# Cytokine analysis script - all rounds - adjusted for tissue weight

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#README: Cytokine expression profiles in Matrinem. Below are 4 sections, each making a table and a plot for individual experimental rounds.

## Data import

```
#_______Import dataset and Transform to adjusted values_______#

cytokines <- read_excel("data/processed/cytokines.xlsx") %>%
  filter(tissue == "ileum") %>%
  pivot_longer(.,
               cols = INFg:TNFa ,
               names_to = "variables",
               values_to = "values"
  ) %>%
   #mutate(values = values/(tissue_weight_mg/1000)) %>% #Adjust for tissue weight / 1000 (grams)
   mutate(values = values/tissue_weight_mg) %>% #Adjust for tissue weight (mg)
  pivot_wider(
    id_cols = c(
      sample_id,exp_number, group, received_antibiotics,maternal_treatment, type_of_feed, tissue,tissue_
    names_from = variables,
    values_from = values)


#____ Make long format _______#
cytokines_long <- cytokines %>%
  pivot_longer(.,
               cols = INFg:TNFa ,
               names_to = "variables",
               values_to = "values"
  )
```

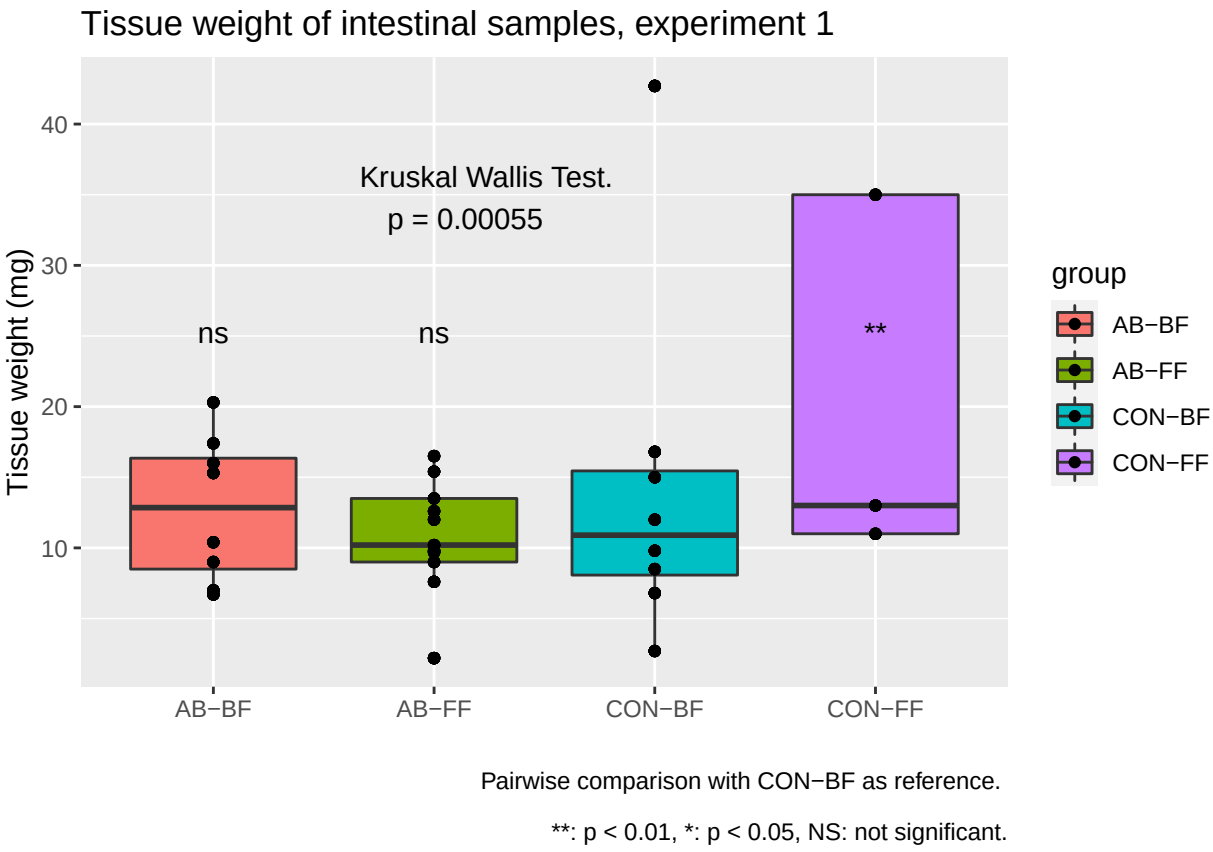
## Exp1

### Tissue weight

| .y. | n | statistic | df | p | method |
|---|---|---|---|---|---|
| tissue_weight_mg | 300 | 17.5374 | 3 | 0.000548 | Kruskal-Wallis |

```
## Coefficient covariances computed by hccm()
```

| Effect | DFn | DFd | F | p | p<.05 | ges |
|---|---|---|---|---|---|---|
| group | 3 | 296 | 11.284 | 5e-07 | * | 0.103 |




## Check for data distribution.

##Table

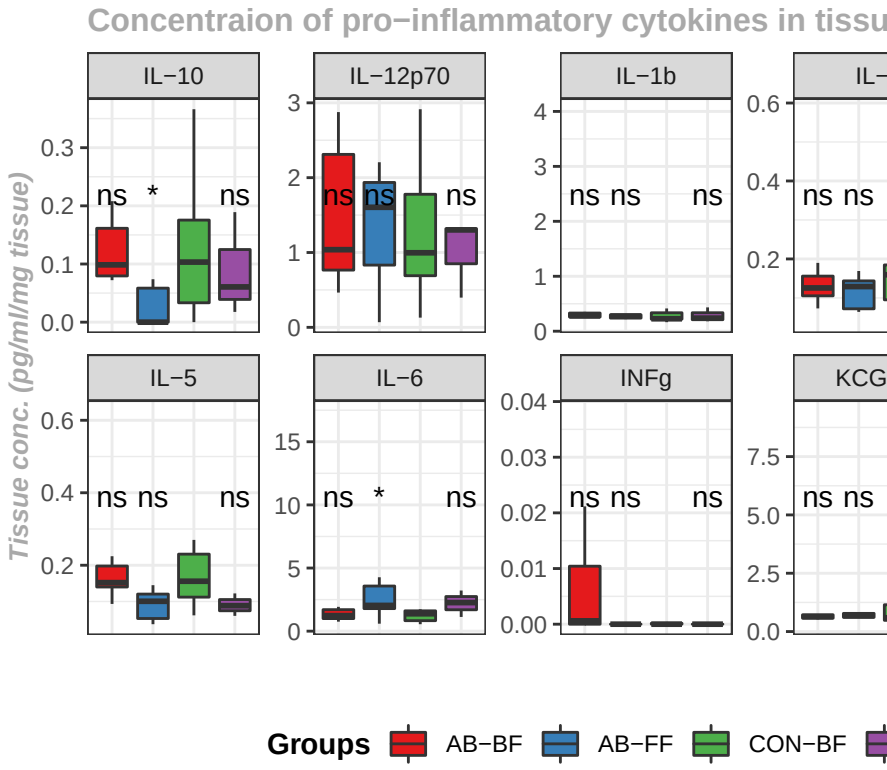
**Tissue concentraions of proinflammatory cytokines** *(pg/ml/mg tissue)*

| **Groups:** | **AB-BF**, N = 8[1] | **AB-FF**, N = 11[1] | **CON-BF**, N = 8[1] | **CON-FF**, N = 3[1] | **p-value**[2] |
|---|---|---|---|---|---|
| IL-10 | 0.10 (0.08, 0.16) | 0.00 (0.00, 0.06) | 0.10 (0.03, 0.18) | 0.06 (0.04, 0.12) | 0.021 |
| IL-12p70 | 1.04 (0.77, 2.31) | 1.60 (0.83, 1.94) | 1.00 (0.69, 1.78) | 1.30 (0.85, 1.32) | 0.8 |
| IL-1b | 0.28 (0.26, 0.33) | 0.28 (0.24, 0.30) | 0.23 (0.22, 0.34) | 0.24 (0.21, 0.34) | 0.6 |
| IL-2 | 0.13 (0.11, 0.16) | 0.13 (0.07, 0.14) | 0.16 (0.10, 0.18) | 0.07 (0.06, 0.08) | 0.2 |
| IL-4 | 0.09 (0.06, 0.11) | 0.05 (0.04, 0.07) | 0.07 (0.05, 0.08) | 0.05 (0.04, 0.06) | 0.15 |
| IL-5 | 0.15 (0.14, 0.20) | 0.10 (0.05, 0.12) | 0.16 (0.11, 0.23) | 0.09 (0.07, 0.11) | 0.031 |

| | | | | | |
|---|---|---|---|---|---|
| IL-6 | 1.23 (1.00, 1.70) | 2.04 (1.79, 3.57) | 1.38 (0.83, 1.61) | 2.26 (1.69, 2.74) | 0.073 |
| KCGRO | 0.64 (0.57, 0.72) | 0.68 (0.61, 0.78) | 0.59 (0.48, 1.15) | 0.69 (0.65, 0.72) | 0.8 |
| TNFa | 0.24 (0.19, 0.27) | 0.15 (0.12, 0.21) | 0.28 (0.18, 0.43) | 0.10 (0.10, 0.12) | 0.051 |

[1]Median (IQR)
[2]Kruskal-Wallis rank sum test
Matrinem Experiment 1

### Plot




To see all the cytokine leves plotted run this code:

## Exp2 exp_number : Matrinem Experiment 1

### Tissue weight

| .y. | n | statistic | df | p | method |
|---|---|---|---|---|---|
| tissue_weight_mg | 170 | 18.65943 | 2 | 8.87e-05 | Kruskal-Wallis |

### Check for data distribution.

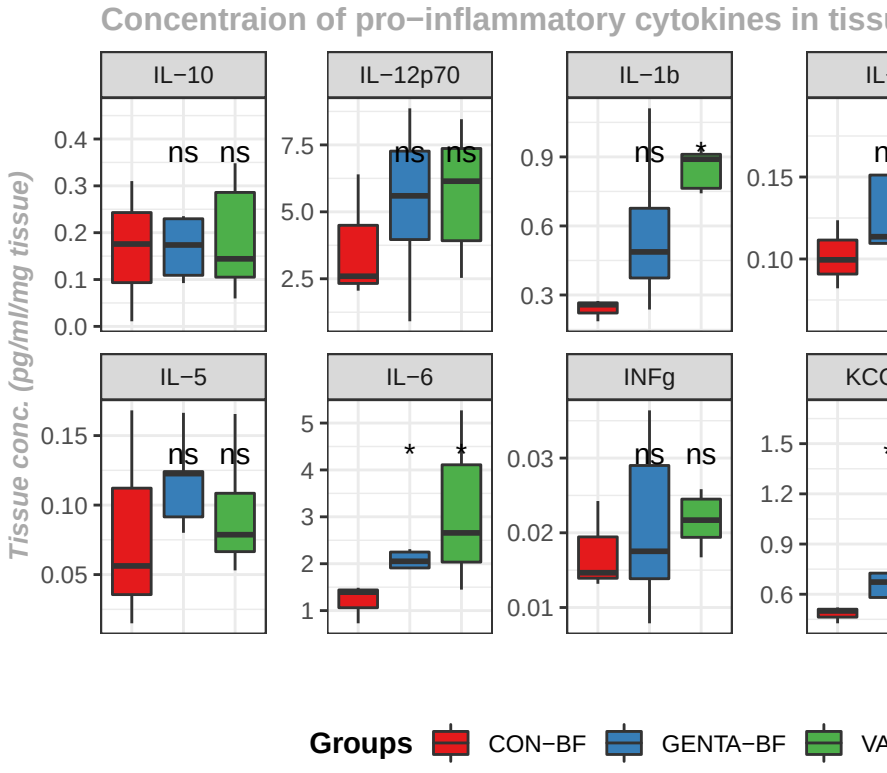
##Table

**Tissue concentraions of proinflammatory cytokines** *(pg/ml/mg tissue)*

| **Groups:** | **CON-BF**, N = 3[1] | **GENTA-BF**, N = 7[1] | **VANCO-BF**, N = 7[1] | **p-value**[2] |
|---|---|---|---|---|
| IL-10 | 0.18 (0.09, 0.24) | 0.17 (0.11, 0.23) | 0.14 (0.11, 0.29) | >0.9 |
| IL-12p70 | 2.59 (2.32, 4.50) | 5.60 (3.96, 7.27) | 6.14 (3.92, 7.37) | 0.5 |
| IL-1b | 0.26 (0.22, 0.27) | 0.49 (0.37, 0.68) | 0.89 (0.76, 0.91) | 0.015 |
| IL-2 | 0.10 (0.09, 0.11) | 0.11 (0.11, 0.15) | 0.13 (0.12, 0.16) | 0.3 |
| IL-4 | 0.08 (0.07, 0.09) | 0.18 (0.14, 0.21) | 0.11 (0.05, 0.31) | 0.4 |
| IL-5 | 0.06 (0.04, 0.11) | 0.12 (0.09, 0.12) | 0.08 (0.07, 0.11) | 0.6 |
| IL-6 | 1.39 (1.06, 1.44) | 2.05 (1.91, 2.25) | 2.66 (2.03, 4.11) | 0.034 |
| KCGRO | 0.50 (0.46, 0.51) | 0.67 (0.58, 0.73) | 0.82 (0.76, 1.10) | 0.007 |
| TNFa | 0.34 (0.32, 0.35) | 0.34 (0.33, 0.37) | 0.43 (0.37, 0.47) | 0.036 |

[1]Median (IQR)
[2]Kruskal-Wallis rank sum test
Matrinem Experiment 2

### Plot




To see all the cytokine leves plotted run this code:

## Exp3 exp_number : Matrinem Experiment 2

### Tissue weight

| .y. | n | statistic | df | p | method |
|---|---|---|---|---|---|
| tissue_weight_mg | 380 | 5.7912 | 2 | 0.0553 | Kruskal-Wallis |

## Check for data distribution.

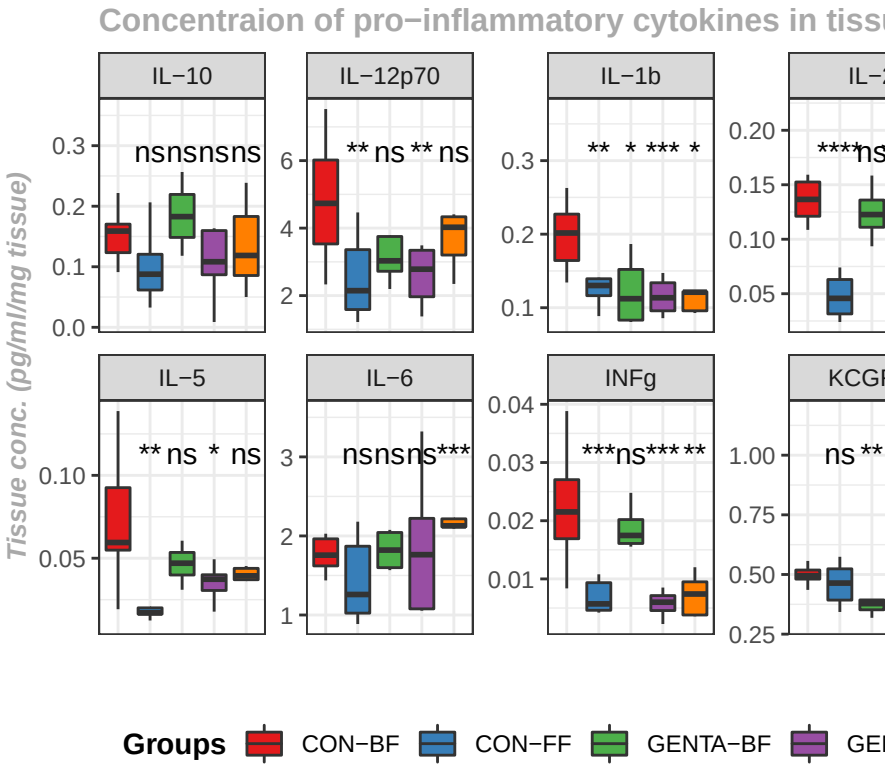
##Table

**Tissue concentraions of proinflammatory cytokines** *(pg/ml/mg tissue)*

| **Groups:** | **CON-BF**, N = 10[1] | **CON-FF**, N = 10[1] | **GENTA-BF**, N = 4[1] | **GENTA-FF**, N = 8[1] | **VANCO-FF**, |
|---|---|---|---|---|---|
| IL-10 | 0.16 (0.12, 0.17) | 0.09 (0.06, 0.12) | 0.18 (0.15, 0.22) | 0.11 (0.09, 0.16) | 0.12 (0.09, 0 |
| IL-12p70 | 4.73 (3.53, 6.02) | 2.15 (1.59, 3.36) | 3.03 (2.72, 3.75) | 2.78 (1.97, 3.34) | 4.03 (3.20, 4 |
| IL-1b | 0.20 (0.16, 0.23) | 0.13 (0.12, 0.14) | 0.11 (0.08, 0.15) | 0.11 (0.10, 0.13) | 0.12 (0.10, 0 |
| IL-2 | 0.14 (0.12, 0.15) | 0.05 (0.03, 0.06) | 0.12 (0.11, 0.14) | 0.05 (0.04, 0.05) | 0.07 (0.07, 0 |
| IL-4 | 0.03 (0.02, 0.05) | 0.02 (0.01, 0.02) | 0.02 (0.02, 0.02) | 0.02 (0.02, 0.03) | 0.03 (0.02, 0 |
| IL-5 | 0.06 (0.05, 0.09) | 0.02 (0.02, 0.02) | 0.05 (0.04, 0.05) | 0.04 (0.03, 0.04) | 0.04 (0.04, 0 |
| IL-6 | 1.76 (1.62, 1.96) | 1.26 (1.02, 1.87) | 1.82 (1.60, 2.04) | 1.76 (1.08, 2.22) | 2.13 (2.12, 2 |
| KCGRO | 0.49 (0.48, 0.52) | 0.46 (0.39, 0.52) | 0.38 (0.35, 0.39) | 0.35 (0.30, 0.46) | 0.60 (0.54, 0 |
| TNFa | 0.31 (0.29, 0.33) | 0.15 (0.10, 0.16) | 0.26 (0.25, 0.28) | 0.17 (0.12, 0.19) | 0.18 (0.16, 0 |

[1]Median (IQR)
[2]Kruskal-Wallis rank sum test
Matrinem Experiment 3

### Plot




To see all the cytokine leves plotted run this code:

## Exp4 : exp_number : Matrinem Experiment 3

### Tissue weight

| .y. | n | statistic | df | p | method |
|---|---|---|---|---|---|
| tissue_weight_mg | 260 | 46.29115 | 2 | 0 | Kruskal-Wallis |

### Check for data distribution.

##Table

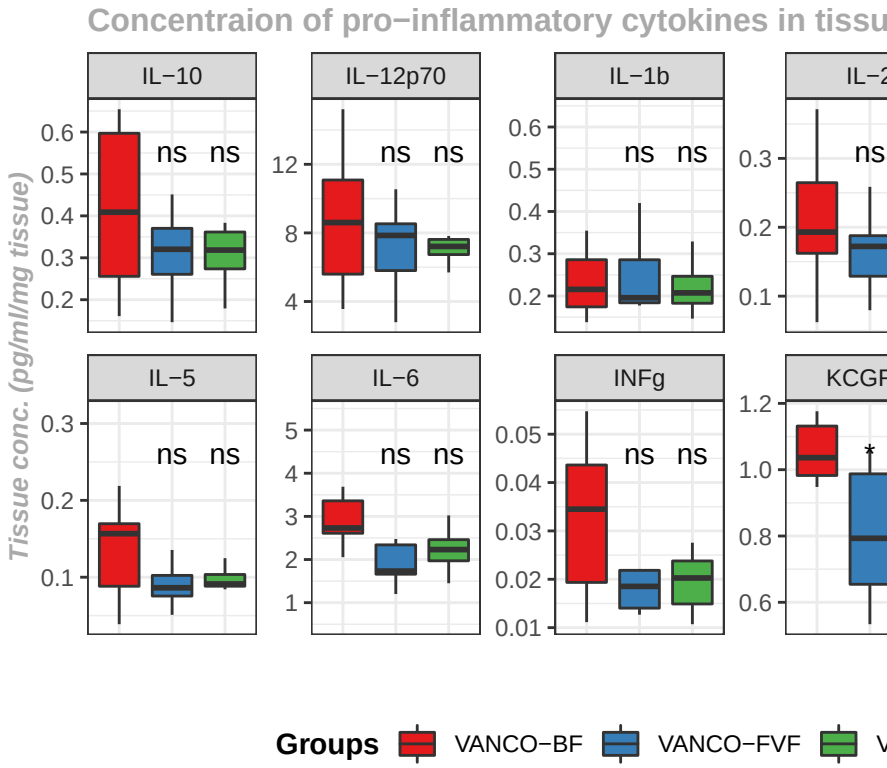
**Tissue concentraions of proinflammatory cytokines** *(pg/ml/mg tissue)*

| **Groups:** | **VANCO-BF**, N = 9[1] | **VANCO-FVF**, N = 9[1] | **VANCO-SM**, N = 8[1] | **p-value**[2] |
|---|---|---|---|---|
| IL-10 | 0.41 (0.26, 0.60) | 0.32 (0.26, 0.37) | 0.32 (0.27, 0.36) | 0.7 |
| IL-12p70 | 8.60 (5.59, 11.08) | 7.85 (5.80, 8.53) | 7.21 (6.74, 7.62) | 0.7 |
| IL-1b | 0.22 (0.17, 0.29) | 0.20 (0.18, 0.29) | 0.21 (0.18, 0.25) | 0.9 |
| IL-2 | 0.19 (0.16, 0.26) | 0.17 (0.13, 0.19) | 0.16 (0.15, 0.17) | 0.4 |
| IL-4 | 0.16 (0.13, 0.19) | 0.10 (0.10, 0.15) | 0.12 (0.11, 0.14) | 0.3 |
| IL-5 | 0.16 (0.09, 0.17) | 0.09 (0.08, 0.10) | 0.09 (0.09, 0.10) | 0.2 |

| | | | | |
|---|---|---|---|---|
| IL-6 | 2.73 (2.61, 3.36) | 1.73 (1.66, 2.34) | 2.23 (1.97, 2.46) | 0.12 |
| KCGRO | 1.04 (0.98, 1.13) | 0.79 (0.65, 0.99) | 0.79 (0.73, 0.92) | 0.020 |
| TNFa | 0.18 (0.15, 0.22) | 0.20 (0.19, 0.23) | 0.17 (0.15, 0.20) | 0.2 |

[1]Median (IQR)
[2]Kruskal-Wallis rank sum test
Matrinem Experiment 4

## Plot




To see all the cytokine leves plotted run this code: