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KRL test to objective evaluation of welfare: sensibility to housing conditions and dietary supplements

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Abstract. The aim of this work was to determine the sensibility of KRL test to evaluate pig welfare in relation to different housing conditions and dietary supplements. It is reported that the response to oxidative stress could be considered as welfare parameter in swine. The KRL test allows the evaluation of total blood antioxidant activity. In the first trial we evaluated the total blood antioxidant activity of 12 swine allotted respectively to solid and totally slatted floor. In the second trial blood samples were obtained at 0, 15 and 60 days from 10 weaned piglets fed control diet and diet supplemented with vitamin E (54 mg/kg) respectively. The first trial showed that the total antioxidant activity of red blood cells resulted higher in pig allotted to the solid floor ($P=0.01$). In the second trial the KRL test evidenced an increase in the total antioxidant activity of whole blood and red blood cells of piglets fed diet supplemented with vitamin E ($P<0.05$). The present research showed that the KRL test is able to discriminate welfare in relation to different housing conditions and dietary supplements.

Keywords. Total blood antioxidant activity – KRL test – Welfare – Pig.

Emploi du test KRL pour une évaluation objective du bien-être : sensibilité aux systèmes d'élevage et à la supplémentation alimentaire

Résumé. La sensibilité du test KRL pour l'évaluation du bien-être des porcs a été étudiée en relation avec le système d'élevage et la supplémentation alimentaire. La réponse au stress oxydatif est un paramètre pour l'évaluation du bien-être chez le porc. Le test KRL permet de mesurer la résistance globale des individus vis-à-vis de l'agression des radicaux libres. Dans un essai d'engraissement le pouvoir de défense antiradicalaire global a été évalué sur 12 porcs élevés sur béton plein ou sur caillebotis intégral. Dans le second essai des prélèvements sanguins sont réalisés au sevrage, à 15 et 60 jours après sevrage sur 10 porcelets allottés en 2 régimes alimentaires identiques, à l'exception de l'ajout de vitamine E (54 mg/kg). L'activité antioxydante totale des hématies est plus élevée chez les porcs élevés sur béton plein ($P=0,010$). Le test KRL démontre une augmentation du pouvoir de défense antiradicalaire global du sang et des hématies chez les porcelets alimentés avec vitamine E ($P<0,05$). En conclusion, le test KRL est capable de discriminer le bien-être en relation avec le système d'élevage et la supplémentation alimentaire.

Mots-clés. Pouvoir de défense antiradicalaire global – Test KRL – Bien-être – Porc.

I – Introduction

The management of production animals has changed radically across the European Union (EU) over the past five decades. Over this time, animal agriculture has intensified and the animals have been moved to indoor housing systems with higher stocking densities. Over the past years a considerable amount of scientific research has focused on animal welfare (Broom 1991; Sandøe *et al.*, 2003). Performance records, behavioural, physiological and clinical parameters are considered as good indicators for assessing animal welfare (McGlone, 2001; Broom 1996). The welfare in pig species could be evaluated through three different basic approaches. The first method is based on the normal biological functioning including the physical and physiological condition of the animal. The second approach regards animal feelings (Broom 1991) and it can be assessed using ethological parameters. The third, called functional approach, is based on

physiological, immunological and pathological indicators, that currently offers interesting data on pig welfare (Barnett *et al.*, 2001).

In the recent years oxidative stress became an important goal in human and animal research as cause of some disease. The “reactive oxygen metabolites” (ROM) are produced endogenously by normal metabolic processes, but amounts may be increased markedly by exogenous factors (Machlin and Bendich 1987). Deficiencies of natural protective substances or excess exposure to stimulators of ROM production may result in oxidative stress, defined as an imbalance between oxidants and antioxidants at the cellular or individual level (Finkel and Holbrook, 2000).

In farm animals, oxidative stress may be involved in several pathological conditions, that are relevant for animal production and the general welfare of the individuals (Lykkesfeldt *et al.*, 2007). Diseases such as pneumonia and sepsis have been shown to involve altered redox balance in pigs (Lauritzen *et al.*, 2003; Basu and Eriksson, 2000). Brambilla *et al.*, (2002) reported that the response to oxidative stress can be utilized as welfare parameters in pigs.

Methods for quantifying oxidative stress include the assessment of the total antioxidant capacity of serum or plasma. The Trolox equivalent antioxidant capacity (TEAC) assay, the oxygen radical absorbance capacity (ORAC) assay, and the ferric reducing ability of plasma (FRAP) assay are commonly used and have been extensively evaluated. These methods allow to measure the total antioxidant capacity of serum or plasma without consider the antioxidant defences in the red blood cells.

The KRL test shows the resistance to free radicals assessed as the time needed to haemolyse 50% of red blood cells exposed to a controlled free radical attack and provides an assessment of total antioxidant defences, since all families of antioxidants present in whole blood are used to fight off the oxidant attack (Stocker *et al.*, 2003; Girard *et al.*, 2005). The KRL test has several applications; in humans KRL is used to study the effectiveness of natural or pharmaceutical treatments and to discriminate welfare conditions depending on medium or high stress.

In the pig production chain, the relationships between housing system and welfare became an important goal of interest (EFSA 2005). The floor type is a determinant factor of certain foot lesions (Gillman *et al.*, 2009) and can affect health, performance and behaviour (Ruiterkamp 1987). In particular on totally slatted floor the animal movement decreases and they spend more time lying down (Rossi *et al.*, 2008). Higher stress during pre-slaughter handling was also observed in pigs reared on totally slatted floor than on solid floor (Nanni Costa *et al.*, 2007).

Another goal of interest are the elevated oxidative stress in the post weaning phase that can lead to increased prevalence of infectious disease. Weaning are reported as one of the critical stages for dietary vitamin E as a nutrient for growth and health status in pigs (Lauridsen *et al.*, 2005). In fact the weaning-induced stress and the decline in the antioxidant level causes an elevated oxidative status in weaned piglets (Rossi *et al.*, 2009; Sauerwein *et al.*, 2005).

The goal of the present experimental work was to determine the sensibility of KRL test to evaluate pig welfare in relation to different housing conditions and dietary supplements.

II – Materials and methods

Two independent trials were performed to test the KRL test sensibility to discriminate the effect of the housing conditions and dietary supplement on total blood antioxidant activity in pigs.

In the first trial 24 barrows of a live weight in the range of 80 to 120 kg, half allotted to solid floor and half to totally slatted floor, were randomly selected. Fasting blood samples were taken by anterior vena cava puncture, collected in 10 mL vacutainer glass tubes containing EDTA (Venoject®, Terumo Europe N.V., Leuven, Belgium) and immediately placed on ice pending analysis. The analysis were performed within 24 h from the sampling procedure.

In the second trial eighty weaned Dalling piglets of an average weight of 7 kg, were assigned, on the basis of weight and sex, to two dietary treatments: control diet (175 mg/kg of vitamin E) and a diet supplemented with the basal level plus 54 mg/kg of vitamin E (225 mg/kg of vitamin E). The experimental diets were formulated to be isoenergetic and to meet or exceed the NRC requirements for all nutrients, and was presented for *ad libitum* consumption. At weaning, 15 and 60 d post weaning, 10 piglets per treatment were randomly selected and fasting blood samples were taken by anterior vena cava puncture, collected in 10 ml vacutainer glass tubes containing EDTA (Venoject®, Terumo Europe N.V., Leuven, Belgium) and immediately placed on ice pending analysis. The analysis were performed within 24 h of collection.

Total antiradical activity of whole blood and red blood cells (RBC) for each pig was evaluated using KRL biological test (Laboratoires Spiral, France). The KRL test is currently used to test the capability of erythrocytes to resist a standardized production of free radicals generated from the thermal decomposition of a 27 mmol/L solution of 2,2'-azobis (2-amidinopropane) hydrochloride at 37 °C (Prost, 1992; Blache and Prost, 1992). Whole Blood and RBC samples diluted to 1/50 were submitted in isotonic saline solution to organic free radicals. Haemolysis was recorded using a 96-well microplate reader by measuring the optical density decay at 450 nm (Fig. 1).



Fig. 1. KRL reader and 96-well microplate.

Results were expressed as the time required to reach 50% of maximal haemolysis (half-haemolysis time - HT₅₀ - in minutes), which refers to the whole blood resistance to free-radical attack.

In the first experimental trial one-way ANOVA was used to determine statistically significant differences between the two housing conditions. In the second experimental trial the data were analyzed using a repeated measure ANOVA with the weaning value entered as a covariate. Data are presented as mean ± SEM. Differences between means were considered significant at $P < 0.05$.

III – Results and discussion

In the first trial no differences in the total blood antioxidant activity were observed in pigs reared on the two different kinds of floor ($P = 0.320$) (Figure 2A). Considering the antioxidant activity of the RBC, an higher value was observed in pigs reared on solid floor than on totally slatted floor ($P = 0.010$) (Fig. 2B).

The RBC value concerns especially the intracellular defence status and it is important for understanding the balance between attack and defence of organism in a medium/long period,

considering that RBC mean life in pig is 60-85 days (Pastorelli *et al.*, 2009). This result allows to hypothesize that the environmental stress, due to the totally slatted floor, cause an intracellular imbalance between the oxidative and antioxidant systems that make the cells more susceptible to oxidative damage (Brambilla *et al.*, 2002). The above findings point out that the adaptive response to a long term stressing condition implies an impairment of this reaction. During the state of chronic stress negative effects on health and the development of pathological conditions involving oxidative stress may occur (Lykkesfeldt *et al.*, 2007).

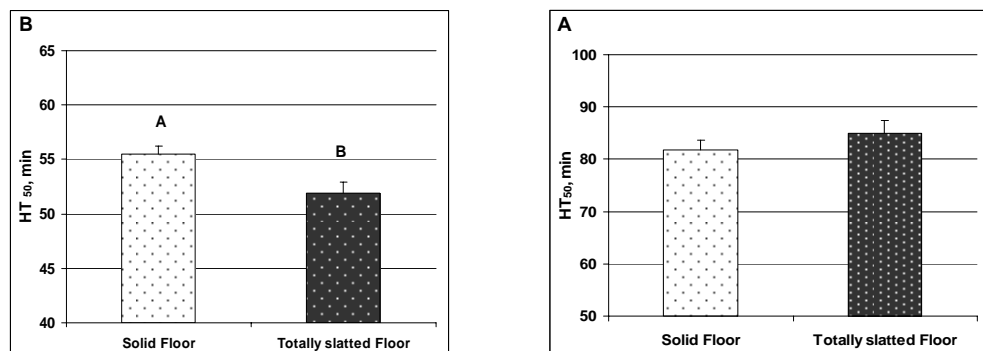


Fig. 2. Whole blood (A) and red blood cells (B) antioxidant activity in pigs reared on solid or totally slatted floor. Data are reported as means \pm SEM; $n=12$; A, B for $P = 0.010$.

The second trial regards the KRL test sensibility to the dietary supplementation of vitamin E in post weaning piglets. The results show that the total antioxidant activity in both whole blood and RBC was enhanced ($P < 0.05$) by a supplemental dosage of dietary vitamin E (Fig. 3).

A higher supplementation with vitamin E in the post weaning phase improves the total blood antioxidant activity, according with an our previous study that reported a decrease in the ROS production after dietary supplementation of natural antioxidant (Corino *et al.*, 2007). These data give clear evidence that dietary antioxidant supplementation after weaning may positively affect the antioxidant status, and therefore improving pig health.

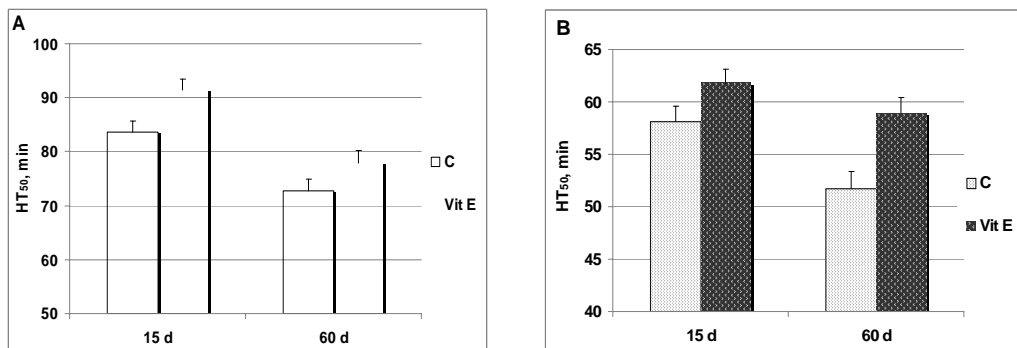


Fig. 3. Whole blood (A) and red blood cells (B) antioxidant activity in piglet fed control or vitamin E supplemented diet. Data are reported as means \pm SEM; $n=10$; C and Vit E treatments differ for $P < 0.05$.

IV – Conclusions

Under the conditions of the present research, the KRL test is able to discriminate pig welfare in relation to different housing conditions and dietary supplements. The housing system with totally slatted floor negatively influenced RBC antioxidant activity, evidencing a sign of a chronic stress. The supplemental dosage of vitamin E are able to increase blood total antioxidant activity, suggesting a better resistance to oxidative stress occurring in the post weaning phase. These results confirm that the KRL test should be consider as a new laboratory analysis to assess welfare in pig specie.

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