

## RESEARCH REGARDING THE INFLUENCE OF ORGANIC SELENIUM ON THE IMMUNE RESPONSE IN SWINE

Bogdan TAŞBAC<sup>1</sup>, Tiberiu BURINARU<sup>1</sup>, Gavrilă ZAGRAI<sup>2</sup>,  
Otilia Cristina MURARIU<sup>3</sup>

<sup>1</sup>University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independenței, District 5, 050097, Bucharest, Romania

<sup>2</sup>University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Faculty of Veterinary Medicine, 3-5 Calea Mănăştur Street, 400372, Cluj-Napoca, Romania

<sup>3</sup>“Ion Ionescu de la Brad” University of Life Sciences of Iași,  
3 Mihail Sadoveanu Alley, Iași, Romania

Corresponding author email: researchfvmb@gmail.com

### Abstract

Currently, immunomodulation is an alternative in the fight against many diseases, being considered a possibility to fight against many infectious diseases that can affect pig herds. Selenium can be used for this purpose, the effect of its administration on the immune response being the main purpose of the present study. Following the administration of organic selenium, we found that in the case of WBC parameters, granulocytes and agranulocytes percentages and lymphoblastic transformation percentages of B lymphocytes, there are no major differences between the values recorded at the beginning of the experiment and the values recorded in the two experimental moments. Instead, following the administration of organic selenium, we observed significant increases in T lymphocytes percentages (by 9.94%, after 21 days, and respectively by 8.18%, after 30 days), percentages of lymphoblastic transformation of T lymphocytes (by 59.59% after 21 days, and respectively by 64.14% after 30 days), as well as the helper T lymphocytes/ suppressor T lymphocytes ratio (by 46.15% after 21 days, and respectively by 65.38% after 30 days). Regarding the percentage of B lymphocytes, a decrease of this parameter is observed by 38.34% after 21 days and by 21.84% after 30 days following the administration of the product based on organic selenium.

**Key words:** immunomodulation, lymphocytes, pigs, selenium.

### INTRODUCTION

Currently, immunomodulation represents an important alternative in combating numerous diseases of pets as well as of economic interest animals, especially in the perspective of banning the use of antibiotics. The importance of the application of immunomodulatory therapies exerts influence on both animal health (Byrne et al., 2014) and public health according to the common desire formulated on the basis of the "One Health" principles (Savu & Petcu, 2002; Petcu, 2006; Petcu et al., 2007).

The use of immunomodulatory preparations in pig herds, both in the case of professional holdings and in the case of households, can increase the subjects' resistance to the action of pathogens, by positively regulating the duration and intensity of the immune response (Găjăilă et al., 2022). Through the development and application of vaccines throughout history,

numerous biological threats have been defeated, but techniques to enhance the non-specific immune response have not benefited the same attention in the research field. Non-specific immunomodulation becomes fundamental alongside the principles of biosecurity in the case of the evolution of infectious pathologies, that, as of now, have no discovered vaccines.

Immunomodulation is still an area of interest for the veterinary medicine research field, being considered a potential weapon in the fight against pathological entities which produce major economic losses in pig herds. If the immunosuppressive therapies address those situations in which it is necessary to suppress a harmful immune response, immune stimulation may represent a prophylactic or therapeutic alternative in case of infectious or parasitic pathologies.

It is known that the use of antibiotics in animal feed can have an immune-stimulating role, but

the legislation of the European Community prohibits them, as factors for preventing antibiotic residues in food products of animal origin (Gallois et al., 2009; Pogurschi et al., 2015; Ghimpețeanu et al., 2022; Pogurschi et al., 2022). Nowadays, the administration of mineral supplements is a common practice in animal husbandry (Ghiță et al., 2021), some of which have an immune-stimulating effect.

From the category of essential minerals for supporting the immune system in pigs, the most cited in the specialized literature are iron and selenium (Marin et al., 2013; Zhou et al., 2021; Falk et al., 2019).

Selenium plays a very important role in the protection of cell membranes against oxidative stress. Numerous selenium-dependent enzyme structures constitute elements of the antioxidant mechanisms so important in the organism defense against pathogens. Organic selenium or inorganic selenium is used as a supplement for animal forage. The inorganic form of selenium, namely selenite or selenate, presents a number of disadvantages compared to organic selenium. Interactions with other minerals in the body, a higher toxicity and a lower storage capacity in the form of reserves are more often discussed in its case. Thus, the organic form of selenomethionine obtained from natural yeast cultures is often preferred in both humans and animals due to its superior bioavailability. Organic forms of selenium are absorbed with an efficiency of about 70-90% while the absorption of selenite does not exceed 60%. Of particular importance is the fact that selenium, among all the elements, has very close values between the threshold characteristic of this mineral's deficiency (<40 µg/day) and the toxic level (>400 µg/day).

The goal of our research was to evaluate by modern techniques the non-specific immune response, in the case of pigs administered organic selenium.

## MATERIALS AND METHODS

In the present study, we verified the effectiveness of a commercial preparation based on organic selenium (Sel-Plex<sup>®</sup>), considered to have an immunomodulatory effect.

Sel-Plex<sup>®</sup>, additive approved at European level, represents a genuine source of organic selenium,

through its composition based on the presence of yeast (*Saccharomyces cerevisiae*). It was administered to pigs by inclusion in the feed in accordance with the manufacturer's recommendations (100 mg/kg feed). At this concentration, the animals in the experimental group did not have a daily intake of selenium higher than 0.5 mg/kg body weight.

In order to pursue the objectives of the present study, a homogeneous batch of 20 pigs of similar ages (4-5 months) belonging to a farm in the area of Cernica, Ilfov county, was established. In choosing the subjects in the study, it was ensured that the individuals were as homogeneous as possible in terms of genetic characteristics, age difference, maintenance conditions and fattening status, considering the influence of some hormones secreted by adipose tissue on lymphocyte populations (Ghiță et al., 2021).

The samples were collected as followed: the first series of samples, prior to the administration of the organic selenium-based product, the subsequent samplings taking place after 21 and 30 days respectively from the time of inclusion of the Sel-Plex<sup>®</sup> product in the feed. We mention that throughout the administration of selenium, the animals were not treated with any medicinal products, taking in consideration their effect (especially anti-inflammatory drugs) on leukocytes and implicitly on the immune response (Codreanu, 2018; Ghiță et al., 2015). The immune status evaluation and the implications of the administration of the product based on organic selenium in the tested pigs batch was carried out by classical haematological and immunological techniques with high applicability in veterinary laboratories.

The chosen haematological analysis consisted in the determination of WBC and granulocytes/agranulocytes ratio using an IDEXX type analyzer. The cellular immunology tests included the separation of the total population of lymphocytes in a density gradient with Ficoll medium, the determination of the percentage of T and B lymphocytes by the EA rosette technique and the establishment of the existing ratio between the subpopulations of helper T lymphocytes and suppressor T lymphocytes by the E rosette technique. To obtain pure populations of T and B lymphocytes, the technique of separation by adhesion to nylon

fibers was used, and the evaluation of the functionality of these cells was performed by the lymphoblastic transformation test (TLT), the variant based on the determination of the glucose consumption index in the reaction medium.

The mitogen used in the test was phytohemagglutinin (PHA). To test the phagocytic functions, the population of polymorphonuclear neutrophils separated from the blood with the Dextran medium was chosen for determining the locomotion capacity in a Boyden type filter

layer with the evaluation of the migration density.

The statistical analysis was performed with the help of the t-test (Student test).

## RESULTS AND DISCUSSIONS

The obtained results will be presented in the form of a centralizing table and some graphs, accompanied by short comments. Table 1 presents analysed parameters for the two experimental moments.

Table 1. The values obtained for the experimental moments

Analysed parameters	Before selenium administration	21 days after selenium administration	30 days after selenium administration
Leukocytes totale ( $10^9/l$ )	15.1	14.9	15.9
Granulocytes, %	41	43	41
Agranulocytes, %	59	57	59
T lymphocytes, %	79.4	87.3*	85.9*
B lymphocytes, %	20.6	12.7*	16.1*
hTL/sTL ratio	2.6	3.8*	4.3*
Lymphoblastic transformation TL, %	39.6	63.2*	65.0*
Lymphoblastic transformation BL, %	41.1	39.2	42.4
Directed migration density ( $\mu$ )	1702	2140*	2215*

\* $p < 0.01$

Studying the data presented in Table 1, it can be observed that in the case of WBC parameters, granulocytes and agranulocytes percentages and lymphoblastic transformation of B lymphocytes percentage, there are no major differences between the values recorded at the beginning of the experiment and the values recorded in the two experimental moments. For the other parameters analysed, it can be observed that their values undergo changes in the case of the two experimental moments, changes that will be presented below.

Following the administration of the Sel-Plex<sup>®</sup> product, there is an increase in % lymphocytes T by 9.94%, after 21 days, and respectively by 8.18%, after 30 days (Figure 1), in both cases the differences being significant from the point of view statistically ( $p < 0.01$ ).

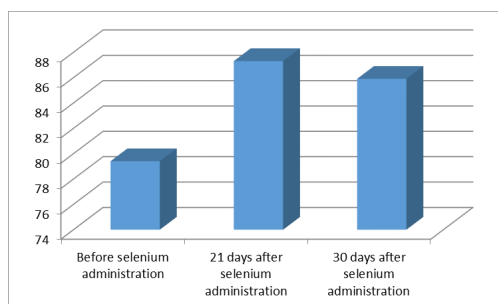


Figure 1. T lymphocytes % during the experiment

Regarding the B lymphocytes percentage, there is a decrease of this parameter by 38.34% after 21 days and by 21.84% after 30 days (Figure 2), in both situations the differences are statistically significant ( $p < 0.01$ ).

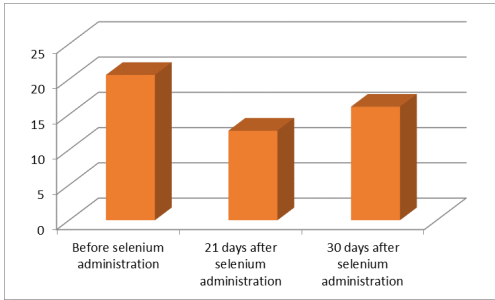


Figure 2. B lymphocytes % during the experiment

The indicated change can be explained by the accelerated proliferation induced by the administration of the Sel-Plex<sup>®</sup> product, of the population of T Lymphocytes, cells responsible for cell-mediated immunity. Therefore, clones of activated T lymphocytes become dominant and the population of B lymphocytes suffers a numerical decline against the background of granulocytes/agranulocytes ratio maintained constant. Such changes in the ratios between the different types of leukocytes are also found in the case of the administration of other products for immunostimulatory purposes (Li et al., 2020; Ioniță et al., 2014).

Regarding the helper TL/suppressor TL ratio, it was found to increase by 46.15% after 21 days, and respectively by 65.38% after 30 days following the administration of the Sel-Plex product (Figure 3), in both cases the differences being significant ( $p < 0.01$ ).

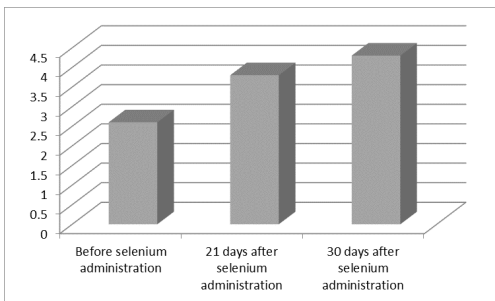


Figure 3. Helper TL/suppressor TL ratio during the experiment

The helper TL/suppressor TL ratio represents the state of balance necessary for the regulatory T lymphocyte subpopulations in order to carry out the immune response mechanisms. The resulting parameters indicate a significant increase in the subpopulation of T helper

lymphocytes, which demonstrates that through their functions these cells non-specifically activate a series of effector cells of cellular type immunity (Gâjailă et al., 2015). The decrease in the proportion of T lymphocytes in this report doesn't reach such values that indicate a state of harmful immunodepression, but a reactivation of some cytotoxic and phagocytic functions independent of the presence of specific antibodies. Regarding the lymphoblastic transformation of TL percentage, it is observed that it increased by 59.59% after 21 days, and respectively by 64.14% after 30 days following the administration of organic selenium (Figure 4), in both cases the differences being significant from a statistical point of view ( $p < 0.01$ ).

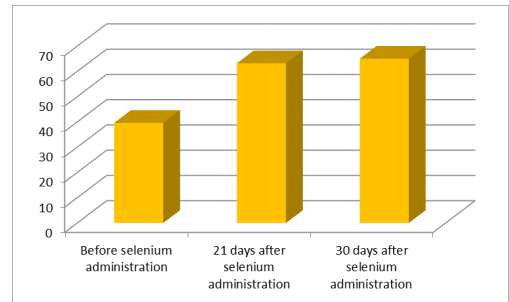


Figure 4. TL lymphoblastic transformation % during the experiment

The lymphoblastic transformation test clearly indicates the reactivity of lymphocyte populations to non-specific activating factors such as selenium. T and B lymphocytes stimulated with selenium and plant mitogens proved to be much more reactive in the case of T lymphocytes, where the transformation indices almost doubled. It is possible that administered selenium acts mainly on T lymphocytes and very little on B lymphocytes, a fact also reported by other authors (Vetvicka et al., 2014; Zavodnik et al., 2011).

In the case of directed migration density, it is observed that this parameter increased by 25.73% after 21 days, and respectively by 30.14% after 30 days from the initiation of the treatment with organic selenium (Figure 5), in both cases the differences being significant from a statistic point of view ( $p < 0.01$ ).

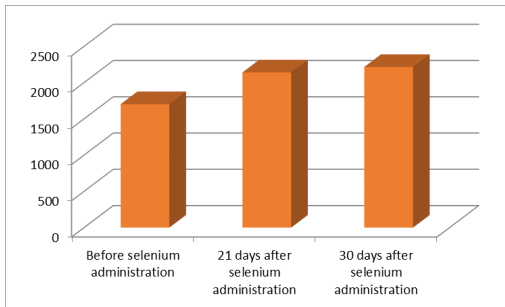


Figure 5. Directed migration density during the experiment

The phagocytic function performed by the population of polymorphonuclear neutrophils is conditioned by the perception of the chemotactic stimulus that triggers the stages of the phagocytosis mechanism (Thacker, 2010). Directed migration demonstrates the activation of the phagocytic cell and constitutes the first step in the evaluation of this function. The results indicate an increase in neutrophil reactivity after the administration of the selenium-based product, an aspect reported in the specialized literature (Dalgaard et al., 2018; Gájáilä et al., 2016).

Following the administration of the Sel-Plex® product, the dominant changes appear in the results obtained in the evaluation of the HhTL/sTL ratio, the index of lymphoblastic transformation in the case of B lymphocytes and the locomotion capacity by applying the phagocyte migration technique under an agarose layer. These results indicate a specific activation of the cell-mediated immune response in the direction of the coordination functions of the subpopulation of hT lymphocytes and the phagocytic function of neutrophils.

The establishment of the values of the T and B lymphocyte populations, together with the evaluation of the ratios between the 2 categories of cells, showed that the Sel-Plex® product has the potential to increase the proportion of T lymphocytes. The orientation of the TL/BL ratio towards high values of 4, with a weight higher than the 80% value of T lymphocytes, can demonstrate an activation of the proliferation process of T lymphocytes and implicitly the switching of the body's defense functions towards a stronger cell-mediated immune response. Evaluating the reaction to the chemotactic stimulus in the Boyden filter and

the locomotion capacity of the circulating neutrophils demonstrated an increase in chemotaxis manifested by an increased migration of neutrophils through the filtering layer. This effect is translated by the optical density values for the directed migration which increased on average by 30%. Similar values were also recorded in the evaluation carried out after 30 days of product administration.

## CONCLUSIONS

The administration of selenium in the form of Sel-Plex®, product with an immunomodulatory effect, did not produce major changes in the leukogram values, the ratio of granulocytes and agranulocytes remaining constant in the reference range characteristic of the species. Major changes were found within the subpopulation of lymphocytes.

A change in the TL/BL ratio was identified in the sense of increasing the subpopulation of T lymphocytes without exceeding the maximum threshold considered normal for the development of an effective immune response. The reaction denotes an activation of cellular immune response mechanisms.

The administration of the Sel-Plex® product has identified implications in the coordination mechanisms of immune processes, organic selenium increasing the helper TL/suppressor TL ratio in favor of helper T lymphocytes, to significant values that don't exceed the maximum physiological threshold allowed in pigs, which eliminates possible hypersensitivity states.

The functional evaluation of T and B lymphocytes carried out by the lymphoblastic transformation test demonstrated that the T lymphocyte transformation index increased significantly, a characteristic element that indicates a non-specific but significant stimulation of T lymphocyte subpopulations participating in cellular immune response mechanism.

The use of the Sel-Plex® product demonstrated that it has also generated a stimulation of the specific phagocytic function of neutrophils by stimulating a higher locomotion activity correlated with an intensified chemotaxis.

The tests carried out indicate the immunostimulatory potential of selenium-based products in the immune reaction in pigs, but

great attention must be paid to the doses of selenium administered and the time suitable for a real stimulation because the toxic effects induced by selenium are also known to take place.

## REFERENCES

- Byrne, A.K., Loving, L.C., & McGill, L.J. (2020). Innate immunomodulation in food animals' evidence for trained immunity? *Front Immunol*, 11, 1099.
- Codreanu, I., (2018). *Textbook of animal physiology*. Bucharest, RO: Printech Publishing House.
- Dalgaard, T.S., Briens, M., Engberg, R.M., Lauridsen, C. (2018). The influence of selenium and selenoproteins on immune responses of poultry and pigs. *Animal feed science and technology*, 238, 73–83.
- Falk, M., Lebed, P., Bernhoft, A., Framstad, T., Kristoffersen, A.B., Salbu, B., & Oropeza-Moe, M. (2019). Effects of sodium selenite and L-selenomethionine on feed intake, clinically relevant blood parameters and selenium species in plasma, colostrum and milk from high-yielding sows. *J. Trace Elem. Med. Biol.*, 52, 176-185.
- Gallois, M., Rathkötter, H.J., Bailey, M., Stokes, C.R., & Oswald, I.P. (2009). Natural alternatives to in-feed antibiotics in pig production: can immunomodulators play a role? *Animal*, 3(12), 1644-1661.
- Găjăilă, G., Ghiță, M., Petcu, C.D., Dobre, R., Botezatu, R., Andrei, C., Mihai (Oprea), O.D., & Cotor, G. (2022). Research on the immunomodulatory effect of levamisole in swine. *Scientific Papers. Series D. Animal Science*, LXV (1), 278-283.
- Găjăilă, G., Găjăilă, I., Cotor, G., & Ioniță, L. (2016). Testing the killing ability of pig neutrophils after stimulation with an ethanalamine derivative. *Journal of Biotechnology*, 231, Supplement, S71.
- Găjăilă, G., Găjăilă, I., Cotor, G., & Ioniță, L. (2015). Influence of incubation length and temperature on the efficiency of magnetic separation of cattle blood neutrophils. *Journal of Biotechnology*, 208S, S23.
- Ghimpețeanu, O.M., Pogurschi, E.N., Popa, D.C., Dragomir, N., Drăgoiu, T., Mihai, O.D., & Petcu, C.D. (2022). Antibiotic use in livestock and residues in food-a public health threat: A Review. *Foods*, 11 (10), 1430. DOI: 10.3390/foods11101430.
- Ghiță, M., Botezatu, R., Coman, C., Vuță, V., Găjăilă G., Nicolae, A.C., Drăgoi C.M., & Cotor, G. (2021). Research regarding the effect of leptin upon the ratio of certain lymphocyte populations in rat. *Revista Farmacia*, 69(6), 1089-1093.
- Ghiță, M., Petcu, C.D., Cotor, G., Zagrai, G., Andrei, C., & Mihai (Oprea), O.D. (2021). Research on the effect of a dietary supplement on growth and erythrogram in pigeons. *Scientific Papers-Series D-Animal Science*. LXIV(1), 142-147.
- Ghiță, M., Cotor, G., Vițălaru, A., & Brășlașu, D. (2015). Comparative study on the effect of prednisone and dexamethasone on leucocytes, in rabbit. *Journal of Biotechnology*, 208, Supplement, S92.
- Ioniță, L., Găjăilă, G., Cotor, G., Ionita, C., Ivana, S., & Vitalaru, B. (2014). Adjustments in neutrophil/lymphocyte ratio by administering a phytoterapeutic extract with an immunomodulatory effect on dairy cows after calving. *Journal of Biotechnology*, 185, Supplement, S45.
- Li, N.Y., Sun, Z.J., Ansari, A.R. et al. (2020). Impact of maternal selenium supplementation from late gestation and lactation on piglet immune function. *Biol. Trace Elem. Res.*, 194, 159–167.
- Marin, M.P., Nicolae, C.G., Tapaloaga, D., Petcu, C., Tapaloaga P.R., & Dinita G. (2013). The metabolic utilization of iron and copper in the piglet organism. *Current Opinion in Biotechnology*, 24 (1), S51-S51.
- Petcu, C.D. (2006). *HACCP-Food safety guarantor*. Bucharest, RO: Idea Design Publishing House.
- Petcu, C.D., Savu, C., Mitrănescu, E., & Chirilă, S. (2007). The implementation of the integrated quality and food safety management system in the food industry units. *Lucrări Științifice Medicină Veterinară*, XL, 545-51, Timișoara.
- Pogurschi, E., Cîric, A., Zugravu, C., & Patrascu, D. (2015). Identification of antibiotic residues in raw milk samples coming from metropolitan area of Bucharest. *Agriculture and agricultural Science Procedia*, (6), 242-245.
- Pogurschi, E.N., Petcu, C.D., Mizeranschi, A.E., Zugravu, C.A., Cîrnatu, D., Pet, I., & Ghimpețeanu, O.M. (2022). Knowledge, Attitudes and Practices Regarding Antibiotic Use and Antibiotic Resistance: A Latent Class Analysis of a Romanian Population. *Int J Environ Res Public Health*, 19(12), 7263. doi:10.3390/ijerph19127263.
- Savu, C., & Petcu, C.D. (2002). *Hygiene and control of products of animal origin*. Bucharest, RO: Semne Publishing House.
- Thacker, E., (2010). Immunomodulators, immunostimulants and immunotherapies in small animal veterinary medicine. *Veterinary clinics of North America: Small animal practice*, 40(3), 473-483.
- Vetvicka, V., Vannucci, L., & Sima, P. (2014). The effects of  $\beta$ -glucans on pig growth and immunity. *Open Biochem J.*, 8, 89-93.
- Zhou, S.Y., Wu, B.X., Liu, Z., & Zhang, T.J. (2021). Effects of different selenium sources on sow reproductive performance and piglet development: a meta-analysis. *J. Anim. Feed Sci.*, 30 (3), 260–270.
- Zavodnik, L.B., Shimkus, A., Belyavsky, V.N., Voronov, D.V., Shimkiene, A., & Voloshin, D.B. (2011). Effects of organic selenium yeast administration on perinatal performance, growth efficiency and health status in pigs. *Arch. Zoot.*, 14(3), 5-20.