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MLA/AWI Animal Welfare Objective Measures Workshop Outcomes and Future steps

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Abstract

The development of improved measures of animal welfare and an enhanced understanding of how animals cope with challenging situations will allow farming industries to optimise practices and provide assurance to consumers, markets and regulatory authorities. The purpose of this project and associated industry Workshop was to develop an integrated research framework that may be utilised for the discovery, refinement and delivery of objective welfare measures for the Australian animal farming industries. This framework will be evaluated by MLA, AWI, and other industry bodies, with the aim of developing and commissioning research in the area. The authors of the report recommend that:

1) An advisory committee be established to guide the development of the integrated framework and engagement of research providers;

2) Phases for research and development of objective welfare measures include

   i) model and method development,
   ii) stress challenge experiments,
   iii) validation of welfare assessments,
   iv) publication and extension of outcomes;

3) Simple, practical measures or assessments, available from current knowledge and amenable to immediate application, be implemented through industry welfare audits currently under development;

4) An implementation strategy for welfare assessment be developed for Australian livestock industries.

Executive Summary

A workshop on Objective Measures of Animal Welfare was convened by MLA/AWI in Sydney on 6 and 7 June 2005. Thirty-nine participants representing farming industries, government, animal welfare interests, and Australian and International research groups met to identify strategies for a coordinated approach to the development of improved and objective measures of animal welfare for the Australian animal agriculture industries.

During the course of the workshop several common themes emerged in the context of objective welfare assessment. Notably, recognition of the need for an integrated approach, recognition of the importance of animal feelings/emotions within any future assessment of animal welfare and the importance of matching the acceptable levels of animal welfare assessments with public attitudes and expectations for farm animal management.

The workshop was designed to facilitate discussion about the key elements of an integrated objective welfare assessment framework including; the experimental challenge models, the measurement approaches and integration of measurement inputs.

Based on the workshop inputs and after considering the criteria of relevance, practicability, repeatability and type of stress response, the authors of this report propose that the following experimental challenge models are the most suitable:

1. Rest deprivation
2. Fear
3. Thermal (hot and cold)
4. Under-nutrition

Emphasis was given by the authors to those challenges that elicit a more chronic stress response as the costs of adaptation are likely to be higher and therefore, more likely to compromise animal welfare. However, these particular models will enable the quantification of both the acute (initial) and chronic responses to the specific stressors.

Assessments of animal cognition and feelings, behaviour, health, physiology (including gene expression), productivity and environment were all considered relevant within the framework. The inclusion of each will ultimately depend on the specific challenge model, the experimental hypotheses and design constraints. For specific measurement approaches, such as those aimed at the quantification of animal feelings and emotions, further development is required to establish their applicability within the framework. An indicative matrix of measurement approaches and experimental challenge models is proposed.

The following recommendations are made by the authors:

1. Establish an advisory committee that would guide the development of the integrated framework and engagement of research providers.

2. The following research and development phases are recommended within the integrated framework:

    - Model and method development

- Stress challenge experiments
- Validation of welfare assessments
- Publication and extension of outcomes

3. Identify simple practical measures/assessments that have immediate application within industry welfare audits currently under development

4. Industry develop an implementation strategy(s) for welfare assessment within livestock industries

Contents

1 Introduction

At the MLA/AWI Animal Welfare Objective Measures Workshop, held in Sydney on 6 and 7 June, thirty-nine participants representing farming industries, government, animal welfare interests, and Australian and International research groups met to identify strategies for a coordinated approach to the development of improved and objective measures of animal welfare for the Australian animal agriculture industries.

As the first step in this process, the Workshop represented a scoping exercise, aimed at capturing ideas for a coordinated approach, rather than a meeting that was destined to arrive at a single conclusion for the research plan. Nonetheless, some important and common themes emerged from the two days of intensive discussions. These themes include:

- An understanding of the current context of the move towards international standards for animal welfare (and hence the need for widely accepted measures).
- A recognition of the importance of an integrated approach to researching improved, objective ways of measuring animal welfare. Specifically, the need to determine environmental inputs, and behavioural, health, physiological, molecular and productivity responses within a common framework to understand the key pathways and overall impact on the animal.
- A recognition of the importance of animal perception, cognition and emotions in our future understanding and assessment of animal welfare.
- The need to identify practical measures that can be cost effectively applied, but which have been validated through research as reflecting the true welfare state of the animal.
- The need to identify simple measures that can be applied now and in the near future for auditing welfare standards, and to develop a pipeline of more detailed objective measures that can be applied in research-based evaluations (e.g. husbandry practice A vs. practice B), and in more comprehensive auditing/assurance programs (such as through automated data capture).
- The need for research to develop objective animal welfare measures to be based on a rigorous, hypothesis-driven approach, with a subsequent integration process to understand the links between the different response systems of the animal.
- The importance of matching the acceptable levels of animal welfare measures with public attitudes and expectations for farm animal management.

The workshop break-out sessions revealed a broad range of proposed situations and contexts within which objective animal welfare measures could be researched and identified. These situations often (but not always) reflected commercial practices or environments that represented a potential animal welfare challenge. In the subsequent section, these situations are described as ‘Experimental Models’, but it is important to appreciate that this can refer equally to a real-life commercial setting in which research is conducted in a controlled, hypothesis-driven approach. Rather than present the entire range of experimental situations that arose from the break-out sessions (these are detailed in Appendix 2), this report suggests several experimental models that arose from the Workshop and which we the authors believe capture the key elements of the complete set.

Similarly, the scoping process at the Workshop identified some strengths and weaknesses of a long list of specific measurement approaches that could be incorporated into a coordinated research approach to develop objective animal welfare measures. The full range is contained within the notes in Appendix 2. This report attempts to refine these approaches into some key principles and options for measurement approaches.

Finally, this report presents some suggestions on how the process, which began with the Workshop on 6th and 7th June 2005 can be developed into a research program that results in improved animal welfare measures that benefit Australian industry, and provide confidence to markets, regulatory authorities, interest groups and the public.

2 Workshop summary

2.1 Workshop Aims Reuben Rose (MLA)

Ideal outcomes

- A clear direction for the future of animal welfare research to identify objective measures for assessing animal welfare with defined time lines and end points
- Facilitate collaboration between research teams and improve understanding of research activities in the field of welfare measurement research
- Development of research projects that would facilitate identification of measurements that are real, workable and science based
- Identification of potential collaborators and networks as part of this research
- Identification of key research areas and suitable measurement techniques to consider when developing projects

2.2 Introduction of an integrated framework – Andrew Fisher (CSIRO)

- Animal welfare represents a complex synthesis of the biological state of an animal and the interpretation of this state by human observers
- In order to capture the mechanisms that contribute to different states of welfare, we should not just rely on one aspect of biology (e.g. behaviour or stress physiology), but rather utilise approaches that represent a range of pathways
- The purpose of this Workshop is not to advocate one or two specific ideas for the development of improved animal welfare measures, but rather to scope the development of an integrated framework, within which different but meritorious approaches can be applied in concert
- An integrated framework for research on objective welfare measures should comprise a number of key aspects:
    - Livestock challenge models of industry relevance and varying stressor components
    - Differing biological mechanisms and assessment approaches that capture the complexity of animal responses
    - Analytical models to identify response signatures indicative of compromised animal welfare, and links to applied measurement platforms
    - International linkages to incorporate key science capabilities

2.3 International perspective – Jeff Rushen (Agriculture & Agrifood Canada)

Why we measure animal welfare:

- Phase 1 – animal welfare assessment driven by European legislation – dependent on qualitative factors
- Phase 2 – animal welfare assessment driven by issues raised by auditable animal welfare standards
    - Measures need to be "uncontroversial"
    - Health most useful
    - Behavioural, physiological, immunological least useful
- Phase 3 – Animal welfare assessment driven pressure to develop internationally accepted measures of animal welfare for global trade e.g. OIE
    - What types of measures?

2.4 Breakout session 1 – measurement types

Key points from workshop groups:

- Animal welfare involves both physical and mental aspects
- There is a dichotomy between research and auditing requirements
- The proposed animal welfare research framework utilises a correlational approach however there is a conflict between integration and specificity. Alternative is hypothesis driven research
- Health and behavioural measures should be added to research framework
- There should be a third dimension to the framework matrix – "animal preparedness" (backgrounding, genotype etc.)
- Productivity and product quality are important drivers for animal welfare at producer level and this should not be ignored.
- An integrative measure of behaviour, metabolic, immunological and adaptive aspects of animal welfare should be considered
- Some practices are non-negotiable for public acceptance
- Should we consider a " Whole of Life" measure of animal welfare rather than a short term approach
- Additional approaches may include:
    - Telemetry/non-invasive,
    - IRT – infrared thermography
    - MRI – brain wave activity
    - NIR – near infrared spectroscopy
    - Ecological approaches with behavioural measures
    - EEG (eg reward pathway approach)
    - Metabolomics

Note that the complete set of information from the Breakout sessions is presented in Appendix 2.

2.5 Challenges and opportunities in behavioural measures – Alain Boissy (INRA)

Welfare – it's all about emotions:

- We should not only measure the reactions of animals, but also question their emotional states to better assess their welfare - We need to access the emotional world of animals
- Current behavioural and physiological indicators offer limited opportunities to assess emotional experience
- New methods to assess affective states have to be developed
- Cognitive ethology has to be developed to better approach animal welfare
- Emotions in animals have cognitive, behavioural, physiological and subjective components
- By combining measurements of the cognitive, behavioural and physiological components in an integrated way, we can get close to assessing the subjective component

2.6 Breakout session 2 – behavioural measures

Key points from workshop groups:

- Validation of behavioural measures is important
- Behavioural measures must be quantifiable
- Health measures could be linked with behavioural measures
- Approaches must be species specific and should also consider genetic background and animal experience
- Need to understand causes and consequences related to behavioural measurements
- Objective tests needed for fear, adaptability, anxiety and cognitive ability
- Techniques for assessing behaviour include: visual appraisal, electronic monitoring, mathematical calculations
- Happy animal = productive animal
- Are the results of current work on cognition well recognised?

Note that the complete set of information from the Breakout sessions is presented in Appendix 2.

2.7 Challenges and opportunities in physiological measures – Dominique Blache (UWA)

- Key physiological approaches
  - Some indicators of pain
  - Immune system measures
  - Enzyme activities
  - Respiration and heart rate
  - Body temperature
  - Hypothalamic-pituitary-adrenal axis activity and other hormones

- Neurotransmitters
- Metabolites

- Current developments and future opportunities
  - Remote blood sampling
  - Microdialysis
  - Free-range physiological monitor (FRPM)
  - Solid state immunosensors
  - Transdermal sampling
- Towards objective measures
  - Integrative physiology
  - New technologies, Modeling, multivariate analysis, Psychoneuroendocrinology, Psychoneurommnunology
  - Homeostasis, Allostasis, Allostatic load and overload

2.8 Breakout session 3 – physiological systems

Key points from workshop groups:

- Key physiological systems are endocrine, immune and metabolic – simultaneous measurement of all may identify interactions between systems
- Need to consider responses to both acute and chronic stress – less able to measure chronic stress
- HPA is still a key system for including in assessment
- The selection of variables and systems depends on the context or challenge
- Measurement redundancy – many parameters tell us the same thing
- Very important to relate production traits (eg reproduction, growth rate) to physiological measures
- Remote measurement is expensive but may be used to validate more practical measures
- Need measures that are interpretable – many are currently not

Note that the complete set of information from the Breakout sessions is presented in Appendix 2.

2.9 Challenges and opportunities in molecular and genomic approaches – John Gibson (UNE)

- Our understanding of the mammalian genome is advancing rapidly
- Bovine genome project

- Molecular genomic knowledge offers new tools and understanding, but can not answer all the questions
- Some of the technologies (e.g. microarrays) are currently expensive but will become cheaper
- Microarray approach – hypothesis testing – specific genes/pathways.
- Candidate gene expression
- Proteomics - what do the proteins do?
- Metabolomics - how it all fits together

2.10 Breakout session 4 – molecular and genomic approaches

Key points from workshop groups:

- Possibly not a currently useable objective measure of AW
- May be useful as a basic research tool to generate hypotheses to increase understanding of animal responses
- Important for identifying different genotypes and their adaptation to particular environments (long term genetic selection for welfare?)
- Experimental design very important when using these techniques.
- Important to conduct studies within currently known physiological framework
- High cost
- May be able to store samples from current studies for future analysis once techniques further developed?
- Animal welfare is involved with understanding the animal's biology – need to incorporate these new tools of biology in order to understand the pathways involved
- Must have very good experimental models and well defined experiments to obtain meaningful results
- Currently most useful for cattle – little available for sheep

Note that the complete set of information from the Breakout sessions is presented in Appendix 2.

2.11 Considerations in selecting challenges and animals – John Barnett (AWSC)

- Type of model?
  - short or long term responses, acute or chronic stress
- What is the challenge to be imposed?
  - Identifying those factors affecting homeostasis
  - social, environmental, nutritional, health, psychological
- Measures

    - physiological (hormonal, immunological), behavioural (observations, tests)
- Species specific attributes
- Decide on the issue
    - what do we want to measure?
    - do we want to measure acute or chronic responses, or both?
- Acute stress model
    - what is the most appropriate model in sheep and cattle?
    - surgical models are generally not repeatable within animals
    - could use transport, novelty, isolation
    - does model have to be relevant to industry? eg. sheep restraint/isolation model, bedding
- Chronic stress models
    - do we have appropriate models in sheep and cattle?
    - undernutrition?
- Measures
    - what are the most appropriate physiological and behavioural measures of acute and chronic stress?

2.12 Breakout session 5 – Selection of challenges and animals

Key points from workshop groups:

- Stressors need to be realistic and relevant to applied needs
- Stressors need to be species and class specific
- Should be able to measure both a physiological response and a behavioural response to the stressor
- Real life situations may involve multiple stressors with cumulative effects
- Hard to differentiate from acute and chronic stressors
- Need to consider both intensive and extensive management systems/stress
- Whole of life" stress measurement??? Is it the "Holy Grail"?
- Difficult to justify use of acute pain stress models (ethics, public perception; large amount of research already carried out on many acute pain inducing procedures)
- Possible stress models include long distance transport, undernutrition, dairy cow model, heat stress

Note that the complete set of information from the Breakout sessions is presented in Appendix 2.

2.13 Integration and application issues to consider – Stephen Page (Advanced Veterinary Services)

- What is being measured?
- What is the Gold standard?
- What does the Customer need?
- Repeatability, Precision, Variability, Robustness, Interpretation
- Practicality, cost, convenience
- Bayesian approaches
- Delphi method
- Strengths of collaborative approach- teamwork, consensus, ownership
- Weaknesses of collaborative approach - group think, inertia, conflict

2.14 Breakout session 6 – Integration and application

The four groups were asked to design detailed research plans around two challenges- heat stress and undernutrition. The complete set of information from the Breakout session is presented in Appendix 2.

2.15 Next steps – Where to from here? – Michelle Kellaway and Reuben Rose (MLA)

- Post-workshop feedback request to be emailed (Jun/Jul)
    - Areas of expertise/interest in integrated research framework
    - Current relevant research experiments
    - Gaps?
- Revised framework document to be circulated to all participants for comments and then circulate finalised report
- Funding body discussion regarding level of interest in research in this area
- Advisory Group?
    - Based on expertise
    - Cover international and national spectrum
- Ongoing collaboration/development of relationships was encouraged. Science teams indicated an interest in them progressing an ARC funding application to assist with this.

3 Author's assessment and Recommendations – Experimental models

A central axis of the integrated framework is the experimental challenge models. The nature of these models will have a large bearing on the choice and application of the various welfare methodologies. This point was emphasised several times during the workshop.

A wide range of challenge models was discussed during the workshop and these included:

Acute stressors
- Pain via husbandry procedures (castration, dehorning, tail docking etc.)
- Handling and restraint

Moderate - chronic stressors
- Transport
- Fear
- Under-nutrition
- Thermal
- Disease/chronic pain (eg. Lameness)
- Lying deprivation
- Space allowance
- Social change (mixing, isolation)

There were several key points that were either emphasised or frequently raised during the workshop session on stress challenges (refer Appendix 2 for more detail):

- Stressors need to be realistic and relevant to applied needs
- Stressors need to be species and class specific
- Should be able to measure both a physiological response and a behavioural response to the stressor
- Real life situations may involve multiple stressors with cumulative or multiplicative effects
- Hard to differentiate from acute and chronic stressors
- Need to consider both intensive and extensive management systems/stress
- "Whole of life" stress measurement??? Is it the "Holy Grail"?
- Difficult to justify use of acute pain stress models (ethics, public perception; large amount of research already carried out on many acute pain inducing procedures)
- Possible stress models include long distance transport, undernutrition, dairy cow rest deprivation model, heat stress.

Noteworthy amongst these, was the discussion regarding the contrast between acute and chronic stressors and their relevance in terms of animal welfare. This point was frequently raised and was also highlighted in Dr John Barnett's presentation on the subject. Within the workshop groups, there was considerable debate about the definition of acute and chronic stress. For example, should they be defined in terms of the duration and intensity of the stressor or the animal's response? There was a shared view that animals essentially adapt to single acute challenges and therefore, these may be less relevant to animal welfare compared to those challenges where the animal fails to adapt i.e. restore homeostasis. Previous studies have focussed largely on the capacity of animals to adapt physiologically to acute stressors, whereas the emotional impact of exposure to an acute stressor has received less attention. The consequences of the emotional impact of an acute stressor on the emotive response to subsequent exposure to the same acute stressor appears to need further exploration. Another important point that was raised was the need to consider combined or cumulative stress challenges as these might be more representative of the "real-life" challenges encountered by production animals. However, some expressed the view that it was probably prudent to initially quantify the impacts of specific stressors before examining the combined or cumulative effects.

As with other components of the framework, there wasn't extensive discussion about the specific merits or disadvantages of each of the above stress challenges at the workshop. There were, however, specific comments directed at the utility of pain as a model. Several participants felt that the animal's response to acute pain (eg. castration, dehorning) was reasonably well characterised and that further investigation was not going to improve the current argument that alternative strategies (genetic, revised on-farm practices or the requirement for anaesthesia and analgesia during surgical husbandry procedures) are clearly required on welfare grounds. In contrast, less was known regarding the industry significance and welfare implications of chronic pain associated with disease or physical injury. Whilst securing ethical clearance will be an issue for all of the above stressors/models, it was recognised that this may be particularly problematic for a chronic pain model. There appears to be no ethical justification for industry adopting practices that invoke chronic pain and so the rationale for studying a chronic pain model would need very careful consideration. In chronic disease states such as acidosis where there may be no clinical signs of pain there could be a need to establish whether an animal is experiencing chronic pain and if so to develop methods to assess and alleviate this state.

3.1 Author's recommended challenge models

The models identified at the workshop were subsequently reviewed by the authors to select those that were considered to have the greatest application within the framework. The following selection criteria identified at the workshop were applied during this assessment:

- Relevance
  The models must be relevant to welfare-related problems within animal production systems in Australia and preferably also in other countries. The latter is quite important in the context of developing measures of welfare outcomes that have international acceptance and/or application.
- Practicability
  Consideration needs to be given to the practical constraints of applying any model such as the capacity to integrate both physiological and behavioural assessment methodologies.
- Repeatability
  It is important to utilize models that can be consistently applied within and between research/field sites.
- Stress response
  Challenges that invoke a more chronic stress response are preferred since the costs of adaptation are likely to be higher and therefore, more likely to compromise animal welfare. In contrast, animals generally adapt readily to more acute stressors. Ultimately, it will be important to evaluate the cumulative impact of chronic and acute challenges. For example, to examine whether an animal's adaptive capacity to respond appropriately to acute challenges is compromised when it is undergoing a chronic challenge. It is also important to recognise that whilst emphasis is given to more chronic stress challenges, by quantifying the temporal responses to the stress challenge, it will in essence facilitate the quantification of both the initial or acute and chronic responses.

After considering these criteria and the workshop outcomes, the models based on pain (acute – surgical husbandry procedures), handling/restraint and social change (isolation/mixing) were excluded on the basis that the stress response is typically acute and transient in nature. Moreover, there is evidence to show that stressors such as mixing, isolation and restraint do not always elicit major behavioural or physiological changes in cattle (eg. Colditz et al 2005). The points raised above in relation to acute pain as a model are also relevant. A model based on restricted space allowance was questioned on its relevance as it applies more to intensively reared livestock. On the other hand, space allowance has clear relevance as one of the many stressors that apply during transport (land or sea). However, since research was already under way with regard to welfare outcomes of livestock transport in Australia and rest deprivation was considered a more robust model, space allowance was not considered further as a preferred model.

Given these points, we propose that the following models are likely to be the most suitable

(i) Rest deprivation
This model has been successfully applied in dairy cattle in New Zealand (eg. Fisher et al 2002). Typically, cattle require 10 – 12 h rest/day. Under this challenge, cattle are deprived of their normal rest period by 30% over several days by placing them on floors that either prevent lying (wooden grids) or are inherently uncomfortable to lie on (concrete). The challenge results in quite pronounced physical (fatigue) and emotional (frustration) costs to the animal.

Rest deprivation has relevance to a number of cattle (beef and dairy) and sheep industry situations including transport (land and sea), lairage and potentially during periods of adverse climatic conditions (eg drought and long term wet periods). The model can be practically and repeatedly applied although some consideration needs to be given to its application under field conditions. Furthermore, it has the potential to allow the integration of both physiological and behavioural demand assessments.

(ii) Chronic fear – negative handling
Subjecting production animals to poor or negative handling results in a chronic fear response to humans and as a consequence, can profoundly reduce welfare and productivity (see review by Hemsworth 2003). The negative handling treatment amplifies the inherent fear of humans by animals. This model has broad application as it has been successfully applied in a range of production animals (chickens, pigs and dairy cattle). Furthermore, it has clear industry relevance as it targets the issue of the variability in the quality of care given to production animals by their handlers and managers.

Notwithstanding the utility of this model to elicit a chronic fear response, it might be valuable to consider whether the same could also be achieved without human intervention. For example, subjecting animals to electric shocks that they are neither able to control nor predict via a remotely controlled device may elicit a fear response of similar magnitude to that achieved through negative handling. Furthermore, this treatment may also be considerably safer to apply particularly for cattle and would allow evaluation in a field setting without being confounded by the effects of stock handling needed to undertake the experiments. As part of this, the validity of relationships between the actual (human) and surrogate (electric shock) model would need to be established.

(iii) Thermal challenge- heat and cold
Thermal stressors represent a continuing practical challenge in Australasian livestock management systems. Examples include heat stress during transport, in feedlots, or at pasture (especially if shade is limited), and cold stress during sudden changes of weather in southern grazing regions. Although there is

some good information on the classical physiological responses of livestock to thermal challenge (such as the research done by LeRoy Hahn and others at the USDA), we do not know how animals perceive their situation in response to the thermal environment, and at what points (short of mortality) animal welfare is progressively compromised. Both commercial settings and experimental facilities (i.e. climate rooms) provide sound research platforms to examine the integrated animal responses to both hot and cold thermal challenge, and to identify the thresholds in either practical animal measures or environmental inputs that represent progressive impacts on animal welfare as evidenced by animal perception, behaviour, physiology, productivity and health.

(iv) Undernutrition
In grazing-based meat and wool production systems, there will be periods where animal body condition declines because of sub-optimal feed levels (which are dependent on the weather). Furthermore, some animals may fail to eat sufficiently for a period within more intensive management environments (such as during adaptation to drought feeding or in a pre-embarkation feedlot). Although it is clearly no longer acceptable to have animals generally decline to the point of death due to undernutrition, we need to develop better measures to validate welfare when animals are in a declining plane of nutrition, and at particular body condition scores. There is a need to know how an animal feels when it is hungry and in light body condition, what the impacts on its immunity and health are, and how an animal that is in a state of undernutrition copes with the advent of an acute challenge (such as transport to the saleyard).

4 Author's assessment and Recommendations – Measurement approaches

4.1 Animal cognition, perception and emotions

A strong message from the workshop was the need to include assessment of animal feelings and emotivity within the objective measures. Discussions encompassed a range of approaches for incorporating the understanding and assessment of animal emotions. We have categorised and presented these approaches below for further feedback and development, rather than advocate any one or two methods at this stage. Given the complexity of the subject, it may be important to utilise several approaches to understand animal feelings within the research program. In addition, some methods may be better suited within some challenges than others. In any case, there is a need to conduct underlying research to better understand and validate methods of measuring animal emotions, along with integrating changes in emotions with other biological and productivity impacts.

a. Modification of animal reactivity
This approach, as presented in detail by Dr Alain Boissy, examines how animals that are undergoing some sort of challenge respond behaviourally (and physiologically) when presented with a sudden and/or novel stimulus. The response to the stimulus is viewed as being influenced by an animal cognitive component (how it perceives the stimulus), and a subjective component (the 'affective' state of the animal- i.e. its emotional state of mind at the time), leading to the behavioural and physiological responses that are measured. The affective state is able to be inferred by understanding the animal's cognitive capacity and measuring how the animal's responses differ under low or high pre-existing challenge environments. As an example, cattle subjected to repeated social regroupings exhibit a hyperreactive response to a sudden jet of water (Boissy et al., 2001).

b. Animal behavioural demand- operant and non-operant

Behavioural preference measures aim to assess an animal's preference (or avoidance) of a certain practice or environment. Behavioural demand or motivation measures aim to assess how much an animal wants (or does not want) a practice or environment. This latter approach has been developed from Marian Dawkins' utilisation of consumer demand theory for animal welfare research. The animal's feelings about its situation are inferred from the measures of how much it wants or does not want the relevant environment, practice or resources being evaluated. Behavioural preference and demand measures are largely used within experimental studies, as they are likely to be difficult to apply in an audit or assurance context. Operant techniques require an animal to perform a specific action (e.g. pressing a lever), and the behavioural demand can be quantified by increasing the number of operant responses required to obtain the desired resource. The capacity to quantify with a high degree of precision the operant response provides great power to this method. However, some caution needs to exercised in the interpretation of behavioural demand as the animal's motivation for change may in fact be "hard-wired" rather than under cognitive control.

c. Neurophysiological methods
In this approach, an animal's state of mind is inferred from changes in brain activity and function, as measured by a range of techniques, including electroencephalogram (EEG- the electrical activity of the brain), microdialysis measurement of changes in neurotransmitters and other brain chemicals, neuroanatomical and neurophysiological imaging techniques (eg. NMR) and (post-mortem) measurement of neurotransmitter receptor populations. Although animals such as sheep can be instrumented to have their EEG recorded while they move around a paddock, the otherwise generally invasive nature of these measurements makes them most suited to detailed research, aimed at trying to understand the linkages between challenges, emotional states and brain function in domestic animals.

4.2 Behavioural responses

Behavioural response measures are those in which the animal exhibits some kind of behaviour indicative of its situation or in attempting to alleviate the impacts of a challenging environment. Examples include the assessment of the incidence of the "hunched immobile" posture following mulesing in lambs or vocalization during the lairage and handling of animals in abattoirs. These measures usually need to be fairly specific to the situation being assessed (i.e. animals tend not to exhibit a generalised behavioural response to all stressors). Behavioural response measures have two potential advantages: 1) they can represent an effective integration of many of the challenge inputs that an animal is receiving; and 2) the behaviour of animals is considered important by the general public. Potential disadvantages include the technical difficulty in objectively measuring behaviour, and a lack of certainty in many cases about what a behavioural response may indicate about the underlying biological state of the animal.

4.3 Animal health

The workshop noted that health has frequently been overlooked as an indicator of welfare. If a welfare challenge or problem produces a typical and specific clinical picture in animals (eg. lameness), then these clinical signs are likely to represent an appropriate measure. Health measures and epidemiology have been used in the broad-scale screening or auditing of the welfare status of farm animals, or occasionally in large, case-controlled experiments. They are of most use in intensive situations where such data is relatively easy to collect (or is collected anyway), and where specific disease conditions tend to occur. A good example is in the application of such techniques to evaluate the welfare status of dairy cows under the UK RSPCA Freedom Food scheme, in comparison with dairy cows in farms not covered by the scheme (Main et al., 2003). It was shown that the Freedom Food farms had better results for some welfare indicators, including mastitis and body condition, but poorer welfare indicators for other measures, including injuries and lameness. A challenge for current application of the epidemiological approach is that by focusing on clinical outcomes, it leaves open the challenge that underlying stress levels are not captured. For example, it is argued by opponents of live export that the mortality, although well quantified and generally at low levels, represents a "tip of the iceberg" as a measure of the overall welfare impacts on the animals shipped. Measurement of biomarkers associated with subclinical or pre-pathological change therefore has much to offer epidemiological studies of welfare.

4.4 Physiology, biochemistry and immunology

This category incorporates a broad range of responses, from hormonal and cardiovascular changes, through to health, disease and metabolism. There are some general physiological responses to stressor challenges that are used in welfare assessment such as the sympatho-adrenal (SA) response (ie. fight or flight). The SA response is characterized by almost instantaneous release of adrenalin and noradrenalin from the adrenal glands into the bloodstream resulting in increased heart rate and cardiac output. The measurements of adrenalin, noradrenalin and their metabolites are not widespread in welfare assessment because these hormones are rapidly broken down and are relatively difficult to analyse. More common is the assessment of heart rate in experimental studies, often using datalogging or telemetry systems. The outputs of the flight or fight response tend to be relatively short lived, and thus are more suited to acute, sudden, and short-term welfare challenges.

The adrenal glands also play an important role in the other key hormonal response to stressors- the response effected through the hypothalamic-pituitary-adrenal (HPA) axis. Activation of this axis results in the release of glucocorticoid hormones from the adrenal glands. These hormones can be measured in blood samples, or in urine, or as their metabolites in faeces. In livestock, cortisol is commonly measured in short- to medium-term experimental welfare studies. Despite its widespread use, there are caveats associated with the interpretation of the cortisol response. Firstly, cortisol concentrations need to be interpreted in context (mating can induce cortisol increases). Secondly, in species such as cattle, longer-term stressors appear to induce a down-regulation of the HPA axis, such that cortisol values can return towards baseline, despite other evidence indicating that the animals have not adapted (Fisher et al., 2002). As a general rule, it is unwise to depend on any one measure, such as cortisol, as the sole determinant of animal welfare, unless that measure represents a clear and specific welfare insult (e.g. lameness). HPA function or sensitivity can also be assessed through the application of CRH and ACTH challenges

There are many physiologically-based analytes that can be used in welfare assessment, often in response to specific situations. For example, metabolites such as non-esterified fatty acids and ketone bodies in blood are used to assess inanition and stress-induced catabolism of body tissues. Plasma protein concentration and osmolality can increase due to dehydration. The packed cell volume (PCV or "haematocrit") of the blood can increase due to dehydration-induced haemoconcentration, or as a result of the flight or fight response.

Immune measures assess the competence of an animal's immune system. Immunocompetence, which is clearly important in resistance against disease, may be compromised by welfare challenges. Immune measures include the relative populations of white blood cells, laboratory-based assays of white blood cell function, and measurement of biochemical analytes produced by or relevant to the immune system and disease (e.g. interleukins, interferons, acute phase proteins). Measurement of immune competence has two potential applications. Firstly, acute changes in immune function following exposure to a stressor provide an additional measure that integrates a number of the homeostatic drivers of the impact of the stressor on the animal. Secondly, basal immune function when an animal is stabilized or adapted within its production environment is likely to be associated with disease risk. Thus immune measures can provide a warning of decreased disease resistance and the pre-pathological consequences of welfare challenges for an animal (Fell et al., 1999); however, to this point they remain difficult to measure outside the laboratory, and there has been insufficient effort to identify widely-applicable and easily measurable markers of immunocompetence.

Brain measures of welfare are based on the discipline of neurophysiology. In live animals, studies have measured the changes in neurotransmitters through microdialysis, or changes in the electrical wave patterns of the brain through EEG (electroencephalogram). Unless there are major developments in technology, such brain-based measures are likely to remain restricted to specialised, controlled experimental research. However, they can be of considerable importance in validating the effect of key challenges, and in linking and validating other potential measures through to the underlying biology and brain state of the animal. As stated above, there is an important role for physiological brain measures in unravelling emotional states of livestock.

One of the challenges in the physiological assessment of animal welfare, particularly outside of controlled experiments, is the sampling and analysis procedures that may be required. It is not always easy to collect a blood sample from an animal, and the act of collecting the sample may in itself induce a stress response. Less invasive sampling media such as saliva, urine or faeces may be used, but there is still the requirement for processing and analysis of the analyte of interest. Accordingly, there has been interest in the development of biosensor or sampling systems that can capture physiological information about animals in a non-invasive and stress-free manner. These systems have not yet been developed to a state for widespread commercial use in animals, but are attracting substantial interest and investment within human medicine.

4.5 Molecular and genomic approaches

One area of biology that has not been thoroughly examined in the context of farm animal welfare is in the understanding of the underlying cellular pathways involved in welfare challenges. Despite the tools of molecular biology being applied to increase our understanding of the key response pathways of animals to specific disease challenges, or reproductive states, we still have little idea of the key genes, their expression and the resultant proteins that are activated in welfare challenges. One reason for this is the high costs associated with quantification of gene expression. Indeed this was noted at the workshop and some felt that in view of this, gene expression technologies were more amenable for more strategic or targeted application. However, Prof John Gibson did emphasise during his presentation that these costs have and continue to decline. Furthermore, the application of gene expression methods to the challenge models undertaken in the research program should provide a valuable insight to regulation of homeostasis and adaptation. The workshop noted that past experience indicates that new regulatory pathways can be revealed by gene expression studies that lead to subsequent hypothesis-driven research. Bioinformatics techniques can identify with increasing facility genetic variations and expression patterns that may have practical value in selection of adaptable animals.

4.6 Productivity

The workshop strongly supported the inclusion of measures of productivity in assessment of welfare. Implicit here is the view that “a happy animal is a growing animal”. Whilst this view is reasonable, there are exceptions to it (eg. overeating in clinically depressed individuals) and some care needs to be exercised in the application of productivity-based assessments of welfare.

5 Author's assessment and Recommendations – Integrated Framework

5.1 The process of integration

Stephen Page from Advanced Veterinary Therapeutics presented an overview of some issues to consider during integration and application of the results of the objective measures framework. Important factors considered in Stephen's presentation included:

1. The role of the formal processes of risk assessment in identifying drivers for welfare assessment
2. A product development approach to appraisal of the need for and type of tests to be developed
3. The value of adopting the rigor of diagnostic and analytical test methodologies including
    a. specificity,
    b. sensitivity
    c. repeatability
    d. practicality
    e. cost
    f. usefulness
4. The importance of an evidence based approach
5. The challenges of sociological team work factors to integration and application of the findings
6. The role of method such as the Delphi method in formation of judgements for thresholds and diagnostic criteria for assessment of welfare states.

5.2 A proposed approach for the integrated framework

From the workshop deliberations of experimental models, measurement methodologies and issues to address in the development of an Integrated Framework for Objective Measures of Livestock Welfare, we propose the following approach. The core of the proposal for the integrated framework is the interrogation of key challenge models with a common suite of measurement methods. An important concept here is the comparison of responses, as assessed by the various measurement methodologies, across a range of challenge models (that compromise welfare through differing aetiologies relevant to industry practices and production systems) in order to identify the strengths of the individual measures and the ways the measures can be combined to identify states of reduced welfare. Rather than analysing the responses to each challenge in isolation from other challenges, the opportunity exists to analyse the responses across the range of challenges to identify commonalities amongst measures in their ability to report on the welfare status of the animals. Having said that, there are likely to remain some unique and important ways that individual challenge models (and industry practices) will modify emotive, cognitive, behavioural and physiological responses of animals that are important elements of and indicators of compromised welfare. Inevitably, some variability will occur in the applicability of each measurement approach to each challenge. The following table explores the potential applicability of the measurement methodologies to the challenge models outlined above.

Measure	Experimental Model			
	Rest Deprivation	**Fear**	**Thermal**	**Under nutrition**
Feelings & Behaviour				
Modification of reactivity				
Behavioural demand (non-operant)				
Behavioural demand (operant)				
Neurophysiology				
Behavioural response				
Physiology				
Neuroendocrine				
Metabolic				
Immune Function				
Gene expression				
Health				
Pathologies				
Predisposition to disease				
Mortality				
Productivity				
Growth				
Reproductive efficiency				
Product quality				

Definite
Probable – may requires initial validation
Not sure
Probably not

The data from each measurement methodology is likely to differ in its relationship to the severity and nature of the stressor. If they didn't, a single measure (say cortisol) would already be able to be applied generically to assess animal welfare. A graphic presentation of how the different types of measurement could differ in their sensitivity to the heat stress model is presented in the figure below (which is easier to interpret if printed in colour). It illustrates how measurement modalities may yield differing indications of the impact of the challenge on an animal. Although this is only an example, it is highly likely that this is what we will observe in response to the major stress challenges listed above. The challenge will be to develop robust analytical techniques that can integrate the different measurement modalities so that a meaningful interpretation of the animal welfare status can be made.

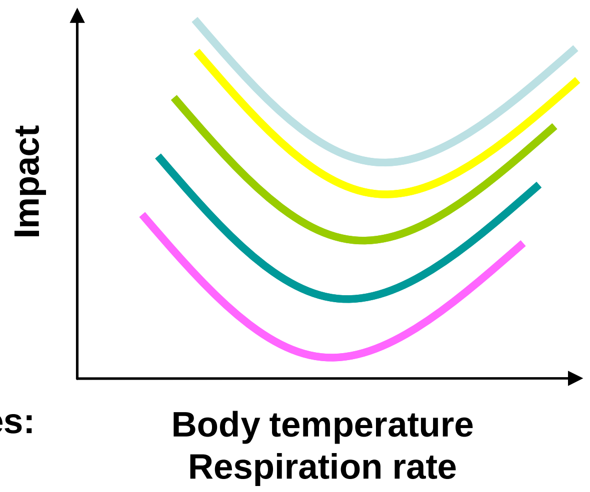
5.3 Data analysis

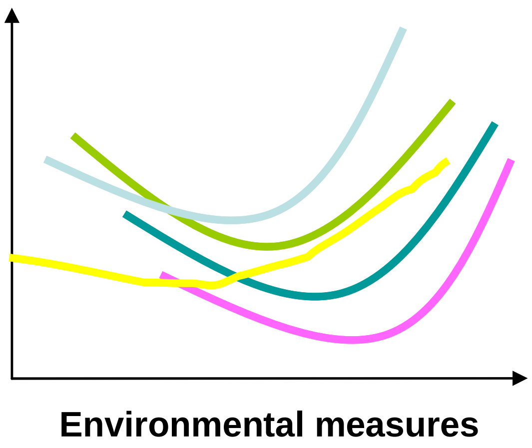
The differing responsiveness of the biological components of the animal responses to the various challenge models highlights the importance of the analytical steps in integrating the data from the challenge models into an interpretation framework. An important feature of the biological responses of animals to stressors, as captured by the concepts of pre-pathological change and allostasis, is that these responses are non-linear. (For a detailed description of non-linear responses using examples from the immune system see Callard and Yates, 2005). Recent developments in analytical methodologies within the discipline being called complex systems science such as neural networks and support vector machine methodologies are proving powerful in identifying the concatenation of variables that diagnose a state or a disease risk. We propose that these new analytical methodologies for interpreting non-linear biological systems be applied in the proposed Objective Welfare Measures research initiative. To our knowledge such methodologies for identifying the emergent property that is known as compromised welfare have not previously been applied to animal welfare studies. Without being dogmatic about the particular analytical approaches that should be employed, the issue of data analysis is raised to flag the importance of including high level statistical expertise in the team in order to capture the potential created by the measurement : challenge model matrix proposed within the Integrated Framework

Thermal

	Animal feelings		Physiology		Molecular	Health	Environment	Productivity
Heat Cold	Modification of animal reactivity	Behavioural demand	Immunocompetence	Metabolic, incl. body temperature	Gene expression (candidates identified from other research)	Thermal biology review	Environmental inputs	Feed itake, weight gain

Indicative practical measures:





6 Author's assessment and Recommendations – Linkages

6.1 CRC for Beef Genetic Technologies

Within the Beef CRC, Program 3 Adaptation and Welfare, has a sub-program which corresponds closely with the goals of the MLA/AWI Objective Welfare Measures initiative. Following the MLA/AWI Workshop on 6 & 7 June, Beef CRC researchers met to plan the CRC welfare research program. The outcomes of this meeting were to have CRC research in the area aligned with the MLA/AWI Objective Welfare Measures research effort. The CRC researchers proposed to focus on two of the four stressors that are outlined in this report, namely fear and deprivation of rest. In a broader context, the overall focus of the Beef CRC on genomics and gene expression offers the opportunity for the CRC program to provide these measurement approaches to the Objective Welfare Measures research effort.

6.2 New Zealand

The attendance of Meat & Wool New Zealand at the workshop, and the participation of a number of New Zealand-based animal welfare scientists provide the opportunity for a trans-Tasman approach to objective welfare measurement development. Both Australia and New Zealand have largely outdoor and pasture-based livestock management systems, and have a number of common animal welfare challenges. There is the opportunity for animal welfare research commissioned by Meat & Wool New Zealand to form part of the Objective Welfare Measures research effort, by aligning and collaborating with the Australian research in the initiative. Meat and Wool New Zealand are also involved within the Beef CRC.

6.3 Other International

It should be recognised that links with the international research community will be important for the optimal performance of the MLA/AWI Objective Welfare Measures initiative. The establishment of collaborative links between Australasian scientists in the welfare measures area and key overseas groups working on the same issues will enhance the research overall, provide greater leverage on investment, and will help ensure that the measures developed through MLA/AWI funding will be relevant and accepted internationally. The European Union funds a major animal welfare research project, through its 6^{th} Framework Program. The aim of the EU Welfare Quality Project is to achieve integration of animal welfare in the food quality chain. The project aims to accommodate societal concerns and market demands, to develop reliable on-farm monitoring systems, product information systems, and practical species-specific strategies to improve animal welfare. Research is focused on three main species and their products: cattle (beef and dairy), pigs, and poultry. The research program is designed to develop European standards for on-farm welfare assessment and product information systems as well as practical strategies for improving animal welfare. The standards for on-farm welfare assessment and information systems will be based upon stringent scientific validation, along with consumer demands and marketing requirements. A dialogue should be established between the MLA/AWI Objective Measures Program and the EU Welfare Quality Initiative with a view to creating an agreement for interactions such as exchange of information, researcher collaborations, etc. It would also be valuable to explore collaborative opportunities in North America. These would include Dr Jeff Rushen and colleagues at Agriculture and Agrifood Canada, Professor David Fraser and colleagues at the University of British Columbia, the scientists at Michigan State University working on stress-induced gene expression and receptor changes (Dr

Jeannie Burton, Dr Adroaldo Zanella and colleagues), and the group headed by Professor Joy Mench at UC Davis.

7 Author's assessment and Recommendations – Future steps

7.1 Welfare advisory committee

It is recommended that an advisory committee of about 4 people (representing Australia and New Zealand interests) be established to guide development of the Integrative Framework for Objective Welfare Measures and the process for engagement of research providers, and implementation of the research program.

7.2 Integrated framework for the development of objective assessments of livestock welfare

The following research and development stages are proposed for developing an integrated framework:

(i) Model and method development

Initial research focused on the development/evaluation of specific methodologies and challenge models is required prior to undertaking the major stress challenge studies. In particular, good progress has been made on the development of methods that potentially quantify an animal's emotional state, however, it is clear that further development is required. This would facilitate confirmation and enable modification and testing of the protocols to ensure wider applicability of the tests. Several of the challenge models (eg chronic fear and rest deprivation) will also need to be tested and possibly modified to confirm their utility in field based cattle research. The period of development of the challenge models would also provide an opportunity for developing some of the measurement methodologies such as gene expression.

(ii) Stress challenge experiments

Controlled studies would be undertaken utilizing the four experimental challenge models (Fear, Rest deprivation, Thermal challenge and Undernutrition) and the agreed suite of measures that quantify changes in the animal's behaviour, emotional and physiological states.

During the design of these studies, it will be important to consider animal factors that can and potentially will influence the magnitude of the response to the challenge (eg. temperament (fearfulness), age, physiological state (pregnancy status)).

Ideally, these studies would be undertaken in both cattle and sheep and replicated to facilitate statistical confidence in the results.

The research will require a strong statistical foundation not only during the experimental design phase, but to develop and apply techniques that can integrate the complex array of inputs to generate generic and context-specific welfare assessments.

(iii) Validation of welfare assessments

During this stage, the most informative welfare assessment measures will be validated within industry situations.

(iv) Publication and extension of results

7.3 Immediate development of auditable welfare assessments

During the workshop, the urgent need for auditable welfare assessments was highlighted. These assessments should be built on existing knowledge and the assessment criteria would include simple practical measures (eg. livestock environment thresholds (temperature, noxious gases) during live shipping, incidence of specific diseases/injuries/mortalities within a herd or flock, Grandin abattoir audits, provision of appropriate stock handling and loading facilities). Depending on the context, these assessments might only provide a rudimentary indication of the welfare status. However, it needs to be emphasised that these would form the foundation on which the new knowledge and research outputs from the livestock welfare measures framework would be built upon.

It is recognised that the MLA strategic plan includes audit development and it is recommended that practical measures be identified from current knowledge and integrated with the MLA process for development of welfare quality assurance in allied parts of its R&D plan.

7.4 Implementation of welfare assessments in industry

Concurrent with the research phase, it is paramount that the animal industries engage in the development of implementation strategies. The industries need to consider several key issues notably:

- What is the best vehicle for the application of welfare assessments within industry (eg. self regulation through QA or independent audit systems?)
- Which agencies need to be involved?
- Revision of welfare related codes of practice
- Resources required to implement these changes

It is important to commence this process sooner rather than later, as it will facilitate more timely adoption of the research outcomes.

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9 Appendices

9.1 Appendix 1 Pre-Workshop Aims and Background

1. Background

The development of improved measures of animal welfare and an enhanced understanding of how animals cope with challenging situations will allow farming industries to optimise practices and provide assurance to consumers, markets and regulatory authorities.

Rather than have research on improved welfare measures occurring as separate projects, with each project examining a different approach or biological system in isolation, the aim of this Workshop is to scope the potential for integration of such research. This would permit the evaluation of how the varying responses are linked, and provide an enhanced understanding of the true welfare costs to the animal.

This facilitated Workshop will bring together Australian and International scientists working in animal welfare and related disciplines, as well as industry stakeholders and research and development bodies. A combination of key briefings, break-out groups and general discussion will be used to examine the differing approaches and biological responses that are considered important for the development of improved welfare measures, and to explore the potential for their integration.

2. Purpose and Outcomes

Purpose
To develop an integrated research framework that may be utilised for the discovery, refinement and delivery of objective welfare measures for the Australian animal farming industries. This framework will be evaluated by MLA, AWI, and other industry bodies, with the aim of developing and commissioning research in the area.

Outcomes

- Identification of the key challenge models and biological response pathways that could be incorporated in the research framework:
    - Livestock challenge models that incorporate key stressors of industry relevance.
    - Biological mechanisms and assessment approaches that capture the complexity of animal responses and biological costs to the animal.
    - Analytical methodologies that convert the complex response inputs into meaningful and applicable welfare measures
- Determination of potential national and international research collaborations to enhance research and optimise cost effectiveness in the welfare measures research area
- Identification of processes by which welfare measures identified from subsequent research can be applied to assess animal welfare for Australian farming industries

3. Framework description

Background and general description
The MLA Strategic Plan for Animal Welfare identifies the need for research to develop improved, objective measures of animal welfare.

Such measures of animal welfare can then be used to: 1) evaluate the welfare status of livestock husbandry practices and environments; 2) address any issues in practices that are revealed; and 3) provide assurance to markets, regulatory authorities and the general public.

Animal welfare represents a complex synthesis of the biological state of an animal and the interpretation of this state by human observers. In order to capture the mechanisms that contribute to different states of welfare, we should not just rely on one aspect of biology (e.g. behaviour or stress physiology), but rather utilise approaches that represent a range of pathways.

The debate central to how animal welfare should be assessed has been both contentious and at times, non-productive. Consequently, there is a lack of agreement amongst scientists and regulatory authorities on what is the most appropriate approach or methodology. The purpose of this Workshop is not to advocate one or two specific ideas for the development of improved animal welfare measures, but rather to scope the development of an integrated framework, within which different but meritorious approaches can be applied in concert.

This intersecting approach is particularly important when one considers that in order to make an objective assessment of an animal's welfare, we need to not just measure a response (which may be adaptive), but be able to interpret changes in terms of the animal's ability to adapt and the cost of adaptation. Applying different scientific approaches within a common framework will permit the understanding of how the different mechanisms interact with each other, and allow the identification of common measures or pathways that may respond to multiple stressors, and which capture the true cost to an animal of the challenges involved.

An integrated framework for research on objective welfare measures should comprise a number of key aspects:

1. Livestock challenge models of industry relevance and varying stressor components.

2. Differing biological mechanisms and assessment approaches that capture the complexity of animal responses.

3. Analytical models to identify response signatures indicative of compromised animal welfare, and links to applied measurement platforms. (For example, a major advantage of conducting cross-disciplinary assessments on common resources would be the opportunity to combine data through complex systems modelling to better describe the state of compromised welfare than that revealed by traditional analytical approaches.)

4. International linkages to incorporate key science capabilities existing outside Australia, to enhance knowledge transfer and avoid duplication of effort, and to enhance the international acceptance of the welfare measures developed.

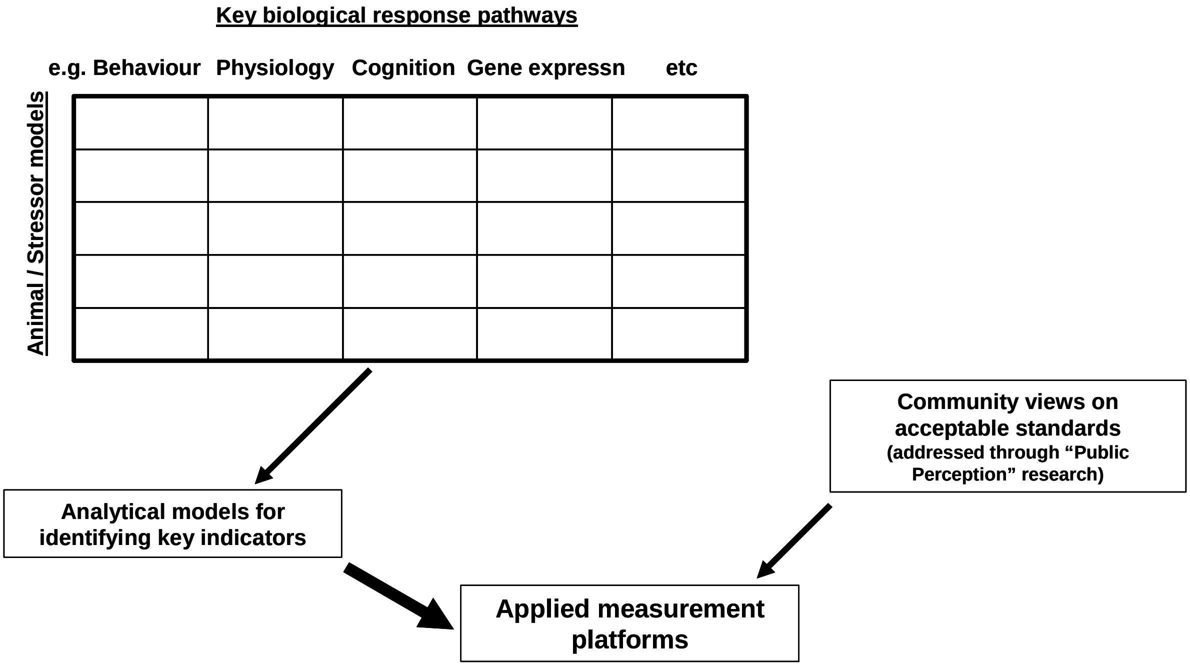


Figure 1. Schematic diagram of integrated framework to develop objective welfare measures

The applied measurement platforms will be based upon the most informative objective welfare indicators, and will be utilised predominantly for welfare auditing and welfare quality assurance, and less so for utilisation in experimental evaluations of practices or environments. The nature of the applied measurement platforms will depend partially on the types of objective indicators identified, but it is likely that measurement systems will be sufficiently generic to enable the use of underpinning technology in this area. An example of the use of an applied measurement platform would be a system that automatically records an animal's stress levels at the time of slaughter, so that Australian meat could be packaged with this assurance along with other quality information.

There is ongoing research from a number of industry and scientific groups on monitoring and understanding public perceptions and opinions in the welfare area. Although such public perception research may not form part of an objective welfare measures project, it would be important to link with this research, so that welfare measures utilised in farm animals and the acceptable thresholds of these measures are in alignment with public expectations.

9.2 Appendix 2 Workshop Output Notes collated by Ruth Davis

<u>Breakout Session 1 - Measurements</u>

Discussion on the core subsets of measurement types (additions/deletions?) and how we identify/interpret measurement thresholds?

1. **What broad measurement approaches and biological response systems should be incorporated in the framework? (NB- avoid focusing on the inclusion/exclusion of specific variables.)**
2. **Are there approaches that need to be added to the framework? (or deleted?)**
3. **How will we determine whether a particular level of response for a variable constitutes an adaptive/mild response or compromised welfare.**

KEY POINTS FROM WORKSHOP GROUPS

- *Animal welfare involves both physical and mental aspects*
- *There is a dichotomy between research and auditing requirements*
- *The proposed AW research framework utilises a correlational approach however there is a conflict between integration and specificity. Alternative is hypothesis driven research*
- *Health and behavioural measures should be added to research framework*
- *There should be a third dimension to the framework matrix – “animal preparedness” (backgrounding, genotype etc.)*
- *Productivity and product quality are important drivers for AW at producer level and this should not be ignored.*
- *An integrative measure of behaviour, metabolic, immunological and adaptive aspects of animal welfare should be considered*
- *Some practices are non-negotiable for public acceptance*
- *Should we consider a “ Whole of Life” measure of AW rather than a short term approach*
- *Additional approaches may include:*
    - *Telemetry/non-invasive,*
    - *IRT – infrared thermography*
    - *MRI – brain wave activity*
    - *NIR – near infrared spectroscopy*
    - *Ecological approaches with behavioural measures*
    - *EEG (eg reward pathway approach)*
    - *Metabolomics*

RED GROUP (1)

<u>Definition of Animal Welfare:</u> “To do with mental or physical aspects”

<u>Main driver:</u> To find ways of linking these two aspects

<u>Split:</u> **<u>Research</u>** ↔ **<u>Audits</u>**

There is a dichotomy between the research agenda and the pressing need for auditable measures NOW! eg. for sea transport. Auditing needs to start now based on current knowledge.

Example: Identify aspects of audit with least science basis.

Audits
- must be based on current understanding and knowledge
- complex
- many pressures
- important not to "over promise" – ensure that it is made clear that audit parameters are based on current knowledge but that research is ongoing to address welfare concerns

Research
- need to integrate with auditing
- the research framework proposed at this meeting is correlational. Risks are associated with this approach as it is difficult to determine what relates to what.
- Conflict between integration and specificity
- The alternative research approach is to test hypotheses
- Research needs time or rubbish will be generated
- International (possible links) approach – already exists to some degree
- There are possible approaches to link physical and mental aspects of AW

Measures to Add to Framework

Physical: Health
Mental: Behavioural approaches

BLUE GROUP (1)

Key Measures:

- Productivity is the main driver for animal welfare (farm profitability)
- Product quality also important – often AW issue if product sub-standard

Then need:
- High throughput technology – must be initially interpretable by researcher, usable by producer and interpretable by the media to communicate to the public.

Integrated measure of:
- behavioural
- metabolic
- immunological
- adaptive (stress physiology)

Processes through:
- gene expression patterns (proteomics)

- need for researcher → time → producer friendly technology→ public

Link to more esoteric issues such as:
- quantifying behaviour
    - telemetry
    - imaging – happy versus sad animals
- “twitchy ears”
- feedlot monitoring
- injuries and health
- developmental programming (dam tries to imprint gene expression patterns in-utero based on own experience)

Need to assess:
- Welfare continuum in relation to production continuum. The community currently dictates the “cut-off” points. Cut-offs should be determined scientifically.
- Metabolomics – have potential to evaluate animal health from a single blood sample

YELLOW GROUP (1)

1. Broad approaches and biological response systems …….

- Animal’s sensory perception – motivation/neurological underpinning of satisfaction and reward pathways
- Human aspects
    - Consumer, producer, community
    - ? what is ethical, what is “natural” practice?
    - what practices are non-negotiable for public acceptance?
- “Whole of Life” measure – may need a model
    - context – time – short = 1-2 weeks or whole of life
    - severity versus duration (of impact)
    - recovery time/breaks between stressors

2. Added approaches

- Telemetry/non-invasive
- IRT – infrared thermography
- MRI – brain wave activity
- NIR – near infrared spectroscopy
- Ecological approaches with behavioural measures
- EEG (eg reward pathway approach)
- Metabolomics

3. Determining adaptation versus consequence to the animal

- Need to consider number of animals impacted

- Trade-off with what's acceptable by public – "Conjoint adaptive analysis" (questionnaire that adapts as you work through it depending on answers; trades off different resources; get a set of different resources for the animal)
- Use scientists/experts to measure using subjective and objective comparisons

GREEN GROUP (1)

Q2.

Add health to framework

Q1 & 2.

Some measures that can be used right now are:

- Mortality
- Lameness
- Bloat

But what levels of the above are acceptable?

Over time other measures may be used depending on:

- New knowledge
- Change in public perceptions

A third dimension should be added to the matrix – "preparation" of animals (backgrounding etc)

Breakout SESSION 2 - BEHAVIOUR

Questions:

1. **What are the key behaviour components that need to be included in the framework?**
2. **How will they be measured?**
3. **Are these more applicable for some animals or challenge types than others?**

KEY POINTS FROM WORKSHOP GROUPS

- *Validation of behavioural measures is important*
- *Behavioural measures must be quantifiable*
- *Health measures could be linked with behavioural measures*
- *Approaches must be species specific and should also consider genetic background and animal experience*
- *Need to understand causes and consequences related to behavioural measurements*
- *Objective tests needed for fear, adaptability, anxiety and cognitive ability*
- *Techniques for assessing behaviour include: visual appraisal, electronic monitoring, mathematical calculations*
- *Happy animal = productive animal*
- *Are the results of current work on cognition well recognised?*

RED GROUP (2)

1. Behavioural observations – qualitative versus quantitative assessment
2. Choice/preference – motivation (addition of a work component)
3. Cognitive ethology eg breaking down emotional states (Alain's work)

- Need to validate measures. Can validate against physiological measurements (or vice versa?)
- Could try to link with health measures (physical health versus mental health)
- Difficult to validate behaviour – what is normal behaviour?
- If interested in physical and mental health could study relationship between behaviour and health in specific systems.

GREEN GROUP(2)

- Emotions are a central part of animal welfare
- A lot of research is currently underway on cognition – is this work getting out?
- Emotional responses more complex than once thought
- Need more understanding of basic mechanisms controlling behaviour such as feeding
- Stress induced immunological suppression may be due to mental or physical stress
- "Natural behaviour" no longer considered
    - Need to better understand why animals perform specific behaviour and the consequences of those behaviours (or lack thereof)
    - Interest in using modern technology to automatically record animal behaviour in normal settings

- Behavioural measurements must be validated if to be used to assess animal welfare. Must try to understand what it means to the animal.
- Types of behaviour:
  - Indicators of fear, pain
  - Changes in maintenance behaviour (rest, eating)
  - Abnormal behaviour
  - Change in reactivity – apathy
  - Choice tests
- Behaviour is a major issue for critics of animal agriculture – therefore must be dealt with:
  - Key way of getting into the minds of animals
  - Problem of concept of natural behaviour – to use behaviour in welfare assessment need to understand the causes and consequences of the behaviour
  - Interest in using modern technology to automate behavioural recording:
  - Image analysis systems
    - o Activity meters
    - o GPS
    - o Automated feeding equipment

BLUE GROUP (2)

Q1:

- Normal (appropriate) behaviour versus abnormal – objective measure in extensive situation.
- Group animals versus individual animals

Need tests for:

- fear
- adaptability
- anxiety eg ASGO – Flight speed
- cognitive ability

Q2.

- Visual appraisal for unusual behaviour
- Electronic eg. drinking behaviour, anthelmintic administration (NLIS)
- Mathematical eg weight gains

Q3.

- Measures need to be species specific.
- Individual tests may need to be developed.
- Do not put human values on animals.
- Happy animal = productive animal

YELLOW GROUP (2)

Q1.

- Ask the animal its view/perception of the situation it's in.
- Quantification of the emotional experience (vs – response to stimulus).

- Animal distributions in relation to habitat:
    - Manipulate availability of resource
    - Grouping using area
- Time budgets (prioritise behaviours)

Q2.
- Operant techniques – aversion to enter
- Manipulate availability and observe
- Observe

Q3.
Yes!
- e.g novelty (physiology) + operant techniques
- Hierarchy of pleasantness and aversiveness
- Measurement in the presence of conflicting motivations
- Must cater for genetics and animal experience

BREAKOUT SESSION 3 - PHYSIOLOGY

Questions:

1. **What are the key physiological systems and variables that need to be included in the framework?**
2. **How will they be measured?**
3. **Are these more applicable for some animals or challenge types than others?**

KEY POINTS FROM WORKSHOP GROUPS

- *Key physiological systems are endocrine, immune and metabolic – simultaneous measurement of all my identify interactions between systems*
- *Need to consider responses to both acute and chronic stress – less able to measure chronic stress*
- *HPA is still a key system for including in assessment*
- *The selection of variables and systems depends on the context or challenge*
- *Measurement redundancy – many parameters tell us the same thing*
- *Very important to relate production traits (eg reproduction, growth rate) to physiological measures*
- *Remote measurement is expensive but may be used to validate more practical measures*
- *Need measures that are interpretable – many are currently not*

BLUE GROUP (3)

Key physiological systems

- Endocrine
- Immune
- Metabolic

Suggest simultaneous measurement of all three to look at interaction between systems.

Key variables

- Body temp, HR, RR (field)
- Hormones – HPA, Leptin etc (research)
- Metabolites – glucose, FFAs etc (research)

How should the systems and variables be measured?

- Methodology is the limitation to physiological measurement
- Interaction between systems - more knowledge needed
- Integrative role of the brain – CNS
- Suggest use of a function test – change from basal to maximal - indicates capacity for animal to cope (thresholds)

Acute versus chronic stress

- depends on stressor, genetics, experience
- some common responses/some differences depending on species although physiology the same

YELLOW GROUP (3)

- HPA - historic model - lends credibility to work being done
- Hormone drivers – sign of what has happened to animal before eg GH
- ANS – more relevance for acute stress; can be measured indirectly eg HR, receptor expression etc
- Metabolites – eg energy balance – consequences of what has happened

Immune function
- Could look at health effects as an indirect measure of immune function but a large number of animals are required for health studies
- Need both for validation of each other
- Innate immunity versus adaptive immunity

Pain versus suffering
- B-endorphins
- EEG
- Neuromas

Metabolism
- Temp
- RR
- HR
- Clinical measures
- Growth?

Productivity
- growth
- reproductive capacity

Neurotransmitters
- link to behaviour and emotions

Basic Physiology
- still not well understand particularly in relation to what we wish to measure
- "shotgun" approach??

RED GROUP (3)

1. Physiological variables and systems
- selection of variables and systems depends on the context or challenge
- prioritisation should be based on the challenge and what you are trying to assess (physical or mental state); short and long term aims eg brain receptor density changes – long term
- measurement redundancy – many parameters tell us the same thing
- physiology responses can be determined from post mortem samples as well as in vivo
- need to integrate understanding of physiological responses to the challenge
- HPA, immunological, metabolic

- context under which being assessed is vital

2. How to measure?
- less invasive measures (faeces, urine, milk, eggs)
- measurement technologies may restrict behaviour
- remote measurement - key limitation is expense; may be used to validate more practical measures

3. Applicability
- Yes – must be challenge and response specific (variables and measurement approach)

<u>GREEN GROUP (3)</u>

Q 1 & 2

- Very important to relate production traits (eg reproduction, growth rate) to physiological measures
- Need something that is interpretable – many are currently not
- Possibly good measures of acute stress
    - T and HR combined with implants and remote sensing
    - Metabolites (NEFA, glucose etc)
    - Skin conductivity
    - Cortisol and response to ACTH (eg negatively related to feed efficiency)

- Measures are not so good for chronic stress:
    - Energy deficit is a good measure of nutritional stress
    - Use of the "-omic approach" – large number of analytes and complex bioinformatics needed (eg glucose, NEFA, urea)
    - Heat shock proteins
    - Nitric oxide
    - Immune system - ? about links with welfare; ? good to have increased or decreased response
    - like to have dimensions of emotional state related to physiological measures – dopamine, catecholamines

Q3.

Yes!
Must consider acute vs chronic stressors and,
Passive vs active responses
Increased catecholamines versus increased cortisol

BREAKOUT SESSION 4 - MOLECULAR AND GENOMIC APPROACHES

Questions:

- **What are the key genomic and molecular components that need to be included in the framework?**
- **How will they be measured?**
- **Are these more applicable for some animals or challenge types than others?**

KEY POINTS FROM WORKSHOP

- *Possibly not a currently useable objective measure of AW*
- *May be useful as a basic research tool to generate hypotheses to increase understanding of animal responses*
- *Important for identifying different genotypes and their adaptation to particular environments (long term genetic selection for welfare?)*
- *Experimental design very important when using these techniques.*
- *Important to conduct studies within currently known physiological framework*
- *High cost*
- *May be able to store samples from current studies for future analysis once techniques further developed?*
- *Animal welfare is involved with understanding the animal's biology – need to incorporate these new tools of biology in order to understand the pathways involved*
- *Must have very good experimental models and well defined experiments to obtain meaningful results*
- *Currently most useful for cattle – little available for sheep*

RED GROUP (4)

- If we accept physiology as a objective measure then must also accept molecular approaches
- However difficult to see how it fits in as an objective measure of AW.
- May be useful if targeted in specific studies.
- Better to look at it as a basic research tool to generate hypotheses to increase understanding of how animals respond to different situations.
- Difficult to come up with a relationship between a behaviour and a genome
- Important for identifying different genotypes and their adaptation to particular environments.

BLUE GROUP (4)

- 2 possible uses - long term genetic selection for welfare or to answer biologically important questions. If the latter this may identify genetic markers for future experiments
- Gene expression patterns may be used in early life to predict future performance
- What tissue would you like at if using this technology – blood? Lymphocyte gene expression
- Amount of time collecting samples may be very small – but statistical support high
- Predictor of life history pre-slaughter – can gene patterns identify life long history of nutrition and/or recent stress?
- Important to conduct studies within currently known physiological framework

- Should not let this constrain our innovative thinking on how this tool may be used.

YELLOW GROUP (4)

- Strategy - How can we capture the opportunities from these technologies?
- Need to work through the strategies of how the technologies would be applied due to:
  1. High Cost
  2. Experimental design – need to have informed design asking valid biological questions eg QTL, gene expression
  3. Limitations of sampling target tissues eg if interested in neural tissues need multiple time points
- May be able to store samples from current experiments for future use eg. gene expression in peripheral leucocytes
- Animal resources for this type of work – must be well phenotyped, cost involved, issues with ownership of information of resource animals eg dairy animal database, opens up issues of collaboration outside the immediate sphere of influence

GREEN GROUP (4)

Q1.
Animal welfare is involved with understanding the animal's biology – need to incorporate these new tools of biology in order to understand the pathways involved

Gene → Protein → physiology and behaviour
↑ ↓
←←←←←←←←←←←←

Validation will still be necessary – just because a gene changes ….????
Target tissue for particular stressor will need to be identified
Must have very good experimental models and well defined experiments

Q2.
- Microarray approach – hypothesis testing – specific genes/pathways. Currently expensive but will become cheaper
- Candidate gene expression
- Proteomics
- Metabolimics

Need to determine what we want out of it or we may get lost in the results

Q3.
Yes
Acute verus chronic
Currently more useful for cattle and technologies less available for sheep.

WORKSHOP 5 – STRESS MODELS

Questions

- **What relevant acute stress models are available?**
- **Is there a relevant acute stress model available for cattle and sheep?**
- **What measures are useful in interpreting responses?**

Key Points

- *Stressors need to be realistic and relevant to applied needs*
- *Stressors need to be species and class specific*
- *Should be able to measure both a physiological response and a behavioural response to the stressor*
- *Real life situations may involve multiple stressors with cumulative effects*
- *Hard to differentiate from acute and chronic stressors*
- *Need to consider both intensive and extensive management systems/stress*
- *"Whole of life" stress measurement??? Is it the "Holy Grail"?*
- *Difficult to justify use of acute pain stress models (ethics, public perception; large amount of research already carried out on many acute pain inducing procedures)*
- *Possible stress models include long distance transport, undernutrition, dairy cow model, heat stress.*

GREEN GROUP (5)

Q1&2

- Stressors should be realistic and relevant
- Acute:
    - castration and tail docking
    - dehorning and branding
- Chronic:
    - Transport
    - Temperature
    - Fear of humans
    - Social environment
    - Poor nutrition? Is this a chronic stressor
    - Disease – lameness, bacterial diseases, flystrike
- Combination of acute stress with chronic application eg use of stray voltage in milking parlours with dairy cattle (may occur 2 or 3 times a day for many months)
- Simultaneous application of acute stresses – long term effect?
- Long term effect of single acute stressor.
- Need to justify use of acute stressors particularly with regard to public and cost versus benefit

Q2

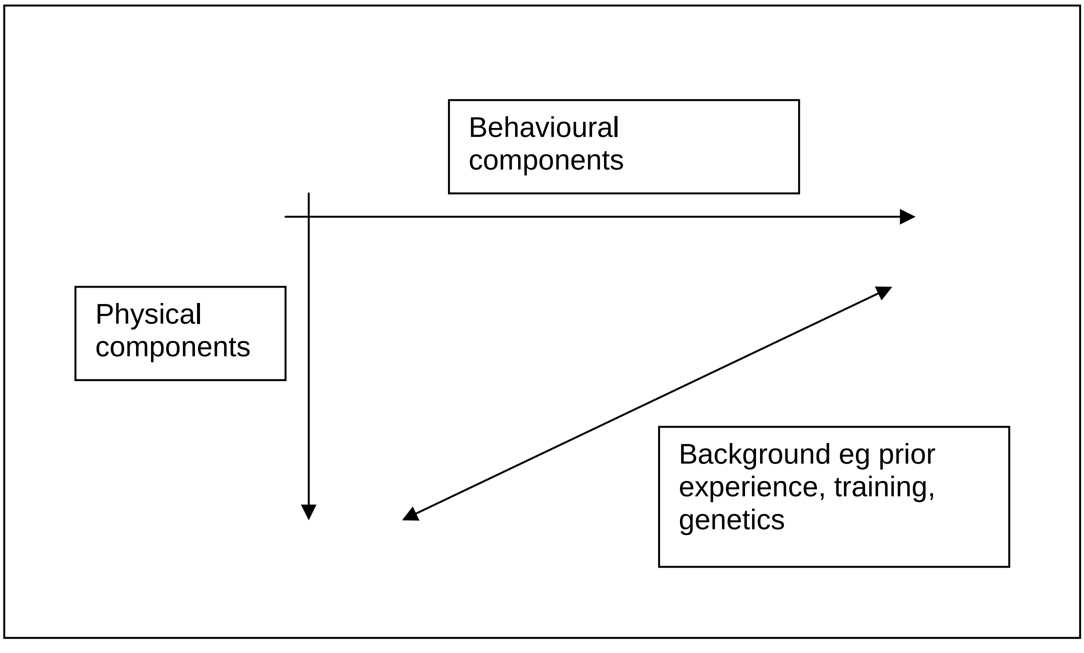
Too hard

Q3

- All food producing species and all ages need to be examined.
- Need to avoid cross species extrapolation
- Need to decide whether you examine extensive or intensive conditions and what would be appropriate for each area

RED GROUP (5)

- Model must be relevant to <u>applied</u> needs.
- Eg under-nutrition – must be able to measure both behavioural response and physiological response when model developed
- ?adaptation to chronic stress
- ? Accumulative stress effects
- ? Affect of repeated stressors
- ?Often complex series of stressors eg dairy cows in NZ winter
- ? Should disease and pain be included as a chronic stressor
- Best to first assess individual stressors then examine in accumulative effects
- Possible to categorise both physical and cognitive responses
- Care needed when measuring acute responses – may be artificial in that there can be adaptive responses. Applied example is how far dairy cows will walk to/from milking as an exercise model
- Possible models
    - Under-nutrition
    - Dairy cow
    - Live export



- "Whole of life" challenge model is the "Holy Grail". This is particularly important in the eye of the community. However group questioned whether this was true?? Animals can have a good life but can still have a single very bad episode. Whole of life issues can also to considered as intensive versus extensive housing for their life.

BLUE GROUP (5)

Q1

- Pain (group did not wish to deal with it – considered as a special case and may complicate the picture, ethical approval difficult to obtain to use pain as an acute stressor). Further discussion suggested that we may already have conducted enough research in this area and it is a matter of applying appropriate anaesthesia; or should select for animals that do not require painful procedures e.g. tail less sheep, less wrinkly sheep, polled cattle. We also do not have good evidence to support the benefits of some of these procedures e.g. tail docking. Less research has been conducted into chronic pathological pain e.g. foot disorders. Do we know enough about the public's perception of the acceptability of these painful procedures?
- Psychological stressor
- Physiological stressor
- Long debate in group on acute and chronic stressor – should it be defined by the duration of the stress or the duration of the response; duration and intensity of stressor need to considered

Q2

- Long distance transport very good as first challenge (but is it acute or chronic?)
- Fear (chronic)
- Heat stress (chronic)
- Nutrition and mixing of groups
- Accumulation of stressors then needs to be considered

Q3

- Sheep and cattle
- Should consider breed differences in ability to adapt

YELLOW GROUP (5)

Q1.

- What is the objective of the research??
- Stressors should be industry relevant but do not discount unrelated measures if it may contribute to the overall outcome of the model.
- Acute versus chronic not that appropriate as they are difficult to define
- Define features of stressor – times between application, recovery of animal between applications.

Q2.

Stressor challenges

- Thermal
- Space
- Mixing
- Isolation (model>)
- Thirst
- Under nutrition
- Handling
- Restraint
- Transport
- Shearing
- Disease
- Surgical intervention
- Deprivation of resting
- Inflammatory stressors
- Hormonal stressors

Overlying the stress challenges are modifiers:

- Combination of stressors
- Genotype
- Epigenetic
- Foetal programming
- Experience

Stressors must be relevant to species and class and of animal
Must also be appropriate to animal's physiological state –lactation, pregnant, high growth

Measures – should include measure of time to recovery. Experiments may not continue long enough to show that animal has recovered (at least from some parameters).

BREAKOUT SESSION 6 - INTEGRATION AND APPLICATION

Stephen Page Presentation

INTEGRATION AND APPLICATION

ISSUES TO CONSIDER

Stephen Page
Advanced Veterinary Therapeutics

DIAGNOSTIC / ANALYTICAL TESTS

Operating characteristics

- Sensitivity [proportion of patients with the condition who have a positive test result]
- Specificity [proportion of patients without the condition who have a negative test result]
- Likelihood ratios
- Repeatability / Precision
- Intra- and inter-observer variability
- Validity
- Robustness
- Accuracy
- Acceptance criteria

RISK ASSESSMENT

- Chemicals, antimicrobial resistance etc
- Science based decisions
- Zealots and decision making
- Risk perception (Paul Slovic, Doug Powell, Peter Sandman)
- Value of information analysis: evaluates the benefit of collecting additional information to reduce or eliminate uncertainty.

Peters et al (2004) An emotion-based model of risk perception and stigma susceptibility: cognitive appraisals of emotion, affective reactivity, worldviews, and risk perceptions in the generation of technological stigma. Risk Analysis 24: 1349-1367

DIAGNOSTIC / ANALYTICAL TESTS

(objective measurement of welfare)

- What is being measured
- What is the "Gold Standard"
  [Pritchard et al 2005. Assessment of the welfare of working horses, mules and donkeys, using health and behaviour parameters. Prev Vet Med 69: 265-283]
- What does the customer need?
- What are the alternatives?
- Practicality, cost, convenience
- Interpretation
- Usefulness

PHARMACOVIGILANCE

- Causality assessment
- Expert opinion
- Global introspection (GI)
- Delphi methods
- VICH (veterinary international harmonisation)

Modus operandi

- MY BACKGROUND
- RISK ASSESSMENT
- PHARMACOVIGILANCE
- PRODUCT DEVELOPMENT
- DIAGNOSTIC TEST ANALOGY
- THOMAS BAYES
- INTEGRATION
  - Strengths, barriers, weaknesses
- MOVING FORWARD

Reverend Thomas Bayes

b. 1702, London - d. 1761, Tunbridge Wells, Kent

Bayes's Theorem

(inductive inference from observation of effects to their cause)

Pre-test odds of a hypothesis being true multiplied by the weight of new evidence generates post-test odds of the hypothesis being true.

In the diagnosis of a state, this refers to the odds of that state being present versus not present.

Gill et al (2005)
Why clinicians are natural bayesians.
BMJ 330: 1080-1083

Goodman SN (1999)
Toward evidence-based statistics. II. The Bayes factor.
Ann Intern Med 130: 1005-1013

PRODUCT DEVELOPMENT

- Satisfying unmet needs.
- Who is the customer?
- Evidence Based Medicine
- Quality and Strength of evidence
- Product life cycle

STRENGTHS

- Increased experience
- Additional expertise
- More ideas
- Team work
- Consensus
- Agreement
- Common goals
- Common methods
- Common interpretation
- Avoid duplication
- Broad participation
- Ownership
- Build strong relationships
- Develop solid communication
- Power in numbers
- Collaboration
- Cooperation
- Partnership
- Sharing information
- Many eyes and ears
- Early alert
- Detection of emerging trends

BARRIERS

- Poor management
- Poor communication
- Poor coordination
- Personal ambition
- Cultural differences
- Time zones
- Distance
- Technology
- Mistrust
- Lack of motivation
- Lack of focus
- Poor leadership
- Low energy
- Ill-defined goals and objectives

WEAKNESSES

- Decreased individualism
- Decreased innovation
- Divergent goals
- Different methods
- Lack of agreement
- "team think"
- Single outcome
- Concessions
- Forceful or dominating personalities
- Individual driver
- Bandwagon effect
- Inertia
- Paralysis / analysis

DELPHI

DELPHI METHOD

- Group communication among a panel of geographically dispersed (anonymous) experts
- Often used to answer single specific question.
- Dialectic process: thesis – antithesis - synthesis
- Structured process for collecting and distilling knowledge from a group of experts by means of a series of questionnaires interspersed with controlled opinion feedback.
- Facilitates formation of group judgment
- Pitfalls well described eg
  - selection of monitors
  - structuring of the questionnaire
  - analysis of responses

Graham B, Regehr G, Wright JG (2003) Delphi as a method to establish consensus for diagnostic criteria. J Clin Epidemiol 6: 1150–1156

- Two stress models proposed (one for each group):
  - Heat stress in sheep eg up to 5-7 days (Red and Blue groups)
  - Under nutrition in cattle eg up to 20% bodyweight loss over 2-3 months (Green and Yellow groups)

- What are the:
  - Experiments
  - Key hypotheses
  - Measurements
  - Integration

HEAT STRESS MODEL – RED GROUP

Hypothesis – *sheep feel crap when they are hot*

How do we know this and how would we quantify it?

Experiments:

1. Temperature gradient room:
    - Observations – where do they want to be; at individual and group level
    - How hard are they willing to work to get to their preferred zone?
    - Impact on animal if they are restricted/thwarted – at different temperatures

2. Measurements:
    - Quantitative and qualitative assessment of behaviour
    - Body temperature (probes), respiratory rate/character and HR (remote)
    - Blood parameters (not specified)

HEAT STRESS MODEL – BLUE GROUP

Hypothesis – *Breed A and Breed B do not differ in suffering adverse effects to heat stress.*

Basis of experiment was to find out which measurements were relevant for future studies related to heat stress.

Experimental design
- Adapt to a climate controlled feel lot situation
- Ad lib access to food and water for 3-4 weeks
- Impose heat stress over 5-7 days
- Need to ensure sufficient statistical power – probably 100+ animals per breed.

Measurements
- Behaviour – time budgets (video, image analysis, loggers}
- Cognitive state – learning tasks/startle response/operant tasks
- Preferences – controllability/predictability
- Physiology – T, HR, RR, endocrine hormones, cytokines
- Gene expression, metabolites
- Intakes – food and water

Integration/analysis

Statistical analysis:
- Principle component/discriminate function

- Relationships among various measures
- New pathways/indicators

UNDER NUTRITION MODEL – GREEN GROUP

Group wanted to determine at what level at which body score does an animal become compromised. This related body condition score to observations in the animal

- Group (individual feeding) or Individuals
  - growing
  - mature
  - cycling females
  - different body scores (select animal type as appropriate for the study)

- Impose treatment (reducing intake)
  - High roughage (full but inadequate energy) or,
  - Not enough food to eat

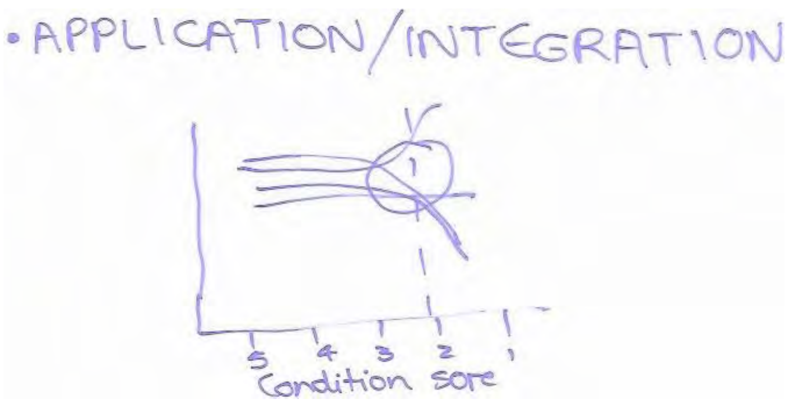
- Measures relative to condition scores
  - Metabolic profile
  - Water intake
  - Behaviour – chewing on bars/social interactions

  Measures depend on the specific question being asked

- Add Stressors/Assess adaptation
  - metabolically effect acute challenge
  - concept of feelings Novel stimulus/response
  - low nutrition susceptible to disease (immunocompetence)

Application/Integration

- Outcome is to find the spot on the graph following animal cost/benefit analysis where a condition score above is acceptable and below is not.



UNDER NUTRITION MODEL – YELLOW GROUP

Assumptions:

- Access to water
- Grazing situation

Null hypothesis – *welfare is not compromised*

Hypothesis:

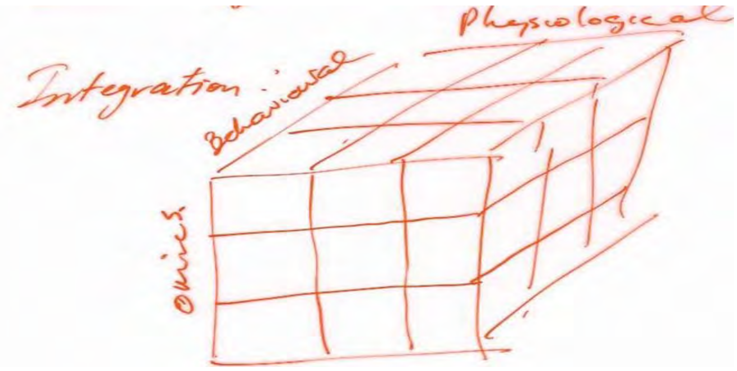
- *Cattle such as these show more abnormal behaviour*
- *Cattle are experiencing suffering, negative mental states*
- *Cattle are unable to cope with additional stressors such as transport*

Experiment
Matched control group (matched by genotype and background)
Contrast treatment 100% weight loss – 75% loss

Behaviour

- Time budgets for abnormal behaviour
- What they are doing at rest
- Operand conditioning (longitudinal) – motivation to feed
- Mental state
    - Test response to novel stimuli at a feed trough – time taken to return
    - Tissue blood samples – metabolites, hormones, leptin, NEFAs, ketones
    - Body condition score
    - Genomic scans – metabolites, proteomics (may store samples for the future), metabolomics
    - Respiration chamber as an indicator of chronic stress; measure heat production as weight loss; serial blood samples; integrate with other measures
- Impose additional stressors eg. transport
- Disease
    - Vaccinate with vaccine that they are naïve to and measure antibody response
    - Worm burdens – elevated?

It was suggested that the measurements be carried on longitudinally as cattle recover their weight.



9.3 Appendix 3 Hard copies of the speaker presentations

MLA and AWI Funded

Animal Welfare Objective Measures Workshop

6th & 7th June 2005

An integrated framework to research and develop Animal Welfare measures

Background

MLA Animal Welfare Plan

- Objective measures of animal welfare
- Land transport
- Development of animal welfare best practice /QA
- Husbandry procedures- validation/alternatives
- Public perceptions of animal welfare
- Training & Education packages

Background

MLA/AWI priority – development of objective measures for the assessment of animal welfare

- **Assess and improve current production/husbandry practices**
- **Provide assurance to consumers of the animal welfare integrity of meat and wool products**
- **Enhance welfare-associated market access**

Animal Welfare Assessment

"...when it comes to 'defining' animal welfare, there is a tendency to rely on too limited a range of measures and a difficulty in dealing conceptually with the multivariate nature of animal welfare assessment."

Rushen (2003)

Animal Welfare Assessment

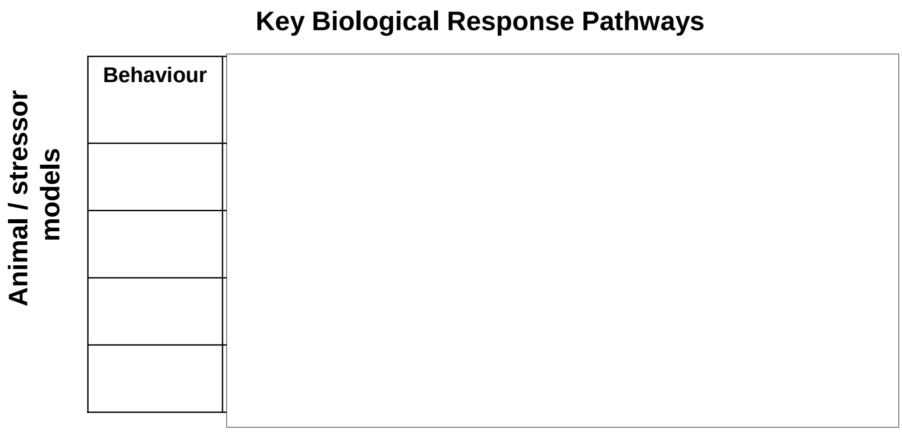
- Different fundamental approaches
  - Biological function
    - Behaviour, neuroendocrinology, immune function etc.
    - Cost of adaptation
  - Animal feelings or consciousness
    - Animal preference/choice
    - More difficult to assess
- Debate regarding these approaches continues

Animal Welfare Assessment

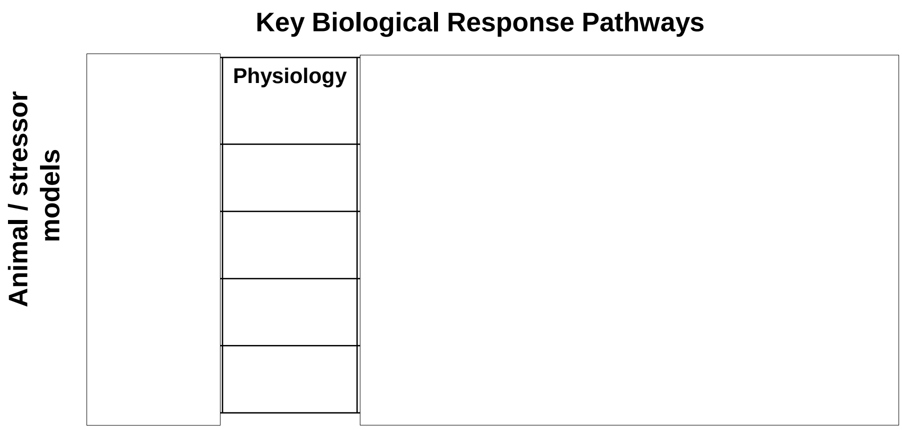
Application Strategies

- Welfare indices
  - Integration of multiple welfare criteria into an overall score
- HACCP based systems
  - Critical control point approach (Grandin welfare audits of abattoirs for McDonalds)
- Measurement technologies
  - Are there automated ways of capturing and using key measures?

Research approaches



Research approaches

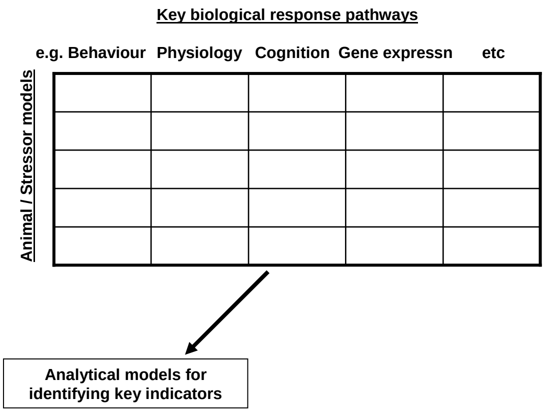


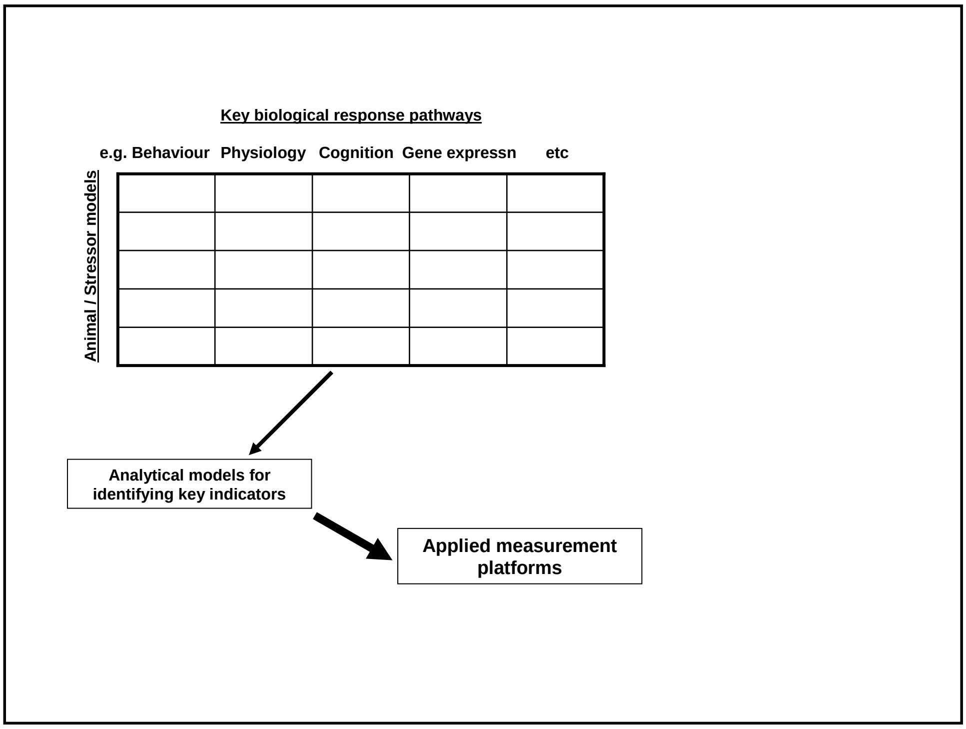
Research approaches

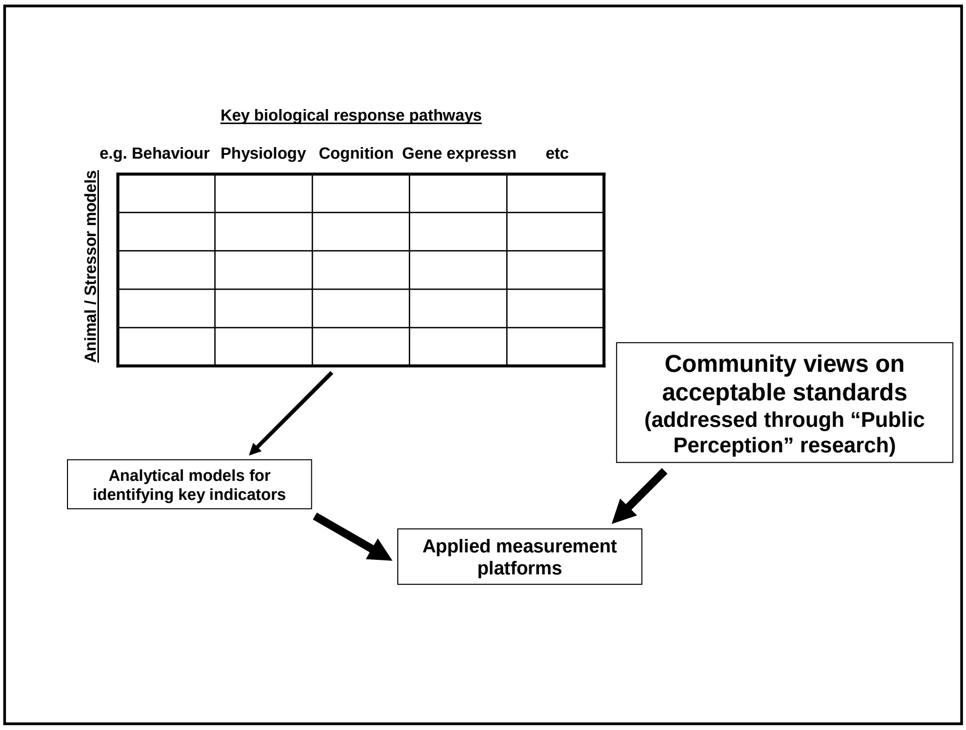
Key Biological Response Pathways

Animal / stressor models

Behaviour	Physiology	Cognition	Gene & Protein expression	Preference	Etc.




Key biological response pathways
e.g. Behaviour
Physiology
Cognition
Gene expressn
etc
Animal / Stressor models
Analytical models for identifying key indicators
Applied measurement platforms


Key biological response pathways
e.g. Behaviour
Physiology
Cognition
Gene expressn
etc
Animal / Stressor models
Community views on acceptable standards (addressed through “Public Perception” research)
Analytical models for identifying key indicators
Applied measurement platforms

Concept

- **Develop an integrated framework for research on objective welfare measures**
- **Framework components**
  - **Integrated, multidisciplinary**
    - **Current - ethology, animal physiology, immunology, neurophysiology**
    - **New - gene expression, cell stress, novel data analysis systems**
  - **Utilise livestock challenge models of industry relevance**
  - **Include different methodologies that capture the complexity of animal responses and welfare assessment**

Concept

- **Develop an integrated framework for research on objective welfare measures**
- **Framework components**
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    - **New - gene expression, cell stress, novel data analysis systems**
  - **Utilise livestock challenge models of industry relevance**
  - **Include different methodologies that capture the complexity of animal responses and welfare assessment**

Integrated Framework

Risks

- Requires commitment from scientists and industry
  - Shared vision and approach
- Difficult goal
- Circularity

Research approaches

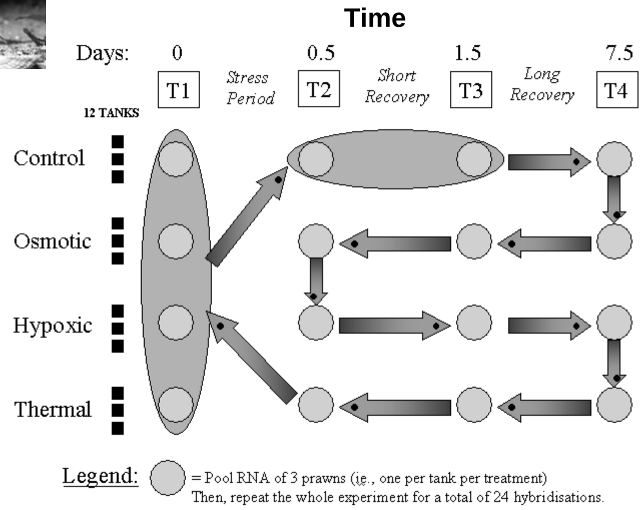
Key Biological Response Pathways

Animal / stressor models

Behaviour	Physiology	Cognition	Gene & Protein expression	Preference	Etc.

Stress research in prawns

Stressor model



Enrique de la Vega & Kate Wilson (Australian Institute of Marine Science)
Toni Reverter (CSIRO LI, design and analysis).

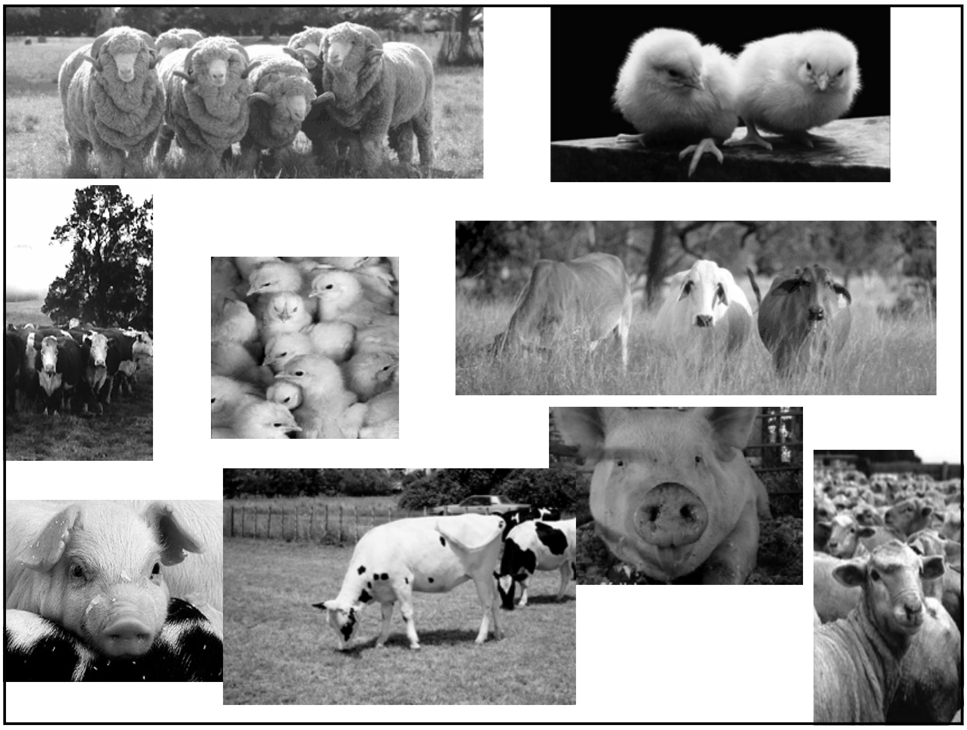
Stress research in prawns

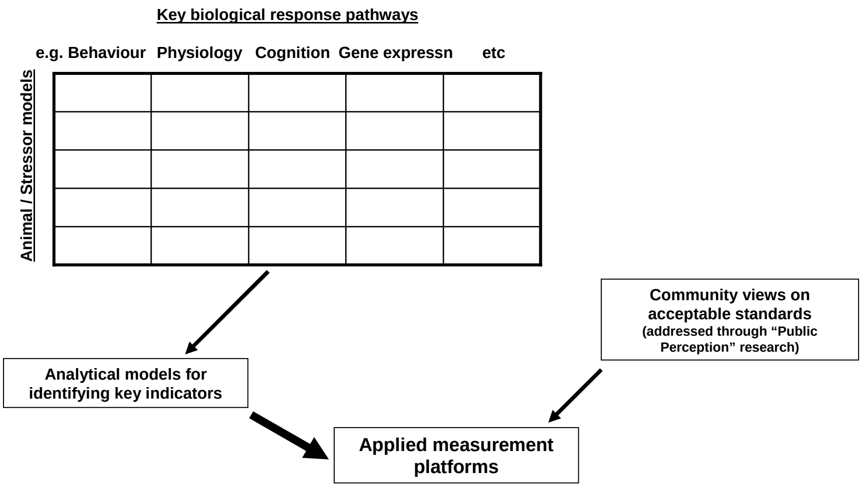
Stressor model

Biological response pathway

	Control pattern	Osmotic pattern	Hypoxic pattern	Thermal pattern
Gene	T1 T2 T3 T4	T1 T2 T3 T4	T1 T2 T3 T4	T1 T2 T3 T4
Lysozyme				
Hemocyanin				
Crustin				
Protease inhibitor				

Enrique de la Vega & Kate Wilson (Australian Institute of Marine Science)
Toni Reverter (CSIRO LI, design and analysis).




Key biological response pathways
e.g. Behaviour Physiology Cognition Gene expressn etc
Animal / Stressor models
Community views on acceptable standards
(addressed through "Public Perception" research)
Analytical models for identifying key indicators
Applied measurement platforms

Animal Welfare Assessment

Summary

- There may be no single best approach or strategy
- Multidisciplinary approach is required
- Need for an integrated research framework

International Context for Animal Welfare by Jeff Rushen

Some unresolved issues

1. How to best assess validity of welfare indicators- relate to some working definition of animal welfare
2. How best to weight and combine multiple indicators to form an overall welfare index (trade-offs)
3. Do we measure the occurrence of threats to welfare or the impact on the animal?

How we measure animal welfare depends on why we measure animal welfare

Definitions of animal welfare need to reflect the full range of concerns of the public / consumers and producers

e.g. an extension of the 5 freedoms

Historical context: Why we measure animal welfare

1. Determine need for legislation (especially within Europe): Phase 1
2. Develop auditable (on-site) animal welfare standards: Phase 2
3. Develop internationally accepted standards and measures (e.g. OIE): Phase 3

Phase 1: Animal welfare assessment driven by issues raised by possible (European) legislation

GOAL: Develop measures of animal welfare in order to see if legislation banning certain practices is justified.

Phase 1: Animal welfare assessment driven by legislation

Test truth of criticisms about the effect of housing systems and management practices on animal welfare:

e.g. do battery cages, tether stalls etc cause laying hens or breeding sows to suffer;

is debeaking of hens, tail docking of pigs, branding of cattle etc. painful

Phase 1: Animal welfare assessment driven by legislation

Research done under controlled, experimental conditions using experimental models

Behavioural and physiological / immunological measures used requiring technical expertise and complex interpretation- not well validated

Phase 1: Animal welfare assessment driven by legislation

Conclusions:

-Criticisms not completely groundless

-Alternatives not always better

-Animal welfare is multidimensional e.g not all welfare measures point in the same direction

Phase 1: Animal welfare assessment driven by European legislation

Conclusions:

-animal welfare very dependent upon “qualitative” factors such as the quality of management, the care that routine procedures performed etc.

-level of animal welfare specific to individual farms (or transporters or slaughter houses)

-need consider the capacity of a practice to cause welfare problems?

Phase 2: Animal welfare assessment driven by issues raised by auditable animal welfare standards (retailers, producer groups, labelling schemes) e.g. McDonalds (USA); RSPCA Freedom Foods (UK)

GOAL: Assure customers that welfare standards are being met on individual farms, slaughter plants etc.

Criteria for animal welfare indicators in auditable animal welfare standards

Need welfare measures that can be used on-site (on-farm, during transport and slaughter)

Measures need to be easily scored during a site visit by auditors with limited training (or recorded by farmers)

Measures need to be "uncontroversial", with a linear relationship with welfare and a clear cut-off point

Phase 2: Most useful welfare measures for auditable animal welfare standards

Most useful

- Health / injury
- Some behavioural (e.g. injurious behaviour, expression of pain)
- Productivity measures (changes in individual animal)
- Other behavioural, physiological, immunological

Least useful

Phase 2: Most useful welfare measures for auditable animal welfare standards

1. Measures of health or injury- mortality rates, bruising and injury; disease incidence:

Least controversial (some face validity)

Most easily scored

Linear relationship with welfare and clear-cut off

Phase 2: Most useful welfare measures for auditable animal welfare standards

2. Some behavioural measures- e.g. lameness, aggression or injurious behaviour, behavioural indicators of pain (e.g. vocalization), slipping while walking,

Less easily scored

More controversial

Linear relationship with welfare and clear cut-off (?)

Phase 2: <u>Less</u> useful welfare measures for auditable animal welfare standards

<u>3. Measures of production</u>

Controversial (Changes in production of individual animals e.g. weight loss of growing animals, drops in egg or milk production , confused with profitability of farm and differences between animals in overall productivity)

Not easily scored

Non-linear relationship with welfare and no clear cut-off

Phase 2: <u>Least</u> useful welfare measures for auditable animal welfare standards

4. Physiological / immunological measures or other behavioural measures ?

Too difficult to measure on-site etc.?

Validity?

Non-linear relationship with welfare?

Clear cut-off?

Phase 3: Animal welfare assessment driven by issues raised by

Pressure to develop “internationally accepted” measures of animal welfare for global trade e.g. OIE

Closer link with food safety

What types of measures?

Phase 3: Animal welfare assessment driven by issues raised by

Pressure to develop “internationally accepted” measures of animal welfare for global trade e.g. OIE

Closer link with food safety

What types of measures?

Phase 1: Animal welfare assessment driven by legislation

2. Determine what is important for good animal welfare:

e.g. is behavioural deprivation a problem for animal welfare?;

do animals need social contact?;

why do animals perform abnormal behaviours?

MLA & AWI - Animal welfare objective measures workshop
Sydney, 6-7 June 2005

Challenges and new opportunities in behavioural measures to assess welfare

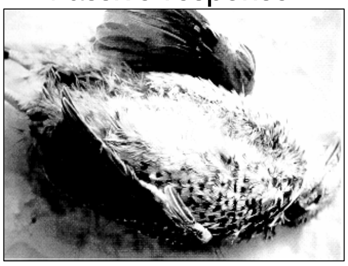
Alain Boissy & Isabelle Veissier
Herbivores Research Unit
Adaptation and Social Behaviours Team

A. Boissy

Variation in behavioural reactivity

acute event
e.g. predator

Passive response



(Faure & Mills 1995)

immobility

Active response

flight / attack

Opposite responses ⇨ ~~Continuum~~

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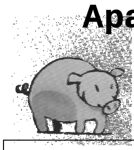
Variation in behavioural reactivity

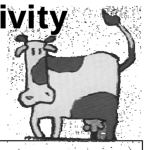
Modification of the reactivity due to chronic situations

After tethering | After repeated regroupings

acute events
e.g. water test

Apathy | **Hyper-reactivity**





Broom (1987)

Animals sitting→standing	
Tethered	Grouped
2 %	29 %

Boissy et al. (2001)

Animals sitting→ standing up	
Unstable	Stable
86 %	25 %

Opposite responses ⇨ ~~Continuum~~

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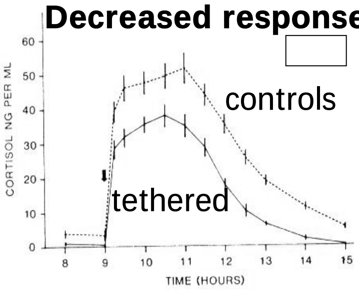
Variation in physiological reactivity

Modification of the reactivity due to chronic situations

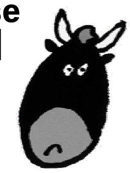
After tethering | After repeated regroupings

acute event
e.g. ACTH test

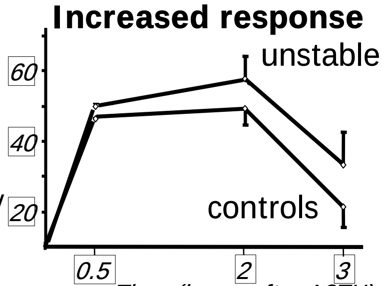
Decreased response | **Increased response**



Ladewig & Smidt (1989)



Blood cortisol (ng/ml)



Veissier et al. (2001)

Opposite responses ⇨ ~~Continuum~~

Boissy

Welfare: the necessity to consider the emotions

Welfare is often assessed on continuous scales

Opposite responses observed:

- ➢ difficulties in interpreting responses in terms of welfare
- ➢ one cannot reason on a continuous axis:

Good environment Poor environment

Welfare <-------------------------------> Stress

We should not only measure the reactions of animals, but also question their emotional states to better assess their welfare

A need to access the emotional world of animals

But what do we really know about animal emotions?

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Definition of emotion

- ➢ Emotion: an intense affective response to an event
- ➢ Emotions belong to the « survival kit » of the species
  i.e. to help to react rapidly to a challenging situation
- ➢ Classically, an emotion is defined by 3 components *(Dantzer 1986)*:
  - **subjective** component (feeling or emotional experience)
  - **behavioural** component (motor response and posture)
  - **physiological** component (activation of the ANS)
- ➢ However,
  - ▪ The subjective component is not directly accessible and can only be inferred from the two other components
  - ▪ Current behavioural and physiological indicators offer limited opportunities to assess emotional experience:
    Even in human, where linguistic reports can be used, behavioural and physiological components appear insufficient to objectively access the emotional experience

* New methods to assess **affective states** have to be developed

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A proposal for better estimate emotions in animals

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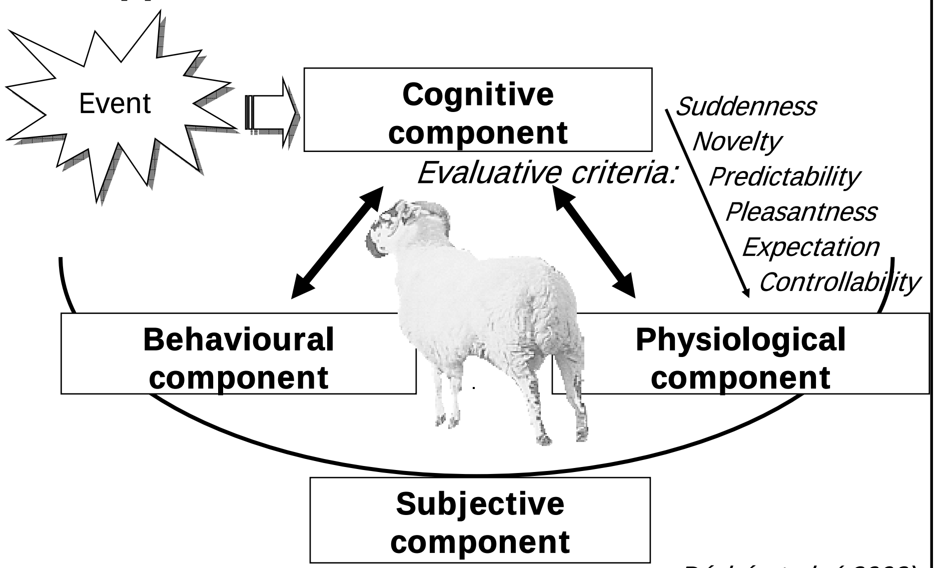
New approach of emotions in animals



Désiré et al. (2002)

Possibility to access emotions in animals according to their cognitive abilities

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Criteria of suddenness

Experimental design

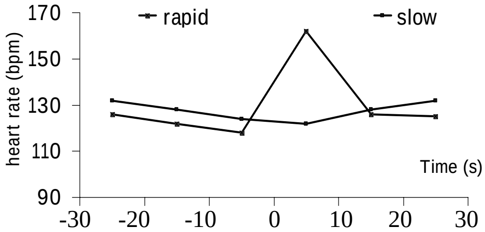
Suddenness:

slow vs. rapid appearance of a scarf behind the trough

startle response

rapid	slow
(n=9)	*(n=10)*
5	1

heart rate



Désiré et al. (2004)

* Suddenness results in startle and rapid-short HR increase

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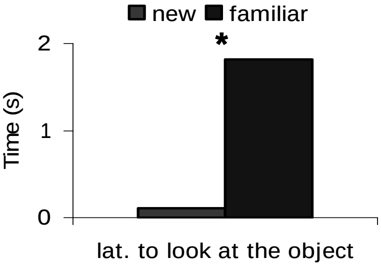
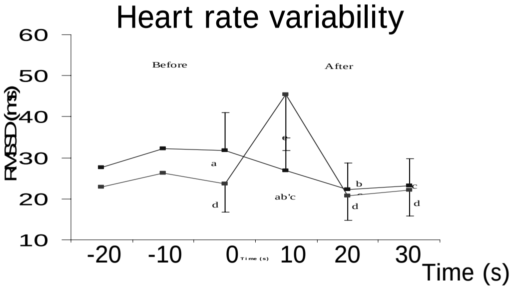
Criteria of novelty

Exposure to familiar vs. novel object

Results





Désiré et al. (2004)

* Novelty results in orientation and vagal tone

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Criteria of predictability

Experimental design

The criterion of predictability is combined with suddenness to estimate if the responses to suddenness (i.e., a startle response and a tachycardia) can be modulated by the possibility to predict the sudden event

A rapid appearance of a plastic backboard (sudden event) is associated with the distribution of a small amount of food only five times out of 10 food distributions

Random treatment: lambs are randomly exposed to the 5 associations (food distribution + sudden event)

Signalled treatment: lambs are randomly exposed to the 5 associations (food distribution + sudden event) but the sudden event was announced by a light

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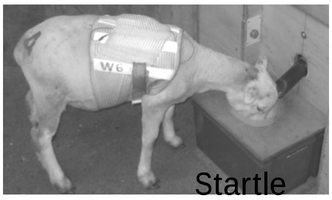
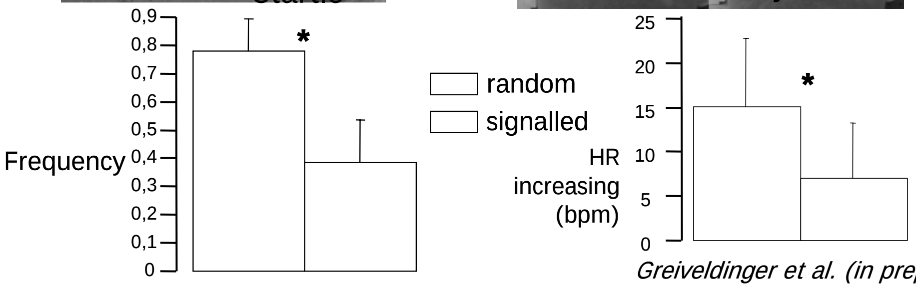
Criteria of predictability





Greiveldinger et al. (in prep.)

Possibility to predict a sudden event
reduces the emotional responses to suddenness

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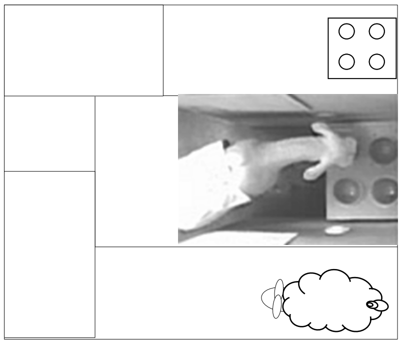
Criteria of expectations

Experimental design



	No. doses of pellets	
	Training	Test
*Sessions**	*11*	*3*
Control -	1	1
Contrast +	1	4
Contrast -	4	1
Control +	4	4

** 1 session = 3 trials*

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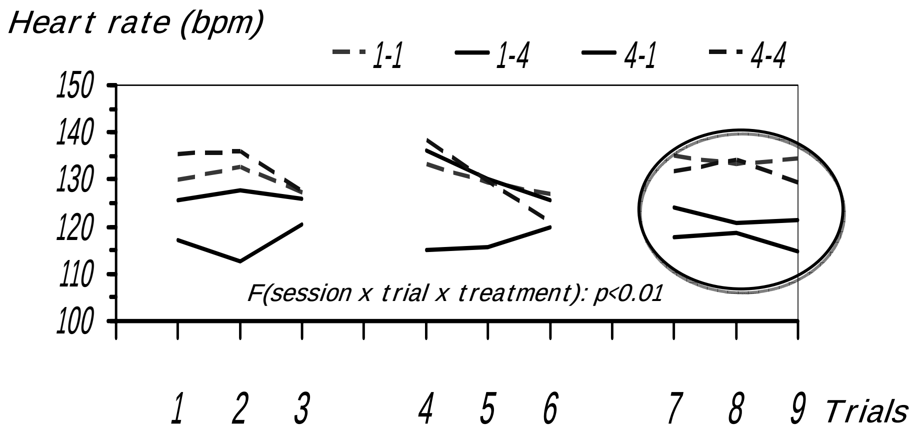
Do lambs react to discrepancy from their expectations?



Désiré et al. (in prep.)

Negative contrast later reduces HR

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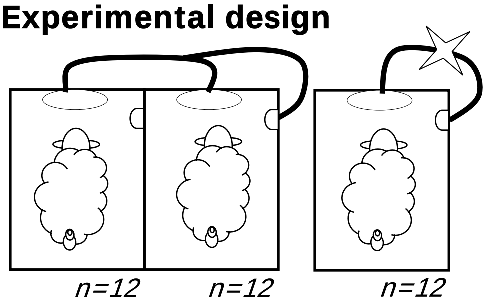
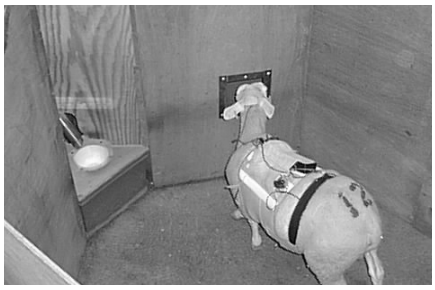
Criteria of controllability

Experimental design





Step 1 *12 sessions*	Step 2 *10 sessions*	treatment
No control	No control	No control
Control	Control	Control
Control	No control	Loss of control

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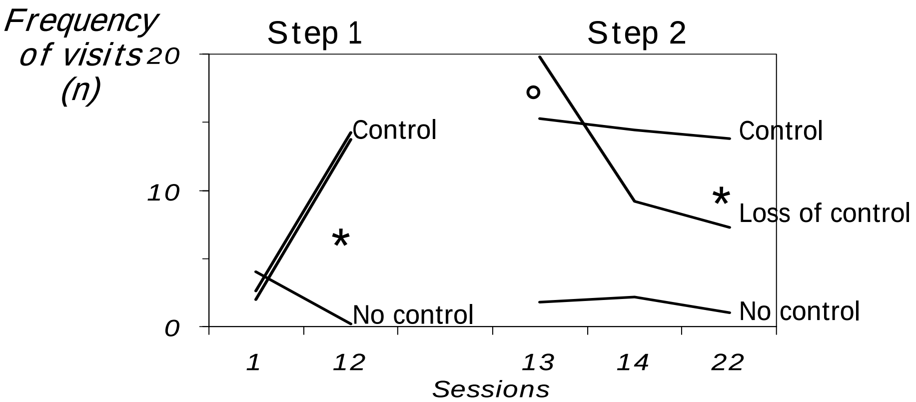
Do lambs perceive the ability to control?



Désiré (2004)

Perception of - the ability to control
- the loss of control

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The combination of suddenness and novelty

Experimental design

Suddenness: slow vs. rapid appearance

Novelty: familiar vs. novel object

Evaluation induced	Training sessions		Test sessions	
	Object	Speed	Object	Speed
Control (n=6)	Scarf	Slow	Scarf	Slow
Suddenness (n=7)	Scarf	Slow	Scarf	Rapid
Novelty (n=7)	Square	Slow	Scarf	Slow
Suddenness * Novelty (n=7)	Square	Slow	Scarf	Rapid

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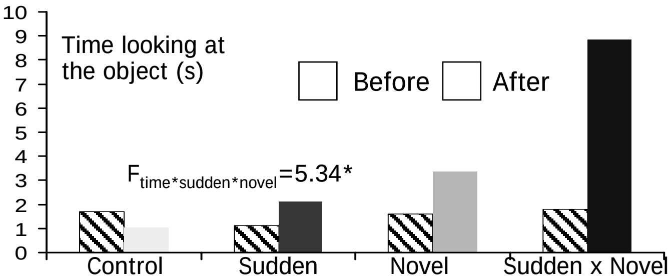
The combination of suddenness and novelty

	Control (n=6)	Sudden (n=7)	Novel (n=7)	Sudden x **Novel** (n=7)
Startle reaction to suddenness (Fischer exact test sudden p=0.0014)	0	5	0	3

Orientation
reaction to novelty



Désiré et al. (submit.)

Synergy effect of suddenness and novelty on orientation

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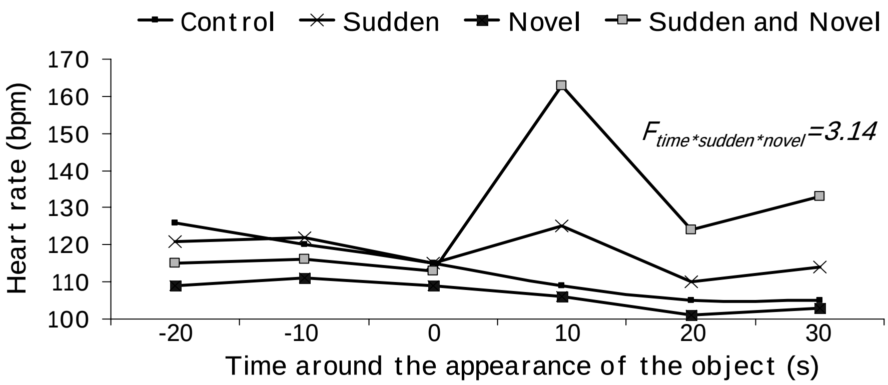
The combination of suddenness and novelty

Cardiac activity
reaction to suddenness



Désiré et al. (submit.)

Synergy effect of suddenness and novelty on tachycardia

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Conclusion

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Welfare, it's all about emotions

- Lambs are capable to evaluate their environment from a set of simple criteria
- The use of complex evaluative criteria is still unclear
- The aggregation of criteria would be at the basis of emotions

Criteria		Evaluation	Emotionnal responses
Suddenness		**YES**	**YES**
Novelty		**YES**	**YES**
Predictability		**YES**	**YES**
Discrepancy from expectation	Negative contrast	**YES**	**YES**
	Positive contrast	**YES**	**?**
Uncontrollability of an appetitive event	Loss of control	**YES**	**?**
Suddenness X Novelty		**YES**	**YES**

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Welfare, it's all about emotions

- **Cognitive ethology** has to be developed to better approach animal welfare:
  research into appraisal mechanisms will further enhance our understanding of animal emotions
- Our **conceptual framework** can be used to coordinate and homogenise the various studies in animals and may provide new information about affective states in animals.
- This framework should help:
  - for a **comparative approach** to assess the range of emotions that different species are able to access,
  - for a **genetic x experience approach** to investigate whether individual variation in evaluative tendencies (predisposition) may render animals more or less vulnerable to affective disorders, such as depression.

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Adaptation and Social Behaviours Team

- 4 researchers, 3 engineers, 2 technicians, and 5 PhD & undergraduate students
- Studies:
  - – Analysis of behavioural processes of adaptation: emotions, learning & attachments
  - – Assessment of animal responses to constraints in farming conditions

- Goal:

  To propose welfare friendly systems

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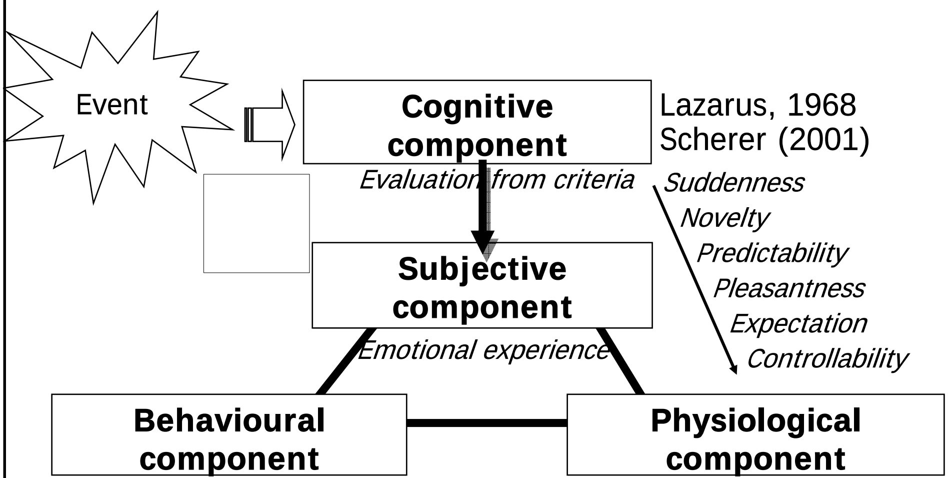
Appraisal theories of emotions in human



The outcome of the evaluation determines the nature of the emotional experience

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DIAPOSITIVE INTERCALAIRE

Application:
Do lambs perceive such evaluative criteria?

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Challenges and New Opportunities in Physiological Systems

Dominique Blache & Christian Cook

- Key physiological approaches
- Current developments and future opportunities
- Towards objective measures

Definition of welfare

"The state of well-being brought about by meeting the physical, environmental, nutritional, behavioural and social needs of the animal or groups of animals under the care, supervision or influence of people"

Key physiological approaches

Production and health

Indicators of pain

- Acetylcholine
- Aspartate
- Adenosine/ATP
- Bradykinin
- Cytokines
- Glutamate
- Histamine
- Lactate
- Leukotriene
- Leukotriene
- Nerve growth factor
- Neurokinin
- Nitric oxide
- Prostaglandins
- Serotonin
- Somatostatin
- Substance P
- Vasoactive intestinal peptide

Key physiological approaches

Immune system

- Immunoglobulins
- Leucocytes subpopulations
- Lymphocytes proliferation
- Interleukins
- Interferons
- Tumor Necrosis Factors
- Transforming Growth Factors

Key physiological approaches

Enzymatic activities

- Plasma creatine kinase
  - Muscle and heart damage
  - Increase after injection of antibiotic - calves
  - Higher in calves kept in large pen
- Blood lactase dehydrogenase (LDH5)
  - Increases with
    - Transport
    - Capture
    - Handling
    - pigs, cattle, deer, baboons

Key physiological approaches

Respiration rate

- Can occur without changes in body activity
- Indicator of emotional disturbance
- Indictor of physiological disturbance

Key physiological approaches

Heart rate

- Tachycardia
  - In response to a stimulus
  - Increase in metabolic rate is needed
  - Changes in behaviour
- Bradycardia
  - Emotional response
  - During orientation response
- Heart rate changes
- Heart rate variability

Key physiological approaches

Temperature

- Increases in response to stress
  - 0.5 to 1.4 °C
  - Storm
  - Unfamiliar human
  - Separation form mother (young macaque)
  - Transport (calves)
- Decreases during “despair” phases
  - Young macaques
  - Tree shrews defeated by another individual
  - Alarming visual stimulus (chickens)

Key physiological approaches

Activity of HPA axis

- AVP, CRH, ACTH, Cortisol

Activity of sympatho-adrenal system

- Catecholamines, heart rate

Key physiological approaches

Other hormonal systems

- Reproductive hormone
  - LH
  - Prolactin
  - Sex steroids

Neurotransmitters

- Dopamine
- Noradrenaline
- Serotonin
- Or their metabolites

Key physiological approaches

Metabolites

- Glucose
- Free fatty acids
- Cholesterol

Key physiological approaches

"The state of well-being brought about by meeting the physical, environmental, nutritional, behavioural and social needs of the animal or groups of animals under the care, supervision or influence of people"

- Limitations in
  - Techniques
  - Understanding of physiological systems

Current developments and future opportunities

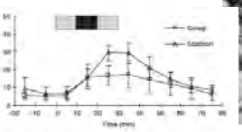
Technologies

- Remote blood sampling
- Microdialysis
- Free-range physiological monitor (FRPM)
- Solid state immunosensors
- Transdermal sampling

Remote blood sampling systems

- Blood parameters
- Hands-off
- Integrative approach

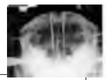
- Invasive
- Reliability
- Stability of the compounds
- Limitation in sample number
- Size

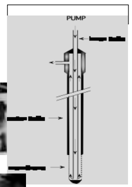


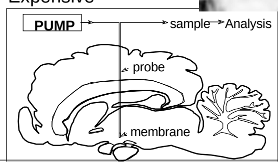
Microdialysis

- Local concentrations of neurotransmitters, neuropeptides, peptides, etc…
- Brain, blood and any tissue
- Good sensitivity
- Reverse microdialysis

- Hands-on
- Invasive
- Expensive







Free-Range Physiological Monitor

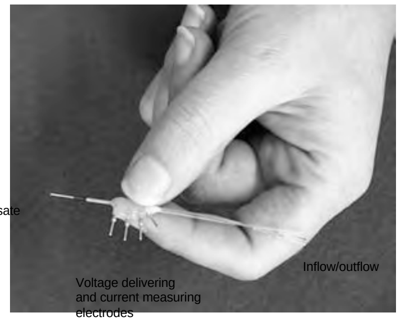
- Temperature, ECG, EEG…etc
- Hands-off
- Custom ambulatory monitor
- Integrative approach

- Invasive
- Reliability
- Lot of potential

Solid state immunosensor

- Built around concept of a dialysis probe
- Size ranging from as small as 50 μm (brain) to 500 μm (tail vein)
- Enables rapid collection of data and sensitivity of localised immunoassay - analytes in blood and brain
- Potentially every minute for up to 1000 minutes continuously allowing frequent measurement
- Disadvantages
  - Sensitive, field robustness a problem
  - Invasive – still requires some surgery

Tail vein implant



Less invasive approaches

- North American and Swiss work suggests that certain combinations of ultrasound & electrical current can dramatically change permeability of skin and in doing so ……

  Set up a potential "flux" of some analytes from blood to skin where they be collected under mild vacuum
- Currently in final development stage for glucose in diabetes and used for delivery of pharmaceutical through skin to circulation.
- Transdermal delivery / collection with linearity to blood changes
- Painless, low volume, repeatable, high frequency of sampling permissible (no blood removal) low invasive and low in stress

Future Potential

- Transdermal collection method giving measures relative to blood change for some analytes

  Painless, low invasive
- Preliminary data suggests feasibility in mammals
- Portable, repeatable, allows frequent sampling
- Still needs much validation across different conditions/species

Towards objective measures

- Integrative physiology
  - New technologies
  - Modeling, multivariate analysis, etc
  - Psychoneuroendo/immnunology
- Dynamic of the physiological systems
  - Time and interaction
  - Variability of threshold
  - History of animals

Towards objective measures

- Can stress be "a low energy availability" challenge?
  - Metabolic hormones
  - Homeostasis
  - Allostasis
  - Allostatic load and overload

The 'omics revolution

MLA/AWI Welfare Workshop

June 6-7, 2005

John Gibson

TIGB
The Institute for Genetics and Bioinformatics

University of New England, Armidale, NSW 2351

The 'omics revolution

1) Molecular genetic markers	Locate and select for regions controlling genetic variation
2) High throughput gene expression	Identify pathways controlling biological systems
3) Genome sequence assembly	Put names on pathways, identify function and locate genes controlling variation
4) High throughput proteomics	Also identify pathways
5) High throughput metabolomics	Describe how pathways interact in metabolism
6) To be announced	More power, more quickly, more cheaply

Example of technology progress

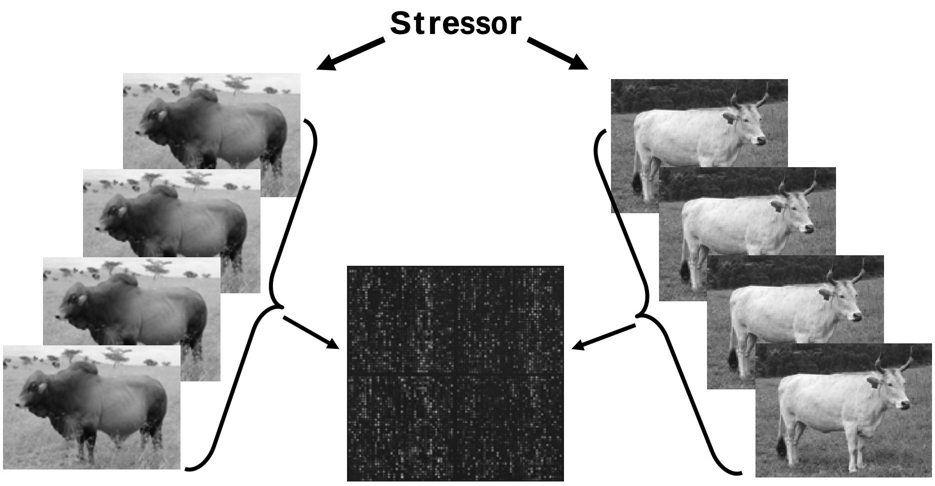
Cattle QTL mapping
expt: 1990-1998

Genome wide scan for QTL: Genotyping took three labs, four people, four years

If repeated in 2003: one lab, one person, 60 days

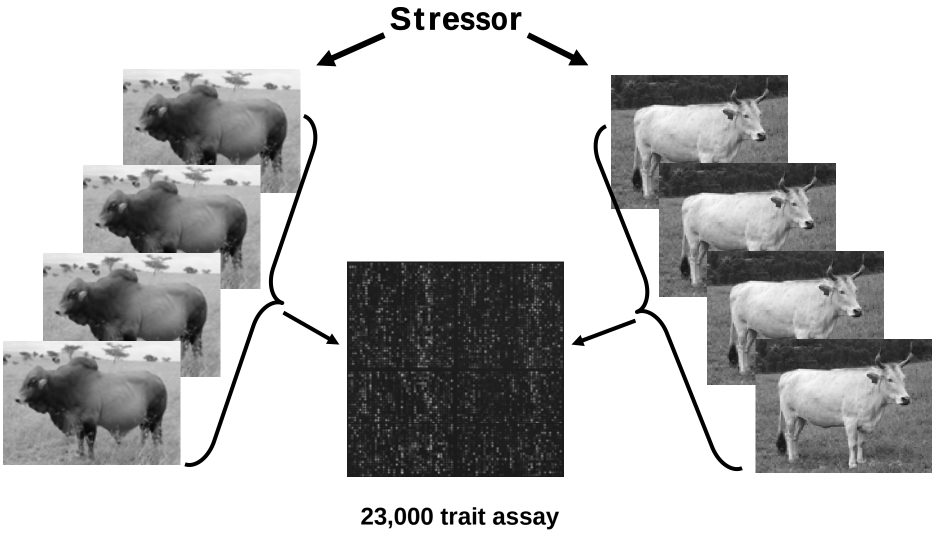
If repeated today: one lab, one person, 5 days, five times more information, half cost

Hypothesis generating: what are the functional differences between genotypes?

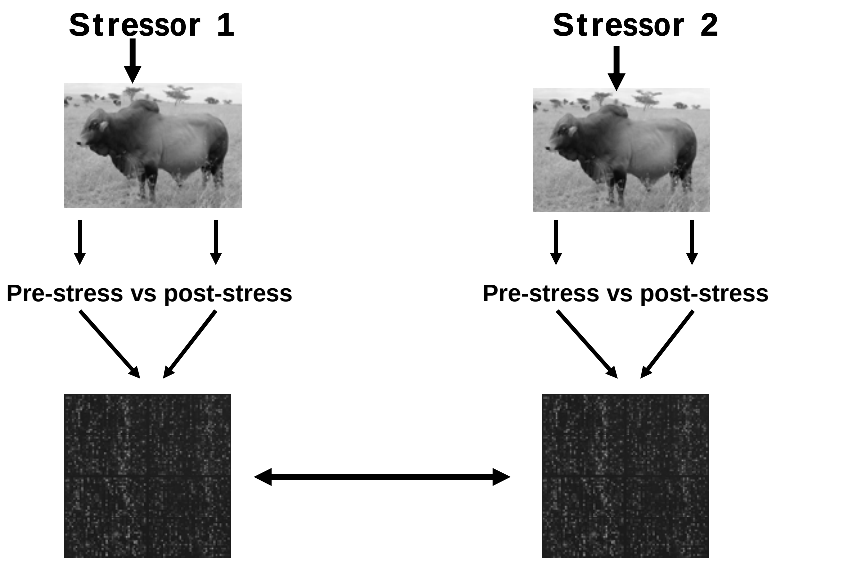


23,000 trait assay

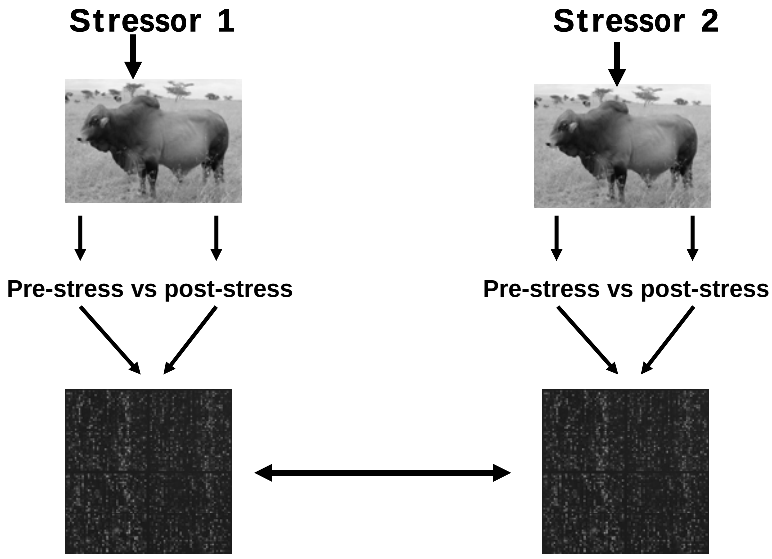
Predicting phenotypes & genotypes



Hypothesis generating: what are the biological effects of different stressors?



Hypothesis testing: what are the biological effects of different stressors?



State of the art for key species

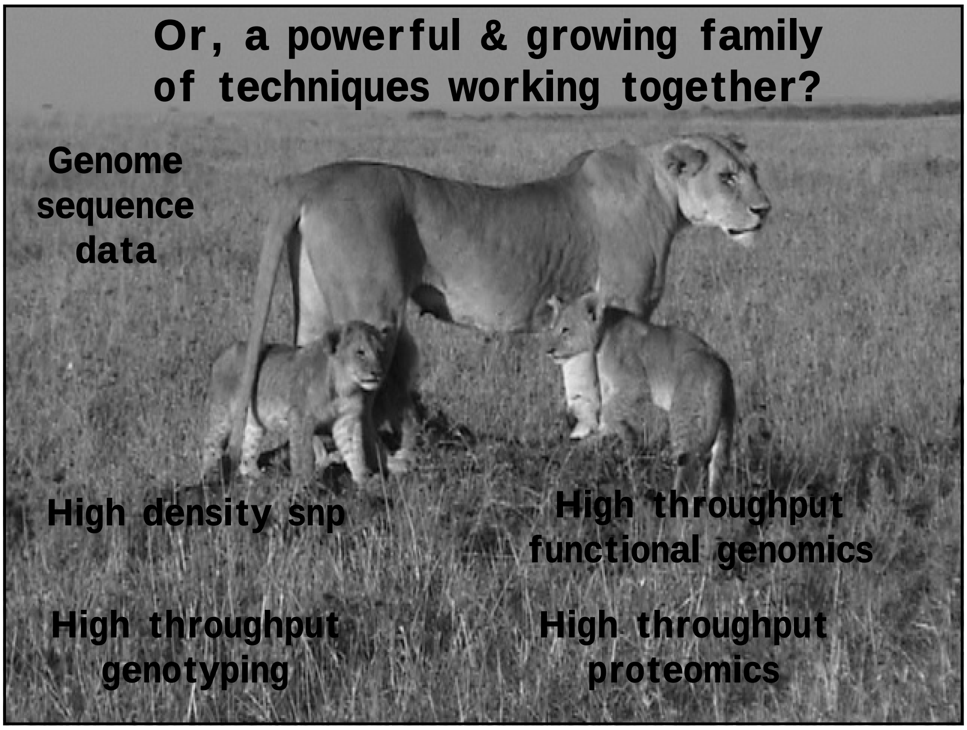
Technology	Cattle	Chicken	pig	sheep
Low density markers	***	***	***	**
High density snp	**(*)	***	No	No
Genome sequence	***	***	2008?	No
Microarrays	***	***	*	*
Proteomics	*(**)	***	2008?	No
Metabolomics	*	*	No	No

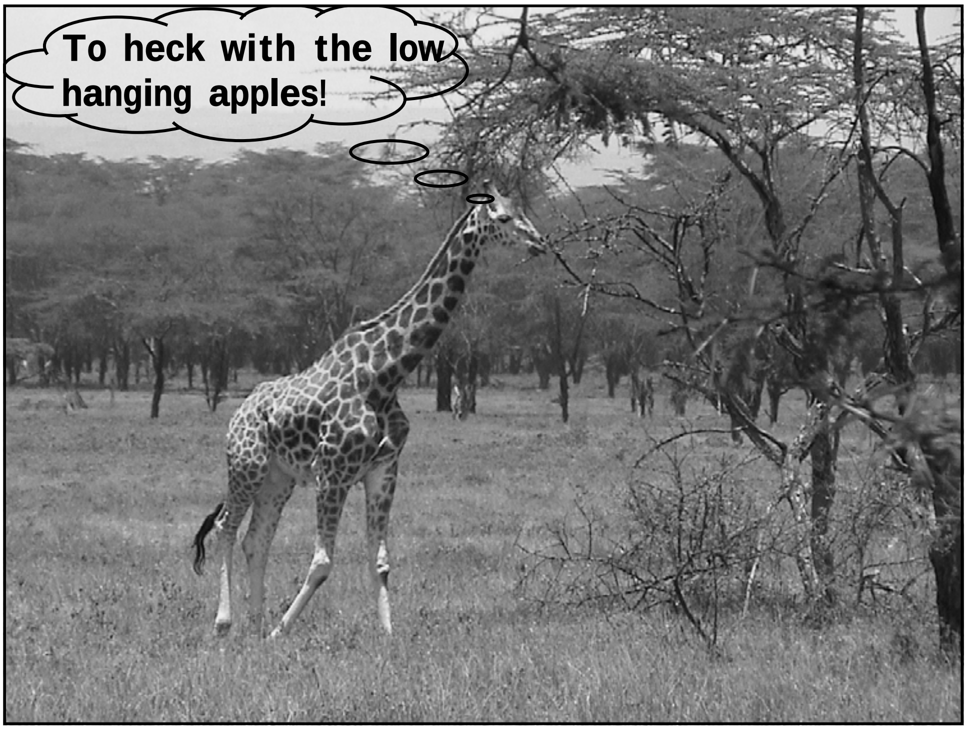

A rapid hunting method when looking for an unknown target
The era of high throughput omic techniques


Powerful gene hunting techniques waiting to be unleashed

Too much data?
The era of system overload?


Powerful techniques fighting for top spot?


Or, a powerful & growing family
of techniques working together?
Genome
sequence
data
High density snp
High throughput
functional genomics
High throughput
genotyping
High throughput
proteomics


To heck with the low hanging apples!


A bit bloody but worth the effort

An early macroarray

Results: load of sh

The Animal Welfare
Science Centre

What are the key factors to consider in selecting challenge and animal models?

John Barnett
Animal Welfare Science Centre, Primary Industries Research, Werribee, Victoria

ANIMAL WELFARE SCIENCE CENTRE · THE UNIVERSITY OF MELBOURNE · MONASH University · State Government Victoria Department of Primary Industries

Selecting animal models

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Science Centre

Key factors:

- Type of model?
  - short or long term responses, acute or chronic stress
- What is the challenge to be imposed?
  - Identifying those factors affecting homeostasis
    - social
    - environmental
    - nutritional
    - health
    - psychological
- Measures
  - physiological (hormonal, immunological), behavioural (observations, tests)
- Species specific attributes

2

Acute stress models

The Animal Welfare Science Centre

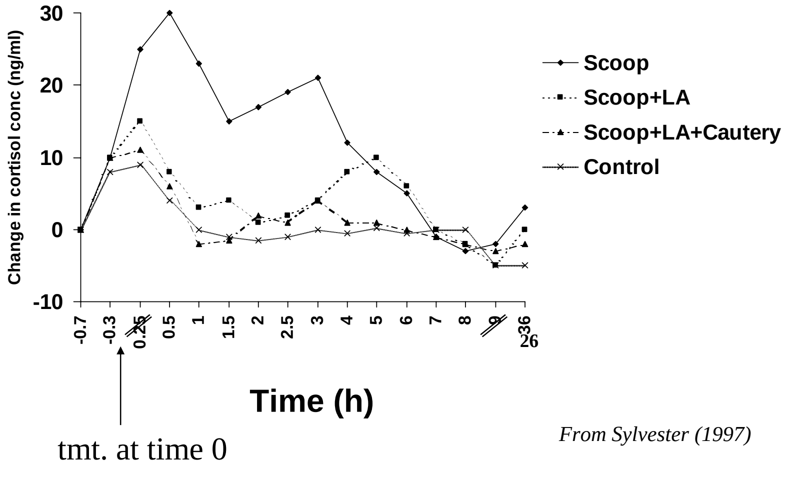
Procedure	Species	Response
Surgical procedures (tail-docking, etc.	Calves, lambs, pigs	Cortisol, acute phase proteins, etc.
Shearing	Sheep	Endorphin, cortisol
Transport	Sheep	Endorphin, cortisol
Transit time through a race	Sheep	Behavioural
Electrical stimulation	Sheep	Behavioural, EEG
Electrical stimulation	Cattle	Meat quality
Exercise	Sheep	Meat quality
Transport		
Exercise		
New pen	Pigs	Cortisol, HR, RR, metabolites, etc.
Electric shock		
Abrupt change in temperature		
Novelty		
Unfamiliar pig		

3

Acute stress

The Animal Welfare Science Centre

Cortisol response in calves to dehorning (ng/ml)



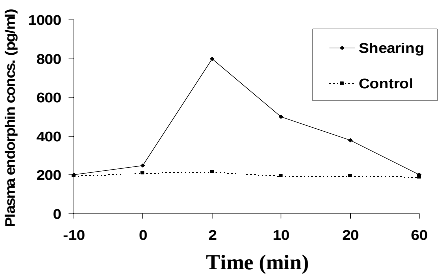
From Sylvester (1997)

4

Endorphin response

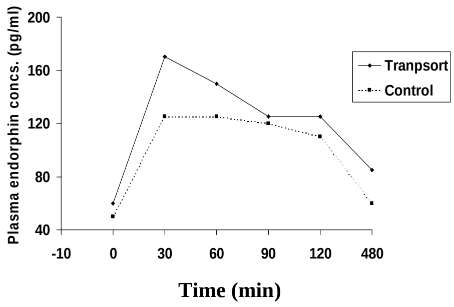
The Animal Welfare
Science Centre

Shearing (sheep)



From Jephcott et al. (1987)

Transport (pigs)



From Bradshaw et al. (1987)

5

Cortisol responses in pigs - transport

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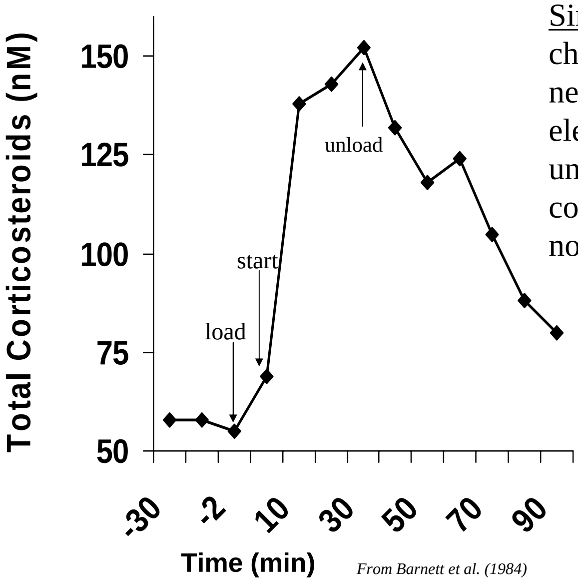
Science Centre



<u>Similar responses to</u>:
chasing,
new pen,
electric shock,
unfamiliar pig,
cold,
novelty, etc.

From Barnett et al. (1984)

6

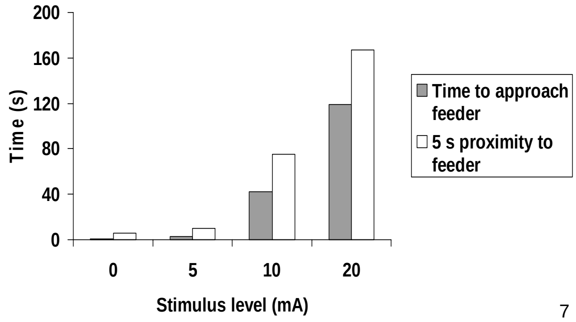
Behavioural response

The Animal Welfare Science Centre

Transit time of sheep through a race after electroimmobilization (s)

Control	Treated
30	140

Response of sheep to electrical stimulation



7

Chronic stress models

The Animal Welfare Science Centre

Procedure	Species	Response
Natural events	*Antechinus agilis*	Cortisol, behaviour, immune
Negative handling (fear)	Pig (young & adult)	Cortisol, N_2 balance, behaviour
Tether housing	Pig (adult)	Cortisol, reproductive, immune
Overcrowding	Pig (adult)	Cortisol, behaviour
Negative handling (fear)	Dairy cow	Cortisol, behaviour
Undernutrition?	Sheep	Cortisol, metabolites
Undernutrition/marketing?	Cattle	Cortisol, meat quality

8

Chronic stress model - I

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Science Centre

Antechinus agilis - brown marsupial mouse

9

Life history of *Antechinus agilis*

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Science Centre

- **dramatic and synchronised**

  all males die at end of breeding season

- **mortality induced by a behaviourally induced** (aggression, competition for females) **stress response**

  ↑ *aggression, intense mating,*
  ↑ *total cortisol,* ↓ *in CBG =* ↑ *in free cortisol*
  →↓ *in weight,* ↓ *in plasma glucose and Na, haemorrhagic ulceration, anaemia and immunosuppression resulting in pathological states and death.*

10

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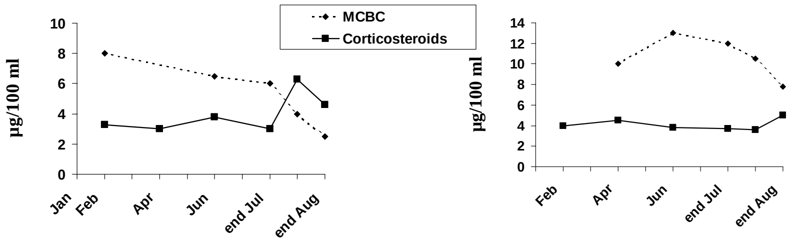
Science Centre

Mean Maximum Corticosteroid Binding Capacity and Mean Total Plasma Corticosteroid Concentrations in *A. agilis* (µg/100 ml)

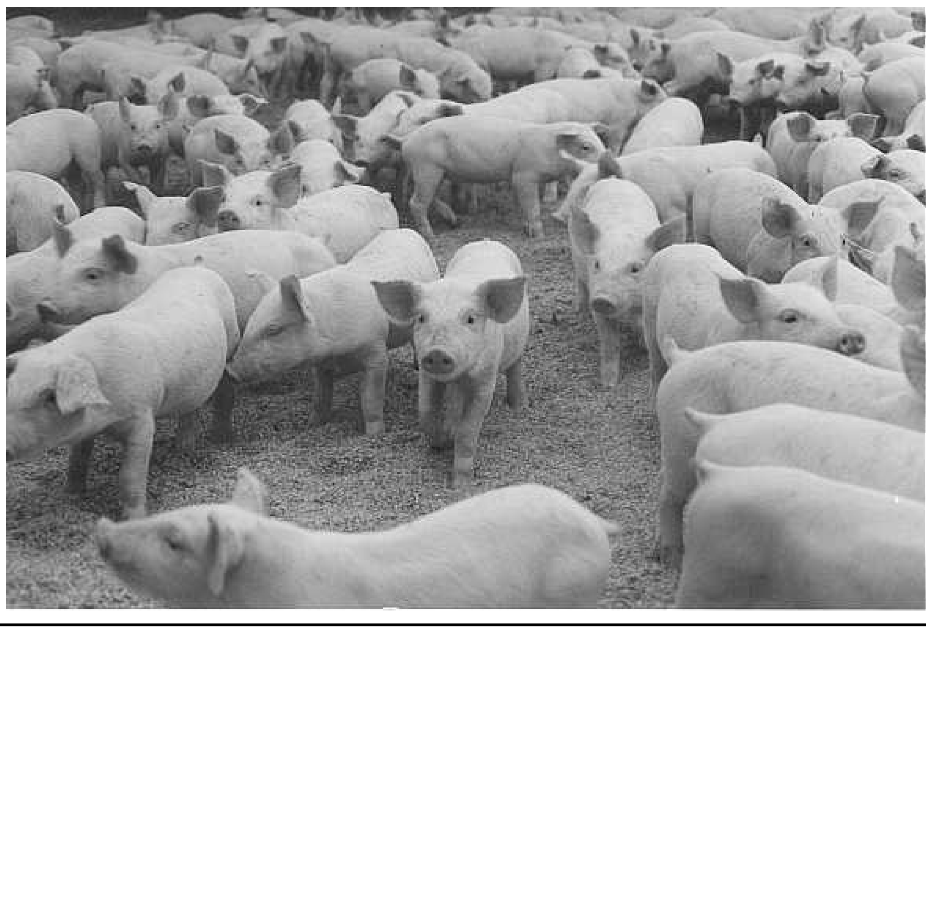
Males | Females



From Lee et al. (1977)

Chronic stress model - II - Pigs

The Animal Welfare
Science Centre



Pigs - negative handling ie. fear of humans

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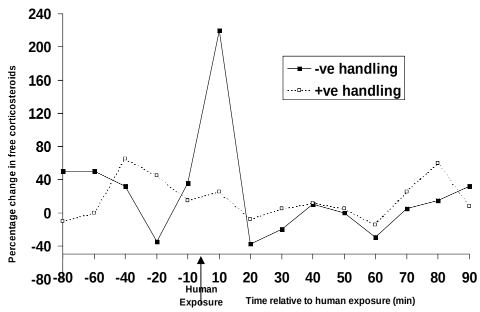
Science Centre

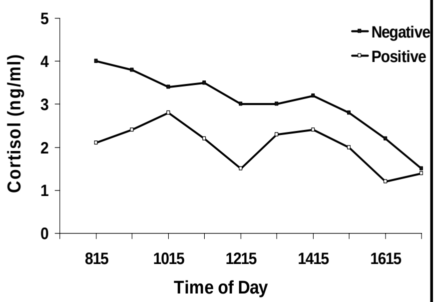
acute response to presence of human *(2 min exposure)*

<u>Cortisol response</u>

chronic response in absence of humans



Percentage change in free plasma corticosteriod concentrations *(Hemsworth et al., 1981)*



Basal plasma cortisol concentrations of gilts handled positively or negatively *(Hemsworth et al., 1981)*

Consequences:
- lower weight gain (negative nitrogen balance)
- lower reproductive performance

13

The Animal Welfare
Science Centre

Behaviour, growth & stress physiology of growing pigs

Variables	Handling Treatment			
	+ve	Control	Inconsistent	-ve
Time to interact with human (s)	**10 [a]**	**92 [b]**	**175 [c]**	**160 [c]**
Growth rate (g/day)	455[b]	458[b]	420[ab]	404[b]
Basal cortisol (ng/ml)	1.6[x]	1.7[x]	2.6[y]	2.5[y]

From Hemsworth et al. (1987)

14

Challenge models

The Animal Welfare Science Centre

- Decide on the issue
  - what do we want to measure?
    - do we want to measure acute or chronic responses, or both?
- Acute stress model
  - what is the most appropriate model in sheep and cattle?
    - surgical models are generally not repeatable within animals
    - could use transport, novelty, isolation
    - does model have to be relevant to industry? eg. sheep restraint/isolation model, bedding
- Chronic stress models
  - do we have appropriate models in sheep and cattle?
    - undernutrition?
- Measures
  - what are the most appropriate physiological and behavioural measures of acute and chronic stress?

15

INTEGRATION AND APPLICATION

ISSUES TO CONSIDER

Stephen Page
Advanced Veterinary Therapeutics

Modus operandi

- MY BACKGROUND
- RISK ASSESSMENT
- PHARMACOVIGILANCE
- PRODUCT DEVELOPMENT
- DIAGNOSTIC TEST ANALOGY
- THOMAS BAYES
- INTEGRATION
  - Strengths, barriers, weaknesses
- MOVING FORWARD

RISK ASSESSMENT

- Chemicals, antimicrobial resistance etc
- Science based decisions
- Zealots and decision making
- Risk perception (Paul Slovic, Doug Powell, Peter Sandman)
- Value of information analysis: evaluates the benefit of collecting additional information to reduce or eliminate uncertainty.

Peters et al (2004) An emotion-based model of risk perception and stigma susceptibility: cognitive appraisals of emotion, affective reactivity, worldviews, and risk perceptions in the generation of technological stigma. Risk Analysis 24: 1349-1367

PHARMACOVIGILANCE

- Causality assessment
- Expert opinion
- Global introspection (GI)
- Delphi methods
- VICH (veterinary international harmonisation)

PRODUCT DEVELOPMENT

- Satisfying unmet needs.
- Who is the customer?
- Evidence Based Medicine
- Quality and Strength of evidence
- Product life cycle

DIAGNOSTIC / ANALYTICAL TESTS

(objective measurement of welfare)

- What is being measured
- What is the "Gold Standard"

  [Pritchard et al 2005. Assessment of the welfare of working horses, mules and donkeys, using health and behaviour parameters. Prev Vet Med 69: 265-283]
- What does the customer need?
- What are the alternatives?
- Practicality, cost, convenience
- Interpretation
- Usefulness

DIAGNOSTIC / ANALYTICAL TESTS

Operating characteristics

- Sensitivity [proportion of patients with the condition who have a positive test result]
- Specificity [proportion of patients without the condition who have a negative test result]
- Likelihood ratios
- Repeatability / Precision
- Intra- and inter-observer variability
- Validity
- Robustness
- Accuracy
- Acceptance criteria

Reverend Thomas Bayes

b. 1702, London - d. 1761, Tunbridge Wells, Kent

Bayes's Theorem

(inductive inference from observation of effects to their cause)

Pre-test odds of a hypothesis being true multiplied by the weight of new evidence generates post-test odds of the hypothesis being true.

In the diagnosis of a state, this refers to the odds of that state being present versus not present.

Gill et al (2005)
Why clinicians are natural bayesians.
BMJ 330: 1080-1083

Goodman SN (1999)
Toward evidence-based statistics. II. The Bayes factor.
Ann Intern Med 130: 1005-1013

INTEGRATION

STRENGTHS BARRIERS WEAKNESSES

STRENGTHS

- Increased experience
- Additional expertise
- More ideas
- Team work
- Consensus
- Agreement
- Common goals
- Common methods
- Common interpretation
- Avoid duplication
- Broad participation
- Ownership
- Build strong relationships
- Develop solid communication
- Power in numbers
- Collaboration
- Cooperation
- Partnership
- Sharing information
- Many eyes and ears
- Early alert
- Detection of emerging trends

BARRIERS

- Poor management
- Poor communication
- Poor coordination
- Personal ambition
- Cultural differences
- Time zones
- Distance
- Technology
- Mistrust
- Lack of motivation
- Lack of focus
- Poor leadership
- Low energy
- Ill-defined goals and objectives

WEAKNESSES

- Decreased individualism
- Decreased innovation
- Divergent goals
- Different methods
- Lack of agreement
- “team think”
- Single outcome
- Concessions
- Forceful or dominating personalities
- Individual driver
- Bandwagon effect
- Inertia
- Paralysis / analysis

MOVING FORWARD

DELPHI

DELPHI METHOD

- Group communication among a panel of geographically dispersed (anonymous) experts
- Often used to answer single specific question.
- Dialectic process: thesis – antithesis - synthesis
- Structured process for collecting and distilling knowledge from a group of experts by means of a series of questionnaires interspersed with controlled opinion feedback.
- Facilitates formation of group judgment
- Pitfalls well described eg
  - selection of monitors
  - structuring of the questionnaire
  - analysis of responses

Graham B, Regehr G, Wright JG (2003) Delphi as a method to establish consensus for diagnostic criteria. J Clin Epidemiol 6: 1150–1156

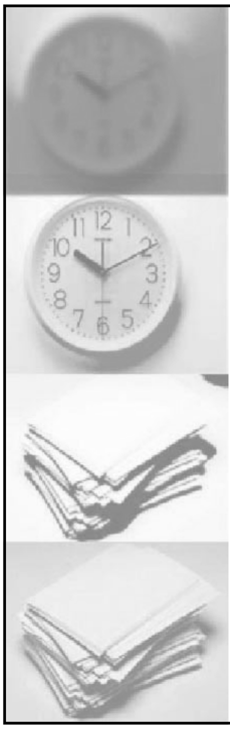
Objective Measures of Animal Welfare

Opportunity or ultimatum?

By Clive Phillips

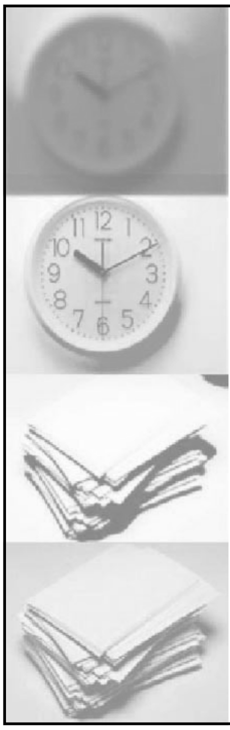
Required outcomes

- Acceptable working definition



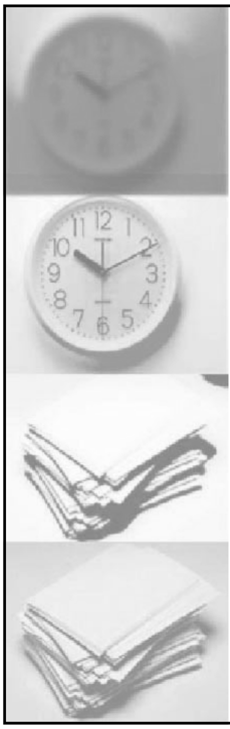
Required outcomes

- Acceptable working definition
  - Five Freedoms ?



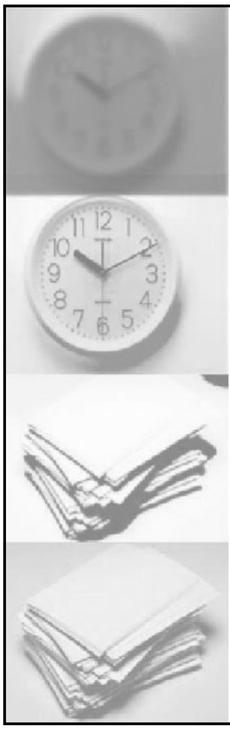
Required outcomes

- Acceptable working definition
  - Five Freedoms ?
- Thresholds for animals



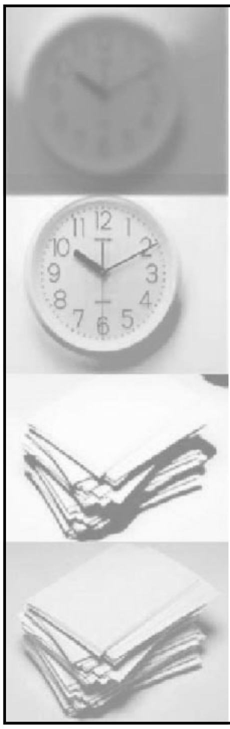
Required outcomes

- Acceptable working definition
  - Five Freedoms ?
- Thresholds for animals
  - but continuum acknowledged



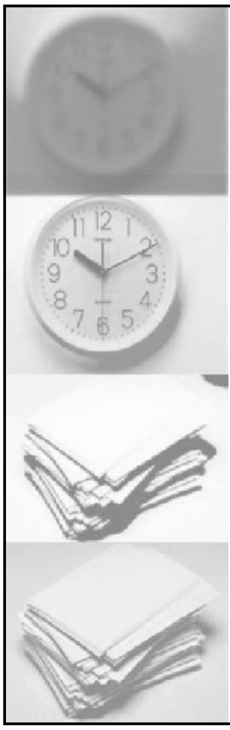
Required outcomes

- Acceptable working definition
  - Five Freedoms ?
- Thresholds for animals
  - but continuum acknowledged
- Public perception considered



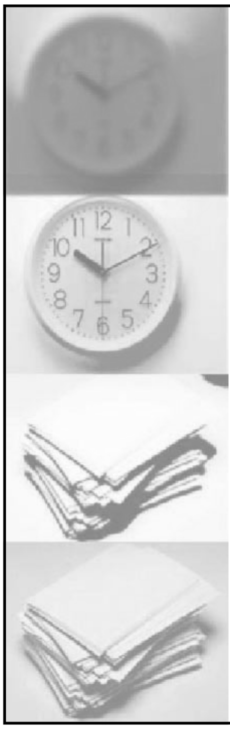
Required outcomes

- Acceptable working definition
  - Five Freedoms ?
- Thresholds for animals
  - but continuum acknowledged
- Public perception considered
- Collaboration
  - Advantages and disadvantages



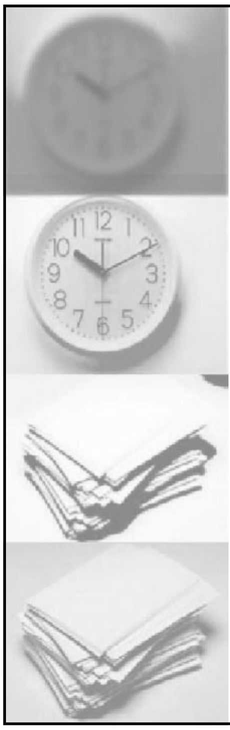
Industry involvement

- Investment in welfare measures must benefit industry



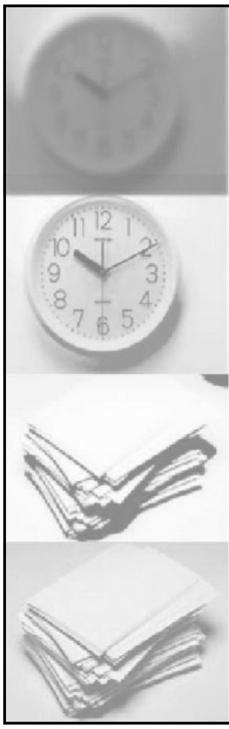
Industry involvement

- Investment in welfare measures must benefit industry
  - More than training



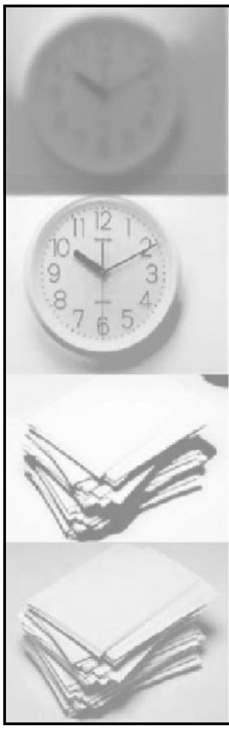
Industry involvement

- Investment in welfare measures must benefit industry
  - More than training
  - More than direct aid



Industry involvement

- Investment in welfare measures must benefit industry
  - More than training
  - More than direct aid
  - More than doing nothing



Application in practice Needed now!

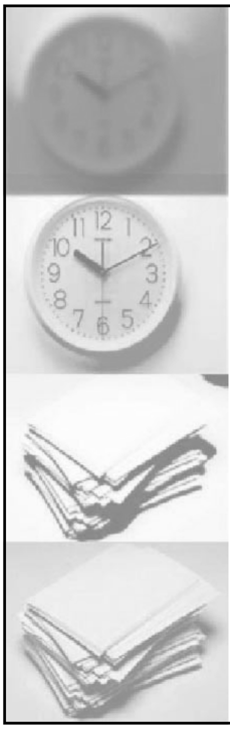
Audits

‘Best guess’

Application in practice Needed now!

- Audits
  - 'Best guess'
- HACCP
- Indices – acknowledge trade-off potential

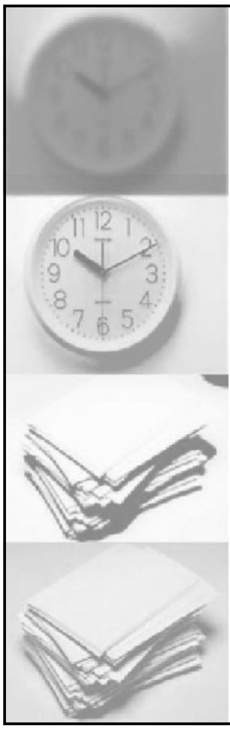
Can high welfare during rearing be offset by poor welfare at life's end?



Application in practice
Needed now!

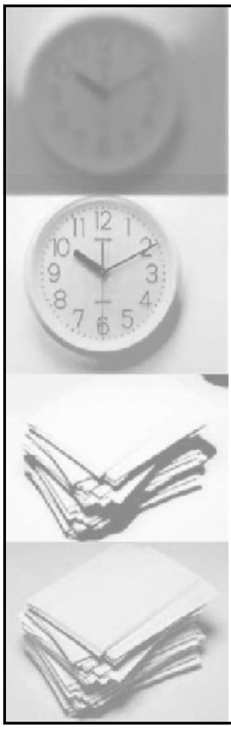
Audits
  'Best guess'
HACCP
Indices – acknowledge trade-off potential

Some focus on areas of public concern
e.g. health (win-win), production?
and some behaviour



Possible measurements

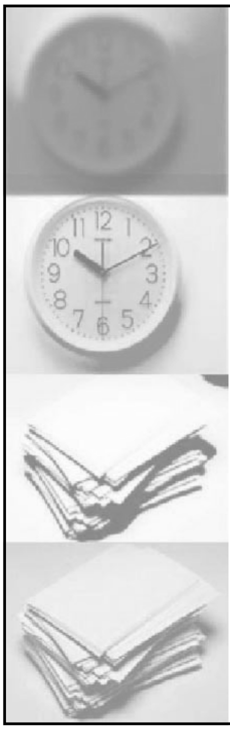
- Behaviour
- Physiology
- Gene expression
- Cognition
- + Health
- + Environment?



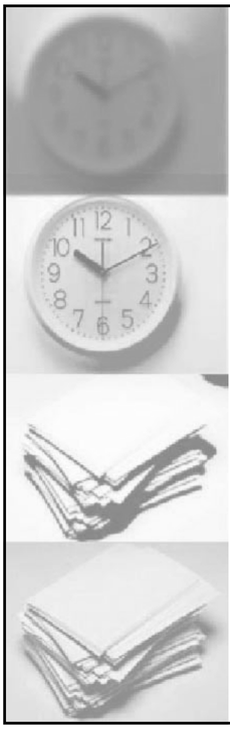
Possible measurements

- Behaviour
- Physiology
- Gene expression
- Cognition
- + Health
- + Environment?

Must be fast, repeatable, reliable and related to welfare concerns

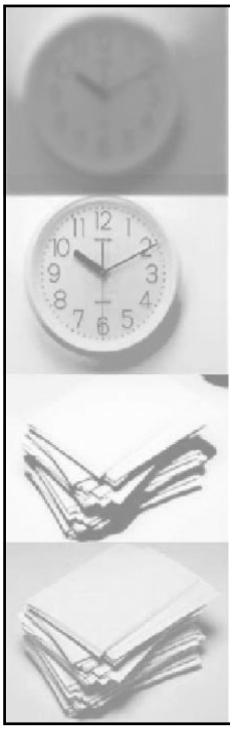


Application in research
Needed yesterday!



Application in research Needed yesterday!

- Behaviour
  - Observations – meaning?
  - Telemetry – time and expense



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- Behaviour
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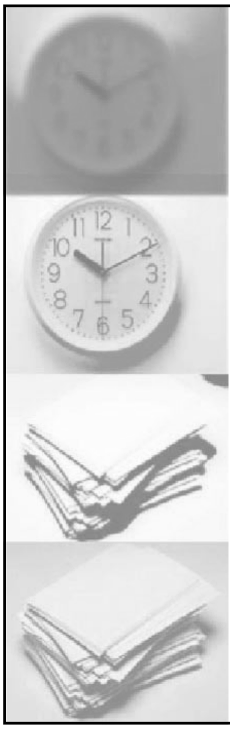
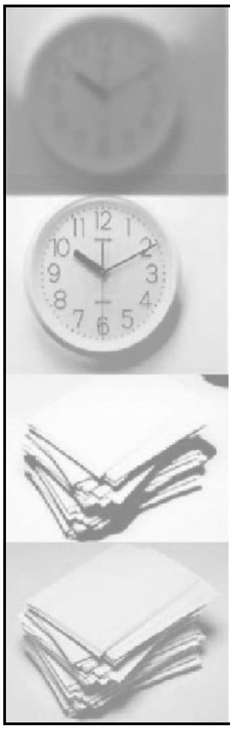
- Physiology
  - Hard to measure
  - Hard to relate to welfare?



Pain responses

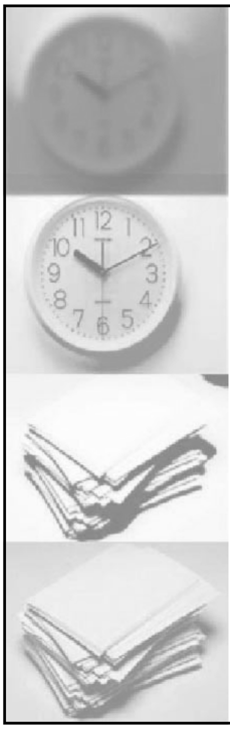
Production and health

- Acetylcholine
- Aspartate
- Adenosine/ATP
- Bradykinin
- Cytokines
- Glutamate
- Histamine
- Lactate
- Leukotriene
- Leukotriene
- Nerve growth factor
- Neurokinin
- Nitric oxide
- Prostaglandins
- Serotonin
- Somatostatin
- Substance P
- Vasoactive intestinal peptide



Application in research Needed yesterday!

- Behaviour
  - Observations – meaning?
  - Telemetry – time and expense
- Physiology
  - Hard to measure
  - Hard to relate to welfare?
  - Need to use developments in other disciplines



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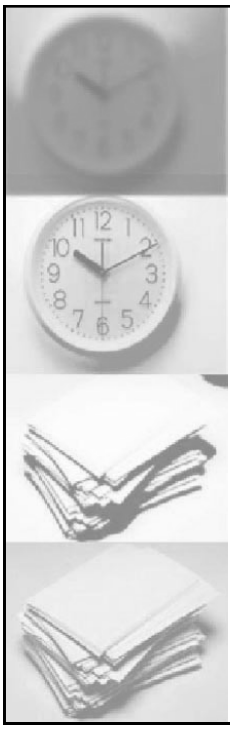
  - Hard to relate to welfare?
  - Need to use developments in other disciplines
- Cognition
  - Key concern for public



Application in research Needed yesterday!

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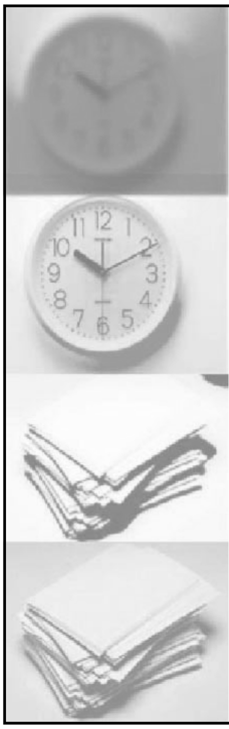
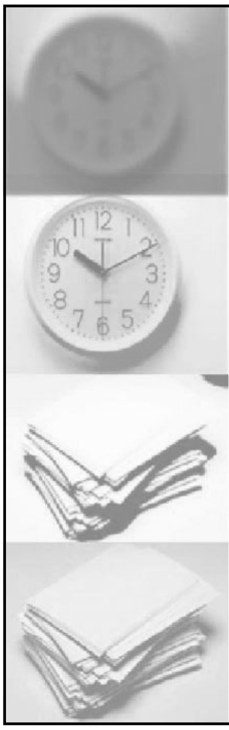
  - Need to use developments in other disciplines
- Cognition
  - Key concern for public
  - Ask the animal !



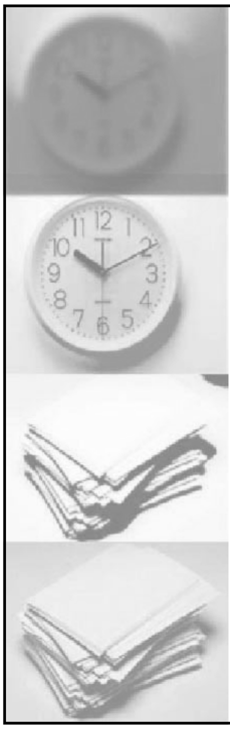
Application in research Needed yesterday!

- Behaviour
  - Observations – meaning?
  - Telemetry – time and expense
- Physiology
  - Hard to measure
  - Hard to relate to welfare?
  - Need to use developments in other disciplines
- Cognition
  - Key concern for public
  - Ask the animal !
- Gene expression
  - Hard to measure, but getting easier
  - Some potential – but how soon and at what cost?



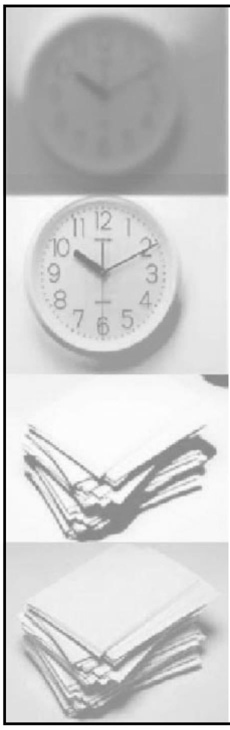
Focus areas

- Dynamic processes
  - Preparation
  - Foetal programming
- Public perception
- Individual responses
- Relation to production
- Chronic stress models
  - Additive stressors



Growth in animal welfare science

- Research focused on legislation
- Auditable standards
- International standards



Key quotes

- Andrew/Kevin/Steve
  ‘*No quick fix!’*
    ‘But no Holy Grail either’

- Michelle

‘*Don’t be overwhelmed*!’