



# **“EFFECT OF ANTIOXIDANT SUPPLEMENTATION ON FAECAL MICROBIOTA OF HORSES AT DIFFERENT EXERCISE LEVELS”**

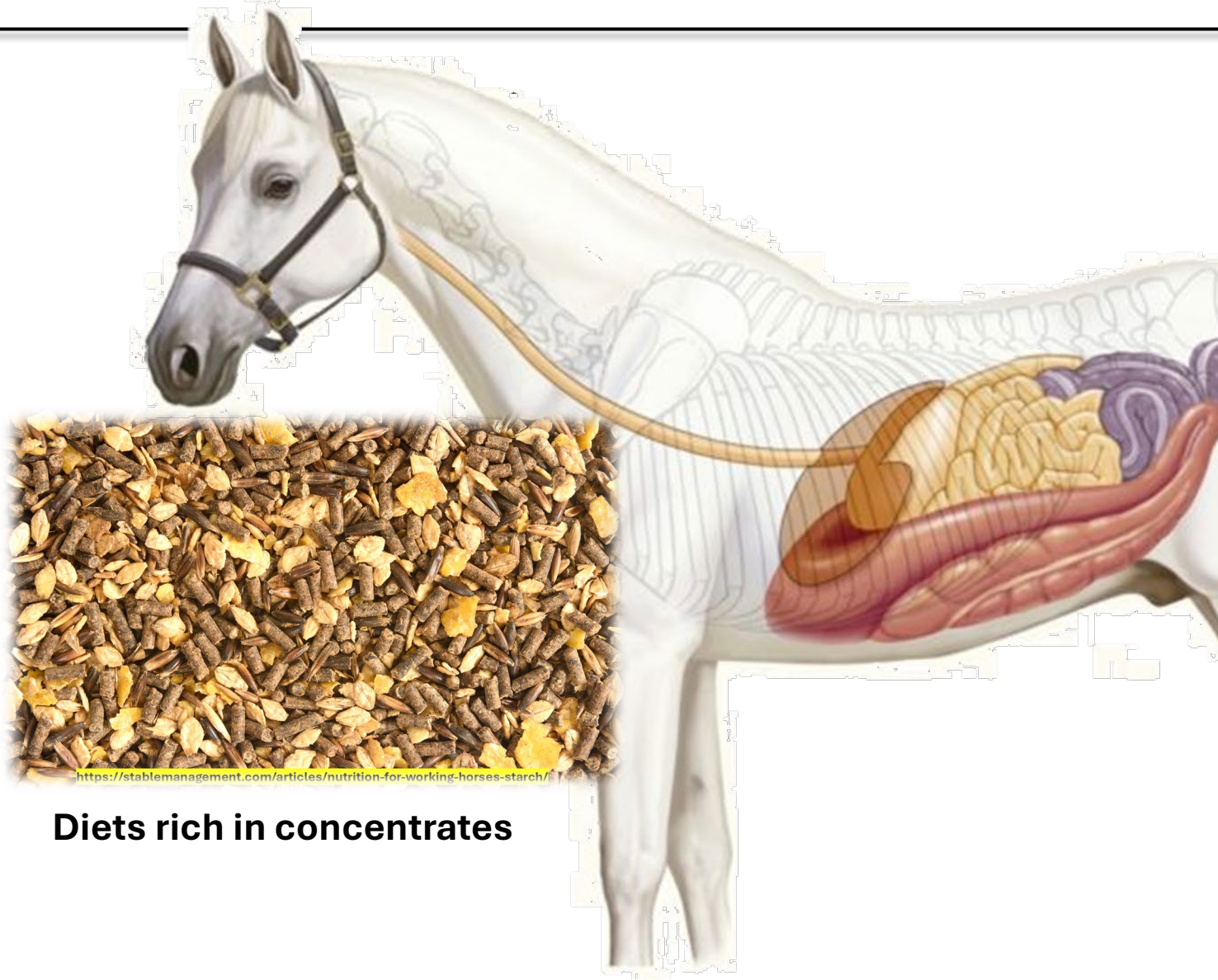


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**Florence, Italy 2024**



# Introduction...



**Diets rich in concentrates**

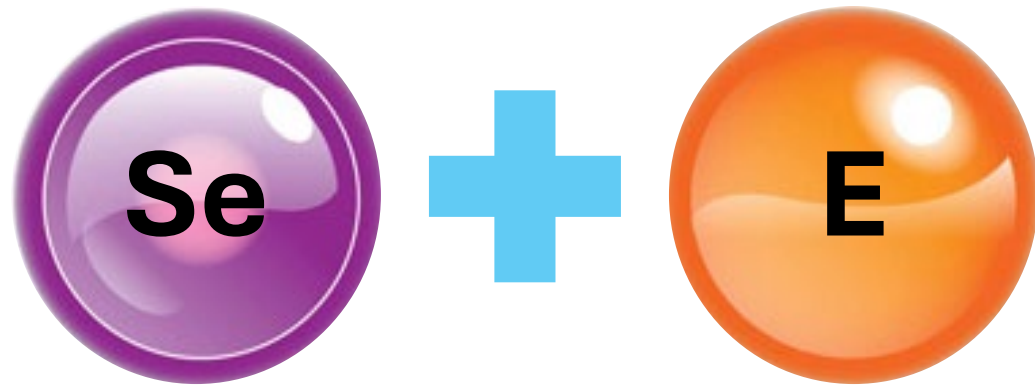


## **Alterations:**

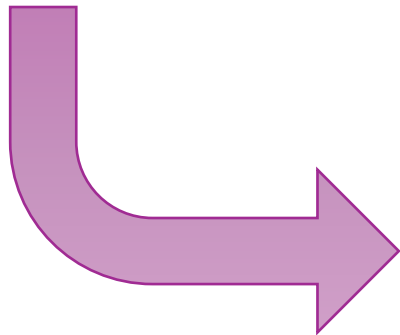
- **in the digestive system**
- **microbiota (dysbiosis)**
- **fermentation patterns**
- **presentation of colic and laminitis**

# Introduction...

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**Antioxidants**



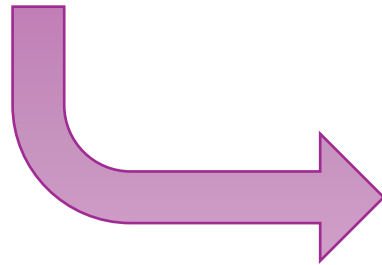
**Selenium improves fiber fermentation**

# Objective and hypothesis...

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Effect on faecal microbiota



Supplementation modifies faecal  
microbiota



# Material & Methods...

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EXPERIMENTAL PROTOCOL APPROVED

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE OF THE FACULTY

VETERINARY MEDICINE OF THE NATIONAL UNIVERSITY OF MEXICO

## **Experimental site:**

Mounted Police Unit of  
Mexico City

## **Animals:**

24 horses (hot blood), 4  
treatments with 6 horses  
each one

5 - 13 years old



Individually stable  
(ventilation, feeding and  
water dispenser)

## **Exercise:**

Sand track

# Material & Methods...

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Factorial arrangement:  $2 \times 2$ ,

- ❖ 2 selenium levels
- ❖ 2 vitamin E levels
- ❖ a positive control group was used

Se and E (NRC, 2007 recommendations)

with repeated measures (0-11 weeks, 77 days)

|          |                       | Vitamin E            |                      |
|----------|-----------------------|----------------------|----------------------|
|          |                       | LE<br>(1.6 UI/kg PV) | HE<br>(2.0 UI/kg PV) |
| Selenium | LSe<br>(0.1 mg/kg MS) | C                    | E                    |
|          | HSe<br>(0.3 mg/kg MS) | Se                   | Se + E               |

# Material & Methods...

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## ANTIOXIDANTS



**Seleno-yeast**  
(2.052 g Se/ kg)  
(Se-met)

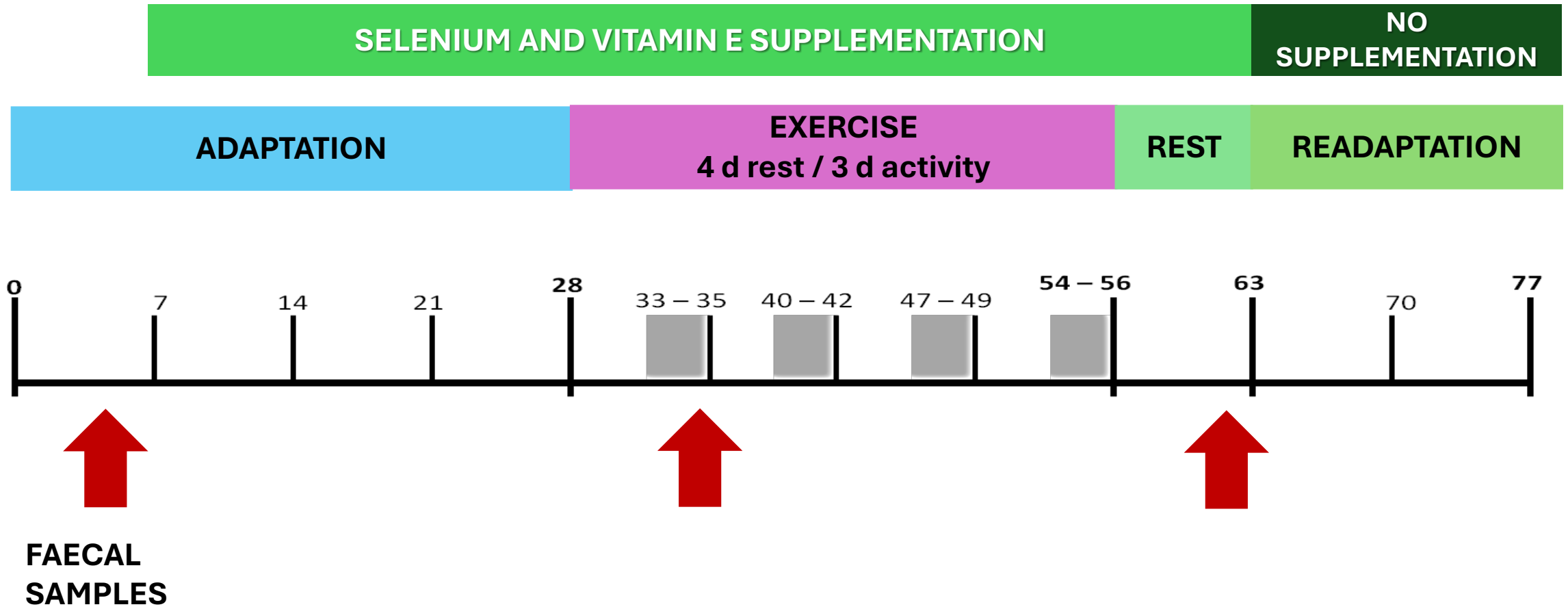


**DL- tocopherol**  
(439 UI E/g)



# Material & Methods...

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# Material & Methods...

| CHEMICAL COMPOSITION OF THE DIET<br>(DRY MATTER, DM) |        |
|------------------------------------------------------|--------|
| Item                                                 |        |
| CP (N x 6.25, g/kg of DM)                            | 103.88 |
| NDF (g/kg of DM)                                     | 389.70 |
| ADF (g/kg of DM)                                     | 342.43 |
| Ca (g/kg of DM)                                      | 5.15   |
| P (g/kg of DM)                                       | 3.58   |
| Se (g/kg of DM)                                      | ND     |
| Vitamin E (IU/kg)                                    | 13.74  |
| E (Mcal/kg of DM)                                    | 5.42   |

ND= No detectable UNDER 2 ppb (2 ng/g or 2 µg/kg)

Total kg offer/day: 8 kg of DM

| Ingredients    | g / kg DM |
|----------------|-----------|
| Oat hay        | 611.50    |
| Lucerne hay    | 195.40    |
| Comercial feed | 193.10    |

❖ Feeding schedule: 0500, 0700, 1400 and 1600 h.

❖ 9.3 kg DM / day

## METHODOLOGY:

- CP (N\*6.25) = Kjeldahl Method
- Gross Energy = Calorimeter
- NDF and ADF = Van Soest (1991)
- Ca = Absorption Atomic Spectrophotometry
- P = Photometric method
- Se = Hydride Generator Coupled to AAS
- Vitamina E = HPLC

# Material & Methods...

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## **THE STUDY OF THE FAECAL MICROBIOTA WAS CONDUCTED WITH THE METHODOLOGY REPORTED IN THE LITERATURE...**

- The V4 hypervariable region of the 16S RNA was used (Caporasso et al., 2011; Gaona et al., 2019).
- Purified rRNA amplicons were pooled and sequenced (Illumina MiSeq platform; Yale Genome Analysis Center, CT, USA).
- Sequences were analyzed with QIIME 2 2018.6 (Bolyen et al., 2018)
- A quality protocol was applied (Callahan et al., 2016).

# Material & Methods...

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## THE STUDY OF THE FAECAL MICROBIOTA WAS CONDUCTED WITH THE METHODOLOGY REPORTED IN THE LITERATURE...

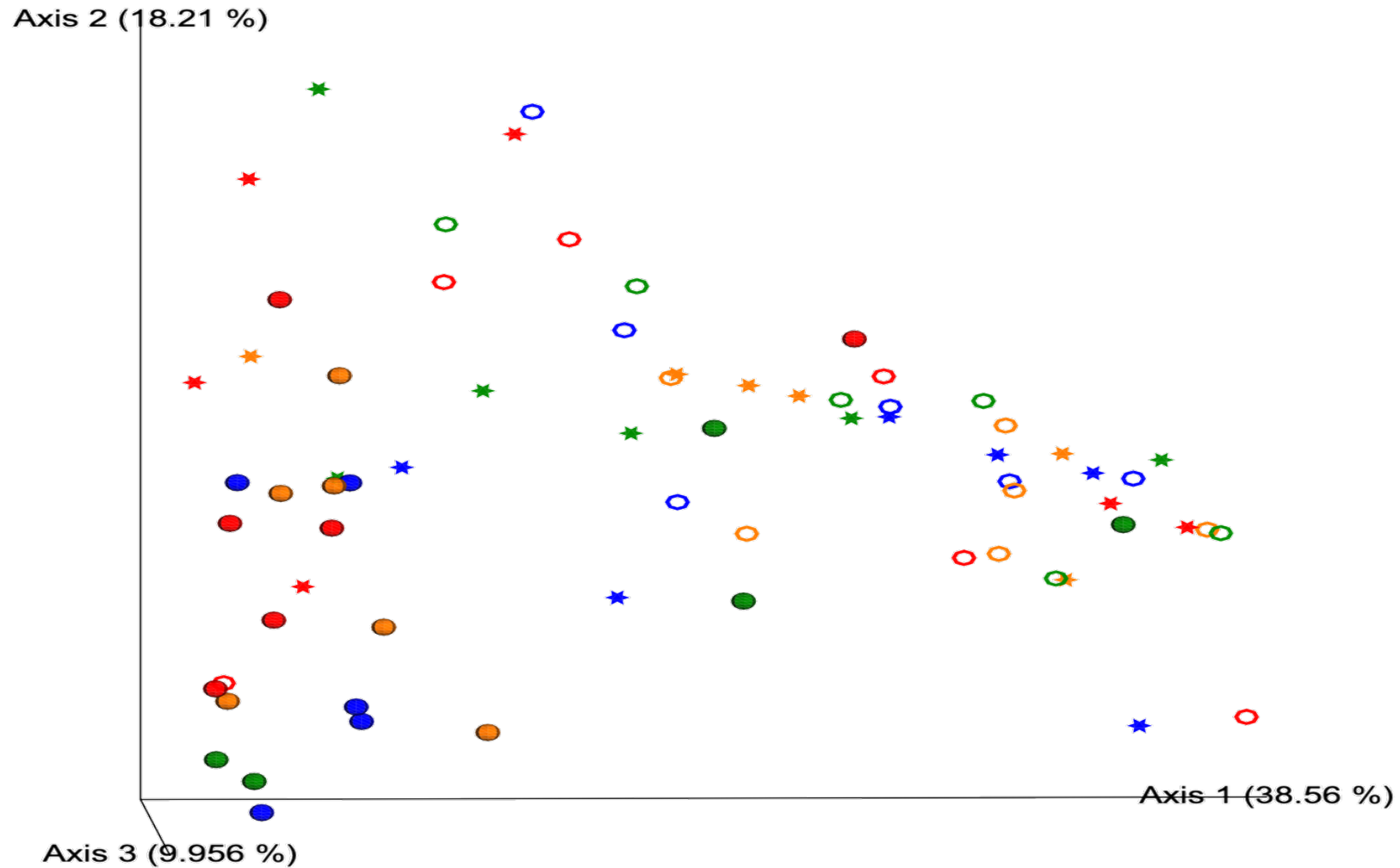
- Taxonomy was assigned to ASVs (Rognes et al., 2016) against the SILVA database, with a percentage identity of 0.95.
- Alpha diversity was calculated (Shannon, Simpson and Observed otus).
- Phylogenetic  $\beta$ -diversity analysis was measured using weighted unifrac (Lozupone et al., 2007) and visualized (principal coordinate analysis (PCoA).
- Complement qiime core-metrics-phylogenetic diversity at 32,184 ASV per sample).

# Results...

**Alpha diversity index in different weeks on horses under treatment with antioxidants (selenium and vitamin E) in different experimental weeks**

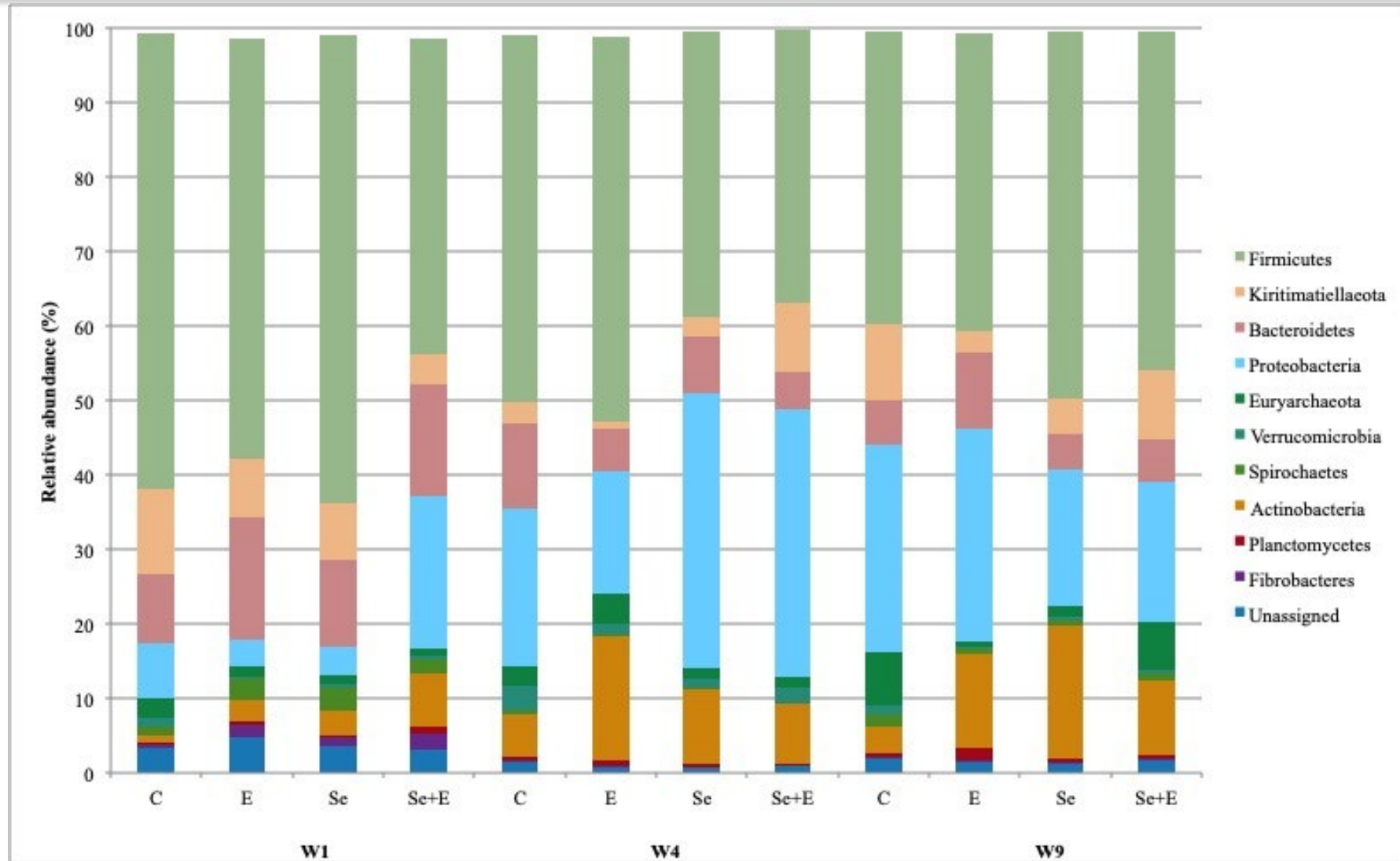
| Diversity index | Week | Treatment       |                 |                 |                 |
|-----------------|------|-----------------|-----------------|-----------------|-----------------|
|                 |      | C               | E               | Se              | Se+E            |
| Simpson         | 1    | 0.9 ± 0.1       | 1.0 ± 0.1       | 0.9 ± 0.1       | 1.0 ± 0.0       |
|                 | 4    | 0.9 ± 0.1       | 1.0 ± 0.0       | 0.9 ± 0.2       | 0.8 ± 0.2       |
|                 | 9    | 0.8 ± 0.3       | 0.9 ± 0.1       | 0.9 ± 0.0       | 0.9 ± 0.0       |
| Shannon         | 1    | 6.9 ± 1.5       | 8.2 ± 0.9       | 7.6 ± 0.8       | 7.2 ± 0.9       |
|                 | 4    | 5.8 ± 1.8       | 6.4 ± 0.5       | 5.7 ± 1.6       | 5.4 ± 1.9       |
|                 | 9    | 6.0 ± 2.8       | 6.1 ± 1.5       | 6.6 ± 0.7       | 6.8 ± 1.0       |
| Observed otus   | 1    | 845.0 ± 229.3 a | 984.6 ± 178.9 a | 888.8 ± 81.3 a  | 632.6 ± 88.3 a  |
|                 | 4    | 503.0 ± 279.1 b | 478.3 ± 38.2 b  | 477.3 ± 182.5 b | 469.3 ± 130.3 b |
|                 | 9    | 710.2 ± 234.9 a | 600.7 ± 272.0 a | 727.3 ± 188.2 a | 723.0 ± 228.3 a |

# Results...



Weighted unifrac of different treatments on horses color red for control, blue for E, orange for Se and Green for Se+E; spheres represent week 1, rings are from week 4 and stars from week 9.

# Results...



Phylum in feces of horses under treatment with antioxidants (selenium and vitamin E) in different experimental weeks

# Conclusion...

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The use of Se + E in the diet as well as physical activity modifies the fecal microbiota of horses.



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