

EFFECTS OF CREATINE AND VITAMIN E ON MUSCLE ENERGETIC METABOLISM, ANTIOXIDANT STABILITY AND MEAT QUALITY OF PIGS

R. Lahučký, O. Bučko, P. Haščík, T. Liptaj

Received: June 1, 2012

Abstract

LAHUČKÝ, R., BUČKO, O., HAŠČÍK, P., LIPTAJ, T.: *Effects of creatine and vitamin E on muscle energetic metabolism, antioxidant stability and meat quality of pigs*. Acta univ. agric. et silvic. Mendel. Brun., 2012, LX, No. 5, pp. 151–160

The effects of supplementing the diet of pigs with creatine monohydrate (CMH) and vitamin E on blood plasma creatine concentration, vitamin E level in muscle, muscle energy metabolism, antioxidant capacity and meat (*musculus longissimus dorsi*) quality of pigs (DNA tested and negative on malignant hyperthermia) were investigated. Two treatments were used: supplementation with CMH alone (2 g.kg⁻¹ of feed, 10 days before slaughter); and supplementation with both vitamin E (500 mg α -tocopherol.kg⁻¹ of feed for minimum of 30 days) and CMH (2 g.kg⁻¹ of feed, 10 days before slaughter). Pigs supplemented with CMH alone showed elevated plasma creatine concentration ($P \leq 0.05$). Phosphorus nuclear magnetic resonance (³¹P NMR) measurements on *post mortem* (15 min.) muscle samples showed the highest phosphocreatine levels and ratio PCr/Pi ($P \leq 0.05$) in CMH supplemented pigs alone and in combination with vitamin E. Dietary supplementation with vitamin E significantly ($P \leq 0.05$) increased the concentration of α -tocopherol in meat. Supplementation with CMH alone or in combination with vitamin E resulted in higher ($P = 0.07$) *a* values of loin chops at 5 days of storage. Antioxidative capacity (measured as MDA production after incubation of *longissimus* muscle homogenates with Fe²⁺/ascorbate) was substantially improved by vitamin E and somewhat by CMH supplementation.

meat quality, pork, creatine monohydrate, vitamin E

Previous research has examined the effects of supplementing swine finishing diets with creatine. Some studies suggested a positive effect (Berg and Alle, 2001; James *et al.*, 2002; Young *et al.*, 2004, 2005), but others found little or no effect on meat quality parameters (O'Quinn *et al.*, 2000; Maddock *et al.*, 2002; Berg *et al.*, 2003; Stahl and Berg, 2003; Rosenvold *et al.*, 2007; Stahl *et al.*, 2007; Young *et al.*, 2007). Dietary creatine (mainly as creatine monohydrate, CMH) supplementation has been studied extensively in humans since creatine (phosphocreatine, PCr) functions in maintaining cellular adenosinetriphosphate (ATP) homeostasis (Harris *et al.*, 1992).

Increasing and maintenance the PCr/Pi ratio and thus the amount of available energy for resynthesis of ATP could also improve some meat quality parameters, as indicated by studies of the metabolism of phosphorus compounds using phosphorus nuclear magnetic resonance spectroscopy, both *ante mortem* (Lahucky *et al.*, 1993, 2002; Kohn *et al.*, 1999) and *post mortem* (Miri *et al.*, 1992; Shen *et al.*, 1992; Moesgaard *et al.*, 1995; Scholz *et al.*, 1995; Lahucky *et al.*, 2000; Bertram *et al.*, 2002). Muscle metabolism around the time of slaughter has a major effect on pork quality. An important genetic factor that alters muscle metabolism is the presence of mutations in the ryanodine receptor gene (RYR1) as was shown in many studies (Lahucky *et al.*, 1993, 2002; Fiedler *et al.*,

1999; Maddock *et al.*, 2002). Carriers (heterozygotes, Nn) produce lower quality meat and it was proposed that carriers should be excluded from the breeding population (and also from welfare point of view). Results of experiment using 25 g CMH per pig and per day for 5 days before slaughter (Maddock *et al.*, 2002) do not provide convincing evidence for an increase the quality of fresh pork of normal and halothane carrier genotypes but some positive data indicated a need for further studies. Young *et al.* (2007) found the plasma content of creatine higher and the phosphorylation of creatine more efficient in Duroc and Landrace pigs but a stronger and more homogenous response to CMH supplementation was seen in the responding Duroc breed compared to the quasi-responding Landrace breed. Lower cooking loss and higher redness (a value) were found on crossbred barrows when 0.92% CMH supplemented in diet (Stahl *et al.*, 2007). Together with widely used antioxidants such as vitamin E (both natural and synthetic forms) alone or in combination with selected plant extracts (Buckley *et al.*, 1995; Lauridsen *et al.*, 1999; Lahucky *et al.*, 2000, 2010; Bolere *et al.*, 2009; Trefan *et al.*, 2011) it seems be supplementation by CMH could also positively influence antioxidant capacity of muscle (Lawler *et al.*, 2002).

The objective of the present experiment was further to evaluate the effects of creatine monohydrate supplementation alone and together with vitamin E on muscle phosphorus metabolism, antioxidant capacity and meat quality parameters of growth-finishing pigs.

MATERIAL AND METHODS

Animal and sample preparations

36 pigs originating from lines of Slovak White Meaty pigs were used in this experiment. The RYR-1 genotype (Fujii *et al.*, 1991) of these animals was determined by a DNA based test (Malignant hyperthermia syndrome; MH) and only homozygous negative (NN) pigs were used. The experiment was conducted in accordance with the

institutional guidelines for animal care (Slovak Agriculture Research Centre, Research Institute of Animal Production, RIAP, Nitra, 1999). The animals were divided into a control group (12 pigs, 6 barrows and 6 gilts) and two experimental groups (12 pigs in each group: 6 barrows and 6 gilts). The pigs were penned in double boxes at the institute facilities. The control group received a standard diet (Tab. I). The first experimental group (group CMH) received supplemental creatine monohydrate (CMH, Sigma, 2 g.kg⁻¹ of feed, 10 days before slaughter) and the second experimental group (group CMH+E) received supplemental vitamin E (ROVOMIX® E-50 SD, 500 mg a-tocopherol.kg⁻¹ of feed for minimum of 30 days) plus supplemental creatine monohydrate (again Sigma, 2 g.kg⁻¹ of feed, 10 days before slaughter). The levels of α -tocopherol in the diets are shown in Tab. I (Protocol 1222-1223/2007, Central Control Institute of Agriculture, Bratislava). The animals were stunned, slaughtered and exsanguinated in the slaughter house of RIAP Nitra (transportation about 200 m) with an average live weight of 105 $\pm$ 6 kg. Blood was collected in heparinized tubes for creatinine estimation. 15 min after slaughter a sample of approx. 1 g was taken from the *longissimus* muscle (last rib) on the right side using a biopsy instrument (Biotech, Slovakia), immediately frozen, and stored in liquid nitrogen until analysed. After chilling the carcass at 3–4 °C for 24 h, the entire *musculus longissimus thoracis* (MLD) was removed from the carcass (right side, 13/14 rib) and then sliced into chops (2.5 cm thick). One wrapped sample was stored in a refrigerator for 5 days at 4 °C.

Chemical analysis

The concentration of vitamin E (a-tocopherol) in muscle was measured by HPLC (Berlin *et al.*, 1994) at FBN Dummerstorf, Germany. The peroxidative stability of *longissimus* homogenates was estimated on the basis of the concentration of thiobarbituric acid reactive substances (TBARS) (Kuechenmeister *et al.*, 1999). TBARS were expressed in equivalents of MDA (nM.mg⁻¹ homogenate protein), a breakdown product formed during peroxidation stimulated

I: Composition of the diet

Item	%	Item	Control	Group CMH+E	Group CMH
Wheat	24.0	Organic matter, %	85.2	85.2	85.2
Barley	40.0	Crude protein, %	14.9	14.9	14.9
Oat	10.0	Crude fat, %	2.4	2.4	2.4
Soybean meal	12.0	Crude fibre, %	4.05	4.05	4.05
Wheat meal	4.0	N-free extract, %	65.4	65.4	65.4
Lucerne meal	3.0	Ash, %	6.63	6.63	6.63
Meat and bone meal	2.0	Metabolisable energy, MJ	12.4	12.4	12.4
Fish meal	1.0	Lysine, %	0.75	0.75	0.75
Mineral supplement	3.0	α -tocopherol-added, mg/kg (30 days)	-	500.0	-
Fodder salt	0.4	- analysed, mg/kg	33.5	514.0	
Biofactor supplement	0.6	Creatinemonohydrate g.kg ⁻¹ (10 days)	-	2	2

II: Plasma creatine level and vitamin E in muscle of pigs ($n = 12$)

Trait	Control LSM $\pm$ SE	Group CMH LSM $\pm$ SE	Group CMH+E LSM $\pm$ SE
Creatine ($\mu\text{mol.l}^{-1}$)	123.42 ^a $\pm$ 4.97	175.54 ^b $\pm$ 4.97	163.40 ^b $\pm$ 4.87
Vitamin E (mg.kg^{-1})	1.58 ^a $\pm$ 0.20	1.63 ^a $\pm$ 0.20	3.96 ^b $\pm$ 0.20

^a Different letters denote significant differences between groups at $P = 0.05$ ^b Different letters denote significant differences between groups at $P = 0.05$ III: Phosphorous compounds by P NMR spectroscopy of longissimus dorsi muscle ($n = 12$)

Trait	Control LSM $\pm$ SE	Group CMH LSM $\pm$ SE	Group CMH+E LSM $\pm$ SE
Sugar phosphate (SP)	32.38 $\pm$ 2.56	36.76 $\pm$ 2.56	37.66 $\pm$ 2.50
Inorganic phosphate (Pi)	25.77 ^a $\pm$ 1.31	22.37 $\pm$ 1.31	20.77 ^b $\pm$ 1.28
Phosphocreatine (PCr)	4.09 ^a $\pm$ 1.17	6.66 $\pm$ 1.17	7.82 ^b $\pm$ 1.14
Adenosinetri-phosphate (ATP)	10.27 $\pm$ 0.73	9.53 $\pm$ 0.73	10.40 $\pm$ 0.72
Ratio PCr/Pi	0.15 $\pm$ 0.09	0.42 $\pm$ 0.09	0.39 $\pm$ 0.09

^a Different letters denote significant differences between groups at $P = 0.05$ ^b Different letters denote significant differences between groups at $P = 0.05$

by Fe^{2+} /ascorbate. The protein content of homogenates was estimated by a modified method of Markwell *et al.* (1978). Creatine was determined by a photometric colorimetric test (Human). Total protein and intramuscular fat were measured using an Infratec Analyzer.

The levels of phosphorus compounds (sugar phosphate – SP, inorganic phosphate – Pi, phosphocreatine – PCr, adenosinetriphosphate – α -, γ -, β ATP) were measured using magnetic resonance spectroscopy. Phosphorus nuclear magnetic resonance spectroscopy (^{31}P NMR) was carried out on the biopsy sample introduced into a 10 mm diameter tube (maintained at 39 °C) filled with deuterated water D_2O). The ^{31}P NMR spectrum was recorded at 121 MHz on a VXR 300 (Varian) spectrometer (NMR laboratory, Slovak University of Technology, Bratislava). The ^{31}P spectra were recorded with a spectral width of 3932.4 Hz and 45° pulses of duration 35.0 μs . The recycle time was 0.8 s. Each spectrum was the result of 512 transients. The time of accumulation per spectrum was 7.6 min. An exponential line broadening of 20 Hz was used prior Fourier transformation and signal of PCr was taken as an internal reference at –2.47 ppm. Out of a number of spectra for calculation only the first and second were used. The levels of the individual phosphorus compounds (SP, Pi, PCr, ATP) were expressed as a percentage of the total content of phosphorus compounds as described previously (Lahucky *et al.*, 1993, 2000) and the ratio of PCr/Pi was calculated.

Meat quality measurements

The pH of the *longissimus* muscle between 13 and 14 rib was determined using a combined pH electrode (Ingold). Instrumental colour measurements were recorded for *L (lightness; 0: black, 100: white), a* (redness/greenness: positive values – red, negative

values – green), and b* (yellowness/blueness: positive values – yellow, negative values – blue) using a spectrophotometer (Hunter Lab MiniScan). Drip loss analysis was performed according to Honikel (1998). Shear force was determined on cooked samples (core temperature of 75 °C) using a Warner-Bratzler apparatus.

Statistical evaluation

Data from the experiment were analysed by a one-way ANOVA with fixed factor treatment (control, CMH, CMH+E) using the statistical software package SAS® Version 9.1 (SAS Institute Inc., Cary, NC, 2004). All pairwise comparisons were done using an F-test with LSD correction. In terms of individual groups within the fixed factor, the gender was balanced using an adequate number of barrows and gilts.

RESULTS AND DISCUSSION

Feeding supplemental CMH at the 2 g.kg⁻¹ level for 10 days before slaughter increased ($P < 0.05$) plasma creatine concentration in both group CMH (175.54 $\mu\text{mol.l}^{-1}$) and group CMH + E (163.4 $\mu\text{mol.l}^{-1}$) if compare to control group (123.42 $\mu\text{mol.l}^{-1}$) (Tab. II). The plasma creatine level in the non-supplemented pigs was higher and levels in the supplemented pigs were lower than the contents found by others (Young *et al.*, 2005, 2007; Rosenfold *et al.*, 2007) using higher level of CMH supplementation. Our measurements were done from blood taken off after slaughter but differences between non-supplemented and supplemented pigs were significant ($P \leq 0.05$). The levels in phosphorus compounds assessed by P NMR spectroscopy in muscle biopsy samples (taken of 15 min after slaughter) are given in Tab. III. The levels of sugar phosphate (SP) were higher in both CMH supplemented when compare to control pigs but differences were not significant ($P \geq 0.05$).

IV: Chemical composition of longissimus dorsi muscle (n = 12)

Trait	Control LSM ± SE	Group CMH LSM ± SE	Group CMH+E LSM ± SE
Total water, %	73.9 ± 0.2	73.5 ± 0.2	74.0 ± 0.2
Protein, %	22.0 ± 0.2	22.2 ± 0.2	22.1 ± 0.2
Intramuscular fat, %	2.9 ± 0.2	2.9 ± 0.2	2.6 ± 0.2

V: Pork quality of longissimus dorsi muscle (n = 12)

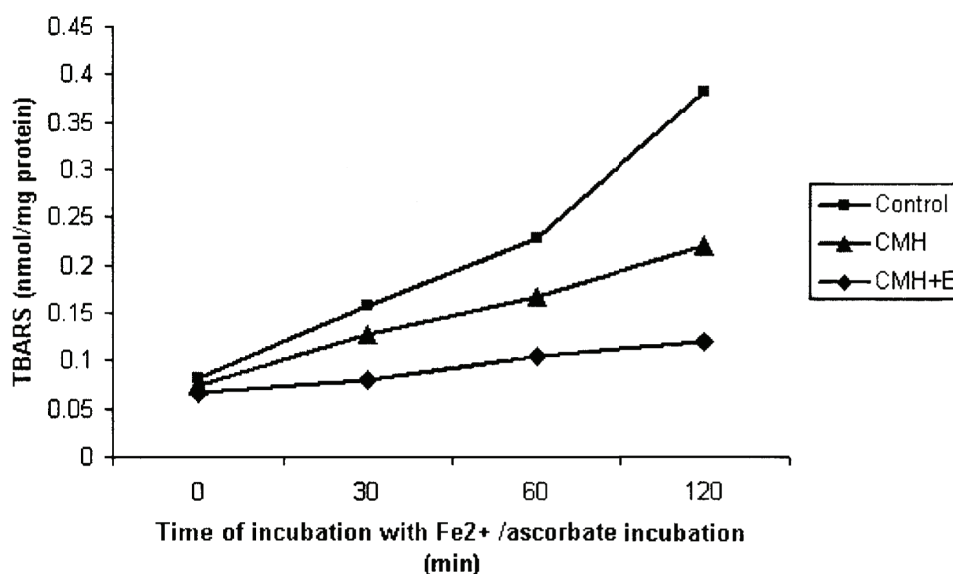
Trait	Control LSM ± SE	Group CMH LSM ± SE	Group CMH+E LSM ± SE
pH ₁	6.24 ± 0.36	6.29 ± 0.36	5.82 ± 0.35
pH ₂₄	5.57 ± 0.03	5.53 ± 0.03	5.56 ± 0.03
Drip loss (24 hours). %	4.33 ^a ± 0.30	3.35 ^b ± 0.30	3.53 ^b ± 0.29
Colour (24 hours)	L*	49.83 ± 0.87	49.43 ± 0.87
	a*	1.82 ± 0.24	1.82 ± 0.24
	b*	7.71 ± 0.23	7.78 ± 0.22
Colour (5 days)	L*	51.50 ± 0.77	50.69 ± 0.77
	a*	2.60 ± 0.36	3.04 ± 0.36
	b*	8.43 ± 0.39	9.00 ± 0.36
Shear force (W-B). kg	5.59 ± 0.29	5.57 ± 0.29	4.54 ± 0.28

^a Different letters denote significant differences between groups at P = 0.05

^b Different letters denote significant differences between groups at P = 0.05

Inorganic phosphate (Pi) tended to be lower in CMH supplemented pigs and differences between control and CMH + vitamin E supplemented groups were significant ($P \leq 0.05$). Opposite tendency were found for phosphocreatine level (PCr) with highest value in pigs supplemented with CMH + vitamin E but differences if compare to control group were significant ($P \leq 0.05$). Values of ATP (γ -ATP) were relatively stable if compare control and experimental groups and differences were not significant ($P \geq 0.05$). Efficiency of the metabolism of muscle energetic compounds evaluated by index PCr/Pi was found higher in groups supplemented with CMH and differences between group supplemented CMH + vitamin E and control pigs were significant ($P \leq 0.05$). Results on P NMR spectroscopy parameters are comparable with earlier results of non mutated genotype (on MH homozygote) of biopsy samples taken of 15 min after slaughter (Lahucky *et al.*, 2004). Higher efficiency of energetic muscle metabolism (reduced PCr breakdown, slower PCr decay and higher PCr/Pi ratio) were found also by others (Moesgaard *et al.*, 1995; Lahucky *et al.*, 2004) in pigs supplemented by magnesium oxide (MgO). It was also shown (Lahucky *et al.*, 2000) that supplementation with vitamin E could improve the efficiency of the metabolism of energetic compounds in *musculus longissimus dorsi*. Total water and total protein contents (Tab. IV) were not influenced and significant differences were not found ($P \geq 0.05$). Intramuscular fat content was higher in group CMH compared to CMH + E and the control group. Berg and Allee (2001) reported 5 days CMH

supplementation had trend to increase crude fat in loins but our results did not support increasing the level of intramuscular fat in group CMH + vitamin E supplementation and the differences between control and both experimental groups were not significant ($P \geq 0.05$). Levels of intramuscular fat are comparable with earlier finding (Lahucky *et al.*, 2005, 2007). Meat quality characteristics from control and experimental pigs are summarized in Tab. V. Feeding supplemental CMH separately or a combination with vitamin E did not affect pH (1 h and 24 h) and differences were not significant ($P \geq 0.05$). Maddock *et al.* (2000), Berg and Allee (2001) and Quinn *et al.* (2001) reported higher pH₁ and pH₂₄ in supplemented pigs with 25 g CMH per pig and 5 days before slaughter. Our results together with others did not support significant influence of pH values (Young *et al.*, 2004, 2010; Rosenvold *et al.*, 2007). The percentage drip loss values in groups supplemented with CMH were tended ($P = 0.05$) to be higher if compare to control pigs (3.5% and 3.4 vs. 4.3%). Our results are comparable with finding of James *et al.* (2000) the supplementation of CMH could positively influence drip loss value (4.1 vs. 5.3%) and as was shown not only CMH supplementation but also genotypes could influence the level of drip loss (Fiedler *et al.*, 1999; Lahucky *et al.*, 2002; Maddock *et al.*, 2002; Young *et al.*, 2007). Our results were done from pigs free on RYR1 gene mutation (negative on MHS) and drip loss value are comparable with Duroc pigs (Young *et al.*, 2004) with and without supplementation of CMH (3.0 vs. 4.3%). The effect of supplementary CMH and combination of CMH and vitamin E on tenderness



1: Effects of dietary creatine (CMH) and vitamin E (CMH + E) supplementation on the antioxidative stability of longissimus dorsi muscle

of LD muscle, determined by the Warner-Bratzler showed the lowest value at group vit. E + CMH but differences are not significant. The results are comparable with others. Shear force method did not indicate significant influence ($P \geq 0.05$) what is comparable with others (Maddock *et al.* 2002). Color data were not different ($P \geq 0.05$) for *L*, *a* and *b* values as estimated 24 h *post mortem*. At 5 days *post mortem* the *a* value (redness) were higher in the *longissimus* muscle for group CMH and group CMH + E ($P = 0.07$). Comparable results were found by Stahl *et al.* (2007) following by feeding diet containing 0.9% CMH. Potentially explanation for higher *a* value observed in pork supplemented diet with CMH could be suggested that creatine posses antioxidant properties, specifically the ability to remove superoxide anions and peroxynitrite (Lawler *et al.*, 2002; Stahl *et al.*, 2007). A possible antioxidant effect of CMH supplementation alone and together with vitamin E was assessed by peroxidative stability of *musculus longissimus dorsi* homogenate stimulated by Fe²⁺/ascorbate. Lower values of peroxidation (lower value of MDA production after incubation with Fe²⁺/ascorbate mixture were found in pigs supplemented with CMH and CMH + E compared to control pigs but at 120 min incubation significant differences between all three groups were found. Supplementation of additives in combination with vitamin E posses the highest antiperoxidative effect and is in agreement as was shown earlier (Lahucky *et al.*, 2000, 2004).

CONCLUSION

In the experiment, we compared the parameters of muscle energy metabolism, antioxidant stability, chemical composition and physical and technological quality of pork of the white meat breed after application of creatine monohydrate and α -tocopherol into the diet of pigs.

In conclusion we can summarize, that pigs fed with a high level of CMH and vitamin E significantly increased the content of α -tocopherol in the muscle creatine monohydrate and antioxidant stability of pork meat and at the same time there was also improvement in parameters of muscle energy metabolism manifested primarily through indicators Inorganic phosphate and Phosphokreatine. Statistically significant difference in these variables between the control and treatment groups CMH + E was detected at $P < 0.05$. Feeding with CMH improved some parameters of muscle energy metabolism and antioxidant stability of meat.

We have also observed better drip loss values, where statistically significant differences between both experimental and control groups at $P < 0.05$ were found. Further researches are needed, to confirm more serious findings of the physical and technological parameter changes of the pig meat.

SUMMARY

The effects of supplementing the diet of pigs with creatine monohydrate (CMH) and vitamin E on blood plasma creatine concentration, vitamin E level in muscle, muscle energy metabolism, antioxidant capacity and meat (*musculus longissimus dorsi*) quality of pigs (DNA tested and negative on malignant hyperthermia) were investigated. The experiment was conducted in accordance with the

institutional guidelines for animal care (Slovak Agriculture Research Centre, Research Institute of Animal Production, RIAP, Nitra, 1999). 36 pigs originating from lines of Slovak White Meaty pigs were used in this experiment. The RYR-1 genotype of these animals was determined by a DNA based test (Malignant hyperthermia syndrome; MH) and only homozygous negative (NN) pigs were used. The animals were divided into a control group (12 pigs, 6 barrows and 6 gilts) and two experimental groups (12 pigs in each group: 6 barrows and 6 gilts). The pigs were penned in double boxes at the institute facilities. The control group received a standard diet. The first experimental group (group CMH) received supplemental creatine monohydrate (CMH, Sigma, 2 g.kg⁻¹ of feed, 10 days before slaughter) and the second experimental group (group CMH+E) received supplemental vitamin E (ROVOMIX® E-50 SD, 500 mg α -tocopherol.kg⁻¹ of feed for minimum of 30 days) plus supplemental creatine monohydrate (again Sigma, 2 g.kg⁻¹ of feed, 10 days before slaughter). The animals were stunned, slaughtered and exsanguinated in the slaughter house of RIAP Nitra (transportation about 200 m) with an average live weight of 105 $\pm$ 6 kg. Blood was collected in heparinized tubes for creatinine estimation. 15 min after slaughter a sample of approx. 1 g was taken from the *musculus longissimus dorsi* (last rib) on the right side using a biopsy instrument (Biotech, Slovakia), immediately frozen, and stored in liquid nitrogen until analysed. The concentration of vitamin E (α -tocopherol) in muscle was measured by HPLC (Berlin *et al.*, 1994) at FBN Dummerstorf, Germany. The peroxidative stability of *longissimus* homogenates was estimated on the basis of the concentration of thiobarbituric acid reactive substances (TBARS) (Kuechenmeister *et al.*, 1999). TBARS were expressed in equivalents of MDA (nM.mg⁻¹ homogenate protein), a breakdown product formed during peroxidation stimulated by Fe²⁺/ascorbate. The protein content of homogenates was estimated by a modified method of Markwell *et al.* (1978). Creatine was determined by a photometric colorimetric test (Human). Total protein and intramuscular fat were measured using an Infratec- Analyzer. The levels of phosphorus compounds (sugar phosphate – SP, inorganic phosphate – Pi, phosphocreatine – PCr, adenosinetriphosphate - α , γ -, β ATP) were measured using magnetic resonance spectroscopy. The pH of the *longissimus* muscle between 13 and 14 rib was determined using a combined pH electrode (Ingold). Instrumental colour measurements were recorded for *L, a* and b* using a spectrophotometer (Hunter Lab MiniScan). Drip loss analysis was performed according to Honikel (1998). Shear force was determined on cooked samples (core temperature of 75 °C) using a Warner-Bratzler apparatus. Feeding supplemental CMH at the 2 g.kg⁻¹ level for 10 days before slaughter increased ($P < 0.05$) plasma creatine concentration in both group CMH (175.54 μ mol.l⁻¹) and group CMH + E (163.4 μ mol.l⁻¹) if compare to control group (123.42 μ mol.l⁻¹). The plasma creatine level in the non-supplemented pigs was higher and levels in the supplemented pigs were lower. Our measurements were done from blood taken off after slaughter but differences between non-supplemented and supplemented pigs were significant ($P < 0.05$). The levels of sugar phosphate (SP) were higher in both CMH supplemented when compare to control pigs but differences were not significant ($P > 0.05$). Inorganic phosphate (Pi) tended to be lower in CMH supplemented pigs and differences between control and CMH + vitamin E supplemented groups were significant ($P < 0.05$). Opposite tendency were found for phosphocreatine level (PCr) with highest value in pigs supplemented with CMH + vitamin E but differences if compare to control group were significant ($P < 0.05$). Values of ATP (γ -ATP) were relatively stabile if compare control and experimental groups and differences were not significant ($P > 0.05$). Efficiency of the metabolism of muscle energetic compounds evaluated by index PCr/Pi was found higher in groups supplemented with CMH and differences between group supplemented CMH + vitamin E and control pigs were significant ($P < 0.05$). Total water and total protein contents were not influenced and significant differences were not found ($P > 0.05$). Intramuscular fat content was higher in group CMH compared to CMH + E and the control group. Feeding supplemental CMH separately or a combination with vitamin E did not affect pH (1 h and 24 h) and differences were not significant ($P > 0.01$). The percentage drip loss values in groups supplemented with CMH were tended ($P = 0.05$) to be higher if compare to control pigs (3.55 and 3.4 vs. 4.3). The effect of supplementary CMH and combination of CMH and vitamin E on tenderness of LD muscle, determined by the Warner-Bratzler shoowed lowest value at group vit. E + CMH but differences are not significant. Shear force method did not indicate significant influence ($P > 0.05$). Color data were not different ($P > 0.05$) for L, a and b values as estimated 24 h *post mortem*. At 5 days *post mortem* the a value (redness) were higher in the *longissimus* muscle for group CMH and group CMH + E ($P = 0.07$). A possible antioxidant effect of CMH supplementation alone and together with vitamin E was assessed by peroxidative stability of *longissimus* homogenate stimulated by Fe²⁺/ascorbate. Lower values of peroxidation (lower value of MDA production after incubation with Fe²⁺/ascorbate mixture were found in pigs supplemented with CMH and CMH + E compared to control pigs but at 120 min incubation significant differences between all three groups were found. In conclusion feeding high level of CMH and vitamin E together to White Meaty pigs substantially improved α -tocopherol content and antioxidative stability in muscle and improved in some extence muscle energetic metabolism. Feeding CMH alone improved in some extence muscle energetic metabolism and antioxidant stability.

Acknowledgement

The authors wish to thank Dr. Katarina Vasickova for DNA analysis, Dr. Ivan Bahelka and Jozef Fogada Research Institute of Animal Production, Nitra for animal care and technical assistance during slaughter and cutting of muscles. For vitamin E analysis and laboratory help with antioxidant activity analysis we thanks Research Institute for the Biology of Farm Animals, Dummerstorf, Germany. This research was partially supported by the Ministry of Agriculture in Bonn (Germany) and the Ministry of Agriculture in Bratislava (Slovak Republic) and DAAD (Deutscher Akademischer Austauschdienst) in Bonn.

REFERENCES

- BERLIN, E., MC CLURE, D., BANKS, M. A., PETERS, R. C., 1994: Heart and liver fatty acid composition and vitamin E content in miniature swine fed diets containing corn and menhaden oils. *Comparison Biochemical and Physiological* 109A, 1, 53–61, ISSN 1095-6433.
- 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, Print ISSN 0021-8812; Online ISSN 1525-3163.
- 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, Print ISSN 0021-8812; Online ISSN 1525-3163.
- BERTRAM, H. CH., STODKILDE-JORGENSEN, H., KARLSSON, A. H., ANDERSEN, H. J., 2002: *Post mortem* energy metabolism and meat quality of porcine *M. longissimus dorsi* as influenced by stunning method – A ³¹P NMR spectroscopic study. *Meat Science*, 62, 113–119, ISSN 0309-1740.
- BOLER, D. D., GABRIEL, S. R., YANG, H., BALSBAUGH, R., MAHAN, D. C., BREWER, M. S., MCKEITH, F. K., KILLEFER, J., 2009: Effect of different dietary levels of natural-source vitamin E in grow-finish pigs on pork quality and shelf life. *Meat Science*, 83, 723–730, ISSN 0309-1740.
- BUCKLEY, D. J., MORRISSEY, P. A., GRAY, J. I., 1995: Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *Journal of Animal Science*, 73, 3122–3131, Print ISSN 0021-8812; Online ISSN 1525-3163.
- FIEDLER, L., ENDER, K., WICKE, M., MAAK, S., LENGGERKEN, V. G., MEYER, W., 1999: Structural and functional characteristics of muscle fibres in pigs with different malignant hyperthermia susceptibility (MHS) and different meat quality. *Meat Science*, 53, 9–15, ISSN 0309-1740.
- FUJII, J., OTSU, K., ZORZATO, F., DE LEON, S., KHANA, V. K., WEILER, J., O BRIEN, P., MAC LENNAN, D. H., 1991: Identification of a mutation in the porcine ryanodine receptor that is associated with malignant hyperthermia. *Science, Washington*, 253, 448–451, ISSN 1095-9203.
- HARRIS, R. C., SODERLUND, K., HULTMAN, E., 1992: Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. *Clinical Science (London)*, 83, 367–374, ISSN 0143-5221.
- HONIKEL, K. O., 1998: Reference methods for the assessment of physical characteristics of meat. *Meat Science*, 49, 447–457, ISSN 0309-1740.
- JAMES, B. W., DRITZ, S. S., TOKACH, M. D., GOODBAND, R. D., NELSEN, J. L., 2000: Margins of safety can be lowered for supplemental copper, zinc, iron, and manganese in finishing pig diets. *Proc. Midwestern Section ASAS*, 160, p. 39. (Abstr.)
- JAMES, B. W., GOODBAND, R. D., UNRUH, J. A., TOKACH, M. D., NELSEN, J. L., DRITZ, S. S., O'QUINN, P. R., ANDREWS, B. S., 2002: Effect of creatine monohydrate on finishing pig growth performance, carcass characteristics and meat quality. *Animal Feed Science and Technology*, 96, 135–145, ISSN 0377-8401.
- KOHN, L. T., CORRIGAN, J. M., DONALDSON, M. S., 1999: *To err is human: Building a Safer Health System*. Washington: National Academy Press; 1999. [cit. 2011-06-10]. <http://books.nap.edu/openbook.php?isbn=0309068371>.
- 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. *Eur. Food Res. Technol.*, 209, 97–103. ISSN 1438-2377 (print), 1438-2385 (online).
- LAHUCKY, R., MOJTO, J., POLTARSKY, J., MIRI, A., RENOU, J. P., TALMANT A., MONIN, G., 1993: Evaluation of halothane sensitivity and prediction of *post mortem* muscle metabolism in pigs from a muscle biopsy using P-31 NMR spectroscopy. *Meat Science*, 33, 3: 373–384, ISSN 0309-1740.
- LAHUCKY, R., KRSKA, P., KUECHENMEISTER, U., NUERNBERG, K., LIPTAJ, T., NUERNBERG, G., BAHNELKA, L., DEMO, P., KUHN, G., ENDER, K., 2000: Effect of Vitamin E on changes in Phosphorus Compounds assessed by P NMR Spectroscopy and ATPase from *post mortem* Muscle samples and Meat quality of Pigs. *Archiv für Tierzucht, Dummerstorf*, 43, 5: 487–497, ISSN 0003-9438.
- LAHUCKY, R., BAULAIN, U., HENNING, M., DEMO, P., KRSKA, P., LIPTAJ, T., 2002: *In vitro* PNMN studies on biopsy skeletal muscle samples compared with meat quality of normal and

- heterozygous malignant hyperthermia pigs. *Meat Science*, 61, 233–241, ISSN 0309-1740.
- LAHUCKY, R., NUERNBERG, K., KUECHENMEISTER, U., BAHNELKA, L., MOJTO, J., NUERNBERG, G., ENDER, K., 2004: The effect of dietary magnesium oxide supplementation on fatty acid composition, antioxidative capacity and meat quality of heterozygous and normal malignant hyperthermia pigs. *Archiv für Tierzucht, Dummerstorf*, 47, 6: 183–191, ISSN 0003-9438.
- LAHUCKY, R., KUECHENMEISTER, U., BAHNELKA, I., NOVOTNA, K., VASICKOVA, K., ENDER, K., 2005: Effects of vitamin E by dietary supplementation and of calcium ascorbate by *post mortem* injection in muscle on the antioxidative status and on meat quality of pigs. *Archiv für Tierzucht, Dummerstorf*, 48, 6: 592–600, ISSN 0003-9438.
- LAHUCKY, R., BAHNELKA, I., KUECHENMEISTER, U., VASICKOVA, E., NUERNBERG, K., ENDER, K., NUERNBERG, G., 2007: Effects of dietary supplementation of vitamins D-3 and E on quality characteristics of pigs and longissimus muscle antioxidative capacity. *Meat Science*, 77, 2: 264–268, ISSN 0309-1740.
- LAHUCKY, R., NUERNBERG, K., KOVAC, L., BUCKO, O., NUERNBERG, G., 2010: Assessment of the antioxidant potential of selected plant extracts – *In vitro* and *in vivo* experiments on pork. *Meat Science*, 85, 779–784, ISSN 0309-1740.
- LAURIDSEN, CH., NIELSEN, J. H., HENCKEL, P., SORENSEN, M. T., 1999: Antioxidative and oxidative status in muscles of pigs fed rapeseed oil, vitamin E, and copper. *Journal of Animal Science*, 77, 105–115, Print ISSN 0021-8812; Online ISSN 1525-3163.
- LAWLER, J. M., BARNES, W. S., WU, G., SONG, W., DEMAREE, S. R., 2002: Direct antioxidant properties of creatine. *Biochemical and Biophysical research Communications*, 290, 1: 47–52, ISSN 0006-291X.
- MADDOCK, R. J., BIDNER, B. S., CARR, S. 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. *Proc. Recip. Meat, Conf., Indianapolis*, 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, Print ISSN 0021-8812; Online ISSN 1525-3163.
- MARKWELL, M. A. K., HAAS, S. M., BIEBER, L. L., TOLBERT, N. E., 1978: A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Analytical Biochemistry*, 87, 206–210, ISSN 0003-2697.
- MIRI, A., TALMANT, A., RENOU, J. P., MONIN, G., 1992: P-31 NMR-study of *post mortem* changes in pig muscle. *Meat Science*, 31, 2: 165–173, ISSN 0309-1740.
- MOESGAARD, B., QUISTORFF, B., CHRISTENSEN, V. G., THERKELSEN, I., JORGENSEN, P. F., 1995: Differences of *post mortem* ATP turnover in skeletal muscle of normal and heterozygote malignant hyperthermia pigs – comparison of P-31-NMR and analytical biochemical measurements. *Meat Science*, 39, 1: 43–57, ISSN 0309-1740.
- O'QUINN, P. R., ANDREWS, B. S., GOODBAND, R. D., UNRUH, J. A., NELSEN, J. L., WOODWORTH, J. C., TOKACH, M. D., OWEN, K. Q., 2000: Effects of modified tall oil and creatine monohydrate on growth performance, carcass characteristics, and meat quality of growing-fmishing pigs. *Journal of Animal Science*, 78, 2376–2382, Print ISSN 0021-8812; Online ISSN 1525-3163.
- PROTOCOL 1222-1223/2007, Central Control Institute of Agriculture, Bratislava.
- 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, ISSN 0309-1740.
- SCHOLZ, A., MITCHELL, A. D., WANG, P. C., HUAFU, S., ZHENJIE, Y., 1995: Muscle metabolism and body composition of pigs with different ryanodine receptor genotypes studied by means of ³¹P nuclear magnetic resonance spectroscopy and H magnetic resonance image. *Archiv für Tierzucht, Dummerstorf*, 38, 5: 539–552, ISSN 0003-9438.
- SHEN, H., LAHUCKY, R., KOVAC, L., O'BRIEN, P. J., 1992: Comparison of Hal gene status with PNMR- determined muscle metabolism and with Ca sequestration activity of anoxia- challenged muscle from pigs homozygous and heterozygous for porcine stress syndrome. *Pig News and Information*, 13, 3: 105–109, ISSN 0143-9014.
- STAHL, C. A., BERG, E. P., 2003: Growth parameters and meat quality of fmishing hogs supplemented with creatine monohydrate and a high glycemic carbohydrate for the 30 days of production. *Meat Science*, 64, 169–174, ISSN 0309-1740.
- 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, ISSN 0309-1740.
- TREFAN, L., BUENGER, L., BLOOM-HANSEN, J., ROOKE, J. A., SALMI, B., LARZUL, C., TERLOUW, C., DOESCHL-WILSON, A., 2011: Meta-analysis of the effects of dietary vitamin E supplementation on the α -tocopherol concentration and lipid oxidation in pork. *Meat Science*, 87, 305–314, ISSN 0309-1740.
- YOUNG, J. F., BERTRAM, H. C., OKSBJERG, N., 2004: Dietary creatine affects meat quality of pure breeds of Duroc and Landrace differently. 50th International Congress of Meta Science and Technology, Helsinki, Finland, Proceeding, Session 2, Meat quality, Abstract, 64.
- YOUNG, J. F., BERTRAM, H. C., ROSENVOLD, K., LINDAHL, G., OKSBJERG, N., 2005: Dietary

- creatine monohydrate affects quality attributes of Duroc but not Landrace pork. *Meat Science*, 70, 4: 717–725, ISSN 0309-1740.
- YOUNG, J. F., BERTRAM, H. C., THEIL, P. K., PETERSEN, A. G., POULSEN, K. A., RASMUSSEN, M., MALMENDAL, A., NIELSEN, N. C., VESTERGAARD, M., OKSBJERG, N., 2007: In vitro and in vivo studies of creatine monohydrate supplementation to Duroc and Landrace pigs. *Meat Science*, 76, 342–351, ISSN 0309-1740.
- YOUNG, J. F., LARSEN, L. B., MALMENDAL, A., NIELSEN, N. CH. IDA K STRAADT, I. K., OKSBJERG, N., HANNE, C., BERTRAM, C. H., 2010: Creatine-induced activation of antioxidative defence in myotube cultures revealed by explorative NMR-based metabonomics and proteomics. *Journal of the International Society of Sports Nutrition* 2010, 7: 9, Abstract, ISSN (Print) 1550-2783 (Online), <http://www.jissn.com/content/7/1/9>.

Address

Ing. Rudolf Lahučký, PhD., Ing. Ondřej Bučko, PhD., Katedra špeciálnej zootechniky, doc. Ing. Peter Haščík, PhD., Katedra hodnotenia a spracovania živočíšnych produktov, Slovenská poľnohospodárska univerzita v Nitre, Tr. A. Hlinku 2, 949 76 Nitra, doc. Ing. Tibor Liptaj, PhD., Ústav analytickej chémie, Slovenská technická univerzita Bratislava, Radlinského 9, 812 37 Bratislava, Slovenská republika, e-mail: rudilahucky@centrum.sk, ondrej.bucko@uniag.sk, peter.hascik@uniag.sk, tibor.liptaj@stuba.sk

