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C-076

Plant secondary metabolites and traceability of dairy products: a key role for livestock production and efficiency in ruminant dairy products

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Primary metabolites, such as amino acids, organic acids and carbohydrates are essential for life and exist in all plants. Secondary metabolites (PSM) are not directly involved in the normal life cycle and they play an important role in plant defence, adaptation to environment, colouring and other metabolic functions. The term ‚Äòsecondary' is based on early research in which metabolism was thought to be partitioned into a primary component that directly supports growth, development, and reproduction, and a secondary component that has no direct role in these primary functions. Traditionally, PSM have been considered as ‚Äòanti-nutritive' since they are often bitter, deter animals from eating the plant, or cause aversion through interfering with animal digestive and fermentation processes or directly affecting host metabolism. However, more recent trends and research for feed in the livestock production have stimulated interest in PSM, as they may benefit animal production when they are fed in the correct form and dose. It is possible to classify secondary metabolites as: phenols, sulphur-containing compounds, terpenes alkaloids, lipids (coronaric acid) and enzymes (polyphenol oxidase, PPO). To improve the nitrogen use efficiency during ensiling the use of tannin containing forages has been beneficial (2-4% DM in tannins contents) with an estimated profit in US dairy farms estimated at $300 million/y with reduced nitrogen losses of 25%. Tannins have shown antimethanogenic properties (-15%) with different tannin sources showing variable results on on palatability with low adverse effects on animal performance and organic matter digestibility. In Italy several PDO cheeses are produced from grazed pastures with strong seasonality, typical of the mountainous nature and Mediterranean climate. The cheeses sourced from grazed herbage are characterized by a higher content of volatile compounds compared to cheese made from animal fed at stall. The volatile compounds, besides giving a characteristic flavour to the cheese, can also be used as bio-markers as indicative compounds pass from the pasture to the cheese. Polyphenols oxidase, tannins, coronaric acid are capable when properly evaluated, to modulate the livestock system making it more efficient and more environmentally sustainable. Finally of particular interest is the role of grazing ruminants in land management and landscape re-evaluation for tourism, a key element to prevent the depopulation of rural areas.

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Cytotoxicity, DNA integrity and methylation in mammary and kidney epithelial cell lines exposed to Ochratoxin A

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Ochratoxin A (OTA) is a secondary metabolite of moulds that may contaminate food and feedstuffs. OTA is recognized as a nephrotoxic, hepatotoxic and teratogenic substance in different animal species. The kidney is the target organ of OTA, whereas lower OTA concentrations could be detected in other tissues, such as adipose tissue, liver and muscles. In addition, OTA transfer to milk has been demonstrated in several species, such as humans, rabbits, rats and ruminants, identifying the mammary gland as one of the potential target of this mycotoxin. This study aimed to investigate the *in vitro* damage induced by OTA using two well established epithelial cell lines. MDCK cells have been used as a model of the kidney epithelium, while BME-UV1 have been employed as a model of the mammary gland epithelium. The effect of OTA on cultured cell lines, with subsequent evaluation of cell viability (MTT test) and membrane stability (LDH test), was assessed. In all experiments performed, control consisted of MDCK and BME-UV1 cells exposed to basal medium alone. After 24h of OTA treatment (concentration range 0.15 up to 10 µg/mL), MDCK and BME-UV1 cell viability was strongly reduced in a dose-dependent way and LC50 has been calculated. LC50 for MDCK cells was 1 µg/mL while, for BME-UV1 cells, LC50 was 0.8 µg/mL. In light of LC50, the range of concentrations for further experiments was determined (0.3 up to 1.25µg/mL). The percentage of LDH released by MDCK and BME-UV1 cells increased in a dose-dependent way. In particular, 1.25 µg/mL of OTA determined a 35% of LDH released in MDCK cells and a 46% of LDH released in BME-UV1 cell line. Subsequently, the effect of the addition of OTA to MDCK and BME-UV1 cells has been evaluated on DNA integrity, which was detected by gel electrophoresis, by the analysis of DNA oxidation biomarker 8-OHdG (8-OHdG adduct) and the global DNA methylation status (5-mC). The level of 8-OHdG adduct was significantly ($P<0.05$) increased in BME-UV1 cells treated with 1.25 µg/ml of OTA. The analysis of 5-mC revealed that in MDCK and BME-UV1 cells, OTA has not induced alterations in the global DNA methylation status. The results obtained herein could represent the basis for the development of future studies investigating the *in vitro* relationship between DNA damage and the global DNA methylation status, promoting new strategies to control OTA cytotoxicity at different tissue level.