RCT Ketosis

Prelim analysis

10/28/2021

## Initial analysis

The analysis was divided into two groups: A) One including only subjects under treatment 1,2 and 3. B) another one including subjects under treatment 1,2,3 or Control. Even though the control group had the same number of subjects as treatments 1,2,3, only 3 subjects were followed for all four points (Day 0, 2, 7, 14). The remaining 22 were evaluated only on day 0.

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

library(readxl)  
library(rstatix)

##   
## Attaching package: 'rstatix'

## The following object is masked from 'package:stats':  
##   
## filter

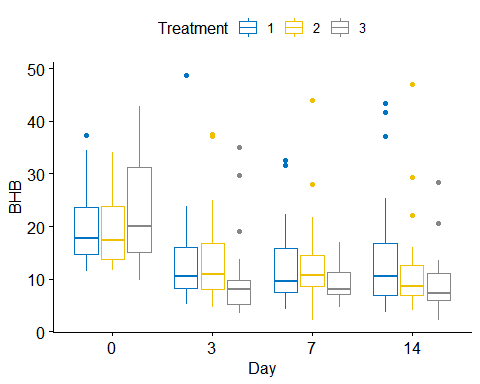
library(tidyverse)

## -- Attaching packages --------------------------------------- tidyverse 1.3.1 --

## v ggplot2 3.3.5 v purrr 0.3.4  
## v tibble 3.1.5 v dplyr 1.0.7  
## v tidyr 1.1.4 v stringr 1.4.0  
## v readr 2.0.2 v forcats 0.5.1

## -- Conflicts ------------------------------------------ tidyverse\_conflicts() --  
## x dplyr::filter() masks rstatix::filter(), stats::filter()  
## x dplyr::lag() masks stats::lag()

library(ggpubr)  
##Read dataset  
ketosis <- read\_excel("datasets/Ketosis\_full.xlsx")  
## crate nominal variables   
ketosis$Treatment <- as.factor(ketosis$Treatment)  
ketosis$Day <- as.factor(ketosis$Day)  
ketosis$Treatment <- as.factor(ketosis$Treatment)  
ketosis$Cow <- as.factor(ketosis$Cow)  
  
  
bxp <- ggboxplot(  
 ketosis, x = "Day", y = "BHB",  
 color = "Treatment", palette = "jco"  
)  
bxp



## Outliers

It is evident in the graph the presence of outliers that is confirmed by an statistical test. These can be due to: 1) data entry errors, measurement errors or unusual values.

library(readxl)  
library(rstatix)  
library(tidyverse)  
library(ggpubr)  
ketosis %>%  
 group\_by(Treatment, Day) %>%  
 identify\_outliers(BHB)

## # A tibble: 19 x 27  
## Day Treatment Cow `Cow ID` Albumin BHB BUN Calcium CHO Glucose  
## <fct> <fct> <fct> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>  
## 1 0 1 38 2486 3.4 37.3 9 8.4 46 35  
## 2 3 1 51 64500 3.2 48.8 8.6 6.2 53 23  
## 3 7 1 6 6158 4.3 32.5 9.9 9.4 70 44  
## 4 7 1 13 476 4 31.6 6.5 8.5 57 38  
## 5 14 1 3 3187 4.7 41.7 10.8 2.7 101 33  
## 6 14 1 6 6158 4.3 43.5 11.6 9.4 66 48  
## 7 14 1 9 713 4.3 37.1 7.8 9.8 118 35  
## 8 3 2 40 50493 4 37.1 12 8.1 49 38  
## 9 3 2 69 85435 4.1 37.5 9 9.7 88 38  
## 10 7 2 40 50493 4.9 43.9 9.8 11.2 49 38  
## 11 7 2 69 85435 4.2 28 13.5 8.9 105 44  
## 12 14 2 40 50493 4.6 47.1 6.7 10.4 53 48  
## 13 14 2 52 260 3.7 22.1 9.9 7.3 61 44  
## 14 14 2 58 599 4 29.4 8.7 8.5 114 50  
## 15 3 3 4 50324 4 29.7 5 9.6 27 45  
## 16 3 3 43 88132 3.9 19 10.7 8 58 46  
## 17 3 3 48 61080 3 35.1 10.6 6.9 62 64  
## 18 14 3 15 8342 4.7 28.3 15.9 8.8 63 44  
## 19 14 3 21 43867 4.1 20.6 4.9 8 79 46  
## # ... with 17 more variables: Magnesium <dbl>, NEFA <dbl>, TP <dbl>,  
## # Triglicerides <dbl>, Milk (lb) <chr>, Precision Xtra mmol/L day 0 <chr>,  
## # DIM <dbl>, Pen <dbl>, Breed <chr>, LACT <dbl>, DDRY <chr>, DOPN PREV <chr>,  
## # Attitude <chr>, Food intake <chr>, Rumination <chr>, is.outlier <lgl>,  
## # is.extreme <lgl>

## Testing Interactions in the analysis for subjects under treatments 1,2 or 3.

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)

## Loading required package: Matrix

##   
## Attaching package: 'Matrix'

## The following objects are masked from 'package:tidyr':  
##   
## expand, pack, unpack

library(lmerTest)

##   
## Attaching package: 'lmerTest'

## The following object is masked from 'package:lme4':  
##   
## lmer

## The following object is masked from 'package:stats':  
##   
## step

library(rstatix)  
library(tidyverse)  
library(ggpubr)  
# Testing interaction between treatment and day.  
lme <- lmer(BHB ~ Treatment\*Day + (1|Cow), data=ketosis)  
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

## Final model in the analysis for subjects under treatments 1,2 or 3.

Then, it is only included treatment and day in the mixed model. The BHB was statistically significantly different at the different time points (0,3,7,14 day), p < 0.0001. The BHB was not statistically significantly different at the different treatments (1,2,3), p > 0.05.

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

## 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

## Adjusting for baseline in the analysis for subjects under treatments 1,2 or 3.

Model adjusting for the baseline observation (day 0).

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

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [  
## lmerModLmerTest]  
## Formula: BHB ~ Treatment:Day + Day + (1 | Cow)  
## Data: ketosis  
##   
## 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 \*   
## Treatment2:Day0 -0.7444 2.9970 153.6413 -0.248 0.80416   
## Treatment3:Day0 2.6693 2.9573 153.6413 0.903 0.36815   
## Treatment2:Day3 0.5722 2.9970 153.6413 0.191 0.84883   
## Treatment3:Day3 -3.6982 2.9573 153.6413 -1.251 0.21301   
## Treatment2:Day7 0.3556 2.9970 153.6413 0.119 0.90572   
## Treatment3:Day7 -4.0553 2.9573 153.6413 -1.371 0.17230   
## Treatment2:Day14 -2.5889 2.9970 153.6413 -0.864 0.38904   
## Treatment3:Day14 -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 Tr2:D0 Tr3:D0 Tr2:D3 Tr3:D3 Tr2:D7  
## Day3 -0.573   
## Day7 -0.573 0.500   
## Day14 -0.573 0.500 0.500   
## Trtmnt2:Dy0 -0.707 0.405 0.405 0.405   
## Trtmnt3:Dy0 -0.717 0.411 0.411 0.411 0.507   
## Trtmnt2:Dy3 -0.243 -0.405 0.000 0.000 0.343 0.174   
## Trtmnt3:Dy3 -0.246 -0.411 0.000 0.000 0.174 0.343 0.507   
## Trtmnt2:Dy7 -0.243 0.000 -0.405 0.000 0.343 0.174 0.343 0.174   
## Trtmnt3:Dy7 -0.246 0.000 -0.411 0.000 0.174 0.343 0.174 0.343 0.507  
## Trtmnt2:D14 -0.243 0.000 0.000 -0.405 0.343 0.174 0.343 0.174 0.343  
## Trtmnt3:D14 -0.246 0.000 0.000 -0.411 0.174 0.343 0.174 0.343 0.174  
## Tr3:D7 T2:D14  
## Day3   
## Day7   
## Day14   
## Trtmnt2:Dy0   
## Trtmnt3:Dy0   
## Trtmnt2:Dy3   
## Trtmnt3:Dy3   
## Trtmnt2:Dy7   
## Trtmnt3:Dy7   
## Trtmnt2:D14 0.174   
## Trtmnt3:D14 0.343 0.507

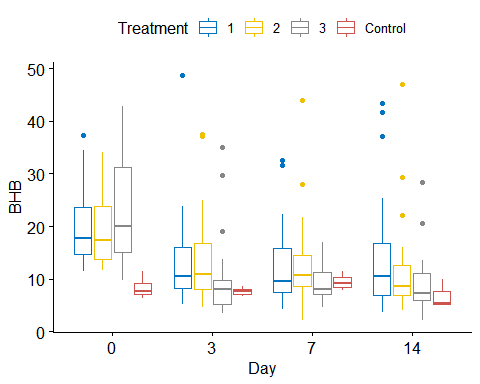
anova(lme)

## 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 \*  
## Treatment:Day 655.21 81.901 8 103.32 1.5430 0.15150   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

# Analysis for subjects under treatments 1,2, 3 or Control.

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

library(readxl)  
library(rstatix)  
library(tidyverse)  
library(ggpubr)  
##Read dataset  
ketosis2 <- read\_excel("datasets/Ketosis\_full\_control.xlsx")  
## crate nominal variables   
ketosis2$Treatment <- as.factor(ketosis2$Treatment)  
ketosis2$Day <- as.factor(ketosis2$Day)  
ketosis2$Treatment <- as.factor(ketosis2$Treatment)  
ketosis2$Cow <- as.factor(ketosis2$Cow)  
  
  
bxp <- ggboxplot(  
 ketosis2, x = "Day", y = "BHB",  
 color = "Treatment", palette = "jco"  
)  
bxp



## 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)  
anova(lme)

## Type III Analysis of Variance Table with Satterthwaite's method  
## Sum Sq Mean Sq NumDF DenDF F value Pr(>F)   
## Treatment 360.08 120.03 3 70.948 2.4031 0.0747 .   
## Day 1312.38 437.46 3 180.792 8.7586 1.872e-05 \*\*\*  
## Treatment:Day 793.95 88.22 9 175.191 1.7662 0.0777 .   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

## Final model for subjects under treatments 1,2, 3 or Control.

Then, it is only included treatment and day in the mixed model. The BHB was statistically significantly different at the different time points (0,3,7,14 day), p < 0.0001. The BHB also was statistically significantly different at the different treatments (1,2,3), p < 0.05.

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

## Type III Analysis of Variance Table with Satterthwaite's method  
## Sum Sq Mean Sq NumDF DenDF F value Pr(>F)   
## Treatment 898.73 299.58 3 70.652 5.8013 0.001328 \*\*   
## Day 2788.04 929.35 3 178.550 17.9968 2.999e-10 \*\*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

## Adjusting for baseline

Model adjusting for the baseline observation (day 0).

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

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [  
## lmerModLmerTest]  
## Formula: BHB ~ Treatment:Day + Day + (1 | Cow)  
## Data: ketosis2  
##   
## REML criterion at convergence: 1614.2  
##   
## Scaled residuals:   
## Min 1Q Median 3Q Max   
## -1.8392 -0.5225 -0.1419 0.2987 3.5385   
##   
## Random effects:  
## Groups Name Variance Std.Dev.  
## Cow (Intercept) 24.50 4.949   
## Residual 49.95 7.067   
## Number of obs: 240, groups: Cow, 66  
##   
## Fixed effects:  
## Estimate Std. Error df t value Pr(>|t|)   
## (Intercept) 20.2833 2.0336 174.6023 9.974 < 2e-16 \*\*\*  
## Day3 -6.3167 2.3558 167.8372 -2.681 0.008064 \*\*   
## Day7 -7.0333 2.3558 167.8372 -2.986 0.003254 \*\*   
## Day14 -4.9222 2.3558 167.8372 -2.089 0.038176 \*   
## Treatment2:Day0 -0.7444 2.8760 174.6023 -0.259 0.796058   
## Treatment3:Day0 2.6693 2.8379 174.6023 0.941 0.348214   
## TreatmentControl:Day0 -12.1106 3.3020 174.6023 -3.668 0.000325 \*\*\*  
## Treatment2:Day3 0.5722 2.8760 174.6023 0.199 0.842521   
## Treatment3:Day3 -3.6982 2.8379 174.6023 -1.303 0.194235   
## TreatmentControl:Day3 -6.4085 5.1957 215.0386 -1.233 0.218764   
## Treatment2:Day7 0.3556 2.8760 174.6023 0.124 0.901751   
## Treatment3:Day7 -4.0553 2.8379 174.6023 -1.429 0.154800   
## TreatmentControl:Day7 -3.8585 5.1957 215.0386 -0.743 0.458511   
## Treatment2:Day14 -2.5889 2.8760 174.6023 -0.900 0.369268   
## Treatment3:Day14 -5.8558 2.8379 174.6023 -2.063 0.040550 \*   
## TreatmentControl:Day14 -8.6697 5.1957 215.0386 -1.669 0.096650 .   
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

##   
## Correlation matrix not shown by default, as p = 16 > 12.  
## Use print(x, correlation=TRUE) or  
## vcov(x) if you need it

anova(lme)

## Type III Analysis of Variance Table with Satterthwaite's method  
## Sum Sq Mean Sq NumDF DenDF F value Pr(>F)   
## Day 542.3 180.77 3 167.84 3.6192 0.014412 \*   
## Treatment:Day 1683.3 140.28 12 134.74 2.8085 0.001858 \*\*  
## ---  
## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1