GENETIC POLYMORPHISM OF ryr 1 GENE FROM TURKEY PSE BREAST MUSCLE.

Fernanda Gonzales Paião[1*], Viviane Ribeirete[2], Rafael Humberto de Carvalho[3], Mayka Reghiany Pedrão[1], Fábio Augusto Garcia Coró[1] and Massami Shimokomaki[1,3]

[1] Federal Technological University - Paraná - Campus Londrina, Estrada dos Pioneiros, 3131,CEP 86036-370, Londrina. Paraná. Brazil.

[2] Graduate Student, Federal Technological University – Paraná – Campus Londrina. Paraná. Brazil

[3] Londrina State University –Londrina, PR 445, s/n, CEP 86051-990, Londrina. Paraná. Brazil

*fergonzalesp@hotmail.com

Abstract – A mutation in ryr 1 gene affect meat quality in pork, causing PSE (*Pale, Soft and Exudative*) meat. The objective of this study was to identify polymorphisms in turkey´s α-ryr gene that could be associated with PSE meat. Because ryr genes are over 100,000 bp long and code for proteins with about 5000 amino acids, the primers used in this work amplified a portion of this gene, corresponding to the hotspot region 1 that contains the known mutation leading to PSE meat in pork. Total blood DNA was extracted from 10 breast muscles, 5 samples from PSE meat and 5 from samples considered normal. These DNA samples were amplified by PCR, cloned, sequenced, and used to identify single nucleotide polymorphisms (SNPs). The amplified fragment of α-ryr was 653 nucleotides in length. A non-synonymous nucleotide substitution (A/G) was identified in 3 PSE meats and 1 normal meat. This SNP caused a change from Met to Val in the α-RYR protein. Since this SNP was identified in both meat samples and due to the fact that this mutation does not change the structure and/or function of the muscle protein, this alteration in DNA sequence is an inappropriate candidate to be used as a genetic marker for turkey PSE meat.

I. INTRODUCTION

Porcine stress syndrome (PSS) leads to meat that is paler, tougher, and have lower water-holding capacity known as PSE meat [1]. The PSE meat results in great losses in the meat industry by offering product with altered colors and flavors and lower yield during cooking [2].

The main cause of PSS is a mutation at nucleotide 1843 of cDNA (GenBank M911451) of the gene encoding the calcium release channel in the skeletal muscle sarcoplasmic reticulum called ryanodine receptor (RYR1) commonly known as the halothane gene (HAL) [3]. Some turkey and chicken breast muscle have been found to be lighter or paler than what is considered to be normal [4, 5]. Similar to PSE pork, turkey pale poultry muscles have reduced water-holding capacity (WHC) and higher drip loss than normal muscles. However, the lighter color poultry may also have normal WHC and drip loss [4,6]. Based on these findings, researchers have adopted the PSE term to describe pale avian muscle. However, the genetic basis for the PSE syndrome remains unknown and the participation of RYR on this abnormality has not yet been fully related to the occurrence of PSE meat in poultry [7,8,9]. The objective of this study was to identify genetic polymorphisms in a fragment of α-ryr homologous to mammalian ryr 1, sequencing a fragment of this amplified gene from normal and PSE turkey breast meats collected in a commercial plant in Brazil.

II. MATERIALS AND METHODS

Turkeys 137- to 145-days-old were slaughtered under commercial processing plant conditions. The color (L values) and pH were measured in 810 breast fillet after 24h postmortem. Muscle samples (0.5 x 2.0 x 1.0 cm^3) from 5 PSE fillets (pH< 5.7 and L*≥55.0) and 5 normal fillets (pH≥ 5.7 and L*<55) were collected and stored in microtubes (1.5 mL) at -20°C. Total DNA was macerated under nitrogen and extracted from *Pectoralis major* m. using the Axyprep multisource Genomic DNA Miniprep Kit (Axygen, Union City, CA) following the manufacturer's instructions. For PCR amplification, the primers were the same used by Droval *et al.* [8]. The PCR amplification consisted of an initial denaturation at 95ºC for 1 min, followed by 35 cycles at 95ºC for 30s, at 52ºC for 30s, at 68ºC for 2 min, and a final extension at 72ºC for 7 min. PCR confirmation was conducted by agarose gel electrophoresis and the amplified fragments were cut from the gel and purified

using the Pure Link Quick Gel Extraction Kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. All PCR products were inserted into the pGEM-T Easy ® vector (Promega, Madison WI) following the manufacturer's instructions and transformed using DH5a eletrocompetent cells. The recombinant clones were isolated and sequenced on both strands using the M13 universal primers and the BigDye® Terminator v3.1. kit (Applied Biosystems, Warrington, UK) for the automatic sequencer ABI 3100 (Applied Biosystems, Warrington, UK). The chromatograms obtained were manually analyzed using the program Vector NTI Suite 8 (InforMax), followed by removal of the vector sequence (Vector Screen (http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html), and finally global alignment using ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html) to obtain the consensus sequence.

III. RESULTS AND DISCUSSION

To identify the possible mutations, a region of 653 pb from ryr gene was amplified (Fig 1). This amplified fragment corresponded to the 3' end of mutation hot spot 1 of human RYR1.

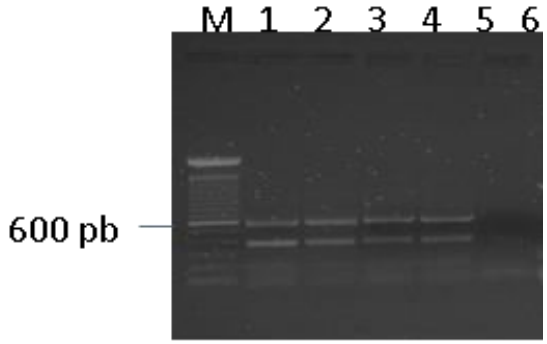

Figure 1:Electrophoresis of amplified products on a 1% agarose gel. *Lane* M=100-bp molecular weight marker (Invitrogen); *lanes 1 and-2:* DNA samples from PSE turkey meat; *lanes 3 and 4*: DNA samples from turkey normal meat, *lane 6:* negative control (no DNA).

The ryr amplified fragment was cut from agarose gel, cloned and sequenced, showing 88% sequence identity with turkey RYR cDNA. Aligning the turkey α-ryr genomic sequence of allele I with the turkey cDNA sequence (EU177005.1) using the bl2seq program [10], it was identified a complete intron localized in nucleotides 46-466. The similarity in 88% occurred because our sequence contains an intron, which is removed from RNAm during transcriptional process. This intron was not similar to ryr cDNA sequence available in Genbank database, because this cDNA was processed and the presence of intron was not observed. The part of our sequence was comparatively 100% in relation to the exons from turkey available in Genbank [11]. It was also identified two sequences similar to α-ryr that were called alleles I and II, respectively. A Single Nucleotide Polymorphism (SNP) was identified at position 612, where it was verified to be a substitution of an A (adenine) for a G (guanidine). Genomic sequence from allele I was more common within the samples with 60% of the birds being homozygous for A at position 612 (Fig. 2). Allele II, which carried the G to A substitution nucleotide at position 612, altered the RYR amino acid sequence relative to the turkey α-ryr, changing a methionine to a valine residue. According to the SIFT program [12] this substitution may cause changes within the protein structure, however this change may be tolerable, because both are hydrophobic amino acids. However, this detected polymorphism cannot be associated as mutation consequently promoting the occurrence of PSE meat, because in muscle that developed normal meat this phenomena was also detected. These results were similar to chickens [8] and corroborated to those reported by Chiang et al [7] suggesting that in poultry the ryanodine genetic basis of PSE meat is rather different from pork as recently suggested by Malila et al., [13].

```
Alelle 1:610 GGAACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653
Alelle 2:610 GGGACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653

Turk PSE 2 clone 1 610 GGAACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653
           Clone 2 610 GGGACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653
           Clone 3 610 GGAACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653

Turk PSE 3 clone 1 610 GGGACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653
           Clone 2 610 GGGACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653
           Clone 3 610 GGGACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653

Turk PSE 4 clone 1 610 GGACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653
           Clone 2 610 GGAACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653
           Clone 3 610 GGGACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653

Turk Nor 2 clone 1 610 GGAACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653
           Clone 2 610 GGAACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653
           Clone 3 610 GGGACGTGGTGCAGCCCCGCATGGCTGCCAGCTTTGTGCCGGAC 653
```

Figure 2. Alignment of the partial nucleotide sequences from five turkey that carried a

polymorphism, both genomic sequences (alleles I and II) at position 612 of the α-RyR gene fragment. The polymorphic nucleotide position within each sequence is shown in bold.

IV. CONCLUSION

A polymorphic SNP was detected in α-ryr DNA sequences from turkey that developed either normal or PSE meat. However, this alteration in ryr gene was not conclusive to explain the turkey PSE meat genetic basis.

ACKNOWLEDGEMENTS

The authors are thankful to CNPQ, CAPES and UTFPR for supporting this work. FGP is pos doctoral CAPES research fellow; FAGC, MRP and MS are CNPq Research fellows. VR holds CNPq/IC scholarship.

REFERENCES

1. Candek-Potokar, M. et al. (1998). Effects of age and/or weight at slaughter on longissimus dorsi muscle: biochemical traits and sensory quality in pigs. Meat Science 48: 287-300.

2. O'Neill, D.J. et al. (2003). Effects of PSE on the quality of cooked hams. Meat Science 64: 113-118. Meat Science 64: 113–118.

3. Fujii, J. et al (1998). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253: 448-451.

4. Owens, C.M. et al. (2000). The characterization and incidence of pale, soft and exudative turkey meat in commercial plant. Poultry Science 79: 553-558.

5. Marchi, D.F. et al. (2009) Sensitivity to halothane and its relationship to the development of PSE (Pale, Soft, Exudative) meat in female lineage broilers. Brazilian Archives of Biotechnology 52: 229-223.

6. Carvalho, R.H. et al. (2013) Commercial turkey PSE (Pale, Soft and Exudative) meat in Brazil during summer season. In Proceedings 59th International Congress of Meat Science and Technology (pp. 138-139), 18-23 August 2013, Izmir, Turkey.

7. Chiang, W. et al., (2003). Identification of two *αRYR* alleles and characterization of *αRYR* transcript variants in turkey skeletal muscle. Gene 330: 177-184.

8. Droval, A.A. (2012) A new single nucleotide polymorphism in the ryanodine gene of chicken skeletal muscle. and Molecular Research 11: 821-829.

9. Paião, F.G, et al. (2013). Skeletal muscle calcium channel ryanodine and the development of pale, soft and exudative meat in poultry. Genetics and Molecular Research 12: 3017-3027.

10. Altschul, S.F., et al. (1997). Basic local alignment search tool. Jourrnal of Molecular Biology 215: 403-410.

11. Genebank site, disponible at: http://www.ncbi.nlm.nih.gov/genbank/.

12. SIFIT program, disponible at: http://sift.bii.a-star.edu.sg/.

13. Malila, Y. et al. (2013). Differential gene expression between normal and pale, soft and exudative turkey meat. Poultry Science 92:1621-1633.