NEW BIOLOGY AS A TOOL FOR CONTROL OF PORK QUALITY

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Objectives

The objectives of these studies are to identify markers of different meat quality traits on the basis of different breeds and raring conditions as well as stress-induced differences in meat quality. Biological markers related to meat quality traits are investigated at gene, protein, and metabolite levels by the use of omics technologies such as microarray, proteomics, and NMR-based metabonomics, and analyzed using bioinformatics tools.

Methodology

Experiment 1: Ten litters of four female pigs were allocated to four treatment groups according to litter: control without stress exposure or treadmill exercise for approximately 30 min. The exercise was followed by either 0, 1, or 3 hours rest before slaughter. Muscle biopsy samples from M. *longissimus dorsi* (LD) and M. *biceps femoris* (BF) and blood samples were taken at the time of sticking and after 24 h samples were analysed for various meat quality traits, proteomics and microarray.
Experiment 2: 50 pigs: 20 Large White (LW) and 30 Basque (B) finishing castrated boars were used in the experiment. In each breed, 10 pigs were reared in a conventional (slatted floor, 1.0 m²/pig) system, and 10 pigs in an alternative (bedding and outdoor area, 2.4 m²/pig) system, at INRA experimental farm. Moreover, 10 Basque pigs were reared in the extensive (free range) production system of the Basque region (south west of France). All animals were slaughtered at the average live weight of 150 kg. *M. longissimus dorsi* (LD) samples were taken 30 minutes after exsanguination, frozen immediately in liquid nitrogen and stored at -80° C until RNA isolation.

Results & Discussion

Exercise stress increased the muscle temperature, reduced creatine phosphate, ATP and glycogen content leading to a reduced pH early pm and increased drip-loss in pigs exercised immediately before slaughter. The microarray data analysis also revealed that exercise-induced changes in the transcription occur in genes involved in oxidative stress response, apoptosis and proteolysis, skeletal muscle development and regeneration, and in genes regulating glucose and fatty acid utilization. The difference between control pigs and exercised pigs slaughtered without rest was confirmed by NMR-based metabonomics of the plasma metabolite profile. This effect was mainly ascribed to plasma lactate which was also the metabolite contributing most to the association between the plasma metabonome and pH at slaughter as well as temperature and drip-loss. After 1 and 3 h of rest the metabolite profile was reversed to that of the control pigs and so was the pH and drip-loss effects. However, the toughness of meat was increased by exercise irrespective of resting hours.

Proteomic analysis produced proteome abundance profiles of the LD and BF muscle samples of each animal. The analysis investigated the relationships between protein abundance and 1. effect of stress and resting, 2. the technological meat quality traits, and 3. The three way

relationship between protein abundance and both stress and resting effect and meat quality together. The results showed that the abundance patterns of several proteins in both muscles could be related to the exercise and resting treatment. Exercise either reduced or increased abundance of the proteins and resting reversed the effect of the exercise. However, some protins showed delayed reaction or no reaction at all, while other proteins showed an overshooting effect to the treatment. Associations between protein abundance and biochemical and meat quality related parameters showed that energy metabolism related parameters associated especially in the BF muscle and less in the LD muscle, which could be related to muscle functionality. In both muscles many proteins associated with pH and Minolta_B. When searching for proteins whose abundance associated with both stress-effect and meat quality it was noted that several proteins associated with more than one trait.

To investigate genetic background underlying differences in pork quality and identifiy markers of meat quality, a microarray experiment was conducted to determine the gene expression profiles in LD of two pure breeds of pigs, i.e. Large White and Basque pigs, differing in muscle and meat characteristics. 98 genes belonging to three main clusters appeared differently expressed between LW and B pigs. Up-regulated genes of polysaccharide metabolism in LW are in accordance with the higher muscle glycolytic potential in modern than indigenous (B) pig breeds. Regulation of transcription and metabolic processes were upregulated in LW, in agreement with their higher growth rate, potential for protein deposition and metabolic rate, whereas the ubiquitin conjugation functional class was downregulated in the LW. Although not clustered, genes connected with lipid metabolism were downregulated in the LW, in accordance with their lower muscle lipid content as compared with the B pigs.
Besides, Pearson correlation coefficients were calculated between gene expression values and drip loss, a major pork quality trait, on LW and B pigs. After Bonferroni correction, a list of 192 genes with either positive (n=100) or negative (n=92) correlations of their transcript abundance with drip loss was obtained, with determination coefficients (R^2) ranging between 22% and 67%. Among the 30 genes with the highest R^2, 10 appear to be components of cell membrane, taking part in ions, proteins and glycerol binding and transport; 9 take part in different stages of protein biosynthesis, and 3 are responsible for cell shape and motility. Very high correlations were found for phosphoglucomutase 1 (PGM1) involved in glycolytic pathway, and for caveolin-3, which are generally more expressed in glycolytic than oxidative muscles; this agrees with the negative correlation found in our study between drip loss and the oxidative phosphorylation pathway.

Conclusions

Large variation in meat quality traits were obtained in both experiments and combined with omics technologies this gave rise to several proteomic peaks and transcripts that can be used as biomarkers to predict meat quality. At the same time proteomic peaks can be used to study the effect of stress and resting time on meat quality traits.
The transcriptomics results are also very promising for development of biomarkers to predict meat quality and to better understand the genetic background affecting pork quality.