

Effects of Creatine Monohydrate Diet on Muscle Metabolism, Quality, Sensory and Oxidative Stability of Pork in Female, Entire and Castrated Male Pigs

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Abstract: Forty-two (42) finishing pigs (entire males—EM, surgical castrates—SC and gilts—G, each of 14)—progeny of Landrace sows and Hampshire × Pietrain boars, were included in the trial. They were randomly divided into control and experimental groups (each of seven). Control groups were fed the standard diet without any supplement. Experimental groups received standard diet with the same composition as control but with supplement of creatine monohydrate (CMH, 2.0 g/kg of feed) for 30 d prior to slaughter. Sex of pigs had significant effect ($p < 0.05$) on drip loss and tenderness of pork when EM showed higher drip loss than SC and lower tenderness compared to other two groups (4.71% vs. 3.80%, resp. 3.23 vs. 3.91 and 4.12). Creatine level in plasma was increased by CMH supplementation in 46% in EM, 43% in SC and 41% in G. Similarly, concentration of phosphocreatine (PCr) in muscle increased in 84% in EM, 88% in SC and 83% in G, respectively. CMH also improved meat colour L^* (50.03 vs. 48.88) and reduced drip loss in both EM (5.24% vs. 4.18%) and G (4.48% vs. 3.60%). Higher tenderness and better oxidative stability of pork after CMH supplementation was found in all three sexes.

Key words: Eating quality, feed additives, oxidative stability, pig, pork quality.

1. Introduction

In recent years, surgical castration of pigs has become a problematic technique from the animal welfare point of view. Based on physiological and ethological investigations, it has been proven that this practice is a painful even when done in very young animals. The primary purpose of surgical castration of male piglets is to prevent the development of firstly, adverse odour or smell, so called “boar taint” and secondly, sexual or aggressive behavior of young boars. On the other hand, a positive effect of “non-castration” is reflected in faster growth, better feed conversion and higher lean meat content in carcasses of entire males (EM) which is desirable for pork producers. In the light of expected ban on surgical castration in the near future, various alternatives such

as rearing the uncastrated males or immunovaccination of boars in order to prevent boar taint are already being applied in and outside the EU (The Netherlands, Spain, Germany, France, Switzerland, Norway). In some other countries, castration is performed using analgesia and/or anesthesia pain relief. Several countries have already committed to a gradual phasing out of the surgical castration of pigs.

After presumed ban on surgical castration, rearing the EM might become the main practice in EU pig husbandry. As mentioned above, EM have several advantages but also some disadvantages compared to surgical castrates (SC). In addition to the increased risk of boar taint, the lower meat quality of EM may be a problem, as some studies have shown [1-5]. Addition of various supplements (vitamins, minerals, trace elements, creatine, betaine, etc.) to pig nutrition may affect positively pork quality, its composition and other attributes.

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Creatine is an amino acid derivative synthesized in liver and kidneys of vertebrates, transported through the blood and taken up by tissues with high energy demands, primarily skeletal muscles and brain. Creatine supplies the muscles with energy, increases their volume by binding the water in muscle cells [6]. It acts at creation of adenosine triphosphate (ATP) which represents immediate source of energy. Creatine monohydrate (CMH) supplementation could affect the glycogen metabolism *post mortem* by buffering lactic acid production which might reduce the rapid pH decline and thereby improve meat quality [7]. Berg and Allee [8], and James *et al.* [9] found postponed pH drop and increased water holding capacity in meat of pigs supplemented with creatine. Positive effect of creatine supplementation on pork color, pH, incidence of pale, soft, exudative (PSE), drip loss was found in several studies [10-16]. On the contrary, some studies suggested no or, even though, negative effect on pork quality [17, 18], and tenderness [19].

The aim of this study was to evaluate the effect of CMH supplementation on plasma creatine, phosphorus compounds levels, meat and eating quality, and antioxidative capacity of EM and compare it to SC and gilts (G).

2. Materials and Methods

2.1 Animals and Treatments

Forty-two (42) finishing pigs (EM, SC and G, each

of 14)—progeny of Landrace sows and Hampshire × Pietrain boars, were included in the trial. Pairs of the same sex were housed in the pens at experimental farm of Research Institute for Animal Production (RIAP). They were randomly divided into control and experimental groups (each of seven). Control (C) groups were fed the standard diet (Table 1) without any supplement. Experimental groups received standard diet with the same composition as control but with supplement of CMH (2.0 g/kg of feed) for 30 d prior to slaughter. Pigs were allowed free access to drinking water during whole experiment. Feed was provided for *ad libitum* consumption. The trial was executed in accordance with Act on animal veterinary care No. 39/2007 of Slovak Republic and approved by Animal Care Committee of the RIAP.

2.2 Slaughtering, Muscle and Fat Sampling

Pigs were slaughtered at 110 ± 5 kg live weight at experimental slaughter house of RIAP by electrical stunning (90-100 V, 0.9-1.0 A, 50 Hz). After that, exsanguination and evisceration were done 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. The second day after slaughter, samples of *longissimus dorsi* muscle (approximately 300 g) were removed from the right side of each carcass and sliced into chops 2.0 cm thick for further

Table 1 Composition and nutritive value of diets.

Ingredients	%	Chemical composition	C	CMH
Barley	42.7	Dry matter, %	86.30	86.30
Wheat	21.0	Crude protein, %	16.84	16.84
Oat	15.0	Crude fat, %	2.43	2.43
Soybean meal	12.0	Crude fibre, %	4.86	4.86
Wheat brans	2.0	N-free extract	41.68	41.68
Meat and bone meal	2.0	Metabolizable energy, MJ	12.31	12.31
Fodder yeast	1.7	Lysine, g/kg	0.86	0.86
Mineral supplement	2.5	CMH, g/kg	-	2.00
Biofactor supplement	0.6			
Fodder salt	0.5			

C: control; CMH: creatine monohydrate supplemented diet.

meat quality (colour, drip loss) analysis, and sensory evaluation. One wrapped sample was stored in the dark for 5 d at 4 °C for shear force and colour analysis.

2.3 Chemical Analyses

Level of creatine in blood plasma of pigs was determined by photometric colorimetric method. The concentrations of phosphocreatine (PCr) and ATP were measured using magnetic resonance spectroscopy. The ^{31}P nuclear magnetic resonance (NMR) spectrum was recorded at 121 MHz using VXR 300 device (Slovak University of Technology, Bratislava). The concentrations of both PCr and ATP were calculated as percentage of the total content of phosphorus compounds as described by Lahučký *et al.* [20].

2.4 Meat Quality Measurements

Two measures of pH—45 min and 24 h after slaughter, were performed from the right side of carcass in the loin between 13th and 14th rib using pH meter (Mettler-Toledo, Greifensee, Switzerland). Colour was measured by Hunter Lab MiniScan spectrometer (L —lightness, a —redness, b —yellowness). Drip loss was evaluated by Honikel method [21]. Shear force was determined on cooked samples using Warner-Bratzler device—Texture Analyser TA-XT2i (Stable Micro Systems Ltd, Surrey, UK).

2.5 Oxidative Stability Analyses

Approximately 30 min *post mortem*, *longissimus dorsi* muscle samples (100 g) were taken, wrapped into aluminium foils and imposed in liquid nitrogen for 5 d for oxidative stability analysis. The oxidative capacity of *longissimus dorsi* muscle homogenate was determined as thiobarbituric acid reactive substance (TBARS) according to Kuechenmeister *et al.* [22]. TBARS values express equivalents of malondialdehyde (MDA, nM/mg homogenate protein) which is breakdown product formed during peroxidation of lipids stimulated by Fe^{2+} /ascorbate.

2.6 Sensory Evaluation

Frozen *longissimus dorsi* muscle samples were thawed for 2 h at room temperature (23 °C). Then they were cut into 50 × 10 mm slides and heated on the contact grill at 180 °C for 4 min. After the heat treatment, pork samples were served separately to the respondents.

Eight trained specialists, both men and women, were to evaluate meat samples from EM, castrates and females. Each respondent evaluated 12 samples. They used 5-point scale where 1 = the worst and 5 = the best quality of the parameter.

2.7 Statistical Analysis

Two-way analysis of variance (ANOVA) with fixed effects of treatment (CMH or none) and sex (EM, SC and G) was used (statistical program SAS-STAT, version 9.1.3, Institute Inc., Cary, NC, USA, 2002-2003). Statistical model was:

$$y_{ijk} = \mu + B_i + D_j + B*D_{ij} + e_{ijk} \quad (1)$$

where y_{ijk} —characteristic of trait selected, μ —intercept, B_i —effect of sex ($i = \text{EM, SC, G}$), D_j —effect of diet ($j = \text{C, CMH}$), $B*D_{ij}$ —two-way interaction effect sex × diet, e_{ijk} —random error ($k = 1, \dots, n_{ijk}$).

Basic statistics was done using MEANS procedure and variability is expressed as the standard error of the means (SEM). Corresponding interactions between treatment and sex were calculated using procedure GLM. Significance of differences was tested using Tukey's test and considered to be statistically significant at the level of $p < 0.05$.

3. Results and Discussion

Sex of pigs had no significant effect ($p > 0.05$) on creatine concentration in plasma and individual phosphorus compounds (PCr, ATP) level in muscle. In contrast, supplementation of diet by CMH had significant effect ($p < 0.05$) on creatine concentration in plasma and PCr in muscles of pigs (Table 2). Li *et al.* [16] also found increased concentration of creatine

and PCr after CMH supplementation. On the other hand, ATP values of experimental and control groups in the study were almost the same and differences were not significant ($p \geq 0.05$). Lahučký *et al.* [14] reported comparable effect of CMH supplementation in experiment with dominant homozygous in *RYR1* gene (NN) pigs when plasma creatine level and PCr concentration in *longissimus dorsi* muscle increased

by 42% and 63%, respectively. They showed that higher efficiency of energetic muscle metabolism (decreased and slower PCr breakdown) was found in pigs supplemented by CMH.

In the study, small effect of sex on pork quality traits was observed when significant differences only for drip loss ($p < 0.05$) between EM and SC were found (Table 3). The same results as the study—higher

Table 2 Plasma creatine, muscle adenosine triphosphate (ATP) and phosphocreatine (PCr) levels of *longissimus dorsi* muscle.

Trait	Sex			Sign.	Diet		Sign.	SEM	Sex × CMH
	EM	SC	G		C	CMH			
Plasma creatine, $\mu\text{mol/L}$	147.3	144.5	145.7	ns	120.6 ^a	171.7 ^b	*	7.54	*
PCr ¹	5.18	5.33	5.10	ns	3.58 ^a	6.77 ^b	*	1.52	*
ATP ²	10.82	10.74	10.96	ns	10.57	11.06	ns	1.19	ns

^{a, b} different letters in row mean significant differences at $p < 0.05$; ^{1, 2} percentage of total content of phosphorus compounds; *significance at $p < 0.05$; ns: non significant; EM: entire males; SC: surgical castrates; G: gilts; C: control group; CMH: creatine monohydrate supplemented group.

Table 3 Pork quality of *longissimus dorsi* muscle.

Trait	Sex			Sign.	Diet		Sign.	SEM	Sex × CMH
	EM	SC	G		C	CMH			
pH ₄₅	6.29	6.35	6.36	ns	6.33	6.33	ns	0.38	ns
pH ₂₄	5.60	5.55	5.56	ns	5.61	5.53	ns	0.09	ns
Colour ₂₄	<i>L</i> [*] 49.48	49.35	49.49	ns	50.03 ^a	48.88 ^b	*	0.78	ns
	<i>a</i> [*] 1.85	1.86	1.86	ns	1.85	1.87	ns	0.24	ns
	<i>b</i> [*] 7.81	7.78	7.81	ns	7.84	7.75	ns	0.19	ns
Colour ₅	<i>L</i> [*] 50.06	51.88	51.26	ns	51.73	50.35	ns	0.84	ns
	<i>a</i> [*] 2.77	2.96	2.79	ns	2.70	2.91	ns	0.45	ns
	<i>b</i> [*] 8.60	8.61	8.65	ns	8.38	8.88	ns	0.28	ns
Drip loss, %	4.71 ^a	3.80 ^b	4.04 ^{ab}	*	4.53 ^a	3.84 ^b	*	0.43	*
Shear force, kg	5.13	5.25	5.08	ns	5.08	5.24	ns	0.22	ns

^{a, b} different letters in row mean significant differences at $p < 0.05$; * significance at $p < 0.05$; ns: non significant; EM: entire males; SC: surgical castrates; G: gilts; C: control group; CMH: creatine monohydrate supplemented group; pH₄₅: trait measured 45 min *post mortem*; pH₂₄: trait measured 24 h *post mortem*; Colour₂₄: trait measured 24 h *post mortem*; Colour₅: trait measured 5 d *post mortem*.

Table 4 Interactive effect of sex and CMH supplementation on phosphorus compounds, pork quality and oxidative stability.

Trait	EM		SC		G		SEM
	C	CMH	C	CMH	C	CMH	
Plasma creatine ¹ , $\mu\text{mol/L}$	118.4 ^a	172.0 ^b	121.3 ^a	172.8 ^b	119.8 ^a	168.5 ^b	7.54
PCr ²	3.64 ^a	6.68 ^b	3.68 ^a	6.90 ^b	3.59 ^a	6.56 ^b	1.52
Drip loss, %	5.24 ^a	4.18 ^b	3.88	3.73	4.48 ^a	3.60 ^b	0.43
Tenderness	2.88 ^a	3.56 ^b	3.70 ^a	4.13 ^b	3.90 ^a	4.29 ^b	0.25
TBARS (30 min)	0.26 ^a	0.10 ^b	0.30 ^a	0.15 ^b	0.26 ^a	0.14 ^b	0.03
TBARS (60 min)	0.35 ^a	0.13 ^b	0.38 ^a	0.19 ^b	0.33 ^a	0.19 ^b	0.02
TBARS (120 min)	0.43 ^a	0.20 ^b	0.45 ^a	0.23 ^b	0.39 ^a	0.25 ^b	0.02

^{a, b} different letters in row mean significant differences at $p < 0.05$; ^{1, 2} percentage of total content of phosphorus compounds; EM: entire males; SC: surgical castrates; G: gilts; C: control group; CMH: creatine monohydrate supplemented group.

Table 5 Sensory properties of *longissimus dorsi* muscle.

Trait	Sex			Sign.	Diet		Sign.	SEM	Sex × CMH
	EM	SC	G		C	CMH			
Juiciness	3.27	3.65	3.49	ns	3.41	3.53	ns	0.36	ns
Tenderness	3.23 ^a	3.91 ^b	4.12 ^b	*	3.50 ^a	4.03 ^b	*	0.25	*

^{a, b} different letters mean significant differences at $p < 0.05$; * significance at $p < 0.05$; ns: non significant; EM: entire males; SC: surgical castrates; G: gilts; C: control group; CMH: creatine monohydrate supplemented group.

Table 6 Oxidative stability of *longissimus dorsi* muscle.

Trait	Sex			Sign.	Diet		Sign.	SEM	Sex × CMH
	EM	SC	G		C	CMH			
TBARS (0 min)	0.06	0.05	0.06	ns	0.06	0.05	ns	0.001	ns
TBARS (30 min)	0.18	0.23	0.20	ns	0.28 ^a	0.12 ^b	*	0.02	*
TBARS (60 min)	0.24	0.29	0.26	ns	0.35 ^a	0.17 ^b	*	0.02	*
TBARS (120 min)	0.31	0.35	0.33	ns	0.43 ^a	0.22 ^b	*	0.02	*

^{a, b} different letters mean significant differences at $p < 0.05$; * significance at $p < 0.05$; ns: non significant; EM: entire males; SC: surgical castrates; G: gilts; C: control group; CMH: creatine monohydrate supplemented group.

drip loss in EM compared to SC were reported by Pauly *et al.* [2] and Aluwé *et al.* [5]. In contrast, several studies reported no significant differences between G, EM and SC in this parameter [23–26]. These discrepancies in results between studies may be due to different pig breeds, genotypes related to *RYR1* or muscles analyzed. Drip loss in the study was also significantly affected by dietary CMH supplementation. EM and G had reduced drip loss ($p < 0.05$) but not SC. Significant interaction between sex and diet was found for this qualitative parameter (Table 4). Similar results were reported by Lahučký *et al.* [14] and Li *et al.* [16]. In another study Li *et al.* [15] suggested that drip loss reduction by CMH may be associated with a delayed early pH decline and a decrease in the rate of glycolysis. In contrast, no effect of CMH on drip loss was reported by other studies [8, 12, 19].

Positive effect of CMH ($p < 0.05$) on meat colour L^* measured 24 h *post mortem* was found. Results of various studies published worldwide are often contradictory when some of them reported positive effect of CMH supplementation [12]—partially, in some muscles; some none effect [14, 19, 27–30] or even though negative effect—reduced colour darkness [13, 31].

Influence of sex and supplementary CMH on sensory characteristics of pork is summarized in Table 5. Juiciness of pork was not affected by sex or creatine ($p > 0.05$). However, tenderness was influenced by both factors—sex and diet. Pork from G was assessed by panellists as the best, followed by SC and EM. Differences between first two groups and EM were significant ($p < 0.05$). Higher tenderness in pork from G compared to EM was also found by Cho *et al.* [32] and Jeleníková *et al.* [33] whilst Kim *et al.* [34] reported no significant differences between these sexes in three-cross Landrace × Yorkshire × Duroc and Landrace × Yorkshire × Woori black pigs, respectively. In contrast, some studies observed better tenderness in SC than G [19, 35] or SC than EM [5, 23, 24]. Supplementary CMH in the presented study significantly improved ($p < 0.05$) tenderness. Improvement was found in all three sexes as also documented by interactions (Table 4). This is in contrast to the findings of Rosenvold *et al.* [19] who did not find any effect of CMH on tenderness of pork.

Effect of sex and CMH supplementation on oxidative stability of pork is shown in Table 6. While the sex did not have any influence ($p > 0.05$), dietary CMH suggested significant reduced peroxidation—lower values of MDA production after

30, 60 and 120 min incubation with Fe^{2+} /ascorbate mixture in EM, SC and G. Interactions between sex and diet were also found for all three time incubations (Table 4). Similar effect of CMH supplementation on oxidative stability of pig muscle was found by Lahučký *et al.* [14]. Also, significant interactions of sex and diet were found in plasma creatine and PCr concentrations, drip loss and tenderness of meat. Creatine level in plasma increased by 45% in EM, 42% in SC and 41% in G. Similarly, concentration of PCr in muscle increased by 84% in EM, 88% in SC and 83% in G, respectively. CMH supplementation reduced drip loss in EM and G, and improved pork tenderness in all the three sexes ($p < 0.05$)

4. Conclusions

The results showed that sex of pigs had significant effect on drip loss and tenderness of pork. EM had higher drip loss than SC and lower tenderness compared to other two groups. These facts may be problematic from the meat industry as well as consumer's point of view after the expected ban on surgical castration of piglets and alternative EM production. Supplementation of diet with CMH increased creatine concentration in plasma and improved some parameters of muscle energy metabolism (PCr) in pork muscle of EM, SC and G. CMH also improved colour of fresh meat (24 h), reduced drip loss in both EM and G which could be interesting from the meat industry point of view. Higher tenderness and oxidative stability of pork after CMH supplementation might be beneficial for consumers. It seems that CMH supplementation may eliminate some worse parameters of meat and eating quality in entire male pigs.

Acknowledgments

The authors thank Jana Zeleňáková (RIAP Nitra) for meat quality analysis. Special thanks also go to Research Institute for the Biology of Farm Animals, Dummerstorf, Germany, for antioxidant stability analysis.

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