Cow's whey proteins involvement in the development of the immunological system and gastrointestinal tract in calves. A review

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ABSTRACT. Milk is a complex biological fluid that ensures the correct growth and development of young mammals. Depending on the stage of lactation, milk is classified as colostrum secreted three days post-parturition and milk produced 72 h after the delivery and they have slightly different nutrient and protein composition. It was shown that the proteome of bovine milk is represented by over 4500 different proteins. In the present paper by using a gene ontology analysis (String analysis), proteins identified in bovine colostrum and milk were grouped into two categories: proteins involved in immunological processes and proteins associated with digestive system development. Among these proteins, proteins characteristic to either colostrum or milk were identified, among others kininogen-2 and cathepsin L1. The constant development of proteomic techniques leads to the identification of novel cow's whey proteins that have not been found yet. The new, low- and medium-abundant proteins help to better understand the physiological process of calf growth and maturation.

Introduction

After birth, the newborn's living environment is completely changed. From the intra-uterine sterile atmosphere, it is transited to the extra-uterine habitat, where neonate is exposed to different pathogens. Due to its unique composition, milk has bioactive properties, which facilitate to exchange of the newborn's life environment (Golinelli et al., 2011). Milk is a complex biological fluid that consists of macronutrients such as proteins, fat, carbohydrates and micronutrients like minerals and vitamins. Milk is essential for young mammals and ensures correct growth and development (Le et al., 2011). The most important components of milk are proteins which provide not only amino acids but also

regulating factors (e.g., growth factors, cytokines, immunoglobulins, enzymes).

Similar to other biological fluids, the protein profile of bovine milk subjects dynamic changes at a time (Le et al., 2011). Depending on the stage of lactation, milk is classified as colostrum secreted up to three days post-parturition and mature milk is produced 72 h after the delivery. Moreover, milk can be divided according to the milking periods: colostrum, early lactation, peak, mid-lactation and dried up period. Each period has a slightly different nutrient and protein composition (Gopal and Gill, 2000; Golinelli et al., 2011).

It was shown recently that the bovine colostrum and milk proteome are represented by over 4500 different proteins (Delosière et al., 2019). Bovine

milk is composed of high-abundant proteins such as caseins (α-, β-, γ- and κ-caseins), representing about 80% of total proteins of bovine milk (Yamada et al., 2002; Le et al., 2011; Zhang et al., 2011; Delosière et al., 2019). Furthermore, in the milk, fractions such as whey proteins, milk fat globule proteins and exosomes proteins can be distinguished.

Unlike bovine milk, the main protein fraction of colostrum is antibodies (immunoglobulins (Ig): IgG, IgA, IgM (Le et al., 2011)). Moreover, colostrum contains other immune-regulating elements, antimicrobial compounds, absent or present in lower concentrations in bovine milk. These colostrum components provide protection for the newborn calf, while the immune system is still developing (Gopal and Gill, 2000).

Basic analysis of the milk proteomics includes investigation of the high-abundant proteins which are present at high levels (caseins, α-lactalbumin, β-lactoglobulin) and are easily detected (Zhang et al., 2015). The low-abundant proteins are difficult to detect under proteomic analysis and require the removal of high-abundant proteins (O'Donnell et al., 2004).

In the current paper basing on available cow's colostrum and milk proteomic research, and using the String bioinformatic tool, whey proteins involved in the development of the immune system (Table 1) and digestive system (Table 2) were choosen.

Methods

Based on the available literature, data about cow's colostrum and early milk proteins was collected. In proteomic studies of bovine milk, the following techniques are applied: western blot (Nemir et al., 2000), ELISA test (Schack et al., 2009), liquid chromatography-tandem mass spectrometry (Zhang et al., 2011, 2015; Tacoma et al., 2016; Sun et al., 2019), as well as 2-D electrophoresis coupled *via* mass spectrometry of MALDI-TOF type (Golinelli et al., 2011). In the present paper, using the String bioinformatic tool, proteins were divided according to their biological role. The analysis showed that the largest group are proteins involved in the immunological process (Table 1). In the milk, there are also proteins associated with the development of the digestive system of the newborn calves. Moreover, basing on the collected data, we determined the presence (or absence) of the proteins in colostrum and milk (Table 3). It allowed for predicting proteins characteristic for colostrum or milk.

Proteins involved in the immune response

After being born, young calves are immunologically 'naïve' and sensitive to pathological microorganisms, which may cause lots of health problems, especially respiratory and bowel diseases. Immunological protection is provided by the ingestion of colostrum (Chase et al., 2008).

The main fraction of colostrum proteins are immunoglobbulins, among which the most prominent are IgG (Yamada et al., 2002). Immunoglobulins are transferred to newborns along with colostrum (milk). It provides passive immunity to the calves until their immune system is fully developed and is necessary for their proper growth (Godden, 2008). The concentration of immunoglobulins in bovine milk decreases during the subsequent days of lactation. The concentration of IgG in cow's milk highly decreases during the first 3 days after calving, while the IgM concentration gradually decreases until day 7 after delivery (Yamada et al., 2002; Zhang et al., 2011, 2015).

Sun et al. (2019) using liquid chromatography with tandem mass spectrometry demonstrated that the cow's whey proteins are mainly involved in complement and coagulation cascades, antigen processing and presentation. Proteomics analysis showed that in cow's colostrum and milk are proteins associated with complement system activation in the classical pathway (complement component 1, C1; complement component 3, C3; complement component 4, C4; complement component 7, C7; complement component 9, C9), alternative pathway (complement factor B, CFB; complement factor D, CFD) and lectin complement pathway (mannose-binding lectin 2, MBL2). Moreover, in the serum of cow's milk are present proteins precipitating in the regulation of complement system such as antithrombin-III (SERPINC1), C1 inhibitor (SERPING1), α-1-antiproteinase (SERPINA1), clusterin (CLU) and vitronectin (VTN), α-2-macroglobulin (A2M) (Table 1). The main role of the complement system is to eliminate pathogens by causing cytolytic membrane damage, opsonization the accelerating process of immunofagocytosis, and initiation and control of the inflammatory response. All three ways of activating the complement system leading to the formation of C3 convertase. Due to activation of the cascade, C3 convertase forms a membrane attack complex (MAC), which creates numerous pores in the cell membrane (Nesargikar et al., 2012; Tacoma et al., 2016; Sun et al., 2019).

Table 1. Proteins involved in the immunological processes in calves.

Protein name	Gene name	Protein molecular weight, kDa	Isoelectric point	Reference
Complement system process				
CD59 molecule	*CD59*	14	7.97	Zhang et al. (2015), Le et al. (2011)
complement C1q A chain / B chain	*C1QA/C1QB*	26	9.11/ 9.53	Le et al. (2011)
complement C1r	*C1R*	80	5.86	Tacoma et al. (2016), Le et al. (2011)
complement C1s	*C1S*	77	4.97	Le et al. (2011)
complement C2	*C2*	83	8.67	Le et al. (2011)
complement C3	*C3*	187	6.41	Zhang et al. (2015), Yamanda et al. (2002)
complement t C4A	*C4A*	102	6.15	Le et al. (2011)
complement C5	*C5*	189	6.19	Le et al. (2011)
complement C6	*C6*	105	6.73	Tacoma et al. (2016), Zhang et al. (2015), Le et al. (2011)
complement C7	*C7*	93	6.91	Tacoma et al. (2016), Zhang et al. (2015)
complement C8	*C8B*	66	6.02	Le et al. (2011)
complement C9	*C9*	62	5.66	Tacoma et al. (2016), Zhang et al. (2015)
complement factor B	*CFB*	85	7.87	Tacoma et al. (2016), Zhang et al. (2015), Le et al. (2011)
complement factor D	*CFD*	28	7.64	Tacoma et al. (2016), Le et al. (2011)
complement factor H	*CFH*	140	6.43	Tacoma et al. (2016), Zhang et al. (2015)
complement factor I	*CFI*	69	8.07	Le et al. (2011)
complement factor properdin	*CFP*	51	8.32	Le et al. (2011)
mannose-binding lectin 2	*MBL2*	26	5.11	Le et al. (2011)
serpin family C member 1 (antithrombin-III)	*SERPINC1*	52	7.01	Tacoma et al. (2016), Zhang et al. (2015)
serpin family G member 1 (C1 inhibitor)	*SERPING1*	52	6.20	Tacoma et al. (2016), Zhang et al. (2015)
vitronectin	*VTN*	54	5.92	Tacoma et al. (2016)
vimentin	*VIM*	54	5.05	Le et al. (2011)
Antibacterial properties				
cathelicidin-1	*CATHL1*	18	7.58	Tacoma et al. (2016), Zhang et al. (2015), Le et al. (2011)
cathelicidin-2	*CATHL2*	20	9.19	Tacoma et al. (2016), Zhang et al. (2015)
cathelicidin-3	*CATHL3*	22	10.87	Tacoma et al. (2016), Zhang et al. (2015), Le et al. (2011)
cathelicidin-4	*CATHL4*	16	6.29	Zhang et al. (2015)
cathelicidin-5	*CATHL5*	18	8.37	Zhang et al. (2015), Le et al. (2011)
cathelicidin-6	*CATHL6*	18	9.39	Tacoma et al. (2016), Zhang et al. (2015), Le et al. (2011)
Immune system process				
orosomucoid 1 (α-1-acid glycoprotein)	*ORM1*	23	5.62	Tacoma et al. (2016), Zhang et al. (2015)
serpin family A member 1 (α-1-antiproteinase)	*SERPINA1*	46	6.05	Zhang et al. (2015), Le et al. (2011)
αlpha-1β-glycoprotein	*A1BG*	54	5.30	Tacoma et al. (2016), Zhang et al. (2015), Golinelli et al. (2011), Le et al. (2011)
α-2-HS-glycoprotein	*AHSG*	38	5.26	Tacoma et al. (2016), Zhang et al. (2015), Le et al. (2011)
α-2-macroglobulin	*A2M*	168	5.71	Tacoma et al. (2016), Zhang et al. (2015), Le et al. (2011)
apolipoprotein A1	*APOA1*	30	5.71	Tacoma et al. (2016), Zhang et al. (2015)
apolipoprotein E	*APOE*	36	5.55	Tacoma et al. (2016), Zhang et al. (2015)
apolipoprotein A2	*APOA2*	11	7.80	Tacoma et al. (2016), Zhang et al. (2015)
β-1,4-galactosyltrasferase 1	*B4GALT1*	45	9.38	Zhang et al. (2015)
β-2-microglobulin	*B2M*	14	7.79	Tacoma et al. (2016), Zhang et al. (2015)
cathepsin L1	*CTSL1*	37	6.55	Tacoma et al. (2016), Le et al. (2011)
cathepsin S	*CTSS*	37	6.85	Tacoma et al. (2016), Le et al. (2011)
chemokine (C-X-C motif) ligand 2	*CXCL2*	11	10.17	Le et al. (2011)
chitinase 3-like 1	*CHI3L1*	43	8.87	Yamanda et al. (2002)
clusterin	*CLU*	51	5.73	Zhang et al. (2015), Le et al. (2011)
coronin 1A	*CORO1A*	51	6.25	Le et al. (2011)
FKBP propyl isomerase 1A	*FKBP1A*	12	7.87	Le et al. (2011)

Table 1. continued

Protein name	Gene name	Protein molecular weight, kDa	Isoelectric point	Reference
haptoglobin	*HP*	45	7.83	Tacoma et al. (2016), Zhang et al. (2015)
hemopexin	*HPX*	52	7.90	Tacoma et al. (2016), Le et al. (2011)
high-mobility group box 1	*HMGB1*	25	5.61	Le et al. (2011)
high-mobility group box 2	*HMGB2*	24	7.62	Tacoma et al. (2016), Le et al. (2011)
joining chain of multimeric IgA and IgM	*JCHAIN (IGJ)*	18	5.07	Zhang et al. (2015), Le et al. (2011)
immunoglobulin kappa chain	*IGK*	27	6.08	Le et al. (2011)
immunoglobulin lambda like polypeptide 1	*IGLL1*	23	10.10	Le et al. (2011)
immunoglobulin M	*IGHM*	50	5.32	Zhang et al. (2014), Yamada et al. (2002)
Inflammatory response				
inter-α-trypsin inhibitor heavy chain 1	*ITIH1*	101	6.98	Tacoma et al. (2016), Zhang et al. (2015)
inter-α-trypsin inhibitor heavy chain 2	*ITIH2*	6	9.23	Tacoma et al. (2016), Zhang et al. (2015)
inter-α-trypsin inhibitor heavy chain 4	*ITIH4*	102	6.22	Tacoma et al. (2016), Zhang et al. (2015)
inter-α-trypsin inhibitor heavy chain 3	*ITIH3*	100	5.59	Tacoma et al. (2016)
inter-α-trypsin inhibitor heavy chain H5	*ITIH5*	104	8.75	Tacoma et al. (2016)
interleukin 10 receptor, subunit β	*IL10RB*	36	5.93	Tacoma et al. (2016)
interleukin 17 receptor E-like	*IL17REL*	35	9.27	Tacoma et al. (2016)
interleukin 34	*IL34*	26	7.56	Tacoma et al. (2016)
kininogen 1	*KNG1* (KNG1)	69	6.14	Zhang et al. (2015)
kininogen 2	*KNG2* (KNG2)	69	6.09	Le et al. (2011)
lactoperoxidase	*LPO*	81	8.83	Zhang et al. (2015), Le et al. (2011)
lactotransferrin	*LTF*	78	8.69	Zhang et al. (2015), Le et al. (2011), Yamada et al. (2000)
lipopolysaccharide-binding protein	*LBP*	54	6.61	Zhang et al. (2015)
mannosidase α class 2B member 1 (lysosomal α-mannosidase)	*MAN2B1*	113	9.16	Le et al. (2011)
macrophage migration inhibitory factor	*MIF*	12	7.68	Le et al. (2011)
mannose-binding lectin 2	*MBL2*	26	5.11	Le et al. (2011)
matrix metalloproteinase 9	*MMP9*	79	5.58	Tacoma et al. (2016), Le et al. (2011)
CD14 molecule	*CD14*	40	5.37	Zhang et al. (2015)
secreted protein acidic and cysteine rich (osteonectin)	*SPARC (ONT)*	35	4.71	Le et al. (2011)
secreted phosphoprotein 1 (osteopontin)	*SPP1 (OST)*	31	4.49	Zhang et al. (2015), Le et al. (2011)
peptidoglycan recognition protein 1	*PGLYRP1*	21	9.59	Zhang et al. (2015), Le et al. (2011)
proliferation-associated 2G4	*PA2G4*	44	6.13	Tacoma et al. (2016)
α-1-microglobulin	*AMBP*	39	7.81	Zhang et al. (2015)
Parkinsonism associated deglycase	*PARK7*	20	6.84	Le et al. (2011)
S100 calcium binding protein A12	*S100A12*	11	5.91	Le et al. (2011)
quiescin sulfhydryl oxidase 1	*QSOX1*	63	9.32	Tacoma et al. (2016), Le et al. (2011)
superoxide dismutase 1	*SOD1*	16	5.85	Le et al. (2011)
transforming growth factor β 1	*TGFB1*	44	8.97	Tacoma et al. (2016)
transforming growth factor β 2	*TGFB2*	48	8.82	Tacoma et al. (2016), Le et al. (2011)

Table 2. Milk proteins involved in the development of the calves digestive system

Protein name	Gene name	Protein molecular weight, kDa	Isoelectric point	Reference
Myostatin (growth/differentiation factor 8)	*MSTN*	43	6.14	Zhang et al. (2015)
Insulin-like growth factor-binding protein 2	*IGFBP2*	34	7.13	Tacoma et al. (2016)
Insulin-like growth factor-binding protein 5	*IGFBP5*	30	8.72	Tacoma et al. (2016)
Insulin-like growth factor-binding protein 7	*IGFBP7*	29	8.25	Tacoma et al. (2016)
Lipoprotein lipase	*LPL*	53	8.77	Zhang et al. (2015)
Ribonuclease, RNase A family, 1 (pancreatic)	*RNASE1*	16	8.93	Zhang et al. (2015), Le et al. (2011)
Transforming growth factor β 1	*TGFB1*	44	8.97	Tacoma et al. (2016)
Transforming growth factor β 2	*TGFB2*	48	8.82	Tacoma et al. (2016), Le et al. (2011)
Xanthine dehydrogenase	*XDH*	147	7.97	Zhang et al. (2015), Le et al. (2011)

As shown in Table 3 the concentration of proteins involved in the complement system activation (C3, C7, C9, CFB) is higher in colostrum in comparison to milk.

Analyzing proteins profile of cow's colostrum and milk (Golinelli et al., 2011; Le et al. 2011; Zhang et al., 2011, 2015; Tacoma et al., 2016; Sun et al., 2019) we observed the presence of a mannose-binding lectin (MBL) (Table 1). MBL2 is a protein that participates in the lectin complement pathway (LCP) and is structurally similar to the C1q factor (Collard et al., 2000). MBL2 belongs to collagenous C-type lectins and binds to mannose and N-acetylglu-cosamine residues present on the surface of bacteria.

Table 3. Comparison of proteins concentrations levels in colostrum and milk (Golinelli et al., 2011; Le et al., 2011; Zhang et al., 2011; Zhang et al., 2015; Tacoma et al., 2016; Sun et al., 2019)

Gene name	Protein concentration		Gene name	Protein concentration	
	colostrum	milk		colostrum	milk
A1BG	++	+	IGFBP2	no data	+
A2M	++	+	IGFBP5	no data	+
AHSG	+	+	IGFBP7	+	+
AMBP	+	+	JCHAIN	++	+
APOA1	++	+	IGK	++	+
APOA2	+	+	IGLL1	++	+
APOE	++	+	IGHM	++	+
B2M	+	+	IL10RB	no data	+
B4GALT1	+	+	IL17REL	no data	+
C1QA/C1QB	+	+	IL34	no data	+
C1R	+	+	ITIH1	++	+
C1S	+	+	ITIH2	++	+
C2	+	+	ITIH3	no data	+
C3	++	+	ITIH4	+	+
C4A	+	+	ITIH5	no data	+
C5	+	+	KNG1	+	+
C6	+	+	KNG2	−	+
C7	++	+	LBP	++	+
C8	+	+	LPL	+	++
C9	++	+	LPO	+	++
CATHL1	++	+	LTF	+	+
CATHL2	+	+	MAN2B1	−	+
CATHL3	+	+	MBL2	+	+
CATHL4	+	+	MIF	+	+
CATHL5	+	+	MMP9	+	+
CATHL6	+	+	MSTN	++	+
CD14	++	+	ORM1	++	+
CD59	++	+	PA2G4	no data	+
CFB	++	+	PARK7	+	+
CFD	+	+	PGLYRP1	++	+
CFH	+	+	QSOX1	−	+
CFI	+	+	RNASE1	++	+
CFP	+	+	S100A12	+	+
CHI3L1	+	−	SERPINA1	++	+
CLU	++	+	SERPINC1	++	+
CORO1A	+	+	SERPING1	+	+
CTSL1	−	+	SOD1	+	+
CTSS	+	+	SPARC (ONT)	+	+
CXCL3	+	+	SPP1 (OST)	++	+
FKBP1A	+	+	TGFB1	no data	+
HMGB1	+	−	TGFB2	+	+
HMGB2	+	+	VIM	+	+
HP	+	+	VTN	no data	+
HPX	++	+	XDH	+	++

'+' presence of protein, '++' high concentration of protein, '−' absence of protein

Activation of the complement system by the MBL complex is related to mannose-binding lectin-associated serine proteases (MASAP-1 and MASAP-2), which allow the cleavage of C2 and C4 factors to form C3 convertase (Matsuhita et al., 2000). In contrast to the classical complement pathway, LCP activation does not require the participation of antibodies or C1 factor (Collard et al., 2000).

Apart from proteins involved in the formation of MAC (C3, C6, C7, C9), in cow's milk protein profile are present proteins protecting against lysis initiated by the membrane attack complex, e.g., CD59 molecule, (CD59) (Table 1). The CD59, also known as a MAC inhibitory factor, is a membrane glycoprotein attached to the cell membrane *via* aglycosylphosphatidylinositol anchor. This protein binds to the complement components Cb5–C8 (C5b–C8) complex preventing the attachment and polymerization of C9, thereby blocking the formation of MAC (Zhang et al., 2015). It has been shown that the concentration of CD59 decreases during infection, allowing the complement system to function properly (Kimberley et al., 2007). Moreover, in colostrum and milk, there is vitronectin (Table 1) involved in inhibiting MAC formation ensuring that the host cells are not lysed.

The concentration of proteins associated with the complement system (A2M, C3, C6, C7, C9, CFB and CD59) decreases with subsequent lactation days. The high concentration of complement system proteins in colostrum determines the specific response (adaptive) and the innate immune response (non-specific), supporting the immune system of newborns (Zhang et al., 2015).

Proteins involved in the immunological processes (complement system proteins and immunoglobulin) are closely related to enzymatic proteins, including protease inhibitors. Zhang et al. (2015) observed a decreasing concentration of several protease inhibitors, such as SERPINA1, α-1-microglobulin (AMBP), inter-α-tryspin inhibitor heavy chain 1–5 (ITIH1–ITIH5) during milk maturation (Table 3). The authors showed a positive correlation between decreasing abundance of enzymatic proteins and decreasing concentration of immunoglobulins in milk during the transformation of colostrum onto early milk. A simultaneous increase in the concentration of IgG and trypsin inhibitors may indicate the protective role of this enzyme protein against proteolytic degradation of IgG.

In the protein profile of cow milk are present cytokines such as interleukins and osteopontin (SPP1), which exhibits cytokine-like properties (Schack et al., 2009). Osteopontin has been shown to affect the function of macrophages, dendritic cells and T cells (Table 1). Osteopontin regulates the process of Th1 lymphocytes immunizing by inducing the interleukin-12 (IL-12) production and interleukin-10 (IL-10) secretion inhibition. According to Schack et al. (2009), a high concentration of this protein in colostrum may indicate the participation of osteopontin in the development and maturation of the newborn's immune system. Comparative analysis of colostrum and milk proteins showed that osteopontin is also present in milk (Table 3).

Moreover, in milk is present transforming growth factor β-1/2 (TGFB1/2). TGF-Bs maintaining immune tolerance and homeostasis against self and foreign antigens. TGF-βs are provided with milk until the development of the immune system of the neonate. This protein may be a key immunoregulatory factor for IgA production (Sanjabi et al., 2017).

In bovine milk serum, there are present proteins characteristic only for colostrum or milk. Among these proteins, we can distinguish kininogen 2 (encoded by *KNG*) and cathepsin L1 (encoded by *CTSL1*) which are characteristic for milk. Kininogen 2 is involved in the inflammatory response and cathepsin L1 participates in the immune system processes of the newborn calf (Tacoma et al., 2016; Le et al., 2011). On the other hand, among proteins that are present in colostrum, chitinase 3-like 1 (encoded by *CHI3L1*) and high-mobility group box 1 (encoded by *HMGB1*) can be mentioned (Yamanda et al., 2002; Le et al., 2011), both also involved in the immunological processes.

The development of the immune system is a long-term process. During the neonatal period, the production of antibodies is limited. Therefore it is necessary to provide simultaneously with milk proteins (Table 1) involved in the stimulation of the newborn's immune system. The milk proteomic research showed the presence of S100A12 protein (Le et al., 2011). Studies by Yang et al. (2001) indicate that S100A12 is a chemotactic factor for neutrophils and monocytes, and therefore is involved in the immune response, which is closely related to the function performed by colostrum.

Proteomic approaches allow for the identification of colostrum and milk low-abundant proteins

that are involved in neonate's protection against pathogens. In colostrum, antibacterial proteins cathelicidin 1–6 (CATHL1–CATHL6) belonging to the cathelicidin protein family were identified (Zhang et al., 2015). Cathelicidins are also present in the milk (Table 3). Those proteins have a capacity of binding lipopolysaccharides thereby interact with Gram-positive and Gram-negative bacteria taking part in innate immune response (Zhang et al., 2015).

Our analysis allowed the selection of proteins associated with the immune process. Collected data showed the pattern of protein concentration in colostrum and milk (Table 3).

Proteins involved in the maturation of the gastrointestinal tract and accompanying organs

Analysis with available bioinformatic tools connected with databases allowed us to indicate proteins involved in the development of the gastrointestinal tract (GIT) in calves (Table 2).

The digestive tract of newborn calves is not fully mature and requires functional and morphological changes (Blum, 2006). Gastrointestinal (GI) developmental changes aimed at undertaking the digestive function occur already during fetal life; however, the largest adaptive changes occur during the first 48 h of non-fetal life. Postnatal development of the GIT continues until the total transition to solid food (Guilloteau et al., 2009). The development of GIT is also associated with the maturation of companion organs, i.e. pancreas and liver (Blum, 2006).

Newborn calves are pseudo-monogastric, and the intestinal absorption of large protein molecules takes place *via* pinocytosis. The intensity of this process decreases within 7 days after birth. Digestion of proteins by lysosomes is replaced by digestion in the light of the GIT (Blum, 2006).

Milk is a complex fluid of the mammary gland containing many compounds supporting the development of the neonate. Growth factors and bioactive peptides may modulate the GI system functions as well as its development (Pihlanto-Leppälä, 2001).

The growth factors that have been demonstrated to be present in cow's milk include (TGF-βs) and insulin-like growth factors (IGFs) (Table 2). According to Playford et al. (2000), high levels of TGF-β in bovine milk may play a key role in maintaining the integrity of the newborn's digestive tract. The TGF-βs group includes cytokine growth/

differentiation factor 8 (MSTN), which is the dominant growth factor in bovine milk. MSTN influences development and growth of the small intestine (Zhang et al., 2015). IGFs are factors that stimulate cell proliferation and differentiation (Playford et al., 2000). IGFs are involved in the differentiation of intestinal epithelial cells in calves (Zhang et al., 2015).

Due to the maturation of the digestive system and accompanying organs, among others pancreas, the calf's digestive capacity is limited. The digestive enzymes present in the colostrum and milk support digestive processes in the newborn shown in Table 2. The use of proteomic analysis allows to demonstrate the presence of digestive enzymes, i.e. ribonuclease pancreatic, xanthine dehydrogenase and lipoprotein lipase in bovine colostrum and milk (Zhang et al., 2015).

Lipases are present in milk to help infants to digest lipids. Lipoprotein lipase (LPL) is an enzyme, the concentration of which is higher in early milk than in colostrum (Table 3). This enzyme is involved in the digestion of triglycerides and the absorption of lipids in calves (Zhang et al., 2015).

The presence of symbiotic microflora in the rumen of ruminants ensures proper utilization of plant food. Bacteria colonizing the rumen produce digestive enzymes (including cellulase), which in the final stage leads to the formation of short-chain fatty acids which are the primary source of energy for ruminants. Then, these microorganisms go along with the rumen content to further parts of the digestive system, providing, among others, large amounts of ribonucleic acids (Sassi and Benner, 2007). The group of proteins involved in the development of the digestive tract are ribonucleases, among which RNASE1 is included. This pancreatic protein is a digestive enzyme involved in the breakdown of bacterial RNA, and the uptake of nutrients in the intestines, therefore it is particularly important for ruminants (Liu et al., 2014). Research on the change of protein profile occurring during early lactation until its end showed this protein is in high concentration during the initial phase of lactation and supports the process of the GIT maturation in calves (Zhang et al., 2015).

The concentration of xanthine dehydrogenase (XDH) in the colostrum and milk showed time-dependent changes (Table 3) (Le et al., 2011; Zhang et al., 2015). XDH is an enzyme belonging to the molybdenum hydrolase family and catalyzes the oxidation of hypoxanthine to xanthine and xanthine

to uric acid. XDH can be easily transformed into xanthan oxidase (XO) through the oxidation of sulfhydryl residues or proteolysis. XO from milk catalyzes hydrogen peroxide biosynthesis, inducing the synthesis of reactive oxygen species (ROS) in response to bacterial infection (Le et al., 2011). Proteomic studies have shown that the presence of XDH in milk increases with subsequent days of lactation (Table 3) (Le et al., 2011; Zhang et al., 2015). In the opinion of Zhang et al. (2015), increased concentration of this protein may be associated with the reduction of oxidative stress in newborn calves. Oxidative stress is the result of a disturbed balance between the production of ROS and antioxidant defense. Research indicates that oxidative stress is an important factor in the occurrence of functional disorders in the calf's digestive system (Kashyap and Farrugia, 2011). The small intestine is especially sensitive to oxidative stress due to the lower concentration of enzymes that protect it from oxidative stress as compared to other segments of the GIT (Van Der Vliet et al., 1989). In addition, the increase in protein content of the XDH is associated with an increase in the concentration of lactoperoxidase (LPO), which has bactericidal properties through the ability to convert hydrogen peroxide to ROS (Table 3). According to Le et al. (2011), the combined interaction of both proteins (XDH and LPO) plays also a key role in the innate immune system of a newborn.

Conclusions

Milk is a complex fluid of the mammary gland, providing essential nutrients and regulating components to newborn calves, determining their proper growth and maturation. Bovine placenta prevents entering antibodies to the developing fetus, so newborn calves are born without innate immunity. In the development of the calf's immune system, the first 24 h of life are especially significant. In that time the intestinal barrier is open. The open barrier enables the entry of immunocompetent proteins in an unchanged form. Such a 'physiological' transfer of proteins determines the proper development of the calf immune system. Among the identified proteins in cow milk involved in immunological response were proteins associated with the complement system, taking part in the inflammatory process and general stimulation of the immune system.

Moreover, after birth ruminant's digestive system is not fully mature and requires functional and morphological changes enabling the proper digestion of nutrients. So, in colostrum and milk, proteins involved in the development of the gastrointestinal tract and competitive organs are present.

The cow's milk proteome is subjected to dynamic changes depending on the time of lactation. Both colostrum and milk contain proteins characteristic only for this given stage of milk maturity. The observed differences result from the changing needs of developing infants.

The constant development of proteomic techniques leads to the identification of novel cow's whey proteins that have not been found yet. The new low-abundant proteins help to better understand the physiological process of calf growth and maturation.

Conflict of interest

The Authors declare that there is no conflict of interest.

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