Original Paper

The analysis of pork quality affected by diet containing organic chromium

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The objective of the experiment was to evaluate the effect of organic chromium on the parameters of the chemical composition and physical quality of the pork. The control group was fed by standard feed mixture. The mineral-protein dough used in feed mixture for experimental group was enriched by chromium-nicotinate, which made 0.75 mg kg^{-1} Cr^{3+} in the complete feed. In the values of pH_1 and pH_{24} parameter, statistically significant differences between groups were not found. We also found statistically non-significant differences in the indicator of electrical conductivity measured 45 minutes p. m and 24 hours p. m. The value of the drip-loss in % in the control group was slightly higher compared with the experimental group, but the results were statistically insignificant. The value of the CIE L* in the control group was 63.17±4.26 compared with the experimental group where we measured the value 61.29±4.11 which means that the control group had slightly lighter colour of meat. In CIE L * value, we found statistically significant difference ($P \leq 0.05$). The value of the Warner-Bratzler shear force in the control group was slightly higher compared with the experimental group, but the results were statistically insignificant. The indicators total water, total protein and intramuscular fat were not affected by organic chromium. We found statistically significant difference ($P \leq 0.05$) in content of monounsaturated fatty acids in favour of the experimental group.

Keywords: granulated vermicompost, maize, potatoes, starch, vitamin C

1. Introduction

The experiment by Matthews et al. (2003) was conducted to evaluate the dietary effects of chromium propionate (CrProp) on pork quality of growing-finishing pigs. In this experiment the values of pH_1 and pH_{24} were not affected by the diet with chromium propionate supplementation. The effects of chromium propionate on growth, carcass traits and pork quality of growing-finishing pigs were investigated by Matthews et al. (2005). Twenty-four-hour loin pH_{24} (pH_{24} = 5.66) was increased in pigs fed by chromium propionate, but 45-min pH_1 (pH_1 = 6.11) were not affected by chromium propionate. In experiment by Matthews et al. (2001), the effect of chromium tripicolinate (CrPic) and chromium propionate (CrProp) on electrical conductivity was not found. Boleman et al. (1995) reported no effects of chromium picolinate on drip loss in their study. On the other side, Matthews et al. (2001) published in their study results saying that Cr supplementation may have an effect on water holding capacity. Similarly, Shelton et al. (2003) reported that chromium propionate increased water-holding capacity in one experiment. Matthews et al. (2003) reported that chromium propionate had no effect on colour scores (subjective or objective) and our data are in agreement with them. There were also Cr responses in colour measures in study by Lindemann et al. (2008). Waylan et al. (2003) reported that supplementing with chromium nicotinate had minimum effects on CIE (Elementary coloured area) L*, a*, b*. Dietary inclusion of chromium in diets of pigs and sheep has potential to improve meat colour, which was presented by Dikeman (2007). Matthews et al. (2003) report that Cr does not affect shear force and our data are in agreement with them. However, Boleman et al. (1995) reported that shear force was increased in pigs fed by chromium picolinate during the growing-finishing period compared to pigs fed by chromium picolinate during the finishing period only. According to study by Jackson et al. (2009), chromium supplementation resulted in a decrease in fresh loin chop shear force, which is not in agreement with the results of Matthews et al. (2003). Boleman et al. (1995) measured the value of *intramuscular fat* 2.98 % in experimental group where chromium picolinate was used, but the differences between groups were statistically insignificant. According to Lien et al. (2001), the carcasses of the pigs that received the chromium supplemented ration contained less oleic acid (C18 : 1) and total unsaturated fatty acids ($P \leq 0.05$). The total saturated fatty acid content was higher than that of the controls ($P \leq 0.05$). Chromium supplemented groups contained less total unsaturated fatty acids (59.30%) compared with the experimental group (63.95%). Cr supplementation may affect the fatty acid profile of fat stores in the pig and this may be the reason for the observed improvement in some aspects of pork quality was published

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in study by Jackson et al. (2009). Adipose tissue and not liver, is the predominant site of fatty acid synthesis in the pig Mersmann (1986); this suggests that chromium would increase fatty acid synthesis in pigs, which, again, should result in an increase in fat deposition. According to Wenk et al. (1995) the results of the carcass composition as well as the fatty acid profile of neutral and complex lipids in the muscle (*M. longissimus dorsi*) at the 10th rib did not indicate a statistically significant effect of the Cr supplements.

2. Material and methods

The experiment was introduced in the experimental centre of the Department of Animal Husbandry of the Slovak University of Agriculture in Nitra. There were 56 Large White Breed pigs used in the 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 the control group of 28 pigs (14 barrows and 14 gilts) and experimental group of 28 pigs (14 barrows and 14 gilts). Feeding and water were *ad libitum*. The control group was fed a standard diet which consisted of three feed mixtures (OŠ) aplicated in various growth phases from 30 to 45 kg OŠ-03, from 45 to 70kg OŠ-04 a from 70 to 100 kg OŠ-05 (Table 1).

The experimental group was fed by the same feed mixture in the same growth phase as the control group at which the mineral-protein dough used in feed mixture for experimental group was enriched by chromium-nicotinate, which made 0.75 mg kg^{-1} Cr^{3+} in the complete feed. Chromium-nicotinate was in form inactivated yeats *Saccharomyces cerevisiae* fermented on medium which was from natural sources with higher content trivalent chromium. All feed was fed from 30 to 100 kg body weight. The growth potential was monitored by weighing the pigs with 0.5 kg accurateness. The weightings were carried out at two week intervals in period from 30–90 kg and at weekly intervals in periods to 30 kg and from 90 to 100 kg live weight of pigs. The found weight was statistically evaluated concurrently with the age of pigs in the days. The date of detection the age of animals and respective weight had to be consistent. Slaughter and dissection of pigs' carcasses were performed at the slaughterhouse experimental center of livestock close to the Department of animal husbandry. Pigs were slaughtered with an average body weight of 102.5 kg. Dissection of carcass was performed according to the methodology STN 466164. The samples for the analysis of the chemical indicators were taken from thighs (*musculus semimembranosus*, *msm*) during dissection of the right half/carcase hold in storage

Table 1 Composition of the diet

Trait	Control			Chromium nicotinate		
	Phase 1 (30–45 kg)	Phase 2 (45–70 kg)	Phase 3 (45–70 kg)	Phase 1 (30–45 kg)	Phase 2 (45–70 kg)	Phase 3 (45–70 kg)
Barley in %	26.5	26.0	26.0	26.5	26.0	26.0
Wheat in %	26.0	24.4	26.0	26.0	24.4	26.0
Corn in %	17.7	26.3	27.0	17.7	26.3	27.0
Soybean meal in %	26.5	20.0	15.2	26.5	20.0	15.2
Wheat bran in %	0.0	0.0	3.0	0.0	0.0	3.0
Mineral and protein supplement in %	3.0	3.0	2.8	3.0	3.0	2.8
Feed acid in %	0.3	0.3	0.0	0.3	0.3	0.0
Dry mater in %	88.73	88.63	88.47	88.73	88.63	88.47
Crude protein in g	202.5	176.39	160.06	202.50	176.39	160.06
Metabolisable energy in MJ	13.12	13.10	13.04	13.12	13.10	13.04
Lysine in g	12.32	10.49	9.13	12.32	10.49	9.13
Methionine + cysteine in g	6.28	5.62	5.18	6.28	5.62	5.18
Threonine in g	7.72	6.68	5.97	7.72	6.68	5.97
Tryptophane in g	2.53	2.14	1.91	2.53	2.14	1.91
Calcium in g	5.90	5.75	5.35	5.90	5.75	5.35
Total phosphorus in g	6.48	6.27	6.21	6.48	6.27	6.21
Digestible phosphorus in g	1.25	1.12	1.12	1.25	1.12	1.12
Chrom – added in µg kg^{-1}	–	–	–	750	750	750
Chrom – analysed in µg kg^{-1}	1.3	1.4	1.3	744	752	724

for 24 hours *post mortem* at the temperature 3–4 °C. The sample from the muscle 100 g *musculus semimembranosus* was taken 23 cm from the centre and it was hold in storage separately for 14 days at the temperature -19±0.5 °C before the analysis was carried out.

The chemical composition indicators of the pork in *musculus semimembranosus* of Large White Breed were measured on the muscular homogenate sample (50 g) by the FT IR method using the device Nicolet 6700. We analyzed the total proteins in g 100 g, the *intramuscular fat* in g 100 g and total water in g 100 g. The infra-red spectrum of the muscular homogenate analysis itself was carried out by the molecular spectroscopy method. The principle of this method is the absorption of the infra-red spectrum during the sample transition where there is a change of the rotary vibrating energetic conditions of the molecule depending on the changes of the dipole momentum molecule. The analytical output is the infra-red spectrum which is a graphic representation of the function dependence of the energy, mostly given in transmittance percentage (T) or absorbance units (A) on wave-length of the incident emission. The transmittance is defined as a ratio of the intensity of the emission which has passed the sample (I) and the intensity of the emission emitted by the source (Io). The absorbance is defined as a decimal logarithm 1/T. The dependence of the energy on the wave-length is logarithmic, so a repetency – defined as a reciprocal of the wave-length – is used; therefore the presented dependence of the energy on the repetency is a linear function. The particular fatty acids were evaluated from the muscular homogenate from *musculus semimembranosus* (50 g) by the gas chromatography (GC) method at the laboratory of the Institute of Chemistry, Faculty of Natural Science, Comenius University in Bratislava.

The colour of the meat was measured on the *musculus semimembranosus* section across the muscular fibres 24 hours *post mortem* by the spectrophotometer CM-2600d. We set the colour spectrum CIE L*a*b* for the color analysis. L* represents lightness and its scale ranges from 0 (black) to 100 (white). a* represents redness/greenness and its scale ranges from + 60 (red) to -60 (green). b* represents yellowness/blueness and its scale ranges from -60 (yellow) to -60 (blue). Because the surface was wet, we evaluated the colour with shine (SCI). The electrical conductivity was determined 45 minutes and 24 hours *post mortem* in *musculus semimembranosus* by Quality-meter PSE in mS cm^{-1}. The muscle pH-log molc. (H^+) was determined 45 minutes and 24 hours *post mortem* in *musculus semimembranosus* using the micro-capillary combined electrode. We used the portable acidometer Sentron-Titan. Drip loss in % was determined according to Honikel (1998) methodology using 50 g muscle of *musculus semimembranosus* in time from 24 to 48 hours after swine slaughter. The muscle was hung in special plastic bags in the fridge at the temperature between 4 and 6 °C. Drip loss was determined in %. Shear force of the pork was determined by Warner-Batzler device. The samples were kept at the temperature 4+/-1 °C for 7 days. After that, they were heated up to the temperature of 71 +/-1 °C during 30 minutes and sample was trimmed like a block 1 × 1 cm cut-through across the fibres. Shear force was determined by Chatillon device.

DNA was isolated by the salting out method according to Miller et al. (1988) that was optimized for the laboratory conditions of the Department of Genetics and Breeding Biology. The samples of DNA were frozen till the beginning of the analyses. Thereafter, the isolated DNA was used for PCR-RFLP analysis of the RYR1 gene. For the amplification of the specific sections of the RYR1 gene were used following oligonucleotide primers FOR and REV that were adapted from Kaminski et al. (2002). The primer FOR 5´-CTGGGA CATCATCCTTCTGG-3´ and the primer REV 5´-GGGTTCTAAGCTCTGGGGTC-3´. The reaction mixture for PCR with total content of 25 µl contained: 1.5 µl × 10 reactive solution, 25 mM $MgCl_2$, 10 mM dNTP mixture, 10 pmol/µl of the primers FOR a REV, 5 U/µl Taq DNA polymerase (Fermentas) and 50 ng/µl DNA. Polymerase chain reaction was performed in the thermal cycler MJ Mini (Biorad) with the following thermal and time regime: denaturation 95 °C/3 min, hybridization 56 °C/20 seconds, polymerization 72 °C/30 seconds. The number of cycles was 30. The acquired PCR product in size 272 bp was consequently cleaved by restriction enzyme FastDigest Hin6 I (Fermentas) at temperature 37 °C and time period 5 minutes. Visualization of products PCR-RFLP was accomplished electrophoretically in 2% agarose gel with addition of *intercalating* dye GelRed (Biotium) with the help of the UV transillumitor.

For the control and the experimental groups, basic statistical variation characteristics were determined. For differences comparison between groups, we used one-way analysis of variance in pursuance of statistical software package SAS® version 9.1 (SAS Institute Inc, Cary, NC, 2004). In terms of individual groups of fixed factor was the balance of adequate number of barrows and gilts in the groups.

3. Results and discussion

In physical indicators of quality pork we reported the following differences in individual parameters between the control and the experimental groups (Table 2). The value of parameter pH_1 was found in the control group at 6.15±0.15 compared with the experimental group, where we found the value of 6.18±0.11. In parameter pH_{24} we found the value 5.66±0.07 in the control group compared with the experimental group where we found the value pH_{24} 5.67±0.08. The significant differences in parameters pH_1 and pH_{24} between the groups were not found ($P \geq 0.05$). Matthews et al. (2003) reported

the value of pH_1 = 5.94 and pH_{24} = 5.58 in the experimental group but the values of pH_1 and pH_{24} were not affected by the diet with supplementation of CrProp. According to Matthews et al. (2005) pH_{24} (pH_{24} = 5.66) was increased in pigs fed by CrProp, but pH_1 (pH_1 = 6.11) were not affected by CrProp. In the parameter electrical conductivity 45 min. *post mortem* was measured the value of 6.68±2.02 in the control group. In the experimental group there was a slightly lower value 5.97±1.65 measured. In electrical conductivity 24 hours *post mortem*, we found a higher value in the control group 11.72±2.49 compared with the experimental group 9.75±3.07. Our results are in agreement with the study by Matthews et al. (2001). The indicator drip loss in % was found in the control group 9.91±3.01 – a slightly higher value compared with the experimental group, where we measured 9.75± 3.07. In indicator drip loss in % we found no statistically significant difference between the groups. Similar results in indicator of drip loss were published by Boleman et al. (1995). The values of drip loss in this study was measured at level of 9.19 in the control group and 8.53 in the experimental group. Boleman et al. (1995) reported no effects of CrPic on drip loss in their study. On the other side Matthews et al. (2001) in their study published results saying that Cr supplementation may have an effect on water holding capacity. Similarly, Shelton et al. (2003) reported that CrProp increased water-holding capacity. Colour data were for CIE L* value in the control group 63.17±4.26 compared with the experimental group, where we measured the value 61.29±4.11 which means that the control group had a slightly paler meat colour. In CIE L* value, we found a statistically significant difference $P \leq 0.05$. The colour of meat for CIE a* value was found in the control group 8.12±4.80 compared with the experimental group, where we measured 8.28±6.25. The value of the colour of meat CIE b* was found in the control group 2.13±10.60 compared with the experimental group, where we measured the value 5.00±10.36. In colour measurements CIE a* and CIE b*, we found no statistically significant differences between the control and the experimental groups. Matthews et al. (2003) reported that CrProp had no effect on colour scores (subjective or objective) and our data are in agreement except of the CIE L*. There were also Cr responses in colour measures in study by Lindemann et al. (2008). The CIE L* scores did not differ statistically, which is not in agreement with our results. In the CIE a* and CIE b* values by Lindemann et al. (2008) there were found statistically significant differences between control and experimental groups. These results are not in agreement with our results. Waylan et al. (2003) reported that supplementing with CrNic had minimal effects on CIE L*, a*,b* and similar results found Matthews et al. (2005). Dietary inclusion of chromium in diets of pigs and sheep has potential to improve meat colour (Dikeman, 2007). The Warner-Bratzler shear force was found in the control group in the values of 3.73±1.29 kg compared with the experimental group where we measured the value 3.61±1.15 and we found no statistically significant difference between the groups. Matthews et al. (2003) reports that Cr does not affect shear force and our data are in agreement. However, Boleman et al. (1995) reported that shear force was increased in pigs fed CrPic during the growing-finishing period compared to pigs fed CrPic during the finishing period only. According to study by Jackson et al. (2009), chromium supplementation resulted in a decrease of shear force in fresh loin chop, which is not in agreement with the results of Matthews et al. (2003). The value of shear force was found 4.12 in control group compared with experimental group where we found the value 3.98 in pigs fed CrProp. Shear force was not affected by CrProp which is consistent with our results according to study Matthews at al. (2005). The indicators of the chemical composition of pork in *musculus semimembranus* were measured with the following values of monitored parameters (Table 3). Total water in % was observed 70.85±1.08% in the control group, while in the experimental group; we measured the value 71.06±1.06%. The values of *intramuscular fat* in homogenates *musculus semimembranus* were measured at 4.60±1.74% in the control group compared with the experimental group, where we measured the value of 4.12±1.11%. Boleman et al. (1995)

Table 2 Pork quality of *semimembranosus* muscle

Trait	Control LSM±SE	Group chromium nicotinate LSM±SE
pH_1 – log molc. (H^+) v MSM	6.15±0.15	6.18±0.11
pH_{24} – log molc. (H^+) v MSM	5.66±0.07	5.67±0.08
E. $conductivity_1$ – ms/cm v MSM	6.68±2.02	5.97±1.65
E. $conductivity_{24}$ –ms/cm v MSM	11.72±2.49	10.94±2.28
Drip loss (24–48 hours *p.m.*) v %	9.91±3.01	9.75±3.07
Colour (24 hours) CIE L* CIE a* CIE b*	63.17±4.26[a] 8.12±4.80 2.13±10.60	61.29±4.11[a] 8.28±6.25 5.0±10.36
Shear force, (W–B) kg	3.73±1.29	3.61±1.15

[a] – different letters denote significant differences between groups at $P \leq 0.05$

Table 3 Chemical composition of *semimembranosus* muscle (g.100 g^{-1}) (n = 56)

Trait	Control LSM±SE	Group chromium nicotinate LSM±SE
Total water in g 100 g^{-1}	70.85±1.08	71.06±1.06
Intramuscular fat in g 100 g^{-1}	4.6±1.74	4.12±1.11
Total Protein in g 100 g^{-1}	21.80±1.01	21.92±0.52

Table 4 Fatty acids composition of the intramuscular fat in *semimembranosus* muscle (g.100 g^{-1} FAME) (n = 56)

Trait	Control LSM±SE	Group chromium nicotinate LSM±SE
Saturated fatty acids	36.40±1.36	36.34±1.28
Monounsaturated fatty acids	53.48±1.54[a]	54.45±1.63[a]
Polyunsaturated fatty acids	7.21±1.50	7.90±1.67

[a] – different letters denote significant differences between groups at $P \leq 0.05$

measured the value of *intramuscular fat* 2.98% in experimental group where was used chromium picolinate but differences between groups were statistically insignificant. Total protein in % was measured in the control group at 21.8±1.01 and in the experimental group, we found 21.92±0.52. In indicators of chemical composition of pork in monitored parameters we found no statistically significant differences between the groups. In the *intramuscular fat* there was investigated the relative composition of fatty acids and we found the following values (Table 4). Saturated fatty acids were 36.40±1.36% in the control group compared with the experimental group, where we found 36.34±1.28%. In the saturated fatty we found no statistically significant difference between groups. Mono unsaturated fatty acids were measured 53.48±1.54% in the control group and 54.45±1.63 in the experimental group, the difference between groups was demonstrated at $P \leq 0.05$. The amount of poly unsaturated fatty acids was measured in the control group at 7.21±1.50 compared with the experimental group, where we found the amount of poly unsaturated fatty acids of 7.9±1.67%, while the difference between the groups was statistically insignificant. Lien et al. (2001) measured the values of total saturated fatty acid 36.15% in the control group compared with the experimental group, where they found 41%. The total saturated fatty acid content was highert than that of the controls ($P \leq 0.05$). Chromium supplemented groups contained less total unsaturated fatty acids (59.30%) compared with the experimental group (63.95%). Cr supplementation may affect the fatty acid profile of fat stores in the pig, and this may be the reason for the observed improvement in some aspects of pork quality was published in study by Jackson et al. (2009). Adipose tissue, and not liver, is the predominant site of fatty acid synthesis in the pig Mersmann (1986); this suggests that chromium would increase fatty acid synthesis in pigs, which, again, should result in an increase in fat deposition. According to Wenk et al. (1995) the results of the carcass composition as well as the fatty acid profile of neutral and complex lipids in the muscle (*M. longissimus dorsi*) at the 10th rib did not indicate a statistically significant effect of the Cr supplements. According to Lien et al. (2001), the carcasses of the pigs that received the chromium supplemented ration contained less oleic acid (C18 : 1) and total unsaturated fatty acids ($P \leq 0.05$).

4. Conclusions

In conclusion we can state that the utilization of feed additives with higher content of bioactive chromium has positive influence on the parameters of physical quality of pork meat. This was mostly proved in parameters such as pale colour of pork, but less in pH values, electrical conductivity, wateriness and firmness. When we dealt with the chemical composition of pork meat we found out that there are no significant differences in individual markers, although the values of averages in marker *intramuscular fat* were a little bit higher in the control group in comparison with the experimental group. The composition of fatty acids in *intramuscular fat* in *musculus semimembranus* proved the fact of higher part of mono-unsaturated acids in group that was fed with additive of bioactive chromium while the amount of saturated fatty acids was approximately the same in both groups. Higher content of half-unsaturated acids was also indicated in the experimental group but this fact was statistically insignificant. Positive data received on improving colour meat L* and monounsaturated fatty acids need further study.

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