Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 29

Projects with a primary purpose of: Translational and Applied Research - Animal Welfare

Project Titles and keywords

1. **Validation of welfare indicators for Laying Hens**
    - Animal welfare, chicken, validation

2. **Validating humane killing of young ungulates**
    - Casualty, Slaughter, Percussion, CPK200, Stun/kill

3. **Monitoring of Disease and Welfare in Production Animals**
    - Farm animal, welfare, disease, production, health

4. **Study to improve the electrical stunning of birds**
    - Electrical stunning, turkeys, ducks, geese, chickens

5. **Nociception in Fish**
    - Animal welfare, behaviour, neurobiology

6. **Welfare of poultry under different housing and management practices**
    - Poultry, Housing, Management, Stress

7. **Pig performance, digestibility and bone strength**
    - Performance, minerals, digestibility, bone strength

8. **Ornamental fish nutrition and health**
    - Fish, nutrition, health

9. **Use of systems and genetics data in dairy management**
    - Dairy cattle genetics systems

10. **Neurophysiological and behavioural determination of effective stunning in fish**
    - Fish, slaughter, stun, EEG

11. **Effects of housing and management on the welfare of dairy cattle and calves**
    - disease, cold stress, stocking density, negative emotional states

Project 1	**Validation of welfare indicators for laying Hens**	
Key Words (max. 5 words)	Animal welfare, chicken, validation	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	As humans, we cannot assume we know what matters to animals of other species. The best approach is to assess the valence (the animal's perception of how positive or negative a situation is) using careful tests of preference or aversion. However, it is not feasible to use the same methods to audit and assess animal welfare on farms as the process would be far too difficult. The aim of this work is to establish which simple measures of behaviour, physiology or body condition are strongly linked with valence perception. These measures can then be used as validated welfare indicators to assess animal welfare in routine situations.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	It is very important to assess animal welfare fairly and objectively so that welfare problems on farms can be identified and solutions can be implanted and tested. This work will provide new tools for people undertaking welfare audit roles, including farmers, vets and people conducting audits on behalf of NGOs or retailers.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will use chickens because the welfare of farmed chickens remains an issue of great concern to the public. We will use approximately 312 chickens over a period of 5 years.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We propose to house a proportion of chickens in environments that approximate to average to poor farming conditions (with stimuli that we know are mildly aversive) and take a full range of measurements of behaviour, physiology and body condition. These will be compared with chickens that are housed in environments that approximate to the best that could possibly be provided under farming conditions. The likely severity is mild. The majority of birds will be re-homed to people who want to keep a small number of backyard chickens. A minority of birds will be killed for post-mortem analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The work is specifically designed to establish indicators of welfare for chickens, and so can only be conducted on chickens.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have conducted previous work and so we know how variable the responses of the chickens are likely to be. This enables us to use the minimum number possible whilst still obtaining meaningful results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The work is specifically designed to establish indicators of welfare for chickens, and we have decided not to use any aversive stimuli that would be harsher or more severe than those likely to be encountered on real commercial farms. Our general aim is to ensure that the aversive environments and stimuli presented remain at a mild level – we do this by introducing stimuli gently and carefully and observing initial reactions. If birds appear overly frightened or distressed we tone down the stimuli but if no reaction, then we increase to point where mild aversion is detected.

Project 2	**Validating humane killing of small ungulates**	
Key Words (max. 5 words)	Casualty, Slaughter, Percussion, CPK200, Stun/kill	
Expected duration of the project (yrs)	1 year	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	√	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	**Summary:** A surplus or unhealthy goat kids and lambs need to be humanely killed on farm. Legislation requires that this is carried out with "no unnecessary suffering pain or distress." Casualty or surplus slaughter of young animals on-farm is usually carried out by administering a blow to the head, which is generally performed by swinging the young animal against the floor or a wall. Previous research has demonstrated that a mechanical stun/kill offers an acceptable method for the humane killing of neonates and that a non-penetrating percussive device shows less variability that penetrating devices. Measurement of neonate skull development also supports the use of a controlled percussive blow to kill neonate piglets. **Project plan:** It is proposed that a cartridge-powered gun (CPK2) that is commercially available will deliver a percussive blow of sufficient force to both stun and kill neonates. The cartridge-powered gun was first tested on neonate cadavers to determine the extent of the trauma produced by the percussive blow (CPK2). Following this initial trial, a total of two hundred animals destined for casualty or surplus slaughter of each species will be shot on-farm with the CPK2 percussive gun and assessed for signs of	

	effective stunning followed by death by experienced researchers. The carcass of each animal will be bagged and tagged and brought back to the laboratory for post-mortem examination. Statistical advice indicates that 200 experimental animals are required to validate the method on each species. The response from farmers has been very positive as they, in particular pig producers, are supportive of this research proposal.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The velocity developed by the gun with a range of cartridges will be measured in the laboratory to calibrate the system and to allow other manufacturers to produce an effective tool based on the experimental results. Following consultation with DEFRA, a final report will be produced and stakeholder meetings organised to promote the uptake of the final tool to farmers. A booklet will be produced outlining the correct use of the CPK2 with each species and distributed through the relevant livestock associations. This research will enable a humane method of slaughter for neonate piglets, lambs and kids to be produced for livestock farmers. The present methods that are available do not meet the requirements of a modern farming industry where the emphasis on animal welfare is paramount.
What species and approximate numbers of animals do you expect to use over what period of time?	The project work on 202 piglets has been completed under the previous project licence. Research on an additional 39 lambs and 108 kids are required to complete the project over the next 4 months.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	No adverse effects are expected as the force delivered should exceed that needed to kill the animal instantly. In the event that an animal is stunned but not killed using the percussive device then the animal will be killed immediately using a schedule 1 technique.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The purpose of the outlined work is to complete the assessment of a device for the killing of neonate pigs, sheep and goats started under PPL 30/2999. It is not possible to complete the study without the use of living animals. All of the animals used in the study will be either animals destined to be killed on the grounds of casualty slaughter or routine farm management.
2. Reduction	In determining the number of animals needed we

Explain how you will assure the use of minimum numbers of animals	have based our assumptions on exceeding a 95% confidence level. To achieve this will require a group size of 200 animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The species (and age) chosen are those where there is a need to improve the current methods of slaughter. All of the animals to be used in this study are destined to be killed on the grounds of casualty slaughter or routine farm culling. Consequently no animals will be killed solely for the purpose of the study. Participating producers will be instructed that animals that are suffering any pain and distress must not be held back and kept alive for this project but must be humanely dispatched as soon as is practicable. Furthermore the method of killing to be used is likely to be more reliable than that which would otherwise be used. Based on the work conducted today all animals are expected to be rendered immediately unconscious by application of the stun, and the vast majority will be killed outright, in a small number of case it may be necessary to kill the animal using a schedule 1 method at the end of the three minute post stun assessment period.

Project 3	**Monitoring of Disease and Welfare in Production Animals**	
Key Words (max. 5 words)	Farm animal, welfare, disease, production, health	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Farm animals kept to produce meat, milk, wool or other fibres, may become diseased and / or subject to welfare compromise during their life on farms. This project aims to provide a service whereby the data collected will allow veterinary surgeons and scientists to gain a better understanding of important (from commercial and animal welfare perspectives) production animal diseases. This may then lead to refinements and improvements in animal management and husbandry policies, treatment regimes and ultimately animal welfare.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	A reduction in disease in farmed animals will improve the efficiency of production, thus improving the returns for farmers and reducing wastage. This is important as efficient farming systems have a reduced impact on the environment in terms of greenhouse gas emissions and are financially more viable. Improved animal welfare and animal comfort will improve production as animals will be more likely to eat and drink normally and be less at risk of disease.	
What species and approximate numbers of animals do you expect to use	Cattle: up to 950 in total (largely dependent on case load in referral clinic)	

over what period of time?	Sheep: 580 Goats: 30 Camelids: 30 Pigs: 30 Poultry: 50
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse effects are expected to be minimal as the techniques proposed to be used are all very minor. However, adverse effects associated with production diseases are to be expected given that these studies will occur in commercial animals. Animal welfare is the paramount consideration and this study will not add to the likely adverse effects normally associated with a particular disease status. Alternatively, more prompt intervention as a result of the data collected may reduce the incidence or severity of adverse effects normally associated with a particular disease status. All animals are intended to remain alive at the site they are held at until they reach the end of their productive lives (i.e . the same as any other production animal species).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no alternatives to the use of live animals for the objectives of the proposed research program because intact, whole animals are required for the study of physiological responses and many body systems are involved which cannot be adequately reproduced in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For surveillance work, animal numbers are variable dependent on case-load. For other studies animal numbers have been calculated based on power calculations that predict sample sizes to obtain sufficient data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The species used are common production animal species in the UK and the wider world and therefore findings from this research are highly applicable internationally as well as nationally. Animal welfare is at the heart of what veterinary surgeons daily work entails. All proposed investigators are mindful of welfare and will take appropriate action if animals under procedure appear to demonstrate adverse reactions to the proposed work. All techniques listed in the project application are minimally invasive and are recognised daily activities of production animal vets in clinical practice.

Project 4	**Study to improve the electrical stunning of birds**	
Key Words (max. 5 words)	Electrical stunning, turkeys, ducks, geese, chickens	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The project aims to assess currently available head-only/head-to-body electrical stunning systems and develop new stunners/electrode types for turkeys, ducks, geese and chickens that improve stun effectiveness with respect to animal welfare, whilst minimising negative impact on meat quality, ensuring industry relevance.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Inadequate stunning resulting in the recovery of consciousness prior to, during or after bleeding will result in unacceptable suffering, pain and distress to the animal. This can be further compromised when an inadequate neck cut fails to sever both carotid arteries. The expected welfare benefits of the proposed study are the development and testing of new head-only stunning systems that improve the quality and duration of unconsciousness following head-only electrical stunning. Furthermore, the proposed study will examine whether or not head-only and/or head-to-body electrical stunning with modification can be a reliable stunning method for waterfowl. Stun guidelines will be developed for existing and new stunner designs that improve effectiveness and animal welfare. Finally, and crucially it will improve validity of practical measures used to assess insensibility in poultry and waterfowl.	

	The results from the proposed project would provide scientific evidence that could directly underpin legislation (UK, EU and worldwide). These findings would also have direct relevance to animal welfare NGOs (development of guidelines and training), other animal charities, poultry producers and stunner manufacturers.
What species and approximate numbers of animals do you expect to use over what period of time?	217 Turkeys 217 Geese 217 Ducks 434 Chickens (layer hens and broilers) Total 1085 birds over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The maximum severity expected is moderate. Potential adverse effects could include: • Stress from handling (low severity, high likelihood), this is unavoidable, but will be minimised when possible. • Pain/discomfort from positioning of electrodes (low severity and likelihood), this will be mediated by use de-sensitising cream. • Pre-stun shocks (medium severity and very low likelihood), which will be continuously examined for. Any shock will be transient before the induction of the unconsciousness. • Spinal seizures where the animal is conscious (moderate severity, very low likelihood). Will be continuously monitored for. If an animal is identified as having a spinal cord seizure it will immediately be euthanatised. • Distress associated with recovery from the stun (high likelihood and mild severity). This is unavoidable in recovery experiments. The assessment period will be kept as short as possible and the birds will be immediately restunned and dispatched after this period. • Infection from surgical implanting of electrodes (OPTIONAL) (low severity, low likelihood). All birds will be operated on using aseptic techniques and will be given antibiotics post procedure. Also the time period between surgery and electrical stunning will be no longer than 1 day, further reducing the risk of infection on welfare.

	Any birds that are showing signs of pain and distress during the instrumentation process will be immediately euthanised. Birds deemed to be in ill health will be removed from the study and euthanised. As the project is examining stunning/slaughter methods, all animals that enter the stunning/slaughter phase of the project will be killed during the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no meaningful way of assessing the performance of electrical stunning equipment with *in vitro*, anaesthetised or already dead bird preparations. Live birds are necessary, so they relate to standard commercial slaughter practices.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The sample sizes for the project are the minimum necessary for meaningful results, this was determined by statistical analysis of the results from a previous project in chickens. Where possible a stepped approach will be used, where the study will be stopped once the required results are generated.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Turkeys, ducks, geese and chickens are being used, as they are the species in which these stunning methods are currently or will be used commercially for slaughter for human consumption. Any birds that experience trauma not associated with the stunning method or have been deemed to not be immediately rendered unconscious will be euthanised.

Project 5	**Nociception in Fish**	
Key Words (max. 5 words)	Animal welfare, behaviour, neurobiology	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Research has sought to understand whether fish are capable of nociception or pain and the present study will determine what precise locations in the brain are active during noxious stimulation, the behavioural signs of pain and whether analgesics reduce these, whether fish seek access to pain killing drugs and whether stress modulates these responses. This project also aims to develop an automated means of assessing fish welfare that can accurately determine the health status of fish.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	How non-mammalian brains process nociception or pain is currently an unanswered question in neurobiology. We need a better understanding of how laboratory practices may result in nociception or pain and these studies provide new information on the detection and alleviation of pain in fish. These results will be relevant to improving fish welfare, developing analgesic protocols and may inform regulations produced by government and public bodies with respect to the treatment of fish.	
What species and approximate numbers of animals do you expect to use over what period of time?	Currently, the precise areas of the fish brain which process nociceptive information have not been characterised. This study will use neuroanatomy to determine specific areas of the brain activated by noxious stimulation. Fish will be noxiously stimulated	

	and humanely killed to obtain whole brain samples (up to 6 hours after treatment). These will be sectioned and stained for candidate genes and compared with non-noxious controls and fish given analgesia (n = 75 trout and zebrafish). Pain is not just a sensory response but is accompanied by feelings of discomfort. Therefore, to understand whether fish feel badly when noxiously stimulated they will be given access to an unfavourable area where they can access pain relief. Therefore, if fish have to pay a cost of being in an undesirable area this means they are willing to bear this cost to seek analgesia providing an insight into the fish's subjective state (n = 30 zebrafish and trout; up to 6 hours). A common phenomenon seen in mammals is stress induced analgesia whereby stressed animals show no signs of pain due to the release of endorphins. This has not been investigated in fish and would provide evidence that the fish nociceptive system is similar to that of mammals. Fish will be given a standard stressor prior to testing. The behaviour and physiology of noxiously stimulated fish will be compared with non-stressed fish as well as those given an opioid antagonist to block endorphins. Hormones and opioids will be compared with behavioural responses to determine whether stress induced analgesia occurs in fish (n = 72 trout and zebrafish). These short experiments (<24 hours) must be conducted on whole animals since they include behavioural reactions, therefore, non-animal alternatives are not viable. Sample sizes have been calculated using statistics to obtain the minimum number of animals needed to provide scientific meaning. The impact of environmental enrichment on the recovery from stress and nociception shall also be explored and may be used to improve laboratory husbandry procedures as well as enhancing welfare (n = 144 zebrafish; 72 guppies; 72 common clownfish and 72 golden sawfin goodeids; from 1 week to 2 years in barren or enriched conditions; experiments may last up to 5 weeks except for nociception which lasts for 6 hours).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Fish will experience mild discomfort or stress and show full recovery between 3 and 6 hours in our previous experiments. Animals resume normal behaviour and feeding upon recovery, therefore, these procedures are considered mild. Animals shall be humanely killed at the end of experiments.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project involves whole animal responses to nociceptive stimulation and as such live animals must be used. In order to investigate brain activity in response to a stimulus, the whole animal must be intact and it is currently not possible to use alternatives such as brain slices etc. Part of the project will assess in vivo neuronal activity in three transgenic lines of zebrafish larvae up to five days post hatch before they become protected. Although not a regulated procedure we aim to show larvae are a valid model for nociceptive testing to ultimately reduce the use of adults in the future.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Sample sizes have been kept to a minimum and have been calculated using Power statistics to ensure maximum scientific validity. We shall use randomisation when selecting individuals, blinding to assess videos and samples, and comply with ARRIVE Guidelines on reporting of experiments. The design is appropriate to test the hypotheses with control, sham treated groups and analgesic or antagonist groups. Principal components analysis shall be used to determine which measured variables are most indicative of the treatment groups then the top 3-4 variables shall be analysed further to understand the impact of treatment, time, enrichment and analgesia where appropriate. Assuming the data is normally distributed we shall employ a general linear model where the independent variables are greater than two factors (one way or two way ANOVA are more suitable if 2 or less) and shall adopt repeated measures to account for the same fish being sampled over time. A statistician has been consulted.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Relatively little is known in fish with respect to their capacity for pain yet they are subject to invasive procedures in a variety of contexts. Therefore, ethically we need to have more information on their capacity for pain perception to inform public and government body regulations with respect to the treatment and welfare of fish. In the behavioural experiments, if any animal shows more than mild responses, they shall be killed immediately by a humane method. With the concentrations of algesics used in past experiments, all animals recovered after the behavioural and physiological effects subsided and resumed normal feeding behaviour. Pain relief

	will be provided for many of the control animals. Therefore, there were no long-term adverse effects on the fish's behaviour but at the termination of all experiments, the fish shall be killed by a humane schedule 1 method. As animals usually recover within 6 hours, resuming normal activity and feeding, these procedures are considered mild.

Project 6	**Welfare of poultry under different housing and management practices**
Key Words	Poultry, Housing, Management, Stress
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Despite large changes in housing methods and legislation that governs the keeping of farmed animals, much is still unknown on how best to keep poultry in ways that both meet their behavioural and biological needs, while still making farming sustainable. For example, laying hens are still not commonly housed without beak trimming; broiler breeders are chronically food deprived (but physically healthy), without definitive evidence as to their overall welfare state; and different housing designs throw up benefits in some aspects of welfare but drawbacks in others, thus objective ways of measuring chronic stress from housing methods could be beneficial. This project licence would enable us to investigate ways in which to house and manage poultry that best meets their welfare needs while still working within viable methods of food production.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

This work may, in the long-term, enable us to house birds with less mutilations (e.g. beak trimming of laying hens), find better ways to feed broiler breeders (if current methods indicate chronic hunger and other methods do not) and identify what types of housing systems are the least chronically stressful for poultry (e.g. indoor versus free range).

What types and approximate numbers of animals do you expect to use and over what period of time?

In 5 years, we would use up to 10,000 birds (domestic chickens and/or turkeys). This is because we sometimes conduct our work under commercial conditions.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Some birds will experience mild to moderate discomfort due to the housing environment, but this will often be short term, or they will be allowed to make a choice of where to be. Short-term food or water deprivation will cause short-term hunger and thirst. Food and/or water composition are designed to restrict growth to reflect commercial levels of restriction in place for parent stock of meat birds, and not more. Mild aversive stimuli are designed to stimulate chronic stress, but this will be designed to reflect stress seen in commercial systems and no more. Ironically, not beak trimming birds is regulated, because it can lead to greater feather pecking, cannibalism, and mortality, but this will be closely monitored and strict control measures will be in place. Where birds are beak trimmed for a study, only commercially-applicable methods will be tested. Physiological manipulations (e.g. hormones) will only be done within the natural range for the species. The maximum severity level is moderate, however in reality most birds will experience mild severity. Some birds will be humanely killed at the end of a procedure for tissue collection. Where possible, birds will be rehomed (e.g. laying hens), or they will be released from the Act and culled as per normal farming practice.

Application of the 3Rs

Replacement

Behaviour and welfare studies need to study the whole animal and their responses to their environment.

Reduction

We will base our animal numbers on prior experience and after discussion with a statistician, to ensure that the number of animals used is neither too high nor too low. We use optimal experimental designs and analyses that take account of all sources of variation, to get the maximum information from our studies. The use of commercial conditions impacts on the number of animals that are used (e.g. they are typically higher than laboratory-based studies), however this means that the relevance of our work to agricultural industry is likely to be greater.

Refinement

Poultry are used because their housing and management systems (and how they respond) are the areas of interest. With every experiment performed, we carry out a harms/benefits analysis, and review these retrospectively to see how measures can be refined for future similar trials. These are requirements of our AWERB. This assists us in planning future trials to minimise welfare costs to animals.

Project 7	**Pig performance, digestibility and bone strength**	
Key Words (max. 5 words)	Performance, minerals, digestibility, bone strength	
Expected duration of the project (yrs)	Two	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	√	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim is to improve knowledge and understanding of how piglets respond to variations in provision of dietary minerals (phosphorus and calcium) and also exogenous enzymes that are added to promote mineral digestibility.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will identify levels of dietary phosphorus that optimise animal performance, without compromising bone strength, thus improving animal welfare and reducing the environmental impact of pig systems	
What species and approximate numbers of animals do you expect to use over what period of time?	Piglets, 128, two years.	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	The adverse effects are considered to be very minor and are associated with keeping animals individually housed, although they will have some visual, auditory, olfactory and tactile contact with each other,	

level of severity? What will happen to the animals at the end?	and withholding bedding during periods of faecal collection for digestibility measurements; the level of severity is mild. Animals will be euthanized at the end of the programme.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	No in vitro model is available allowing the evaluation of performance, mineral digestibility and bone strength in pigs fed specific diets. We have considered the use of less sentient animals (e.g. rodents) but data generated would not be applicable to pigs.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use power functions to estimate the minimum number of replicates necessary
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Pigs are the chosen species as the main objective of the programme is an assessment of performance, mineral digestibility and bone strength in this species; extrapolation from another species is not possible. This program of work will require the use of individual housing in pens, a procedure that has been used frequently in the past. Experience gained reveals that pigs acclimatise rapidly to confinement in these pens whose dimensions allow expression of normal behaviour Pigs will consume particulate bedding material that, because of its fibrous nature, will be substantially undigested and will accordingly be present in faeces thus compromising diet digestibility measurements. Thus, for that phase of the programme where digestibility is to be assessed, no particulate bedding material will be provided. Floors of pens are however considered to be sufficiently comfortable so as not to compromise health and welfare, as confirmed in previous studies. A rubber mat or alternative may be added to pens to provide a resting/lying area (e.g. when collection of urine and faecal output are not required).

	Welfare costs will be minimised by providing suitable conditions (e.g. temperature) and environmental enrichment. Access to both feed and water will be ad libitum.

Project 8	**Ornamental fish nutrition and health**	
Key Words (max. 5 words)	Fish; Nutrition; Health;	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project is focussed on enhancing the quality of nutrition and care given to ornamental fish, to result in an improvement in the health and well-being of these animals. Nutritional studies conducted with fish have primarily used commercially farmed fish species due to their economic value. Consequently there is a paucity of data on the nutritional requirements of ornamental species. This area warrants further investigation particularly when the life style, feeding strategy and physiology can be vastly different when compared to farmed species. There are three primary objectives of the programme: 1) the evaluation of food stuffs and ingredients through parameters such as feed acceptance, feed conversion, digestibility, growth rate, pigmentation, feeding behaviour and impact on water quality, and the effect of product on markers of fish health such as plasma biochemistry, haematology, immunology, longevity and reproductive output; 2) to aid development of new food stuffs and water treatment products that are	

	supplemented with functional ingredients; and 3) to develop novel measures of nutritional status and health in ornamental fish.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Findings from this project will advance the scientific understanding of the nutritional requirements, health and care of ornamental fish. This will result in the development of suitable food stuffs and water treatment products. The knowledge will be shared through scientific publications, scientific congresses and in the general aquarists literature so making it available to as wide an audience of scientists and fish keepers as possible.
What species and approximate numbers of animals do you expect to use over what period of time?	Ornamental fish species (multiple) 4000 over 5 years Fish will not be endangered/I critically threatened species.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All procedures are considered to be mild. Procedures involve manipulation of the external environment e.g. the addition of water additives, limited cessation of the water supply and diet manipulation. Others include mild stressors (e.g. netting, aerial exposure) and some, using light anaesthesia for restraint purposes only, include blood sampling, faecal collection, scale and fin sampling, colour measurement, and mucus collection. It is planned that no animals will be euthanised as a result of this project and fish will be homed. However, if exceptional circumstances dictate humane killing would be employed as a control measure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have available a number of alternatives, i.e. tissue cell lines, including epithelial and fin fibroblast cells to using live fish and use these where appropriate and review the literature to keep abreast of developments. However, the majority of measures apply to whole body systems that are difficult to replicate in an in vitro situation. This is particularly true where the aquatic environment in which the fish live can exert a significant influence on health.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical power calculations are used in the design of all experimental trials to ensure that the appropriate number of animals are utilised; variance data will be used where available from historical studies and applicable publications.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Since the term 'fish' represents a highly diverse number of species, it is important to conduct the studies with the specific species of interest. The knowledge outcomes of these studies will be applied to the species studied and so the research is in direct service of the species. The procedures employed are considered primarily in the context of achieving the objectives while not exceeding the mild severity threshold. An Animal Welfare and Ethical Review Body and appropriate staff training will ensure that every effort is taken to minimise potential pain, distress or lasting harm.

Project 9	**Use of systems and genetics data in dairy management**	
Key Words (max. 5 words)	Dairy cattle genetics systems	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	√	Translational and applied research
		Regulatory use and routine production
	√	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Dairy farming systems and dairy genetics are changing rapidly. This project will have two contrasting systems and two genetic lines, selected for fat and protein yield. Detailed monitoring and genotyping of the animals will provide data which will be used to improve genetic selection in the national herd and help design new systems.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The project will provide data from controlled systems which will be used to improve selection in the national herd. This improved selection will be combined with refinements is systems to improve the sustainability of dairy farming systems with improved animal health and reduced environmental impact	
What species and approximate numbers of animals do you expect to use over what period of time?	720 cattle over 5 years.	
In the context of what you	Blood samples will be taken from the animals; there	

propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	may be occasional adverse reactions to this (medium severity). At the end of the time animals spend on the project they will be returned to the rest of the herd and may be used for other experiments
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Basis of the research is to collect and analyse data from actual dairy farming systems, these data cannot be obtained without animals in the systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Each lactating group (system x genetic merit) will consist of 50 cows, the group size will be kept at approximately 50 by replacing animals which are either culled, in normal farm practice or are replaced at the end of 3rd lactation. Previous systems work has shown that 50 animals is the minimum to represent a farm system and obtain reliable data especially on health and fertility.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Having a dairy systems approach is integral to the study as the scientific questions relate to dairy production. All animals will be kept under normal farm management routines and veterinary attention will be sought for any animals which are ill and these animals will be treated as in normal farm practice.

Project 10	Neurophysiological and behavioural determination of effective stunning in fish
Key Words	Fish, Slaughter, Stun, EEG
Expected duration of the project	3 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

There are two major scientific unknowns that are being addressed by this project

1. Determination of changes in brain activity and behaviour that are commensurate with an effective stun in fish

2. To use these behavioural and neurophysiological measures to evaluate the electrical field strength required for an effective electrical stun in fish

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Awareness and understanding of and concern for fish welfare have continued to grow over recent years. The European Food Safety Authority (EFSA) concluded in 2009, that “the balance of evidence indicates that some fish species have the capacity to experience pain” these include commonly farmed species. Therefore, as for terrestrial mammals, adequate stunning prior to slaughter should be considered a prerequisite for good welfare of fish species. This work is worthwhile because: 1) There is currently a lack of scientifically validated information on the behavioural and neurophysiological signs that indicate adequate stunning in fish – making it impossible to evaluate the efficacy of new and existing electrical stunning systems 2) The electrical parameters require to effectively stun fish have not been determined scientifically, therefore it is currently unclear as to whether equipment currently in use is fully ablating consciousness This study will be of value to engineers designing

commercial stunning equipment, legislators involved in setting minimum standards for equipment used for electrical stunning of fish at a national and EU level and to fish farmers assessing the effectiveness of the equipment they are using. In so doing the study will make a marked contribution to the welfare of farmed fish at the time of slaughter

What types and approximate numbers of animals do you expect to use and over what period of time?

We anticipate using a maximum of 150 Trout over a maximum period of 3 years.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

This project will run as two sequential steps; in step 1 changes in brain activity and the behavioural signs of an effective electrical stun will be determined. This information will be used in step 2 to investigate the electrical parameters required to fully ablate consciousness in fish. Measurement of brain activity requires electrodes to be implanted onto the surface of the brain under general anaesthesia. Following surgery local anaesthetic will be used to minimise any pain. The initial study will use 30-40. The effect of the electrical stunning is not thought to be painful as generalised pain is not reported by people who have undergone Electrical Convulsive Treatment on recovery from anaesthesia. In the second step of the study there is the potential for fish to be inadequately stunned by the electrical field strength under test. In order to minimise the impact of inadequate stunning fish will be stunned individually and if two fish fail to be stunned effectively the electrical stunning parameters will be changed to prevent this occurring (e.g. to a higher current or application of the electrical field for a longer period of time). The expected severity for these studies is moderate. At the end of the study all fish will be killed.

Application of the 3Rs

Replacement

Studies investigating effective stunning prior to slaughter require assessment of consciousness. Consciousness is a complex phenomenon, the mechanisms of which are not fully understood in animals or people, and there is no alternative to using living animals in order to measure consciousness in animals.

Reduction

We have consulted a Chartered Statistician (Professor Toby Knowles) to obtain advice about likely numbers of fish needed for this experiment. Based on his advice and the numbers of animals used in similar types of experiments in fish we intend to use approximately 30-40 animals for step 1 . The number of animals required for step 2 is difficult to ascertain in advance because it is impossible to perform a power analysis for this type of study. However fish will be studied in pairs to minimise the

number of animals that could be exposed to an ineffective stun. It is estimated that similarly 30-40 animals will be required for this part of the programme of work.

Refinement

We wish to investigate behavioural and brain correlates of effective stunning in fish, therefore we have chosen to use fish as a model species. It would not be appropriate to use mammals or a species of lower sentience (e.g. crabs, molluscs) to answer this research question because there is no scientific basis on which to extrapolate data from other species to fish. It is likely that species specific changes in brain activity and behaviour occur during stunning.

Although implantation of electrodes to measure brain activity (EEG) is invasive it will be carried out under general anaesthesia and local anaesthetic solution will be injected into the subcutaneous tissues around the site of the incision through the skin to minimise pain post surgery. Electrode implantation will also be practiced on dead Trout (obtained from a supermarket) before commencing studies in living animals. In order to preserve the electrodes fish will be isolated following placement; although this might be a stressor, the risks of electrodes becoming dislodged if fish were allowed to swim together justifies this short term measure. If possible fish will remain in visual contact with tank mates by partitioning the tank using a Perspex material. An alternative to implanted electrodes would be to use needle EEG electrodes placed subcutaneously, which would be less invasive to place, but there is a high likelihood that this type of electrode would be displaced as a result of muscular activity following stunning necessitating use of a greater number of animals in order to obtain sufficient brain activity data.

We debated whether to anaesthetise the fish prior to stunning in order to reduce potential suffering resulting from the stun, particularly with respect to the first batch of fish that will be allowed to recover consciousness following stunning. However anaesthesia in fish significantly reduces the magnitude of any brain activity and also reduces the ability to detect behavioural changes (e.g. loss of the righting reflex) as a result of the stun and so would not be appropriate in this case.

The number of fish that will be allowed to recover post stun is relatively few and although potentially distressing to the fish involved, in terms of the overall benefit to fish species in terms of improved stunning conditions at slaughter the benefits significantly outweigh the harm to the few numbers of animals concerned.

Project 11	Effects of housing and management on the welfare of dairy cattle and calves
Key Words	disease, cold stress, stocking density, negative emotional states
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

(b) translational or applied research with one of the following aims:

Yes

(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

There are three major objectives of this project: 1) to investigate the effects of high stocking density on the feeding, activity and frequency of experiencing aggression in dairy cattle; 2) to determine what combinations of temperatures and wind-speed produce cold stress in calves; 3) to determine whether new approaches to detecting disease early in its progression are better for calves than the current methods. One of these includes a specialised ear tag which is attached to the calf's ear to assess changes in temperature,

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

There are a number of benefits from each part of the project: 1. Stocking density in cows: Trials on the effects of high stocking density will produce information on how to best to design cow housing systems to optimise cow welfare in a farm system, where the production of milk is the main aim. This information can be used by farmers or farm advisors when building new cow sheds or by policy makers when producing national guidelines. 2. Cold stress in calves: Although the advice on best practice for housing for calves is to provide good ventilation, it is becoming apparent that the combination of cold temperatures and high wind-speed can cause cold stress in the calves. Quantifying the point at which calves start to experience cold stress would provide guidelines on when farmers need to take action. 3. Early disease detection: Finding new methods to detect illness in calves in its early stages would be beneficial for a number of reasons. Firstly, farmers could use these methods to identify individual animals suffering from disease more quickly than

previously, allowing earlier treatment. This would promote good health and growth in the animals. As low growth and poor health is associated with higher greenhouse gas emissions overall, early disease detection will also have a societal benefit.

What types and approximate numbers of animals do you expect to use and over what period of time?

Adult cows and calves will be used in this project. We will use up to 1500 animals over a 5 year time period.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Stocking density in cows: under high stocking density conditions, cows may not be able to feed at peak times, and some cows may be pushed out of the feed troughs by other cows. They may also find it difficult to find a place to lie down. However, the actual amount of food is not restricted, and lying areas are not always occupied, so all cows will be able to return to feed or lie down later in the day. We are taking a blood sample, faecal or saliva sample to assess stress levels. Taking these samples can cause short-term discomfort, but care will be taken when taking the sample. These procedures are of mild severity. Cold stress in calves: calves are very likely to experience some discomfort when they are exposed to high wins-speeds and low temperatures. However, each calf will be monitored continuously and the wind and cold air application will be stopped once the calf shows signs of experiencing cold. We will take the temperature of the calf with a rectal thermometer twice a day. It is unlikely, but this may cause mild discomfort. Care will be taken to avoid this. These procedures are of mild severity. Early disease detection: the likelihood is low, but some animals may experience inflammation or irritation in the ear due to the FeverTag. Likewise, the likelihood is low, but some animals may experience inflammation of the anal area due to the temperature being taken using a rectal thermometer twice per day. If any inflammation is detected then the calf will be treated and removed from the experiment if does not resolve. These procedures are of mild severity. At the end of all trials, the animals will be returned to the non-experimental herd on the farm.

Application of the 3Rs

Replacement

All of the issues that we wish to investigate are problems that directly affect dairy cows and calves, so the greatest experimental validity is gained by carrying out the experiments directly on these animals. No viable alternatives exist for the systems we wish to investigate. There are no computer models or other model species available, but we will investigate potential new ones that arise during the course of the project.

Reduction

We will take advice from statisticians to make sure that we have the best experimental design and use statistical techniques to determine what is the minimum number of animals that can be used.

Healthy animals will be used to ensure that the results are valid.

Samples will be stored carefully to make sure that none are lost or spoiled before they can be analysed.

Refinement

We have chosen to use dairy cattle, as the issues being investigated are quite specific to this species and their current management systems.

Animals will be carefully monitored throughout any procedure and for an appropriate period afterwards to ensure a full recovery. For the stocking density trial, it will be necessary to use housing conditions that will not comply with the Home Office Code of Practice, but this is necessary for the purpose of the experiment. In other cases, animals will receive good housing and care.