Study on the relation between dietary Cr (III) and the metabolism of other trace minerals (Cu, Fe, Mn, Zn) in growing pigs

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SUMMARY

Trivalent chromium is essential to normal carbohydrate, lipid and protein metabolism. The objective of this study was to evaluate the interrelations between Cr (III) and trace minerals (Cu, Fe, Mn, Zn) in growing pigs fed with chromium picolinate supplemented diets.

A 6 week study was conducted on 8 castrated Topigs male pigs assigned to 2 groups (C and E), housed in individual metabolic cages. The pigs had an initial bodyweight of 17.16 ± 0.62 kg and 49.93 ± 2.3 kg (C) respectively 48.75 ± 5.3 kg (E) final weight. The pigs were fed on corn-soybean meal-based diets (18.75% CP; 3063 kcal/kg ME). The diet of E group was supplemented with 200 ppm CrPic. Samples of ingesta, faeces and urine were collected in 3 balance periods of 5 days each. At the end of experiment, all pigs were slaughtered and meat (tenderloin; loin; ham; shoulder; belly; scruff) and organ (liver, spleen, kidney) samples were collected. The levels of Cu, Fe, Mn and Zn were measured by FAAS in the samples of ingesta, faeces, urine and meat.

Chromium supplements decreased, but not significantly (P>0.05) trace minerals apparent absorption coefficients for all of them: Cu (32.11%, C *vs.* 32.05%, E); Fe (52.47%, C *vs.* 53.70%, E); Mn (35.24%, C *vs.* 32.76%, E); Zn (47.56%, C *vs.* 44.30%, E). Trace elements (Cu, Fe, Mn, Zn) concentrations were determined in samples of: tenderloin, loin, ham, shoulder, belly and scruff. Significant increased concentrations (P≤0.05) were obtained for Zn in: loin (4.38 ± 0.29 ppm, C *vs.* 5.19 ± 0.29 ppm, E), shoulder (8.21 ± 0.41 ppm, C *vs.* 9.74 ± 0.40 ppm, E), belly (3.78 ± 0.54 ppm, C *vs.* 5.04 ± 0.82 ppm, E) and scruff (4.89 ± 0.29 ppm, C *vs.* 6.09 ± 0.84 ppm, E). The deposits of Cu, Fe, Mn and Zn in the main organs (liver, spleen and kidney) were also evaluated and

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significant increased concentrations (P≤0.05) of Zn in the liver samples of E group (18.40 ± 0.14 ppm C *vs.* 20.02 ± 0.56 ppm E) were noticed.

The synergy of Cr and Zn was highlighted in this study by the results obtained in determinations on anatomical parts.

Keywords: trivalent chromium, pigs, trace minerals, metabolism

INTRODUCTION

The biological role of Cr (III) as a trace element for animals has been already suggested in the late 50's of the 20th century (Schwartz and Mertz, 1959). In human nutrition, chromium is used as a nutritional supplement recommended in impaired carbohydrate metabolism characterised by reduced glucose tolerance and impaired insulin action, and for weight reduction (Pechova, 2007). Currently, the predominant hypothesis on the Cr(III) action is the chromodulin-mediated role on the insulin-activated glucose uptake by cells (EFSA 2009).

In the late 1990's, chromium started to be studied as an essential mineral in livestock animals (cattle, sheep, pigs and poultry) (Pechova, 2007). Several authors have reported improvements in the carcass composition of pigs fed diets supplemented with chromium. Page et al. (1993) concluded that providing 200 ppb of Cr as picolinate (CrPic) reduced 10th rib backfat thickness and increased longissimus muscle area and total muscling. Improvement in gain/feed and improvement in carcass traits were reported by Lindemann et al. (1995) for pigs fed diets containing 200 ppb Cr as CrPic. Mooney and Cromwell (1995) did not find 200 ppb Cr from CrPic to be effective in increasing absolute longissimus muscle area or decreasing backfat thickness. Wang, (2004), has shown that chromium nanoparticles have a beneficial influence on the quality and quantity of pig skeleton muscles.

There is lack of data to support the interactions between chromium and other trace minerals from animal chromium supplemented diets. The biological value of minerals depends mainly on the degree of absorption from the digestive tract (Korniewicz et al., 2007). Trace elements may compete or support each other in their activity and absorption from the gastrointestinal tract and may be stored in organs or meat. There are interactions between elements: synergistic or antagonistic.

The objective of this study was to evaluate the existing interrelations between Cr (III) and trace minerals (Cu, Fe, Mn, Zn) in growing pigs fed with chromium picolinate supplemented diets.

MATERIAL AND METHODS

The experiment was performed comply with Directive 2010/63/EU on the protection of animals used for scientific purposes and all procedures described, were approved by Ethical Commission of National Research and Development Institute for Biology and Animal Nutrition.

Experimental design

The experiment was conducted on 8 growing castrated TOPIGS male pigs, under balance experiment conditions and it ran for 45 days. Throughout the experimental period, the piglets were randomly assigned to 2 groups, a control group (C) and an experimental group (E), kept in individual metabolic cages (Agrico, Rybarska, Czech Republic) with an area of 0.87 m^2, placed in an experimental hall under controlled environmental conditions (temperature of 24^0C, humidity 50-60 %). The pigs were fed on corn-soybean meal-based diets (18.75% CP; 3063 kcal/kg ME). The diet of E group was supplemented with 200 ppm CrPic. The piglets were fed the respective diets daily, at 8.00 a.m., *ad libitum*. Water was supplied *ad libitum* via drinking nipples. The pigs had an initial bodyweight of 17.16 ± 0.62 kg. The source of Cr^{3+} was Chromium picolinate (Sigma Aldrich, Germany) and it brought the chromium level to 200 µg Cr/kg feed in E group. At the end of experiment, all pigs were slaughtered and meat (tenderloin; loin; ham; shoulder; belly; scruff) and organ (liver, spleen, kidney) samples were collected. The samples were frozen at -80^0C and kept until mineral analysis.

The productive parameters were calculated from the records of the body weights and feed intake.

Sample collection

After a period of 5 days for accommodation, the mineral balance was determined during 3 periods of 5 days each. The amount of feed given to each pig was weighed daily, as well as the leftovers (collected each morning). During the 3 periods of balance (5 days/ week) samples of ingesta and excreta (faeces and urine) were collected daily from each animal and average weekly samples were formed. The faeces were collected once a day and stored at about 3^0C. At the end of the collection period the faeces were weighed and homogenised; the feed and faeces samples were dried at 65^0C in stove BMT model ECOCELL Blueline Comfort (Neuremberg, Germany) and ground using a GRINDOMIX GM 200 mill (Retsch, Germany). The urine volume was recorded daily and 10% of it was kept in containers in refrigerator (4^0C). Sulphuric acid, 10 mL 10% was added to the container holding the urine. Average weekly samples of faeces/urine per piglet were formed at the end of each 3 periods of balance.

The coefficients of apparent absorption of the dietary minerals, the retention coefficient and the utilization coefficient were calculated using the data from the chemical analysis on the feeds, urine and faeces, corroborated with the daily records of the intake and excreta. Trace minerals balance parameters, were determined using digestibility equations (Schiemann, 1981).

Chemical analysis

The samples (feed, faeces, meat, organ) were dried at 65^0C using a stove BMT model ECOCELL Blueline Comfort (Nuremberg, Germany) and grounded using laboratory mill Grindomix GM 200 (Retsch, Germany).

Trace mineral concentrations were determined in feed, faeces, meat and organ samples applying flame atomic absorption spectrometry (FAAS) as described by Untea et al., (2012) after microwave digestion. The urine was prepared by diluting the samples with distilled water and FAAS determination.

The used equipment was as follows: Atomic absorption spectrometer Thermo Electron – SOLAAR M6 Dual Zeeman Comfort (Cambridge, UK), with deuterium lamp for background correction and air-acetylene flame and microwave digestion system with remote temperature measurement, BERGHOF, Speedwave MWS-2 Comfort (Eningen, Germany). Stock solutions of Cu, Fe, Mn, Zn, 1000 ppm traceable to SRM from NIST, were used to standardize the flame atomic absorption spectrometer. Class A glassware was used for transvasation, dilution and storage.

Statistics

The analytical data were compared performing analysis of variance (ANOVA), using STATVIEW for Windows (SAS, version 6.0). The differences between mean values in the groups were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Performance parameters (published data by Untea et al., 2014) showed no significant differences between groups regarding the final weight of pigs: 49.93 ± 2.3 kg (C) respectively 48.75 ± 5.3 kg (E).

The results of the balance study showed that Cr(III) supplemented in the diet did not influence the apparent absorption, retention and utilization of copper, iron, manganese and zinc (tables 1 and 2). The observed differences between groups were not statistically significant ($P>0.05$).

The mineral balance data (Tables 1 and 2) for Cu, Fe, Mn, Zn, showed that the absorption coefficients are in agreement with the literature for pigs with respect for average age and weight. In the previous studies, Veum (2004), reported absorption coefficients about 24.6% Cu and 32.9% Zn for piglets with

average weight of 6.31 kg/pig. Rincker (2005) studied the mineral balance on piglets with 8.2 kg/pig average initial weight and obtained retention coefficients of 39.54% Cu, 53.17% Fe, 46.04% Mn and 41.46% Zn.

Table 1. Balance data obtained for Cu and Fe

	Copper		Iron	
	C	E	C	E
Intake (mg/day)	52.91 ± 7.7	55.80 ± 9.8	615.0 ± 89.3	644.2 ± 91.5
Faeces (mg/day)	36.77 ± 8.5	40.47 ± 9.9	267.0 ± 57.4	299.4 ± 69.3
Urine (mg/day)	0.19 ± 0.1	0.17 ± 0.1	3.1 ± 1.0	2.9 ± 0.8
Absorption (%)	32.11 ± 3.3	32.05 ± 1.4	52.47 ± 5.9	53.7 ± 6.7
Retention (%)	31.74 ± 2.2	31.77 ± 1.5	56.0 ± 6.2	53.1 ± 5.8
Utilization (%)	98.83 ± 0.4	99.15 ± 0.7	99.0 ± 0.2	99.1 ± 0.4

Table 2. Balance data obtained for Mn and Zn

	Manganese		Zinc	
	C	E	C	E
Intake (mg/day)	172.84 ± 23.4	180.37 ± 29.8	231.89 ± 31.1	241.85 ± 39.7
Faeces (mg/day)	115.42 ± 24.0	123.8 ± 27.8	119.72 ± 24.9	123.05 ± 22.2
Urine (mg/day)	0.5 ± 0.08	0.7 ± 0.1	3.26 ± 1.0	3.22 ± 1.0
Absorption (%)	35.24 ± 3.0	32.76 ± 4.6	47.56 ± 4.6	44.30 ± 9.1
Retention (%)	34.91 ± 2.8	32.33 ± 4.3	46.00 ± 4.7	43.08 ± 9.4
Utilization (%)	99.1 ± 0.4	98.7 ± 0.8	96.58 ± 1.0	96.68 ± 2.2

Results are expressed as a mean ± SD.

The deposits of Cu, Fe, Mn and Zn in the main organs (liver, spleen and kidney) were evaluated and the results are presented in table 3. Comparing the experimental findings, the balance data (Table 2) showed similar Zn utilisation coefficients for the studied groups and it can be noticed that the CrPic, had a positive effect on Zn deposition in liver (the main mineral deposit organ).

Table 3. Mineral concentrations in organs

		Cu, (mg/kg)	Fe, (mg/kg)	Mn, (mg/kg)	Zn, (mg/kg)
Liver	C	19.31 ± 1.59	342.41 ± 33.82	9.28 ± 0.79	183.90 ± 1.44^{b}
	E	20.00 ± 3.43	334.82 ± 45.05	10.12 ± 0.60	222.38 ± 8.71^{a}
Spleen	C	3.25 ± 0.34	480.53 ± 64.6	1.79 ± 0.55	104.90 ± 1.82
	E	3.29 ± 0.31	448.26 ± 73.18	1.95 ± 0.19	107.89 ± 2.30
Kidney	C	30.80 ± 2.05	205.59 ± 33.5	6.84 ±0.67	109.41 ±5.91
	E	29.93 ± 3.13	220.50 ± 16.04	6.96 ± 0.90	107.91 ± 6.43

In the same column, different superscripts mean significantly different ($P<0.05$) from C (a) respective E (b). Results are expressed as a mean ± SD.

Similar range of values of mineral organs concentrations in pigs were reported in the scientific literature (Apgar et al., 1995; Luo and Dove, 1996; Jondreville et al., 2005; Hun and Thaker, 2009).

For evaluation of chromium effect on carcass mineral properties, six anatomical parts were considered: tenderloin; loin; ham; shoulder, belly; scruff. The trace mineral concentrations obtained are presented in table 4.

Table 4. Mineral concentrations in meat samples

		Cu, (mg/kg)	Fe, (mg/kg)	Mn, (mg/kg)	Zn, (mg/kg)
Tenderloin	C	1.97 ± 0.42	45.32 ± 1.72	N.D.	50.58 ±4.22
	E	1.52 ± 0.62	42.75 ± 5.05	N.D.	47.46 ± 0.76
Loin	C	0.13 ± 0.01	1.77 ± 0.17	0.03 ± 0.01	4.38 ± 0.29^{b}
	E	0.16 ± 0.07	2.07 ± 0.47	0.03 ± 0.01	5.19 ± 0.29^{a}
Ham	C	0.94 ± 0.12	40.43 ± 3.5	N.D.	52.64 ±4.47
	E	1.04 ± 0.49	36.83 ± 6.9	N.D.	50.93 ± 5.79
Shoulder	C	0.30 ± 0.05	3.64 ± 0.42	0.04 ± 0.00	8.21 ± 0.41^{b}
	E	0.34 ± 0.07	3.88 ± 0.20	0.05 ± 0.03	9.74 ± 0.40^{a}
Belly	C	0.04 ± 0.02	2.30 ± 0.47	0.03 ± 0.01	3.78 ± 0.54^{b}
	E	0.03 ± 0.02	2.33 ± 0.19	0.06 ± 0.03	5.04 ± 0.82^{a}
Scruff	C	0.07 ± 0.03	3.81 ± 1.27	0.07 ± 0.01	4.89 ± 0.29^{b}
	E	0.09 ± 0.05	3.63± 0.35	0.08 ± 0.01	6.09 ± 0.84^{a}

In the same column, different superscripts means significantly different ($P<0.05$) from C (a) and E (b) respectively. Results are expressed as a mean ± SD.

N.D. – not detected (levels were below 0.02 ppm, the detection limit for Mn determination by FAAS)

In the case of zinc, significant differences ($P<0.05$) were noticed between groups for loin, shoulder, belly and scruff. These results sustain the previous observation (Table 3) about the synergistic relation between chromium and zinc even though there were no differences ($P>0.05$) regarding Zn balance parameters between groups.

The relation between Cr and Fe has been mostly investigated due to the fact that both these minerals are transported as transferrin-bound. At low Fe saturation, Cr and Fe bind preferentially to different binding sites. When the Fe concentration is higher, the two minerals compete for the same binding sites. Evidence that Cr may impair Fe metabolism has been published by Ani and Mostaghie (1992). Copper is considered an indirect chromium antagonist due to its close relationship with oestrogens (Watts, 1989) but also due to its synergism with iron. In our study, no significant difference was noticed for Cu and Fe concentrations in the considered anatomical parts.

CONCLUSIONS

No effect of Cr(III) supplementation was observed on Fe, Cu and Mn metabolism in this study.

The results obtained in zinc determinations on liver and four anatomical parts, highlighted that Cr (III) supplements potentiate Zn deposition in the organism of animals. These findings support the hypothesis of a synergistic relation between these two trace elements.

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