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[1]Research Institute of Animal Production, Nitra, Slovak Republik; [2]Research Institute for the Biology of Farm Animals, Dummerstorf, Germany

RUDOLF LAHUCKY[1]; KARIN NÜRNBERG[2]; ULRICH KÜCHENMEISTER[2]; IVAN BAHELKA[1]; JOZEF MOJTO[1],GERD NÜRNBERG[2] and KLAUS ENDER[2]

The effect of dietary magnesium oxide supplementation on fatty acid composition, antioxidative capacity and meat quality of heterozygous and normal malignant hyperthermia (MH) pigs

Abstract

The objective of this research was to examine the impact of supplementation with magnesium oxide (MgO) on the fatty acid composition, antioxidative capacity and quality parameters, determined on 24 pork *longissimus* muscles (LD). Crossbred pigs equal for halothane genotypes (12 normal, nonmutant – NON, 12 heterozygous, monomutant - MON) and sex, were fed a diet supplemented with MgO (3.6 g magnesium daily) for 5 days prior to the slaughter. There was a tendency of higher intramuscular fat, a significant higher concentration of C17:0 and C18:2n-6, C22:5n-3 and the total amount of n-3 fatty acids ($P<0.05$) in LD of supplemented pigs. A higher resistance to in vitro stimulation of peroxidation in muscle with MgO supplementation was observed. Increasing dietary level of MgO resulted in higher concentration ($P<0.05$) of magnesium in plasma. Genotype had significant effects on some quality indicators . Pigs fed the MgO supplemented diet had higher muscle pH compared to pigs fed the control diet. Significant differences ($P<0.05$) were received between control heterozygous pigs and MgO normal pigs. The LD of pigs fed diets supplemented with MgO had lower percentage of drip loss compared to pigs fed the control diet. Significant differences ($P<0.05$) were found between heterozygotes control group and the other three groups. In conclusion MgO supplementation could not only improve post mortem pH rate breakdown and water holding capacity but also the antioxidant stability.

Key Words: pig, MH status, muscle, magnesium, fatty acid, antioxidant capacity, meat quality

Zusammenfassung

Titel der Arbeit: **Auswirkungen einer Zufütterung von Magnesiumoxyd auf die Fettsäurezusammensetzung, die antioxidative Kapazität und die Fleischqualität bei Schweinen mit unterschiedlichem MH-Status**

Der Einfluss einer Zufütterung von Magnesiumoxid auf die Fettsäurezusammensetzung, die antioxidative Kapazität und die Fleischbeschaffenheit bei Schweinen wurde untersucht. Kreuzungstiere (n=24), gleichverteilt nach Gechlecht und Halothangenotyp (12 normal, NON, 12 heterozygot, MON) wurden entweder mit Kontrollfutter oder mit Kontrollfutter, ergänzt durch MgO (3,6 g täglich) 5 Tage vor dem Schlachten gefüttert. Der intramuskuläre Fettgehalt war in der Tendenz erhöht, die Konzentration der C17:0, C18:2n-6, C22:5n-3 und die Summe der n-3 Fettsäuren waren signifikant ($P<0,05$) höher im Schweinemuskel bei MgO Ergänzung. Im Muskel dieser Tiere wurde auch eine erhöhte antioxidative Kapazität gegenüber einer stimulierten Oxidation festgestellt. Der Genotyp hatte einen signifikanten Einfluss auf einige Fleischbeschaffenheitsmerkmale. Die MgO Supplementierung führte zu eienem erhöhten pH Wert gegenüber den durch Kontrollfütterung erhaltenen. Signifikante Differenzen ($P<0,05$) ergaben sich zwischen den heterozygoten Kontrolltieren und den normalen MgO Tieren. Die MgO Supplementierung führte auch zu einem geringeren Dripverlust gegenüber der Kontrollgruppe. Die Differenzen zwischen der heterozygoten Kontrollgruppe und den anderen drei Gruppen war signifikant. Eine Futtergänzung mit MgO kann nicht nur den pH Wert günstiger gestalten, sondern auch die Wasserhaltekapazität erhöhen sowie eine erhöhte antioxidative Kapazität herbeiführen.

Schlüsselwörter: Schwein, MH Status, Muskel, Magnesium, Fettsäuren, antioxidative Kapazität, Fleischqualität

Introduction

Numerous nutritional and processing treatments have been evaluated for their effectiveness to improve pork quality. Pig finishing diets supplemented with vitamins

(specifically vitamin E) and minerals helped to improve meat quality (RAMSEY et al., 1995). Research have been shown that Mg-supplementation may have beneficial effects on the behavior (KUHN et al., 1981), stress response (LUDVIGSEN, 1985; KIETZMANN and JABLONSKI, 1985; D´SOUZA et al., 1998), skeletal muscle metabolism, and meat quality (SCHAEFER et al., 1993; D´SOUZA et al., 1999; LAHUCKY et al., 2004; BAHELKA et al., 2004) of swine. It has been reported that the inclusion of Mg in finishing diets reduces plasma cortisol and catecholamine concentrations during transportation (KIETZMANN and JABLONSKI, 1985) and influences positively the Mg^{2+} inhibition of Ca^{2+} release in muscle of MH pigs as an important factor in the development of the MH syndrome (OWEN et al., 1997; LAHUCKY et al., 2004). However, there are also contradictory results concerning the improvement in pork quality (KUHN et al., 1982; LUDVIGSEN, 1985; OTTEN et al., 1992; SCHAEFER et al., 1993; D´SOUZA et al., 1998; APPLE et al., 2001; LAHUCKY et al., 2004; BAHELKA et al., 2004). Long-term supplementation of swine diets with magnesium fumarate resulted in higher muscle pH values and less pale, more desirable-coloured pork (OTTEN et al., 1992), but supplementing diets of finishing pigs with magnesium aspartate for only 5 days prior to slaughter also reduced drip loss, improved pork colour, and reduced the incidence of pale, soft, exudate pork (SCHAEFER et al., 1993; D´SOUZA et al., 1998). Positive effects on muscle metabolism and on meat quality with supplementation magnesium oxide were recently received using MH pigs (LAHUCKY et al., 2004; BAHELKA et al., 2004). Supplementing the diets of growing-finishing pigs with MM (Magnesium mica, silicate product containing 8% Mg) reduced the number of carcasses with a pale pinkish-grey colour and increased the number of carcasses with pink to purplish-red colour (WATSON et al., 1999), but residual effects of Mg-supplementation (MM) on pork quality during refrigerated stored were also studied (APPLE et al., 2001) and the data indicated inclusion of MM in swine diets may retard the onset of oxidative rancidity in vacuum-packaged pork loins.

The objective of this study was to investigate the additional effects of supplementing diets of growing-finishing heterozygote and normal MH pigs with magnesium oxide on fatty acids composition, antioxidant stability and meat quality attributes.

Material and Methods

Animals and diets

A total of 24 pigs were used in the study. Pigs were the progeny of crossbred sires (Pietrain x Hampshire tested by DNA probe as heterozygote on MH) mated to dams (Large White x White Meaty tested as homozygote negative on MH). Barrows and gilts were balanced and tested by DNA probe (FUJII et al., 1991). Equal numbers of heterozygotes (n = 12) and normal on MH (n = 12) pigs were used in the experiment. At 80 ± 5 kg live weight heterozygote pigs were biopsied (using biopsy instrument, BIOTECH, Slovakia) tested on meat quality prediction (pH from bioptate after 1 hour incubation at $39^{O}C$) as was described earlier (LAHUCKY et al., 2001). No significant differences in pH bioptate values (6.14 ± 0.11 vs. 6.12 ± 0.10) were found in heterozygotes between control (n = 6) and Mg supplemented pigs (n = 6). Pigs were divided into two groups consisting of 12 pigs each (6 heterozygotes, monomutant – MON and 6 normal, nonmutant – NON on MH gene). Animals were housed in the

control station of RIAP Nitra and penned in pairs. They were fed with commercial feed mixture (Table 1 and 2) ad libitum and had free access to water via a nipple drinker. Pigs in the control diet were fed 3 kg of finisher feed per pig per day for 5 days prior to the slaughter. Pigs in Mg-diet group were fed 3 kg of the same feed supplemented with magnesium oxide (provided by BIOFACTORY, Slovakia) at a level of 3.6 g additional magnesium per pig per day for 5 days prior to the slaughter.

Table 1
Formulation and nutritive value of finisher diet (Futtermittelanteile und Inhaltsstoffe in %)

Ingredients	%	Ingredients	%
Barley	42.7	Dry matter	86.30
Wheat	21.0	Crude protein	16.84
Oat	15.0	Crude fat	2.43
Soybean meal	12.0	Crude fibre	4.86
Wheat brans	2.0	N-free extract	41.68
Meat and bone meal	2.0	Metabolizable energy (MJ)	12.31
Fodder yeast	1.7	Lysine	0.86
Mineral supplement	2.5	Calcium	0.96
Biofactor supplement	0.6	Phosphorus	0.71
Fodder salt	0.5	Magnesium	0.22

Table 2
Fatty acid composition of the basal diet (g/100 g FAME) (Fettsäurezusammensetzung des Grundfutters)

(%)	basal diet
C14:0	0.21
C16:0	13.39
C18:0	2.07
C18:1c9	30.94
C18:2c	43.49
C18:3n3	5.32
n-6/n-3	7.82

Slaughter and sample collection

Pigs were fasted for 20 h with access to water, transported (about 200 m) to the slaughterhouse of the institute in spring at a temperature ranging from 7 to 22^0 C. The animals were killed at 105 (± 5) kg live weight by electro stunning (90-100 V, 0.9-1.0 Amps, 50 Hz, application time 5-7 s). The animals were exsanguinated and scalded (10 min, 62^0 C). Evisceration was completed about 20 min post mortem. Chilling of the carcasses (air temperature 2 to 4^o C, air velocity 0.5-1.0 m/s) started approximately at 70 min post mortem and continued overnight.

Blood samples (4 ml) were collected at the time of exsanguination in blood collection tubes to determine calcium and magnesium concentrations. Approximately 20 g muscle was collected from (LD) between the 13^{th} and 14^{th} rib at 30 min post mortem. Muscle samples were frozen (liquid nitrogen) and stored at $–70^o$ C before analysing for fatty acid composition and antioxidative capacity.

Sample analysis

Fatty acid composition of muscle (control, n=12 and Mg supplemented, n=11) was determined by capillary gas chromatography (NÜRNBERG et al., 1998). As an internal standard nonadecanoic acid methyl ester was used. Lipid extraction was done with chloroform/methanol (2:1 v/v). After 18 h at 5 °C, extracts were filtered, washed

with 0.02 % $CaCl_2$ in water, dried with Na_2SO_4 and $CaCO_3$ (10:1, wt/wt) and filtered again. The fatty acid composition was analysed by gas chromatography after preparing methyl esters. Methyl esters were prepared via saponification with 0.5 N methanolic NaOH (5 minutes at 60 °C) and subsequent methylation with boron trifluoride/methanol (14 %, wt/vol) at 60 °C for 5 minutes. The fatty acid composition was then analysed with a Perkin Elmer gas chromatograph (PERKIN ELMER, Überlingen Germany) with a flame ionisation detector, on a 0.25µm DB 23 fused silica capillary column (J&W SCIENTIFIC, Fisons, 60 m x 0.25 mm i.d.).

The sum of n-3 fatty acids was calculated using the equation:

C18:3 n-3 + C18:4 n-3 + C20:5 n-3 (EPA) + C22:5 n-3 (DPA) + C22:6 n-3 (DHA).

The sum of n-6 fatty acids was calculated using the equation:

C18:2 n-6 + C20:4 n-6 + C20:3 n-6 + C22:4 n-6.

The sum of the saturated fatty acids was calculated using the equation:

C14:0 + C16:0 + C17:0 + C18:0 + C20:0.

For evaluating the stability of the skeletal muscle lipids (antioxidative capacity, peroxidative stability) against stimulated lipid peroxidation, a determination of thiobarbituric acid reactive substances (TBARS) was performed (BUEGE and AUST, 1978). To stimulate lipid peroxidation, 3 ml of muscle homogenates were incubated in 0.1 mM ascorbate and 5 µM $FeSO_4$ for different time intervals. Volumes of 0.5 ml were withdrawn and pipetted into 0.25 ml of 20 % trichloroacetic acid (TCA) in 100 mM KCl at 0, 30, 60, and 120 minutes incubation time. These samples were centrifuged at 10,000 x g for 10 min and 0.5 ml of the supernatants were mixed with 0.5 ml thiobarbituric acid (0.67 %) and boiled for 15 min in a waterbath. The absorbance at 535 nm was determined immediately after cooling. At 45 min after slaughter the pH (portable pH meter, model 3071, JENWAY, England) was determined in the *longissimus* muscle (13^{th} /14^{th} rib) using a combined glass electrode (P19/BNC). The day after slaughter (24 h) conductivity (TECPRO GmbH, Germany), colour (CIE LAB, MINISCAN, Lightness) and drip loss (HONIKEL, 1998) were also measured. Analysis of intramuscular fat was done by INFRATEC (Germany).

Statistical analysis

All data were analysed by the least-squares method using the GLM procedures (SAS). All tables contain the least squares means (LSM) and the standard error (SEM) of the LSM. All statistical tests of LSMs were performed for a significance level p=0.05.

Results

The quantitatively intamuscular fat and fatty acid composition of muscle are shown in Table 3. No significant (P>0.05) differences were observed among genotypes for total intramuscular fat and fatty acid composition, therefore the factor genotype was not included in the variance analysis. There was only a tendency to higher levels of intramuscular fat in pigs supplemented with magnesium.

In tendency the muscle of Mg supplemented pigs had the highest intramuscular fat content and the highest concentration of all saturated fatty acids. The increase of C17:0 was significant (P<0.05). The polyunsaturated fatty acids content of C18:2n-6, C18:3n-3 and C22:5n-3 were higher (P>0.05) in Mg supplemented pigs.

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Table 3
Fatty acid composition (mg/100 g muscle) of *longissimus* muscle in pigs (Fettsäurezusammensetzung (mg/100 g Muskel) des *m. longissimus dorsi* der Schweine)

Trait	Control (n=12)		MgO (n=11)	
	LSM	SEM	LSM	SEM
Intramuscular fat (%)	1.39	0.2	1.76	0.2
C12:0	0.46	0.2	0.65	0.2
C14:0	16.29	3.3	23.84	3.4
C16:0	330.83	59.5	475.76	62.4
C16:1	50.77	7.3	62.09	7.7
C17:0	1.64[a]	0.3	2.77[b]	0.3
C17:1	1.66	0.3	2.64	0.4
C18:0	154.93	33.2	243.96	34.8
C18:1trans-11	1.20	0.4	2.07	0.4
C18:1cis-9	563.67	109.5	799.33	114.8
C18:2n-6	155.76[a]	8.5	181.22[b]	8.9
C18:3n-3	5.61[a]	0.8	8.61[b]	0.8
C20:3n-6	8.17	0.5	8.54	0.5
C20:4n-6	75.75	3.2	75.85	3.3
C20:5n-3	3.52	0.3	3.60	0.3
C22:5n-3	10.77[a]	0.3	11.53[b]	0.4
C22:6n-3	3.36	0.3	3.22	0.3
SFA	505.62	96.5	749.76	101.2
UFA	949.10	137.8	1250.00	144.5
PUFA	263.76	11.6	293.34	12.1
Sum of n-3 FAs	23.26[a]	1.2	26.96[b]	1.2
Sum of n-6 FAs	239.67	10.7	265.61	11.2
n-6/n-3 ratio	10.40	0.4	9.99	0.4
UFA/SFA	1.90	0.05	1.76	0.05
UFA/PUFA	0.58	0.05	0.47	0.05

a, b - $P<0.05$

No differences in fatty acid composition between heterozygote and homozygote genotypes as well as between control and Mg supplemented pig were observed. Nevertheless, there was an increase in antioxidative capacity in muscle of MgO supplemented pigs compared to control pigs (Table 4).

Table 4
The effect of dietary magnesium oxide (MgO) supplementation on TBARS values by stimulation for up to 120 min with Fe^{2+} /ascorbate muscle homogenates (Einfluss der Futtersupplementierung mit Magnesiumoxyd auf die TBARS-Werte von Muskelhomogenaten nach einer Stimulation der Peroxidation bis zu 120 min durch Fe2+/Ascorbat)

Time		Control	MgO	Significance P
0 min	Mean	0.154	0.132	*
	S.E.	0.006	0.006	
30 min	Mean	0.622	0.458	*
	S.E.	0.050	0.052	
60 min	Mean	1.048	0.800	*
	S.E.	0.067	0.070	
120 min	Mean	1.646	1.358	-
	S.E.	0.120	0.125	

*$P<0.05$

Plasma calcium and magnesium concentrations are presented in Table 5. Plasma magnesium concentrations were higher ($P<0.05$) in pigs fed the MgO supplemented diet then in pigs fed the control.
Muscle pH and meat quality results are shown in Table 6. Pigs that were fed the MgO supplemented diet had higher muscle pH compared with pigs fed the control diet.

Significant differences (P<0.05) we received between control heterozygous pigs and MgO normal pigs.

Table 5
Least squares means for the effects of diet (D) and genotype (G) on plasma calcium and magnesium concentration at slaughter (Einfluss des Futters (D) und des Genotyps (G) auf die Konzentration von Calcium und Magnesium im Plasma des Schlachtblutes)

Item		Control		MgO		SE	Interaction
		MON	NON	MON	NON		G x D
Calcium, mg/l	LSM	25.10	24.90	24.50	24.70	0.48	-
Magnesium, mg/l	LSM	8.43	8.63	9.60	9.25	0.42	-

G – genotype (MON – monomutant, NON – nonmutant)
D – diet (magnesium oxide – MgO supplementation)

Table 6
Least squares means for the effects of diet (D) and genotype (G) on meat quality traits of *longissimus dorsi* muscle (Einfluss des Futters (D) und des Genotyps (G) auf die Fleischqualität *des m. longissimus dorsi*)

Item		Control		MgO		SE	Interaction
		MON	NON	MON	NON		G x D
pH 45 min	LSM	6.20^{a}	6.50^{b}	6.43^{b}	6.59^{b}	0.07	-
Conductivity 24 h, μS	LSM	5.93	4.52	4.77	4.95	0.49	-
Lightness (L) 24 h	LSM	48.19	49.00	48.83	47.38	1.28	-
Drip loss 24 h, %	LSM	6.23^{a}	4.82^{bc}	5.02^{c}	4.13^{b}	0.28	-

Within a row and comparison, means with different superscript letter differ (P<0.05)

The LD of pigs fed diets supplemented with magnesium had lower percentage drip loss compared to pigs fed the control diet. No significant (P>0.05) differences were found in electrical conductivity and lightness between control and with magnesium supplemented pigs.

Discussion

The present study examined meat quality, fatty acids composition and the antioxidative stability in muscle of heterozygous (monomutant – MON) and normal MH (nonmutant – NON) of control and Mg supplemented pigs. Finding no significant differences among genotypes for total intramuscular fat and fatty acid composition is in agreement with results introduced earlier on British Landrace pigs by FLETCHER et al. (1988) and later on German Saddle Back pigs by NÜRNBERG et al. (1999). However, there was only a tendency to higher levels of intramuscular fat in pigs supplemented with magnesium. COFFEY and BRAZLE (1995) reported also an increased intramuscular fat and USDA quality grade after the inclusion of MM (Magnesium mica) in finishing diets for beef cattle but in pigs SCHAEFER et al. (1993) reported that intramuscular fat content and marbling score (APPLE et al., 2001) were not significantly affected by supplementing swine diets with Mg.

The accumulation of saturated fatty acid in pork is not desirable because of the negative effects on blood lipids (WOLFRAM, 2003). The higher linolenic acid concentration is positive for human nutrition. The increase of PUFA is connected with the higher intramuscular fat level in Mg supplemented pigs. Arachidonic acid and C20:5n-3 are precursors of eicosanoids. They are not affected by feeding. However, considering ratio n-6/n-3 fatty acids (Table 3) differences between control and Mg supplemented pigs were not significant. In pig, deposition of linoleic acid and linolenic acid is proportional to the level in the feed (ENSER et al., 1996, NÜRNBERG et al.,

2000). The elevating effect of Mg supplementation on n-3 fatty acid seems to be interesting from the nutritional point of view.

No differences in fatty acid composition between heterozygote and homozygote genotypes as well as between control and Mg supplemented pig were observed. Nevertheless, there was an increase in antioxidative capacity in muscle of MgO supplemented pigs compared to control pigs (Table 4). MgO seems to have a stabilizing effect on oxidation and this is possibly related to the development of meat quality (Table 6). Further explanation for Mg antioxidant properties was discussed by APPLE et al. (2001). They observed reduction of TBARS, albeit small, from supplementing swine diets with MM (Magnesium mica) and possibly an associciation with Mg ions replasing manganese ions in the activation of superoxide dismutase or in scavenging free radicals was introduced.

As was shown (NÜRNBERG et al., 1999) MHS (malignant hyperthermia susceptible) muscle obtained at 45 min had not only significant ($P<0.05$) lower pH but were more susceptible to peroxidation compared with MHR (malignant hyperthermia resistent) pigs.The effect of low pH has long been attributed to myoglobin denaturation and pale colour of PSE pork. Low pH has been shown to reduce the stability constant for the haemoglobin linkage and increase the autooxidation rate (RENERRE et al., 1992, TAM et al., 1998). They stated that the accumulation of metmyoglobin depends on several contradictory mechanisms, such as the rate of oxygen diffusion and consumption, pigment autoxidation and enzymatic reduction of metmyoglobin. Many different factors could ultimately affect the action of these mechanisms on fresh meat colour stability, breed and pre-slaughter handling. From this point of view studies on changing fatty acid composition in fresh and aged muscles of malignant hyperthermia pigs (with different pH decline and different water holding capacity) at defined feeding condition would be worth for further research.

Plasma magnesium concentrations were higher ($P<0.05$) in pigs fed the MgO supplemented diet then in pigs fed the control, comparable with results of D‘SOUZA et al. (1999).

D’SOUZA et al. (1998) found higher ($P<0.05$) muscle pH at 40 min and 24 h after slaughter in pigs that were fed the MgAspartate supplemented diet. In contrast, other authors (KUHN et al., 1981, APPLE et al., 1999) did not find significant differences in muscle pH with Mg supplemented pigs. These discrepancies, possibly because of differences in animal testing (genotype, DNA test, biopsy) were discussed in different papers (LAHUCKY et al., 2004, BAHELKA et. al., 2004). Significant differences in drip loss ($P<0.05$) were found between heterozygotes control group and the other three groups which is in agreement with SCHAEFER et al. (1993), D’SOUZA et al. (1998, 1999), and HAMILTON et al. (2002). As was stated, pigs with a high content of fatty tissue are not guarantee for good meat quality and also n-3 fatty acid enriched diet fed to pigs did not affect the meat quality (NÜRNBERG et al., 1999) but as follows from our results the supplementation with Mg can reduce the percentage of drip loss and this appears to be beneficial in reducing exudative muscle of PSE pork.

Conclusion

Supplementation of pigs with MgO could not only improve the post mortem pH rate breakdown and water holding capacity but also improve antioxidant stability. The concentration of linolenic acid in muscles of pigs was increased by feeding MgO.

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Author's addresses
Dr. RUDOLF LAHUCKY*, IVAN BAHELKA, JOZEF MOJTO
Research Institute of Animal Production
Hlohovska 2
94991 Nitra
Slovak Republik

E-Mail: lahucky@vuzv.sk

Dr. KARIN NÜRNBERG, Dr. ULRICH KÜCHENMEISTER, Dr. GERD NÜRNBERG,
Prof. Dr. Dr. h.c. KLAUS ENDER
Research Institute for Biology of Farm Animals Dummerstorf
Wilhelm-Stahl-Allee 2
D-18196 Dummerstorf
Germany

*Corresponding Author