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

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Abstract

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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 (^{31}P 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 Fe^{2+}/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 creatinemonohydrate, 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 ± 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±SE	Group CMH LSM±SE	Group CMH+E LSM±SE
Creatine (μmol.l^{-1})	123.42^{a} ± 4.97	175.54^{b} ± 4.97	163.40^{b} ± 4.87
Vitamin E (mg.kg^{-1})	1.58^{a} ± 0.20	1.63^{a} ± 0.20	3.96^{b} ± 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 ± SE	Group CMH LSM ± SE	Group CMH+E LSM ± SE
Sugar phosphate (SP)	32.38 ± 2.56	36.76 ± 2.56	37.66 ± 2.50
Inorganic phosphate (Pi)	25.77^{a} ± 1.31	22.37 ± 1.31	20.77^{b} ± 1.28
Phosphocreatine (PCr)	4.09^{a} ± 1.17	6.66 ± 1.17	7.82^{b} ± 1.14
Adenosinetri-phosphate (ATP)	10.27 ± 0.73	9.53 ± 0.73	10.40 ± 0.72
Ratio PCr/Pi	0.15 ± 0.09	0.42 ± 0.09	0.39 ± 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 μmol.l^{-1}) and group CMH + E (163.4 μmol.l^{-1}) if compare to control group (123.42 μmol.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; Rosenvold *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)*

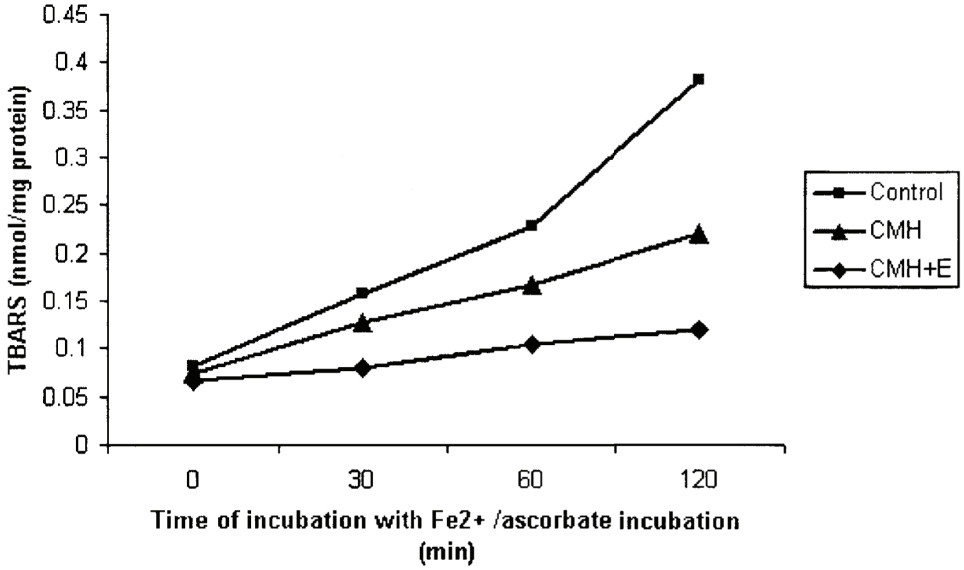
Trait	Control LSM ± SE	Group CMH LSM ± SE	Group CMH+E LSM ± SE
Total water, %	73.9 ± 0.2	73.5 ± 0.2	74.0 ± 0.2
Protein, %	22.0 ± 0.2	22.2 ± 0.2	22.1 ± 0.2
Intramuscular fat, %	2.9 ± 0.2	2.9 ± 0.2	2.6 ± 0.2

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	48.62 ± 0.85
	a*	1.82 ± 0.24	1.82 ± 0.24	2.14 ± 0.23
	b*	7.71 ± 0.23	7.71 ± 0.23	7.78 ± 0.22
Colour (5 days)	**L***	51.50 ± 0.77	50.69 ± 0.77	50.04 ± 0.75
	a*	2.60 ± 0.36	3.04 ± 0.36	3.69 ± 0.35
	b*	8.43 ± 0.39	9.00 ± 0.36	8.65 ± 0.38
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 ≤ 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). 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 ≥ 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 ≥ 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 ≥ 0.05). Maddock *et al.* (2000), Berg and Allee (2001) and Quinn *et al.* (2001) reported higher pH_1 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 géne 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 shoowed 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 Fe^{2+}/ascorbate. Lower values of peroxidation (lower value of MDA production after incubationwith Fe^{2+}/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 a-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 alpha-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 ± 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 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. 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 μmol.l^{-1}) and group CMH + E (163.4 μmol.l^{-1}) if compare to control group (123.42 μmol.$^{-1}$1). The plasma creatine level in the non-supplemented pigs was higher and levels in the supplemented pigs were lower. Our measurements were done from blood taken off after slaughter but differences between non-supplemented and supplemented pigs were significant ($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 Fe^{2+}/ascorbate. Lower values of peroxidation (lower value of MDA production after incubationwith Fe^{2+}/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.

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