

Does the 1 Gy dose of gamma radiation impact the pork quality?

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Abstract: A nuclear accident (e.g., Fukushima), and, in particular, the transport of animals within a radiation-affected area can lead to a whole-body, or partial external irradiation, followed by oxidative stress, which could result in subsequent meat quality changes. In this experiment, live pigs were exposed to half-body irradiation by an external dose of 1.0 Gy. The caudal half of the animal's body was irradiated. After their slaughter, samples from the muscle tissue of *musculus semimembranosus* and *musculus longissimus lumborum et thoracis* at the upper margin of *musculus gluteus medius* (irradiated body half) and at the 3rd–4th thoracic vertebra (non-irradiated half) were collected to determine the meat quality parameters. A significant difference ($P < 0.05$) was observed only in the meat colour parameter (a^*) in the irradiated group of pigs. If there is no internal contamination, and the half-body exposure to the external radiation dose does not exceed 1 Gy, pigs from an irradiation-affected area may be used for human consumption.

Keywords: drip loss; ionising radiation; meat colour; pH; pig

The external radiation dose and radiation dose from internal contamination may mainly affect farm animals near damaged nuclear facilities (e.g., Fukushima) (Ohmori et al. 2014). Due to modern technologies in pig farming, it is possible to significantly reduce the internal contamination of farmed animals by radionuclides for a certain time. Especially, the radical theory of ionising radiation on living organisms presupposes the development of oxidative stress in the irradiated individuals.

As studies have shown, external exposure to ionising radiation causes oxidative stress that accelerates the lipid peroxidation of the polyunsaturated fatty acids, generating alkanes, and alkane metabolites (Phillips et al. 2015). In particular, by the oxidation-reduction process, the meat colour and the levels of myoglobin, haemoglobin, and cytochromes are affected (Bekhit and Faustman 2005).

Meat represents muscle tissue in a state of degradation. Degradation reactions generate free radicals

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highly reactive with tissue components, especially unsaturated fatty acids. These processes accelerate the metmyoglobin formation (Young and West 2001). As demonstrated in the experiments with the irradiation of pigs, the cell membranes' integrity is directly associated with drip loss, as well (Dvorak et al. 2004).

However, meat quality is affected by many other intravital factors, such as nutrition (Lindhahl et al. 2006), the structure of the muscle fibres (Ryu and Kim 2005), and, in particular, by stress during the transport and pre-slaughter treatment (D'Souza et al. 1998).

This study aimed to determine whether radiation stress would have a negative impact on the selected pork quality parameters.

MATERIAL AND METHODS

Animals and sampling

Twenty Slovak Large White pig breed individuals (*Sus scrofa domestica*) were used in the study. The pigs were divided randomly into two groups (one experimental and one control group) of 10 animals each. Both groups consisted of gilts and barrows in a ratio of 1 : 1. At the beginning of the experiment, the pigs were 2 months of age and weighed 30 kg. The pigs were kept at the University Farm in Zemplinska Teplica (The University of Veterinary Medicine and Pharmacy in Kosice) and were housed in separate holding pens (experimental and control groups) with access to daylight and were raised under standard conditions. During the experiment, a standard pig feed mixture OS 03 (PD Podunajske Biskupice, Bratislava, Slovakia) for the corresponding age category was fed to the pigs. Water and food were available *ad libitum* at nipple drinkers and food dispensers. The pig handling and transport in both the control and experimental groups were identical, except for the irradiation. To reduce the effects of other stress factors, two weeks before the irradiation, the pigs of both groups were repeatedly placed in the irradiation cages and to a transport lorry for adaptation. During both experiments, no animal died. The experiments were performed in August. Anaesthetics were not used during experiments. The irradiation was performed at the Faculty of Science of the Pavol Jozef Safarik University in Kosice, Slovakia located 35 km away

from the farm by a CHISOSTAT device (Chirana, Prague, Czech Republic). Three days post-irradiation, the pigs were transported to the slaughterhouse in Zemplinska Teplica (The University of Veterinary Medicine and Pharmacy in Kosice) located just 1 km from the farm. After a short 3-hour rest period at the slaughterhouse, the animals were slaughtered. Electric prods were neither used during the pigs' housing nor during the loading and unloading. In the case of an accident, the pigs' evacuation must be completed within three days. The same evacuation time was set for the experiment.

Ten experimental pigs were irradiated by an external dose of 1.0 Gy gamma radiation ^{60}Co . The pigs were placed in cages and the caudal half of the body from the eighth to the ninth thoracic vertebra was irradiated by a dose of 1.0 Gy at a dose wattage of 0.97 Gy/h.

The upper half of the cage was shielded by 50 mm thick lead bricks. The pigs had no possibility of moving forward, backward, or sideways during the irradiation in the cage. They could only stand in an upright position or lay down. This ensured the homogeneity of the irradiated area. The pigs were slaughtered and meat samples were collected from the *musculus semimembranosus* and *musculus longissimus thoracis et lumborum* at the last rib, and additionally, from two other parts – the *musculus longissimus lumborum* at the upper margin of the *musculus gluteus medius* (irradiated half of the pig) and the *musculus longissimus thoracis* at the 3rd–4th thoracic vertebra (non-irradiated half of the pig).

Meat quality

The muscle (meat) pH and colour were measured after 45 min and 24 h *post-mortem*. The pH was measured using an Orion 250 A+ digital pH meter equipped with an Orion puncture electrode (Thermo Electron Corporation, Waltham, USA). The calibration was performed on three buffers with a pH of 4.01, 7.00, and 9.00. The pH value was recorded automatically after the measured values had stabilised.

The meat colour was determined in the CIELAB (CIE 1976 L*a*b* colour space) system using the portable Colour-guide sphere specs spectrophotometer (BYK-Gardner, Geretsried, Germany) (Dvorak et al. 2004). The instrument was calibrated before the measurements.

The meat colour at the perpendicular cut to the muscle fibres was calculated as an average value of three separate measurements (Commission Internationale de l'Eclairage 1986). A similar method was used in determining the pH (Henckele et al. 2000). For comparative studies, it is essential to maintain precise procedures (Brewer et al. 2001).

To compare the results, further CIELAB parameters were calculated (Honikel 1998).

The determination of the drip loss was carried out by a standard method within the interval from 24 h to 48 h *post-mortem*, and also by a modified method using a container (Honikel 1998) in the interval from 0 h to 24 h *post-mortem*. The lactate content was determined spectrophotometrically (340 nm) using a commercial kit for the determination of lactic acid. Approximately 500 mg of muscle was homogenised for 30 s in 2 ml of 1 M PCA. Potassium hydroxide (2 M) was added to neutralise the solution, and the final volume was made to 10 ml with distilled water. Following 20 min of refrigeration and centrifugation, the lactic acid concentration was measured (Choe and Kim 2014). The determination of the water content and fat content was performed according to the Association of Official Agricultural Chemists (AOAC 2003).

Statistical analysis

A two-sample *t*-test with unequal variances was used for the average significance difference testing in both the experimental and control groups.

A paired *t*-test was used for comparing the average values at different locations of the *musculus longissimus thoracis et lumborum* to each other or compared to the *musculus semimembranosus*. The paired *t*-test was also applied to test the differences of the values 45 min and 24-h *post-mortem*. Due to the expected variability, a significance level $P = 0.05$ was determined in the planning of the experiments for all the hypotheses. The statistical values were calculated by means of MS Excel.

RESULTS

Although only half of the pig's body was exposed to radiation, the overall response of the organism to irradiation was studied in the experiment.

No significant ($P < 0.05$) differences were observed in the basic meat indicators between the experimental and control groups (Table 1).

The values of the lactic acid concentration in both the *musculus longissimus thoracis et lumborum* and *musculus semimembranosus* were higher in the irradiated pigs. The pH₂₄ value results in the control and experimental groups were almost identical for both muscle groups, and a significant pH decrease after 24 h can be considered a standard.

The irradiated and control groups showed significant ($P < 0.05$) differences in the meat colour b^*_{45} between the cranial (3rd–4th thoracic vertebra) and caudal (at the cranial margin of *musculus gluteus medius*) part of the *musculus longissimus thoracis et lumborum* (Table 2).

Table 1. Meat quality parameters of the *musculus longissimus thoracis et lumborum* at the last rib and the *musculus semimembranosus* in the control and experimental groups after the half-body (partial) irradiation of 1.0 Gy

Parameter	<i>Musculus longissimus thoracis et lumborum</i>	<i>Musculus longissimus thoracis et lumborum</i>	<i>Musculus semimembranosus</i>	<i>Musculus semimembranosus</i>
	irradiated group mean ± SEM	control group mean ± SEM	irradiated group mean ± SEM	control group mean ± SEM
pH ₄₅	6.14 ± 0.104	6.22 ± 0.044	6.11 ± 0.104	6.22 ± 0.113
pH ₂₄	5.66 ± 0.020	5.67 ± 0.016	5.76 ± 0.032	5.74 ± 0.025
Water (%)	73.54 ± 0.214	74.53 ± 0.228	73.07 ± 0.775	73.75 ± 0.842
Fat (%)	5.10 ± 0.425	3.46 ± 0.207	6.97 ± 0.811	5.70 ± 0.876
Remise (%)	17.88 ± 0.633	19.70 ± 0.779	17.84 ± 1.255	18.60 ± 1.149
Drip losses 24–48 h (%)	1.97 ± 0.220	2.06 ± 0.263	2.34 ± 0.321	2.00 ± 0.232
Lactic acid (g/kg)	15.75 ± 0.201	14.77 ± 0.448	16.35 ± 0.691	15.60 ± 0.510

Subscript 24 = 24 h *post-mortem*; subscript 45 = 45 min *post-mortem*; mean = arithmetical mean; SEM = standard error of the mean

*Statistical significance ($P < 0.05$)

Table 2. Meat quality parameters of the *musculus longissimus thoracis et lumborum* in the control and experimental groups after the half-body (partial) irradiation of 1.0 Gy

Parameter	<i>Musculus longissimus thoracis</i>		<i>Musculus longissimus lumborum</i>	
	experimental group mean \pm SEM	control group mean \pm SEM	experimental group mean \pm SEM	control group mean \pm SEM
pH ₄₅	6.09 \pm 0.084	6.18 \pm 0.039	6.32 \pm 0.093	6.35 \pm 0.099
pH ₂₄	5.74 \pm 0.026	5.66 \pm 0.026	5.73 \pm 0.031	5.60 \pm 0.046
L* ₄₅	45.99 \pm 0.953	47.11 \pm 1.044	45.07 \pm 0.578	45.47 \pm 1.135
L* ₂₄	54.30 \pm 1.062	54.78 \pm 0.533	54.86 \pm 0.750	56.43 \pm 0.670
a* ₄₅	-0.42 \pm 0.156	-0.43 \pm 0.387	-0.11 \pm 0.272	-0.19 \pm 0.442
a* ₂₄	0.96 \pm 0.209	0.45 \pm 0.416	0.30 \pm 0.207*	0.62 \pm 0.368
b* ₄₅	3.41 \pm 0.254	3.30 \pm 0.220	4.28 \pm 0.236*	4.24 \pm 0.280*
b* ₂₄	5.88 \pm 0.294	5.32 \pm 0.312	5.92 \pm 0.282	6.80 \pm 0.237*
ΔE^* ₄₅	29.44 \pm 0.952	30.56 \pm 1.044	28.64 \pm 0.584	28.86 \pm 1.176
ΔE^* ₂₄	38.47 \pm 0.533	37.93 \pm 1.065	38.55 \pm 0.759	40.24 \pm 0.680
C* ₄₅	3.46 \pm 0.260	3.50 \pm 0.251	4.36 \pm 0.223	4.44 \pm 0.285
C* ₂₄	5.98 \pm 0.389	5.49 \pm 0.292	5.96 \pm 0.285	6.91 \pm 0.261
Drip losses 0–24 h (%)	2.14 \pm 0.365	1.92 \pm 0.209	1.81 \pm 0.224	2.44 \pm 0.380

Subscript 24 = 24 h *post-mortem*; subscript 45 = 45 min *post-mortem*; L*, a*, b*, ΔE^* , C* = meat colour parameters (CIELAB); mean = arithmetical mean; SEM = standard error of the mean

*Statistical significance ($P < 0.05$) at the 3rd–4th thoracic vertebra and the upper margin of the *m. gluteus medius*

Interestingly, 24 h *post-mortem*, a similar difference of the b*₂₄ indicator was reported in the control group only, while, in the experimental group, the difference between the *musculus longissimus thoracis* (3rd–4th thoracic vertebra) and *musculus longissimus lumborum* was not significant ($P > 0.05$).

The difference in the b*₂₄ parameter value in the *musculus longissimus lumborum* in the experimental vs the control group can be associated with the irradiation of the rear half of the body.

The meat colour parameter a*₂₄ measured 24 h *post-mortem* in the group of experimental pigs represents another important result. The a*₂₄ value of the irradiated part – *m. longissimus lumborum* was three times lower compared to the non-irradiated part – *m. longissimus thoracis* (Table 2). It means that the irradiated part of the *m. longissimus thoracis et lumborum* is a brighter red. In the control group, the situation is *vice versa*, although without any statistical significance ($P > 0.05$). This corresponds, though not statistically significant ($P > 0.05$), with the increase of the a*₄₅ parameter in the irradiated pigs.

DISCUSSION

The significant differences ($P < 0.05$) in the meat colour parameter b*₄₅ between the cranial and caudal part of the *musculus longissimus thoracis et lumborum* (Table 2) are in accordance with other authors. The colour differences of the muscle fibres in the meat can be observed not only between different muscles, but also within the muscle fibre, both on the cross-section and the longitudinal axis (Ryu and Kim 2005). A darker meat colouration occurs as a consequence of impaired oxygen diffusion, which makes the myoglobin colour to dominate, changing the light reflection from the surface, and is visually registered as a dark red colour (Lawrie and Ledward 2006).

The exposure of pigs to radiation had no significant ($P > 0.05$) effect on the value of the ΔE^* and C* parameters. The higher values of these parameters after 24 h correspond to the meat ageing of the *m. longissimus thoracis et lumborum*. In the fresh red meat, myoglobin occurs in three chemical forms. The surface colour changes of the meat are initiated by the exposure of the meat to oxygen,

and among others, are caused by changes in the content of the chemical forms of the myoglobin, e.g., oxygenated myoglobin (oxymyoglobin), oxidised myoglobin (metmyoglobin), and reduced myoglobin (deoxymyoglobin) (Karamucki et al. 2011).

Pink deoxymyoglobin, after its exposure to air, is rapidly oxidised to a red-coloured oxymyoglobin, which is sequentially oxidised to brown a metmyoglobin (Bekhit and Faustman 2005; Pavelkova and Flimelova 2012).

It was considered that the brown metmyoglobin pigment can be reduced by the metmyoglobin reductase activity to a pinkish-red deoxymyoglobin.

As in identical experiments, we managed to prove the certain impact of ionizing radiation on enzymes' activity (Smutna et al. 2013), the reduction in metmyoglobin reductase activity cannot be excluded. Scheffler and Gerrard (2007) also pointed out the crucial importance of enzymes in the *post-mortem* energy metabolism.

The significant ($P < 0.05$) difference in the a^*_{24} parameter of the cranial area of the *musculus longissimus dorsi* between the experimental and control groups can be attributed to the abscopal effect (Frey et al. 2012) of the body's overall response to the local (half body) irradiation (Table 2). Other numerical differences are not significant ($P > 0.05$) due to the variability, and probably also to the lower sensitivity to the oxidative stress. It also shows that a higher local radiation dose can affect the meat colour, which may be considered as a minor one considering the meat quality. On the other hand, consumers prefer more reddish coloured meat, which can be achieved by the irradiation of the meat by a dose 2 500–5 000 Gy.

In our study, the colour parameters or lactic acid concentration values differ from the findings of other authors (Tomko 1992; Choe et al. 2008), which can be explained by the age and weight of the pigs. Different results in the lactic acid and lactate dehydrogenase concentrations were also reported in pigs with muscle diseases, e.g., *myoclonia congenita* (Tomko 1993).

Advanced technologies of pig raising should prevent the significant internal contamination of pigs with radionuclides. However, even in pigs contaminated after a nuclear accident, there is a possibility to reduce the internal contamination by conventional procedures (Jandl et al. 1989). The affected pigs can be transported to distant

slaughterhouses and used for further processing after standard decontamination by water showering (Petaja et al. 1992).

In the case of the half-body irradiation by a single dose of 1 Gy, we observed local colour changes in the *musculus longissimus thoracis et lumborum*, which, however, is of no significance when considering the meat production. In the case of a nuclear accident, if there is no internal contamination, and the half-body exposure to the external radiation dose does not exceed 1 Gy, pigs from the affected area may be used for human consumption.

Conflict of interest

The authors declare no conflict of interest.

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