

## Extracts from wine by-products affect sheep cell proliferation and cytokine production

Cristina Inghese (1), Maria Giovanna Ciliberti (1), Matteo Francavilla (1), Marzia Albenzio (1), Rosaria Marino (1), Antonella Santillo (1), and Mariangela Caroprese (1)

(1) Università degli Studi di Foggia, Dipartimento SAFE, Italia.

The Wine sector is one of the most important compartments of the Italian economy; different by-products originate from wine production, among which wine lees. Several studies indicated the possibility of using the different wine by-products in animal feeding as they contain bioactive compounds such as polyphenols [1-2]. Recently, authors demonstrated the ability of these compounds from wine by-products to interfere in the inflammatory cytokines pathways [3], and, therefore, to up-regulate the expression of antiinflammatory cytokines such as IL-10 [4]. The aim of this study was to evaluate the effects of bioactive compounds extracted from three different wine lees from white (Wh), rosè (Ro) and red wine (Re), on sheep peripheral blood mononuclear cell (PBMC) proliferation and cytokine production. Wine lees extracts were obtained by microwave-assisted extraction with three different solvents: water (W), water/ethanol 1:1 (W-Et) and ethanol (Et), with or without  $\text{Na}_2\text{CO}_3 \times 10 \text{ H}_2\text{O}$  as catalyzer (W/k, W-Et/k, Et/k). Subsequently, total phenols, anthocyanins and flavonoids content and antioxidant capacity in term of ABTS and FRAP on wine lees extracts were determined using an UV-spectrometer. Sheep PBMC, stimulated with Concanavalin A and LPS, were cultivated for 24 h at 37°C with 5 % of  $\text{CO}_2$  and treated with each wine lees extracts at two different concentrations (0.4 ng/mL and 0.8 ng/mL). The free cells supernatant was collected for ELISA interleukin (IL)-6, IL-1 $\beta$ , IL-10 and IFN- $\gamma$  analysis; on cells, Bromodeoxyuridine proliferation assay was performed. Data were analyzed using ANOVA for mixed models using the MIXED procedure of SAS. PBMC treated with wine lees extracts registered a marked reduction of cells proliferation compared to stimulated cells ( $P < 0.001$ ). The levels of IL-10 and IFN- $\gamma$  were affected by wine lees extracts ( $P < 0.001$ ). In particular, the ReW at 0.8 ng/mL led to higher production of both cytokines, probably due to the higher scavenging capacity as demonstrated by ABTS. The proinflammatory cytokines IL-6 and IL-1 $\beta$  were affected by wine lees extracts ( $P < 0.001$ , and  $P < 0.01$ , respectively). Even if any sharp variation among extracts was recorded, the ReW/k at 0.8 ng/mL exerted an increment of IL-6 compared to not stimulated cells. Results from the present experiment demonstrated the ability of wine lee by-products to affect the immune responses of sheep PBMC.