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Genetic parameter estimates for plasma oxidative status traits in slaughter pigs

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Abstract

The aim of the present study was to assess the effect of sex and to estimate genetic parameters for several traits related to plasma oxidative status in slaughter pigs, i.e., ferric reducing ability of plasma (FRAP), concentrations of α -tocopherol and malondialdehyde (MDA), and glutathione peroxidase (GPx) activity. Blood samples were collected at slaughter from 477 Piétrain \times (Landrace \times Large White intercross) pigs of 2 performance test stations. Heritabilities (\pm SE) of plasma oxidative status traits as well as their phenotypic and additive genetic correlations with animal performance traits were estimated with multiple-trait REML animal models using VCE software. Results displayed no significant difference between barrows and gilts for FRAP and α -tocopherol in plasma. However, gilts had a significantly higher concentration of MDA and lower GPx activity compared with barrows. Heritability estimates were high for GPx (0.55 ± 0.05), and medium to low for α -tocopherol (0.30 ± 0.06), FRAP (0.22 ± 0.05), and MDA (0.15 ± 0.04). Estimated additive genetic and phenotypic correlations between these four traits were generally low, except for a negative additive genetic correlation between FRAP and GPx of $-0.45 (\pm 0.23)$. Additive genetic correlations between plasma oxidative status traits and animal performance traits were also generally absent or low with maximum values of ~ 0.3 . Parameter estimates in this study have to be interpreted with caution because of the small size of the dataset. Nevertheless, it may be concluded that there is considerable additive genetic variance for plasma oxidative status traits in slaughter pigs. More research is warranted on the genetic determination of oxidative stress in farm animals and its relevance in breeding programs.

Key words: genetic correlation, heritability, oxidative status, pig, plasma, sex

Introduction

Oxidative stress in biological systems refers to a disturbance in the balance between pro-oxidants and antioxidants in favour of the former, leading to potential damage of biomolecules (Trevisan et al., 2001; Halliwell and Whiteman, 2004). Although living organisms have developed complex antioxidant mechanisms to counteract metabolic reactive species production (Prior and Cao, 1999; Katalinic et al., 2005), oxidative stress may occur and is thought to be involved in the development of chronic diseases.

Also in farm, animals are there indications that oxidative stress is involved in some production diseases (Miller et al., 1993), but the occurrence and implications are not clear at this time.

Several zootechnical factors were reported to be directly or indirectly associated with oxidative stress in farm animals. Some of these factors are considered as inner factors, e.g., animal species and muscle type (Janssen et al., 1993; Decker and Mei, 1996), sex (Godin and Garnett, 1992; Katalinic et al., 2005), and age (Miller et al., 1993; Cao et al., 1996; Cejková et al., 2004). Other factors are associated with environmental conditions,

e.g., season (Bernabucci et al., 2002), rearing conditions and handling (Aourousseau, 2002), physical activity (Balogh et al., 2001; Rush et al., 2003), nutrition (Nemec et al., 1994; Lauridsen et al., 1999; Castillo et al., 2006), and diseases (Gümüzlü et al., 2002; Lykkesfeldt and Svendsen, 2007).

Although oxidative stress is a very complex and tissue-dependent phenomenon, measuring biomarkers of oxidative stress in plasma to assess the body “oxidative status” is considered useful (Bernabucci et al., 2002). Little information is available on the genetic determination of the oxidative status of slaughter pigs, and its association with performances. It is however likely that there is genetic variation in the susceptibility to oxidative stress. For example, physical activity during transport stress of slaughter pigs may be associated with excess production of radical oxygen species, which is a characteristic of oxidative stress, and generally results in elevated levels of neuroendocrine stress parameters. The latter are known to differ among pig genetic lines (Foury et al., 2007). In a recent study, it was shown that pigs selected for improved feed efficiency showed a lower susceptibility to oxidative stress induced by poor hygiene conditions (Sierzant et al., 2019).

Therefore, the present study was conducted to estimate the effect of sex and genetic variability of plasma oxidative status traits, and the genetic relationship between the latter and some important animal and carcass performance traits of slaughter pigs.

Material and Methods

The experiment was carried out according to the guidelines of the Ethics Committee of Ghent University (Belgium) for the humane care and use of animals in research.

Animals and sampling

For the current study, 477 animals from the “Flanders Pig Herdbook Association” progeny test program were sampled at slaughter in the period January to July 2007. The pigs, 236 barrows and 241 gilts, were three-way crossbred offspring of 93 herdbook Piétrain sires (average number of animals per sire = 5.10; range 2 to 13) and 237 Landrace × Large White intercross dams (average number of piglets per litter = 2.00; range 1 to 8). All sires were homozygous positive, and all dams were homozygous negative for the porcine stress syndrome (RYR1 gene), hence the slaughter pigs in the present study were all heterozygous for RYR1 (Fujii et al., 1991). Animals originated from 2 finishing test station farms (Rumbeke, $n = 196$ and Scheldewindeke, $n = 281$), where they were fed a commercial diet ad libitum in conventional troughs. Average (SD) live weight was 24.8 (3.26) kg at arrival and 110.3 (5.19) kg the day before slaughter, allowing calculation of the ADG in the test period (average duration in test 117 ± 13.7 d). In the progeny test program, 3 litters per sire are followed up with 6 or 7 full sib littermates penned together. Diets and housing conditions were highly standardized. Animals in the present study were a random subset of test animals. In the progeny test program, animals are selected and delivered to the slaughterhouse at 2-wk intervals when approaching the target slaughter weight of 110 kg.

Pigs were slaughtered in a commercial slaughterhouse (Covameat, Wijtschate, and Belgium), situated at 38 and 79 km from Rumbeke and Scheldewindele stations, respectively, on 10 different slaughter days (average number of animals sampled per slaughter day = 49; range 26 to 67). The animals were bled following electrical stunning, after ~18 h fasting on average and 1 to 3 h lairage. At slaughter, ~65 mL blood was collected in tubes

containing 1.3 mL of an anticoagulant solution (191 mM EDTA, 77 mM NaN₃, and 1.24 mM Thimerosal). Plasma samples were obtained after centrifugation at $810 \times g$ and 25 °C for 15 min and were stored at -18 °C until analysis.

Carcass data were collected at the end of the slaughter line, including warm carcass weight (kg), carcass yield (%), carcass lean meat (CLM, %), and carcass conformation score. The CLM (%) was estimated using a linear regression equation including loin thickness (mm) and backfat thickness (mm) that were measured using the CGM-Sydel apparatus according to the approved procedure for pig carcass classification in Belgium (Commission Decision 97/107/EC). The carcass conformation score is based on a linear regression equation including measurements of the ham angle (°) and ham width (mm) by the PIC 2000 video image analysis system (Rovi-Tech, Presles, Belgium), and the CLM content from the CGM device (De Smet et al., 2000). A lower score corresponds to a more round-shaped carcass conformation.

Oxidative status analyses

The ferric reducing ability of plasma (FRAP) assay is considered as a measure of “the total antioxidant power”, referred analogously to as the “ferric reducing ability of plasma”, according to the method described by Benzie and Strain (1996). The test is based on the reduction of the ferric-tripyridyltriazine (Fe^{III}-TPTZ) complex to the ferrous (Fe^{II}) form at low pH. The FRAP medium solution was prepared prior to the assay performance, by mixing 10 mM Fe^{III}-TPTZ diluted in 40 mM HCl and 20 mM FeCl₃ 6H₂O in a 300 mM acetate buffer (pH 3.6). Measurements were performed on 100 µL of sample diluted with 300 µL HPLC water. The reaction is monitored for 20 min at 593 nm and 37 °C, resulting in an increase in absorbance and a development of an intense blue chromogen. FRAP values are obtained by comparing the absorbance change at 593 nm in the test reaction mixtures with a standard regression curve using ferrous ions in known concentration, in the range of 0 to 1,000 µM FeSO₄ 7H₂O. Results are expressed in µmol Fe²⁺ L⁻¹.

The α -tocopherol concentration in plasma was determined by HPLC using α -tocopherol as standard solution, as described by Desai (1984). From 1 mL plasma, α -tocopherol was separated by HPLC through a Supelcosil LC18 (25 mm × 4.6 mm × 5 µm) column, using methanol/water (97/3, v/v) as isocratic elution phase. The elution flow rate was maintained at 2 mL/min during 25 min at UV wavelength $\lambda = 292$ nm. Results are expressed as µg of α -tocopherol mL⁻¹ plasma.

The malondialdehyde (MDA) concentration in plasma was measured by the TBARS method (thiobarbituric acid (TBA) reactive substances), as described by Grotto et al. (2007), to assess lipid oxidation. Plasma was submitted to an alkaline hydrolysis at 60 °C during 30 min, and then MDA was derivatized by 2-TBA in acidic medium to form TBARS (pink coloured substances) at 90 °C for 45 min. The medium solution comprised of 450 µL plasma, 300 µL 1.5 M NaOH, 750 µL 85% H₃PO₄, 750 µL 0.8% TBA, and 300 µL 10% SDS. In parallel, the same reaction was carried out with TMP (1, 1, 3, 3, -tetramethoxypropane) in a concentration range of 0 to 30 nmol mL⁻¹ as standard. The formed TBARS were extracted with 3 mL *n*-butanol, and their absorbance was measured immediately with a spectrophotometer at 532 nm. Results were expressed as nmol MDA mL⁻¹ plasma.

Glutathione peroxidase (GPx; EC 1.11.1.9) activity was determined in plasma according to the method described by Hernandez et al. (2004). The assay mixture (3 mL) consisted of 5 µL plasma diluted to 300 µL with phosphate buffer (pH 7; 50 mM; 25 °C), 2,650 µL of the medium solution, 26 µL of NADPH-solution

(17.3 mM), and 20 μL of H_2O_2 (22.5 mM). The medium solution was composed of 1.13 mM of reduced glutathione, 0.57 mM EDTA, 1.13 mM NaN_3 , and 1.7 Units of glutathione reductase. All the reagent reactions were diluted in phosphate buffer. The decrease in absorbance was kinetically monitored at 340 nm for 5 min, and an extinction coefficient of $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$ was used to calculate the NADPH concentration. One unit (U) of GPx was defined as the amount of enzyme required to oxidise 1 μmol of NADPH $\text{min}^{-1} \text{ mL}^{-1}$ plasma, at 25 °C.

Statistical analysis

Results for the plasma oxidative status and animal performance traits were first analysed by Linear Mixed Model ANOVA to test the fixed effects of sex and station. The models included the fixed effects of sex and station and sire as random factor. For the oxidative status traits, the day of laboratory analysis was included as an additional random factor. This analysis was performed using SPSS version 15.0 for Windows.

Genetic parameter estimates were obtained using the VCE-5 software and multi-trait mixed animal model methodology (Kovac and Groeneveld, 2003). Models (1) and (2) were used for animal performance and oxidative status traits, respectively.

$$Y_{ijkl} = \mu_i + \text{Sex}_{ij} + \text{Station}_{ik} + a_{il} + \varepsilon_{ijkl}$$

$$Y_{ijklm} = \mu_i + \text{Sex}_{ij} + \text{Station}_{ik} + d_{im} + a_{il} + \varepsilon_{ijklm}$$

where Y_{ijkl} or Y_{ijklm} is observation for trait i , μ_i is a common constant for trait i , Sex_{ij} is a fixed effect of sex j ($j = 1, 2$) for trait i , station_{ik} is a fixed effect of station k ($k = 1, 2$) for trait i , d_{im} is the random effect of analysis date m ($m = 1, \dots, 10$) for trait i , a_{il} is the random additive genetic effect of animal l ($l = 1, \dots, 2,090$) for trait i , ε_{ijklm} is the random residual effect for trait i . It has to be mentioned that non-additive genetic effects were not accounted for in the present analysis. The laboratory analyses for oxidative status traits were performed per slaughter batch,

hence the random effect of analysis date also includes possible slaughter day variance.

A 4-trait analysis was performed to estimate heritabilities and genetic correlations for oxidative status traits. To estimate genetic and phenotypic correlations between oxidative status and animal performance traits, a 5-trait analysis was performed including the 4 oxidative status traits and each one of the performance traits. Five generations of pedigree information was available on the paternal and maternal side, totalizing 2,081 animals.

Litter was not included in the model due to the low average number of samples per litter. If there would be common litter environmental variance, then a proportion of this variance might be included in the estimates of the additive genetic variance. However, we assume that with the low number of samples per litter, this contribution will be very small.

Results

Fixed effects

The effect of sex on animal performance and plasma oxidative status traits is shown in Table 1. As expected, a highly significant effect of sex and sire on most animal performance traits was found, with higher values for live weight at slaughter, ADG, conformation score, loin thickness and back fat thickness for barrows compared with gilts, and lower values for carcass yield and CLM content. An effect of sex was observed on plasma MDA concentration ($P = 0.002$) and GPx activity ($P < 0.001$), but not for FRAP and α -tocopherol, with barrows showing a higher GPx activity in plasma compared with gilts, and vice versa for MDA.

No difference between test stations was observed for animal performance traits (results not shown). However, there was an effect of test station ($P < 0.01$) on FRAP ($226.5 \pm 18.7 \mu\text{M/L}$ plasma for Rumbek Station vs. $197.5 \pm 18.6 \mu\text{M/L}$ plasma for Scheldewindeke station) and α -tocopherol ($3.34 \pm 0.118 \mu\text{L/mL}$ plasma vs. 3.03 ± 0.107 , respectively).

Table 1. Least square means (SE) for the effect of sex on animal performance and plasma oxidative status traits

	Sex			Sire	
	Barrows (n = 236)	Gilts (n = 241)	P-value	V-estimates ⁴	P-value ⁵
Animal performance traits					
Live weight at slaughter, kg	110.9 (0.370)	109.6 (0.364)	0.004	1.95	0.049
ADG, g d ⁻¹	762.5 (6.71)	710.3 (6.65)	<0.001	2371.84	<0.001
Carcass weight, kg	87.6 (0.315)	87.5 (0.310)	0.74	1.122	0.106
Carcass yield, %	79.0 (0.278)	79.8 (0.276)	0.002	4.467	<0.001
CLM, %	60.2 (0.630)	62.4 (0.629)	<0.001	1.001	<0.001
Conformation score	1.97 (0.029)	1.77 (0.028)	<0.001	0.025	<0.001
Loin thickness, mm	123.3 (0.713)	121.5 (0.723)	0.014	12.928	0.001
Back fat thickness, mm	13.7 (0.256)	10.3 (0.252)	<0.001	1.705	0.003
Ham angle, degrees	44.7 (0.596)	43.6 (0.588)	0.104	12.644	<0.001
Ham width, mm	203.5 (0.786)	204.8 (0.776)	0.119	23.346	<0.001
Plasma oxidative status traits					
FRAP ¹ , $\mu\text{mol Fe}^{2+} \text{ L}^{-1}$	211.2 (18.6)	212.7 (18.5)	0.77	256.034	0.034
α -Tocopherol, $\mu\text{g mL}^{-1}$	3.20 (0.104)	3.16 (0.104)	0.59	0.051	0.057
MDA ² , nmol mL^{-1}	6.77 (0.499)	6.26 (0.498)	<0.003	0.208	0.054
GPx, U ³	0.487 (0.029)	0.423 (0.028)	<0.001	0.002	0.006

¹FRAP, Ferric reducing ability of plasma.

²MDA, Malondialdehyde.

³GPx, Glutathione peroxidase; U: amount of enzyme required for oxidizing 1 μmol of NADPH $\text{min}^{-1} \text{ mL}^{-1}$ plasma at 25 °C.

⁴Variance estimate component for sire (random factor).

⁵Estimated using the restricted maximum likelihood ratio test.

Estimated additive genetic variability and genetic relationships among plasma oxidative status parameters

Estimated genetic parameters for the plasma oxidative status traits are given in Table 2. Heritability estimates were high for GPx, and medium to low for α -tocopherol, FRAP, and MDA. Genetic correlations between these four traits are difficult to evaluate because of the large standard errors of estimate. Genetic and phenotypic correlations were generally low, except for a rather high negative genetic correlation between FRAP and GPx, and a medium positive phenotypic relationship between FRAP and MDA. There were further weak negative genetic relationships between FRAP and MDA and between GPx and α -tocopherol, but with large standard errors of estimate.

Estimated genetic relationship between oxidative status parameters and animal performance traits

In Table 3, estimated genetic and phenotypic correlations between plasma oxidative status and animal performance traits are shown. The phenotypic correlations were all very low and never exceeded $|\pm 0.15|$. Genetic correlations were higher, but overall also low ($<|\pm 0.40|$) and accompanied by relatively large standard errors of estimate. FRAP was not genetically related to ADG, but showed a medium-to-weak positive genetic relationship with back fat thickness and conformation score, and a weak negative relationship with loin thickness and CLM. The α -tocopherol concentration showed a genetically medium-to-weak positive relationship to ADG and back fat and loin thickness and was weakly negatively related to CLM and conformation score. Genetic correlations between MDA and GPx on the one hand and the performance traits on the other hand

were lower than for the other two oxidative status traits, with only a medium-to-weak positive relationship between MDA and loin thickness and a weak positive relationship between GPx and back fat thickness.

Discussion

To our knowledge, this is the first study that provides estimates for genetic parameters specific to oxidative status biomarkers in farm animals. Parameters measured in plasma included FRAP, α -tocopherol, GPx, and MDA, representing different levels or outcomes of the antioxidant defence system. The FRAP assay is considered a useful measure of "the total antioxidant power" of plasma, reflecting the combined antioxidant effect of non-enzymatic compounds of endogenous and exogenous origin in biological fluids (Benzie and Strain, 1996). α -Tocopherol is the main lipophilic antioxidant, which acts by breaking the propagation chains occurring during lipid peroxidation of polyunsaturated fatty acids in membranes and lipoproteins (Thérond et al., 2000). Although α -tocopherol contributes to the overall non-enzymatic defence assessed by the FRAP measurement, its individual contribution, as well as that from many single antioxidants, was found to be very low compared with that of bilirubin, and no apparent interaction was found between different antioxidants (Benzie and Strain, 1996). Hence, the FRAP assay and the α -tocopherol concentration can be seen as relatively independent measures of the oxidative status in plasma (Benzie and Strain, 1996; Thérond et al., 2000). GPx represents a second group of oxidative status parameters, i.e., the endogenous antioxidant enzymes. It is the most extensively characterized selenoprotein antioxidant enzyme

Table 2. Estimated genetic parameters for plasma oxidative status traits^{1,2,3}

	FRAP	α -Tocopherol	MDA	GPx
FRAP, $\mu\text{mol Fe}^{2+} \text{L}^{-1}$	0.222 (0.051)	-0.045 (0.170)	-0.247 (0.227)	-0.451 (0.227)
α -Tocopherol, $\mu\text{g mL}^{-1}$	-0.034	0.299 (0.057)	0.047 (0.120)	-0.189 (0.136)
MDA, nmol mL^{-1}	0.364	0.008	0.150 (0.042)	-0.098 (0.175)
GPx, U	0.043	0.085	0.091	0.550 (0.052)

¹Heritabilities on diagonal, genetic correlations above diagonal and phenotypic correlations below diagonal.

²Estimates (SE) from a 4-trait animal model analysis.

³FRAP, ferric reducing ability of plasma; MDA, malondialdehyde; GPx, glutathione peroxidase activity.

Table 3. Estimated genetic and phenotypic correlations between animal performance traits and plasma oxidative status traits^{1,2}

	h^2 estimates ¹	FRAP, $\mu\text{mol Fe}^{2+} \text{L}^{-1}$	α -Tocopherol, $\mu\text{g mL}^{-1}$	MDA, nmol mL^{-1}	GPx, U
Genetic correlations					
ADG, g d^{-1}	0.975 (0.022)	-0.040 (0.122)	0.371 (0.110)	0.062 (0.105)	-0.052 (0.068)
Back fat thickness, mm	0.440 (0.058)	0.355 (0.113)	0.361 (0.069)	-0.060 (0.184)	0.169 (0.085)
Loin thickness, mm	0.623 (0.066)	-0.363 (0.182)	0.319 (0.159)	0.344 (0.167)	0.175 (0.082)
Lean meat, %	0.441 (0.063)	-0.354 (0.137)	-0.244 (0.066)	-0.071 (0.178)	-0.024 (0.089)
Conformation score	0.472 (0.053)	0.414 (0.117)	-0.249 (0.076)	-0.045 (0.177)	0.107 (0.098)
Phenotypic correlations					
ADG, g d^{-1}		0.019	0.153	0.007	-0.002
Back fat thickness, mm		0.056	0.109	-0.039	0.000
Loin thickness, mm		0.073	0.065	0.056	0.067
Lean meat, %		-0.054	-0.095	0.000	-0.048
Conformation score		0.002	-0.040	-0.046	0.022

¹Estimates (SE) from a 5-trait animal model analysis including the 4 oxidative status traits and the respective animal performance trait.

²FRAP, ferric reducing ability of plasma; MDA, malondialdehyde; GPx, glutathione peroxidase activity.

(Czuczejko et al., 2003). It reacts within cells to detoxify H_2O_2 by reducing it to water and oxygen, thus playing a preventive role against oxidative stress (Davies, 1995; Benzie, 2000). Finally, the MDA concentration in plasma reflects one outcome of oxidative stress. MDA is a secondary product generated by lipid peroxidation (Thérond et al., 2000; Wood et al., 2006). It reacts, together with other aldehydes, with TBA to give a pink and fluorescent chromogen that is measured in the TBARS assay as the most frequently used method to assess lipid peroxidation (Wood et al., 2006; Grotto et al., 2007). Although the TBARS assay is criticised for several reasons and the direct measurement of MDA is likely to improve the analytical specificity and validity (Therasse and Lemonnier, 1987; Pincemail et al., 1996; Wood et al., 2006), it is still widely used and considered appropriate for assessing the oxidative status in living animal tissue along with other methods (Wood et al., 2006).

In the present study, the oxidative status was assessed in plasma taken at slaughter from progeny test station pigs that had been reared and slaughtered according to commercial, yet standardized, practices. This approach was intended to reflect the in vivo oxidative status. However, an effect of stress associated with transport and slaughter on the oxidative status traits cannot be excluded. Oxidative stress is a complex phenomenon and is determined by many factors. Genotype, environmental conditions, and genotype \times environment interactions are all considered important in determining stress states and animal welfare in pigs (Fabrega et al., 2002; Avero et al., 2007; Foury et al., 2007). Stress-related states, referred to as emotional and physical stress, have been documented to contribute to enhancing in vivo reactive oxygen species production and increasing the risk for oxidative stress (Davies et al., 1982; Harmon et al., 1997; Aourousseau, 2002). Evidence of handling-induced (immobilization, temperature variation, and brutal handling) oxidative stress by stimulating the flux of free radicals in the organism and weakening the red blood cells has been reported for lambs (Aourousseau, 2002) and rats (Kondo et al., 1992; Kizaki et al., 1997). Such handling-induced oxidative stress resulted in rats in decreased values for GPx activity and increased concentrations of TBARS in muscle tissue (Kondo et al., 1992). In a related study of Costantini et al. (2008), it was found that serum antioxidant capacity was higher in selected lines of non-aggressive mice vs. aggressive mice.

No difference was found between barrows and gilts for FRAP and α -tocopherol, but the MDA concentration was slightly but significantly higher in gilts compared with barrows, and the opposite was found for GPx activity. This would point to a small difference, if biologically relevant at all, in plasma oxidative status in favour of the barrows. Earlier studies have revealed gender differences in FRAP values in rat (Katalinic et al., 2005; Vipin et al., 2007) and in human (Benzie and Chung, 1999), with higher values for males. Our results do thus not correspond to these findings.

So far, there is scant information on oxidative status variability in farm animals. The current results do suggest considerable genetic variability for plasma oxidative status traits, particularly the heritability for the activity of the endogenous enzyme GPx was rather high. Hence, animals may differ in their genetic predisposition to oxidative stress and their antioxidant defence potential. Estimates were, however, relatively low compared with those observed for some other stress-related parameters in pigs; e.g., total number of polymorphonuclear leukocytes as an immune responsiveness parameter in swine ($h^2 = 0.87$) (Edfors-Lilja, 1994); resistance to *Trichuris suis* infections ($h^2 = 0.32$ to 0.73) (Nejsun et al., 2009), creatine kinase in landrace pigs as

stress resistance measurement ($h^2 = 0.73$) (Schwörer et al., 1980). The heritability estimate for GPx activity was 2- to 3-fold higher compared with the value for the other oxidative status traits. This is not unexpected because endogenous GPx activity reflects the expression of a gene, whereas FRAP values and α -tocopherol concentrations evidently are more determined by nutritional factors.

Overall, the estimated genetic correlations among oxidative status traits and between these traits and animal performance traits were rather low and difficult to interpret. Caution is needed in view of the relatively large standard errors of estimate. The sample size in the present study is small for allowing accurate estimation of genetic parameters. The SE values of h^2 estimates for the plasma oxidative status traits varied between 9% (for GPx activity) and 28% (for MDA level) of the h^2 estimates and were thus only slightly higher than the SE values of the h^2 estimates for animal performance traits (between 2% and 13% of the estimated h^2 values). Since the h^2 estimates for the performance traits are in line with literature data, except for the extremely high value for ADG, it may be assumed that the h^2 estimates for oxidative status traits in the present study are reasonable. On the other hand, relative errors for the genetic correlation estimates were much larger, hence these estimates are not very reliable and need to be confirmed in a larger study.

Among the oxidative status traits, only a moderately strong negative genetic association between FRAP and GPx activity was found. The biological significance of this relationship is not fully clear, although one might argue that a higher concentration of non-enzymatic compounds circulating in the plasma, reflected in the FRAP values, reduces the need for a high endogenous antioxidant enzyme activity. The absence of clear relationships among oxidative status traits supports their partly independent contribution to and distinct nature in the oxidative status biological network. However, a negative relationship between plasma α -tocopherol concentrations protecting against and MDA concentrations reflecting lipid peroxidation could be expected, which was not apparent in the present study. Similarly, one might expect a negative relationship between FRAP values and MDA concentrations. A positive phenotypic correlation was found in our study, but no relationship at the genetic level. In other reports, oxidative stress was associated with increased values of both FRAP and MDA (Allen and Foegeding, 1981; Benzie and Chung, 1999; Vipin et al., 2007), corresponding to our observation. Nevertheless, as mentioned above, the plasma FRAP value has been reported to be higher in males than in females in oxidative stress conditions (Benzie and Chung, 1999; Katalinic et al., 2005) and vice versa for MDA (Allen and Foegeding, 1981). It thus remains difficult to interpret the link between different parameters of the oxidative stress response. In a human study, also no relationship was found between different markers of the antioxidant status (FRAP and uric acid) and outcomes of oxidative stress (protein carbonyls and 8-iso-prostaglandine F_{2a}), all measured in plasma (Garcia-Larsen et al., 2009).

Investigating the genetic relationships between plasma oxidative status traits and some important growth and carcass composition traits allows making inferences about the genetic trend in oxidative status following selection for increased growth rate and decreased carcass fatness that has taken place over the last decades in modern pig production. Overall, the relationships were weak and not very consistent, not pointing to any clear negative or positive evolution in oxidative status in relation to the performance traits. However, ADG appeared to be positively related to the plasma α -tocopherol concentration. In a similar way, α -tocopherol seemed to be positively related with

back fat thickness and loin thickness, and negatively with CLM at the genetic level. ADG was positively related to back fat and loin thickness and negatively related to CLM in the present study (data not shown), in line with general expectations, hence the genetic relationships between the plasma α -tocopherol concentration and the performance traits seem to be consistent. This was less the case for the other traits. FRAP, MDA, and GPx displayed all a moderate correlation with only one or two performance traits, which are in addition difficult to assign a biological significance. Carcass leanness was slightly negatively related to the plasma α -tocopherol concentration and FRAP value, but the absence of a correlation with plasma MDA concentration and with GPx activity does not point to a lower protection against oxidative stress in leaner pigs. Based on MDA measurements, meat from animals with an overall leaner carcass was found to be more prone to oxidation (Nilzen et al., 2001). More research is needed on larger datasets to explore the genetic relationships between oxidative status and performance traits.

At the phenotypic level, only a weak positive relationship between the plasma α -tocopherol concentration and ADG was found, and this value was lower than the genetic correlation. Apart from the growth-promoting effect of supplementing vitamin E in deficient diets, it is to our knowledge not evident from the literature that higher circulating plasma α -tocopherol concentrations in a range of normal values are linked to growth rate. This interesting finding remains to be further investigated.

In conclusion, there appears to be considerable additive genetic variance for plasma oxidative status traits in pigs, particularly for the activity of the endogenous enzyme GPx. This should be confirmed in other and large populations. More research is warranted on the genetic determination of oxidative stress in farm animals and its relationships with performance and reproduction traits that largely make up current breeding objectives.

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Conflict of interest statement

None declared.

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