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# Selected Factors Determining the Content of Lactoferrin, Lysozyme and Immunoglobulins G in Bovine Milk

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## 1. Introduction

According to the statement "prevention is better than cure" the consumers' concern about their health increases, which is reflected in the sales of products supporting the immune system. Such products are made of milk and colostrum, especially the immune proteins isolated from them. These proteins are increasingly used to enrich baby food, dietary medications or high-protein formulas recommended for convalescents and athletes. Furthermore, these proteins are used in pharmacology and cosmetology. They play a major role in the transmission of passive immunity to the offspring and protect the host mammary gland (Gapper et al., 2007; Stelwagen et al., 2009). This group of proteins include immunoglobulins, lactoferrin, lactoperoxidase and lysozyme. Increasingly, these milk components are used by humans for the prophylactic or therapeutic aims.

Content of antibacterial proteins in the diet is one of the factors determining the normal immune response of the organism. Lactoferrin, lysozyme and immunoglobulins are therefore essential components of milk from the consumers' viewpoint. In order to meet the demands of the consumers, the dairy industry should seek high quality raw materials tested for biologically active compounds. It is therefore important to know the factors that determine their content in cow milk. In this study the influence of selected factors on content of lactoferrin, lysozyme and immunoglobulins G in cow milk is discussed.

## 2. Characteristics of milk proteins with the antibacterial properties

### 2.1 Immunoglobulins

Very important group of proteins exhibiting antimicrobial activity are the immunoglobulins. These compounds are high molecular globulins, which are present in plasma and body fluids. Depending on the physicochemical structure and biological activity, three major classes of immunoglobulins are distinguished, i.e. IgG, IgM and IgA. IgG dominate (approximately 80 %) in milk of ruminants, while in other mammalian milk, including

human, – IgA (approximately 90 %). They determine the specific humoral immunity of a body (El-Loly & Farrag, 2007; Pakkanen & Aalto, 1997). During the process of antigens binding as well as phagocytosis or complement activation these proteins are involved in the destruction of pathogenic microorganisms, i.e. *Escherichia coli, Candida albicans, Clostridium difficile, Shigella flexneri, Streptococcus mutans* and *Helicobacter pylori* (Gapper et al., 2007; Korhonen, 2004). Furthermore, the immunoglobulins block the action of toxins and viruses. In many countries, the specimens on the basis of immunoglobulins are commercially available. They are meant for the livestock, mainly for newborn calves and pigs in order to prevent gastro-intestinal infections (El-Loly, 2007). Other increasingly popular products are also Ig-based products used as food additives (Gapper et al., 2007; Stelwagen et al., 2009; Struff & Sprotte, 2008). It has been shown that the supplementation use is beneficial for immunity system and prevents diseases of the digestive system (Mehra et al., 2005; Struff & Sprotte, 2008). The reduction of diarrhea incidence caused by rotavirus was noted in the infants and children up to four years (El-Loly, 2007; Gapper et al., 2007; Rawal et al., 2008). Clinical studies have confirmed the efficacy of these specimens in the analgesic therapy in patients with fibromyalgia syndrome (Goebel et al., 2008; Struff & Sprotte, 2008).

### 2.2 Lactoferrin

Lactoferrin is a glycoprotein with a molecular weight of about 80 kDa. Due to the size and construction, it belongs to the transferrin family, which has a specific ability to bind iron (Baker & Barker, 2005; Legrant et al., 2008). It occurs as a single polypeptide chain which consists of about 690 amino acids (Baker & Barker, 2005, Pakkanen & Aalto, 1997). This protein was first isolated from milk in the 60s of the previous century. Lactoferrin also occurs in other secretions such as saliva, tears, semen, bronchial mucous secretion, digestive and genital tract. It is also a component of neutrophil secondary granules from which, during an injury, infection and inflammation, it is released into the blood (Artym, 2010, Baker & Barker, 2005; Garcia-Mantoya, 2011; Małaczewska & Rotkiewicz, 2007). This protein is an essential element of non-specific innate immunity of humans and other mammals (Kruzel et al., 2007; Legrant et al., 2008). By binding and sequestrating of iron, lactoferrin exhibits antibacterial properties against Gram-positive and Gram-negative bacteria, non-capsular and capsular viruses as well as various types of fungi and parasites (Małaczewska & Rotkiewicz, 2007; Orsi, 2004; Steijns & Hooijdonk, 2000; Wakabayashi et al., 2006). This action partially results from the ability of protein to chelate iron ($Fe^{3+}$ ions), thereby removing this element from the environment of microbial growth (Andersen et al., 2003). Other mechanisms of the lactoferrin antimicrobial action include direct destruction of sheaths and disturbance of bacterial cell metabolism, inhibition of the processes of bacterial adherence to body tissues of the host (Hendrixon et al., 2003), inhibition of biofilm formation by some bacteria (Singh et al., 2002) and stimulation of the immune system of host to fight against pathogens (Artym, 2006). Lactoferrin protects the intestinal epithelial cells, and at the same time inhibits the growth of *E. coli* and other pathogenic intestinal bacteria, mainly *Enterobacteriaceae*, while stimulating the growth of useful intestinal microflora of the *Bifidobacterium genus* (Wakabayashi et al., 2006). This is particularly crucial in the case of neonates in whom there is a gradual colonization of the alimentary canal by diverse microflora. The development of normal bacterial flora ensures the efficient digestion, protects against the pathogenic bacteria development and increases immunity (Actor et al., 2009, Griffiths et al., 2003). These factors determined the use of lactoferrin in

food for infants (Satue-Gracia et al., 2000; Wakabayashi et al., 2006). An additional advantage of lactoferrin in the fight against bacterial infections is the possibility of increasing a bacteria sensitivity to certain antibiotics (vancomycin, penicillin) and lowering their effective doses. The combination of penicillin with lactoferrin doubles an inhibitory activity of antibiotic against *Staphylococcus aureus* (Diarra et al., 2002). Lactoferrin also shows an antiviral activity (Van der Strate et al., 2001; Zimecki & Artym, 2005). In clinical trials, it proved to be effective in inhibiting of hepatitis C and B type virus infections (Ikeda et al; 2000; Ishii et al., 2003; Okada et al., 2002), herpes (*Herpes simplex virus*) (Andersen et al., 2003; Jenssen et al., 2004), HIV (Berkhout et al., 2002; Semba et al., 1998) and rotavirus, which are the most important etiological factor for acute diseases running with a diarrhea – major cause of mortality of infants and young children in developing countries (Brock, 2002; Superti et al., 1999; Van der Strate et al., 2001). Moreover, the synergistic effect with antiviral drugs, including interferon, acyclovir and cidofovir, has been observed (Andersen et al., 2003; Ishii et al., 2003). This effect allows the reduction of the doses of used drugs, characterized by high toxicity for the organism. Lactoferrin also has antioxidant capacity and prevents the formation of free radicals, as well as regulates the production and release of cytokines and tumor necrosis factor – TNF, secreted by macrophages (Małaczewska & Rotkiewicz, 2007). *In vitro* and *in vivo* research showed that lactoferrin applied direct antitumor effect on melanoma and colon cancer cells (Spadaro et al., 2008). Moreover, the results suggest that lactoferrin accelerates the formation of bone tissue, and therefore can be used in the prevention and treatment of the bone diseases, including osteoporosis. Due to cytoprotective properties, it may be involved in slowing down the development of neurodegenerative diseases, such as Alzheimer's or Parkinson's disease or multiple sclerosis (Blains et al., 2009, Cornish et al., 2004).

Very important properties of lactoferrin is its resistance to heat and proteolytic enzymes (Małaczewska & Rotkiewicz, 2007; Steijns & Hooijdonk, 2000; Wakabayashi et al., 2006). In veterinary medicine, what is particularly promising is the use of lactoferrin in aquaculture as an agent safe for the environment and human health (Małaczewska et al., 2009).

### 2.3 Lysozyme

Lysozyme (N-acetylmuramide glycanhydrolase, E.C.3.2.1.17) is a low molecular weight (14.4 kDa) enzymatic protein from the hydrolase group. It is widely distributed in nature, occurring in many body fluids and tissues of living organisms (Fox & Kelly, 2005). The highest concentration of the enzyme was found in tears and egg white protein, which is currently the basic source of its obtaining on an industrial scale (Cegielska-Radziejewska et al., 2008; Chiang et al., 2006; Liśnierowski, 2009; Malicki et al., 2003). The relatively large quantities were also noted in human milk (Benkerroum, 2008; Pakkanen & Aalto, 1997; Shah, 2000). Lysozyme is natural defense mechanism of an organism. Its action is based on disintegrating the bacteria by dissolving the polysaccharide-peptide complex (peptidoglycan), which is the main component of the cell wall of numerous bacteria (Masschalck & Michiels, 2003). Under natural conditions, the antibacterial activity of lysozyme (monomer form) is limited to Gram-positive bacteria, and only after modification (despite the reduction of hydrolytic activity) its bactericidal activity extends to Gram-negative bacteria, including many pathogenic bacteria (Benkerroum, 2008; Chang & Li, 2002; Ibrahim et al., 1996; Liśnierowski, 2009; Liśnierowski et al., 2004; Masschalck et al., 2001;

Masschalck & Michiels, 2003). Lysozyme is also one of the mechanisms of non-specific, humoral immune response (Benkerroum, 2008; Montagne et al., 1998; Pakkanen & Aalto, 1997). Antibacterial properties of lysozyme causes a considerable interest in its practical utilization in many sectors of food industries. It is used primarily as an additive to food, showing the preservative properties (Leśnierowski, 2009; Malicki et al., 2003; Proctor and Cunningham, 1988; Rosiak & Kołożyn-Krajewska, 2003). During rennet cheese production lysozyme limits the growth of butyric fermentation bacteria, especially *Clostridium tyrobutyricum*, causing cheese bloating (Danyluk & Kiev, 2001; Proctor & Cunningham, 1988). Lysozyme is also used in medical diagnostics, pharmacology and veterinary medicine. The enzyme has found a wide application in the therapies of viral and bacterial infections, treatment of skin as well as eye diseases, periodontitis, leukemia and cancer (Benkerroum, 2008; Proctor & Cunningham, 1988; Zimecki & Artym, 2005). Lysozyme exerts an antibiotic adjuvant mechanism of action, and therefore it is often called the endogenous antibiotic. It has been shown that the administration of lysozyme enriched milk for the premature has a positive influence on their development and speeds up the fight against an infection (Zimecki & Artym, 2005).

## 3. Antibacterial proteins content in milk of different animal species

Antibacterial proteins content in milk of different species of livestock is highly variable (table 1). The greatest amount of lactoferrin and lysozyme contains human milk, however, it is a poor source of immunoglobulins G. The milk of mares, similar to human milk, is characterized by a high content of lysozyme, at lower level of lactoferrin. Milk of other livestock species contains considerably less of these proteins, however, in this group of animals the camel and cow milk is distinguished by a higher content of lactoferrin and the ovine milk – lysozyme. The richest source of IgG is the camel milk. It is worth noting that the lactoferrin derived from human milk reveals the lowest antibacterial activity, while the highest from the camel milk (Coness et al., 2008). A changeability within the species is primarily due to the differences in lactation period, feeding regimen, number of analyzed samples, breeds, and methods of analysis.

| Specification | Lactoferrin | Lysozyme | Immunoglobulins G |
|---|---|---|---|
| Human | 700-2000 | 100-890 | 40-54 |
| Cow | 80-500 | 0.37-0.60 | 100-800 |
| Buffalo | 50-320 | 0.13-0.15 | 460-1300 |
| Camel | 200-728 | 0.73-5.00 | 2000 |
| Goat | 98-150 | 0.25 | 100-400 |
| Ewe | 140 | 1-4 | 500 |
| Mare | 820 | 400-890 | 390 |

Table 1. Average concentrations of lactoferrin, lysozyme and immunoglobulins G in milk of different species (mg/l) (Dračková et al., 2009; El-Hatmi et al., 2007; Konuspayeva et al., 2008; Liu et al., 2009; Pandya & Khan, 2006; Park et al., 2007; Stelwagen et al., 2009; Wheeler & Hodgkinson, 2007)

## 4. Materials and methods

### 4.1 Materials

#### 4.1.1 Animals

The studies were conducted throughout four successive years (2006-2009) on milk samples collected from seven different breeds of dairy cows maintained in Poland, i.e. three breeds with an international meaning (Polish Holstein-Friesian, Simental and Jersey) as well as four local breeds (Polish Red, Whitebacks, Polish Black and White and Polish Red and White). The Polish Holstein-Friesian, Simental and Jersey cows were managed under an intensive system in free-stall barns. An animal feeding system, established over both the winter and summer season, was based on a Total Mixed Ration – TMR (corn silage, haylage and feed concentrate). It should be also mentioned that one group of the Simental cows, included into the research, was maintained in the Southern Poland and a conventional feeding system was used. In the summer the animals grazed pasture *ad libitum*, and in the winter they received haylage, hay, and concentrate. However, the local breeds of cows are predominantly managed in small-sized farms in South-Eastern Poland (mountain, submountain or boggy terrains). Owing to small number and genetic distinction, the animals of these breeds are included into the genetic resources conservation programme (Litwińczuk et al., 2006). These cows were housed in a conventional system, i.e. in tie-stall barns. The summer feeding was based on pasture forage, i.e. green fodder comprising grasses and legumes supplemented by hay or straw, while the winter feeding on haylage and hay with fodder beet additive. In all the farms, a feed ration was supplemented with a feed concentrate.

#### 4.1.2 Milk samples collection

Milk samples were collected individually from each cow during trial milking which occurred twice a year, once in the summer period and again in the winter. A total of 3,105 milk samples were examined (table 2).

| Breed | Summer season | Winter season |
|---|---|---|
| Intensive system | | |
| Polish Holstein-Friesian | 502 | 539 |
| Simental | 215 | 242 |
| Jersey | 184 | 192 |
| Total | 901 | 973 |
| Conventional system | | |
| Simental | 221 | 207 |
| Polish Red | 159 | 126 |
| Whitebacks | 133 | 117 |
| Polish Black and White | 91 | 75 |
| Polish Red and White | 54 | 48 |
| Total | 658 | 573 |

Table 2. Number of milk samples taken to the research

### 4.2 Chemical analysis

All milk samples were transported to the laboratory of the Department of Commodity Science and Processing of Animal Raw Materials, University of Life Sciences in Lublin (Poland). Each milk sample was analyzed for somatic cell count (SCC) using flow cytometry technology – Somacount 150 apparatus (Bentley, USA). In 2,662 samples the SCC did not exceed 400,000 cells/ml, i.e. the standard accepted by the European Parliament and the Council of 29 April 2004 (EC Regulation No. 853/2004 on the specific hygiene rules for food of animal origin). These milk samples were included solely into the research evaluating an effect of cow breed, age of cows and stage of lactation and feeding system.

Lactoferrin and lysozyme contents were determined using the reversed-phase high-performance liquid chromatography (RP-HPLC) with UV-Vis detector. From each sample of raw milk was taken 50 ml and adjusted to pH 4.6 with 0.1 mol/l HCl, and allowed to stand at room temperature for about one hour to allow for the acid precipitation of caseins. Consequently, whey (7 ml) was taken from each of the samples separately and then centrifuged at 10,000 rpm for 15 min. Finally, whey solutions were filtered through paper quality filter discs (diameter: 125 mm, density: 65 g/m$^2$, grade: 3 hours (Munktell, Germany)) and 0.20-µm disposable sterile filters (Millipore type GSTF, USA). The supernatants in vials were kept refrigerated until further analysis, and were injected into the chromatograph at the suitable time (in the amount of 20 µl). Protein separation was performed on liquid chromatography ProStar 210 model and UV-Vis ProStar 325 detector (Varian, USA). The measurements were carried out using the water/acetonitrile mobile phase at gradient elution and column NUCLEOSIL 300-5 C18 (Varian, USA) of 250 mm length and 4.6 mm diameter. The mobile phase was solvent A (90% water, 10% acetonitryle) and solvent B (90% acetonitryle, 10% water), purchased from Sigma (Germany). The solvents were filtered through 0.45-µm filters (Millipore, USA) and degassed by using ultrasounds. The total analysis time for a single sample was 35 min at 205 nm wavelength with column temperature of 37°C. The analyses of reference substances were conducted under the same conditions. On the grounds of the obtained chromatograms, using program Star 6.2 Chromatography Workstation (Varian, USA), the qualitative and quantitative identification of each substance was performed followed by their concentration determination. Calibration of the chromatographic system for whey proteins determination was carried out by the external standard method. For this purpose, each protein was calibrated individually by injecting solutions of the standards (20 µl). The standards were purified proteins, i.e. lactoferrin (90 %) from bovine milk and lysozyme (95 %) from hen egg whites, which were purchased from Sigma (Germany). All chemicals were of HPLC analytical grade. Concentrations of lactoferrin and lysozyme solutions ranged from 0 to 200 mg/l and from 0 to 20 µg/l respectively, and were prepared to create the calibration curves. The limits of quantification LOQ (for lactoferrin – 40 mg/l and for lysozyme – 2.8 µg/l) and detection LOD (for lactoferrin – 8.7 mg/l and lysozyme – 0.9 µg/l) were determined. The immunoglobulin G (IgG) levels were established by the aid of radial immunodiffusion technique with Bovine IgG LL tests (The Binding Site, Birmingham, UK). Control samples, provided in the IgG LL set, were used before the test samples.

### 4.3 Statistical analysis

Data are given as mean ± standard deviation. The obtained results were analyzed by the General Linear Model (GLM) – factorial ANOVA procedures of Statsoft Inc. Statistica ver.6

(Statsoft Inc. 2003). It was done on the grounds of one-way and multi-way analysis of variance with interaction, using Tukey's HSD procedure.

The following factors were taken into consideration:

- cow breed

Seven breeds of cows kept in Poland, i.e. three breeds of international importance (Polish Holstein-Friesian, Jersey and Simmental) and four local breeds, kept only in Poland (Polish Red, Whitebacks, Polish Black and White and Polish Red and White).

- age of cows and stage of lactation

Age classes, mostly noticed as subsequent lactation: I, II, III and IV.

Stage of lactation, i.e. up to 120 days, from 121 to 200 and from 201 to 305 days of lactation.

- feeding system

Two feeding systems solely for the Simental cows were distinguished:

  - conventional (in the summer cows grazed the pasture (ad libitum), in the winter cows were fed with haylage and hay),
  - intensive (total mixed ration (TMR) feeding system was used throughout the year).
- somatic cell count.

The research material for each breed was split into four groups according to somatic cell count detected in the milk samples: Group I – up to 100,000 cells/ml, Group II – 101,000-400,000 cells/ml, Group III – 401,000-500,000 cells/ml, and Group IV – 501,000-1,000,000 cells/ml. Next, to regulate the SCC distribution, the SCC data were transformed into log 10 SCC for each sample before statistical analysis could take place.

## 5. Factors affecting the content of lactoferrin, lysozyme and immunoglobulins G

### 5.1 Cow breed

It has been shown that among the many breeds of cows involved in milk production in Poland, the cows of local breeds produce a raw material with a higher content of antibacterial proteins, such as lactoferrin and immunoglobulins G. The highest concentration of these proteins was found in the milk of Polish Red cows (128.7 and 558.1 mg/l). A slightly lower level of them was established in milk of Polish Red and White (120.9 and 545.6 mg/l) as well as Whitebacks (115.2 and 540.2 mg/l). Significant amounts of lactoferrin and immunoglobulins G were also reported in the milk of Simental cows (116.74 and 579.9 mg/l), managed under an intensive production system. However, the milk obtained from two breeds of cows with the greatest importance in milk production, both in Poland and the world, i.e. Holstein-Friesian and Jersey, was a poorer source of these proteins (table 3). Simultaneously, milk of Jersey cows characterized by a high content of lysozyme (13.02 μg/l). Milk of local cow breeds contains only slightly less lysozyme (from 10.79 to 12.42 μg/l). The comparable to the present research lactoferrin content in raw milk from Swedish farms, i.e. 90 mg/l (70-110 mg/l) was obtained by Lindmark-Månsson et al. (2003). In another Swedish study (Wedholm et al., 2006), both in the milk of Swedish

Holstein cows, and Swedish Red-White, established 120.0 mg/l of lactoferrin. Higher level of this protein (145.66-204.89 mg/l) reported Wielgosz-Groth et al. (2009) in the milk of Polish Holstein-Friesian cows. A substantially lower this protein content (7.30-14.73 mg/l) in milk from Black-White variety cows with 50-75 % share of Holstein-Friesian genes obtained Reklewska et al. (2003). The authors, however, noted the closest to the present study lysozyme concentration. That milk, depending on the feeding system, contained from 12.54 to 16.43 µg/l of lysozyme. Significantly more of this protein (70 µg/l) found Elegamy et al. (1996). Tsuji et al. (1990), analyzing lactoferrin concentration in the colostrum obtained from four breeds of cows in Japan, showed a higher share of this protein in the colostrum produced by Holstein-Friesian and Jersey cows (1.96-2.11 mg/ml), compared to Japanese Brown and Japanese Black (0.40-0.56 mg/ml). Newstead (1976) determined IgG concentration in milk of Jersey and Friesian cows. The author found (compared to the present research) a substantially lower level of this protein in milk of Jersey cows, i.e. 0.32 g/l. While milk gained from Friesian cows contained 0.46 g/l IgG. In the research of Levieux and Ollier (1999) revealed the comparable to the present study concentration of IgG. Krukowski et al. (2006) in the milk from Black and White variety cows with 50-75 % Holstein-Friesian genes reported higher level of these proteins – 628 mg/l. Whereas, in milk from the Polish Holstein-Friesian Black and White variety cows, the differences in IgG content reached over 200 mg/l. Murphy et al. (2005) showed a breed influence on concentration of IgG in colostrum of beef cows (from 75.7 mg/ml for the Limousin to 95.5 mg/ml for Charolais).

| Specification | Lactoferrin (mg/l) | Lysozyme (µg/l) | Immunoglobulins G (mg/l) |
|---|---|---|---|
| Polish Holstein-Friesian | $88.42^{A}$<br>14.72 | $8.22^{A}$<br>0.99 | $423.6^{A}$<br>18.4 |
| Simental | $116.74^{BC}$<br>16.54 | $9.84^{AB}$<br>1.88 | $579.9^{B}$<br>16.5 |
| Jersey | $103.48^{AB}$<br>15.68 | $13.02^{B}$<br>2.21 | $508.6^{AB}$<br>29.1 |
| Polish Red | $128.7^{C}$<br>17.35 | $12.17^{B}$<br>5.76 | $558.1^{B}$<br>22.4 |
| Whitebacks | $115.2^{BC}$<br>21.01 | $12.42^{B}$<br>3.38 | $540.2^{B}$<br>46.1 |
| Polish Black and White | $105.9^{AB}$<br>19.37 | $10.79^{AB}$<br>4.82 | $530.2^{B}$<br>29.3 |
| Polish Red and White | $120.9^{BC}$<br>19.22 | $11.51^{AB}$<br>5.23 | $545.6^{B}$<br>48.3 |

A, B, C – differences significant at P≤0.01

Table 3. Effect of breed on the antimicrobial protein content in bovine milk (mean ± standard deviation) (Król et al., 2007, 2010a, 2010b; unpublished data)

### 5.2 Age of cows and stage of lactation

Age of cows (usually referred to as the subsequent lactation) and stage of lactation are the main physiological factors affecting the productivity and chemical composition of cow milk. It has been shown that the poorest source of analyzed antimicrobial proteins proved to be

the milk obtained from primiparous cows (table 4). The lowest level of IgG was found in I lactation (454.8 mg/l). In subsequent lactations share of these compounds increased gradually, with significant differences (P≤0.01) occurred only in the IV lactation. Lysozyme content progressed successively with the lactations, with peaks reported in IV lactation. Whereas, the highest lactoferrin concentration was observed in milk from cows at II and IV lactation, 107.18 and 115.61 mg/l, respectively. In the studies of Levieux & Ollier (1999), similarly to the present research, the primiparous cows produced significantly less IgG, as compared to cows at II-IV lactation (P≤0.05) and older (P≤0.01). The results of Guliński et al. (2006) and Mian-Bin Wu & Xu Yin-Jun (2009) confirmed the higher concentration of these proteins in milk and colostrum produced by the multiparous cows. It was also noted that in the subsequent lactations were significant differences in lactoferrin content (Back & Thompson, 2005; Hagiwara et al., 2003; Liu et al., 2009; Tsuji et al., 1990). Older cows, i.e. in IV lactation, produced milk with significantly higher concentration of this protein, compared to younger ones (Back & Thompson, 2005; Liu et al., 2009; Tsuji et al., 1990). Whereas, Hagiwara et al. (2003) reported significantly lower lactoferrin concentration in V lactation in relation to II (P≤0.01) and III (P≤0.05) lactation.

| Specification | Subsequent lactation | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Lactoferrin (mg/l) | 89.39$^{A}$ 17.89 | 107.18$^{B}$ 18.26 | 99.50$^{AB}$ 16.32 | 115.61$^{B}$ 19.33 |
| Lysozyme (µg/l) | 7.56$^{a}$ 2.11 | 9.29$^{ab}$ 1.68 | 9.87$^{b}$ 2.13 | 10.28$^{b}$ 2.36 |
| Immunoglobulins G (mg/l) | 454.8$^{A}$ 16.4 | 511.7$^{AB}$ 21.5 | 513.2$^{AB}$ 19.7 | 541.1$^{B}$ 25.9 |

a, b – differences significant at P≤0.05; A, B – differences significant at P≤0.01

Table 4. Effect of subsequent lactation on the antimicrobial protein content in bovine milk (mean ± standard deviation) (Król et al., 2010a)

Antibacterial protein level also underwent significant changes throughout the lactation period (table 5). Milk collected during the first months of lactation was characterized by the lowest lactoferrin and lysozyme content. Significantly more of these proteins amounts were determined in the late stage of lactation, i.e. by 48.11 mg/l (P≤0.01) and 3.12 mg/l (P≤0.05). Milk obtained from this lactation stage also contained the highest immunoglobulins G level (553.8 mg/l). During the first stages of lactation, these proteins content was significantly lower (P≤0.05), i.e. ranged from 70.6 mg/l (early stage) to 78.4 mg/l (middle stage). Concentration of IgG investigated by Caffin et al (1983) was also noted to change (alike in the present study) during the lactation course. In the early (30 days) and the middle stage (150 days) of lactation, there were found comparable values, i.e. 0.37 and 0.38 mg/ml. The significantly higher IgG content was reported in milk obtained in late lactation (270 days) – 0.60 mg/ml. Changes in IgG level in milk during lactation also confirmed the research of Liu et al. (2009). Wielgosz-Groth et al. (2009) showed an increase in the content of lactoferrin in milk of Jersey cows during lactation. Its concentration ranged from 51.91 µg/ml in the first month of lactation to 259.43 µg/ml in the tenth one. The changes in these protein content thought the lactation were also reported by Hiss et al. (2008), who analyzed goat milk. Until 32$^{nd}$ lactation week the lactoferrin level maintained between 10 to 28 mg/ml,

then it successively progressed to reach over 100 μg/ml in 44th lactation week. In human milk also stated that the lactoferrin and lysozyme concentration increase in the duration of lactation (Hennart et al., 1991; Montagne et al., 2001). Cheng et al. (2008) found a high correlation coefficient between the content of lactoferrin and lactation stage (r = 0.557).

| Specification | Lactation stage | | |
|---|---|---|---|
| | <120 days | 121-200 days | 201-305 days |
| Lactoferrin (mg/l) | 76.12$^{A}$ 15.69 | 109.50$^{AB}$ 19.58 | 124.23$^{B}$ 21.36 |
| Lysozyme (μg/l) | 8.16$^{a}$ 2.25 | 9.18$^{ab}$ 2.64 | 11.28$^{b}$ 3.29 |
| Immunoglobulins G (mg/l) | 483.2$^{a}$ 21.3 | 475.4$^{a}$ 19.8 | 553.8$^{b}$ 23.6 |

a, b – differences significant at P≤0.05; A, B – differences significant at P≤0.01

Table 5. Effect of lactation stage on the antimicrobial protein content in bovine milk (mean ± standard deviation) (Król et al., 2010a)

### 5.3 Feeding system

Among the environmental factors, the feeding system has the major impact on the milk yield and its chemical composition. For example the Simental cows, for which it was possible to distinguished two groups of feeding system, the significant differences in the content of these proteins were showed between the groups (table 6). Milk of cows grazing the pasture characterized by a higher content of lactoferrin (by 27.9 mg/l), lysozyme (by 0.65 mg/l), as well as IgG (by 39.6 mg/l). Higher level of the functional whey proteins in milk of cows grazing the pasture also obtained King et al. (2007) and Reklewska et al. (2003). Different results reported Wielgosz et al. (2009). The authors found lower levels of lactoferrin in the milk of cows kept on the pasture (145.66-148.83 mg/l) in comparison with milk of cows fed in barns (174.63-204.89 mg/l). Turner et al. (2003) also reported higher levels of lactoferrin in milk of cows fed the TMR system in relation to milk of cows grazing the pasture. In the subsequent study (Turner et al., 2007) the content of lactoferrin in milk of cows using the pasture in various degree was compared. Significantly (P≤0.05) higher lactoferrin yield (in g per day) was found in the milk of cows having unrestricted access to pasture (*ad libitum*). Mackle et al. (1999) showed that a pasture supplementing with maize grain and silage led to slightly decreasing of IgG content.

| Specification | Feeding system | |
|---|---|---|
| | Traditional system | Intensive system |
| Lactoferrin (mg/l) | 127.52$^{B}$ 19.1 | 110.71$^{A}$ 17.8 |
| Lysozyme (μg/l) | 10.90$^{b}$ 2.4 | 10.25$^{a}$ 2.4 |
| Immunoglobulins G (mg/l) | 602.4$^{B}$ 40.1 | 562.8$^{A}$ 31.6 |

a, b – differences significant at P≤0.05; A, B – differences significant at P≤0.01

Table 6. Effect of feeding system on the antimicrobial protein content in bovine milk (mean ± standard deviation) (Król et al., 2008; Brodziak, 2011; unpublished data)

### 5.4 Somatic cell count

Somatic cell count is a commonly recognized indicator of bovine udder health, milk quality and its technological usability. SCC is also one of the criteria for admission to the purchase of milk. According to many authors and bovine quarter producing milk with the SCC over 200,000 cells/ml shows the symptoms of subclinical mastitis. In Poland and other EU countries in accordance with the applicable decree, i.e. Regulation (EC) No. 853/2004, raw cow milk should not contain more than 400,000 somatic cells/ml. SCC has been shown to influence the immunoactive protein content. With the growth of SCC significantly increased the concentration of lactoferrin, lysozyme and IgG (table 7). Milk with the highest number of somatic cells (group IV) contained most of these proteins. In comparison to milk of cows belonging to I group, these differences were for lactoferrin – 26.66 mg/l (i.e. 32.6 % value of group I), lysozyme – 5.14 µg/l (60.2 %) and IgG – 219.4 mg/l (42.6 %). A substantial effect of SCC on immunoactive proteins content was confirmed by relatively high positive values of calculated correlation coefficients. For the content of lactoferrin: r = 0.65, for lysozyme r = 0.63 and for immunoglobulins G r = 0.79. Urech et al. (1999), in the study of quarter milk, indicated similar tendencies when 100,000 cells/ml were recognized as the threshold of somatic cell count. Quarter milk obtained from clinically healthy mammary glands contained an average of 84,000 of somatic cells/ml, whereas the milk from infected glands had 293,000 cells/ml ($P<0.001$). The authors showed the significant growth of lactoferrin (by 0.45 %) in milk from the affected udder. Similarly, Hamann (2002) defined "the gold standard" for a cell count to be up to 100,000 somatic cells/ml. Counts that reached above this point provided evidence of disturbed milk secretion that would lead to a reduced production of daily milk, changes in its chemical composition, and a deterioration of the processing properties of the milk. According to Lindmark-Månsson et al. (2000, 2006) an exceeding 5,000 cells/ml for somatic cell count limit leads to increase of lactoferrin content in milk, and a close relationship between this milk component and the status of udder health has been confirmed by very high correlation coefficients between milk lactoferrin concentration and somatic cell counts ($r = 0.962$ and $r = 0.918$ at $P<0.001$), obtained in two independent studies. A significant increase ($P<0.05$) of lactoferrin content with concurrent progression of a mammary gland disease was also noted in goat and buffalo milk (Leitner et al., 2004; Piccinini et al., 2006). The effect of SCC growth on the content of IgG was reported also by other authors (Caffin et al., 1983; Liu et al., 2009).

| Specification | SCC group | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Lactoferrin (mg/l) | $81.84^{a}$<br>17.2 | $82.56^{a}$<br>18.4 | $91.07^{ab}$<br>19.6 | $108.5^{b}$<br>20.2 |
| Lysozyme (µg/l) | $8.65^{A}$<br>1.74 | $9.68^{AB}$<br>1.46 | $11.54^{AB}$<br>2.05 | $13.79^{B}$<br>2.68 |
| Immunoglobulins G (mg/l) | $514.6^{a}$<br>18.9 | $520.9^{a}$<br>22.7 | $587.1^{ab}$<br>35.5 | $734.0^{b}$<br>54.3 |

a, b – differences significant at P≤0.05; A, B, C – differences significant at P≤0.01

Table 7. Effect of SCC on the antimicrobial protein content in bovine milk (mean ± standard deviation) (Litwińczuk et al., 2011; unpublished data)

Summarized in table 8 the results of two-way analysis of variances indicate significant influence of the analyzed factors (breed, age of cows, stage of lactation, feeding system, SCC) on the content of individual proteins. For lactoferrin content the significant interactions between breed of cows and stage of lactation as well as breed and SCC were also found. In the case of the following interactions: breed and age of cows as well as age of cows x stage of lactation the significant correlations have been shown for lysozyme content.

| Factor | Lactoferrin | Lysozyme | Immunoglobulins G |
|---|---|---|---|
| Breed | 0.004 | 0.010 | 0.002 |
| Age of cows | 0.000 | 0.000 | 0.002 |
| Stage of lactation | 0.000 | 0.021 | 0.030 |
| Feeding system | 0.001 | 0.031 | 0.008 |
| SCC | 0.015 | 0.000 | 0.025 |
| Breed x age of cows | 0.521 | 0.015 | 0.150 |
| Breed x stage of lactation | 0.029 | 0.365 | 0.131 |
| Breed x SCC | 0.000 | 0.553 | 0.777 |
| Age of cows x stage of lactation | 0.075 | 0.003 | 0.778 |
| Age of cows x SCC | 0.363 | 0.470 | 0.951 |
| Stage of lactation x SCC | 0.893 | 0.560 | 0.817 |

Table. 8. Results of one-way and two-way variances analysis for chosen milk proteins (P values) (Litwińczuk et al., 2011; unpublished data)

## 6. Conclusion

Content of the antibacterial proteins is determined by the genetic factors (breed of cows), environmental (feeding system and SCC) as well as physiological (age of cows and stage of lactation). Significant increase of these proteins concentration could be achieved by a production system changing, i.e. the introduction of pasture.

## 7. References

Actor, J.K., Hwang, S., Kruzel, M.L. (2009). Lactoferrin as a natural immune modulator. *Curr. Pharm. Des.*, Vol. 15, No. 17, pp. 1956-1973

Andersen, J.H., Jenssen, H., Gutteberg, T.J. (2003). Lactoferrin and lactoferricin inhibit Herpes simplex 1 and 2 infection and exhibit synergy when combined with acyclovir. *Antivir. Res.*, No. 58, pp. 209-215

Artym, J. (2010). Role of lactoferrin in iron administration in the organism. Part II. Antimicrobial and antiinflammatory action by an iron sequestration. *Postepy Hig. Med. Dosw.*, No. 64, 604-616

Back, P.J, Thomson, N.A. (2005). Exploiting cow genotype to increase milk value through production of minor milk components. *Proceedings of the New Zealand Society of Animal Production*, Vol. 65, pp. 53-58

Baker, E.N., Baker, H.M. (2005). Molecular structure, binding properties and dynamics of lactoferrin. *Cell Mol Life Sci.*, Vol. 62, pp. 2531-2539

Benkerroum, N. (2008). Antimicrobial activity of lysozyme with special relevance to milk. *African Journal of Biotechnology*, Vol. 7, No. 25, pp. 4856-4867

Berkhout, B., Van Wamel, J.L., Belijaars, L., Meijer, D.K., Visser, S., Floris, R. (2002). Characterization of the anti-HIV effects of native lactoferrin and other milk proteins and protein-derived peptides. *Antiviral Res.*, Vol. 55, pp. 341-355

Blais, A., Malet, A., Mikogami, T., Martin-Rouas, C., Tomé, D. (2009). Oral bovine lactoferrin improves bone status of ovariectomized mice. *Am. J. Physiol. Endocrinol. Metab.*, No. 296, pp. 1281–1288

Brodziak, A. (2011). Influence of the chosen environmental and genetic factors on the content and functional properties of milk whey proteins. *Doctoral thesis*. University of Life Sciences in Lublin

Caffin, J.P., Poutrel, B., Rainard, P. (1983). Physiological and pathological factors influencing bovine immunoglobulin $G_1$ concentration in milk. *J. Dairy Sci.*, Vol. 66, No. 10, pp. 2161-2166

Cegielska-Radziejewska, R., Leśnierowski, G., Kijowski, J. (2008). Properties and application of egg white lysozyme and its modified preparations – a review. *Pol. J. Food Nutr. Sci.*, Vol. 58, No. 1, pp. 5-10

Chang, J.Y., Li, L. (2002). The unfolding mechanism and disulfide structures of denatured lysozyme. *FEBS Letters*, No. 511, pp. 73–78

Cheng, J.B., Wang, J.Q., Bu, D.P., Liu, G.L., Zhang, C.G., Wei, H.Y., Zhou, L.Y., Wang, J.Z. (2008). Factors affecting the lactoferrin concentration in bovine milk. *J. Dairy Sci.*, Vol. 91, No. 3, pp. 970-976

Chiang, B.H., Su, C.K., Tsai, G.J., Tsao, G.T. (2006). Egg white lysozyme purification by ultrafiltration and affinity chromatography. *J. Food Sci.*, Vol. 58, pp. 303-306

Conesa, C., Sánchez, L., Rota, C., Pérez, M.D., Calvo, M., Farnaud, S., Evans, R.W. (2008). Isolation of lactoferrin from milk of different species: Calorimetric and antimicrobial studies. *Comparative Biochemistry and Physiology*, Part B, No. 150, pp. 131–139

Cornish, J., Callon, K.E., Naot, D., Palmano, K.P., Banovic, T., Bava, U., Watson, M., Lin, J.M., Tong, P. C., Chen, Q., Chan, V.A., Reid, H.E., Fazzalari, N., Baker, H.M., Baker, E.N., Haggarty N.W., Grey, A.B., Reid, I.R. (2004). Lactoferrin is a potent regulator of bone cell activity and increases bone formation *in vivo. Endocrinology*, Vol. 145, No. 9, pp. 4366-4374

Danyluk, B., Kijowski, J. (2001). The effect of lysozyme monomer on the growth of *Clostridium tyrobutyricum*. *Przem. Spoż.*, No. 12, pp. 16-19 (in Polish)

Diarra, M.S., Petitelere, D., Lacasse, P. (2002). Effect of lactoferrin in combination with penicillin on the morphology and the physiology of *Staphylococcus aureus* isolated from bovine mastitis. *J. Dairy Sci.*, Vol. 85, pp.1141-1149

Dračková, M., Borkovcová, I., Janštová, B., Naiserová, M., Přidalová, H., Navrátilová, P., Vorlová, L. (2009). Determination of lactoferrin in goat milk by HPLC method. *Czech J. Food Sci.*, Vol. 27, pp. 102-104

Elagamy, E.I., Ruppanner, R., Ismail, A., Champagne, C.P., Assaf, R. (1996) Purification and characterization of lactoferrin, lactoperoxidase, lysozyme and immunoglobulins from camel's milk. *Inter. Dairy J.*, Vol. 6, No. 2, pp. 129-145

El-Hatmi, H., Girardet, J.M., Gaillard, J.L., Yahyaoui, M.H., Attia, H. (2007). Characterisation of whey proteins of camel (*Camelus dromedarius*) milk and colostrums. *Small Ruminant Research*, Vol. 70, pp. 267-271

El-Loly, M.M. (2007). Bovine milk immunoglobulins in relation to human health. *Inter. J. Dairy Sci.*, Vol. 2, No. 3, pp. 183-195

El-Loly, M.M., Farrag, A.F. (2007). Isolation of immunoglobulin-rich fractions from whey. *Milchwissenschaft*, Vol. 62, No. 2, pp. 199-202

Fox, P.F., Kelly, A.L. (2005). Indigenous enzymes in milk: Overview and historical aspects – Part 2. *Inter. Dairy J.*, No. 16, pp. 517-532

Gapper, L.W., David, E.J., Copestake, D.E.J., Otter, D.E., Indyk, H.E. (2007). Analysis of bovine immunoglobulin G in milk, colostrum and dietary supplements: a review. *Anal. Bioanal. Chem.*, Vol. 389, pp. 93-109

Garcia-Mantoya, I.A., Cendon, T.S., Arevalo-Gallegos, S., Rascon-Cruz, Q. (2011). Lactoferrin a multiple bioactive protein: An overview. *Biochemica et Biophysica Acta*, doi:10.1016/j.bbagen.2011.06.018, pp. 1-11

Goebel, A., Buhner, S., Schedel, R., Lochs, H., Sprotte, G. (2008). Altered intestinal permeability in patients with primary fibromyalgia and in patients with complex regional pain syndrome. *Rheumatology*, Vol. 47, No. 8, pp. 1223-1227

Griffiths, E.A., Duffy, L.C., Schanbacher, E.L., Dryja, D., Leavens, A., Neiswander, R.I., Oiao, H., DiRienzo, D., Ogra, P. (2003). In vitro growth responses of bifidobacteria and enteropatogenes to bovine and human lactoferrin. *Dig. Dis. Sci.*, Vol. 48, pp. 1324-1332

Guliński, P., Niedziałek, G., Salmończyk., E, Górski T. (2006). Immunoglobulin content in colostrum of cows within select genetic and environmental factors (in Polish). *Medycyna Wet.*, Vol. 62, No. 3, pp. 339-342

Hagiwara, S., Kawai, K., Anri, A., Nagahata, H. (2003). Lactoferrin concentrations in milk from normal and subclinical mastitic cows. *J. Vet. Med. Sci.*, Vol. 65, No.3, pp. 319-323

Hamann, J. (2002). Relationship between somatic cell count and milk composition. *Bulletin FIL-IDF*, Vol. 372, pp. 56-59

Hendrixson, D.R., Qiu, J., Shewry, S.C., Fink, D.L., Petty, S., Baker, E.N., Plaut, A.G., St Geme, J.W. (2003). Human milk lactoferrin is a serine protease that cleaves Haemophilus surface proteins at arginine-rich sites. *Mol. Microbiol.*, Vol. 47, pp. 607–617

Hennart, P.F., Brasseur, D.J., Dologne-Desnoeck, J.B., Dramaix, M.M., Robyn, C.E. (1991). Lysozyme, lactoferrin, and secretory immunoglobulin A content in brest milk: influence of duration of lactation, nutrition status, prolactin status, and parity of mather. *Am. J. Clin. Nutr.*, Vol. 53, pp. 32-39

Hiss, S., Meyer, T., Sauerwein, H. (2008). Lacyoferrin concentrations in goat milk throughout lactation. *Small Ruminant Research*, Vol. 80, pp. 87–90

Ibrahim, H.R., Higashiguchi, S., Juneja, L.R., Kim, M., Yamamoto, T., Sugimato, Y., Aoki, T. (1996). Partially unfolded lysozyme at neutral pH agglutinates and kills Gram-negative and Gram-positive bacteria through membrane damage mechanism. *J. Agric. Food Chemistry*, Vol. 44, pp. 3799–3806

Ikeda, M.A., Nozaki, K., Sugiyama, T., Tanaka, A., Naganuma, K., Tanaka, H., Sekihara, K., Shimotohno, M., Saito, Kato. N. (2000). Characterization of antiviral activity of lactoferrin against hepatitis C virus infection in human cultured cells. *Virus Res.*, Vol. 66, pp. 51-63

Ishii, K., Takamura, N., Shinohara, M., Wakui, N., Shin, H., Sumino, Y., Ohmoto, Y., Teraguchi, S., Yamauchi, K. (2003). Long-term follow-up of chronic hepatitis C patients treated with oral lactoferrin for 12 months. *Hepatol. Res.*, Vol. 25, pp. 226-233

Jenssen, H., Andersen, J.H., Uhlin-Hansen, L., Gutteberg, T.J., Rekdal, Ø. (2004). Anti-HSV activity of lactoferricin analogues is only partly related to their affinity for heparan sulfate. *Antivir. Res.*, Vol. 61, pp. 101-109

Konuspayeva, G., Loiseau, G., Levieux, D., Faye, B. (2008). Lactoferrin and immunoglobulin content in camel milk from Bactrian, Dromedary and hybrids in Kazakhstan. *Journal of Camelid Sciences*, No. 1, pp. 54-62

Korhonen, H. (2004). Isolation of immunoglobulins from colostrums. *Bulletin of the IDF*, Vol. 389, pp. 78-84

Korhonen, H., Marnila, P., Gill, H.S. (2000). Milk immunoglobulins and complement factors. *British Journal of Nutrition*, Vol. 84, pp. 75-80

Król, J., Brodziak, A., Litwińczuk, Z., Szwajkowska, M. (2011). Whey proteins use in health promotion. *Żywienie Człowieka i Metabolizm*, Vol. XXXVIII, No. 1, pp. 36-45

Król, J., Litwińczuk, Z., Barłowska, J., Kędzierska-Matysek, M. (2007). Initial results on casein and whey protein content in milk of Polish Red and Whitebacked cows. *Ann. Anim. Sci.*, No. 1, pp. 207-211

Król, J., Litwińczuk, Z., Brodziak, A, Barłowska, J. (2010a). Lactoferrin, lysozyme and immunoglobulin G content in milk of four breeds of cows manager under intensive production system. *Polish Journal of Veterinary Science*, Vol. 13, No. 2, pp. 357-361

Król, J., Litwińczuk, Z., Brodziak, A, Sawicka-Zugaj, W. (2010b). Bioactive protein content in milk from local breeds of cows included in the genetic resources conservation programme. *Ann. Anim. Sci.*, Vol. 10, No. 3, pp. 213-221

Król, J., Litwińczuk, Z., Litwińczuk, A., Brodziak, A. (2008). Content of protein and its fractions in milk of Simmental cows with regard to a rearing technology. *Ann. Anim. Sci.*, Vol. 8, No.1, pp. 57-61

Krukowski, H., Lisowski, A., Różański, P., Polkowska, I. (2006). IgG level in mastitis cows' milk (in Polish). *Roczn Nauk PTZ*, Vol. 2, No. 2, pp. 65-69

Kruzel, M.L., Actor, J.K., Boldogh, I., Zimecki, M. (2007). Lactoferrin in health and disease. *Postepy Hig. Med. Dosw.*, Vol. 61, pp. 261-267

Legrand, D., Pierce, E., Carpentier, M., Mariller, C., Mazurier, J. (2008). Structure and function lactoferrin. *Adv. Exp. Med. Biol.*, Vol. 606, No. 94, pp. 163

Leitner, G., Merin, U., Silanikove, N. (2004). Changes in milk composition as affected by subclinical mastitis in goats. *J. Dairy Sci.*, Vol. 87, pp. 1719-1726

Leśnierowski, G. (2009). New manners of physical-chemical modification of lysozyme. *Nauka Przyroda Technologie*, Vol. 3, No. 4, pp. 1-18

Leśnierowski, G., Cegielska-Radziejewska, R., Kijowski, J. (2004). Thermally and chemical-thermally modified lysozyme and its bacteriostatic activity. *World Poult. Sci. J.*, Vol. 60, pp. 303–310

Levieux, D., Ollier, A. (1999). Bovine immunoglobulin G, β-lactoglobulin, α-lactalbumin and serum albumin in colostrum and milk during the early post partum period. *J. Dairy Res.*, Vol. 66, pp. 421-430

Lindmark-Månsson, H., Sensson, U., Paulsson, M., Alden, G., Frank, B., Johnson, G. (2000). Influence of milk components, somatic cells and supplemental zinc on milk processability. *Inter. Dairy J.*, Vol. 10, pp. 423-433

Lindmark-Månsson, H., Fonden, R., Pettersson, H.E. (2003). Composition of Swedish dairy milk. *Inter. Dairy J.*, Vol. 13, pp. 409-425

Lindmark-Månsson, H., Brånning, C., Alden, G., Paullsson M. (2006). Relationship between somatic cell count, individual leukocyte populations and milk components in bovine udder quarter milk. *Inter. Dairy J.,* Vol. 16, pp. 717-727

Litwińczuk, Z., Chabuz, W., Stanek, P., Sawicka, W. (2006). Genetic potential and reproductive performance of Whitebacks – Polish native breed of cows. *Arch. Tierz., Dummerstorf,* Vol. 49, pp. 289–296

Litwińczuk, Z., Król, J., Brodziak, A, Barłowska, J. (2011). Changes of protein content and its fractions in bovine milk from different cow breeds subject to somatic cell count. *J. Dairy Sci.*, Vol. 94, No. 2, pp. 684-691

Liu, G.L., Wang, J.Q., Bu, D.P., Cheng, J.B., Zhang, C.G., Wei, H.Y., Zhou, L.Y., Zhou, Z.F., Hu, H., Dong, X.L. (2009). Factors affecting the transfer of immunoglobulin G1 into the milk of Holstein cows. *The Veterinary Journal*, Vol. 182, pp. 79-85

Mackle, T.R., Bryant, A.M., Petch, S.F., Hill, J.P., Auldist, M.J. (1999). Variation in the composition of milk protein from pasture-fed dairy cows in the late lactation and the effect of grain and silage supplementation. *New Zealand Journal of Agricultural Research*, Vol. 42, pp. 147-154

Małaczewska, J., Rotkiewicz, Z. (2007). Lactoferrin as a multipotential protein. *Medycyna Wet.*, Vol. 63, No. 2, pp. 136-139

Małaczewska, J., Wójcik, R., Siwicki, A.K. (2009). Possibilities of lactoferrin using as an immune stimulator in fish and shellfish. *Medycyna Wet.*, Vol. 65, No. 2, pp. 95-98

Malicki, A., Jarmoluk, A., Brużewicz, S. (2003). Effect of lysozyme additive on the durability and microbial safety of the sausage in barrier sheath. *Medycyna Wet.*, Vol. 2, No. 2, pp. 29-36

Masschalack, B., Van Houdt, R., Van Haver, E.G., Michiels, C.W. (2001). Inactivation of gram-negative bacteria by lysozyme, denatured lysozyme, and lysozyme-derived peptides under high hydrostatic pressure. *Appl. Environ. Microbiol.*, Vol. 67, No. 1, pp. 339-344

Masschalck, B., Michiels, C.W. (2003). Antimicrobial properties of lysozyme in relation to foodborne vegetative bacteria. *Microbiol. Rev. Crit.*, Vol. 29, No. 3, pp. 191-214

Mehra, R., Marnila, P., Korhonen, H. (2005). Milk immunoglobulins for health promotion. *Inter. Dairy J.*, Vol. 16, pp. 1262-1271

Mian-bin, W., Yin-jun, X. (2009) Isolation and purification of lactoferrin and immunoglobulin G from bovine colostrum with serial cation-anion exchange chromatography. *Biotechnology and Bioprocess Engineering*, Vol. 14, pp. 155-160

Montagne P., Cuillière M.L., Molé C., Béné M.C., Faure G. (1998). Microparticle-enhanced nephelometric immunoassay of lysozyme in milk and other human body fluids. Clinical Chemistry, Vol. 44, pp. 1610-1615

Montagne, P., Cuillière, M.L., Molé, C., Béné, MC., Faure, G. (2001). Changes in lactoferrin and lysozyme levels in human milk during the first twelve weeks of lactation. *Adv. EXp. Med. Biol.*, Vol. 501, pp. 241-247

Murphy, B.M., Drennan, M.J., O'Mara, F.P., Earley, B. (2005). Cow serum and colostrum immunoglobulin (IgG1) concentration of five suckler cow breed types and subsequent immune status of their calves. *Irish Journal of Agricultural and Food Research,* Vol. 44, pp. 205–213

Newstead D.F. (1976) Carotene and immunoglobulin concentrations in the colostrums and milk of pasture-fed cows. *J. Dairy Res.,* Vol. 43, pp. 229-237

Okada, S., Tanaka, K., Sato, T., Ueno, H., Saito, S., Okusaka, T., Sato, K., Yamamoto, S., Kakizoe, T. (2002). Dose-response trial of lactoferrin In patients with chronie hepatitis C. *Jpn. J. Cancer Res*., Vol. 93, pp. 1063-1069

Orsi, N. (2004). The antimicrobial activity of lactoferrin: current status and perspectives. *Biometals*, Vol. 17, pp. 189–196

Pakkanen, R., Aalto, J. (1997). Growth factors and antimicrobial factors of bovine colostrum. *Inter. Dairy J.*, Vol. 7, pp. 285-297

Pandya, A.J., Khan, M.M.H. (2006). Buffalo milk. In: *Handbook of milk of non-bovine mammals*. Park Y.W., Haenlein G.F.W. (Eds), Iowa, Blackwell Publishing Professional, pp. 195-273

Park, Y.W., Juarez, M., Ramos, M., Haenlein, G.F.W. (2007). Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research*, Vol. 68, pp. 88–113

Piccinini, R., M. Mirelli, B. Ferri, C. Tripaldi, M. Belotti, V. Dapra, S. Orlandini, and A. Zecconi. 2006. Relationship between cellular and whey components in buffalo milk. *J. Dairy Res*., Vol. 73, pp. 129-133

Proctor, V.A., Cunningham, F.E. (1988). The chemistry of lysozyme and its use as a food preservative and a pharmaceutical. *Crit. Rev. Food Sci. Nutr*., Vol. 26, No. 4, pp. 359-395

Rawal, P., Gupta, V., Thapa, B.R. (2008). Role of colostrum in gastrointestinal infections. *Indian J. Pediatr*., Vol. 75, No. 9, pp. 917-921

Reklewska, B., Bernatowicz, E., Reklewski, Z., Nałęcz-Tarwacka, T., Kuczyńska, B., Zdziarski, K., Oprządek, A. (2003). Biological active proteins content in milk of cows in relations to feeding system and season. *Zesz. Nauk. Przeg. Hod*. 68, 85-98

Satue-Gracia M.T., Frankel E.N., Rangavajhyala N., German J.B. (2000). Lactoferrin in infant formulas: effect on oxidation. *J. Agric. Food Chem*., Vol. 48, pp. 4984-4990

Semba, R.D., Miotti, P.G., Lan, Y., Chiphangwi, J.G., Hoover, D.R., Dallabetta, G.A., Yang, L.P., Saah, A.J. (1998). Maternal serum lactoferrin and vertical transmission of HIV. *AIDS*, Vol. 12, pp. 331-332

Shah, N.P. (2000). Effects of milk-derived bioactives: an overview. *British Journal of Nutrition*, Vol. 84, Suppl. 1, pp. 3-10

Singh, P.K., Parsek, M.R., Greenberg, E.P., Welsh, M.J. (2002). A component of innate immunity prevents bacterial biofilm development. *Nature*, Vol. 417, pp. 552–555

Spadaro, M., Caorsi, C. Ceruti, P., Varadhachary, A., Forni, G., Pericle, F. Giovarelli, M. (2008). Lactoferrin, a major defense protein of innate immunity, is a novel

maturation factor for human dendritic cells. *The FASEB Journal*, Vol. 22, pp. 2747-2757

Steijns, J.M., Hooijdonk, A.C.M. (2000). Occurrence, structure, biochemical properties and technological characteristics of lactoferrin. *British Journal of Nutrition*, Vol. 84, Suppl. 1, pp. 11-17

Stelwagen, K., Carpenter, E., Haigh, B., Hodgkinson, A., Wheeler, T.T. (2009). Immune components of bovine colostrum and milk. *J. Anim Sci.*, Vol. 87, pp. 3-9

Struff, W.G., Sprotte, G. (2008). Bovine colostrum as a biologic in clinical medicine: a review – part II: clinical studies. *Int. J. Clin. Pharmacol. Ther.*, Vol. 46, No 5, pp. 211-225

Sung, K., Khan, S.A., Nawaz, M.S., Cerniglia, C.E., Tamplin, M.L., Philips, R.W., Kelley, L.C. (2011). Lysozyme as a barrier to growth of Bacillus anthracis strain Sterne in liquid egg white, milk and beef. *Food Microbiology*, Vol. 28, pp. 1231-1234

Superti, F., Ammendolia M.G., Valenti P., Seganti L. (1997). Antirotaviral activity of milk proteins: lactoferrin prevents rotavirus infection in the enterocyte-like cell line HT-29. *Med. Microbiol. Immunol.*, Vol. 186, pp. 83-91

Szwajkowska M., Wolanciuk A., Barłowska J., Król J., Litwińczuk Z. (2011) Bovine milk proteins as the source of bioactive peptides influencing the consumers' immune system – a review. *Animal Science Papers and Reports*, Vol. 29, No. 4, pp. 269-280

Tsuji, S, Hirata, Y, Mukai, F, Ohtagaki, S (1990). Comparison of lactoferrin content in colostrum between different cattle breeds. *J. Dairy Sci.*, Vol. 73, pp. 125-128

Turner, S.A., Williamson, J.H., Roche, J.R., Kolver, E.S. (2003). Diet and genotype affect milk lactoferrin concentrations in late lactation. *Proceedings of the New Zealand Society of Animal Production*, Vol. 63, pp. 87-90

Turner, S.A., Thomson, N.A., Auldist, M.J. (2007). Variation of lactoferrin and lactoperoxidase in bovine milk and the impact of level of pasture intake. New *Zealand Journal of Agricultural Research*, Vol. 50, pp. 33-40

Urech, E., Z. Puha, and M. Schallibaum (1999). Changes in milk protein fraction as affected by subclinical mastitis. *J. Dairy Sci.*, Vol. 82, pp. 2402-2411

Van der Strate, B.W.A., Beljaars, L., Molema, G., Harmsen, M.C., Meijer, D.K.F. (2001). Antiviral activities of lactoferrin. *Antiviral Res.*, Vol. 52, pp. 225–239

Wakabayashi, H., Yamauchi, K., Takase, M. (2006). Lactoferrin research, technology and applications. *Inter. Dairy J.*, Vol. 16, pp. 1241-1251

Wedholm, A., Hallén, E., Larsen, L.B., Lindmark-Månsson, H., Karlsson, A.H., Allmere, T. (2006). Comparison of milk protein composition in a Swedish and a Danish dairy herd using reversed phase HPLC. *Acta Agriculturae Scand Section A*, Vol. 56, 8-15

Wheeler, T.T., Hodgkinson, A.J. (2007). Immune components of colostrums and milk – a historical perspective. *Journal Mammary Gland Biol. Neoplasia*, Vol. 12, 237-247

Wielgosz-Groth, Z., Sobczuk-Szul, M., Wroński, M., Rzemieniewski, A. (2009). Season and production level on composition of proteins in milk of Polish Holstein-Friesian. *Biul. Nauk.*, Vol. 30, pp. 135-139

Zimecki, M., Artym, J. (2005). Therapeutic properties of proteins and peptides from colostrum and milk. *Postepy Hig. Med. Dosw.*, Vol. 59, pp. 309-323

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