

# *In vivo* approaches for Allergenicity

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Pictures are created with BioRender.com

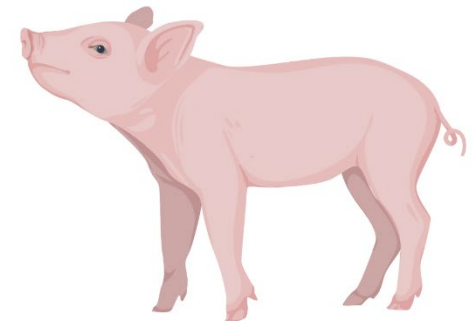
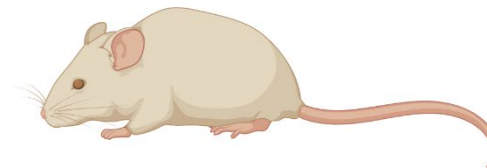
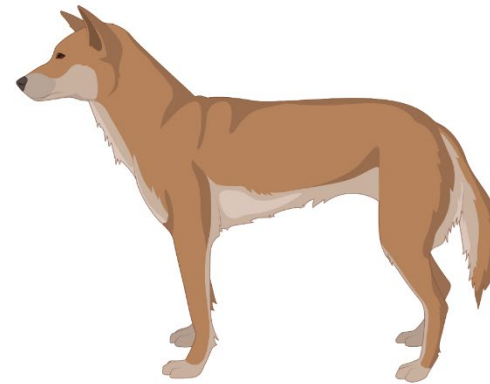
# Background

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Several animals have been used in the study of food allergy – mice, rats, piglets and dogs

Main reasons for using animal models in the study of food allergy:

- To increase our knowledge on food allergy and the underlying mechanisms
- We can study food allergy in a controlled environment without any confounding factors
- Food allergy is a complex disease and animal models allow us to study food allergy in a model eg. containing an intact gut, comprising full functional digestion, allergen absorption, immune regulation and microbiota.



# IgE vs. non-IgE mediated animal models

## Non-IgE mediated food allergy

- Food protein-induced enterocolitis syndrome (FPIES)
- Food protein-induced allergic proctocolitis (FPIAP)
- Enteropathy (FPE)
- Food-induced pulmonary haemosiderosis (Heiner's Syndrom)
- Allergic eosinophilic esophagitis (EoE)\*
- Celiac disease (CD)



Common for these diseases is that the exact pathogenic mechanisms are insufficiently understood, and the diagnosis mostly relies on positive food challenges. Thus, there are no real biomarkers and diseases are difficult to investigate in animal models.

Several animal models have been developed – but is there a need for such models in the prediction of allergy to new proteins/foods?

## IgE mediated food allergy



\*Reviewed in Lozano-Ojalvo et al. Drug Discovery Today: Disease models. 2015

# Animal models of IgE mediated allergy

## Two main types of animal models:

- Predicting sensitisation
  - Predicting sensitisation and clinical relevance
- 
- The animal models are mostly distinguished by:
    - Animal strain
    - Route of administration
    - Use of adjuvant
      - Non-adjuvantic
      - Adjuvantic
      - Genetically modified
      - Humanised



C57BL/6 mice



Balb/C mice



C3H/HeJ mice

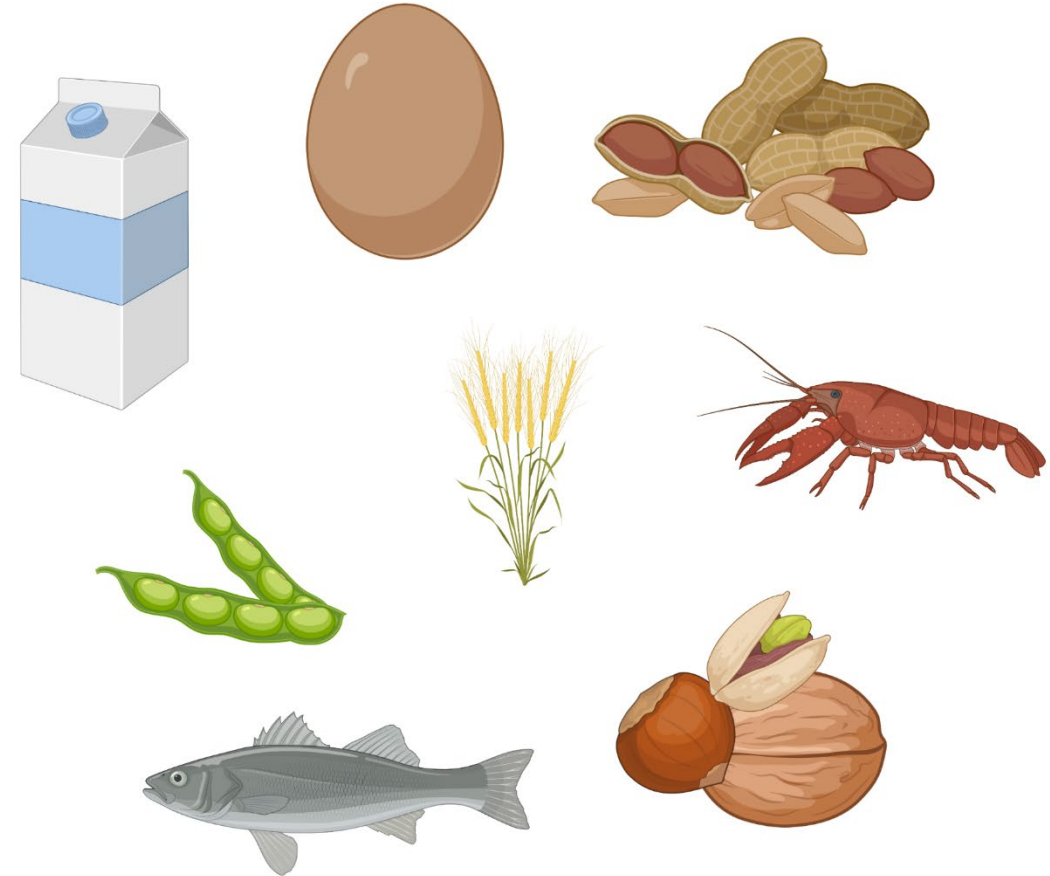


Brown Norway rats

# A predictive animal model

## What do we need from a predictive animal model?

- Translational - representative for the human situation
- Sensitive
- Robust
- Cost-effective
- Fast
- Easy
- Reliable



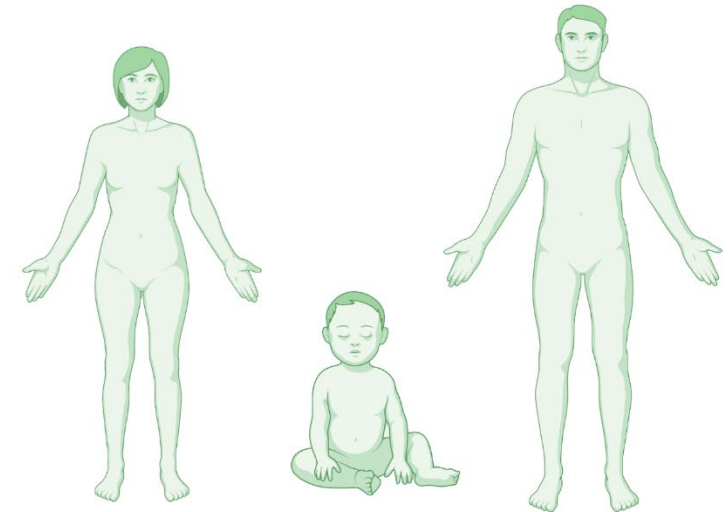
# Sensitisation and elicitation in humans

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Food allergy is a complex and not fully understood disease.

Especially sensitisation is a complex and only poorly understood process and there are things that we don't know about sensitisation to food allergens in humans, like:

- The sensitisation route – oral, dermal, respiratory
- The dose-response relationship
- The impact of the frequency of exposure
- The impact of tolerance to cross-reacting allergens
- The role of bystander effect from other allergens
- The role of digestion
- The role of matrix
- The role of processing
- The role of infection
- The role of microbiota



What are the exact mechanisms at play – and are they always the same?

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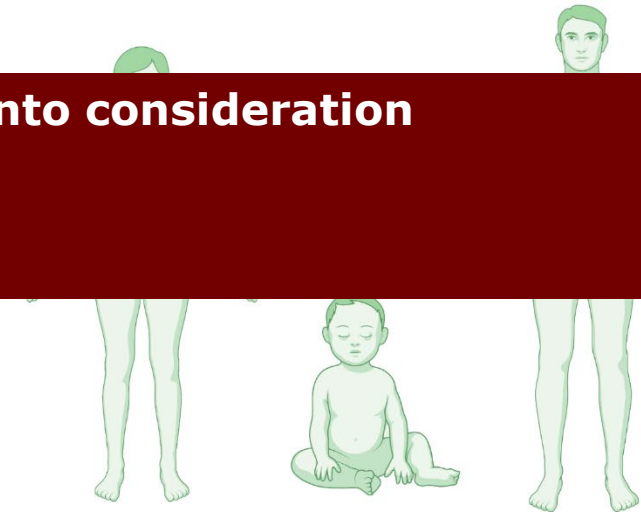
- The sensitisation route – oral, dermal, respiratory

**There is a lot of different parameters to take into consideration**



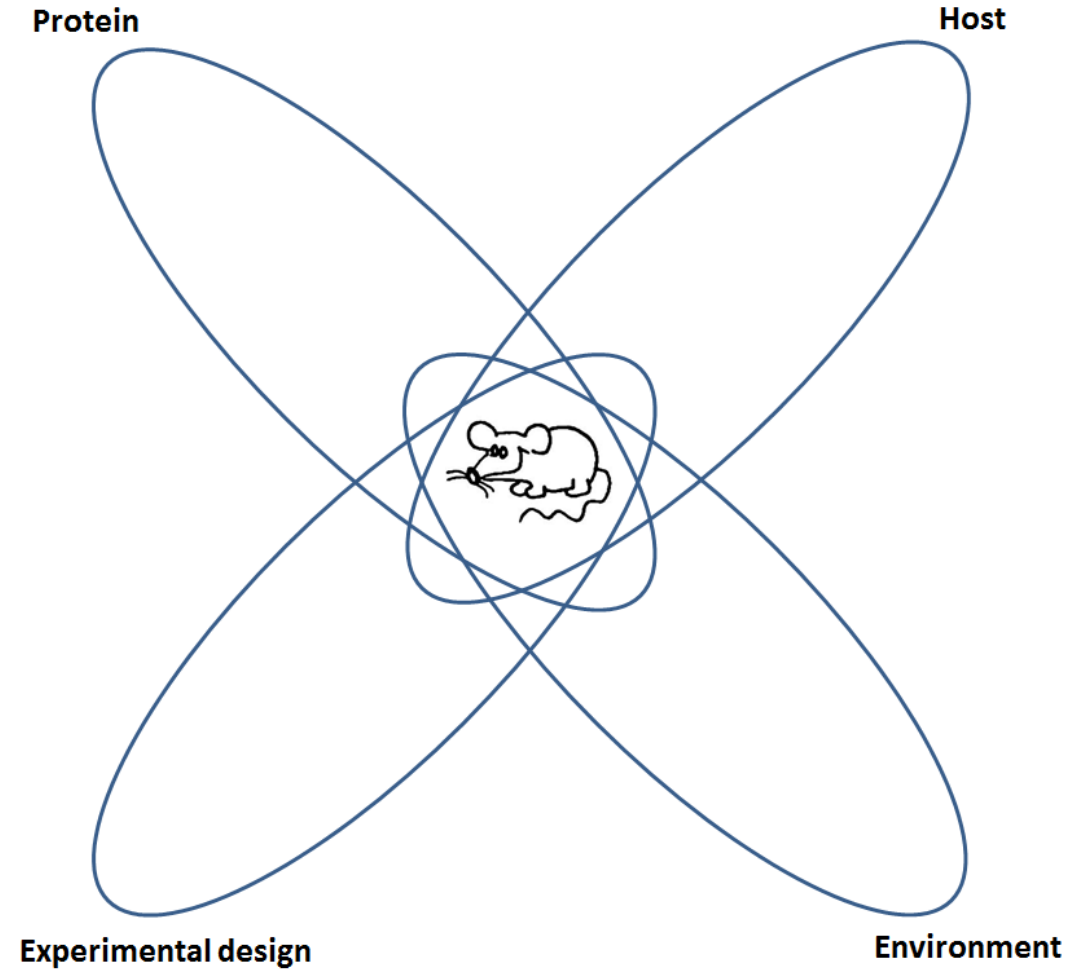
**Study design is important**

- The role of bystander effect from other allergens
- The role of digestion
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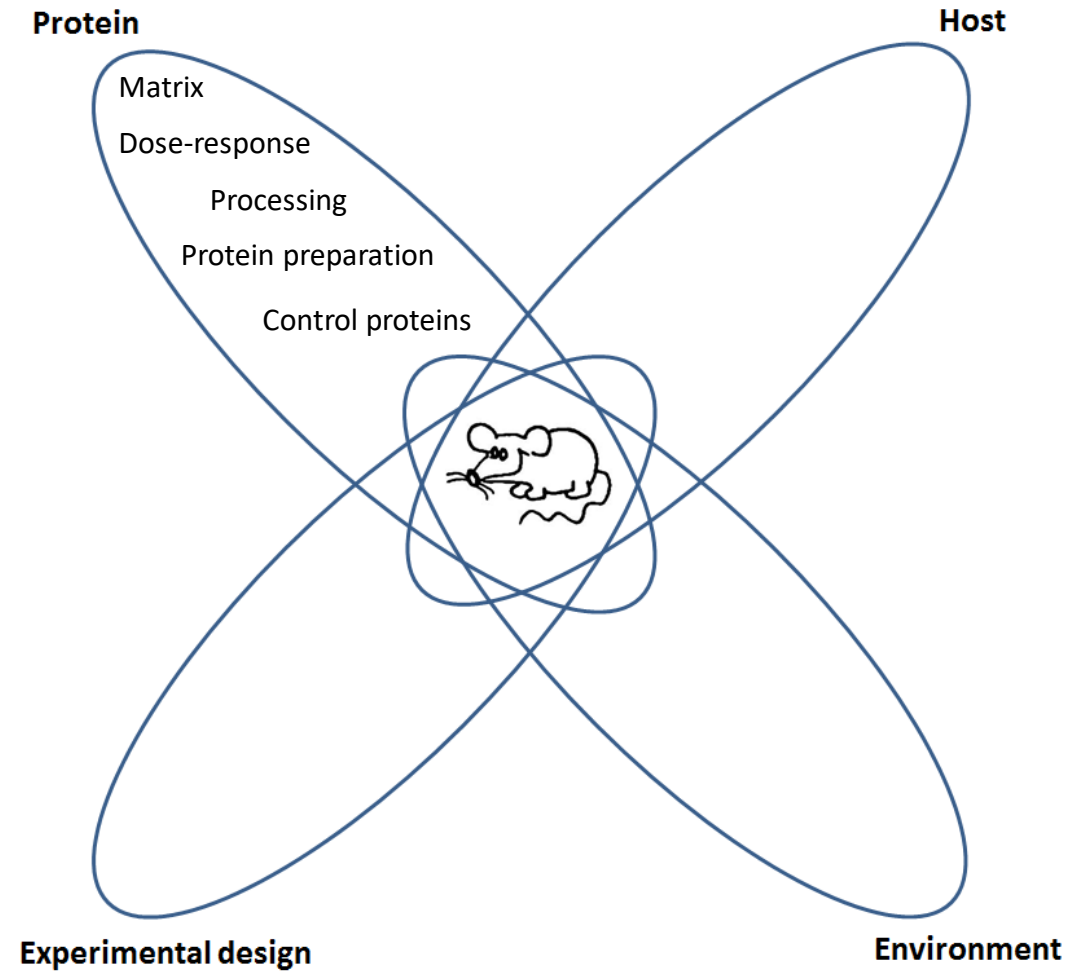
# Factors influencing the animal model



Bøgh et al, Clin Transl Allergy. 2016

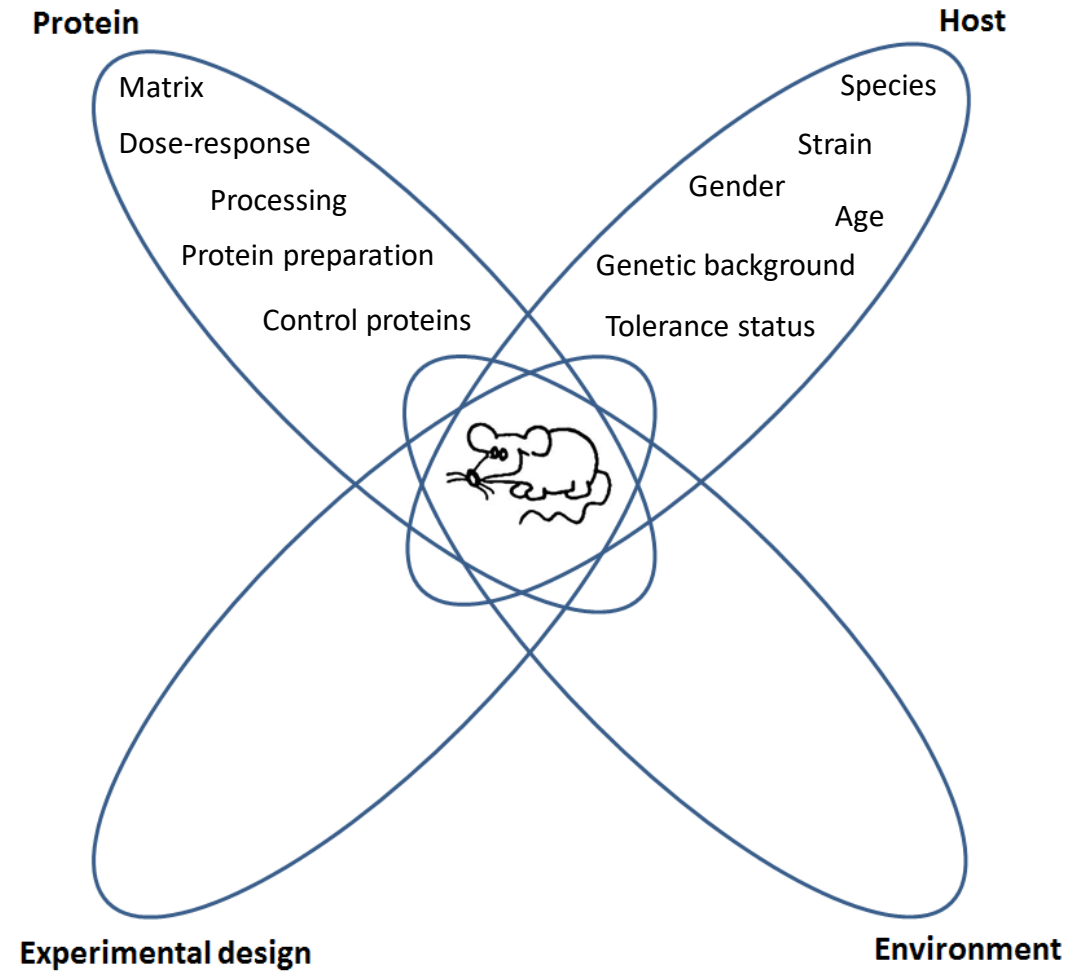


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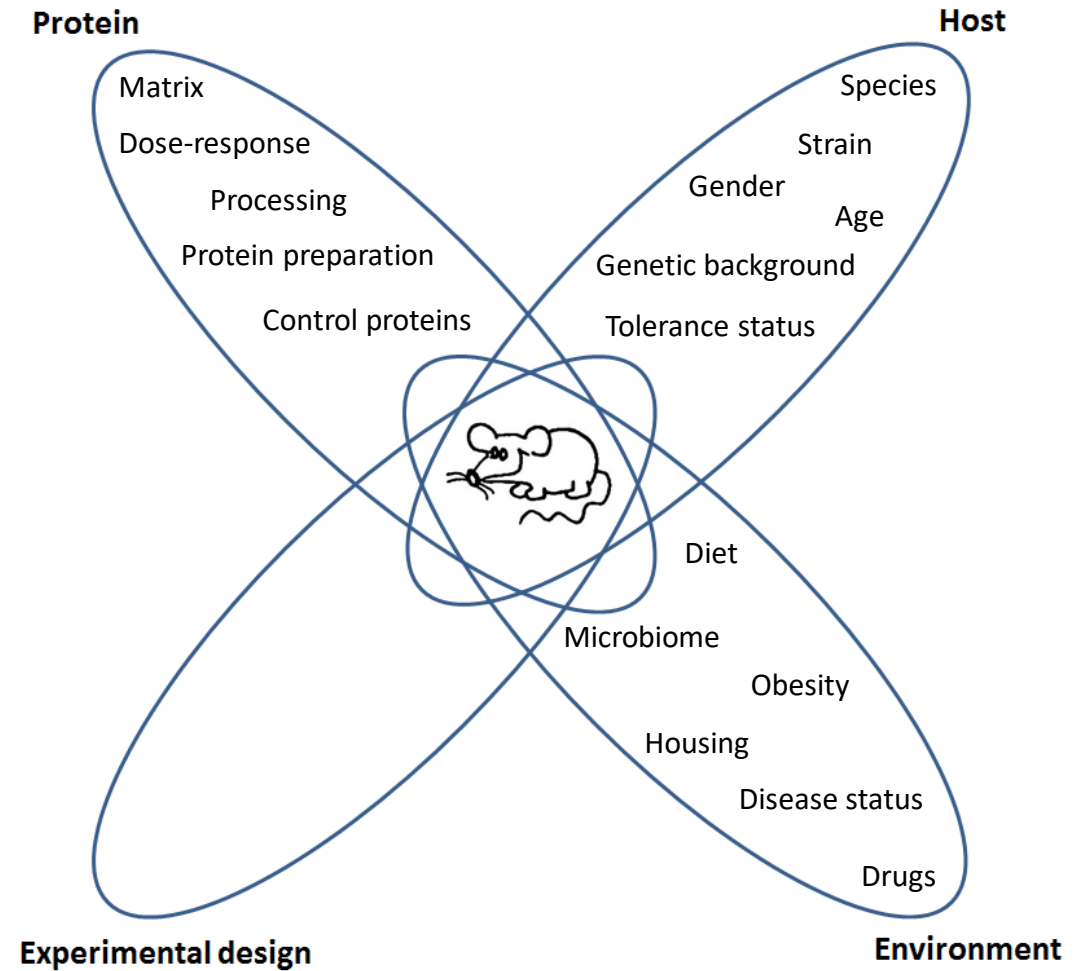
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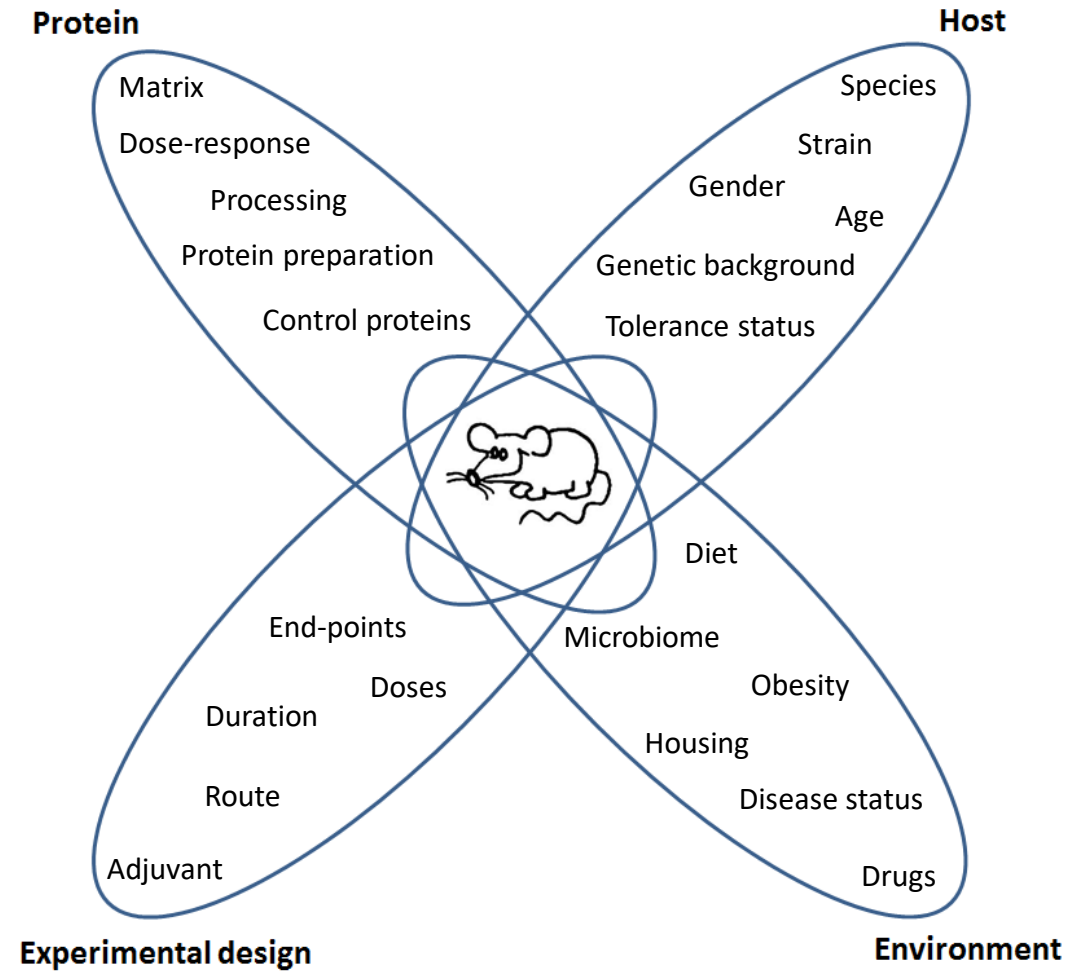
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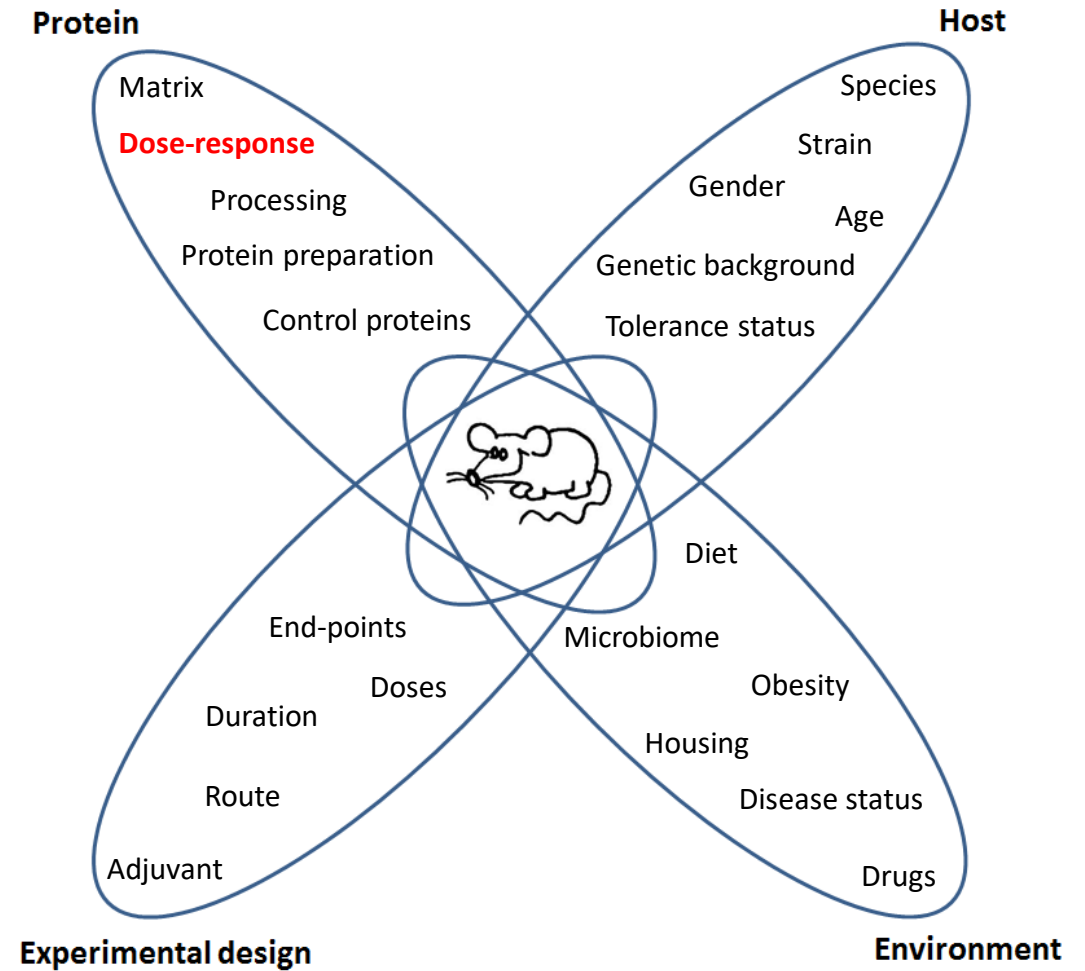
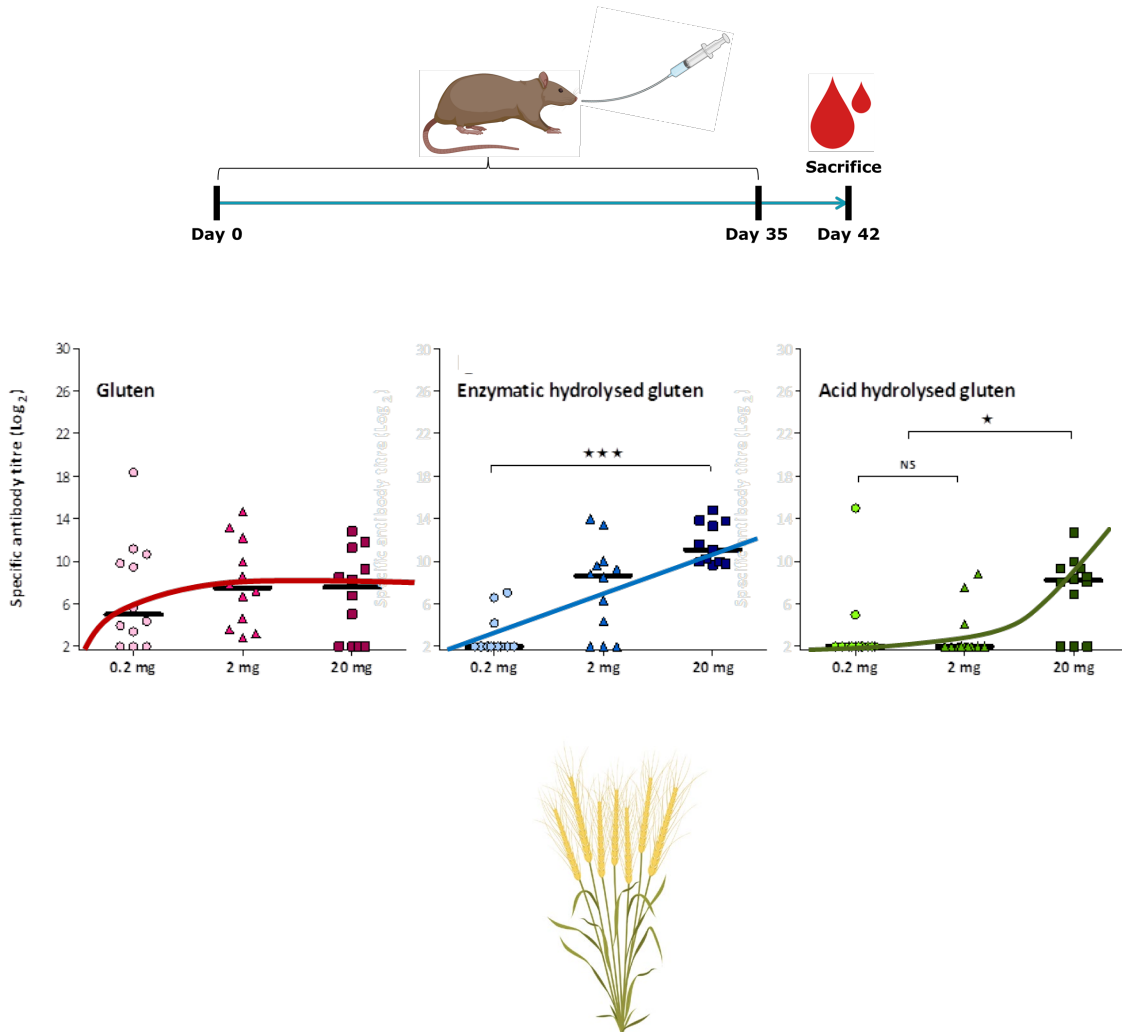
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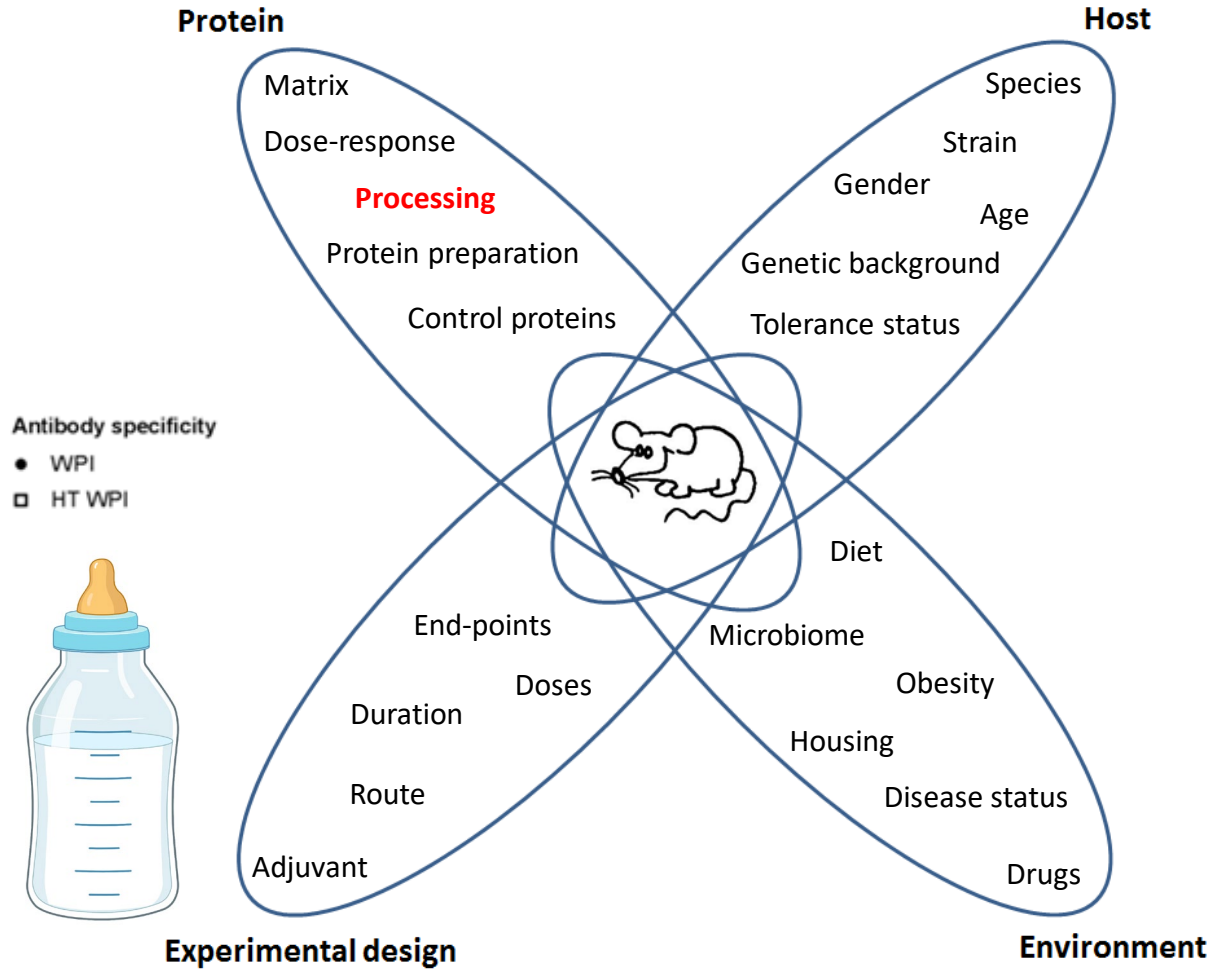
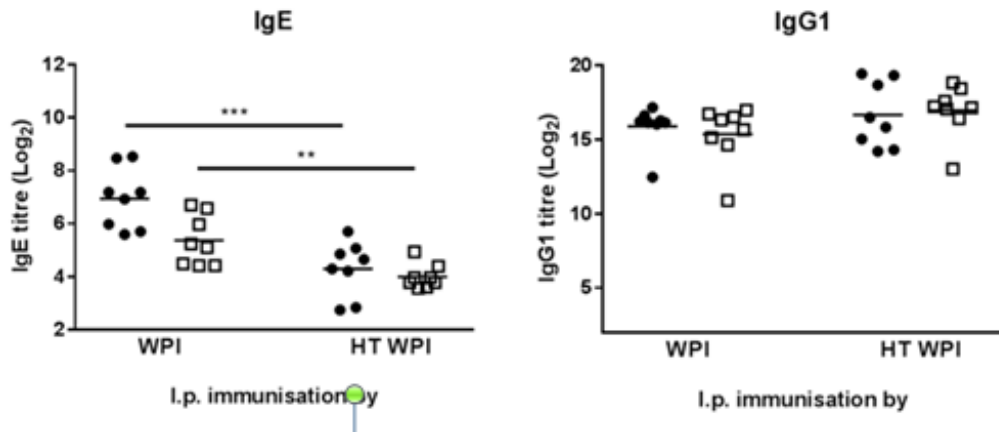
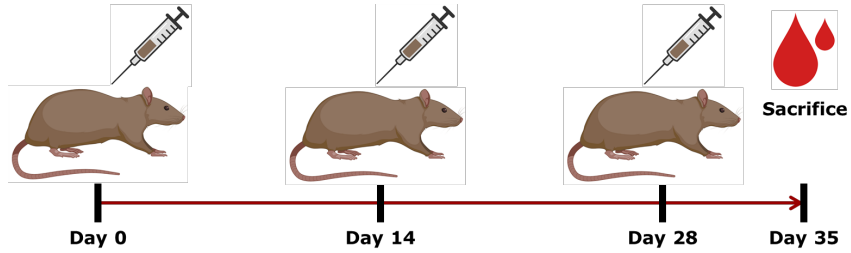


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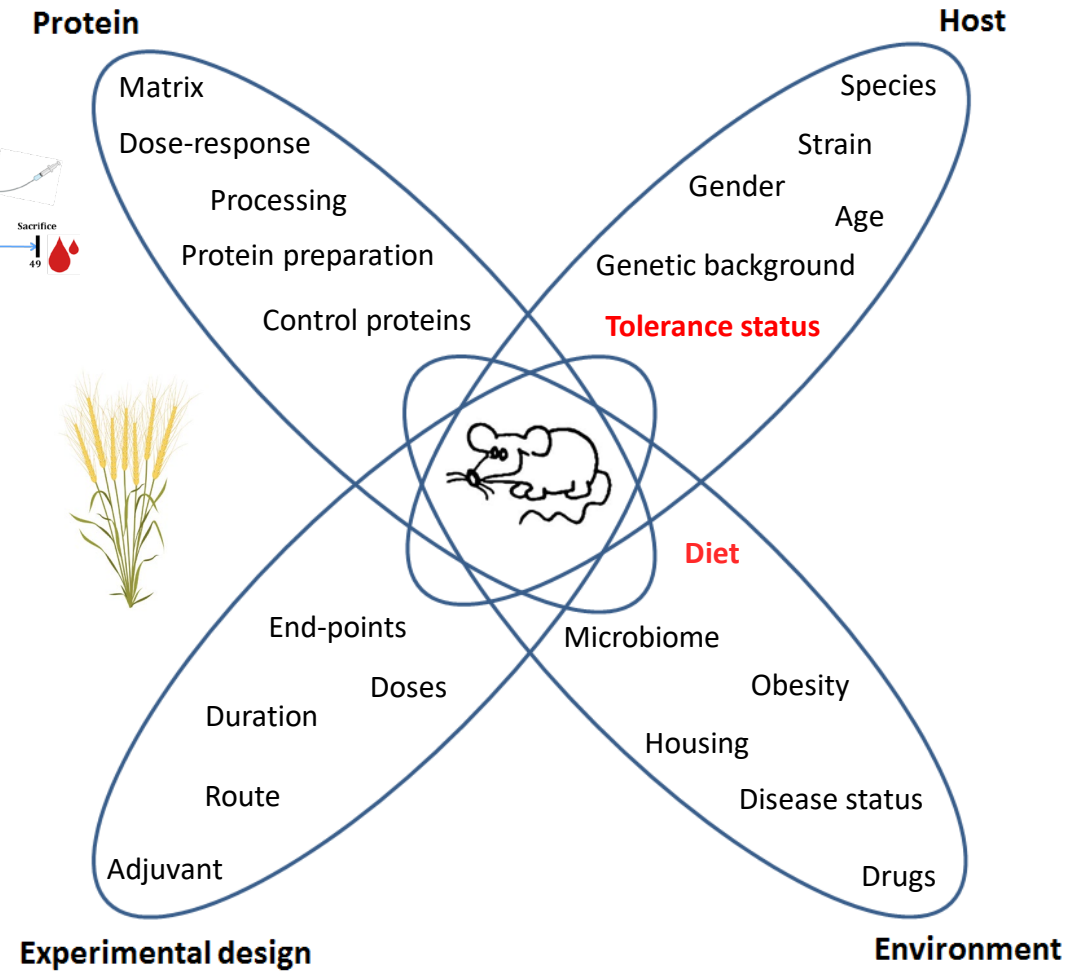
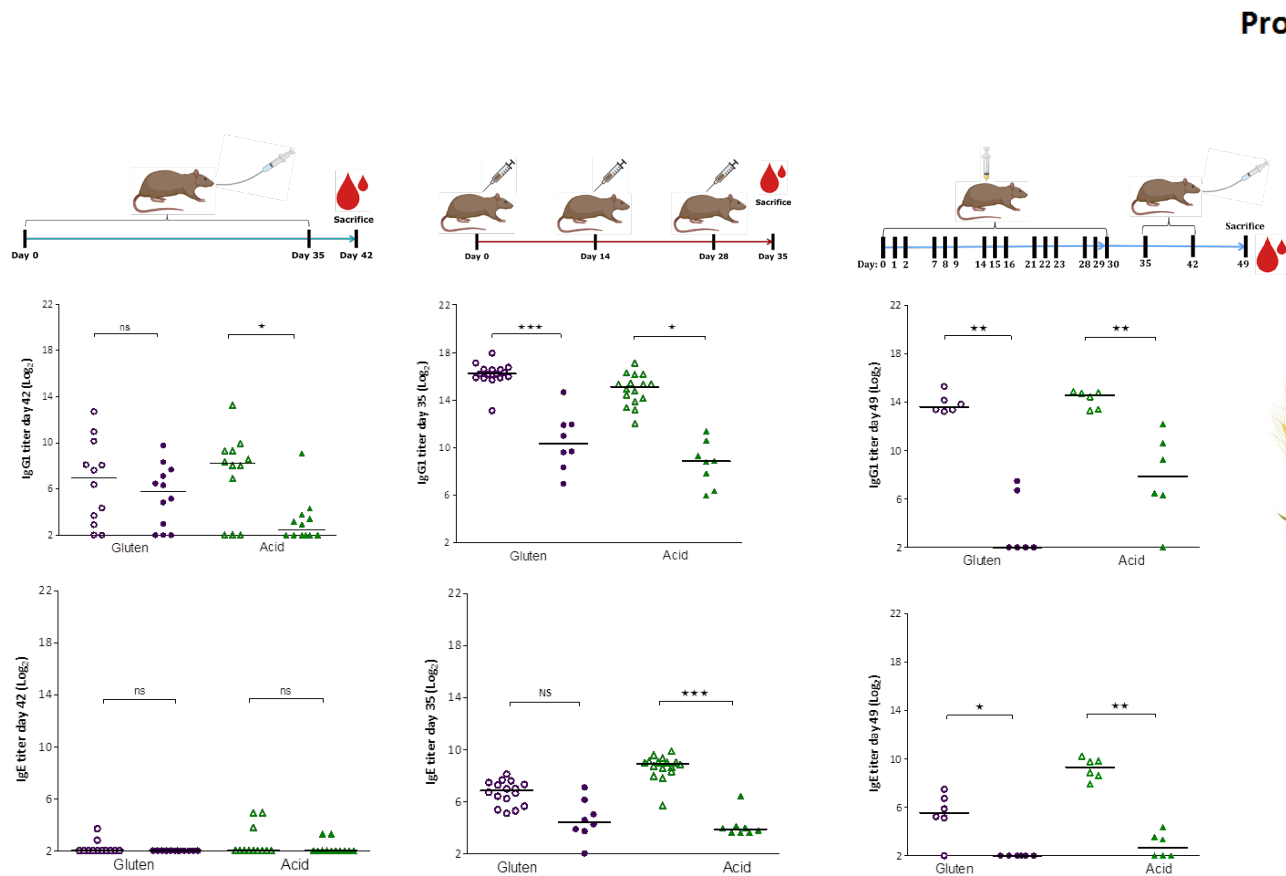
# Protein – Dose-response



# Protein – Processing

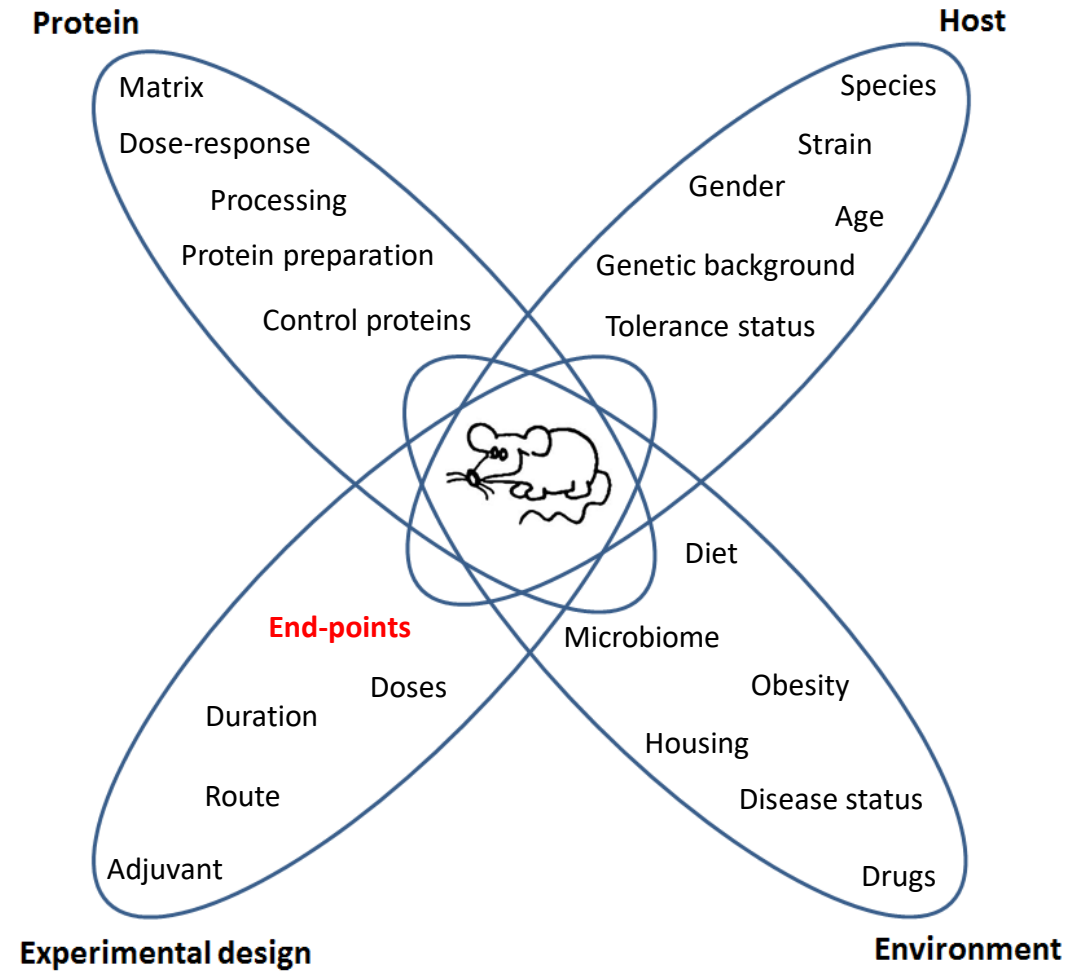


# Environment – Diet/Tolerance status



Ballegaard et al., Int Arch of Allergy Immunol. 2019/Madsen & Bøgh, PlosOne. 2020

# Experimental design – End point





# Experimental design – End point

**Antibody levels:**

- Specific IgG1 serum levels
- Specific IgE serum levels
- Specific IgA serum levels
- Total IgE serum levels
- Specific Ig Avidity measures

Total IgA faecal levels  
Specific IgA faecal levels



**Clinical reactions:**

- Ear swelling test
- Temperature drop
- Skin prick test
- Symptom scoring
- Histamin levels

BAT assay



**Gut-related changes:**

- Histology
- Specific uptake (LP vs. PP vs. EP)
- Permeability
- Tight-junction gene expression
- Tight-junction proteins
- Tight junction re-location



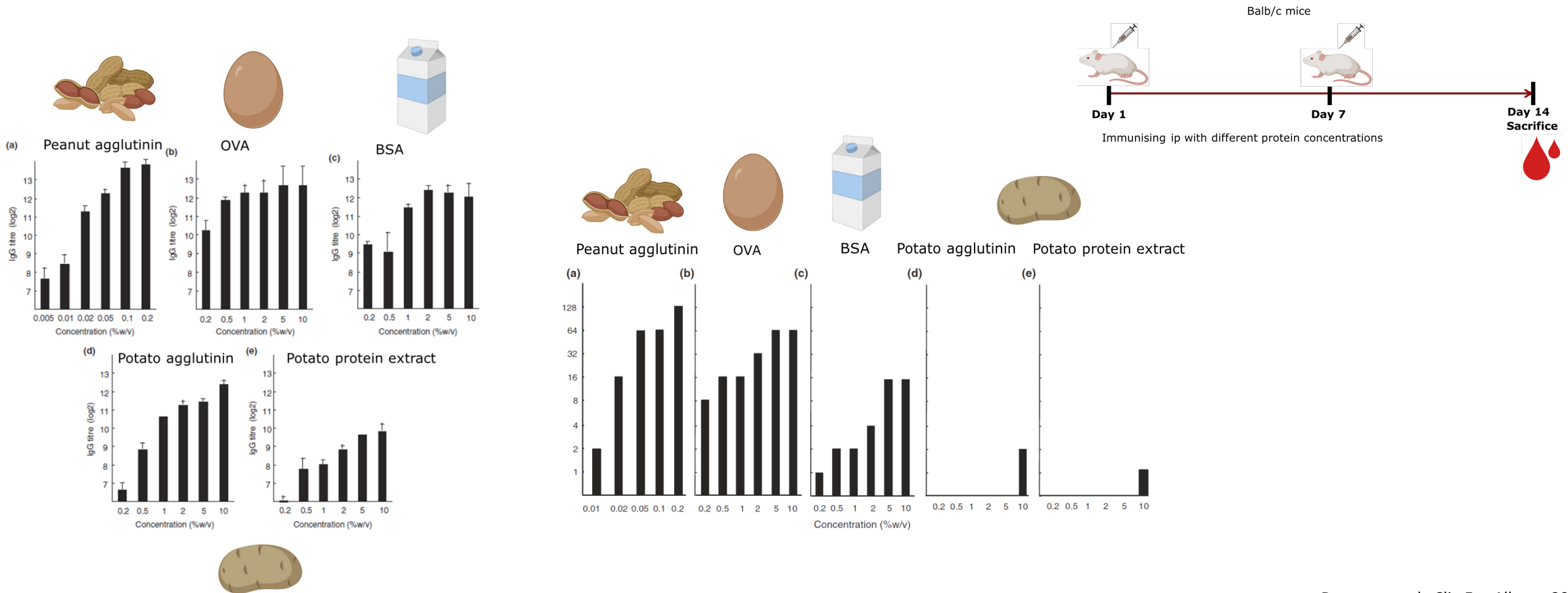
**Immune regulation:**

- Immune cell profiling
- Cytokine
- Gene expression
- Secretion
- Cell homing



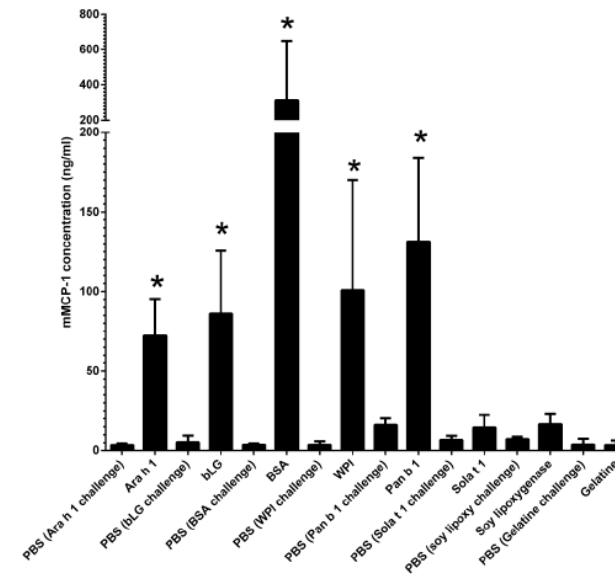
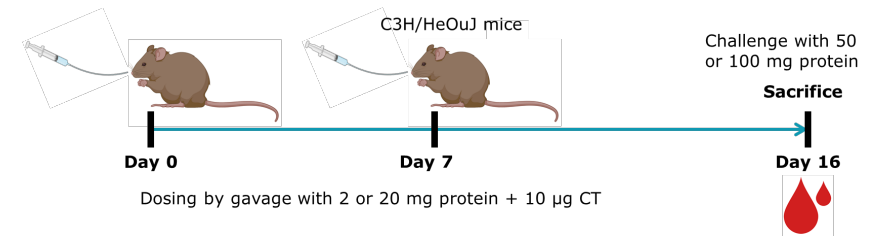
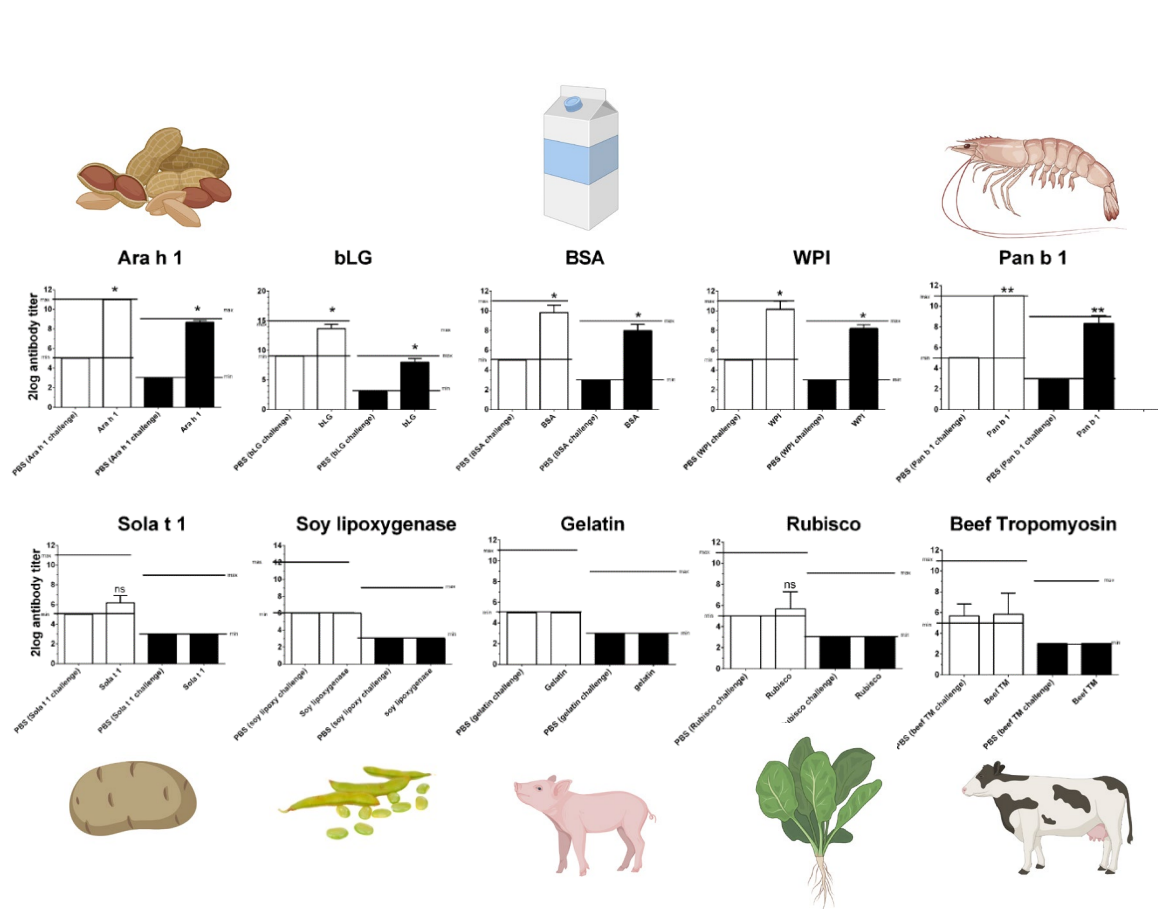
# Predictive animal models of IgE mediated allergy

Should be able to predict and distinguish allergenic food/proteins from low/non-allergenic foods/proteins:



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# Do we need *in vivo* models for predicting allergenicity

**Food allergy is a complex disease and the underlying mechanisms are not fully understood!**

**Do we need *in vivo* models for both**

- ***De novo* sensitisation – development of potential new allergies**
- **Cross-reactivity – elicitation of reactions in already allergic individuals**

**Animal models are valuable tools, especially in the prediction of the sensitisation phase as we presently have no other tools. However, the exact purpose and thus the experimental design will need to be decided.**

**Subsequently, the animal models will need to be tested in a dose-response related manner and validated in a ring trial.**

**Animal models may be used to validate future predictive *in vitro* models.**



# Acknowledgement



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Improving Allergy Risk Assessment Strategy for new food proteins

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