CHICKEN PSE (*PALE, SOFT, EXUDATIVE*) MEAT. MUTATIONS IN THE RYANODINE RECEPTOR GENE

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Background

Poultry genetic improvement has been traditionally devoted to the increase of meat production taking into consideration the relationship between weight gain and feed conversion ratio. One of the consequences of this intense selection is the occurrence of the PSE meat (Pale, Soft, Exudative) due to the miofibrilar proteins denaturation that affects fresh meat and meat products qualities. In pigs, it has been demonstrated the existence of a point mutation in the ryanodine receptor 1 gene (*ryr1*) which alters the function of the ryanodine receptor type 1 protein (RYR1). This protein controls Ca^{+2} flux between sarcoplasmatic reticulum and sarcoplasm, and has direct relationship to the PSE meat origin (2). Therefore, there is possibility of this gene *ryr* to be potential candidate for originating poultry PSE meat.

In poultry, besides RYR1 there is the presence of the ryanodine type 3 protein (RYR3), being the first voltage-dependent and the last calcium-dependent, both having the fundamental role of calcium flux control within muscle fiber. Both proteins are present at equal concentration in the muscle cell, differing from mammal that contains only 10% of RYR3 (9). Thus, because of this relative higher concentration, RYR3 must play an important role towards calcium movement in relation to development of poultry PSE meat in comparison to pork.

Objective

The objective of this work was to search for mutations in ryanodine 3 (*ryr3*) gene regions related to the occurrence of chicken PSE meat.

Material and methods

The experiment was conducted in four consecutive steps:

1. Raising, slaughtering and functional properties measurement (pH and color) of *Pectoralis major* m. from 1494 chickens sampled from a population developed by Embrapa Swine and Poultry, Concordia, Brazil, for genomic studies (4).

2. Ten birds were selected based on the functional properties as typical PSE (+) meat and PSE (-) meat: $pH < 5.8$ and $L^* > 52.0$ and $pH > 5.8$ and $L^* < 49.0$, respectively (7) (Table 1).

3. DNA extraction from 20 blood samples being 10 PSE (+) and 10 PSE (-) collected at the slaughtering step and design for PCR primers for gene *ryr3* regions.

4. Cloning using *E. coli* as vector and DNA sequencing.

The selected regions for amplification through PCR methodology corresponded to a *ryr3* hypothetic exons deducted from human specie information. This strategy was necessary because of chicken *ryr 3* available information was originated from RNAm and this fact obviously would affect the direct primers design for genomic DNA samples. A possible presence of introns would increase the region size to be amplified therefore inhibiting polymerization activity. For each of the 20 DNA samples, 5 regions were amplified successfully by PCR and currently three of them were cloned and sequenced. The obtained results were compared to the database BLAST (1). A comparative primary structure was carried out by the same program on the resultant RYR3 from transcription and translation regions. The secondary structure was predicted by the *PSIPRED V2.4* program (5).

Results and discussions

The sequenced 3 regions presented the size of 158 bp (reg 1), 173 bp (reg 2) and 149 pb (reg 3). In Figure 1 is shown the electrophoresis chromatography of PSE (+) (P) and PSE (-) (N) samples for PCR product bands from *ryr3* reg 1. The same Molecular Weight indicates that the region presents the same size in every sample suggesting the absence of nucleotides deletions or insertions within these regions. Although not shown, regions 2 and 3 presented similar pattern. Table 2 shows mutations within *ryr3* regions after sequencing. In region 3, a mutation was found with a timine residue instead of adenine residue in 100% of the population differing from the available sequence on the BLAST database (Table 2)

Other mutations were observed in only one chicken sample as shown in Table 2. Primary structure analysis revealed some of these mutations within aminoacid sequence alteration in the RYR3. However, there was no variation in the secondary structure as predicted by the PSIPRED V2.4 program. Although in 480 nucleotides of *ryr3* gene evidence of mutation was not found related to PSE meat, other regions are currently being investigated at gene region specific RYR3 domain.

In addition, other candidate genes can be related to the origin of chicken PSE meat, such as, phospholipase A_2 (8) and *ryr1* genes (3) which would make this study more complex. On the other hand, QTLs mapping and gene expression studies will help to elucidate the fundamental mechanisms underling PSE occurrence in poultry.

Conclusion

Chicken PSE meat has so far no association with genetic mutation in ryr3 at the regions analyzed in this study.

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References

1. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., Lipman, D. J. J. Mol. Biol. 990, 215: 403-410.

2. Fujii J., Otsu, K., Zorzato, F, Leon, S., Khanna, V. K. Science 1991, 253: 448-451.

3. Lara J. A. F., Ninov K. et al. Brazilian J. Poultry Sci. 2002; suppl. 4: 15.

4. Ledur, M. C., Lara, J. A F.,et al. ISAG-2002. International Society of Animal Genetics Conference, Germany, 2002.

5. Mcguffin, L. J., Bryson, K. Jones, D. Bioinformatics 2000, 16: 404-405.

6. Olivo, R., Soares, A.L., Ida, E. I., Shimokomaki M. J. Food Biochemistry 2001, 25: 271-283.

7. Soares, A. L., Lara, J. A. F., Ida, E. I., Guarnieri, P. D., Olivo, R., Shimokomaki, M. Proc. International Congress of Meat Science Technology, Roma, 2002, 48: 432-433.

8. Soares, A. L., Ida, E.I., Miyamoto, S., et al.. J. Food Biochemistry 2003: 27(4): in press.

9. Sutko, J. I., Airey, J. A. Physiological Reviews 1996, 76: 1027-1071.

Table 1 – Selected chicken samples for DNA analysis

Samples	L* values	pH
PSE (+)		
6125	54.3	5.62
5794	55.0	5.46
4470	56.5	5.55
4348	60.6	5.50
4347	58.1	5.64
4416	59.1	5.46
4970	59.2	5.55
5140	56.0	5.45
5155	55.3	5.71
5180	59.4	5.42
PSE (-)		
5444	45.8	5.84
5420	43.2	5.92
5732	47.4	5.82
4669	47.7	5.87
4360	48.1	5.87
4447	47.6	5.92
4460	47.8	5.77
4415	47.0	5.85
5105	48.9	5.80
4484	44.7	5.76

Table 2- Mutations at *ryr3* gene after DNA sequencing

ryr3 regions	Mutations	Samples occurence (n=20)	Primary structure
3	T replaced A at TTT TTC AGA	100%	Phe replaced Tyr
3	G replaced A at TTT GAG GCT	5%	No alteration
2	A replaced G at CTG AGC CAA	5%	Ser replaced Gly
2	G replaced T at ACT CGC CTG	5%	Arg replaced Leu

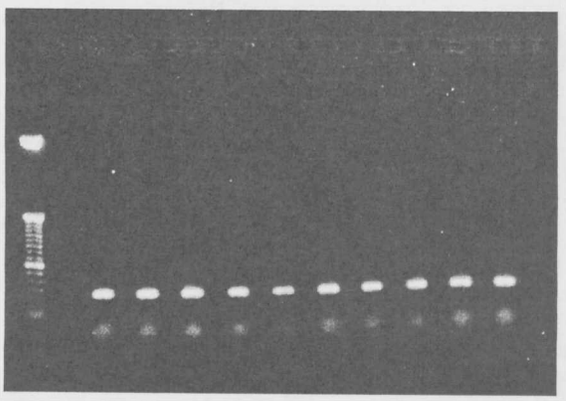

Fig. 1. Agarose-gel electrophoresis pattern of the PCR products from *ryr3 gene* region 1 (158 pb) of chicken blood samples: P (PSE+), N (PSE -).