Short and long-term effect on feed efficiency, plasma metabolome and gut microbiota of oregano essential oil (Carvacrol) in dairy calves

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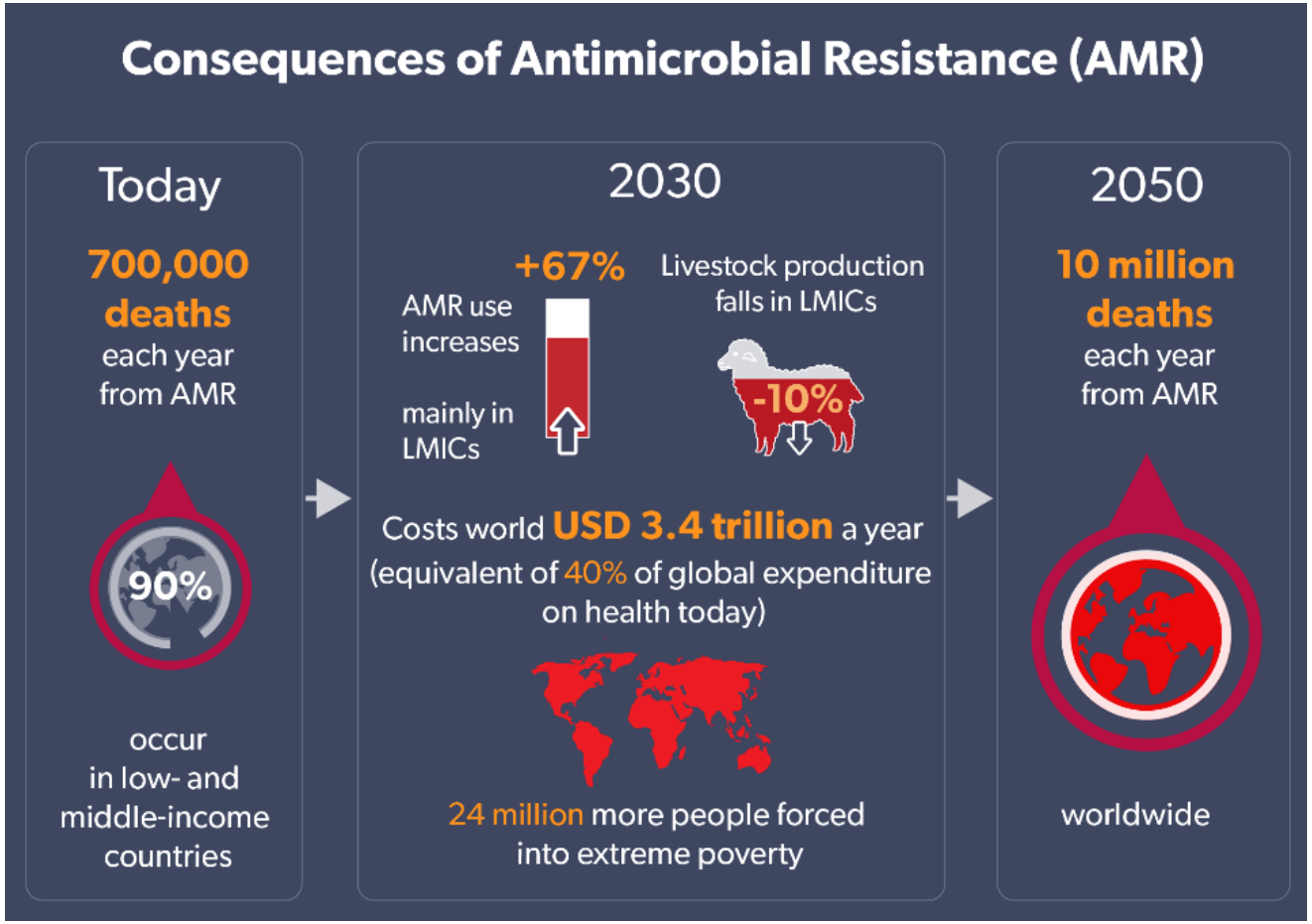
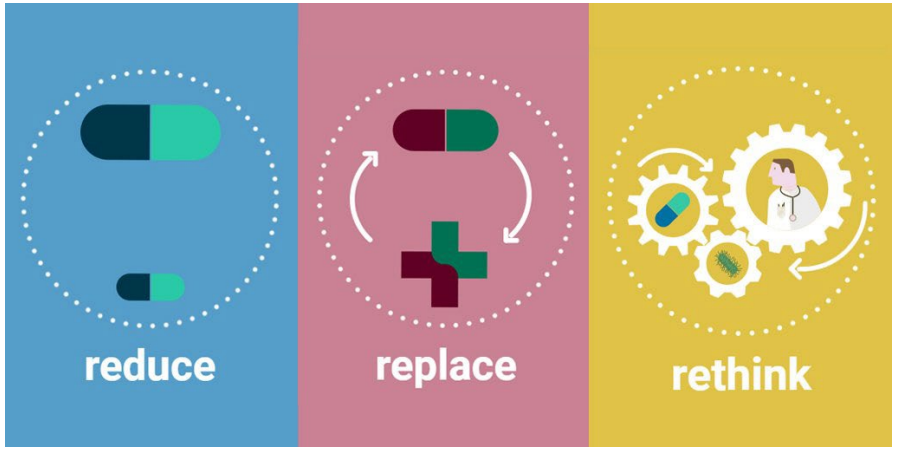
divas
DIPARTIMENTO DI MEDICINA VETERINARIA E SCIENZE ANIMALI

PRIMA
PARTNERSHIP FOR RESEARCH AND INNOVATION IN THE MEDITERRANEAN AREA

UNIVERSITÀ DEGLI STUDI DI MILANO

THE ISSUE OF ANTIMICROBIAL RESISTANCE



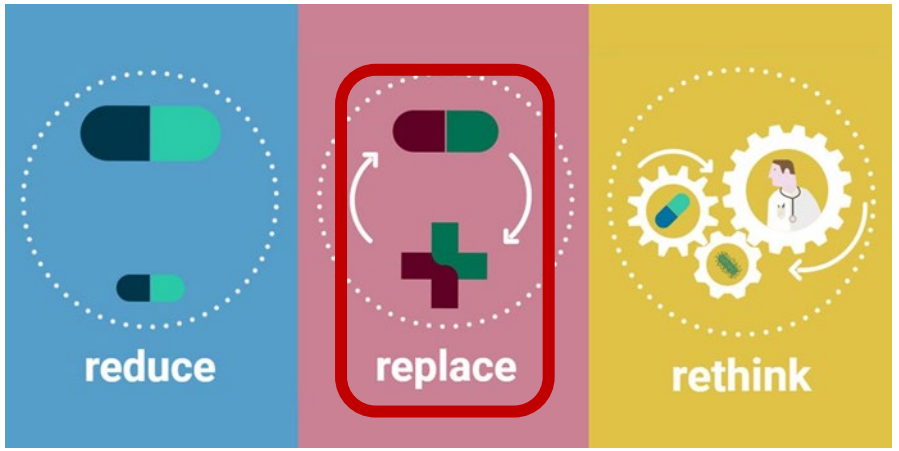


PNAS . March 19, 2015 112 (18) 5649-5654

Milk quality along the dairy chain for a safe and sustainable milk (MILKQUA)(2019-2023)

MilkQua

Find alternatives to the use of antibiotics in dairy cattle farms



MILKQUA explored Essential Oils as possible alternatives to antibiotics in dairy cattle

The use of Essential oils to improve the health and productivity of livestock



Why Essential Oils?

- Aromatic lipophilic liquids, derived from aromatic plants.
- Have antibacterial and anti-inflammatory activities
- Already tested (72% of the organic cattle farmers in France use aroma therapy)

Still:

- The real effectiveness of EOs and the mechanisms of action in cattle farming remain largely unknown
- No information about possible effects on feed efficiency
- Having antibacterial and anti-inflammatory activities is not “per se” always beneficial

Nehme, R. Et al. . (2021). **Essential Oils in Livestock: From Health to Food Quality**. Antioxidants. DOI:10.3390/antiox10020330

(Colombo, 2022; Guénic & Coll, 2014)

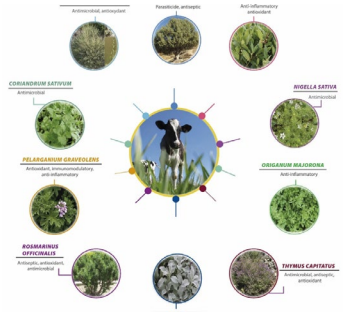
Research question

Does essential oil (EO) supplementation during the perinatal period impact feed efficiency in dairy calves and strengthen their long-term immunity, gut microbiome and blood metabolome?

Experimental Design

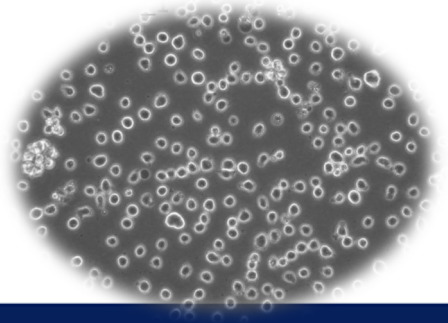
Selection of plants, extraction of the EO, and in vitro testing against major milk pathogens

- Antibacterial activity



Test of selected EO on an in vitro leukocyte model

- Immunomodulatory activity



In vivo validation

Feed efficiency
Gut microbiome
Plasma metabolome

Sub-clinical mastitis

- Mammary gland health
- OMICS

Experimental Design

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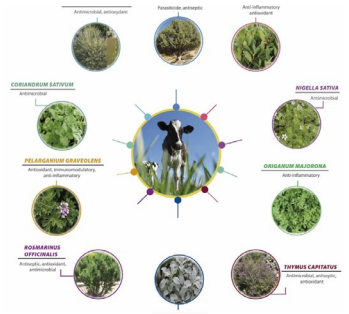
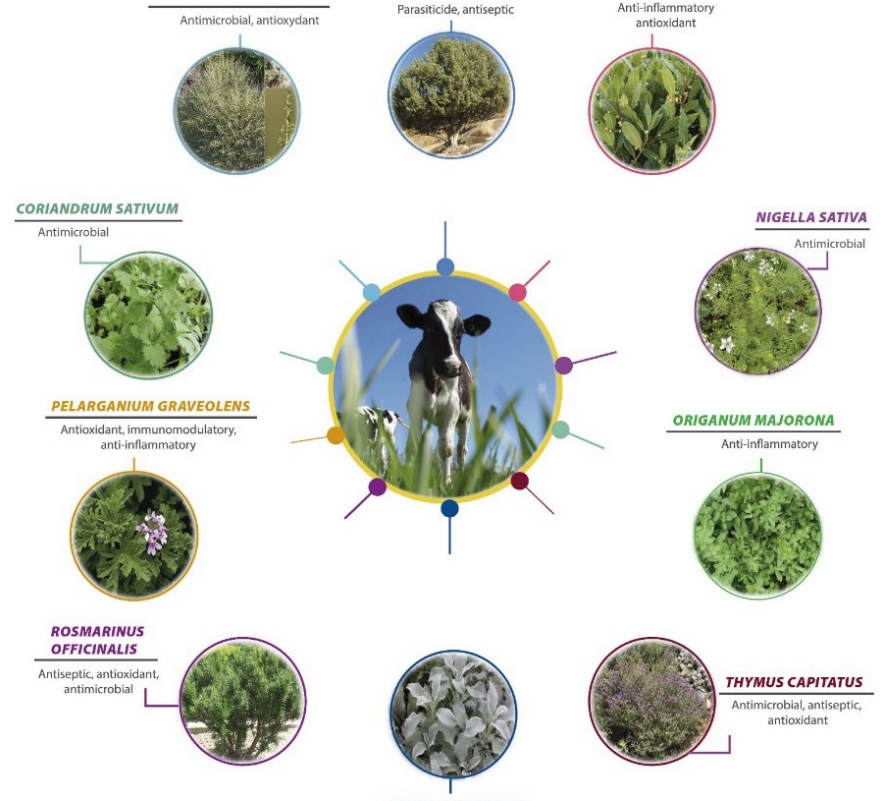


Evaluation of EO antibacterial activity



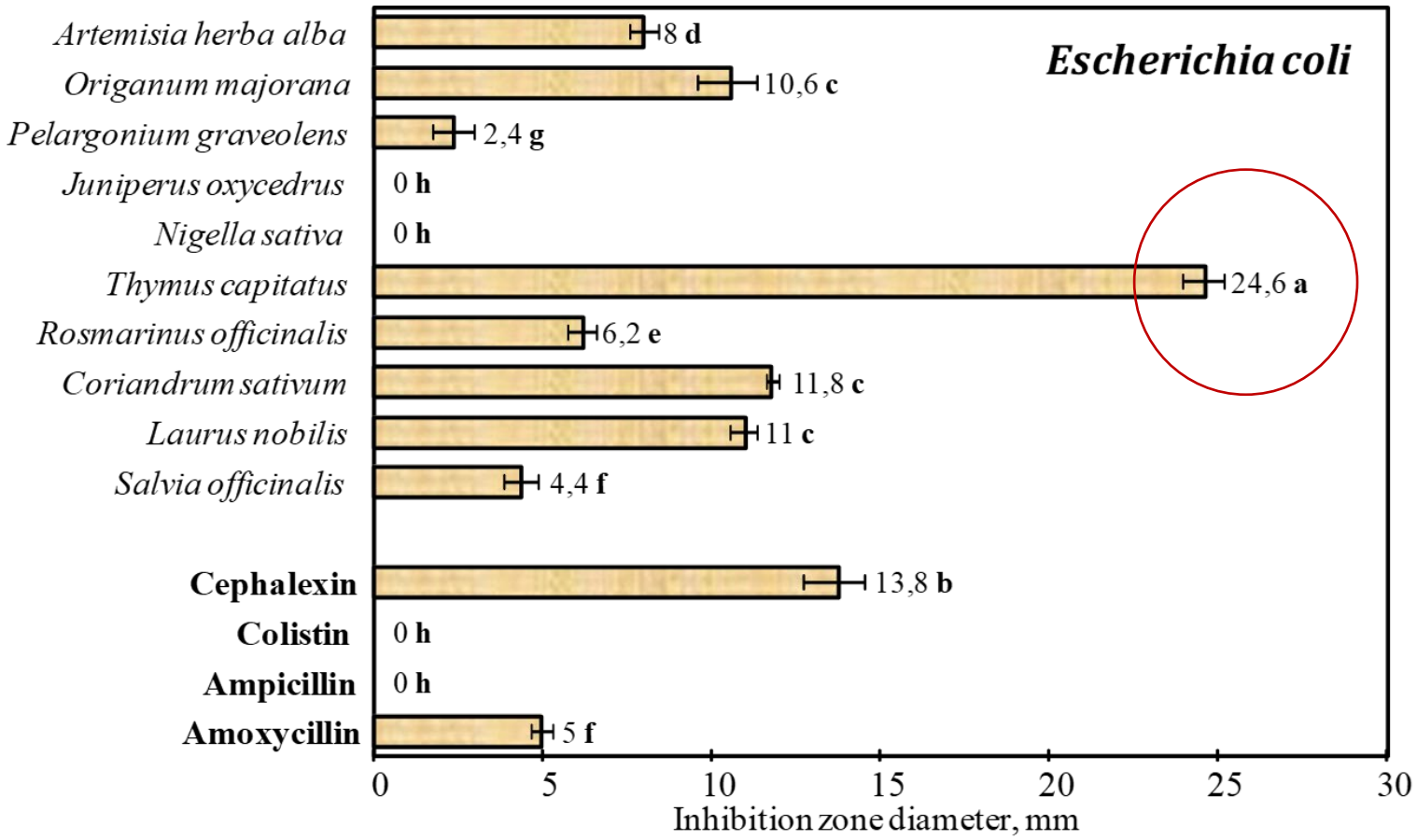
EO1 - *Salvia officinalis*
EO2 - *Laurus nobilis*
EO3 - *Coriandrum sativum*
EO4 - *Rosmarinus officinalis*
EO5 - *Thymus capitatus*
EO6 - *Nigella sativa*
EO7 - *Juniperus oxycedrus*
EO8 - *Pelargonium graveolens*
EO9 - *Origanum vulgare*
EO10 - *Artemesia herba-alba*

Each essential oils were:

1. Extracted
2. Analysed
3. Tested in vitro against major mammary gland pathogens

Centre de Biotechnologie, Technopole Borj Cédria
Hannen Falleh, Rihad Khsouri

Screening of the antibacterial activity of EOs against *E.coli* and *S.aureus* isolated from the cow's mammary gland



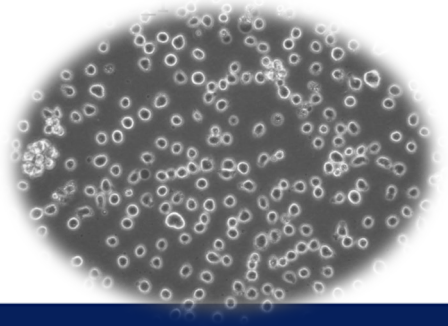
Similar results against *S.aureus*

***Thymus capitatus* EO features the major anti-bacterial activity**

Experimental Design

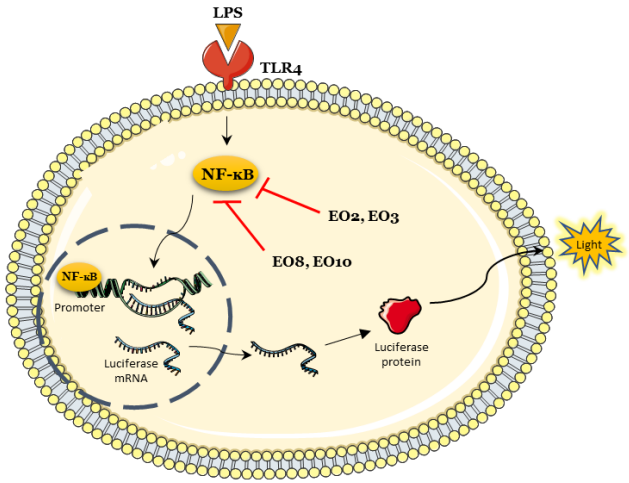
Test of selected EO on an in vitro leukocyte model

- Immunomodulatory activity



Assessment of EO anti/pro-inflammatory activity

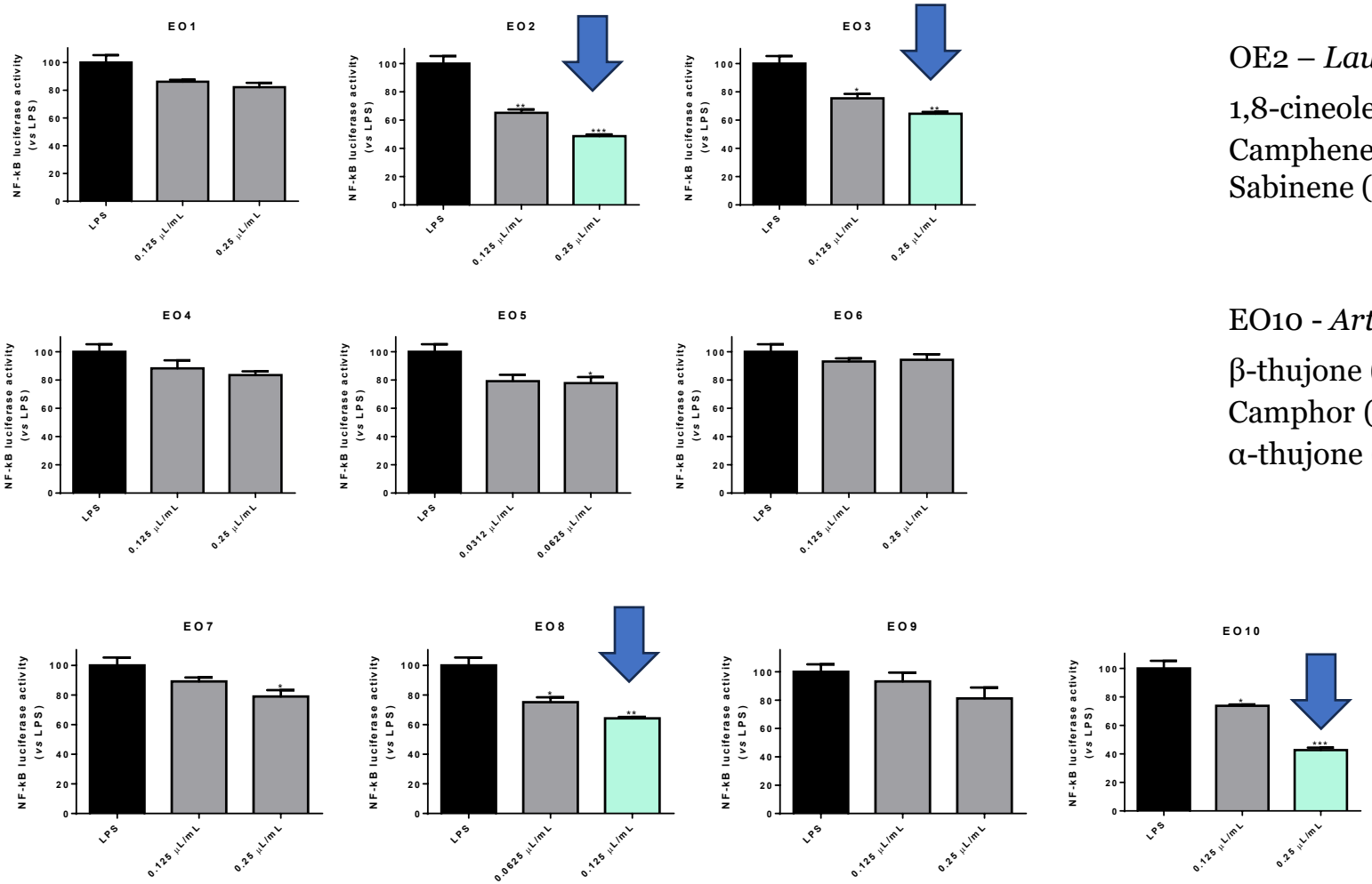
- Measurement of NFkB production
- Pro-inflammatory cytokine abundance (mRNA)



THP-1 Lucia cells

David Pereira – University of Porto

Screening of essential oils against LPS-induced NFkB activation in THP-1 macrophages



OE2 – *Laurus nobilis*

1,8-cineole (43.85%)
Camphene (13.82%)
Sabinene (7.03%)

OE3 – *Coriandrum sativum*

Linalool (64.10%)
α-Pinene (7.26%)
o-Cymene (5.91%)
Camphor (5.50%)

EO10 - *Artemesia herba-alba*

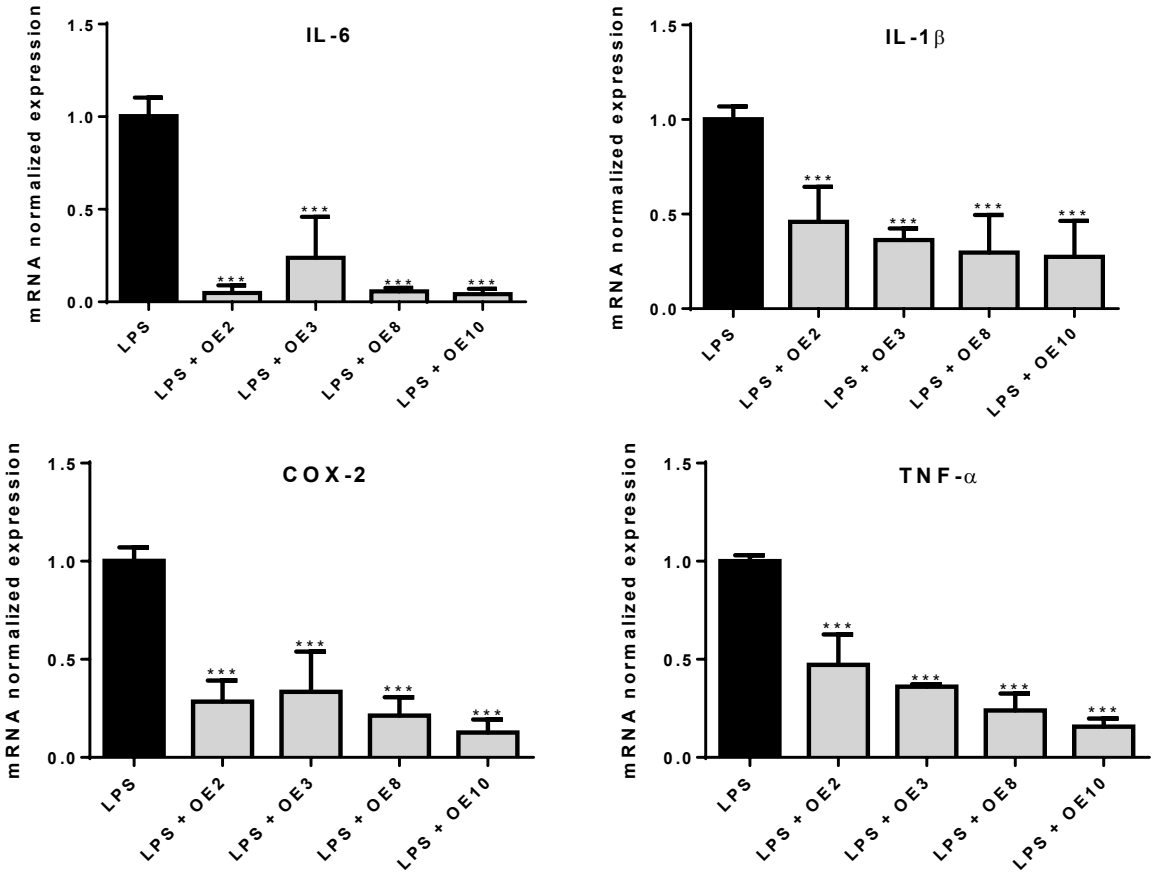
β-thujone (38.27%)
Camphor (11.88%)
α-thujone (11.58%)

OE8 – *Pelargonium graveolens*

β-citronellol (36.88%)
Geraniol (15.02%)
Citronellyl formate (8.74%)

Thymus capitatus EO had no NFkB activations

THP-1 macrophages: qPCR analysis of selected EO on pro-inflammatory cytokines and COX-2



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***Thymus capitatus* EO had no immunomodulatory activity**

- Pereira, R. B et al (2023). **Anti-inflammatory activity of essential oils from Tunisian aromatic and medicinal plants and their major constituents in THP-1 macrophages**. Food Research International

Experimental Design

Feed efficiency
Gut microbiome
Plasma metabolome

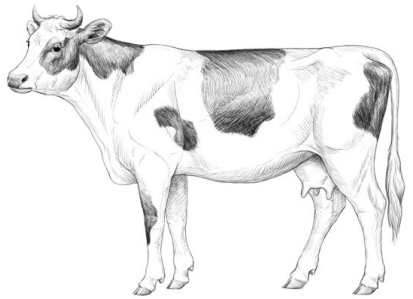
In vivo study

Experimental protocol

n=8 (CTRL group)

n=8 (**EO** group, approx. 200 mg of (T. capitatus) **daily during 45 days**)

(EO supplementation during the liquid feeding period)
D3 to D45

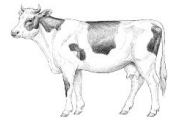


(Adult Phase, D370)

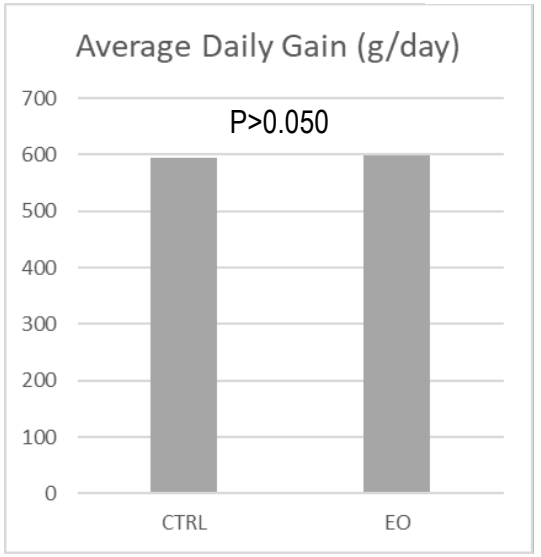
- Individual food intake/Blood/Faeces: T0 (D3), T1 (D45), T2 (D370) of age
- Weight development and feed efficiency measurement
- Biochemical parameters, peripheral blood mononuclear cell (PBMC) differential count,
- Faecal (Gut) microbiota
- Plasma untargeted Metabolome

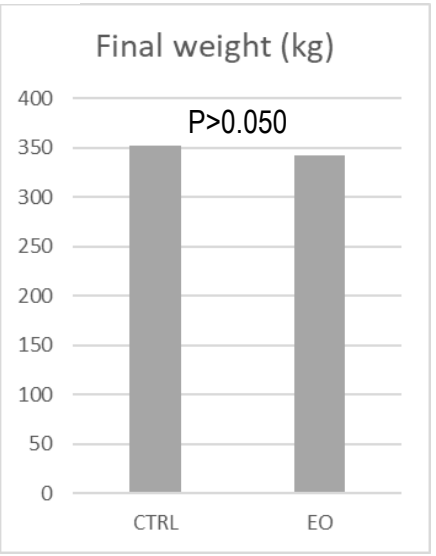
Sonia Andres
CSIC – Leon
University of Leon

Results feed gain, blood parameters, Ox stress



D45

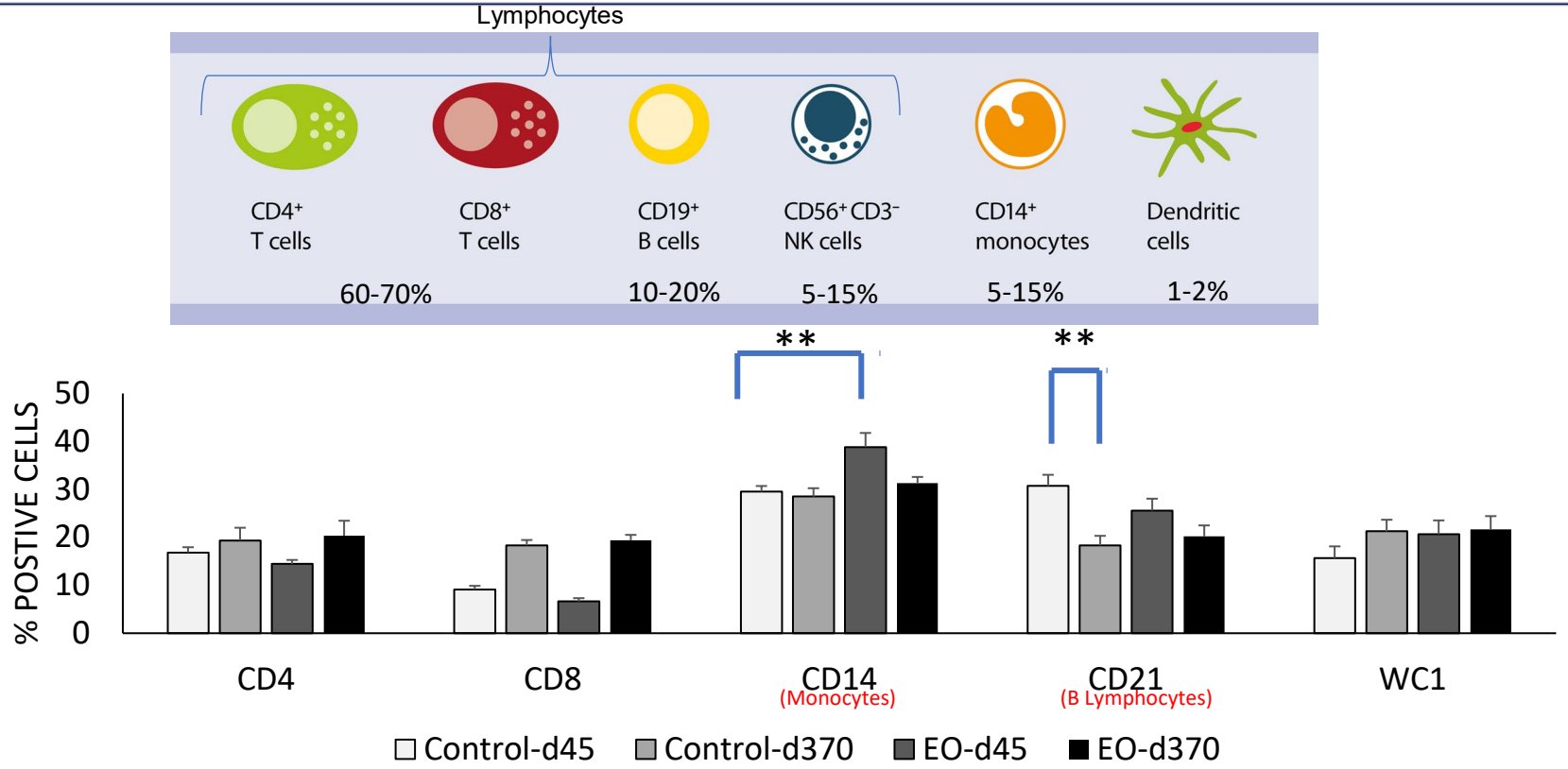




D370

- Better feed gain and higher weight in the EO group at D45
- No significant difference (weight and weight gain) between the 2 long-term D370 groups
- No significant difference for all blood parameters measured (Cholesterol, Insulin, TG, Glucose, minerals...)
- No effect of EOs on oxidative stress (SOD measurement)

Changes in mononuclear cell profile



- ✓ No significant difference between groups for T cell count (CD8+), γδ T cell count (WC1+)
- ✓ Decrease of CD21+ lymphocyte counts between D45 and D370 in the control group
- ✓ Increase of monocytes in the EO treatment on the monocyte population (CD14+) at day 45

EO effects on feed efficiency and blood parameters

- Limited (negligble?)
- Related to the first 45 days (no long-term effects)

Changes in the microbiota (16S)

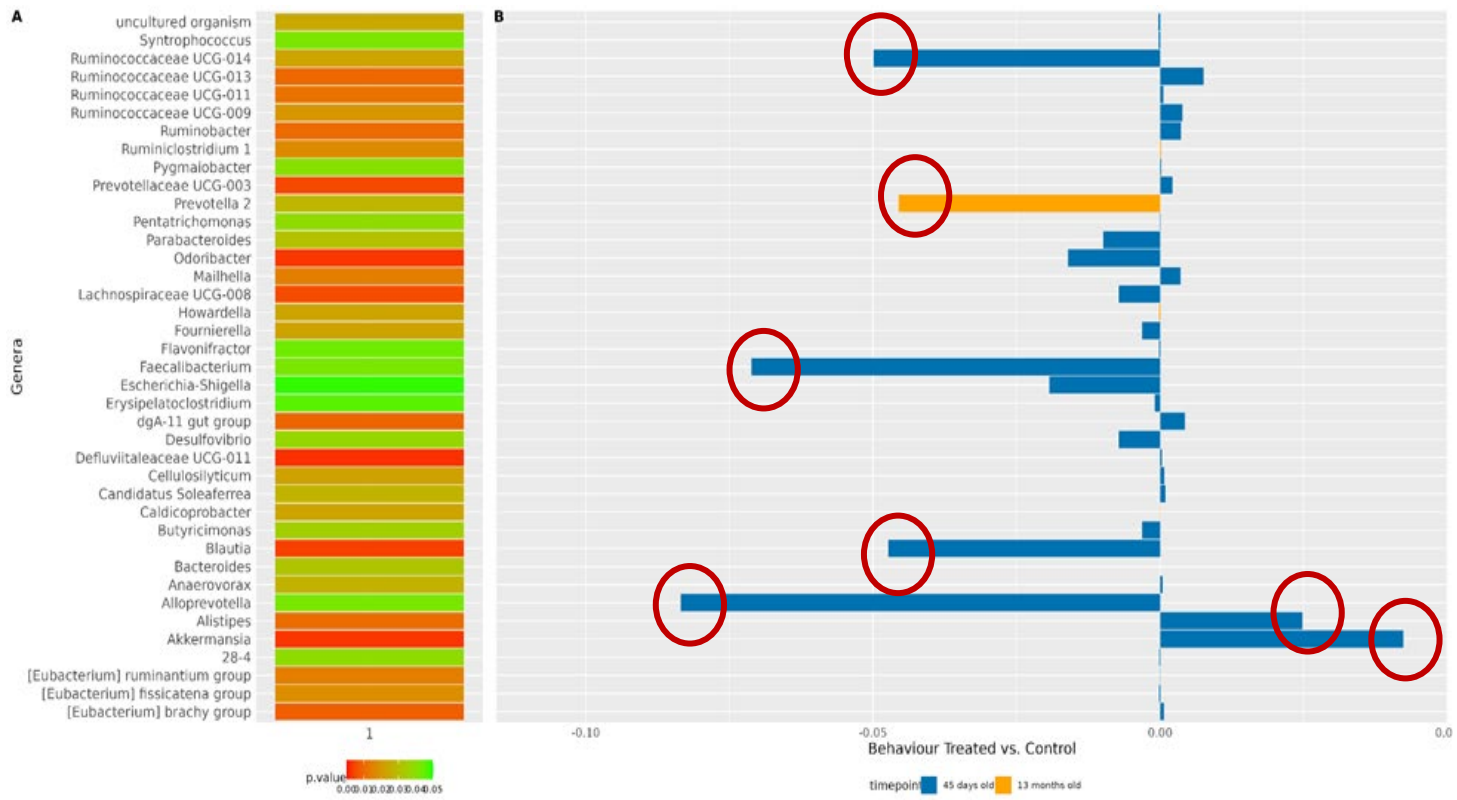
Changes at D45

↓
- Ruminococcaceae UCG-014
- Faecalibacterium
- Blautia
- Alloprevotella

↑
- Allistipes
- Akkermansia

Changes at D370

↓
- Prevotella



Changes at 45

Changes at D370

Changes in the microbiota (16S)

Changes at D45

Ruminococcaceae UCG-014 → Marker of gut health

↓ Faecalibacterium → Associated to diarrhea

Blautia

Alloprevotella → Marker of gut health

↑ Allistipes

Akkermansia → Probiotics (protection against several intestinal diseases)

Changes at D370

↓ Prevotella → Reduced during diarrhea

EO effects on gut microbiome

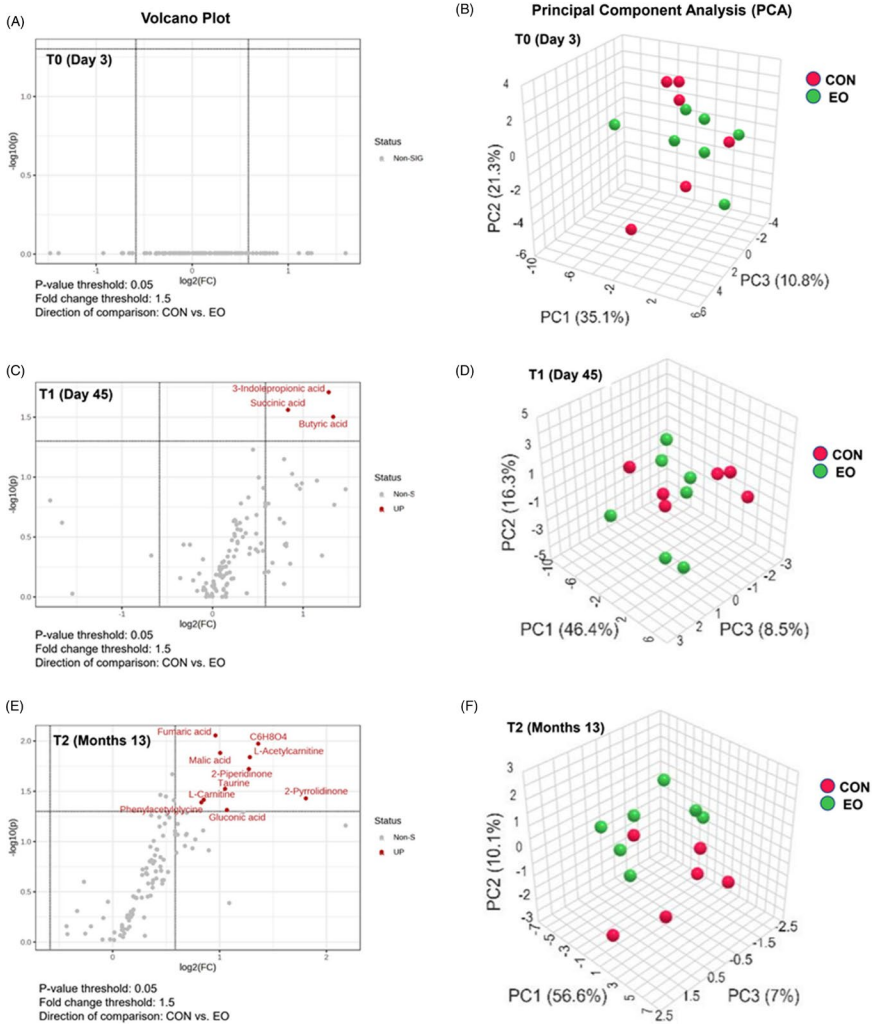
- Limited effects
- More evident after the first 45 days
- Still present after 13 months
- With contradictory effects (potentially dangerous)

Blood Metabolome changes

T0

D45

D360



At D45

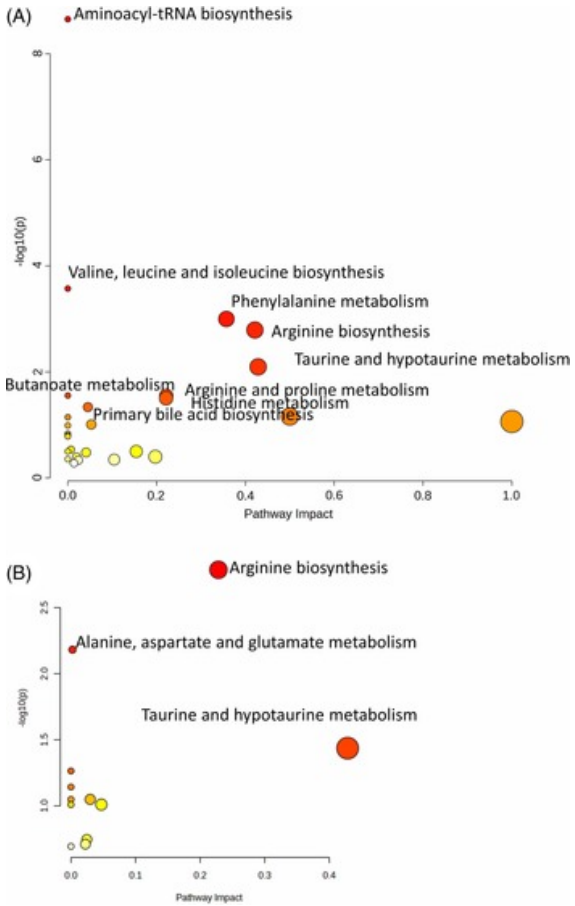
- Changes in metabolites that are produced only by bacteria
- < Butyric acid, that is produced by intestinal bacteria (Blautia, Ruminococcaceae and Faecalibacterium)
- < 3-indole-propionic acid produced by Faecalibacterium

inhibitor of gut dysbiosis, related to endotoxin leakage and hydroxyl radical scavenger

The metabolic pathway changes

Control calves

D360



At D360

- ✓ Decreased metabolism of amino acid synthesis in the EO group.
- ✓ Decreased energy production (fumaric acid, malic acid, and carnitines, such as L-acetyl carnitine and L-carnitine)
- ✓ Decrease in biogenic amines, such as taurine and citrulline, indicates a decrease in antioxidant activity

Take home message(s)

1 Carvacrol-based EOs have a strong anti-bacterial but not anti-inflammatory activity in vitro

2 Early supplementation with carvacrol improves calves' weight gain at 45 days of age.

3 Carvacrol intake induces early changes in the gut microbial population, consistent with changes in the plasma metabolome,

Not necessarily “desirable” from the point of view of gut health
Not positive for feed efficiency

4 Limited long-term effect of carvacrol supplementation on animal performance and immunity was observed

Where to find more details

BenJemaa M, Rahali FZ, Falleh H, Beji-Serairi R, Megdiche-Ksouri W, Hamrouni I, Hammami M, Nehme R, Pereira RB, Andrés S, Bouhallab S, Pereira DM, Ceciliani F, Abdennebi-Najar L, Ksouri R. Essential Oil Stabilisation by Response Surface Methodology (RSM): Nanoemulsion Formulation, Physicochemical, Microbiological, and Sensory Investigations. Molecules. 2022 Oct 28;27(21):7330. doi: 10.3390/molecules27217330. PMID: 36364161; PMCID: PMC9655826.

Ranilla MJ, Andrés S, Gini C, Biscarini F, Saro C, Martín A, Mateos I, López S, Giráldez FJ, Abdennebi-Najar L, Pereira D, Falleh H, Ksouri R, Cremonesi P, Castiglioni B, Ceciliani F. Effects of Thymbra capitata essential oil on in vitro fermentation end-products and ruminal bacterial communities. Sci Rep. 2023 Mar 13;13(1):4153. doi: 10.1038/s41598-023-31370-9. PMID: 36914736; PMCID: PMC10011596.

Pereira RB, Rahali FZ, Nehme R, Falleh H, Jemaa MB, Sellami IH, Ksouri R, Bouhallab S, Ceciliani F, Abdennebi-Najar L, Pereira DM. Anti-inflammatory activity of essential oils from Tunisian aromatic and medicinal plants and their major constituents in THP-1 macrophages. Food Res Int. 2023 May;167:112678. doi: 10.1016/j.foodres.2023.112678. Epub 2023 Mar 15. PMID: 37087210.

Andrés S, Gini C, Ceciliani F, Gutiérrez-Expósito D, Arteche-Villasol N, Martín A, Cremonesi P, Faré F, Ghaffari MH, Giráldez FJ, Abdennebi-Najar L. Essential oil supplementation in milk replacers: short- and long-term impacts on feed efficiency, the faecal microbiota and the plasma metabolome in dairy calves. J Dev Orig Health Dis. 2024 Apr 2;15:e5. doi: 10.1017/S2040174424000084. PMID: 38563206.

Thanks for your attention

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Thanks for listening