EXPERIMENT BASICS

CLASS 2 OPENING ACTIVITIES

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CLASS 1 RECALL

12:30-12:40

EXPERIMENTAL UNIT

> The **experimental unit** is the smallest unit to which a treatment can be applied independently.

For each case, identify:

- what receives the treatment
- the experimental unit
- the number of experimental units

CALVES

Twenty-four calves are housed individually. Each calf receives one of three dietary supplements for six weeks. Body mass is recorded every week.

Identify the treatment structure.

Question	Answer
What receives treatment?	
Experimental unit?	
Number of units?	
What is repeated?	

PIG PENS

Twenty-four pigs live in six pens, with four pigs in each pen. Feed is supplied through one shared trough in every pen. Pens receive one of two diets, and every pig is weighed weekly.

Identify the treatment structure.

Question	Answer
What receives treatment?	
Experimental unit?	
Number of units?	
What sits within a unit?	

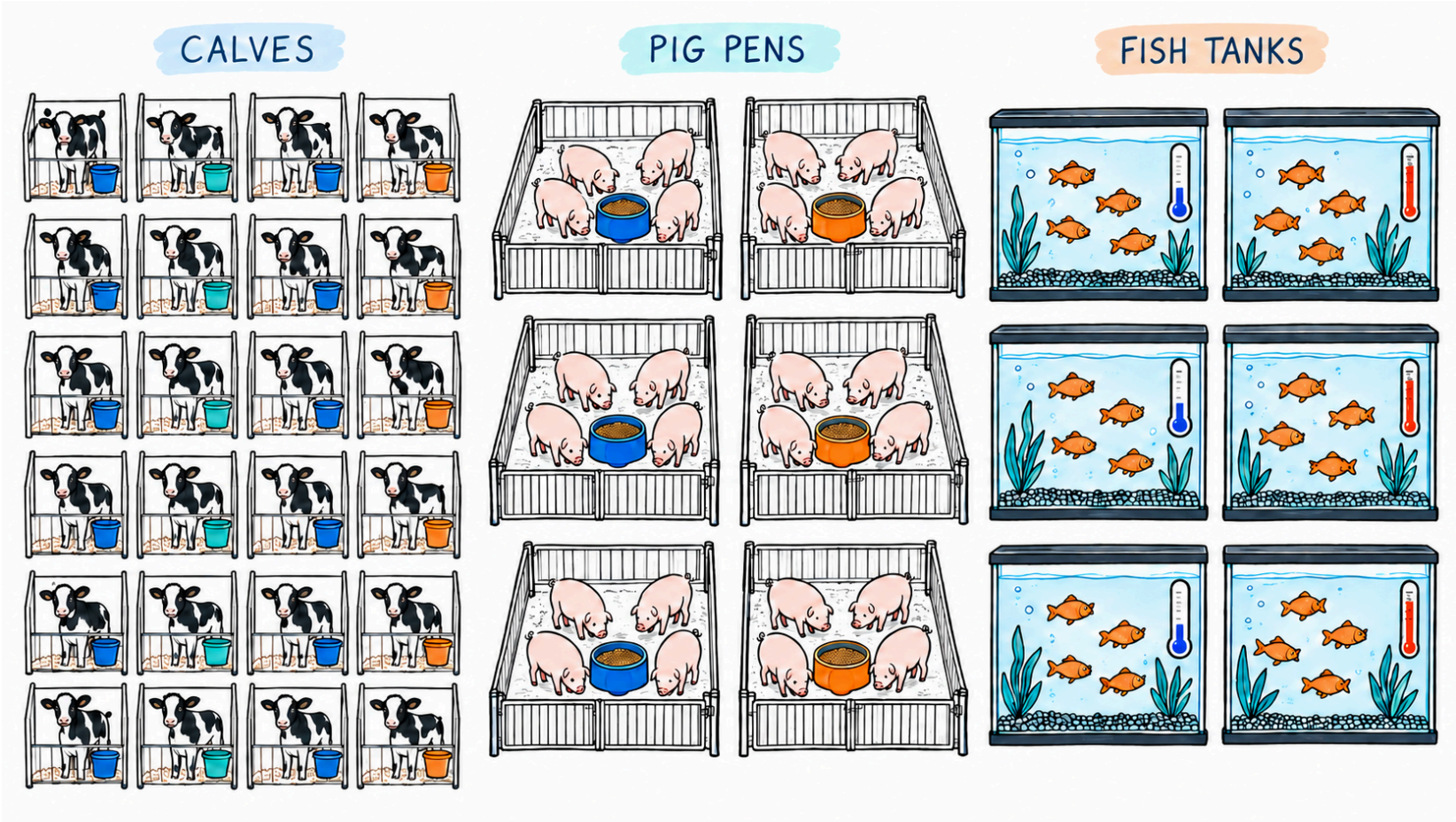
FISH TANKS

Thirty fish are distributed among six tanks, with five fish in each tank. Water temperature is controlled separately for each tank. Three tanks receive warm water and three receive cool water.

Identify the treatment structure.

Question	Answer
What receives treatment?	
Experimental unit?	
Units per temperature?	
What sits within a unit?	

TREATMENT STRUCTURE



The treatment assignment determines the experimental unit. The number of measurements does not determine it.

EXPERIMENTS

12:40-13:10

RESEARCH QUESTION

> Does increasing dietary protein improve growth rate in growing pigs?

A useful research question identifies:

- the biological system of interest
- the factor that may explain variation
- the response used to evaluate the explanation

DESIGN ELEMENTS

Design element	Pig growth example
Research question	Does dietary protein affect growth rate?
Hypothesis	Dietary protein affects growth rate
Treatment factor	Dietary protein concentration
Factor levels	Standard and increased protein
Response variable	Growth rate over a defined period

Does the proposed comparison provide evidence about the stated hypothesis?

TREATMENT FACTOR

The **treatment factor** is the explanatory variable deliberately manipulated in the experiment.

In the pig example:

- factor: dietary protein concentration
- standard level: the current diet
- experimental level: the increased-protein diet
- treatment: the condition applied to an experimental unit

RESPONSE VARIABLE

The **response variable** records the outcome used to evaluate the treatment effect.

For growth rate, the study must specify:

- the measurement used to calculate growth
- the period over which growth is assessed
- the units of measurement
- whether measurements occur at animal or pen level

NO COMPARISON

Twenty pigs receive a new feed. Their mean growth rate is **800 g per day**.

What does this tell us?

- it describes growth under the new feed
- it does not show what growth would have been under another condition
- it cannot estimate the effect of changing feed

CONTROL GROUP

Add a contemporaneous group of pigs receiving the current diet.

The control group provides:

- the relevant alternative condition
- an estimate of outcomes over the same period
- a basis for estimating the treatment effect

The appropriate control depends on the research question.

RIVAL EXPLANATIONS

Barn 1

- increased-protein diet
- one ventilation system
- one management team

Barn 2

- standard diet
- a different ventilation system
- a different management team

If growth differs, which feature caused the difference?

The design changes the diet, barn environment and management at the same time.

DESIGN CHECK

Before interpreting an experiment, identify:

- the research question and biological hypothesis
- the treatment factor and its levels
- the response variable and measurement schedule
- the experimental units receiving each condition
- the comparison required to estimate the treatment effect

Describe the proposed experiment using all five items.

REPLICATION

13:10-13:30

WHICH N?

Diet A: 20 pigs
Diet B: 20 pigs

The researcher reports **n = 20 per treatment**.

Before accepting that value, ask:

- how the pigs are housed
- where the feed is delivered
- which units receive diets independently

TWO KINDS OF UNIT

Term	Working definition	Pig example
Experimental unit	Smallest unit receiving treatment independently	Pen when feed goes to a shared trough
Observational unit	Unit on which the response is measured	Pig when each pig is weighed

The units may be the same, but they do not have to be.

SUBSAMPLES

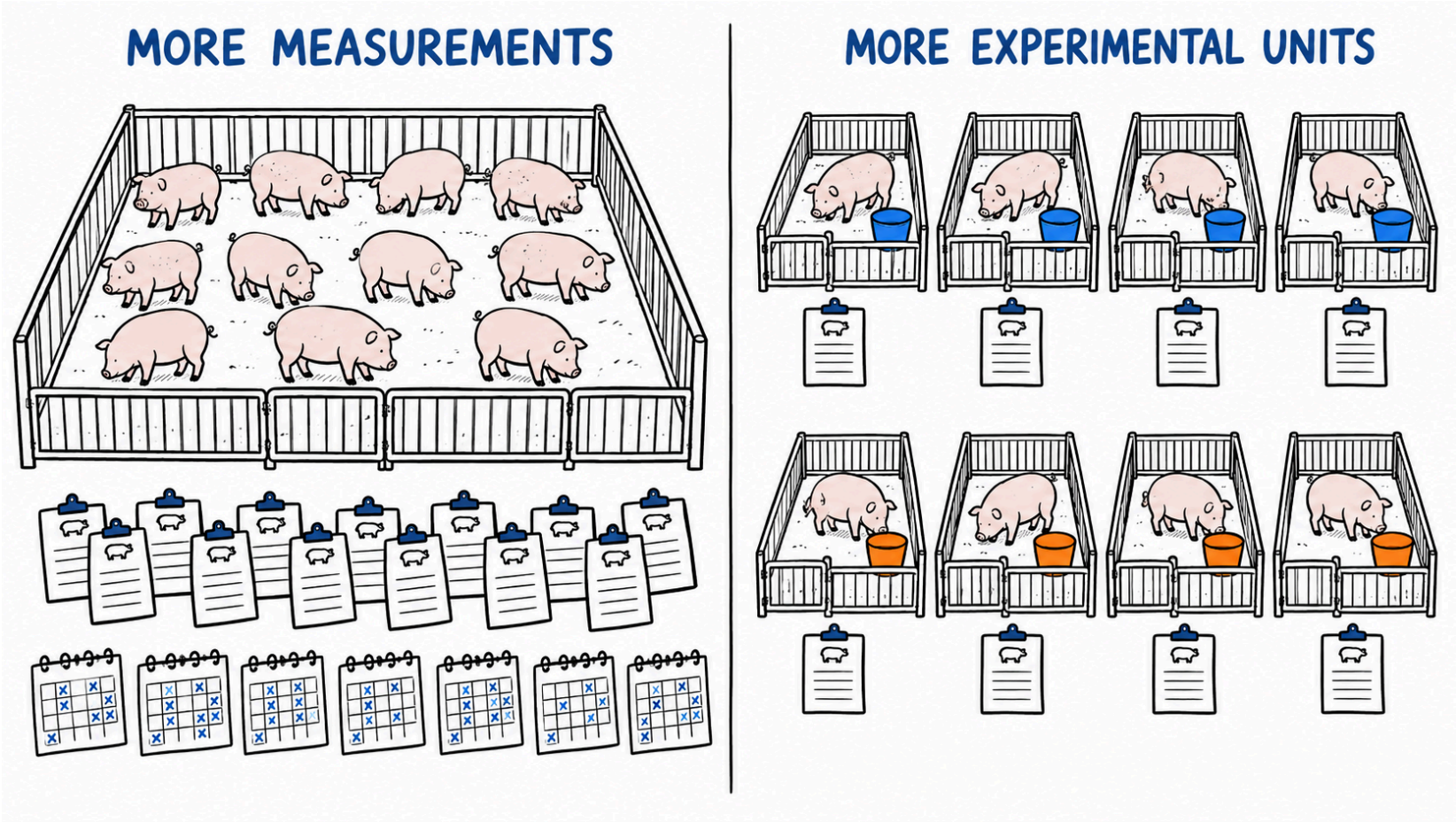
A **subsample** is an observation taken from within an experimental unit.

Subsamples can:

- describe variation within the experimental unit
- improve estimation of an experimental-unit response
- reveal individual responses within a treated group

Subsamples do not add independent treatment allocations.

MEASUREMENTS OR UNITS?



Repeated weighing improves information about a pen. Additional independently treated pens increase treatment replication.

THREE QUICK CASES

1. Six pens receive each diet. One mean growth value is calculated for every pen.
2. One pen receives each diet. Twelve pigs are weighed in every pen.
3. One cow is measured weekly for ten weeks.

> Classify the extra information as **replication**, **subsampling**, or **repeated measurement**.

PSEUDOREPLICATION

> **Pseudoreplication** occurs when observations that are not independent treatment replicates are analysed or interpreted as though they were independent replicates.

Common sources include:

- several animals sharing one treated enclosure
- repeated observations from the same animal
- several samples or technical measurements from one unit

INDEPENDENT UNITS

When evaluating replication:

- identify the experimental unit from treatment application
- count units that could receive treatments separately
- keep subsamples and repeated observations linked to their unit
- report the treatment sample size using independent experimental units

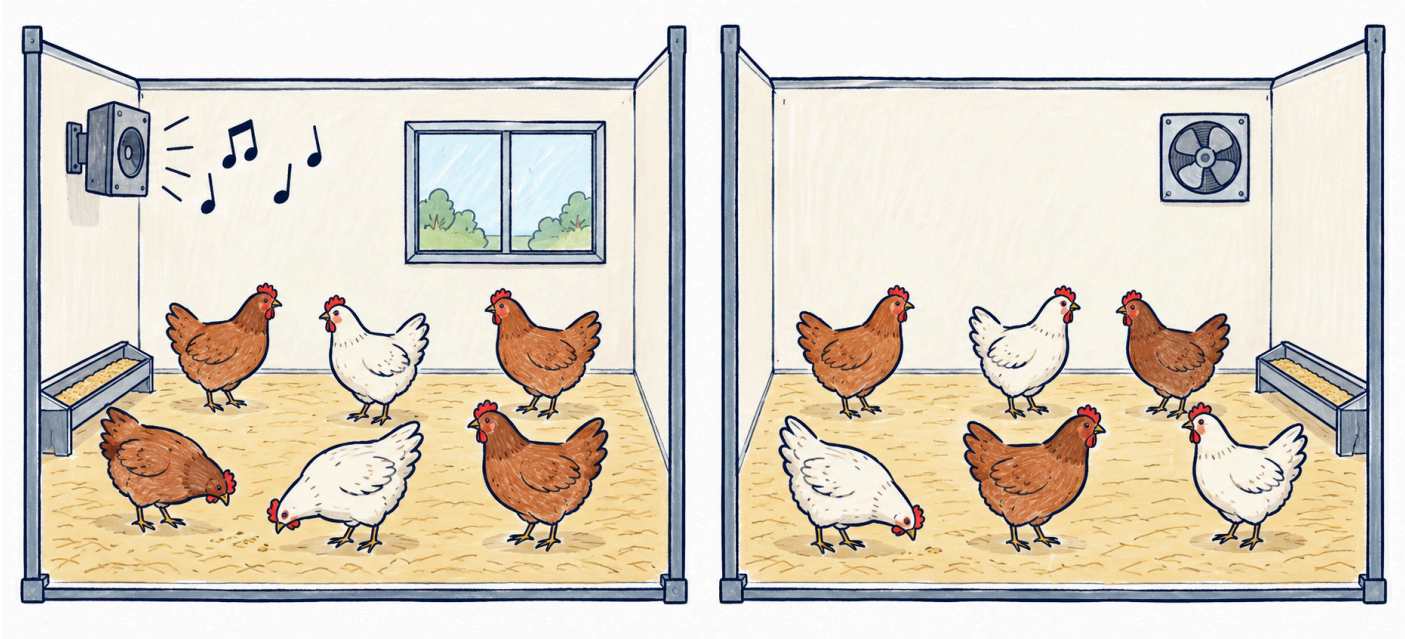
What was independently treated, and how many times was each treatment applied?

MINI-SURGERY

13:30-13:45

MUSIC AND HENS

Room A contains 30 hens and receives classical music for four weeks. Room B contains 30 hens and receives no music. Corticosterone concentration is measured in every bird.



YOUR DIAGNOSIS

Work in groups. Complete the diagnosis before suggesting a repair.

Question	Working answer
What biological question is being asked?	
What is the treatment factor?	
What is the response variable?	
What receives treatment independently?	
How many independent units receive each condition?	
What threatens the comparison?	

TREATMENT STRUCTURE

Design element	Hen study
Research question	Does classical music affect stress in hens?
Treatment factor	Sound condition
Factor levels	Classical music and no music
Response variable	Corticosterone concentration in each hen
Experimental unit	Room, because music is applied to a whole room
Treatment replication	One room per condition

The 30 measurements in a room describe hens exposed together. They do not create 30 independent treatment assignments.

TWO PROBLEMS

Pseudoreplication

- Hens share a room-level treatment.
- Treating 30 hens as 30 treatment replicates would overstate the independent information.

Room–treatment confounding

- Music occurs only in Room A.
- Every persistent difference between the rooms varies with treatment.

Would perfect measurement of every hen solve either problem?

MINIMUM REPAIR

If music must be applied to a whole room:

- include several independent rooms for each sound condition
- randomly allocate rooms to classical music or no music
- measure hens within every room and retain their room membership in the analysis

If treatments can be isolated within smaller enclosures, those enclosures could become the experimental units, provided several receive each condition independently.

> **Which part of the repair adds genuine treatment replication?**

ALLOCATION

13:45-14:00

RANDOM ALLOCATION

Random allocation uses a random process to decide which experimental units receive each treatment.

It:

- starts from the eligible experimental units
- prevents investigator preference from determining treatment groups
- gives each unit a known opportunity to receive each condition
- supports an unbiased comparison of treatment effects

What would appear as one row in the allocation list?

SAMPLING OR ALLOCATION?

Question	Random sampling	Random allocation
What is decided?	Which units enter the study	Which treatment each unit receives
Starting point	A target population	The available experimental units
Main purpose	Support generalisation to the population	Support a causal treatment comparison
Farm example	Select farms from a region	Assign enrolled farms to protocols

An experiment may use one, both or neither process. They answer different questions.

RESEARCHER CHOICE

Forty dog owners choose whether their dog receives a new anxiety supplement or remains on its usual diet. After eight weeks, anxiety scores are lower in the supplement group.

The groups may have differed before treatment because of:

- baseline anxiety severity
- owner expectations and motivation
- home routines or other care

Could owner choice explain part of the observed difference?

CONFOUNDING

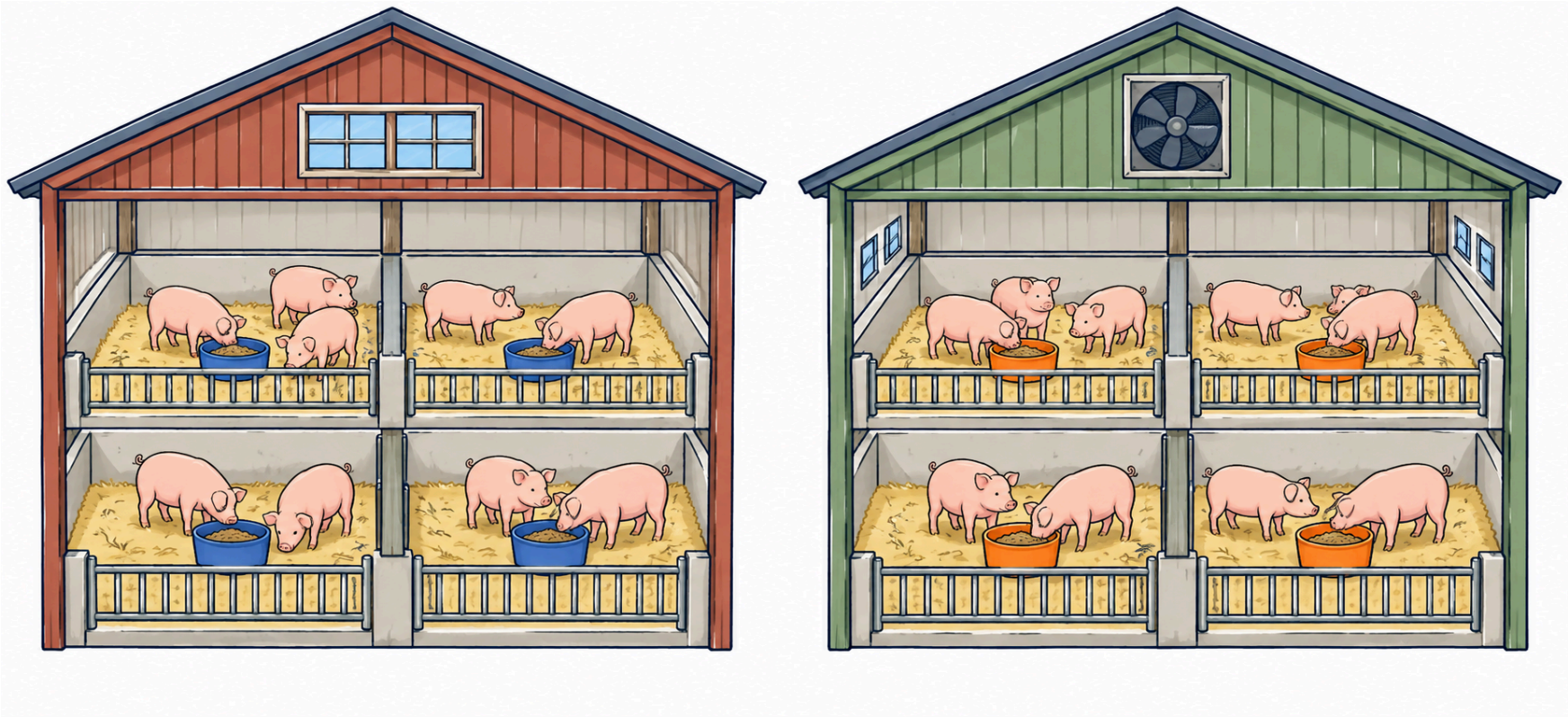
> **Confounding** occurs when the treatment effect cannot be distinguished from the effect of another variable that changes with treatment.

Confounding can arise when treatment levels occur:

- in different rooms, barns or tanks
- during different periods
- in groups formed by systematic choice

More measurements within the same experimental units do not separate the effects.

DIET OR BARN?



If growth differs, can the experiment distinguish a diet effect from a barn effect?

DESIGN REPAIRS

How treatment is applied	A defensible allocation
Each pen can receive either diet	Include both diets in each barn and randomly allocate pens
A whole barn must receive one diet	Include several barns per diet and randomly allocate barns
Only one barn is available per diet	Redesign the study or limit the conclusion to a descriptive comparison

The allocation must create independent treatment comparisons at the experimental-unit level.

SURGERY QUESTIONS

For every proposed experiment, ask:

- What is the experimental unit?
- Could each unit have received any treatment?
- How was the random allocation implemented?
- Does another variable change systematically with treatment?
- What alternative explanation remains inseparable?
- Does the comparison support the proposed causal conclusion?

Name the allocation problem before proposing a repair.

SOURCES

Ruxton, Graeme D., and Nick Colegrave. 2016. *Experimental Design for the Life Sciences*. 4th ed. Oxford: Oxford University Press. ISBN 978-0-19-871735-5. Publisher page.

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