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Short Report

Influence of selected factors upon the blood loss from the carcasses of pigs free of the stress susceptibility gene (*RYR1*^{T})

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The aim of the study was to determine the effect of road transport, lairage time, meat % and carcass weight, sex, and *PPARGC1A* gene polymorphism on the degree of blood loss after slaughter. The study was conducted on 350 hybrid finishers from three farms located at different distance from the meat processing plant, but with the same methods of keeping animals and dietary treatment. The degree of blood loss was measured in the *obliquus internus abdominis* muscle using the compressor method. The results showed that the degree of blood loss from carcasses was influenced by the ambient temperature before slaughter, lairage time and the meat percentage in finishers. Finishers slaughtered without a pre-slaughter rest, with the shortest fasting time and the highest meat % of carcasses (>60%), were characterized by the lowest blood loss. In addition, blood loss was significantly related to ambient temperatures during the pre-slaughter handling, when pigs were transported for a distance of 232 km. Summarising, animal welfare proved to be significant for blood loss in pigs free of stress sensitivity gene (*RYR1*^{T}), especially in those with high meat %.

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Appropriate transport conditions and a sufficiently long lairage time may help in obtaining higher post slaughter blood loss.

KEY WORDS: blood loss rate / pigs / *PPARGC1A* gene / pre-slaughter handling

The primary purpose of exsanguination, apart from killing an animal, is to obtain edible products, especially muscles, with the best possible quality which largely depends on the level of blood loss in the carcass [Warriss 1977, Griffiths *et al.* 1985]. When blood loss is not sufficient or does not occur at all, residual blood in the muscle tissue acts as a buffer, preventing the development of glycogenolysis and the acidification of muscle tissue. A lack of post-mortem acidification causes increased susceptibility of the muscle tissue to degradation, prevents the enzymatic processes of maturation and thus the obtaining of positive sensory attributes of the meat. For these reasons, the greatest possible blood loss is favorable for the usefulness and quality of the meat [Szkucik 2004].

Sanitary research developed on the basis of the annual reports of the Chief Veterinary Inspectorate in Poland 2001-2011 showed that meat being disqualified for human consumption because of insufficient blood loss accounted for 3.78% of all non-accepted carcasses [Szkucik *et al.* 2012].

To date there has been no uniform and clear method for detecting different levels of blood loss. In most cases, it is based on subjective perception by the inspector, *i.e*. an assessment of the appearance of muscle tissue and sometimes internal organs. The applicable regulations include no indication regarding necessary measurements or tests for the determination of the level of residual blood in muscle tissue. Nonetheless, to objectify the degree of blood loss, one can use a number of methods, including macroscopic examination and more detailed diagnostic tests [Szkucik 2004].

Proper determination of blood loss should include macroscopic post-mortem examination of carcasses and internal organs, and appropriately selected exploratory tests, especially a hemoglobin diffusion test [Beutling 1984] or compressor test [Szkucik 1996]. The most useful muscles for blood loss determination are the *supraspinatus*, *obliquus internus abdominis* and *musculi colli* [Szkucik 2004]. However, due to their availability at the time of the post-mortem examination, only the *obliquus internus abdominis* and *musculi colli* have practical importance [Szkucik 1996]. This is confirmed by the results of research on cattle and pig carcasses originating from industrial and sanitary slaughter, as well as those delivered to the slaughterhouse as slaughtered in transport [Szkucik *et al.* 2001].

The aim of this study was to determine the effect of road transport distance, lairage time, meat % and weight of carcasses, sex, and *PPARGC1A* gene polymorphism on the degree of blood loss from the carcasses of finishers.

Material and methods

The study was conducted during summer on 350 PIC hybrid finishers, the offspring of paternal line PIC337 and maternal line Camborough22. The finishers were transported from three farms located at different distances from the meat processing plant: 67 km (1 h 50 min), 232 km (5 h 30 min) and 306 km (6 h 50 min). At all farms pigs had been kept and fed in the same manner.

All the examined pigs, regardless of the length of road transport, had been transported with not less than 180 in a truck to the meat processing plant after reaching a body weight of around 110 kg, and being deprived of feed for 12 hours. After arrival and unloading, finishers were divided into three groups (similar share of sexes) with different lairage time (0 h, 6 h, 12 h) during which they were not fed.

During slaughter of pigs, in accordance with current industrial technology, after stunning them with CO_2 (BUTINA stunner), stinging and blood loss in the upright position, the sex of the finishers was determined. After evisceration and veterinary inspection, a meat % was measured and hot weight of carcasses determined; at 40 min after slaughter, samples were taken from the *obliquus internus abdominis* muscle in the right half-carcasses, in order to determine the degree of blood loss and for genetic determinations.

Meat % of the carcass was determined in the left half-carcass by CGM apparatus [Borzuta 2004]. The carcasses were separated into three meat % classes according to the EUROP system: S (>60%), E (55-60%) and U (50-54.9%).

Non-skinned carcass hot weight without fat and kidneys was measured on Q electronic scales to an accuracy of 100 g. Carcasses were divided into three groups depending on their hot weight: 70-80.0 kg, 80.1-90 kg, and 90.1-100.0 kg.

The degree of blood loss was determined by compressor method as described by Szkucik [1996]. 300 mg of meat sample was placed on a 70x70 mm square of blotting paper (WHATMAN #2) saturated with KCl solution. Then the paper along with the sample was placed between the plates of the compressor, pressed with screws and left for 5 minutes. After removing paper from the compressor, the outlines of the sample and extruded liquids were drawn on the paper. Then, the surface areas of both stains were measured. The difference between the surface areas, as well as the colour of the extruded fluid, was the final result of the test. The results are given in a 3-point scale: 1 point (correct bleeding) – 4 cm^2 yellow stain; 2 points (insufficient bleeding) – 5 cm^2 pale red stain; 3 points (no bleeding) – >6 cm^2 dark red stain.

DNA was isolated from muscle tissue using a High Pure PCR Template Preparation Kit (ROCHE) according to the manufacturer's recommendations. *RYR1* and *PPARGC1A* genotypes were determined by PCR-RFLP. *RYR1* gene polymorphism was identified using *Hin*6I restriction enzyme according to Brening and Brem [1992]. All finishers were free of the stress susceptibility gene *RYR1* with the allele *T*. *PPARGC1A* gene polymorphism was identified by endonuclease *Alu*I according to the method of Kunej *et al.* [2005]. The tested finishers had three genotypes of the *PPARGC1A* gene – *AA*, *AT* and *TT*.

The obtained results were statistically analysed using Statistica 9.1 PL software. To establish the effect of selected factors on the degree of blood loss of finishers, statistical analysis was performed using the method of least squares (LSQ) based on the GLM procedure according to the following linear model:

$$y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + e_m + f_{ijklmn}$$

where:

y_{ijklmn} – trait measured;
μ – overall mean;
a_i – effect of the length of road transport (i =1, 2, 3);
b_j – effect of lairage time (j =1, 2, 3);
c_k – impact of sex (k =1, 2);
d_l – effect of meat % (l=1, 2, 3);
e_m – impact of carcass weight (m=1, 2, 3);
f_{ijklmn} – random error.

The results were statistically analysed by calculating the arithmetic means and standard deviations. Significance of differences between factors of variation was tested using Tukey's test.

Results and discussion

Within the analysed finishers there were significant differences in blood loss depending on the distance of road transport, lairage time and meat % of carcasses in EUROP classes (Tab. 1), confirming that the condition of the animals in the period prior to slaughter may significantly influence blood loss [Szkucik *et al.* 2001].

The main factor determining the level of residual blood is stress related to the handling of the animal immediately before slaughter, including transport [Warriss and Leach 1978]. According to the presented results, the pigs transported 232 km were characterized by a significantly ($P \leq 0.01$) lower blood loss compared to those transported 67 km or 306 km, where the level of blood loss was similar. Different results were presented by Tereszkiewicz [2005] who reported that pigs transported 200 km had a significantly lower blood loss than pigs transported 50 km. In the present study pigs transported 232 km had been exposed to the highest temperature amplitude during pre-slaughter handling, especially during lairage (Fig. 1). The ambient temperature experienced by pigs transported 232 km increased significantly during lairage (20-30°C), what was not observed in pigs transported over 67 km or 306 km. The ambient temperature has a big impact on the level of stress during transport and lairage [Warriss and Brown 1994]. According to Bąk [1995] the effect of season of the year on the marked deterioration in quality of meat not depend so much on the temperature, as on its variation throughout the day. High amplitude of temperature

Table 1. Factors affecting the blood loss rate (scores in 3 point scale) in finishers

Item	Blood loss rate	
	mean	SD
Distance of transport (km)		
67 (n=114)	1.54^{B}	0.40
232 (n=118)	1.75^{A}	0.50
306 (n=118)	1.50^{B}	0.43
Lairage time (h)		
0 (n=1180	1.69^{A}	0.46
6 (n=115)	1.61^{AB}	0.49
12 (n=117)	1.49^{B}	0.41
EUROP carcass class		
S (n=27)	1.70^{A}	0.47
E(n=216)	1.63^{AB}	0.48
U (n=107)	1.49^{B}	0.40
PPARGC1A gene		
AA (n=117)	1.60	0.44
AT (n=197)	1.57	0.45
TT (n=36)	1.72	0.53
Hot carcass weight (kg)		
70-80 (n=65)	1.62	0.51
80-90 (n=185)	1.58	0.46
90-100 (n=100)	1.60	0.42
Sex		
barrows (n=151)	1.59	0.46
gilts (n=199)	1.60	0.46

^{AB}Means in rows bearing the same superscripts differ significantly at P≤0.01.

during the day in the summer has a negative impact on animals, especially during transport and the entire period of pre-slaughter handling.

According to literature, the highest blood loss is obtained from healthy animals slaughtered in accordance with regulations concerning lairage time [Szkucik *et al.* 2001]. There is a view that animals tired after prolonged or strenuous transport should have a longer lairage time, what is confirmed by the results of this study. Regardless of the transport distance, carcasses of finishers with the longest fasting period (24 h) slaughtered after 12 h of lairage had a significantly (P≤0.01) higher blood loss than finishers fasting for 12 h and slaughtered immediately after transport. However, the conditions of lairage before slaughter often do not ensure rest, but rather exacerbate stress [Warriss and Brown 1985], as confirmed by Tereszkiewicz *et al.* [2004] with finishers transported 200 km. In that study, blood loss from finishers slaughtered immediately after transportation was higher than those of finishers with 24 h lairage. Importantly, blood loss of pigs subjected to 8 h lairage was similar to pigs slaughtered immediately after transport.

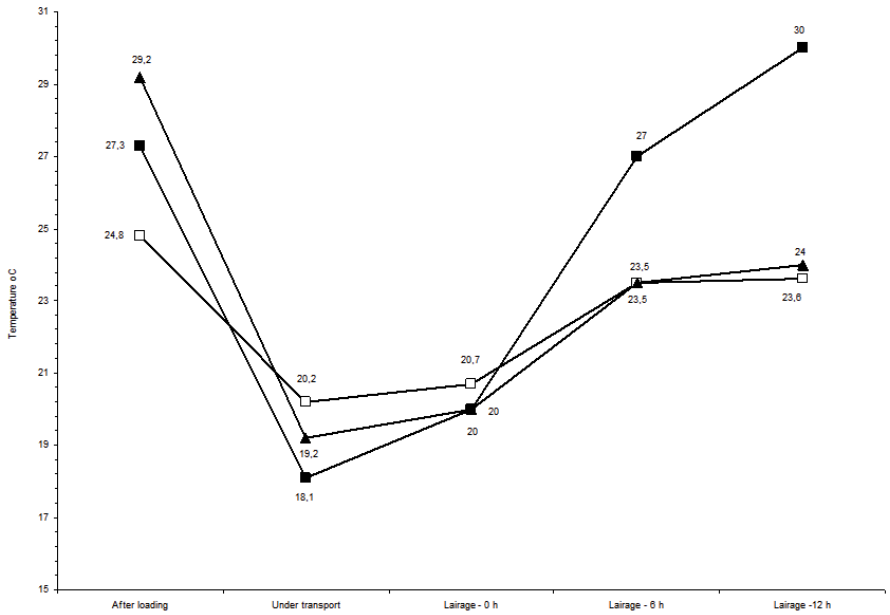

Fig. 1. Ambient temperatures (°C) during preslaughter handling of finishers in relation to transport distances; □ – 67 km, ■ – 232 km, ▲– 306 km.

An unintended consequence of improved meat % of pigs is their decreased resistance to stressors that are inherent in meat production and pre-slaughter handling [Michell and Hefron 1982]. This is confirmed by the results of this study in which pigs with high meat % (>60%, class S) were more prone to stress, resulting in a significantly lower blood loss (P≤0.01) than finishers with lower meat % (50-55%, class U). It should be noted that in this study all pigs were free from the stress susceptibility gene (*RYR1*^{T}), which according to many authors has a beneficial effect on the musculature of the carcass, but increases the sensitivity to stress and also deteriorates the quality of meat and reproductive traits of pigs [Biedermann *et al.* 2000].

The peroxisome proliferator-activated receptor-gamma coactivator-1 (*PPARGC1A*, *PGC*-1α) is a transcriptional coactivator which regulates genes associated with energy metabolism. It influences thermogenesis, mitochondrial biogenesis, adipogenesis and muscle fibre-type conversion [Lin *et al.* 2002, Puigserver and Spiegelman 2003]. However, in the analyzed material there was no association between *PPARGC1A* gene polymorphism and the degree of blood loss in pig carcasses.

In the present study no effect of carcass weight and sex on the degree of blood loss was found. According to literature, blood volume obtained during exsanguination diminishes with the age of animal (of the same body weight) [Warriss and Rhodes 1977]. Males and neuters lose less blood than females. Only in poultry sex have no significant effect on the volume of blood lost during exsanguination [Newell and Shaffner 1950]. Body weight influences the level of bleeding mainly in pigs, in which

weight gains consist principally in the deposition of adipose tissue, characterized by a relatively small blood circulation [Prost 1985].

Research on finishers free of the stress sensitivity gene (*CC/RYR1*) showed that the degree of blood loss was influenced by ambient temperatures during pre-slaughter handling, lairage time and by the meat % of finishers. Pigs with the shortest fasting time, slaughtered without lairage and with the highest meat % (>60%) were characterized by the worst level of blood loss. In addition, lower blood loss was related to a greater amplitude of ambient temperature during the pre-slaughter handling of pigs transported 232 km. There were no differences in blood loss depending on *PPARGC1A* gene polymorphism, hot weight range and sex. In summary, animal welfare during pre-slaughter handling is important for blood loss of pigs free of the gene susceptibility to stress (*RYR1*^{T}).

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