

# Antibacterial and anti-inflammatory effects of *Thymus Capitatus* and its major components against subclinical mastitis in dairy cows



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IDELE / STLO  
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- Subclinical mastitis (SM) → inflammation of the mammary tissue
- High cost
- Complicated treatment (using antibiotics)
- Antibiotic resistance
- Studies show the anti-inflammatory antibacterial effectiveness of essential oils



Study the antibacterial and the anti-inflammatory effects of *Thymus Capitatus* (TC) against subclinical mastitis

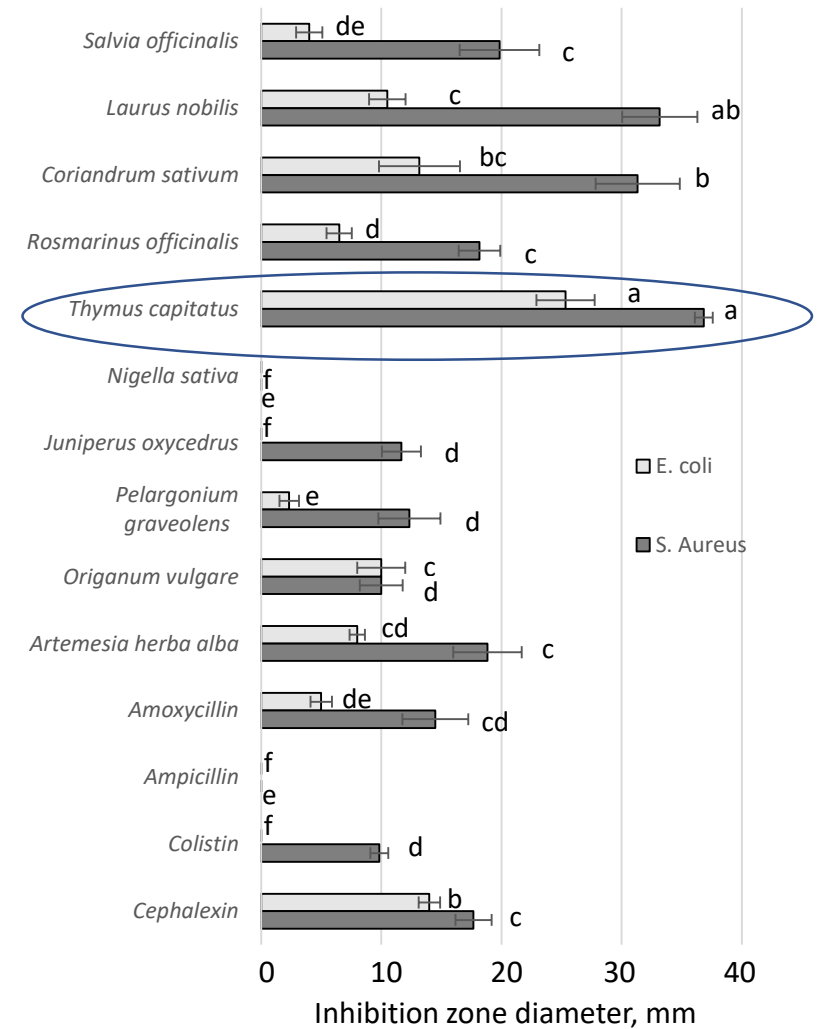


EO	Species
1	<i>Salvia officinalis</i>
2	<i>Laurus nobilis</i>
3	<i>Coriandrum sativum</i>
4	<i>Rosmarinus officinalis</i>
5	TC
6	<i>Nigella sativa</i>
7	<i>Juniperus oxycedrus</i>
8	<i>Pelargonium graveolens</i>
9	<i>Origanum vulgare</i>
10	<i>Artemesia herba alba</i>

## Disc diffusion method

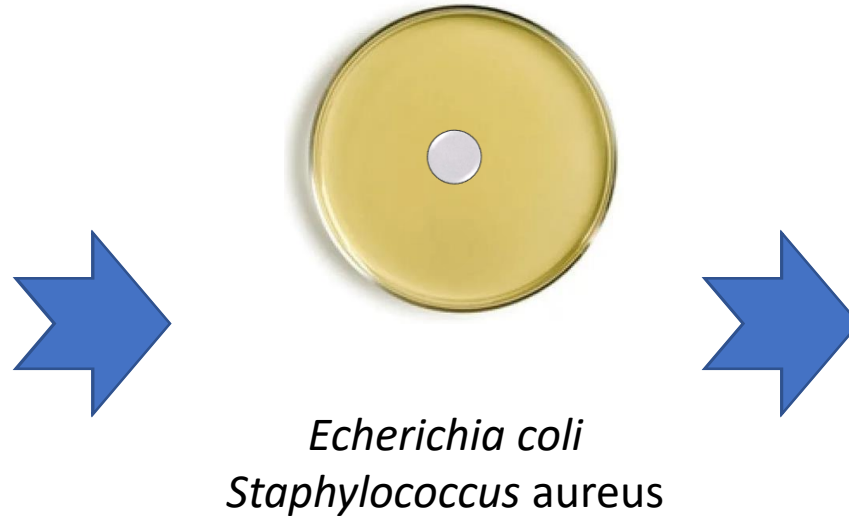


*Echerichia coli*  
*Staphylococcus aureus*



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## Disc diffusion method



## TC composition using GC MS

N°	Compound	% of total volatiles
1	$\alpha$ -thujene	1.49 $\pm$ 0.06
2	$\alpha$ -pinene	0.71 $\pm$ 0.01
3	Camphene	0.21 $\pm$ 0.00
4	$\beta$ -myrcene	1.42 $\pm$ 0.05
5	l-Phellandrene	0.30 $\pm$ 0.00
6	$\alpha$ -terpinene	1.84 $\pm$ 0.07
7	p-cymene	7.06 $\pm$ 0.15
8	$\beta$ -phellandrene	0.55 $\pm$ 0.01
9	$\gamma$ -terpinene (T)	7.58 $\pm$ 0.16
10	Linalool	1.95 $\pm$ 0.07
11	Borneol	0.57 $\pm$ 0.01
12	Cyclohexen-1-ol	0.91 $\pm$ 0.02
13	Thymol	0.24 $\pm$ 0.00
14	Carvacrol (C)	70.62 $\pm$ 1.07
15	Caryophyllene	4.55 $\pm$ 0.08

## *EX VIVO*

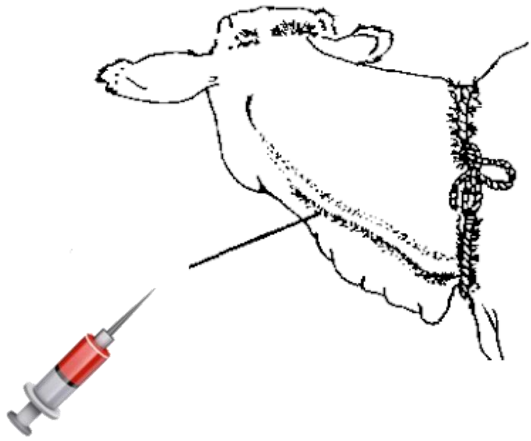
- Peripheral blood mononuclear cell (PBMC) stimulated with toxins and *TC*



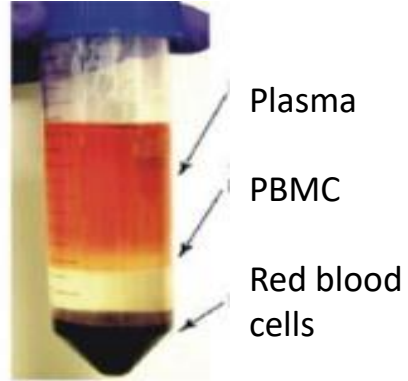
## *IN VIVO*

- Application of *TC* on the udder.
  - Anti-inflammatory
  - Anti-bacterial
  - milk quality and properties

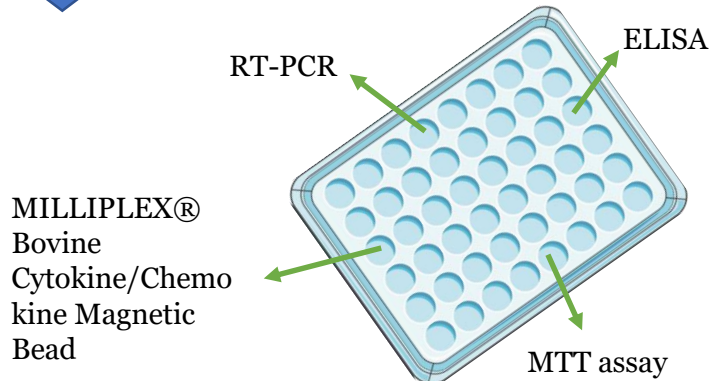
## 1 Fresh blood from jugular vein



## 2 PBMC isolation

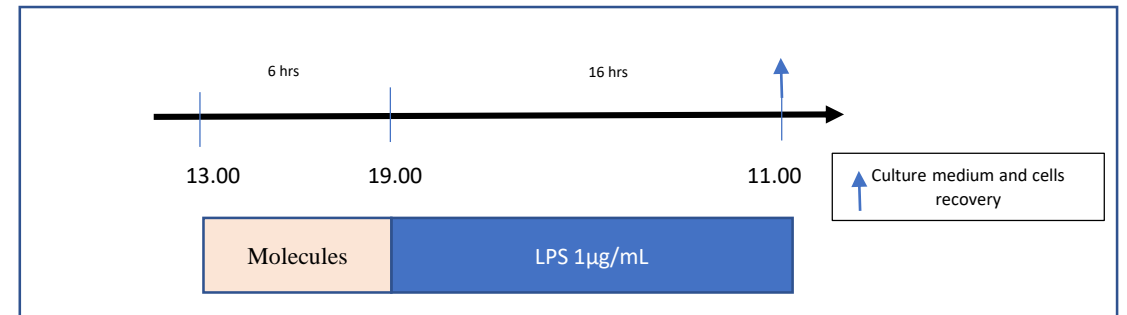


## 3 PBMC stimulation with LPS (*E. coli* 0111:B4 ; L4391)

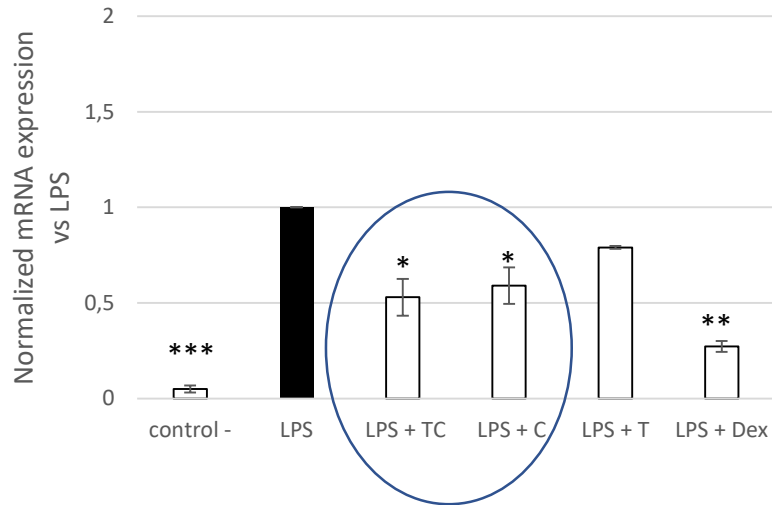


## Stimulation with TC and its major components :

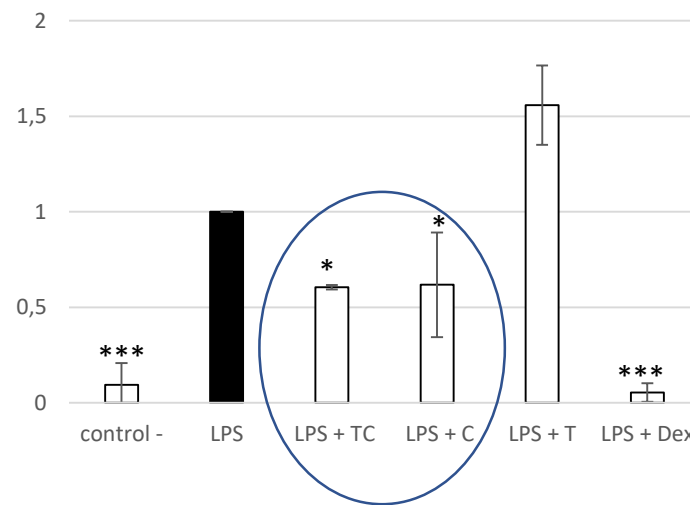
Molecules	Final concentration
T	0,5 µg/mL
C	0,5 µg/mL
TC	3 µg/mL
Dexamethasone (Dex)	19.6µg/mL



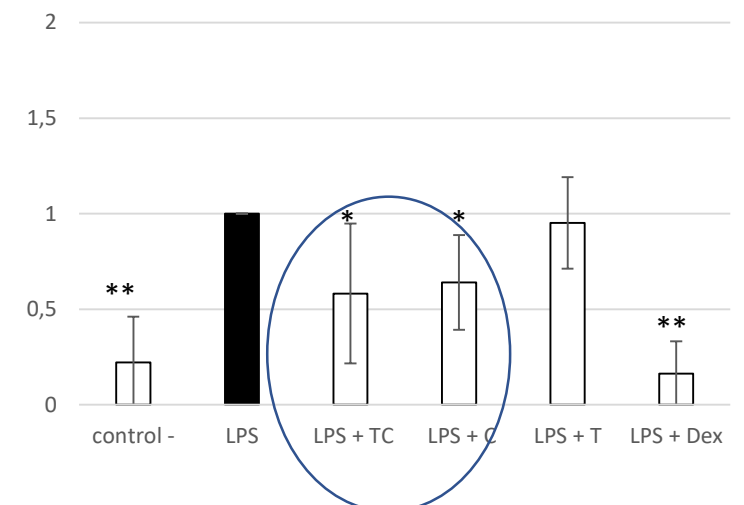
### Cyclooxygenase-2 (COX-2)



### Interleukin 6 (IL6)



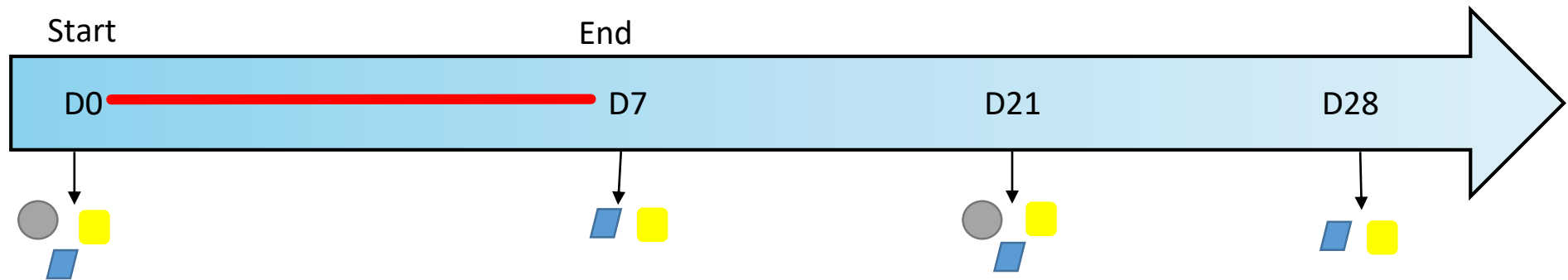
### Tumour Necrosis Factor alpha (TNFα)



- *Thymus capitatus* and carvacrol decrease the expression of COX2, IL6 and TNF α inflammation by 2



- 12 cows (Prim'Holstein) with SM
- SM is detected when : Successive somatic cell count (SCC) >  $10^5$  cells/mL **and** presence of pathogens in milk
- Treatment group (n=6) : milking grease + TC (10%)
- Control group (n=6) : milking grease
- Application : 2 times per day



- Milk quality : Sensory and Technological analysis (zetasizer) ●
- Marker of Inflammation (Interleukin 8 (IL8) in milk using ELISA) ▭
- Microbiological analysis ■
- SCC: flow cytometer (2 times per week)

## Bacteriological

Coagulase-negative staphylococci (85% of infections)

Evolution of the number of sterile milk from affected quarters

Day	D0	D8	D21	D28
Control	0/8	1/8	1/8	2/8
Treatment	0/8	1/8	2/8	2/8

## SCC

Nonsignificant difference between the 2 groups  
p=0.766

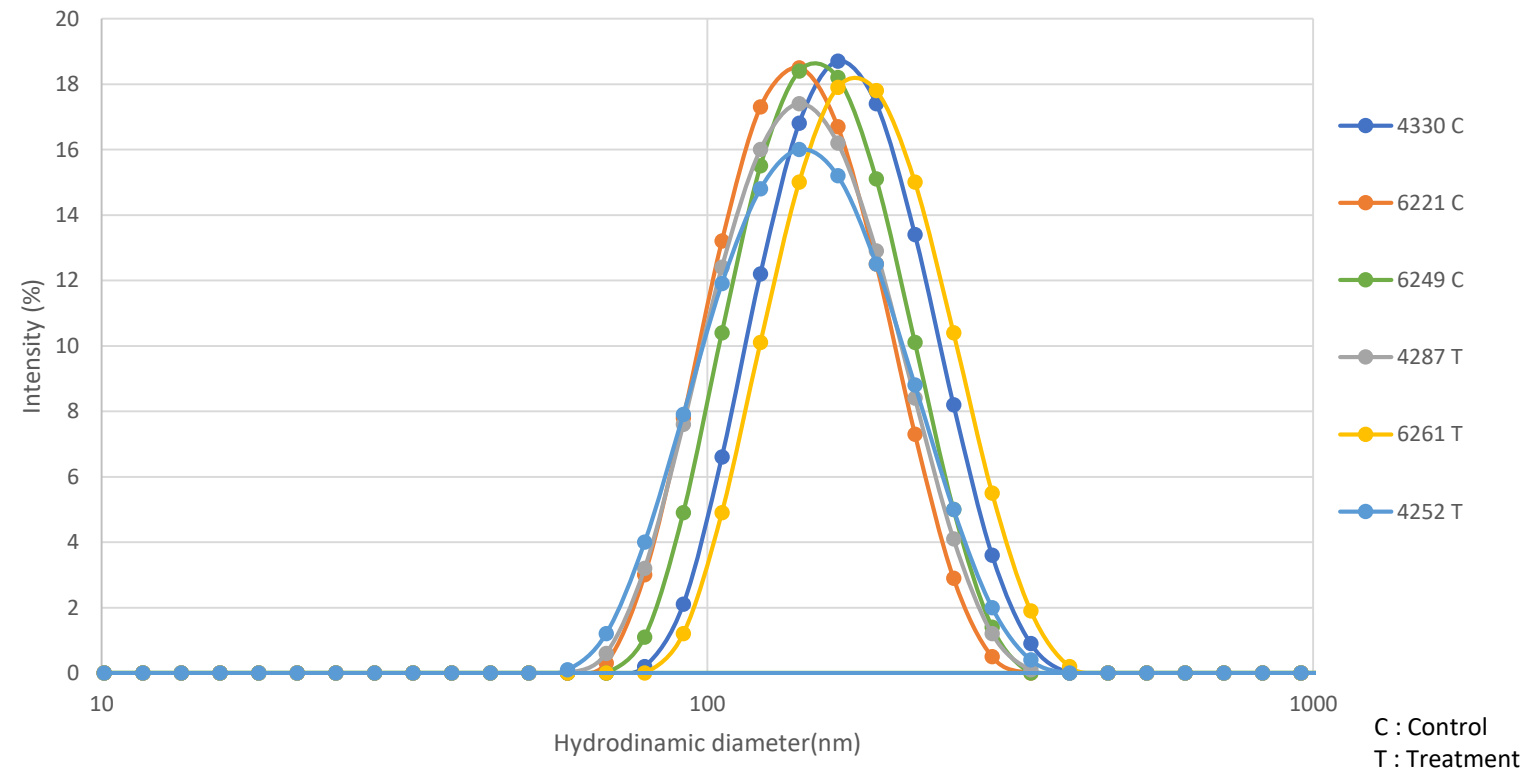
Evolution of the SCC

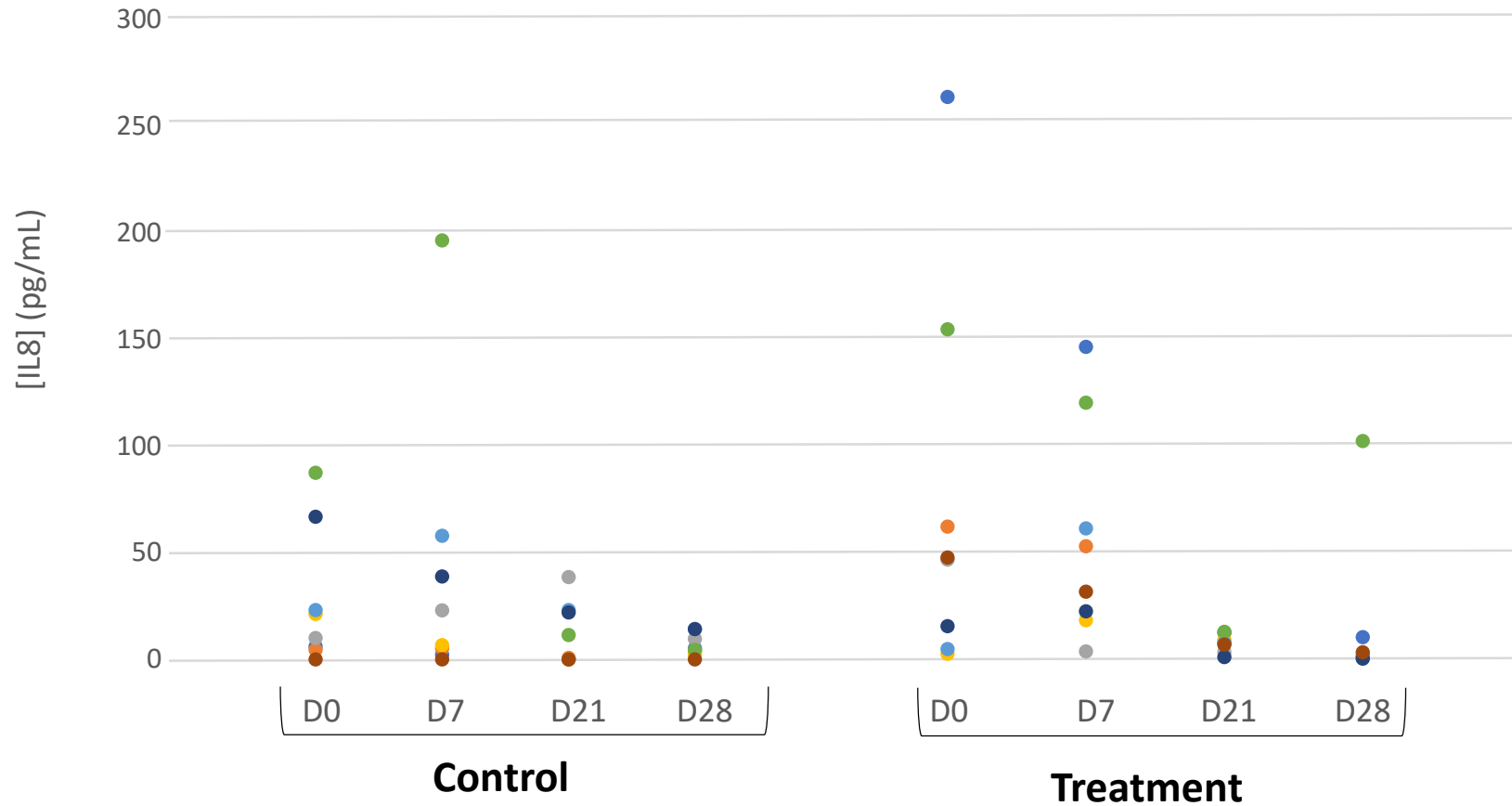
Day	Before (*10 <sup>3</sup> cells/mL)	After (*10 <sup>3</sup> cells/mL)
Control	138	137
Treatment	147	141

- Nutritional properties
- Sensory analysis

Global smell
Acidity
Aromas
Sweetness
Color
Bitterness
Creaminess
TC's smell

Distribution of the hydrodynamic diameters of casein micelle in milk in both groups





No evidence that *TC* affect the presence of the inflammatory cytokine IL8 in milk

- *TC* presents:
    - Antibacterial properties;
    - Anti-inflammatory properties as its main component carvacrol
  - *TC* doesn't affect the milk properties and quality
  - Inconclusive *in vivo* results
- ↓
- Dose of the *TC* in the *in vivo* essay
  - Frequency of the application
  - Oxidation of the *TC* during its application
  - *TC* and milk interaction → alteration of antibacterial properties (Lefèvre 2008)



**Further studies are needed to test these hypothesis**



# THANK YOU FOR YOUR ATTENTION

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