ANTIOXIDANT AND IMMUNE STATUS OF PIGS SUPPLEMENTED WITH SELENIUM AND VITAMIN-E

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ABSTRACT

Oxidation and production of free radicals are integral parts of cell metabolism in animals. Selenium (Se), a component of glutathione peroxidase enzyme, destroys free radicals in the cytoplasm, while vitamin E (vit-E), a non-enzyme scavenger of free radicals acts as a lipid soluble antioxidant in cell membrane. Previous studies have indicated improvement in antioxidant activity and immune status of lambs and calves through dietary supplementation of Se and vit-E, while little information is available in pigs (*Sus domesticus*). The present study was conducted to assess the effect of dietary supplementation of Se and vit-E in seclusion and in conjugation on the antioxidant enzyme levels and immune status in pigs, for which twenty male large white Yorkshire pigs of similar age (2-3 months) and body weight (14.96 ± 0.68 kg, average) were randomly divided into four groups with equal number of animals in each group. Group I served as control (basal diet without any supplementation). Group II animals were given basal diet, supplemented with 0.3 mg selenium as sodium selenite. Group III animals were given basal diet, supplemented with 100 mg of vitamin E as DL-α-tocopheryl acetate. Group IV animals were given basal diet, supplemented with both Se and vit-E at the doses given to group II and group III animals. Blood was collected at the outset (0 day) and at the end (120 days) of experimental feeding and analyzed for activity of antioxidant enzymes, *viz*., catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) concentration as a measure of lipid per-oxidation (LPO). At 120 days, cellular and humoral immunity responses were determined. The results revealed that supplementation of Se alone could significantly ($P \leq 0.05$) enhance SOD, supplementation of Se and vit-E in combination enhanced ($P \leq 0.05$) CAT and GSH-Px, while supplementation of Se and / or vit-E reduced ($P \leq 0.05$) LPO. Cellular and humoral immune responses were significantly ($P \leq 0.05$) enhanced with supplementation of Se and / or vit-E. It is concluded that Se and vit-E significantly augmented the levels of antioxidant enzymes in pigs with concomitant upsurge in immune status.

KEY WORDS

Antioxidant enzymes, Immunity, Pigs, Selenium, Vitamin E

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INTRODUCTION

Selenium (Se), a component of glutathione peroxidase enzyme, destroys free radicals in the cytoplasm. Vitamin E, a non-enzyme scavenger of free radicals (Mac Pherson, 1994), acts as a lipid soluble antioxidant in cell membrane (Noguchi et al., 1973). Oxidation and production of free radicals are integral parts of cell metabolism in animals, which are destroyed by glutathione peroxidase, whereas vitamin E reacts with peroxide radicals to yield stable hydroperoxide.

NRC (1998) recommended a dietary level of 0.3 mg Se and 100 IU of vitamin E / kg DM for growing pigs. Previous studies have reported that, Se and vitamin E supplementation improved antioxidant activity and immunity in lambs (Kumar et al., 2009) and buffalo calves (Sandhu and Singha, 2003). However, little information is available on the effect of supplementation of selenium and vitamin E on antioxidant and immune status of pigs (*Sus domesticus)*. Therefore, the present study was conducted in male large white Yorkshire pigs to assess the effect of selenium and vitamin E supplementation on their antioxidant enzymes and immune status.

MATERIALS AND METHODS

Experimental animals, their feeding and management: Twenty (20) male large white Yorkshire piglets of similar age (2-3 months) and body weight (14.96 ± 0.68 kg, average) reared at Instructional Livestock Farm, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha were selected for the current study. The piglets were then divided into four groups of five animals each based on their body weights following randomized block design and were kept in a well-ventilated shed. Pigs in all the four groups were fed on concentrate mixture to meet their nutrient requirements (NRC, 1998).

The constituents of concentrate mixture were crushed maize (62%), deoiled soyabean meal (15%), wheat bran (15%), fish meal (06%), mineral mixture (1.5%), and common salt (0.5%). The chemical composition of feed on dry matter (DM) basis was organic matter (89.28%), crude protein (18.3%), ether extract (2.18%), crude fibre (5.63%), total ash (10.72%), nitrogen free extract (63.17%), calcium (1.47%), phosphorus (0.8%), and selenium (0.02 ppm). The crude protein content of concentrate mixture was 18.3%, whereas the Se concentration was 0.02 mg / kg.

The feeding treatments of different groups were, group I (basal diet, i.e. control), group II (basal diet supplemented with 0.3 ppm Se as sodium selenite), group III (basal diet supplemented with 100 mg vitamin E as DL-α-tocopheryl acetate), and group IV (basal diet supplemented with 0.3 ppm inorganic Se and 100 mg vitamin E). The supplements were given along with the concentrate mixture. Clean and fresh drinking water was provided *ad lib* twice a day to all the animals. This feeding experiment lasted for 120 days.

Blood was collected at the outset (0 day), and after 120 days of experimental feeding to estimate antioxidant enzymes.

Processing of blood samples and preparation of erythrocyte pellet: Five (5) ml whole blood was collected from all the groups at 0 day and at 120 days of experimental feeding in sterilized micro-centrifuge tubes containing 0.75 ml of acid citrate dextrose (ACD) constituting citric acid 8 g, sodium citrate 22 g and dextrose 25 g, and volume made to 1 litre with addition of distilled water as anticoagulant. Subsequently, the blood samples were centrifuged at 3000 rpm for 10 min at 4°C followed by separation of plasma and the buffy coat.

The resulting erythrocyte pellet was washed thrice with phosphate buffer saline (PBS) containing disodium hydrogen phosphate 13.65 g, sodium dihydrogen phosphate 2.43 g and sodium chloride 10 g dissolved in 800 ml distilled water, pH adjusted to 7.4 and volume made to 1 litre with addition of distilled water (Yagi et al.,1989). The pellet was then diluted to 1:1 in PBS and was used for the estimation of haemoglobin. However for the estimation of catalase (CAT), superoxide dismutase (SOD), lipid per-oxidation (LPO) and glutathione peroxidase (GSH-Px), 1 ml of the 1:1 diluted RBCs were mixed with 9 ml distilled water to prepare RBC haemolysate of 1:20 dilution.

Estimation of antioxidant enzymes: Lipid peroxidation in RBC hemolysate was determined as per Placer et al. (1966), and the concentration of malondialdehyde (MDA) in nmol of MDA / mg haemoglobin was calculated using the extinction coefficient of 1.56×108 / M / cm (Utley et al., 1967). CAT was assayed in erythrocytes by the method of Bergmeyer et al. (1983). SOD activity of RBC haemolysate samples was measured using nitro blue tetrazolium as a substrate with suitable dilution (Marklund and Marklund, 1974) and modifcation as per Minami and Yoshikawa (1979). GSH-Px activity was determined as per Paglia and Valentine (1967).

Immune status: At 120 days of the experiment, all the pigs were injected intradermally in the flank region with 100 micro gram of Phytohaemaglutinin-P (PHA-P) in 0.1 ml of normal saline to measure the cellular immune response. The humoral immunity was assessed from the antibody production against sheep red blood cell (SRBC) by using micro titration hemagglutination (HA) technique.

Statistical analysis: Data were subjected to the test of significance between the different groups of animals using two way analysis of variance techniques (Snedecor and Cochran, 1980) and means were compared using Duncan's multiple range tests (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The results revealed significant ($P \leq 0.05$) difference between different treatment groups with respect to the levels of different antioxidant enzymes, *viz*., glutathione peroxidase (GSH-Px),

superoxide dismutase (SOD), lipid peroxidation (LPO), and catalase (CAT) in the RBC of pigs after the completion of 120 days period of feeding trial (Table-1).

Antioxidant enzymes: The mean GSH-Px (µmole NADPH oxidized /g Hb/ min) of group II, group III, and group IV at 120 days were significantly ($P \leq 0.05$) higher than group I (control). The differences between group II, group III, and group IV were non-significant ($P \geq 0.05$). This indicated that supplementation of the combination of both Se and vit-E enhanced the level of GSH-Px.

The mean SOD (U/mg Hb) of group II and group IV at 120 days were significantly ($P \leq 0.05$) higher than group I (control). The differences between group II, group III, and group IV, and the difference between group I and group III were non-significant ($P \geq 0.05$). This indicated that supplementation of Se alone could enhance SOD.

The mean of LPO (µmol MDA formed / mg Hb) of group II, group III, and group IV at 120 days were significantly ($P \leq 0.05$) lower than group I (control). The differences between group II, group III, and group IV were non-significant ($P \geq 0.05$). This indicated that supplementation of Se and/ or vit-E could reduce LPO.

The mean of CAT (U/mg Hb) of group II, group III, and group IV at 120 days were significantly ($P \leq 0.05$) higher than group I (control). The differences between group I, group II, and group III, and the differences between group II, group III and group IV were non-significant ($P \geq 0.05$). This indicated that supplementation of the combination of both Se and vit-E enhanced the level of CAT.

Immune response: Our results indicated that supplementation of Se and/ or vit-E can trigger better ($P \leq 0.05$) cellular as well as humoral immune response compared to the normal pigs (Table-2).

Table-1. Antioxidant enzyme status of pigs in different experimental groups.

Parameter	Group I	Group II	Group III	Group IV	P value
GSH-Px (µmole NADPH oxidized /g Hb/min)					
0 Day	103.79 ± 4.74	102.25 ± 8.04	104.57 ± 6.55	101.86 ± 7.20	0.991
120 Day	117.68^{a} ± 9.33	156.66^{ab} ± 15.09	153.95^{ab} ± 8.74	187.91^{b} ± 6.78	0.002
SOD (U/mg Hb)					
0 Day	16.10 ± 0.71	15.92 ± 0.63	15.86 ± 0.74	16.14 ± 0.44	0.731
120 Day	16.65^{a} ± 0.54	18.93^{b} ± 0.47	17.75^{ab} ± 0.91	19.20^{b} ± 0.97	0.037
LPO (µmol MDA formed / mg Hb)					
0 Day	1.50 ± 0.14	1.54 ± 0.09	1.56 ± 0.18	1.48 ± 0.14	0.982
120 Day	2.61^{a} ± 0.30	2.26^{b} ± 0.21	2.29^{b} ± 0.19	1.42^{b} ± 0.15	0.011
CAT (U/mg Hb)					
0 Day	1.55 ± 0.10	1.47 ± 0.29	1.51 ± 0.20	1.49 ± 0.15	0.993
120 Day	1.61^{a} ± 0.34	2.59^{ab} ± 0.16	2.65^{ab} ± 0.58	3.40^{b} ± 0.51	0.039

^{a,b,c}Means bearing different superscripts in the same row differ significantly ($P<0.05$).

Table-2. Immune response of pigs to selenium and vitamin E supplementation.

Attribute	Group I	Group II	Group III	Group IV	P value
Skin fold thickness (cm)					
0 hr	0.190 ± 0.004	0.205 ± 0.006	0.198 ± 0.004	0.191 ± 0.007	0.223
24 hr	0.280 ± 0.007	0.288 ± 0.0025	0.284 ± 0.009	0.297 ± 0.005	0.421
48 hr	0.289^{a} ± 0.002	0.322^{b} ± 0.005	0.326^{b} ± 0.004	0.379^{c} ± 0.003	0.000
72 hr	0.206 ± 0.004	0.285 ± 0.0015	0.302 ± 0.007	0.327 ± 0.006	0.451
Antibody production against SRBC					
HA titre	5.67^{a} ± 0.30	7.32^{b} ± 0.23	7.30^{b} ± 0.33	8.33^{b} ± 0.4	0.003

^{a,b,c}Means bearing different superscripts in the same row differ significantly (P<0.05)

Discussion: Our studies indicated that dietary supplementation of Se and vit-E significantly augmented the antioxidant status of pigs in terms of GSH-Px, SOD, CAT and LPO activities. It conforms to previous studies regarding increase in the levels of RBC enzyme (GSH-Px) due to selenium supplementation in lambs (Kumar et al., 2009).

It is also akin to the report of Sandhu and Singha (2003), who have reported significant ($P \leq 0.05$) reduction in the levels of GSH-Px, CAT, SOD, and MDA in buffalo calves supplemented with 250 mg vitamin E and 7.5 mg Se, compared to the calves with high levels of these enzymes under induced endotoxin shock due to infusion of lyophilized *E coli* endotoxin indicating potent antioxidant properties of selenium and vitamin E.

Our results with respect to the cellular and humaoral responses was a little deviation from the report of Shinde et al. (2007), who have observed that Se supplementation improved ($P \leq 0.008$) the humoral immune response, while vitamin E supplementation improved ($P \leq 0.064$) cell mediated immune response in buffalo male calves.

CONCLUSION

It is concluded that dietary supplementation of Se and vit-E significantly augmented the antioxidant status of pigs in terms of GSH-Px, SOD, CAT and LPO activities besides concomitant rise in the level of immunity.

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