Biotechnology in Animal Husbandry 29 (3), p 431-440 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.04
DOI: 10.2298/BAH1303431K

SKELETAL RYANODINE RECEPTOR GENE ASSOCIATED WITH CHEMICAL COMPOSITIONS OF MEAT AND PHYSIOCHEMICAL PARAMETERS OF BLOOD

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Original scientific paper

Abstract: Objectives of the current study were to segregate single nucleotide polymorphism of the ryanodine receptor gene (RyR1) gene as well as to evaluate its effects on blood parameters and chemical composition traits of meat. For these, 90-American Yorkshire (AY), 85-American Landrace (AL) and 162-Australian unrelated commercial Yorkshire x Landrace (YL) pigs were genotyped for the locus C1843T (Arg615Cys) corresponding to alleles "N" and "n". In relation to the RyR1 polymorphism, a frequency of 0.09 was observed for the "n" allele in the YL breed but not in the AY and AL. Therefore, only the YL pigs were phenotyped for physiochemical properties and chemical compositions of meat. A significant association was found between genotypes and observed traits, assessed by [1]WBC_{30}, WBC_{100}, PLT_{30}, DM_L, CP_L, and P_L with genotype NN being higher than Nn as well as by GLU_{60} and Ash_L ($P<0.05$) with NN being lower than Nn. The allele "N" showed great potential in the genetic improvement for health and metabolism in pigs.

[1] **Abbreviations**
$WBC_{30, 60 \text{ or } 100}$: total number of white blood cells at 30, 60 or 100kg of live weight of pig
$RBC_{30, 60 \text{ or } 100}$: total number of red blood cells at 30, 60 or 100kg of live weight of pig
$PLT_{30, 60 \text{ or } 100}$: total number of platelets at 30, 60 or 100kg of live weight of pig
$HCT_{30, 60 \text{ or } 100}$: hematocrit percentage at 30, 60 or 100kg of live weight of pig
$GLU_{60 \text{ or } 100}$: glucose concentration at 60 or 100kg of live weight of pig
$UR_{60 \text{ or } 100}$: urea concentration at 60 or 100kg of live weight of pig
$DM_{L \text{ or } H}$: percentage of dry matter in loin or ham
$EE_{L \text{ or } H}$: percentage of ether extract in loin or ham
$CP_{L \text{ or } H}$: percentage of crude protein in loin or ham
$Ash_{L \text{ or } H}$: percentage of ash in loin or ham
$Ca_{L \text{ or } H}$: percentage of calcium in loin or ham
$P_{L \text{ or } H}$: percentage of phosphorus in loin or ham

Key words: RyR1 gene, allelic frequency, blood parameters, chemical compositions of meat, association

Introduction

One of the major genes affecting meat quality traits, performance and other economic traits in different populations of pig is the RyR1 sensitivity gene, also known since 1991 as a mutated skeletal RYR1 *(Fujii et al. 1991, Leach et al. 1996, Larzul et al. 1997, Pommier et al. 1998, Miller et al. 2000, Hamilton et al. 2000)*. The RyR1 gene related to porcine stress syndrome (PSS), which develops in genetically predisposed individuals, upon exposure to halogenated anesthetics *(Obi et al. 2010)*. It resulted from a single nucleotide substitution in the RyR1 gene *(Fujii et al. 1991)*. Its sensitive allele "n" was determined to be implicit cause of PSE (pale, soft, and exudative) leading to poor quality and low commercial value of pork *(Simpson et al. 1989, Sather et al. 1991a,b,c, Kallweit et al. 2007)*. RyR1 genotypes can be easily identified by many different methods *(Fujii et al. 1991, Dalens & Runavot 1993, Pommier et al. 1993, Thuy et al. 2005, Marini et al. 2012)*. Additionally, all three-stress syndromes PSS, PSE and MH (malignant hyperthermia) are associated with similar physiological-biochemical and metabolic changes, and all three forms produce PSE musculature *(Mitchell & Heffron 1982, Schaefer et al. 1990)*. Therefore, blood components genetically known lines of 8-9 week-old pigs NN and nn were tested. Significant differences in protein, RBC, cortisol, creatinine, bilirubine, aminotransferase, actate dehydrogenase, creatine phoshokinase levels among genotypes were demonstrated *(Schaefer et al. 1990,* Popovski *et al.*, 2012*)*. It was implied that identification of stress-susceptible and carrier pigs may be based on some properties of blood (Schaefer *et al*. 1990, Popovski *et al.*, 2012). In this study, PCR-RFLP/*Hin*6I was used to identify RyR1 genotypes at position 1843. Subsequently, blood indexes and chemical compositions of meat were analyzed and compared between negative and positive pigs.

Materials and methods

The study was conducted at the Experimental Animal Unit of Can Tho University (CTU farm). Pigs were given commercial feed of the GreenFeed Joint Stock Company (Table 1).

Experimental animals consisted of 90-American Yorkshire (AY), 85-American Landrace (AL) and 162-Australian unrelated commercial Yorkshire x Landrace pigs (YL).

Table 1. Nutrient value of the experiment feed

Composition	Grower (30-60kg BW)	Finisher (60-100kg BW)
ME, kcal/kg	3,000	2,900
CP min, %	15	13
P min, %	0,6	0.6
EE min, %	3	2
Ca, %	0.8-1.2	0.8-1.2
NaCl, %	0.2-0.8	0.2-0.8
CF max, %	5.5	5.5
BMD max, mg/kg	35	-
Source: www.greenfeed.com.vn		

Blood were taken from jugular vein of YL pigs at three various time points 30, 60 and 100 kg of live weight and contained in a tube with either EDTA for physical analysis (white blood cells, red blood cells, platelets, hematocrit) using the Cell-DYN 1700 Hematology Analyzer *(Abbott, USA)* or Heparine for chemical analysis (glucose and urea) using the TC-3300 *(Teco Diagnostics, Anaheim, California, USA)*. Then, blood samples were analyzed within 2 hours after taking. Additionally, when pigs reached a live weight of about 100 kg, they were slaughtered. Samples of longissimus dorsi muscle (loin) and ham were collected to analyze their chemical compositions (dry matter, crude protein, ether extract, ash, calcium, and phosphorus) according to protocols of *the AOAC international (1990)*.

Genomic DNA was extracted from tail samples. In order to discriminate alleles "N" and "n" at locus C1843T (Arg615Cys), materials and procedures including PCR-RFLP/*Hin*6I were used as described by *Thuy et al. (2005)*.

A possible association of polymorphism with the observed traits was statistically analyzed upon the model $y_{ij} = \mu + \alpha_i + \varepsilon_j$ (μ: overmean, α: effects of genotypes and ε: standard error) using the Minitab 13 (GLM procedure, Tukey test, confidence level 95% in pairwise comparisons).

Results and discussion

Genotype frequency

None of homozygous genotype nn was found in all populations. Animals originating from America only had genotype NN. Segregating alleles "N" and "n" was identified in YL pigs (Table 2). It was known that YL has been imported since 1900s, while AY and AL animals just entered the breeding system of the CTU farm last year. This may be due to RyR1 removal from pigs in modern breeding programs. Similarly, it was also found out very low frequencies of recessive allele "n" in different populations. In Yorkshire and White Meaty pigs, genotype nn was

absent. The frequencies were 0.09, 0.13 and 0.16 in Yorkshire, White Meaty and Landrace, respectively *(Omelka et al., 2006)*. However, the Nn genotype was not detected in normal meat, but was detected at a rate of 9.3% in PSE meat in a crossbreed population Duroc x (Yorkshire x Landrace). This could be due to the low prevalence of mutation in reproductive pigs *(Obi et al. 2010)* because of the absence of the mutation in Duroc boars *(Kitsutaka et al., 2008)*.

Table 2. Contribution of genotypes and alleles in experimental populations

Breeds	Genotypic frequency		Total	Allelic frequency		χ^2
	NN	Nn		N	n	(HWE)
AY	90 (1.0)	0 (0.00)	90	180 (1.00)	0 (0.00)	Fixed
AL	85 (1.0)	0 (0.00)	85	170 (1.00)	0 (0.00)	Fixed
YL	132 (0.81)	30 (0.19)	162	264 (0.91)	30 (0.09)	>0.05

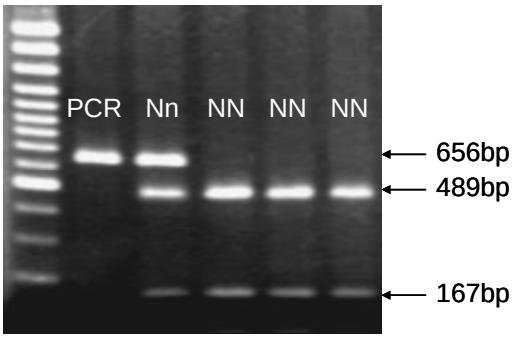

Figure 1: Patterns of polymorphism detected using PCR-RFLP/Hin6I

Polymorphic association with blood parameters

In this study, an increase of the age was synonymous with (i) reduction of WBC and PLT concentration and (ii) slight fluctuation of RBC and HCT. The WBC_{30}, RBC_{30} and PLT_{30} increased significantly from the negative pigs NN to the positive pigs Nn ($P<0.05$), while HCT was in contrast. In addition, almost observed physiochemical indexes of both genotypes closed together at 60 kg of live weight, except GLU_{60} ($P<0.05$). Furthermore, when pigs reached a live weight of about 100 kg, the homozygotes were significantly different from the heterozygotes for WBC_{100} and RBC_{100} ($P<0.05$). Generally, a significant difference for physiochemical parameters was found between the positive and negative, especially at two time points of 30 and 100 kg of live weight. Analyzing biochemical components of blood in 8-9 week-old pig lines indicated that pigs with homologous nn showed WBC (16.54±0.55 vs 18.32±0.61), HCT (41.27±0.55 vs 41.29±0.62) and glucose (4.70±0.13 vs 4.95±0.14) lower than ones with

homologous NN. Significant effects for RBC (7.43±0.15 vs 8.01±0.13) and protein (65.00±0.88 vs 68.78±0.79) were found between genotypes NN and nn ($p<0.01$) but not significant for urea (5.12±0.22 vs 5.31±0.20) *(Schaefer et al., 1990)*. Based on obtained results, *Schaefer et al. (1990)* implied that identification of RyR1 genotypes might be established based on differences in the circulation levels of metabolites.

Polymorphic association with chemical compositions of pork

Investigation has showed up some differences of chemical compositions of pork. The biggest DM (24.98) was in meat of YL combination. Protein, fat and ash contents were respectively 22.33, 1.50 and 1.15 in meat of YL *(Jukna & Jukna, 2005)*. Authors also summarized that the different meat quality indexes varied more inside the breeds than their differences among the breeds. In this study, most of statistical differences between genotypes were found for chemical composition traits of loin such as DM_L (25.17 vs 24.64), Ash_L(1.35 vs 1.50), CP_L (21.22 vs 20.56), P_L (0.19 vs 0.15) ($P<0.05$) in which homozygotes were better than heterozgotes.

Protein is the worthiest in the nutrient contents of meat, whereas DM might indirectly reflect to driploss level or the water holding capacity of pork (meat with low DM will become much more water). Actually, if DM of Nn was lower than that of NN, it was because meat of Nn pigs tended to be PSE (Table 3).

Table 3. Effect of genotypes on physiochemical properties of blood

	NN	Nn	Avg SEM	P
At 30 kg of live weight				
WBC_{30}, $x10^9$/l	24.00	20.07	1.05	0.014
RBC_{30}, $x10^9$/l	5.89	4.74	0.19	0.000
PLT_{30}, $x10^{12}$/l	306.63	210.00	17.63	0.000
HCT_{30}, %	0.39	0.41	0.01	0.088
At 60 kg of live weight				
WBC_{60}, $x10^9$/l	18.16	16.38	0.67	0.083
RBC_{60}, $x10^9$/l	5.45	4.01	0.64	0.133
PLT_{60}, $x10^{12}$/l	206.82	193.33	7.80	0.252
HCT_{60}, %	0.37	0.38	0.01	0.433
GLU_{60}, mmol/L	4.32	4.92	0.10	0.000
UR_{60}, mmol/L	5.73	6.02	0.24	0.431
At 100 kg of live weight				
WBC_{100}, $x10^9$/l	12.16	8.80	0.93	0.018
RBC_{100}, $x10^9$/l	4.74	6.33	0.25	0.000
PLT_{100}, $x10^{12}$/l	213.93	247.17	11.80	0.063
HCT_{100}, %	0.37	0.39	0.01	0.415
GLU_{100}, mmol/L	4.39	4.62	0.09	0.092
UR_{100}, mmol/L	5.98	5.95	0.39	0.962
WBC: white blood cells, RBC: red blood cells, PLT: platelets, HCT: hematocrit, GLU: glucose, UR: Urea				

Moreover, no significant dominance was found for all of the chemical components of ham although the negative seemed to be higher for costly contents (DM_H and CP_H) than the positive (Table 3). It was also reported that the effect of RyR1 genotypes resulted in (i) a significantly a greater proportion of protein (22.65 vs 22.31, respectively) ($P< 0.01$), (ii) a smaller proportion of fat (4.98 vs 5.85, respectively) ($p<0.01$), and (iii) a smaller proportion of ash (1.10 vs 1.09, respectively) ($P< 0.01$) in the loin muscle of Nn animals compared with that of NN pigs *(Pommier et al., 1998)*.

Fat also is one of the important chemical components to evaluate meat quality because it contributes to flavor and tenderness of meat, especially during grilling. However, in this study there was no significant difference between genotypes for EE of both loin and ham. Some authors indicated that the RyR1 genotype did not cause any significant change in the fatty acid composition (C14:0; C16:0; C16:1; C18:0; C18:1; C18:2; C18:3; C20:0; C20:1; C20:2 and C20:3) in subcutaneous backfat and intramuscular fat of *Musculus semimembranosus (Tor et al., 2001)*. This was in strong agreement with report of *García-Macíasa et al. (1996)* but not with one of *Biedermann et al. (2000)* because polyunsaturated fatty acids increasing in Pietrain pigs with the allele "n". The earlier reports also showed that there was not any effect of the RyR1 genotype on meat colour traits and on chemical composition (dry matter, organic matter, crude protein and intramuscular fat *(García-Macíasa et al., 1996; Leach et al., 1996)*. However, in the report of *Thaller et al. (2000)*, the authors demonstrated that pigs with genotype NN had intramuscular fat value higher than ones with the other genotypes.

Table 4. Effect of genotypes on chemical compositions of meat

	NN	Nn	Avg SEM	P
Longissimus dorsi				
DM_L, %	25.17	24.64	0.17	0.036
Ash_L, %	1.35	1.50	0.04	0.012
CP_L, %	21.22	20.56	0.15	0.004
Ca_L, %	0.23	0.27	0.01	0.078
P_L, %	0.19	0.15	0.01	0.000
EE_L, %	2.04	2.04	0.08	0.942
Ham				
DM_H, %	24.70	24.37	0.20	0.272
Ash_H, %	1.32	1.34	0.03	0.666
CP_H, %	20.68	20.59	0.14	0.672
Ca_H, %	0.20	0.22	0.21	0.352
P_H, %	0.19	0.17	0.01	0.062
EE_H, %	1.94	1.89	0.14	0.798
DM: dry matter, CP: crude protein, Ca: calcium, P: phosphorus, EE: ether extract (fat)				

Conclusion

RyR1 genotypes partly affected the physiochemical parameters as well as the chemical compositions of loin but not those of ham. This may be due to differences in the structural characteristics of the two kinds of muscle fibres. The study provides further information for RyR1 roles in controlling economic traits. This is very meaningful for the programs of pig breeding because the use of genetic markers associated with economic traits can lead to increased rates of genetic response and bring more economic profit to pig production industry.

Acknowledgements

This work was supported by the grant Can Tho University (project T2011-71) and GreenFeed Viet Nam Joint Stock Company (www.greenfeed.com.vn).

Skeletni rijanodin receptora gen povezan sa hemijskim sastavom mesa i fizičko hemijskim parametrima krvi

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Rezime

Ciljevi ove studije bili su da se odvoji polimorfizma nukleotida rijanodin receptor gena (RIR1) gena, kao i da se proceni njihov uticaj na parametara krvi i osobine hemijskog sastava mesa. U tu svrhu, 90 grla američkog jorkšira (AY), 85 američkog landrasa (L) i 162 grla australijskog komercijalnog jorkšira x landras (YL) su genotipizirane za lokus C1843T (Arg615Cis) odgovarajući alel "N" i "n" . U odnosu na RyR1 polimorfizam, učestalost 0.09 je registrovana za "n" alel kod YL rase, ali ne u AY i AL. Dakle, samo su svinje YL fenotipizirane za fizičko-hemijske osobine i hemijski sastav mesa.Značajna povezanost je utvrđena između genotipova i ispitivanih osobina, procenjena WBC_{30}, WBC_{100}, PLT_{30}, DM_L, CP_L, i P_L gde je genotipom NN bio viši od Nn, kao i preko GLU_{60} i Ash_L ($p < 0,05$) sa NN nižim nego što je Nn .Alel "N" pokazao veliki potencijal u genetskom unapređenjm u pogledu zdravlja i metabolizma kod svinja.

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Received 03 May 2013; accepted for publication 26 June 2013