

# Enzymatic Breakdown of Lactose into Glucose in Cows Milk

Final Report

STAT 368

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Contributors:

Milagros N. Cortez  
Sam Ulan  
Siyi Chen  
Denzheng Kong

## Background and Introduction

Lactose is a common disaccharide found in dairy products, and it is composed of the monosaccharides glucose and galactose and has the molecular formula  $C_{12}H_{22}O_{11}$ . Enzymes are the main substances which can catalyze the breakdown of molecules in the body of organisms, and their effectiveness is dependent on the temperature, pH and time. With lactose, the enzyme lactase is responsible for its breakdown into its constituent sugars which are easily transported and absorbed by the body to use as a source of energy. When the organ responsible for lactase production – the small intestine – underproduces or fails to produce the enzyme it leads to the common symptoms of lactose intolerance such as bloating, diarrhea, and gas. Known to develop over time, in particular once individuals reach adulthood, or at the early stages of an individual's life. Raw and pasteurized milk and milk products contain some amount of lactose in them; with milk being one of the most common sources of dairy in the human diet. Pasteurized cow's milk is readily available in stores and is purchasable in varying fat percentages to suit different preferences.

To perform the experiment, stock enzyme solutions are created to simulate the process of mechanical digestion which are then able to be added to test cups that have been assigned various combinations of milk fat level, enzyme type, and a time value. After the time value elapses, a measurement of glucose content (which indicates the level of enzymatic activity) is taken and recorded using a human blood glucose monitor. This process is then replicated to get a total of four replicates for each of the assigned combinations of factors.

## Objective

Through performing this experiment we aim to establish whether there is a particular brand that is more effective at enzymatically catalyzing the lactose present in milk under different types of milk fat content, and time constraints.

## Choice of Factors and Levels

We decided to use milk as a blocking factor with 4 different levels of pasteurized milk: skim (0%), 1%, 2%, and 3.25%. We also used another 4-level blocking factor for time with levels being 1min, 5min, 15min, and 20min. In a preliminary test, the level of glucose after 20 minutes exceeded the level the glucose monitor was able to measure, which is why 20 minutes was the maximum time we used. For our treatment factor, we have four factors which are shown in Figure 1:Lacteeze (A), Webber Naturals (B), Lactaid (C), and Compliments (D). Table 1 in the Appendix presents the variables used in the experiment in columns, and their levels:

## Measuring Equipment

For this experiment we used blood glucose monitors manufactured by Ascensia Diabetes Care Holdings AG (Figure 2, Appendix) along with compatible blood glucose test strips from the same manufacturer. We also selected to record the amount of glucose produced in mmol/L. Additional information of the blood glucose monitors used for this experiment are that these monitors are intended to measure quantitatively glucose from a range of 0.6 mmol/L to 33.3 mmol/L. Measurements outside of this range cause the monitor to present a message of the result being over or under the range as seen in Figure 3 of the Appendix. The monitor has been designed to give accurate results under temperatures from 5°C to 45°C.

## Choice of Experimental Design

As the objective of this experiment is to test the effectiveness of lactase in these tablets to break down lactose into glucose under different types of milk, and time constraints, for this experiment we will use Latin Square Design since it allows us to test and control for two blocking factors and one treatment factor. Since we decided on four levels for each of the block factors (milk and time), we need  $4 \times 4 = 16$  treatments as we can observe in Table 2 (Appendix). We chose to complete 4 replicates, making for 64 total treatments. We assumed no interactions for this experiment, and our statistical model to be:

$$y_{ijkl} = \mu + \alpha_i + \tau_j + \beta_k + \delta_l + \varepsilon_{ijkl}; i, j, k, l = 1, 2, 3, 4.$$

## Methodology

For this experiment, we performed a Latin square design with four replications with three factors of four levels. Moreover, to conduct this experiment we used sixty-eight sterile cups, sixty-eight spoons, two blood glucose monitors, digital chronometers, the four brands of lactase, four 2L cartons of milk (one carton for each type of milk used), 320 ml of water, and blood glucose test strips.

Prior to performing the experiment, the cartons of milk were set in a 22°C room to change to room temperature (22°C), the amount of lactase per tablet was calculated for each of the brands used from which we calculated the number of tablets to use, we pulverized in separate containers the tablets to create individual solutions of each, and the cups were labelled according to the design in Table 2 (Appendix), leaving four cups per treatment as seen in Figures 4 and 5 (Appendix).

The way we calculated the number of tablets to use for the solutions in the experiment was by using the Food Chemical Codex Lactase Units (FCCLU) value provided by the brands in the packages of the tablets which establishes the activity levels of the enzymes per tablet. We found that each brand had different FCCLU per tablet, with Lacteeze containing 4,000 FCCLU, Lactaid containing 4,500 FCCLU, Compliments containing 4,500 FCCLU, and Webber Naturals containing 9,000 FCCLU. Based on this number we decided that we would use 4,500 FCCLU per cup with half a cup of milk. Since each brand is present four times in one replication of the design as seen in Table 2 (Appendix), that would mean sixteen cups would need 4,500 FCCLU of a particular brand of lactase from the four we chose to test, which equated to a total of 72,000

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FCCLU for the 80ml stock solutions. Table 3 of the Appendix summarizes our calculations for the amount of tablets per solution of 80 ml.

In separate containers we pulverized the tablets of lactase, forming a fine powder which we mixed with 80 ml of water to form the enzyme solution. We used filtered water for each solution. The solutions were made in separate labelled cups. 5 ml of solution would be used to add to each cup requiring the enzyme.

After preparing the solutions and pouring the room temperature milk (verified with a thermometer) into its respective cups, we added 5ml of the corresponding enzyme solution to each cup tested and set the timers to the corresponding time for each cup (1min, 5min, 15min, or 20min).

To measure the response variable we used a spoon to take a sample of milk from the cup when the time elapsed and with fresh test strips, recorded the value. Final measurements for each replication and treatment are presented in Table 4 of the Appendix.

## Statistical Analysis

For the statistical analysis, we used R Studio to conduct an analysis of variance and proceed with the analysis of significant factors through pairwise comparisons. We generated a linear model that included the replications, milk, time and lactase brand and an ANOVA for a Latin square with replications (replicated squares) as seen in Figure 5 of the Appendix, with our hypothesis for this ANOVA being  $H_0: \alpha_i = 0$  and  $H_a: \alpha_i \neq 0$ .

From Figure 5 we can see that at  $\alpha = 0.05$ , the two blocking factors of milk and time, as well as the treatment factor of lactase brand were significant to the concentration of glucose present. Replications were not significant, which indicates that there are no significant variations between the replications. We proceed first to examine the levels of our treatment factor lactase brand.

To examine the levels of our treatment factor, we first calculated the mean concentration of glucose for each lactase brand and got an ANOVA test for the variances as seen in Figure 6 of the Appendix. The test hypothesis of the ANOVA for the variances was set to:

$$H_0: \sigma_A^2 = \sigma_B^2 = \sigma_C^2 = \sigma_D^2$$

We obtained the mean concentration of glucose for each brand: Lacteeze (A)=26.16975 mmol/L, Webber Naturals (B) = 19.29375 mmol/L, Lactaid (C) = 20.025 mmol/L, and Compliments (D) = 20.39375 mmol/L. From Figure 5, we can see that at  $\alpha = 0.05$  we fail to reject the null hypothesis that the variances of the lactase brands are equal, furthermore, we can proceed to test for multiple comparisons and use Tukey's test for multiple comparisons.

We obtained  $T_\alpha = 3.771107$ , and for the Tukey test of multiple comparisons we can see that at  $\alpha = 0.05$  Brand A (Lacteeze) is significantly different from the rest of the brands B (Webber Naturals), C (Lactaid), and D (Compliments). Additionally, Lacteeze has the greatest mean glucose concentration indicating a greater efficiency at breaking down lactose.

We next analyzed the two block factors, as they were significant, starting with milk. We obtained the mean glucose concentration of each milk used in the experiment to be skim = 1, 1% = 2, 2% = 3, 3.25% = 4. We also found that the mean concentration of glucose to be: skim milk =

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21.0562 mmol/L, 1% = 19.35625 mmol/L, 2% = 23.48125 mmol/L, and 3.25% = 21.98750 mmol/L.

From the Tukey test, we found that at  $\alpha = 0.05$ , 2% milk was different from 1% milk, but not skim or 3.25% milk. Likewise, 1% milk is not different from skim or 3.25% milk. Additionally, we can see that 2% milk has a greater mean concentration of glucose than 1% milk.

Lastly, we analyzed the time factor as seen in Figure 9 of the Appendix. For this factor, we did the same procedure as the two factors above and found the mean glucose concentration of each level to be 1 min = 8.6625 mmol/L, 5 min = 19.66875 mmol/L, 15 min = 28.11875 mmol/L, and 20 min = 29.43125 mmol/L.

From the Tukey test we found that at  $\alpha = 0.05$ , 1 min and 5 min were different from each other and different from 15min and 20 min, while 15 min was not significantly different from 20 min, but 15 min and 20 min were different from other time levels. We can see as well that the mean glucose concentrations for 15 min and 20 min were higher, which tells us that at that time lactase breaks significantly more lactose.

Additionally to these tests for the significant factors, we also created scatter plots of the glucose concentration against each factor to visualize the results which can be seen in figures 10, 11, and 12.

Lastly, we tested if the model fits the assumptions of Linearity, Independence, Normality and Equal variances (LINE). We visually confirmed that the data did not violate these assumptions by generating Figure 13. The underlying assumption for using a Latin Square design is that none of the factors have interaction with each other. In Figures 14, 15 and 16, we can see that there are some interactions between the factors, which is indicated by a crossover of the lines with each other. Milk and time have the least amount of interaction with each other, while time and brand have the most and show more linearity with each other. From this we can see that the assumption of no interactions does not hold completely, and therefore the conclusions we draw from this testing may not be fully representative of the true relationship due to this assumption not being fully satisfied.

## Conclusions and Recommendations

In conclusion, our test results showed plausible interactions among factors. We found that time, milk, and lactase brand affect the breakdown of lactose into glucose and galactose. We found that Lacteeze (brand A) significantly increased the concentration of glucose, for which it is the most efficient brand of the four tested. We found that 1% and 2% milk were significantly different from other types of milk, with 1% milk giving the smallest mean concentration of glucose, and 2% milk giving the greatest mean concentration of glucose. We also found that after 15 minutes of lactase activity, the concentration of glucose in milk would not significantly increase, for which we consider 15 minutes the time when most lactose is broken down by lactase. Moreover, we recommend for future experiments to consider interactions between these factors.

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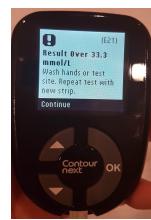
## Appendix



**Figure 1.** Presentation of four lactase tablets found in pharmacies. (a) Lacteeze, (b) Webber Naturals, (c) Lactaid, and (d) Compliments.



**Figure 2.** Blood glucose monitors manufactured by Ascensia Diabetes Care Holdings AG.



**Figure 3.** Message displayed when measurements are over 33.3 mmol/L.



**Figure 4.** Labelling of cups. Each treatment has four cups, one for each replication, labelled. Additionally, the single cups at the front are cups for the enzyme solutions.

```
Analysis of Variance Table
Response: mmolL
DF Sum Sq Mean Sq F value Pr(>F)
Replication 3 53.9 17.96 1.1134 0.35229
Milk 3 143.2 47.74 2.9605 0.04082 *
Time 3 4397.8 1465.94 90.8997 < 2.2e-16 ***
Brand 3 481.0 160.32 9.9413 2.882e-05 ***
Residuals 51 822.5 16.13
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

**Figure 5.** ANOVA results.

```
A 19.29375 20.02500 20.39375
B 26.16875 19.29375 20.02500 20.39375
> glucosendiff <- vector(0)
> for (i in 1:(length(glucosendiff)-1)) {
+   glucosendiff[i+1] <- abs(glucosendiff[i]-glucosendiff[i+1])
+ }
> glucosendiff[1] <- median(glucosendiff)
> glucosendiff <- 1*(glucosendiff-glucosendiff[1])
> anova(glucosendiff)
```

**Figure 6.** Means and ANOVA of lactase brand variances.

```
$parameters
  test name.t ntr StudentizedRange alpha
  Tukey Brand 4 3.755879 0.05
$means
  mmolL std r Min Max Q25 Q50 Q75
  A 26.16875 7.629130 16 9.5 32.3 24.300 29.50 31.575
  B 19.29375 10.334955 16 5.6 32.3 9.875 19.70 29.275
  C 20.02500 9.181975 16 3.9 31.7 11.350 21.25 28.025
  D 20.39375 10.575284 16 4.4 33.2 14.000 20.15 29.775
$comparison
NULL
$groups
  mmolL groups
  A 26.16875 a
  D 20.39375 b
  C 20.02500 b
  B 19.29375 b
```

**Figure 7.** Tukey test value and Tukey test for Lactase brands.

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```
> qtukey(0.95,4,51)*sqrt(16.13/16)
[1] 3.771107
```

```
1   2   3   4
21.05625 19.35625 23.48125 21.98750
```

```
> tukeyTest
$statistics
  MSerror Df    Mean      CV      MSD
  16.12702 51 21.47031 18.70418 3.770758

$parameters
  test name.t ntr StudentizedRange alpha
  Tukey Milk  4       3.755879  0.05

$means
  mmoll    std   r Min Max   Q25   Q50   Q75
1 21.05625 6.484851 16 9.5 29.6 16.75 21.25 26.225
2 19.35625 10.567117 16 4.4 32.3 9.80 23.05 28.025
3 23.48125 10.938996 16 5.6 33.2 17.15 29.45 32.100
4 21.98750 10.502246 16 3.9 32.3 14.65 25.40 31.450

$comparison
NULL

$groups
  mmoll groups
3 23.48125    a
4 21.98750    ab
1 21.05625    ab
2 19.35625    b
```

**Figure 8.** Tukey test value and Tukey test for milk type.

```
> timebl.means
1   2   3   4
8.66250 19.66875 28.11875 29.43125
```

```
> tukeyTest
$statistics
  MSerror Df    Mean      CV      MSD
  16.12702 51 21.47031 18.70418 3.770758

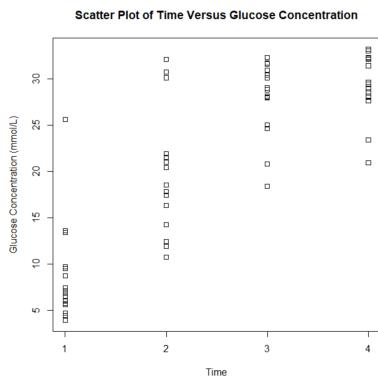
$parameters
  test name.t ntr StudentizedRange alpha
  Tukey Time  4       3.755879  0.05

$means
  mmoll    std   r Min Max   Q25   Q50   Q75
1 8.66250 5.350748 16 3.9 25.6 5.675 6.95 9.550
2 19.66875 6.552630 16 10.7 32.1 15.775 18.15 21.600
3 28.11875 4.067795 16 18.4 32.3 27.175 28.90 31.075
4 29.43125 3.452867 16 20.9 33.2 28.075 29.50 32.200

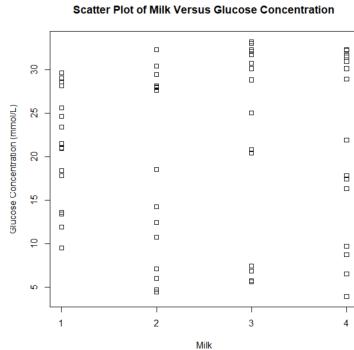
$comparison
NULL

$groups
  mmoll groups
4 29.43125    a
3 28.11875    a
2 19.66875    b
1 8.66250    c
```

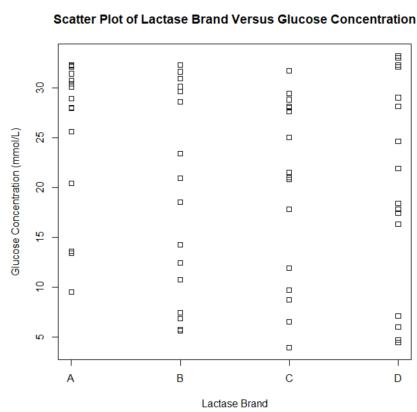
**Figure 9.** Tukey test value and Tukey test for time factor.



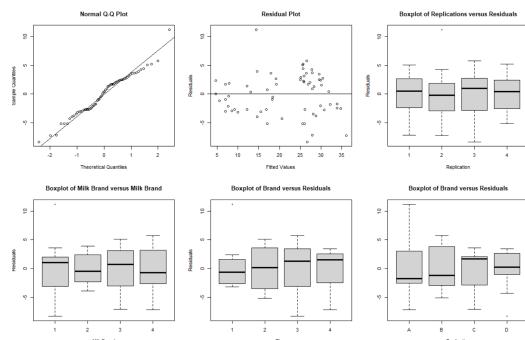
**Figure 10.** Scatter plot of Glucose concentration (mmol/L) versus Time. As we can observe, as time increases from 1 min, 5 min, 15 min to 20 min, the concentration of glucose increases.



**Figure 11.** Scatter plot of Glucose concentration (mmol/L) versus Milk.

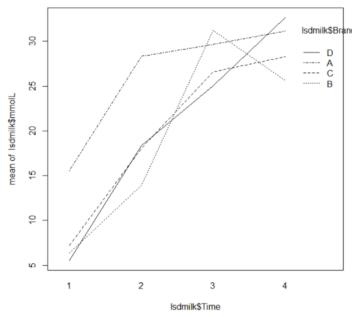


**Figure 12.** Scatter plot of Glucose concentration (mmol/L) versus Time.

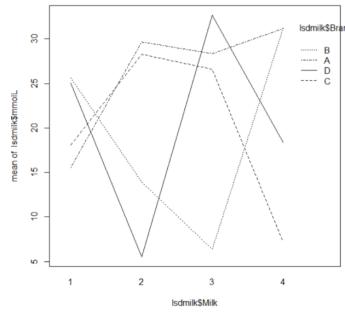


**Figure 13.** Q-Q plot, residuals plot, and boxplots of factors against residuals

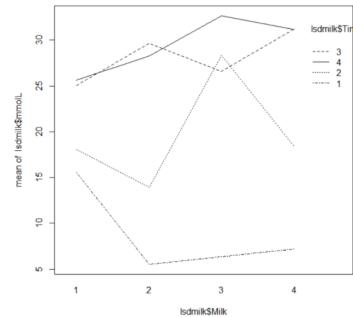
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**Figure 14:**Interaction between Time and brand



**Figure 15:**Interaction between Milk and Brand



**Figure 16:**Interaction between Milk and Time

Type of Factor	Treatment	Blocking	Blocking
Factor	Lactase Brand	Time	Milk
Levels	Lacteeze	1 min	Skim
	Webber Naturals	5 min	1%
	Compliments	15 min	2%
	Lactaid	20 min	3.25%

**Table 1.** Table presenting the three factors used in the experiment, and their respective four levels. Additionally, we provide information on the type of factor each factor is in the experiment.

↓ Time \ Milk→	Skim	1%	2%	3.25%
1 min	A	D	B	C
5 min	C	B	A	D
15 min	D	A	C	B
20 min	B	C	D	A

**Table 2.** Table showing our experimental design, each columns presents a level from the milk blocking factor, each row presents a level from the time blocking factor, and each letter presents a level from our lactase brand treatment factor: A = Lacteeze, B = Webber Naturals, C = Lactaid, and D = Compliments.

Brand	FCCLU per tablet	Number of tablets needed
Webber Naturals	9,000	$\frac{72,000}{9,000} = 8$ tablets
Lacteeze	4,000	$\frac{72,000}{4,000} = 18$ tablets
Lactaid	4,500	$\frac{72,000}{4,500} = 16$ tablets
Compliments	4,500	$\frac{72,000}{4,500} = 16$ tablets

**Table 3.** Table showing the brands of lactase tablets used in the experiment, the FCCLU in each tablet, and the number of tablets needed to make a solution of 80ml for the experiment.

#	Milk	Time (min)	Brand	Amount of Glucose (mmol/L)			
				Replication I	Replication II	Replication III	Replication IV
1	Skim	1	Lacteeze	13.6	25.6	13.4	9.5
2	Skim	5	Lactaid	11.9	21	21.5	17.8
3	Skim	15	Compliments	29	24.6	18.4	28.1
4	Skim	20	Webber Naturals	23.4	29.6	28.6	20.9
5	1%	1	Compliments	7.1	6	4.4	4.7
6	1%	5	Webber Naturals	10.7	14.2	12.4	18.5
7	1%	15	Lacteeze	27.9	30.4	28	32.3
8	1%	20	Lactaid	28	29.4	28.1	27.6
9	2%	1	Webber Naturals	7.4	6.8	5.7	5.6
10	2%	5	Lacteeze	30.7	32.1	30.1	20.4
11	2%	15	Lactaid	20.8	31.7	25	28.8
12	2%	20	Compliments	32.1	32.3	33.2	33
13	3.25%	1	Lactaid	8.7	6.5	9.7	3.9
14	3.25%	5	Compliments	17.8	16.3	21.9	17.4
15	3.25%	15	Webber Naturals	30.1	31.6	32.3	30.9
16	3.25%	20	Lacteeze	32.7	28.9	32.7	31.4

**Table 4.** Table of experiment results. The recorded results for each replication are shown below. Each replication contains sixteen recorded observations, for the sixteen combinations of treatments.

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### R-code:

```
# Stat 368 Final Project Data Analysis
# Milagros N. Cortez
# Opening the data:
library(openxlsx)
library(agricolae)
lsdmilk = read.xlsx("stat368_project.xlsx", cols = 2:6, rows = 1:65)
lsdmilk$Replication = as.factor(lsdmilk$Replication)
lsdmilk$Milk = as.factor(lsdmilk$Milk)
lsdmilk$Time = as.factor(lsdmilk$Time)
lsdmilk$Brand = as.factor(lsdmilk$Brand)
# since replications are all the same
linmodmilk = lm(mmolL ~ Replication + Milk + Time + Brand, data = lsdmilk)
# Latin Square Design Analysis
anova(linmodmilk)

# finding the treatment factor means:
treatment.means <- vector()
for (i in c("A", "B", "C", "D")) treatment.means[i] <-
mean(lsdmilk$mmolL[lsdmilk$Brand==i])
treatment.means

# Analyzing the Treatment Variable varances
glucosendiff = vector()
for (i in c("A", "B", "C", "D")) glucosendiff[lsdmilk$Brand==i] <-
abs(lsdmilk$mmolL[lsdmilk$Brand==i])-median(lsdmilk$mmolL[lsdmilk$Brand==i])
glucosendiffmod = lm(glucosendiff~lsdmilk$Brand)
anova(glucosendiffmod)

qtukey(0.95,4,51)*sqrt(16.13/16)

milkdata = aov(mmolL ~ Replication + Milk + Time + Brand, data = lsdmilk)
TukeyHSD(milkdata, "Brand")
tukeyTest = HSD.test(milkdata, "Brand")
tukeyTest

# Checking differences in the milk block
milkbl.means <- vector()
for (j in c("1", "2", "3", "4")) milkbl.means[j] <-
mean(lsdmilk$mmolL[lsdmilk$Milk==j])
milkbl.means

# Analyzing the milk Variable variances
glucmildiff = vector()
for (j in c("1", "2", "3", "4")) glucmildiff[lsdmilk$Milk==j] <-
abs(lsdmilk$mmolL[lsdmilk$Milk==j])-median(lsdmilk$mmolL[lsdmilk$Milk==j])
glucosendiffmilk = lm(glucmildiff~lsdmilk$Milk)
anova(glucosendiffmilk)

qtukey(0.95,4,51)*sqrt(16.13/16)

TukeyHSD(milkdata, "Milk")
tukeyTest = HSD.test(milkdata, "Milk")
tukeyTest

# Checking the differences in the Time block
timebl.means <- vector()
```

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```
for (k in c("1", "2", "3", "4")) timebl.means[k] <-  
mean(lsdmilk$mmoll[lsdmilk$Time==k])  
timebl.means  
  
# Analyzing the time Variable variances  
gluctimdiff = vector()  
for (k in c("1", "2", "3", "4")) gluctimdiff[lsdmilk$Time==k] <-  
abs(lsdmilk$mmoll[lsdmilk$Time==k])-median(lsdmilk$mmoll[lsdmilk$Time==k])  
glucosedifftime = lm(gluctimdiff~lsdmilk$Time)  
anova(glucosedifftime)  
  
qtukey(0.95,4,51)*sqrt(16.13/16)  
  
TukeyHSD(milkdata, "Time")  
tukeyTest = HSD.test(milkdata, "Time")  
tukeyTest  
  
# Checking the appropriateness of the model  
windows()  
par(mfrow = c(1,2))  
qqnorm(linmodmilk$residuals)  
qqline(linmodmilk$residuals)  
plot(linmodmilk$fitted.values, linmodmilk$residuals, main = 'Residual Plot',  
xlab = 'Fitted Values', ylab = 'Residuals')  
abline(h=0)  
  
windows()  
par(mfrow = c(1,4))  
plot(c(lsdmilk$Replication), linmodmilk$residuals, main = 'Boxplot of  
Replications versus Residuals', xlab = 'Replication', ylab = 'Residuals')  
plot(c(lsdmilk$Milk), linmodmilk$residuals, main = 'Boxplot of Milk Brand  
versus Residuals', xlab = 'Milk Brand', ylab = 'Residuals')  
plot(c(lsdmilk$Time), linmodmilk$residuals, main = 'Boxplot of Time versus  
Residuals', xlab = 'Time', ylab = 'Residuals')  
plot(c(lsdmilk$Brand), linmodmilk$residuals, main = 'Boxplot of Lactase Brand  
versus Residuals', xlab = 'Brand', ylab = 'Residuals')  
  
# Checking treatment efficiency:  
windows()  
stripchart(lsdmilk$mmoll~lsdmilk$Time, main = "Scatter Plot of Time Versus  
Glucose Concentration", xlab = "Time", ylab = "Glucose Concentration (mmol/  
L)", vertical = TRUE)  
  
windows()  
stripchart(lsdmilk$mmoll~lsdmilk$Milk, main = "Scatter Plot of Milk Versus  
Glucose Concentration", xlab = "Milk", ylab = "Glucose Concentration (mmol/  
L)", vertical = TRUE)  
  
windows()  
stripchart(lsdmilk$mmoll~lsdmilk$Brand, main = "Scatter Plot of Lactase Brand  
Versus Glucose Concentration", xlab = "Lactase Brand", ylab = "Glucose  
Concentration (mmol/L)", vertical = TRUE)  
  
windows()  
interaction.plot(lsdmilk$Milk, lsdmilk$Time, lsdmilk$mmoll)  
  
interaction.plot(lsdmilk$Milk, lsdmilk$Brand, lsdmilk$mmoll)  
interaction.plot(lsdmilk$Time, lsdmilk$Brand, lsdmilk$mmoll)  
  
interacMilklm = lm(lsdmilk$mmoll ~ (lsdmilk$Milk + lsdmilk$Time +  
lsdmilk$Brand)^2)  
summary(interacMilklm)  
anova(interacMilklm)
```