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Research Article

EFFECT OF DIETARY SUPPLEMENTATION OF VITAMIN E AND SELENIUM ON TOTAL ANTIOXIDANT STATUS AND *IN VITRO* IMMUNE COMPETENCE IN GROWER GHUNGROO PIGS

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ABSTRACT: The current study aimed to assess the effects of dietary vitamin E and selenium supplementation on antioxidant status and *in vitro* immune competence in grower Ghungroo pigs. Eighteen healthy female Ghungroo pigs (*Sus scroffa domestica*) between the ages of two and three months were divided into three groups at random: the control group (n = 6) was fed only the basal diet, the treatment-1 (T1) group (n = 6) was fed the basal diet with vitamin E at a rate of 11 IU/kg feed, and the treatment-2 (T2) group (n = 6) was supplemented with selenium at a rate of 0.15 mg/kg feed every day, according to NRC recommendations. Blood samples were collected from all the experimental animals on days 15, 30, 45, and 60 of supplementation for analysis. Total antioxidant status was assessed using kits available commercially following the manufacturer's instructions. Colorimetric NBT and MTT assays were done to evaluate *in vitro* phagocytic activity and lymphocyte proliferation response respectively. This study is the first to evaluate the total antioxidant status and *in vitro* immune function of grower Ghungroo pigs. Both vitamin E and selenium supplementation increased overall antioxidant status, with vitamin E having a stronger effect than selenium. The vitamin E-treated group exhibited the highest lymphocyte proliferation response, followed by control and selenium-treated groups. Neutrophils from the vitamin E-treated pigs showed significantly ($p<0.001$) higher phagocytic activity compared to the control and selenium-treated groups. So, it can be concluded from the above findings that both vitamin E and selenium improved the body's overall antioxidant status and immune function by enhancing lymphocyte proliferation response and neutrophil phagocytic activity, with vitamin E showing a more pronounced effect than selenium.

Keywords: Ghungroo, Antioxidant status, Lymphocyte proliferation response, Phagocytic activity of neutrophils, Vitamin E, Selenium.

INTRODUCTION

Stressful conditions in animals generate reactive oxygen species (ROS) and reactive nitrogen species (RNS) as free radicals that negatively affect production performance [1]. In swine species, oxidative stress leads to anorexia and negative weight gain by disrupting intestinal structure, altering gastrointestinal microbiota, and hindering absorption [2], along with impaired immune responses [3]. Several studies in cross-bred and exotic pigs have confirmed increased free radical production and a poor antioxidant defense system after weaning [4]. Antioxidants play a pivotal role against oxidative stress by preventing oxidation and neutralizing free radicals [5]. Vitamin E and selenium are potential antioxidants. in growing pigs [6] as their deficiency can lead to liver and muscle degeneration. Supplementation of vitamin E and selenium in combination reduced the clinical effects of swine dysentery caused by *Treponema hyodysenteriae* [7]. In recent years, the supplementation of vitamin E and

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selenium in weaner diets has gained considerable attention for two reasons. Firstly, piglets are susceptible to vitamin E deficiencies due to limited placental transfer of vitamin E [8]. Secondly, selenium concentration in the plasma is reported to decline within 7 days post-weaning [9]. Therefore, supplementing vitamin E and selenium in the feed may alleviate the negative effects of oxidative stress during the post-weaning phases of growth. The indigenous pig breed "Ghungroo," discovered in the eastern Sub-Himalayan region of West Bengal state, India [10], is a valuable local genetic resource that performs well in low-input production systems [11]. Ghungroo pigs are known for their high fertility, body weight gain, and feed conversion capacity [11]. However, there is a lack of research on the assessment of antioxidant status in Ghungroo pigs during their accelerated growth phase by supplementing dietary antioxidants. Therefore, the present investigation was carried out to evaluate the effect of dietary vitamin E and selenium on antioxidant status and *in vitro* immune competence in growing Ghungroo pigs.

MATERIALS AND METHODS

All the experiments were approved by the Institutional Animal Ethics Committee, Indian Veterinary Research Institute (IVRI), ERS, Kolkata. (vide No. F.42(A)/ERS/Kol/2022-23/386).

Experimental animals

The experiment involved the random selection of eighteen healthy female growers Ghungroo pigs (*Sus scroffa domestica*) aged two to three months. The experimental pigs had an average weight of 19.5 ± 0.36 kg. All the animals were housed in clean, dry, and hygienic sheds with concrete floors. They received routine vaccinations and anthelmintic treatment. The study trial was continued for 2 months. The pigs were fed commercially produced starter feed from the 4th to 8th weeks and grower ration from the 9th to 18th weeks with unlimited access to water. The animals were grouped into control (n=6) supplemented with basal diet, treatment-1 (T1) group (n=6) fed with vitamin E (in the form of α tocopherol acetate) @11IU/ Kg feed on daily basis treatment (T2) group of animals (n=6) fed with selenium (as sodium selenite) @0.15mg/ Kg feed daily as recommended by NRC [33].

The mean environmental temperature during the study period was 31°C (21°C to 41°C) with a mean relative humidity of 78% (50% to 93%) respectively. The mean temperature humidity index (THI) was 85.

Collection of blood samples

Blood (7mL/animal) was collected from the anterior vena-cava using a sterile syringe (5mL and 10mL) and needle in the EDTA-coated vacutainer tubes after proper restraining of the animals prior to morning feeding on day 15, day 30, day 45, and day 60 of supplementation. The blood samples were put in an ice box and carried to the laboratory for further processing.

Measurement of total antioxidant status

The total antioxidant status in the plasma was estimated using a commercially available ELISA-based antioxidant kit (Sigma-Aldrich, Saint Louis, MO, USA) as per the manufacturer's protocol.

Evaluation of *in vitro* immune competence

The proliferative response of lymphocytes was estimated using the colorimetric MTT (tetrazolium) according to the procedure given by Mosmann [12]. The phagocytic activity of neutrophils was estimated by quantitative Nitroblue Tetrazolium (NBT) assay as per the methods of Abuharfeil and coworkers [13].

Statistical analysis

The statistical analysis was done by the SYSTAT software package. Two-way ANOVA was applied to test the level of significance considering groups and days as factor. Data from different experiments are presented as mean ± SE. The statistical model was

$Yij = \mu + Gi + Dj + (GD)ij + eij$

Where, μ = Population means, Gij = Effect of the group (Control, T1, and T2), Dij = Effect of days of supplementation, (GD)ij = Interaction, eij = Random error.

RESULTS AND DISCUSSION

Total antioxidant status in the plasma

Total antioxidant status (mM relative to the concentration of the Trolox standard) in the plasma in grower Ghungroo pigs supplemented with vitamin E (T1), and selenium (T2) along with control during different days of supplementation have been presented in Table 1.

The total antioxidant status (TAS) of Ghungroogrower pigs significantly varied between treatments ($p<0.001$) but remained stable throughout the supplementation period. Vitamin E and selenium supplementation both increased overall antioxidant status, with vitamin E showing a stronger impact than selenium. The selenium-treated group initially had the highest TAS, which decreased by the 45th day

Table 1. Total antioxidant status (mM relative to the concentration of the Trolox standard), *in vitro* lymphocyte proliferation response and phagocytic activity of neutrophils in control, vitamin E (T1) and selenium (T2) supplemented grower Ghungroo pigs.

Parameters	Group	Days					
		15 day	30 day	45 day	60 day	Mean	p Value
Total antioxidant status (mM relative to the concentration of the Trolox standard)	Control	$14.18^{x}\pm1.02$	$12.15^{x}\pm0.68$	$14.15^{x}\pm0.96$	15.25±2.27	$13.93^{x}\pm0.68$	0.466
	T1	$15.24^{ax}\pm0.87$	$24.07^{cz}\pm0.64$	$20.91^{by}\pm0.68$	$21.22^{b}\pm1.31$	$20.36^{y}\pm0.79$	0.000
	T2	$19.63^{by}\pm1.55$	$15.55^{ay}\pm0.49$	$14.47^{az}\pm0.71$	$21.55^{b}\pm1.74$	$17.26^{z}\pm0.80$	0.002
	p Value	0.013	0.000	0.000	0.061	0.000	
In vitro lymphocyte proliferation response (SI)	Control	$0.88^{by}\pm0.06$	$0.74^{ax}\pm0.03$	$0.77^{ax}\pm0.02$	$0.66^{ax}\pm0.03$	$0.76^{a}\pm0.02$	0.002
	T1	$0.74^{ax}\pm0.04$	$1.33^{by}\pm0.09$	$1.24^{cz}\pm0.04$	$0.97^{cy}\pm0.06$	$1.07^{b}\pm0.04$	0.000
	T2	$1.01^{z}\pm0.04$	$1.01^{z}\pm0.07$	$0.97^{y}\pm0.03$	$1.05^{y}\pm0.08$	$1.01^{c}\pm0.03$	0.790
	p Value	0.000	0.000	0.000	0.000	0.000	
In vitro phagocytic activity of neutrophils	Control	$0.21^{b}\pm0.02$	$0.15^{a}\pm0.01$	$0.17^{a}\pm0.01$	$0.14^{ax}\pm0.01$	$0.17^{b}\pm0.01$	0.002
	T1	0.20±0.02	0.23±0.04	0.18±0.02	$0.21^{y}\pm0.04$	$0.21^{c}\pm0.02$	0.700
	T2	$0.18^{b}\pm0.02$	$0.14^{ab}\pm0.02$	$0.13^{ab}\pm0.01$	$0.11^{ax}\pm0.01$	$0.14^{a}\pm0.01$	0.031
	p Value	0.445	0.051	0.094	0.018	0.000	

*Values are expressed as mean ± SE. Means with common superscript within a row (a, b, c, d) do not differ significantly between days of supplementation. Means with common superscript within column (x, y) do not differ significantly between groups.

and peaked by the 60th day of supplementation. In contrast, the vitamin E-treated group maintained a constant TAS level from day 30 until the completion of the experiment.

The accelerating phage of growth in pigs is characterized by high oxidative stress resulting due to generation of high reactive oxygen species (ROS) [14] together with decreased activity of antioxidant enzymes such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) [2]. The TAS in relation to age and physiological stages in Large-White Yorkshire pigs [4] and crossbred pigs [15]. However, the TAS in grower Ghungroo pigs has not been investigated to date.

The treatment of selenium and vitamin E significantly improved TAS in grower Ghungroo pigs in this investigation. These findings were in accordance with the earlier reports of pigs [16, 17]. Vitamin E and selenium, known for their antioxidant properties, were shown to mitigate the harmful effects of ROS during the growth phase [18] to maintain a balanced redox state essential for proper growth and development [19]. Studies [16, 17] have reported that vitamin E supplementation significantly increased the plasma glutathione (GSH) to glutathione oxidized (GSSG) ratio (GSH/GSSG) and reduced plasma malondialdehyde (MDA) concentration. Selenium supplementation also enhanced the activity of Selenium-dependent liver glutathione peroxidase (GSH-Px) post-weaning [20]. In our, investigation TAS level was increased from day 30 until 2 months of supplementation. Similar findings were also reported in pigs [21] with no change in TAS level till 35 days of supplementation in post-weaning piglets whereas, continual supplementation of Selenium can maintain the antioxidant status from 50 days post-mating upto 90 days of gestation in multiparous sows [22]. However, the effect of selenium on antioxidant status depends on its source. The organic Se was reported to have a limited effect on antioxidant activity in post-weaning piglets [21] and multiparous sows [22].

In vitro lymphocyte proliferation response

The *in vitro* lymphocyte proliferation response (expressed as stimulation index, SI) of grower Ghungroo pigs supplemented with vitamin E (T1), selenium (T2), and control during different days of supplementation have been presented in Table 1.

The *in vitro* lymphocyte proliferation response in grower Ghungroo pigs differed significantly between treatments ($p<0.001$) and days of supplementation ($p<0.05$). The stimulation index was highest in the vitamin E-treated group, followed by the control and selenium-treated groups. There was no clear pattern of alteration in the lymphocyte proliferation response between different days of supplementation.

***In vitro* phagocytic activity of neutrophils**

The *in vitro* phagocytic activity of neutrophils of grower Ghungroo pigs supplemented with vitamin E (T1), and selenium (T2) along with control during different days of supplementation have been presented in Table 1.

The *in vitro* phagocytic activity of neutrophils varied significantly ($p<0.001$) among the different treatment groups, but there were no significant changes observed between the days of supplementation. Neutrophils isolated from the vitamin E-treated group of pigs showed significantly higher phagocytic activity compared to the control and selenium-treated groups. There were no changes in the *in vitro* phagocytic activity of neutrophils observed across the different days of supplementation.

Lymphocyte stimulation is employed to evaluate the proliferating potential of lymphocytes in response to mitogens such as phytohemagglutinin [23]. The phagocytic activity of neutrophils and lymphocyte proliferation response were reported to alter significantly among different age groups in Ghungroo piglets [24] and lactating Ghungroo sows [25]. In our current study, both vitamin E and selenium treatments stimulated lymphocyte proliferation response but the *in vitro* phagocytic activity of neutrophils was highest in the vitamin E-treated groups only. Similar to our findings, Larsen and Tollersud [26] showed that mitogen (phytohaemagglutinin) induced lymphocyte proliferation response was enhanced by vitamin E and selenium in pigs and Burkholder and Swecker [27] noted decreased phagocytic activity in vitamin E deficient pigs. A decrease in lymphocyte proliferation response *in vitro* activity of natural killer (NK) cells and antibody-dependent cell-mediated cytotoxicity (ADCC) in pigs fed a vitamin E and selenium-deficient diet were also reported [28]. The immune-modulating properties of vitamin E and selenium are attributed to their antioxidant properties, which protect cell membranes from free radicals [29] and neutralize oxidative free radicals generated during phagocytosis for pathogen killing [30]. The stimulation of lymphocyte proliferation response may be due to the fact that selenium stimulates the expression of the IL-2 receptor on immune cells and promotes antibody production by B-lymphocytes [31]. Suppression of immune activity suppression in lymphocytes and polymorphonuclear cells in pigs with vitamin E deficiency [3]. Contrary to our findings Meeker *et al.* [32] found impaired NK cell activity in rats deficient in vitamin E and selenium. In another study, Lessard *et al.* [28] noted decreased peripheral blood lymphocyte blastogenesis when sera from vitamin E and selenium-deficient pigs were added to the culture.

CONCLUSION

Both vitamin E and selenium improved the body's overall antioxidant state and immune competence by modulating lymphocyte proliferation response and neutrophil phagocytic activity. However, the effects of vitamin E supplementation were stronger than selenium.

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