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Significance of Preslaughter Stress and Different Tissue PUFA Levels on the Oxidative Status and Stability of Porcine Muscle and Meat

JETTE F. YOUNG,* KATJA ROSENVOLD, JAN STAGSTED, CHARLOTTE L. STEFFENSEN, JACOB H. NIELSEN, AND HENRIK J. ANDERSEN

Department of Animal Product Quality, Danish Institute of Agricultural Sciences, P.O. Box 50, DK-8830 Tjele, Denmark

Polyunsaturated fatty acids (PUFAs) and exercise-induced stress are known to increase the oxidative susceptibility of lipids in muscle tissue. In contrast, antioxidative enzymes, e.g., catalase, superoxide dismutase, and glutathione peroxidase, are known to help sustain the delicate oxidative balance in biological tissue upon the application of stressors. The present study investigates the combined effect of different diet-induced muscle PUFA contents and preslaughter stress on the activity of antioxidative muscle enzymes and the oxidative stability of cooked meat. An increased content of unsaturated fatty acids in the tissue led to a decreased activity of lactate dehydrogenase in the plasma, indicating increased cell integrity. Catalase activity in the muscle tissue increased with increasing PUFA levels. However, this upregulation in antioxidative status of the muscle could not counteract the subsequent development of accelerated lipid oxidation in cooked meat as measured in terms of thiobarbituric acid reactive substances. Moreover, preslaughter stress induced increasing oxidative changes with elevated PUFA levels in the muscle tissue.

KEYWORDS: Pork; lipid oxidation; antioxidative enzymes; catalase; exercise

INTRODUCTION

The oxidative stability of muscle is critical for the quality of both fresh and processed meat (*1*, *2*). In particular, oxidative alterations of the lipid fraction in porcine meat is an important parameter discriminating the sensory quality of the meat (*3*).

Both increased polyunsaturated fatty acids (PUFAs) (*1*, *2*) and physical stress (*4*) are known to increase the oxidative susceptibility of lipids in muscle tissue. The inherent antioxidative defense system, including regulation of antioxidative enzymes, e.g., catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), normally maintains the oxidative balance in biological tissues through controlled quenching of reactive oxygen species (*5*). Diet-induced elevation of unsaturated fatty acids, which are more prone to oxidation, can represent a stressor requiring improved antioxidative capacity, as shown by the induction of antioxidative enzyme activity in chicken muscles (*6*). An understanding of stressor-induced changes in the antioxidative status of muscles in relation to the oxidative susceptibility of meat from slaughter animals is, at present, unexplored, even though antioxidative enzyme activity has been indicated to play a role in the oxidative stability of meat (*7*).

The aim of the present study was to investigate the effects of diets with different PUFA contents in combination with the effects of exercise-induced stress in relation to parameters affecting the oxidative stability of meat. The exercise was used as a standardized model of preslaughter stress, simulating the physical and emotional stress imposed on pigs during transport and handling at the slaughter plant immediately prior to slaughter.

MATERIALS AND METHODS

Animals and Management. In the present study, 72 pigs from 18 four-animal litters (crossbreed Danish Landrace and Danish Yorkshire female pigs and noncarriers of the Halothane gene) were reared at the experimental farm of The Research Centre Foulum, The Danish Institute of Agricultural Sciences.

The pigs were allocated into three dietary treatments, and within each litter, two pigs were exercised for 10 min on a treadmill immediately prior to slaughter to simulate stress, resulting in a total of six experimental groups with 12 pigs in each group. The pigs were distributed between the different treatments in blocks of three litters.

The three different diets were (i) a standard grower-finishing diet produced at The Research Center Foulum (control diet); (ii) a diet with high levels of grass meal, rape seed meal, and dried sugar beet pulp (GRASB diet); and finally, (iii) a diet with high contents of inulin (Raftiline HP, Orafti, Belgium), rape seed meal, and animal fat (INURA diet). All pigs were housed individually and fed standard grower-finishing diet semi ad libitum from 25 kg until reaching a live weight of approximately 70 kg according to a standard scale of The Research Centre Foulum (*8*, *9*).

* Address correspondence to this author at the Danish Institute of Agricultural Sciences, Department of Animal Product Quality, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark. Telephone: + 45 89 99 11 68. Fax: + 45 89 99 15 64. E-mail: jettef.young@agrsci.dk.

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Table 1. Ingredients[a] and Chemical Compositions[b] of Control, GRASB, and INURA Diets

ingredient	control[c]	GRASB	INURA
rape seed cake		36.0	55.5
soybean meal	18.5	7.5	6.0
dried sugar beet pulp		25.0	
grass meal		24.5	
raw potato starch			3.0
inulin			25.0
barley	57.3		
sugar beet molasses	1.0		
wheat	20.9		
pig fat and soya bean oil (1:1)	2.1	6.0	10.0
vitamin mineral mixture[d]	0.2	0.2	0.2
α-tocopherol (mg/g)	3.0	2.9	3.1
chemical composition			
gross energy (MJ/kg)	19.3	21.7	21.3
net energy (MJ/kg)	8.8	9.3	9.5
ash (%)	5.0	7.5	5.1
crude protein (*N* x 6.25) (%)	19.9	23.6	22.4
crude fat (%)	5.6	18.0	16.9
starch (%)	46.2	1.2	5.1
mono- and disaccharides	2.2	4.9	4.1
raffinose oligosaccharides	0.6	1.2	2.0
inulin (%)			26.6
DF[e] (%)	16.4	39.6	18.4
NCP[f] (%)	11.3 (4.5)	21.2 (9.8)	9.4 (4.0)
cellulose (%)	3.0	12.2	3.6
klason lignin (%)	2.0	6.3	5.4

[a] Ingredients in %. [b] Chemical composition in % of dry matter. [c] Supplemented with 0.16% lysin in the form of 40% lysin + 60% wheat bran and 0.04% D,L-methionin in the form of 40% D,L-methionin + 60% wheat bran. [d] Solovit Mikro 106 containing 2 500 000 IU of vitamin A, 500 000 IU of vitamin D_3, 30 000 mg of vitamin E, 1100 mg of vitamin K_3, 1100 mg of vitamin B_1, 2000 mg of vitamin B_2, 1650 mg of vitamin B_6, 5500 mg of D-pantotensyre, 11 000 mg of niacin, 27.5 mg of biotin, 11 mg of vitamin B_{12}, 25 000 mg of Fe, 40 000 mg of Zn, 13 860 mg of Mn, 10 000 mg of Cu, 99 mg of J, and 150 mg of Se per kilogram. [e] DF, dietary fiber, the sum of noncellulose polysaccharides, cellulose, and klason lignin. [f] NCP, noncellulose polysaccharides; values in parentheses denote contents of soluble NCP.

Subsequently, the experimental diets were introduced through a 1-week acclimatization period (days 1−7) gradually changing from the standard grower-finishing diet into 100% of either of the experimental diets, followed by 2 weeks (days 8−22) on 100% experimental diet. Control pigs were given standard grower-finishing diet during the whole test period (days 1−22). Ingredients and compositions of the diets, analyzed as described in (*10*), are presented in Table 1. The pigs were slaughtered on day 22, 12−25 h after feed withdrawal.

Slaughter Procedure. On the day of slaughter, the pigs were transported from the rearing house to the experimental slaughter plant (200 m). Immediately prior to slaughter, half of the pigs were exercised on a treadmill at a speed of 3.8 km/h for 10 min. The pigs were stunned by 85% CO_2 for 3 min and exsanguinated. The carcasses were then scalded at 62 °C for 3 min, cleaned, and eviscerated within 30 min.

Muscle Biopsy and Blood Sampling. Muscle biopsies of *m. longissimus dorsi* (LD) were taken above the last rib on day 1 prior to the diet change and at a site approximately 5 cm from the first biopsy in the cranial direction on day 21 and on day 22 immediately prior to slaughter, i.e., after exercise for exercised pigs. Sampling 1 min post mortem (after initial sticking) was performed between the two earlier biopsies. The LD muscle biopsy samples (300−500 mg) were taken with a spring-loaded biopsy instrument (Biotech PPB-U, Nitra, Slovakia). Immediately after sampling, the biopsies were frozen in liquid nitrogen and stored at −80 °C until further analysis. Activities of CAT, GSH-Px, and SOD were determined using biopsies from day 1, day 22, and 1 min post mortem.

Blood was sampled from the jugular vein into heparinized vacuum tubes on days 1 and 21, as well as during exsanguination at the abattoir. Plasma was prepared by centrifugation at 1000*g* for 15 min at 4 °C and stored at −80 °C. Lactate dehydrogenase (LDH) was determined on plasma samples from days 1 and 22 and from exsanguinated blood.

After 45 min, the carcasses were placed in a chill room at 4 °C.

Eight-centimeter LD samples were taken 24 h post mortem at the last rib, going in the cranial direction. The first 3-cm portions of the samples were used for other purposes, and the following 5-cm portions were prepared for subsequent determination of thiobarbituric acid reactive substances (TBARS). Lipid oxidation was induced by heating balls of meat formed from minced LD muscle (10 g) in polypropylene plastic bags in a water bath at 70 °C for 10 min, which were then allowed to develop through storage for 1, 3, or 6 days at 4 °C in oxygen-permeable polyethylene film. At a distance of 6 cm from the last rib in the cranial direction, 1-cm-thick LD samples were taken (24 h post mortem) and stored at −20 °C until analysis. These samples were used for determination of the fatty acid composition.

The experiments were approved by The Danish Inspectorate of Animal Experimentation, and the pigs were treated in accordance with the guidelines outlined by the same authority.

Lactate Dehydrogenase Activity. The activity of LDH was determined using pyruvate and NADH as substrates according to principles outlined by Passonneau and Lowry (1993) (*11*). LDH activity in plasma is expressed in terms of millimoles of NADH oxidized per minute per liter.

Tocopherol in the Diet. The tocopherol contents in the diets were determined as described by Jensen and co-workers (*12*).

Fatty Acid Composition. Fatty acids were determined by gas chromatographic separation and quantification as described by Lauridsen and co-workers (*13*).

Catalase, Glutathione Peroxidase, and Superoxide Dismutase Activities. Frozen tissue (100 mg) in 0.8 mL of homogenization buffer (0.05 M Tris-HCl, pH 7.4, 1mM EDTA, 0.25 M sucrose) was homogenized on ice with an Ultraturrax for 5 s at 13 500 rpm. The homogenate was centrifuged at 10.000*g* for 30 min at 4 °C, and the supernatant was stored at −80 °C until use. The antioxidative enzymes were all assayed in microtiter plates essentially as described here: CAT activity was measured spectrophotometrically at 240 nm as a decrease in the H_2O_2 concentration (*14*); GSH-Px activity was measured as the rate of NADPH consumption at 340 nm through coupling with added glutathione reductase (*15*); and SOD activity was measured at 560 nm as inhibition of xanthine/xanthine oxidase-mediated oxidation of cytochrome C (*16*). All samples were measured in triplicate at appropriate dilutions in homogenization buffer to give activities of the enzymes in the linear range of standard curves constructed with pure enzymes (Sigma, St. Louis, MO). Protein contents of the homogenates were determined using the BCA assay (Pierce, Rockford, IL) with bovine serum albumin as the standard. Activities of CAT and GSH-Px are expressed in terms of units defined as 1 μmol of substrate converted per minute per milligram of protein homogenate. One unit of SOD is defined as 50% inhibition of the absorbance at 560 nm.

Thiobarbituric Acid Reactive Substances. Lipid oxidation was assessed as TBARS as described by Young and co-workers (*17*).

Data Analyses. The statistical analysis was carried out with the Statistical Analysis System version 8.00 (SAS Institute, Cary, NC). The MIXED procedure was applied when calculating the least-squares means and standard errors of all of the variables. A model including the individual effects of diet, exercise, and time, as well as their interactions; the random effect of litter; and the repeated effect of time (immediately prior to stunning and 1 min post mortem) with animal as the subject was applied for the variables LDH, GSH-Px, SOD, and CAT. The enzyme activities measured on day 1 were included as a covariate in the models. A model including the fixed effects of diet and exercise, as well as their interactions, and the random effect of litter was applied for the variables TBARS and fatty acids. Least-squares means were calculated and considered to be significantly different if $p < 0.05$.

RESULTS

Fatty Acid Composition and α-Tocopherol Content. The α-tocopherol contents of the diets were similar (Table 1). Pigs fed the GRASB diet had an increased PUFA content in the

Table 2. LS Means ($n = 24$) of Fatty Acid Composition and Calculated PUFA Contents in LD of Pigs Fed Control, GRASB, and INURA Diets

	control	GRASB	INURA	SEM	p
C8	0.081[a]	0.061[b]	0.053[b]	0.006	0.0045
C10	0.515[a]	0.352[b]	0.270[b]	0.036	<0.0001
C12	0.134[a]	0.115[b]	0.101[b]	0.008	0.0021
C14	1.034	0.947	0.980	0.048	0.219
C14:1	0.031[a]	0.034[a]	0.014[b]	0.004	0.0054
C15	0.098	0.092	0.088	0.007	0.522
C16	24.05[a]	22.45[b]	22.32[b]	0.226	<0.0001
C16:1	3.15[a]	2.47[b]	2.38[b]	0.107	<0.0001
C17	0.23[a]	0.23[a]	0.28[b]	0.009	<0.0001
C17:1	0.24[a]	0.19[b]	0.22[c]	0.007	<0.0001
C18	10.08	10.39	10.23	0.140	0.198
C18:1ω9	36.59	34.69	37.05	1.00	0.205
C18:1ω7	4.20	4.23	4.14	0.064	0.413
C18:2	13.04[a]	17.40[b]	15.69[b]	0.82	0.0017
C18:3ω6	0.136[a]	0.076[b]	0.076[b]	0.008	<0.0001
C18:3ω3	0.444[a]	1.223[b]	1.202[b]	0.043	<0.0001
C18:4	0.180[a]	0.158[a,b]	0.146[b]	0.010	0.0202
C20	0.114[a]	0.118[a]	0.138[b]	0.006	0.0019
C20:1	0.560[a]	0.602[a]	0.675[b]	0.027	0.0047
C20:2	0.330	0.344	0.322	0.013	0.317
C20:3ω6	0.495[a]	0.381[b]	0.356[b]	0.029	0.0012
C20:4	2.79	2.38	2.18	0.216	0.101
C20:3ω3	0.064[a]	0.127[a]	0.124[b]	0.006	<0.0001
C20:5	0.461	0.426	0.381	0.033	0.231
C22	0.120[a]	0.074[b]	0.057[b]	0.009	<0.0001
C22:1	0.066[a]	0.030[b]	0.028[b]	0.006	0.0014
C22:6ω3	0.414[a]	0.292[b]	0.283[b]	0.027	0.0005
C24	0.326	0.344	0.285	0.046	0.231
PUFAs	18.34[a]	22.79[b]	20.76[a,b]	1.11	0.0062

[a,b] Within a row, means without a common superscript letter differ ($p < 0.05$).

Table 3. LS Means ($n = 12$) of TBARS[a] in Heat-Treated Meatballs Made from LD of Pigs Fed Control, GRASB, or INURA Diets of Which Half Were Exercised (Ex)

	control		GRASB		INURA		
day	non-ex	ex	non-ex	ex	non-ex	ex	SEM
1	0.05[b]	0.06[b]	0.07[b]	0.09[b]	0.06[b]	0.07[b]	0.018
3	0.19[b]	0.21[b]	0.29[c]	0.29[c]	0.26[c]	0.30[c]	
6	0.31[b]	0.32[b]	0.39[c]	0.43[c]	0.39[c]	0.41[c]	

[a] TBARS, expressed in nanomoles of malondialdehyde equivalents per 100 mg of tissue, were determined in samples stored for 1, 3, and 6 days. [b,c] Within a row, means without a common superscript letter differ ($p < 0.05$).

muscle tissue compared to the control pigs, whereas the increased PUFA content in pigs fed INURA was not significantly different from the content in the controls ($p = 0.13$) (Table 2). The fatty acid mainly contributing to this difference was linoleic acid (C18:2).

Thiobarbituric Acid Reactive Substances. TBARS in heat-treated meatballs increased as expected with increasing time of storage ($p < 0.001$), and after 3 and 6 days of storage, the TBARS levels had increased more in meatballs from both GRASB- and INURA-fed pigs than in meatballs from control pigs (Table 3). Although no significant stress effect was noticed within each individual feeding group, an overall estimation of preslaughter stress resulted in higher TBARS levels in the meatballs compared to those from nonstressed pigs ($p = 0.04$).

Lactate Dehydrogenase. Overall, the LDH activity in plasma was higher in the control group ($p = 0.02$) than in the pigs fed the high-PUFA diets GRASB and INURA (Table 4). Preslaughter stress did not affect the control group, whereas pigs on the diets causing high PUFA levels in the tissue showed increased LDH activities after preslaughter stress ($p = 0.06$ and 0.006 for GRASB and INURA, respectively). In general, LDH activities were increased in plasma ($p < 0.001$) as a consequence of slaughter.

Table 4. LS Means ($n = 10$) of LDH[a] in Plasma from Pigs Fed Control, GRASB, or INURA Diets of Which Half Were Exercised (Ex)

	control		GRASB		INURA		
time	non-ex	ex	non-ex	ex	non-ex	ex	SEM
1 day before slaughter	1.83[b]	1.59[b,c]	1.47[b,c]	1.55[b,c]	1.32[c]	1.59[b,c]	0.17–0.18
immediately after slaughter	3.01[b]	2.79[b]	2.32[c,d,e]	2.78[b,c,e]	2.14[d]	2.83[b]	

[a] LDH activity is expressed in millimoles of NADH oxidized per minute per liter of plasma. Data were corrected for differences in basic levels of LDH (1 day prior to diet change). [b−d] Within a row, means without a common superscript letter differ ($p < 0.05$). [e] $p = 0.06$ for difference between non-ex and ex pigs fed GRASB diet.

Table 5. LS Means ($n = 12$) of Activities (U/mg of protein) of the Antioxidative Enzymes Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (mU/mg of Protein) (GSH-Px) in LD

		control		GRASB		INURA		
enzyme	time[a]	non-ex	ex	non-ex	ex	non-ex	ex	SEM
SOD	1	0.51	0.58	0.56	0.48	0.51	0.63	0.080–0.106
	2	0.49[b]	0.66[b,c]	0.49[b]	0.57[b,c]	0.79[c]	0.55[b]	
CAT	1	8.2[b]	9.4[c]	13.2[e]	12.4[e]	10.3[c,d]	10.9[d]	0.39–0.62
	2	7.7[b]	8.5[b,c]	12.4[e]	12.4[d,e]	9.7[c]	10.0[c,d]	
GSH-Px	1	8.5	9.5	8.8	8.6	9.6	9.0	0.84–1.17
	2	6.4	7.0	8.3	8.3	7.8	7.5	

[a] Data from biopsies taken the day before (time 1) and immediately after (time 2) slaughter were corrected for differences in basic levels of respective enzyme activities (1 day prior to diet change). [b−e] Within a row, means without a common superscript letter differ ($p < 0.05$).

Activities of Antioxidative Enzymes. The activities of SOD and GSH-Px in muscle tissue were not affected by any of the feeding regimes used, whereas the activity of muscle CAT increased in INURA-fed pigs and more so in GRASB-fed pigs compared to the control (Table 5). Neither of the studied enzymes was affected by preslaughter stress, but both CAT ($p = 0.03$) and GSH-Px ($p = 0.003$) activities were decreased upon slaughter (time 2 in Table 5).

DISCUSSION

The applied diets resulted in three different PUFA levels in the meat within standards obtained in most commercial production systems, mainly due to differences in linoleic acid (C18:2). The increased PUFA content of the meat resulted in decreased oxidative stability, in accordance with previous studies on both fresh (*1*, *2*) and cooked pork (*2*).

During the preslaughter exercise used in the present study, the pigs are expected to increase the oxygen intake, as oxygen intake has been reported to increase 10−15-fold above rest for exercised humans (*18*). This is assumed to increase the oxidative stress in muscle (*18*, *19*) and serum (*20*) and, simultaneously, to introduce an ischemic-like situation as also occurs post mortem. Both situations are known to increase oxidative stress through increased formation of reactive oxygen species (*5*, *21*).

Extended exposure to stressors, e.g., training, has previously been reported to affect the antioxidative status of biological fluids and tissues; thus, SOD activities have been reported to increase in the coronary arterioles of pigs (*22*), and CAT activity has been shown to increase in muscle tissue of rats (*23*) upon

exposure to training. Similar training-induced responses are most likely to be ascribed to adaptation to the stressor. Likewise, adaptation to other stressors, e.g., diet change over an extended period of time, can induce changes in the antioxidative status of muscle, even though this area is poorly investigated. The observed increase in CAT activity in the meat of GRASB- and INURA-fed pigs might be an example of this.

In the present study, a dramatic increase in LDH activity in plasma was obtained upon slaughter, as also has been reported previously (*24*), most probably as a result of ongoing muscle damage (spasms) triggered by the CO_2 stunning procedure (*25*).

An effect of preslaughter stress was registered in the plasma of slaughtered pigs raised on the GRASB and INURA diets, which resulted in an additional increase in LDH activity compared to the respective levels in nonstressed pigs. Consequently, the LDH activities in plasma from these preslaughter-stressed pigs reached levels similar to those of nonstressed control pigs. This result agrees with previous reports of increased LDH activity in blood upon exposure of a physical stressor (exercise) (*26*, *27*), most possible with simultaneous formation of reactive radical species, which induce oxidative stress (*26*). Preslaughter stress increased TBARS in cooked meats in accordance with a study showing that preslaughter stress (exercise) of rats also increased TBARS values in both liver and muscles (*4*).

In conclusion, the present study shows that an increased content of unsaturated fatty acids in muscle increases the antioxidative potential of the muscle (increased catalase activity), which decreases the oxidative deterioration (lipid oxidation) upon cooking of the meat.

ABBREVIATIONS USED

CAT, catalase; GSH-Px, glutathione peroxidase; GRASB, grass meal, rape seed meal, and dried sugar beet pulp diet; INURA, inulin, rape seed meal, and animal fat diet; LD, *m. longissimus dorsi*; LDH, lactate dehydrogenase; PUFAs, polyunsaturated fatty acids; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

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LITERATURE CITED

(1) Rhee, K. S.; Ziprin, Y. A.; Ordonez, G.; Bohac, C. E. Fatty acid profiles of the total lipids and lipid oxidation in pork muscles as affected by canola oil in the animal diet and muscle location. *Meat Sci.* **1988**, *23*, 201−210.

(2) Monahan, F. J.; Buckley, D. J.; Morrissey, P. A.; Lynch, P. B.; Gray, J. I. Influence of dietary fat and alpha-tocopherol supplementation on lipid oxidation in pork. *Meat Sci.* **1992**, *31*, 229−241.

(3) Byrne, D. V.; Bredie, W. L. P.; Bak, L. S.; Bertelsen, G.; Martens, H.; Martens, M. Sensory and chemical analysis of cooked porcine meat patties in relation to warmed-over flavour and pre-slaughter stress. *Meat Sci.* **2001**, *59*, 229−249.

(4) Sen, C. K.; Atalay, M.; Agren, J.; Laaksonen, D. E.; Roy, S.; Hanninen, O. Fish oil and vitamin E supplementation in oxidative stress at rest and after physical exercise. *J. Appl. Physiol.* **1997**, *83*, 189−195.

(5) Halliwell, B. Free Radicals and Antioxidants: A Personal View. *Nutr. Rev.* **1994**, *52*, 253−265.

(6) Maraschiello, C.; Sárraga, C.; Regueiro, G. Glutathione peroxidase activity, TBARS, and α-tocopherol in meat from chickens fed different diets. *J. Agric. Food Chem.* **1999**, *47*, 867−872.

(7) Decker, E. A.; Xu, Z. Minimizing rancidity in muscle foods. *Food Technol.* **1998**, *52*, 54−59.

(8) Madsen, A.; Petersen, J.; Søgaard, A. *Anatomic content of the female and castrated male pig fed according to scale or ad libitum and slaughtered at 20, 50, 80 or 110 kg liveweight*; Report No. 769; National Institute of Animal Science: Tjele, Denmark, 1990.

(9) Madsen, A.; Østerballe, R.; Mortensen, H. P.; Bejerholm, C. *Meat quality. The influence of feeds on meat quality of growing pigs*; Report No. 673; National Institute of Animal Science: Tjele, Denmark, 1990.

(10) Rosenvold, K.; Petersen, J. S.; Laerke, H. N.; Jensen, S. K.; Therkildsen, M.; Karlsson, A. H.; Moller, H. S.; Andersen, H. J. Muscle glycogen stores and meat quality as affected by strategic finishing feeding of slaughter pigs. *J. Anim. Sci.* **2001**, *79*, 382−391.

(11) Passonneau, J. V.; Lowry, O. H. *Enzymatic Analysis: A Practical Guide*. The Human Press Inc.: Clifton, NJ, 1993.

(12) Jensen, S. K.; Engberg, R. M.; Hedemann, M. S. All-rac-alpha-tocopherol acetate is a better vitamin E source than all-rac-alpha-tocopherol succinate for broilers. *J. Nutr.* **1999**, *129*, 1355−1360.

(13) Lauridsen, C.; Nielsen, J. H.; Henckel, P.; Sørensen, M. T. Antioxidative and oxidative status in muscle of pigs fed rapeseed oil, vitamin E and cupper. *J. Anim. Sci.* **1999**, *77*, 105−115.

(14) Abei, A. Catalase in vitro. *Methods Enzymol.* **1984**, *105*, 121−126.

(15) St. Clair, D. K.; Chow, C. K. Glutathione peroxidase: activity and steady-state level of mRNA. In *Free Radicals: A Practical Approach;* Punchard, N. A.; Kelly, F. J., Eds.; Oril Press: Oxford, U.K., 1996; pp 227−240.

(16) Goldstein, S.; Czapski, G. Superoxide dismutase. In *Free Radicals: A Practical Approach;* Punchard, N. A.; Kelly, F. J., Eds.; Oril Press: Oxford, U.K., 1996; pp 241−255.

(17) Young, J. F.; Stagsted, J.; Jensen, S. K.; Karlsson, A. H.; Henckel, P. Ascorbic acid, α-tocopherol, and oregano supplements reduce stress-induced deterioration of chicken meat quality. *Poult. Sci.* **2003**, *82*, 1343−1351.

(18) Alessio, H. M. Exercise-induced oxidative stress. *Med. Sci. Sports Exercise* **1993**, *25*, 218−224.

(19) Davies, K. J.; Quintanilha, A. T.; Brooks, G. A.; Packer, L. Free radicals and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun.* **1982**, *107*, 1198−1205.

(20) Ashton, T.; Rowlands, C. C.; Jones, E.; Young, I. S.; Jackson, S. K.; Davies, B.; Peters, J. R. Electron spin resonance spectroscopic detection of oxygen-centred radicals in human serum following exhaustive exercise. *Eur. J. Appl. Physiol. Occup. Physiol.* **1998**, *77*, 498−502.

(21) Huk, I.; Brovkovych, V.; Nanobash Vili, J.; Weigel, G.; Neumayer, C.; Partyka, L.; Patton, S.; Malinski, T. Bioflavonoid quercetin scavenges superoxide and increases nitric oxide concentration in ischaemia-reperfusion injury: an experimental study. *Br. J. Surg.* **1998**, *85*, 1080−1085.

(22) Rush, J. W.; Laughlin, M. H.; Woodman, C. R.; Price, E. M. SOD-1 expression in pig coronary arterioles is increased by exercise training. *Am. J. Physiol. Heart Circ. Physiol.* **2000**, *279*, H2068−H2076.

(23) Somani, S. M.; Husain, K. Exercise training alters kinetics of antioxidant enzymes in rat tissues. *Biochem. Mol. Biol. Int.* **1996**, *38*, 587−595.

(24) Kallweit, E.; Sye, Y. S. Relationship between LDH (lactate dehydrogenase) isoenzyme distribution in the blood serum and meat quality charcteristics of pigs. *Fleischwirtschaft* **1971**, *51*, 1797−1800.

(25) Forslid, A. Muscle spasms during pre-slaughter CO_2-anaesthesia in pigs. Ethical considerations. *Fleischwirtschaft* **1992**, *72*, 167−168.

(26) Itoh, H.; Ohkuwa, T.; Yamazaki, Y.; Shimoda, T.; Wakayama, A.; Tamura, S.; Yamamoto, T.; Sato, Y.; Miyamura, M. Vitamin E supplementation attenuates leakage of enzymes following 6 successive days of running training. *Int. J. Sports Med.* **2000**, *21*, 369−374.

(27) Chen, M. T.; Lin, S. S.; Lin, L. C. Effect of stress before slaughter on changes to the physiological, biochemical and physical characteristics of duck muscle. *Br. Poult. Sci.* **1991**, *32*, 997−1004.

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