

Tooth Growth in Guinea Pigs from Vitamin C Supplements

Introduction

The ToothGrowth data set considers the effects of Vitamin C on tooth length in guinea pigs administered through two different supplements. The supplements are administered to 10 subjects in three different doses (0.5 mg, 1 mg and 2 mg) for a total of 60 observations.

Examination of the help file for the data set reveals that the observations come from a study by C. I. Bliss published in 1952 called *The Statistics of Bioassay*. Unfortunately this document is not available online for free for further examination. Instead, we use `?ToothGrowth` for information.

The help file provides very little information. We have no information about control groups for example. We do not know the expected tooth length in guinea pigs who did not receive supplements.

Now, we'll perform our own exploratory analysis of the data, to address the following questions:

- What is the average tooth length observed in the subjects for each dosage? How are the averages different for each supplement?
- Was there enough data collected to believe that the mean observations are statistically significant at the 95% confidence level?
- Is there evidence to support the conclusion that Vitamin C delivered through Orange Juice has the same impact as Vitamin C delivered in the form of Ascorbic Acid?

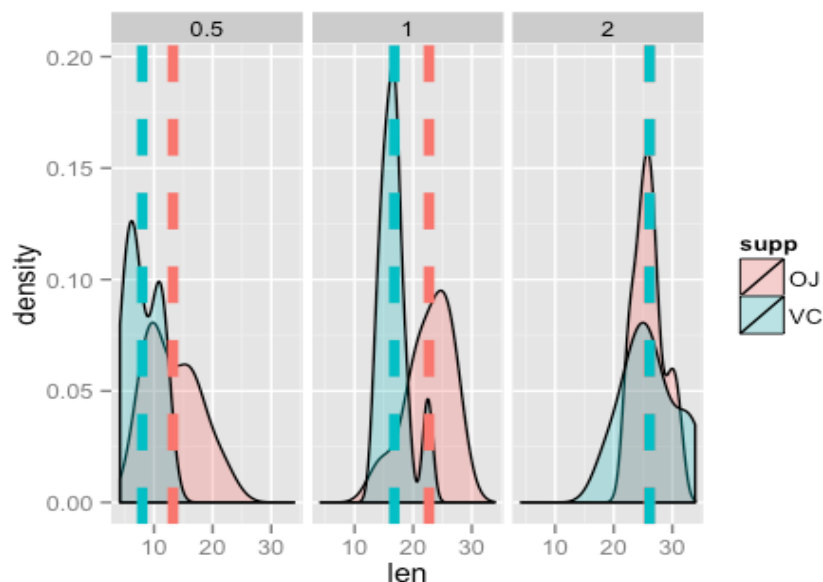
Exploratory Analysis

To get a sense of how the dosage level and supplement type interact, we plot the density of the sample means on an overlapped graph where the distributions and means can be easily understood visually.

```
# gather summary statistics on tooth length as it varies by dosage and supplement type
means <- ddply(ToothGrowth, c("supp", "dose"), summarize, sample.mean = mean(len), sample
.sd = sd(len), sample.var = var(len))
means

##   supp dose sample.mean sample.sd sample.var
## 1   OJ  0.5      13.23      4.460      19.889
## 2   OJ  1.0      22.70      3.911      15.296
## 3   OJ  2.0      26.06      2.655       7.049
## 4   VC  0.5       7.98      2.747       7.544
## 5   VC  1.0      16.77      2.515       6.327
## 6   VC  2.0      26.14      4.798      23.018

# how are the samples distributed and are the means correlated?
ggplot(ToothGrowth, aes(x = len, fill = supp)) + geom_density(alpha = 0.3) +
  geom_vline(data = means, aes(xintercept=sample.mean, color = supp),
    linetype = "dashed", size = 2) + facet_grid(~ dose)
```



Summary of Data Set

Analysis shows that the differences in the sample means of the tooth lengths in guinea pigs appears to trend toward zero as the dosage administered increases. A simple visual analysis suggests that at the **2 mg** dosage, Vitamin C has the same effect on tooth length whether delivered through Orange Juice or Ascorbic Acid.

what are the differences in the average tooth length?

```
deltas <- ddply(means, c("dose"), summarize, OJ.vs.VC = paste(abs(round(diff(sample.mean), 3)), "mg difference in sample means"))
deltas
```

```
##   dose      OJ.vs.VC
## 1  0.5 5.25 mg difference in sample means
## 2  1.0 5.93 mg difference in sample means
## 3  2.0 0.08 mg difference in sample means
```

However, we must demonstrate that the data collected is statistically significant enough to support this hypothesis. For that, we turn to our confidence tests.

Confidence Tests

The goal is to determine whether or not the data shows strong evidence that the method of delivery of Vitamin C, either through Orange Juice or Ascorbic Acid, makes no difference to the effects on tooth growth in guinea pigs. First, let's review our assumptions:

- **The 60 observations are drawn from 6 groups of 10 guinea pigs.** Since we are talking about tooth length, we must presume that in each of the experiments, guinea pigs were chosen at random and at birth and then observed over time as they matured. Because the groups under observation are all of the same size, in our significance test we can assume that the *pooled variance estimate* is the *weighted average of the group variances*.
- **Our sample sizes are small, 10 subjects each, and the variance of the population is unknown.** Therefore, we will use a *two-tailed test of the population mean* using the sample standard deviation and sample mean to estimate the population.

- **Different doses of Vitamin C were not given to the same guinea pigs.** Again, since different experiments on tooth growth cannot be observed in the same subjects, we will use the method of *independent (unpaired) sample t-tests* for our analysis.
- **We will ignore the obvious confounder in the study, namely, that Vitamin C may have no significant impact on tooth length at all.** Since we don't have any data from a control group where no Vitamin C was administered, we can't really say whether or not the substance plays any meaningful role in the dental development of these rodents.

Stated more formally:

$$H_0 = \mu_{OJ_2mg} - \mu_{VC_2mg} = 0$$

$$H_a = \mu_{OJ_2mg} - \mu_{VC_2mg} \neq 0$$

$$\alpha = 0.05$$

Null Hypothesis, confidence

```
H0 <- 0
```

```
significance.level <- 1 - 0.95
```

Isolate the subjects given supplements at the 2 mg dosage level

```
X.OJ <- ToothGrowth %>% filter(supp == "OJ" & dose == 2) %>% select(len) %>% .[[1]]
```

```
X.VC <- ToothGrowth %>% filter(supp == "VC" & dose == 2) %>% select(len) %>% .[[1]]
```

Sample means

```
MU.OJ <- mean(X.OJ)
```

```
MU.VC <- mean(X.VC)
```

```
N.OJ <- length(X.OJ)
```

```
N.VC <- length(X.VC)
```

sample standard deviations

```
SD.OJ <- sd(X.OJ)
```

```
SD.VC <- sd(X.VC)
```

Pooled standard error

```
SE <- sqrt((SD.OJ^2/N.OJ) + (SD.VC^2/N.VC))
```

Degrees of freedom

```
DF <- SE^2 / ((SD.OJ^2/N.OJ)^2/(N.OJ-1) + (SD.VC^2/N.VC)^2/(N.VC-1))
```

T statistic, manually computed

```
T <- ((MU.OJ - MU.VC) - H0)/SE
```

two-tailed t-test, assuming equal variance

```
T.test <- t.test(X.OJ, X.VC, conf.level = 1 - significance.level, alternative = "two.sided", var.equal = FALSE)
```

First, check our manually computed *t-statistic* versus the easy one we get from **R**. If they are the same, then their differences will be zero (rounded at 10 digits).

Correctly computed t statistic? (rounded to 10 digits)

```
round(T - T.test$statistic, 10) == 0
```

```
##      t
```

```
## TRUE
```

Now, we check our computed probability score. We need a *p-value* of less than 0.05 (95% confidence) in order to reject the null hypothesis that the means should be zero.

```
# Reject the null hypothesis?
T.test$p.value < significance.level

## [1] FALSE
```

Our computed *p-value*, 0.9639 is not less than the significance level 0.05. Therefore, we fail to reject the null hypothesis H_0 as expected.

Conclusions

Summary of Analysis

By plotting the densities from the outside, we had an intuitive notion that the differences in the sample means between the groups of guinea pigs would trend towards zero as the dosage increased. We isolated two small independent groups of guinea pigs, who each received the same dosage of Vitamin C but through two different supplements.

We set our null hypothesis to be that the differences in the population means between the two groups would be zero. We computed the sample means and used that to estimate the population means. We further computed our sample means and calculated a weighted pooled variance based on the assumption of unequal variance between the samples.

Finally, we computed our probability score using a *t-test* at the 95% confidence level, finding a *p-value* that was greater than the expected significance level. Therefore, we were unable to reject the null hypothesis.

This matches our observed intuition from the sample densities in the exploratory analysis. We do not try to conclude whether the Vitamin C impacts tooth length. Instead, we only conclude that the *form of the supplement* makes no difference on the effectiveness of Vitamin C, if that substance has any effect at all.

Multiple comparisons and other considerations

We noted in the assumptions that because we have no control groups among these guinea pigs, it's quite possible that Vitamin C has no effect at all on tooth length, and that the differences observed at various dosage level could be the product of random chance.

To see intuitively what is meant by **the problem of multiple comparisons**, check out this story on Wired [Scanning Dead Salmon in fMRI Machine Highlights Risk of Red Herrings](#).

The problem of multiple comparisons is probably relevant in the case of **pigs who drink orange juice** but from the point of view of this study, beyond the scope of what we can analyze with this limited data set.

Note:

The code presented above relied on several installed packages, the list given below. If you are knitting this document yourself from the original source, use `load("statinference-006-pt2.RData")` to load the environment into **R**.

```
# show required packages
required.packages

## [1] "dplyr" "ggplot2" "plyr"
```