Veterinary World, EISSN: 2231-0916
Available at www.veterinaryworld.org/Vol.7/December-2014/8.pdf

RESEARCH ARTICLE
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Effect of supplementation of different levels of selenium as nanoparticles/sodium selenite on blood biochemical profile and humoral immunity in male Wistar rats

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Received: 31-07-2014 **Revised:** 06-11-2014 **Accepted:** 11-11-2014, **Published online:** 11-12-2014

doi: 10.14202/vetworld.2014.1075-1081. **How to cite this article:** Bunglavan SJ, Garg AK, Dass RS, Shrivastava S (2014) Effect of supplementation of different levels of selenium as nanoparticles/sodium selenite on blood biochemical profile and humoral immunity in male Wistar rats, *Veterinary World* 7(12): 1075-1081.

Abstract

Aim: To study the effect of supplementation of different levels of selenium as nanoparticles/sodium selenite on blood biochemical profile and humoral immunity in male Wistar rats.

Materials and Methods: The experimental research was conducted at Division of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar. 63 male Wistar rats were divided into 9 equal groups on the basis of their mean body weight (BW) (124.3±3.1 g BW) following completely randomized design. Experimental feeding was similar in all the groups except for the source and level of selenium (Se) in the diet. While Group 1 (control) was fed a basal diet with no Se supplementation, in Groups 2 and 3, 150 ppb Se was supplemented either as sodium selenite or Se nanoparticles, respectively. In Groups 4, 5, 6 and 7, Se was supplemented as its nanoparticles at 50%, 25%, 12.5% and 6.25% levels respectively i.e. at 75 ppb, 37.5 ppb, 18.75 ppb and 9.375 ppb levels respectively. In Groups 8 and 9, 300 ppb Se was supplemented either as Se nanoparticles or sodium selenite, respectively. Experimental feeding was conducted for a period of 91 days. At the end of the experimental trial, blood samples were collected to analyze the blood serum biochemical profile (serum glucose, serum total protein (TP), serum albumin, serum globulin, serum albumin: globulin ratio [A:G ratio], serum total cholesterol) and humoral immunity.

Results: The levels of serum glucose, serum TP and serum albumin were comparable ($p>0.05$) among the nine groups of male Wistar rats. The mean serum total cholesterol was significantly ($p<0.001$) lowered in all the Se supplemented Wistar rats compared to the control group. The mean serum globulin level was significantly ($p<0.05$) higher and A:G ratio was significantly ($p<0.05$) lowered in Group 3 (supplemented with 150 ppb selenium nanoparticles) followed by Groups 2, 4, 5, 6, 8, and 9 as compared to the control group. The mean serum antibody titer was significantly ($p<0.001$) higher in all the Se supplemented groups with the highest value in Group 3 (supplemented with 150 ppb selenium nanoparticles) followed by Groups 4, 5, 8 and 9 compared to the control group.

Conclusion: Supplementation of selenium nanoparticles at the level of 150 ppb gave the best performance in terms of increased serum globulin level, reduced A:G ratio, and improved humoral immune status in male Wistar rats.

Keywords: humoral immunity, nanoparticles, selenium, serum cholesterol, serum globulin, serum glucose, serum total protein, Wistar rats.

Introduction

Selenium, which was earlier classified as a toxic element, has now been proved to be an essential mineral required for proper health, immunity, and reproductive functions of animals [1]. More than thirty different selenium containing enzymes have been defined in animals [2]. It has an important role in the generation of resistance to disease either through enhancing the immune response, leukocyte function or specific immunity of the animals [3]. It is a component of glutathione peroxidase (POD) enzyme [4], which destroys free radicals in the cytoplasm [5]. It has been demonstrated that selenium protects the tissues against oxidative damage [6]. Selenium improves immune responses [7] as it is required for the development and expression of non-specific humoral and cell-mediated immune responses. Selenium is also a component of the enzyme Type I deiodinase, which is required for the conversion of thyroxine (T_4) into more active tri-iodothyronine (T_3) [8]. Selenium deficiency results in immune suppression and reduced resistance to infection, neutrophil function, antibody production, proliferation of T and B cells in response to mitogens, and cytodestruction by T-lymphocytes and natural killer cells. Probably the effects on function of glutathione POD and ability to interact with cell membranes represent the immuno-enhancing role of selenium [9,10].

Presently, sodium selenite is the usual Se source as a supplement in animal feeds [11]. However, it has the disadvantage in animal nutrition that it is a potential toxin at higher dietary levels [12], has lower absorption efficiency [11] and exhibits pro-oxidation defects [12]. Moreover being carcinogenic in nature its use is being discouraged. In recent years, organic

forms of Se such as Se enriched yeast [13] or selenomethionine [14] have also been tried and were reported to be a better and safer source of Se for animal feeding as compared to inorganic Se sources [15]. Elemental selenium powder in the redox state of 0 is not soluble and generally considered to be biologically inert. Recently, nanotechnology holds promise for medication and nutrition because materials at the nanometer dimension exhibit novel properties such as high-surface activity, great specific surface area, high catalytic efficiency, a lot of surface active centers, and a strong adsorbing ability [16-18]. Previous researches have reported that Se nanoparticles possessed comparable efficiency to selenite, selenomethionine, and methylselenocysteine in up regulating selenoenzymes in mice and rats in having higher bioavailability and lower acute toxicity [19-22]. However, reports on the effect of Se nanoparticles on the blood serum biochemical profile and humoral immune response of the animals are scanty.

Hence, an experiment was designed to study the effect of supplementation of different levels of selenium as selenium nanoparticles/sodium selenite on blood biochemical profile and humoral immunity in male Wistar rats.

Materials and Methods

Ethical approval

The present study was conducted at Division of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar. The experimental protocol was approved by Institutional Animal Ethics Committee.

Animals, selection and grouping

Sixty-three male Wistar rats were procured from Laboratory Animal Resource Section of Indian Veterinary Research Institute, Izatnagar. For acclimatizing to the new environment, these animals were maintained for 7 days on a standard diet comprising of concentrate mixture before the start of the proper experiment. These animals were then divided into nine groups of 7 animals in each group on the basis of their mean body weight (BW) (124.3±3.1 g BW) following completely randomized design.

Housing and management

All the experimental animals were housed in a well-ventilated room. Strict management and hygienic practices were adopted throughout the experimental period. Clean drinking water was provided *ad libitum* twice a day at about 9:30 A.M. and 3:30 P.M. daily.

Feeds and feeding

Experimental feeding was similar in all the groups except for the source and level of Se in the diet. The male Wistar rats were offered a basal diet to meet their nutrient requirements as per NRC [23] recommendations for rats. The basal diet consisted of ground maize grain (*Zea mays*) (27.5%), Bengal gram (*Cicer arietinum*) (25%), soybean (*Glycine max*) meal (21%), wheat (*Triticum aestivum*) bran (24%), mineral mixture (2%) with vitamin A, and common salt (0.5%). Weighed amount of the basal diet was provided at 9.00 A.M. daily to meet the nutrient requirements. The daily nutrient requirement was adjusted every week based on the BW of the animals While Group 1 (control) was fed a basal diet with no Se supplementation, in Groups 2 and 3, 150 ppb Se was supplemented either as sodium selenite or Se nanoparticles, respectively. In Groups 4, 5, 6 and 7, Se was supplemented as its nanoparticles at 50%, 25%, 12.5% and 6.25% levels respectively i.e., at 75 ppb, 37.5 ppb, 18.75 ppb and 9.375 ppb levels respectively. In Groups 8 and 9, 300 ppb Se was supplemented either as Se nanoparticles or sodium selenite, respectively. Experimental feeding was conducted for a period of 91 days.

Chemical analysis of feed samples

Samples of feed offered were analyzed for proximate principles, phosphorous (P) [24] and calcium (Ca) [25].

Estimation of Se in feed samples

Weighed amount of dried feed samples were taken in different 100 mL Kjeldahl flasks, soaked overnight in 10-20 mL triple acid mixture of nitric acid, sulfuric acid and perchloric acid (4:2:1), and digested. Selenium was estimated in the triple acid digested samples using atomic absorption spectrophotometer model 4141 (Electronic Corporation of India Limited, Hyderabad, India) in air acetylene flame along with vapor generation (hydride generator) assembly.

Preparation of selenium nanoparticles

Selenium nanoparticles were synthesized as per the methods described by Ingole *et al* [26] and Chen *et al* [27]. The size of the obtained Se nanoparticles ranged from 35 nm to 50 nm as determined by transmission electron microscopy (TEM). TEM characterization was performed with a JEOL-JEM-1011 electron microscope, by dropping the sample suspension in ethanol on copper (Cu) grid coated with a thin amorphous carbon film [28]. The concentration of Se was estimated using atomic absorption spectrophotometer model 4141 (ECIL, Hyderabad, India) along with vapor generation assembly.

Blood analysis

Collection of blood and separation of serum and plasma

After the completion of 70 days of experimental feeding, about 2 mL of blood was collected from each animal through cardiac puncture in the morning (before watering and feeding) and was taken into clean and dry test tube and kept in slanting position for 45 min to separate out the serum.

Estimation of blood biochemical constituents

All the blood biochemical parameters were estimated using diagnostic kits manufactured by Span Diagnostic Limited, Surat, India.

Serum glucose

Serum glucose was estimated by glucose oxidase (GOD) and POD method as described by Henry [29]. Glucose was oxidized by GOD to gluconic acid and hydrogen peroxide. In a subsequent POD catalyzed reaction, the oxygen liberated was accepted by the chromogen system to give a red colored quinone amine compound. The red color so developed was measured at 505 nm and was directly proportional to glucose concentration (mg/dL in blood serum).

Serum total protein (TP) and albumin

Serum TP and albumin were estimated by Biuret and bromocresol green (BCG) dye binding method [30]. Serum protein binds to copper ions in an alkaline medium of Biuret reagent and produced a purple color complex, whose absorbance at 555 nm was proportional to protein concentration. Serum albumin binds to BCG in acidic condition and produced a green color, whose absorbance was measured at 630 nm and the concentration, was expressed as g/dL in blood serum.

Serum globulin

It was calculated by subtracting serum albumin from TP and expressed as g/dL in blood serum.

Serum total cholesterol

Cholesterol in the blood serum was determined by the method of Wybenga *et al.* [31]. Cholesterol present in serum reacted with a hot solution of the ferric perchlorate, ethyl acetate and sulfuric acid and gave a lavender colored complex, whose absorbance was measured at 560 nm.

Monitoring of the immune response

At the end of 91 days of experimental feeding, humoral immune responses of the animals in the different groups were assessed by standard tube agglutination test (STAT).

Assessment of humoral immune response

Seven animals from each group were inoculated intramuscularly with a single dose (0.5 mL) of *Pasteurella multocida* oil adjuvant vaccine. The blood samples were collected at 0, 7, 14, 21 and 28 days of post vaccination for humoral immune response. About 1.0 mL of blood was collected from each animal through cardiac puncture in clean labeled tubes and the harvested serum was carefully transferred to the clean labeled plastic vials and stored in deep freezer (−20°C) for further analysis.

STAT

Serial two fold dilutions of serum samples were prepared in normal saline. 0.5 mL of formalinized plain antigen of which the optical density was adjusted to brown opacity tube No.: 2 was added to 0.5 mL of diluted test samples in test tubes. The tubes were shaken well, and overnight incubation was done at 37°C. STAT titer was expressed as the reciprocal of the highest dilution showing 50% clearing.

Statistical analysis

All the data generated in the above experiments were statistically analyzed using 17th version of Statistical Package for Social Science (SPSS), (SPSS USA) [32] computer package. For comparison of groups, Generalized linear model ANOVA procedure and Duncan's multiple range tests [33] were used.

Results

Chemical composition of the basal diet

The chemical composition of the basal diet offered to male Wistar rats is presented in Table-1. The percent of different nutrients in the basal diet on dry matter basis was almost similar to the National Research Council [23] recommendations for rats. The selenium content in the basal diet was very low being 0.08 ppm.

Blood biochemical parameters

The mean blood serum glucose, serum total cholesterol, serum TP, serum albumin, serum globulin, and A:G ratio after 70 days of experimental feeding in different groups are presented in Table-2.

The mean values of serum glucose and serum TP were comparable ($p>0.05$) in the nine groups and were within the normal range, indicating that the supplementation of selenium nanoparticles up to 300 ppb had no effect on these parameters.

The mean values of serum total cholesterol were significantly ($p<0.001$) lowered in all the Se supplemented Wistar rats compared to control the group. However, they were within the normal range (40-130 mg/dL) in rats.

The mean values of serum albumin were comparable ($p>0.05$) in the nine experimental groups and also within the normal range. However, the mean serum globulin level was significantly ($p<0.05$) higher in Group 3 followed by Groups 2, 4, 5, 6, 8, and 9 as compared to control the group. The A:G ratio was also significantly ($p<0.05$) lowered in Group 3 compared to other experimental groups.

Humoral immune response

The serum antibody production against formalin inactivated *P. multocida* vaccine in Wistar rats at different periods (days post vaccination) as measured by STAT is presented in Table-3.

Table-1: Chemical composition of the basal diet offered to male Wistar rats.

Nutrients	% DMB
Organic matter	95.28
Crude protein	22.66
Ether extract	4.56
Total ash	4.72
Crude fiber	7.75
Nitrogen-free extract	60.31
Calcium	1.50
Phosphorus	0.66
Selenium	0.08 ppm

DMB=Dry matter basis

Table-2: Blood serum biochemical profile in different groups of male Wistar rats.

Attributes	Groups									SEM	p value
	1	2	3	4	5	6	7	8	9		
Glucose (mg/dL)	84.0	86.0	85.5	83.5	84.6	85.7	83.8	84.1	85.3	0.47	0.866
Total cholesterol (mg/dL)*	120^{c}	95^{b}	85^{a}	84^{a}	92^{b}	94^{b}	101^{b}	88^{a}	97^{b}	2.28	<0.001
Total protein (g/dL)	6.55	6.64	6.85	6.24	6.76	6.87	6.31	6.59	6.63	0.11	0.598
Albumin (g/dL)	4.47	3.84	3.82	3.84	3.87	4.11	4.32	4.24	4.18	0.23	0.001
Globulin (g/dL)*	2.08^{a}	2.80^{b}	3.03^{c}	2.40^{b}	2.89^{b}	2.76^{b}	1.99^{a}	2.35^{b}	2.45^{b}	0.21	0.002
A:G ratio*	2.15^{e}	1.37^{b}	1.26^{a}	1.60^{c}	1.34^{b}	1.49^{c}	2.17^{e}	1.80^{d}	1.71^{d}	0.14	0.021

*Means bearing different superscripts in a row differ significantly (p<0.05), A:G=Albumin: globulin ratio, SEM=Standard error of mean

Table-3: Anti-*P. multocida* (P_{52}) $\log_{10}$ STAT titer in the sera of Wistar rats in different groups.

Group (G)	Period (days post vaccination) (P)						p value
	0	7	14*	21*	28*	Mean	
1	ND	1.23^{a}	1.75^{a}	1.98^{a}	1.90^{a}	1.71^{a}	G<0.001
2	ND	1.75^{c}	1.98^{a}	2.20^{c}	2.05^{b}	2.00^{b}	P<0.001
3	ND	1.98^{c}	2.51^{c}	2.73^{d}	2.58^{c}	2.45^{d}	G×P<0.001
4	ND	1.82^{c}	2.20^{c}	2.46^{c}	2.38^{b}	2.20^{c}	
5	ND	1.83^{c}	2.05^{b}	2.43^{c}	2.35^{c}	2.17^{c}	
6	ND	1.60^{b}	1.98^{a}	2.16^{b}	2.06^{b}	1.94^{b}	
7	ND	1.68^{c}	1.90^{a}	2.13^{b}	2.04^{b}	1.94^{b}	
8	ND	1.70^{c}	2.10^{c}	2.30^{c}	2.20^{c}	2.09^{c}	
9	ND	1.72^{c}	2.20^{c}	2.32^{c}	2.26^{c}	2.13^{c}	
SEM	ND	0.05	0.05	0.04	0.04	0.04	

SEM=Standard error of mean, ND=Not detectable as there was absence of agglutination in the test tubes. *Means bearing different superscripts in a column differ significantly (p<0.001), STAT=Standard tube agglutination test, *P. multocida=Pasteurella multocida*

It was observed that the serum antibody titer was significantly (p<0.001) higher in all the Se supplemented groups compared to control the group. It was further observed that on 21st day, the antibody titer values were significantly (p<0.001) higher in Group 3 followed by Groups 4, 5, 8 and 9 compared to control and other Se supplemented groups, indicating that selenium nanoparticles supplementation at 150 ppb had highest effect on improving the humoral immune response in male Wistar rats.

Discussion

Blood serum biochemical profile

The supplementation of selenium either as Se nanoparticles or sodium selenite in the basal diet had no effect on serum glucose, serum TP and serum albumin in male Wistar rats. Contrary to our findings, Singh *et al*. [34] observed low blood glucose concentration in buffalo calves fed wheat straw containing high Se (8.54 ppm) throughout the experiment. Ebrahimi *et al*. [35] also reported decreased plasma glucose concentration in Holstein calves fed with 0.3 ppm of selenium as Sel-plex for 120 days. On the contrary, Nayyar *et al.* [36] reported significantly higher level of blood glucose in anestrous buffalo heifers supplemented with vitamin E + Se as compared to control. Similarly Mohapatra *et al*. [37] reported that supplementation of 0.3 ppm nano Se in layer chicks up to 8 weeks post feeding significantly increased serum glucose levels compared to control.

However our results are supported by Shinde *et al*. [38] who observed that supplementation of vitamin E (300 IU) and Se (0.3 ppm) or both in the diet had no effect on serum glucose levels in buffalo calves. Yang et *al.* [39] too observed that serum glucose and TP levels were non-significant in 0.3 ppm organic Se supplemented broiler chicks after 42 days post feeding compared to control the group. Similarly, Chung *et al*. [40] reported that the supplementation of 0.25 ppm of organic or inorganic selenium in Korean goats for 5 weeks had no effect on plasma glucose level. Mudgal *et al*. [41] also reported that feeding of 0.3 ppm of selenium for 120 days in buffalo calves had no effect on serum glucose concentrations. Dominguez-vara *et al*. [42] too observed that feeding of 0.3 ppm organic Se to Rambouillet sheep for 95 days had no effect on plasma glucose concentration.

It was further observed that Se supplementation remarkably lowered serum total cholesterol levels in Wistar rats with the highest impact in Se nanoparticles supplemented groups (Table-2), which was also dose dependent. Similar to our results, in rats, Se supplementation has been reported to increase low-density lipoprotein (LDL) receptor activity [43] and decrease the 3-OH-methyl-glutaryl CoA reductase expression [44] leading to decreased plasma LDL cholesterol and total cholesterol levels [45]. Also, Mohapatra *et al.* [37] reported that supplementation of 0.3 ppm nano Se in layer chicks up to 8 weeks post feeding significantly lowered serum cholesterol levels compared to control. On the contrary, Yang *et al.* [39] reported that total cholesterol level was non-significant in 0.3 ppm organic Se supplemented broiler chicks after 42 days post feeding compared to control the group.

The lowered A:G ratio and increased serum globulin levels are indicative of immunity status of the animals, and therefore the addition of selenium nanoparticles at 150 ppb level and other Se supplemented groups had an enhanced effect on the immune status of the animals with the best results with 150 ppb Se nanoparticles.

In contrast to the present findings, no effect was observed in serum globulin levels which might

be due to either very low (0.1 ppm) [46] or very high (8.54 ppm) levels of Se supplementations [34]. Similarly Yang *et al.* [39] reported that serum globulin level was non-significant in 0.3 ppm organic Se supplemented broiler chicks after 42 days post feeding compared to control the group.

However, similar to our results, supplementation of 0.2 ppm Se in buffalo heifers increased serum globulin levels and reduced albumin level and A:G ratio [47]. Similar to our results, an increased globulin and reduced levels of albumin and A:G ratio was observed in male buffalo calves supplemented with 0.3 ppm of Se [41]. Similarly, Mohapatra *et al.* [37] reported that supplementation of 0.3 ppm nano Se in layer chicks up to 8 weeks post feeding significantly increased TP and serum globulin levels and also significantly lowered A:G ratio compared to control.

Humoral immune response

Selenium supplementation in the basal diet as either Se nanoparticles or sodium selenite enhanced the humoral immune response of Wistar rats compared to control the group. The highest immune response in 150 ppb Se nanoparticles supplemented group indicated its supremacy among different levels of nanoparticles supplemented groups and also indicated that Se nanoparticles had a better efficacy than sodium selenite in enhancing the immune status of Wistar rats.

Contrary to our observations, there was no effect on the immunological response against *Pasteurella haemolytica* vaccination in steers given a single intramuscular dose of 25 mg Se [48]. Similarly, 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 cattle [49]. However, in these experiments, the basal diet itself had a quite high level of Se, and probably for that reason, they did not get any effect due to further Se supplementation. Basal diet in our experiment had comparatively lower levels of Se (0.08 ppm), which might have been responsible for the positive effect on humoral immune response in the rats on different levels of Se nanoparticle supplementation.

Similar to our results, it was observed that antibody mediated immune response was significantly higher in 0.15 and 0.30 ppm Se supplemented groups as compared to control group in growing lambs [50], but there was no significant difference between the two Se-supplemented groups. It was also observed that both cellular and humoral immunity were significantly increased in layer chicks supplemented with 0.3 ppm nano Se after 8 weeks of post feeding [37]. Similarly Cai *et al.* [51] reported that supplementation of nano Se up to the level of 0.3ppm in broiler chicks 42 days post feeding resulted in significant increase in the IgG and IgM levels compared to control. Male buffalo calves supplemented with 0.3 ppm Se exhibited significantly higher humoral immune response compared to control the group [41,52].

Conclusion

Supplementation of Se nanoparticles at the level of 150 ppb in the basal diet significantly improved the mean serum globulin level and reduced A:G ratio and also improved the humoral immune response in male Wistar rats compared to 150 ppb and 300 ppb levels of sodium selenite and 300 ppb of Se nanoparticles respectively. It was concluded that among the different Se sources (inorganic and nanoparticles) and levels (0-300 ppb), its supplementation at 150 ppb level as nanoparticles gave the best performance in terms of improved humoral immune status in male Wistar rats.

Author's Contributions

SJB, AKG, RSD and SS designed the experiment. SJB conducted the research experiment. SJB, AKG, RSD and SS drafted and revised the manuscript. All the authors read and approved the final manuscript.

Acknowledgment

The authors are thankful to the Director, Indian Veterinary Research Institute, Izatnagar, India, for providing all the necessary facilities to carry out this research work. Financial assistance from Indian Council of Agricultural Research provided to the first author (SJB) in the form of Senior Research Fellowship is also gratefully acknowledged.

Competing Interests

The authors declare that they have no competing interests.

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