control groups should be compared. Any significant deviations should be recorded.

Source	(Noble et al., 2018) RSPCA 2018

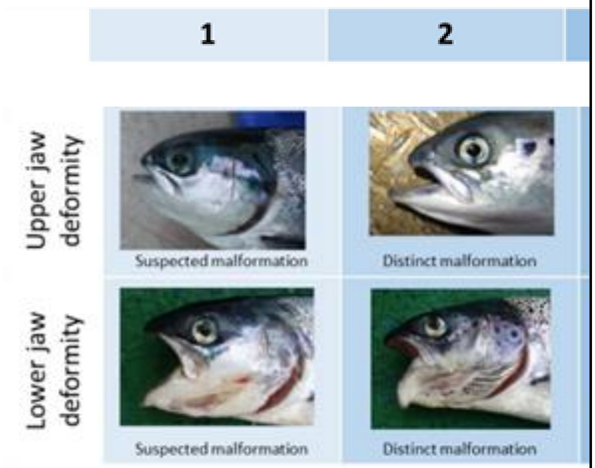
Mouth/jaw deformities and wounds -basic

<table>
<tr><td>Life stage</td><td>Parr, smolt, adult</td></tr>
<tr><td>Aim of indicator</td><td>

This measure should be used to assess mouth deformities and wounds.

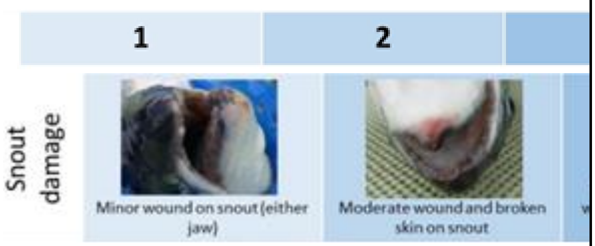
Severity

1: Suspected malformation of jaw
2: Distinct malformation
3: Major malformation



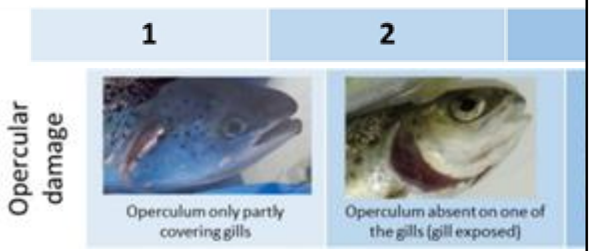
Severity

1: Minor wound on snout
2: Moderate wound and broken skin on snout
3: Large deep and extensive wound. Can cover the whole head



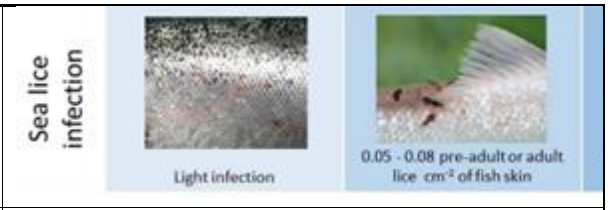
</td></tr>
<tr><td>Scoring</td><td>Mouth/ jaw deformity scores between PB and control groups should be compared. Any significant deviations should be recorded.</td></tr>
<tr><td>Source</td><td>(Noble et al., 2018; RSPCA, 2018)</td></tr>
</table>

Opercular damage -basic

Life stage	Parr, smolt, adult
Aim of indicator	This measure should be used to assess opercular damage and gill status, and includes shortening, lack of opercula, warped opercula and “soft” opercula. It is particularly applicable to early life stages in fresh water phase and can be caused by suboptimal rearing conditions and dietary deficiency. Gill bleaching and gill status should also be monitored in relation to turbidity and TSS. **Severity** 1: Operculum only partly covering gills 2: Operculum absent on one of the gills 3: Both operculum absent (both gills exposed) 
Scoring	Opercular scores between PB and control groups should be compared. Any significant deviations should be recorded.
Source	(Noble et al., 2018) RSPCA 2018

Disease: Sea lice infestation –basic

Life stage	Parr, smolt, adult
Aim of indicator	This measure should be used to assess the prevalence of sea lice infestation. **Severity** 1: Light infection 2: 0.05 – 0.08 pre-adult or adult lice cm^2 fish skin 3: ≥ 0.08 pre-adult or adult lice cm^2 fish skin 

	
Scoring	Sea lice scores between PB and control groups should be compared. Any significant deviations should be recorded.
Source	(Noble et al., 2018)

Pathogen/ bacterial challenge test - Enhanced

Life stage	Parr/ Smolt/ Adult
Aim of indicator	The aim of this measure is to determine whether PB fish are more or less resistant to common diseases of fish compared to the control group. Pathogen challenge tests are commonly performed in salmon production to assess the resistance and immune response to specific pathogens. These tests help identify the presence of diseases and evaluate the

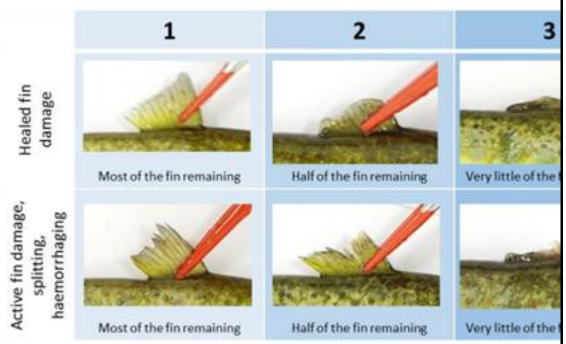
	effectiveness of preventive measures, such as vaccines or management strategies. Used here, this test would assess the immune response, disease resistance and the survival of PB fish exposed to specific pathogens, compared to control group responses. Challenge tests are conducted under controlled laboratory or research conditions to ensure accurate and reproducible results. Fish groups are typically exposed to a controlled dose of the pathogen, either by injection, immersion, or cohabitation with infected fish. Control groups are also included to compare the response of unchallenged fish. Definitive diagnosis often entails tissue sampling and off site analyses, but some of the external signs of disease or conditions that pose a welfare risk can also be diagnosed on farm by experienced personnel.
Scoring	Record mortality rates, signs of disease, immune response markers, and pathogen load in different tissues for each animal in the PB and control group. Identify any significant deviations between the two groups.
	(Noble et al., 2018)

Behavioural interactions

This domain refers to the ability of the animal to interact appropriately to the environment and to other animals.

Although included under the Environment domain, swimming performance and the startle test will also form part of the assessment of behaviour.

Dorsal fin damage - basic

Life stage	Parr, smolt, adult
Aim of indicator	The aim of this measure is to determine whether fish have suffered from dorsal fin damage, resulting from aggression from conspecifics, and whether this is more or less likely in the PB salmon compared to controls. Aggression and biting can be a problem in salmon parr and can be qualitatively or quantitatively monitored by visual observation of the fish. A better, less labour intensive proxy for monitoring aggression is to note the number of fish with dorsal fin damage, as there is a clear correlation between biting and dorsal fin damage in salmon parr. Changes in the levels of dorsal fin damage are most likely related to inappropriate feeding regimes or underfeeding, although other factors may play a role (e.g. handling, water velocity and flow). **Severity** 0: Normal 1: Slight damage, most of the fin remaining 2: Moderate damage, half of the fin remaining 3: Severe damage, very little of the fin remaining 
Scoring	Dorsal fin damage scores between PB and control groups should be compared. Any significant deviations should be recorded
Source	(Noble et al., 2018)

Additional behavioural tests that require further development for use in a Welfare Assessment setting (see Appendix IIIa for further details):

Foraging behaviour – Enhanced+

Food Anticipatory Behaviour – Enhanced+

Mental state

This domain refers to the quality of the emotional state of the animal and its ability to experience positive and negative emotional states. Several of the assessments in the 'Behavioural interactions' section

Qualitative Behavioural Analysis (QBA) - enhanced

Life stage	Smolt/ Adult
Aim of indicator	QBA is used to assess the mental experience of animals. Groups of salmon are observed for 1 minute and scored according to a species specific list of terms (Jarvis et al., 2021) QBA uses an observer assessment of the overall behavioural profile of the animal to form a judgment about its welfare (Wemelsfelder et al., 2001) . The method has recently been successfully used in salmon (Jarvis et al., 2021, Weise et al., In prep) . If this method can be further explored and validated for fish, it could form a powerful on-farm method of assessing fish welfare.
Scoring	Use terms from Jarvis et al 2021/Wiese et al (in prep) and compare scores between PB and control groups. Report any significant deviations from control groups.
Source	(Jarvis et al., 2021; Wemelsfelder et al., 2001; Weise et al., In prep)

Further work

Further work is required to determine how many animals to assess in both the precision-bred and control line groups. It is possible that differences in scores will be detected between the precision-bred and control groups. Consideration is needed to determine the limit at which any negative effects on the precision-bred population is acceptable, and how positive effects (e.g., reductions in disease) might be traded off against any potential negative effects (e.g., improved disease resistance but reduced activity).

The Precision Breeding Act states that 'An application under this section must include a declaration that the notifier does not expect the health or welfare of the relevant animal or its qualifying progeny to be adversely affected...by any precision bred trait ("an animal welfare declaration"). How many generations of animals that should be assessed, and how and where they are assessed needs to be addressed but is outside the scope of this project.

The longer-term monitoring of welfare and performance of animals in commercial settings where the environment and management maybe different to nucleus herds or research facilities, and the impact of the crossing the gene into different genetic backgrounds also needs to be considered but is beyond the scope of this project.

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(16) (PDF) Salmon Welfare Index Model (SWIM 1.0): A semantic model for overall welfare assessment of caged Atlantic salmon: Review of the selected welfare indicators and model presentation. Available from: https://www.researchgate.net/publication/236270790_Salmon_Welfare_Index_Model_SWIM_10_A_semantic_model_for_overall_welfare_assessment_of_caged_Atlantic_salmon_Review_of_the_selected_welfare_indicators_and_model_presentation [accessed Jun 29 2023].

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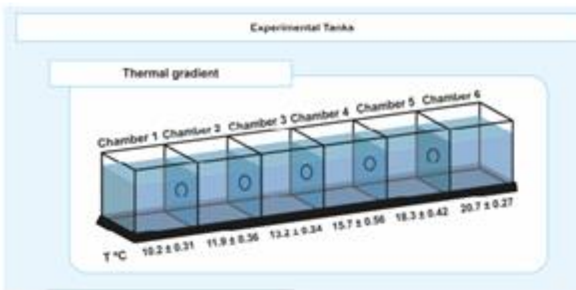
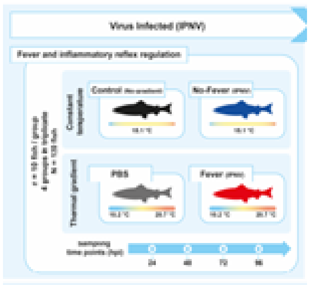
Appendix IIIa. Additional tests that may require further research to be practicable in an on-farm welfare assessment context

Environment Domain

Behavioural thermoregulation - Behavioural fever. Enhanced+

Life stage	
Aim of indicator	The aim is to determine whether fish are responding appropriately to thermal changes/ temperature gradients in the presence of a pathogen. This test will cross over with the Health domain, as this also a measure of pathogen response/ susceptibility. Fish will move to warmer water when they detect a pathogen, thereby increasing their body temperature (otherwise known as behavioural fever); this can stimulate a strong immune response, allowing the fish effectively to cure themselves of the responsible pathogen. Under controlled laboratory settings, PB and control groups are provided with a thermal gradient environment during infection (with e.g., Infectious pancreatic necrosis virus (IPNV)). Thermal tanks (between 10 and 20 ◦C) are provided to infected fish. Uninfected fish are used as a control   (adapted from Sanhueza et al., 2021)
Scoring	Following Sanhueza et al,. 2021, infected salmon parr shift thermal preference to 18 and

	20 ºC over a 96 h period (compared to 13 to 15 ºC in uninfected fish). Compare the response in infected PB and control groups and check for any deviations from this expected thermal preference range in response to infection. Note the number of mortalities (if any) in infected groups.
Source	(Sanhueza et al., 2021)

Behavioural interactions domain

Foraging behaviour – Enhanced+

Life stage	Parr, smolt, adult
Aim of indicator	The aim of this test is to assess foraging behaviour of fish. Currently, no test exists that has been validated for use in an on-farm setting. However, this could be part of sibling group testing, in a similar manner to the disease resistance/susceptibility testing. This test measures the speed with which fish resume feeding after a stressor (e.g., moving/ handling/ transportation/ grading). This can be used as a foraging-related welfare indicator of stress responsiveness, or ability to recover from stress. This has been conducted in the salmonid rainbow trout, and similar indications would be expected in salmon (Øverli et al., 2006) .
Scoring	Expose groups of fish to stressor and record time to resume normal feeding. Time to resume normal feeding of PB population is compared to that of control groups, check for any significant deviations from base line/ control groups
Source	(Øverli et al., 2006)

Food Anticipatory Behaviour – Enhanced+

Life stage	Parr, smolt, adult
Aim of indicator	The aim of this test is to assess anticipatory behaviour in response to feeding, or to sounds or signals associated with feeding. A good feed anticipatory response and feed intake can be signs of high feeding motivation indicating unstressed fish. Anticipatory behaviour is therefore accepted as a good indicator of health and coping ability (Martins et al., 2012) . In aquaculture cages and tanks, this can be shown as a high concentration of fish near the feeding area and increased schooling activity (Juell et al. 1994, Chen and Purser 2001). Currently, no test exists that has been validated for use in an on-farm setting, as this test requires husbandry staff to train the fish over time to associate a cue with access to food. The following procedure could be used in a lab under controlled conditions to quantify and compare anticipatory behaviour between PB and control groups. To assess anticipatory behaviour, fish are conditioned using a delay conditioning regime. For example, flashing lights can be used as a CS (conditioned stimulus) (Bratland et al., 2010; Folkedal et al., 2011) , and a food reward is used as the US (unconditioned stimulus). A flashing light is delivered, followed by the delivery of the food reward. This regime is conditioned over several trials across several days (e.g., 7 trials per day, over 8 days). Alternative cues could be used as the CS, for example visual or acoustic cues.
Scoring	Fish density in the feeding area can be quantified and compared between groups. And/ or, number of trails to reach learning association can be compared between groups. Following Bratland et al.,2010; Folkedal et al., 2011

Source	(Bratland et al., 2010; Chen et al., 2019; Folkedal et al., 2011; Juell et al., 1994; Martins et al., 2012)

Appendix IV

Case Study: Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) resistance

Site of edit: Cluster of differentiation 163 (CD163) gene

Role in PRSSV infection: PRRSV infects alveolar macrophages by binding to CD169 viral receptors. The virus is transferred into the cell by receptor-mediated endocytosis and then 'uncoated' by CD163 in the endosome, which releases the virus into the cytoplasm. Subsequently CD163 has been shown to be defined as the receptor/gate-keeper for PRSSV, as absence of CD163 confers PRRSV resistance (Whitworth et al., 2016, Nature Biotechnology 34, 20-22).

CD163 gene: Macrophage scavenger receptor for haemoglobins, exclusively expressed by cells of monocyte/macrophage heritage. In humans CD163 has been shown to be involved in clearing haemoglobin from circulation following tissue injury, increased CD163 expression is induced by anti-inflammatory mediators, such as interleukin-10 and glucocorticoids, suggesting an anti-inflammatory role and is important in wound-healing. The virus binds to the cysteine-rich domain 5 region of the receptor, and editing in the exon 7 region of the gene deletes subdomain 5 and prevents PRRSV binding. This is suggested not to affect other functions of CD163 (Burkard et al., 2017). CD163 has 8 other domains, which play a role in anti-inflammatory processes and infection with other pathogens but no other function has yet been shown to be associated with domain 5 except PRRSV infection.

Comparison of CD163 domain 5 edited pigs with wild-type: Growth rates (birth weight and 60 d weight) and blood counts (hematocrit, red blood count, mean corpuscular volume, serum haptoglobin are considered normal (2 homozygote/biallelic and 2 heterozygotes compared: Burkard et al., 2018; 6 wild type and 6 CD163-modified animals compared, Chen et al., 2019). It is stated that size, stature and morphology were equivalent to wild-type siblings but no data shown (photos of wild type and edited pigs are shown). The study suggests that no adverse effects are seen in edited pigs when maintained under standard husbandry conditions (Burkard et al., 2018), although this was a specific pathogen free unit from 6 weeks of age. This research group also state that they have bred three generations of edited animals over 10 litters and not observed breeding abnormalities.

Potential specific impacts on GE CD163 pigs: Although no detrimental phenotypic outcomes have been reported in homozygote pigs derived from edited animals, the data suggest that a comprehensive screening for specific issues that might be related to the CD163 edit has not been carried out. The proposed list of welfare indicators for pigs (Appendix A) include health screening, which would be useful in detecting an increased risk of specific types of disease or injury in these pigs. The assessments of growth, development and health outcomes could detect if there has been a significant change in the incidence of important welfare conditions in GE pigs. In addition, it may be necessary to investigate if the known biological functions of CD163 have been impaired by edits to domain 5. Since the gene is implicated in inflammatory disease and wound healing these may need to be specifically considered in a welfare declaration, and indicators included that allow these potentially deleterious impacts to specifically ruled out.

1. *Reduced ability to deal with inflammatory diseases*

Pigs are susceptible to a number of endemic inflammatory diseases. These include pleuritis and pneumonia-like respiratory disease, mastitis and metritis, joint ill (mycoplasma arthritis in young pigs) and osteoarthritis, the most common cause of painful lameness in adult pigs.

The proposed basic measures of growth and body condition would indicate if there was a pronounced and persistent difference between edited pigs and their wildtype conspecifics, and the gait scoring proposed as a measure of mobility would also detect increased lameness in adult pigs. For CD163 edited pigs assessing lameness in younger pigs (using a similar score), and assessment of coughing, respiratory difficulty (shallow, rapid breathing) and loss of appetite would be required to assess risks of inflammatory respiratory disease. Presence of respiratory disease and reproductive inflammation should be checked in sows. Specific indicators for these conditions are generally presence/absence of clinical conditions but should be scored frequently (5 minutes, at least weekly) to determine the severity and duration of the condition. Proposed indicators are (after Welfare Quality Pigs®):

Indicators of inflammatory endemic disease in pigs

Condition	**Animal class**	**Description**	**Scored**
Coughing	Sows, piglets, growers/finishers	Coughing more than once in a 5 minute observations period	Individual animal: 0: no evidence of coughing 1: persistent coughing
Respiratory difficulty	Sows, piglets, growers/finishers	Rapid shallow breathing (more than 28 breaths per minute in sows, or 55 beaths per minute in piglets), or heavy and laboured where movement of chest easy to detect, observed for 5 minutes	Individual animal: 0: normal breaths 1: rapid shallow breaths or laboured

Loss of appetite	Sows, piglets, growers/finishers	Feed intake measures as described in indicator list	Individual animal:
Mastitis	Sows	Presence of swelling and redness, udder is hot and hard on palpation	Individual animal:
Metritis	Sows	Presence of milky white discharge at the vulva.	Individual animal:

2. *Impaired wound healing*

CD163 plays a role in wound healing and gene-edited pigs may have an impaired and delayed wound healing. Lesion scoring as identified in the main welfare declaration document should be carried out but where a lesion is detected then this should be inspected daily until the wound has healed and compared to similar assessments of non-edited pigs.

If tail docking is carried out then Wound Freshness scoring should be carried out daily until wounds have healed as:

Wound Freshness

0: No Wound
1: Intact scab or healed old wound.
2: Not intact scab – older blood, red tissue
3: Fresh bite/scratch or wound, not bleeding or weeping
4: Fresh bite/scratch or wound – weeping but not bleeding (can include bloodied, blood stuck to tail hair)
5: Fresh bite/scratch or wound – bleeding – dripping with blood & splattering own bottom or other pigs.

3. *Greater carcase bruising and/or response to soft tissue injuries*

As CD163 is involved in clearing haemoglobin from circulation following tissue injury potentially edited pigs may have slower healing from soft tissue injuries that may occur during life or during transport, handling and slaughter. In live pigs the lesion scoring scheme should be carried out in grower and finisher pigs weekly, and daily if a lesion of score 1 or 2 is detected to determine the time taken for lesions to disappear:

- **0** No Lesion - Pigs without any of the below body marks
- **1** Mild - Pigs with mild body marks. Linear lesion longer than 10cm or if there are 3 or more 3cm lesions or if there is a circular area larger than 1cm diameter
- **2** Severe - Pigs with severe body marks. Lesion is larger than 5x5cm diameter, or lesion extends into deeper layers of skin, or lesions cover a large percentage of skin (>25%).

Carcase bruising post-mortem can be scored by standardised scoring methods (Faucitano, 2001), scoring from 0 (no blemishes) to 4 (severe). Comparing the severity of carcase lesions from pigs carrying the CD163 edit with their wildtype counter parts will help to determine if edited pigs have a higher frequency of lesions or if these persist for longer.

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Appendix V

Case Study: Avian Influenza Virus (AIV) resistance

Site of edit: *Acidic nuclear phosphoprotein 32 family member A (ANP32A) gene*

Role in AIV infection: AIV infects the cell by binding to the cell surface and using the host cellular proteins to evade host restriction factors to replicate its genome. ANP32 genes are evolutionarily widespread but species differences exist between mammals and avians (Long et al., 2016). In chickens the AIV relies on ANP32A to support activity of AIV polymerase and hence its replication (Park et al. 2019). In vitro studies in primordial germline chicken cells demonstrate that gene-edits to remove a 33 amino acid insertion in the ANP32A gene prevented replication of AIV (Long et al. 2019). No published studies of birds with these edits have yet been produced.

ANP32A gene: Proteins derived from the ANP32 family of genes are implicated in a number of processes including intracellular signal transduction, nucleocytoplasmic transport and apoptotic processes (https://www.ncbi.nlm.nih.gov/gene/8125#pathways). They are widespread in eukaryotes, and have broad cellular distribution – leading to proposed broad range of biological functions: cell signalling and transduction, transcriptional regulation, intracellular transport regulation, proliferation, apoptosis, neuronal development and cancer (Yu et al., 2022). In mammals ANP32A is related to occurrence of nervous system disease (e,g, elevated in Alzheimer's disease in humans), and to failure of embryonic development in mice. ANP32A is also implicated in tumorigenesis and inhibits the activity of oncogenes in rat embryos, although it may also be a pro-oncogene in some cancers.

Comparison of ANP32A chickens with wild-type: To date there is no published literature on the development of live birds following gene-edits for ANP32A although this seems the logical next steps in developing AIV resistant birds.

Potential specific impacts on ANP32A gene-edited chickens: As summarised above, and discussed in more detail in Yu et al. (2022), proteins produced by ANP32A are widespread and involved in many biological processes, in addition to their role in the replication of AIV. This makes it challenging to suggest specific welfare measures or indicators that could be required to ensure that gene-edited birds are able to maintain appropriate standards of welfare. The broad approach we have taken in suggesting indicators across all Five Domains of welfare is therefore important as a targeted approach to focus on any potential welfare challenges is not possible.

Given the known impacts of ANP32A in neurological development and early life development (albeit in a mammalian model) then the behavioural indicators of early life development and cognitive abilities seem appropriate to ensure that PB chicks are not developmentally impaired in comparison to their wildtype counterparts (e.g. hatchability, crop fill, chick viability measures, thermal competence, activity/resting patterns, growth). The role of

ANP32A in tumorigenesis suggests that health checks, maintenance of body weight and mortality records, particularly of older adult birds (e.g. broiler breeders or laying hens) should also be relevant to ensure there is not higher mortality and morbidity from these causes in gene-edited birds.

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Appendix VI

Case Study: Myostatin disruption using CRISPR/Cas9 to enhance muscle mass in Atlantic salmon (*Salmo salar)*

Introduction: Myostatin (MSTN), which suppresses muscle growth and is famous for its role in 'double-muscled' cattle (such as the Belgian Blue), has been edited by several research groups in many species, including fish, although much less focus has been applied to fish models. Here we use what is known in the current literature and present a hypothetical case study to investigate the possible welfare outcomes of disruption of candidate MSTN gene/s (currently unidentified) affecting muscle growth in Atlantic salmon.

Role of myostatin: Myostatin (MSTN), also known as Growth and Differentiation factor 8 (GDF-8), is a member of the transforming growth factor β (TGF-β) superfamily, and functions as a negative regulator of skeletal muscle mass through the cell surface receptor, activin receptors type II (ACVR2) (1,2) . Mammalian MSTN is exclusively expressed from one gene copy and limited to skeletal muscle. In contrast, two or four copies have been found in fish (3) and these genes are differentially expressed in many tissues such as muscle, eye, stomach, skin, brain, gonads, kidney, intestine, liver, spleen, gill and heart. It could therefore be expected that MSTN plays a more diverse role in fish than in mammalian species. For example, in some fish species MSTN is reportedly involved in both growth performance and the immune response (4,5) .

Naturally occurring mutations of myostatin are observed in many mammalian species (e.g., sheep (6) , goats (7) , chickens (8) , and dogs (9)). Gene editing of the myostatin gene in mammalian models inhibits the production or function of myostatin using gene knockout (10) or overexpression of inhibitors (13) , which results in increased muscle mass. MSTN-deficient animals exhibit visibly distinct muscular hypertrophy or hyperplasia, commonly known as the double-muscled phenotypes (10) , typified by the conformation of Belgian Blue and Piedmontese cattle (11,12) .

Comparison of MSTN gene-edited salmon with wild-type: In comparison to mammalian models, there has been much less focus on the editing of myostatin genes in fish. To date, there has been no literature published on the development of salmon following gene-edits for MSTN. Several research groups have edited MSTN in other fish species, including (but not limited to) sea bream (14,16) , catfish (17–19) , common carp (15) , and flounder (20) . CRISPR/Cas9 editing of the myostatin gene (mstnba) in the common carp, showed increased muscle mass in F0 founders (15). In contrast, inactivation of mstna (19) and mstnb (18) in yellow catfish displayed no significant increase in muscle growth in either F0 or F1 generations. The discrepancy in response between common carp and yellow catfish suggests functional variation in the gene among different species.

In sea bream, myostatin mutations (Pm-mstn) using CRISPR/Cas9 were formed by deletions in the first exon of the Pm-mstn, which cause disruption of the C-terminal active domain of MSTN. The edited animals exhibited a 16% increase of skeletal muscle mass. The authors established a homozygous gene-disrupted strain of fish in 2 years, which is far shorter than conventional breeding methods allow. Their study suggests that genome editing can accelerate the speed of aquaculture fish breeding in this species (14) . The authors further showed that important characteristics of aquaculture production, such as weight gain, specific growth rate, and feed efficiency were significantly higher in the edited animals compared to wild-type fish, with no significant difference in the daily feed intake (16) . Edited fish in this study fed similarly to that of wild-type fish during the juvenile stage, showing a higher ability to convert feed efficiently and accumulate ingested protein, resulting in better overall growth.

Potential welfare impacts in MSTN gene-edited salmon:

In terms of increasing commercial productivity, increasing growth is valuable in economic terms (e.g., reduction in feed costs, reduced time to slaughter for example), but could have negative implications for fish welfare. Disruption of myostatin can result in a number of health and welfare complications leading to potential welfare concerns in mammals (21), such as compromised mobility, increased dystocia, and musculoskeletal issues.

It would seem intuitive that enhanced growth could have some similar associated developmental complications in fish to those observed in mammal models (21) . In common carp, for example, analysis showed that myostatin knockout mutants had severe bone defects (15) , which would impact the welfare of these animals throughout various life stages. In CRISPR/Cas9 MSTN-edited zebrafish (mstna and mstnb), deletion of mstnb gene enhanced growth performance, however mortality following a stressor event (exposure to an immunosuppressant) increased by ∼30% compared to wild-type fish. This resulted from decreased transcription of several critical immune-related genes in the edited zebrafish. The edited fish also exhibited lower pathogen tolerance (towards pathogenic *E. tarda*) compared to wild-type fish. These results indicate that mstnb plays a key role in zebrafish muscle growth, while knocking out the gene had severe effects on immune function.

Potential welfare indicators:

The potential impacts of MSTN gene edited salmon have not been explored to date. This makes it challenging to suggest specific welfare measures or indicators that could be used to ensure that appropriate standards of welfare can be maintained for gene-edited fish. Based on findings from other fish species, we can suggest targeted approaches for welfare assessment of gene edits of MSTN in the future. In addition, we also recommend taking a complimentary broad approach and suggest assessment of welfare indicators across all Five Domains of welfare for completeness (see Welfare Indicator List for Salmon from the present project).

Given the known impacts of gene-edited myostatin in other species of fish, (e.g., bone development and immune function), and the outlined issues in mammalian models (muscloskeletal issues), enhanced use of indicators from the health and nutrition domains seem appropriate to ensure precision-bred fish are not developmentally impaired in comparison to their wild-type counterparts (e.g., standard growth rate, feed conversion rate, swimming performance, morphological welfare indicators (with emphasis on spine development), and pathogen load tests). As the response to an immunological challenge specifically indicated an impact on immune function in zebrafish, the response to disease challenge should be specifically monitored, as well as conducting frequent health checks, and monitoring mortality and maintenance of body weight to ensure that mortality and morbidity has not been increased in precision-bred fish.

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