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**REVIEW PAPER**

# AQUAOPORIN-2 WATER CHANNEL IN THE KIDNEY OF FARM ANIMALS: AN OVERVIEW*

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**Abstract**

The Aquaporin 2 (AQP2) water channel is a small-molecule transmembrane protein expressed in mammalian kidneys that plays a crucial role in the renal water retention and production of concentrated urine. AQP2 is a vasopressin-regulated water channel and is excreted into urine. When AQP2 was discovered in rat renal tubules, intensive research began, which was soon reflected in the literature by numerous articles, both research reports and reviews, which provided more details on the localization of AQP2 and its expression regulation factors. Most of the research in this field, however, has been carried out on humans and laboratory animals, with very few reports concerning livestock animals. This is the first review that summarizes our current knowledge on renal AQP2 in such groups of animals as cattle, small ruminants, horses and pigs. This article presents the recent findings concerning the structure and function of AQP2 in the renal water reabsorption. This review will mainly focus on: (1) the distribution of AQP2 in the renal tubule cells in cattle and horses; (2) changes in urinary AQP2 excretion during osmotic diarrhea in calves; (3) the effect of dietary supplementation on the renal expression of AQP2 by growing piglets and young goats; (4) AQP2 expression changes during the fetal and neonatal life in sheep. The information and prospects in the research on renal AQP2 in farm animals presented in this article will hopefully be a contribution to further studies in both basic and clinical research.

**Keywords:** aquaporins, aquaporin 2, renal function, kidney, farm animals.

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## INTRODUCTION

Appropriate distribution and proper osmolality of systemic fluids, both within and outside cells, is crucial for sustaining the homeostasis of any living system. Water transport across biological membranes is fundamental for this process. There are two mechanisms of water transport through lipid barrier membranes. One is simple diffusion, with a relatively sluggish and limited water flow. Another mechanism relies on specialized channels referred to as aquaporins (AQPs). Aquaporin water transfer is both massive and rapid. A single aquaporin channel is able to transport nearly $2\text{-}3^9$ water molecules per second. AQPs, first identified and described by Peter Agre and co-workers in the 1990s, represent the main route for water entering or leaving the cell (Agre, Kozono 2003).

Aquaporins belong to a large family of small, transmembrane hydrophobic proteins with a molecular weight from 27 to 37 kDa. In all, 13 aquaporin isoforms (AQP0–AQP12) have been identified and localized in all types of mammalian cells of various tissues and organs (Holmes 2012). Given the structure and function of each AQP isoform, the proteins were divided into three groups. The first group (i) comprises so-called classic aquaporins, permeable to water molecules only (AQP0, AQP1, AQP2, AQP4 and AQP5). The second group (ii) is composed of so-called aquaglyceroporins, which, apart from water, transport also other small molecules such as glycerol, urea, ammonia and nitrate (AQP3, AQP7, AQP9, AQP10). The last group (iii) involves unorthodox aquaporins (AQP6, AQP8, AQP11 and AQP12), which share low homology with other proteins from this family (Park, Kwon 2015, Michałek 2016). The kidneys, which are the main organ regulating the water-electrolyte equilibrium and acid-base management, were revealed to comprise 9, precisely localized aquaporin isoforms, i.e. AQP1, AQP2, AQP3, AQP4, AQP5, AQP6, AQP7, AQP8 and AQP11 (Holmes 2012, Kortenoeven, Fenton 2014, Michałek 2016). AQP2 plays a key role in the regulation of renal retention of water in response to the needs of the body. This protein is localized in the principal cells of the connecting tubules (CNT) and collecting duct (CD). With an increased demand for water, AQP2 contributes to an increase in water renal reabsorption, resulting in excretion of a small volume of concentrated urine (Sasaki 2012). Water reabsorption *via* AQP2 in the final part of the renal tubule is crucial to maintaining a proper water balance, and any disturbances in this process can lead to severe diseases (Kitchen et al. 2015). Therefore, there has been tremendous interest in this protein in recent years, especially in the context of water-electrolyte management disorders. In this respect, the literature brings a plurality of original research papers and reviews on this topic in humans and laboratory animals. There are few data, however, on renal AQP2 in farm animals. Advances in the physiology of laboratory animals and human medicine are accompanied by the development of veterinary medicine. Frequently, new technologies and proce-

dures implemented in human medicine become later applied in the treatment of animal diseases. Therefore, this review was aimed at collecting and analyzing all available data on renal AQP2 in farm animals, which should probably encourage new research involving this group of animals.

## STRUCTURE AND FUNCTION OF AQP2

AQP2, first identified by Fushimi and coworkers (1993) in the rat renal CD, is a 29-kDa protein with additional higher molecular mass of 35-50 kDa, which corresponds to the N-glycosylated form (Fushimi et al. 1993). AQP2 is a homotetrameric protein, with each of he four monomers composed of six highly hydrophobic transmembrane (H1, H2, H3, H4, H4, H5 and H6) domains connected by five loops (A, B, C, D and E.) Two loops (B and D), which contain a characteristic motif of three amino acids (NPA, Asp-Pro-Ala), form a selective permeation pore inside the channel (Binesh, Kamali 2015). Water transport through AQPs is bidirectional and its driven by the osmotic gradient (Figure 1*d*) (Kitchen et al. 2015). Abundance and cellular localization of AQP2 are mainly regulated by vasopressin (AVP). Under water saturating condition, AQP2 is stored in intracellular vesicles of the collecting tubule and collecting duct principal cells – Figure 1*b*, *e* (Kortenoeven, Fenton 2014). In response to an increase in the plasma sodium concentration and a decrease of water in the vascular bed, AVP is released to blood from the posterior pituitary gland. Vasopressin-dependent renal water reabsorption occurs by short-term and long-term regulation. In either case, finally increased cell membrane permeability of the CNT and CD for water and then enhanced it absorption to the peritubular space and to the capillary (Kortenoeven, Fenton 2014). The short-term regulation process occurs over a period of minutes as a result of the regulation of the trafficking of intracellular vesicles containing AQP2 to and from the apical plasma membrane in response to vasopressin (Figure 1*b*). The long-term regulation process takes hours or days and is a result of the regulation of the whole-cell AQP2 abundance by vasopressin (Wilson et al. 2013). In the short-term regulation, shuttling of AQP2 between intracellular vesicles and apical plasma membrane requires a functional AVP–AC–cAMP–PKA signalling cascade (Wilson et al. 2013). Binding of AVP to vasopressin type 2 receptor ($V_2$) induces an activation of the Gs protein alpha subunit, which in turn stimulates adenylate cyclases (AC) – Figure 1*b*. Then, the production of intracellular cyclic adenosine monophosphate (cAMP) is increased, which in turn causes the activation of protein kinase A (PKA). As a result of PKA activation, Ser256 located in the cytoplasmic C-terminal region of AQP2 monomers undergoes phosphorylation. AQP2 phosphorylation triggers the transport and fusion of the protein with the apical plasma membrane, which again causes massive water inflow to cells of CNT and CD. The basement membranes of these cells contain AQP3 and AQP4, through which water flows along the osmotic gradient into the peritubular space (Figure 1c). After the fusion with the cell membra-

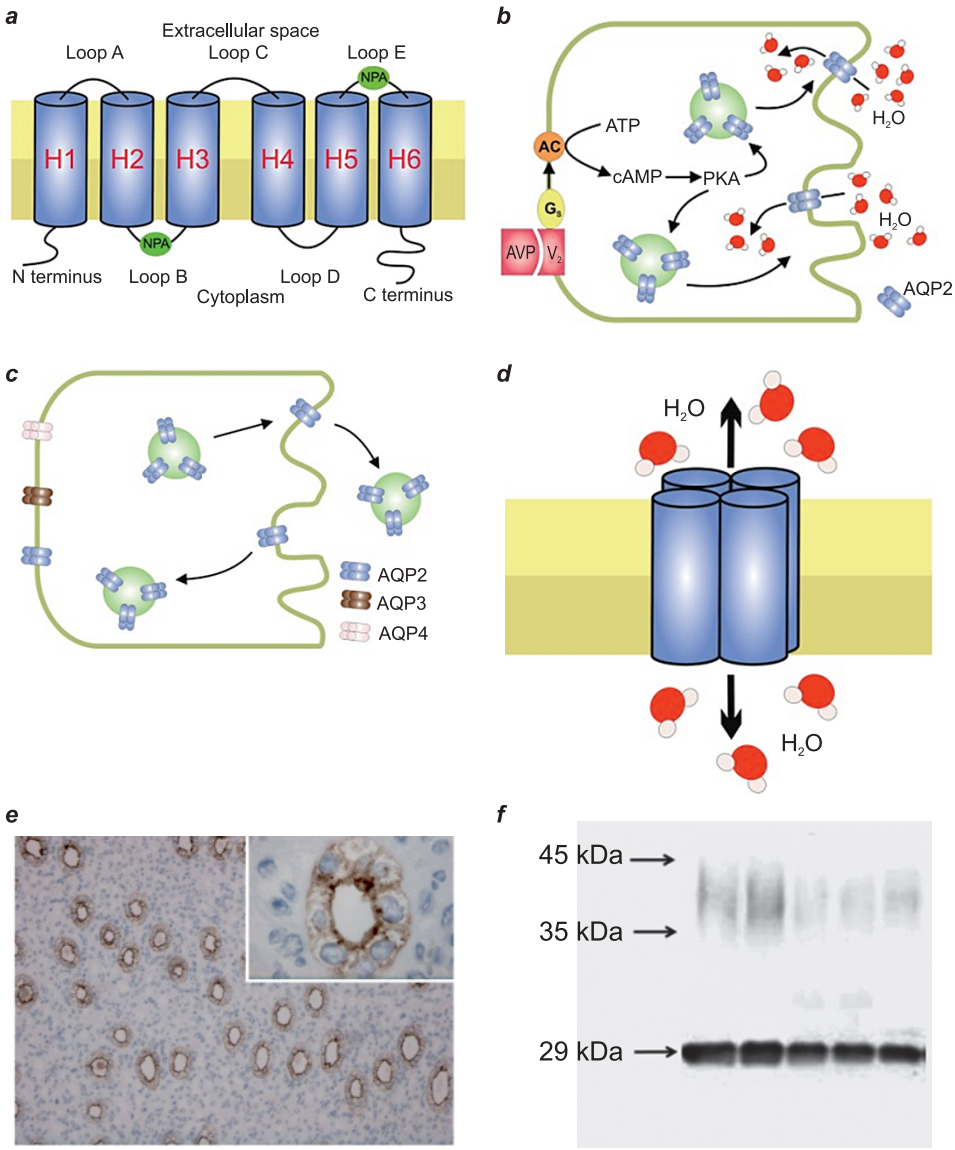


Fig. 1. AQP2 in the kidney and urine: *a* – structure of AQP2, *b* – activation of Gs protein alpha subunit, stimulation of adenylate cyclases (AC), increased production of intracellular cAMP, protein kinase A (PKA) activation, phosphorylation and fusion of AQP2 with the apical plasma membrane of the collecting ducts principal cell in response to AVP stimulation, *c* – AQP2 after fusion with cell membrane is excreted into urine or undergo endocytosis, *d* – the flow of water through AQP2 may occur in either direction, depending on the osmotic pressure on both sides of the cell membrane, *e* – the immunohistochemistry of AQP2 in the kidney of young beef cattle (Michałek et al. 2014*b*), *f* – western blot of AQP2 in calves urine (Michałek et al. 2014*a*)

ne, AQP2 is excreted into urine or undergoes endocytosis (Moeller, Fenton 2012) – Figure 1*c*, *f*. According to Brown (2008), the amount of AQP2 in the apical plasma membrane is a result of the balance between continuing endocytosis and exocytosis of AQP2, both in the presence and absence of AVP. The process of long-term regulation is associated with changes in the total amount of AQP2 in the kidney, which arise from the balance between the production of AQP2 by translation and its removal from the cell by either degradation or exosomal secretion into urine (Wilson et al. 2013).

Beside AVP, several other hormones and signalling molecules are involved in the expression and cellular localization of AQP2. These include prostaglandins, angiotensin II (ANG II), aldosterone, insulin, cytoskeleton and intracellular $Ca^{2+}$ concentration (Kortenoeven, Fenton 2014). Prostaglandins antagonize the action of AVP trough retrieval of AQP2 from the apical plasma membrane. ANG II and insulin increase AQP2 protein expression and mRNA levels. Long-term stimulation of aldosterone induced decreased apical but increased basolateral expression of AQP2. A critical role in the trafficking of AQP2 to the apical plasma membrane is also played by the microtubular network. Cytoskeletal reorganization, including depolymerization of F- actin, increased osmotic water permeability of the collecting duct cells (Kwon et al. 2013). It has been shown that the intracellular $Ca^{2+}$ concentration is involved in the vasopressin-induced increase in water permeability. A transient increase in intracellular $Ca^{2+}$ as a result of the stimulation of $V_2$ receptors is necessary to start translocation of AQP2 to the apical plasma membrane (Kwon et al. 2013).

## THE CLINICAL RELEVANCE OF AQP2

Several clinically important water-balance disorders are associated with dysregulation of AQP2. These include central diabetes insipidus (CDI), nephrogenic diabetes insipidus (NDI), syndrome of inappropriate antidiuresis (SIADH), congestive heart failure (CHF), hepatic cirrhosis, preeclampsia and nephrotic syndrome – NS (Wilson et al. 2013). Despite different etiology, changes in the expression and localization of AQP2, and thereby a change in water permeability of the CNT and CD, are observed in each of these diseases. As a result, there is excessive water loss with urine or excessive water retention in the body, which are always accompanied by electrolyte disturbances. Diabetes insipidus-specific symptoms, i.e. polyuria and polydipsia, are common in everyday clinical practice (Sasaki et al. 2012). In humans, the most common form of this disorder is acquired NDI, which is most often seen in association with sustained ureteral obstruction, sustained hypokalemia, hypercalcemia or sustained lithium intake (Marples et al. 1996, Sasaki et al. 2012). Since it is impossible to directly determine the renal expression of AQP2 in patients, an attempt has been made to identify and determine possible changes in the expression of this protein in urine. The experiments revealed that in healthy adults from 3 to 6% of total kidney AQP2 is excreted

in urine and AQP2 excretion closely parallels changes in the action of vasopressin in the kidney (Wilson et al. 2013). It has also been found that urinary excretion is increased in patients with SIADH, congestive heart failure, hepatic cirrhosis and in pregnant women (Martin et al. 1999, Ivarsen et al. 2003). In this context, a concept has been proposed that urine AQP2 expression may be a biomarker of the antiduretic activity in the CNT and CD, as well as a useful parameter to diagnose water imbalance and in pharmacotherapy monitoring (Elliot et al. 1996). Currently, the most important goal is to measure urinary AQP2 expression in the diagnosis of NDI and to assess the effectiveness of desmopressin treatment (Sasaki et al. 2012, Salih et al. 2014). Unfortunately, this does not apply to neonates, in whom – in the face of numerous differences in renal regulation and function – no close relationship between renal excretion of AQP2 and renal vasopressin action has been found (Zelenina et al. 2006, Michałek et al. 2014*a*).

## AQP2 IN THE KIDNEY OF FARM ANIMALS

### Expression of AQP2 in bovine kidney and urine

According to Monaghan and Hannan (1983), acute renal diseases affect 4% of slaughter animals on average. It may be presumed, however, that such cases are much more common, since in large-scale cattle farming the sings of renal diseases, such as polyuria and polydipsia often remain unnoticed (Mueller 2007). Less interest in the cattle renal disorders, as compared to dogs or cats, underlies the fact that only four articles can be found in the literature that deal with bovine renal aquaporins (Mobasheri et al. 2011, Altunbans et al. 2013, Michałek et al. 2014*a*, *b*). AQP2 has been detected in bovine kidneys, although data as to its localization are somehow divergent. Mobasheri et al. (2011) found that AQP2 was present in the kidney of adult lactating cows only in the apical plasma membrane of the principal cells of the CD. In the kidney of 7-month-old calves, however, the protein was found in the apical membrane, intracellular vesicles and in the basolateral membrane of the collecting duct principal cells – Figure 1*e* (Michałek et al. 2014*b*). Strong expression of AQP2 was found in the apical membrane, whereas poor AQP2 expression was observed in numerous intracellular vesicles. We also found weak but distinct AQP2 staining on the basolateral membrane. The observed localization, distribution and expression of AQP2 in young beef cattle was typical and characteristic for humans and animal species (Nielsen et al. 1993, Kishore et al. 1996, Loffing et al. 2000, Bauchet et al. 2011). It is only the expression of AQP2 in the basolateral membrane that seems higher in bovine kidneys compared to humans and laboratory animals. Stronger AQP2 staining in the basolateral membrane in cattle may imply a much more extensive participation of AQP2 in the water transport from principal cells to the peritubular space. These findings seem to conform with the experiments carried out by Altunbas et al. (2013). As previously mentioned, both in humans and animals, AQP3 and AQP4 provide an exit pathway

for the water that enters the cell *via* AQP2. Altunbas et al. (2013) identified AQP4 only in the cytoplasm of proximal tubule epithelial cells. To maintain the efficient water transport from the luminal fluid across the tubular epithelium, lack of AQP4 in the basolateral membrane of the bovine collecting duct principal cells must be compensated for by another AQP. Because we found a distinct expression of AQP2 in the basolateral membrane, we have proposed that this AQP probably mimics the role of AQP4 in the principal cells of the bovine collecting duct (Michałek et al. 2014*b*). The localization, expression and role of AQP2, AQP3 and AQP4 in the bovine kidney, however, are disputable and require further studies. Namely, in contrast to Altunbas et al. (2013), Mobasheri et al. (2011) identified both AQP3 and AQP4 in the basolateral membrane of the CD principal cells of the kidney of adult lactating cows.

High newborn calf mortality rates, which cause significant economic loss to commercial animal farming, result mostly from diarrhea occurring within the first 2-3 weeks of age (Singh et al. 2009). An additional loss of water with feces, with a still limited ability to produce concentrated urine, quickly leads to serious disturbances of the water-electrolyte balance, with a resulting mortality reaching 15% of calves during the first two weeks of life (Lorenz et al. 2011). Among the factors limiting the production of concentrated urine in rat and human neonates is a AQP2-participated reduction of water reabsorption (Bonilla-felix 2004). Therefore, the aim of our study included identification of AQP2 and an analysis of changes in its expression in urine of calves during the first month of life with an induced controlled, transient osmotic diarrhea (Michałek et al. 2014*a*). Using Western blot analysis, two forms of AQP2 were identified in the studied calves, which are also characteristic for humans and laboratory animals (Figure 1f), namely 29 kDa unglycosylated form and 35-45 kDa glycosylated form. Thus far, these have been reports concerning renal excretion of AQP2 in cattle. We have found that in response to additional fecal loss of water under diarrhea, the expression of AQP2 in urine in calves increases nearly three-fold. The distinct increase in AQP2 expression in the apical plasma membrane of the collecting duct principal cells, which results in an apparent increase in renal excretion of this protein with urine, does not seem to cause increased tubular water retention, as is evident from the lack of changes in urine osmolality in calves. The absence of any increase in the osmotic pressure and higher expression of AQP2 has also been observed in human neonates (Zelenina et al. 2006). The studied group of calves, like neonates of other species, did not demonstrate any close relationship between an increase in AQP2 expression and urine osmolality. This is most likely due to the immature structure of their kidneys and the limited ability to produce a high osmotic gradient in the medulla (Michałek et al. 2014*a*). Nevertheless, the results indicate that during the first weeks of life, there is an efficient mechanism functioning in cattle which leads to an increased renal expression of AQP2 in response to additional water loss (Michałek et al. 2014*a*). This mechanism has not been

fully explained in this species so far, and it seems somewhat different compared to humans and laboratory animals. In fact, studies on calves revealed an association between the plasma concentration of $Ca^{2+}$ and renal excretion of AQP2 (Michałek et al. 2014*a*). As it was previously mentioned for humans and laboratory animals, vasopressin is the main factor leading to an increase in AQP2 expression in the apical membrane of the principal cells and increased urinary excretion of the protein (Holmes 2012). During transient diarrhea in calves, an increased level of AQP2 renal excretion was observed; however, there were no accompanying changes in the plasma osmolality or AVP concentration. On the other hand, there was a significant decrease in the plasma levels of $Ca^{2+}$ (Michałek et al. 2014*a*). There are authors who observed a relationship between changes in the plasma $Ca^{2+}$ concentration and renal AQP2 expression (Valenti et al. 2000, Nèmeth-Cahalan et al. 2004). An experiment on rats, for example, revealed that an increase in the plasma calcium levels caused a reduction of nearly 50% in total AQP2 in the kidneys, reduced AQP2 expression in the apical plasma membrane of the principal cells in the CD, and decreased urinary excretion of this protein (Earm et al. 1998). According to some authors, elevated levels of $Ca^{2+}$ in the blood plasma most probably reduce AVP-stimulated activation of adenylate cyclase and also reduce cAMP production (Earm et al. 1998). Reduced plasma calcium concentrations in the studied calves (possibly caused by accelerated passage of the intestinal contents and reduced calcium absorption in the small intestine during osmotic diarrhea) probably contributed to an increased AQP2 expression in the apical plasma membrane and its elevated renal excretion. More accurate explanation of this process, however, needs further detailed studies.

**Renal AQP2 expression in small ruminants**

Renal disorders and clinical manifestations of renal failure are poorly documented in small ruminants. There are no precise data on the role of AQP2 in the renal regulation of water balance in this group of animals. There is only one article available in the literature that deals with renal AQPs in goats. The authors studied effects of dietary N reduction on the expression of renal AQP1, AQP2 and CaR (calcium-sensing receptor, a potential modulator of AQP2) in young goats (Elfers et al. 2014). The experiment was carried out on twenty male White Saanen, 3-month-old goats divided into two feeding groups, offered either a diet with an adequate or a reduced N supply. The results allowed the authors to conclude that amino acid sequences of caprine AQP1 and AQP2 showed a high homology to humans, cows and sheep. It has also been demonstrated that a long-term alimentary N reduction leads to increased AQP1 mRNA expression in the renal cortex and an increase in AQP2 expression on mRNA in the outer medulla of young goats. The authors propose that the observed increase in AQP2 expression was caused by increased AVP concentrations during dietary reduction.

The experiment did not confirm the possible role of CaR in the regulation of AQP2 expression in young goats. It was noticed, however, that up-regulation of AQP1 and AQP2 could be connected with the up-regulation of urea transporter UT-A1 to increase the possibility of absorbing urea in times of N scarcity in young ruminants (Elfers et al. 2014).

According to Butkus et al. (1999), AQP2 in adult sheep is present in the principal cells of the collecting ducts in the cortex, the outer stripe and inner stripe of the outer medulla and in the inner medulla. The authors also tried to evaluate AQP2 expression in the kidneys of ovine fetuses, neonates, and over the first weeks of lambs' life. The study substantiated a conclusion that the level of AQP2 mRNA at about 100 days of gestation (65% of gestation) is 17% of that in the adult kidney. Later in gestation, close to parturition, the AQP2 mRNA is 41% of that in the adult. With the growth and development, the sensitivity of a fetus's kidneys to AVP changes as well. With the progress in gestation, the fetal kidney response to AVP increases, but is still low compared to an adult sheep (Wintour et al. 1982). The limited production of concentrated urine, which results from low AQP2 expression and a weak response of the renal tubules to AVP stimulation, is undoubtedly related to the fact that the fetus plays a vital role in the production of amniotic fluid in sheep. In order to maintain an adequate volume of amniotic fluid, the fetus must excrete appropriate amounts of dilute urine (Butkus et al. 1999). The experiment also revealed that a treatment with dexamethasone or with angiotensin I results in an increase in the fetal expression of AQP2 mRNA. In the kidneys of newborn sheep, expression of AQP2 nearly doubles during 1-2 weeks of life, at which stage the levels of AQP2 were about 50% of the adult values in kidneys of pregnant sheep. The results reported by Butkus et al. (1999) brought new data on the physiology of the ovine kidney, but just as importantly contributed to our better understanding of the mechanism of urine production and the role of AQP2 in utero life and after birth. The changes in AQP2 expression in the ovine fetal kidney were repeatedly cited by other authors interested in the renal function in the fetal and perinatal period of life.

Sheep were also used in studies investigating the role of AQP2 and AQP1 in the vesicoureteral reflux, VUR (Gobet et al. 2008). For this purpose, VUR was surgically induced in male sheep fetuses at 95 days of gestation. After birth, the animals were sacrificed at age 6 months, and their kidneys were collected for AQP2 and AQP1 expression analysis. The results clearly demonstrated severe down-regulation of AQP2 and AQP1 expression under reflux with impaired urinary concentrating capacity. According to the authors, reduced expression of these proteins also proves their importance in the pathophysiological changes involved in urinary concentrating capacity during VUR. Once again, it has been noticed that urinary levels of AQP2 and AQP1 can be a useful parameter that facilitaes a diagnosis of early tubular damage in conditions associated with bilateral VUR.

## Localization and expression of AQP2 in equine kidneys

According to Floyd et al. (2007), the total body water of a fully grown 600 kg equid is 360 L, divided between intracellular (200 L), extracellular (100 L), and intravascular (60 l) compartments. An adult horse's body produces urine in an amount of 15-30 ml $kg^{-1}$ of body weight daily, which translates into 5 to 15 L of urine per day (Groendyk et al. 1988). In the first 6-8 weeks of life, foals produce greater urine volumes, up to 148 ml $kg^{-1}$ of body weight (Brewer et al. 1991). Given such a tremendous amount of urine produced and the volume of each water space, maintaining the correct water--electrolyte balance requires extremely effective mechanisms involved in the renal water excretion and retention. Despite this, renal disorders in horses, nephrogenic diabetes insipidus and urinary obstruction, are rare conditions. Common are, on the other hand, cases of psychotic polyuria, which often leads to loss in the ability of urine concentration. Horses also quite often suffer dehydration resulting from intestinal obstruction, endotoxemia or excessively intense work under improper conditions of the environment (Cohen et al. 1993).

Despite the fact that an equine kidney filters on average 1.6 L blood plasma per minute, which translates into 2000 L per day (Gleadhill et al. 2000), and that the amount of water reabsorbed in the renal tubules is equally large, there is little information on AQPs in horses. Literature brings only one report in this field, by Floyd et al. (2007), who evaluated the expression and nephron segment-specific distribution of major AQPs, including AQP2 in the kidney of the domestic horse. Western blotting revealed AQP2 in the cortex and medulla of the equine kidney, as a single band of about 30 kDa in molecular weight. The presence of AQP2-characteristic bands was also observed in the equine papilla. AQP3 and AQP4 were also identified in the equine cortex, medulla and papilla. A detailed immunohistochemical analysis revealed that AQP2 is localized in the apical plasma membranes of principal cells lining the cortical, medullary and papillary collecting ducts. In the basal membranes of principal cells, AQP3 and AQP4 were identified. This expression and localization AQP2, AQP3 and AQP4 are typical and characteristic for humans and animals. Presence of AQP2 in the apical membranes of CNT and CD suggests that this protein in horses plays an important role in renal water excretion regulation.

## AQP2 in the kidney of pigs

As is the case of other species of farm animals, renal disorders appear to be rare in swine. There are, however, cases of glomerular and tubular diseases, e.g. amyloidosis, glomerulonephritis, ischemic or nephrotoxic tubular necrosis. Pigs may also suffer from polyuria and polydipsia (Drolet 2007). Excessive thirst or abnormally large passage of urine are more common in sows compared to other animals. Dysfunctions in the renal regulation of the water-electrolyte balance in pigs are rare, which is probably a reason

of stagnation in the advances in this field observed in recent years. On the other hand, deeper knowledge of the physiology of the porcine kidney is desirable, since – besides pork production and genetic improvement – pigs seem to represent an important animal model of human diseases. Nephrology and renal physiology may be of special importance, as it has been demonstrated that the porcine kidney is morphologically and functionally similar to human one (Eskild-Jensen et al. 2007). The mini-pig is the breed most commonly used in medical research. A relatively recent article by Eskild-Jensen et al. (2007) provides an analysis of AQP2 expression in mini-pigs with the neonatal induced partial unilateral ureteral obstruction (PUUO). Luo et al. (2013), however, proposed a AQP2-Cre transgenic mini-pigs, which may be used as an object in investigations of gene functions in kidney development and the mechanism of human renal disease. The use of pigs as a research model motivated us to undertake further research aimed to analyze changes in renal AQP2 expression under varied nutrition regimes. Given a growing interest in the use of inulin and probiotics in diets of both humans and livestock animals, it was decided to identify in detail the expression and immunolocalization of AQP2 in the kidneys of growing piglets fed a diet supplemented this way (Michałek et al. 2016*a*, *b*). The piglets were offered a diet containing varied levels of inulin-type fructans or inulin with probiotics, and subsequently they showed an increased total AQP2 expression and an increase in the abundance of this protein in the apical plasma membrane of the CD principal cells. Changes in localization and expression of AQP2 in the piglets were most probably related to the homeostatic response of the kidneys to increased absorbtion of many micro- and macronutrients resulting from the supply of the supplemented diet. Namely, as is generally known, in order to sustain the proper water-electrolyte balance, an increased absorption and, in consequence, increased concentration of many components must be accompanied by increased renal water retention. Among the few papers about AQP2 in swine, there is a research report on the identification and expression analysis of AQP2 in the kidneys of free-living wild boars, *Sus scrofa* (Michałek et al. 2015). The presence of AQP2 in the renal tubules was also confirmed in this animal species. Similarly to humans and laboratory animals, AQP2 in wild boars is localized in the apical, basolateral and intracellular vesicles in the kidney collecting duct principal cells.

## CONCLUSIONS

Our current knowledge and understanding of AQP2 in the kidney of farm animals are still incomplete, despite the substantial progress in research methods and the knowledge gathered with respect to the role of AQP2 in the renal regulation of water retention in humans and laboratory animals. In this context, it is now difficult to propose the level of AQP2 as a measure

of specific renal functions. However, the knowledge already gained in this field indicates that AQP2 analysis in kidneys and urine may prospectively be used in farm animals, similarly as it is used in humans and laboratory animals. The data on the effect of a diet on changes in AQP2 expression and localization seem to be particularly promising in this respect. Therefore, it appears that a challenge for researchers in fields of modern biology and veterinary medicine is to determine the precise localization of AQP2 and species-specific factors regulating its expression. Such information will most likely help to explain the physiological roles of AQP2 and to attain deeper understanding of the renal function in farm animals. Not only may future studies on livestock animals broaden the knowledge of renal water retention in a particular species, but they can also bring new data to be applied in both animal husbandry and in the diagnosis and treatment of kidney diseases and disorders of the water-electrolyte balance in this group of animals.

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