Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

Transcriptome analysis of skeletal muscle tissue to identify genes involved in pre-slaughter stress response in pigs

Roberta Davoli, Paolo Zambonelli, Jakob Hedeegard, Henrik Hornshoj, Leonardo Nanni Costa, Alessandra Stella, Luca Fontanesi, Michela Colombo, Christian Bendixen & Vincenzo Russo

To cite this article: Roberta Davoli, Paolo Zambonelli, Jakob Hedeegard, Henrik Hornshoj, Leonardo Nanni Costa, Alessandra Stella, Luca Fontanesi, Michela Colombo, Christian Bendixen & Vincenzo Russo (2009) Transcriptome analysis of skeletal muscle tissue to identify genes involved in pre-slaughter stress response in pigs, Italian Journal of Animal Science, 8:sup2, 69-71, DOI: 10.4081/ijas.2009.s2.69

To link to this article: https://doi.org/10.4081/ijas.2009.s2.69

Transcriptome analysis of skeletal muscle tissue to identify genes involved in pre-slaughter stress response in pigs

Roberta Davoli[1], Paolo Zambonelli[1], Jakob Hedeegard[2], Henrik Hornshoj[2], Leonardo Nanni Costa[1], Alessandra Stella[3], Luca Fontanesi[1], Michela Colombo[1], Christian Bendixen[2], Vincenzo Russo[1]

[1]DIPROVAL, Sezione di Allevamenti Zootecnici, Università di Bologna, Italy
[2]Faculty of Agricultural Sciences, University of Aarhus, Denmark
[3]Parco Tecnologico Padano, Lodi, Italy

Corresponding author: Roberta Davoli. DIPROVAL, Sezione di Allevamenti Zootecnici, Facoltà di Agraria, Università di Bologna. Via F.lli Rosselli 107, 42100 Reggio Emilia, Italy - Tel. +39 0522 290507 - Fax: +39 0522 290523 - Email: roberta.davoli@unibo.it

ABSTRACT - The knowledge of genes and molecular processes controlling stress reactions and involved in the genetic system determining resistance to stress in pigs could be important for the improvement of meat quality. This research aimed to compare the expression profiles of skeletal muscle between physically stressed and not stressed pigs of different breeds immediately before slaughter. DNA microarray analysis showed that different functional categories of genes are up-regulated in stressed compared to not stressed pigs and relevant differences among breeds were found.

Key words: Stress genes, Skeletal muscle, Pig, Gene expression.

Introduction - Stress is a body reaction to *stimuli* that disturb the normal physiological steadiness influencing metabolism and behaviour. Stress response is likely to have adverse effects on growth, reproductive performance, and disease resistance in animals (Johnson *et al*., 1992; Hazard *et al*., 2008). Terlouw (2005) reported that in pigs exposed to stress conditions, metabolites and hormones are released into the blood stream leading to negative effects on the meat quality. The knowledge of the metabolic processes that are influenced by stress and the identification of genes responsible for stress susceptibility/tolerance are presently quite limited. The aim of this study was to compare by microarray analysis the transcription profile of skeletal muscle genes between stressed and not stressed pigs of different breeds (Italian Large White, ILW; Italian Duroc, ID; Pietrain, PI).

Material and methods - A total of 28 unrelated castrated pigs of three breeds ILW (n=10), ID (n=10), PI (n=8) were reared at the experimental research farm of DIPROVAL until approx. 120 kg of live weight, fed the same diet (commercial feeding *semi ad libitum*) and slaughtered in a single day on the same abattoir. The experimental protocol fully complies with the rules approved by the Ethical Committee of University of Bologna. The pigs were genotyped for SNP g.1843C>T in Halothane gene (*RYR1)* by PCR-RFLP (Russo *et al*., 1993). The pigs of each breed were separated into two groups and only the pigs of one group were subjected to a physical stress immediately before slaughtering. The stress conditions were performed by pushing the pigs to run 4 times along a raceway of 25 metres using an electric goad that was applied at least one time on all subjects. After slaughtering, the meat quality traits pH at 45 min and 24 h *post mortem*, colour (CIE L*, a*, b* system), drip loss were measured (ASPA, 1996), and muscle samples for further analyses were collected. Total RNA was isolated from *Semimembranosus* muscle samples using TRIzol reagent and its integrity was assessed on Agilent 2100 Bioanalyzer. The reverse transcribed RNA samples were labelled with fluorescent Alexa

Fluor 555 dye and individually hybridised with the Operon/Qiagen pig 11k Oligo set (version 1.0) together with an Alexa Fluor 647 labelled common reference sample consisting of RNA from skeletal and heart muscle of an unrelated pig. On the whole, 28 slides were hybridised and the biological replicates considered for each breed and stress treatment are shown in Table 1.

Table 1. Number of stressed and not stressed pigs of each breed and their genotype for SNP RYR1 g.1843C>T.

Breeds	RYR1 g.1843C>T	Stressed	Not stressed
Italian Large White	CC	5	5
Italian Duroc	CC	5	5
Pietrain	CC	4	4

Scan Array Express HT system scanner was used for image acquisition and images were analysed using GenePix Pro. The background subtracted and lowess normalised median values of the foreground florescence intensity of each spot were analysed by SAS package using a mixed procedure that considered fixed (treat, breed, scan, slide (breed)) and random (subarray (slide)) factors and adjustments for overall effects of array and dye across genes (P<0.001; FDR=5%). Differences in the least square means were obtained and tested for significant differences by t-test (Cui and Churchill, 2003). Functional annotation and classification of differentially expressed (DE) genes were performed by DAVID bioinformatic resources (Dennis *et al*., 2003).

Results and conclusions - The total number of DE genes and the number of those that are up- and down-regulated in stressed pigs from ILW, ID, and PI are reported in Table 2.

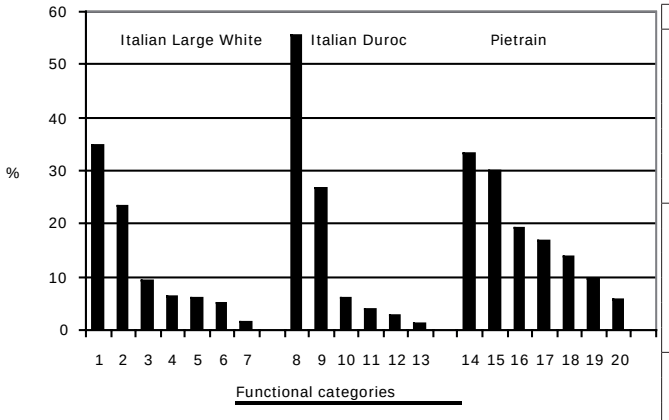
The result could suggest that a relevant number of genes determine the response to stress conditions before slaughter. Several authors (Elsasser *et al*., 2000; Terlouw, 2005; Hazard *et al*., 2008; Terlouw and Rybarczyk, 2008) reported that stress reactions in pigs influence various metabolic and physiological processes. The large number of differentially expressed genes that we found could be indicative that for stress response a network of interactions between cells and integration of many metabolic signals should be developed. In the analysed animals of the three breeds, the number of up-regulated genes in stressed pigs is lower than that of down-regulated genes. It could be supposed that in order to elaborate the response/resistance to stress only the expression of specific genes are enhanced to trigger the more specific and essential metabolic activities useful to sustain the physiological reactions to stress. In Figure 1, the main functional categories of up-regulated genes identified in the stressed pigs of breeds are reported. Only the categories signal transduction and stress response are present in all breeds and it is remarkable that the different breeds show different specific functional categories of genes. In ID pigs, there is a large number of over-expressed genes involved in metabolic processes concerning mainly proteins and carbohydrates. In stressed ILW pigs, genes of the lipid metabolism and genes coding for proteolytic and hydrolase activities are up-regulated. In stressed PI pigs, the number of up-regulated genes is very low and one of the main categories is represented by genes involved in nuclear activities like transcription factors, DNA binding, histone deacetylases. In PI pigs compared to ILW and ID there is a higher percentage of ove-

Table 2. Number of up- and down-regulated genes in stressed pigs and total number of differentially expressed (DE) genes. The number of unique transcripts are indicated in parenthesis.

Breeds	Up-regulated genes in stressed pigs	Down-regulated genes in stressed pigs	Total number of DE genes
Italian Large White	441 (414)	539 (499)	980
Italian Duroc	594 (553)	794 (762)	1,388
Pietrain	75(72)	112 (104)	187

rexpressed genes coding for several chaperone proteins specific to stress response. Moreover, in this sample of PI pigs we did not find genes coding for proteins with metabolic functions. This result could suggest a delayed activation of physiological/metabolic processes to stress response in the sample of considered PI pigs and a slower reaction to stress *stimulus*, compared to ILW and ID.

Figure 1. Main functional categories of the up-regulated genes in the stressed pigs of each breed.



Breed	N	Functional categories
ILW	1	Cytoplasm
	2	Hydrolase activity / Proteolysis
	3	Protein transport
	4	Enzyme regulator activity
	5	Lipid metabolic process
	6	Signal transduction
	7	Stress response
ID	8	Metabolic process
	9	Protein metabolic process
	10	Carbohydrate metabolism
	11	Signal transduction
	12	Response to stimulus
	13	Stress response
PI	14	Regulation of cellular processes
	15	Nucleus
	16	Response to stimulus
	17	Intracellular signal cascade
	18	Stress response
	19	Signal transduction
	20	Blood vessels angiogenesis

The differences in gene expression between breeds will be considered to evaluate the presence of relationships with the meat quality traits measured on the same animals. Moreover, a subset of genes with highly significant differences of expression level and/or some genes that might be interesting for the function of the coded protein will be selected for further analysis by qRT-PCR. In conclusion, the use of DNA microarray expression profiling revealed variations in gene regulation between stressed and not stressed pigs and among breeds. Moreover, the results are suggestive that several biochemical and physiological processes seem to be activated or/and regulated to develop the stress response in pigs.

This work was supported by Italian MIPAF SelMol project.

REFERENCES - **ASPA,** 1996. Metodiche per la determinazione delle caratteristiche qualitative della carne. Università di Perugia. **Cui,** X., Churchill, G.A., 2003. Statistical tests for differential expression in cDNA microarray experiments. Genome Biol. 4:210. **Dennis,** G. Jr., Sherman, B.T., Hosack, D.A., Yang, J., Gao, W., Lane, H.C., Lempicki, R.A., 2003. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol. 4:P3. **Elsasser,** T.H., Klasing, K.C., Filipov, N., Thompson, F., 2000. **The metabolic consequences of** stress: targets for stress and priorities of nutrient use. In: The biology of animal stress. CAB International, pp. 77-110. **Hazard,** D., Liaubet, L., Sancristobal, M., Mormède, P., 2008. Gene array and real time PCR analysis of the adrenal sensitivity to adrenocorticotropic hormone in pig. BMC Genomics. 9:101. **Johnson,** E.O., Kamilaris, T.C., Chrousos, G.P., Gold, P.W., 1992. Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. **Neurosci. Biobehav. Rev. 16:115. Russo,** V., Davoli, R., Tagliavini, J., Dall'Olio, S., Bigi, D., Costosi, E., Coscelli, M.B., Fontanesi, L., 1993. Identificazione del genotipo dei suini per la sensibilità all'alotano a livello del DNA mediante PCR. **Zoot. Nutr. Anim., 19:89. Terlouw**, C., 2005. Stress reactions at slaughter and meat quality in pigs: genetic background and prior experience. A brief review in recent findings. Livest. Prod. Sci. 94:125-135. **Terlouw**, E.M.C., Rybarczyk, P., 2008. Explaining and predicting differences in meat quality through stress reactions at slaughter: the case of Large White and Duroc pigs. Meat Sci. 79:795-805.