



Effects of terpenes oral administration in blood plasma and milk concentration and some physicochemical characteristics of sheep milk and cheese

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Introduction

- 📖 Food crises → ↑ consumers' interest
- ✓ feeds
 - ✓ farming system
 - ✓ animal products
- 📖 Certification schemes on animal products involve,
- ✓ administrative controls
 - ✓ on site inspections at the farm

Introduction

📖 Food tracers used to

- ✓ certify quality aspects and origin
- ✓ control public health risks

📖 BIOMARKERS

- ✓ not synthesized by animals
- ✓ occurrence in animal products or tissues is unambiguously due to the feed they have consumed



Introduction

The criteria for a substance to be classified as biomarker :

- ✓ robustness of the related qualitative and quantitative analytical methods
- ✓ sensitivity to changes in intake of the diet of interest
- ✓ specificity to the ration of interest
- ✓ linear relationship between dietary intake and biomarker concentration in animal tissues

Materials and Methods

- ♦ 8 adult sheep (crossed indigenous)
- ♦ 20 days experiment
- ♦ Two equal groups: Control (C)
Treatment (T)
- ♦ Ration: alfalfa hay
straw
commercial pelleted concentrate mixture
- ♦ Diet  cover the animals' requirements
for maintenance and milk production

Materials and Methods

- T group animals  Oral administration of 1g of each terpene

•  α -pinene,

•  limonene

•  b-caryophyllene

} Dilution in 10ml vegetable oil

- C group animals 10 ml of plain vegetable oil.
- 18 days oral administration

Materials and Methods

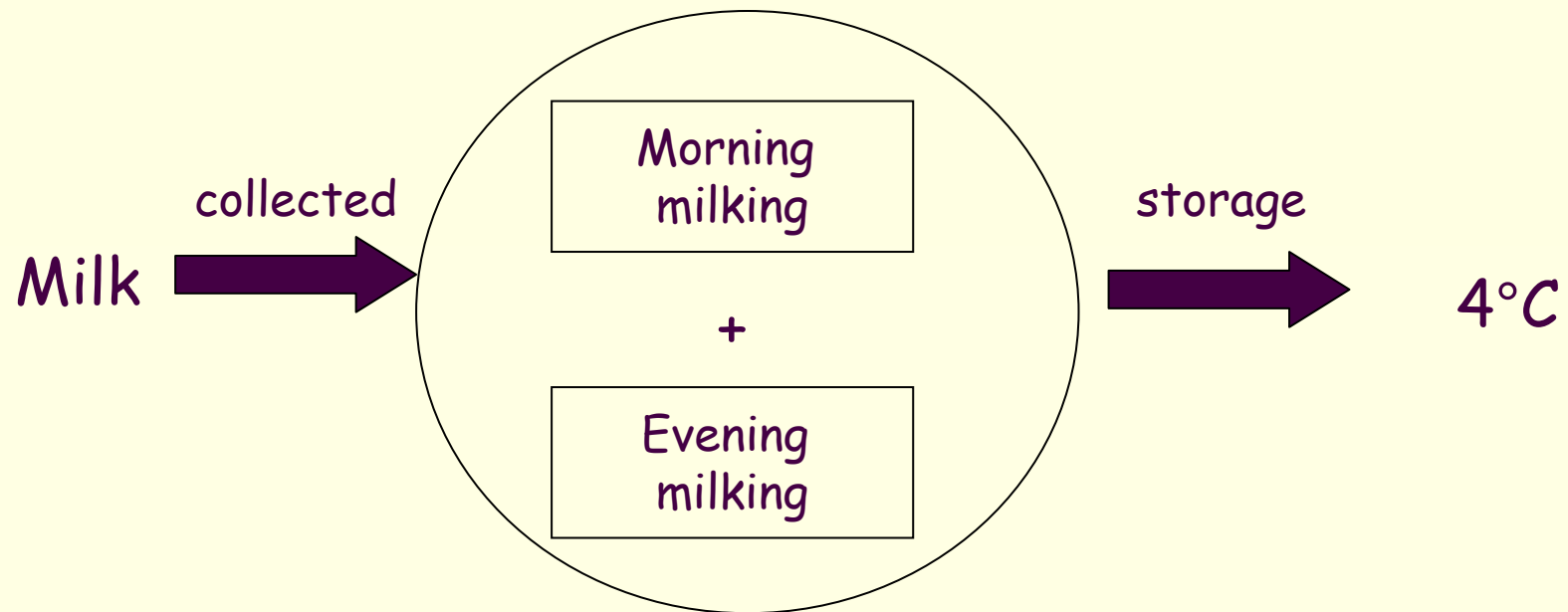
- ◆ Milk production recorded
 - daily until 3rd day
 - every other day
- ◆ Blood samples collected
 - short intervals for 24 hours
 - following milk schedule
- ◆ Milk chemical characteristics through MILCOSCAN
- ◆ Milk minerals through atomic absorption spectroscopy
- ◆ Terpenes determination in
 - ✓ blood plasma
 - ✓ milk samples

Materials and Methods

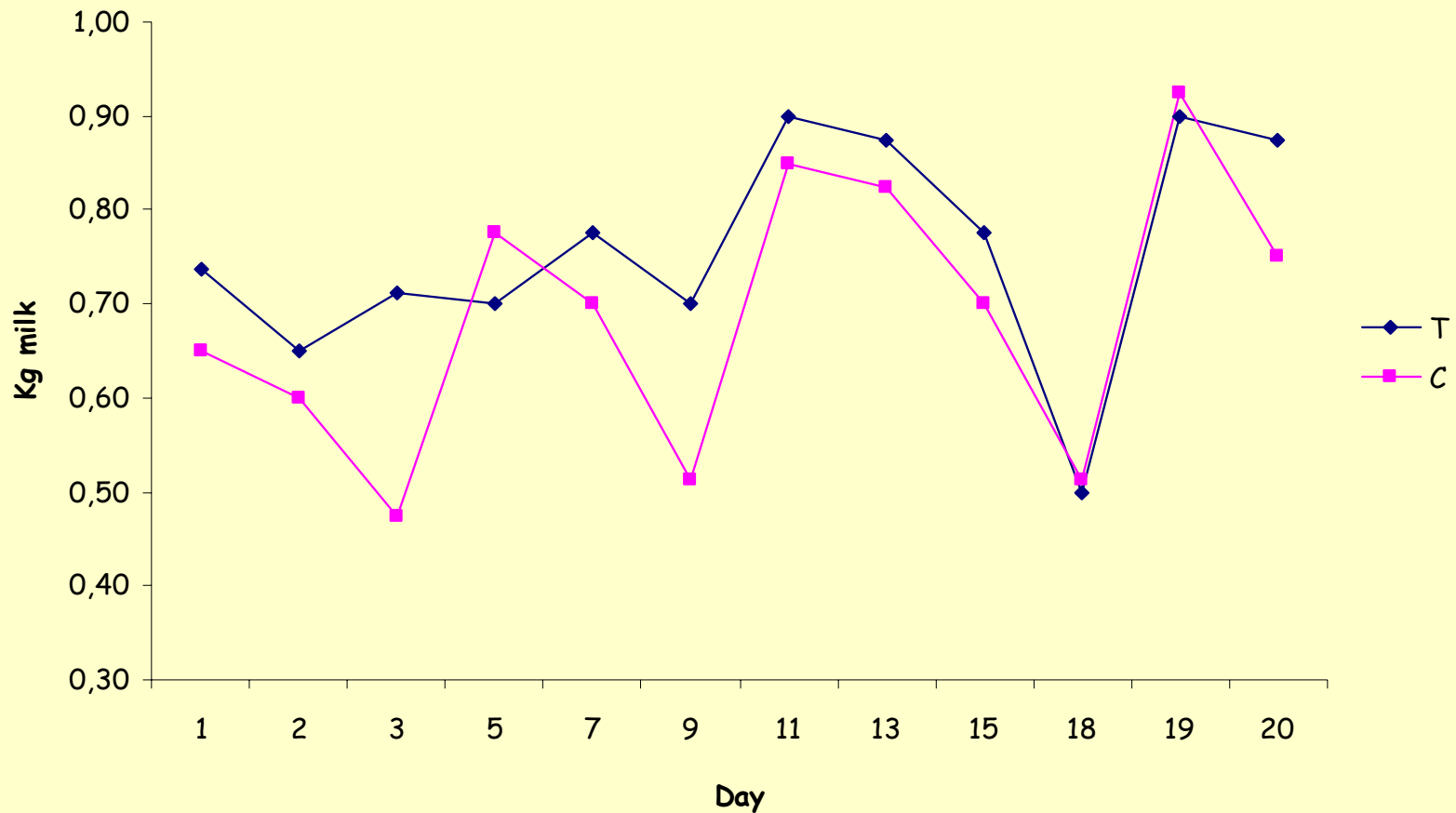
- Blood plasma samples were extracted using petroleum ether
- Solid Phase Micro-extraction Method using PDMS/CAR fiber was used for milk samples
- Terpenes were identified on a GC-MS

Materials and Methods

- ◆ Kefalotiri" cheese preparation
 - beginning of the experimental period
 - middle period of terpenes administration

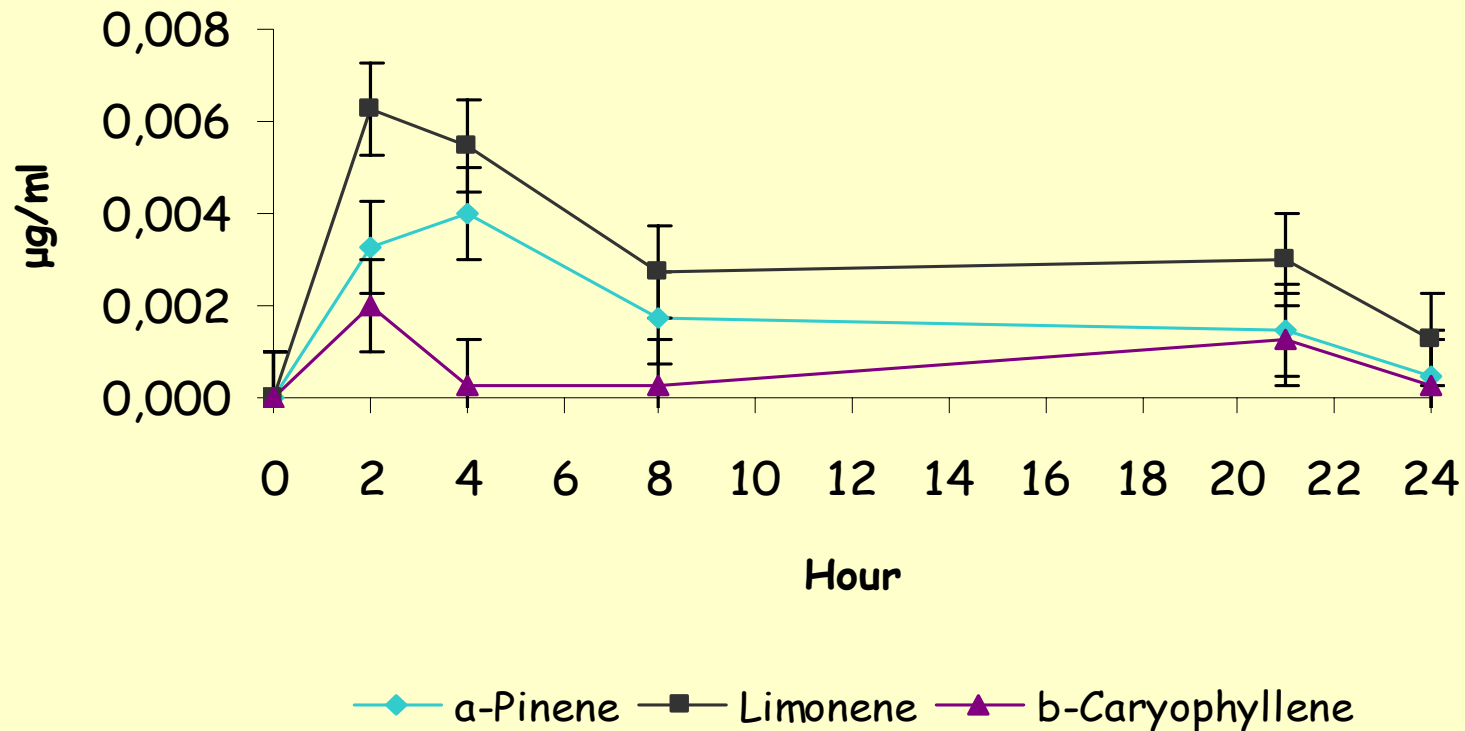


Results



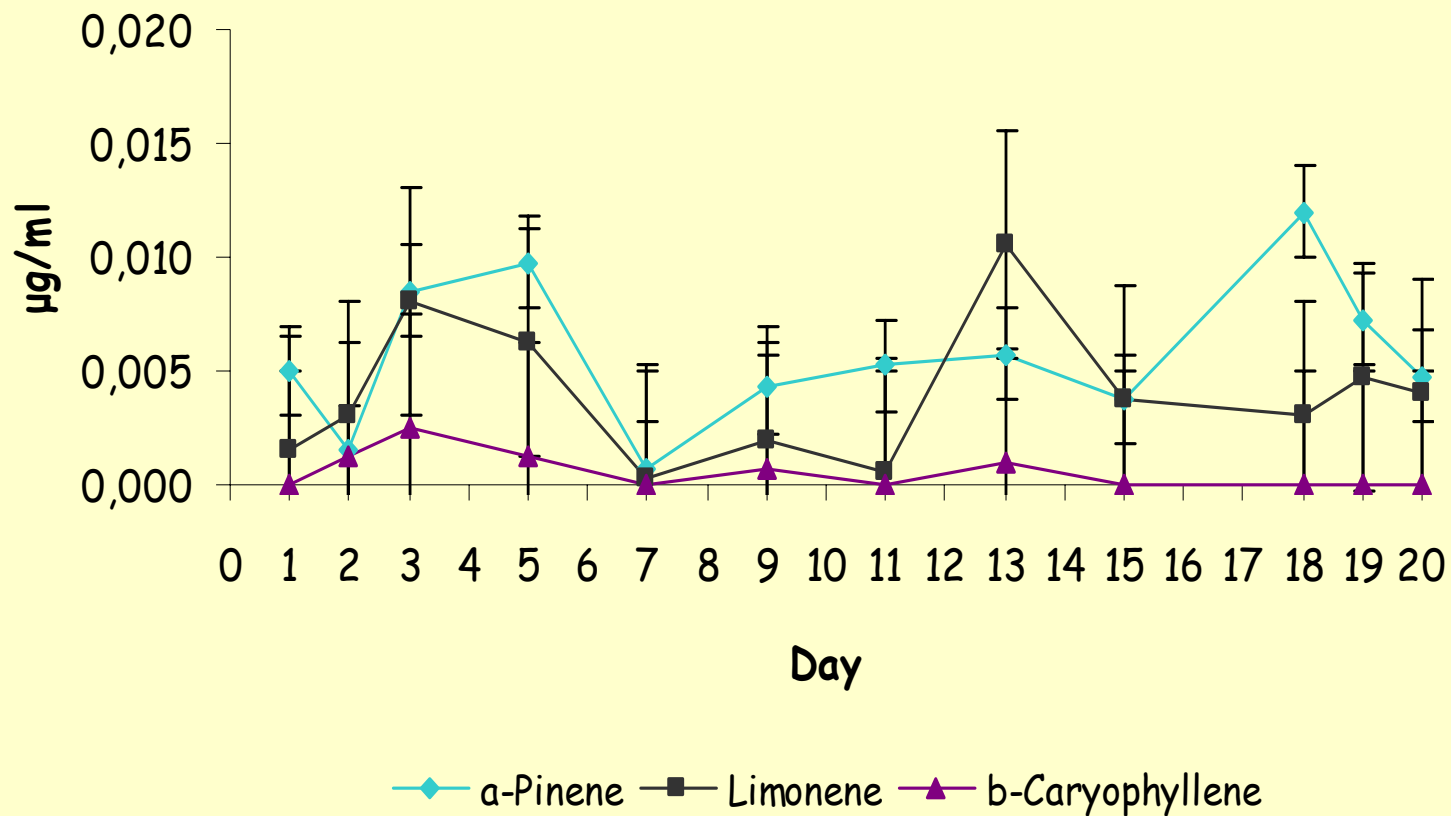
Evolution of sheep's milk production (Kg/head/day) for the Control (C) and Treatment (T) groups of animals

Results



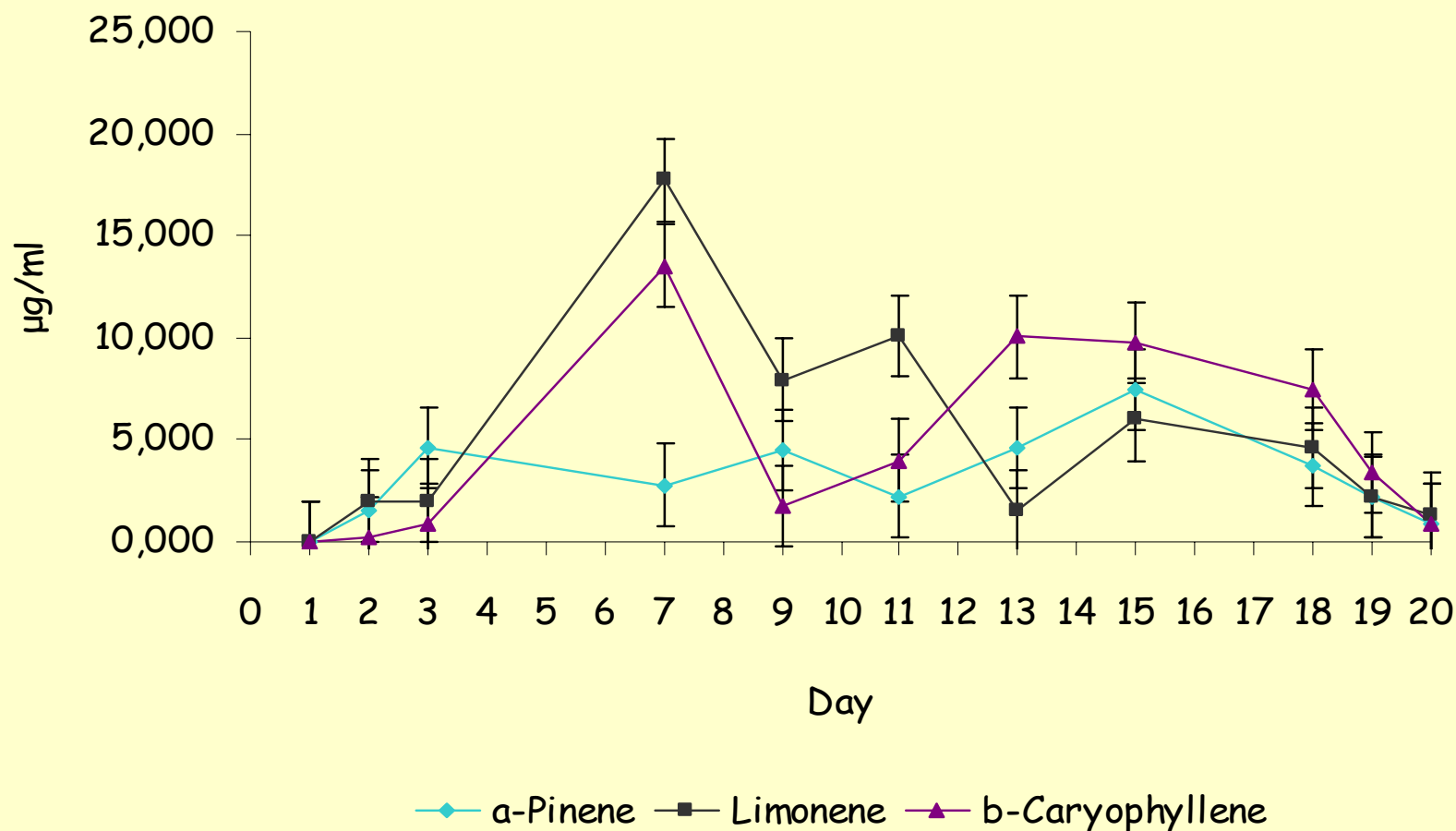
Mean α-pinene, limonene and b-caryophyllene concentration (µg/ml) for the first 24 hours in sheep blood plasma receiving 1g/head/day of each substance

Results



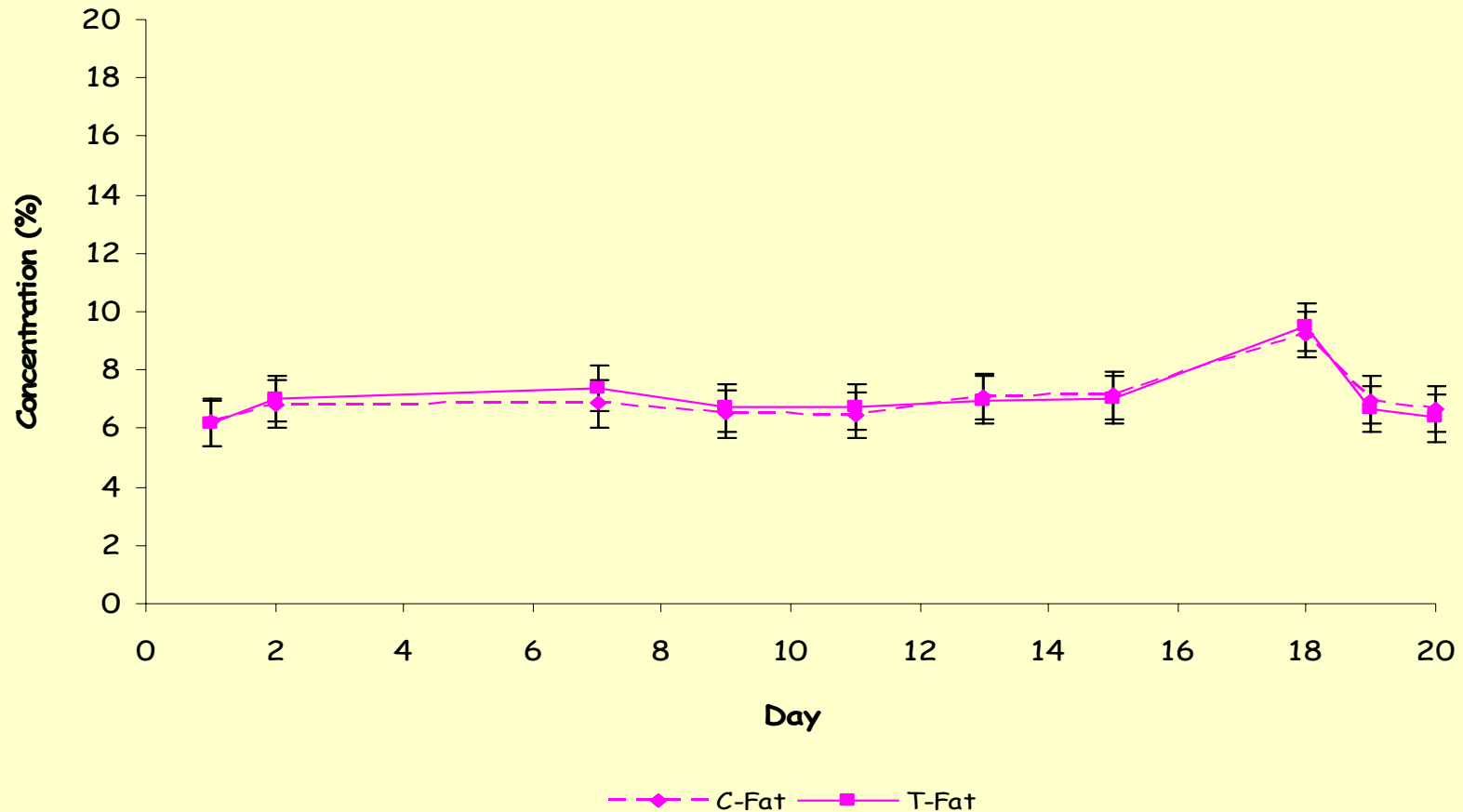
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Results



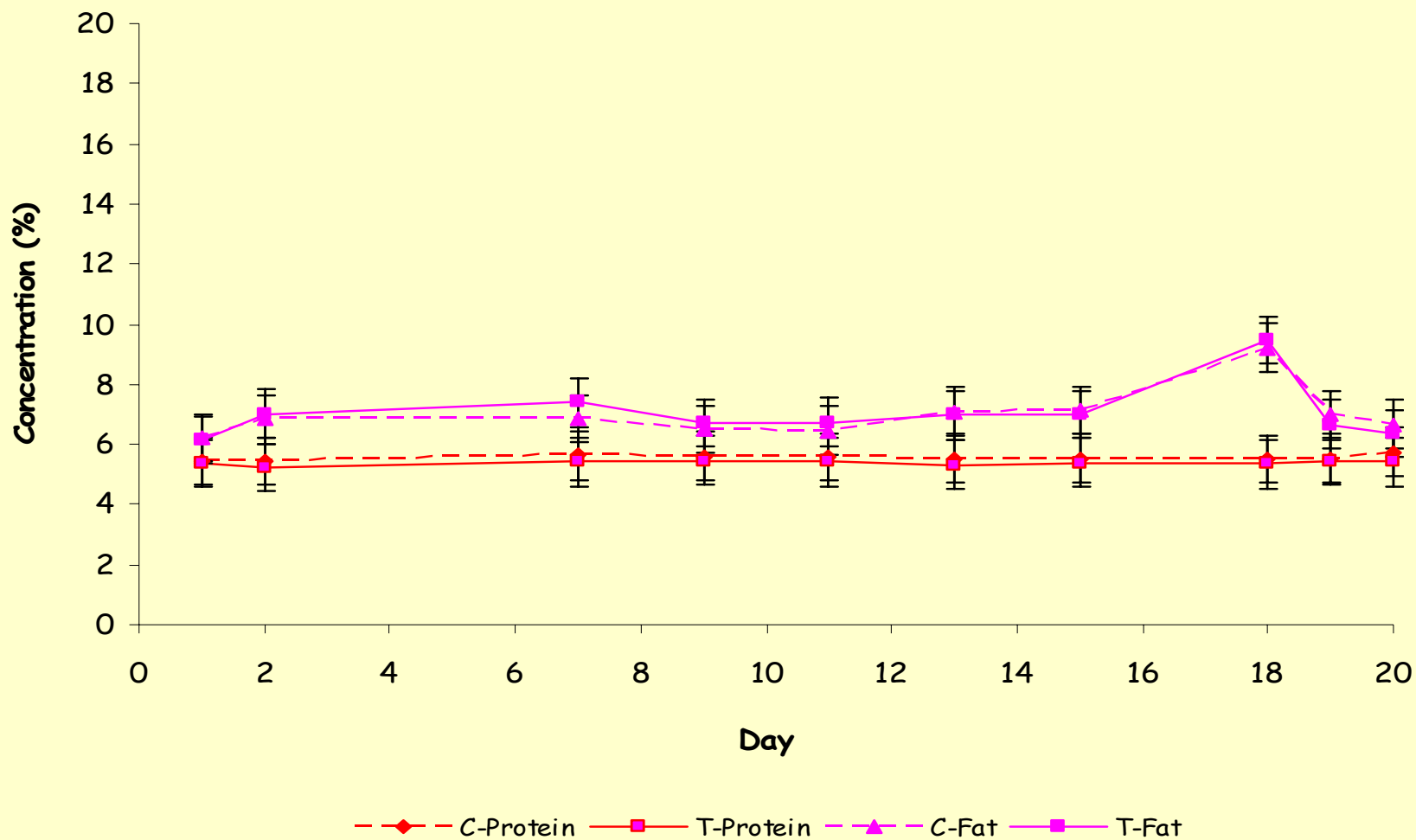
Mean α-pinene and limonene concentration (µg/ml) in sheep milk receiving 1g/head/day of each substance

Results



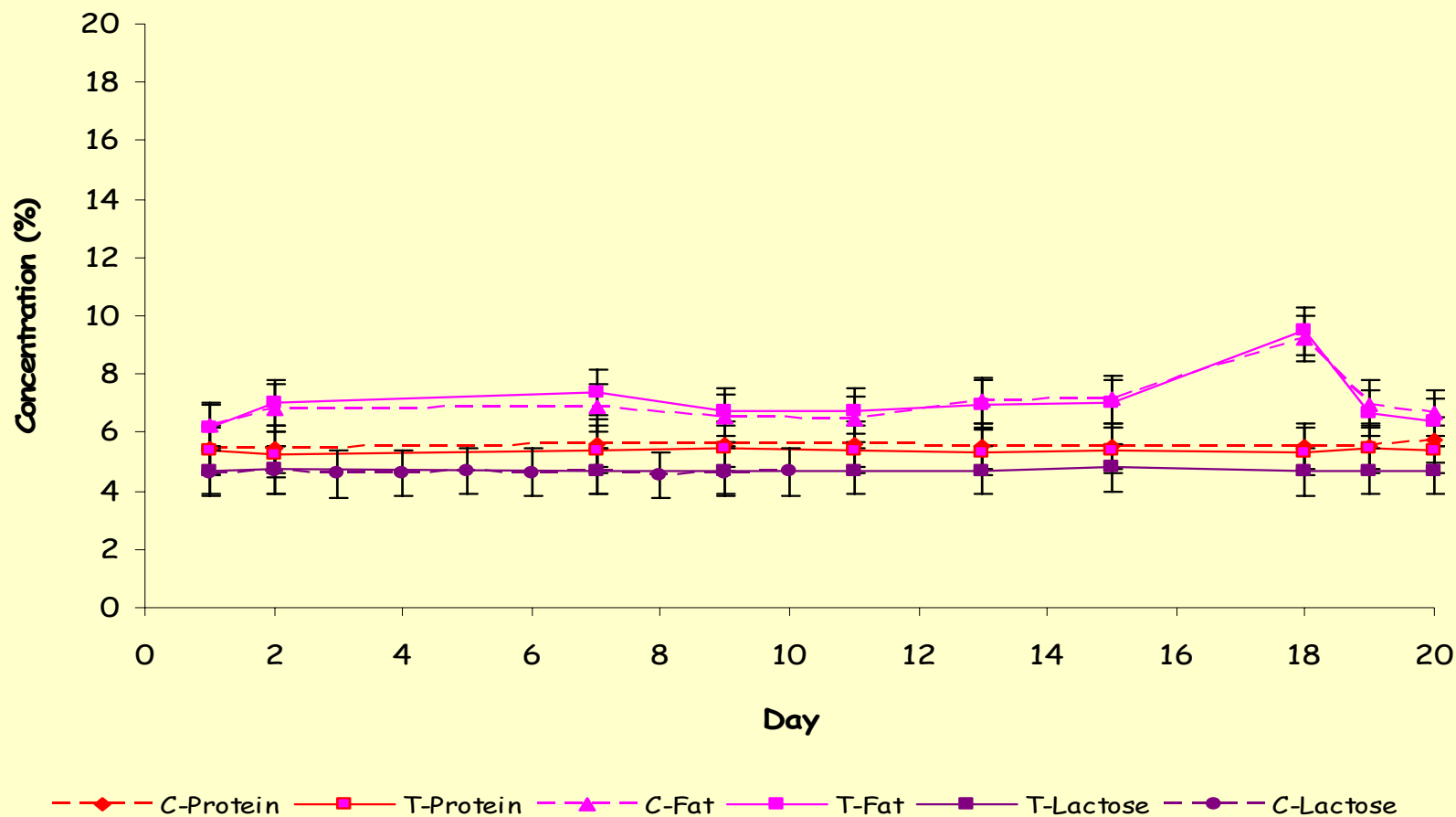
Chemical composition of ewe 's milk produced by Control (C) and Treatment (T) groups of animals at different days during the trial

Results



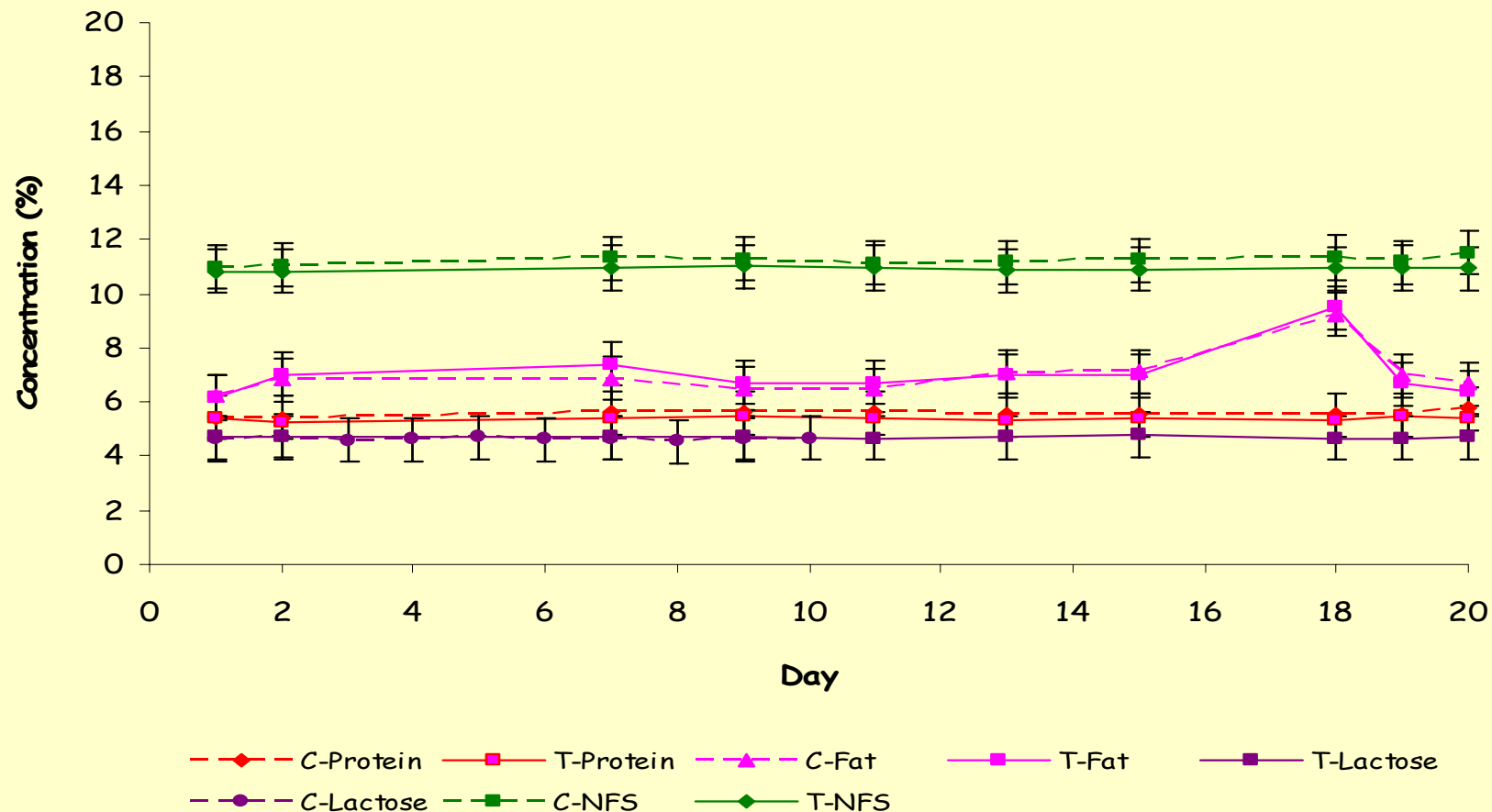
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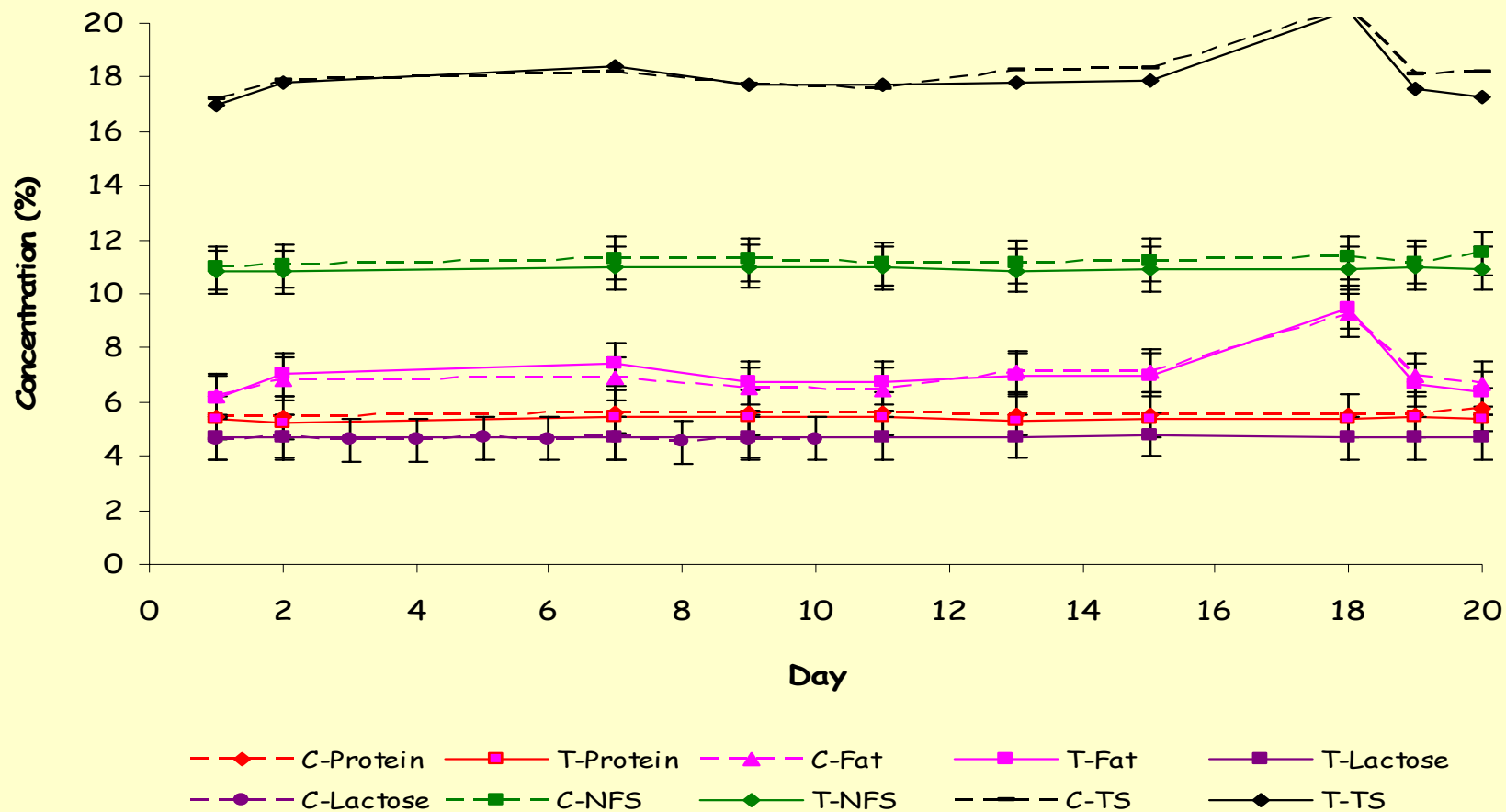
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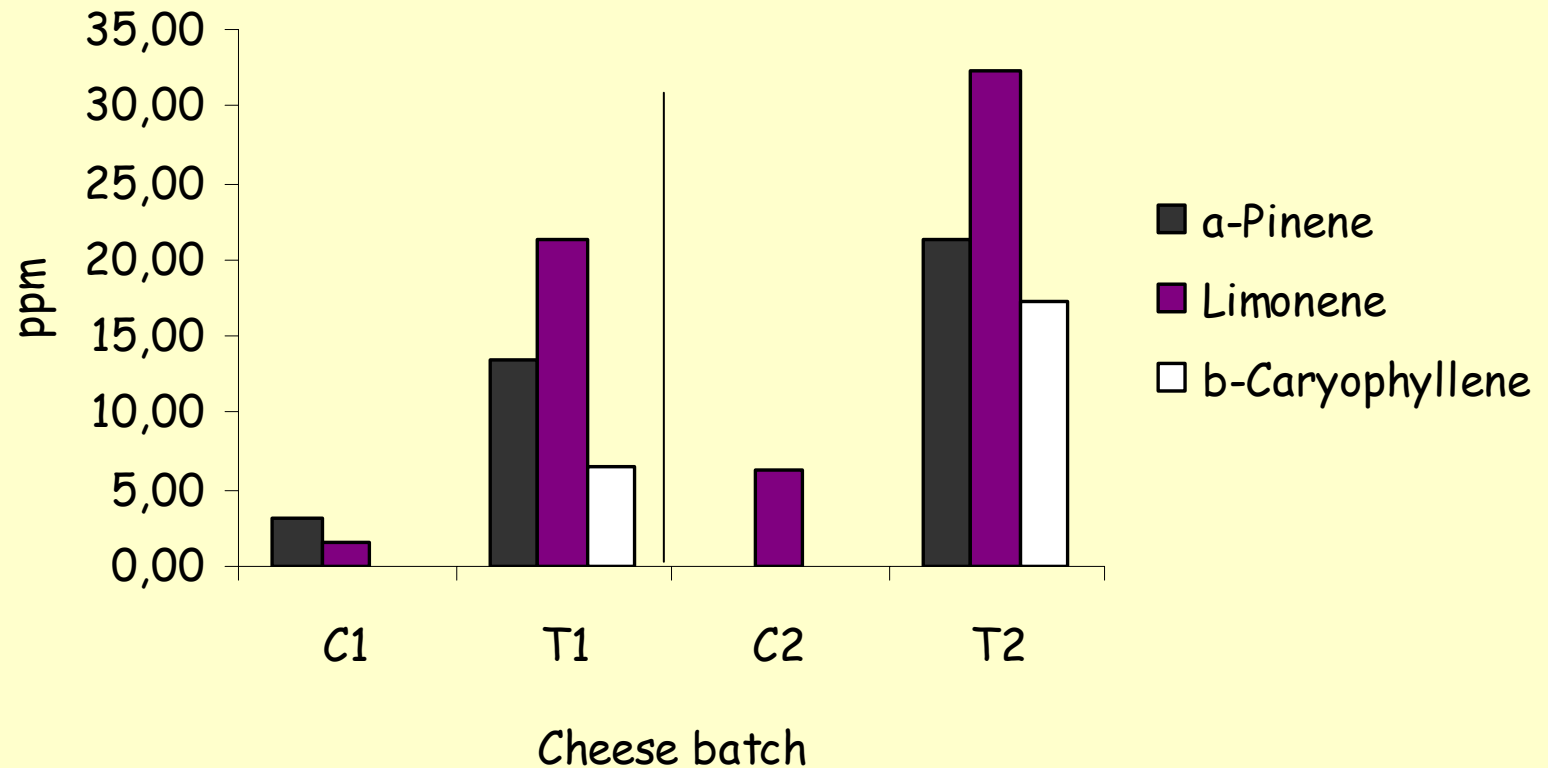
Chemical composition of ewe 's milk produced by Control (C) and Treatment (T) groups of animals at different days during the trial

Results

Treatment	2	7	13	20	S.E	P
Ca (mg/100g milk)						
C	262.84	283.96	275.30	276.51	18.21	ns
T	248.41	250.81	263.54	252.02	18.21	ns
Mg (mg/100g milk)						
C	23.78	22.22	22.34	23.62	1.01	ns
T	21.31	22.25	22.19	21.63	1.01	ns
Na (mg/100g milk)						
C	87.70	70.54	60.35	63.85	8.54	ns
T	67.32	76.95	78.28	63.14	8.54	ns
K (mg/100g milk)						
C	232.96	161.85	153.28	157.96	12.42	ns
T	196.27	178.91	153.98	159.57	12.42	ns

Ewe's milk salts produced by Control (C) and Treatment (T) groups of animals at different days during the trial

Results



Concentration (ppm) of α -pinene, limonene and β -caryophyllene in ripe cheese produced from sheep milk of Control (C) and Treatment (T) groups of animals.

Results

Treatment	Moisture (%)	Fat (%)	Total protein (%)	Water soluble protein (%)
1 st Cheese batch				
C	37.45	33.00	24.45	8.73
T	34.28	35.17	24.45	6.99
2 nd Cheese batch				
C	33.83	34.00	24.38	6.03
T	34.24	35.00	24.85	6.11

Chemical characteristics of ewe 's cheese produced by Control (C) and Treatment (T) groups of animals



Conclusions

- ◆ Terpenes can be integrated as additional parameters in certification schemes as biomarkers in animal products.
- ◆ Terpenes appear appropriate biomarkers towards certification of ewe milk samples, though with limitations due to unexplained variation.
- ◆ Terpenes profile can authenticate grazing sheep-cheese at a limited capacity.



Acknowledgements

The financial support of BIO-HELLAS (organic certification organization), towards this contribution is gratefully acknowledged.

Thank you for your attention !!!!