THE ROLE OF CREATINE IN THE ORGANISM OF PIGS AND ITS EFFECT ON THE QUALITY OF PORK: A REVIEW*

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Abstract

In pigs, creatine is synthesized mainly in the liver, kidneys and pancreas from amino acids such as glycine, arginine and methionine. It is located mainly in skeletal muscle (approximately 95–98%) in the form of phosphocreatine. It is a source of energy for muscles, thus delaying the postmortem metabolism of glycogen and lactate formation and a decrease in pH during conversion of muscle to meat. Use of supplemental creatine monohydrate in pig feed may contribute to the improvement of pork quality parameters such as pH, colour, water holding capacity and drip loss. These parameters are also improved in pigs carrying the *RN*⁻ and *RYR1*^T gene. Creatine contained in meat can also be a precursor of heterocyclic aromatic amines mutagenic to humans, formed during thermal processing of meat.

Key words: pig, meat, muscle, creatine, creatine monohydrate, heterocyclic aromatic amines

Creatine (gr. *kreas*) was discovered in 1832 in beef meat by the French chemist Michel Chevreul (Shao and Hathcock, 2006; Wyss and Kaddurah-Daouk, 2000). Creatine (N-aminoiminomethyl-N-methylglycine) is a guanidino compound with a structure similar to the amino acids with a molecular weight $131 \cdot 10^3$ Da and a positive charge at physiological pH (Andres et al., 2008; Orsenigo et al., 2005).

The muscle creatine occurs mainly in the form of phosphocreatine, which is the main source of energy for contracting muscle fibres. It belongs to one of the eight phosphagenes occurring in animals and has its own enzyme creatine kinase (EC 2.7.3.2), discovered by Lohman in 1934. This is a key enzyme that controls the energy economy of the cell. Creatine kinase is also essential for the effective operation of pumps including calcium, providing calcium homeostasis in activated muscle cells (Grzyb and Skorkowski, 2008).

^{*}This study was supported by statutory activity.

Scientific reports show that creatine used as feed additive, primarily in the form of creatine monohydrate in pigs, can not only improve the functioning of the body, but also help to improve the quality of pork (James et al., 2002).

The aim of this study was to provide the characteristics of creatine and the effect of its use in the diet of pigs on pork quality.

Biosynthesis of creatine

Creatine is synthesized in the body from the amino acids glycine, arginine and methionine (Andres et al., 2008; Brosnan et al., 2011; Moret et al., 2011; Shao and Hathcock, 2006). It contains an entire glycine molecule and amidino group of arginine and a methyl group from S-adenosylmethionine, which is produced by its activation by the ATP. Amidine group of arginine is transferred by the enzyme Larginine:glycine amidinotransferase (EC 2.1.4.1) to glycine molecules, forming ornithine and guanidinoacetate. Next the S-adenosylmethionine passing the methyl group with the participation of glycine N-methyltransferase enzyme (EC 2.1.1.2) to guanidinoacetate creates creatine and S-adenosylhomocysteine (Brosnan et al., 2011, 2007; Verhoeven et al., 2005). About 40% of all methyl groups from S-adenosylmethionine are used for the synthesis of creatine. They are derived from the diet (i.e. methionine, betaine or choline) or produced endogenously in the methylneogenesis, which depends on the share of B vitamins (Brosnan and Brosnan, 2010; Brosnan et al., 2011; Szramko et al., 2010). During the synthesis of creatine in the body, about 40% of homocysteine is also produced. Dietary creatine, reducing the endogenous synthesis of creatine, may lower plasma homocysteine. Feeding rats a diet containing 0.4% creatine supplement for 14 days resulted in a decrease in plasma homocysteine levels by about 25%, without affecting the concentration of methionine (Brosnan et al., 2007). Thus, addition of creatine may have antitumour, antiviral and neuroprotective activity, among others (Ipsiroglu et al., 2001; Sestili et al., 2011). It can also prevent the formation of many chronic diseases, including diseases of the cardiovascular system (Brosnan et al., 2007; Moret et al., 2011). Creatine synthesis occurs primarily in the kidneys, liver and pancreas and to a lesser extent in brain and testis (Andres et al., 2008; Brosnan et al., 2011; Gualano et al., 2010; Shao and Hathcock, 2006; Snow and Murphy, 2001). Creatine is then transported through the blood and stored in cells of tissues with high energy requirements (Verhoeven et al., 2005). A significant part of it, about 95-98%, reaches skeletal muscle. The remaining amount of creatine is, inter alia, in the brain, heart, sperm, and smooth muscle (Gualano et al., 2010; Snow and Murphy, 2001). Creatine enters the cells via protein acting as a creatine transporter (CreaT) (approximately 70.5 · 10³ Da) dependent on the Na⁺ and Cl⁻ in the cell membranes (Brosnan et al., 2007; Orsenigo et al., 2005; Snow and Murphy, 2001; Verhoeven et al., 2005). Once entering the cell, creatine is phosphorylated to phosphocreatine. This reaction takes place with the participation of the enzyme creatine kinase, which controls energy cells (Brosnan et al., 2011; Orsenigo et al., 2005; Snow and Murphy, 2001). In the final stage about 1.7% of the creatine is converted by non-enzymatic removal of water for creatinine ring structure. The vast majority of this compound is excreted by the kidneys in the urine. The amount of creatinine removed is proportional to total creatine

and creatine phosphate in the body, and consequently also the total mass of muscle. A small part of creatinine may be converted to arginine or guanidinobutyrate (Brosnan et al., 2011, 2009; Dragsted, 2010; Mora et al., 2008; Snow and Murphy, 2001; Wyss and Kaddurah-Daouk, 2000).

Creatine in piglets

The bioavailability of creatine in the diet of newborn piglets may be close to 100% because they have greater activity of creatine in the intestines than adult animals (Brosnan and Brosnan, 2010; Brosnan et al., 2009). Probably the demand for creatine is proportionately greater in young animals than in adults, because in addition to covering losses resulting from the conversion of creatine to creatinine, it must also be delivered to the growing tissues. Creatine concentration in milk (about 529 μ mol/litre) is about two-fold higher than in plasma (about 292 μ mol/litre) of lactating sows (Brosnan et al., 2009). In piglets between 4 and 11 days old, the demand for total creatine is approximately 12.5 μ mol/week, of which approximately 23% (2.8 μ mol/week) is supplied with sow's milk, and the remaining 77% (9.7 μ mol/week) is synthesized *de novo* by piglets (Brosnan et al., 2011). The synthesis of creatine uses up to 63–77% of all labile methyl groups used by piglets (Brosnan et al., 2011, 2009).

Creatine also affects the proper development and functioning of the central nervous system in piglets. It plays a very important role in energy processes in the brain. Providing energy homeostasis by buffering the ATP has a direct impact on its proper functioning (Andres et al., 2008).

The metabolism of creatine in the muscle of pigs

The primary source of energy in the cell is ATP whose concentration is relatively low (2-5 mM), which allows for contraction of the muscles for only a few seconds. Therefore, additional processes must occur to maintain metabolic homeostasis allowing ATP in activated muscle cell. This status can be maintained due to the high resources of phosphocreatine (20-35 mM) in the cell, which allows regeneration of ATP as a result of reversible reaction catalyzed by creatine kinase. This enzyme transfers a phosphate group from phosphocreatine to ADP, resulting in the synthesis of cellular ATP. During the regeneration of ATP, creatine kinase using ADP and H⁺ prevents the acidification of the muscles and increases the concentration of ADP. A decrease in ATP may cause ADP conversion to ATP and AMP through the adenylate kinase (EC 2.7.4.3) and IMP formation from AMP and ammonia. The creatine kinase reaction prevents the increase of free ADP, which could inhibit the ATP-dependent processes. The increase in AMP during extended and exhausting muscle work causes the activation of activated protein kinase (AMPK), which inactivates the enzymes by their phosphorylation, and stimulates fatty acid oxidation and glucose transport and uptake in muscle (Dziewulska et al., 2010; Grzyb and Skorkowski, 2008; Scheffler and Gerrard, 2007; Westerblad et al., 2010).

Phosphocreatine represents about 2/3 of creatine in muscle. High concentrations of phosphocreatine occur in tissues with high energy requirements. Fast-glycolytic muscles contain very high phosphocreatine resources compared with slowoxidative muscles (Grzyb and Skorkowski, 2008; Scheffler and Gerrard, 2007). It was shown that meat from Duroc pigs has a higher content of phosphocreatine (4.2–23.4 µmol/g of meat) than the meat of Landrace pigs (7.0–18.7 µmol/g of meat) (Pfau et al., 2006). Phosphocreatine in pig longissimus muscle is present in higher concentrations (18-19 µmol/g) than ATP (6.6-6.8 µmol/g) (Grzyb and Skorkowski, 2008; Scheffler and Gerrard, 2007). Phosphocreatine and oxidative metabolism are able to satisfy the energy needs of muscles when the levels of oxygen are adequate and the muscles contract slowly. Otherwise the muscles begin to contract fast, which leads to oxygen depletion and degradation of phosphocreatine, causing a sharp decline of ATP in the muscles. This is followed by lysis of anaerobic glycolysis of muscle glycogen to lactic acid and hydrogen ions to rephosphorylate ADP to ATP, which prevents the formation of a lasting actomyosin bond (Scheffler and Gerrard, 2007). The distribution of glycogen in muscle is regulated by glycogen phosphorylase. The rest is glucose release from glycogen, which in the process of glycolysis is converted to pyruvate. During intense exercise lactate dehydrogenase converts pyruvate and NADH⁺ H⁺ into lactic acid and NAD⁺. Regeneration of NAD⁺ is essential to the process of glycolysis, while the rate of lactic acid formation depends on the availability of oxygen in relation to energy demand. The accumulation of lactic acid in muscle occurs primarily during large muscle activity when the rate of ATP consumption is high. In parallel, the accumulation of hydrogen ions with ions of lactic acid during growth of muscle activity lowers the pH value (Westerblad et al., 2010).

The rate and extent of pH decrease during conversion of muscle to meat has no significant impact on meat quality characteristics (Lindahl et al., 2006; Scheffler and Gerrard, 2007). Under conditions of normal metabolic processes muscle pH decreases progressively from 7.4 in the muscle after slaughter to about 5.6–5.7 within 6–8 hours, and then to about 5.3–5.7 after 24 hours. Faster decrease in pH below 6.0 during the first hours after slaughter and ultimate pH 5.3–5.7 in the muscles is caused by rapid glycolysis and produces large quantities of heat, which is important during the chilling of carcasses. The rate and extent of pH decline after slaughter also has a significant influence on the properties of proteins, and thus the quality of pork. High muscle temperature after slaughter and low pH cause denaturation of about 20% of myofibrils and sarcoplasmic proteins and reduce the distance between myosin heads, affecting water loss by increasing muscle. A significant reduction in sarcoplasmic proteins myofibrils may hamper the ability to maintain water in PSE-type meat (pale, soft, exudative) (Scheffler and Gerrard, 2007).

The meat of pigs carrying the $RYRI^{T}$ gene undergoes a faster process of glycogenolysis and glycolytic changes after slaughter. Reducing the concentration of ATP, phosphocreatine and glycogen leads to accumulation of lactic acid, which causes a rapid reduction in pH and increase in temperature of the meat after slaughter. The excessive protein denaturation myofibrils adversely affect water holding capacity and colour of meat, which is defined as PSE. Technological efficiency of meat from pigs carrying the gene $RYRI^{T}$ can be reduced by 2–3% compared to the meat from individuals not carrying that gene. The muscles of pigs carrying the $RYRI^{T}$ gene are subject to problems with normal regulation of Ca²⁺ ion concentration in muscle cells. Abnormal movement of Ca^{2+} through calcium channels in the cell, resulting in immobilization of the calcium pump in the endoplasmic reticulum to stop the calcium ions in sarcoplasm, leads to sustained muscle contraction, which affects the meat quality characteristics (Berg and Allee, 2001; Rosenvold and Andersen, 2003; Scheffler and Gerrard, 2007).

Addition of creatine monohydrate to feed for pigs may be a buffer against the lactic acid produced, reducing the sharp decrease in pH of PSE meat, thereby changing its colour (Berg and Allee, 2001; Stahl et al., 2005; Young et al., 2005). The loin muscles of Duroc pigs had darker colour and a smaller proportion of redness (a*) and vellowness (b*) compared to Landrace pigs, which could be due to a slower decrease in pH value and ultimately a higher pH in the meat (Lindahl et al., 2006). The addition of 12.5, 25 or 50 g creatine monohydrate/day for 5 days before slaughter resulted in an increase of pH value and water absorption in the longissimus dorsi muscle of Duroc pigs and lower colour parameters (L^* – colour lightness, a* and b* – chromaticity coordinates) and improved meat juiciness in Landrace pigs. After the addition of creatine monohydrate water content in meat was similar in Landrace and Duroc pigs at 74.6 and 74.1%, respectively (Young et al., 2005). The drip loss was lower in the Duroc meat breed (3.5%) compared to the Landrace meat breed (6.1%) (Lindahl et al., 2006; Young et al., 2005). The pH_{as} value increased by 0.27 units (P<0.05) in the semimembranosus muscle of pigs receiving supplemental creatine monohydrate. This supplement, however, did not affect the drip loss and composition of muscles. The addition of creatine monohydrate has also led to lower values for colour lightness (L*) in two hams: semitendinosus (5.15 units) (P<0.05) and semimembranosus (1.95 units) from the carcasses of animals that carry the $RYR1^T$ gene (Maddock et al., 2002). The supplementation of 25 g/day of creatine monohydrate for 10 days before slaughter in barrows did not significantly affect the meat quality parameters such as pH, colour, water holding capacity and drip loss (O'Quinn et al., 2000). The addition of 0.92% creatine monohydrate and 2.75% dextrose to feed for barrows and gilts did not cause statistically significant changes in the parameters of pork quality by reducing only the values of redness (a*) and yellowness (b*) in the sirloin (Berg et al., 2011).

An important factor of genetic changes in muscle metabolism is the presence of the Rendement Napole (RN^{-}) gene in pigs (Maddock et al., 2002; Scheffler and Gerrard, 2007). This single point mutation of the gene is responsible for increased muscle glycolytic potential (> 180–200 µmol/g muscle), which delays the decrease in pH postmortem. It was shown that Hampshire pigs, in which RN^{-} gene was first described, had more than twice the muscle glycogen compared with other breeds. Animals carrying the RN^{-} gene were reported to have higher levels of phosphocreatine in the muscles than pigs with other genotypes (Scheffler and Gerrard, 2007). The meat of pigs with the RN^{-} gene is often referred to as "acidic meat" due to low pH, which lowers its technological quality. The RN^{-} gene does not affect the initial pH after slaughter, but results in a lower final pH value, which is associated with higher reflectance (lighter meat) and poor water absorption of the meat. Differences in water holding capacity of pork meat in carriers and non-carriers of the RN^{-} gene may be due to a much higher denaturation of contractile and sarcoplasmic proteins of pork. The RN^{-} gene increases the loss of only about 1%, while the technological capacity of meat is reduced by 5–6% in carriers of RN^{-} gene, compared with individuals not carrying this gene (Rosenvold and Andersen, 2003 a).

Effect of creatine on body weight growth of pigs

The use of creatine monohydrate added at 50 g/day resulted in increased levels of creatine in the blood plasma of pigs from about 85 μM to 174 μM in females and 195 μ M in castrates (Rosenvold et al., 2007). A study with Duroc and Landrace pigs showed that creatine levels in plasma increased in a dose-dependent manner. The supplement may also contribute to the improvement of weight growth of pigs (Young et al., 2007). Before the feed supplement was provided, the average daily growth in Duroc pigs (598 g/day) was significantly higher than in Landrace pigs (555 g/day). The administration for 5 days of creatine monohydrate supplement increased body weight with increasing supplement in the diet. At 50 g/day of creatine monohydrate, weight growth increased by about 2 kg in Duroc pigs and by about 3 kg in Landrace pigs. In these breeds the supplement of 12.5, 25 and 50 g/day caused an increase in body weight (1.91, 1.48 and 2.53 kg, respectively) relative to the control group (Young et al., 2005). In turn, the supplementation of 25 g/day of creatine monohydrate to the feed for barrows for 10 days before slaughter did not significantly affect the body weight gains (O'Quinn et al., 2000). Pigs resistant to stress, and carriers of the $RYRI^T$ gene received the supplement of creatine monohydrate in an amount of 25 g/day for 5 days before slaughter. Pigs receiving the supplement gained 2.26 kg more body weight (P<0.05) within 5 days of supplementation (Maddock et al., 2002). Body weight growth may be due to improved retention of muscle proteins and water in the muscles, because the intracellular creatine and phosphocreatine may increase the intracellular osmotic pressure, causing water penetration into the muscle cells (Berg and Allee, 2001; Stahl et al., 2001; Young et al., 2005; 2007). Creatine also has antioxidant activity. As a low molecular weight antioxidant it protects the cells in the aquatic environment against oxidative stress (Lawler et al., 2002; Sesili et al., 2011). The increase in creatine phosphate content in muscle of pigs following 5-day administration of creatine monohydrate may depend on the breed, because there were only Duroc and not Landrace pigs (Young et al., 2007).

Heterocyclic aromatic amines

The presence of creatine and its anhydride can have a negative impact on meat, because they may be precursors of heterocyclic aromatic amines, which are formed on the surface of meat during thermal processing (Mora et al., 2008; Pfau et al., 2006). In animal studies they cause cancer of various organs. A diet rich in meat, due to the possibility of heterocyclic aromatic amines, may increase the risk of human cancers, including colon, prostate and pancreas (Pfau et al., 2006; Polak et al., 2009). Their concentration occurs at the level of ppb (parts per billion) and depends mainly on the amount of heating time and temperature, but also on water content, the proportion of amino acids and type of meat. The content of the precursors of heterocyclic aromatic amines is greater in PSE meat than in normal pork, because it increases the drip loss (7.6 compared with 4.7 g/100 g) (Polak et al., 2009). The

addition of creatine monohydrate to feed for pigs, increasing the concentration of creatine in meat, may contribute to greater human exposure to the mutagenic effects (Pfau et al., 2006; Polak et al., 2009). However, creatine monohydrate supplemented to feed at up to 50 g/day for 5 days before slaughter does not increase the levels of heterocyclic aromatic amines, which are formed during cooking of pork (Pfau et al., 2006).

In conclusion, creatine is found in the muscles mainly as phosphocreatine, providing a source of energy necessary for contraction of skeletal muscles. The high content of creatine phosphate, especially in meat rich in glycolytic fibres, can delay the formation of lactic acid, thus lowering the pH during conversion of muscle to meat. The supplementation of creatine monohydrate to the feed for pigs may contribute to the improvement of pork quality parameters such as pH, colour, water holding capacity and drip loss. These parameters are also improved in pigs carrying the RN^- and $RYRI^T$ gene. Creatine found in meat may also be a precursor of heterocyclic aromatic amines which have carcinogenic properties to humans and are formed during thermal processing of pork.

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Accepted for printing 10 I 2013

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Rola kreatyny w organizmie świń i jej wpływ na jakość mięsa wieprzowego: artykuł przeglądowy

STRESZCZENIE

U świń kreatyna jest syntetyzowana głównie w wątrobie, nerkach i trzustce z takich aminokwasów, jak: glicyna, arginina i metionina. Znajduje się ona głównie w mięśniach szkieletowych (około 95–98%) w postaci fosfokreatyny. Stanowi źródło energii dla mięśni, a tym samym opóźnia metabolizm glikogenu *post mortem* oraz tworzenie mleczanu i spadek pH podczas konwersji mięśni do mięsa. Stosowanie dodatku monohydratu kreatyny do pasz dla świń może przyczynić się do poprawy takich parametrów jakości wieprzowiny, jak: pH, barwa, wodochłonność czy wielkość swobodnego wycieku. Parametry te ulegają również poprawie u świń będących nosicielami genów *RN*⁻ i *RYR1*^T. Zawarta w mięsie kreatyna może być także prekursorem heterocyklicznych amin aromatycznych o właściwościach mutagennych dla ludzi, powstających podczas obróbki termicznej mięsa.