



EFFECT OF DIFFERENT DIETARY LEVELS OF SELENIUM ON IMMUNITY IN GROWING NELLORE RAM LAMBS

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ABSTRACT

An experiment was conducted on 24 male growing Nellore lambs with uniform body weight (15.75 ± 0.47 kg) which were randomly divided into four groups of six animals each. These animals were supplemented with 0, 0.45, 0.9 and 1.8 ppm Selenium, respectively by adding inorganic selenium in the form of sodium selenite in concentrate mixture. The humoral immune response against enterotoxaemia was higher in the selenium supplemented lambs than the unsupplemented lambs. There was a significant difference ($p < 0.05$) among the groups for the humoral immune response. The titre values were highest in the group supplemented with selenium at 1.8 ppm (T4). The cell mediated immune response was significantly different ($p < 0.05$) among the groups and the skin fold thickness (mm) was highest in the group supplemented with selenium at 1.8 ppm (T4) after 24 h of injection.

KEYWORDS: Selenium, lambs, humoral and cell mediated immunity.



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INTRODUCTION

Sheep and goat are the species of economic value to the small and marginal farmers and landless labourers in India. Sheep are traditionally managed on grazing lands. It provides a dependable source of income to the shepherds through sale of animals for the meat purpose. Hence, sheep and goat continue to play an important role in the livestock production systems in tropics. India is having 74 millions of sheep and 154 millions of goats and these animals produce 26.98 x10⁴ MT mutton and 66.63 x 10⁴ MT chevon per year¹. Contribution of sheep and goat sector to the Indian economy is estimated to Rs. 2, 900 crores per annum. Their contribution to the economy is quite substantial and constitutes about 5.40 percent of 'Gross National Product' (GNP) of agriculture sector. Earlier, National Research Council² recommended a dietary level of 0.1 to 0.2 ppm of Se for sheep, but in its later report, supplementation of 0.3 ppm of Se has been recommended in the diet of cattle, sheep, and pigs³. Selenium's role in animal health is based on the functions of seleno proteins, many of which have antioxidant activities⁴. Although reactive oxygen species and free radicals are a natural result of the body's normal metabolic activity, excessive stress as a result of disease, environmental extremes, and nutritional imbalances can lead to over production of free radicals. Therefore, it is imperative that micronutrients involved in antioxidant functions be present in tissues to provide oxidant-antioxidant balance. Selenium enhances the ability of lymphocytes to respond to the cytokine IL-2 by increasing the expression of IL-2 receptors on lymphocytes. Enhancement of these interactions leads to increased numbers of lymphocytes, increased cytotoxicity of killer cells, and increased antibody production by B cells⁵. The goal of enhancing immunity is to increase resistance to disease. A decreased incidence of metritis in Se-treated dairy cows provides a good example of an association between Se deficiency and decreased disease resistance⁶. It has been observed in cattle herds, with long-standing annual problems with foot rot and pink eye, that there is a

markedly reduced incidence (seasonal) of these diseases once exposed to continuous Se supplementation⁷. Hence, in this study an attempt has been made to determine the possible strategic role of selenium at different levels of supplementation.

MATERIALS AND METHODS

Humoral Immunity

To assess the humoral immune response in the experimental animals the antibody titres in the blood were measured. For this blood collection and vaccination of the animals has been done. For the development of antibodies the enterotoxaemia vaccine of batch no.06 which was manufactured by M/S Veterinary Biological Research Institute, Hyderabad was given. The vaccine was given subcutaneously 1ml as the dose per animal. The booster dose of the vaccine was given on the 14th day. Then about 10 ml of blood was collected from the jugular vein for collection of serum from each lamb on the 0th day and also post vaccination of 14, 21 and 28 days. All these samples were preserved at -20°C. All the samples were then analysed for antibody titres by using the procedure of an indirect ELISA.

Indirect ELISA

100µl of Ag diluted to 1:100 dilution using coating buffer was added to each well of 96 well ELISA plate and plate was incubated at 4°C overnight and washed thrice with washing buffer. The plate was incubated at 37°C for 1 h after adding 50 µl of blocking buffer and washed thrice with washing buffer. 100 µl of strong positive, negative serum controls were added in quadruplicate to the respective wells. Similarly 100 µl of test serum samples (1:100 dilution) were added, incubated at 37°C for 1 h and then washed thrice with washing buffer. 100 µl of anti sheep IgG HPRO concentrate diluted in blocking buffer was added to all the wells in plate and incubated at 37°C for 1h washed and 100 µl of freshly prepared substrate solution (TMB) was added to each well and kept at room temperature for 10-15 minutes. Finally the reaction was stopped by adding 50 µl of 1M H₂SO₄ to each well and

absorbance values were recorded by using ELISA plate reader at 450 nm.

$$\% \text{ positivity} = \frac{\text{O.D value of test sample} - \text{O.D value of -ve sample}}{\text{O.D value of +ve sample} - \text{O.D value of -ve sample}} \times 100$$

Cell Mediated Immunity

To estimate the cell mediated immunity sheep pox vaccine, which is the live attenuated lamb testicular cell culture freeze dried vaccine, was given to the animals (as PHA-P is the non specific mitogen). This belongs to VBRI, Shantinagar, Hyd-28. Phytohaemagglutinin Phosphate (PHA-P) was used as a non-specific mitogen to evaluate cellular immunity. An area of approximately 6×6 cm was clipped on both sides of the neck of the sheep and approximately 2×2 cm was delineated with an indelible marker. The initial skin-fold thickness at each site was measured using vernier callipers. Sheep were intradermally inoculated on one side of the neck with 100 µl of PHA-P diluted to 1 mg/ml in distilled water. Distilled water (100 µl) was injected into the opposite side of the neck to serve as a negative control. Skin fold thickness was measured again 24h after injection.

RESULTS

Humoral Immune Response

The serum antibody production against injection of enterotoxaemia vaccine in Nellore ram lambs was assessed by ELISA which was measured as absorbance at 450 nm and expressed as percent positivity values. The percent positivity values on 0th day i.e. before giving the vaccine were 9.25±0.79, 12.43±1.27, 7.56±2.27 and 11.56±2.49 in T1, T2, T3 and T4, respectively. On 14th day before giving the booster dose the per cent values were 51.43±4.04, 48.55±3.63, 54.77±10.08 and 62.23±5.08. The supplementation of selenium has shown the effect on immune response and the percent positivity values were significantly different on 21st and 28th day of post sensitization and on those 2 days the response was increased linearly.

Effect of supplementation of different levels of selenium on humoral immune response against Enterotoxaemia titers assayed by ELISA in growing Nellore Ram lambs(% positivity values)

Day	Diet				SEM
	T1	T2	T3	T4	
0th d	9.25±0.79	12.43±1.27	7.56±2.27	11.56±2.49	0.95
14th d	51.43±4.04	48.55±3.63	54.77±10.08	62.23±5.08	3.11
21st d	54.71±4.32 ^b	59.85±6.38 ^b	66.93±9.85 ^b	95.96±5.58 ^a	4.61
28th d	54.09±5.17 ^b	59.98±5.73 ^b	68.54±10.07 ^b	99.07±6.76 ^a	4.93

Each value is the average of six observations. ^{a, b} values bearing different superscripts in a row differ significantly (P<0.05)

The values on 21st and 28th day were 54.71±4.32, 59.85±6.38, 66.93±9.85 and 95.96±5.58 and 54.09±5.17, 59.98±5.73, 68.54±10.07 and 99.07±6.76 in T1, T2, T3 and T4 groups.

Cell Mediated Immunity

Cell Mediated Immune response was assessed by DTH assay by injecting PHA-P. The assay was directly proportional to increase in the skin fold thickness of the animal after challenge. Before inoculation of PHA-P the initial thickness (mm) in groups T1, T2, T3 and T4 were 4.07±0.26, 3.48±0.24, 3.93±0.44 and 3.13±0.26. After inoculation of

PHA-P the skin fold thickness was increased and it was highest in T4 group followed by T3, T2, and T1 group. There was a significant difference (P<0.05) among the groups. The skin fold thickness (mm) after inoculation of PHA-P i.e., after 24 hours of duration in T1, T2, T3 and T4 8.63±0.43, 9.47±0.70, 10.04±0.72 and 12.07±0.78

Effect of supplementation of different levels of selenium on the cell mediated immune response (CMI) in growing Nellore ram lambs in terms of skin fold thickness by injecting PHA-P antigen

Skin Thickness	Diet				SEM
	T1	T2	T3	T4	
Initial test	4.07±0.26	3.48±0.24	3.93±0.44	3.13±0.26	0.16
Final test	8.63±0.43 ^b	9.47±0.70 ^{ab}	10.04±0.72 ^{ab}	12.07±0.78 ^a	0.51

Each value is the average of six observations. ^{a, b} values bearing different superscripts in a row differ significantly (P<0.05)

DISCUSSION

Humoral Immune Response

The activity of humoral immune response may be due to the GHPx enzymes. Although Se deficiency does not affect the number of neutrophils, it does impair aspects of their function. Neutrophils and macrophages kill bacteria by generating superoxide and hydrogen peroxide in the respiratory burst process⁸. Selenium deficiency impairs the ability of certain GHPx enzymes ability to metabolize peroxides and prevent self-inflicted damage⁹. The serum antibody response against *P. multocida* P52 antigen in the lambs as measured by absorbance at 492 nm in ELISA on different days of collection in different groups has been observed that antibody mediated immune response was significantly (P<0.01) higher in both the selenium-supplemented groups as compared to control group, but there was no significant (P>0.05) difference between the two Se supplemented groups¹⁰. In agreement with our results, an experiment on beef cows¹¹, which showed significantly higher concentration of serum IgG with the increasing levels of Se in the salt mix. Similarly, enhanced antibody response to *tetanus toxoid*, *parainfluenza-3* virus, and *Corynebacterium pseudotuberculosis* was observed in the lambs fed diets supplemented with 0.1, 0.5, and 1 ppm Se as compared to control group¹². Significantly higher humoral immunity has been reported in male buffalo calves supplemented with 0.3 ppm Se compared to control group reported^{13,14}. Although Se deficiency does not affect the number of neutrophils, it does impair aspects of their function. Neutrophils and macrophages kill

bacteria by generating superoxide and hydrogen peroxide in the respiratory burst process^{5,8}. The Se supplementation led to a significant increase of *Chlamydia* antibody response (P<0.05)¹⁵. The specific antibody titres against *E. coli* were determined in serum samples by ELISA and the results showed that the injection of selenium significantly improved the production of specific antibodies against *E. coli*, and that the production of specific antibodies was greater after the administration of selenium¹⁶. Significantly higher antibody titres were observed in Japanese quail chicks of 0-6 week fed Se supplemented diets when inoculated with sheep red blood cells¹⁷. Contrary to this finding, there was no effect on immunological response against *Pasteurella haemolytica* vaccination in steers given a single intramuscular dose of 25 mg Se¹⁸. There was no effect of supplementation of 1 ppm Se over control diet (0.41 ppm Se) on antibody titer against sheep red blood cells in crossbred beef cattle¹⁹. Calves deficient in Se had lower antibody titers than Se-treated calves²⁰.

Cell Mediated Immunity

The increased Cell-mediated immune response may be due to the reason that interactions between antigens and immune cells. Signaling molecules such as cytokines bind to target receptors on other immune cells⁸. Selenium enhances the ability of lymphocytes to respond to the cytokine IL-2 by increasing the expression of IL-2 receptors on lymphocytes⁵. Enhancement of these interactions leads to increased numbers of lymphocytes, increased cytotoxicity of killer

cells, and increased antibody production by B cells^{5,8}. Selenium supplementation tended to increase *in vivo* cell-mediated immune response ($P=0.12$) compared with control calves²¹. There has been little research conducted to determine the effects of Se supplementation on *in vivo* cell-mediated immunity, but it appears the Se deficiency may impair the ability of the immune system to respond to mitogens. Ewes of group

given 5 mg of Se on day 30 before lambing showed a greater ($P < 0.01$) response to PHA 6 h after injection than ewes of non treated group²². During week 4, the response to intradermally injected phytohaemagglutinin, an index of the *in vivo* cell-mediated immune response, was shown to be increased in the groups fed on the Se-supplemented diets of Japanese quail chicks of 0-6 week²³.

CONCLUSION

Selenium supplementation improved the cell mediated and humoral immunity response in the lambs when compared to the unsupplemented lambs.

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