



Original Contribution

SEL-PLEX® AND SODIUM SELENITE DIETARY SUPPLEMENTS WITH RESULTING SERUM LYSOZYME AND COMPLEMENT ACTIVITIES IN SOWS AND PROGENY DURING POST PARTUM PERIODS

L. Sotirov^{1*}, D. Goundasheva², M. Andonova², S. Denev³, M. Dimitrov⁴, N. Vassilev⁴

¹Department of Genetics; Faculty of Veterinary Medicine, Trakia University,

²Department of General and Clinical Pathology; Faculty of Veterinary Medicine, Trakia University, ³Department of Microbiology; Agrarian Faculty, Trakia University, ⁴Department of Obstetrics, Gynaecology and Andrology; Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria

ABSTRACT

The aim of this study was to investigate the potential immunomodulating effects of selenium added to diets in an organic (Sel-plex®) or a mineral form (Na selenite) in sows and in their progenies. For that, concentrations of these substances were studied in blood samples from 14 sow dams before and after parturition. The same procedure was done in the progenies, 20-35 days, post-partum. In spite of the large dispersion of lysozyme concentrations, this parameter was markedly depressed at parturition only in untreated sows, whereas in supplemented females, lysozyme concentrations remained close to basal values. By contrast, the alternative pathway of complement activation (APCA) values increased at parturition and immediately after, then slowly decreased in the 3 groups, but in the Sel-plex® group, these variations were lower than in the 2 other groups. Variations of the classical pathway of complement activation (CPCA) were characterised by gradual decreases and then recovery in all the groups, the lowest decreases being in the Na selenite group. Furthermore, treatment of sow dams by Sel-plex® promoted increases of lysozyme concentrations and complement activities in piglets. In essence, organic selenium supplementation in sows during pregnancy and post-partum stimulates innate immune responses.

Key words: sows, piglet, Sel-plex®, Sodium selenite, complement, lysozyme

INTRODUCTION

The animal health protection is of primary importance in the animal breeding industry. Multiple factors have a negative impact on immune system condition and therefore, on animal health. One of these factors is pregnancy. Numerous authors (1 - 4) have shown that the concentrations of oestrogens in pregnant guinea pigs, cows and sheep increased with advancing gestation and reached a peak at farrowing. At this time, they observed that the concentrations of several important immune parameters were the lowest and thus, the risk of infection – the highest. In order to decrease this negative effect some authors recommend inclusion of microelements to animal foods. Micronutrients, zinc, selenium, iron, copper,

vitamins A, C, E and B-6, and folic acid have important effects on immune responses. The immuno-stimulating effects of selenium were reported by Mishanin et al. (5) in cows fed with diets enriched with sodium selenite. They observed marked increases of phagocytic index, serum lysozyme concentrations and bactericidal activity in treated animals and in their offspring compared to control groups. The results obtained by Ndiveni and Finch (6) in vitro indicated the potential benefits of in vivo supplementation of dairy cows with vitamin E and selenium in terms of enhancing their natural resistance to mastitis. The paper of Finch and Turner (7) provided a comprehensive review of the effects of selenium and vitamin E on the immune responses of domestic animals and discussed their effects with respect to the differences in the basal nutritional status of the animals concerned, the type of supplements used, the route and timing of their administration and

* **Correspondence to:** Assoc. Prof. Dr. L. Sotirov, Department of Genetics; Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria; E-mail: sotirov20@yahoo.com

the different agents which have been used to stimulate an immune response.

With this study we aimed to investigate the influence of selenium on lysozyme concentration and complement activity in pregnant sows.

MATERIALS AND METHODS

Animals

Three groups of sow dams (English Large White) were used: group I (n = 5), whose daily diet was supplemented with 0,9 ppm Sodium selenite; group II (n = 6), treated with the same dose of organically bound selenium via the preparation Sel-Plex® (Alltech Inc., USA) and group III (n = 3) that was not treated with selenium preparations and served as control. The daily ration of the animals consisted of 2% corn, 22% wheat, 12% sunflower meal, 8% soybean meal, 12% bran, 21% barley and 5% PRP (protein rich product). The used premix was P 2005 No. 3 and 60 mg Sodium selenite per 1 kg premix were added. The supplemented premix was mixed to forage at a ratio of 5 kg premix per 1 tonne forage, i.e. the selenite concentration in the forage was 0,3 ppm/kg. Each animal received 3 kg forage daily, equal to 0,9 ppm sodium selenite each day. The progeny of untreated sows (control group, n = 9) and the progenies of sows, treated with either sodium selenite (n = 12) or Sel-plex® (n = 11) have been tested as well.

METHODS

Blood for analysis was sampled from the medial *sinus ophthalmicus* of sow dams prior to selenium administration, at the day of parturition, 3h and 6h post parturient and in 20th and 35th day. Blood was also obtained from newborn piglets at the age of 20 and 35 days. The blood samples were allowed to clot for one hour at room temperature (25°C) and then were centrifuged for 10 min at 2000 g. The blood sera were poured in small tubes and tested for lysozyme concentrations, APCA and CPCA activity immediately.

The selenium was determined by atom absorption spectrophotometry (Spectr-AA220z) with a graphite furnace and a Zeeman corrector of the rest background absorption. The preparation method was validated with a standard reference material CKM of the Community Bureau of Reference-BKR-476 Commission of the European Communities. The sows were artificially inseminated and the pregnancy was ultrasonographically detected (Aloka-SSD-

500).

Serum lysozyme concentrations were determined by the method of Lie (8). Briefly 20 ml of 2% agarose (ICN, UK, Lot 2050) dissolved in phosphate buffer (0,07 M Na₂HPO₄ and NaH₂PO₄, pH = 6.2) was mixed with 20 ml suspension of 24-hour culture of *Micrococcus lysodeicticus* at 67°C. This mixture was poured out in Petri's dish (14 cm diameter). After solidifying at room temperature 32 wells were made (5 mm diameter). Fifty microlitre of undiluted sera was poured out in each well. Eight standard dilutions (from 0,025 to 3,125 µg/ml) of lysozyme (Veterinary Research Institute, Veliko Tirnovo) were used in the same quantity as well. The samples were incubated for 20 hours at 37°C and lytic diameters were measured.

The alternative pathway of complement activation (APCA) was studied by the method of Sotirov (9). Each serum sample was first diluted by mixing 200µl serum with 300µl veronal - veronal Na buffer (in final concentrations: 146 mM NaCl, 1,8 mM 5,5-diethylbarbituric acid sodium salt, 3,2 mM 5,5-diethylbarbituric acid, 1 mM EGTA and 0,8 mM MgCl₂). In U bottomed plates (Flow Laboratories, UK), 7 other dilutions from each diluted serum were again prepared in veronal-veronal Na buffer: 80 µl diluted serum + 20 µl buffer, 70 µl diluted serum + 30 µl buffer, 60 µl diluted serum + 40 µl buffer, 50 µl diluted serum + 50 µl buffer, 40 µl diluted serum + 60 µl buffer, 30 µl diluted serum + 70 µl buffer and 20 µl diluted serum + 80 µl buffer. The final serum dilutions were respectively 8/45, 7/45, 6/45, 5/45, 4/45, 3/45 and 2/45. Then, 50 µl buffer and 100 µl of 1% rabbit erythrocyte suspension were added to each well. After incubation for 1 hour at 37°C, samples were centrifuged at 150 g for 3 minutes at room temperature (23°C). Thereafter, 150 µl of each supernatants were removed and placed in flat bottomed plates for measurement of optical density at 540 nm by "Sumal-PE2" ELISA reader (Karl Zeiss, Germany). The final APCA activity was calculated using special computer program developed in the Trakia University, and expressed as CH50 units (CH50 units correspond to 50% of complement-induced haemolysis of applied erythrocytes).

The classical pathway of complement activation (CPCA) was tested by method of Stelzner and Stein (10). Each serum sample (30µl) was first diluted with 170 µl veronal-veronal Na buffer (in final concentrations: 182 mM NaCl, 2,3 mM 5,5-diethylbarbituric

sodium salt, 3,9 mM 5,5-diethylbarbituric acid, 19 mM CaCl₂ and 125 mM MgCl₂). In U bottomed plates (Flow Laboratories, UK) five other dilutions from each diluted serum were prepared in veronal-veronal Na buffer (final concentrations: 1/5,6; 1/11,3; 1/22,6; 1/45,3; 1/90,6). Then 100 µl buffer and 100 µl of 1% haemolytic antibodies sensitised with sheep erythrocyte suspension in each dilution were dropped and were incubated at 37°C for 1 hour. Optical density were measured by "Sumal-PE2" ELISA reader (Karl Zeiss, Germany) at 540 nm. CPCA activity was calculated using special computer program developed in Trakia University, and expressed as CH50 units (CH50 units correspond to 50% of complement-induced haemolysis of applied

erythrocytes).

Statistical analysis:

Data were analysed using the fixed effect MANOVA model (Program STATISTICA, StatSoft, Inc., USA).

RESULTS

Serum Selenium concentrations have increased in the Na Selenite and in the Sel-Plex[®] treated sows since the parturition and this parameter remained strongly elevated on day 35, whereas, selenium concentrations have not varied in control (not treated) animals (**Figure 1**).

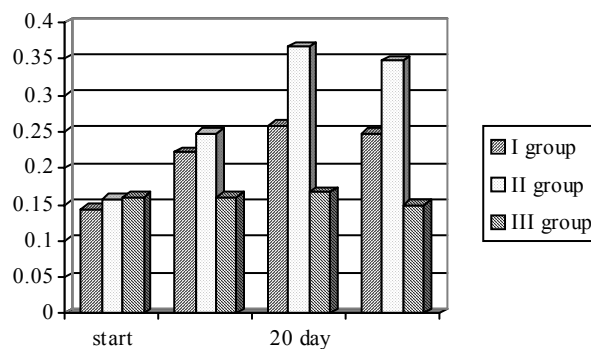


Figure 1. Selen concentrations in blood serum of pregnant sows non treated and treated with Sodium selenide and Sel-plex

Table 1. Lysozyme concentrations (µg/ml) in untreated sows (control group) and sows, treated with Sel-Plex[®] or Na selenite (0,3 ppm/kg) according to time. Results are expressed as Means ± Standard errors. CV(%) – Coefficient of variations

| Groups | Prior to Treatment | Parturition | Times after parturition | | | |
|-------------------------------|-----------------------|-----------------------|-------------------------|-----------------------|-----------------------|------------------------|
| | | | 3 hours | 6 hours | Day 20 | Day 35 |
| Control (n = 3) | 0,43 ± 0,09 32,74% | 0,29 ± 0,02 10,23% | 0,35 ± 0,03 10,27% | 0,54 ± 0,15 38,11% | 0,25 ± 0,02 10,29% | 0,36 ± 0,07 27,27% |
| Na Selenite (n = 5) | 0,53 ± 0,07 32,79% | 0,58 ± 0,11 46,43% | 3,28 ± 2,21 178,49% | 0,65 ± 0,19 78,85% | 0,28 ± 0,07 43,94% | 0,29 ± 0,04 24,62% |
| Sel-plex [®] (n = 6) | 0,49 ± 0,04 20,23% | 0,42 ± 0,07 40,04% | 2,11 ± 1,53 177,44% | 0,58 ± 0,14 60,39% | 0,30 ± 0,06 42,23% | 5,17 ± 4,36 188,69% |

Baseline lysozyme concentrations (**Table 1**) were quite comparable between groups and the intra-group coefficients of variations were low (20,23% to 32,79%), but after parturition, a great dispersion of lysozyme concentrations was noticed for all the groups (coefficients of variations fluctuated from 10,27% to 178,49%). Because of this large distribution of values, no significant difference according to time between the treated and the control

groups was observed, except at the time of parturition. At this particular time, a significant increase of lysozyme concentrations in sows receiving dietary sodium selenite supplementation was evidenced compared to untreated females ($p < 0,05$). However, lysozyme concentrations tended to increase but not significantly from the beginning of the study to the 3 hours post-parturition in treated groups, whereas they

were significantly lowered the 20th day post-partum compared to initial values ($p < 0,05$). Indeed, they rapidly increased again on day 35 in the Sel-plex[®] treated sows, while they remained depressed in the sodium selenite treated group (**Table 1**).

The low intra-group coefficients of variations of complement activities (for APCA: from 5,26% to 34,27%; for CPCA: from 3,64% to 51,75%) indicated that the dispersion of APCA and CPCA values in same group according to time was reduced compared to lysozyme concentrations (**Tables 2 and 3**). Prior to treatment, APCA and CPCA activities were comparable between groups. In untreated sows, APCA markedly increased at parturition (control group vs. treated groups: $p < 0,05$), then gradually decreased between the 3rd and the 6th hours, were comparable to basal values on the 20th day, and finally strongly declined on the 35th day (6th hour vs. 35th day: $p < 0,05$). In contrast, in treated sows, APCA remained stable at the time of parturition, increased 3 and 6 hours after (prior treatment vs. 6 hours:

$p < 0,001$ for sows treated by sodium selenite), then decreased on the 20th and the 35th day post-partum (6 hours vs. day 35: $p < 0,001$ and $p < 0,01$ for sows treated by sodium selenite and Sel-plex[®] respectively)(**Table 2**). On the other hand, CPCA tended to decrease, but not significantly, at parturition and immediately after (3 to 6 hours), were depressed on the 20th day and returned to basal values on day 35 in control animals (**Table 3**). The variations of CPCA activities in treated sows were parallel to those of untreated animals: they declined from parturition to the 20th day, and finally were similar to baseline values on day 35 (in the sodium selenite treated group: day 35 vs. 3 and 6 hours and day 35 vs. day 30: $p < 0,01$) (**Table 3**). Consequently, variations of APCA values were less intense in treated animals (particularly in the Sel-plex[®] treated group) than in untreated females, and CPCA activities did not exhibit any significant variation between groups according to time after parturition.

Table 2. Alternative pathway of complement activation (APCA) activities (CH50) in untreated sows (control group) and sows, treated with Sel-Plex[®] or Na selenite (0,3 ppm/kg) according to time. Results are expressed as Means \pm Standard errors. CV(%) – Coefficient of variations

| Groups | Prior to Treatment | Parturition | Times after parturition | | | |
|-------------------------------|---------------------------|---------------------------|----------------------------|-----------------------------|----------------------------|---------------------------|
| | | | 3 hours | 6 hours | Day 20 | Day 35 |
| Control (n = 3) | 93,24 \pm 4,66 7,07% | 112,5 \pm 3,3* 4,20% | 93,5 \pm 9,2 13,9% | 100,4 \pm 3,9*** 5,26% | 95,8 \pm 7,4 10,88% | 70,7 \pm 6,7 13,52% |
| Na Selenite (n = 5) | 82,04 \pm 7,4 9,07% | 91,6 \pm 6,16 16,48% | 112,3 \pm 15,7 34,27% | 104,9 \pm 3,1*** 7,27% | 86,9 \pm 10,06 23,14% | 63,9 \pm 4,9 16,42% |
| Sel-Plex [®] (n = 6) | 91,1 \pm 4,2 12,19% | 88,6 \pm 6,2 17,06% | 92,03 \pm 3,03 8,71% | 98,1 \pm 2,07** 5,59% | 86,1 \pm 5,96 15,47% | 78,03 \pm 5,7 17,29% |

* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$

In piglets, no significant difference in lysozyme concentrations, in APCA and CPCA activities according to dietary treatment has been evidenced on days 20 and 35 (**Table 4**). Lysozyme concentrations and CPCA values tended to increase with age in progenies. Increases of lysozyme concentrations were however significant in piglets from treated sows ($p < 0,05$ and $p < 0,01$ for piglets stemming from dams treated by Sel-plex[®] and sodium selenite, respectively), but not in

progenies of control females. By contrast, significant elevations of CPCA activities were obtained on day 35 in piglets from untreated and Sel-plex[®] treated females ($p < 0,01$). On the other hand, the APCA values of progenies from treated and untreated dams markedly decreased with age. The 35-day old piglets showed lower APCA activities in the 3 groups ($p < 0,05$ for piglets from control dams, $p < 0,01$ for piglets from Sel-plex[®] and sodium selenite treated dams).

Table 3. Classical pathway of complement activation (CPCA) activities (CH50) in untreated sows (control group) and sows, treated with Sel-Plex® or Na selenite (0,3 ppm/kg) according to time. Results are expressed as Means ± Standard errors. CV(%) – Coefficient of variations

| Groups | Prior to Treatment | Parturition | Times after parturition | | | |
|------------------------|-------------------------|-----------------------|-------------------------|-----------------------|-------------------------|-------------------------|
| | | | 3 hours | 6 hours | Day 20 | Day 35 |
| Control (n = 3) | 270,1 ± 29,9 15,68% | 254,9 ± 11,3 6,29% | 254,1 ± 5,05 2,81% | 234,5 ± 6,04 3,64% | 221,5 ± 27,3 17,45% | 270,9 ± 16,2 8,44% |
| Na Selenite (n = 5) | 286,06 ± 11,5 10,66% | 254,9 ± 8,66 8,32% | 243,5 ± 8,9 9,02% | 250,9 ± 8,02 7,81% | 242,9 ± 4,53 4,17% | 298,7 ± 11,4** 8,54% |
| Sel-Plex® (n = 6) | 277,9 ± 15,5 14,74% | 254,7 ± 10,3 9,93% | 250,8 ± 7,7 8,14% | 235,4 ± 9,8 10,98% | 181,07 ± 46,9 51,75% | 275,2 ± 10,5 8,51% |

** $p < 0,01$

Table 4. Lysozyme concentrations (µg/ml), APCA and CPCA activities (CH50) in progenies stemming from untreated sows (control group) or from treated sows with Sel-Plex® or Na selenite (0,3 ppm/kg). Results are expressed as mean ± standard errors. CV: coefficients of variations (%) in parenthesis

| Parameters | Progenies for | | | | | |
|---------------------------------|----------------------------|--------------------------|--------------------------------------|---------------------------|------------------------------------|----------------------------|
| | Control sows (n = 9) | | Na selenite treated sows (n = 12) | | Sel-Plex® treated sows (n = 12) | |
| | 20D | 35D | 20D | 35D | 20D | 35D |
| Lysozyme Concentrations (µg/ml) | 0,33 ± 0,04 (35,60%) | 1,26 ± 0,82 (183,78%) | 0,31 ± 0,04 (38,58%) | 1,07 ± 0,25** (76,85%) | 0,41 ± 0,15 (120,33%) | 1,64 ± 0,46* (93,07%) |
| APCA activities (CH50) | 106,3 ± 7,2*** (19,12%) | 82,6 ± 4,7 (16,25%) | 98,2 ± 4,4** (14,72%) | 80,6 ± 3,02 (12,49%) | 101,2 ± 4,2** (13,21%) | 72,8 ± 4,2 (19,28%) |
| CPCA activities (CH50) | 254,1 ± 8,05 (8,96%) | 284,6 ± 5,6** (5,54%) | 239,9 ± 5,4 (7,05%) | 261,3 ± 11,9 (15,15%) | 240,04 ± 7,5 (10,41%) | 286,9 ± 11,8** (13,61%) |

** $p < 0,01$; *** $p < 0,001$

DISCUSION

In this study, lysozyme concentrations greatly fluctuated in all the 3 groups after parturition, indicating that strong individual reactions due to differences in the animal genotypes occurred in response to dietary selenium supplementation and/or to parturition. However, they were markedly depressed at parturition and remained low until the 20th day in control (untreated) sows. By contrast, in dietary selenium supplemented sows, they remained close to baseline values or increased at parturition and immediately after (3 and 6 hours after), and decreases of lysozyme concentrations were only observed on the 20th day. They increased again on day 35 only in the Sel-plex® treated group. According to Sotirov (9), the normal average lysozyme concentrations in sow dams was 1.83 ± 0.05 µg/ml (calculated from the values obtained in 562 sows from different breeds). Consequently, the present study showed that

immuno-suppressive effects of gestation and parturition were stronger in the control group than in the dietary selenium supplemented groups. Moreover, the negative effects were the lowest in the Sel-plex® treated group, i.e. this group had the highest potential for defence against infectious agents. In this group, a significant positive effect was achieved between serum selenium concentrations and lysozyme concentrations, suggesting that elevated selenium concentrations for a long time would improve lysozyme synthesis (11 - 15). Furthermore, although significant differences were not being evidenced between progenies stemming from untreated and treated sows, dietary selenium supplementation in sow dams, particularly with Sel-plex®, has also promoted lysozyme production in 35 day old piglets.

At parturition and 6 hours later, APCA activities increased in the untreated animals. Although slight elevations of APCA values

were recorded in the sodium selenite treated sows at 3 and 6 hours, they appeared more stable in the treated groups, particularly in the Sel-Plex® group. Thereafter, APCA activities decreased on days 20 and 35 in the 3 groups. On the other hand, CPCA activities slowly decreased from parturition to the 20th day and returned to basal values on day 35 in the 3 groups. Nevertheless, this drop was less marked and the recovery more intense in the sodium selenite group. Taken together, and despite a relatively high degree of dispersion of the 3 parameters tested, these data indicated that dietary selenium supplementation, particularly with the organic form (Sel-plex® treatment) would attenuate the immunosuppressive effects of parturition, and reduce susceptibility to infections. According to Chandra (16) nutrition is a critical determinant of immunocompetence and risk of illness and death largely due to infectious disease. It is now established that undernourished individuals have impaired immune responses. The most consistent abnormalities are seen in cell-mediated immunity, complement system, phagocytes, mucosal secretory antibody response and antibody affinity. These changes, together with other handicapping factors observed in underprivileged societies, lead to more infections. It is now recognized that deficiencies of single nutrients also impair immune responses (16). The best studied are zinc, iron, vitamin B-6, vitamin A, copper and selenium. If malnutrition occurs during foetal life, as epitomized in small-for-gestational age infants, the effects on cell-mediated immunity are very significant and long lasting. Later studies of the same author (17) confirmed that protein-energy malnutrition is associated with a significant impairment of cell-mediated immunity, phagocyte function, complement system, secretory immunoglobulin A antibody concentrations, and cytokine production. Deficiency of single nutrients also results in altered immune response: this is observed even when the deficiency state is relatively mild. The micronutrients, zinc, selenium, iron, copper, vitamins A, C, E and B-6, and folic acid have important influences on immune responses (17). Overnutrition and obesity also reduce immunity. Hawkes et al. (18) reported that antibody titres against diphtheria vaccine were 2.5-fold greater after reinoculation in the high selenium group. White blood cell counts decreased in the high-selenium group and increased in the low-selenium group, resulting primarily from changes in granulocytes. Apparent increases in cytotoxic T-

lymphocytes and activated T-cells in the high-selenium group only approached significance. Lymphocyte counts increased on day 45 in the high-selenium group. In vitro proliferation of peripheral lymphocytes in autologous serum in response to pokeweed mitogen was stimulated in the high-selenium group by day 45 and remained elevated throughout the study, whereas proliferation in the low selenium group did not increase until day 100. This study indicates that the immune-enhancing properties of selenium in humans are the result, at least in part, of improved activation and proliferation of B-lymphocytes and perhaps enhanced T-cell function.

The immuno-stimulating effects of selenium were reported by Mishanin et al. (5) in cows fed with diets enriched with sodium selenite. They observed marked increases of phagocytic index, serum lysozyme concentrations and bactericidal activity in treated animals and in their offspring compared to control groups. The results obtained by Ndiveni and Finch (6) in vitro indicate the potential benefits of in vivo supplementation of dairy cows with vitamin E and selenium in terms of enhancing their natural resistance to mastitis. The paper of Finch and Turner (7) provides a comprehensive review of the effects of selenium and vitamin E on the immune responses of domestic animals and discusses their effects with respect to the differences in the basal nutritional status of the animals concerned, the type of supplements used, the route and timing of their administration and the different agents which have been used to stimulate an immune response. According to Ferencik and Ebringer (19) almost all nutrients in the diet play a crucial role in maintaining an "optimal" immune response, and both insufficient and excessive intakes can have negative consequences on the immune status and susceptibility to a variety of pathogens. As a constituent of selenoproteins, selenium is needed for the proper functioning of neutrophils, macrophages, NK cells, T lymphocytes and some other immune mechanisms. Elevated selenium intake may be associated with reduced cancer risk and may alleviate other pathological conditions including oxidative stress and inflammation. Selenium appears to be a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS. It is required for sperm motility and may reduce the risk of miscarriage. Broome et al. (20) found that selenium supplements augmented the cellular

immune response through an increased production of interferon gamma and other cytokines, an earlier peak T cell proliferation, and an increase in T helper cells. Milad et al. (21) tested the effect of vitamin E and selenium administration on selected parameters of cellular immunity in pregnant sheep with body weight of 42 to 66 kg. The administered preparation led to significant effects ($p < 0.001$; $p < 0.05$) on phagocytic activity index of leukocytes and phagocytic activity index of neutrophils. Giadinis et al. (22) pointed that Se alone led to a significant increase of Chlamydia antibody response ($p < 0.05$), but not when it was given in combination with vit E. Animals that received vit. E had much lower titres, just above of those of the controls.

Some studies have previously shown that in various animal species (guinea pigs, cows and sheep) serum lysozyme concentrations and complement activities decreased at the time of parturition and slowly recovered within 30 - 40 days (1 - 4), and the infectious risk for females and their progenies was greater in post-partum period. Consequently, it is out of interest to achieve a reduction of this risk through dietary selenium supplementation, particularly in sows, in which intensive breeding conditions would promote susceptibility to infections.

CONCLUSIONS

The preparation Sel-Plex[®] applied at the specified doses and according to the used schedule demonstrated the highest immunostimulating effect on lysozyme and both the alternative and the classical pathways of complement activation compared to Sodium selenite and control groups.

REFERENCES

1. Boulanger, P., Rice, C. E. and Plummer, P. J. G. Parallel studies of complement and blood coagulation. XII. Effect of pregnancy and sex hormones. *The Cornell Veterinarian*, 44 (1): 191-198, 1954.
2. Iliev, Ya. and Sotirov, L. Activity of complement and lysozyme in cows and their young under conditions of industrial raising. *Veterinarna sbirka*, 9-10: 19-22, 1988.
3. Detilleux, J. C., Kehrl, M. E. Jr., Stabel, J. R., Freeman, A. E. and Kelly D. H. Study of immunological dysfunction in periparturient Holstein cattle selected for high and average milk production. *Veterinary Immunology and Immunopathology*, 44: 251-267, 1995.
4. Sotirov, L., Bivolarski, B., Dzhorbineva, M. and Dimitrov, I. Level of the alternative pathway of complement activity and lysozyme in pregnant ewes before and after delivery. *Bulgarian Journal of Agricultural Science*, 2: 229-233, 1996.
5. Mishanin, Yu. F. Immunobiological blood parameters in cows and calves supplemented with selenium. *Vestnik Selskohozyaistvennoi Nauki*, Moskva, 2: 86-91, 1992.
6. Ndiweni, N. and Finch, J. M., 1996. Effects of in vitro supplementation with alpha-tocopherol and selenium on bovine neutrophil functions: Implications for resistance to mastitis. *Veterinary Immunology and Immunopathology*, 51 (1-2): 67-78, 1996.
7. Finch, J. M. and Turner, R. J. Effects of selenium and vitamin E on the immune responses of domestic animals. *Research in Veterinary Science*, 60 (2): 97-106, 1996.
8. Lie, O. Markers of Resistance to Infection in Dairy Cattle. PhD Tesiss, National Veterinary Institute, Oslo, Norway, 1985.
9. Sotirov, L. K. Phenotype characteristic and inheritance of lysozyme and complement activity in swine. PhD Thesis, Trakia University, Stara Zagora, Bulgaria, 1991.
10. Stelzner, A. and Stein, G. Moglichkeiten zur hamolytischen Aktivitätsmessung von Gesamtkomplement. Theoretische Grundlagen und metodische Hinweise. *Wissenschaftliche Zeitschrift der Friedrich Schiller Universitat Jena*. Mathematisch Naturwissenschaftliche Reihe 20, Jg H6: 933-941, 1971.
11. Turner, R. J. and Finch, J. M. Immunological malfunctions associated with low selenium-vitamin E diets in lambs. *Journal of Comparative Pathology*, 102 (1): 99-109, 1990.
12. Wolska, K., Bukowski, K., Anusz, Z. and Jakubczak, A. Bactericidal activity of human, swine and cattle serum against pseudomonas aeruginosa strains. *Med Dosw Mikrobiol*, 51 (3-4): 339-45, 1999.
13. Wagstrom, E. A., Yoon, K. J. and Zimmerman, J. J. Immune components in porcine mammary secretions. *Viral Immunology*, 13 (3): 383-97, 2000.
14. Krakowski, L., Krzyzanowski, J., Wrona, Z., Kostro, K., Siwicki, A. K. The influence of nonspecific

- immunostimulation of pregnant sows on the immunological value of colostrum. *Veterinary Immunology and Immunopathology*, 87 (1-2): 89-95, 2002.
15. Gupta, S., Kumar, H. and Soni, J. Effect of Vitamin E and selenium supplementation on concentrations of plasma cortisol and erythrocyte lipid peroxides and the incidence of retained fetal membranes in crossbred dairy cattle. *Theriogenology*, 66 (2): 493, 2006.
 16. Chandra, R. K. Nutrition and immunoregulation - significance for host-resistance to tumors and infectious diseases in humans and rodents. *Journal of Nutrition*, 122, (3): 754-757, 1992.
 17. Chandra, R. K. Nutrition and the immune system from birth to old age. *European Journal of Clinical Nutrition*, 56: 73-76, Suppl. 3, 2002.
 18. Hawkes, W. C., Kelley, D. S. and Taylor, P. C. The effects of dietary selenium on the immune system in healthy men. *Biological Trace Element Research*, 81 (3): 189-213, 2001.
 19. Ferencik, M. and Ebringer, L. Modulatory effects of selenium and zinc on the immune system. *Folia Microbiologica*, 48 (3): 417-426, 2003.
 20. Broome, C. S., McArdle, F., Kyle, J. A. M., Andrews, F., Lowe, N. M., Hart, C. A., Arthur, J. R. and Jackson, M. J. An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status. *American Journal of Clinical Nutrition*, 80 (1): 154-162, 2004.
 21. Milad, K., Racz, O., Sipulova, A., Bajova, V. and Kovac, G. Effect of vitamin E and selenium on blood glutathione peroxidase activity and some immunological parameters in sheep. *Veterinarni Medicina*, 46 (1): 1-5, 2001.
 22. Giadinis, N., Koptopoulos, G., Roubies, N., Siarkou, V. and Papasteriades A. Selenium and vitamin E effect on antibody production of sheep vaccinated against enzootic abortion (*Chlamydia psittaci*). *Comparative Immunology Microbiology and Infectious Diseases*, 23 (2): 129-137, 2000.