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**Guest Editors: Fulvia Bovera (Coordinator),
Marzia Albenzio, Mariangela Caroprese, Rosaria Marino,
Gianluca Neglia, Giovanni Piccolo, Angela Salzano.**

In Italy, 'Mozzarella di Bufala Campana' cheese, gained a very well-defined profile in the market, enhanced and protected by a Protected Designation of Origin (PDO) trademark. Since attempts are regularly made to place non-authentic PDO mozzarella in premium markets as a substitute for real PDO buffalo mozzarella, the aim of the present study was to combine advanced GC-MS and metabolite identification in a robust and repeatable technology platform for characterisation of the metabolome of buffalo milk and mozzarella. The study utilised 20 commercial buffalo dairies. Eleven dairies were located in a PDO area and nine dairies were located in non-PDO area in Italy. All dairies had a processing facility that produced mozzarella cheese exclusively from their own milk. Pooled samples of raw milk (100 mL) and mozzarella cheese (100 g) were obtained from each dairy. A total of 185 endogenous metabolites were consistently detected in both milk and mozzarella cheese. The PLS-DA score plots clearly differentiated PDO and non-PDO milk and mozzarella samples. Regarding milk samples, it was possible to divide metabolites into two class according to area: those with lower concentrations in the PDO (Galactopyranoside, hydroxybutyric acid, allose, citric acid) and those which have lower concentrations in non-PDO samples (Talopyranose, pantothenic acid, mannobiose, maltose, phosphate, mannofuranose, dodecanoic acid, lactose, palmitic acid, N-acetyl glutamic acid, N-acetyl glucosamine). The same was recorded in mozzarella samples with some metabolites (Talopyranose, 2,3-dihydroxypropyl icosanoate, Sorbose, 4-pnehl glutamic acid, Oxalic acid, Galactose) higher in PDO samples while others (Tagatose, Lactic acid dimer, Ribitol, Dodecyl thio-glycolate, N-acetyl glucosamine, Valine, Diethylene glycol) higher in non-PDO samples. At present, more studies and efforts should be done on the applicability of the use of a metabolomic approach for legal purposes.

O095

Antioxidant and anti-inflammatory activities of buffalo milk bioactive metabolites

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The interest on developing foods with health promoting and disease preventing properties is still increasing. Buffalo milk (*Bubalus bubalis*), the second most consumed milk worldwide, is a good source of substances with high nutritional value which

are necessary for body maintenance and to fight against several disease conditions. The health promoting and functional ability of buffalo milk bioactive metabolites have been recently expanded. Indeed, recent results showed peculiar profile of betaines and short-chain acylcarnitines of buffalo milk, with regard to δ -valerobetaine (δ VB), γ -butyrobetaine (γ BB), acetylcarnitine, and propionylcarnitine.

Buffalo milk also shows high antioxidant and anti-inflammatory activities in counteracting the high-glucose (hGluc)-induced endothelial damage. These effects were amplified by δ VB enrichment, resulting in decreased reactive oxygen species, lipid peroxidation, and cytokine release during hGluc treatment ($p < .05$). Of interest, δ VB counteracted the hGluc activated inflammatory signal by modulating the expression levels of SIRT1, SIRT6 and NF- κ B ($p < .05$). In conclusion, this study shows the first evidence of buffalo milk properties in reducing endothelial oxidative stress and inflammation, suggesting a potential role of δ VB as a novel dietary compound with health-promoting properties.

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O096

Study of nutraceutical substances (Rutin) in sheep's milk fed with buckwheat hay

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Different ruminants' diet can change milk chemical and nutritive composition. Forages, such as buckwheat, could change the nutritive properties of milk, being a significant source of bioactive compounds, metabolites that are thought to have an important role of both human and animal diets. Recent research has demonstrated that flavonoids play a synergic antioxidant role against free radical damage. Flavonoids, such as rutin, are an important class of phytochemicals products found in most fruits, vegetables, herbs and forages.

Rutin is the dominant flavonol glycoside in common buckwheat and is well known that is a powerful antimicrobials and antioxidants with anti-inflammatory and immune system benefits. Two groups each with ten Massese sheep (body weight 60 ± 5 kg) at the same stage of lactation were studied. Blood samples of the two groups were collected before and at end of the trial in order to monitor the metabolic profile. Each animal was fed daily with 1 kg of buckwheat hay and 1 kg of concentrate. The rutin content of buckwheat hay was 2.26 g/kg.

The animals were monitored in order to ensure that the hay was completely ingested. Individual milk samples were collected at 0, 15, 20, 25, 30, 35, 40 days after the first buckwheat hay administration.

The rutin determination in buckwheat hay and milk was performed by a HPLC method. After 15 days of buckwheat hay administration, the lower rutin content in milk was 0.89 mg/kg. The presence of rutin in milk may constitute a non-hydrolysed aliquot or may indicate a difference between monogastrics and ruminants. This also could be in agreement with the literature which indicates that urinary and biliar excretion of flavonoids and their metabolites may be the main metabolic pathways for such compounds.

It is well known that flavonoids have biological activities and they could be used for preparing fortified foods, these compounds could be added to milk and other food.

The study in progress will be useful to evaluate the rutin carry-over from buckwheat hay to milk and the possible nutraceutical applications of this milk to improve health benefits in humans.

0097

Use of donkey milk in infant feeding

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There are still few literatures about the role of donkey milk (DM) in human nutrition and increasing knowledge is crucial in order to provide practical advice for DM consumption. The aim of this study was to monitor nutritional quality, hygiene and health risks, and the impact of DM in the feeding of children with cow's milk protein allergy (CMPA). DM was supplied by a farm located in central Italy, conforming with EU regulation 853/2004. Eighteen pasteurised milk samples (at 65 °C for 30 min) were taken monthly. Pasteurised DM showed a total average viable count of 4332.22 CFU/mL (± 3046.78), a slightly alkaline pH (7.12 ± 0.17), a lactose percentage of 6.83 ± 0.34 , a total protein percentage of 1.63 ± 0.19 , while casein was $0.81\% \pm 0.11$. Fat percentage (0.51 ± 0.52) was lower compared to ruminant milk and about 48% of the total milk fatty acids were unsaturated. In addition,

DM contained 7.52 ± 2.49 g/100 g of fat of n3 linolenic acid. Eighty-one children with CMPA referred to the Allergy Unit of the Anna Meyer Children's Hospital were recruited. They underwent to an allergological work-up including an oral food challenge (OFC) with DM; during the OFC the palatability of the milk was also evaluated. In children ≥ 3 years of age, DM palatability was assessed with a specific Wong-Baker modified pain scale, while in children < 3 years of age it was assessed through the physician's judgment. The results of the allergological work-up showed that DM did not cause allergic reactions in the 98.7% of patients, in addition, a good palatability of the milk was found. DM was included for six months in nutritional plans for 16 children with IgE-mediate CMPA (mean age of 20 ± 18.4 months at the beginning of the study) and six with Food Protein-Induced Enterocolitis Syndrome (mean age of 5.33 ± 1.75 months). The daily dose of DM varied from a maximum of 1000 mL to a minimum 200–250 mL according to the age of the children. Given the low fat of DM, the diet of the children was supplemented with extra virgin olive oil (EVO) according to the age (from a minimum of 1.5 g of EVO and 1.5 g of Medium Chain Triglycerides vegetable oil in each 100 mL of milk to a maximum of 8–10 mL of EVO added to the daily meals). All the children that underwent to the nutritional plans were monitored twice (at the beginning and at the end of the study) for the auxological parameters. The results showed that DM did not change the normal growth rate of allergic children.

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ANIMAL PHYSIOLOGY, HEALTH AND WELFARE – REPRODUCTION I

0098

Quality of chicken semen cryopreserved with different N-methylacetamide concentrations combined with trehalose

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The study aimed to assess the effect of different N-methylacetamide (MA) concentrations combined with trehalose on the quality of post-thawing chicken semen. Forty adult Hubbard male chicken breeders were used. All chickens were housed in individual cages at 20 °C, exposed to a 14L:10D photoperiod and fed a commercial standard chicken diet. Males were divided into four different