Traceability of Inbred and Crossbred Cinta Senese Pigs by Evaluating the Oxidative Stress

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Summary

Previous studies on the oxidative stress in swine indicated a strong link between the values of reactive oxygen metabolites (ROMs), the subsequent antioxidant adaptive response (OXY) and the genetic selection. Such findings, mainly related to a cardiovascular inadequacy in lean, large muscle blocks and fast growing breeds, is associated with specific metabolic diseases such as porcine stress syndrome and mulberry heart disease. In this study, we investigated the oxidative stress parameters to trace the genetics of Cinta Senese (CS) pigs, a historical breed free-range reared in Siena countryside. Sera from CS (n = 24) and Large White × CS (LW × CS) (n = 24) groups around 120 kg body weight fed the same diet were sampled at slaughter. Sera from wild boars (WB) (n = 24) hunted in the same district were also considered. ROMs and OXY were evaluated in the three groups of swine. Significant differences by one-way ANOVA (P < 0.05) between groups were found for both procedures. ROM levels were lower in WB (9.79 ± 5.76 mmol H₂O₂) and CS (18.02 ± 7.42 mmol H₂O₂), and highest in LW × CS (42.78 ± 8.61 mmol H₂O₂). OXY values ranged from 271.37 ± 50.90 µmol neutralized HOCl (WB) to 343.21 ± 57.45 µmol neutralized HOCl (LW × CS). Results indicated that the evaluation of the oxidative stress can effectively trace the CS pigs, thus aiding in preserving the overall specific traits of such a historical animal.

Introduction

In past years, pig husbandry has been addressed for the selection of better genotypes to reduce fat and to increase growth, thus improving the feed conversion (Cameron, 1994). Although such criteria can lead to an increased economic performance, they may be detrimental to animal welfare. In some inbred lines, the possible deficient oxygen supply in tissues can determine the systematic release of reactive oxygen species, such as superoxide ion, nitrogen oxide and hydroxyl radical, naturally confined in distinct cell compartments. Such free radicals are involved in the pathogenetic mechanism of the following metabolic diseases: (a) mulberry heart disease in weanling pigs, caused by a cardioangiopathy due to lipid peroxidation (Rice and Kennedy, 1989); (b) porcine stress syndrome, determined by an abnormal accumulation of lactic acid in the cell compartments (Christian and Lundstrom, 1992); and (c) Osteochondrosis, due to an altered metabolism of bone growth (Stern et al., 1995). Recently, our group evaluated the oxidative stress in piglets to predict the outbreaks of the mulberry heart disease (Brambilla et al., 2001) and in low and high inbred swine (Brambilla et al., 2002).

The aim of this work was to study the oxidative stress as a consequence of the different breeding practices in two different populations, inbred and crossbred Cinta Senese (CS), an ancient suilline race native of the Siena countryside still reared in extremely limited numbers. Because its growth rate is very low, the CS race can be crossbred with Large White (LW) male in order to improve the weight gain and feed conversion of the crossbred offspring (LW × CS).

Materials and Methods

Animals

Wild boars (WB) (n = 24), about 60 kg body weight (BW), shot during the serological campaign for classical swine fever in the autumn of 2001. CS pigs (n = 24), 120 kg BW, were sampled at farm Belsedere, at a small slaughterhouse (Trequanda, Siena, Italy). LW × CS pigs (n = 24) were sampled at Arezzo slaughterhouse.

Blood sampling

Veterinary personnel from the Local Veterinary Unit of Torrita di Siena for CS and LW × CS, drew blood samples from jugular vein by Veno Jet tube (Terumo, Leuven, Belgium) with a 19-Gauge pin according to Good Veterinary Practices. WB were shot by hunters in the countryside between Siena and Grosseto districts in the autumn of 2001. The presence of classical swine fever in wild animals was investigated. Blood samples were collected from WB pigs within 3 min after death and brought to the laboratory. After refrigeration at 4°C, sera were recovered by gentle centrifugation (800 × g for 5 min at 4°C) and tested for haemolysis. Sera
with a haemoglobin content <0.3 mg/ml were considered suitable for reactive oxygen metabolites (ROMs) and antioxidant (OXY) determinations, labelled and then stored at −20°C until use. Veterinary personnel recorded the anamnesis about farm management, with respect to breed, stocking rates, weight gain and feed composition. The clinical status and the welfare of pigs sampled were evaluated in the ante mortem inspection, while the possible presence of pathological lesions due to previous diseases was checked in the post mortem inspection, at slaughter.

Measurement of ROMs and OXY
The following laboratory facilities were used: disposable 1.5 ml polypropylene vials from Eppendorf Italia (Milan, Italy), micropipettes 5–50 μl, 50–200 μl and 200–1000 μl from Gilson Italia (Milan, Italy), 96-wells plates from Nunc-immuno plate (Nalge Nunc Italia, Roma, Italy), ROMs and OXY kit from Diacron (Grosseto, Italy) able to measure ROM and OXY power in serum, respectively. The former determines the early reagents, such as hydroperoxides, induced by the reactive oxygen species following the oxidation of proteins, lipids and nucleic acids (ROMs) (Alberti et al., 2000); the latter determines the total amount of free radical scavengers, in terms of capability to neutralize a titred HOCl solution in animal sera (OXY) (Prior and Cao, 1999).

For determination of ROMs, swine sera were diluted 1:10 in distilled water before analysis. Briefly, 5 μl of diluted samples were incubated for 75 min at 37°C with 200 μl of a chromogen solution 100:1 (0.01 M acetic acid/sodium acetate buffer pH 4.8: N,N-diethylparaphenylendiamine). Absorbances were read at 520 nm in end point mode. After 15 min a reference standard 4.5 mM H₂O₂ and reagents blank were used for the calibration. For internal quality control, titred sera in the interval 0.56–4.5 mM H₂O₂ were inserted in the procedure. Results were expressed as mM H₂O₂.

For the determination of OXY, sera to be tested were prediluted 1:100 with distilled water. Two hundred microlitres 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 absorbances read at 520 nm as endpoint. Calibration was achieved by using a reference serum which is able to neutralize 440 μM HOCl. Appropriate internal quality controls were inserted in the procedure. Results were expressed as μM neutralized HOCl.

Statistical analysis
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 ROMs 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
Growth and performance traits of the three different swine groups, WB, CS and LW × CS during the period 30–100 kg are shown in Table 1.

Absolute values of ROMs and OXY measured in sera of the investigated animals determined by groups (breed) are reported in Figs. 1 and 2, respectively. The differences between groups (one-way ANOVA) were significant for both parameters investigated ($P < 0.05$).

A high positive and significant correlation between ROMs and OXY values was shown only in the CS group ($P = 0.002$; $r = +0.619$) (Fig. 3 top). The correlation between ROMs and OXY values was not significant for the LW × CS ($r = +0.007$) (Fig. 3 bottom) and WB ($r = -0.013$) groups.

Discussion
We decided to investigate oxidative stress because of the strong correlation between genetic selection and impairment of the cardiovascular system in swine (Dämmrich, 1987). In the LW breed selected for muscle block, Dämmrich and colleagues showed that in the large skeletal muscle, the capillary-to-type II fibre distance is too great for adequate metabolic removal.

![Fig. 1. Serum levels of ROMs in the investigated pigs determined by groups. Mean values with different letters differ significantly ($P < 0.001$). Values are expressed as mean ± SD ($n = 24$ each), in mM H₂O₂. The error bars represent the SD.](image-url)

Table 1. Growth and performance traits of the investigated animals determined by groups (breed)

<table>
<thead>
<tr>
<th>Groups</th>
<th>$n$</th>
<th>Average age (days)</th>
<th>Average body weight (kg)</th>
<th>Average daily weight gain (g) during the period 30–100 kg</th>
<th>Average feed consumption (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>24</td>
<td>300 ± 58</td>
<td>60 ± 17</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CS</td>
<td>24</td>
<td>420 ± 36</td>
<td>120 ± 5</td>
<td>370 ± 21</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>LW × CS</td>
<td>24</td>
<td>270 ± 12</td>
<td>120 ± 4</td>
<td>480 ± 22</td>
<td>2.4 ± 0.2</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. ND: Not Determined.
In these large fibres, lactic acid will accumulate. Selection for heart muscle is more likely in modern breeds because the heart is smaller in relation to BW: 0.21% of the BW in the LW but 0.38% in a WB. In order to confirm this hypothesis, we selected CS groups (similar BW and feeding) with a different genetic background, and we evaluated both the oxidative stress and the ability of coping. The absence of clinical or pathological findings after the slaughter inspection indicates that infectious pressure would not influence, to a large extent, the observed differences. Instead, these can be reasonably traced back to genetic-based factors, related to body mass and weight gain.

Significant differences were noted for both ROMs and OXY values between free-range CS group and LW x CS (Figs. 1 and 2) group, indoor reared, and with a faster evolution of the weight gain (Table 1). This could be addressed reasonably to a better oxygen supply to the muscular mass in LW x CS than in CS, and to a more equilibrated ratio between muscular mass and the cardiovascular system.

We showed a significant positive correlation between ROMs and OXY values (Fig. 3) in the CS group but not in the LW x CS group. These findings clearly point out a fundamental difference between stress and distress situations, whereby the former can be effectively counteracted by an adaptive response, the latter implies an impairment of such a response. Owing to the above, CS in good conditions should show a proportional and positive OXY response to ROM release; LW x CS group, forced to cope with a genetic-based oxidative stress should show a non-proportional and positive OXY response, with OXY values set at the plateau level that could represent the maximum adaptive response, as noted in weaning pigs susceptible to mulberry heart disease (Brambilla et al., 2001) and in high inbred LW x Landrace pigs even if reared as specific pathogen-free subjects (Brambilla et al., 2002).

Data from WB, because reasonably affected by the prolonged stress situation determined by their hunting and by the presence of tissue damage after the shooting, should be mainly considered as an useful indication of the possible basal OXY and ROM values in swine not undergoing any genetic selection.

The cross-breeding of CS with fast growth pig lines, such as LW and Landrace, could expose such animals to a distress status, thus compromising their welfare and, possibly, the nutritional quality of fats and meat due to lipid peroxidation, if not counterbalanced by an appropriate antioxidant supply in the feed. Nevertheless, such exogenous antioxidant supply should be prolonged in time and, if not dosed appropriately, could provoke a pro-oxidant effect, thus worsening the distress situation (Podmore et al., 1998). So, the oxidative stress as welfare parameter can really improve the pig management by predicting the coping ability of pigs intended for meat production. In our case, because mainly correlated with genetics, and not influenced to a great extent by different feeding, the oxidative stress parameters could be a reliable tool to reinforce the overall traceability of not crossbred CS production, from farm to fork.

Last but not least, our data may be relevant to the choice of pig breeds intended as organ donors for xenotransplantation, which should not be submitted to a major oxidative stress. In such a frame, CS would be proposed as breed of selection, with respect to LW x Landrace ones.

Acknowledgements
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Fig. 2. Serum levels of OXY in the investigated pigs determined by groups. Mean values with different letters differ significantly (P < 0.001). Values are expressed as mean ± SD (n = 24 each), in µM neutralized HOCl. The error bars represent the SD.

Fig. 3. Correlation (r) between ROMs and OXY values in CS (top) and LW x CS (bottom) groups.

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References


