Research article

Response to oxidative stress as a welfare parameter in swine

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In pigs, the genetic selection for lean, large muscle blocks and fast growth has been linked to an increased prevalence of metabolic diseases such as porcine stress syndrome and mulberry heart disease. These diseases are associated with cardiovascular inadequacy, which may lead to oxidative stress. In the present study, reactive oxygen metabolites (ROMs) and the anti-oxidant power (OXY) in sera of different swine groups were investigated. The following groups were selected (each around 80 kg body weight): wild boars (WB), Cinta Senese (CS), and Landrace × Large White (LxLW), the latter as both specific pathogen-free (SPF) and intensively farmed animals. In addition, a group of LxLW agonic sows (AS) was also investigated; this group is known to be under oxidative stress. Two colorimetric micro-methods were used to measure ROMs and OXY; ROMs were expressed as mM H_2O_2 and OXY as µM HOCl neutralised. Between groups, average ROM and OXY values were found to be significantly different by one-way ANOVA ($P < 0.001$). ROM levels were lower in WB (13.41 ± 1.85) and CS (19.27 ± 1.68), and highest in LxLW (42.00 ± 1.36). OXY values ranged from 260.10 ± 22.13 (WB) to 396.90 ± 9.83 (LxLW). Only one swine group (the CS group) showed a significant, positive correlation between ROM and OXY values. The AS group even showed a negative correlation between ROM and OXY values. These results imply satisfactory environmental coping occurred only within the CS group. Results are discussed in the light of animal welfare legislation, food safety and consumers' protection.

Introduction

In pig meat production over the last 50 years, genotypes have been intensively selected for fat reduction and fast growth in order to improve feed conversion.[1] Although such selection criteria can lead to increased productivity and profitability, they may be detrimental to animal welfare. The indiscriminate over-selection for single traits in pigs has caused many problems in lean, fast-growing pigs.[2] In the Landrace breed selected for muscle block, Dämmrich[3] showed that, in the larger muscle (type II) fibres, the capillary-to-fibre distance is too great for adequate metabolic removal, causing accumulation of lactic acid. Heart strain is more likely in modern breeds because the heart is smaller in relation to body weight: 0.21% of the body weight in the Landrace but 0.38% in a wild boar. In some in-bred lines, the possible deficient oxygen supply in tissues can determine the systematic release, within distinct cell compartments, of reactive oxygen species, such as superoxide anions, nitrogen oxide and hydroxyl radicals. Such free radicals are involved in the pathogenetic mechanism of the following metabolic diseases: (i) mulberry heart disease in weaning pigs caused by a cardio-angiopathy due to lipid peroxidation;[4] (ii) porcine stress syndrome, determined by an abnormal accumulation of lactic acid in the cell compartments;[5] and (iii) osteochondrosis, due to an altered metabolism of bone growth.[6]

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Abbreviations: ROMs, reactive oxygen metabolites; OXY, anti-oxidant power; MHD, mulberry heart disease; PSS, porcine stress syndrome; WB, wild boars; CS, Cinta Senese; LxLW: Landrace x Large White; SPF, specific pathogen-free; AS, agonic sows

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Recommendations for welfare conditions of intensively kept pigs are laid down in a report of the Scientific Veterinary Committee of the European Union.[7] However, there is a lack of data about biochemical parameters which may be used to evaluate acceptable welfare conditions. The standard of acceptable welfare condition is defined by a satisfactory coping ability.

Recently, our group developed and validated two convenient micromethods based on the Fenton reaction.[8] The first method determines early products of oxidation derived from proteins, lipids and nucleic acids (ROMs), such as hydroperoxides, induced by exposure to reactive oxygen species. The second method determines the total amount of free radical scavengers in animal sera, defined by the capability to neutralise a titred HOCl solution (OXY).[9] Because of their reduced cost, high throughput, and simplicity, the veterinary field has already applied such micromethods to the analysis of animal sera in order to evaluate the oxidative stress in farmed animals.[10,11]

In this study, the aforementioned micromethods were used to investigate oxidative stress in the following groups of swine: wild boars (WB), Cinta Senese, a low-inbred free-range pig population reared in a restricted area of the Tuscany region (CS), and three groups of highly in-bred Large White × Landrace pigs: the first reared in an intensive farm (LxLW), the second reared in specific pathogen-free conditions (SPF), and the third reared under agonic conditions due to a feed-borne intoxication (AS).

Materials and Methods

Animals

Wild boars (WB; $n = 16$; 60–80 kg body weight) were shot during the Sardinian serological campaign for classical swine fever, in Autumn 2000. Cinta Senese (CS) pigs ($n = 23$; 80–100 kg body weight) were sampled at farm Belsedere (Trequanda, Siena, Italy). Large White × Landrace pigs were sampled from three different environments: (i) an intensive pig farm (L×LW; $n = 23$; 80–100 kg body weight); (ii) the Laboratory Animals Unit of Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna, rearing specific pathogen-free subjects for xeno-transplantation (SPF; $n = 8$; 80–100 kg body weight); and (iii) a population of 830 pregnant sows suffering acute intoxication due to the presence of oxidised fried oils in their feed stock (AS; $n = 9$; 200 kg body weight).

Blood sampling

Veterinary personnel from the Local Veterinary Unit of Torrita di Siena for CS, from Istituto Zooprofilattico della Sardegna, Sassari for WB, and from Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia, Brescia for LxLW, SPF and AS, drew blood samples from the jugular vein using a Veno Jet tube (Terumo, Leuven, Belgium) with a 19 gauge pin, according to Good Veterinary Practices. After refrigeration at 4°C overnight, sera were recovered by centrifugation (800 *g*, 5 min, 4°C) and tested for the presence of haemolysis. Sera with a haemoglobin content < 0.3 mg/ml were considered suitable for ROM and OXY determinations, labelled and then stored at –20°C until use. Veterinary personnel evaluated the clinical status and the welfare of the pigs sampled, and recorded anamnesis regarding farm management, with respect to breed, stocking rates, weight gain, feed composition. CS and LxLW pigs, found to be healthy at farm, were also inspected at slaughter to check for the presence of pathological lesions due to previous diseases.

Measurement of ROMs and OXY

The following apparatus was used: disposable 1.5 ml polypropylene vials (Eppendorf Italia, Milan, Italy); micropipettes of 5–50 µl, 50–200 µl and 200–1000 µl capacity from Gilson Italia (Milan, Italy); and an automatic analyser for clinical chemistry – the Synchron clinical CX 5 Delta (Beckmann Coulter Italia, Milan, Italy). ROM and OXY kits from Diacron (Grosseto, Italy) were used to determine reactive oxygen metabolites and anti-oxidant power in serum, respectively. For determination of ROMs, swine sera were diluted 1:20 in distilled water before automatic analysis. Briefly, 5 µl of diluted samples were incubated for 5 min at 37°C with 200 µl of a 100:1 mixture containing 0.01 M acetic acid/sodium acetate buffer pH 4.8 and *N,N*-diethyl-*p*-phenylenediamine as chromogen. Absorbances were read at 520 nm in the kinetic mode, at 3, 4 and 5 min. To calibrate the system, a reference standard of 4.5 mM H_2O_2 and a reagent blank were used. For internal quality control, titred sera in the range 0.56–4.5 mM H_2O_2 were inserted as blind samples in the procedure. Results were expressed as mM H_2O_2.

For the determination of OXY, sera to be tested were prediluted 1:100 with distilled water. A 200 µl aliquot of a titred HOCl solution as oxidant was incubated with 5 µl of diluted sera for 10 min at 37°C. Then, 5 µl of the specific chromogen solution was added and the absorbance read at 520 nm. Calibration was achieved by using a reference serum able to neutralise 440 µM HOCl. Appropriate internal quality controls were inserted in the procedure. Results were expressed as µM HOCl neutralised.

Statistical analysis

Based on the previous evidence that ROM and OXY values are normally distributed ($n > 100$) among cattle and

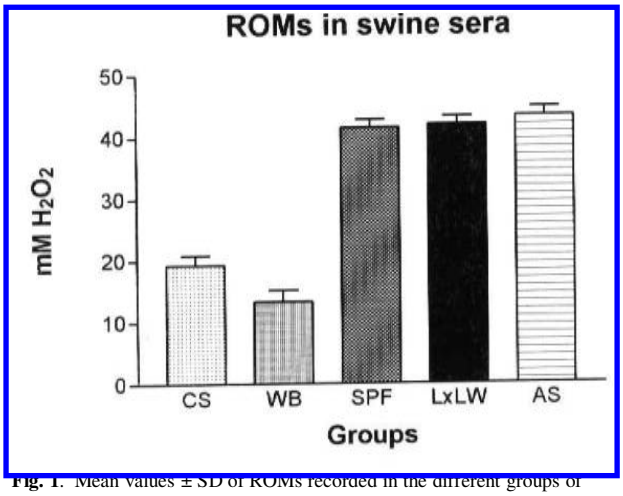

Fig. 1. Mean values ± SD of ROMs recorded in the different groups of swine.

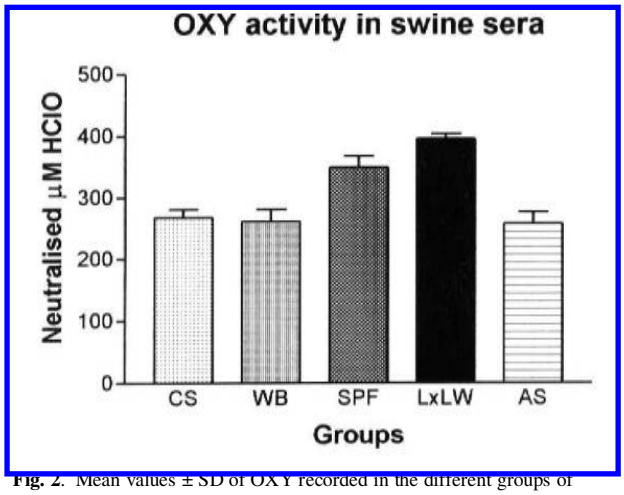

Fig. 2. Mean values ± SD of OXY recorded in the different groups of swine.

pig populations, averaged data from each group were checked for statistical significance by one-way ANOVA; the Newman-Keuls post-test was adopted to compare different pairs of groups. The co-variation of ROM and OXY content within each group was evaluated by assessing the two-tail Pearson correlation value. The threshold for statistical significance was set at $P \leq 0.05$.

Results and Discussion

Absolute values of ROMs and OXY dosed in sera of the 5 different pig groups are reported in Figure 1 and 2, respectively. The differences between groups (one-way ANOVA) were significant for both parameters under study ($P < 0.001$); the differences related to each group pair (Newman-Keuls post-test) are reported in Table 1.

A significant correlation between ROM and OXY content was shown only in the CS group ($P = 0.002$); accordingly, the r value for the CS group is higher (+0.623) than groups WB (+0.162), SPF (+0.055) and LxLW (+0.224). Interestingly, a negative correlation value (–0.533) was found in the AS group. The distributions of test data in the CS and LxLW groups are shown in Figure 3.

Table 1. Differences of ROM and OXY values related to each group pair (Newman-Keuls post-test)

Group pairs	ROMs *P* value	OXY *P* value
CS versus WB	< 0.05	> 0.05
CS versus SPF	< 0.001	< 0.001
CS versus LxLW	< 0.001	< 0.001
WB versus SPF	< 0.001	< 0.01
WB versus LxLW	< 0.001	< 0.001
SPF versus LxLW	> 0.05	< 0.05
AS versus LxLW	> 0.05	< 0.001
AS versus SPF	> 0.05	< 0.01
AS versus CS	< 0.001	> 0.05
AS versus WB	< 0.001	> 0.05

Different approaches have been proposed for the evaluation of welfare in farmed animals: behavioural,[12,13] clinical,[14] zootechnical,[15] hormonal,[16] biochemical and immunological.[18] Clinical chemistry is preferable due to the robustness of the main parameters measured, the reproducibility of the results, the opportunity for evalu-

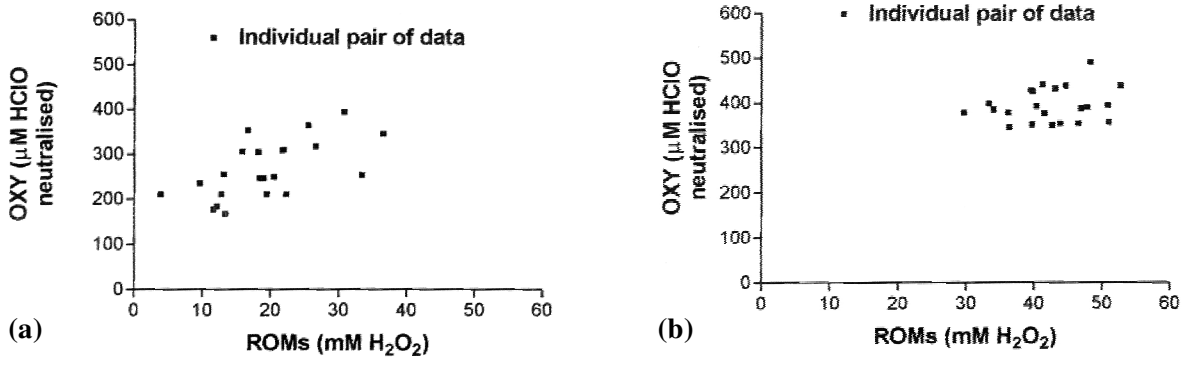

Fig. 3. Correlation between ROM and OXY values in the CS (a) and LxLW (b) groups.

resources and time requested, and the compatibility with animal handling procedures, *i.e.* during blood drawing.[18]

The decision to investigate oxidative stress in genetically distinct groups of swine was prompted by the known correlation between genetic selection and impairment of cardiovascular systems in swine and the possible association between the latter and oxidative stress. Previous studies have shown the serious pig metabolic diseases can be traced back to oxidative stress and that basal ROM levels in resting pigs, determined by the same micromethod used in the present study, may be 10–15 times higher than those of humans and cattle when a human reference serum was used as calibrator.[19] Such differences between human and swine values could be partially accounted for by pre-analytical error, because of the possible instability of analytes in swine sera stored at 4°C overnight (clotting time). On the other hand, the threshold for acceptance (< 3 mg/ml haemoglobin) in sera to be analysed, and the reproducibility of the results in pig and cattle sera stored at 4°C for 24 h and 48 h,[11] suggest that such a source of variability should marginally affect the comparison between the different animal species under study. Therefore, errors introduced by the clotting procedure and the storage conditions of serum samples may only marginally contribute to the dramatic difference observed in serum samples of ROMs and OXY between humans and swine.

In the present study, pig groups were selected with a strongly different genetic background, basically homogeneous for the body weight.

Both ROM and OXY values varied significantly between swine groups (Figs 1 & 2). Since there are no major differences between the SPF and LxLW groups, we conclude that the importance of infectious pressure in determining the observed variations would not be large; instead, these variations can be reasonably traced back to genetically-based factors, related to body mass and weight gain. Our findings also indicate that the coping ability of swine varied significantly among groups: this was probably optimal in the CS group which mounted a proportional OXY response to ROMs in a reasonable range of the dose-response curve (Fig. 3). In contrast, the response was skewed in groups WB, SPF and LxLW: it should be noted that samples from WB were drawn from shot animals, *i.e.* in the presence of tissue damage. No coping was evidenced in the AS group; its negative correlation value (Table 1) implies a major impairment of the anti-oxidative defence mechanism. In addition, by comparing ROMs in the SPF, LxLW and AS groups, we conclude that the response was probably set at a plateau level (Fig. 1, Table 1).

The above findings clearly point out a fundamental difference between stress and distress situations, whereby the former can be effectively counteracted by an adaptive response, but the latter implies an impairment of such a response. Consequently, pigs in good welfare conditions should show proportional and positive OXY response to ROM release; pigs forced to cope with a prolonged oxidative stress should show a non-proportional and positive OXY response; pigs with major impairment would show a negative correlation, due to inadequate production or renewal of antioxidant molecules and/or their progressive depletion by increases in the formation of ROMs.

Conclusions

The assessment of the coping ability of farmed pigs by the methods used in the present study may provide objective biochemical data relevant to the possible improvement of animal welfare assessment. In particular, such data may provide a basis for predicting oxidative stress coping ability according to genetics. Within the frame of consumer protection in a 'from farm-to-fork' scheme, consumers are paying increasing attention to informative labelling about animal keeping and quality of edible fats. A reliable oxidative stress evaluation at the farm level may constitute an effective source of information to meet such demands placed by consumers. Such evaluations may also be relevant to the choice of organs for xenotransplantation, which should not be submitted to a major oxidative stress.

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