

Briefly, write up your strategy in designing your experiment.

- My aim was to design an experiment that could determine the optimum amount of copper units to add to two types of feed (wheat or maize) to optimise growth rate of pullets.
- Apart from the effects of the treatments (copper unit level and type of feed given), other sources of variation I had to consider were 1) difference in conditions across the 4 different brooders, 2) position of the chicks in each brooder as there are 4 tiers in every brooder
- To control for these sources of variation I: Used blocking by replicating the same experimental design in each brooder. In each brooder, I fed 1 nest a wheat diet and 1 nest maize on each tier, alternating left and right. I did not randomize this allocation, as I wanted to be sure each tier had both wheat and maize treatments. I chose 4x treatments of copper unit application: 0 units, 100 units, 200 units and 300 units. We were told that it was known that 150 units would increase pullet growth rate, so I wanted to compare doses higher and lower than 150 to look at growth rate trend, and compare this to a control (0 units). Each tier was assigned one treatment. To increase sample size I replicated this design in each of the 4 brooders. However, to account for difference in tiers, in each brooder a given copper treatment was completed on a different tier.
- Sources of variation I couldn't control for were genetic relatedness of pullets, original size and weight of pullets or effect of individual nest conditions (for example, condition of nest, competition for feed between the birds sharing the same nest and therefore which pullet ate more food).

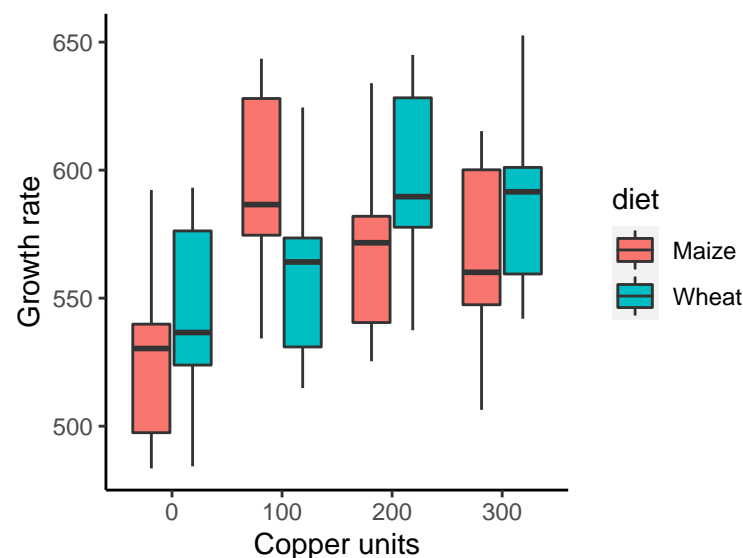


Figure 1: Box plots showing effect of quantity of copper units added to two different feed types (maize and wheat) on growth rate of pullets. Experiment created and run on vested Rshiny application (<https://stats-uoa.shinyapps.io/vested/>), virtual experiment 'Miracle grow for chooks'

Analyse your data

- It seemed necessary to include random effects of brooder into the model, as conditions may have varied between the four brooders, so I initially fitted a linear mixed-effect model (with an interaction between copper and diet) using brooder as a random effect. However coefficients of this model showed that the intercept was almost the same for every brooder, meaning there was little effect of brooder on the model and therefore I removed this term.
- I then fitted a basic linear model with an interaction between copper and diet. Diagnostic plots showed that assumptions were met.
- There was strong evidence that for both diets, all copper treatments increased growth rate in comparison to controls ($p < 0.001$). However, this relationship differed between diets: for the maize diet,

the 100 unit copper treatment increased growth the most, followed by the 200 and 300 copper unit treatments. However, for the wheat diet, overall increase in growth rate was less, and growth rate was highest for the 200 unit treatment.

- Anova showed that there was strong evidence against the null hypothesis that growth rate was not affected by diet, copper units or the interaction between diet and copper ($p = <0.01$). To investigate this further I used the predict means package to carry out multiple comparisons.
- Using the pm output, I found strong evidence of differences within all maize copper treatments and within all wheat copper treatments ($p = <0.001$). The predict means plot shows that there is an interaction between diet and copper.
- The highest growth rates were from Maize:100, wheat:200, wheat:300 treatments. However, there was no evidence for differences between these treatments in the predict means output ($p > 0.2$), and therefore I would recommend the farmer applying a Maize:100 treatment to reduce cost of copper for the same increase in growth rate (assuming wheat and maize are the same price).
- Future improvements to this model could include fitting the model with nest as a random effect.

```
library(readr) # Call packages needed for analysis
library(ggplot2)
library(tidyverse)
library(predictmeans)
library(lme4)
# Read in data from github
data<-read.csv("https://raw.githubusercontent.com/menzian/BIOSCI_738_Labs/main/Labs/chick_data-2022-04-04.csv")
data_1 <- data %>%
  dplyr::select(c("copper", "growth", "diet"))%>%
  group_by(diet) #grouping relevant data
data_1$copper <- as.factor(data_1$copper) #Changing variables to factors for analysis
data_1$diet <- as.factor(data_1$diet)

##Creating box plot showing data
ggplot(data_1, aes(x=copper, y=growth, fill=diet)) +
  geom_boxplot() + ylab("Growth rate") + xlab("Copper units") +
  theme(panel.border = element_blank(), panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        axis.line = element_line(colour = "black"), panel.background = element_blank())

##Analysis
data_pm <- data %>%
  mutate(copper = as.factor(copper)) %>%
  mutate(diet = as.factor(diet)) #making copper a factor so that I can use predict means later
##Creating linear model with interaction between copper and diet, and adding random effect of block ID
lmerTest_mod <- lmerTest::lmer(growth ~copper*diet + (1|brooder), data = data_pm)
coefficients(lmerTest_mod) #extracting coefficients from lm
##Creating linear model with interaction between copper and diet
lm_1 <- lm(growth ~ copper*diet, data = data_pm)
gglm::gglm(lm_1) # checking model assumptions
summary(lm_1)$coef # extracting coefficients from lm
anova(lm_1) #anova
##Multiple comparisons using predict means
pm <- predictmeans(lm_1, modelterm = "copper:diet", pairwise = TRUE, plot = TRUE)
plot <- pm$predictmeansPlot
plot
##Using Charlotte's function to extract coefficients of interest
url <- "https://gist.github.com/cmjt/72f3941533a6bdad0701928cc2924b90"
devtools::source_gist(url, quiet = TRUE)
comparisons(pm)
```