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The influence of betaine and creatine monohydrate supplementation on chemical composition, oxidative stability and pork quality in RYR1 heterozygous pigs

Vplyv suplementácie betaínu a kreatínmonohydrátu na chemické zloženie, oxidačnú stabilitu a kvalitu mäsa ošípaných heterozygótnych v géne RYR1

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ABSTRACT

The aim of this study was to evaluate an effect of betaine and creatine monohydrate supplementation on chemical composition, meat quality, and oxidative stability of pork in pigs genotyped on mutation in *RYR1* gene. Totally, sixty pigs – crosses of Lx(HAxPN) were involved in the experiment. Pigs were divided to one homozygous (NN) – control (C1), and three heterozygous (Nn) groups, each group including 15 animals. One group of Nn pigs was the control (C2) and two Nn groups were experimental. Control groups received a standard diet without supplement. Experimental groups were fed the same composition as controls but with supplement of betaine – BET (1.25 g/kg of feed) or creatine monohydrate - CMH (2.0 g/kg of feed) for thirty days prior to slaughter. Pigs C2 had worse meat quality, especially pH_{45}, colour L^* and drip loss ($P<0.05$), than homozygous C1 pigs. Dietary supplementation with betaine increased initial pH and decreased drip loss in Nn group ($P<0.05$) compared to C2 group. Supplementary CMH also increased pH_{45} and had a tendency to lowering the drip loss ($P=0.058$). Both feed additives substantially improved oxidative stability of pork, especially after 120 min incubation ($P<0.01$), compared to control C2 pigs.

Keywords: pig, betaine, creatine monohydrate, pork, meat quality, oxidative stability

ABSTRAKT

Cieľom štúdie bolo zhodnotiť vplyv suplementácie kŕmnej dávky betaínom a kreatínmonohydrátom na chemické zloženie, kvalitu a oxidačnú stabilitu mäsa ošípaných genotypovaných na mutáciu v géne *RYR1*. Do pokusu bolo zaradených 60 ošípaných – hybridov Lx(HAxPN). Ošípané boli rozdelené do jednej homozygótnej (NN) kontrolnej skupiny (C1), a troch heterozygótnych (Nn), pričom v každej bolo po 15 jedincov. Jedna skupina heterozygótov bola kontrolná (C2) a dve Nn skupiny boli experimentálne. Kontrolné skupiny (C1, C2) boli kŕmené štandardnou zmesou bez akéhokoľvek prídavku. Pokusné skupiny boli kŕmené tou istou zmesou ako kontrolné, avšak s prídavkom betaínu – BET (1,25 g/kg krmiva) alebo kreatínmonohydrátu – CMH (2,0 g/kg krmiva) po dobu 30 dní pred porážkou. Ošípané C2 mali horšiu kvalitu mäsa, najmä pH_{45}, farbu L^* a straty odkvapom ($P<0,05$) než skupina C1. Suplementácia betaínom zvýšila počiatočné pH a znížila straty odkvapom ($P<0,05$) v porovnaní s C2 skupinou. Prídavok CMH taktiež zvýšil pH_{45} a mal tendenciu znížiť straty odkvapom ($P=0,058$) oproti C2 skupine. Obe kŕmne aditíva výrazne zlepšili oxidačnú stabilitu mäsa, obzvlášť po 120 min inkubácii ($P<0,01$), v porovnaní s kontrolnou C2 skupinou.

Kľúčové slová: ošípaná, betaín, kreatínmonohydrát, bravčové mäso, kvalita mäsa, oxidačná stabilita

INTRODUCTION

The main target of current pig production has been to increase lean meat content and growth intensity. However, selection on these parameters has led to a decreasing pork quality and higher stress susceptibility of pigs. Relationship between increased meatiness and deteriorated pork quality has been explained mainly by the appearance of the recessive „*n*" allele of the *RYR1* gene. This allele causes a rapid pH decline in skeletal muscle *post mortem* leading to the formation of pale, soft, exudative (PSE) meat (Pommier and Houde, 1993; De Smet et al., 1996; Fiedler et al., 2001; Marini et al., 2012; Rey-Salgueiro et al., 2018). The recessive homozygous pigs (*nn*) have been found to show higher lean meat content in carcass but at the same time greater incidence of PSE meat compared to pigs free of „*n*" allele (NN genotype). Studies on carcass and meat quality of heterozygous pigs (Nn genotype) are inconsistent. Some of them reported only little or no effect on pork quality (e.g. Kortz et al., 2003; de Oliveira Band et al., 2005; Rybarczyk et al., 2016) while others showed a great impact (Silveira et al., 2011; Tomažin et al., 2017; Čobanović et al., 2019, etc.). Therefore, improving pigmeat parameters and oxidative stability of pork using different feed additives has been investigated worldwide in many studies. Especially for the last ten to -fifteen years, research on dietary betaine and creatine monohydrate effects in swine industry has been observed. Most of these studies have been concerned with carcass and fattening properties (O´Quinn et al., 2000a, 2000b; Yu et al., 2004; Feng et al., 2006; Stahl et al., 2007; Yang et al., 2009; Rojas-Cano et al., 2011) but little research on pork quality has been performed.

Betaine is known as a product of choline degradation. It occurs naturally in many tissues where it plays a role in various metabolic processes as a methyl donor (e.g., formation of methionine) or its demethylation creates glycine. Betaine might be useful for improving the meat quality and sensory properties of pork assuming that it is accumulated in muscles (Matthews et al., 2001b). Some reports have shown positive effect of dietary betaine supplementation on pork quality, especially on its pH (Matthews et al., 2001a,b; Lindahl et al., 2006), and colour (Yang et al., 2009; Su et al., 2013) whereas others showed no such effects (Øverland et al., 1999; Madeira et al., 2015).

Creatine is an amino acid derivative occuring mainly in skeletal muscles where 2/3 is stored as creatine phosphate. It supplies the muscles with energy, increases their volume by binding the water in muscle cells, acts at formation of adenosine triphosphate (ATP) which represents immediate source of energy. The increase of energy stores in pig muscles by creatine monohydrate supplementation could affect the glycogen metabolism post mortem (buffering lactic acid production). This might reduce the rapid pH decline and occurrence of PSE meat (Prevost et al., 1997). Some studies found a positive effect of creatine supplementation on pH, water holding capacity (WHC), colour or drip loss (Maddock et al., 2000, 2002; Stahl et al., 2001; Berg and Allee, 2001; James et al., 2002; Lahučký et al., 2012; Li et al., 2015, 2018). On the other hand, some studies suggest no or even a negative effect on pork quality (O´Quinn et al., 2000a; Stahl and Berg, 2003).

Discrepancies between the impact of betaine and creatine on pork quality might be due to incidence of „*n*" allele of the *RYR1* gene of experimental pigs included in several studies. However, only a small number of studies have been performed regarding incidence of *RYR1* gene in pigs supplemented with betaine or creatine monohydrate. Therefore the aim of this study was to evaluate the effect of betaine and creatine supplementation on chemical composition, meat quality and oxidative stability of pork in pigs genotyped on mutation in *RYR1* gene.

MATERIAL AND METHODS

Animals and treatments

Totally, sixty pigs – crosses between Landrace (L) sows and Hampshire x Pietrain (HAxPN) boars were involved in the experiment. Animals were housed in the pens (1.75 m^2 per pig) at experimental farm of Research Institute for Animal Production in Nitra, Slovakia (RIAP).

Pigs were tested for occurrence of mutation in Halothane gene by DNA based test (Bauerová et al., 1996) and divided to one homozygous (NN) – control 1 (C1), and three heterozygous (Nn) groups, each of 15. One group of Nn pigs was control 2 (C2) and two Nn groups were experimental. Control groups received the standard diet (Table 1) without any supplement. Experimental groups were fed standard diet with the same composition as controls but with supplement of betaine (1.25 g/kg of feed) – group BET or creatine monohydrate - CMH (2.0 g/kg of feed) – group CMH for thirty days prior to slaughter. All feed mixtures including those with supplements were supplied by a specialized company.

Table 1. Composition and nutritive value of the diets

Ingredients, g/kg	C1 + C2	BET	CMH
Barley	427	427	427
Wheat	210	210	210
Oat	150	150	150
Soybean meal	120	120	120
Wheat brans	20	18.75	18
Meat and bone meal	20	20	20
Fodder yeast	17	17	17
Mineral supplement	25	25	25
Biofactor supplement	6	6	6
Fodder salt	5	5	5
Betaine	-	1.25	-
Creatine monohydrate	-	-	2
Analysed, g/kg			
Dry matter	863.4	864.2	864.5
Crude protein	168.4	168.8	170.5
Crude fat	24.3	25.2	25.5
Crude fibre	48.6	50.3	50.1
N-free extract	577.8	574.3	575.2
Metabolizable energy (MJ/kg)	12.3	12.4	12.4
Lysine, in DM	8.6	9.0	8.9
Betaine, in DM	-	1.38	-
Creatine monohydrate, in DM	-	-	2.25

C1, C2 – control groups, BET – betaine supplemented group, CMH - creatine monohydrate supplemented group

Pigs were allowed free access to drinking water during the whole experiment. Feed was provided for *ad libitum* consumption with recording of consumption. The experiment was performed in accordance with the Act on animal veterinary care No. 39/2007 of Slovak Republic and approved by the Animal Care Committee of the Research Institute for Animal Production.

Slaughter and muscle sampling

Animals were slaughtered at 110 ± 5 kg live weight at the experimental slaughterhouse of RIAP by electrical stunning (90 - 100 V, 0.9 - 1.0 A, 50 Hz) followed by exsanguination. Evisceration was completed about 20 min post mortem. Chilling of the carcasses (air temperature 2 - 4 °C, velocity 0.5 – 1.0 m/s) started approximately 60 min after slaughter and was continued overnight. After 24-hours chilling of carcasses at 4 °C, the *longissimus dorsi* (LD) muscle (150 g of sample) was removed from the right side of carcass and sliced into chops 2.5 cm thick for further meat quality (colour, drip loss) and chemical composition analysis. One wrapped sample was stored in the dark for 5 days at 4 °C for shear force and colour analysis.

Chemical analyses

Approximately 30 min post mortem, *longissimus dorsi* muscle samples (100 g) were taken, wrapped into aluminium foil and imposed in liquid nitrogen for 5 days for oxidative stability analysis. The oxidative capacity of *longissimus muscle* homogenate was determined as thiobarbituric acid reactive substances (TBARS) according to Kuechenmeister et al. (1999). TBARS values were expressed as equivalents of malondialdehyde (MDA, nM/mg homogenate protein) which is a breakdown product formed during peroxidation of lipids stimulated by Fe^{2+}/ascorbate. To stimulate lipid peroxidation, 3 ml of muscle homogenate was incubated in 0.1 mM ascorbate and 5 mM $FeSO_4$. From this, 0.5 ml was immediately removed and pipetted into 0.25 ml of 20% trichloroacetic acid in 100 mM KCl. The remaining homogenate was placed in a water bath at 37 °C and after 30, 60, and 120 min, 0.5 ml each of this incubated homogenate were transferred into the trichloroacetic acid. Subsequently, the samples were centrifuged at 10 000 g for 10 min and 0.5 ml of the supernatants were mixed with 0.5 ml of thiobarbituric acid (0.67%) and boiled for 15 min in a water bath. The absorbance at 535 nm was determined immediately after cooling.

Chemical composition of pork – total water, protein and intramuscular fat content were determined using Infratec Analyzer.

Meat quality measurements

Approximately 45 min and 24 h after slaughter, pH values were taken from the right side of carcass in the loin between 13th and 14th rib. The pH was measured using pH meter Mettler-Toledo (Switzerland). Colour was measured by Hunter Lab MiniScan spectrometer (L-lightness, a-redness, b-yellowness). Drip loss was assesed by Honikel method (1998), shear force was determined as a tenderness on cooked samples (core temperature 80 °C, time 20 min) using Warner-Bratzler apparatus (Texture Analyser TA-XT2i, Stable Micro Systems Ltd, Survey, UK).

Statistical analysis

Data from the experiment were analysed by a two-way ANOVA with fixed effects of treatment (betaine, creatine monohydrate or none) and *RYR-1* genotype (NN, Nn) and corresponding two-way interactions using procedure GLM of the statistical program SAS-STAT, version 9.1.3 (SAS Institute Inc., Cary, N.C., USA, 2002-2003). Data in tables are expressed as least square means (LSM) ± standard error (SE). Significance of differences between groups was tested by a Tukey-test. Whilst no significant interactions between genotype and diet were found, we did not show them in the tables.

RESULTS AND DISCUSSION

Effect on chemical composition

Chemical composition of pigmeat was not affected by the *RYR1* genotype (Table 2). This is in agreement with other studies (Kortz et al., 2003; Škrlep et al., 2010; Rybarczyk et al., 2016; Tomažin et al., 2017). Feeding

Table 2. Chemical composition of *longissimus dorsi* muscle

Trait	C1	C2	BET	CMH	SE
Total water, %	73.85	73.72	73.60	73.61	0.22
Total protein, %	22.53	22.32	22.48	22.21	0.19
Intramuscular fat, %	2.65	2.58	2.69	3.03	0.11

C1 – NN without supplement, C2 – Nn without supplement, BET – Nn with betaine, CMH – Nn with creatine monohydrate, SE – standard error

supplemental betaine at level 1.25 g/kg for thirty days before slaughter did not have any significant effect on chemical composition (P>0.05). The same result of betaine on total water was detected by Matthews et al. (2001a). Rojas-Cano et al. (2011), Su et al. (2013), and Madeira et al. (2015, 2016) did not find any effect on intramuscular fat. Supplementation of diet with creatine monohydrate had also no influence on chemical composition of pork in Nn heterozygous pigs of our study (P>0.05). These findings are comparable with other authors (Stahl et al., 2001; Berg and Allee, 2001; Berg et al., 2003, 2011, Lahučký et al., 2012; Li et al., 2015).

Effect on pork quality

Meat quality characteristics of control and experimental groups are summarized in Table 3. Pigs heterozygous in *RYR1* had lower initial pH compared to NN ones (P<0.05) whereas ultimate pH was almost the same in both groups (P>0.05). These findings correspond with other studies (de Oliveira Band et al., 2005; Otto et al., 2007; Škrlep et al., 2010; Rybarczyk et al., 2016; Tomažin et al., 2017; Čobanović et al., 2019). Feeding supplemental betaine in our study significantly increased initial pH but not ultimate in Nn pigs. The same effect of betaine was reported by Matthews et al. (2001b) and Yang et al. (2009). Supplementation of CMH in our study also positively influenced pH_{45} but not pH_{24}. This is comparable with the results of Stahl et al. (2001, 2007), Berg et al. (2003, 2011), Lindahl et al. (2006), Rosenvold et al. (2007), and Li et al. (2015, 2018). The increase in initial pH may indicate that the rate of pH decline after slaughter is slower in pigs fed diets supplemented with betaine or creatine monohydrate. Moreover, supplementation of CMH can increase the content of creatine phosphate, especially in meat rich in glycolytic fibres, and delay the formation of lactic acid lowering pH early post mortem in pigs carrying mutation in *RYR1* gene (Janicki and Buzała, 2013). The effect of this gene on pork colour parameter L* 24 h post mortem was detected (P<0.05). Pigs free on mutation (NN) had darker meat than Nn pigs (49.31 vs. 50.67, P<0.05). These results are comparable with other studies (Otto et al., 2007; Škrlep et al., 2010). The values of colour lightness (L*) after betaine and/or CMH adding were lower than in Nn pigs fed without additives, however differences were not significant (P>0.05). Our findings confirmed the results of other investigations on betaine (Matthews et al., 2001b; Feng et al., 2006; Yang et al., 2009) or creatine supplementation (Stahl et al., 2001, 2007; Rosenvold et al., 2007). Other colour parameters (a*, b* 24 h or 5 days as well as L* 5 days after slaughter) were not affected by feed additives.

Heterozygous pigs had higher drip loss compared to NN pigs (4.37 vs 2.34 %). As known, drip loss is related to faster pH drop and worse water holding capacity in muscles of heterozygous Nn pigs. Significant differences between both genotypes are well-documented in the literature (e.g., Kortz et al., 2003; de Oliveira Band et al., 2005; Tomažin et al., 2017). Betaine in our study significantly reduced drip loss in heterozygous pigs (3.05 vs 4.37 %, P>0.05), comparable with Matthews et al. (2001b) who found decrease of drip loss by 11 %. Supplementation of CMH showed tendency to significance (P=0.058) compared to C2 pigs. Results of other studies are contradictory. Lahučký et al. (2012) and Li et al. (2015, 2018) reported reducing the drip loss whereas some studies did not show any effect of CMH (Berg and Allee, 2001; Young et al., 2005; Rosenvold et al., 2007; Berg et al., 2011).

Table 3. Pork quality of *longissimus dorsi* muscle

Trait	C1	C2	BET	CMH	SE
pH_{45}	6.43[a]	6.05[b]	6.31[a]	6.33[a]	0.09
pH_{24}	5.54	5.50	5.52	5.53	0.05
Colour (24 h) L*	49.31[a]	50.67[b]	50.37[ab]	49.58[ab]	0.84
a*	1.81	1.65	1.88	1.87	0.39
b*	7.74	7.85	7.67	7.54	0.26
Colour (5 days) L*	51.48	51.75	51.12	51.72	1.05
a*	2.55	2.36	2.44	2.37	0.24
b*	8.81	8.96	8.62	8.88	0.29
Drip loss (24 h), %	2.34[a]	4.37[b*]	3.05[a]	3.52*	0.27
Shear force (5 days), kg	3.55	4.88	4.32	4.21	0.36

C1 – NN without supplement, C2 – Nn without supplement, BET – Nn with betaine, CMH – Nn with creatine monohydrate, SE – standard error
[a,b] Different letters mean significant differences between treatments (P<0.05)
*P=0.058

The influence of *RYR1* gene on tenderness of *longissimus dorsi* muscle determined by the Warner-Bratzler shear force showed lower value in Nn pigs compared to NN ones but differences were insignificant (P>0.05). The same result was found by de Oliveira Band et al. (2005) while Tomažin et al. (2017) found higher value in heterozygous Nn pigs. Although, both feed additives – betaine and CMH – in our study reduced shear force, differences were not significant compared to control Nn pigs (4.32, 4.21 vs 4.88). Comparable results were found by Matthews et al. (2001a,b), Stahl et al. (2001), Berg et al. (2003, 2011) and Lahučký et al. (2012). Yang et al. (2009) and Li et al. (2018) suggested reduced shear force value in pork loin and semitendinosus muscle after betaine supplementation.

Effect on oxidative stability

Oxidative stability of *longissimus dorsi* muscle is presented in Table 4. This parameter was not affected by *RYR1* genotype. This is in agreement with Tomažin et al. (2017). Significantly lower values of MDA production after 30, 60 and 120 min incubation with Fe^{2+}/ascorbate mixture were found in Nn pigs supplemented by betaine or creatine monohydrate. Tendency of reducing lipid oxidation in pork stored for 13 days after betaine supplementation was only outlined in the study of Hur

Table 4. Oxidative stability of *longissimus dorsi* muscle

Trait	C1	C2	BET	CMH	SE
TBARS (0 min), nM/mg	0.06	0.06	0.06	0.06	0.001
TBARS (30 min), nM/mg	0.18[a]	0.16[ab]	0.19[a]	0.12[b]	0.02
TBARS (60 min), nM/mg	0.36[a]	0.34[a]	0.25[b]	0.22[b]	0.02
TBARS (120 min), nM/mg	0.49[A]	0.52[A]	0.32[B]	0.29[B]	0.03

C1 – NN without supplement, C2 – Nn without supplement, BET – Nn with betaine, CMH – Nn with creatine monohydrate, [a,b] Different letters mean significant differences between treatments (P<0.05), [A,B] Different letters mean significant differences between treatments (P<0.01)

et al. (2007). Su et al. (2013) suggested great reduction of TBARS values in pork stored for 4, 7, 10 or 13 days after betaine adding. Lahučký et al. (2012) found positive effect of CMH on oxidative stability of pork. Possible explanation of positive effect of betaine or creatine could be decreasing the concentration of polyunsaturated fatty acids in muscle that are susceptible to lipid oxidation as reported by Su et al. (2013) in case of betaine.

CONCLUSIONS

Our results confirmed poorer pork quality of pigs with a mutation in the *RYR1* gene, especially initial pH, colour lightness L^* and drip loss ($P<0.05$). Supplementation of diet by betaine or creatine monohydrate significantly increased pH_{45}. Betaine supplementation also reduced drip loss ($P<0.05$) whereas in case of creatine there was a tendency to significance. Moreover, feeding with betaine or creatine monohydrate improved oxidative stability of pigmeat ($P<0.05$). Further research is needed to determine the optimal level and time of supplementation of both additives for a more beneficial effect.

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DECLARATION OF COMPETING INTEREST

The authors confirm that there is no competing financial or any other interest in this work.

REFERENCES

Bauerová, M., Vašíček, D., Uhrín, P., Chrenek, P., Mlynek, J., Bulla, J. (1996) Detection of malignant hyperthermia in pigs by DNA-test using plucked hair as a source of DNA. Pig News Information, 16, 109N-111N.

Berg, E.P., Allee, G.L. (2001) Creatine monohydrate supplemented in swine finishing diets and fresh pork quality: I. A controlled laboratory experiment. Journal of Animal Science, 79, 3075-3080. DOI: https://doi.org/10.2527/2001.79123075x

Berg, E.P., Maddock, K.R., Linville, M.L. (2003) Creatine monohydrate supplemented in swine finishing diets and fresh pork quality: III. Evaluating the cumulative effect of creatine monohydrate and alpha-lipoic acid. Journal of Animal Science, 81, 2469-2474. DOI: https://doi.org/10.2527/2003.81102469x

Berg, E.P., Stahl, C.A., Shannon, M.S., McNamara-Perry, D.L., Schmidt, T.B., Wiegand, B.R. (2011) The influence of dietary protein on market barrows and gilts supplemented creatine monohydrate in conjunction with a high glycemic carbohydrate. Meat Science, 88, 429-433. DOI: https://doi.org/10.1016/j.meatsci.2011.01.022

Čobanović, N., Stajković, S., Grković, N., Suvajdžić, B., Vasilev, D., Karabasil, N. (2019) Effects of *RYR1* gene mutation on the health, welfare, carcass and meat quality in slaughter pigs. Proceedings of 60th International Meat Industry Conference MEATCON 2019, IOP Conference Series: Earth and Environmental Science, 333. DOI: https://doi.org/10.1088/1755-1315/333/1/012051

de Oliveira Band, G., Guimarães, S.E.F., Lopes, P.S., Schierholt, A.S., Silva, K.M., Pires, A.V., Gomide, L.A. M. (2005) Relationship between porcine stress syndrome gene and pork quality traits of F_2 pigs resulting from divergent crosses. Genetics and Molecular Biology, 28, 1. DOI: http://dx.doi.org/10.1590/S1415-47572005000100015

De Smet, S.M., Pauvels, H., De Bie, S., Demeyer, D.I., Callewier, J., Eeckhout, W. (1996) Effect of halothane genotype, breed, feed withdrawal and lairage on pork quality of Belgian slaughter pigs. Journal of Animal Science, 74, 1854-1863. DOI: https://doi.org/10.2527/1996.7481854x

Feng, J., Liu, X., Wang, Y.Z., Xu, Z.R. (2006) Effects of betaine on performance, carcass characteristics and hepatic betaine-homocysteine methyltransferase activity in finishing barrows. Asian-Australasian Journal of Animal Science, 19, 402-405. DOI: https://doi.org/10.5713/ajas.2006.402

Fiedler, I., Kuhn, G., Hartung, M., Kuechenmeister, U., Nuernberg, K., Rehfeldt, C., Kłosowska, D. (2001) Effects of the malignant hyperthermia syndrome (MHS) on meat quality, muscle fibre characteristics and metabolic traits of the *longissimus muscle* in Pietrain pigs. Archiv für Tierzucht, 44, 203-217. DOI: https://doi.org/10.5194/aab-44-203-2001

Honikel, K.O. (1998) Reference methods for the assessment of physical characteristics of meat. Meat Sciene, 49, 447-457. DOI: https://doi.org/10.1016/S0309-1740(98)00034-5

Hur, S.J., Yang, H.S., Park, G.B., Joo, S.T. (2007) Effects of dietary glycine betaine on pork quality in different muscle types. Asian-Australasian Journal of Animal Science, 20, 1754-1760.

James, B.W., Goodband, R.D., Unruh, J.A., Tokach, M.D., Nelssen, J.L., Dritz, S.S.A. (2002) Review of creatine supplementation and its potential to improve pork quality. Journal of Applied Animal Resources, 21, 1-16. DOI: https://doi.org/10.1080/09712119.2002.9706352

Janicki, B., Buzała, M. (2013) The role of creatine in the organism of pigs and its effect on the quality of pork: A review. Annals of Animal Science, 13, 207-215.

Kortz, J., Szaruga, R., Kapelański, W., Kurył, J., Rybarczyk, A., Natalczyk-Szymkowska, W. (2003) Effect of *RYR1* genotype on carcass leanness and pork quality. Electronic Journal of Polish Agricultural Universities, 6, 2. DOI: http://www.ejpau.media.pl/volume6/issue2/animal/art-07.html

Kuechenmeister, U., Nuernberg, K., Fiedler, I., Kuhn, G., Nuernberg, G., Ender, K. (1999) Cell injury and meat quality of pig in the time period post mortem from two genotypes susceptible or resistant to malignant hyperthermia. European Food Research and Technology, 209, 97-103.

Lahučký, R., Bučko, O., Haščík, P., Liptaj, T. (2012) Effects of creatine and vitamin E on muscle energetic metabolism, antioxidant stability and meat quality of pigs. Acta universitatis agriculturae et silviculturae Mendelianae Brunensis, 60, 151-160.

DOI: https://doi.org/10.11118/actaun201260050151
Li, J.L., Guo, Z.Y., Li, Y.J., Zhang, L., Gao, F., Zhou, G.H. (2015) Effect of creatine monohydrate supplementation on carcass traits, meat quality and postmortem energy metabolism of finishing pigs. Animal Production Science, 56, 1. DOI: https://doi.org/10.1071/AN14017.
Li, J.L., Zhang, L., Fu, Y., Li, Y., Jiang, Y., Zhou, G., Gao, F. (2018) Creatine monohydrate and guanidinoacetic acid supplementation affects the growth performance, meat quality, and creatine metabolism of finishing pigs. Journal of Agricultural and Food Chemistry, 66, 9952-9959. DOI: https://doi.org/10.1021/acs.jafc.8b02534
Lindahl, G., Young, J.F., Oksbjerg, N., Andersen, H.J. (2006) Influence of dietary creatine monohydrate and carcass cooling rate on colour characteristics of pork loin from different pure breeds. Meat Science, 72, 624-634. DOI: https://doi.org/10.1016/j.meatsci.2005.09.024
Maddock, R.J., Bidner, B.S., Carr, N., McKeith, F.K., Berg, E.P., Savell, J.W. (2000) Supplementation with creatine monohydrate improved the lean quality of fresh pork of two different genotypes. Proceedings of Recipient Meat Conference, Indianapolis, USA, 53, 118.
Maddock, R.J., Bidner, B.S., Carr, S.N., McKeith, F.K., Berg, E.P., Savell, J.W. (2002) Creatine monohydrate supplementation and the quality of fresh pork in normal and halothane carrier pigs. Journal of Animal Science, 80, 997-1004.
DOI: https://doi.org/10.2527/2002.804997x
Madeira, M.S., Alfaia, C.M., Costa, P., Lopes, P.A., Martins, S.V., Lemos, J.P.C., Prates, J.A.M. (2015) Effect of betaine and arginine in lysine-deficient diets on growth, carcass traits, and pork quality. Journal of Animal Science, 93, 10, 4721-4733.
DOI: https//doi.org/10.2527/jas.2015-9117.
Madeira, M.S., Rolo, E.A., Alfaia, C.M., Pires, V.R., Luxton, R., Doran, O., Prates, J.A.M. (2016) Influence of betaine and arginine supplementation of reduced protein diets on fatty acid composition and gene expression in the muscle and subcutaneous adipose tissue of cross-bred pigs. British Journal of Nutrition, 115, 937-950.
DOI: https//doi.org/10.1017/S0007114515005218
Marini, S.J., Vanzetti, L.S., Borelli, V.S., Villareal, A.O., Denegri, G.D., Cottura, G.A., Franco, R. (2012) *RYR1* gene variability and effect on meat pH in Argentinean hybrids swines. Investigación Veterinaria, 14, 19-23.
Matthews, J.O., Southern, L.L., Bidner, T.D., Persica, M.A. (2001a) Effects of betaine, pen space, and slaughter handling method on growth performance, carcass traits, and pork quality of finishing barrows. Journal of Animal Science, 79, 967-974.
DOI: https://doi.org/10.2527/2001.794967x
Matthews, J.O., Southern, L.L., Higbie, A.D., Persica, M.A., Bidner, T.D. (2001b) Effects of betaine on growth, carcass characteristics, pork quality, and plasma metabolites of finishing pigs. Journal of Animal Science, 79, 722-728.
DOI: https://doi.org/10.2527/2001.793722x
Otto, G., Roehe, R., Looft, H., Thoelking, L., Knap, P.W., Rothschild, M.F., Kalm, E. (2007) Associations of DNA markers with meat quality traits in pigs with emphasis on drip loss. Meat Science, 75, 185-195.
DOI: https://doi.org/10.1016/j.meatsci.2006.03.022
O´Quinn, P.R., Andrews, B.S., Goodband, R.D., Unruh, J.A., Nelssen, J.L., Woodworth, J.C., Owen, K.Q. (2000a) Effects of modified tall oil and creatine monohydrate on growth performance, carcass characteristics, and meat quality of growing-finishing pigs. Journal of Animal Science, 78, 2376-2382.
DOI: https://doi.org/10.2527/2000.7892376x
O´Quinn, P.R., Nelssen, J.R., Goodband, R.D., Unruh, J.A., Woodworth, J.C., Tokach, M.D. (2000b) Effects of modified tall oil versus a commercial source of conjugated linoleic acid and increasing levels of modified tall oil on growth performance and carcass characteristics of growing-finishing pigs. Journal of Animal Science, 78, 2359-2368. DOI: https://doi.org/10.2527/2000.7892359x
Øverland, M., Rorvik, A.K., Skrede, A. (1999) Effect of trimethylamine oxide and betaine in swine diets on growth performance, carcass characteristics, nutrient digestibility, and sensory quality of pork. Journal of Animal Science, 77, 2143-2153.
DOI: https://doi.org/10.2527/1999.7782143x
Pommier, S., Houde, A. (1993) Effect of genotype for malignant hyperthermia as determined by restriction endonuclease assay on the quality characteristics of commercial pork loins. Journal of Animal Science, 71, 420-425. DOI: https://doi.org/10.2527/1993.712420x
Prevost, M.C., Nelson, A.G., Morris, G.S. (1997) Creatine supplementation enhances intermittent work performance. Research Quarterly for Exercise and Sport, 68, 233-240.
DOI: https://doi.org/10.1080/02701367.1997.10608002
Rey-Salgueiro, L., Martinez-Carballo, E., Fajardo. P., Chapela, M.J., Espiñeira, M., Simal-Gandara J. (2018) Meat quality in relation to swine well-being after transport and during lairage at the slaughterhouse. Meat Science, 142, 38-43.
DOI: https://doi.org/10.1016/j.meatsci.2018.04.005
Rojas-Cano, M.L., Lara, L., Lachica, M., Aguilera, J.F., Fernández-Fígares, I. (2011) Influence of betaine and conjugated linoleic acid on development of carcass cuts of Iberian pigs growing from 20 to 50 kg body weight. Meat Science, 88, 525-530.
DOI: https://doi.org/10.1016/j.meatsci.2011.02.004
Rosenvold, K., Bertram, H.C., Young, J.F. (2007) Dietary creatine monohydrate has no effect on pork quality of Danish crossbred pigs. Meat Science, 76, 160-164.
DOI: https://doi.org/10.1016/j.meatsci.2006.10.023
Rybarczyk, A., Terman, A., Żak, G., Kumalska, M., Polasik, D. (2016) Associations of *CAST* and *RYR1* genes polymorphism with carcass and meat quality in crossbreed pigs with a share of Pietrain breed. Annals of Warsaw University of Life Sciences – SGGW, Animal Science, 55, 2, 253-260.
Silveira, A.C.P., Freitas, P.F.A., Cesar, A.S.M., Antunes, R.C., Guimarães, E.C., Batista, D.F.A., Torido, L. (2011) Influence of the halothane gene (HAL) on pork quality in two commercial crossbreeds. Genetics and Molecular Research, 10, 1479-1489.
DOI: https://doi.org/10.4238/vol10-3gmr925
Stahl, C.A., Allee, G.L., Berg, E.P. (2001) Creatine monohydrate supplemented in swine finishing diets and fresh pork quality: II. Commercial applications. Journal of Animal Science, 79, 3081-3086. DOI: https://doi.org/10.2527/2001.79123081x
Stahl, C.A., Berg, E.P. (2003) Growth parameters and meat quality of finishing hogs supplemented with creatine monohydrate and a high glycemic carbohydrate for the last 30 days of production. Meat Science, 64, 169-174.
DOI: https://doi.org/10.1016/s0309-1740(02)00176-6
Stahl, C.A., Carlson-Shannon, M.S., Wiegand, B.R., Meyer, D.L., Schmidt, T.B., Berg, E.P. (2007) The influence of creatine and a high glycemic carbohydrate on the growth performance and meat quality of market hogs fed ractopamine hydrochloride. Meat Science, 75, 143-149. DOI: https://doi.org/10.1016/j.meatsci.2006.06.023
Su, B., Wang, L., Wang, H., Shi, B., Shan, A., Li, Y. (2013) Conjugated linoleic acid and betain prevent pork quality issues from diets containing distillers´ dried grains with solubles. Canadian Journal of Animal Science, 93, 477-485.
DOI: https://doi.org/10.1139/CJAS2013-056
Škrlep, M., Kavar, T., Čandek-Potokar, M. (2010) Comparison of PRKAG3 and *RYR1* gene effect on carcass traits and meat quality in Slovenian

commercial pigs. Czech Journal of Animal Science, 55, 4, 149-159. DOI: https://doi.org/10.17221/6/2009-CJAS

Tomažin, U., Batorek-Lukač, N., Škrlep, M., Prevolnik-Povše, M., Ogorevc, J., Dovč, P., Čandek-Potokar, M. (2017) Meat quality of Krškopolje pigs as affected by *RYR1* genotype. Proceedings of the 11th International Symposium Modern Trends in Livestock Production, Belgrade, Serbia, October 11-13, 2017.

Yang, H.S., Lee, J.I., Joo, S.T., Park, G.B. (2009) Effects of dietary glycine betaine on growth and pork quality of finishing pigs. Asian-Australasian Journal of Animal Science, 22, 706-711. DOI: https://doi.org/10.5713/ajas.2009.80645

Young, J.F., Bertram, H.C., Rosenvold, K., Lindahl, G., Oksbjerg, N. (2005) Dietary creatine monohydrate (CMH) affects quality attributes of Duroc but not Landrace pork. Meat Science, 70, 717-725. DOI: https://doi.org/10.1016/j.meatsci.2005.03.008

Yu, D.Y., Xu, Z.R., Li, W.F. (2004). Effects of betaine on growth performance and carcass characteristics in growing pigs. Asian-Australasian Journal of Animal Science, 17, 1700-1704. DOI: https://doi.org/10.5713/ajas.2004.1700