



NUTRITIONAL AND HEALTH BENEFITS OF ESSENTIAL OILS (EO) IN DAIRY COWS



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Pereira DM, Andrés S, Pereira RB, Nehme R, Vambergue E, Gini C, Even S, Sabbah M, Falleh H, Hamrouni I, Ksouri R, BenJemaa M., Rahali F.Z, Giráldez FJ, López S, Ranilla M.J, Cremonesi P, Castiglioni B, Bouhallab S, Ceciliani F.



The PRIMA programme is supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation

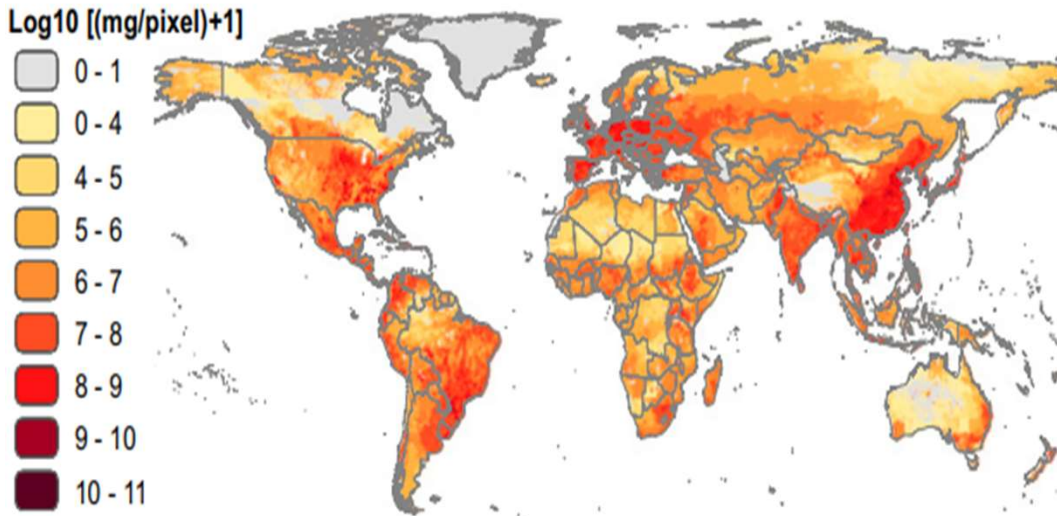


MilkQua

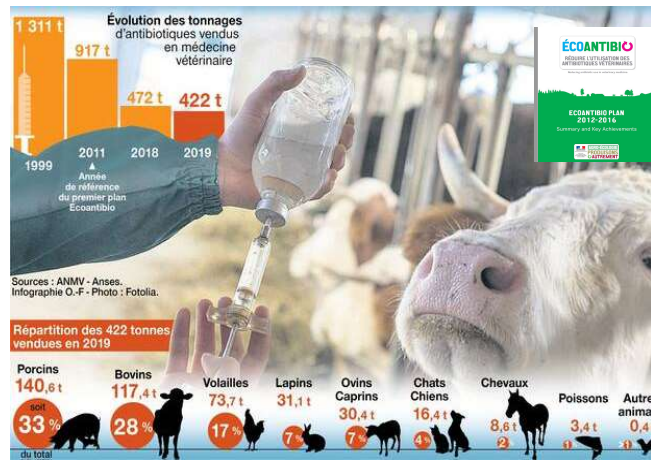
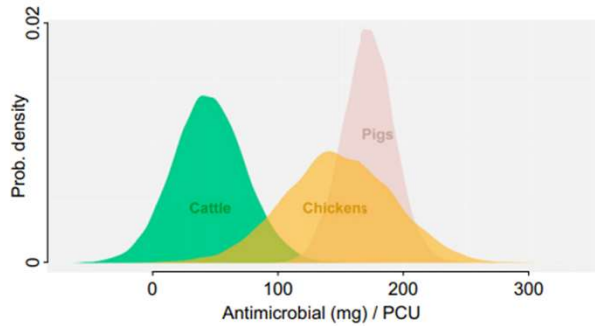


I. ANTIMICROBIAL IN LIVESTOCK

The global consumption of antimicrobials in animals is twice that of humans
Nature, 2012, 486, 465–466



Global antimicrobial consumption in livestock in milligrams per 10 km2



In France antimicrobial consumption decreased by almost 30% in livestock during the last decade

The Antibiotic Alarm. Nature. 2013;495(7440); Bartlett JG and al. Clin Infect Dis. 2013;56(10):1445–145; Gross M. Antibiotics in Crisis. Curr Biol. 2013;23(24):R1063–R1065; Public Health. 2014;2:145 ; T.P. Van Boeckel et al. PNAS . March 19, 2015 112 (18) 5649-5654

I. ANTIMICROBIAL IN LIVESTOCK

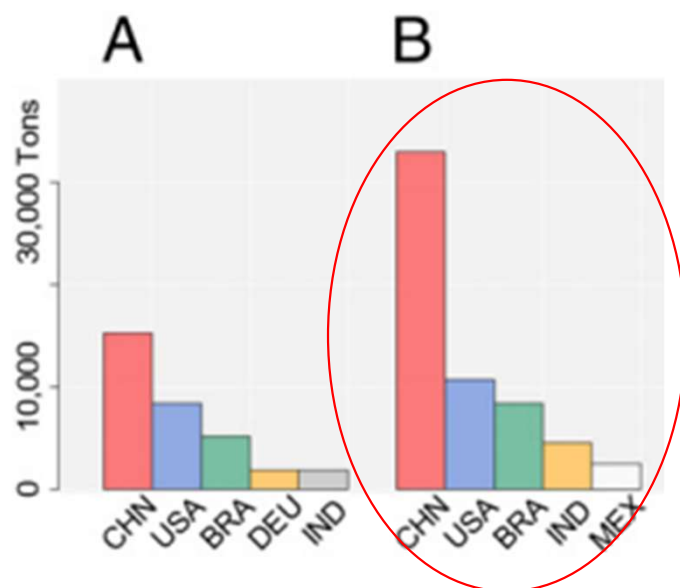
ANTIMICROBIAL RESISTANCE



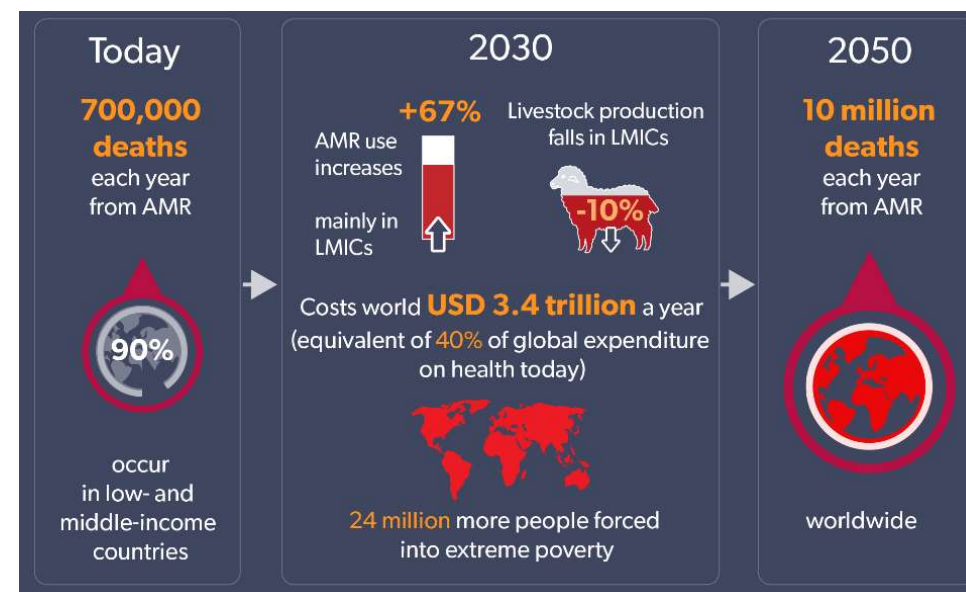
April 11, 2012 | US News | Isaac
 FDA Seeks to Limit Antibiotic Use in Livestock



I guess it was after I started abusing antibiotics that I noticed my four extra teats...



Largest 5 consumers of antimicrobials in livestock in 2030 (predicted) (B)





USING EO AS ALTERNATIVES TO ANTIMICROBIALS

A MYTH OR REALITY?



II. EO characterization and biological activities



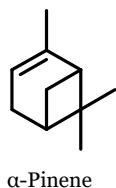
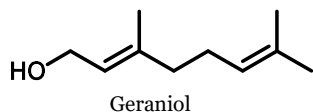
- Volatile
- Lipid soluble
- Density - generally lower than water
- Mostly obtain by steam distillation
- Chemical variation even for the same plant species



Terpene type compounds

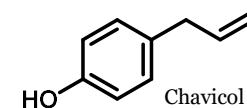
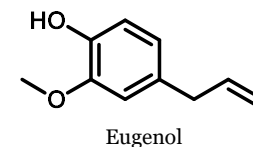
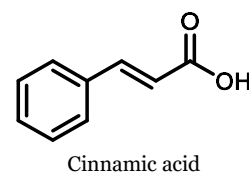
Monoterpenes

- Acyclic
- Cyclic



Aromatic type compounds

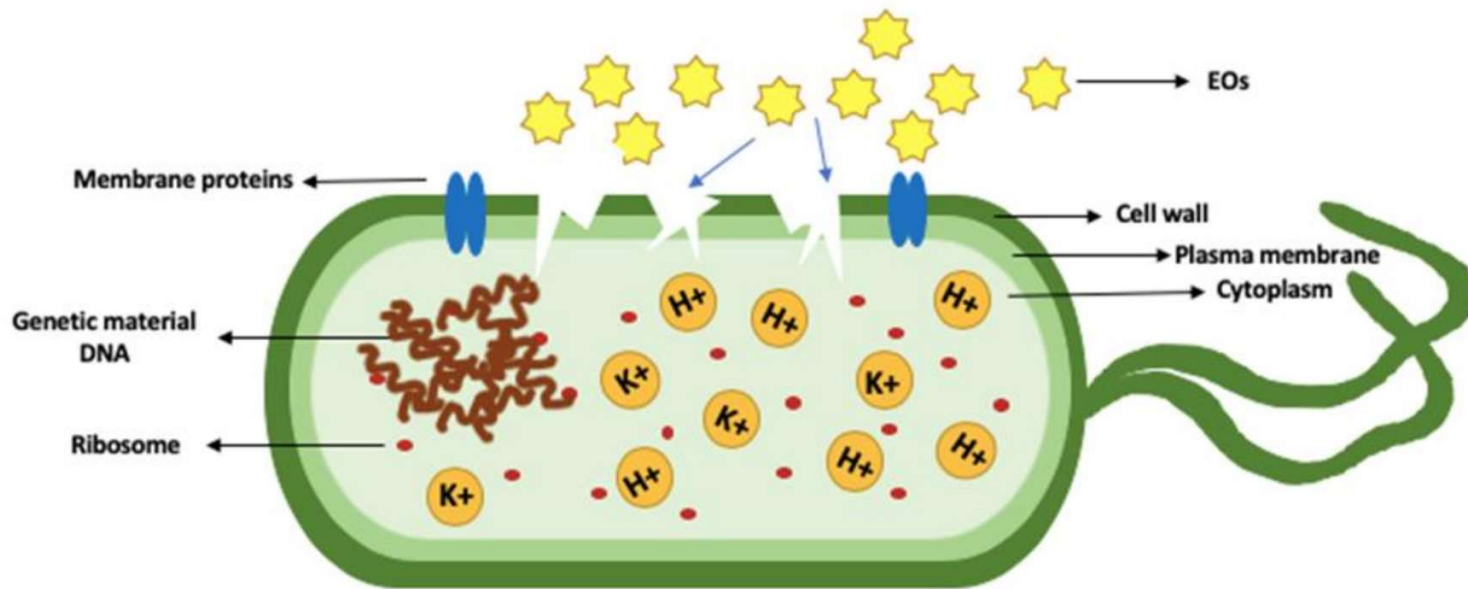
Phenylpropanoids



➤ Esters, ethers, aldehydes, lactones, ketones and alcohols are all found in essential oils.

II. EO characterization and biological activities

ANTIBACTERIAL ACTIVITY : MECHANISMS OF ACTION



Damage to the cell wall;
Degradation to the cell membrane;
Coagulation of the cytoplasmic protein;
Leakage of the cell contents and **reduction** of the proton-motive force

Minimum inhibition concentration of essential oils against various Bacterial pathogens (adapted from yang et al., 2015)

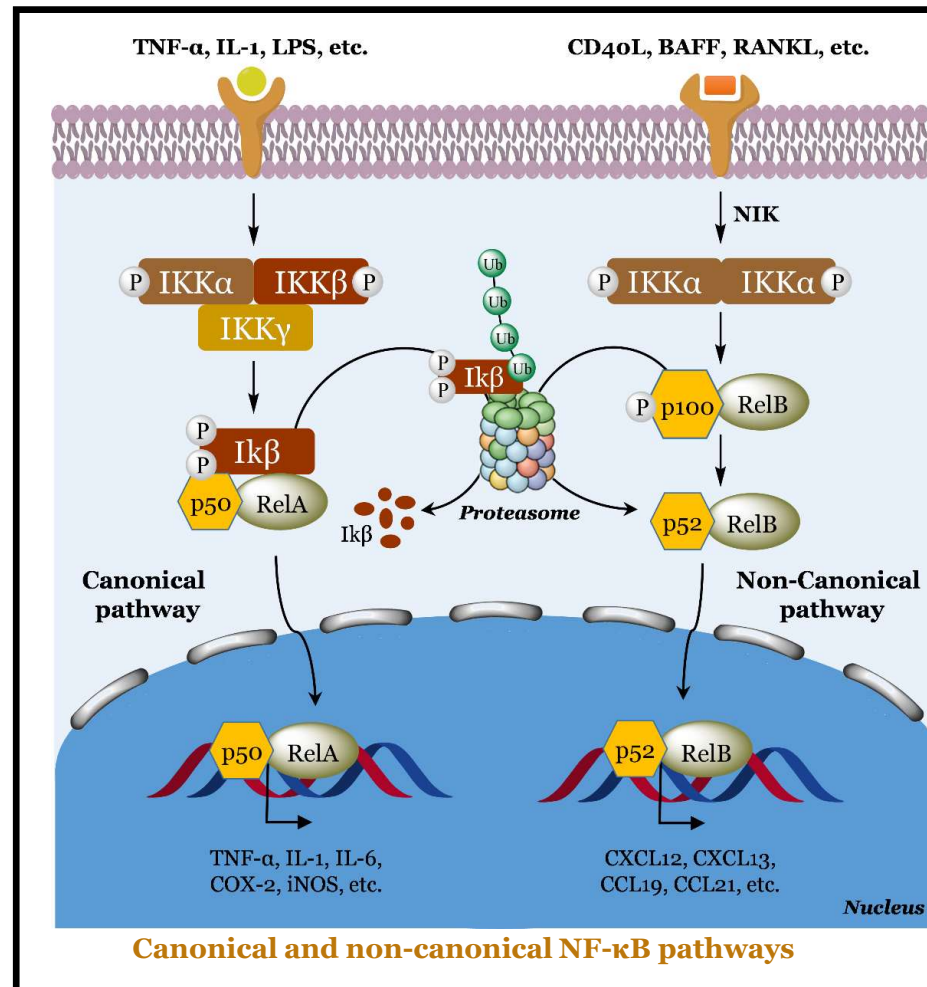
- ✓ A strong bactericidal activity against pathogenic bacteria such as Escherichia coli O157: H7 and Salmonella spp evidenced by MIC in vitro test

Product	Pathogenic microbe	Gram	MIC (unit)	MIC (#)	Reference
Thymol	<i>Lactococcus piscicum</i>	+	mg/L	320	Navarrete et al., 2010
	<i>Streptococcus phocae</i>	+	mg/L	640	Navarrete et al., 2010
	<i>Flavobacterium psychrophilum</i>	-	mg/L	320	Navarrete et al., 2010
	<i>Vibrio anguillarum</i>	-	mg/L	80	Navarrete et al., 2010
	<i>Vibrio parahaemolyticus</i>	-	mg/L	320	Navarrete et al., 2010
	<i>Pseudomonas sp.</i>	-	mg/L	640	Navarrete et al., 2010
	<i>Brachyspira hyodysenteriae</i>	-	mmol/L	1.25	Vande Maele et al., 2016
	<i>Escherichia coli</i> O157:H7	-	µg/mL	166	Si et al., 2006
	<i>Salmonella typhimurium</i> DT104	-	µg/mL	233	Si et al., 2006
	<i>Escherichia coli</i> K88	-	µg/mL	100	Si et al., 2006
Eugenol	<i>Lactococcus lactis</i>	+	mg/L	1,280	Navarrete et al., 2010
	<i>Vibrio sp.</i>	-	µg/mL	156	Seongwei et al., 2009
	<i>Escherichia coli</i>	-	µg/mL	625	Seongwei et al., 2009
	<i>Salmonella</i>	-	µg/mL	156	Seongwei et al., 2009
	<i>Pseudomonas sp.</i>	-	µg/mL	325	Seongwei et al., 2009
	<i>Edwardsiella tarda</i>	-	µg/mL	56 to 125	Seongwei et al., 2009
	<i>Aeromonas hydrophilla</i>	-	µg/mL	625	Seongwei et al., 2009
	<i>Brachyspira hyodysenteriae</i>	-	mmol/L	2.5	Vande Maele et al., 2016
	<i>Escherichia coli</i> O157:H7	-	µg/mL	466	Si et al., 2006
	<i>Salmonella typhimurium</i> DT104	-	µg/mL	400	Si et al., 2006
Carvacrol	<i>Escherichia coli</i> K88	-	µg/mL	300	Si et al., 2006
	<i>Listonella anguillarum</i>	-	µg/mL	25	Volpatti et al., 2013
	<i>Brachyspira hyodysenteriae</i>	-	mmol/L	1.25	Vande Maele et al., 2016
	<i>Escherichia coli</i> O157:H7	-	µg/mL	283	Si et al., 2006
	<i>Salmonella typhimurium</i> DT104	-	µg/mL	167	Si et al., 2006
Cinnamaldehyde	<i>Escherichia coli</i> K88	-	µg/mL	100	Si et al., 2006
	<i>Brachyspira hyodysenteriae</i>	-	mmol/L	0.31	Vande Maele et al., 2016
	<i>Escherichia coli</i> O157:H7	-	µg/mL	133	Si et al., 2006
	<i>Salmonella typhimurium</i> DT104	-	µg/mL	100	Si et al., 2006

II. EO characterization and biological activities

ANTI-INFLAMMATORY ACTIVITIES: MECHANISM OF ACTION

NF- κ B pathway



Adapted from Periera et al. MILKQUA steering board, LODI, september, 2021

**III. NUTRITIONAL
EFFECTS OF ESSENTIAL
OILS IN THE DAIRY
RUMINANT SECTOR**



III. Nutritional effects : in vitro studies

Effects of EO on ruminal microbiota



Study 1

Rumen simulation technique (Rusitec) and a dual-flow (DF) fermenter

Essential oil from cinnamon leaf (500mg/L) → a decrease in Protozoa abundance

Study 2

Strained rumen fluid for 12 and 24 h incubation periods:

- Mixture of essential oils (1g/kg of substrate) : Protozoa abundance *Selenomonas* and *Ruminococcus albus* ↗
- Mixture of essential oils (2g/Kg of substrate): *Butyrivibrio fibrisolvens*, fungi and *Ruminococcus flavefaciens* ↗

III. Nutritional effects : in vitro studies

Effects of EO on ruminal fermentation and methane production



Corn silage + Oregano, thyme, cinnamon and orange peel EOs

- ✓ Decreasing in Acetate and in the acetate/propionate ratio
- ✓ Selective toxicity against rumen bacterial strains that grow at low rumen pH.
- ✓ No effects on *Volatile fatty acid*

Joch et al , 2016

Eugenol, carvacrol, citral, limonene, 1,4-cineole, p-cymene, linalool, bornyl acetate, α -pinene, and β -pinen were incubated at a dose of a **dose of 1,000 μ L/L** for **24 h** in diluted rumen fluid

- ✓ Eeugenol, carvacrol, citral, -cineole, p-cymene, linalool and β -pinen inhibit volatile fatty acid
- ✓ bornyl acetate only decreases methane production per mol of VFA and may help to improve the efficiency of energy use in the rumen.

III. Nutritional effects : in vitro studies

Effects of EO on ruminal fermentation, methane production and microbiota



J. Dairy Sci. 103:2303–2314
<https://doi.org/10.3168/jds.2019-16611>
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Effects of oregano essential oil on in vitro ruminal fermentation, methane production, and ruminal microbial community

Rui Zhou,¹ Jianping Wu,^{1*} Xia Lang,² Lishan Liu,² David P. Casper,³ Cailian Wang,² Liping Zhang,¹ and Sheng Wei¹

¹College of Animal Science and Technology, Gansu Agricultural University, No. 1 Yingmen Village Anning, Lanzhou, Gansu, People's Republic of China, 730070

²Animal Husbandry, Pasture, and Green Agriculture Institute, Gansu Academy of Agricultural Sciences, No. 1 Nongkeyuan Village Anning, Lanzhou, Gansu, People's Republic of China, 730030



- Oregano supplementation (from 13 to 1200 mg during 24 h, **modify VFA concentrations and reduce methane emissions** by extensively altering the ruminal bacterial community.
- An optimal feeding rate for future animal studies of approximately **52 mg/L for mature ruminants is recommended**

III. Nutritional effects : in vivo studies

Effects of EO on ruminal microbiota



Alfalfa + corn silage +750 mg/day of Crina Ruminant supplement® (thymol, eugenol, vanillin and limonene)

- ✓ No effects on the abundance of ruminal protozoa, bacteria and fungi.
- ✓ A possible adaptation of rumen microbes to the compounds.

Benchaar C et al (2007). J Dairy Sci 90:886–897



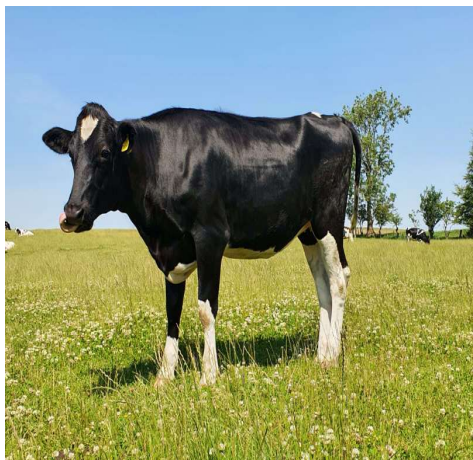
Corn silage + Supplying OEO 4-7 g/d (carvacrol, thymol, γ -terpinene, *p*-cimene and linalool)

- ✓ No effects on the ruminal pH but it could shift the rumen microbial population to one with less protozoa.
- ✓ Enhances the growth of certain rumen microbial populations, but the shifts were influenced by the supply rate.
- ✓ Low amount (4 g•d⁻¹) of OEO could have positive effects on ruminal microbial populations, whereas supplying elevated doses could be detrimental to those same ruminal microbial populations

Zhou R et al (2019). PLoS ONE 14:e0217054.

III. Nutritional effects : in vivo studies

Effects of EO on dairy cow performances



Randomized Controlled Trial

> J Dairy Sci. 2013;96(12):7892-903. doi: 10.3168/jds.2013-7128.

Epub 2013 Oct 11.

Effect of essential oils on ruminal fermentation and lactation performance of dairy cows

J A Tekippe ¹, R Tacoma, A N Hristov, C Lee, J Oh, K S Heyler, T W Cassidy, G A Varga, D Bravo



- ✓ A marginal effects of the tested EO product fed at 525 mg/d f Xtract 6965) on ruminal fermentation and productivity of lactating dairy cows but do show a trend for a consistent increase in total-tract NDF digestibility



Concentrates, seasonal green forage + clove and rosemary EOs (4 g/d) in heifers feeding

- ✓ A decrease in the digestibility of the dry matter and in NDF
- ✓ Inclusion of EO in the diet increases rumination rate

IV . Effects of EO on animal health : antihelminthic, antiparasitic and against mastitis



➤ Parasitol Res. 2014 Dec;113(12):4431-7. doi: 10.1007/s00436-014-4121-4. Epub 2014 Sep 10.

Acaricidal properties of the formulations based on essential oils from *Cymbopogon winterianus* and *Syzygium aromaticum* plants

Valéria de Mello ¹, Márcia Cristina de Azevedo Prata, Márcio Roberto da Silva, Erik Daemon, Luciane Santos da Silva, Flávia del Gaudio Guimarães, Alessandra Esther de Mendonça, Evelize Folly, Fernanda Maria Pinto Vilela, Lilian Henriques do Amaral, Lucio Mendes Cabral, Maria da Penha Henriques do Amaral

Repellent and acaricidal activity of essential oils and their components against *Rhipicephalus* ticks in cattle.

Chohan M, Abbas RZ, Israr M, Abbas A, Mehmood K, Khan MK, Sindhu ZUD, Hussain R, Saleemi MK, Shah S.

Parasitol. 2020 Jul;283:109178. doi: 10.1016/j.vetpar.2020.109178. Epub 2020 Jun 29.

ID: 32652458 Review.

➤ J Helminthol. 2019 Dec 17;94:e111. doi: 10.1017/S0022149X19001081.

Effect of essential oils on cattle gastrointestinal nematodes assessed by egg hatch, larval migration and mortality testing

S Saha ^{1 2}, S Lachance ²

Development of essential oil-based phyto-formulations to control the cattle tick *Rhipicephalus microplus* using a mixture design approach.

Lazcano Díaz E, Padilla Camberos E, Castillo Herrera GA, Estarrón Espinosa M, Espinosa Andrews H, Paniagua Buelnas NA, Gutiérrez Ortega A, Martínez Velázquez M.

Exp Parasitol. 2019 Jun;201:26-33. doi: 10.1016/j.exppara.2019.04.008. Epub 2019 Apr 25.

PMID: 31029699

AROMAM Field trial: Efficiency of an essential oil-based mixture to cure mild and severe clinical mastitis in lactating dairy cows

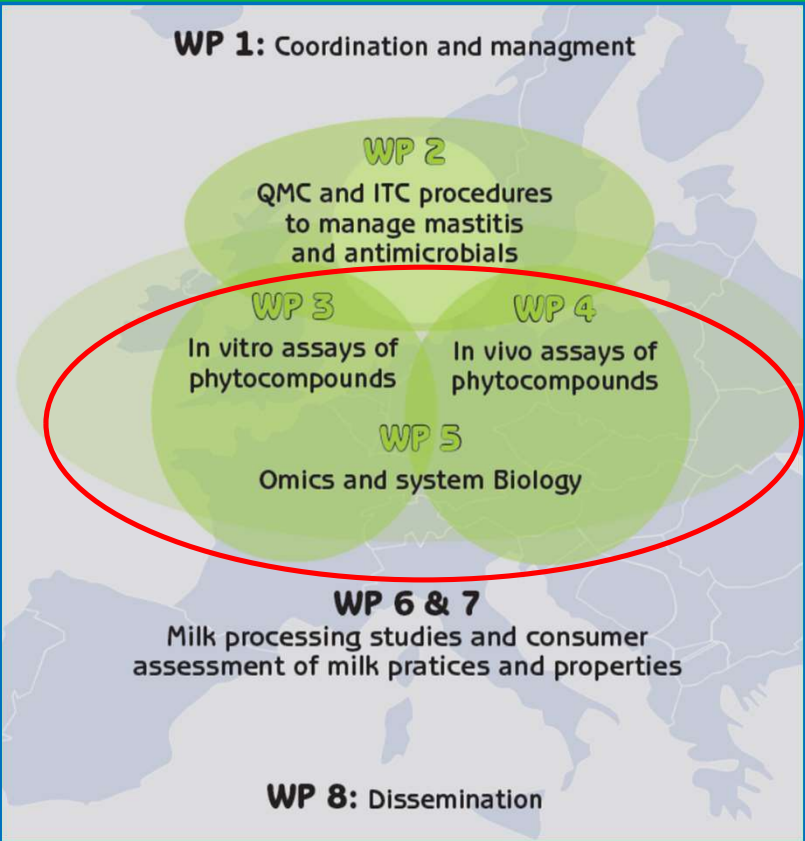
GUIADEFUR M. (1), BALLOT N. (2), BELLENOT D. (3), HARDIT V. (1), SULPICE P. (4), MARTIN G. (1), LAMARLE F. (1), JOUET L. (5), FAURIAT A. (4).

(1) Institut de l'Elevage, 149 rue de Bercy, 75012 PARIS

SUMMARY – The objective of this study was to evaluate an essential oil mixture (EO) for the treatment of bovine mild and moderate clinical mastitis in lactating dairy cattle. In dairy herds in Bretagne Pays de la Loire (B-PdL) and Auvergne Rhône Alpes (AURA), a total of 131 clinical cases were randomly assigned to essential oil (HE, n = 72) or antibiotic (ATB, n = 63) group. EO was applied topically on infected quarter during 14 consecutive milking. Results showed that clinical cure rate was lower in the HE group than in ATB group (72.3 % vs 88.1 %, P < 0.05). Cure rate based on 2 consecutive individual SCC < 300 000 cell/ml after occurrence was higher in HE group only in B-PdL (95.0 % vs 60.0 %, P < 0.05). Bacteriological cure rate was lower in HE group compare to ATB group only in AURA (96.0 % vs 53.4 %, P < 0.05). In the growing context of antimicrobial resistance, results showed that there is an interest to consider EO as a complement to antibiotics to evaluate new treatment strategies of dairy cows mammary infections.



Part B – MILKQUA ongoing results



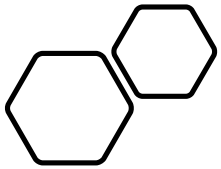
MilkQua

Coordination : Latifa Abdennebi-Najar (Idele)

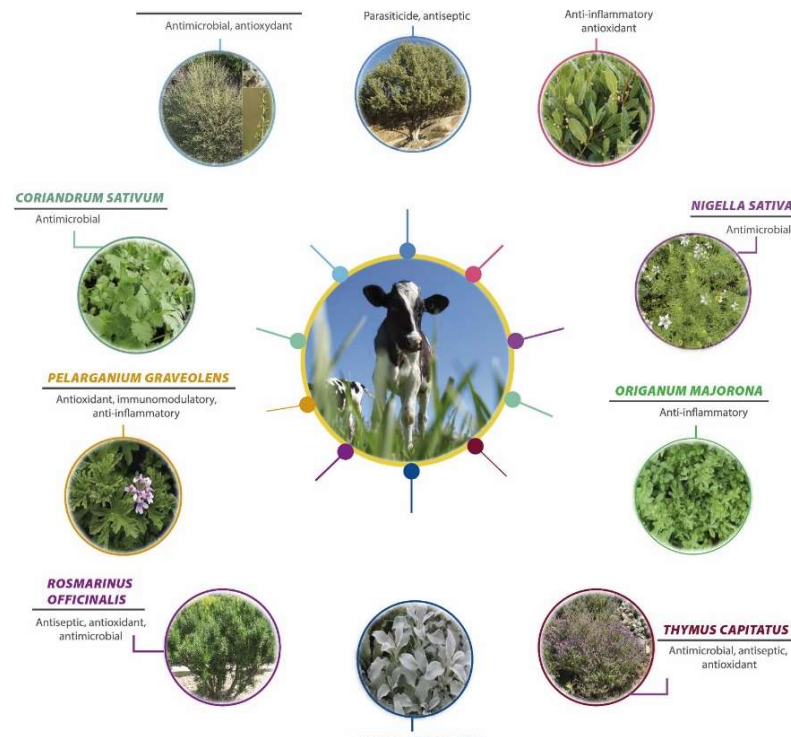


The PRIMA programme is supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation

I. AIMS AND APPROCHES

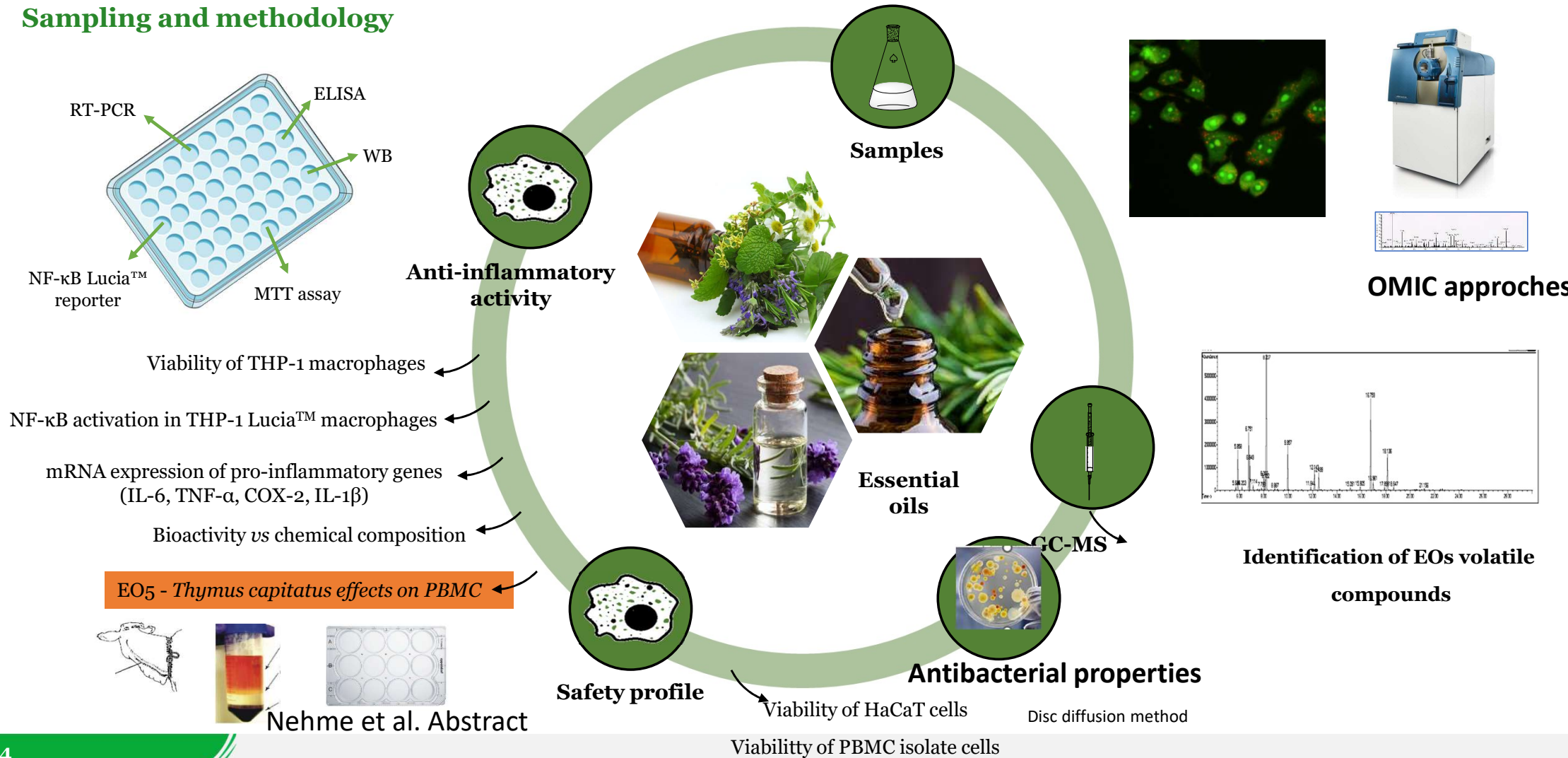


- Screening of promising natural extracts from 10 Mediterranean plants
- Evaluating their antimicrobial anti-inflammatory and antioxidant activities (in vitro)
- Evaluating their nutritional effects both in vitro and in vivo
- Evaluation of health effects of Tymus Capitatus and its major components on Mastitis (Ralph Nehme, Abstract nr. 39489)

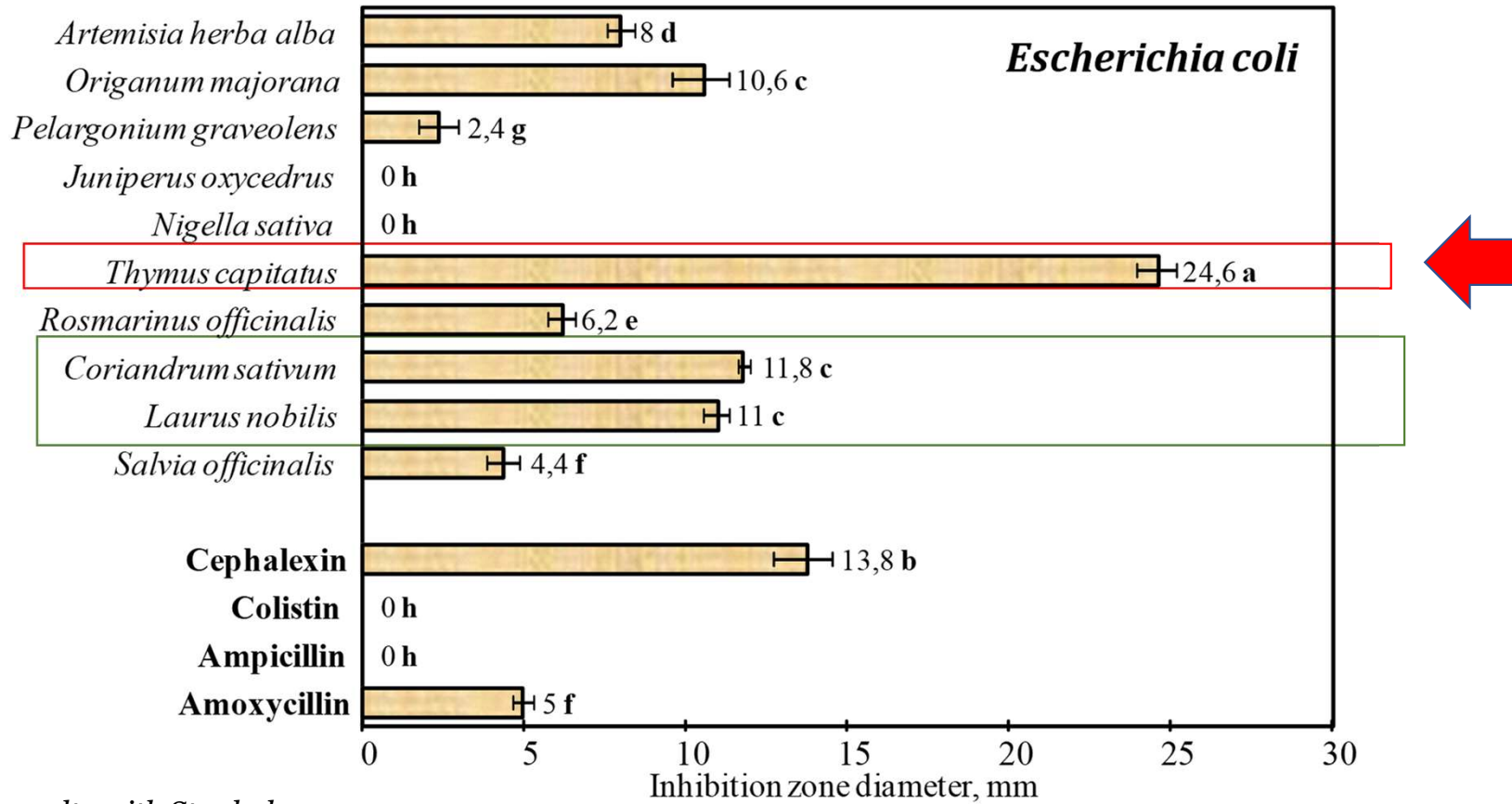


- EO1 - *Salvia officinalis*
- EO2 - *Laurus nobilis*
- EO3 - *Coriandrum sativum*
- EO4 - *Rosmarinus officinalis*
- EO5 - *Thymus capitatus* (TC)**
- EO6 - *Nigella sativa*
- EO7 - *Juniperus oxycedrus*
- EO8 - *Pelargonium graveolens*
- EO9 - *Origanum vulgare*
- EO10 - *Artemesia herba-alba*

Sampling and methodology



Antibacterial effects of EO against isolated E. Coli and Staphylococcus Aureus bacteria from the cow udder



Same results with *Staphylococcus aureus*

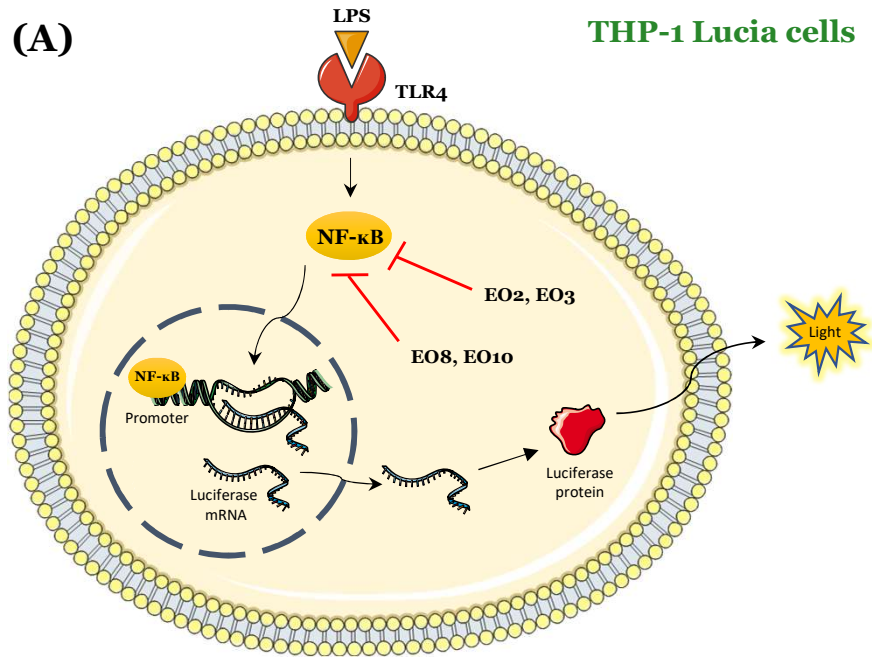
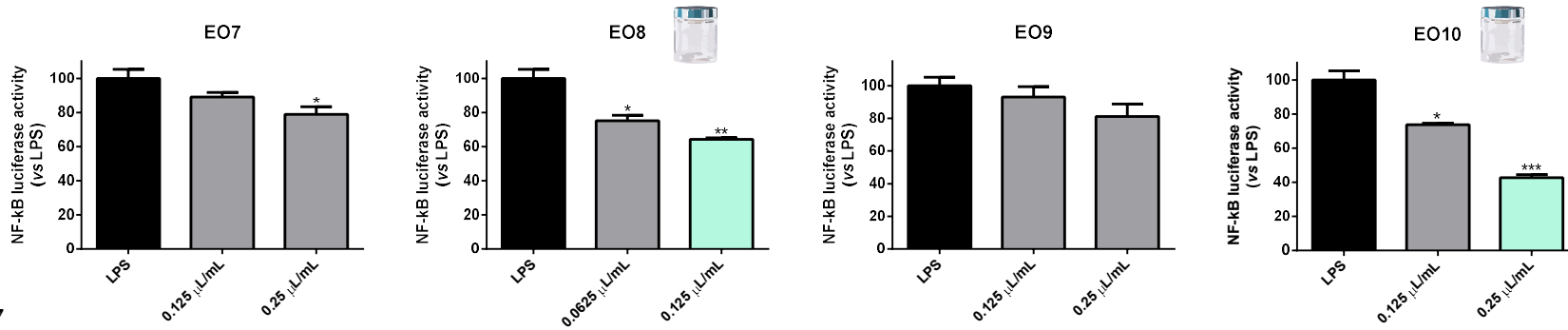
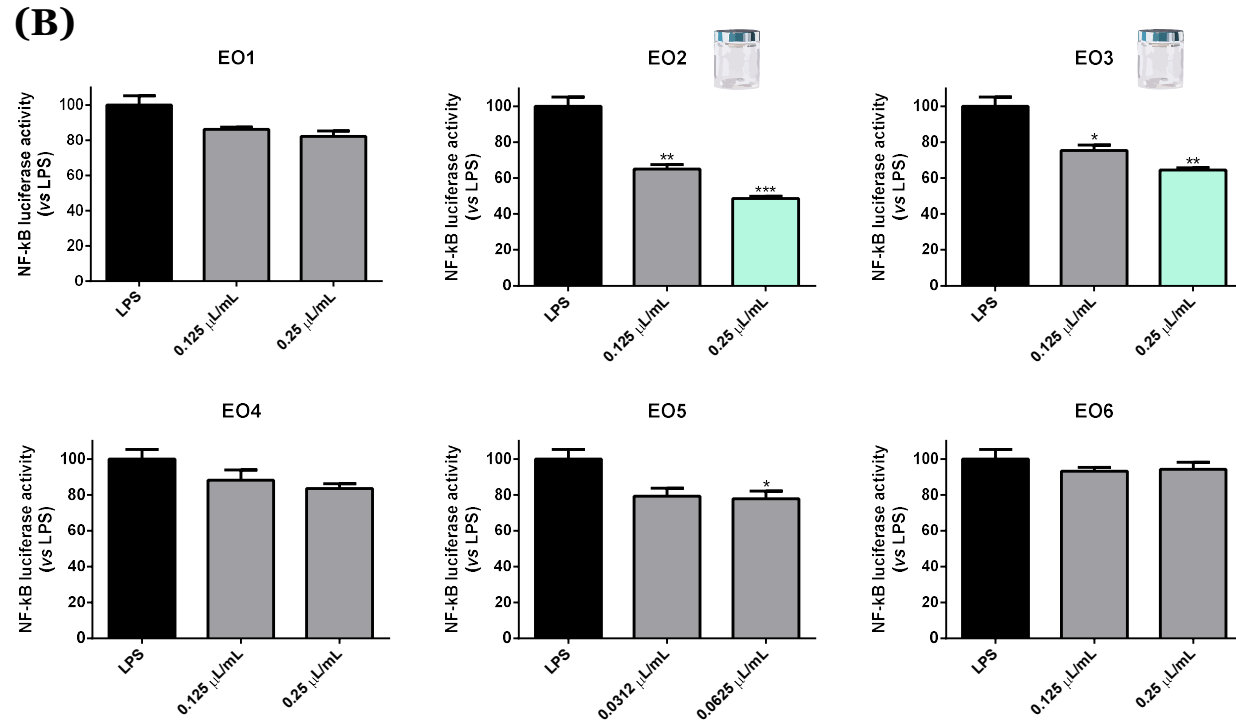


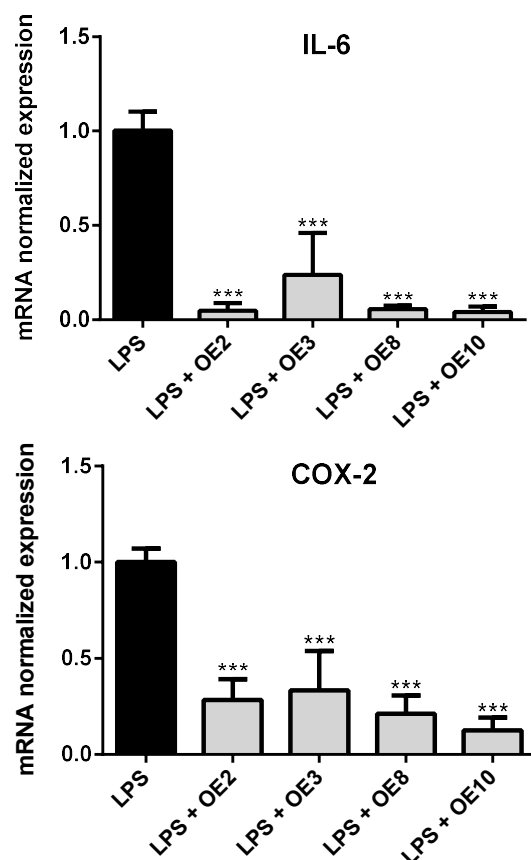
Fig. 3A: Experimental model used for the assessment of NF-κB.



→ **RT-PCR**

NF-κB activation in LPS-challenged THP-1 Lucia cells in the presence of EOs 1–10 for 24 h.

THP-1 macrophages: qPCR analysis of selected EOs



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%)

OE8 – *Pelargonium graveolens*

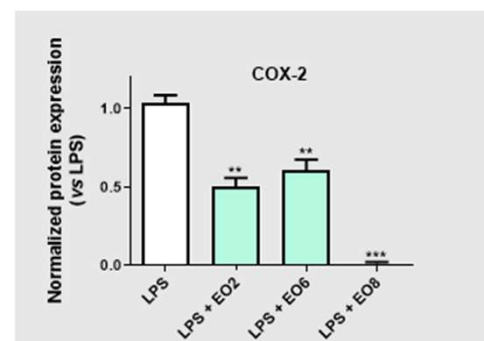
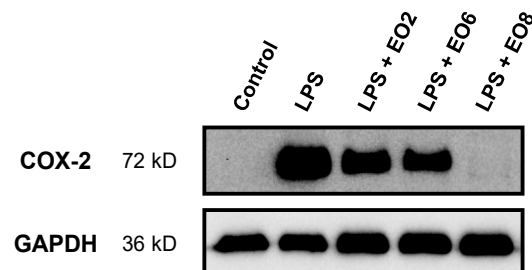
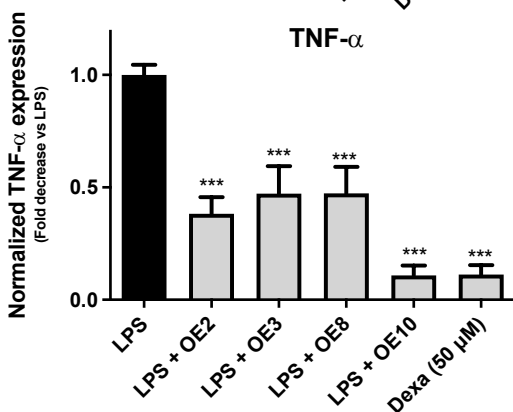
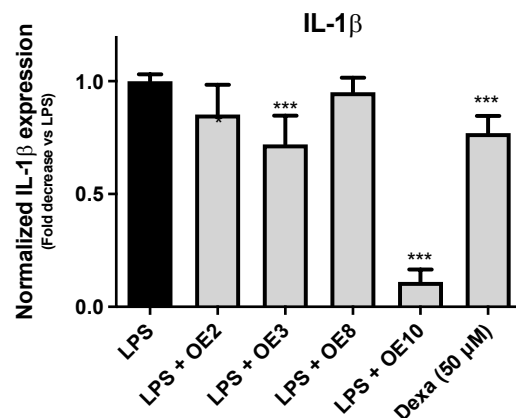
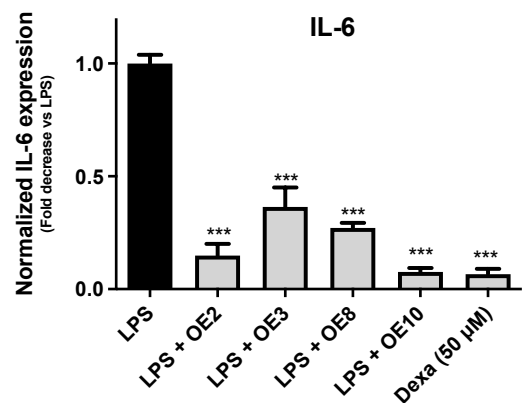
β-citronellol (36.88%)
Geraniol (15.02%)
Citronellyl formate (8.74%)

EO10 – *Artemisia herba-alba*

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

IL-6, IL-1β, COX-2 and TNF-α mRNA expression in THP-1 cells, pre-treated for 2h with EOs 1-10 followed by 22h co-treatment with LPS (1 μg/mL). mRNA expression was determined by qPCR after normalization with GAPDH reference gene. EO2, EO3, EO10 – 0.25 μL/mL; EO8 – 0.125 μL/mL.

THP-1 macrophages: ELISA and Western Blot Analysis of selected EOs



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%)

OE8 – *Pelargonium graveolens*

β-citronellol (36.88%)

Geraniol (15.02%)

Citronellyl formate (8.74%)

EO10 - *Artemisia herba-alba*

β-thujone (38.27%)

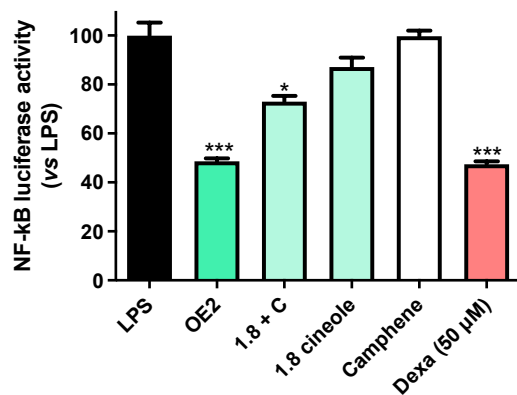
Camphor (11.88%)

α-thujone (11.58%)

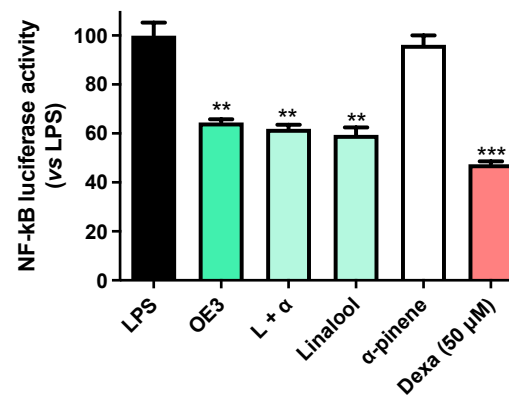
IL-6, IL-1β, TNF-α and Cox-2 protein levels in THP-1 cells, pre-treated for 2h with selected EOs, followed by 22h co-treatment with LPS (1 µg/mL).

Evaluation of bioactivity of EO versus chemical composition on THP-1 macrophages

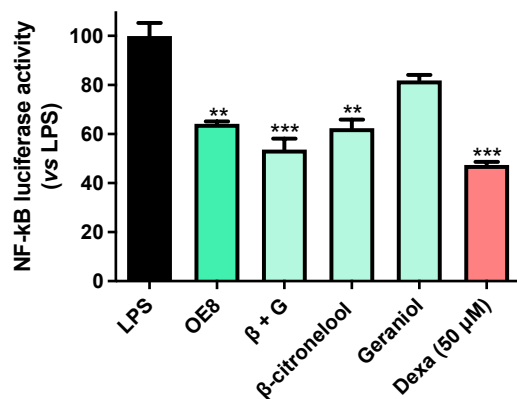
Major components



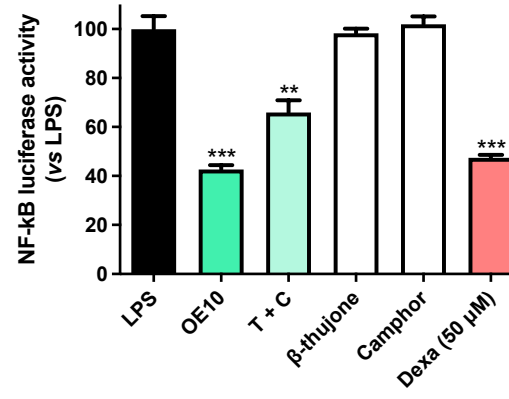
Major components

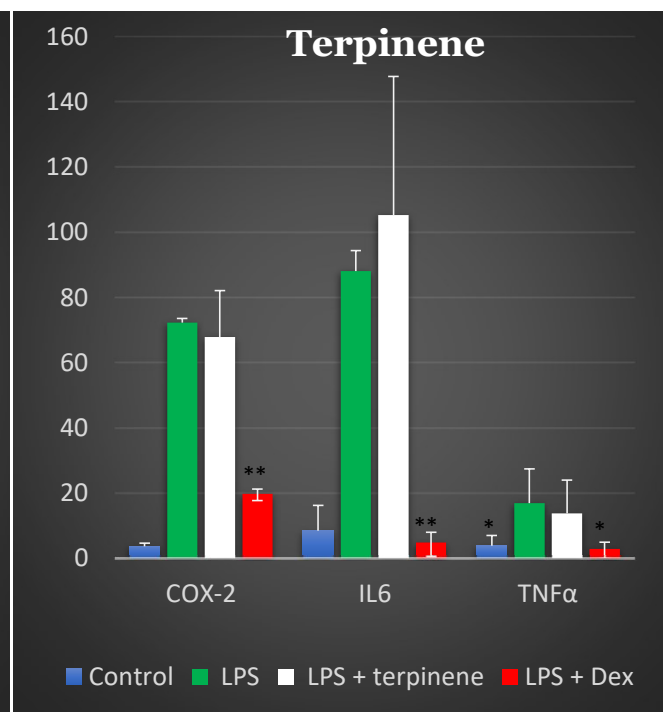
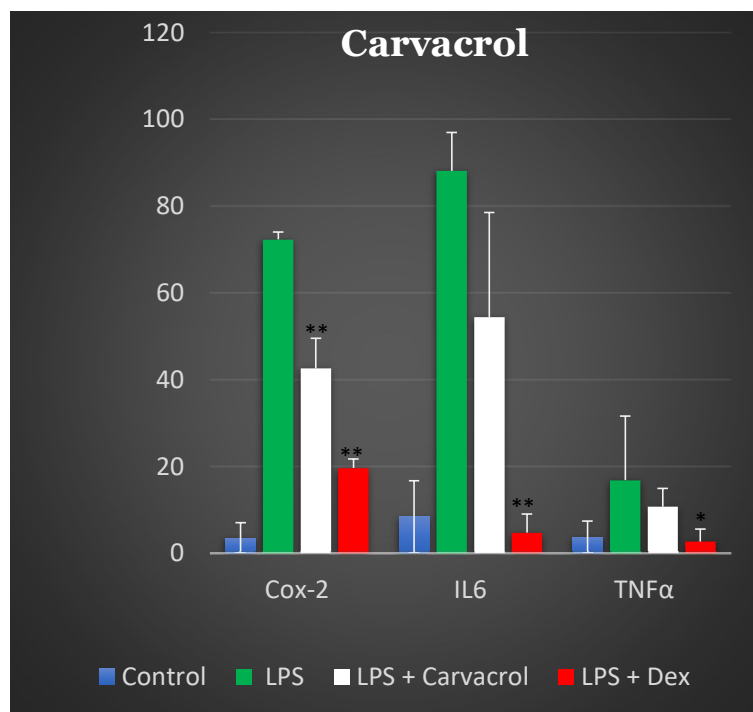
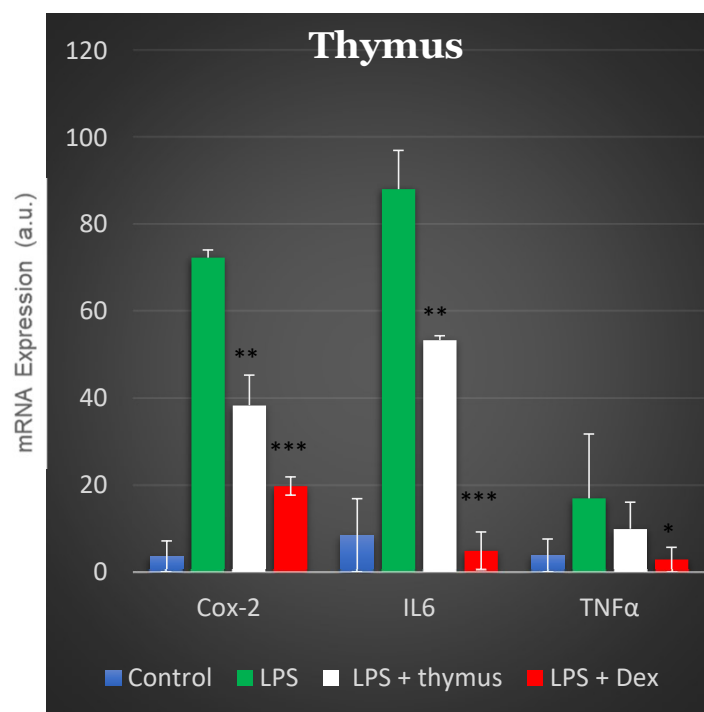
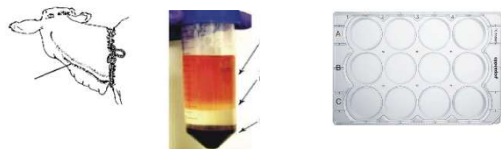


Major components



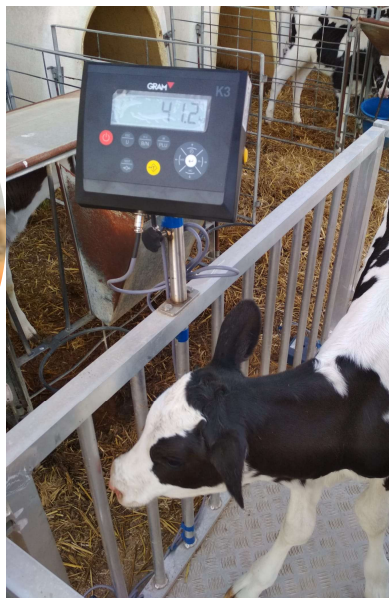
Major components





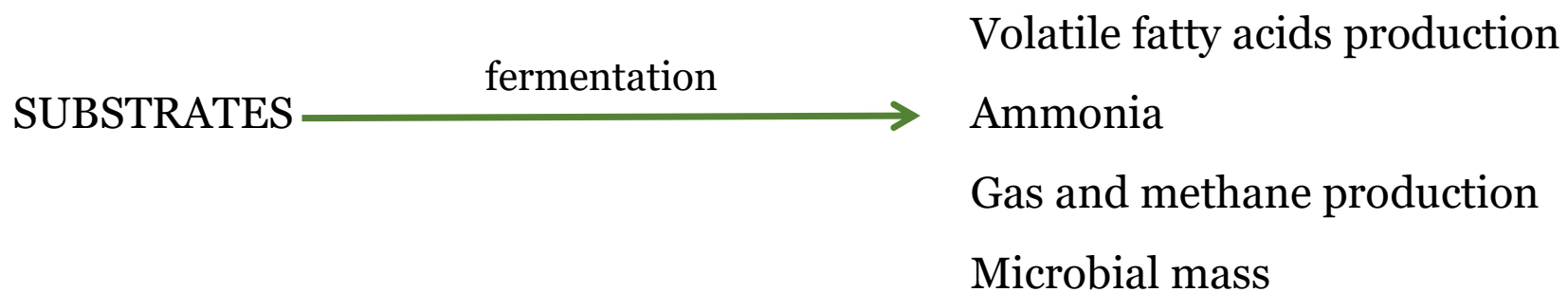
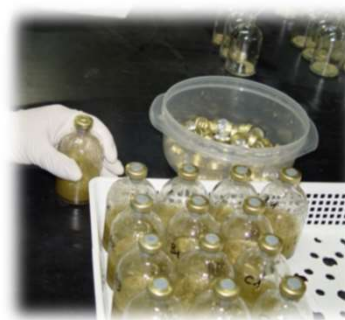
IL-6, IL-1 β , TNF- α and Cox-2 protein levels in PBMC, pre-treated for 6h with TC, followed by 16h co-treatment with LPS (1 μ g/mL).

*: p<0.05; ** p<0.01; *** p<0.001 treatment VS LPS



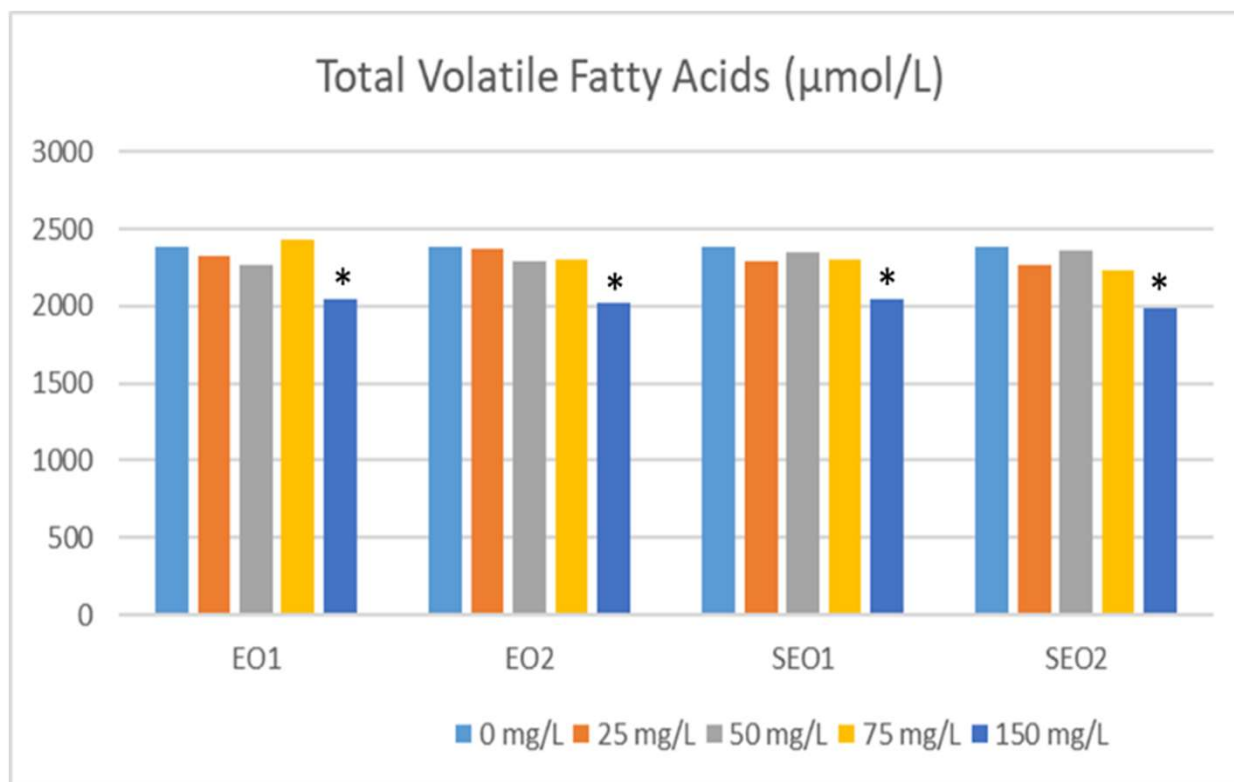
Batch cultures

Culture media + inoculum Substrate Additive
Incubation at 39 °C



Major results

- ✓ Synthetized EOs have similar effects to natural Thymus. All of them inhibited ruminal fermentation above 75 mg/L.



Major results

- ✓ No positive effects on ruminal fermentation parameters were detected with any of the five compounds (tested at the equivalent dose of 75 mg carvacrol/L) when using F or C diets.
- ✓ Thymus EO decreased propionate, and carvacrol was the main responsible
- ✓ Methane production was not affected
- ✓ An increase of NH₃ was observed when adding EO1 to the F diet

DIET C									
variable	Control	EO1	SEO1	Carvacrol	Cymene	Terpinene	SEM	P-value	Root MSE
Gas (ml)	146	144	144	150*	150*	149	0.82	<0,001	1.641
Methane (μmol)	586	564	538	554	601	569	45.82	0.937	91.638
Total VFA (μmol/L)	2675	2529*	2628	2679	2692	2575	33.50	0.020	67.007
Molar proportions (μmol/100 μmol)									
Acetate	59.5	57.8*	59.4	59.5	59.6	58.7	0.421	0.056	0.842
Propionate	13.7	12.2	12.0	12.4	13.0	12.9	0.575	0.374	1.149
Butyrate	18.5	19.6	18.7	18.3	18.6	19.0	0.370	0.281	0.741
Valerate	2.29	2.49	2.43	2.46	2.34	2.40	0.054	0.152	0.109
Caproate	3.98	6,02*	5,61*	5.40	4.42	4.98	0.373	0.014	0.747
Isoacids	2.03	1.88	1.90	1.87	1.99	1.97	0.048	0.153	0.096
Ac:Pr (mol/mol)	5.14	5.75	5.87	5.69	5.48	5.47	0.28	0.518	0.559
methane/VFA (mol/mol)	0.223	0.228	0.204	0.207	0.225	0.225	0.017	0.857	0.034
NH ₃ -N (mg/L)	242	255	244	244	249	266	9.82	0.533	19.640

DIET F									
variable	Control	EO1	SEO1	Carvacrol	Cymene	Terpinene	SEM	P-value	Root MSE
Gas (ml)	146	140	139	139	140	141	1.92	0.222	3.847
Methane (μmol)	579	583	516	507	488	528	29.42	0.181	58.847
Total VFA (μmol/L)	2662	2579	2495*	2459*	2621	2629	40.00	0.016	79.999
Molar proportions (μmol/100 μmol)									
Acetate	66.0	66.4	65.7	66.1	66.4	66.1	0.291	0.494	0.583
Propionate	15.0	13.6*	14.2	13.8*	14.2	14.4	0.239	0.015	0.478
Butyrate	12.3	12.9	13.05*	12.9	12.8	12.6	0.170	0.081	0.340
Valerate	2.14	2.23	2.24*	2.32*	2.21	2.23	0.024	0.004	0.049
Caproate	2.22	2.78*	2.73*	2.76*	2.30	2.36	0.077	<0.001	0.153
Isoacids	2.35	2.09*	2.15	2.21	2.17	2.29	0.052	0.032	0.104
Ac:Pr (mol/mol)	4.78	5.37*	5.22*	5.30*	5.15	5.13	0.106	0.021	0.213
methane/VFA (mol/mol)	0.217	0.226	0.205	0.207	0.185	0.202	0.013	0.381	0.026
NH ₃ -N (mg/L)	235	259*	265*	225	225	217	5.584	<0,0001	11.168

Trial 1: EOs supplementation in dairy calves

Two groups of New born calves (n=16)

- **Control (CON):** Milk replacer twice a day just after parturition during the first 45 days (4 to 6 liters)
- **EO experimental group (EO) :** Milk replacer twice a day just after parturition during the first 45 days + progressive increasing dose of Commercial EOs (88% carvacrol content) from 130 to 200 mg



Andrés et al, manuscript in preparation



Trial 2 : EO supplementation in lactating dairy cows

Two groups of dairy cattle (n = 18) (Initial milk yield 30.3 ± 3.6 kg/d , average BW 550 ± 42.3 kg) / 15 days adaptation + 30 days measurements / Receiving Total Mixed Diets

- **Diet control (CON):** Oat hay (2.68 kg DM) + Silages (9.24 kg DM) + Concentrate (10.8 kg DM)
- **Diet experimental (EOT):** CON + 6 ml/day of essential oil



Ben salem et al, manuscript in preparation

MAJOR RESULTS

Trial 1:

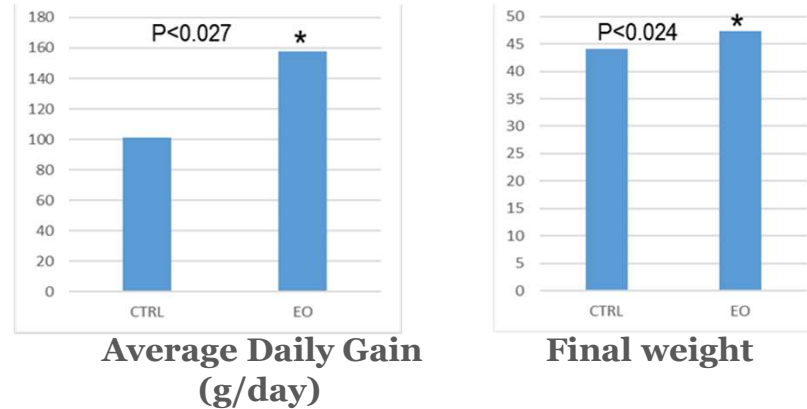
- Newborn dairy calves being fed EO during the suckling period were more efficient than the control Group
- No long-term effects of EOs effects during the replacement phase
- Long term effect on monocytes populations (*Andres et al. EAAP 2022 Abstract nr. 39164*)

Trial 2

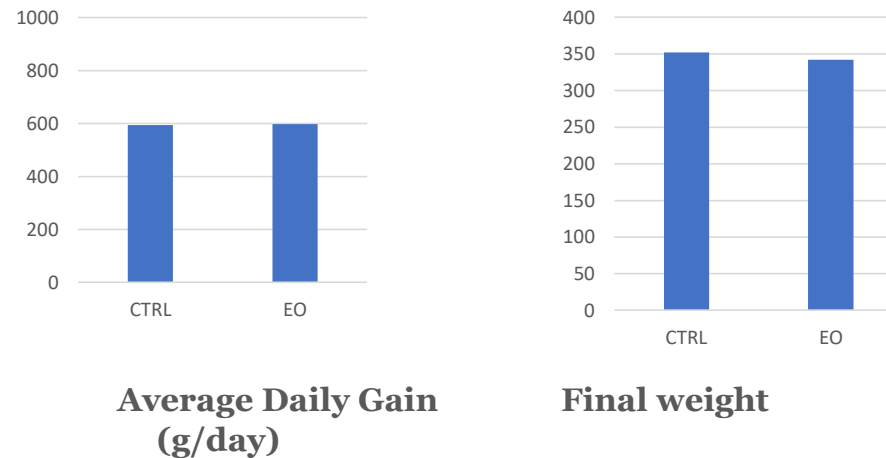
- Numerical increase ($P > 0.05$) of milk yield in EOT-group (CON = 30.0; EOT = 30.3 kg/cattle/day)
- EOT administration had no effect on SFA, MUFA, PUFA
- **EOT decreased fat & protein contents and w3/ w6**



New born Calves (45 days)



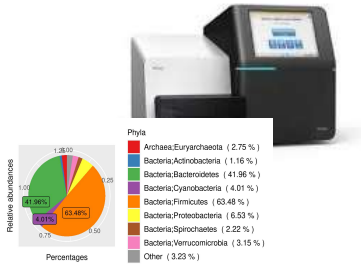
Adult Cow (12 month)



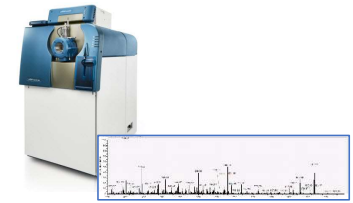
OMICS ongoing results



MICROBIOMICS Milk/Skin/Faeces/Rumen

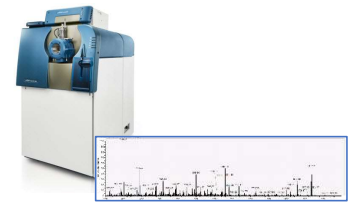


LIPIDOMICS Milk



Diacylglycerols (Differences at T21)
Ceciliani et al. EAAP 2022 Abstract nr. 39922

METABOLOMICS Plasma



ongoing

MICRO-RNAOMICS Milk (exosomal component)

127 miRNAs with an upmodulated trend and 62 with a downmodulation trend
18 upmodulated and 32 downmodulated at T8

Ceciliani et al. Manuscript in preparation

Milk – Changes in abundance in 190 genera
Gini et al. Manuscript in preparation

Skin –

Faeces – Changes in abundance in 11 genera
Llorente et al., manuscript in preparation

Rumen – Changes in abundance of 39 genera
Gini et al. EAAP 2022 Abstract nr. 39033



Conclusion

Effects on ruminal fermentation

- Changing in microbiota → changes in end- products fermentation
- Changes in Volatile fatty acid (contradictory results)
- Decreases in ruminal ammonia (contradictory results)

Effects on methane production

- Inhibition of archeal community reducing methanogens activity
- Suppression the growth of ruminal fungi and protozoa harbouring methanogens
- **Results depends on the concentration of EO**

Effects on animal performance

- Improve calves feed efficiency and growth performance
- Improve animal immunity and decrease morbidity of neonatal diarrhea
- Affect milk production and composition (contradictory results)
- Depends largely on the concentration of EO and other animal factors (physiological status..)
- **The dose and routes of administration deserve further studies, allowing a better animal performance and health to be achieved**

Limits and obstacles of EO use



Composition and biological activities associated to the extraction process, type of solvent and storage condition



Possible adverse effects, toxicology needs to be studied in details



Access to a large amount of plant biomass is required which constitutes an obstacle to the commercial applicability



Differences in the mode of action of the essential oils *in vitro* and *in vivo*, which merits attention for future research
Lack of internationally standardized techniques to evaluate the antimicrobial activities

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