61st Annual Meeting of the European Association for Animal Production (EAAP 2010)
23- 27 August 2010, Heraklion, Crete Island, Greece
Session 16 "Product quality (milk, meat) in small ruminants" (poster 15)

In vitro evaluation of terpene's effects on aspects of animal physiology: cytotoxicity and rumen degradation

Poulopoulou I. [1*], Pitulis N. [2], Mountzouris K.C. [1], Hadjigeorgiou I. [1] and Xylouri E. [2]

[1] *Department of Nutrition Physiology and Feeding,* [2] *Department of Anatomy and Physiology of Farm Animals*
Faculty of Animal Science and Aquaculture, Agricultural University of Athens, Iera Odos 75, 118 55, Athens, Greece
**gpoulop@hotmail.com*

INTRODUCTION

Herbivores often consume, through foraging, substantial amounts of plant secondary metabolites (PSM), which, at certain levels can lead to toxicity. The aim of this study was to evaluate the potential cytotoxic effects of three terpenes, α-pinene, limonene and β-caryophyllene using in vitro techniques and to assess the detoxification capacity of rumen for these substances.

MATERIALS AND METHODS

♣ Cytotoxicity test: Rabbit kidney cell line RK13, grown in DMEM containing 10% FBS, was used as a substrate model. A methylthiazolyldiphenyl-tetrazolium bromide (MTT) reduction assay was used to assess cell metabolic activity. Cells were treated for 24 hours with each terpene, as well as their combination in a range of concentrations (100 to 3.1 μg/mL). Tests run in triplicate (Photo 1).

♣ Rumen degradation test: the 3 terpenes were incubated in sheep and goat rumen fluid at a concentration of 100 μg/mL and degradation rate was measured at 0, 2, 4, 8, 20 and 24 hours. Degradation was also assessed in supernatant and microbial cell fractions after centrifugation.

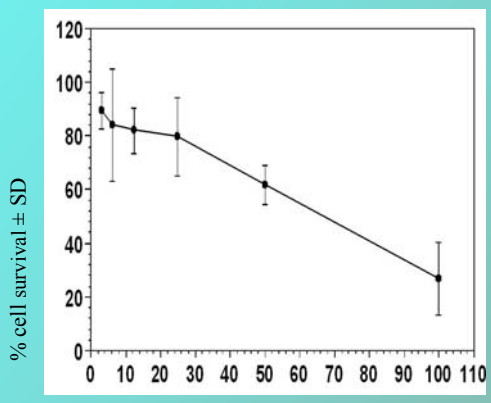
RESULTS

Limonene, β-caryophyllene and α-pinene arrested RK-13 cell proliferation. The combination of three had a pronounced effect indicating a synergistic inhibition of cell proliferation.

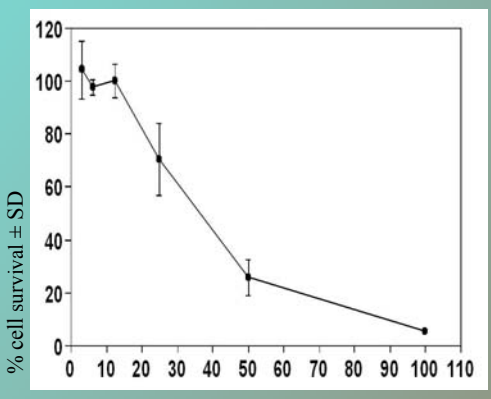
All three terpenes were degraded by rumen fluid liquor forms, but to different extend. Between species degradation in sheep or goat rumen fluid reached for: a) α-pinene 80% and 60% respectively, b) limonene 50% and 45% respectively and c) b-caryophyllenne 55% for both species.

CONCLUSIONS

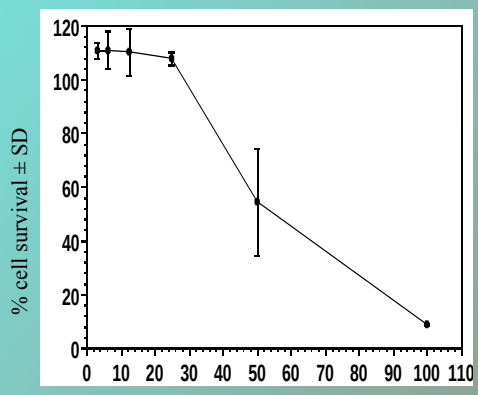
The results indicate terpenes might have a toxic effect on animal cells, but rumen microbial activity can reduce quantities available for absorption at ruminant's lower intestine.



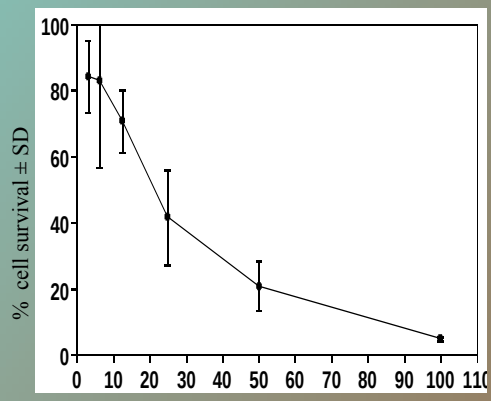
α -pinene % survival concentration in RK cells



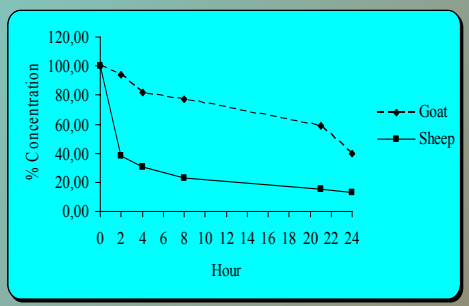
Limonene % survival concentration in RK cells



β -caryophyllene % survival concentration in RK cells



Terpenes combination % survival



α -pinene % concentration in sheep and goat rumen fluid

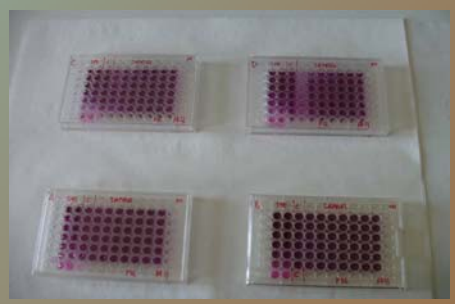
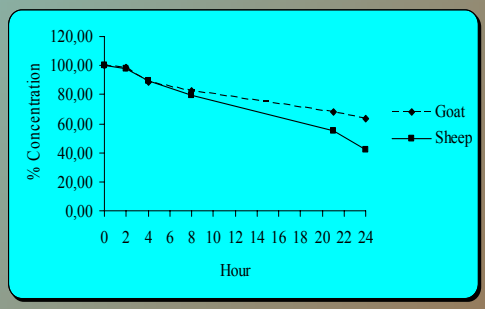
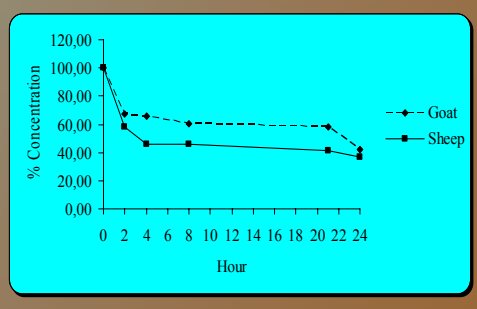


Photo 1: Treated RK 13 cells



Limonene



β -caryophyllene

% concentration in sheep and goat rumen fluid