Scientific Papers. Series D. Animal Science. Vol. LXV, No. 2, 2022
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

REVIEW OF THE HEALTH BENEFITS OF LACTOFERRIN

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Abstract

Lactoferrin (LF) is a protein contained in raw milk. It was found that the highest content of lactoferrin is found in human milk, but the milk of other species also contains this protein (cow, goat, buffalo, sow, mare, mouse). Lactoferrin may improve the immune system or provide an iron supplement. It has the task of transporting iron, appreciated for the first time as a mediator of iron absorption. Potential applications of lactoferrin have prompted scientists to develop this nutraceutical protein for use in animal feed, human food and pharmaceutical applications. It is known that there are currently concerns in the world about obtaining a higher amount of lactoferrin from the milk of different animal species. This paper is a review that aims to highlight the biologically active role of lactoferrin.

***Key words**: composition, lactoferrin, lactoferic milk, role.*

INTRODUCTION

Due to the high biological value of milk, the mammary gland of domestic animals has received an economic and social importance humanity continues to remain dependent on breastfeeding (Cotor & Ghita, 2012). Its nutritional value is attributed by large compounds including lactoferrin (Bondoc, 2007). Lactoferrin (LF) is found in the milk of pets. This is a glycoprotein that can achieve several biological functions (Karav et al., 2017). Lactoferrin was first detected in 1939 by Sorensen & Sorensen (1939) and later identified in human and bovine milk in 1960 by Johanson, (1939); Groves, (1960); Montreuil et al., (1960). In recent years, research has been conducted on the function of lactoferrin, much of it in areas unrelated to the areas of iron absorption and antimicrobial activity (Sanchez et al., 1992). Lactoferrin applications express importance for thermal stability, pH sensitivity, iron release and susceptibility to enzyme degradation. Lactoferrin acts as an iron transporter, considered for the first time as an intermediate in the absorption of intestinal iron or as an antimicrobial agent (Brock, 2012). Lactoferrin has the ability to supply iron to cells that need it or restricts it when it is in excess, maintaining the homeostasis of iron in the body. According to Kruzel et al. (2002), the only source of iron in milk is lactoferrin, which is a key protein for the healthy development of infants. Lactoferrin helps maintain the balance of free iron in the body to prevent the occurrence of pathological conditions. Cornish et al. (2004) through studies on the role of lactoferrin in stimulating the growth of osteoblasts, give it the probability of being used in the future in the therapy of bone diseases (osteoporosis).

In 2014, Siqueiros-Cendón et al. published a review of recent advances in understanding the mechanisms underlying the multifunctional roles of lactoferrin and provide a future perspective on its potential prophylactic and therapeutic applications. It has been established that lactoferrin is a versatile molecule that has been shaped by natural selection to be a first-line defence for mammals.

MATERIALS AND METHODS

Bibliographic sources, scientific databases, relative available articles from the literature that discuss the comparative values of lactoferrin concentrations were consulted. Graphically interpreted and analyzed data from the literature on lactoferrin content detected in milk samples from different animal species (cattle, goats, sheep, donkey and breast milk) were graphically interpreted and analyzed.

Lactoferrin from milk from different animal species as well as human milk were tested by a fast High-performance liquid chromatography (HPLC) method. The lactoferrin concentration was calculated by the standard curve method. Gas chromatography is an important fulcrum in modern chemical analysis. The method is an evolution of an older method, classical column chromatography, which was primarily used for the preparative isolation of natural compounds.

RESULTS AND DISCUSSIONS

There are a number of factors that influence the concentration of milk components in predictable ways (Cheng et al., 2008), these factors can be grouped into genetic and environmental factors. The category of genetic factors includes: breed, species, age, parity, conformation and body development, diet, lactation, lactation routine, physiological type and stage of lactation. The category of environmental factors includes: regional differences, season, climatic factors and temperatures (Welty et al., 1976; Cheng et al., 2008). Cheng et al., in 2008, stated that lactoferrin was associated with lactation (r = 0.555) and milk production per day (r = -0.472). Tsakali et al. (2014) used the main categories of milk (goat and sheep milk, 70:30) to obtain Feta cheese, the milk of these species having a high concentration of lactoferrin. An average amount of lactoferrin of 272 ± 24 µg/ml was reported in Feta whey.
Conesa et al. (2008) used a rapid method, chromatography (SP-Sepharose) to identify the purification of lactoferrin in milk from animal species: sheep, goat, camel, alpaca, elephant and gray seal but also human milk. The results of the research showed that there are some differences in the structure of lactoferrin proteins among mammalian species. The differences are observed in the behavior under heat treatment and in their antibacterial activity. The thermal behavior of lactoferrins isolated from milk was obtained by differential scanning calorimetry. The thermograms obtained the results of the values of the maximum heat absorption temperature and the change in enthalpy of denaturation were found to be higher than lactoferrins, saturated with iron as when they were analyzed as isolated, because in milk, lactoferrin is present with low iron saturation (Bezwoda & Mansoor, 1989; Nam et al., 1999). The results of lactoferrin with the highest antibacterial activity were camel lactoferrin, followed by goat lactoferrin and sheep lactoferrin. The tested concentrations that did not show antimicrobial activity were in the alpa and human lactoferrin species (Conesa et al., 2008).
The results of the highest concentrations detected in milk samples from animal species found by Tsakali et al. (2019) were found for goat milk (927.3 ± 52.1 µg/ml) and human milk (512.0 ± 35.7 µg/ml) (Figure 1).

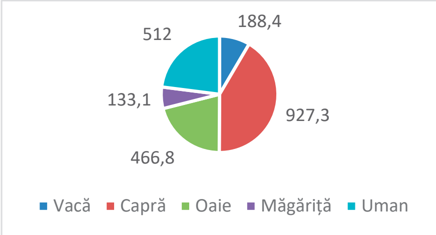

Figure 1. Mean detected lactoferrin values in milk samples from different animal species (µg/ml) (Tsakali et al., 2019)

The lowest detected results were found for donkey milk (133.1 ± 12.9 µg/ml) and cow's milk (188.4 ± 13.2 µg/ml), sheep's milk was found with a concentration of 466.8 ± 23.1 µg/ml.

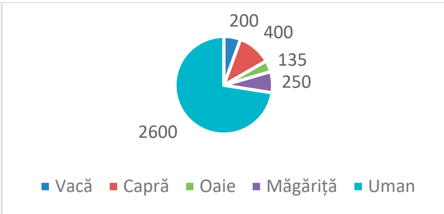

Figure 2. Mean values of lactoferrin concentrations detected in milk samples from different animal species in the literature (µg/ml) (Polidori & Vincenzetti, 2012; Queiroz et al., 2013; Gubid et al., 2015)

The results of the reference values in the literature were also found in high concentrations for goat milk (60-400 µg/ml) (Polidori & Vincenzetti, 2012) and human milk (200-2600 µg/ml) (Queiroz et al., 2013). Detected low results ranged from 20-200 µg/ml for cow's milk and 135 µg/ml for sheep's milk (Polidori & Vincenzetti, 2012). Concentrations between

120-250 µg/ml were found in donkey milk (Gubid et al., 2015)(Figure 2).
The results for sheep and goat milk were much higher than in the literature, due to re-testing with the addition of the lactoferrin standard in different concentrations, this giving a linear increase in lactoferrin and the difference in milk composition. The difference between the values obtained by Tsakali et al. (2019) and those of the literature have been reported for free fatty acids in animal milk but also human milk (Santillo et al., 2018). The results found by Tsakali et al. (2019) were satisfactory within the mentioned limits and the quantification of lactoferrin was possible, although additional tests should be performed to estimate its accuracy in each species. This rapid HPLC method could be used as a qualitative screening for the presence or absence of lactoferrin.

Uses of Lactoferrin
The use of lactoferrin in infant nutrition is an area that reflects an impressive number of studies, but the role of lactoferrin in the feeding of newborns in animals is less studied. The studies were performed generally for calves, having a role in regulating the intestinal flora (a calf that sucks 2 liters of colostrum, ingests about 2 g of lactoferrin per day).
Lactoferrin can also be used in food for fish farming, it can influence the defense system by regulating the activity of macrophages and the
Data from the literature, articles, patents with different topics on the structure, composition and properties of lactoferrin, its obtaining and purification, preclinical and clinical studies were consulted in order to obtain information on the use of lactoferrin in various fields. Commercial applications of bovine lactoferrin are shown in Table 1.

Table 1. Commercial applications of bovine lactoferrin (Miron & Macovei, 2006)

The product	Functionality
Infant formulas	Camouflage for human milk, improved resistance to pathogens
Nutritional supplements	Adjuvant in iron absorption, adjuvant to increase immunity
Functional drinks	Increases the solubility and absorption of iron
Beauty	antioxidants
Oral hygiene products	Improving oral hygiene

The best method of administering lactoferrin to infants is breastfeeding, which regulates the intestinal flora which is associated with an increased resistance to colonization by pathogens, activity of lymphocytes (Miron & Macovei, 2006). Lactoferrin is suspended in fish oil which is dispersed in the granules of the feed given to the alvevins. An experiment was performed by Kussendrager (1994), in which such feed was administered to salmon contaminated with Aeromonas salmonicide. The results showed that mortality decreased from 14% to 2% after feeding with 100 mg lactoferrin/day/kg of fish (Berkhout et al., 2003). Currently, the world produces about 20-30 tons of lactoferrin, being extracted from whey from cheese or skim milk. Active lactoferrin may be antimicrobial agent for poultry, pork, fish and marine life.

Antibacterial activity
Research by Valenti & Antonini (1998) has shown that lactoferrin has, independently of antibacterial activity and anti-invasive action represented by the ability to inhibit the penetration of bacteria into host cells. Thus, studies on *S. pyogenes* and *S. aureus* have shown that lactoferrin can be used as an adjunct in antimicrobial therapy, inhibiting the internalization of bacteria and allowing drugs to eliminate and kill bacteria on the studied pharyngeal epithelium. Wakabayashi et al. (2003), comparing the antimicrobial spectrum of bovine lactoferrin and human lactoferrin, found that bovine lactoferrin has strong antimicrobial activity, especially against bacteria and yeasts. Jahani et al. (2015), evaluating the effect of lactoferrin on two different species of Gram-negative and Gram-positive bacteria, showed that lactoferrin was more effective for Gram-positive bacteria (*Staphylococcus epidermidis, Bacillus cereus*) than for Gram-negative (*Campylobacter jejuni, Salmonella*).
A study by Lizzi et al. (2016) on the antibacterial activities of undigested and hydrolyzed lactoferrin with trypsin in the form of lactoferrin (apo-bLf) and undigested and differentiated bovine lactoferrin (bLf) were evaluated against different species bacterial. Trypsin is an enzyme needed for bowel physiology. The results of trypsin apo-bLf

hydrolysis products showed broad-spectrum antibacterial properties with various bacterial strains. Apo-bLf was less susceptible to trypsin hydrolysis compared to the different form. Its triptych fragments had a higher activity than those obtained from diferric-bLf. Embleton et al. (2013) reviewed the antimicrobial activity and therapeutic potential of lactoferrin.

Lactoferrin is found in most body fluids. Three isoforms of lactoferrin were isolated, i.e. two with RNase activity (lactoferrin-β and lactoferrin-γ) and one without RNase activity (lactoferrin-α). Lactoferrin receptors can be found on intestinal tissue, monocytes, macrophages, neutrophils, lymphocytes, platelets, and certain bacteria (Levay & Viljoen, 1995).

Antifungal activity

Numerous studies have been performed on the antifungal activity of lactoferrin. Nikawa et al. (1993) studied the effect of human lactoferrin on 5 oral isolates of *Candida albicanis* and *Candida krusei*, costing a higher sensitivity to lactoferrin for *Candida krusei*. Most studies have been done on *Candida albicans*. Lactoferrin has been shown to inhibit the growth of *Candida albicans in vitro*, including strains resistant to azole antifungal agents, not only in the form of yeast but also in the form of hyphal (Wakabayashi et al., 1998).

Takakura et al (2003) demonstrated the antifungal activity of lactoferrin and pepsin hydrolyzate of lactoferrin, administered orally against oral candidiasis for a model of immunocompromised mice. Lactoferrin and its derivatives were added to drinking water contaminated with hydrochloric tetracillin. Mice showed oral symptoms similar to human canker sores. Lactoferrin from bovine milk can be used as a dietary supplement to support antifungal drug therapy, to prevent side effects but also to prevent oral infections, by using mouthwash and lactoferrin toothpaste (Miron & Macovei, 2006).

Antiviral activity

Research in recent years has shown that lactoferrin has antiviral activity against a large number of RNA and DNA viruses. Bovine and human lactoferrin have been shown to be potential inhibitors of human immunodeficiency virus (HIV) infections in vitro (Miron & Macovei, 2006). The antiviral action of lactoferrin against HIV is manifested in the early stages of the infection, most likely during the absorption of the virus into the host cells. Ando & Kishimoto (1996), cited by Macovei et al. (2006), patented a series of lactoferrin preparations for the prevention and treatment of opportunistic infectious diseases as a result of HIV infection. It has a synergistic action with the drugs used against cytomegavirus (CMV) and HIV.

Berlutti et al. (2011) published an article on the antiviral properties of lactoferrin. The protective effect of lactoferrin against microbial infections has been widely demonstrated in a large number of in vitro studies. However, it has been established that the antiviral activity of lactoferrin detected in monolayers of cultured cells infected with coated and empty viruses is not related to the degrees of lactoferrin iron saturation, while Zn and Mn saturated lactoferrin exerted a strong antiviral capacity against HSV (herpesvirus), HIV infection and poliovirus.

Anticarcinogenic activity

An exceptional use of lactoferrin is a non-toxic adjuvant in the treatment of cancer. Thus, lactoferrin is effective in suppressing the in vitro growth of human pancreatic cancer cells. Research by Tsuda et al. (2002) found that bovine lactoferrin (bLF) inhibits the development of colon cancer, esophageal, lung and bladder cancer in rats being administered orally in the post-initiation stage.

Japanese researchers at the National Cancer Center Research Institute in Tokyo have analyzed in vivo and in vitro animal studies showing that lactoferrin is an anticarcinogenic agent (Brik, 2000). Japanese researchers also looked at the effects of bovine lactoferrin in mice that had been inoculated with skin cancer cells or leukemia. When lactoferrin was administered, even after tumor formation, the growth of tumor cells was suppressed, and the spread of cancer cells in the lungs and liver was significantly lower than in control animals that did not take lactoferrin (Miron & Macovei, 2006).

Immunomodulatory and anti-inflammatory activity of lactoferrin

The property of lactoferrin to interact with many cell types, including most leukocytes, makes it play an excellent role in immunity. Research on various animals has shown that ingestion of lactoferrin has a direct protective effect on the regulation and modulation of the immune system. Lactoferrin can affect both the proliferation and differentiation of immune system cells (Miron & Macovei, 2006).

Lactoferrin has been shown to be essential in preventing allergic inflammation of the lungs and skin, with an increase in lactoferrin levels in the lungs of rats exposed to metal pollutants and bronchial secretions during stable asthma. Lactoferrin is a regulator of allergic skin inflammation (Cumberbatch et al., 2000), being produced in the cells of the epidermis, it has been observed that this protein can increase during the attack of allergens. The productive effect of lactoferrin against allergen-induced skin inflammation has also been increased in humans (Griffiths et al., 2000).

Prevention of bacterial colonization by lactoferrin

Specific inactivation of colonization conditions and moderation of the pathological potential of some bacteria can be observed as a third mechanism of action of lactoferrin (Shi et al., 2000). This assumption came from research on Gram-negative pathogenic bacteria *Haemophilus influenzae* (Qiu et al., 1998; Hendrixon et al., 2003).

Hendrixon et al. (2003) proved that lactoferrin has serine-protease activity and is a member of the protease family. They investigated the activity of human lactoferrin against IgA1 protease and Hap protein adhesion, with a role in the colonization of the respiratory tract by H. influenzae. It has been found that lactoferrin breaks down the two proteins in the region rich in arginine residues, thus proving to be a serine protease (Miron & Macovei, 2006). Lactoferrin is considered an important part in the prevention of dental caries in its inhibitory effect on the colonization of the tooth surface with *S. mutans*.

CONCLUSIONS

Lactoferrin has become an important topic in current research, due to its unique antimicrobial, anti-infective, anti-cancer and anti-inflammatory properties, having a special role in current medical practice. Lactoferrin has many health benefits, added in some functional foods but also in other products. The consequences of bovine lactoferrin have been shown in clinical trials in animals and humans. Favorable actions have been shown against digestive and non-digestive infections, following ingestion of lactoferrin, performed on various species of animals.

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