Performance of the antioxidant protection in blood of highly prolific sows before and after farrowing

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SUMMARY

Increased metabolic burdens put on highly prolific sows during late gestation and lactation cause their exposition to elevated systemic oxidative stress, i.e. uncontrolled imbalance between production and neutralization of reactive oxygen species (ROS). Formation of active free radicals and thus lipid per oxidation (LPO) processes in the body are regulated by the functioning of antioxidant protection (AOP) system.

The aim of presented study was to examine performance of oxidative stress in the blood of highly prolific sows before after farrowing.

The experiment was conducted on Large White sows (n = 20) of the second farrowing with live weight ranging from 180 to 220 kg. The study started on the 100th day of the farrowing period (pregnancy) and continued until the 25th day after farrow. The material for the study was sows blood obtained from the ocular vein 14 days before (-14d) and on the 10th and 25th days after farrowing (+10d and +25d).

All the biochemical researches were conducted spectrophotometrically. The intensity of oxidative stress in the blood plasma was estimated by the content of lipid hydroperoxides (LHP) using ammonium thiocianate; thiobarbituric acid reactive substances (TBARS) and carbonylproteins (CP) with using Lushchak et al. method (2004). The state of AOP in the erythrocytes was determined by the level of enzymes activity, such as: superoxide dismutase (SOD) with using of nitroblue tetrazolium in the system of NADH-pfenazine methosulfate; catalase (CAT) according to the ability of H₂O₂ to make a complex with ammonium molybdate (VI) tetrahydrate; glutathione peroxidase (GP) by the speed of glutathione oxidation in the presence of tert-butylhydroperoxide; glutathione reductase (GR) by the content reduction of NADPH. The amount of reduced glutathione (GSH) was found by the reaction with 5,5-dithiobis-(2-nitrobenzoic) acid.

In research, sharp dynamics of oxidative stress indices in the blood of sows was established. Before farrows, the level of LHP was 1.5- times lower while concentrations of TBARS and CP were about 40% higher in comparison to the +25d (P ≤ 0.05). Simultaneously, in relation to the -14d activity in the erythrocytes of SOD and CAT increased about 10% (P ≤ 0.05), while GP and GR and also concentration of GSH decreased about 1.5- times (P ≤ 0.05) on the +10d.

In conclusion, changes detected in the blood of sows in critical periods of ontogenesis show development of stress condition in their body and the increase of disease appearance risk due to the weakening of adaptation mechanisms.

KEY WORDS

Oxidative stress, antioxidant enzymes, swine, lactation, gestation.

INTRODUCTION

The basis of successful pigs production is high reproductive efficiency of sows. It can be achieved by continuous improvement of genotype, nutrition and housing. However, increased metabolic burdens put on highly prolific sows during late gestation and lactation cause their exposition to elevated systemic oxidative stress.

Oxidative stress is caused by uncontrolled imbalance between production and neutralization of reactive oxygen species (ROS). Formation of active free radicals and thus lipid per oxidation (LPO) processes in the body are regulated by the functioning of antioxidant protection (AOP) system. AOP is located in all cells and biological fluids. It protects biologically important proteins and other macromolecules from peroxidative damage caused by ROS. It is conditioned by enzymatic and non-enzymatic reactions that eliminate initial and final products of free radical processes and inhibit the emergence of oxidation chains. The AOP not only prevents development of free radical reactions and accumulation of superoxide anions and peroxides but also supports high activity of redox processes and ensures elimination of final oxygen metabolites involving them in energy metabolism and the process of synthesis activation.

It seems that the measures of APO may be very useful in animals health protection. It is known that oxidative stress can be involved in variety of pregnancy complications (such as preterm labor, fetal growth restriction, preeclampsia and miscarriage) and also plays a role in the pathophysiology of infertility. Moreover, it has been shown in humans and ruminants that an antioxidative status of the
mother influences the quality of colostrums and milk and in this way the antioxidant defense of a newborn. Therefore, disturbance of AOP during pregnancy and lactation cannot only affect well-being of sows but also the piglets' 2,3. In the above context, it seems interesting to define some parameters of antioxidant protection and lipid peroxidation in the blood of highly productive sows. The research was carried out for better understanding of adaptive processes of formation of anti-radical and anti-peroxide protection mechanisms of highly productive animals’ bodies.

MATERIALS AND METHODS

Experimental design
The experiment was conducted on a private farm on Large White sows (n = 20) of the second farrowing with live weight ranging from 180 to 220 kg. The ration of the animals contained premix Monix 4% (on the basis of French vitamin and mineral mixes) that is used on the farm for pregnant and lactating sows feeding. The study started on the 100th day of the farrowing period (pregnancy) and continued until the 25th day after farrow. Initially the animals were fed in accordance with the diet suitable for the period of farrowing; then it was conducted according to free technology with unrestricted access of the sows and piglets to food and water. The piglets were kept under sows in stalls, separately for each sow. The material for the study was composed of sows blood samples obtained from the ocular vein using 9 ml blood collection system S-Monovette (SARSTEDT, Germany) with 18 gauge ×100 mm needle) 14 days before (-14d) and on the 10th and 25th days after farrow (+ 10d and + 25d). The blood samples were kept in room temperature for about 30 min and centrifuged at 3,000 rpm for 15 min. Then plasma and erythrocytes samples were stored at -23°C for subsequent analysis.

Processing
All the biochemical researches were conducted spectrophotometrically. The intensity of oxidative stress in the blood plasma was estimated by the content of primary lipid peroxidation (LPO) products - lipid hydroperoxides (LHP) using ammonium thiocianate, while the concentration of terminal LPO products such as thiobarbituric acid reactive substances (TBARS) and the content products damage protein molecules - carbonylproteins (CP) were determined by the Lushchak et al. method.

The state of AOP in the erythrocytes was determined by the level of enzymes activity. Superoxide dismutase (SOD) was estimated with help of nitroblue tetrazolium in the system of NADH-pfenazine methosulfate; catalase (CAT) - according to the ability of H2O2 to make a complex with ammonium molybdate (VI) tetrahydrate; glutathione peroxidase (GP) - by the speed of glutathione oxidation in the presence of tert-butylhydroperoxide; glutathione reductase (GR) - by the content reduction of NADPH. The amount of reduced glutathione (GSH) was found by the reaction with 5,5-dithiobis-(2-nitrobenzoic) acid.

Statistical analysis
The analysis of the data was performed by one-way analysis of variance (ANOVA) followed by Tukey’s test, using a computer program SPSS 10.1 (SPSS). The value of the results was ascertained at P ≤ 0.05. The figures were prepared using Grapher 7.0 (Golden Software Inc., USA).

RESULTS
In the course of our research, sharp dynamics of oxidative stress indices in the blood of sows was established. During prenatal period, various concentrations of primary and terminal LPO products in blood plasma were observed. The level of LHP in the -14d and +10d was 1.5- and 1.3- times lower in comparison to the +25d, respectively (P ≤ 0.05, Fig. 1A). During that time, concentration of TBARS and CP was highest before farrow and then decreased on +10d to 74.6% (P ≤ 0.01) and 95.7% (P > 0.05) and 73.4% and 65.4% (P ≤ 0.01) of level at -14d, respectively (Fig. 1B, C). Simultaneously, activity of SOD and CAT in the erythrocytes increased on the +10d by 12.3% (P ≤ 0.05) and 9.6% (P ≤ 0.01) in relation to the -14d and decreased by 13.9% (P ≤ 0.05) and 21.8% (P ≤ 0.01) on the +25d (Fig. 2A, B). However, in comparison to the before-farrowing period, GP activity in erythrocytes decreased by 1.6 - and 1.5 - times (P ≤ 0.05; Fig. 3A) and activity GR 1.6 - and 2.2 times (P ≤ 0.01; Fig. 3B), while concentration of GSH decreased in the blood of sows 1.5-timmes (P ≤ 0.001) and increase to 1.3-times (P ≤ 0.001) on the +10d and +25d, respectively (Fig. 3C).

DISCUSSION
During our research it was found that sows’ farrow is accompanied by high intensity of oxidative processes in their blood. Thus 14 days before farrowing (-14d) high concentrations of TBARS - LPO metabolites as well as the products of free radical damage to protein molecules (CP) were set in the animals’ plasma. Already on the 10th day after farrow the (+ 10d), content decrease of the above-mentioned metabolites of oxidative processes was observed, their concentration being still lower on the 25th day after farrow (+25d). However, even at the end of the study it is too early to talk about the complete normalization of peroxidation processes in the sows’ organisms because concentration of lipid hydroperoxides was still increasing. As it is known from the literature, the second period of farrowing in sows (from the 85th to the 114th day) and lactation period are characterized by increased metabolism and the growth of energy needs. These changes in their organisms are explained by the fact that much more energy and nutrients in sows are spent on the formation of a foetus. This period in mammals is characterised by large consumption associated with the use of substrates and energy matters while the deficit of the required amount of energy in the diet leads to the metabolic disturbances in the organism of dam and as a result the activation of the destructive action of free-radical processes in the cells. It results in oxidative stress which ultimately may cause the decrease of fertility and increase of non-viable offspring. Enzymatic antioxidant defence system at the level of the whole body is represented by erythrocytic enzymatic system, including SOD and CAT as the original chain of protection from superoxide radicals and hydrogen peroxide as well as...
Glutathione final link - GP and GR as protection from both hydrogen peroxide and organic hydroxides. GR provides glutathione regeneration from the oxidized form into the reduced one. Thus, glutathione as an acceptor of ROS is capable to inhibit free radical oxidation. Our study confirmed that the decrease of both TBARS and CP concentrations in the blood of sows on the 10th day after farrowing is accomplished by the increased activity of SOD and catalase.

**Figure 1** - The concentration of products of oxidative stress: A) lipid hydroperoxides (LHP), B) thiobarbituric acid reactive substances (TBARS) and C) carbonylproteins (CP) in the blood plasma of highly prolific sows (n = 20) 14 days before farrowing (-14d) and 10 and 25 days after farrowing (+14d and +25d).

**Figure 2** - The activity of A) superoxide dismutase (SOD) and B) catalase (CAT) in the erythrocytes of highly prolific sows (n = 20) 14 days before farrowing (-14d) and 10 and 25 days after farrowing (+14d and +25d).
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...ver, on the +25d the decline in SOD activity to the baseline was observed and that of catalase fell even lower in relation to the level before giving birth which may indicate strain in the antioxidant body system resulting in oxidative stress. This assumption is proved by the reduction of all studied parameters of ADS glutathione link (GP, GR and GSH) in the sows blood on the +10d as to the beginning of the experiment. On +25d the growth of GSH content was set in the blood of animals probably due to compensatory increase of its synthesis but not due to GR reduction, activity of which was too low. The GSH concentration increase partly explains the growth of GP activity during this period; though its activity was still lower than the level before farrow. The increasing LHP concentration in the blood of sows during all the period of research may be explained by GP low activity in their blood.

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The established decrease in the enzyme link activity of ADS and LHP concentration in the blood of sows on +25d may also be due to reorientation of metabolism in their organisms which coincides with the beginning of the abrupt decrease in milk formation processes from the twenty-first day after farrowing.

Thus, the research on sows showed that during farrowing period and especially after farrowing the activation of free radical processes takes place in their organisms. During the first 25 days after farrowing, ADS of sows does not have time to restore completely and overcome oxidative stress occurs. It is advisable to strengthen dams when necessary with help of biologically active substances prevention, adaptation, immune-stimulating and antioxidant action as well as using the corresponding physiologically optimal conditions of maintenance and animal nutrition.

Summing up, changes detected in the blood of highly prolific sows in critical periods of ontogenesis show the development of stress condition in their body and the increase of disease appearance risk due to the weakening of adaptation mechanisms. The researches of the influence of natural antioxidant importance to the metabolism of high productive animal with the aim of their safety, adaptability and productivity increase should be conducted.

ACKNOWLEDGEMENT

This study was financed by Ukrainian Government and as part of the project DS 3210/KHDZFIZ of University of Agriculture in Krakow, Poland.

References


Figure 3 - The activity of A) glutathione peroxidase (GP), B) glutathione reductase (GR) and C) concentration of reduced glutathione (GSH) in the erythrocytes of highly prolific sows (n = 20) 14 days before farrowing (- 14d) and 10 and 25 days after farrowing (+ 14d and + 25d).