

SUPPLEMENTARY DATA

Manuscript:

de5177f7-b566-402e-a355-5f598d939c92

The TOR regulatory mechanism controls the metabolism of nitrate and the fermentation activity in the yeast Dekkera bruxellensis

RT-qPCR of *D. bruxellensis* genes

Test condition: NH ₄ +rapamycin			Reference condition: NH ₄					
	rep 1	rep 2	rep 3	rep 4	rep 5	rep 6	mean	SD
GAP1	18.7766	19.9032	19.9232	20.6543	21.3178	18.9728	19.9246	0.9693
PUT4	6.9811	7.4000	7.4074	7.6793	7.9259	7.0541	7.4080	0.3604
MEP1	-7.5184	-7.9695	-7.9775	-8.2702	-8.5359	-7.5969	-7.9781	0.3881
YNT1	1.3923	1.4759	1.4773	1.5316	1.5808	1.4069	1.4775	0.0719
YNR1	-2.4473	-2.5941	-2.5967	-2.6920	-2.7785	-2.4729	-2.5969	0.1263
YNA1	1.4904	1.5798	1.5814	1.6394	1.6921	1.5060	1.5815	0.0769
ATP1	-1.5087	-1.5992	-1.6008	-1.6596	-1.7129	-1.5244	-1.6009	0.0779
GDH1	-1.3652	-1.4471	-1.4486	-1.5017	-1.5500	-1.3795	-1.4487	0.0705
ALD5	-1.3707	-1.4529	-1.4544	-1.5077	-1.5562	-1.3850	-1.4545	0.0708
PDC1	-1.7901	-1.8975	-1.8994	-1.9691	-2.0323	-1.8088	-1.8995	0.0924
YNI1	-1.7963	-1.9041	-1.9060	-1.9759	-2.0394	-1.8151	-1.9061	0.0927
ADH1	-2.5298	-2.6816	-2.6843	-2.7828	-2.8722	-2.5563	-2.6845	0.1306
ALD3	-7.2690	-7.7051	-7.7129	-7.9959	-8.2528	-7.3449	-7.7134	0.3752
GDH2	-7.3219	-7.7612	-7.7690	-8.0541	-8.3128	-7.3984	-7.7696	0.3780
GLT1	-32.7840	-34.7511	-34.7859	-36.0625	-37.2209	-33.1266	-34.7885	1.6924

Test condition: NO ₃			Reference condition: NH ₄					
	rep 1	rep 2	rep 3	rep 4	rep 5	rep 6	mean	SD
GAP1	-1.1728	-1.2432	-1.2444	-1.2901	-1.3315	-1.1851	-1.2445	0.0605
PUT4	5.0404	5.3429	5.3482	5.5445	5.7226	5.0931	5.3486	0.2602
MEP1	1.8408	1.9512	1.9532	2.0249	2.0899	1.8600	1.9533	0.0950
YNT1	71.2582	75.5337	75.6093	78.3841	80.9020	72.0028	75.6150	3.6785
YNR1	60.0802	63.6850	63.7488	66.0882	68.2112	60.7079	63.7535	3.1015
YNI1	207.3392	219.7796	219.9996	228.0731	235.3995	209.5056	220.0161	10.7032
YNA1	25.2838	26.8008	26.8276	27.8121	28.7055	25.5479	26.8296	1.3052
GLT1	202.4339	214.5799	214.7947	222.6773	229.8304	204.5490	214.8109	10.4500
GDH1	4.6758	4.9564	4.9613	5.1434	5.3086	4.7247	4.9617	0.2414
GDH2	4.5315	4.8034	4.8082	4.9847	5.1448	4.5789	4.8086	0.2339
PDC1	7.2424	7.6769	7.6846	7.9666	8.2225	7.3181	7.6852	0.3739
ADH1	1.2467	1.3215	1.3228	1.3714	1.4154	1.2597	1.3229	0.0644
ALD3	-2.6463	-2.8050	-2.8078	-2.9109	-3.0044	-2.6739	-2.8080	0.1366
ALD5	3.0010	3.1810	3.1842	3.3011	3.4071	3.0323	3.1844	0.1549
ATP1	-1.8914	-2.0049	-2.0069	-2.0805	-2.1474	-1.9112	-2.0070	0.0976

Test condition: NO3+rapamycin			Reference condition: NH4					
	rep 1	rep 2	rep 3	rep 4	rep 5	rep 6	mean	SD
GAP1	16.9719	17.9902	18.0082	18.6691	19.2688	17.1493	18.0096	0.8761
PUT4	6.3301	6.7099	6.7166	6.9631	7.1867	6.3962	6.7171	0.3268
MEP1	1.0007	1.0607	1.0618	1.1007	1.1361	1.0111	1.0618	0.0517
YNT1	9.1671	9.7171	9.7268	10.0838	10.4077	9.2629	9.7276	0.4732
YNR1	4.7029	4.9851	4.9901	5.1732	5.3394	4.7520	4.9904	0.2428
YNI1	49.8713	52.8636	52.9165	54.8585	56.6207	50.3924	52.9205	2.5745
YNA1	3.2008	3.3928	3.3962	3.5208	3.6339	3.2342	3.3965	0.1652
GLT1	112.4239	119.1694	119.2887	123.6663	127.6389	113.5986	119.2976	5.8035
GDH1	2.2472	2.3820	2.3844	2.4719	2.5513	2.2707	2.3846	0.1160
GDH2	-2.4868	-2.6360	-2.6387	-2.7355	-2.8234	-2.5128	-2.6389	0.1284
PDC1	-1.3880	-1.4713	-1.4728	-1.5268	-1.5759	-1.4025	-1.4729	0.0717
ADH1	2.5887	2.7440	2.7467	2.8475	2.9390	2.6157	2.7470	0.1336
ALD3	-2.8123	-2.9810	-2.9840	-3.0935	-3.1929	-2.8417	-2.9842	0.1452
ALD5	1.0994	1.1654	1.1665	1.2093	1.2482	1.1109	1.1666	0.0568
ATP1	-1.6572	-1.7566	-1.7584	-1.8229	-1.8815	-1.6745	-1.7585	0.0855

Test condition: NO3+rapamycin			Reference condition: NH4					
	rep 1	rep 2	rep 3	rep 4	rep 5	rep 6	mean	SD
GAP1	19.9000	21.0940	21.1151	21.8900	22.5932	20.1079	21.1167	1.0273
PUT4	1.3081	1.3865	1.3879	1.4389	1.4851	1.3217	1.3880	0.0675
MEP1	-1.8444	-1.9551	-1.9570	-2.0289	-2.0940	-1.8637	-1.9572	0.0952
YNT1	-7.6910	-8.1525	-8.1606	-8.4601	-8.7319	-7.7714	-8.1612	0.3970
YNR1	-12.5005	-13.2506	-13.2638	-13.7506	-14.1923	-12.6312	-13.2648	0.6453
YNI1	-4.1291	-4.3768	-4.3812	-4.5420	-4.6879	-4.1722	-4.3815	0.2131
YNA1	-7.9330	-8.4090	-8.4174	-8.7264	-9.0067	-8.0159	-8.4181	0.4095
GLT1	-1.7746	-1.8811	-1.8830	-1.9521	-2.0148	-1.7932	-1.8831	0.0916
GDH1	-2.0825	-2.2074	-2.2096	-2.2907	-2.3643	-2.1042	-2.2098	0.1075
GDH2	-11.2691	-11.9452	-11.9572	-12.3960	-12.7942	-11.3868	-11.9581	0.5817
PDC1	-10.0089	-10.6095	-10.6201	-11.0098	-11.3635	-10.1135	-10.6209	0.5167
ADH1	2.0930	2.2186	2.2208	2.3023	2.3763	2.1149	2.2210	0.1080
ALD3	-1.0676	-1.1317	-1.1328	-1.1744	-1.2121	-1.0788	-1.1329	0.0551
ALD5	-2.7181	-2.8811	-2.8840	-2.9899	-3.0859	-2.7465	-2.8843	0.1403
ATP1	1.1435	1.2121	1.2133	1.2579	1.2983	1.1555	1.2134	0.0590

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4		NO3/NH4		NO3RP/NH4		NO3RP/NO3	
Data: gene ADH1	-2.5298	-2.6816	1.2467	1.3215	2.5887	2.7440	2.0930	2.2186
	-2.6843	-2.7828	1.3228	1.3714	2.7467	2.8475	2.2208	2.3023
	-2.8722	-2.5563	1.4154	1.2597	2.9390	2.6157	2.3763	2.1149
Skewness:	-0.236199		0.235878		0.236458		0.236452	
Excess kurtosis:	-1.009176		-1.011762		-1.009262		-1.008669	
Normality	0.8356		0.8341		0.8345		0.8352	
Outliers								
Mean	-2.6845		1.32292		2.74693		2.22098	
S	0.13059		0.06436		0.13362		0.10805	

Calculate

Validate

Insert column

Delete column

Clear

Load last run

Header: You may change groups' names to the real names.

Data: When entering data, press **Enter** or **,** (comma) after each value.
You may paste full column from excel.

The calculator ignores empty cells or non-numeric cells.

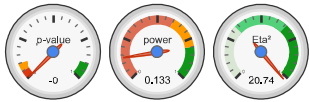
Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values

Group4 contains 6 values
validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	109.1019	36.3673	2867.7683	-2.22e-16
Error (within groups)	20	0.2536	0.01268		
Total	23	109.3555	4.7546		

[R code](#)



One Way ANOVA test, using F distribution df(3,20) (right tailed).

1. H₀ hypothesis

Since p-value< α , H₀ is rejected.
Some of the groups' averages consider to be not equal.
In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals **-2.22045e-16**, [p(x ≤ F) = 1]. It means that the chance of type1 error (rejecting a correct H₀) is small: -2.22e-16 (-2.2e-14%)
The smaller the p-value the stronger it support H₁

3. The statistics

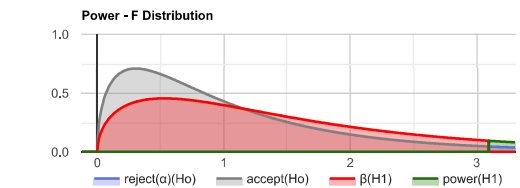
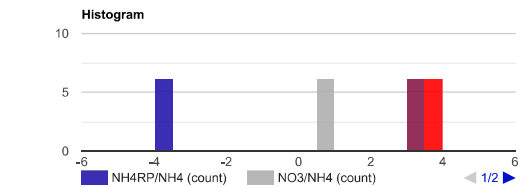
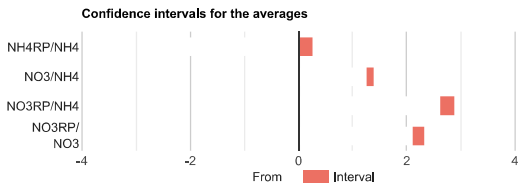
The test statistic F equals **2867.768261**, which is not in the 95% region of acceptance: [0 : 3.0984]

4. Effect size

The observed effect size f is **large** (20.74). That indicates that the magnitude of the difference between the averages is large.
The η^2 equals 1. It means that the **group** explains 99.8% of the variance from the average (similar to R² in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4**.



Validation

● Test power

Equality of variances

The tool used the Levene's test to assess the equality of variances.
The population's variances consider to be **equal**. (p-value = 0.557).
Levene's test power consider to be weak (0.13).
The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)
The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

Normality assumption

The assumption was checked based on the [Shapiro-Wilk Test](#). (α=0.05)
It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	4.0074	0.04597	87.1679	3.8254	4.1894	0.182	5.11e-13
x1-x3	5.4314	0.04597	118.1426	5.2495	5.6134	0.182	5.11e-13
x1-x4	4.9055	0.04597	106.7023	4.7235	5.0875	0.182	5.11e-13
x2-x3	1.424	0.04597	30.9747	1.242	1.606	0.182	5.221e-13
x2-x4	0.8981	0.04597	19.5344	0.7161	1.08	0.182	6.355e-11
x3-x4	0.5259	0.04597	11.4403	0.344	0.7079	0.182	5.565e-7

Group	x2	x3	x4
x1	4.01	5.43	4.91
x2	0	1.42	0.9
x3	1.42	0	0.53

ANOVA
What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data.
ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result.
The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group.
The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields
H₀: The average weight of crops per square meter is equal in all fields.
H₁: At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

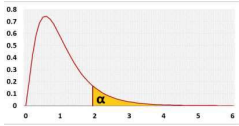
Hypotheses

H₀: μ₁ = ... = μ_k
H₁: not(μ₁ = ... = μ_k)

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples
- Normal distribution of the analyzed population
- Equal standard deviation, σ₁=σ₂=...=σ_k
The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- **k** - Number of groups.
- **n_i** - Sample side of group i
- **n** - Overall sample side, includes all the groups ($\sum n_i$, i=1 to k)
- **$\bar{x}_i$** - Average of group i.
- **$\bar{x}$** - Overall average ($\sum x_{ij} / n$, i=1 to k, j=1 to n_i)
- **S_i** - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	k - 1	$SSG = \sum_{i=1}^k n_i (\bar{x}_i - \bar{x})^2$	MSG = SSG / (k - 1)	F = MSG / MSE	P(x > F)
Error (within groups)	n - k	$SSE = \sum_{i=1}^k (n_i - 1) S_i^2$	MSE = SSE / (n - k)		
Total	n - 1	SS(total) = SSG + SSE	Sample Variance = SS(total) / (n - 1)		

R Code

The following R code should produce the same results

```
value<-  
c(-2.8722,-2.7828,-2.6843,-2.6816,-2.5563,-2.5298,1.2467,1.2597,1.3215,1.3228,1.3714,1.4154,2.5887,2.6157,2.7440,2.7467,2.8475,2.9390,2.0930,2.1149,2.2186,2.2208,2.3023,2.3763)  
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))  
my.dataframe<-data.frame(value, group1)  
res.aov <- aov(value ~ group1, data = my.dataframe)  
summary(res.aov)  
TukeyHSD(res.aov)
```

[Tutorial](#)
[ANOVA](#)

Calculators

[Kruskal-Wallis test](#)
[Two way ANOVA](#)
[Levene's test](#)

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4	NO3/NH4	NO3RP/NH4	NO3RP/NO3
Data: gene ALD3	-7.2690 -7.7051 -7.7129 -7.9959 -8.2528 -7.3449	-2.6463 -2.8050 -2.8078 -2.9109 -3.0044 -2.6739	-2.8123 -2.9810 -2.9840 -3.0935 -3.1929 -2.8417	-1.0676 -1.1317 -1.1328 -1.1744 -1.2121 -1.0788
Skewness:	-0.236214	-0.236777	-0.236535	-0.235622
Excess kurtosis:	-1.009626	-1.009744	-1.009026	-1.01023
Normality	0.835	0.8345	0.8352	0.8353
Outliers				
Mean	-7.71343	-2.80805	-2.98423	-1.1329
S	0.37526	0.1366	0.14517	0.055113

Calculate

Validate

Insert column

Delete column

Clear

Load last run

Header: You may change groups' names to the real names.

Data: When entering data, press Enter or , (comma) after each value.
You may paste full column from excel.

The calculator ignores empty cells or non-numeric cells.

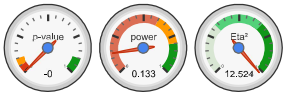
Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values
validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
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Groups (between groups)	3	143.9942	47.9981	1045.7459	-2.22e-16
Error (within groups)	20	0.918	0.0459		
Total	23	144.9122	6.3005		

[R code](#).



One Way ANOVA test, using F distribution df(3,20) (right tailed).

1. H₀ hypothesis

Since p-value< α , H₀ is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals **-2.22045e-16**, [$p(x \leq F) = 1$]. It means that the chance of type1 error (rejecting a correct H₀) is small: -2.22e-16 (-2.2e-14%)

The smaller the p-value the stronger it support H₁

3. The statistics

The test statistic F equals **1045.745851**, which is not in the 95% region of acceptance: [0 : 3.0984]

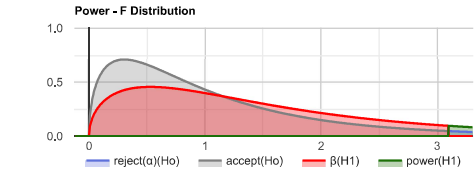
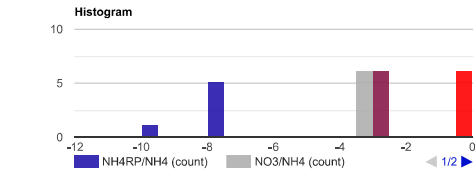
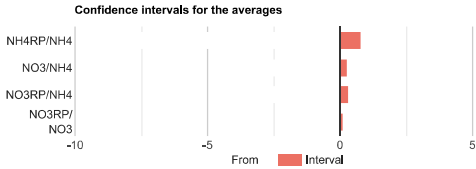
4. Effect size

The observed effect size f is **large** (12.52). That indicates that the magnitude of the difference between the averages is large.

The η^2 equals 0.99. It means that the **group** explains 99.4% of the variance from the average (similar to R² in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x1-x4, x2-x4, x3-x4**.



Validation

Test power

Although the priori power is low (0.1335), the H₀ is rejected.

Equality of variances

The tool used the Levene's test to assess the equality of variances.

The population's variances consider to be **not equal**. (p-value = 0.0303).

Levene's test power consider to be weak (0.13).

The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)

The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

Normality assumption

The assumption was checked based on the [Shapiro-Wilk Test](#). ($\alpha=0.05$)

It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	4.9054	0.08746	56.0854	4.5592	5.2516	0.3462	5.11e-13
x1-x3	4.7292	0.08746	54.071	4.383	5.0754	0.3462	5.11e-13
x1-x4	6.5805	0.08746	75.2381	6.2343	6.9267	0.3462	5.11e-13
x2-x3	0.1762	0.08746	2.0144	-0.17	0.5224	0.3462	0.4995
x2-x4	1.6752	0.08746	19.1527	1.3289	2.0214	0.3462	9.043e-11
x3-x4	1.8513	0.08746	21.1671	1.5051	2.1975	0.3462	1.531e-11

Group	x2	x3	x4
x1	4.91	4.73	6.58
x2	0	0.18	1.68
x3	0.18	0	1.85

ANOVA

What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data. ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result. The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group. The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields
H₀: The average weight of crops per square meter is equal in all fields.
H₁: At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

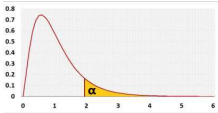
Hypotheses

H₀: $\mu_1 = \dots = \mu_k$
H₁: not($\mu_1 = \dots = \mu_k$)

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples
- Normal distribution of the analyzed population
- Equal standard deviation, $\sigma_1=\sigma_2=\dots=\sigma_k$
The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- k** - Number of groups.
- n_i** - Sample side of group i
- n** - Overall sample side, includes all the groups ($\sum n_i$, i=1 to k)
- $\bar{x}_i$** - Average of group i.
- $\bar{x}$** - Overall average ($\sum x_{i,j} / n$, i=1 to k, j=1 to n_i)
- S_i** - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	k - 1	$SSG=\sum_{i=1}^k n_i (\bar{x}_i-\bar{x})^2$	MSG = SSG / (k - 1)	F = MSG / MSE	P(x > F)
Error (within groups)	n - k	$SSE=\sum_{i=1}^k (n_i-1) S_i^2$	MSE = SSE / (n - k)		
Total	n - 1	SS(total) = SSG + SSE	Sample Variance = SS(total) / (n - 1)		



R Code

The following R code should produce the same results

```
value <-  
c(-8.2528,-7.9959,-7.7129,-7.7051,-7.3449,-7.2690,-3.0044,-2.9109,-2.8078,-2.8050,-2.6739,-2.6463,-3.1929,-3.0935,-2.9840,-2.9810,-2.8417,-2.8123,-1.2121,-1.1744,-1.1328,-1.1317,-1.0788,-1.0676)  
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))  
my.dataframe<-data.frame(value, group1)  
res.aov <- aov(value ~ group1, data = my.dataframe)  
summary(res.aov)  
TukeyHSD(res.aov)
```

[Tutorial](#)
[ANOVA](#)

Calculators

[Kruskal-Wallis test](#)
[Two way ANOVA](#)
[Levene's test](#)

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4		NO3/NH4		NO3RP/NH4		NO3RP/NO3	
Data: gene ALD5	-1.3707	-1.4529	3.0010	3.1810	1.0994	1.1654	-2.7181	-2.8811
	-1.4544	-1.5077	3.1842	3.3011	1.1665	1.2093	-2.8840	-2.9899
	-1.5562	-1.3850	3.4071	3.0323	1.2482	1.1109	-3.0859	-2.7465
Skewness:	-0.236765		0.236317		0.236578		-0.23665	
Excess kurtosis:	-1.007707		-1.010405		-1.007378		-1.009945	
Normality	0.8353		0.8346		0.8348		0.835	
Outliers								
Mean	-1.45448		3.18445		1.16662		-2.88425	
S	0.070751		0.15492		0.056748		0.1403	

- Calculate
- Validate
- Insert column
- Delete column
- Clear
- Load last run

Header: You may change groups' names to the real names.
Data: When entering data, press [Enter](#) or [⏎](#) (comma) after each value.
You may paste full column from excel.
[The calculator ignores empty cells or non-numeric cells.](#)

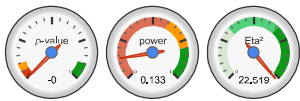
Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values
validation:success

Sample Variance = $SS(\text{total}) / (n - 1)$
Regular sample variance for all observations (not group related)
▼

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	131.6166	43.8722	3380.7497	-4.441e-16
Error (within groups)	20	0.2595	0.01298		
Total	23	131.8761	5.7337		

[R code](#)



One Way ANOVA test, using F distribution df(3,20) (right tailed)

1. H₀ hypothesis

Since p-value < α, H₀ is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals **-4.44089e-16**, [p(x ≤ F) = 1]. It means that the chance of type1 error (rejecting a correct H₀) is small: -4.441e-16 (-4.4e-14%)

The smaller the p-value the stronger it support H₁

3. The statistics

The test statistic F equals **3380.749725**, which is not in the 95% region of acceptance: [0 : 3.0984]

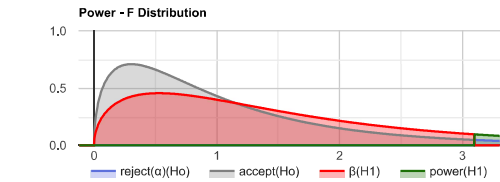
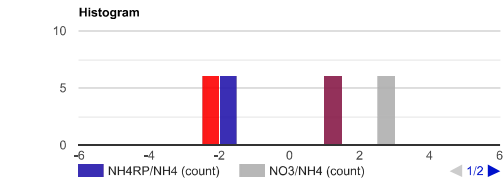
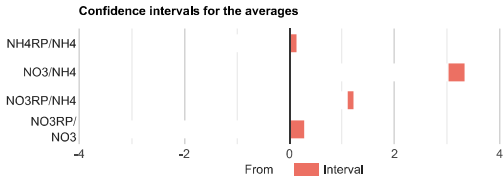
4. Effect size

The observed effect size f is **large** (22.52). That indicates that the magnitude of the difference between the averages is large.

The η² equals 1. It means that the **group** explains 99.8% of the variance from the average (similar to R² in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4**.



Validation

Test power

Although the priori power is low (0.1335), the H₀ is rejected.

Equality of variances

The tool used the Levene's test to assess the equality of variances.

The population's variances consider to be **equal**. (p-value = 0.211).

Levene's test power consider to be weak (0.13).
The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)
The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

- Normality assumption**
The assumption was checked based on the [Shapiro-Wilk Test](#). (α=0.05)
It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	4.6389	0.04651	99.7483	4.4548	4.823	0.1841	5.11e-13
x1-x3	2.6211	0.04651	56.36	2.437	2.8052	0.1841	5.11e-13
x1-x4	1.4298	0.04651	30.7435	1.2457	1.6139	0.1841	5.237e-13
x2-x3	2.0178	0.04651	43.3883	1.8337	2.2019	0.1841	5.11e-13
x2-x4	6.0687	0.04651	130.4918	5.8846	6.2528	0.1841	5.11e-13
x3-x4	4.0509	0.04651	87.1035	3.8668	4.235	0.1841	5.11e-13

Group	x2	x3	x4
x1	4.64	2.62	1.43
x2	0	2.02	6.07
x3	2.02	0	4.05

ANOVA
What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data.
ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result.
The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group.
The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields
H₀: The average weight of crops per square meter is equal in all fields.
H₁: At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

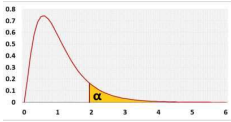
Hypotheses

H₀: μ₁ = ... = μ_k
H₁: not(μ₁ = ... = μ_k)

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples
- Normal distribution of the analyzed population
- Equal standard deviation, σ₁=σ₂=...=σ_k
The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- k** - Number of groups.
- n_i** - Sample side of group i
- n** - Overall sample side, includes all the groups (Σn_i, i=1 to k)
- $\bar{x}_i$** - Average of group i.

- $\bar{x}$ - Overall average ($\sum x_{ij} / n$, $i=1$ to k , $j=1$ to n_i)
- S_i - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	$k - 1$	$SSG = \sum_{i=1}^k n_i (\bar{x}_i - \bar{x})^2$	$MSG = SSG / (k - 1)$	$F = MSG / MSE$	$P(x > F)$
Error (within groups)	$n - k$	$SSE = \sum_{i=1}^k (n_i - 1) S_i^2$	$MSE = SSE / (n - k)$		
Total	$n - 1$	$SS(\text{total}) = SSG + SSE$	Sample Variance = $SS(\text{total}) / (n - 1)$		

R Code

The following R code should produce the same results

```
value<-
c(-1.5562,-1.5077,-1.4544,-1.4529,-1.3850,-1.3707,3.0010,3.0323,3.1810,3.1842,3.3011,3.4071,1.0994,1.1109,1.1654,1.1665,1.2093,1.2482,-3.0859,-2.9899,-2.8840,-2.8811,-2.7465,-2.7181)
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))
my.dataframe<-data.frame(value, group1)
res.aov <- aov(value ~ group1, data = my.dataframe)
summary(res.aov)
TukeyHSD(res.aov)
```

[Tutorial](#)
[ANOVA](#)

Calculators

[Kruskal-Wallis test](#)
[Two way ANOVA](#)
[Levene's test](#)

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4	NO3/NH4	NO3RP/NH4	NO3RP/NO3
Data:	-1.5087 -1.5992	-1.8914 -2.0049	-1.6572 -1.7566	1.1435 1.2121
gene ATP1	-1.6008 -1.6596	-2.0069 -2.0805	-1.7584 -1.8229	1.2133 1.2579
	-1.7129 -1.5244	-2.1474 -1.9112	-1.8815 -1.6745	1.2983 1.1555
Skewness:	-0.236463	-0.236446	-0.236615	0.23718
Excess kurtosis:	-1.010833	-1.00788	-1.008795	-1.009087
Normality	0.8339	0.8355	0.8352	0.8356
Outliers				
Mean	-1.60093	-2.00705	-1.75852	1.21343
S	0.077905	0.097632	0.085554	0.05904

Calculate

Validate

Insert column

Delete column

Clear

Load last run

Header: You may change groups' names to the real names.

Data: When entering data, press Enter or , (comma) after each value.

You may paste full column from excel.

The calculator ignores empty cells or non-numeric cells.

Group1 contains 6 values

Group2 contains 6 values

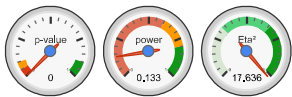
Group3 contains 6 values

Group4 contains 6 values

validation:success

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	41.0643	13.6881	2073.4568	0
Error (within groups)	20	0.132	0.006602		
Total	23	41.1963	1.7911		

[R code](#)



One Way ANOVA test, using F distribution df(3,20) (right tailed).

1. H₀ hypothesis

Since p-value< α , H₀ is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals 0, [p($x \leq F$) = 1]. It means that the chance of type1 error (rejecting a correct H₀) is small: 0 (0%)

The smaller the p-value the stronger it support H₁

3. The statistics

The test statistic F equals **2073.456849**, which is not in the 95% region of acceptance: [0 : 3.0984]

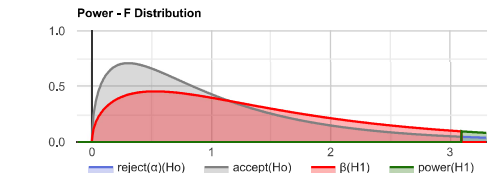
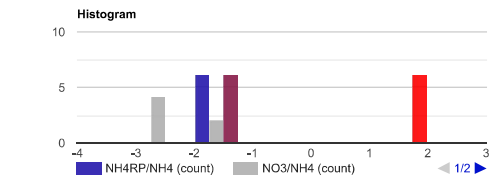
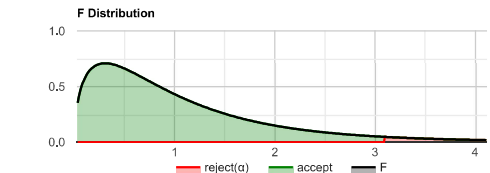
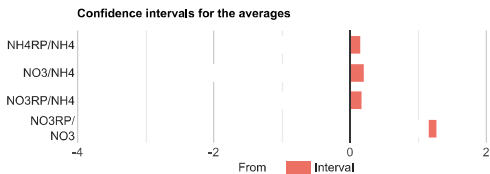
4. Effect size

The observed effect size f is **large** (17.64). That indicates that the magnitude of the difference between the averages is large.

The η^2 equals 1. It means that the **group** explains 99.7% of the variance from the average (similar to R² in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4.**



Validation

Test power

Although the priori power is low (0.1335), the H₀ is rejected.

Equality of variances

The tool used the Levene's test to assess the equality of variances.

The population's variances consider to be **equal**. (p-value = 0.789).

Levene's test power consider to be weak (0.13).

The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)

The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

● **Normality assumption**

The assumption was checked based on the [Shapiro-Wilk Test](#). ($\alpha=0.05$)

It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	0.4061	0.03317	12.2434	0.2748	0.5374	0.1313	1.918e-7
x1-x3	0.1576	0.03317	4.7508	0.02629	0.2889	0.1313	0.01519
x1-x4	2.8144	0.03317	84.8462	2.6831	2.9457	0.1313	5.11e-13
x2-x3	0.2485	0.03317	7.4927	0.1172	0.3798	0.1313	0.0001893
x2-x4	3.2205	0.03317	97.0896	3.0892	3.3518	0.1313	5.11e-13
x3-x4	2.972	0.03317	89.5969	2.8407	3.1032	0.1313	5.11e-13

Group	x2	x3	x4
x1	0.41	0.16	2.81
x2	0	0.25	3.22
x3	0.25	0	2.97

ANOVA

What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data.

ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result.

The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group.

The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields

H₀: The average weight of crops per square meter is equal in all fields.

H₁: At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

Hypotheses

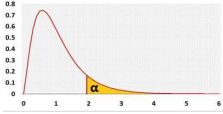
H₀: $\mu_1 = \dots = \mu_k$

H₁: $\text{not}(\mu_1 = \dots = \mu_k)$

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples
 - Normal distribution of the analyzed population
 - Equal standard deviation, $\sigma_1=\sigma_2=\dots=\sigma_k$
- The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- **k** - Number of groups.
- **n_i** - Sample side of group i
- **n** - Overall sample side, includes all the groups ($\sum n_i, i=1 \text{ to } k$)
- **$\bar{x}_i$** - Average of group i.
- **$\bar{x}$** - Overall average ($\sum x_{ij} / n, i=1 \text{ to } k, j=1 \text{ to } n_i$)
- **S_i** - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
--------	--------------------	----------------	-------------	-------------	---------

Groups (between groups)	k - 1	$SSG = \sum_{i=1}^k n_i (\bar{x}_i - \bar{x})^2$	MSG = SSG / (k - 1)	F = MSG / MSE	P(x > F)
Error (within groups)	n - k	$SSE = \sum_{i=1}^k (n_i - 1) s_i^2$	MSE = SSE / (n - k)		
Total	n - 1	SS(total) = SSG + SSE	Sample Variance = SS(total) / (n - 1)		

R Code

The following R code should produce the same results

```
value<-
c(-1.7129,-1.6596,-1.6008,-1.5992,-1.5244,-1.5087,-2.1474,-2.0805,-2.0069,-2.0049,-1.9112,-1.8914,-1.8815,-1.8229,-1.7584,-1.7566,-1.6745,-1.6572,1.1435,1.1555,1.2121,1.2133,1.2579,1.2983)
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))
my.dataframe<-data.frame(value, group1)
res.aov <- aov(value ~ group1, data = my.dataframe)
summary(res.aov)
TukeyHSD(res.aov)
```

[Tutorial](#)
[ANOVA](#)

Calculators

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[Two way ANOVA](#)
[Levene's test](#)

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4	NO3/NH4	NO3RP/NH4	NO3RP/NO3
Data: gene GAP1	18.7766	-1.1728	16.9719	19.9000
	19.9032	-1.2432	17.9902	21.0940
	19.9232	-1.2444	18.0082	21.1151
	20.6543	-1.2901	18.6691	21.8900
	21.3178	-1.3315	19.2688	22.5932
	18.9728	-1.1851	17.1493	20.1079
Skewness:	0.23613	-0.23567	0.236198	0.236177
Excess kurtosis:	-1.009695	-1.010387	-1.009628	-1.009677
Normality	0.835	0.8352	0.835	0.8349
Outliers				
Mean	19.92465	-1.24452	18.00958	21.1167
S	0.9693	0.06053	0.87611	1.02729

Calculate

Validate

Insert column

Delete column

Clear

Load last run

Header: You may change groups' names to the real names.

Data: When entering data, press Enter or , (comma) after each value.
You may paste full column from excel.

The calculator ignores empty cells or non-numeric cells.

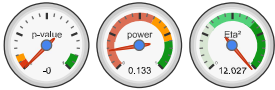
Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values
validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	2000.431	666.8103	964.2594	-2.22e-16
Error (within groups)	20	13.8305	0.6915		

Total	23	2014.2615	87.5766		
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[R code.](#)



One Way ANOVA test, using F distribution df(3,20) (right tailed)

1. H₀ hypothesis

Since p-value< α , H₀ is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals **-2.22045e-16**, [p($x \leq F$) = 1]. It means that the chance of type1 error (rejecting a correct H₀) is small: -2.22e-16 (-2.2e-14%)

The smaller the p-value the stronger it support H₁

3. The statistics

The test statistic F equals **964.259421**, which is not in the 95% region of acceptance: [0 : 3.0984]

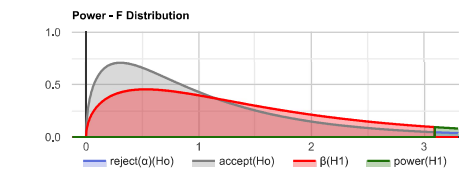
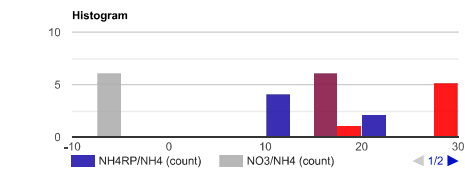
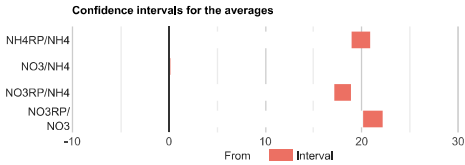
4. Effect size

The observed effect size f is **large** (12.03). That indicates that the magnitude of the difference between the averages is large.

The η^2 equals 0.99. It means that the **group** explains 99.3% of the variance from the average (similar to R² in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x2-x3, x2-x4, x3-x4**.



Validation

● **Test power**

Although the priori power is low (0.1335), the H₀ is rejected.

● **Equality of variances**

The tool used the Levene's test to assess the equality of variances.

The population's variances consider to be **equal**. (p-value = 0.0791).

Levene's test power consider to be weak (0.13).

The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)

The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

● **Normality assumption**

The assumption was checked based on the [Shapiro-Wilk Test](#). (α =0.05)

It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
------	------------	----	---	----------	----------	---------------	---------

x1-x2	21.1692	0.3395	62.3556	19.8254	22.513	1.3438	5.11e-13
x1-x3	1.9151	0.3395	5.641	0.5713	3.2589	1.3438	0.003709
x1-x4	1.1921	0.3395	3.5113	-0.1518	2.5359	1.3438	0.09352
x2-x3	19.2541	0.3395	56.7146	17.9103	20.5979	1.3438	5.11e-13
x2-x4	22.3612	0.3395	65.8668	21.0174	23.705	1.3438	5.11e-13
x3-x4	3.1071	0.3395	9.1523	1.7633	4.4509	1.3438	0.00001455

Group	x2	x3	x4
x1	21.17	1.92	1.19
x2	0	19.25	22.36
x3	19.25	0	3.11

ANOVA

What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data.

ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result.

The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group. The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields

H₀: The average weight of crops per square meter is equal in all fields.

H₁: At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

Hypotheses

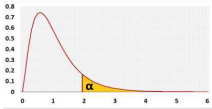
H₀: μ₁ = ... = μ_k

H₁: not(μ₁ = ... = μ_k)

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples
 - Normal distribution of the analyzed population
 - Equal standard deviation, σ₁=σ₂=...=σ_k
- The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- k** - Number of groups.
- n_i** - Sample size of group i
- n** - Overall sample size, includes all the groups (Σn_i, i=1 to k)
- x̄_i** - Average of group i.
- x̄** - Overall average (Σx_{ij} / n, i=1 to k, j=1 to n_i)
- S_i** - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	k - 1	$SSG=\sum_{i=1}^k n_i (\bar{x}_i-\bar{x})^2$	$MSG = SSG / (k - 1)$	$F = MSG / MSE$	P(x > F)
Error (within groups)	n - k	$SSE=\sum_{i=1}^k (n_i-1) S_i^2$	$MSE = SSE / (n - k)$		
Total	n - 1	SS(total) = SSG + SSE	Sample Variance = SS(total) / (n - 1)		

R Code

The following R code should produce the same results

```
value<-
c(18.7766,18.9728,19.9032,19.9232,20.6543,21.3178,-1.3315,-1.2901,-1.2444,-1.2432,-1.1851,-1.1728,16.9719,17.1493,17.9902,18.0082,18.6691,19.2688,19.9000,20.1079,21.0940,21.1151,21.8900,22.5932)
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))
```

```
my.dataframe<-data.frame(value, group1)
res.aov <- aov(value ~ group1, data = my.dataframe)
summary(res.aov)
TukeyHSD(res.aov)
```

[Tutorial](#)

[ANOVA](#)

Calculators

[Kruskal-Wallis test](#)

[Two way ANOVA](#)

[Levene's test](#)

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4		NO3/NH4		NO3RP/NH4		NO3RP/NO3	
Data: gene GDH1	-1.3652	-1.4471	4.6758	4.9564	2.2472	2.3820	-2.0825	-2.2074
	-1.4486	-1.5017	4.9613	5.1434	2.3844	2.4719	-2.2096	-2.2907
	-1.5500	-1.3795	5.3086	4.7247	2.5513	2.2707	-2.3643	-2.1042
Skewness:	-0.236601		0.236032		0.236365		-0.23663	
Excess kurtosis:	-1.007548		-1.009748		-1.00941		-1.009537	
Normality	0.8361		0.835		0.8352		0.8344	
Outliers								
Mean	-1.44868		4.9617		2.38458		-2.20978	
S	0.070476		0.24137		0.11599		0.1075	

Calculate

Validate

Insert column

Delete column

Clear

Load last run

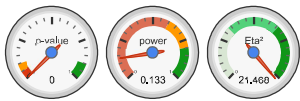
Header: You may change groups' names to the real names.
Data: When entering data, press Enter or , (comma) after each value.
You may paste full column from excel.
The calculator ignores empty cells or non-numeric cells.

Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values
validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	203.3192	67.7731	3072.4188	3.331e-16
Error (within groups)	20	0.4412	0.02206		
Total	23	203.7603	8.8591		

[R code](#)



One Way ANOVA test, using F distribution df(3,20) (right tailed)

1. H₀ hypothesis

Since p-value < α , H₀ is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals **3.33067e-16**, [$p(x \leq F) = 1$]. It means that the chance of type1 error (rejecting a correct H₀) is small: 3.331e-16 (3.3e-14%)

The smaller the p-value the stronger it support H₁

3. The statistics

The test statistic F equals **3072.418771**, which is not in the 95% region of acceptance: [0 : 3.0984]

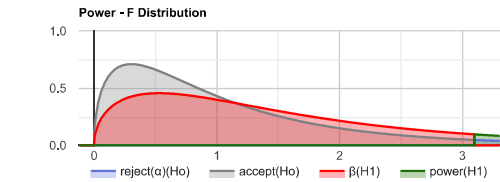
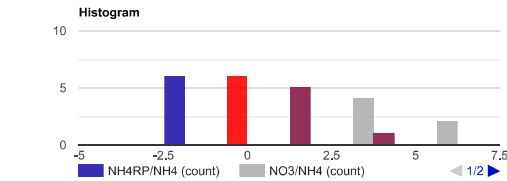
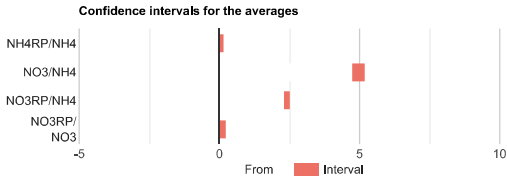
4. Effect size

The observed effect size f is **large** (21.47). That indicates that the magnitude of the difference between the averages is large.

The η^2 equals 1. It means that the **group** explains 99.8% of the variance from the average (similar to R² in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4**.



Validation

Test power

Although the priori power is low (0.1335), the H₀ is rejected.

Equality of variances

The tool used the Levene's test to assess the equality of variances.

The population's variances consider to be **equal**. (p-value = 0.118).

Levene's test power consider to be weak (0.13).
The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)
The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

- Normality assumption**
The assumption was checked based on the [Shapiro-Wilk Test](#). (α=0.05)
It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	6.4104	0.06063	105.7234	6.1704	6.6504	0.24	5.11e-13
x1-x3	3.8333	0.06063	63.2203	3.5933	4.0733	0.24	5.11e-13
x1-x4	0.7611	0.06063	12.5525	0.5211	1.0011	0.24	1.287e-7
x2-x3	2.5771	0.06063	42.5032	2.3371	2.8171	0.24	5.11e-13
x2-x4	7.1715	0.06063	118.2759	6.9315	7.4115	0.24	5.11e-13
x3-x4	4.5944	0.06063	75.7727	4.3544	4.8344	0.24	5.11e-13

Group	x2	x3	x4
x1	6.41	3.83	0.76
x2	0	2.58	7.17
x3	2.58	0	4.59

ANOVA
What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data.
ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result.
The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group.
The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields
H₀: The average weight of crops per square meter is equal in all fields.
H₁: At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

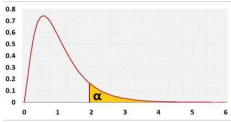
Hypotheses

H₀: μ₁ = ... = μ_k
H₁: not(μ₁ = ... = μ_k)

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples
- Normal distribution of the analyzed population
- Equal standard deviation, σ₁=σ₂=...=σ_k
The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- k** - Number of groups.
- n_i** - Sample side of group i
- n** - Overall sample side, includes all the groups (Σn_i, i=1 to k)
- $\bar{x}_i$** - Averse of group i.

- $\bar{x}$ - Overall average ($\sum x_{ij} / n$, $i=1$ to k , $j=1$ to n_i)
- S_i - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	$k - 1$	$SSG = \sum_{i=1}^k n_i (\bar{x}_i - \bar{x})^2$	$MSG = SSG / (k - 1)$	$F = MSG / MSE$	$P(x > F)$
Error (within groups)	$n - k$	$SSE = \sum_{i=1}^k (n_i - 1) S_i^2$	$MSE = SSE / (n - k)$		
Total	$n - 1$	$SS(\text{total}) = SSG + SSE$	Sample Variance = $SS(\text{total}) / (n - 1)$		

R Code

The following R code should produce the same results

```
value<-
c(-1.5500,-1.5017,-1.4486,-1.4471,-1.3795,-1.3652,4.6758,4.7247,4.9564,4.9613,5.1434,5.3086,2.2472,2.2707,2.3820,2.3844,2.4719,2.5513,-2.3643,-2.2907,-2.2096,-2.2074,-2.1042,-2.0825)
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))
my.dataframe<-data.frame(value, group1)
res.aov <- aov(value ~ group1, data = my.dataframe)
summary(res.aov)
TukeyHSD(res.aov)
```

[Tutorial](#)
[ANOVA](#)

Calculators

[Kruskal-Wallis test](#)
[Two way ANOVA](#)
[Levene's test](#)

Statistics Kingdom

[Home](#) > [M/ANOVA](#) > One-Way ANOVA

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4	NO3/NH4	NO3RP/NH4	NO3RP/NO3
Data:	-7.3219 -7.7612	4.5315 4.8034	-2.4868 -2.6360	-11.2691 -11.9452
gene GDH2	-7.7690 -8.0541	4.8082 4.9847	-2.6387 -2.7355	-11.9572 -12.3960
	-8.3128 -7.3984	5.1448 4.5789	-2.8234 -2.5128	-12.7942 -11.3868
Skewness:	-0.236103	0.236203	-0.236202	-0.236186
Excess kurtosis:	-1.009842	-1.009742	-1.00934	-1.009752
Normality	0.835	0.8351	0.8354	0.8349
Outliers				
Mean	-7.76957	4.80858	-2.63887	-11.95808
S	0.37797	0.23393	0.12839	0.58174

Calculate

Validate

Insert column

Delete column

Clear

Load last run

Header: You may change groups' names to the real names.

Data: When entering data, press Enter or , (comma) after each value.
You may paste full column from excel.

The calculator ignores empty cells or non-numeric cells.

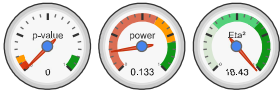
Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values
validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	938.2665	312.7555	2264.3548	0

Error (within groups)	20	2.7624	0.1381		
Total	23	941.0289	40.9143		

[R code](#)



One Way ANOVA test, using F distribution df(3,20).(right tailed).

1. H₀ hypothesis

Since $p\text{-value} < \alpha$, H_0 is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals 0, $[p(x \leq F) = 1]$. It means that the chance of type1 error (rejecting a correct H_0) is small: 0 (0%)

The smaller the p-value the stronger it support H_1

3. The statistics

The test statistic F equals **2264.354792**, which is not in the 95% region of acceptance: [0 : 3.0984]

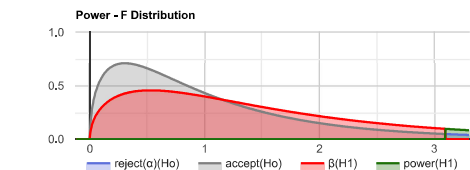
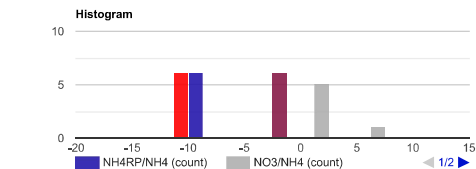
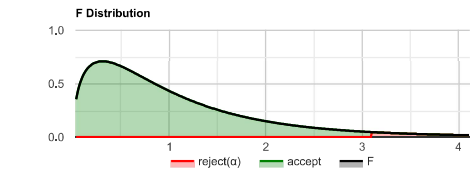
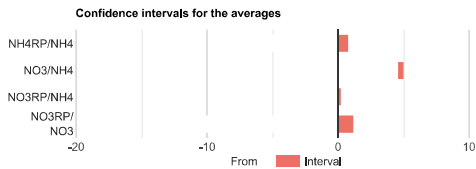
4. Effect size

The observed effect size f is **large** (18.43). That indicates that the magnitude of the difference between the averages is large.

The η^2 equals 1. It means that the **group** explains 99.7% of the variance from the average (similar to R^2 in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4**.



Validation

● **Test power**

Although the priori power is low (0.1335), the H_0 is rejected.

● **Equality of variances**

The tool used the Levene's test to assess the equality of variances.

The population's variances consider to be **equal**. (p-value = 0.0922).

Levene's test power consider to be weak (0.13).

The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)

The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

● **Normality assumption**

The assumption was checked based on the [Shapiro-Wilk Test](#). ($\alpha=0.05$)

It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	12.5782	0.1517	82.9015	11.9776	13.1787	0.6006	5.11e-13
x1-x3	5.1307	0.1517	33.816	4.5301	5.7313	0.6006	5.137e-13
x1-x4	4.1885	0.1517	27.6061	3.5879	4.7891	0.6006	5.979e-13
x2-x3	7.4474	0.1517	49.0855	6.8469	8.048	0.6006	5.11e-13
x2-x4	16.7667	0.1517	110.5076	16.1661	17.3672	0.6006	5.11e-13
x3-x4	9.3192	0.1517	61.4221	8.7186	9.9198	0.6006	5.11e-13

Group	x2	x3	x4
x1	12.58	5.13	4.19
x2	0	7.45	16.77
x3	7.45	0	9.32

ANOVA

What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data. ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result. The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group. The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields
H₀: The average weight of crops per square meter is equal in all fields.
H₁: At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

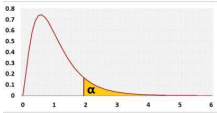
Hypotheses

H₀: μ₁ = ... = μ_k
H₁: not(μ₁ = ... = μ_k)

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples
- Normal distribution of the analyzed population
- Equal standard deviation, σ₁=σ₂=...=σ_k
The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- k** - Number of groups.
- n_i** - Sample side of group i
- n** - Overall sample side, includes all the groups (Σn_i, i=1 to k)
- $\bar{x}_i$** - Average of group i.
- $\bar{x}$** - Overall average (Σx_{i,j} / n, i=1 to k, j=1 to n_i)
- S_i** - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	k - 1	$SSG = \sum_{i=1}^k n_i (\bar{x}_i - \bar{x})^2$	MSG = SSG / (k - 1)	F = MSG / MSE	P(x > F)
Error (within groups)	n - k	$SSE = \sum_{i=1}^k (n_i - 1) S_i^2$	MSE = SSE / (n - k)		
Total	n - 1	SS(total) = SSG + SSE	Sample Variance = SS(total) / (n - 1)		

R Code

The following R code should produce the same results

```
value<-  
c(-8.3128,-8.0541,-7.7690,-7.7612,-7.3984,-7.3219,4.5315,4.5789,4.8034,4.8082,4.9847,5.1448,-2.8234,-2.7355,-2.6387,-2.6360,-2.5128,-2.4868,-12.7942,-12.3960,-11.9572,-11.9452,-11.3868,-11.2691)  
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))  
my.dataframe<-data.frame(value, group1)  
res.aov <- aov(value ~ group1, data = my.dataframe)  
summary(res.aov)  
TukeyHSD(res.aov)
```

[Tutorial](#)

[ANOVA](#)

Calculators

[Kruskal-Wallis test](#)

[Two way ANOVA](#)

[Levene's test](#)

Statistics Kingdom

[Home](#) > [M/ANOVA](#) > One-Way ANOVA

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4	NO3/NH4	NO3RP/NH4	NO3RP/NO3
Data: gene GLT1	-32.7840 -34.7511 -34.7859 -36.0625 -37.2209 -33.1266	202.4339 214.5799 214.7947 222.6773 229.8304 204.5490	112.4239 119.1694 119.2887 123.6663 127.6389 113.5986	-1.7746 -1.8811 -1.8830 -1.9521 -2.0148 -1.7932
Skewness:	-0.236113	0.236155	0.236143	-0.235964
Excess kurtosis:	-1.009757	-1.009702	-1.009686	-1.009549
Normality	0.835	0.835	0.835	0.8357
Outliers				
Mean	-34.7885	214.81087	119.29763	-1.88313
S	1.69239	10.45003	5.80354	0.091612

Calculate

Validate

Insert column

Delete column

Clear

Load last run

Header: You may change groups' names to the real names.

Data: When entering data, press **Enter** or **,** (comma) after each value.
You may paste full column from excel.

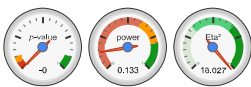
The calculator ignores empty cells or non-numeric cells.

Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values
validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	236833.4837	78944.4946	2166.47	-2.22e-16
Error (within groups)	20	728.7846	36.4392		
Total	23	237562.2683	10328.7943		

[R code.](#)



One Way ANOVA test, using F distribution df(3,20) (right tailed)

1. H0 hypothesis

Since p-value< α , H0 is rejected.
Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals **-2.22045e-16**, [$p(x \leq F) = 1$]. It means that the chance of type1 error (rejecting a correct H0) is small: -2.22e-16 (-2.2e-14%)
The smaller the p-value the stronger it support H1

3. The statistics

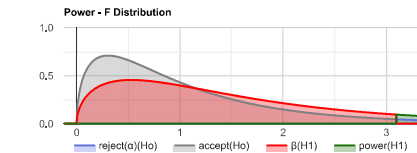
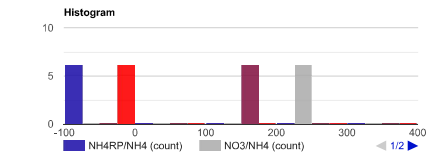
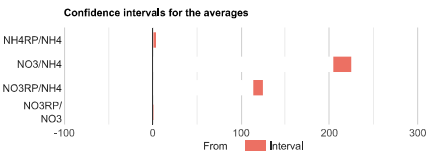
The test statistic F equals **2166.46997**, which is not in the 95% region of acceptance: [0 : 3.0984]

4. Effect size

The observed effect size f is **large** (18.03). That indicates that the magnitude of the difference between the averages is large.
The η^2 equals 1. It means that the **group** explains 99.7% of the variance from the average (similar to R² in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4**.



Validation

Test power

Although the priori power is low (0.1335), the H0 is rejected.

Equality of variances

The tool used the Levene's test to assess the equality of variances.
The population's variances consider to be **not equal** (p-value = 0.00807).
Levene's test power consider to be weak (0.13).
The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)
The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**, non-parametric test.

Normality assumption

The assumption was checked based on the [Shapiro-Wilk Test](#), ($\alpha=0.05$)
It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	249.5994	2.4644	101.2825	239.8446	259.3541	9.7548	5.11e-13
x1-x3	154.0861	2.4644	62.5251	144.3314	163.8409	9.7548	5.11e-13
x1-x4	32.9054	2.4644	13.3524	23.1506	42.6601	9.7548	4.711e-8
x2-x3	95.5132	2.4644	38.7574	85.7585	105.268	9.7548	5.113e-13
x2-x4	216.694	2.4644	87.9302	206.9392	226.4488	9.7548	5.11e-13
x3-x4	121.1808	2.4644	49.1728	111.426	130.9355	9.7548	5.11e-13

Group	x2	x3	x4
x1	249.6	154.09	32.91
x2	0	95.51	216.69

x3	95.51	0	121.18

ANOVA

What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data.

ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result.

The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group.

The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields

H₀: The average weight of crops per square meter is equal in all fields.

H₁: At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

Hypotheses

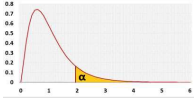
H₀: $\mu_1 = \dots = \mu_k$

H₁: **not**($\mu_1 = \dots = \mu_k$)

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



- ## Assumptions
- Independent samples
 - Normal distribution of the analyzed population
 - Equal standard deviation, $\sigma_1=\sigma_2=\dots=\sigma_k$
- The assumption is more important when the groups' sizes not similar

- ## Required Sample Data
- Sample data from all compared groups

- ## Parameters
- k** - Number of groups.
 - n_i** - Sample side of group i
 - n** - Overall sample side, includes all the groups ($\sum n_i$, i=1 to k)
 - $\bar{x}_i$** - Average of group i.
 - $\bar{x}$** - Overall average ($\sum x_{ij} / n$, i=1 to k, j=1 to n_i)
 - S_i** - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	k - 1	$SSG=\sum_{i=1}^k n_i (\bar{x}_i-\bar{x})^2$	$MSG = SSG / (k - 1)$	$F = MSG / MSE$	$P(x > F)$
Error (within groups)	n - k	$SSE=\sum_{i=1}^k (n_i-1) S_i^2$	$MSE = SSE / (n - k)$		
Total	n - 1	$SS(total) = SSG + SSE$	$Sample\ Variance = SS(total) / (n - 1)$		

R Code

The following R code should produce the same results

```
value<-
c(-37.2209,-36.0625,-34.7859,-34.7511,-33.1266,-32.7840,202.4339,204.5490,214.5799,214.7947,222.6773,229.8304,112.4239,113.5986,119.1694,119.2887,123.6663,127.6389,-2.0148,-1.9521,-1.8830,-1.8811,-1.7932,-1.7744)
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))
my.dataframe<-data.frame(value, group1)
res.aov <- aov(value ~ group1, data = my.dataframe)
summary(res.aov)
TukeyHSD(res.aov)
```

- [Tutorial](#)
- [ANOVA](#)
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 - [Two way ANOVA](#)
 - [Levene's test](#)

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4		NO3/NH4		NO3RP/NH4		NO3RP/NO3	
Data: gene MEP1	-7.5184	-7.9695	1.8408	1.9512	1.0007	1.0607	-1.8444	-1.9551
	-7.9775	-8.2702	1.9532	2.0249	1.0618	1.1007	-1.9570	-2.0289
	-8.5359	-7.5969	2.0899	1.8600	1.1361	1.0111	-2.0940	-1.8637
Skewness:	-0.236107		0.23613		0.236475		-0.235772	
Excess kurtosis:	-1.009664		-1.010952		-1.008603		-1.011117	
Normality	0.8349		0.8347		0.8346		0.8347	
Outliers								
Mean	-7.97807		1.95333		1.06185		-1.95718	
S	0.38812		0.095028		0.05165		0.095211	

- Calculate
- Validate
- Insert column
- Delete column
- Clear
- Load last run

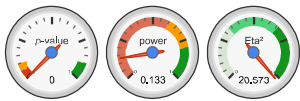
Header: You may change groups' names to the real names.
Data: When entering data, press [Enter](#) or [, \(comma\)](#) after each value.
You may paste full column from excel.
[The calculator ignores empty cells or non-numeric cells.](#)

Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values
validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	362.7079	120.9026	2821.5648	1.11e-16
Error (within groups)	20	0.857	0.04285		
Total	23	363.5649	15.8072		

[R code](#)



One Way ANOVA test, using F distribution df(3,20) (right tailed)

1. H₀ hypothesis

Since p-value < α , H₀ is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals **1.11022e-16**, [$p(x \leq F) = 1$]. It means that the chance of type1 error (rejecting a correct H₀) is small: 1.11e-16 (1.1e-14%)

The smaller the p-value the stronger it support H₁

3. The statistics

The test statistic F equals **2821.56482**, which is not in the 95% region of acceptance: [0 : 3.0984]

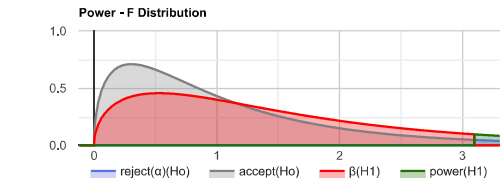
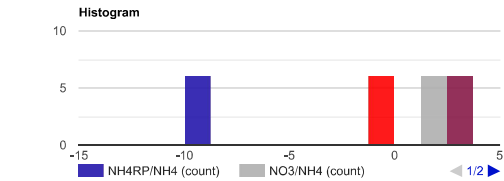
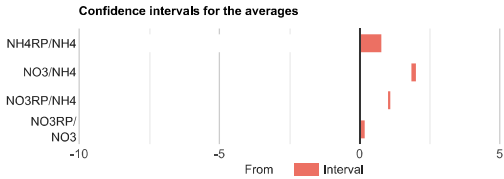
4. Effect size

The observed effect size f is **large** (20.57). That indicates that the magnitude of the difference between the averages is large.

The η^2 equals 1. It means that the **group** explains 99.8% of the variance from the average (similar to R² in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4**.



Validation

Test power

Although the priori power is low (0.1335), the H₀ is rejected.

Equality of variances

The tool used the Levene's test to assess the equality of variances.

The population's variances consider to be **not equal**. (p-value = 0.00984).

Levene's test power consider to be weak (0.13).
The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)
The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

- Normality assumption**
The assumption was checked based on the [Shapiro-Wilk Test](#). ($\alpha=0.05$)
It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	9.9314	0.08451	117.5204	9.5969	10.2659	0.3345	5.11e-13
x1-x3	9.0399	0.08451	106.9713	8.7054	9.3744	0.3345	5.11e-13
x1-x4	6.0209	0.08451	71.2464	5.6864	6.3554	0.3345	5.11e-13
x2-x3	0.8915	0.08451	10.5491	0.557	1.226	0.3345	0.000001905
x2-x4	3.9105	0.08451	46.274	3.576	4.245	0.3345	5.11e-13
x3-x4	3.019	0.08451	35.7249	2.6845	3.3535	0.3345	5.121e-13

Group	x2	x3	x4
x1	9.93	9.04	6.02
x2	0	0.89	3.91
x3	0.89	0	3.02

ANOVA

What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data.
ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result.
The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group.
The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields
 H_0 : The average weight of crops per square meter is equal in all fields.
 H_1 : At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

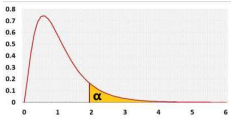
Hypotheses

$H_0: \mu_1 = \dots = \mu_k$
 $H_1: \text{not}(\mu_1 = \dots = \mu_k)$

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples
- Normal distribution of the analyzed population
- Equal standard deviation, $\sigma_1=\sigma_2=\dots=\sigma_k$
The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- k - Number of groups.
- n_i - Sample side of group i
- n - Overall sample side, includes all the groups ($\sum n_i, i=1$ to k)
- $\bar{x}_i$ - Averse of group i .

- $\bar{x}$ - Overall average ($\sum x_{ij} / n$, $i=1$ to k , $j=1$ to n_i)
- S_i - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	$k - 1$	$SSG = \sum_{i=1}^k n_i (\bar{x}_i - \bar{x})^2$	$MSG = SSG / (k - 1)$	$F = MSG / MSE$	$P(x > F)$
Error (within groups)	$n - k$	$SSE = \sum_{i=1}^k (n_i - 1) S_i^2$	$MSE = SSE / (n - k)$		
Total	$n - 1$	$SS(\text{total}) = SSG + SSE$	Sample Variance = $SS(\text{total}) / (n - 1)$		

R Code

The following R code should produce the same results

```
value<-
c(-8.5359,-8.2702,-7.9775,-7.9695,-7.5969,-7.5184,1.8408,1.8600,1.9512,1.9532,2.0249,2.0899,1.0007,1.0111,1.0607,1.0618,1.1007,1.1361,-2.0940,-2.0289,-1.9570,-1.9551,-1.8637,-1.8444)
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))
my.dataframe<-data.frame(value, group1)
res.aov <- aov(value ~ group1, data = my.dataframe)
summary(res.aov)
TukeyHSD(res.aov)
```

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[ANOVA](#)

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[Two way ANOVA](#)
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[Home](#) > [M/ANOVA](#) > One-Way ANOVA

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4	NO3/NH4	NO3RP/NH4	NO3RP/NO3
Data:	-1.7901 -1.8975	7.2424 7.6769	-1.3880 -1.4713	-10.0089 -10.6095
gene PDC1	-1.8994 -1.9691	7.6846 7.9666	-1.4728 -1.5268	-10.6201 -11.0098
	-2.0323 -1.8088	8.2225 7.3181	-1.5759 -1.4025	-11.3635 -10.1135
Skewness:	-0.235642	0.236222	-0.236126	-0.236065
Excess kurtosis:	-1.010786	-1.009586	-1.008597	-1.009615
Normality	0.8349	0.8351	0.8353	0.835
Outliers				
Mean	-1.89953	7.68518	-1.47288	-10.62088
S	0.092389	0.37384	0.071667	0.51669

Calculate

Validate

Insert column

Delete column

Clear

Load last run

Header: You may change groups' names to the real names.

Data: When entering data, press Enter or , (comma) after each value.
You may paste full column from excel.

The calculator ignores empty cells or non-numeric cells.

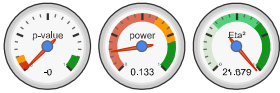
Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values
validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	1006.1683	335.3894	3191.1934	-2.22e-16

Error (within groups)	20	2.102	0.1051		
Total	23	1008.2703	43.8378		

[R code](#)



One Way ANOVA test, using F distribution df(3,20).(right tailed).

1. H₀ hypothesis

Since $p\text{-value} < \alpha$, H_0 is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals **-2.22045e-16**, $[p(x \leq F) = 1]$. It means that the chance of type1 error (rejecting a correct H_0) is small: -2.22e-16 (-2.2e-14%)

The smaller the p-value the stronger it support H_1

3. The statistics

The test statistic F equals **3191.193402**, which is not in the 95% region of acceptance: [0 : 3.0984]

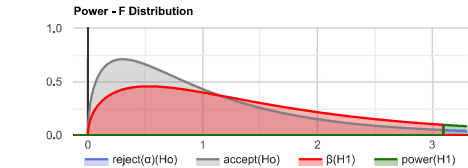
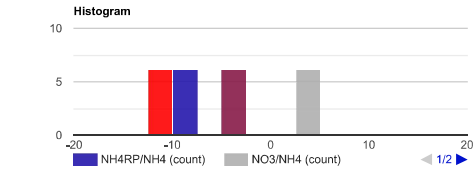
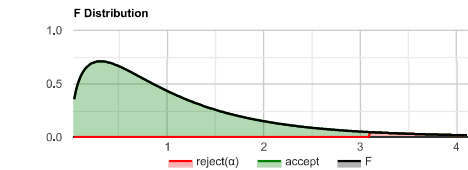
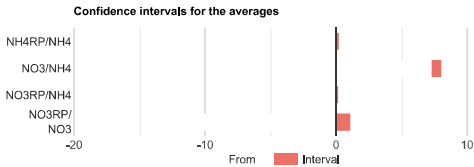
4. Effect size

The observed effect size f is **large** (21.88). That indicates that the magnitude of the difference between the averages is large.

The η^2 equals 1. It means that the **group** explains 99.8% of the variance from the average (similar to R^2 in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x4, x2-x3, x2-x4, x3-x4**.



Validation

Test power

Although the priori power is low (0.1335), the H_0 is rejected.

Equality of variances

The tool used the Levene's test to assess the equality of variances.

The population's variances consider to be **not equal**. (p-value = 0.0223).

Levene's test power consider to be weak (0.13).

The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)

The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

Normality assumption

The assumption was checked based on the [Shapiro-Wilk Test](#). ($\alpha=0.05$)

It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	9.5847	0.1323	72.4197	9.0608	10.1086	0.5239	5.11e-13
x1-x3	0.4266	0.1323	3.2237	-0.09723	0.9505	0.5239	0.1366
x1-x4	8.7213	0.1323	65.8963	8.1975	9.2452	0.5239	5.11e-13
x2-x3	9.1581	0.1323	69.196	8.6342	9.6819	0.5239	5.11e-13
x2-x4	18.3061	0.1323	138.316	17.7822	18.8299	0.5239	5.11e-13
x3-x4	9.148	0.1323	69.12	8.6241	9.6719	0.5239	5.11e-13

Group	x2	x3	x4
x1	9.58	0.43	8.72
x2	0	9.16	18.31
x3	9.16	0	9.15

ANOVA

What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data.

ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result.

The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group. The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields

H₀: The average weight of crops per square meter is equal in all fields.

H₁: At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

Hypotheses

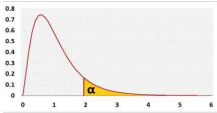
H₀: μ₁ = ... = μ_k

H₁: not(μ₁ = ... = μ_k)

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples
 - Normal distribution of the analyzed population
 - Equal standard deviation, σ₁=σ₂=...=σ_k
- The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- k** - Number of groups.
- n_i** - Sample side of group i
- n** - Overall sample side, includes all the groups (Σn_i, i=1 to k)
- $\bar{x}_i$** - Average of group i.
- $\bar{x}$** - Overall average (Σx_{i,j} / n, i=1 to k, j=1 to n_i)
- S_i** - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	k - 1	$SSG=\sum_{i=1}^k n_i (\bar{x}_i-\bar{x})^2$	$MSG = SSG / (k - 1)$	F = MSG / MSE	P(x > F)
Error (within groups)	n - k	$SSE=\sum_{i=1}^k (n_i-1) S_i^2$	$MSE = SSE / (n - k)$		
Total	n - 1	SS(total) = SSG + SSE	Sample Variance = SS(total) / (n - 1)		

R Code

The following R code should produce the same results

```
value<-  
c(-2.0323,-1.9691,-1.8994,-1.8975,-1.8088,-1.7901,7.2424,7.3181,7.6769,7.6846,7.9666,8.2225,-1.5759,-1.5268,-1.4728,-1.4713,-1.4025,-1.3880,-11.3635,-11.0098,-10.6201,-10.6095,-10.1135,-10.0089)  
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))  
my.dataframe<-data.frame(value, group1)  
res.aov <- aov(value ~ group1, data = my.dataframe)  
summary(res.aov)  
TukeyHSD(res.aov)
```

[Tutorial](#)

[ANOVA](#)

Calculators

[Kruskal-Wallis test](#)

[Two way ANOVA](#)

[Levene's test](#)

Statistics Kingdom

[Home](#) > [M/ANOVA](#) > One-Way ANOVA

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):	0,05
Outliers:	Included
Effect:	Medium
Effect type:	f
Effect Size:	0.25
Rounding:	4

- ☒ Enter raw data directly
☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4		NO3/NH4		NO3RP/NH4		NO3RP/NO3	
Data: gene PUT4	6.9811	7.4000	5.0404	5.3429	6.3301	6.7099	1.3081	1.3865
	7.4074	7.6793	5.3482	5.5445	6.7166	6.9631	1.3879	1.4389
	7.9259	7.0541	5.7226	5.0931	7.1867	6.3962	1.4851	1.3217
Skewness:	0.236004		0.235999		0.235954		0.237282	
Excess kurtosis:	-1.010107		-1.009801		-1.010246		-1.01063	
Normality	0.835		0.835		0.8348		0.8339	
Outliers								
Mean	7.40797		5.34862		6.7171		1.38803	
S	0.36038		0.26021		0.32675		0.06753	

Header: You may change groups' names to the real names.

Data: When entering data, press or (comma) after each value.
You may paste full column from excel.

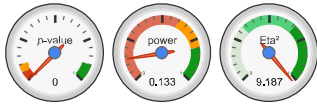
The calculator ignores empty cells or non-numeric cells.

Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values
validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	130.3736	43.4579	562.7186	0
Error (within groups)	20	1.5446	0.07723		
Total	23	131.9182	5.7356		

[R code.](#)



One Way ANOVA test, using F distribution df(3,20) (right tailed)

1. H₀ hypothesis

Since p-value< α , H₀ is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals 0, [p(x ≤ F) = 1]. It means that the chance of type1 error (rejecting a correct H₀) is small: 0 (0%)

The smaller the p-value the stronger it support H₁

3. The statistics

The test statistic F equals 562.718572, which is not in the 95% region of acceptance: [0 : 3.0984]

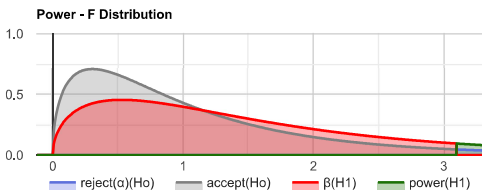
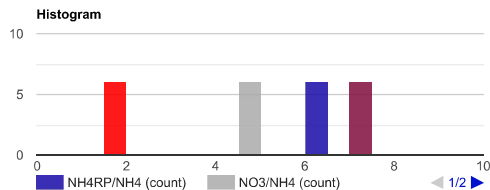
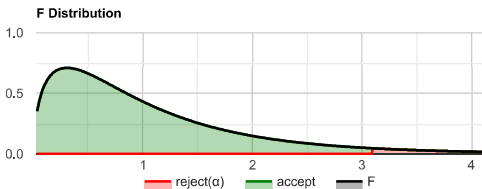
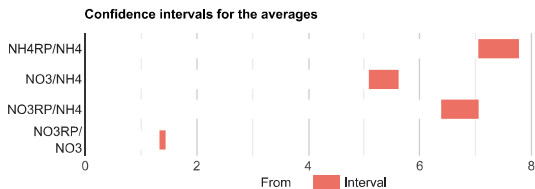
4. Effect size

The observed effect size f is large (9.19). That indicates that the magnitude of the difference between the averages is large.

The η^2 equals 0.99. It means that the group explains 98.8% of the variance from the average (similar to R² in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4.



Validation

●

Test power

Although the priori power is low (0.1335), the H_0 is rejected.

●

Equality of variances

The tool used the Levene's test to assess the equality of variances.
The population's variances consider to be **equal**. (p-value = 0.153).
Levene's test power consider to be weak (0.13).
The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)
The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

●

Normality assumption

The assumption was checked based on the [Shapiro-Wilk Test](#). ($\alpha=0.05$)
It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	2.0594	0.1135	18.1517	1.6103	2.5084	0.4491	2.363e-10
x1-x3	0.6909	0.1135	6.0895	0.2418	1.1399	0.4491	0.001801
x1-x4	6.0199	0.1135	53.0614	5.5709	6.469	0.4491	5.11e-13
x2-x3	1.3685	0.1135	12.0622	0.9194	1.8176	0.4491	2.431e-7
x2-x4	3.9606	0.1135	34.9097	3.5115	4.4097	0.4491	5.126e-13
x3-x4	5.3291	0.1135	46.9719	4.88	5.7781	0.4491	5.11e-13

Group	x2	x3	x4
x1	2.06	0.69	6.02
x2	0	1.37	3.96
x3	1.37	0	5.33

ANOVA

What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data.
ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result.
The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group.
The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example:

Compare four fertilizers used in four fields

H_0 : The average weight of crops per square meter is equal in all fields.
 H_1 : At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

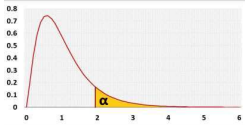
Hypotheses

$H_0: \mu_1 = \dots = \mu_k$
 $H_1: \text{not}(\mu_1 = \dots = \mu_k)$

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples

- Normal distribution of the analyzed population
 - Equal standard deviation, $\sigma_1=\sigma_2=...=\sigma_k$
- The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- **k** - Number of groups.
- **n_i** - Sample side of group i
- **n** - Overall sample side, includes all the groups ($\sum n_i$, i=1 to k)
- **$\bar{x}_i$** - Average of group i.
- **$\bar{x}$** - Overall average ($\sum x_{ij} / n$, i=1 to k, j=1 to n_i)
- **S_i** - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	k - 1	$SSG=\sum_{i=1}^k n_i (\bar{x}_i-\bar{x})^2$	MSG = SSG / (k - 1)	F = MSG / MSE	P(x > F)
Error (within groups)	n - k	$SSE=\sum_{i=1}^k (n_i-1) S_i^2$	MSE = SSE / (n - k)		
Total	n - 1	SS(total) = SSG + SSE	Sample Variance = SS(total) / (n - 1)		



R Code

The following R code should produce the same results

```
value<-
c(6.9811,7.0541,7.4000,7.4074,7.6793,7.9259,5.0404,5.0931,5.3429,5.3482,5.5445,5.7226,6.3301,6.3962,6.7099,6.7166,6.9631,7.1867,1.3081,1.3217,1.3865,1.3879,1.4389,1.4851)
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))
my.dataframe<-data.frame(value, group1)
res.aov <- aov(value ~ group1, data = my.dataframe)
summary(res.aov)
TukeyHSD(res.aov)
```

[Tutorial](#)
[ANOVA](#)

Calculators

[Kruskal-Wallis test](#)
[Two way ANOVA](#)
[Levene's test](#)

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4		NO3/NH4		NO3RP/NH4		NO3RP/NO3	
Data:	1.4904	1.5798	25.2838	26.8008	3.2008	3.3928	-7.9330	-8.4090
gene YNA1	1.5814	1.6394	26.8276	27.8121	3.3962	3.5208	-8.4174	-8.7264
	1.6921	1.5060	28.7055	25.5479	3.6339	3.2342	-9.0067	-8.0159
Skewness:	0.236662		0.236139		0.23622		-0.236218	
Excess kurtosis:	-1.008084		-1.009742		-1.009637		-1.00986	
Normality	0.8357		0.8349		0.8348		0.8349	
Outliers								
Mean	1.58152		26.82962		3.39645		-8.41807	
S	0.076925		1.30517		0.1652		0.40955	

Calculate

Validate

Insert column

Delete column

Clear

Load last run

Header: You may change groups' names to the real names.

Data: When entering data, press Enter or , (comma) after each value.
You may paste full column from excel.

The calculator ignores empty cells or non-numeric cells.

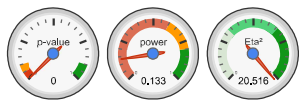
Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values

validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	4007.7714	1335.9238	2805.9393	3.331e-16
Error (within groups)	20	9.5221	0.4761		
Total	23	4017.2935	174.6649		

[R code](#)



One Way ANOVA test, using F distribution df(3,20) (right tailed).

1. H₀ hypothesis

Since p-value < α, H₀ is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals **3.33067e-16**, [p(x ≤ F) = 1]. It means that the chance of type1 error (rejecting a correct H₀) is small: 3.331e-16 (3.3e-14%)

The smaller the p-value the stronger it support H₁

3. The statistics

The test statistic F equals **2805.939274**, which is not in the 95% region of acceptance: [0 : 3.0984]

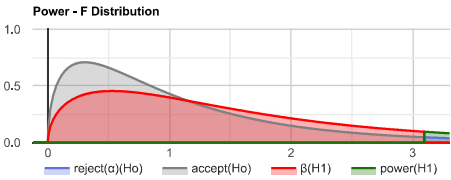
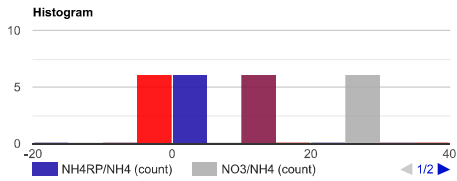
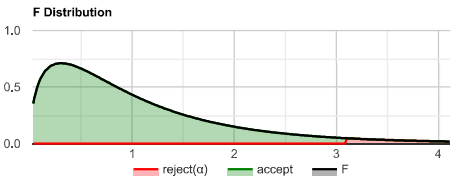
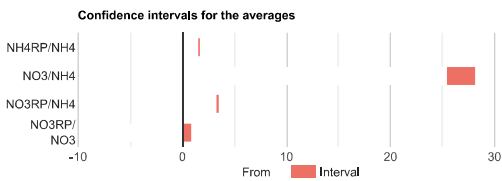
4. Effect size

The observed effect size f is **large** (20.52). That indicates that the magnitude of the difference between the averages is large.

The η² equals 1. It means that the **group** explains 99.8% of the variance from the average (similar to R² in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4**.



Validation

Test power

Although the priori power is low (0.1335), the H₀ is rejected.

Equality of variances

The tool used the Levene's test to assess the equality of variances.

The population's variances consider to be **not equal**. (p-value = 0.00486).

Levene's test power consider to be weak (0.13).

The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)

The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

● **Normality assumption**

The assumption was checked based on the [Shapiro-Wilk Test](#). ($\alpha=0.05$)
It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	25.2481	0.2817	89.6298	24.1331	26.3631	1.115	5.11e-13
x1-x3	1.8149	0.2817	6.4429	0.6999	2.93	1.115	0.001018
x1-x4	9.9996	0.2817	35.4982	8.8846	11.1146	1.115	5.121e-13
x2-x3	23.4332	0.2817	83.1869	22.3181	24.5482	1.115	5.11e-13
x2-x4	35.2477	0.2817	125.128	34.1327	36.3627	1.115	5.11e-13
x3-x4	11.8145	0.2817	41.9411	10.6995	12.9295	1.115	5.11e-13

Group	x2	x3	x4
x1	25.25	1.81	10
x2	0	23.43	35.25
x3	23.43	0	11.81

ANOVA

What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data.
ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result.
The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group.
The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields
 H_0 : The average weight of crops per square meter is equal in all fields.
 H_1 : At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

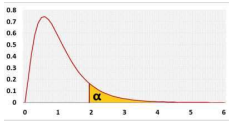
Hypotheses

$H_0: \mu_1 = \dots = \mu_k$
 $H_1: \text{not}(\mu_1 = \dots = \mu_k)$

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples
- Normal distribution of the analyzed population
- Equal standard deviation, $\sigma_1=\sigma_2=\dots=\sigma_k$
The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- k** - Number of groups.
- n_i** - Sample side of group i
- n** - Overall sample side, includes all the groups ($\sum n_i$, $i=1$ to k)
- $\bar{x}_i$** - Average of group i.
- $\bar{x}$** - Overall average ($\sum x_{ij} / n$, $i=1$ to k , $j=1$ to n_i)
- S_i** - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	k - 1	$SSG=\sum_{i=1}^k n_i (\bar{x}_i-\bar{x})^2$	MSG = SSG / (k - 1)	F = MSG / MSE	P(x > F)
Error (within groups)	n - k	$SSE=\sum_{i=1}^k (n_i-1) S_i^2$	MSE = SSE / (n - k)		
Total	n - 1	SS(total) = SSG + SSE	Sample Variance = SS(total) / (n - 1)		



R Code

The following R code should produce the same results

```
value<-
c(1.4904,1.5060,1.5798,1.5814,1.6394,1.6921,25.2838,25.5479,26.8008,26.8276,27.8121,28.7055,3.2008,3.2342,3.3928,3.3962,3.5208,3.6339,-9.0067,-8.7264,-8.4174,-8.4090,-8.0159,-7.9330)
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))
my.dataframe<-data.frame(value, group1)
res.aov <- aov(value ~ group1, data = my.dataframe)
summary(res.aov)
TukeyHSD(res.aov)
```

[Tutorial](#)
[ANOVA](#)

Calculators

[Kruskal-Wallis test](#)
[Two way ANOVA](#)
[Levene's test](#)

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4		NO3/NH4		NO3RP/NH4		NO3RP/NO3	
Data: gene YNI1	-2.4473	-2.5941	60.0802	63.6850	49.8713	52.8636	-4.1291	-4.3768
	-2.5967	-2.6920	63.7488	66.0882	52.9165	54.8585	-4.3812	-4.5420
	-2.7785	-2.4729	68.2112	60.7079	56.6207	50.3924	-4.6879	-4.1722
Skewness:	-0.236677		0.236148		0.236151		-0.236314	
Excess kurtosis:	-1.008809		-1.009695		-1.009726		-1.009831	
Normality	0.8353		0.835		0.835		0.8348	
Outliers								
Mean	-2.59692		63.75355		52.9205		-4.38153	
S	0.12632		3.10147		2.57447		0.21316	

Calculate

Validate

Insert column

Delete column

Clear

Load last run

Header: You may change groups' names to the real names.

Data: When entering data, press Enter or , (comma) after each value.
You may paste full column from excel.

The calculator ignores empty cells or non-numeric cells.

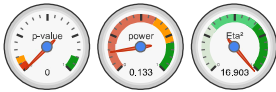
Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values
validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	23296.5306	7765.5102	1904.6683	0

Error (within groups)	20	81.5419	4.0771		
Total	23	23378.0725	1016.4379		

[R code.](#)



One Way ANOVA test, using F distribution df(3,20) (right tailed).

1. H₀ hypothesis

Since $p\text{-value} < \alpha$, H_0 is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals 0, [$p(x \leq F) = 1$]. It means that the chance of type1 error (rejecting a correct H_0) is small: 0 (0%)

The smaller the p-value the stronger it support H_1

3. The statistics

The test statistic F equals **1904.668257**, which is not in the 95% region of acceptance: [0 : 3.0984]

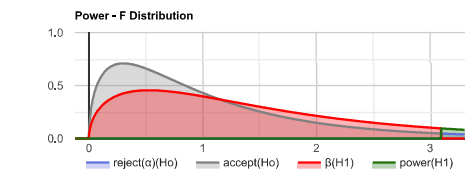
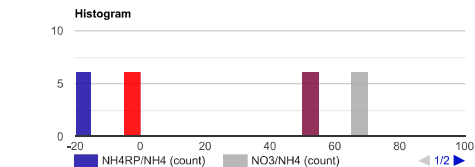
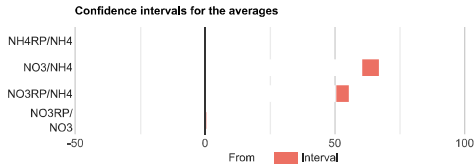
4. Effect size

The observed effect size f is **large** (16.9). That indicates that the magnitude of the difference between the averages is large.

The η^2 equals 1. It means that the **group** explains 99.7% of the variance from the average (similar to R^2 in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x2-x3, x2-x4, x3-x4.**



Validation

● **Test power**

Although the priori power is low (0.1335), the H_0 is rejected.

● **Equality of variances**

The tool used the Levene's test to assess the equality of variances.

The population's variances consider to be **not equal**. (p-value = 0.00782).

Levene's test power consider to be weak (0.13).

The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)

The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

● **Normality assumption**

The assumption was checked based on the [Shapiro-Wilk Test](#). ($\alpha=0.05$)

It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	66.3505	0.8243	80.4904	63.0875	69.6134	3.2629	5.11e-13
x1-x3	55.5174	0.8243	67.3488	52.2545	58.7803	3.2629	5.11e-13
x1-x4	1.7846	0.8243	2.1649	-1.4783	5.0475	3.2629	0.4387
x2-x3	10.8331	0.8243	13.1417	7.5701	14.096	3.2629	6.115e-8
x2-x4	68.1351	0.8243	82.6554	64.8722	71.398	3.2629	5.11e-13
x3-x4	57.302	0.8243	69.5137	54.0391	60.565	3.2629	5.11e-13

Group	x2	x3	x4
x1	66.35	55.52	1.78
x2	0	10.83	68.14
x3	10.83	0	57.3

ANOVA

What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data. ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result. The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group. The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields
H₀: The average weight of crops per square meter is equal in all fields.
H₁: At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

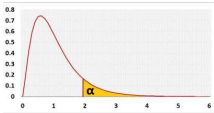
Hypotheses

H₀: μ₁ = ... = μ_k
H₁: not(μ₁ = ... = μ_k)

ANOVA formula

$F = \frac{MSG}{MSE}$

F distribution



Assumptions

- Independent samples
- Normal distribution of the analyzed population
- Equal standard deviation, σ₁=σ₂=...=σ_k
The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- k** - Number of groups.
- n_i** - Sample side of group i
- n** - Overall sample side, includes all the groups (Σn_i, i=1 to k)
- $\bar{x}_i$** - Average of group i.
- $\bar{x}$** - Overall average (Σx_{ij} / n, i=1 to k, j=1 to n_i)
- S_i** - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	k - 1	$SSG=\sum_{i=1}^k n_i (\bar{x}_i-\bar{x})^2$	$MSG = SSG / (k - 1)$	F = MSG / MSE	P(x > F)
Error (within groups)	n - k	$SSE=\sum_{i=1}^k (n_i-1) S_i^2$	$MSE = SSE / (n - k)$		
Total	n - 1	SS(total) = SSG + SSE	Sample Variance = SS(total) / (n - 1)		

R Code

The following R code should produce the same results

```
value<-  
c(-2.7785,-2.6920,-2.5967,-2.5941,-2.4729,-2.4473,60.0802,60.7079,63.6850,63.7488,66.0882,68.2112,49.8713,50.3924,52.8636,52.9165,54.8585,56.6207,-4.6879,-4.5420,-4.3812,-4.3768,-4.1722,-4.1291)  
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))  
my.dataframe<-data.frame(value, group1)  
res.aov <- aov(value ~ group1, data = my.dataframe)  
summary(res.aov)  
TukeyHSD(res.aov)
```

[Tutorial](#)

[ANOVA](#)

Calculators

[Kruskal-Wallis test](#)

[Two way ANOVA](#)

[Levene's test](#)

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4		NO3/NH4		NO3RP/NH4		NO3RP/NO3	
Data: gene YNR1	-2.4473	-2.5941	60.0802	63.6850	4.7029	4.9851	-12.5005	-13.2506
	-2.5967	-2.6920	63.7488	66.0882	4.9901	5.1732	-13.2638	-13.7506
	-2.7785	-2.4729	68.2112	60.7079	5.3394	4.7520	-14.1923	-12.6312
Skewness:	-0.236677		0.236148		0.23602		-0.236126	
Excess kurtosis:	-1.008809		-1.009695		-1.009809		-1.009648	
Normality	0.8353		0.835		0.8348		0.835	
Outliers								
Mean	-2.59692		63.75355		4.99045		-13.26483	
S	0.12632		3.10147		0.24279		0.6453	

Calculate

Validate

Insert column

Delete column

Clear

Load last run

Header: You may change groups' names to the real names.

Data: When entering data, press Enter or , (comma) after each value.
You may paste full column from excel.

The calculator ignores empty cells or non-numeric cells.

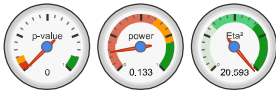
Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values
validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	21437.9185	7145.9728	2827.1763	0

Error (within groups)	20	50.552	2.5276		
Total	23	21488.4705	934.2813		

[R code](#).



One Way ANOVA test, using F distribution df(3,20) (right tailed).

1. H₀ hypothesis

Since p-value< α , H₀ is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals 0, [$p(x \leq F) = 1$]. It means that the chance of type1 error (rejecting a correct H₀) is small: 0 (0%)

The smaller the p-value the stronger it support H₁

3. The statistics

The test statistic F equals **2827.176321**, which is not in the 95% region of acceptance: [0 : 3.0984]

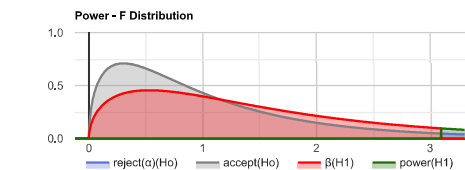
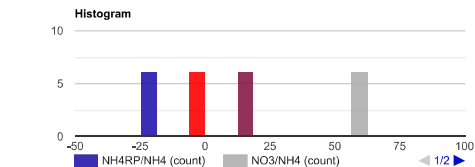
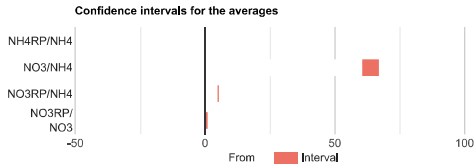
4. Effect size

The observed effect size f is **large** (20.59). That indicates that the magnitude of the difference between the averages is large.

The η^2 equals 1. It means that the **group** explains 99.8% of the variance from the average (similar to R² in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4**.



Validation

Test power

Although the priori power is low (0.1335), the H₀ is rejected.

Equality of variances

The tool used the Levene's test to assess the equality of variances.

The population's variances consider to be **not equal**. (p-value = 0.00235).

Levene's test power consider to be weak (0.13).

The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)

The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

Normality assumption

The assumption was checked based on the [Shapiro-Wilk Test](#). ($\alpha=0.05$)

It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	66.3505	0.6491	102.2269	63.7813	68.9196	2.5691	5.11e-13
x1-x3	7.5874	0.6491	11.6899	5.0182	10.1565	2.5691	3.979e-7
x1-x4	10.6679	0.6491	16.4362	8.0988	13.237	2.5691	1.373e-9
x2-x3	58.7631	0.6491	90.537	56.194	61.3322	2.5691	5.11e-13
x2-x4	77.0184	0.6491	118.6631	74.4492	79.5875	2.5691	5.11e-13
x3-x4	18.2553	0.6491	28.1261	15.6861	20.8244	2.5691	5.717e-13

Group	x2	x3	x4
x1	66.35	7.59	10.67
x2	0	58.76	77.02
x3	58.76	0	18.26

ANOVA

What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data. ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result. The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group. The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields
H₀: The average weight of crops per square meter is equal in all fields.
H₁: At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

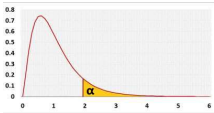
Hypotheses

H₀: μ₁ = ... = μ_k
H₁: not(μ₁ = ... = μ_k)

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples
- Normal distribution of the analyzed population
- Equal standard deviation, σ₁=σ₂=...=σ_k
The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- k** - Number of groups.
- n_i** - Sample side of group i
- n** - Overall sample side, includes all the groups (Σn_i, i=1 to k)
- $\bar{x}_i$** - Average of group i.
- $\bar{x}$** - Overall average (Σx_{ij} / n, i=1 to k, j=1 to n_i)
- S_i** - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	k - 1	$S SG = \sum_{i=1}^k n_i (\bar{x}_i - \bar{x})^2$	$MSG = SSG / (k - 1)$	F = MSG / MSE	P(x > F)
Error (within groups)	n - k	$S SE = \sum_{i=1}^k (n_i - 1) S_i^2$	$MSE = SSE / (n - k)$		
Total	n - 1	SS(total) = SSG + SSE	Sample Variance = SS(total) / (n - 1)		

R Code

The following R code should produce the same results

```
value<-  
c(-2.7785,-2.6920,-2.5967,-2.5941,-2.4729,-2.4473,60.0802,60.7079,63.6850,63.7488,66.0882,68.2112,4.7029,4.7520,4.9851,4.9901,5.1732,5.3394,-14.1923,-13.7506,-13.2638,-13.2506,-12.6312,-12.5005)  
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))  
my.dataframe<-data.frame(value, group1)  
res.aov <- aov(value ~ group1, data = my.dataframe)  
summary(res.aov)  
TukeyHSD(res.aov)
```

[Tutorial](#)

[ANOVA](#)

Calculators

[Kruskal-Wallis test](#)

[Two way ANOVA](#)

[Levene's test](#)

ANOVA Calculator

One-Way ANOVA Calculator and Tukey HSD

Significance level (α):

0,05

Outliers:

Included

Effect:

Medium

Effect type:

f

Effect Size:

0.25

Rounding:

4

- ☒ Enter raw data directly
- ☐ Enter raw data from excel

Enter sample data directly

Groups:	NH4RP/NH4	NO3/NH4	NO3RP/NH4	NO3RP/NO3
Data: gene YNT1	1.3923 1.4759 1.4773 1.5316 1.5808 1.4069	71.2582 75.5337 75.6093 78.3841 80.9020 72.0028	9.1671 9.7171 9.7268 10.0838 10.4077 9.2629	-7.6910 -8.1525 -8.1606 -8.4601 -8.7319 -7.7714
Skewness:	0.236429	0.236155	0.236228	-0.23622
Excess kurtosis:	-1.009392	-1.009715	-1.009693	-1.009429
Normality	0.8349	0.835	0.835	0.835
Outliers				
Mean	1.47747	75.61502	9.72757	-8.16125
S	0.071894	3.67849	0.47321	0.39703

Calculate

Validate

Insert column

Delete column

Clear

Load last run

Header: You may change groups' names to the real names.

Data: When entering data, press Enter or , (comma) after each value.
You may paste full column from excel.

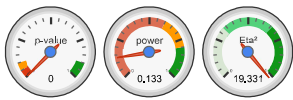
The calculator ignores empty cells or non-numeric cells.

Group1 contains 6 values
Group2 contains 6 values
Group3 contains 6 values
Group4 contains 6 values
validation:success

Hover over the cells for more information.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	3	26005.4611	8668.487	2491.2934	0
Error (within groups)	20	69.5903	3.4795		
Total	23	26075.0514	1133.6979		

[R code.](#)



One Way ANOVA test, using F distribution df(3,20) (right tailed).

1. H₀ hypothesis

Since p-value < α , H₀ is rejected.

Some of the groups' averages consider to be not equal.

In other words, the difference between the sample averages of some groups is big enough to be statistically significant.

2. P-value

p-value equals 0, [p($x \leq F$) = 1]. It means that the chance of type1 error (rejecting a correct H₀) is small: 0 (0%)

The smaller the p-value the stronger it support H₁

3. The statistics

The test statistic F equals **2491.293441**, which is not in the 95% region of acceptance: [0 : 3.0984]

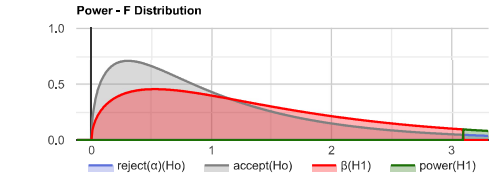
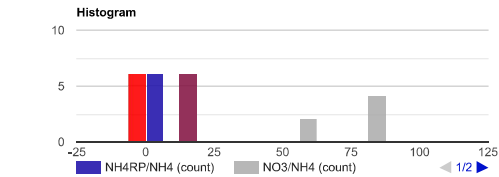
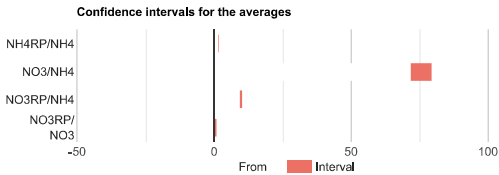
4. Effect size

The observed effect size f is **large** (19.33). That indicates that the magnitude of the difference between the averages is large.

The η^2 equals 1. It means that the **group** explains 99.7% of the variance from the average (similar to R² in the linear regression)

5. Tukey HSD / Tukey Kramer

The means of the following pairs are significantly different: **x1-x2, x1-x3, x1-x4, x2-x3, x2-x4, x3-x4.**



Validation

Test power

Although the priori power is low (0.1335), the H₀ is rejected.

Equality of variances

The tool used the Levene's test to assess the equality of variances.

The population's variances consider to be **not equal**. (p-value = 0.00176).

Levene's test power consider to be weak (0.13).

The groups' size consider similar. (The ratio between the bigger group and the smaller group is: 1)

The ANOVA test consider to be robust to the homogeneity of variances assumption when the groups' sizes are similar.

It is suggested to consider the **Kruskal-Wallis ANOVA**. non-parametric test.

● **Normality assumption**

The assumption was checked based on the [Shapiro-Wilk Test](#). ($\alpha=0.05$)

It is assumed that all the groups distribute normally or have a big sample size, at least 30.

Tukey HSD / Tukey Kramer

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	74.1375	0.7615	97.3542	71.1232	77.1519	3.0143	5.11e-13
x1-x3	8.2501	0.7615	10.8337	5.2358	11.2644	3.0143	0.000001279
x1-x4	9.6387	0.7615	12.6571	6.6244	12.6531	3.0143	1.126e-7
x2-x3	65.8875	0.7615	86.5205	62.8731	68.9018	3.0143	5.11e-13
x2-x4	83.7763	0.7615	110.0113	80.7619	86.7906	3.0143	5.11e-13
x3-x4	17.8888	0.7615	23.4908	14.8745	20.9032	3.0143	2.643e-12

Group	x2	x3	x4
x1	74.14	8.25	9.64
x2	0	65.89	83.78
x3	65.89	0	17.89

ANOVA

What is the ANOVA?

The ANOVA test checks if the difference between the averages of two or more groups is significant, using sample data. ANOVA is usually used when there are at least three groups since for two groups, the *two-tailed* pooled variance t-test and the *right-tailed* ANOVA test have the same result. The basic ANOVA test contains only one categorical value, one-way ANOVA. For example, if you compare the performance of three schools, the categorical variable is school, and the possible values of the categorical variable are School-A, School-B, School-C. There are more complex ANOVA tests that contain two categorical variables ([Two-way ANOVA calculator](#)), or more. When performing a one-way ANOVA test, we try to determine if the difference between the averages reflects a real difference between the groups, or is due to the random noise inside each group. The F statistic represents the ratio of the variance between the groups and the variance inside the groups. Unlike many other statistic tests, the smaller the F statistic the more likely the averages are equal.

Example: Compare four fertilizers used in four fields
H₀: The average weight of crops per square meter is equal in all fields.
H₁: At least one field yields a different average per square meter.

Right-tailed, for ANOVA test you can use only the right tail. [Why?](#)

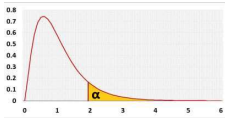
Hypotheses

H₀: $\mu_1 = \dots = \mu_k$
H₁: $\text{not}(\mu_1 = \dots = \mu_k)$

ANOVA formula

$$F = \frac{MSG}{MSE}$$

F distribution



Assumptions

- Independent samples
- Normal distribution of the analyzed population
- Equal standard deviation, $\sigma_1=\sigma_2=\dots=\sigma_k$
The assumption is more important when the groups' sizes not similar

Required Sample Data

- Sample data from all compared groups

Parameters

- k** - Number of groups.
- n_i** - Sample side of group i
- n** - Overall sample side, includes all the groups ($\sum n_i, i=1 \text{ to } k$)
- $\bar{x}_i$** - Average of group i.
- $\bar{x}$** - Overall average ($\sum x_{ij} / n, i=1 \text{ to } k, j=1 \text{ to } n_i$)
- S_i** - Standard deviation of group i

Results calculations

Source	Degrees of Freedom	Sum of Squares	Mean Square	F statistic	p-value
Groups (between groups)	$k - 1$	$SSG = \sum_{i=1}^k n_i (\bar{x}_i - \bar{x})^2$	$MSG = SSG / (k - 1)$	$F = MSG / MSE$	$P(x > F)$
Error (within groups)	$n - k$	$SSE = \sum_{i=1}^k (n_i - 1) S_i^2$	$MSE = SSE / (n - k)$		
Total	$n - 1$	$SS(\text{total}) = SSG + SSE$	$\text{Sample Variance} = SS(\text{total}) / (n - 1)$		



R Code

The following R code should produce the same results

```
value<-  
c(1.3923,1.4069,1.4759,1.4773,1.5316,1.5808,71.2582,72.0028,75.5337,75.6093,78.3841,80.9020,9.1671,9.2629,9.7171,9.7268,10.0838,10.4077,-8.7319,-8.4601,-8.1606,-8.1525,-7.7714,-7.6910)  
group1 <- c(rep("NH4RP/NH4", 6), rep("NO3/NH4", 6), rep("NO3RP/NH4", 6), rep("NO3RP/NO3", 6))  
my.dataframe<-data.frame(value, group1)  
res.aov <- aov(value ~ group1, data = my.dataframe)  
summary(res.aov)  
TukeyHSD(res.aov)
```

[Tutorial](#)
[ANOVA](#)

Calculators

[Kruskal-Wallis test](#)
[Two way ANOVA](#)
[Levene's test](#)