

PHYSICO-CHEMICAL COMPOSITION AND ANTIOXIDANT CAPACITY OF BUFFALO MILK

Aurelia COROIAN¹, Camelia RĂDUCU¹, Vioara MIREȘAN¹, Daniel COCAN¹,
Igori BALTA¹, Adina Lia LONGODOR¹, Luisa ANDRONIE¹, Mircea MUNTEAN²,
Zamfir MARCHIȘ¹

¹University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Animal
Science and Biotechnology, Mănăștur Str. 3-5, 400272, Cluj-Napoca, Romania

²University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture,
Mănăștur Str. 3-5, 400272, Cluj-Napoca, Romania

Corresponding author email: lia_adina@yahoo.com

Abstract

Buffalo milk due to its high fat content from the energy point of view is more valuable compared to cow's milk. The percentage of fat varies depending on the stage of lactation, season, individual, health, animal age and diet. The study of the chemical composition and the antioxidant capacity of buffalo milk is required for both scientific and technological considerations, given the importance of this type of milk in the consumer's diet. The purpose of this study was to evaluate the antioxidant capacity and the physico-chemical composition of buffalo milk by lactation. Buffalo milk can have different qualities, so the quality of buffalo milk is also determined by its content in its components (protein, fat, lactose, vitamins, fatty acids, water content, antioxidant capacity). The physicochemical parameters and antioxidant capacity were influenced by lactation, presenting the highest values in lactations III and IV. During lactation I, parameters such as fat, protein and lactose showed a content of 7.88, 4.35 and 4.71%, respectively. Furthermore, in lactation IV, fat, protein and lactose had increased, showing the content of these parameters as follows 9.53, 4.68 and 4.77%. Antioxidant capacity of buffalo milk showed the highest numbers in lactation III (360.1) and IV, 358.9 µg/ml. From all of the analyzed parameters, only total dry substance content presented the most increased values 18.9% in lactation I.

Key words: buffalo milk, antioxidant capacity, lactation, fat.

INTRODUCTION

Nowadays, more and more consumers are concerned in nutritious, good-tasting and high-quality food products containing bioactive compounds that ensure beneficially health impact (Shah et al., 2000). Generally, milk is regarded as an indispensable food product particularly for children and infants diet (Koletzko et al., 2011.) From all of the ruminant species, buffaloes are taken into consideration as second main milk producers at the global scale after cows, respectively. The significance of the Buffalo is attributed by prolonged longevity of the animals, an increased dry matter of milk, bioactive fatty acids content and development of nutritionally dairy products on markets compared with cows. (Bainbridge et al., 2016; Coroian et al., 2013; Cazacu et al., 2014; Diaconescu et al., 2002; Diaconescu et al., 2013). In addition,

buffalo milk is appearing rarely in the market squares and is a potentially valuable source of essential minerals and vitamins that have a positive effect on human health (Ahmad et al., 2013; Pasquini et al., 2018; Cazacu et al., 2014). Natural antioxidants from milk, are a valuable asset for a healthy alimentation and represents a basic concern of interest for researchers from different fields such as pharmacology, biotechnology, biochemistry, physiology and so on. Antioxidants are acting as chemical scavengers neutralizing free radicals. Reactive oxygen species (ROS) are precursors of oxidative stress and are commonly associated to induce cell damage, altering DNA, proteins, and triggering various human diseases (Lobo et al., 2010; Mann et al., 2016). Sources of antioxidants include tocopherols, polyphenols, vitamins, flavonoids, carotenoids, amino acids, fatty acids, minerals, proteins, some Maillard reaction products,

sterols, peptides and phospholipids (Carocho et al., 2016). Considering antioxidant characteristics, buffalo milk has a higher antioxidant capacity compared to cow milk, meaning that buffalo milk is rich in bioactive compounds that can benefit a healthier diet (Khan et al., 2017). In addition, milk possess two antioxidant systems, lipophilic and hydrophilic. Lipophilic antioxidant fraction also known as fat soluble, is mostly consisted of vitamins A, E, phospholipids and fatty acids (Khan et al., 2017). Both antioxidant systems perform an essential role for the human body namely supporting antioxidant and pro-oxidant homeostasis by disrupting the activity of reactive oxygen species (Grażyna et al., 2017). Also, fat-soluble antioxidants present higher thermal stability, remaining active in most of the milk products, compared to hydrophilic antioxidants. Hydrophilic antioxidant system (water soluble) is mainly comprised of minerals, trace elements, proteins, vitamins and bioactive peptides (Basilicata et al., 2018; Grażyna et al., 2017). On one hand elements from milk such as zinc (Zn), copper (Cu), selenium (Se), iron (Fe), and magnesium (Mg) play a crucial role in the development of human growth. On another hand trace elements, such as mercury (Hg), lead (Pb), cadmium (Cd) and aluminum (Al), in high concentrations can affect human wellbeing. These heavy metals, exert a serious threat due to their toxicity, bioaccumulation in food products (Babu et al., 2018). Unfortunately, ruminants that are raised especially near industrial polluted sectors can cumulate high concentrations of heavy metals through the intake of contaminated feeds (Meshref et al., 2014). Thereupon, animals are secreting those contaminants in milk, presenting a hazard for human health. (Tunegova et al., 2016; Younus et al., 2016). Heavy metals have a bioaccumulation potential, inducing harmful effects in living organisms as chronic toxicity, decreasing fetal development, damaging the DNA and so forth (Zhang et al., 2019; Govind et al., 2014). Interestingly, the absorption of Pb occurs faster in children compared to adults, accumulating mainly in soft tissues and bones (Norouzirad et al., 2018). The element as trivalent Cr (III) presents low toxicity, while hexavalent Cr (VI) is considered carcinogenic, associated with embryotoxicity

and fetotoxicity in animals. (Govind et al., 2014; Samiee et al., 2019). Moreover, the composition of milk is rich in antioxidant enzymes and non-enzymatic antioxidants. Superoxide dismutase (SOD), catalase, glutathione peroxidase (GSHPx) are antioxidative enzymes that have the potential to prevent the formation of radicals such as hydrogen peroxide, superoxide anion, and other peroxides (Lindmark-Månsson et. al., 2000). Based on these findings and to the fact that Buffalo milk has a rich source of antioxidants, proteins and other bioactive compounds that may exert beneficial effects on human health, photochemiluminescence method was applied in order to evaluate antioxidant capacity of milk samples. Therefore, the aims of this study were to evaluate the physicochemical composition and antioxidant capacity of buffalo milk influenced by lactation period.

MATERIALS AND METHODS

Milk sampling

Samples of buffalo milk were individually harvested from buffaloes according to lactation. Five samples were collected for each lactation. It was taken from a small farm in Buciumi commune, Salaj county. Samples were harvested in sterile containers and stored at 4°C until analyzes were performed (Coroian et al., 2013; Marchis et al., 2018). The buffaloes in the study are of Romanian buffalo breed and are in lactation (I-IV). The buffaloes received the same feed and had the same maintenance site.

Physico-chemical analysis

Lactoscan (Milk analyzer Lactoscan) device was used for physico-chemical analysis, a method also reported by Marchis et al. (2018). The following parameters were determined: fat, protein, lactose and total dry substance %.

Antioxidant capacity analysis

Antioxidant capacity of milk samples was done by the photochemiluminescence method, according to (Popov & Lewin, 1996) and protocol of (Photochem producer), for the measurement of lipidsoluble substances (ACL). PHOTOCHEM® instrument (Analytik Jena AG, Jena, Germany) was used to measure the

antioxidant capacity. The principle of the ACL method: free radicals are produced by irradiating a photosensitizing substance (luminol). Then they are partially removed from the sample by the chemical reactions that occur between the existing antioxidants in the sample and free radicals released by the photosensitizer. TROLOX equivalent in the case of lipid soluble and in ascorbic acid equivalent in the case of water soluble antioxidant capacity. Thus, the antioxidant capacity obtained is measured in equivalent standard units. The reagents used for this assay were the following: reagent 1 - methanol, reagent 2 - buffer solution, reagent 3-250 µl/ bottle stock solution PS-2 (photosensitizer and detection agent), and reagent 4-standard calibration solution for the quantification of lipid-soluble antioxidants, equivalent to TROLOX. The working solutions were prepared according to the following protocol: 1st reagent - methanol without dilutions, 2nd reagent - ready to use, 3rd reagent is obtained by defrosting the vial with the basic solution and adding an amount of 750 µl of 2nd reagent, 4th reagent - stock solution - is obtained by adding 500 µl of 1st reagent to 4th reagent, and 5th reagent- The previously obtained reagent is diluted with the 1st reagent in a ratio of 1: 100; 10 µl of the solution thus obtained will contain 1 nmol of standard TROLOX calibration solution. All calculations were performed automatically using a software program called PCL soft. All measurements were done in triplicate. Five samples were analyzed for the antioxidant capacity of buffalo milk for each lactation. The ANOVA (JMP 12, SAS) program for data analysis was used.

RESULTS AND DISCUSSIONS

Different versatile methods of measuring the antioxidant capacity of milk products are presented in the literature and the most mentioned are FRAP and DPPH methods. Moreover (Zulueta et al., 2009) using ORAC_{FL} assay, observed that total antioxidant capacity is especially attributed to casein. Deproteinized milk and whey showed statistically significant differences of (TAC) obtained from UHT-treated and pasteurized milk. Total antioxidant capacity values were not significantly different for UHT and pasteurized milk.

Photochemiluminescence assay used in that study demonstrated many advantages among the other techniques, due to the fact that is fully automatic, sensitive and quick. Moreover, this methodology doesn't need complicated sample preparation steps or time-consuming procedures that are required for example in the case of ORAC_{FL} assay, DPPH method or FRAP method (Sielicka et al., 2014). Photochemiluminescence method is widely used in various studies for antioxidant capacity analysis of samples such as blood, fruits, berries, various plants as well as honey, dairy products and so on (Moffarts et al., 2005; Hegedüs et al., 2011; Balogh et al., 2010; Prasad et al., 2012; Zielińska et al., 2007; Wesołowska et al., 2017). Figure 1 shows the mean values for the antioxidant capacity of buffalo milk by lactation. The lactation stimulates both the physico-chemical composition of buffalo milk and the antioxidant capacity. The antioxidant capacity in lactation 1 showed the lowest mean values of 325.6 (µg/ml). As the number of lactations increases, it can be noticed that the values for antioxidant capacity are higher. Lactation 3 and 4 show the highest values, 360.1 (µg/ml) and 358.9 (µg/ml). These values are similar to those reported in the literature: Physical and chemical parameters for buffalo milk are shown in Figure 2. Studies on the composition of buffalo milk and cow milk were in line with Hamad and Baiomy in 2010.

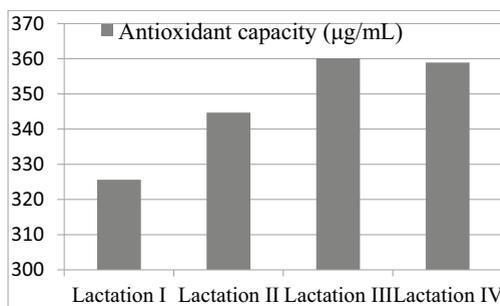


Figure 1. Antioxidant capacity (µg/ml) of buffalo milk by lactation (lactation I-IV)

Many studies have shown that milk proteins have antioxidant action, for example, peptic digestion of casein exhibited to the release of radical scavenging active peptides (Suetsuna et al., 2000; Virtanen et al., 2006). In addition,

peptides derived from whey proteins have also exhibit antioxidant activity. They can be released through fermentation of the milk and enzymatic hydrolysis (Park et al., 2007). Figure 2 shows the mean values for physicochemical profile of buffalo milk influenced by lactation. Buffalo milk fat fraction presented decreased mean values for the lactation I 7.88 (%), it can be observed as the number of lactations grows up, the means are increasing constantly presenting values for lactation II 8.52 (%), lactation III 9.02 (%) and the lactation IV comprising 9.53 (%) of fat, respectively. The same observation is attributed to the protein content, presenting low protein values in the lactation I 4.35 (%) and the highest in the last lactation IV 4.68 (%). Lactose variable presented the highest values during the lactation III with the mean values 4.8 (%) followed by lactation IV 4.77 (%), lactation I 4.71 (%) and lactation II with the lowest content of lactose 4.69 (%). Banu et al., in 1998, in the study of buffalo in our country obtained a mean value for fat of 7.80%, similar values being reported by Georgescu et al., in 2000.

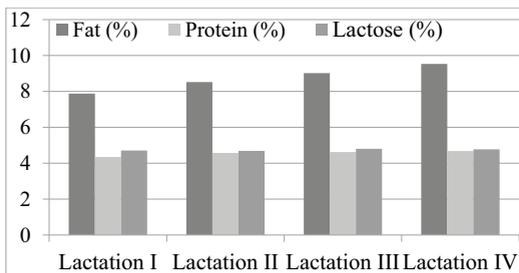


Figure 2. Physico-chemical composition of buffalo milk by lactation

Physico-chemical composition of milk is reported in various studies. The results of the present study are in line with (Smet et al., 2008) showing fat, protein and lactose content of different types of milk. Full-cream milk presented a fat content of 3.26 (%), protein 3.18 (%) and an elevated level of lactose 5.47 (%). PUFA enriched full-cream milk presented the highest fat fraction (3.73%), followed by lactose (4.64%) and protein content (3.35%). Low-fat milk, have the lowest content of fat presenting values of 1.61 (%), lactose 4.72 (%)

and having the most increased protein values 3.38 (%) compared to full-cream milk and enriched full-cream milk (Smet et al., 2008). The chemical composition of raw, pasteurized and boiled buffalo and cow milk was reported by Khan et al., 2017. The fat content of raw, pasteurized and boiled buffalo milk showed values (6.45 ± 0.16), (6.42 ± 0.08) and (6.53 ± 0.07), respectively. Cow milk had a lower content of fat comprising values (4.17 ± 0.13), (4.14 ± 0.05) and (4.21 ± 0.11). The protein concentration of raw, pasteurized and boiled of buffalo milk presented values (3.82 ± 0.14), (3.80 ± 0.05) and (3.88 ± 0.12), whereas cow samples revealed significantly lower protein content (3.22 ± 0.09), (3.19 ± 0.03) and (3.26 ± 0.02), respectively. Lactose differences among the milk samples were not so significant depending on the thermal treatment applied. Presenting values for cow milk as follows (4.54 ± 0.19), (4.52 ± 0.23) and (4.61 ± 0.17), whereas buffalo milk showed (4.85 ± 0.26), (4.87 ± 0.12) and (4.94 ± 0.25), respectively. Our results regarding proteins, fat, lactose and total dry substances are in concordance with Khan et al. (2017) presenting similarities of measurements with those from our study. In addition, the same method was used for physicochemical determination of samples (Lactoscan). Figure 3 indicates the total dry substance from buffalo milk samples according to lactation, revealing the most elevated dry content during the lactation I 18.9 (%), it can be noticed that over lactation II this variable is decreasing presenting the lowest values 18.55 (%). Furthermore, during the progression of lactation III and IV, the content of this parameter is increasing again presenting values of 18.64 (%) and 18.72 (%), respectively.

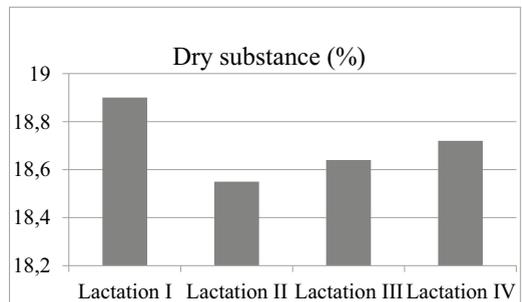


Figure 3. Dry substance level according to different stage of lactation

According to Khan et al. (2017) findings, total dry substances from cow and buffalo raw, pasteurized and boiled milk presented an average means of (12.7 ± 0.34) , (12.6 ± 0.28) and (12.9 ± 0.30) (%) instead, the buffalo milk demonstrates the significantly higher values (16.21 ± 0.43) , (16.05 ± 0.24) and (16.29 ± 0.18) (%) compared to cow milk.

Interestingly, according to Khan et al. (2017) remarked a suggestion that pasteurized milk preferentially may be consumed within three days for better antioxidant activity effects, also according to their results buffalo milk has a higher antioxidant capacity than cow milk. Animal diet is influencing directly antioxidative properties of milk, increasing compounds as α -Tocopherol and β -Carotene.

Grass-clover silage fed animals excreted milk with higher concentrations of α -Tocopherol and β -Carotene, comprising values of (472 ± 33) g/l and (440 ± 23) g/l whereas cows that received hay roughage diet had a lower means of these antioxidants (504 ± 48) and a decrease (from 445 to 264) g/l, respectively (Havemose et al., 2006).

The fatty acid content of milk is well-known to impact oxidative balance stability (Barrefors et al., 1995; Havemose et al., 2004, 2006). In addition, fatty acids unsaturation degree contributes to lipid hydroperoxides cumulation (Havemose et al., 2006). Unfortunately, goats infected with mastitis demonstrated decreased values of antioxidative properties of milk. Using FRAP method (Silanikove et al., 2014) reported lower values of antioxidant properties in contaminated goat milk (305 ± 36) μ M compared to uninfected samples with significantly increased values (427 ± 35) μ M. Moreover, subclinical mastitis affects antioxidant/oxidant balance of milk, reducing the total antioxidant capacity (TAC), presenting values of (mmol Trolox Equv./l) 0.54 ± 0.051 for healthy cows and 0.42 ± 0.047 for mastitis infected, as well as (TOC) total oxidant capacity (Imol H₂O₂ Equv./l) 15.91 ± 0.57 for healthy animals and 20.88 ± 0.90 for infected (Atakisi et al., 2010).

Interestingly, that administration of selenium in the nutrition of dairy cows significantly increases catalase activity (CAT) and (TAC), compared with inorganic selenium supplementation (Gong et al., 2014). Furthermore, the

mentioned mineral is elevating selenium levels in the blood and milk of the animals. Additionally, vitamin A administrated at the doses of 220 IU/kg of BW to the cows, can significantly raise the total antioxidant capacity and hydroxyl radical inhibition capability enhancing milk production performance (Jin et al., 2014).

CONCLUSIONS

The buffalo milk has a high fat content in all four lactations. This is influenced by the number of lactations and shows the highest values in lactation IV.

The buffalo milk is a food beneficial to the human body due to its high level of fat, protein, lactose and antioxidant capacity. Antioxidant capacity has the highest content in lactation III and IV.

ACKNOWLEDGEMENTS

This project is funded by the Ministry of Research and Innovation through Program 1-Development of the National Research and Development System, Subprogram 1.2-Institutional Performance-Projects for Financing the Excellence in CDI, Contract no. 37PFE/06.11.2018. Title of the project: "Increasing the institutional performance through consolidation and development of research directions within the USAMVCN".

REFERENCES

- Ahmad, S. (2013). Buffalo Milk, In *Milk and Dairy Products in Human Nutrition, Production, Composition and Health*. In Park Y.W. and Haenlein G.F.W. editors.
- Analytik Jena AG manual (2005). *Determination of the Lipid Soluble Antioxidative Capacity (ACL) of Heating Oil*. Leipzig, Germany.
- Atakisi, O., Oral, H., Atakisi, E., Merhan, O., Metin Pancarci, S., Ozcan, A., Marasli, S., Polat, B., Colak, A., Kaya, S. (2010). Subclinical mastitis causes alterations in nitric oxide, total oxidant and antioxidant capacity in cow milk. *Research in Veterinary Science*, 89(1), 10–13.
- Babu, A. J., Supriya, R. A., Swetha, C. S., Suganya, G., Vijayabhaskar, R. G., Sasikala, V., Surendra, R., Yeshwanth Sreenivas, K. (2018). Estimation of chromium, copper and lead in milk by inductively coupled plasma-optical emission spectrometry in Tirupati, Andhra Pradesh. *Pharma Innovation Journal*, 7(8), 90–93.

- Banu, C., Vizireanu, C. (1998). *Procesarea industrială a laptelui*. Editura Tehnică București.
- Bainbridge, M. L., Cersosimo, L. M., Wright, A. D. G., Kraft, J. (2016). Content and Composition of Branched-Chain Fatty Acids in Bovine Milk Are Affected by Lactation Stage and Breed of Dairy Cow. *PLoS ONE*, 11(3), pp.??
- Balogh, E., Hegedus, A., Banyai, E. S. (2010). Application of and correlation among antioxidant and antiradical assays for characterizing antioxidant capacity of berries. *Scienia Horticulturae*, 125(3), 332–336.
- Basilicata, M. G., Pepe, G., Adesso, S., Ostacolo, C., Sala, M., Sommella, E., Scala, M. C., Messore, A., Autore, G., Marzocco, S., Campiglia, P. (2018). Antioxidant Properties of Buffalo-Milk Dairy Products: A β -Lg Peptide Released after Gastrointestinal Digestion of Buffalo Ricotta Cheese Reduces Oxidative Stress in Intestinal Epithelial Cells. *Int. J. Mol. Sci.*, 19, 1955.
- Carocho, M., Morales, P., Ferreira, I. C. F. R. (2018). Antioxidants: Reviewing the chemistry, food applications, legislation and role as preservatives. *Trends in Food Science & Technology*, 71, 107–120.
- Cazacu, S., Rotsios, K., Moshonas, G. (2014). Consumers' Purchase Intentions towards Water Buffalo Milk Products (WBMPs) in the Greater Area of Thessaloniki, Greece. *Procedia Economics and Finance*, 9, 407–416.
- Coroian, A., Erler, S., Matea, C. T., Miresan, V., Raducu, C., Bele, C., Coroian, C. O. (2013). Seasonal changes of buffalo colostrum: physicochemical parameters, fatty acids and cholesterol variation. *Chemistry Central Journal*, 7(40), pp.??
- Diaconescu, A., Săndulescu, D., Nicolae, C. (2002). Metode fizico-chimice moderne folosite în analiza laptelui de vacă. *Lucrări științifice. Seria D, XLV, Seria Zootehnie*, București, 93–95.
- Diaconescu, S., Nicolae, C. (2013). *Tehnologia creșterii animalelor - ediție reeditată*. Centrul poligrafic USAMV București.
- EFSA (2009). Cadmium in food. *Scientific opinion of the Panel on Contaminants in the Food Chain*.
- Georgescu, G. (2000). *Laptele și produsele lactate*. Editura Ceres, București.
- Gond, J., Ni, L., Wang, D., Shi, B., Yan, S. (2014). Effect of dietary organic selenium on milk selenium concentration and antioxidant and immune status in midlactation dairy cows. *Livestock Science*, 170, 84–90.
- Govind, P., Madhuri, S. (2014). Heavy Metals Causing Toxicity in Animals and Fishes. *Research Journal of Animal, Veterinary and Fishery Sciences*, 2(2), 17–23.
- Grazyna, C., Hanna, C., Adam, A., Magdalena, B. M. (2017). Natural antioxidants in milk and dairy products. *Int J Dairy Technol.*, 70, 165–178.
- Havemose, M. S., Weisbjerg, M. R., Bredie, W. L. P., Poulsen, H. D., Nielsen, J. H. (2006). Oxidative Stability of Milk Influenced by Fatty Acids, Antioxidants, and Copper Derived from Feed. *J. Dairy Sci.*, 89, 1970–1980.
- Hegedüs, A., Pfeiffer, P., Papp, N., Abranko, L., Blazovics, A., Pedryc, A., Banyai, E. S. (2011). Accumulation of Antioxidants in Apricot Fruit through Ripening: Characterization of a Genotype with Enhanced Functional Properties. *Biol. Res.*, 44(4), 339–344.
- Jin, L., Yan, S., Shi, B., Bao, H., Gong, J., Guo, X., Li, J. (2014). Effects of vitamin A on the milk performance, antioxidant functions and immune functions of dairy cows. *Animal Feed Science and Technology*, 192, 15–23.
- Khan, I. T., Nadeem, M., Imran, M., Ayaz, M., Ajmal, M., Ellahi, M. Y., Khalique, A. (2017). Antioxidant capacity and fatty acids characterization of heat treated cow and buffalo milk. *Lipids in Health and Disease*, 16, 163.
- Koletzko, B., Agostoni, C., Bergmann, R., Ritzenthaler, K., Shamir, R. (2011). Physiological aspects of human milk lipids and implications for infant feeding: a workshop report. *Acta Paediatrica*, 100, 1405–1415.
- Lindmark-Månsson, H., Åkesson, B. (2000). Antioxidative factors in milk. *British Journal of Nutrition*, 84(1), 103–110.
- Lobo, V., Patil, A., Phatak, A., Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev.*, 4(8), 118–126.
- Mann, S., Shandilya, U. K., Sodhi, M., Kumar, P., Bharti, V. K., Verma, P., Sharma, A., Mohanty, A., Mukesh, M. (2016). Determination of Antioxidant Capacity and Free Radical Scavenging Activity of Milk from Native Cows (*Bos indicus*), Exotic Cows (*Bos taurus*), and Riverine Buffaloes (*Bubalus bubalis*) Across Different Lactation Stages. *Int J. Dairy Sci. Process*. 3(4), 66–70.
- Marchis, Z., Odagiu, A., Coroian, A., Oroian, I., Mirza, M., Burduhos, P. (2018). Analysis of Environmental Factors' Impact on Donkeys' Colostrum Quality. *Sustainability*, 10(9), 2958.
- Meshref, A. M. S., Moselhy, W. A., Hassan, N. E. H. (2014). Heavy metals and trace elements levels in milk and milk products. *Journal of Food Measurement and Characterization*, 8, 381–388.
- Moffarts, B., Kirschvink, N., Art, T., Pincemail, J., Lekeux, P. (2005). Effect of oral antioxidant supplementation on blood antioxidant status in trained thoroughbred horses. *Veterinary Journal*, 169(1), 65–74.
- Norouzirad, R., Gonzalez-Montaña, J. R., Martinez-Pastor, F., Hosseini, H., Shahrouzian, A., Khabazkhoob, M., Ali Malayeri, F., Moallem Bandani, H., Paknejad, M., Foroughi-Nia, B., Fooladi Moghaddam, A. (2018). Lead and cadmium levels in raw bovine milk and dietary risk assessment in areas near petroleum extraction industries. *Science of Total Environment*, 635, 308–314.
- Park, Y. W., Juarez, M., Ramos, M., Haenlein, G. F. W. (2007). Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research*, 68, 88–113.
- Pasquini, M., Osimani, A., Tavoletti, S., Moreno, I., Clementi, F., Trombetta, M. F. (2018). Trends in the quality and hygiene parameters of bulk Italian

- Mediterranean buffalo (*Bubalus bubalis*) milk: A three year study. *Anim. Sci J.*, 89, 176–185.
- Popov, I. N., Lewin, G. (1996). Photochemiluminescent detection of antiradical activity; IV: Testing of lipid soluble antioxidant. *J. Biochem. Biophys. Methods*, 31, 1–8.
- Prasad, R., Kamal, S., Sharma, P. K., Oelmüller, R., Varma, A. (2013). Root endophyte *Piriformospora indica* DSM 11827 alters plant morphology, enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*. *J. Basic Microbiol.*, 53, 1016–1024.
- Samiee, F., Vahidinia, A., Javad, M.T., Leili, M. (2019). Exposure to heavy metals released to the environment through breastfeeding: A probabilistic risk estimation. *Science of Total Environment*, 650(2), 3075–3083.
- Shah, N. (2000). Effects of milk-derived bioactives: An overview. *British Journal of Nutrition*, 84(S1), 3–10.
- Sielicka, M., Malecka, M., Purlan, M. (2013). Comparison of the antioxidant capacity of lipid soluble compounds in selected cold pressed oils using photochemiluminescence assay (PCL) and DPPH method. *Eur. J. Lipid Sci. Technol.*, 116, 388–394.
- Silanikove, N., Merin, U., Shapiro, F., Leitner, G. (2014). Subclinical mastitis in goats is associated with upregulation of nitric oxide-derived oxidative stress that causes reduction of milk antioxidative properties and impairment of its quality. *Journal of Dairy Science*, 97(6), 3449–3455.
- Smet, K., Raes, K., Block, J. D., Herman, L., Dewettinck, K., Coudijzer, K. (2018). A change in antioxidative capacity as a measure of onset to oxidation in pasteurized milk. *International Dairy Journal*, 18(5), 520–530.
- Suetsuna, K., Ukeda, H., Ochi, H. (2000). Isolation and characterization of free radical scavenging activities peptides derived from casein. *J. Nutr. Biochem.*, 11, 128–131.
- Tunegova, M., Toman, R., Tancin, V. (2016). Heavy metals - environmental contaminants and their occurrence in different types of milk. *Slovak J. Anim. Sci.*, 49(3), 122–131.
- Virtanen, T., Pihlanto, A., Akkanen, S., Korhonen, H. (2006). Development of antioxidant activity in milk whey during fermentation with lactic acid bacteria. *Journal of Applied Microbiology*, 102, 106–115.
- Wesołowska, M., Dżugan, M. (2017). The Use of the PHOTOCHEM Device in Evaluation of Antioxidant Activity of Polish Honey. *Food Analytical methods*, 10(5), 1568–1574.
- Younus, M., Abbas, T., Zafar, M., Raza, S., Khan, A., Saleem, A. H., Idrees, M. A., Nisa, Q. U., Akhtar, R., Saleem, G. (2016). Assessment of heavy metal contamination in raw milk for human consumption. *South African Journal of Animal Science*, 46(2), pp. ??
- Zhang, T., Ruan, J., Zhang, B., Lu, S., Gao, C., Huang, L., Bai, X., Xie, L., Gui, M., Qiu, R. (2019). Heavy metals in human urine, foods and drinking water from an e-waste dismantling area: Identification of exposure sources and metal-induced health risk. *Ecotoxicology and Environmental Safety*, 169, 707–713.
- Zielinska, D., Szawara-Nowak, D., Michalska, A. (2007). Antioxidant capacity of thermally-treated buckwheat. *Polish Journal of Food and Nutrition Sciences*, 57(4), pp.??
- Zulueta, A., Maurizi, A., Frigola, A., Esteve, M. J., Coli, R., Burini, G. (2009). Antioxidant capacity of cow milk, whey and deproteinized milk. *International Dairy Journal*, 19(6), 380–385.