Journal of Elementology ISSN 1644-2296

Wysocka D., Snarska A., Sobiech P. 2020.
Iron in cattle health.
J. Elem., 25(3): 1175-1185. DOI: 10.5601/jelem.2020.25.2.1960

RECEIVED: 22 January 2020
ACCEPTED: 23 June 2020

REVIEW PAPER

IRON IN CATTLE HEALTH

Dominika Wysocka, Anna Snarska, Przemysław Sobiech

Department and Clinic of Internal Diseases
University of Warmia and Mazury in Olsztyn, Poland

Abstract

Iron is one of the most abundant elements on Earth, a vital component of every living organism, and an essential micronutrient for all animal species. The primary source of iron for calves and other new-born ruminants is milk or a milk replacer, while for adults it is forage. Drinking water can contain various amounts of Fe ions and can be a source of this element as well. Systemic iron homeostasis in vertebrates is mostly regulated by hepcidin (HEP), a peptide synthesized and secreted by hepatocytes. The largest amounts of iron in the body are incorporated into proteins, mainly haemoglobin and myoglobin. This element participates in several biochemical processes including blood production, transport of oxygen, energy metabolism and immune processes. Iron deficiency can result in so-called iron deficiency anaemia, mostly occurring in young calves due to low iron content in cow's milk and high demand for this element by calf's organism. Iron poisoning mostly occurs due to excess iron intake caused by an inappropriate dose of iron given to animals (injection or *per os* administration) or accidental consumption of high doses of supplements. Excess iron intake can cause overload and damage of internal organs. Oxidation of ferrous iron in haemoglobin can result in methemoglobinemia and an inability of erythrocytes to carry oxygen. In recent years, scientists have implicated some questions regarding iron and ruminants that need re-evaluation or further investigation, e.g. the upper tolerable concentration of Fe in drinking water, the relationship between inflammation and iron status, serum Fe as an indicator of inflammation, or ferritin as a predictive factor for iron deficiency anaemia in cattle.

Keywords: cattle, iron, trace element homeostasis.

Anna Snarska, PhD DSc, Department and Clinic of Internal Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland, phone: +48 89 523 32 94, e-mail: anna.snarska@uwm.edu.pl

INTRODUCTION

Microelements, also known as trace elements, trace minerals or micronutrients, are defined as chemical elements required only in small amounts (less than 100 mg kg^{-1} of dry matter) by living organisms (Yatoo et al. 2013). They are essential to ensure the physiological course of various biochemical processes, to maintain human and animal health, and to support an animal's growth and productivity. Fifteen elements meet the above criteria: arsenic (As), chromium(Cr), copper (Cu), cobalt (Co), fluoride (F), iron (Fe), iodine (I), manganese (Mn), molybdenum (Mo), nickel (Ni) selenium (Se), silicon (Si), tin (Sn), vanadium (V) and zinc (Zn) (Radwińska and Żarczyńska 2014). Iron, assigned the symbol Fe in the periodic table of elements, with the atomic number of 26 and atomic weight of 55.847, is not only one of the most abundant elements on Earth (it is the fourth most common element in the Earth's crust), but also a vital component of every living organism, and an essential nutrient for all animal species (Lieu et al. 2001).

This element participates in several biochemical processes: blood production, transport of oxygen, energy metabolism, immune processes, and many others. Despite its crucial role in living organism, the excessive iron intake has harmful effects and induce poisoning symptoms.

The aim of this article is to review current knowledge on the roles and metabolism of iron in ruminants, consequences of its deficiency and poisoning in cattle, and current trends regarding research on this element.

Iron sources

Sufficient iron content in a diet is necessary for the physiological levels of red blood cells (RBC) and haemoglobin (Hb) – Mohri et al. (2010). The iron requirements for cattle were first established by the NRC in 1971 (Weiss 2017). Age, sex, growth rate and bioavailability of dietary iron source are factors affecting cattle's Fe demand (Heidarpour Bami et al. 2008, Prodanovic et al. 2014). Iron requirements for calves are estimated to be around 100 ppm and it is generally believed that young cattle need more iron than mature animals (Volker, Rotermund 2000).

The primary source of iron for calves and other new-born ruminants is milk or a milk replacer. Since dairy milk contains rather low amounts of iron, calves are susceptible to the occurrence of iron deficiency.

The basic and the most important source of minerals for adult ruminants is forage. Several factors related to the soil and plants determine mineral composition of forages. The most important ones are the mineral composition of soil, bioavailability of each element, soil pH, moisture, plant species, and stage of maturity (Gupta et al. 2008, Marijanušić et al. 2017). According to Marijanušić et al. (2017), iron deficiency in soils has become an unsolved global problem in recent years. However, forages used in ruminant diet are usually rich in Fe, for example alfalfa contains 300 mg kg^{-1} of DM, corn gluten feed – 400 mg kg^{-1} of DM, dried distillers grains with solubles – 600 mg kg^{-1} of DM, soyhulls – 600 mg kg^{-1} of DM (Kerr et al. 2008).

Drinking water can contain various amounts of Fe ions, mainly in the form of ferrous ion (Fe^{2+}). Genther and Beede (2013) point to D. K. Beede's personal observation that drinking water may negatively affect the cow's health and milk production when Fe concentrations are equal or greater than 2 mg l^{-1}. It may be related to the changes in Fe or antioxidant status and oxidative stress, or reduced water intake resulting from reduction in water palatability. The current recommendations for Fe concentration in drinking water for cattle (up to 0.3 mg l^{-1}) are based on guidelines for human palatability (WHO 2006, Genther, Beede 2013). However, the upper tolerable concentration of Fe in drinking water for cattle are suggested to be re-evaluated due to the observation that total recoverable Fe concentration of 4 mg l^{-1} did not negatively affect the drinking water preference of lactating dairy cows in comparison to water containing no supplemental Fe (Genther, Beede 2013). Re-evaluation of the parameters of drinking water for cows (mostly regarding Fe) is undoubtedly interesting from scientific point of view. In practice, such re-evaluation seems to make sense only in cases of really bad quality of water or when only water rich in Fe is available to livestock (when the dose of iron in water would negatively affect drinking water preference of lactating dairy cows), since water cannot be treated as a primary iron source for cattle.

Iron status in ruminant's organism

The iron homeostasis in the body is complex and could be divided into intake, transport, utilization, storage and excretion.

Normally, iron is poorly absorbed from most diets (5%-15%), but its absorption can double in states of iron deficiency. The amount of dietary iron that is absorbed from the gastrointestinal tract is usually determined by individual factors (animal's age, iron status, and health) and is inversely related to serum ferritin concentrations. Other factors that influence iron absorption include the environment of the gastrointestinal tract, chemical form and amount of iron, other components of the diet which can enhance or reduce intestinal Fe absorption. Two proteins present in the duodenum play an important role in modulation of Fe absorption, i.e. the cellular Fe importer, which is divalent metal transporter 1 (DMT1), and the cellular Fe exporter, that is ferroportin (FPN) – Hansen et al. (2010).

Once absorbed, the body retains iron unless bleeding occurs. The largest amount of iron in the body is incorporated into proteins, mainly haemoglobin and myoglobin. Serum iron is primarily bound to transferrin and secondarily to ferritin. Knowles et al. (2000) and Egli and Blum (1998) stated that serum iron levels in calves were lower than in adult cows. Such differences might be associated with the fact that growing calves have large demand for iron due to their growth, increasing body weight, blood production (haemoglobin and erythrocyte production), while obtaining an insufficient amount of iron with feed (whole milk or a milk replacer without additional iron).

Milk iron is bound to lactoferrin. Apart from previously mentioned proteins, iron in the body is also bound to hemosiderin (up to 35% of iron) and ferritin (up to 20% of iron), mainly concentrated in the bone marrow, liver and spleen.

Systemic iron homeostasis in vertebrates is mostly regulated by hepcidin (HEP), a peptide, which freely circulates in the blood, weakly binds to albumins and α2-macroglobulin and is filtrated in kidneys (Peslova et al. 2009, Itkonen et al. 2012). The process of iron release from cells is regulated by HEP, by its interaction with a transmembrane protein called ferroportin. Hepcidin is synthesized and secreted by hepatocytes, but it can also be found in other tissues and organs, e.g. lungs, kidneys, skeletal muscles (Michels et al. 2015, Basbugan et al. 2019). Its production depends on the amount of iron in the body, namely it increases when iron storage in the body is elevated or sufficient, but decreases when iron storage in the body is low (e.g. during, hypoxia and anaemia). In the last two years, studies on hepcidin in cattle have opened some new research perspectives. Basbugan et al. (2019) studied the relation between hematologic parameters and hepcidin levels in cattle with theileriosis and concluded that the determination of hepcidin levels in cattle with theileriosis may be useful in a diagnosis, treatment decisions and prognosis. Rajamanickam et al. (2020) in their study on the influence of hepcidin on iron homeostasis during the last trimester of gestation in *Bos indicus* stated that that an increase in hepcidin reduces during pregnancy the maternal serum iron, as well as being a biomarker for iron bioavailability to the developing foetus. These matters demand more in-depth research and a growing interest in the research on hepcidin in cattle can be expected.

Lactoferrin is a multifunctional protein, which was first isolated in 1939 from bovine milk (Sorensen, Sorensen 1940). Lactoferrin is capable of binding to two ferric ions (Fe^{3+}) together with two CO_3^{2-} anions, creating very close but reversible bonds. The ferric ion binds to lactoferrin at basic pH and dissociates at acidic pH (Rastogi et al. 2016) Iron saturation determines the form of lactoferrin: apolactoferrin is iron-free, a monoferric form contains one ferric ion, and hololactoferrin contains two ions (Steijns, van Hooijdonk 2000).

The body treats Fe very frugally. Hem from nonviable erythrocytes is broken down in the liver, spleen and bone marrow, and the iron released in this process is recycled for further use.

Functions of iron in the ruminant's organism – the role of iron in inflammatory response

Inflammation is a complex, acute or chronic response to injurious factors affecting cells or tissues (Baydar, Dabak 2014). Despite the fact that most frequently used inflammation indicators in cattle are WBC (white blood cell count) and APPs (acute phase proteins), e.g. alpha (1)-acid glycoprotein, haptoglobin (Hp), lipopolysaccharide binding protein, serum amyloid A (SAA), and transferrin, some researchers have demonstrated that the serum Fe concentration could also be used as an indicator of acute inflammation in humans and some animal species, including cats, dogs and horses (Neumann 2003, Borges et al. 2007, Ceciliani et al. 2012, Idoate et. al. 2015). This issue in cattle requires more in-depth research but might bring some valuable contribution to veterinary practice.

Many microbials, when attacking the host, have a high demand for Fe, which is essential for bacterial virulence and replication (Ma et al. 2015). In response, the host lowers the serum Fe concentration in order to make this element unavailable to the invading microorganisms. Baydar and Dabak (2014) concluded that determination of the serum Fe concentration in conjunction with other parameters, i.e. WBC and APPs, could be a useful diagnostic measure for acute inflammation in cattle and the assessment of a herd's health, since the serum Fe concentration may reflect the body's inflammatory response, for instance it decreases in acute traumatic reticuloperitonitis and mastitis (Erskine et al. 1993, Baydar, Dabak 2014). According to Tsukano et. al. (2019), a decrease in the serum Fe concentration may also be a useful inflammation marker, especially in cases of non-infectious inflammation, e.g. in young cattle after dehorning. Even though the serum Fe concentration is a cheap and easily performed test, further investigation into the relationship between inflammation and iron status, in particular the expression of hepcidin, responsible for Fe homeostasis, is needed.

Functions of iron in the ruminant's organism – the role of iron in oxidative stress

Some metals demonstrating toxic effects share one common feature, that is they can induce oxidative stress. Iron alongside copper participates in the Fenton reaction, which is the production of hydroxyl radicals from hydrogen peroxide, and in other reactions leading to the formation of other reactive oxygen species (ROS). Accumulation of ROS can contribute to substantial damage of mammalian tissues.

During increased metabolic demands, e.g. during the transition period between gestation and lactation in dairy cows, there is a considerable increase in oxygen requirements, which results in enhanced ROS production. Dietary changes during the transition period and infectious diseases can facilitate the release of Fe for the ROS-generating Fenton reaction. Despite the fact that free Fe is effectively sequestered by various metal-binding proteins under physiological conditions (Kakhlon, Cabantchik 2002), an imbalance between increased production of ROS and the availability of antioxidant defences needed to reduce ROS accumulation during the periparturient period may expose dairy cows to increased oxidative stress (Sordillo, Aitken 2009). There is an established relationship between the loss in antioxidant potential and the physiological changes associated with parturition in dairy cows (Bernabucci et al. 2005, Castillo et al. 2005).

Transition cows are not the only ones susceptible to the negative consequences of oxidative stress. Rajabian et al. (2017) in their study on the relationship between oxidative stress, haematology and iron profile in anaemic and non-anaemic calves concluded that iron insufficiency could be linked to the impairment of antioxidant defence systems in the neonatal calves, and defective antioxidant capacity may induce oxidative damage of their erythrocytes.

Iron deficiency anaemia

Calves in the early stages of life are most susceptible to iron deficiency because dairy milk contains quite small amounts of iron. However, neonates do have some iron reserves in their body. Atyaby et al. (2006) reported that new-born calves before drinking the colostrum had similar blood iron levels as their mothers. Those levels subsequently began to drop after 24 hours. Since iron is necessary for bacterial development, low iron content in milk could be considered as a protective mechanism against bacterial infections in calves (Messenger, Barclay 1983), nevertheless iron deficiency is associated with increased infection rates, reduced growth, and loss of appetite (Mohri et al. 2010).

Iron deficiency anaemia cannot be solely associated with the low iron content in bovine milk. New-born animals are particularly susceptible to the occurrence of anaemia, but nutritional causes of iron deficiency anaemia occur more often in humans. Other factors that contribute to iron deficiency are bleeding gastrointestinal ulcers (causing chronic blood loss), infestation with bloodsucking parasites, haemorrhagic diseases, and malnutrition (Ramin et al. 2012). According to the study by Waldner and Blakley (2014), calves from heifers had lower liver iron concentrations (105 ppm) than calves from older cows (165 ppm).

Iron reservoirs deplete in three stages in the development of iron deficiency. At the beginning, the serum iron remains constant, while the iron storage in the bone marrow, liver and spleen decreases in parallel to a decrease in the serum ferritin. Then, lowering of the serum iron level occurs. The last stage is the development of anaemia (typically, microcytotic and hypochromic anaemia). A decreasing trend in values of haemoglobin (HGB) and haematocrit (HCT) due to iron deficiency has also been reported in neonatal calves during the first weeks of life (Kurtoglu et al. 2003, Mohri et al. 2007).

In differentiational diagnosis, deficiencies of copper and selenium should be taken into consideration (Herdt, Hoff 2011).

Traditionally, iron deficiency anaemia in cattle is diagnosed based on RBC, HGB, HCT and serum iron levels. Recent preliminary study by Joerling and Doll (2019) point out that the serum ferritin may be a useful diagnostic tool in the diagnosis of iron deficiency anaemia in calves. However, this issue in cattle needs additional trials and validation of reference values since the serum ferritin may be increased due to mastitis and metritis (Furugouri et al. 1982).

The most common way to treat and/or prevent iron deficiency anaemia is iron supplementation, e.g. by supplying a milk replacer with higher doses of iron to young calves, feed additives with higher iron content to adults, and injectable iron. However, since too much iron can cause poisoning, any supplementation should be cautious and monitored.

Iron toxicity

Iron poisoning mostly occurs due to excess iron intake caused by an inappropriate dose of iron given to animals (injection or *per os* administration) or accidental consumption of high doses of supplements. The chemical form of iron affects its absorption and can play an important role in the development of iron poisoning. Water soluble iron (ferrous compounds, i.e. ferrous chloride, ferrous sulphide, ferrous carbonate) are more bioavailable than ferric ones.

Acute iron poisoning occurs in calves after administration of a high dose of iron during the first days of life. This can manifest by sudden death shortly after an iron injection or severe depression and coma preceding death.

According to Jenkins and Hidiroglou (1987), a dose of 2000 ppm of iron in the form of sulphate fed in a milk replacer for 6 weeks did not cause any signs of chronic iron poisoning in calves without the fully developed rumen. A dose of 5000 ppm of iron caused signs of chronic iron poisoning, such as reduced weight gains, dry matter intake and digestibility of dry matter and protein. Thompson et al. (1991) addressed the issue of chronic iron poisoning in adult cattle and concluded that subsequent administration of ferric iron at doses of 15, 30 and 60 g per day for 10 days could cause a decrease in bodyweight, milk production and milk fat.

Excess iron intake affects the cow's health mainly due to the interference with the absorption of other minerals and the formation of an oxidation environment (Hansen et al. 2010). When the dose of iron is excessive, the binding capacity of transferrin, the protein that carries iron in the serum, is exceeded and the serum iron concentration increases rapidly.

Excessive iron in a cow's diet can lead to decreased immunity and increased cases of bacterial infections, mastitis and retention of foetal membranes. Even though negative changes in the health status of a dairy cow immediately depresses milk production and alter milk composition, the effect of iron-induced oxidative stress on milk protein synthesis in lactating dairy cows has not been fully studied (Wang et al. 2016). Excess dietary Fe can also negatively affect the intestinal environment by causing damage to the intestinal epithelium in calves. Hansen et al. (2010) hypothesized that the mechanism of epithelium injury induced by high levels of Fe could be due to oxidative damage and subsequent lipid peroxidation, but the exact mechanism remains unknown.

Many studies conducted on laboratory animals demonstrate that excessive iron accumulation in the brain and disturbances in iron homeostasis play an important role in neurodegenerative diseases. Even though there are many neurodegenerations in cattle, they are often not diagnosed and are not such an important matter in ruminants as they are in humans (Zecca et al. 2004, Ward et al. 2014).

CONCLUSIONS

To conclude, iron is essential for cows. Since dairy milk contains quite small amounts of iron and forages used in adult ruminant diet are usually rich in Fe, calves are more susceptible to the occurrence of iron deficiency anaemia than mature cows. In recent years, scientists have implicated several issues regarding iron and ruminants that need further investigation or re-evaluation, e.g. the upper tolerable concentration of Fe in drinking water for cattle, the relationship between inflammation and iron status (hepcidin expression), serum Fe as an indicator of inflammation or ferritin as a predictive factor for iron deficiency anaemia in cattle.

REFERENCES

Atyabi N., Gharagozloo F., Nassiri S. M. 2006. *The necessity of iron supplementation for normal development of commercially reared suckling calves.* Comp. Clin. Pathol., 15: 165-168. DOI: 10.1007/s00580-006-0624-4

Basbugan Y., Yuksek N., Kilinc O. O. 2019. *Relation between hepcidin levels and hematologic parameters in cattle with theileriosis.* Med. Wet., 75(6):355-359. DOI: dx.doi.org/10.21521//mw.6205

Baydar E., Dabak M. 2014. *Serum iron as an indicator of acute inflammation in cattle.* J. Dairy Sci., 97: 222-228. DOI: 10.3168/jds.2013-6939

Bernabucci U., Ronchi B., Lacetera N., Nardone A. 2005. *Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows.* J. Dairy Sci., 88: 2017-2026.

Borges A.S., Divers T.J., Stokol T., Mohammed O.H. 2007. *Serum iron and plasma fibrinogen concentrations as indicators of systemic inflammatory diseases in horses.* J. Vet. Intern. Med., 21(3): 489-494. DOI: 10.1892/0891-6640(2007)21[489:siapfc]2.0.co;2

Castillo C., Hernandez J., Bravo A., Lopez-Alonso M., Pereira V., Benedito J.L. 2005. *Oxidative status during late pregnancy and early lactation in dairy cows.* Vet. J., 169: 286-292.

CECILIANI F., CERON J. J., ECKERSALL P. D., SAUERWEIN H. 2012. *Acute phase proteins in ruminants*. J. Proteomics, 75: 4207-4231. DOI: 10.1016/j.jprot.2012.04.004

EGLI C.P., BLUM J.W. 1998. *Clinical, haematological, metabolic and endocrine traits during the first three months of life of suckling Simmental calves held in a cow-calf operation.* Zentralbl Veterinarmed A, 45: 99-118. DOI: 10.1111/j.1439-0442.1998.tb00806.x

ERSKINE R. J., BARTLETT P. C. 1993. *Serum concentrations of copper, iron, and zinc during Escherichia coli-induced mastitis*. J. Dairy Sci., 76(2): 408-413. DOI: 10.3168/jds.S0022--0302(93)77360-9

FURUGOURI K., MIYATA Y., SHIJIMAYA K. 1982. *Ferritin in blood serum of dairy cows*. J. Dairy Sci., 65(8): 1529-1534. DOI: 10.3168/jds.S0022-0302(82)82377-1

GENTHER O.N., BEEDE D.K. 2013. *Preference and drinking behavior of lactating dairy cows offered water with different concentrations, valences, and sources of iron*. J. Dairy Sci., 96: 1164-1176. DOI: 10.3168/jds.2012-5877

GUPTA U.C., KENING W.U., SIYUAN L. 2008. *Micronutrients in soils, crops, and livestock*. Earth Sci. Front., 15(5): 110-125. DOI: 10.1016/S1872-5791(09)60003-8

HANSEN S. L., ASHWELL M. S., MOESER A. J., FRY R. S., KNUTSON M. D., SPEARS J. W. 2010. *High dietary iron reduces transporters involved in iron and manganese metabolism and increases intestinal permeability in calves*. J. Dairy Sci., 93(2): 656-665. DOI: 10.3168/jds.2009-2341.

HEIDAPOUR BAMI M. H., MOHRI M., SEIFI H. A., TABATABAEE A.A. 2008. *Effects of parenteral supply of iron and copper on hematology, weight gain, and health in neonatal dairy calves.* Vet. Res. Commun., 32(7): 553-556. DOI: 10.1007/s11259-008-9058-6

HERDT T.H., HOFF B. 2011. *The use of blood analysis to evaluate trace mineral status in ruminant livestock*. Vet. Clin. North Am. Food Anim. Pract., 27(2): 255-283. DOI: 10.1016/j.cvfa.2011.02.004

IDOATE I., VANDER LEY B., *Schultz* L., HELLER M. 2015. *Acute phase proteins in naturally occurring respiratory disease of feedlot cattle.* Vet. Immunol. Immunopathol., 163(3-4): 221-226. DOI: 10.1016/j.vetimm.2014.12.006

ITKONEN O., STENMAN U.H., Parkkinen J., Soliymani R., Baumann M., Hämäläinen E. 2012. *Binding of hepcidin to plasma proteins*. Clin. Chem., 58(7): 1158-60. DOI: 10.1373//clinchem.2012.186916.

JENKINS K. J., HIDIROGLOU M. 1987. *Effect of excess iron in milk replacer on calf performance.* J Dairy Sci, 70: 2349-2354.

JOERLING J., DOLL K. 2019. *Monitoring of iron deficiency in calves by determination of serum ferritin in comparison with serum iron: A preliminary study*. Open Vet. J., 9(2): 177-184. DOI: 10.4314/ovj.v9i2.14

KAKHLON O., CABANTCHIK Z.I. 2002. *The labile iron pool: characterization, measurement, and participation in cellular processes (1)*. Free Radic. Biol. Med., 33(8): 1037-1046. DOI: 10.1016/s0891-5849(02)01006-7

KERR B. J., ZIEMER C. J., WEBER T. E., TRABUE S. L., BEARSON B. L., SHURSON G. C., WHITNEY M. W. 2008. *Comparative sulfur analysis using thermal combustion or inductively coupled plasma methodology and mineral composition of common livestock feedstuffs*. J. Anim. Sci., 86(9): 2377-2384. DOI: 10.2527/jas.2007-0811

KNOWLES T.G., EDWARDS J.E., BAZELEY K.J., BROWN S.N., BUTTERWORTH A., WARRISS P.D. 2000. *Changes in the blood biochemical and haematological profile of neonatal calves with age.* Vet. Rec., 147(21): 593-598. DOI: 10.1136/vr.147.21.593

KURTOGLU E., UGUR A., BALTACI A.K., UNDAR L. 2003. *Effect of iron supplementation on oxidative stress and antioxidant status in iron-deficiency anemia*. Biol. Trace Elem. Res., 96: 117-124. DOI: 10.1385/BTER:96:1-3:117

LIEU PT, HEISKALA M, PETERSON PA, YANG Y. 2001. *The roles of iron in health and disease*. Mol. Aspects Med., 2: 1-87.

Ma L., Terwilliger A., Maresso A.W. 2015. *Iron and Zinc Exploitation during Bacterial Pathogenesis*. Metallomics, 7(12): 1541-1554. DOI: 10.1039/c5mt00170f.

Marijanušić K., Manojlović M., Bogdanović D., Čabilovski R., Lombnaes P. 2017. *Mineral composition of forage crops in respect to dairy cow nutrition*. Bulg. J. Agric. Sci., 23(2): 204-212.

Messenger A.J.M., Barclay R. 1983. *Bacteria, iron and pathogenicity*. Biochem. Educ., 11(2): 54-63. DOI: 10.1016/0307-4412(83)90043-2

Michels K., Nemeth E., Ganz T., Mehrad B. 2015. *Hepcidin and host defence against infectious disease*. Plos. Pathog., 11(8): e1004998. DOI: 10.1371/journal.ppat.1004998

Mohri M., Poorsina S., Sedaghat R. 2010. *Effects of parenteral supply of iron on RBC parameters, performance, and health in neonatal dairy calves*. Biol. Trace Elem. Res., 136: 33-39. DOI 10.1007/s12011-009-8514-7

Mohri M., Sharifi K., Eidi S. 2007. *Hematology and serum biochemistry of Holstein dairy calves: age related changes and comparison with blood composition in adults*. Res. Vet. Sci., 83(1): 30-39. DOI: 10.1016/j.rvsc.2006.10.017

Neumann S. 2003. *Serum iron level as an indicator for inflammation in dogs and cats*. Comp. Clin. Pathol., 12(2): 90-94. DOI: 10.1007/s00580-003-0481-3

Peslova G., Petrak J., Kuzelova K., Hrdy I., Halada P., Kuchel P.W., Soe-Lin S., Ponka P., Sutak R., Becker E., Huang M.L., Suryo Rahmanto Y., Richardson D.R., Vyoral D. 2009. *Hepcidin, the hormone of iron metabolism, is bound specifically to alpha-2-macroglobulin in blood*. Blood, 113(24): 6225-6236. DOI: 10.1182/blood-2009-01-201590

Prodanovic R., Kirovski D., Vujanac I., Dodovski P., Jovanovic L., Šamanc H. 2014. *Relationship between serum iron and insulin-like growth factor-I concentrations in 10-day-old calves*. Acta Vet. Brno, 83(2): 133-137. DOI: 10.2754/avb201483020133

Radwińska J., Żarczyńska K. 2014. *The effects of mineral deficiency on the health of young ruminants*. J. Elem., 19(3): 915-928. DOI: 10.5601/jelem.2014.19.3.620

Rajabian F., Mohri M., Heidarpour M. 2017. *Relationships between oxidative stress, haematology and iron profile in anaemic and non-anaemic calves*. Vet. Rec., 181(10): 265 DOI: 10.1136//vr.104179

Rajamanickam K., Ali M. S., Leela V. 2020. *Influence of hepcidin on iron homeostasis during last trimester of gestation in Bos indicus (cattle)*. Ind. J. Anim. Res., 54(2):160-162. DOI : 10.18805/ijar.B-3743

Ramin A.G., Asri-Rezaei S., Paya K., Eftekhari Z., Jelodary M., Akbari H., Ramin S. 2012. *Evalutation of anemia in calves up to 4 months of age in Holstein dairy herds*. J. Fac. Vet. Med. Istanbul Univ., 7: 87-92.

Rastogi N., Singh A., Singh P.K., Tyagi T.K., Pandey S., Shin K., Kaur P., Sharma S., Singh T.P. 2016. *Structure of iron saturated C-lobe of bovine lactoferrin at pH 6.8 indicates a weakening of iron coordination*. Proteins, 84(5): 591-599. DOI: 10.1002/prot.25004

Sordillo L. M., Aitken S. L. 2009. *Impact of oxidative stress on the health and immune function of dairy cattle*. Vet. Immunol. Immunopathol., 128: 104-109. DOI:i:10.1016/j.vetimm.2008.10.305

Sorensen M., Sorensen S.P.L. 1940. *The proteins in whey*. Comptes-Rendus des Travaux du Laboratoire Carlsberg, 23: 55-99.

Steijns J.M., van Hooijdonk A.C. 2000. *Occurrence, structure, biochemical properties and technological characteristics of lactoferrin*. Br. J. Nutr., 84(1): 11-17. DOI: 10.1017/s0007114500002191

Thompson L.J., Hall J.O., Meerdink G.L. 1991. *Toxic effects of trace element excess*. Vet. Clinics North America: Food Animal Practice, 7(1): 277-306. DOI: 10.1016/s0749-0720(15)30818-5

Volker H., Rotermund L. 2000. *Possibilities of oral iron supplementation for maintaining health status in calves*. Dtsch. Tierarztl. Wochenschr, 107: 16-22.

Waldner C. L., Blakley B. 2014. *Evaluating micronutrient concentrations in liver samples from* *abortions, stillbirths, and neonatal and postnatal losses in beef calves.* J. Vet. Diagn. Invest., 26(3): 376-389. DOI: 10.1177/1040638714526597jvdi.sagepub.com

Wang A., Duncan S. E., Knowlton K. F., Ray W. K., Dietrich A. M. 2016. *Milk protein composition and stability changes affected by iron in water sources* J. Dairy Sci., 99: 4206-4221. DOI:10.3168/jds.2015-10481

Ward R.J., Zucca F.A., Duyn J.H., Crichton R.R, Zecca L. 2014. *The role of iron in brain ageing and neurodegenerative disorders.* Lancet Neurol., 13(10): 1045-1060. DOI: 10.1016/S1474--4422(14)70117-6

Weiss W. P. 2017. *A 100-Year Review: From ascorbic acid to zinc – Mineral and vitamin nutrition of dairy cows.* J. Dairy Sci., 100: 10045-10060. DOI: 10.3168/jds.2017-12935

WHO (World Health Organization). 2006. *Guidelines for drinking water quality.* WHO, Geneva, Switzerland.

Yatoo M.I., Saxena A., Deepa P.M., Habeab B.P., Devi S., Jatav R.S., Dimri U. 2013. *Role of trace elements in animals: a review.* Vet. World, 6(12): 963-967. DOI: 10.14202/vetworld.2013.963-967

Zecca L., Youdim M. B. H., Riederer P., Connor J.R., Crichton R.R. 2004. *Iron, brain ageing and neurodegenerative disorders.* Nat. Rev. Neurosci., 5: 863-873.