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## **EFFECT OF THE *STAPHYLOCOCCUS AUREUS* INFECTION ON THE CALCIUM, PHOSPHORUS AND IRON LEVEL IN RABBITS**

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### **ABSTRACT**

Bacterial skin diseases in rabbits are common and represent an interesting model for studying the relationship between infection and changes in metabolism. However, information on the dynamics of calcium, phosphorus and iron levels, during infection, is too scarce. The experiment was carried out with 12 male New Zealand white rabbits, which were divided in 2 groups-experimental and control. The rabbits in the experimental group were infected intradermally with 100 µL of the bacterial suspension of *S. aureus* field strain (density:  $8 \times 10^8$  cfu/mL). Blood samples from each rabbit were taken before euthanasia from *v. auricularis externa* as follows: on hours 6, 24, 48, 72 and days 7, 14 and 21 after *S. aureus* infection in sterile heparinized tubes. The determination of calcium (Ca, mg/dl), phosphorus (P, mg/dl) and iron (Fe, µg/dl) concentrations was carried out by a biochemical analyzer at Olympus (Spain) at the Faculty of Veterinary Medicine at the Autonomous University of Catalonia, Barcelona, Spain. The amount of calcium in infected rabbits decreased significantly by the 48<sup>th</sup> hour ( $p < 0.01$ ) after infection with bacterial suspension. A significant increase of phosphorus in the experimental group was observed at 24<sup>th</sup> hr and on the 7<sup>th</sup> day after infection, reaching a value of  $5.64 \pm 0.19$  mg/dL. The amount of iron at the 24<sup>th</sup> hour ( $p < 0.01$ ) and at the 14<sup>th</sup> day ( $p < 0.05$ ) after infection in the infected rabbits decreased significantly compared to the control group, reaching a value of  $178.8 \pm 87.2$  µg/dL at 24 hours, and at day 14  $123.33 \pm 17.8$  µg / dL ( $p < 0.05$ ) after the onset of infection.

**Key words:** *Staphylococcus aureus*, calcium, phosphorus, iron, rabbit.

### **INTRODUCTION**

Bacterial skin diseases in rabbits and dogs are common and represent an interesting model for studying the relationship between infection and changes in metabolism. These changes, associated with rapid acute-phase response by the body, are a very important protective response of the body because they limit or stop the development of pathogenic microorganisms.

We reported about changes in total protein and protein fractions, lipid profile, dynamics of creatinine and urea and the activities of some enzymes and also concentrations of haptoglobin, fibrinogen and ceruloplasmin in rabbits during *S. aureus* infection as representatives of acute phase proteins (APP) in other our publications (Dishlyanova *et al.*, 2012; Georgieva *et al.*, 2016; Georgieva *et al.*, 2017; Georgieva *et al.*, 2017; Georgieva *et al.*, 2018; Georgieva *et al.*, 2019; Georgieva and Georgiev, 2019).

However, information on macro- and microelements such as calcium, phosphorus and iron levels, during infection, is too scarce.

## MATERIALS AND METHODS

### Animals and experimental design

The experimental procedure was approved by the Ethic Committee at the Faculty of Veterinary Medicine. The experiment was carried out with 12 male New Zealand white rabbits, which were divided in 2 groups-experimental and control. They were placed in individually disinfected metal cells with a grill floor and housed in a room with a temperature (20-22°C). They were fed pelleted feed according to their age and had free access to tap water.

The rabbits were infected intradermally with 100 µL of the bacterial suspension of *S. aureus* field strain (density:  $8 \times 10^8$  cfu/mL) as described by Wills *et al.* (2005). 21 days after infection, rabbits from the experimental group were euthanized by injection with a lethal dose of (100 mg/kg.b.w.) of Thiopental (Sandoz GmbH Austria). The carcasses of all rabbits were subjected to pathoanatomical, pathohistological and bacteriological control.

### Chemical and bacteriological Analyses

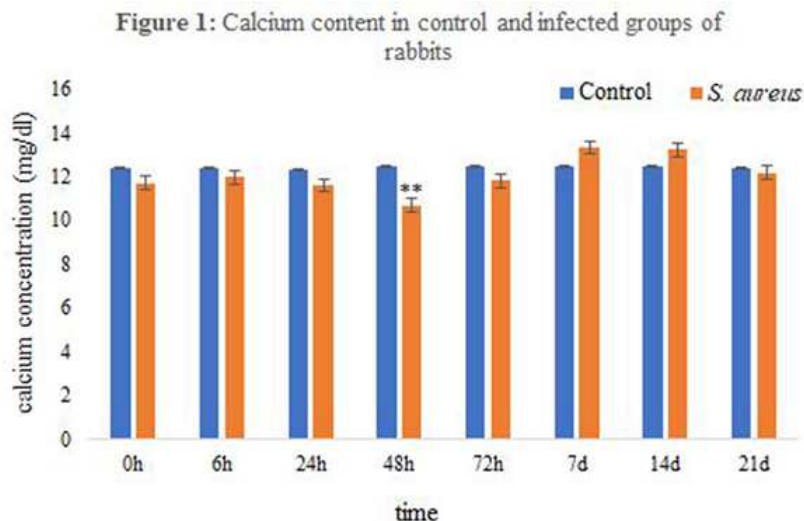
Blood samples from each rabbit were taken before euthanasia from *v. auricularis externa* as follows: on hours 6, 24, 48, 72 and days 7, 14 and 21 after *S. aureus* infection in sterile heparinized tubes. They were centrifuged immediately (1500 g, 10 min, 4 °C) and plasma was obtained. The plasmas were then decanted and stored at -20°C until definitions were performed.

The determination of calcium (Ca, mg/dl), phosphorus (P, mg/dl) and iron (Fe, µg/dl) concentrations was carried out by a biochemical analyzer at Olympus (Spain) at the Faculty of Veterinary Medicine at the Autonomous University of Catalonia, Barcelona, Spain.

### Statistical Analysis

The statistical analysis of the data was performed using ANOVA (Statistics for Windows, Stat Soft Ins., USA, 1993). The statistical significance of intra- and intergroup differences was determined by the Posthoc procedure LSD test (Stat Soft Ins., USA, 1993). The level of statistical significance of the differences was at  $p < 0.05$ .

## RESULTS AND DISCUSSION

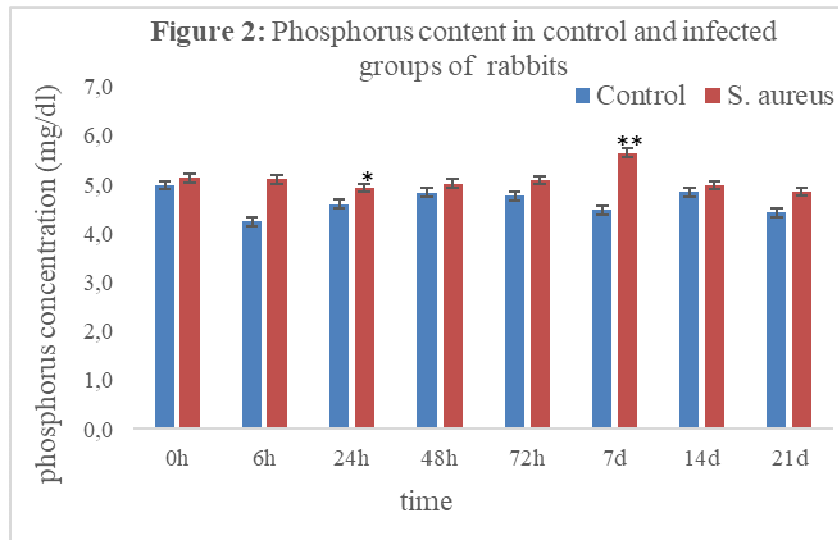


Significance of differences between groups: \* $p < 0,05$ , \*\* $p < 0,01$

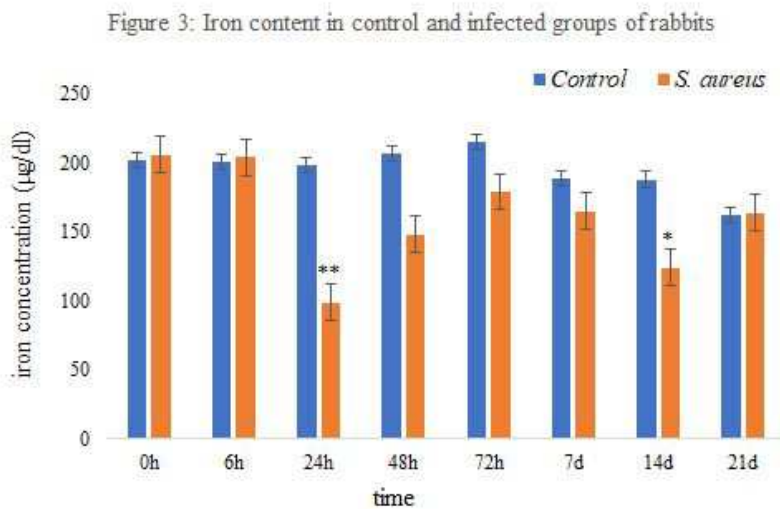
The amount of calcium in infected rabbits decreased significantly by the 48<sup>th</sup> hour ( $p < 0.01$ ) after infection with bacterial suspension (Fig. 1). Da Silva *et al.*, (2011) found a significantly decreased potassium, sodium, calcium (on days 35, 50, 80 and 118) and phosphorus (35 day) following trypanosome invasion rabbits. Gundasheva and Georgieva (2012) found a significant decrease in calcium on days 2 and 4 after exercise of

horses, linking this to hypoalbuminemia, since calcium binds to albumin in the blood, which is in agreement with our data.

A significant increase of phosphorus in the experimental group was observed at 24<sup>th</sup> hr and on the 7<sup>th</sup> day after infection, reaching a value of  $5.64 \pm 0.19$  mg/dL (Fig.2). Parker (1991) finds transit hyperphosphatemia in acute pathological conditions and associates them with extrahepatic infections, which is also in agreement with our data for elevation of the phosphorus after *S. aureus* infection.



Significance of differences: \*p<0.05; \*\*p<0.01



The amount of iron at the 24<sup>th</sup> hour (p<0.01) and at the 14<sup>th</sup> day (p<0.05) after infection in the infected rabbits decreased significantly compared to the control group, reaching a value of 178.8 ± 87.2 µg/dL at 24 hours, and at day 14 - 123.33 ± 17.8 µg/dL (p<0.05) after the onset of infection (Fig.3)

Haptoglobin, which increased at 24<sup>th</sup> hr after infection (Dyshlianova *et al.*, 2011) binding hemoglobin and

respectively iron, removing it from the blood, largely prevents the formation of oxygen radicals, which is stimulated by iron, thereby resulting in its role as an antioxidant (Gutteridge, 1987). On the other hand, iron is one of the essential elements required for bacterial growth. In our opinion haptoglobin which increased during infection caught free iron that is why its concentration during our experimental infection is decreased. The combination of blood flow and tissue infection is also unfavorable because hemoglobin iron creates conditions for the growth of microorganisms (Eaton *et al.*, 1982). Hence the irreplaceable role of haptoglobin, once bound to hemoglobin, to make iron inaccessible to microorganisms (Barklay, 1985).

### CONCLUSIONS

During infection with *S.aureus* in rabbits we established a significant decrease in the concentration of calcium at 48 hour after infection, a significant elevation of phosphorus at 24<sup>th</sup> hr and on day 7 and decreased level of iron 24<sup>th</sup> hr after infection.

### ACKNOWLEDGEMENTS

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## REFERENCES

- Barclay, R. 1985. The role of iron in infection. *Medicine and Laboratory Science*, 42, 166-172.
- Da Silva, A.S., Costa, M.M., Moreira, C.M., Zanette, R.A., Thomé, G.R., Otto, M.A., Moraes, É.M., Flores, Lopes, S.T., Monteiro, S.G., 2011. Experimental infection by *Trypanosoma evansi* in rabbits: Levels of sodium, potassium, calcium and phosphorus in serum. *Acta Scientiae Veterinariae*, 39, 2, 959-967.
- Dyshlianova E., Georgieva, T.M., Petrov, V., Zapryanova, D., Marutsov, Pl., Dinev, I., Nikiforov, I., Georgiev, I.P. 2011. Blood haptoglobin response in rabbits with experimentally induced *S.aureus* infection. *Revue de Medecine Veterinaire*, 162, 11, 514-518.
- Eaton, J.B., Brandt, P., Mahoney, J.R. 1982. Haptoglobin: a natural bacteriostat. *Science*, 215, 691-693.
- Georgieva T.M., Georgiev, I.P., Vachkova, E., Petrov, V., Luytzkanov, M., Petrova, Y. 2018. Effect of *Staphylococcus aureus* infection on the dynamics of creatinine and urea concentrations in the blood plasma of rabbits. *International Scientific Conference "Tradition and modernity in Veterinary Medicine"*, 2018, 3, 2, 5, 3-8.
- Georgieva T.M., Petrov, V., Georgiev, I.P., Zapryanova, D., Marutsov, P., Rusenova, N., Parvanov, P. 2016. Effect of *Staphylococcus Aureus* infection on plasma creatine kinase activity in rabbits. *Trakia Journal of Science*, 14, 1, 52-60.
- Georgieva, T.M., Georgiev, I.P. 2019. Haptoglobin-one of the family of the acute phase proteins in rabbits-clinical applications, structure, function and methods for the determination. *2<sup>nd</sup> Annual International Conference Veterinary Biochemistry and Clinical Biochemistry VBCB, Fac. Vet. Med., Ankara, 24-26 October, 2019, Abstract book*, 13.
- Georgieva, T.M., Georgiev, I.P., Markov, N., Markov, I., Bozakova, N., Petrov, V., Nikolova, N. 2019. Effect of *Staphylococcus aureus* infection on the activities of the alkaline phosphatase, aspartate and alanine transferases in the blood plasma of rabbits. *Trakia Journal of Sciences*, 4, 334-339.
- Georgieva, T.M., Petrov, V., Georgiev, I., Nikolova, P.N., Lyutskanov, M., Bozakova, N. 2017. Study on the total proteins and main protein fractions in rabbits experimentally infected with *Staphylococcus Aureus*, *Trakia Journal of Sciences*, 1, 42-49.
- Georgieva, T.M., Petrov, V., Georgiev, I.P., Zapryanova, D., Lazarov, L., Christov, Ts., Petrova, Y.P., Iliev, P., Cecilliani, F. 2017. Changes in blood lipid profile in rabbits after experimental infection with *Staphylococcus aureus*. *International Scientific Conference Veterinary Medicine in Service in People, 6-7 October, 2017, Stara Zagora, BG*
- Gundasheva, D., Georgieva, T.M. 2012. Influence of physical exercise on the total calcium, albumin and the total protein levels in horses with booster vaccination against influenza and equine herpes virus 4/1. *Trakia Journal of Sciences*, 10, 1, 348-352.
- Gutteridge, J.M. 1987. The antioxidant activity of haptoglobin towards haemoglobin-stimulated lipid peroxidation. *Biochimica Biophysica Acta*, 917, 219-223.
- Parker, S.G. 1991. Transient hyperphosphatasaemia in association with acute infection in adults. *Postgrad Med. J.*, 67, 789, 638-642.
- Wills, Q.F., Kerrigan, C., Soothill, J.S. 2005. Experimental bacteriophage protection against *Staphylococcus aureus* abscesses in a rabbit model. *Antimicrob. Agents Chemother.*, 49, 3, 1220-1221.