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Original article

Effect of supplementation with vitamins E, C and β-carotene on antioxidative/oxidative status parameters in sows during the postpartum period

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Abstract

The effect of vitamins E, C and β -carotene supplementation in sows on the parameters of antioxidative/oxidative status during the postpartum period was investigated. Twenty four primiparous sows, divided into two groups (experimental and control), were included in the study. After the half-way point of pregnancy until farrowing, each experimental sow received feed supplemented twice a week with 200 mg of vitamin E and 1000 mg of vitamin C, and additionally, 70 mg of β-carotene were administered via intramuscular injection, on day 14 and day 7 before farrowing. The control group was not supplemented. Blood samples were collected before supplementation (gestational day 57-58), 48 hours and 7 days after parturition. The following antioxidative and oxidative parameters were measured using spectrophotometric methods: glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), vitamin C, vitamin E, thiobarbituric acid reactive substances (TBARS), and sulfhydryl groups (SH groups). In supplemented sows the erythrocyte activity of GSH-Px and CAT was found to be significantly higher on day 7 after farrowing and the activity of SOD was significantly higher at 48 hours postpartum, compared to the control group. The concentration of vitamins C and E in plasma of the supplemented group was found to be significantly higher and the content of TBARS was found significantly lower at both postpartum measurement points, compared to the control group. The content of SH groups was significantly higher on day 7 postpartum, compared to the control group. The study findings indicate that supplementation of pregnant sows with vitamins E, C and β-carotene in the second half of pregnancy has beneficial effects on the antioxidative/oxidative balance in the postpartum period by increasing the antioxidative potential and reducing lipid and protein peroxidation.

Key words: sows, antioxidants, oxidative stress, postpartum period

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Introduction

A balance between production and neutralisation of reactive oxygen species (ROS) is essential for maintaining homeostasis and the good health of humans and animals. The antioxidant defense system, involving antioxidant enzymes and numerous non-enzymatic endogenous and exogenous antioxidants, is responsible for safe levels of ROS (Halliwell 2006). Antioxidative/oxidative imbalance, caused by excessive production of ROS or/and inefficient antioxidant mechanisms, induces oxidative stress, which is harmful as it leads to oxidative damage to macromolecules, impairs the metabolism and immunity, and can ultimately result in the development of many diseases (Halliwell 2006, Lykkesfeldt and Svendsen 2007, Sordillo and Aitken 2009).

The results of numerous studies indicate that ROS are overproduced during pregnancy (Casanueva and Viteri 2003), delivery (Mocatta et al. 2004) and postpartum (Markiewicz et al. 2005, Kankofer et al. 2010). ROS overproduction causes oxidative stress in mothers and newborns, which is likely to lead to various postpartum diseases (Miller et al. 1993, LeBlanc et al. 2004, Kankofer et al. 2010).

Since ROS have been implicated in the aetiology of many diseases in humans and animals, prevention of these diseases by the administration of various antioxidant compounds arouses much interest. Amongst the factors that can modify oxidative stress, special attention has been paid to vitamin E, vitamin C and β-carotene, whose antioxidative properties have been well documented and which can be easily and safely used as dietary and parental supplements. All three vitamins belong to the main non-enzymatic antioxidants (Chan 1993). They have been demonstrated to reduce oxidative damage to lipids, proteins and nucleic acids (Sies and Murphy 1991). These vitamins are known to show a synergic effect if they exist together as antioxidants (Chan 1993). The synergy between vitamin E and C consists in regeneration of vitamin E by vitamin C (Chan 1993). Unlike vitamin E, vitamin C is capable of scavenging the hypochlorous acid and the tyrosyl radical and protects lipids against reactive nitrogen species-induced oxidation (Carr et al. 2000). Vitamin E protects the double bonds of β-carotene against peroxidation; β-carotene also has the ability to break the chain reaction of lipid peroxidation. Unlike vitamin E, which acts more efficiently at higher oxygen concentrations, β-carotene shows better action at low partial oxygen pressure (Stahl and Sies 1997). Taking into account the synergy between these vitamins we may expect better effects of supplementation of all three vitamins than one individual vitamin on antioxidative/oxidative status of humans and animals.

Numerous studies have shown beneficial effects of supplementation of individual vitamins on pig health and reproductive performance (Mahan 1994, Kostoglou et al. 2000, Pinelli-Saavedra and Scaife 2005); however, the data regarding effects of administration of all three vitamins to pregnant sows on their antioxidative/oxidative status are lacking.

The aim of the present study was to determine the effect of vitamin E, C and β-carotene supplementation in sows on parameters of antioxidative/oxidative status during the postpartum period.

Materials and Methods

Animals and material collection

The study design was approved by the Local Ethics Committee for Experiments with Animals appointed by the University of Life Sciences in Lublin.

Twenty-four Polish Large White and Polish Large White x Polish Landrace primiparous sows were included in the study. All the animals came from one closed-cycle production farm. Once the pregnancy was diagnosed, the sows were penned in individual crates. Approximately 8-10 days before the expected farrowing date, the animals were moved to the farrowing unit and kept in individual farrowing creates. During pregnancy, the sows were fed according to the changing demands for nutrients and energy. The feed was prepared in the farm's mixer and contained: barley, oats, wheat bran, rapeseed cake, soybean meal, mineral lick, and premix for pregnant sows. The study animals were divided into two groups – experimental (group A) and control (group B), 12 sows each. After the half-way point of pregnancy (gestational day 57-58) until farrowing, each experimental sow received feed supplemented twice a week with 200 mg of vitamin E and 1000 mg of vitamin C (Polfasol® EC (vitamin E 0.02g, vitamin C 0.1g, vehicle ad 1.0g), Polfa Kutno, Poland). Polfasol was added to the trough individually for each sow during feeding. Additionally, the experimental animals were administered intramuscular β-carotene (Carofertin® (β-carotene 10 mg, solvent ad 1 ml), Werfft-Chemie GmBH, Vienna, Austria) at a dose of 70 mg/sow at 14 and 7 days before farrowing. The control group was not supplemented. All sows included in the study were clinically healthy, farrowings occurred spontaneously (not induced) on gestational day 115-116. Furthermore, no obstetrical assistance was given, and no drugs were administered throughout the study period.

Blood samples were collected from the cranial vena cava before supplementation (gestational day 57-58), 48 hours and 7 days after farrowing. Plasma

was obtained by blood centrifugation. After haemoglobin determination in the suspension of erythrocytes, which remained after plasma collection, haemolysis was performed with cold distilled water. The prepared plasma and haemolysates were immediately frozen at – 76°C and stored deeply frozen until determinations. Haemolysates were used for antioxidative enzyme activity determinations (glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT)). In the blood plasma, the concentrations of vitamin E, vitamin C, products of lipid peroxidation (thiobarbituric acid reactive substances – TBARS) and protein peroxidation (SH groups) as well as total protein were determined.

Biochemical analyses

The erythrocyte activity of antioxidant enzymes in erythrocytes was determined spectrophotometrically using the Ultrospec 2000 (Pharmacia, Uppsala, Sweden). The activity of GSH-Px was measured at 340 nm according to the Paglia and Valentine (1967) method. The results were expressed in nanokatals per gram of haemoglobin (nkat/g Hb). The activity of SOD was determined at 340 nm using the method described by Sun and Zigman (1978) and expressed in SOD units per gram of haemoglobin (U-SOD/g Hb). CAT activity was measured at 480 nm according to the Cohen et al. (1970) method. The results were presented in nkat/g Hb.

The concentration of vitamin E was estimated using the spectrophotofluorimetric method described by Hansen and Warwick (1966) using a LS 30 Spectrophotofluorimeter (Parkin Elmer Inc., Massachusetts, USA) at wave lengths of 280 nm and 310 nm. The results were expressed in μg/ml.

The concentration of vitamin C was determined spectrophotometrically at 520 nm (Ultrospec 2000, Pharmacia, Uppsala, Sweden) according to the Omaye et al. (1979) method. The results were expressed in mmol/g protein.

The concentration of TBARS was measured spectrophotometrically at 532 nm (Ultrospec 2000, Pharmacia, Uppsala, Sweden) according to the method described by Ledwozyw et al. (1986) and expressed in μmol/g protein.

The content of SH groups was determined spectrophotometrically according to the Rice-Evans et al. (1991) method. The reaction mixture absorbance was measured at 412 nm (Ultrospec 2000, Pharmacia, Uppsala, Sweden). The results were expressed in μmol/g protein.

The protein contents of plasma samples were measured by the biuret method using commercial assay kits (Cormay, Lublin, Poland) based on spectrophotometric measurement (Ultrospec 2000, Pharmacia, Uppsala, Sweden). These values were only used for recalculations of examined parameters for more objective comparisons between different sources of samples.

Statistical analysis

Statistical analysis was performed using Statistica 6.0 (Statistica Software, Poland). The data were found to be normally distributed in accordance with the Kolmogorov-Smirnov test. The Student's *t*-test was used for analysis of statistical significances. All data were presented as means ± standard deviation of the means (SD). The level of significance was set at $P<0.05$.

Results

Before supplementation the mean values of the examined parameters did not differ significantly ($p>0.05$) between the examined groups of sows. The activity of GSH-Px significantly increased ($p<0.001$) after farrowing both in the experimental and control group. GSH-Px activity in the experimental group was found significantly higher ($p<0.05$) compared to the control group only at day 7 postpartum. The activity of SOD in both groups increased after farrowing compared to the pre-supplementation period. A significant difference was found in the experimental group at 48 h ($p<0.001$) and at day 7 ($p<0.01$) after farrowing and in the control group only at day 7 postpartum ($p<0.01$). At 48 h postpartum the activity of SOD was significantly higher ($p<0.001$) in the experimental group than in the control group. CAT activity in the experimental group was significantly higher at 48 h ($p<0.01$) and at day 7 ($p<0.001$) after farrowing compared to the pre-supplementation period. In the control group, at both postpartum measurement points CAT activity did not differ significantly ($p>0.05$) compared with the pre-supplementation period. At day 7 after farrowing CAT activity in the experimental group was significantly higher ($p<0.01$) compared to the control group (Table 1).

In the experimental group, the concentration of vitamin E significantly increased ($p<0.001$) at both postpartum measurement points whereas in the control group, this concentration significantly decreased ($p<0.01$) at 48 h after farrowing compared to the pre-supplementation period. The concentration of vitamin E was significantly higher ($p<0.001$) in the experimental group than in the controls both 48 h and 7 days after farrowing (Table 2).

Table 1. Erythrocyte activity of GSH-Px, SOD and CAT in the examined groups of sows (means ± SD).

Parameter	Group	Pre-supplementation period (pregnancy day 57-58)	48 h after farrowing	7 d after farrowing
GSH-Px	A	199.21 ± 11.34	293.39 ± 13.84^{c}	297.59 ± 11.67^{*c}
(nkat/g Hb)	B	197.04 ± 9.67	285.39 ± 14.00^{c}	286.06 ± 10.17^{c}
SOD	A	5080.19 ± 231.49	5579.27 ± 149.32^{***c}	5826.31 ± 575.08^{b}
(U-SOD/g Hb)	B	4988.73 ± 378.75	5127.62 ± 322.58	5537.97 ± 388.59^{b}
CAT	A	335.90 ± 48.51	391.91 ± 33.51^{b}	429.42 ± 35.84^{**c}
(nkat/g Hb)	B	316.56 ± 67.51	355.90 ± 47.00	369.24 ± 33.84

A – experimental (supplemented) group; B – control group; a – p<0.01 compared to baseline (before supplementation); b – p<0.001 compared to baseline (before supplementation); * – p<0.05 compared to control group; ** – p<0.01 compared to control group; *** – p<0.001 compared to control group

Table 2. Plasma concentration of vitamin E and vitamin C in the examined group of sows (means ± SD).

Parameter	Group	Pre-supplementation period (pregnancy day 57-58)	48 h after farrowing	7 d after farrowing
Vitamin E	A	1.97 ± 0.21	2.71 ± 0.16^{***c}	2.81 ± 0.15^{***c}
(μg/ml)	B	1.86 ± 0.35	1.36 ± 0.28^{b}	1.99 ± 0.34
Vitamin C	A	0.54 ± 0.11	0.44 ± 0.18**	0.27 ± 0.12^{*c}
(mmol/g protein)	B	0.50 ± 0.16	0.21 ± 0.11^{c}	0.17 ± 0.05^{c}

A – experimental (supplemented) group; B – control group; a – p<0.01 compared to baseline (before supplementation); b – p<0.001 compared to baseline (before supplementation); * – p<0.05 compared to control group; ** – p<0.01 compared to control group; *** – p<0.001 compared to control group

Table 3. Plasma concentration of TBARS and SH groups in the examined groups of sows (means ± SD).

Parameter	Group	Pre-supplementation period (pregnancy day 57-58)	48 h after farrowing	7 d after farrowing
TBARS	A	0.082 ± 0.029	0.087 ± 0.014***	0.076 ± 0.008***
(μmol/g protein)	B	0.098 ± 0.024	0.139 ± 0.028^{b}	0.098 ± 0.014
Grupy SH	A	3.00 ± 0.89	3.50 ± 0.25^{b}	3.81 ± 0.21^{**b}
(μmol/g protein)	B	2.95 ± 0.62	3.54 ± 0.28	3.24 ± 0.42

A – experimental (supplemented) group; B – control group; a – p<0.01 compared to baseline (before supplementation); b – p<0.001 compared to baseline (before supplementation); * – p<0.05 compared to control group; ** – p<0.01 compared to control group; *** – p<0.001 compared to control group

The concentration of vitamin C decreased in both groups of sows after farrowing compared to the pre-supplementation period. Significant differences (p<0.001) were found at 48 h postpartum in the control group and at day 7 in both groups. The concentration of vitamin C was significantly higher in the experimental group than in the control group both 48 h (p<0.01) and 7 days (p<0.05) after farrowing (Table 2).

In the experimental group, TBARS concentrations at 48 h after farrowing were similar to the pre-supplementation values whereas 7 days after parturition they decreased markedly but no significant difference was found (p>0.05). In the control group, TBARS concentrations at 48 h after farrowing significantly increased (p<0.01) compared to pre-supplementation values. At both measurement points after farrowing, the concentration of TBARS in the experimental group was significantly lower (p<0.001) than that in the control group (Table 3).

In the experimental group the concentration of SH groups increased significantly (p<0.01) after farrowing compared with the pre-supplementation period. At day 7 after parturition, the concentration of SH groups was significantly higher (p<0.01) in the supplemented group than in the control (Table 3).

Discussion

The aim of the study was to determine the effect of supplementation of the pregnant sows with vitamin

E, C and β-carotene on their antioxidative/oxidative status in the postpartum period. Our results indicate that combined use of these vitamins in the second half of pregnancy exerted beneficial effects on postpartum antioxidative-oxidative status.

The key elements of cell antioxidant defense are antioxidant enzymes. SOD together with GSH-Px and CAT, are the first-line antioxidant protection of any cell (Halliwell 2006). Our findings demonstrated that the combination of antioxidant vitamins stimulated the activity of antioxidant enzymes in erythrocytes of the supplemented sows, which was consistent with the earlier studies of other authors (Rodrigez-Porcel et al. 2002, Tauler et al. 2002). Rodrigez-Porcel et al. (2002) observed increased activities of GSH-Px, SOD and CAT in pig myocardial tissues after a 12-week supplementation with vitamin E at a dose of 100 mg/kg fodder and vitamin C at a dose of 1g per animal. Tauler et al. (2002) showed that supplementation with an antioxidant cocktail of vitamin E, vitamin C and β-carotene for 90 days significantly increased the activity of SOD and CAT in neutrophils of sportsmen. In turn, Zaidi and Banu (2004) found that both vitamin E alone and in combination with vitamins A and C was effective in enhancing the activities of SOD and CAT in stressed rats. The mechanisms through which exogenous vitamins affect the endogenous system of antioxidant defense have not been fully explained. Their effects on genes encoding the synthesis of antioxidant enzymes are considered to be likely (Franco et al. 1999).

Vitamin E is the most important fat-soluble antioxidant and the main element of the first-line defence against fat-soluble peroxyl radicals (Stahl and Sies 1997). Our findings showed that administration of vitamin E with vitamin C and β-carotene to sows during pregnancy resulted in higher vitamin E concentrations in the plasma of the supplemented sows after farrowing, compared to the sows without supplementation. The above results correlate with those presented by Pinelli-Saavedra and Scaife (2005) as well as Pinelli-Saavedra et al. (2008). These authors observed that vitamin E and C supplements (vitamin E 200mg/kg feed + vitamin C 1g/day) received throughout the pregnancy resulted in a significantly higher serum concentration of vitamin E in the supplemented sows at 21 day postpartum compared to the controls and the group supplemented only with vitamin E (200 mg/kg feed). Chao et al (2002) investigated the effects of the supplementation with vitamin C or/and E on the antioxidant system in the hemodialysis of humans and demonstrated that after a 6-week supplementation, the vitamin C+E -supplemented group had significantly higher plasma vitamin E compared with the individual vitamin supplemented groups and the control group. Similarly, Tauler et al. (2002) showed that a combined supplementation with vitamin E, vitamin C and β-carotene significantly increased the plasma levels of vitamin E in humans.

Vitamin C belongs to water-soluble antioxidants and is considered the major antioxidant in extracellular fluids (Stahl and Sies 1997). It is believed that pigs synthesise sufficient amounts of vitamin C to cover their body demands under normal conditions and they do not require its supplementation. However, oxidative stress as well as stress situations during the perinatal period and possible transfer of vitamin C to foetuses and milk increase vitamin C requirements (Ching et al. 2001, Zaidi and Banu 2004). Our study demonstrated that despite the administration of vitamin C together with vitamin E and β-carotene to the pregnant sows, its concentration in the postpartum period was lower than the pre-supplementation values. However, in the supplemented sows, the vitamin C concentration was significantly higher than in the sows without supplementation. It is difficult to determine whether higher concentrations of vitamin C in the supplemented sows were affected by its administration combined with other antioxidants. Previous studies in which pregnant sows were supplemented with vitamin C alone at a dose 1g per sow daily found an increase in its plasma concentration after farrowing compared to the control group, but no significant difference was found (Yen and Pond 1983, Pinelli-Saavedra and Scaife 2005). The results presented by Lauridsen et al. (1999) revealed that high doses of vitamin E administered to sows did not affect the plasma vitamin C concentrations. Likewise, Pinelli-Saavedra and Scaife (2005) noted that the combined administration of vitamin E and C to sows did not cause an increase in the vitamin C concentrations at day 21 postpartum compared to the pre-supplementation values and those in the controls. However, combined use of vitamin E and C in humans resulted in double increases in their concentrations compared to administration of each vitamin separately (Huang et al. 2002). Also Chao et al (2002) demonstrated that humans supplemented with vitamin C and E had significantly higher plasma vitamin C levels compared with humans supplemented with only one of these vitamins.

The study findings demonstrated significantly lower concentrations of TBARS and significantly higher concentrations of SH groups in the supplemented group than in the group without supplementation. This indicates that vitamin E, C and β-carotene supplied to pregnant sows increased the ability to protect lipids and proteins against ROS attacks in the postpartum period. Reduced plasma concentrations of TBARS in the sows supplemented only with vit-

amin E at a dose of 235 mg/kg fodder were earlier demonstrated by Duthie et al. (1989). However, such effects were not observed in piglets receiving vitamin E at a dose of 100 and 200 mg/kg fodder (Lauridsen et al. 1999). Our results confirm findings of similar studies in humans (Bolisetty et al. 2002, Chao et al. 2002, Huang et al. 2002). Bolisetty et al. (2002) supplemented pregnant women with vitamin E, vitamin C and β-carotene, and found that the plasma level of malondialdehyde (MDA) at delivery was significantly lower in the supplemented group compared to the control group. Chao et al (2002) reported lower plasma lipid peroxides in the vitamin C+E -supplemented group of humans compared with the individual vitamin supplemented groups and the control group. Huang et al. (2002) showed that supplementation with a combination of vitamins E and C reduced lipid peroxidation measured based on an evaluation of the urinary 8-isoprostaglandin $F_{2\alpha}$, MDA and 4-hydroxyalkenals.

Data regarding effects of supplementation of vitamin E, C and β-carotene on protein peroxidation in sows are lacking. Studies in humans show that vitamin E supplementation reduces oxidative damage to proteins (Reznick et al. 1992). Likewise, supplementation with vitamin C reduces protein peroxidation in humans and experimental animals (Barja et al. 1994, Carty et al. 2000).

In conclusion, our study findings indicate that supplementation of pregnant sows with vitamins E, C and β-carotene in the second half of pregnancy has beneficial effects on the antioxidative/oxidative balance in the postpartum period by increasing the antioxidant potential and reducing lipid and protein peroxidation.

References

Barja G, Lopez-Torres M, Perez-Campo R, Rojas C, Cadenas S, Prat J, Pamplona R (**1994**) Dietary vitamin C decreases endogenous protein oxidative damage, malondialdehyde, and lipid peroxidation and maintains fatty acid unsaturation in the guinea pig liver. Free Radic Biol Med 17: 105-115.

Bolisetty S, Naidoo D, Liu K, Koh TH, Watson D, Whitehall J (**2002**) Antenatal supplementation of antioxidant vitamins to reduce the oxidative stress at delivery – a pilot study. Early Hum Dev 67: 47-53.

Carr AC, McCall MR, Frei B (**2000**) Oxidation of LDL by myeloperoxidase and reactive nitrogen species: reaction pathways and antioxidant protection. Arterioscler Thromb Vasc Biol 20: 1716-1723.

Carty JL, Bevan R, Waller H, Mistry N, Cooke M, Lunec J, Griffiths HR (**2000**) The effects of vitamin C supplementation on protein oxidation in healthy volunteers. Biochem Biophys Res Commun 273: 729-735.

Casanueva E, Viteri FE (**2003**) Iron and oxidative stress in pregnancy. J Nutr 133: 1700S-1708S.

Chan AC (**1993**) Partners in defense, vitamin E and vitamin C. Can J Physiol Pharmacol 71: 725-731.

Chao JC, Yuan MD, Chen PY, Chien SW (**2002**) Vitamin C and E supplements improve the impaired antioxidant status and decrease plasma lipid peroxides in hemodialysis patients small star, filled. J Nutr Biochem 13: 653-663.

Ching S, Mahan DC, Ottobre JS, Dabrowski K (**2001**) Ascorbic acid synthesis in fetal and neonatal pigs and in pregnant and postpartum sows. J Nutr 131: 1997-2001.

Cohen G, Dembiec D, Marcus J (**1970**) Measurement of catalase activity in tissue extracts. Anal Biochem 34: 30-38.

Duthie GG, Arthur JR, Bremner P, Kikuchi Y, Nicol F (**1989**) Increased peroxidation of erythrocytes of stress-susceptible pigs: an improved diagnostic test for porcine stress syndrome. Am J Vet Res 50: 84-87.

Franco AA, Odom RS, Rando TA (**1999**) Regulation of antioxidant enzyme gene expression in response to oxidative stress and during differentiation of mouse skeletal muscle. Free Radic Biol Med 27: 1122-1132.

Halliwell B (**2006**) Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol 141: 312-322.

Hansen LG, Warwick WJ (**1966**) A fluorometric micro method for serum tocopherol. Tech Bull Regist Med Technol 36: 131-136.

Huang HY, Appel LJ, Croft KD, Miller ER 3rd, Mori TA, Puddey IB (**2002**) Effects of vitamin C and vitamin E on *in vivo* lipid peroxidation: results of a randomized controlled trial. Am J Clin Nutr 76: 549-555.

Kankofer M, Albera E, Feldman M, Gundling N, Hoedemaker M (**2010**) Comparison of antioxidative/oxidative profiles in blood plasma of cows with and without retained fetal placental membranes. Theriogenology 74: 1385-1395.

Kostoglou P, Kyriakis SC, Papasteriadis A, Roumpies N, Alexopoulos C, Saoulidis K (**2000**) Effect of β-carotene on health status and performance of sows and their litters. J Anim Physiol Anim Nutr 83: 150-156.

Lauridsen C, Hojsgaard S, Sorensen MT (**1999**) Influence of dietary rapeseed oil, vitamin E, and copper on the performance and the antioxidative and oxidative status of pigs. J Anim Sci 77: 906-916.

LeBlanc SJ, Herdt TH, Seymour WM, Duffield TF, Leslie KE (**2004**) Peripartum serum vitamin E, retinol, and beta-carotene in dairy cattle and their associations with disease. J Dairy Sci 87: 609-619.

Ledwozyw A, Michalak J, Stepien A, Kadziołka A (**1986**) The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human artheriosclerosis. Clin Chim Acta 155: 275-284.

Lykkesfeldt J, Svendsen O (**2007**) Oxidants and antioxidants in disease: oxidative stress in farm animals. Vet J 173: 502-511.

Mahan DC (**1994**) Effects of dietary vitamin E on sow reproductive performance over a five-parity period. J Anim Sci 72: 2870-2879.

Markiewicz H, Gehrke M, Malinowski E, Kaczmarowski M (**2005**) Evaluating the antioxidant potential in the blood of transition cows. Med Weter 61: 1382-1384.

Miller JK, Brzezinska-Slebodzinska E, Madsen FC (**1993**) Oxidative stress, antioxidants and animal function. J Dairy Sci 76: 2812-2823.

Mocatta TJ, Winterbourn CC, Inder TE, Darlow BA (**2004**) The effect of gestational age and labour on markers of lipid and protein oxidation in cord plasma. Free Radic Res 38: 185-191.

Omaye ST, Turnbull JD, Sauberlich HE (**1979**) Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods Enzymol 62: 3-11.

Paglia DE, Valentine WN (**1967**) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70: 158-169.

Pinelli-Saavedra A, Scaife JR (**2005**) Pre- and postnatal transfer of vitamins E and C to piglets in sows supplemented with vitamin E and vitamin C. Livest Prod Sci 97: 231-240.

Pinelli-Saavedra A, Calderon de la Barca AM, Hernandez J, Valenzuela R, Scaife JR (**2008**) Effect of supplementing sows; feed with alpha-tocopherol acetate and vitamin C on transfer of alpha-tocopherol to piglet tissues, colostrum, and milk: aspects of immune status of piglets. Res Vet Sci 85: 92-100.

Reznick AZ, Cross CE, Hu M, Suzuki YJ, Khwaja S, Safadi A, Motchnik PA, Packer L, Halliwell B (**1992**) Modification of plasma proteins by cigarette smoke as measured by protein carbonyl formation. Biochem J 286: 607-611.

Rice-Evans CA, Diplock AT, Symons MC (**1991**) Techniques in free radical research. In: Burdon RH, van Knippenberg PH (eds) Laboratory techniques in biochemistry and molecular biology. Elsevier Science Publishers BV, Amsterdam.

Rodriguez-Porcel M, Lerman LO, Holmes DR Jr, Richardson D, Napoli C, Lerman A (**2002**) Chronic antioxidant supplementation attenuates nuclear factor-kappa B activation and preserves endothelial function in hypercholesterolemic pigs. Cardiovasc Res 53: 1010-1018.

Sies H, Murphy ME (**1991**) Role of tocopherols in the protection of biological systems against oxidative damage. J Photochem Photobiol B 8: 211-224.

Sordillo LM, Aitken SL (**2009**) Impact of oxidative stress on the health and immune function of dairy cattle. Vet Immunol Immunopathol 128: 104-109.

Stahl W, Sies H (**1997**) Antioxidant defense: vitamins E and C and carotenoids. Diabetes 46 (Suppl 2): S14-S18.

Sun M, Zigman S (**1978**) Determination of superoxide dismutase in erythrocytes using the method of adrenaline autooxidation. Anal Biochem 90: 81-89.

Tauler P, Aguilo A, Fuentespina E, Tur JA, Pons A (**2002**) Diet supplementation with vitamin E, vitamin C and beta-carotene cocktail enhances basal neutrophil antioxidant enzymes in athletes. Pflugers Arch 443: 791-797.

Yen JT, Pond WG (**1983**) Response of swine to periparturient vitamin C supplementation. J Anim Sci 56: 621-624.

Zaidi SMKR, Banu N (**2004**) Antioxidant potential of vitamins A, E and C in modulating oxidative stress in rat brain. Clin Chim Acta 340: 229-233.