RCT Ketosis

Prelim analysis

10/28/2021

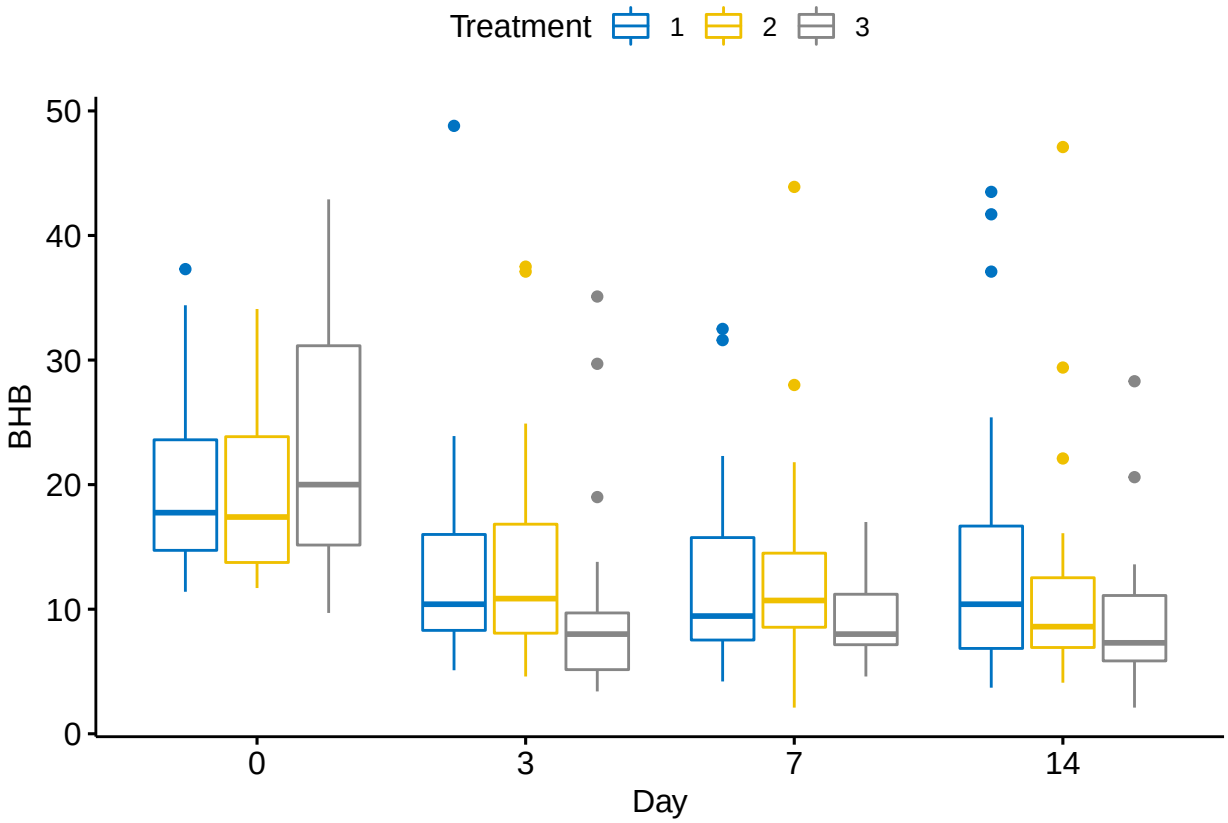
Analysis for animals under treatments 1,2, 3.

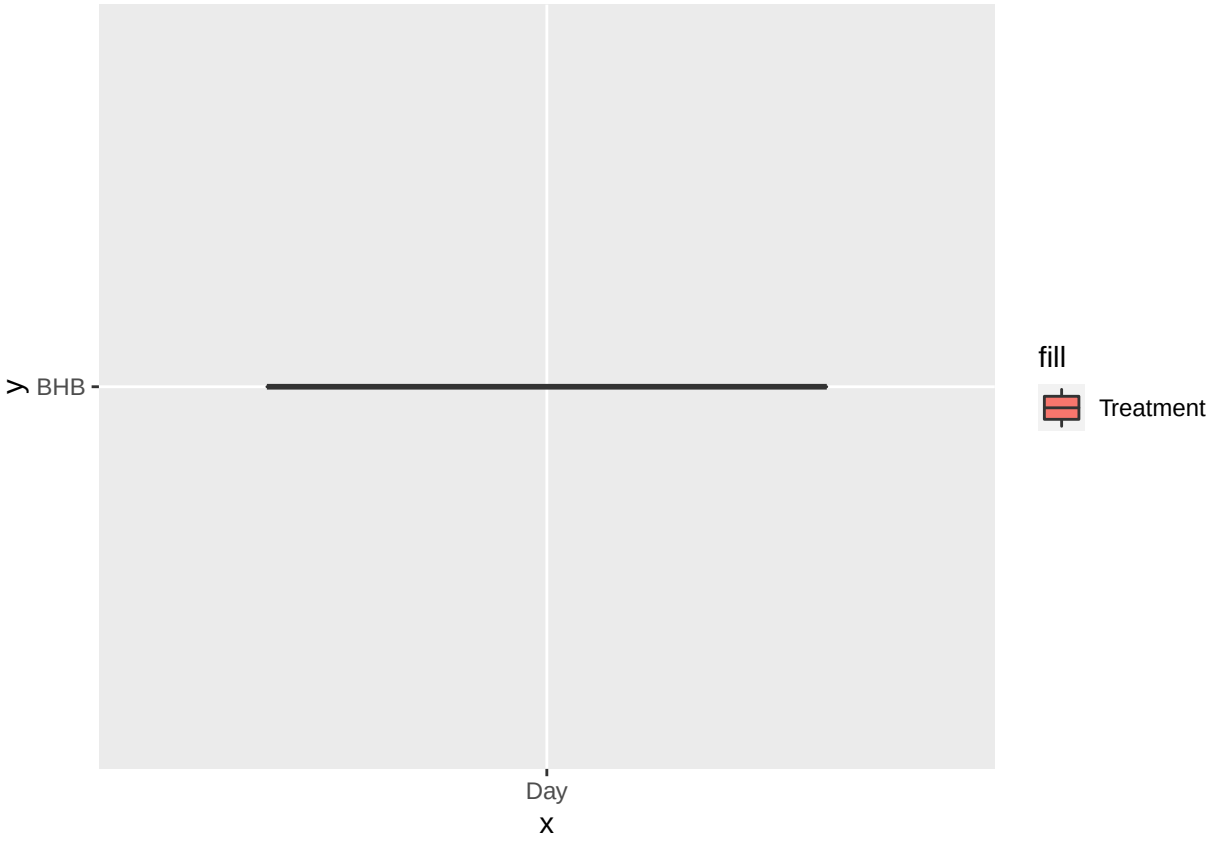
The graph shows the distribution of BHB for subjects under treatments 1,2, 3 or Control.

For this analysis animals labeled as controls were removed because they were not truly controls of the treatments. This analysis is focused on animal under treatment 1, 2, 3. First, we tested if the interaction Day*Treatment is significant. The model shows that interaction is not significant.

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: BHB ~ Treatment * Day + (1 | Cow)
##    Data: ketosis2
##
## REML criterion at convergence: 1510
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.8060 -0.5215 -0.1648  0.3433  3.4240
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  Cow      (Intercept) 27.76    5.269
##  Residual             53.08    7.285
## Number of obs: 220, groups:  Cow, 55
##
## Fixed effects:
##                  Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)       20.2833     2.1192 153.6413   9.571  < 2e-16 ***
## Treatment2        -0.7444     2.9970 153.6413  -0.248  0.80416
## Treatment3         2.6693     2.9573 153.6413   0.903  0.36815
## Day3              -6.3167     2.4285 156.0000  -2.601  0.01019 *
## Day7              -7.0333     2.4285 156.0000  -2.896  0.00432 **
## Day14             -4.9222     2.4285 156.0000  -2.027  0.04438 *
## Treatment2:Day3    1.3167     3.4344 156.0000   0.383  0.70196
## Treatment3:Day3   -6.3675     3.3889 156.0000  -1.879  0.06212 .
## Treatment2:Day7    1.1000     3.4344 156.0000   0.320  0.74918
## Treatment3:Day7   -6.7246     3.3889 156.0000  -1.984  0.04898 *
## Treatment2:Day14  -1.8444     3.4344 156.0000  -0.537  0.59200
## Treatment3:Day14  -8.5251     3.3889 156.0000  -2.516  0.01290 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```
## Correlation of Fixed Effects:
##             (Intr) Trtmn2 Trtmn3 Day3   Day7   Day14  Tr2:D3 Tr3:D3 Tr2:D7
## Treatment2  -0.707
## Treatment3  -0.717  0.507
## Day3        -0.573  0.405  0.411
## Day7        -0.573  0.405  0.411  0.500
## Day14       -0.573  0.405  0.411  0.500  0.500
## Trtmnt2:Dy3  0.405 -0.573 -0.290 -0.707 -0.354 -0.354
## Trtmnt3:Dy3  0.411 -0.290 -0.573 -0.717 -0.358 -0.358  0.507
## Trtmnt2:Dy7  0.405 -0.573 -0.290 -0.354 -0.707 -0.354  0.500  0.253
## Trtmnt3:Dy7  0.411 -0.290 -0.573 -0.358 -0.717 -0.358  0.253  0.500  0.507
## Trtmnt2:D14  0.405 -0.573 -0.290 -0.354 -0.354 -0.707  0.500  0.253  0.500
## Trtmnt3:D14  0.411 -0.290 -0.573 -0.358 -0.358 -0.717  0.253  0.500  0.253
##             Tr3:D7 T2:D14
## Treatment2
## Treatment3
## Day3
## Day7
## Day14
## Trtmnt2:Dy3
## Trtmnt3:Dy3
## Trtmnt2:Dy7
## Trtmnt3:Dy7
## Trtmnt2:D14  0.253
## Trtmnt3:D14  0.500  0.507

## Type III Analysis of Variance Table with Satterthwaite's method
##                Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Treatment       99.55   49.77     2    52  0.9377    0.3980
## Day           2951.74  983.91     3   156 18.5370 2.453e-10 ***
## Treatment:Day  555.66   92.61     6   156  1.7448    0.1141
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```


Treatment
1
2
3
BHB
50
40
30
20
10
0
0
3
7
14
Day

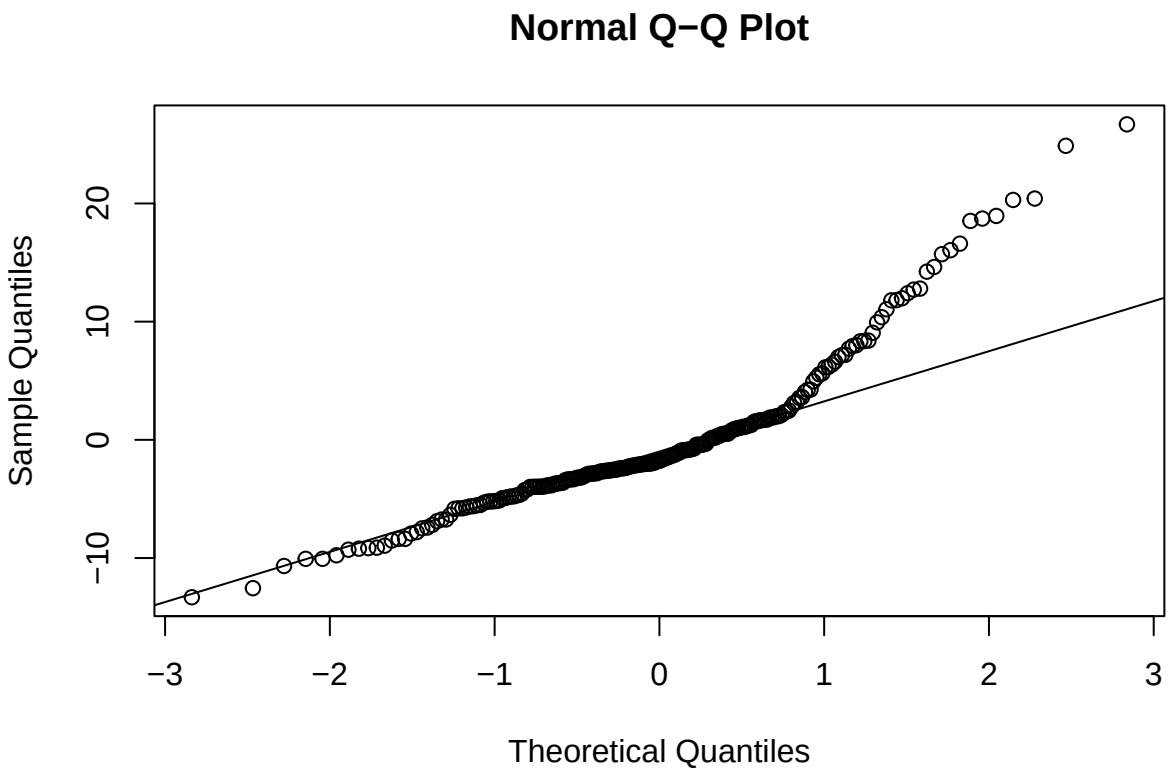


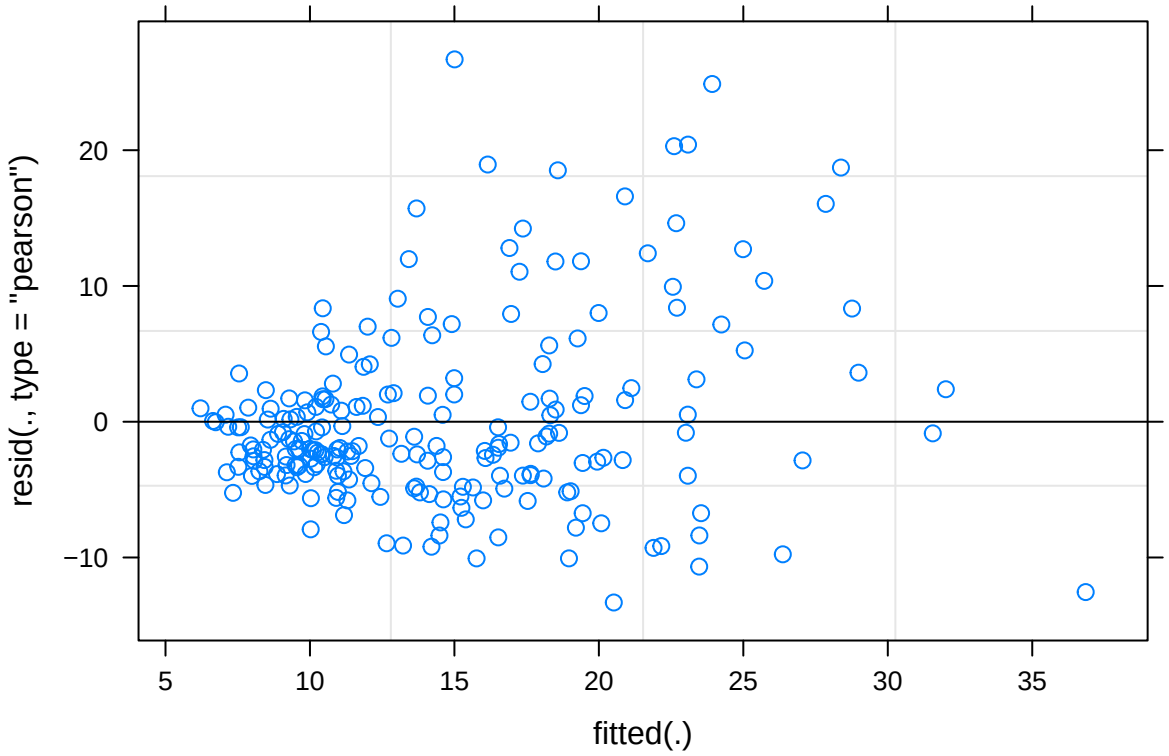

```
## # A tibble: 19 x 6
##    Day   Treatment Cow     BHB is.outlier is.extreme
##    <fct> <fct>     <fct> <dbl> <lgl>      <lgl>
##  1 0     1         38     37.3 TRUE       FALSE
##  2 3     1         51     48.8 TRUE       TRUE
##  3 7     1         6      32.5 TRUE       FALSE
##  4 7     1         13     31.6 TRUE       FALSE
##  5 14    1         3      41.7 TRUE       FALSE
##  6 14    1         6      43.5 TRUE       FALSE
##  7 14    1         9      37.1 TRUE       FALSE
##  8 3     2         40     37.1 TRUE       FALSE
##  9 3     2         69     37.5 TRUE       FALSE
## 10 7     2         40     43.9 TRUE       TRUE
## 11 7     2         69     28   TRUE       FALSE
## 12 14    2         40     47.1 TRUE       TRUE
## 13 14    2         52     22.1 TRUE       FALSE
## 14 14    2         58     29.4 TRUE       TRUE
## 15 3     3         4      29.7 TRUE       TRUE
## 16 3     3         43     19   TRUE       FALSE
## 17 3     3         48     35.1 TRUE       TRUE
## 18 14    3         15     28.3 TRUE       TRUE
## 19 14    3         21     20.6 TRUE       FALSE
```

This are the results for the model without interaction.

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
```

```
## lmerModLmerTest]
## Formula: BHB ~ Treatment + Day + (1 | Cow)
##    Data: ketosis2
##
## REML criterion at convergence: 1543.9
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.8031 -0.5217 -0.2390  0.2535  3.6142
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  Cow      (Intercept) 27.40    5.234
##  Residual             54.54    7.385
## Number of obs: 220, groups:  Cow, 55
##
## Fixed effects:
##             Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)  22.1035     1.7388  88.4466  12.712  < 2e-16 ***
## Treatment2   -0.6014     2.1352  52.0000  -0.282    0.779
## Treatment3   -2.7350     2.1069  52.0000  -1.298    0.200
## Day3         -8.0855     1.4083 162.0000  -5.741 4.52e-08 ***
## Day7         -8.9964     1.4083 162.0000  -6.388 1.71e-09 ***
## Day14        -8.4709     1.4083 162.0000  -6.015 1.16e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            (Intr) Trtmn2 Trtmn3 Day3   Day7
## Treatment2 -0.614
## Treatment3 -0.622  0.507
## Day3       -0.405  0.000  0.000
## Day7       -0.405  0.000  0.000  0.500
## Day14      -0.405  0.000  0.000  0.500  0.500

## Type III Analysis of Variance Table with Satterthwaite's method
##            Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Treatment  102.29   51.15     2    52  0.9377     0.398
## Day       3015.65 1005.22     3   162 18.4299 2.452e-10 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```


Normal Q−Q Plot
Sample Quantiles
Theoretical Quantiles
−10
0
10
20
−3
−2
−1
0
1
2
3



Testing Interactions for the model with subjects under treatments 1,2, 3 or Control.

When the interaction between treatment and day is included in a mixed model that account for the repeated measure, we can see that there is not a statistically significant interactions between treatment and day. This means that effect of the treatments is consistent over time.

```
library(readxl)
library(lme4)
library(lmerTest)
library(rstatix)
library(tidyverse)
library(ggpubr)
# Testing interaction between treatment and day.
lme <- lmer(BHB ~   Treatment*Day + (1|Cow), data=ketosis2)
summary(lme)
```

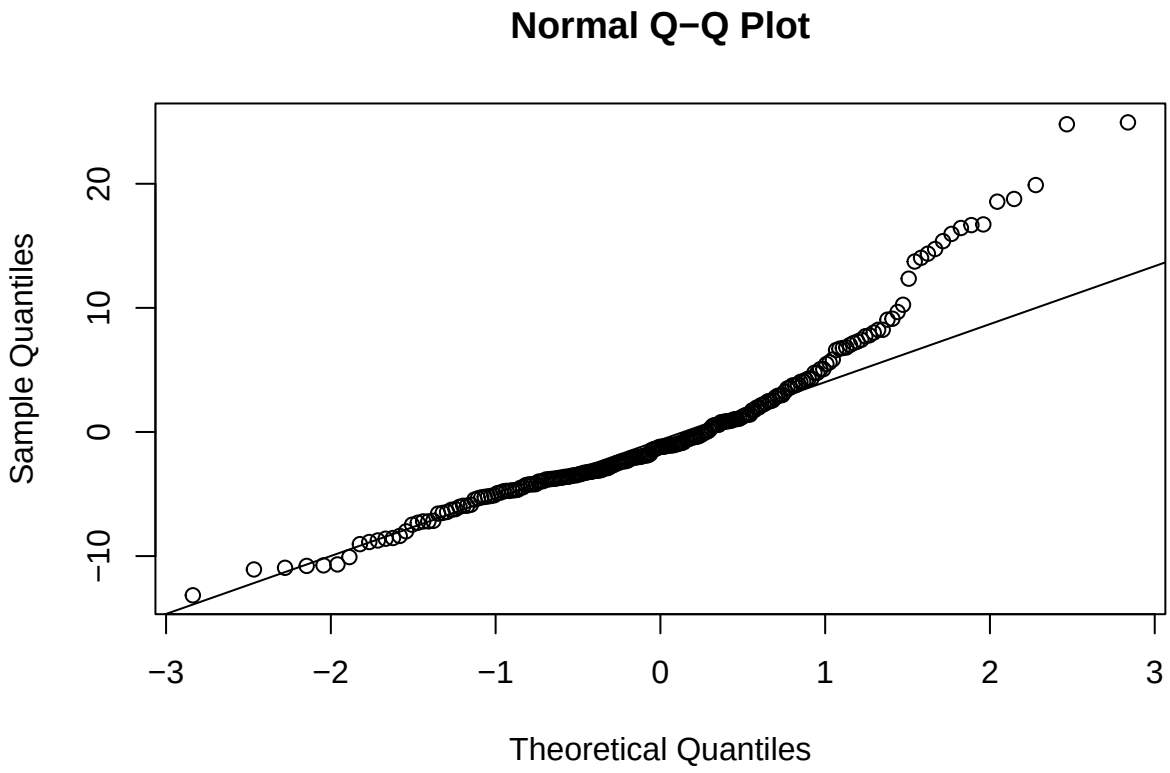
```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: BHB ~ Treatment * Day + (1 | Cow)
##    Data: ketosis2
##
## REML criterion at convergence: 1510
##
```

```
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.8060 -0.5215 -0.1648  0.3433  3.4240
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  Cow      (Intercept) 27.76    5.269
##  Residual             53.08    7.285
## Number of obs: 220, groups:  Cow, 55
##
## Fixed effects:
##                   Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)       20.2833     2.1192 153.6413   9.571  < 2e-16 ***
## Treatment2        -0.7444     2.9970 153.6413  -0.248  0.80416
## Treatment3         2.6693     2.9573 153.6413   0.903  0.36815
## Day3              -6.3167     2.4285 156.0000  -2.601  0.01019 *
## Day7              -7.0333     2.4285 156.0000  -2.896  0.00432 **
## Day14             -4.9222     2.4285 156.0000  -2.027  0.04438 *
## Treatment2:Day3    1.3167     3.4344 156.0000   0.383  0.70196
## Treatment3:Day3   -6.3675     3.3889 156.0000  -1.879  0.06212 .
## Treatment2:Day7    1.1000     3.4344 156.0000   0.320  0.74918
## Treatment3:Day7   -6.7246     3.3889 156.0000  -1.984  0.04898 *
## Treatment2:Day14  -1.8444     3.4344 156.0000  -0.537  0.59200
## Treatment3:Day14  -8.5251     3.3889 156.0000  -2.516  0.01290 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) Trtmn2 Trtmn3 Day3   Day7   Day14  Tr2:D3 Tr3:D3 Tr2:D7
## Treatment2  -0.707
## Treatment3  -0.717  0.507
## Day3        -0.573  0.405  0.411
## Day7        -0.573  0.405  0.411  0.500
## Day14       -0.573  0.405  0.411  0.500  0.500
## Trtmnt2:Dy3  0.405 -0.573 -0.290 -0.707 -0.354 -0.354
## Trtmnt3:Dy3  0.411 -0.290 -0.573 -0.717 -0.358 -0.358  0.507
## Trtmnt2:Dy7  0.405 -0.573 -0.290 -0.354 -0.707 -0.354  0.500  0.253
## Trtmnt3:Dy7  0.411 -0.290 -0.573 -0.358 -0.717 -0.358  0.253  0.500  0.507
## Trtmnt2:D14  0.405 -0.573 -0.290 -0.354 -0.354 -0.707  0.500  0.253  0.500
## Trtmnt3:D14  0.411 -0.290 -0.573 -0.358 -0.358 -0.717  0.253  0.500  0.253
##             Tr3:D7 T2:D14
## Treatment2
## Treatment3
## Day3
## Day7
## Day14
## Trtmnt2:Dy3
## Trtmnt3:Dy3
## Trtmnt2:Dy7
## Trtmnt3:Dy7
## Trtmnt2:D14  0.253
## Trtmnt3:D14  0.500  0.507
```

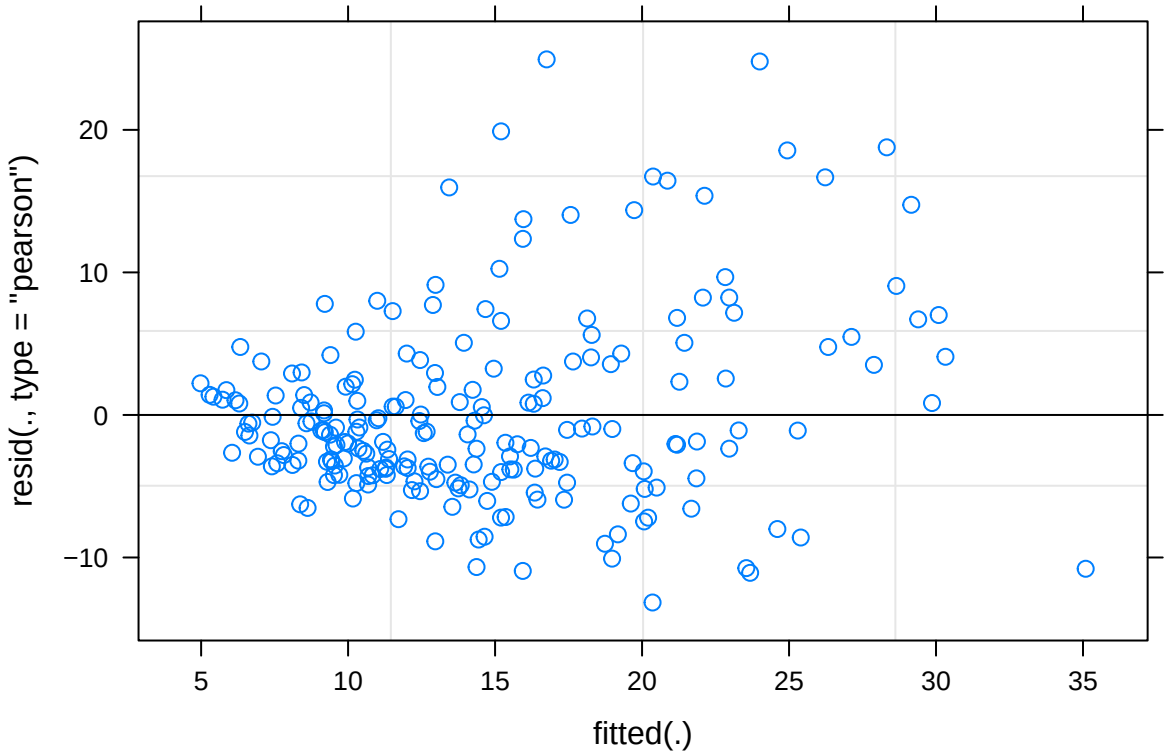
```r
anova(lme)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Treatment       99.55   49.77     2    52  0.9377    0.3980
## Day           2951.74  983.91     3   156 18.5370 2.453e-10 ***
## Treatment:Day  555.66   92.61     6   156  1.7448    0.1141
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

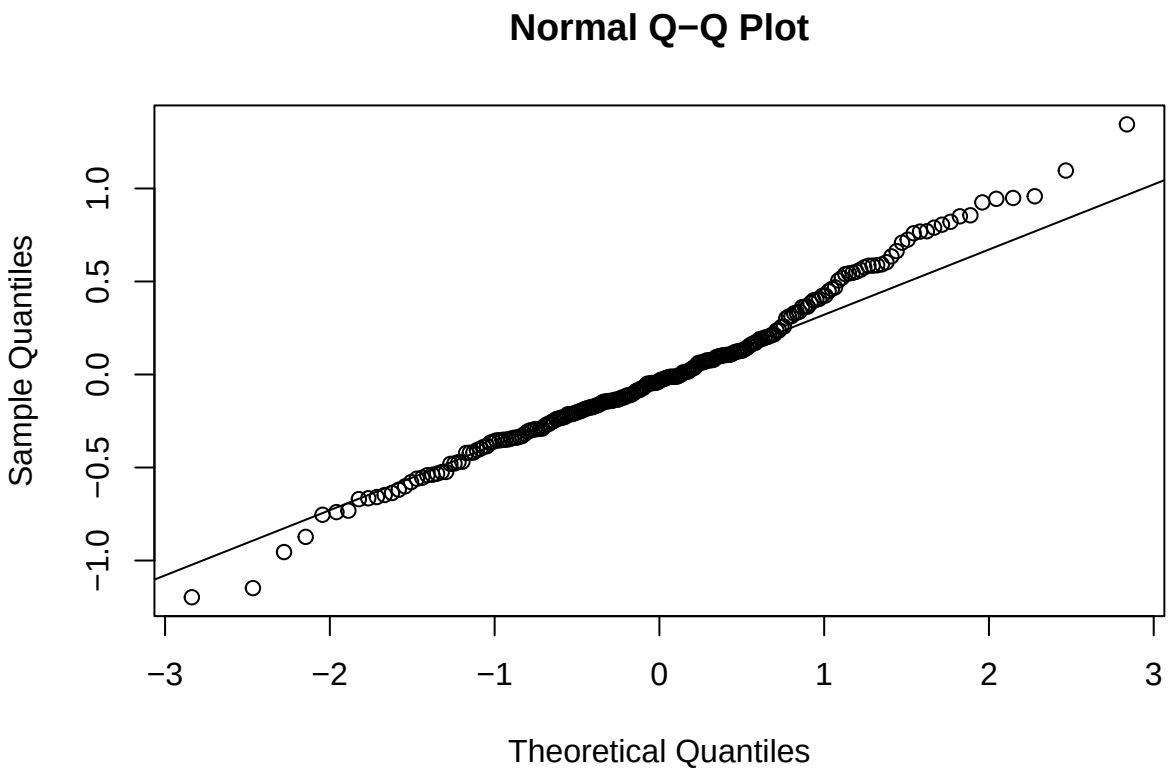
```r
lmeLog <- lmer(log(BHB) ~   Treatment + Day + (1|Cow), data=ketosis2)

qqnorm(resid(lme))
qqline(resid(lme))
```



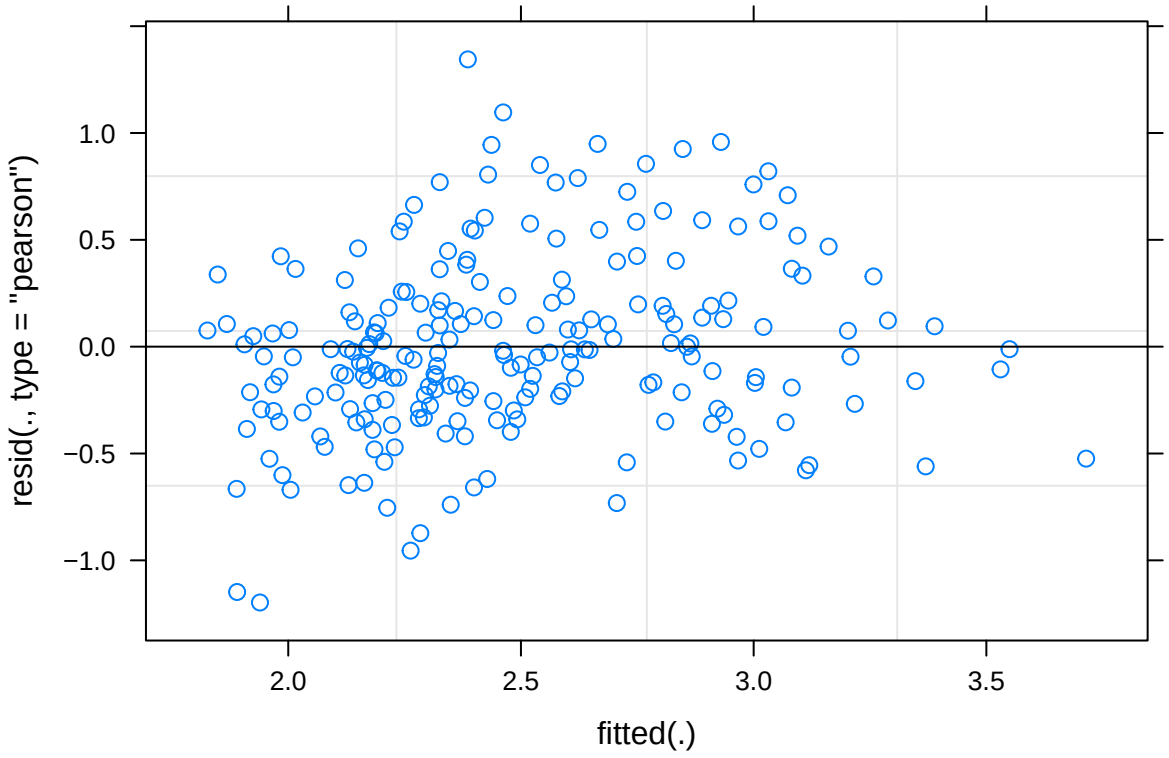

```r
plot(lme)
```




```
qqnorm(resid(lmeLog))
qqline(resid(lmeLog))
```


Normal Q−Q Plot
Sample Quantiles
−1.0
−0.5
0.0
0.5
1.0
−3
−2
−1
0
1
2
3
Theoretical Quantiles

```
plot(lmeLog)
```




```
summary(lmeLog)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: log(BHB) ~ Treatment + Day + (1 | Cow)
##    Data: ketosis2
##
## REML criterion at convergence: 353.4
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.59515 -0.57471 -0.07406  0.44946  2.91449
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  Cow      (Intercept) 0.09612  0.3100
##  Residual             0.21285  0.4614
## Number of obs: 220, groups:  Cow, 55
##
## Fixed effects:
##              Estimate Std. Error        df t value Pr(>|t|)
## (Intercept)   3.05652    0.10582  91.16909  28.883  < 2e-16 ***
## Treatment2   -0.03075    0.12881  51.99999  -0.239   0.8123
## Treatment3   -0.22178    0.12710  51.99999  -1.745   0.0869 .
## Day3         -0.62015    0.08798 162.00001  -7.049 4.92e-11 ***
```

```
## Day7         -0.64120    0.08798 162.00001  -7.288 1.31e-11 ***
## Day14        -0.68283    0.08798 162.00001  -7.761 8.94e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            (Intr) Trtmn2 Trtmn3 Day3   Day7
## Treatment2 -0.609
## Treatment3 -0.617  0.507
## Day3       -0.416  0.000  0.000
## Day7       -0.416  0.000  0.000  0.500
## Day14      -0.416  0.000  0.000  0.500  0.500
```

```
anova(lmeLog)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##            Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Treatment  0.7673  0.3837     2    52  1.8024     0.175
## Day       17.4361  5.8120     3   162 27.3055 2.405e-14 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Average differences in treatments for animals under treatments 1,2, 3 or Control.

The BHB was statistically significantly different at the different treatments (1,2,3, control), $p < 0.05$.

It can be seen that Treatment 1 is significantly associated with an average increase of 7.641 in BHB compared to Controls.

It can be seen that Treatment 2 is significantly associated with an average increase of 7.040 in BHB compared to Controls.

It can be seen that Treatment 3 is significantly associated with an average increase of 4.906 in BHB compared to Controls.

```
library(readxl)
library(rstatix)
library(tidyverse)
library(lmerTest)
library(lme4)
library(ggpubr)
# Testing interaction between treatment and day.
lme <- lmer(BHB ~   Treatment + (1|Cow), data=ketosis2)
summary(lme)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: BHB ~ Treatment + (1 | Cow)
##    Data: ketosis2
##
## REML criterion at convergence: 1599.2
##
## Scaled residuals:
```

```
##     Min      1Q  Median      3Q     Max
## -1.6777 -0.6402 -0.3059  0.5078  3.2088
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  Cow      (Intercept) 23.07    4.804
##  Residual             71.83    8.475
## Number of obs: 220, groups:  Cow, 55
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)  15.7153     1.5098 52.0000  10.409 2.56e-14 ***
## Treatment2   -0.6014     2.1352 52.0000  -0.282    0.779
## Treatment3   -2.7350     2.1069 52.0000  -1.298    0.200
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            (Intr) Trtmn2
## Treatment2 -0.707
## Treatment3 -0.717  0.507
```

```
anova(lme)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##           Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Treatment 134.71  67.354     2    52  0.9377  0.398
```

Average differences in days for animals under treatments 1,2, 3 or Control.

The BHB was statistically significantly different at the different days (0, 3, 7, 14), $p < 0.05$.

It can be seen that Day 3 is significantly associated with an average decrease of 6.753 in BHB compared to Day 0.

It can be seen that Day 7 is significantly associated with an average decrease of 7.522 in BHB compared to Day 0.

It can be seen that Day 14 is significantly associated with an average decrease of 7.164 in BHB compared to Day 0.

```
library(readxl)
library(rstatix)
library(tidyverse)
library(lmerTest)
library(lme4)
library(ggpubr)
# Testing interaction between treatment and day.
lme <- lmer(BHB ~   Day + (1|Cow), data=ketosis2)
summary(lme)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
```

```
## Formula: BHB ~ Day + (1 | Cow)
##    Data: ketosis2
##
## REML criterion at convergence: 1552.2
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.7776 -0.5137 -0.2206  0.2560  3.6659
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  Cow      (Intercept) 27.30    5.225
##  Residual             54.54    7.385
## Number of obs: 220, groups:  Cow, 55
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)   20.962      1.220 161.941  17.184  < 2e-16 ***
## Day3          -8.085      1.408 162.000  -5.741 4.52e-08 ***
## Day7          -8.996      1.408 162.000  -6.388 1.71e-09 ***
## Day14         -8.471      1.408 162.000  -6.015 1.16e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##       (Intr) Day3   Day7
## Day3  -0.577
## Day7  -0.577  0.500
## Day14 -0.577  0.500  0.500
```

```
anova(lme)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##     Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Day 3015.7  1005.2     3   162   18.43 2.452e-10 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

with adjustment to baseline (make sence if "controls" were included)

```
library(readxl)
library(lme4)
library(lmerTest)
library(rstatix)
library(tidyverse)
library(ggpubr)
# Testing interaction between treatment and day.
lme <- lmer(BHB ~   Day + Treatment:Day + (1|Cow), data=ketosis2)
summary(lme)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
```

```
## lmerModLmerTest]
## Formula: BHB ~ Day + Treatment:Day + (1 | Cow)
##    Data: ketosis2
##
## REML criterion at convergence: 1510
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.8060 -0.5215 -0.1648  0.3433  3.4240
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  Cow      (Intercept) 27.76    5.269
##  Residual             53.08    7.285
## Number of obs: 220, groups:  Cow, 55
##
## Fixed effects:
##                   Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)       20.2833     2.1192 153.6413   9.571  < 2e-16 ***
## Day3              -6.3167     2.4285 156.0000  -2.601  0.01019 *
## Day7              -7.0333     2.4285 156.0000  -2.896  0.00432 **
## Day14             -4.9222     2.4285 156.0000  -2.027  0.04438 *
## Day0:Treatment2   -0.7444     2.9970 153.6413  -0.248  0.80416
## Day3:Treatment2    0.5722     2.9970 153.6413   0.191  0.84883
## Day7:Treatment2    0.3556     2.9970 153.6413   0.119  0.90572
## Day14:Treatment2  -2.5889     2.9970 153.6413  -0.864  0.38904
## Day0:Treatment3    2.6693     2.9573 153.6413   0.903  0.36815
## Day3:Treatment3   -3.6982     2.9573 153.6413  -1.251  0.21301
## Day7:Treatment3   -4.0553     2.9573 153.6413  -1.371  0.17230
## Day14:Treatment3  -5.8558     2.9573 153.6413  -1.980  0.04948 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) Day3   Day7   Day14  Dy0:T2 Dy3:T2 Dy7:T2 D14:T2 Dy0:T3
## Day3        -0.573
## Day7        -0.573  0.500
## Day14       -0.573  0.500  0.500
## Dy0:Trtmnt2 -0.707  0.405  0.405  0.405
## Dy3:Trtmnt2 -0.243 -0.405  0.000  0.000  0.343
## Dy7:Trtmnt2 -0.243  0.000 -0.405  0.000  0.343  0.343
## Dy14:Trtmn2 -0.243  0.000  0.000 -0.405  0.343  0.343  0.343
## Dy0:Trtmnt3 -0.717  0.411  0.411  0.411  0.507  0.174  0.174  0.174
## Dy3:Trtmnt3 -0.246 -0.411  0.000  0.000  0.174  0.507  0.174  0.174  0.343
## Dy7:Trtmnt3 -0.246  0.000 -0.411  0.000  0.174  0.174  0.507  0.174  0.343
## Dy14:Trtmn3 -0.246  0.000  0.000 -0.411  0.174  0.174  0.174  0.507  0.343
##             Dy3:T3 Dy7:T3
## Day3
## Day7
## Day14
## Dy0:Trtmnt2
## Dy3:Trtmnt2
## Dy7:Trtmnt2
## Dy14:Trtmn2
```

```
## Dy0:Trtmnt3
## Dy3:Trtmnt3
## Dy7:Trtmnt3  0.343
## Dy14:Trtmn3  0.343  0.343
```

```
anova(lme)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##               Sum Sq Mean Sq NumDF  DenDF F value  Pr(>F)
## Day           542.30 180.767     3 156.00  3.4057 0.01919 *
## Day:Treatment 655.21  81.901     8 103.32  1.5430 0.15150
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```