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Effects of RYR1 gene mutation on the health, welfare, carcass and meat quality in slaughter pigs

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Abstract. This study assessed the effects of RYR1 mutation on the health, welfare, and carcass and meat quality in slaughter pigs. Any signs of pneumonia, pleurisy, pericarditis, and liver milk spots were recorded as present or absent. At exsanguination, blood samples were collected and RYR1 genotype, blood lactate and glucose concentrations were determined. The following carcass quality traits were measured: live, hot and cold carcass weights, backfat thickness, loin muscle thickness, lean meat content and skin lesion score. pH and temperature of *M. longissimus dorsi* and *M. semimembranosus* were measured 45 minutes postmortem. Nn pigs were more affected by pneumonia, had higher blood lactate and glucose concentrations and more developed *rigor mortis* than NN pigs. NN pigs had lower daily weight gain, produced lighter carcasses, more fat and less meat than Nn pigs. Meat obtained from Nn pigs was of a lower quality class than meat obtained from NN pigs, as shown by the lower pH and higher temperatures measured 45 minutes *post mortem* in both muscles and higher prevalence of pale, soft and exudative meat. In conclusion, the presence of a mutant n allele in pigs positively affected carcass quality traits, but had a deleterious effect on health, welfare and meat quality.

1. Introduction

The major goals of modern pig production have been increased lean meat content and growth rate, which led to a considerable increase in stress susceptibility, decrease in resistance to diseases and impaired meat quality. Besides ordinary selection for higher meatiness, in many pig populations, the improved meatiness is also a result of a high frequency of the ryanodine receptor (RYR1) gene (n), the major gene giving positive effects on meat quantity, but negative effects on pork quality [1-2]. The pigs with RYR1 gene in the form of a recessive homozygote (nn genotype) have been characterized by better feed conversion efficiency, faster growth, superior lean content and conformation compared with pigs free of this mutation (NN genotype), as a result of lower fat and bone proportions and better carcass weight distribution. Nonetheless, nn pigs have also been found to show higher mortality rates during pre-slaughter period, and be more prone to produce pale, soft and exudative (PSE) meat [3-5]. The scientific literature on the characteristics of heterozygous pigs (Nn genotype) is not consistent. Some authors reported that the RYR1 gene in its heterozygous form (Nn genotype) has certain beneficial effects such as a higher lean meat content with little or no effect on pork quality [6]. Other studies indicate the stress carriers (Nn genotype) do have some advantages compared to stress-



negative (NN genotype) pigs, such as better feed efficiency, greater carcass yield and higher meatiness, but at the same time, they do have a higher prevalence of PSE meat [7-9]. Therefore, the aim of this study was to determine the effects of RYR1 mutation on the health, welfare, and carcass and meat quality in slaughter pigs.

2. Materials and Methods

2.1. Animals and management procedures

The study was conducted in 2017 on 60 slaughter pigs (31 barrows and 29 gilts) with average live weight of approximately 112 kg and 6 months old. All pigs were of the same genetics ([Yorkshire × Landrace] sows sired with Pietrain boars) and originated from the same farm. The farm was a conventional farrow-to-finish herd practicing an all in/all-out management with confined (i.e. indoor) sows, weaners and fattening pigs. The farm has 650 breeding sows and produces about 12,500 fattening pigs a year. Pigs were housed in a finishing facility on a slatted floor, in groups of 20 animals per pen, at an average density of 1.0 m² per pig. Animals were fed *ad libitum* with liquid feed during the entire fattening period. The treatment conditions, both before and after slaughter, were identical for all pigs and in accordance with the conventional industrial practice. Pig slaughter and carcass processing were performed at the same small-scale commercial slaughterhouse with a daily slaughter rate of approximately 35 pigs.

2.2. RYR1 genotype determination

To identify RYR1 genotype, the blood sample of each pig was collected in a plastic cup from the bleeding wound at exsanguination. The blood was sampled to vacutainers containing K3EDTA anticoagulant. DNA was isolated using the KAPA Express Extract Kit (Kapa Biosystems, Wilmington, Massachusetts, USA) according to the manufacturer's instructions. After extraction of DNA, the RYR1 genotype of the pigs was determined using PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism) method. After PCR, DNA fragments with 134 base pairs (bp) were amplified using the KAPA2G Robust HotStart ReadyMix (Kapa Biosystems, Wilmington, Massachusetts, USA) and the following primer sequences [10]: primer 1: 5'-GTG CTG GAT GTC CTG TGT TCC CT-3' and primer 2: 5'-CTG GTG ACA TAG TTG ATG AGG TTT G-3'. The PCR reaction mixture with final reaction volume of 25 µl contained: 1X Master Mix (KAPA2G Robust HotStart ReadyMix, 2X) and 0.2 µM of each of the primers. The amplification reaction was carried out in a FlexCycler (Analytic Jena, Germany) under the following conditions [10]: denaturation at 94°C for 5 min; 35 cycles comprising denaturation DNA at 94°C for 40 s, annealing at 59°C for 40 s, complementary strand polymerization at 72°C for 40 s, and; final extension at 72°C for 5 min. The amplified PCR products were digested with the restriction enzyme FastDigest Hin6I (Fermentas, Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C for 5 min according to the manufacturer's instructions. Reaction mixture with final reaction volume 30 µl contained: 10 µl PCR product (~0.1-0.5 µg DNA), 1X buffer and 1 U/µL of the restriction enzyme FastDigest Hin6I. Digestion of this product by FastDigest Hin6I yields two fragments of 84 and 50 bp for stress-resistant (NN genotype), three fragments of 134, 84, and 50 bp for heterozygotes – the stress-carrier (Nn genotype) and only the 134 bp DNA fragment for mutant homozygous – the stress-susceptible (nn genotype) individuals. The digested PCR products were separated by horizontal electrophoresis (Carl ROTH N817.1 minieasy Electrophoresis Unit, Carl Roth, Germany) on 2% agarose gel (NipponGenetics, Tokyo, Japan), stained with ethidium bromide (Sigma-Aldrich Chemie GmbH P.O., Steinheim, Germany). After electrophoresis, gels were analyzed by using a transilluminator (Vilber Lourmat – ETX-20.C 254 nm, Vilber Lourmat, France) and, then, the size of the fragments was compared with a standard of known molecular weight (Gene Ruler 50 bp DNA ladder, Thermo Fisher Scientific, Lithuania).

2.3. Slaughterline examination

The plucks (heart, lung and liver set from each pig) of slaughtered pigs were removed from the slaughterline and visually appraised then palpated for macroscopically visible lesions of pneumonia, pleurisy, pericarditis and liver milk spots according to the Welfare Quality® protocol [11]. A positive case for each pathological lesion was defined as a pig organ affected with any degree of lesion (score 2) and as negative when lesions were absent (score 0). The complete assessment of pathology scores was performed by the three trained investigators.

2.4. Welfare indicators

Blood samples were collected in a plastic cup from the bleeding wound at exsanguination and lactate and glucose concentrations were immediately determined in duplicate (two strips/pig) by dipping the test strips into a blood sample. Blood lactate and glucose concentration were measured using a handheld lactate analyzer (Lactate Scout, EKF Diagnostic, Magdeburg, Germany) and glucometer (GlucoSure AutoCode, ApexBio, Taiwan).

Rigor mortis intensity was determined on the left carcass side 45 minutes *post-mortem* by measuring the degree of angle between body axis and foreleg according to the Davis *et al.* [12]. For that purpose, photographic images of carcasses were taken, at a distance of approximately 2 m and a height of 160 cm, parallel to the plane in which the carcasses were held. The angle was calculated in AutoCAD program. Angle size and rigor intensity are inversely proportional, e.g. a smaller angle means a higher degree of *rigor mortis*. Subjective assessments of the rigor development were made 45 minutes postmortem on the *M. semimembranosus* in the split carcass using a three-point scale according to [13]: 1) muscle not in rigor; 2) muscle partly in rigor; and 3) muscle in full rigor. Semimembranosus muscle is normally exposed on the carcass medial surface and can be assessed by gauging its surface firmness using finger pressure, whereby in a muscle not in rigor the surface feels soft, while a muscle in rigor feels quite firm.

2.5. Growth performance

Average lifetime daily weight gain was derived from live weight at slaughter (carcass weight divided by 0.74 to allow for guts, etc.), minus 1.1 kg (typical weight of newborn piglet), and the total divided by the average age at slaughter, as described by Jaeger *et al.* [14].

2.6. Carcass and meat quality analyses

The carcasses were weighed immediately after splitting and final washing to obtain the hot carcass weight, and re-weighed 24 h after chilling at 4°C to determine the cold carcass weight. Backfat and loin muscle (*M. longissimus dorsi*) thickness were measured with a stainless steel ruler on the midline of the split carcass in millimeters: a fat measurement taken as the minimum fat thickness of the visible fat including rind covering the *M. gluteus medius* and a muscle measurement taken at the shortest connection between the front (cranial) end of the *M. gluteus medius* and the upper (dorsal) edge of the vertebral canal. The lean meat content (%) was calculated using ZP (Zwei-Punkte Messverfahren) method [15] based on the thickness of the backfat and loin depth according to the following formula: $y = 65,93356 - 0,17759 * x_1 + 0,00579 * x_1 - 52,54737 * x_1/x_2$, with y = estimated lean meat content of the carcass (kg); x_1 = backfat depth (mm) and x_2 = loin muscle depth (mm). The number of skin lesions on the left side of the carcasses was counted by three trained observers in the chilling room 45 mins *post-mortem* using the Welfare Quality® protocol [11] as described in Čobanović *et al.* [16]. Carcass skin lesions were also classified as human-inflicted type bruises, fighting-type bruises and mounting-type bruises by visual assessment of shape and size to recognize their origin as described in Čobanović *et al.* [16]. The pH and temperature of the *M. longissimus dorsi* (pH_{45LD}; T_{45LD}) and *M. semimembranosus* (pH_{45SM}; T_{45SM}) were measured 45 minutes after slaughter using a pH-meter Testo 205 (Testo AG, Lenzkirch, Germany). Pork quality classes (pale, soft and exudative – PSE meat; normal meat; and dark, firm and dry – DFD meat) were determined according to Čobanović *et al.* [16] using pH_{45LD} value. The carcasses showing pH_{45LD} values lower than 6.0 were classified as PSE meat,

while the carcasses showing pH_{45LD} values higher than 6.4 were classified as DFD meat. The carcasses with pH_{45LD} between 6.0 and 6.4 were classified as normal pork quality.

2.7. Statistical analysis

Statistical analysis of the results was conducted with SPSS software version 23.00 for Windows. According to RYR1 genotype, the pigs were allocated to two groups: Nn = stress-carriers (n=21) and NN = stress-resistant pigs (n=39). Student's t-test was used to examine the effects of RYR1 gene mutation on the growth performance, welfare indicators, and carcass and meat quality traits of slaughter pigs. Data were described by descriptive statistical parameters as the mean value and standard error of the mean (SE). The effects of RYR1 gene mutation on health indicators, type of carcass lesions and pork quality classes were determined by Fisher's exact test. A value of $P < 0.05$ was considered significant.

3. Results and Discussions

Of the 60 examined slaughter pigs, 65.00% contained the normal RYR-1 genotype (NN genotype), 35.00% individuals contained stress-susceptible n allele (Nn genotype), while none of the animals had a double recessive RYR-1 genotype (nn genotype).

Effects of RYR1 genotype on health and welfare indicators in slaughter pigs are shown in Table 1. Stress-carrier pigs were more affected ($P < 0.05$) by pneumonia than stress-resistant pigs. Pig producers and meat companies aim to produce pigs with high meatiness and good meat quality traits at the same time. However, although the genetic selection of pigs significantly improved meatiness, they became less resistant to diseases [17-18], which can explain a higher predisposition for the occurrence of pathological lesions in stress-carrier pigs observed in this study. In stress-carrier pigs, stressful situations presumably induce changes in the number and proportions of white blood cells, mitogen-induced cell proliferation, natural killer cell cytotoxicity and circulating inflammatory factors, plus suppression of cytokines and immunoglobulin production, which can result in increased susceptibility to any infectious disease [19]. Moreover, in the case of an existing disease, stressful factors might predispose Nn pigs to the emergence of various diseases that further complicate the process [19].

Table 1. Effects of RYR1 genotype on health and welfare indicators in slaughter pigs

RYR1 genotype	Nn	NN	P-value	Significance
Number of pigs	21	39		
<i>Pathological lesion</i>				
Pneumonia (%)	61.90 ^a	28.21 ^b	0.0145	*
Pleurisy (%)	28.57	23.08	0.7569	NS
Liver milk spots (%)	38.10	35.90	>0.9999	NS
Pericarditis (%)	9.52	7.69	>0.9999	NS
<i>Blood parameters</i>				
Lactate (mmol/l)	12.64 ^a ±1.99	7.86 ^b ±0.74	0.0125	*
Glucose (mmol/l)	9.18 ^a ±0.99	5.13 ^b ±0.39	0.0001	*
<i>Rigor mortis</i>				
Foreleg angle (°)	115.20 ^a ±0.78	120.80 ^b ±0.59	<0.0001	*
Rigor scores	1.05 ^a ±0.15	0.49 ^b ±0.09	0.0010	*

Nn = stress-carrier; NN = stress-resistant; * Statistical significance at ($P < 0.05$); NS: not significant ($P > 0.05$); ^{a, b} Different letters in the same row indicate a significant difference at $P < 0.05$

In this study, higher ($P < 0.05$) blood lactate and glucose concentrations, more developed *rigor mortis*, as well as higher rigor scores were recorded in Nn pigs, which confirmed that the heterozygous individuals containing n allele are more sensitive to stress [20]. Genetic selection of pigs for improved meatiness has led to increased numbers of white muscle fibers that are extremely rich in glycogen,

which could explain 2-3 times higher glycogen contents in stress-carrier pigs than in stress-resistant pigs [21], and, therefore, great potential for accumulating lactate and glucose in the circulation after exposure to stress factors. The mechanism of stress in Nn pigs involves an unusual sympatho-adrenomedullary system response, resulting in increased release of catecholamines (adrenaline and noradrenaline) that bind to specific β -receptors on the cell membrane of skeletal muscles, activating the endocyclase enzymes, and thereby causing depletion of muscle glycogen. This contributes to the increased blood lactate and glucose concentrations and rapid development and higher intensity of *rigor mortis* after exposure to stressful circumstances on the day of slaughter [13, 20].

Effects of RYR1 genotype on growth performance, carcass and meat quality traits in slaughter pigs are shown in Table 2. Nn pigs had higher ($P<0.05$) average lifetime daily weight gain, live, hot and cold carcass weights than NN pigs. In addition, Nn pigs had lower ($P<0.05$) backfat thickness, but higher ($P<0.05$) loin muscle thickness and lean meat content, which confirmed the positive influence of the recessive n allele on the carcass quality [22].

Table 2. Effects of RYR1 genotype on growth performance, carcass and meat quality traits in slaughter pigs

RYR1 genotype	Nn	NN	P-value	Significance
Number of pigs	21	39		
<i>Growth performance</i>				
Average lifetime daily weight gain (kg)	0.660 ^a ±0.01	0.596 ^b ±0.01	<0.0001	*
<i>Carcass quality parameters</i>				
Live weight (kg)	114.90 ^a ±1.70	109.90 ^b ±0.73	0.0026	*
Hot carcass weight (kg)	94.86 ^a ±1.66	90.13 ^b ±0.69	0.0032	*
Cold carcass weight (kg)	93.31 ^a ±1.66	88.10 ^b ±0.72	0.0014	*
Backfat thickness (mm)	10.86 ^a ±1.57	14.75 ^b ±0.98	0.0316	*
Loin muscle thickness (mm)	67.33 ^a ±1.30	67.03 ^b ±1.43	0.8876	NS
Lean meat content (%)	56.71 ^a ±1.00	51.99 ^b ±0.80	0.0007	*
Skin lesion score	0.52 ^a ±0.15	1.03 ^b ±0.13	0.0224	*
<i>Type of skin lesions (%)</i>				
Human-inflicted bruises	38.10 ^a	10.26 ^b	0.0169	*
Fighting-type bruises	4.76 ^a	43.59 ^b	0.0024	*
Mounting-type bruises	0.00	12.82	0.1519	NS
<i>Meat quality parameters</i>				
pH _{45LD}	6.01 ^a ±0.03	6.36 ^b ±0.03	<0.0001	*
T _{45LD}	37.23 ^a ±0.07	36.52 ^b ±0.05	<0.0001	*
pH _{45SM}	6.07 ^a ±0.07	6.30 ^b ±0.02	0.0002	*
T _{45SM}	37.07 ^a ±0.06	36.45 ^b ±0.04	<0.0001	*
<i>Pork quality classes (%)</i>				
PSE meat	42.86 ^a	2.56 ^b	0.0002	*
Normal meat	57.14 ^a	84.62 ^b	0.0289	*
DFD meat	0.00	12.82	0.1519	NS

Nn = stress-carrier; NN = stress-resistant; * Statistical significance at ($P<0.05$); NS: not significant ($P>0.05$); ^{a, b} Different letters in the same row indicate a significant difference at $P<0.05$

The results obtained in this study could be explained by the facts that Nn pigs have a more favorable daily feed intake, increased feed conversion efficiency, and, consequently, higher growth rate and meatiness compared to NN pigs [7,22]. Better feed utilization and the higher carcass lean content could be attributed to the faster metabolism, as well as greater ability of stress-carrier pigs to assimilate proteins, and a lower predisposition for fat deposition [22]. In the present study, NN pigs

had greater ($P<0.05$) overall skin lesion score and fighting-type bruises than Nn pigs (Table 2). The higher predisposition of stress-resistant pigs to severe skin lesions could be explained by intense exploratory behavior, which increases contact with pen mates and resulting in confrontations and fights between pigs [23]. In contrast, Nn pigs exhibit greater fear and less curiosity to explore, and, therefore, rarely come into conflict with pen mates [23]. However, the frequency of carcass lesions caused by rough handling was higher ($P<0.05$) in Nn pigs (Table 2), which could be preferentially attributed to their greater sensitivity to stressful stimuli [23]. Due to the higher sensitivity to stress and, consequently, the greater anxiety of Nn pigs, they were more difficult to handle and needed more force, as well as excessive use of sticks and electric prods at loading, unloading and through the lairage raceways, which resulted in a higher frequency of carcass lesions indicating rough handling during the pre-slaughter period [23].

In this study, stress-carrier pigs had lower pH and higher temperature values measured 45 mins *post-mortem* in both muscles, as well as a higher prevalence of PSE meat ($P<0.05$; Table 2). Conversely, stress-resistant pigs had a higher ($P<0.05$; Table 2) prevalence of normal meat. Although skeletal muscles from pigs containing the mutant n allele (Nn genotype) have lower than normal contractile thresholds and an enhanced sensitivity as a consequence of abnormal intracellular calcium homeostasis [6], under normal conditions, the muscles function undisturbed [1]. However, exposure of stress-carrier pigs to stressful conditions just prior to slaughter leads to increased accumulation of calcium ions due to a genetic mutation in the skeletal muscle calcium release channel. Once opened, the mutated channel is unresponsive to Ca^{2+} - and Mg^{2+} -induced closing, thereby provoking muscle contracture, hypermetabolism and hyperthermia [1]. This causes extreme muscle glycogen depletion, resulting in a rapid fall in meat pH, which in combination with high carcass temperature leads to the myofibrillar and sarcoplasmic protein denaturation, subsequently affecting water-holding capacity of pork, which increasing the tendency towards PSE meat [3-5,7].

4. Conclusion

The results of this study showed that pigs containing the mutant n allele (Nn genotype) produced better carcass quality (higher live weight, carcass weight and meatiness, but less fat). However, the presence of the mutant n allele in these pigs had a deleterious effect on animal welfare and meat quality showed by the increased blood lactate and glucose concentrations, more developed *rigor mortis* and higher prevalence of PSE meat. In addition, pigs containing the mutant n allele (Nn genotype) had a higher predisposition for pneumonia, indicating that they are more prone to infectious diseases. Accordingly, further selection towards the elimination of the mutant allele of the RYR1 gene from pig populations would result in an improvement of health, welfare, and pork quality.

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