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Short communication: Technological and seasonal variations of vitamin D and other nutritional components in donkey milk

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ABSTRACT

Vitamin D is an essential nutrient that plays a crucial role in calcium homeostasis and bone metabolism and also acts as a hormone. Although several studies on the content of vitamin D in bovine milk have been conducted, little information is available regarding donkey milk. In the context of the nutritional assessment of donkey milk, the aim of this study was to assess the vitamin D content in donkey milk and its chemical profile, with particular reference to seasonal and technological modifications after pasteurization. The study was conducted on a dairy farm that produces donkey milk for human consumption located in central Italy. At sampling time, an aliquot of total bulk milk production was sampled before and after pasteurization (63°C for 30 min without homogenization) with a total of 20 raw and 20 pasteurized milk samples. The samples were collected for 10 mo, every 15 d, from May to February 2017. All the samples were analyzed for the chemical composition and vitamin D_2 and D_3 content by HPLC after saponification. The donkey milk analyzed showed a higher average vitamin D content (raw milk: vitamin D_2 = 1.68, vitamin D_3 = 0.60 μg/100 mL; pasteurized milk: vitamin D_2 = 1.38, vitamin D_3 = 0.30 μg/100 mL) than reported for bovine and human milk. The results of the effect of pasteurization on milk did not highlight significant differences in the total content of vitamin D. However, vitamin D_3 has a poor thermal stability, which led to a significant reduction in content in pasteurized milk compared with raw milk. The total vitamin D content of donkey milk did not show significant variations between seasons; however, a higher concentration of vitamin D_3 was found in spring and summer. In conclusion, raw and pasteurized donkey milk showed a high content of vitamin D, which could be useful in meeting the deficiencies of this vitamin in humans. Further investigations are needed to improve the vitamin D content in donkey milk by increasing its endogenous synthesis or its transfer in milk and to clarify other variability factors.
Key words: donkey milk, vitamin D, seasonal variability, pasteurization

Short Communication

Vitamin D plays a key role in calcium homeostasis and bone metabolism and acts as a hormone (Müller et al., 2011). Vitamin D_2 (ergocalciferol) is derived from the UV radiation of ergosterol (in particular UV-B radiation), which is a vitamin D precursor naturally found in plants, fungi, and invertebrates. Vitamin D_3 (cholecalciferol) is derived by sunlight exposure from 7-dehydrocholesterol, which is a precursor of cholesterol and also acts as a provitamin D_3 (Schmid and Walther, 2013).

The major source of vitamin D for children and adults is exposure to natural sunlight that is required for UV-B–induced vitamin D production in the skin. Vitamin D that comes from the skin or diet is biologically inert and requires its first hydroxylation in the liver to 25(OH)D_3. The latter requires a further hydroxylation in the kidneys to form the biologically active form of vitamin D1,25$(OH)_2$D.

An oral intake of vitamin D may be an important source in winter, when the UV-B–related synthesis is limited and for people who are not exposed to sunlight (Gill et al., 2016). Vitamin D deficiency is well known, and the concentration in blood serum of the hydroxylated form of calciferol [25(OH)D_3] is recognized as a sensitive accurate indicator of the functional status of vitamin D (Heaney, 2004). The Institute of Medicine (IOM, 2011) defined a vitamin D deficiency as a content of 25(OH)D_3 less than 20 ng/mL in serum. In addition, the widespread risk of deficiency and insufficiency worldwide has been reported (Holick et al., 2011), due to the current mostly indoor life style. In Italy, vitamin D deficiency and insufficiency were detected in 49.9 and 32.3% of adolescents, respectively, and 8.9% of Italian

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adolescents were severely deficient (Vierucci et al., 2014).

Vitamin D dietary intake is also of great importance, and animal foodstuffs (e.g., fish, meat, offal, eggs, and dairy) are the main sources for naturally occurring cholecalciferol (vitamin D_3). However, it is difficult to cover the requirements of vitamin D solely by foodstuffs (Schmid and Walther, 2013). Milk mainly contains 2 forms of vitamin D (D_3 and D_2).

Research has highlighted the variability factors of vitamin D in milk by analyzing endogenous, exogenous, and technological factors, such as season, age of the animal, treatment, and conservation (Jakobsen and Saxholt, 2009; Weir et al., 2017). However, most of the studies focus on bovine milk, whereas little information regarding donkey milk is available (Gentili et al., 2013; Bulgari et al., 2015). The properties of donkey milk have been known since ancient times, and in the last few decades there has been renewed interest from the scientific community due to donkey milk's use as a therapeutic product for children with bovine milk protein allergy (Martini et al., 2017).

Despite having a low bacterial count, the thermal treatment of donkey raw milk is recommended to avoid the risk of food-borne diseases (Martini et al., 2016). Pasteurization is known to eliminate pathogenic microorganisms that could be present in milk and guarantees its preservation. Furthermore, the literature on the effects of pasteurization on nutritional characteristics of donkey milk is not yet exhaustive. In addition, unlike cow milk that is mostly standardized, donkey milk has a certain variability in terms of its components.

Nutritional characteristics of donkey milk are especially interesting, as this milk is targeted for consumption by children for its similarity to human milk. In addition, consumption in the elderly has been proposed given that donkey milk has good calcium content, is low in fat, and easily digestible. Children and the elderly are at particular risk for developing nutritional deficiencies; therefore, they require careful nutrition management, from a quantitative and qualitative point of view, to avoid undernourishment and malnutrition. In the context of the nutritional assessment of donkey milk, the aim of our paper was to carry out an evaluation of the vitamin D content in donkey milk and its chemical profile, with particular reference to seasonal and technological modifications after pasteurization.

The study was conducted on dairy farm that produces donkey milk for human consumption. The farm, located in central Italy, raised about 160 Amiata donkeys, reared outdoors, in a semi-intensive system. The animals were fed about 2.5 kg/d per head of concentrate for dairy donkeys (Progeo S.C.A., Masone, Italy) and polyphite hay ad libitum. The jennies were routinely machine milked twice a day.

At sampling time, an aliquot of total daily bulk milk production was sampled before and after pasteurization (63°C for 30 min without homogenization). The samples were collected for 10 mo, every 15 d, from May to February 2017 (20 raw and 20 pasteurized milk samples), for a total of 40 samples.

All the samples were analyzed for DM, fat, and lactose content by infrared analysis (Milkoscan, Italian Foss Electric, Padova, Italy) as well as protein, casein, and ash (AOAC International, 2006). Individual mineral content (Ca, P, Mg, K, Na, and Zn; mg/L) was determined by atomic absorption spectroscopy and UV-visible spectroscopy according to Horwitz (2000) and Murthy and Rhea (1967). Milk fat extraction was performed following the Röse-Gottlieb method (933.05, AOAC International, 1995), and FAME were prepared using methanolic sodium methoxide according to Christie (1982). A Perkin Elmer Clarus 480 (Perkin Elmer, Norwolk, CT) equipped with a flame ionization detector and a capillary column (60 m × 0.25 mm; film thickness 0.25 mm; ThermoScientific TR-FAME 60 m × 0.25 mm ID; film thickness 0.25 μm, Thermo Fisher Scientific, Waltham, MA) were used.

The helium carrier gas flow rate was 1 mL/min. The oven temperature program level 1 was 50°C held for 5 min; level 2 was 50 to 140°C at 3°C/min, then held for 2 min; and level 3 was 140 to 240°C at 1°C/min, then held for 10 min. The injector and detector temperatures were set at 270 and 300°C, respectively. The peak areas of individual fatty acids (**FA**) were identified using an FA standard injection (Food Industry FAME Mix – Restek Corporation, Bellefonte, PA) and quantified as the percentage of total FA. In addition, nonadecanoic acid methyl ester (C19:0, Restek Corporation) was also used as an internal standard.

Milk fatty acids were grouped as SFA, MUFA, PUFA, and short-chain (**SCFA**), medium-chain (**MCFA**), and long-chain fatty acids (**LCFA**). The SFA included ΣC4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, and C24:0. The MUFA included ΣC14:1, C15:1, C16:1, C17:1, C18:1 *trans*-9, C18:1 *trans*-11, C18:1 *cis*-9, C20:1, C22:1, and C24:1. Thee PUFA included ΣC18:2 tranis-9,12, C18:2 *cis*-9,12, C18:3 n6, C18:3 n3, C20:2, C20:3 n6, C20:4, C20:3 n3, C20:5, C22:2, C22:5, and C22:6. The SCFA included the sum of FA from 4 to 10 C, MCFA included the sum of FA from 11 to 17 C, and LCFA included the sum of FA from 18 to 24 C.

For the determination of vitamin D, 75 mL of donkey milk were saponified by adding KOH pellets directly to the milk according to Perales et al. (2005). As a