REPEATED MEASUREMENTS AND CROSSOVER STUDIES

CLASS 10: ONE ANIMAL, SEVERAL OBSERVATIONS

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Wednesday, 11 November 2026

TODAY: TWO DIFFERENT WAYS TO REPEAT

Time	Work
12:30–12:40	Opening recall: allocation and repeated measurements
12:40–13:15	Calves: one diet, several weights
13:15–13:40	Cows: both treatments, in a random order
13:40–13:55	Break
13:55–14:55	Fit and check the crossover model
14:55–15:10	Break
15:10–15:35	Save a project analysis plan
15:35–16:00	Compare designs, report and exit

OPENING RECALL: HOW DID RESEARCHERS ASSIGN DIETS?

Last class we saw animals sharing pens. A shared feeder means a whole pen may receive one diet.

Today, **32 individually housed calves** each receive their own randomly assigned diet, within four rooms. Each calf is weighed five times.

Discuss with your seated group: did researchers choose a diet for each calf or for a whole room? If they assigned 16 calves to each diet, does weighing every calf five times create more diet assignments?

THE CALF STUDY: WHAT DO WE WANT TO KNOW?

Control and Enhanced diets start **after the baseline weight**.

Calves are weighed at weeks 0, 2, 4, 6 and 8.

Our first question: **how much heavier, on average, are Enhanced calves at week 8?**

These are synthetic teaching data. We are practising analysis, not making a feeding recommendation.

READ THE ACTUAL OBSERVATIONS

Run `in-class-student.R` from the project root. These packages supply the tools we use directly.

```
library("lme4")
library("performance")
library("marginaleffects")
d <- readr::read_csv("data/calf-growth.csv")
head(d, 3)
```

One row is one weight. `calf` identifies repeated observations of the same animal.

READ THE ACTUAL OBSERVATIONS

```
# A tibble: 3 × 5
  room  calf  diet    time_week weight_kg
  <chr> <chr> <chr>       <dbl>     <dbl>
1 R1    C01   Control         0      47.9
2 R1    C01   Control         2      58.6
3 R1    C01   Control         4      65.8
```

TELL R WHICH COLUMNS ARE CATEGORIES

A category is a label rather than a numerical trend.

```
library("dplyr")

d <- d |>
  mutate(
    room = factor(room),
    calf = factor(calf),
    diet = factor(diet, levels = c("Control", "Enhanced")),
    time_factor = factor(time_week, levels = c(0, 2, 4, 6, 8))
  )
levels(d$time_factor)
```

```
[1] "0" "2" "4" "6" "8"
```

Here we estimate a mean at each visit. We do not yet choose a growth curve between visits.

COUNT CALVES, THEN COUNT WEIGHTS

`distinct()` keeps each calf's allocation once.

```
library("dplyr")

allocations <- d |> distinct(calf, room, diet)
allocations |> count(room, diet)
```

```
# A tibble: 8 × 3
  room  diet         n
  <fct> <fct>    <int>
1 R1    Control      4
2 R1    Enhanced     4
3 R2    Control      4
4 R2    Enhanced     4
5 R3    Control      4
6 R3    Enhanced     4
7 R4    Control      4
8 R4    Enhanced     4
```

```
c(calves = nrow(allocations), weights = nrow(d))
```

```
 calves weights
     32     160
```

Four calves per diet per room: **16 calves assigned to each diet**, not 80.

LET EACH CALF HAVE ITS OWN USUAL LEVEL

Some calves tend to be heavier at every visit. `(1 | calf)` lets the model represent that shared pattern.

```
calf_fit <- lmer(
  weight_kg ~ room + diet * time_factor + (1 | calf),
  data = d,
  REML = TRUE
)
summary(calf_fit)
```

`room` allows different room means. `diet * time_factor` allows the diet gap to differ between visits.

LET EACH CALF HAVE ITS OWN USUAL LEVEL

```
Linear mixed model fit by REML ['lmerMod']
Formula: weight_kg ~ room + diet * time_factor + (1 | calf)
   Data: d

REML criterion at convergence: 701.4

Scaled residuals:
     Min       1Q   Median       3Q      Max
-2.31755 -0.51062  0.00236  0.64098  1.95874

Random effects:
 Groups   Name        Variance Std.Dev.
 calf     (Intercept) 29.094   5.394
 Residual              2.541   1.594
Number of obs: 160, groups:  calf, 32

Fixed effects:
                        Estimate Std. Error t value
(Intercept)             38.43207    2.18001  17.629
roomR2                   0.05863    2.72041   0.022
roomR3                   2.40960    2.72041   0.886
roomR4                   4.03348    2.72041   1.483
dietEnhanced             0.02500    1.98857   0.013
time_factor2            10.71919    0.56358  19.020
time_factor4            19.11194    0.56358  33.911
time_factor6            28.42313    0.56358  50.433
time_factor8            36.45800    0.56358  64.690
```

ASK FOR THE WEEK-8 COMPARISON DIRECTLY

`avg_comparisons()` changes the diet label and compares the resulting predicted weights. `by` requests a row for each week.

```
calf_gaps <- avg_comparisons(
  calf_fit,
  variables = "diet",
  by = "time_factor",
  re.form = NA
)
calf_gaps
```

Positive means **Enhanced minus Control** in kg. The comparison gives each of the four study rooms equal weight. `re.form = NA` sets each calf's fitted weight difference to zero.

ASK FOR THE WEEK-8 COMPARISON DIRECTLY

```
 time_factor Estimate Std. Error      z Pr(>|z|)   S  2.5 % 97.5 %
           0    0.025       1.99 0.0126    0.990 0.0 -3.872   3.92
           2    0.471       1.99 0.2367    0.813 0.3 -3.427   4.37
           4    3.245       1.99 1.6320    0.103 3.3 -0.652   7.14
           6    2.626       1.99 1.3206    0.187 2.4 -1.271   6.52
           8    3.222       1.99 1.6204    0.105 3.2 -0.675   7.12

Term: diet
Type: response
Comparison: Enhanced - Control
```

CHECK BEFORE TRUSTING THE COMPARISON

```
check_convergence(calf_fit)
```

```
[1] TRUE
attr(,"gradient")
[1] 2.904132e-09
```

```
check_singularity(calf_fit)
```

```
[1] FALSE
```

First result: did the fitting calculation finish successfully?

Second result: did the estimated between-calf variation collapse to zero? Here `FALSE` means it did not.

A successful calculation cannot tell us whether nearby weights share additional influences. We return to that issue in Class 11.

A DIFFERENT STUDY: EVERY COW RECEIVES BOTH TREATMENTS

A researcher compares two rations, **A and B**, using daily feed intake.

In a **crossover study**, each cow receives A in one treatment period and B in another.

The same cow provides the two measurements. This may help when cows differ greatly in appetite.

It works only if a cow can return to a suitable starting condition between treatments.

RANDOMISE THE ORDER, NOT JUST THE FIRST RATION

Assigned order, called the sequence	First treatment period	Second treatment period
AB: 12 cows	A	B
BA: 12 cows	B	A

Randomly assign **24 cows** to these two orders.

A **period** is a scheduled treatment phase. Both treatments appear in both periods, so a general change over time can be separated from the treatment comparison.

PLAN THE GAP BETWEEN TREATMENTS

This synthetic example records average intake over the final three days of each ten-day treatment period. A ten-day treatment-free gap is specified between periods.

A **washout** is time for the earlier treatment's influence to wear off. **Carryover** means that influence remains.

The stated ten days is a teaching assumption. A real study needs evidence about the particular treatment and outcome.

DECIDE WHETHER CROSSOVER IS SUITABLE

A ration may have a short-lived effect on intake. A vaccine may cause a lasting change. Growth and recovery may also be irreversible.

For each example, discuss: **could the first treatment change the cow's later outcome even after treatment stops?**

If so, extending the gap needs scientific justification; assigning separate animals to treatments may be more appropriate.

BEFORE THE BREAK: CHECK THE DESIGN TOGETHER

Our cow study has two periods and two assigned orders.

1. Why would giving **all cows A then B** prevent a fair treatment comparison?
2. What evidence would justify the treatment-free gap?
3. Why are the two observations from one cow related?

After the break, we fit a model that allows for period and cows' usual differences in appetite.

Break 13:40–13:55.