

**EFFECT OF DIFFERENT DIETARY LEVELS OF  
SELENIUM ON IMMUNITY AND KEEPING QUALITY  
OF MEAT IN GROWING NELLORE RAM LAMBS**

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## INTRODUCTION

- Sheep and goat are the species of economic value to the small and marginal farmers and landless labour in India.
- Sheep and goats with its multi-facet utility for meat, milk, wool, skins and manure form an important component of rural economy particularly in the arid and semi-arid areas of the India.



- India is having 74 millions of sheep and 154 millions of goats and these animals produce  $26.98 \times 10^4$  MT mutton and  $66.63 \times 10^4$  MT chevon per year (FAO, 2010).
- Contribution of sheep and goat sector to the Indian economy is estimated to Rs. 2, 900cr. per annum.
- Their contribution to the economy is quite substantial and constitutes about 5.40 per cent of 'Gross National Product' (GNP) of agriculture sector.



➤ In addition to nutritional insufficiency, the sheep is more prone to various diseases like parasitic infestation, viral infections like “Peste des petits” (PPR), blue tongue and several respiratory infections.

➤ Thus the major goal of sheep rearers for obtaining maximum profits is to minimize the losses from disease and attain good flock viability, which could be obtained by improving the disease resistance capacity of the flock.



- Nutrition is the major decisive factor, which determines the expression of genetic potential of animal in terms of growth and immunity (Klasing and Barnes, 1988).
- The nutrients recognized as having an important role in immunity are energy, protein, vitamins (A, E, C, B<sub>6</sub>, B<sub>12</sub>, folic acid and choline) and minerals (Cu, Se, Zn, Co, Mn and Fe).



Earlier, National Research Council ([NRC 1985](#)) 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 pigs ([NRC 2001](#)).

Salt Institute (2005) has recommended 30 ppm selenium to be added in the salt mineral mixture for sheep as compared to 20 ppm for cattle, indicating that Se requirements of sheep might be higher than cattle.



- Selenium's role in animal health is based on the functions of selenoproteins, many of which have antioxidant activities (Fairweather-Tait *et al.*, 2010).
- 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 cells (Rooke *et al.*, 2004).



- 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 resistance (Suttle and Jones, 1989).
- 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 supplementation (Koller *et al.*, 1983).
- 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

### Experimental Animals

- Twenty four Nellore ram lambs (3-5 months old) with an average body weight of  $15.45 \pm 0.06$  kg were purchased from local market and used for the study.
- These lambs were then randomly allotted to 4 groups (6 in each treatment) in a completely randomized design.
- The animals of group T1 (basal) were offered basal diet, group T2, T3 and group T4 were offered basal diet supplemented with selenium at 0.45, 0.9 and 1.8 ppm, respectively by adding inorganic selenium in the form of sodium selenite.



- The four diets (T1, T2, T3 and T4) were randomly assigned to four groups of animals in a 120 d growth trial.
- Concentrate mixture @ 1 per cent body weight was offered along with the ad libitum of green roughage as APBN 1 and dry roughage as sorghum daily in the morning at 8 AM.
- Residues, if any were weighed on the next morning. Thus, the exact quantity of feed consumed daily by the experimental animals was recorded throughout the experimental period.



## **IMMUNOLOGICAL STUDY**

### **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 14<sup>th</sup> day.



➤ Then about 10 ml of blood was collected from the jugular vein for collection of serum from each lamb on the 0<sup>th</sup> 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.



## 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.
- Phytohaemagglunin 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.



## TBA Procedure

- To estimate the quality of meat TBARS procedure is established.
- In this 4 g of meat sample is taken in a test tube and then 20 ml of TCA (Tri chloro acetic acid) was added to it. Then it was homogenized and filtered through the filter paper.
- Then 3 ml of it was taken in another test tube. Then added 2 ml of TBA to it.
- Then covered with aluminium foil and vortexed the tube, kept in hot water bath for 30 min. Again vortexed the tube again. Then the readings were taken in UV Spectrophotometer at 340 nm.



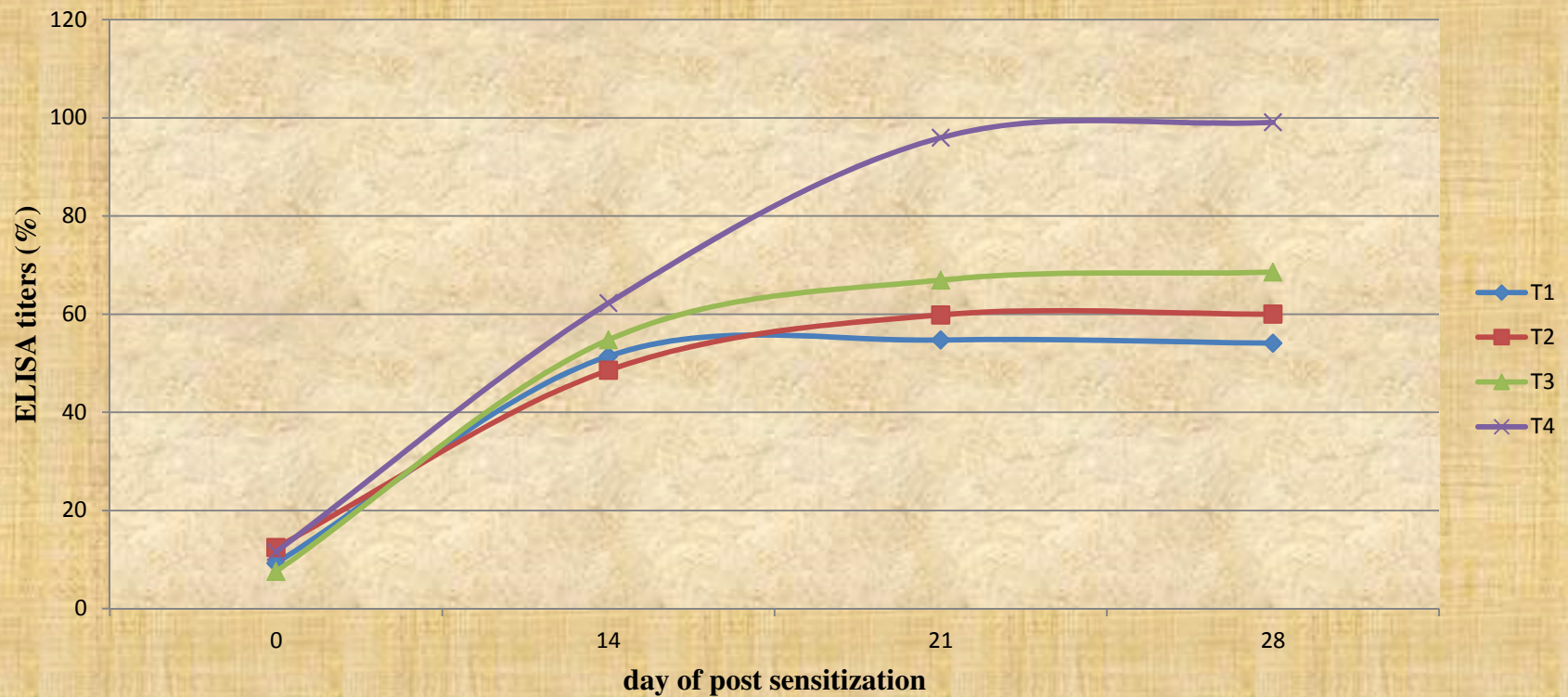
- Effect of supplementation of different levels of selenium on humoral immune response against Enterotoxaemia titers assayed by ELISA in growing Nellore Ram lambs(% positivity values).

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 <sup>b</sup>	59.85±6.38 <sup>b</sup>	66.93±9.85 <sup>b</sup>	95.96±5.58 <sup>a</sup>	4.61
28th d	54.09±5.17 <sup>b</sup>	59.98±5.73 <sup>b</sup>	68.54±10.07 <sup>b</sup>	99.07±6.76 <sup>a</sup>	4.93

Each value is the average of six observations <sup>a, b</sup>values bearing different superscripts in a row differ significantly (P<0.05)



# Effect of supplementation of different levels of selenium on humoral immune response against enterotoxaemia titers assayed by ELISA in growing Nellore ram lambs





## DISCUSSION

### HUMORAL IMMUNE RESPONSE

- The supplementation of selenium has shown the effect on immune response and the per cent positivity values were significantly different on 21<sup>st</sup> and 28<sup>th</sup> day of post sensitization and on those 2 days the response was increased linearly
- Neutrophils and macrophages kill bacteria by generating superoxide and hydrogen peroxide in the respiratory burst process (Rooke *et al.*, 2004; Tizard, 2009).
- Selenium deficiency impairs the ability of certain GHPx enzymes ability to metabolize peroxides and prevent self-inflicted damage (Arthur, 2003).



➤ Kumar *et al.* (2008) reported that 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 groups.



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 <sup>b</sup>	9.47±0.70 <sup>ab</sup>	10.04±0.72 <sup>ab</sup>	12.07±0.78 <sup>a</sup>	0.51



# 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





## Cell Mediated Immunity

- The assay was directly proportional to increase in the skin fold thickness of the animal after challenge. After inoculation of PHA-P the skin fold thickness increased and it was highest in T4 group.
- 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 cells (Tizard, 2009).
- Selenium enhances the ability of lymphocytes to respond to the cytokine IL-2 by increasing the expression of IL-2 receptors on lymphocytes (Rooke *et al.*, 2004).



- Enhancement of these interactions leads to increased numbers of lymphocytes, increased cytotoxicity of killer cells, and increased antibody production by B cells (Rooke *et al.*, 2004; Tizard, 2009).
- 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 week (Biswas *et al.*, 2006).

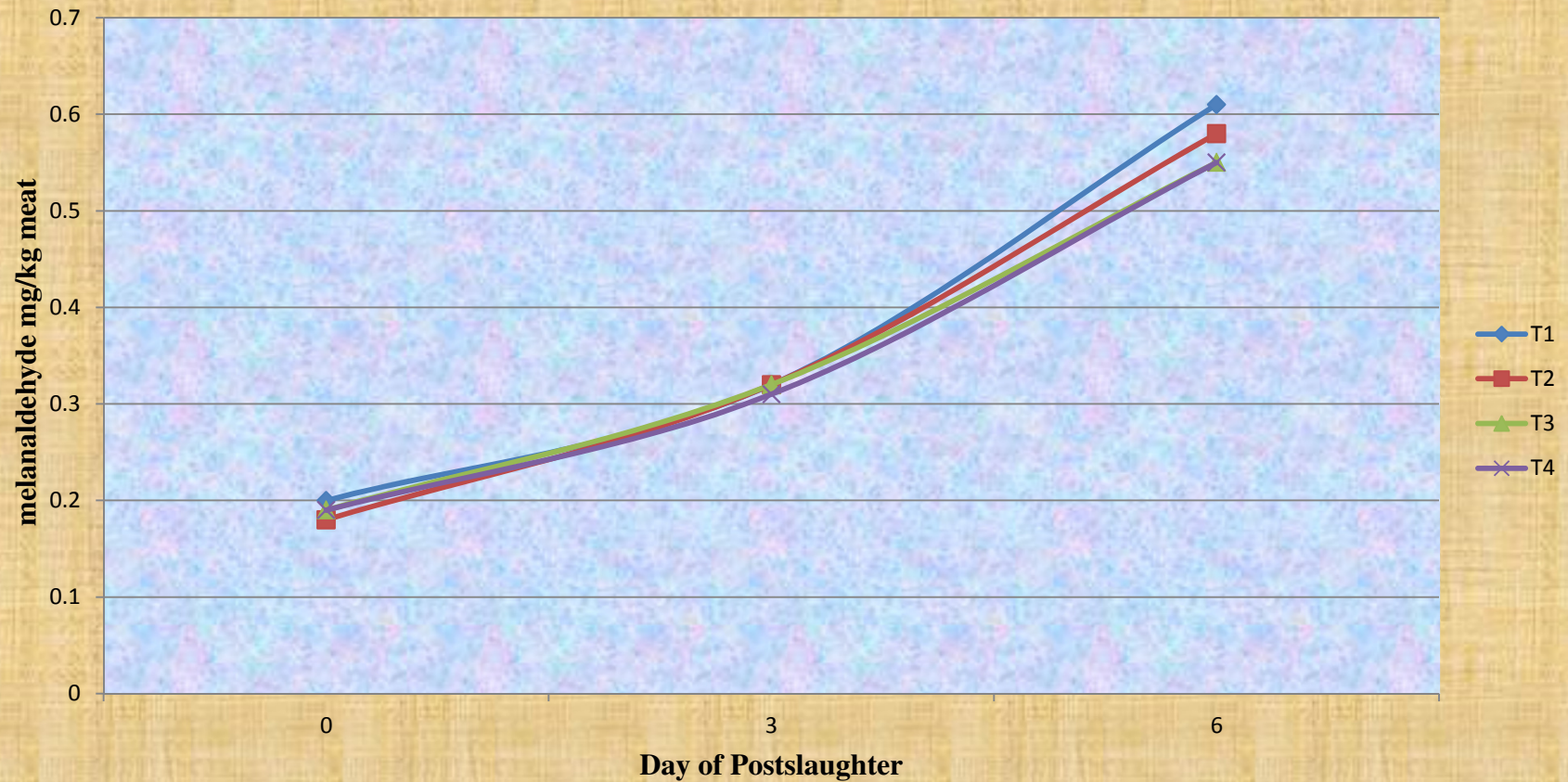


Effect of supplementation of different levels of selenium on keeping quality of meat measured in terms of TBARS (mg melanaldehyde/kg meat) in growing Nellore ram lambs.

Day	Diet				SEM
	T1	T2	T3	T4	
0th d	0.20±0.01	0.18±0.01	0.19±0.02	0.19±0.01	0.01
3 <sup>rd</sup> d	0.32±0.01	0.32±0.02	0.32±0.02	0.31±0.02	0.01
6 <sup>th</sup> d	0.61±0.03	0.58±0.04	0.55±0.01	0.55±0.05	0.02



# Effect of supplementation of different levels of selenium on keeping quality of meat measured in terms of TBARS (mg melanaldehyde/kg meat) in growing Nellore ram lambs





## MEAT QUALITY

- Supplementation of selenium at different levels did not influence the keeping quality of meat. Vignola *et al.* (2009) reported that selenium supplementation in either forms organic and inorganic sources did not show significant differences between the treatments in the meat quality assessed by oxidative stability of meat.
- Skrivanova *et al.* (2007) found that dietary Se had no effect on the fatty acid profile of the *Longissimus thoracis* muscle in calves that had received diets containing either basal Se or Se-enriched yeast.
- O'Grady *et al.* (2001) suggested that dietary Se has limited potential for increasing the oxidative stability of meat. Taylor (2008) reported that beef steaks from cattle supplemented with Se had similar shelf life attributes to those from unsupplemented animals despite having greater Se contents. These results were in agreement with Juniper *et al.* (2009).



**THANK YOU**