Glucose transporters in cattle - a review*

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Glucose is the major energy source for the animal cells. It is important substrate for protein and lipid synthesis. This sugar is absorbed into the cells from body fluids via glucose transporters – structurally related trans-membrane proteins. There are two types of glucose transport - passive and active. The facilitative-glucose-transporter family of proteins (solute co-transporters GLUT, encoded by \textit{SLC2A} genes) participates in energy-independent process of glucose transport. The Na\(^+\)/glucose co-transporter family proteins SGLT (solute carriers, encoded by \textit{SLC5A} genes) mediate the Na\(^+\)-linked transport of glucose against the electrochemical gradient. In this review, we describe genomic structure and function of the bovine glucose transporters. Intra-species comparative analyses of the amino acid identities of glucose transporter proteins is also described, as well as the information on the nucleotide sequence polymorphisms in the bovine glucose transporter genes.

KEY WORDS: cattle / glucose transporters / intra-species comparison

Introduction

Glucose, a monosaccharide is an important carbohydrate substrate for both protein and lipid synthesis. It derives directly from hydrolysis of ingested disaccharides and polysaccharides or is synthesized from other substrates in animals’ organs, mostly in liver. Cells use glucose as a primary source of energy and as a metabolic intermediate. Glucose may be converted either into glycogen or triacylglycerols which are

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subsequently stored within tissues or, in the mammary gland, into lactose. Glucose is a ubiquitous fuel in biology. It is used as an energy source in most organisms, from bacteria to humans. Through glycolysis and later in the reactions of the citric acid cycle, glucose is oxidized to eventually form CO$_2$ and water, yielding energy sources, mostly in the form of ATP, and reducing power in the form of NADPH, through the pentose phosphate.

The insulin actions, hormonal changes and other mechanisms, regulate the concentration of glucose in the blood. A high-fasting blood sugar level is an indication of pre-diabetic and diabetic conditions. Glucose is a primary source of energy for the brain, and transport of this nutrient from blood to the brain is limited by the blood-brain barrier glucose transport system [Takata et al. 1990]. Regulation of glucose transport in response to environmental signals is complex and varies according to cell type and to the external stimuli.

Skeletal muscle is a major consumer of glucose in the body. In the muscle tissue, glucose may be either oxidized or stored as glycogen. Adipose tissue in ruminants represents only a minor target for glucose disposal accounting for some 1% total glucose utilization.

Glucose is a principal precursor of lactose, the major milk carbohydrate. The mammary gland itself cannot synthesize glucose from other precursors because of the lack of glucose-6-phosphatase enzyme [Threadgold and Kuhn 1979]. Therefore, the mammary gland is totally dependent on the blood supply for its glucose needs; during lactation it utilizes 60-85% of the total glucose that enters blood. In lactating cow, 72 g of glucose is required to produce 1 kg of milk [Kronfeld 1982]. The increased glucose demand for lactation is accomplished by increased glucose transporter expression in mammary gland tissues from pregnancy to early lactation [Zhao and Keating 2007].

Glucose transport across the plasma membranes of mammalian cells is carried out by two distinct processes employing the passive, facilitative, energy-independent glucose transporters GLUT, encoded by SLC2A genes, and the active sodium-dependent and energy-dependent glucose transporters SGLT, encoded by SLC5A genes. Moreover, the facilitative glucose transporters can be divided into two families: insulin-sensitive (GLUT4) and insulin non-sensitive (GLUTs: 1, 2, 3 and 5) [Mueckler 1994]. Each glucose transporter plays specific role in cellular metabolism, which is determined by its tissue and substrate-specificity and expression in different physiological states [Thorens 1996].

Most of the studies of the structure and function of glucose transporters and their genes were carried out with humans or laboratory animals – mice and rats. There are relatively little studies carried out of the bovine glucose transporters and the genes encoding these proteins.

In this review, we describe genomic structure, protein structure and function of the bovine glucose transporters as well as their intra-species comparative analyses with the pairwise basic local alignment search tool (BLAST, (http://blast.ncbi.nlm.nih.gov/Blast.cgi).) used for analysis the amino acid identities of glucose transporter proteins.
In addition, the information is given on the nucleotide sequence polymorphisms in the bovine glucose transporter genes as well as some basic information about regulation of these genes in ruminants. This information could be useful for further studies of glucose transporter genes as molecular markers of production and functional traits in farm animals.

**Facilitative-glucose-transporters (GLUT)**

The family of facilitative energy-independent glucose transporters GLUTs (solute carriers; gene symbol *SLC2A*) consists of proteins which utilize the diffusion gradient of glucose (and other sugars) across the cell plasma membrane in the energy-independent process. They exhibit different substrate specificities and tissue expression profiles [Wood and Trayhurn 2003]. These transporters are structurally conserved and related, consisting of 12 trans-membrane domains with both amino and carboxy-terminals located in the cytoplasm (Fig. 1), and N-glycosylation sites located on either

![Fig. 1. Schematic membrane topology of GLUTs.](image)

![Fig. 2. Unrooted phylogenetic tree of the 12 bovine members of the GLUT family transporters (generated with the use of phylogeny program - http://www.ebi.ac.uk/ Tools/phylogeny/ clustalw2_phylogeny/).](image)
the first or ninth extracellular loop. In humans there have been found 13 functional facilitative glucose transporters, described as GLUT1 – 12 and \( \text{H}^+/\text{myo-inositol} \) cotransporter (HMIT) [Joost and Thorens 2001]. Also in the cattle, 13 analogous GLUT proteins and SLC2A genes, as well the SLC2A13 gene encoding HMIT protein, have been identified. Some properties of 11 bovine GLUTs and HMIT are presented in Tables 1 and 2. The GLUT family of sugar transports is divided into three classes. Class I includes GLUT1, GLUT4, GLUT3, and GLUT2 which are 65, 66 and 54% identical in bovine. Class II is comprised of GLUT9, GLUT11 and GLUT5 (fructose transporter), which are 56% and 43% identical. Class III is composed of GLUT6, GLUT8, GLUT10, GLUT12 and HMIT, with their amino acid sequences identical in 43, 63, 26 and 29%, respectively. All of them have a characteristic glycosylation site on loop 9. The phylogenetic tree of 12 members of the bovine SLC2A family of glucose transporters is shown in Figure 2.

Table 1. Summary of the properties of facilitative glucose transporter and \( \text{Na}^+/\text{Glucose} \) co-transporter family members

<table>
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<th>Gene length (bp)</th>
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**GLUT1**

GLUT1 is the insulin-independent glucose transporter. The bovine SLC2A1 gene encoding GLUT1 protein is composed of 10 exons and localizes to the bovine chromosome 3 (BTA3). Its sequence is available in the GenBank database under Acc. No. AC_000160. In the GenBank SNP database are recorded 45 SNPs (single nucleotide polymorphisms) for SLC2A1 gene: 41 in introns, one in 5'-untranslated region, and 3 in exons. The full-length of the bovine GLUT1 transcript has 2,533 nucleotides (nt). The GLUT1 protein consists of 492 amino acids (a.a), with a predicted molecular weight of 54 kDa [Zhao et al. 1996]. GLUT1 was detected with different molecular weights in lactating mammary gland and in dry stages, probably due to differential posttranslational modifications. In a pair wise basic local alignment search tool (BLAST) analysis the amino acid identities relative to GLUT1 varies from 54% for GLUT2 to 31% for GLUT12 and 29% for HMIT (Suppl. Fig. S1).The deduced amino acid sequence of bovine GLUT1 (Acc. No. NP_777027) is 99% identical to that of ovine, 97% with human, 97% with mouse, 97% with rat, 97% with dog, and 88% with chicken (Suppl. Fig. S2).
In bovine GLUT1 the unique proline-rich sequence was found between the putative first two transmembrane domains, near to the N-glycosylation site, at Asn\(^{45}\) [Boado and Pardridge, 1990]. As learned from the topology model, bovine GLUT1 has modified a.a. residues - phosphothreonine Thr\(^{234}\) between 6 and 7 transmembrane domains and phosphoserine Ser\(^{490}\) in the cytoplasmic domain (Fig. 3).

In the bovine, the GLUT1 has been detected in the mammary gland, kidney, omental fat and skeletal muscle [Zhao et al. 1999], in fetal tissues [Hocquette et al. 2006], follicle and corpus luteum [Nishimoto et al. 2006]. The expression level of GLUT1 in bovine ovary was comparable to its expression in brain. In bovine cortical arteries, GLUT1 was shown to be responsible for basal glucose transport [Nishizaki and Matsuoka 1998].

**GLUT2**

The bovine SLC2A2 gene encoding GLUT2 protein is composed of 11 exons and localizes to the chromosome 1 (BTA1). Its sequence is available in the GenBank database under Acc. No. AC_000158. In the GenBank SNP database are recorded 216 SNPs for SLC2A2 gene: 202 in introns, 1 in 5’-untranslated region, 3 in exons, and 10 in 3’-untranslated region. The full-length bovine GLUT2 transcript has 2,707 nt; GLUT2 protein contains 510 a.a. with a predicted molecular weight of 56 kDa. The deduced amino acid sequence of bovine GLUT2 (NP_001096692) is 97, 90, 81, 81, 80 and 62% identical to that of ovine, porcine, human, mouse, rat and chicken, respectively. (Suppl. Fig. S3). With the topology model, bovine GLUT2 has N-glycosylation site at Asn\(^{62}\) between transmembrane domains 2 and 3 (Fig. 4).

In cattle, the low affinity glucose transporter GLUT2 was shown to be involved in the regulation of insulin secretion from β-cells, in the release of hepatic glucose, in the release of absorbed and reabsorbed glucose in the small intestine jejunal region, and kidney [Zhao et al. 1993, 1998, Liao et al. 2010].
The bovine *SLC2A3* gene, coding for the GLUT3 protein, is composed of 11 exons and localizes to the chromosome 5 (BTA5). Its sequence is available in the GenBank database under Acc. No. NP_777028. In the GenBank SNP database 26 SNPs for *SLC2A3* gene are recorded: 17 in introns, 1 in 5'-untranslated region, 1 in exon, and 7 in 3'-untranslated region. The full-length bovine GLUT3 transcript has 4,066 nt; it encodes the 494-a.a. protein, with the predicted molecular weight of 56 kDa. The deduced amino acid sequence of bovine GLUT3 (NP_777028) is 99, 99, 90, 86, 83, 83 and 75% identical, respectively, with that of ovine, caprine, dog, human, mouse, rat and chicken (Suppl. Fig. S4). As shown in the topology model (Fig. 5),
bovine GLUT3 has two N-glycosylation sites, between transmembrane domains 1 and 2 (Asn$^{43}$) and transmembrane domains 9 and 10 (Asn$^{358}$), and another modified a.a. residue - phosphoserine Ser$^{485}$ in the cytoplasmic domain (not shown).

GLUT3 is a high affinity transporter; it is a major brain neuronal glucose transporter. The mRNA encoding GLUT3 was detected in bovine follicles and corpus luteum. The expression level of GLUT3 in bovine ovary was shown comparable to that in brain [Nishimoto et al. 2006].

GLUT4

GLUT4 is an insulin-responsive glucose transporter. The bovine GLUT4-encoding SLC2A4 gene is composed of 11 exons and localizes to the chromosome 19 (BTA19). Its sequence is available in the GenBank database under Acc. No. AC_000176. In the GenBank SNP database are recorded 5 SNPs for SLC2A4 gene, all of them located in introns. The full-length bovine GLUT4 transcript has 1,543 nt; the protein contains 509 a.a., with a predicted molecular weight of 55 kDa [Abe et al. 1997]. Western blot analyses showed the difference in the GLUT4 molecular weight between skeletal muscle (48 kDa) and adipose tissue (53-55 kDa), probably due to different post-translation modifications, such as glycosylation [Zhao et al. 1993]. The deduced amino acid sequence of the bovine GLUT4 (NP_777029) is 93, 93, 93 and 93% identical, respectively, with that of human, pig, dog, mouse and rat GLUT4 (Suppl. Fig. S5). In the bovine GLUT4 there is an N-linked glycosylation site at Asn$^{57}$, highly conserved with human GLUT4, located between putative transmembrane domains 1 and 2 (Fig. 6). The nucleotide sequence of mRNA coding for the 38 a.a. of the terminal domain of insulin-responsive glucose transporter GLUT4 has 91-97% identity with that in sheep, goat and pig. There was found one amino acid conversion

Fig. 6. Structure of GLUT4 – red balls represent N-glycosylated Asn$^{57}$ and phosphorylated Ser$^{274}$ amino acids; grey ribbon shows secondary structure of GLUT4 protein (on the basis of sequence NP_777029 available in GenBank, generated with the use of ViwerLite 4.2 program).
Asn\textsuperscript{508} to His in the C-terminal domain between these species [Abe \textit{et al.} 1997, 1998]. With the topology model, bovine GLUT4 is shown to have the modified a.a. residues between 6 and 7 transmembrane domains - phosphoserine Ser\textsuperscript{274}, and in cytoplasmic domain - phosphoserine Ser\textsuperscript{488} (not shown).

The GLUT4 protein is insulin-sensitive glucose transporter and was first detected in goat adipose tissue [Trayhurn \textit{et al.} 1993] and bovine skeletal muscle [Mandarino \textit{et al.} 1994]. In cattle, GLUT4 was detected in all insulin-responsive tissues: skeletal muscle, heart and adipose tissues [Zhao \textit{et al.} 1993, Abe \textit{et al.} 1997]. The GLUT4 protein levels are significantly higher in oxidative than in glycolytic muscles [Hocquette \textit{et al.} 1995, Duhlmeier \textit{et al.} 2005]. GLUT4 content is higher in perirenal and omental adipose tissues than in subcutaneous adipose tissues in growing calves [Hocquette \textit{et al.} 1996, 2006]. The mRNA encoding GLUT4 was detected in bovine follicles and corpus luteum, the expression level of GLUT4 in bovine ovary was much lower than in muscle and adipose tissue [Nishimoto \textit{et al.} 2006]. The insulin-dependent glucose transporter GLUT4 recycles between the muscle cell membrane and an intracellular tubulo-vesicular pool. In hyperglycemic states, insulin is secreted from endocrine pancreas and stimulates myocyte glucose uptake by increasing the translocation of intracellular GLUT4 vesicles into the plasma membrane [Kahn 1996, Duhlmeier \textit{et al.} 2005].

### GLUT5

The bovine GLUT5 gene \textit{SLC2A5} is composed of 7 exons and localize to the chromosome 16 (BTA16). Its sequence is available in the GenBank database under Acc. No AC\textunderscore 000171. In the GenBank SNP database are recorded 20 SNPs in \textit{SLC2A5} gene introns. The bovine GLUT5 mRNA is 2,140 nt long, and the protein contains 501 a.a. with molecular weight of 55 kDa. The deduced amino acid sequence of bovine GLUT5 (NP\textunderscore 001094512) is 98, 80, 78 and 78% identical to that of ovine, human, mouse and rat, respectively (Suppl. Figure S6). There is N-linked glycosylation site at Asn\textsuperscript{51} located between putative first two transmembrane domains of GLUT5.

The GLUT5 has higher affinity for fructose than glucose and it is localized in the apical brush border membranes of the small intestine and is also expressed in testes, kidney, muscle, brain and adipose tissue [Davidson \textit{et al.} 1992].

### GLUT6

The bovine GLUT6 gene (symbol \textit{SLC2A6}) is composed of 10 exons and localize to the chromosome 11 (BTA11). Its sequence is available in the GenBank database under Acc. No. AC\textunderscore 000168. In the GenBank SNP database are recorded 29 SNPs for \textit{SLC2A6} gene: 21 in introns, 7 in exons, and 1 in 3’-untranslated region. The full-length bovine GLUT6 transcript has 2,112 nt; the protein contains 507 a.a. with molecular weight of 54 kDa. The deduced amino acid sequence of bovine GLUT6
(NP_001073725) is 88, 80 and 78% identical to that of human, mouse and rat, respectively (Suppl. Fig. S7). GLUT6 mRNA is expressed in the spleen, peripheral leukocytes and brain [Doege et al. 2000a].

**GLUT8**

The bovine GLUT8 gene (symbol *SLC2A8*) is composed of 10 exons and localizes to the chromosome 11 (BTA11). Its sequence is available in the GenBank database under Acc No. AC_000168. Thirty three SNPs were identified in the bovine *SLC2A8* gene: 7 in exons and 26 in introns.

The bovine GLUT8 mRNA is 2,073 nt long; the encoded protein contains 478 a.a. with a predicted molecular weight of 51 kDa [Zhao et al. 2004]. The deduced bovine GLUT8 a.a. sequence (Acc. No. NP_963286) is 26 and 24% identical to bovine GLUT1 and GLUT4, respectively. It is also 89, 88, 84, 83 and 58% identical to that of human, dog, rat, mouse and chicken GLUT8, respectively (Suppl. Fig. S8). The bovine GLUT8 has an N-linked glycosylation site on loop 9 and putative di-leucine internalization motif. The exoplasmic loop between transmembrane 9 and 10 is longer in GLUT8 (30 vs. 9 a.a.), and has a glycosylation site that is not present in GLUT1. The sequence of bovine GLUT8 contains several sugar transporter signatures, characteristic for GLUT family. The sugar transport protein signatures 1 are located between a.a. 87 and 104 (GGwILDrAGRKLslvlcA) in loop 2 and between a.a. 310 and 327 (AAliMDrAGRRllltlsG) in loop 8. The sugar transport protein signature 2 (LtGLacGiaslYisEIaypevR) is located between a.a. 129 and 154 in transmembrane domain 4 and loop 4 (Fig. 7). Other motifs found in GLUT8 are: PETPR in loop 6, QQLSGVN in helix 7, GWGIPW in helix 10 and PETKG in C-terminal tail [Doege et al. 2000b, Joost and Thorens, 2001]. Bovine GLUT8 also contains an N-terminal di-leucine motif that has been shown to direct the protein to intracellular storage compartments [Uldry et al. 2001].
The bovine GLUT8 transcript is predominantly expressed in testes, mammary gland, kidney, lung, spleen, intestine epithelia, skeletal muscle and liver [Zhao et al. 2004]. The expression pattern of GLUT8 mRNA is comparable to that of GLUT1 [Zhao and Keating, 2007]. GLUT8 expression in mammary gland may be tissue-specifically regulated and stimulated by the mammogenic and lactogenic hormones - progesterone and prolactin [Zhao et al. 2004]. Zhao et al. [2004] have shown, that the rapid increase of GLUT8 expression in the mammary gland and rise of blood estrogen levels during lactation indicate that GLUT8 expression is not suppressed by estrogen in the mammary tissues. Insulin stimulates GLUT8 translocation in blastocyst but not in fat cells and neuroblasts.

GLUT9

The bovine GLUT9 gene (symbol SLC2A9) is composed of 15 exons and localize to the chromosome 6 (BTA6). Its sequence is available in the GenBank database under Acc. No AC_000163. Six SNPs were identified in the bovine SLC2A9 gene (encoding GLUT9), three located in exons and three in introns, which were significantly associated in with milk traits in German brown cattle [Seefried 2008].

For the bovine GLUT9 six different transcripts were detected, the longest mRNA has 2,515 nt and the shortest – 1,646 nt; protein contains 409 a.a. The deduced amino acid sequence of bovine GLUT9 (XP_002688502) is 86, 84, 84 and 83% identical to that of dog, human, rat and mouse, respectively (Suppl. Fig. S9). GLUT9 have been detected in kidney and liver [Phay et al. 2000].

GLUT10

The bovine GLUT10 gene (symbol SLC2A10) is composed of 5 exons and localize to the chromosome 13 (BTA13). Its sequence is available in the GenBank database under Acc. No. AC_000170. In the GenBank SNP database are recorded 24 SNPs for SLC2A10 gene: 17 in exons, 6 in 3’-untranslated region and 1 in 5’-untranslated region. The bovine GLUT5 transcript is 2,257 nt long, and protein contains 536 a.a. with molecular weight of 56 kDa. The deduced amino acid sequence of bovine GLUT10 (Acc. No. NP_001179368) is 82, 76 and 75% identical to that of human, rat and mouse, respectively (Suppl. Fig. S10). GLUT10 is expressed in the liver and pancreas [McVie-Wylie et al. 2001].

GLUT11

The bovine GLUT11 gene (symbol SLC2A11) is composed of 13 exons and localize to the chromosome 17 (BTA17). Its sequence is available in the GenBank database under Acc. No. AC_000174. In the GenBank SNP database are recorded 17 SNPs for
**SLC2A11** gene: 6 in exons and 11 in 3’- untranslated region. The bovine GLUT11 transcript is 2,178 nt long; protein contains 496 a.a. and has molecular weight of 53 kDa. The deduced amino acid sequence of bovine GLUT11 a.a. (NP_001180026) is 83% identical to that of human (Suppl. Fig. S11).

There have been described two splice variants of GLUT11 transcript and protein, formed through the skipping of exon 2, which results in long 503 a.a. and short 493 a.a. forms. The short form is expressed in heart and skeletal muscle [Doege *et al.* 2001]; the long form is detected in liver, lung, trachea and brain [Wu *et al.* 2002].

**GLUT12**

The bovine GLUT12 gene (symbol *SLC2A12*) is composed of 5 exons and localizes to the chromosome 9 (BTA9). Its sequence is available in the GenBank database under Acc. No. AC_000166. In the GenBank SNP database were identified 31 SNPs in *SLC2A12* gene introns.

The bovine GLUT12 mRNA is 2,423 nt long; protein contains 621 a.a. with a predicted molecular weight of 67 kDa [Miller *et al.* 2005]. The deduced bovine GLUT12 a.a. sequence (Acc. No. NP_001011683) is 88, 83 and 82% identical to that of human, rat and mouse, respectively (Suppl. Fig. S12). With the topology model, bovine GLUT12 has few N-glycosylation sites, between transmembrane domains 5 and 6 (Asn<sup>195</sup>) and transmembrane domains 9 and 10 (Asn<sup>375</sup>). The major structural differences unique to bovine GLUT12 are longer loop between the putative transmembrane domains TM 9 and 10 and longer N- and C-termini [Miller *et al.* 2005]. The large loop 9 contains glycosylation sites Asn<sup>387</sup>, Asn<sup>400</sup> and Asn<sup>405</sup> (Fig. 8). The sequence of bovine GLUT12 has several characteristically conserved sugar transporter family signatures. The sugar transport proteins signature 1 (GGVL1DRYGRRAaiiLSS) is located between a.a. 101 and 118, GRK/R in loop 2, GR in loop 3, E-RG in loop 4, PXXPR in loop 6, GXGPXXW in helix 10 and PETKG in C-terminal tail [Joost and Thorens 2001].

Fig. 8. Structure of GLUT12 – red balls represent N-glycosylated Asn<sup>195</sup>, Asn<sup>375</sup>, Asn<sup>387</sup>, Asn<sup>400</sup> and Asn<sup>405</sup> amino acids; grey ribbon shows secondary structure of GLUT12 protein (on the basis of sequence NP_001011683 available in GenBank, generated with the use of ViwerLite 4.2 program).
GLUT12 mRNA is expressed in the spleen, skeletal muscle, kidney, testes, mammary gland, liver, lungs, and intestine [Miller et al. 2005]. The mRNA of GLUT12 level in the mammary gland of lactating cows is lower than that in non-lactating. The increment of GLUT12 expression in non-lactating mammary gland indicates its possible role in regulating insulin-dependent glucose uptake and development of the mammary secretory epithelium [Komatsu et al. 2007].

**HMIT**

The bovine HMIT gene (symbol SLC2A13) proton myo-inositol cotransporter is composed of 10 exons and localize to the chromosome 5 (BTA5). Its sequence is available in the GenBank database under Acc. No. AC_000162. In the GenBank SNP database are recorded 5 SNPs in the exons of SLC2A13 gene. The bovine HMIT transcript is 1,947 nt long; protein contains 648 a.a. with molecular weight of 70,5 kDa. The deduced amino acid sequence of bovine HMIT (Acc. No. NP_001179892) is 97, 89 and 89% identical to human, mouse) and rat HMIT (Suppl. Fig. S13). HMIT is expressed mostly in brain [Uldry et al. 2001].

**Na\(^+\)/GLUCOSE COTRANSPORTERS SGLT**

The Na\(^+\)/glucose cotransporter family (gene symbol SLC5A, protein symbol SGLT), consists of low-affinity glucose transporters. SGLT is a family of solute-linked carriers that contain sodium-coupled transporters for several nutrients. The Na\(^+\)-electrochemical gradient provided by the Na\(^+\)-K\(^+\) ATPase pump is utilized to transport substrates into cells against its concentration gradient. The cotransported substrates are sugars, inositol, proline, pantothenate, iodide, urea and other undetermined solutes. This family contains 12 genes (SLC5A1-SLC5A12) coding for 12 SGLT proteins. Six most commonly expressed SGLT family transporters and their genes are presented in Tables 1 and 2. The nutrients that are transported via SGLT proteins include glucose (SGLT1, 2, 4 and 5) [Hediger et al. 1989, Wells et al. 1992], myo-inositol (SMIT1 and 2; sodium-dependent myo-inositol transporters 1 and 2) [Berry et al. 1996, Roll et al. 2002], iodide (SLC5A5/NIS Na\(^+\)/iodide cotransporter) [Smanik et al. 1996], biotin and pantothenate (SLC5A6/SMVT sodium-coupled multivitamin transporter) [Wang et al. 1999], choline (SLC5A7/CHT1 choline transporter) [Apparsundaram et al. 2001] and short chain fatty acids/lactate/nicotinate (transporters SLC5A8, 12/SMCT1,2 sodium-coupled monocarboxylate transporter) [Ganapathy et al. 2005].

These proteins are mainly located in the brush-border membranes of intestinal epithelial cells in the small intestine and in the proximal tubules of the kidney [Wright and Turk, 2004]. SGLT transporters contain several characteristic and conserved sodium solute symporter family signatures and have 14 transmembrane domains (Fig. 9). The first cotransporter proteins, Na\(^+\)/glucose and Na\(^+\)/proline cotransporters, were identified in the rabbit intestinal brush border [Peerce and Wright, 1985]. In a pair
wise basic local alignment search tool (BLAST) analysis the amino acid identities relative to SGLT1 (encoded with \textit{SLC5A1} gene) are 60\% for SGLT2, 55\% for SGLT4, 57\% for SGLT5, 51\% for SMIT2 and 24\% for SMCT2 (Suppl. Fig. S14). Unrooted phylogenetic tree of 6 bovine members of the SGLT family transporters is shown in Figure 10.

\textbf{SGLT1}

The bovine SGLT1 gene (symbol \textit{SLC5A1}) is composed of 15 exons and localizes to the chromosome 17 (BTA17). Its sequence is available in the GenBank database under Acc. No. AC_000174. In the GenBank SNP database were deposited 400 SNPs of the bovine \textit{SLC5A1} gene: 1 in 3’-untranslated region, 385 in introns, and 14 in exons. Bovine SGLT1 transcript length is 2,248 nt; protein contains 664 a.a. with molecular weight of 73 kDa [Zhao \textit{et al.} 2005]. Amino acid sequence of SGLT1 protein is 58\% identical to SGLT2. The deduced amino acid sequence of bovine SGLT1 (Acc. No. NP_777031) is 98, 89, 89, 88, 87 and 86\% identical to that of ovine, swine, dog, mouse, rat and human, respectively (Suppl. Fig. S15).
Glucose transporter SGLT1 is responsible for the high-affinity, conservative uptake of most monosaccharides (glucose and galactose), except fructose. SGLT1 mRNA was shown most abundant in bovine intestinal tissues, in the jejunal region [Liao et al. 2010], intermediate in kidney, low in mammary gland, liver, lungs, and not detectable in spleen, skeletal muscle and testes [Zhao et al. 2005]. In bovine cortical arteries SGLT1 plays the role of a molecular sensor for coping with stress such as hypoglycaemia [Nishizaki and Matsuoka 1998].

**SGLT2**

The bovine SGLT2 gene (symbol *SLC5A2*) is composed of 14 exons and localizes to the chromosome 25 (BTA25). Its sequence is available in the GenBank database under Acc. No. AC_000182. In the GenBank SNP database are recorded 33 SNPs of the bovine *SLC5A2* gene: 6 in 3’-untranslated region, 26 in introns, and 1 in an exon.

The full-length bovine SGLT2 transcript has 2,261 nt; protein contains 673 a.a. with a predicted molecular weight of 73 kDa [Zhao et al. 2005]. The deduced bovine SGLT2 a.a. sequence (Acc. No. NP_976236) is 58% and 48% identical to bovine SGLT1 and SGLT5, respectively. It is 92%, 92%, 91% and 91% identical to dog, human, mouse and rat SGLT2 sequence, respectively (Suppl. Fig. S16). The major differences are in the last loop region between the putative transmembrane domains 13 and 14 and in the N-terminal region. There are several signatures in the SGLT2 protein. The sodium solute symporter family signature 1 (GwnlyasVIALLGrmiYTvtGGLaA) is located between a.a. 171 and 196 and the sodium solute symporter family signature 2 (ALfvpRvnekGAfwGLIGGIL) – between a.a. 474 and 494 (Fig. 11) – Zhao et al. [2005].

The SGLT2 is predominantly expressed in bovine kidney and its mRNA is at lower levels in mammary gland, liver, lung, spleen, intestine, and skeletal muscle [Zhao et al. 2005]. Expression of SGLT2 mRNA in bovine mammary gland increases more than 10-fold from late pregnancy to early lactation, similar to SGLT1. This indicates that SGLT2 may play a role in milk synthesis in the lactating mammary gland.
SGLT4

The bovine SGLT4 gene (symbol SLC5A9) is composed of 18 exons and localizes to the chromosome 3 (BTA3). Its sequence is available in the GenBank database under Acc. No. AC_000160. The full-length bovine SGLT4 transcript has 2,418 nt, although 3 other transcripts were detected in bovine tissues: 4,147 nt (X1), 3,119 nt (X2) and 4,150 nt (X3); the protein contains 705 a.a. The deduced bovine SGLT4 a.a. sequence (Acc. No. NP_001192865) is 86, 85 and 80% identical to that of human, rat and mouse (Suppl. Fig. S17). SGLT4 is widely expressed in the body.

SGLT5

The bovine SGLT5 gene (symbol SLC5A10) is composed of 15 exons and localizes to the chromosome 19 (BTA19). Its sequence is available in the GenBank database under Acc. No. AC_000176. In the GenBank SNP database are recorded 174 SNPs of the bovine SLC5A10 gene: 167 in introns, 7 in exons. The bovine SGLT5 transcript is 2,042 nt long; the protein contains 597 a.a. with a predicted molecular weight of 64.7 kDa. The deduced bovine SGLT5 a.a. sequence (Acc. No. NP_001001442) is 86%, 85% and 84% identical to that of human, mouse and rat, respectively (Suppl. Fig. S18). With the topology model, bovine SGLT5 has two N-glycosylation sites in extracellular domain (Asn\(^5\)) and in transmembrane domains 2 and 3 (Asn\(^97\)), and three modified residue in transmembrane domain 4 - phosphoserine Ser\(^{142}\), phosphoserine Ser\(^{142}\) and phosphothreonine Thr\(^{142}\). SGLT5 is expressed in small intestine, brain, kidney, liver and lung in human.

SMIT2

The bovine SMIT2 gene (symbol SLC5A11) sodium/myo-inositol cotransporter 2 is composed of 17 exons and localizes to the chromosome 25 (BTA25). Its sequence is available in the GenBank database under Acc. No. AC_000182. In the GenBank SNP database are recorded 332 SNPs of the bovine SLC5A11 gene: 318 in introns, 11 in exons, 2 in 5'-untranslated region, and 1 in 3'-untranslated region.

The full-length bovine SMIT2 transcript has 2,277 nt; protein contains 674 a.a. with a predicted protein molecular weight of 73.9 kDa. The deduced bovine SMIT2 a.a. sequence (Acc. No. NP_001029832) is 95, 88, 84 and 84% identical to that of caprine, human, mouse and rat (Suppl. Fig. S19).

SMCT2

The bovine SMCT2 gene (symbol SLC5A12) sodium-coupled monocarboxylate transporter 2 is composed of 15 exons and localizes to the chromosome 15 (BTA15).
Its sequence is available in the GenBank database under Acc. No. AC_000172. In the GenBank SNP database are recorded 105 SNPs of the bovine SLC5A12 gene: 103 in introns, 1 in exon, and 1 in 5’-untranslated region. The bovine SMCT2 transcript is 2,924 nt long; protein contains 617 a.a. with a predicted molecular weight of 67.4 kDa. The deduced bovine SMCT2 a.a. sequence (Acc. No. NP_001094529) is 90%, 83% and 80% identical to the sequence of human, rat and mouse SMCT2, respectively (Suppl. Fig. S20). The SMCT2 protein is highly hydrophobic with 13 putative transmembrane domains (instead of 14 domains typical for other SGLTs) with N-terminus at the exoplasmic side of membrane and C-terminus the cytoplasmic side of membrane. With the topology model, bovine SMCT2 has two N-glycosylation sites, between transmembrane domains 6 and 7 (Asn\textsuperscript{219}) and in transmembrane domains 12 and 13 (Asn\textsuperscript{480}).

The SMCT2 is expressed predominantly in the cortical portion of the kidney, at lower level in in small intestine, skeletal muscle and it is a low-affinity transporter [Srinivas et al. 2005].

**Conclusion and perspectives**

There are two types of glucose transporters: passive, facilitative glucose transporters GLUT (encoded by SLC2 genes) and active Na\textsuperscript{+}/glucose cotransporters SGLT (gene symbol SLC5), which participate in the Na\textsuperscript{+}-linked transport process against electrochemical gradient. Both types of glucose transporters are expressed in cattle. Their protein and genomic sequences are highly related among ruminants, the sequence identity being in the range of 91 to 99% between different ruminant species, and in the range 75 to 99% with that of other mammals. Multiple glucose transporter proteins are present in bovine cells and all bovine tissues express more than one of these transporters in order to efficiently obtain sufficient glucose from the extracellular fluid for cell metabolism. Each glucose transporter has different transport regulatory properties and plays specific roles in maintenance of whole body glucose homeostasis.

Due to their function genes encoding glucose transporters are considered possible candidate markers for production traits of farm animals, including cattle. Genetic variation - nucleotide sequence polymorphisms of SLC2A and SLC5A genes like SNPs, InDels or STRs can influence gene expression or functions of glucose transporter proteins. This, in turn, can decrease or increase glucose supply for animals’ tissues and organs, such as muscles and mammary gland and thus greatly influence milk or meat yield or quality.

In humans it was shown that DNA sequence variations in glucose transporter genes may influence the development of certain diseases, like diabetes or cancer [Ng et al. 2002; Grabelius et al. 2010]. Association of genetic polymorphism of glucose transporters with differential glucose uptake has also been reported in several studies [Mueckler et al. 1994, Ng et al. 2002, Grabelius et al. 2010]. Glucose transporter-1
GLUT1) deficiency is leading to a reduced glucose transport into the brain [Seidner et al. 1998]. It is highly expressed in the endothelial cells and in the blood-brain barrier and is exclusively responsible for glucose transport into the brain [Vannucci et al. 1997, Barros et al. 2007]. Glucose transporter-1 deficiency syndrome is caused by mutations in the SLC2A1 gene in humans and results in impaired glucose transport into the brain which causes mental retardation, and epilepsy. It can be diagnosed by a low concentration of glucose in the spinal fluid and by testing of glucose transport in red blood cells. Genetic testing for the SLC2A1 gene confirms this syndrome.

Very little studies have been carried out of polymorphism of glucose transporter genes in livestock. Although in the GenBank SNP database hundreds of single nucleotide substitutions (SNPs) in bovine glucose transporter genes are recorded, mostly derived from next generation sequencing (NGS) studies, they have not been validated with other methods and their association with production or functional traits was not studied. Seefried [2008] in his doctoral thesis has analyzed polymorphisms in different candidate genes for milk production traits in three cattle breeds. In his study, six polymorphic markers were identified in the SLC2A9 gene (encoding GLUT9) which were significantly associated with milk traits (milk yield, fat content, protein content) in German brown cattle. Three of the significantly associated markers were located in a translated region (exons) and three have an intronic position. Recently, a project has been established at the University of Vermont, Burlington entitled, “Single Nucleotide Polymorphisms of Glucose Transporters as Breed Selection Markers for Milk Productivity in Dairy Cattle”, but the results have not been published yet.

Apart from variations in coding regions of genes, focus has also been oriented towards studying the variations in regulatory regions, mainly in promoter regions which regulate a gene transcriptional rate and thus determine the amount of transcripts [Adamowicz et al. 2006, Martin et al. 2002, Szymanowska et al. 2004, Szreder et al. 2008]. Such variations (SNPs, deletion/insertions) in the gene promoter may be located within the potential transcriptional factor binding sites or cis-regulatory sequences; they can modulate (increase or decrease) the efficiency of the transcription and influence gene expression levels. Similarly, nucleotide sequence variations in 3'-UTR regions could influence binding of the regulatory micro RNA molecules, and thus influencing stability of target mRNAs and expression of the encoded proteins (Chekulaeva and Filipowicz, 2009). Nucleotide sequence polymorphisms in the regulatory regions can influence expression levels of glucose transporter genes, and thus influence glucose transport efficiency. However, up to now, very little is known about the regulation of glucose transporter genes in cattle, and more generally, in ruminants. In the sheep, the SGLT1 gene promoter was shown to contain a 16-bp element that binds members of the Sp1 family of transcription factors that enhance basal expression. Also in the sheep, it was shown that SGLT1 promoter contains HNF-1 binding site that appears to be involved in the increase of intestinal SGLT1 gene expression in response to dietary glucose. HNF-1 and two GC boxes are critical for basal expression. [Martin et al. 2000]. Recently, in our laboratory at IGAB PAS in Jastrzębiec, a project have been
carried out aiming at discovering functional polymorphisms in the bovine glucose transporter genes. SNP and InDel polymorphisms were identified in SLC2A and SGLT genes, some of them located in the regulatory sequences – promoter and 3’-UTR regions. The effect of these mutations on the expression levels of glucose transporter genes has been experimentally confirmed. The results will be published soon.

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Glucose transporters in cattle - a review


