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Original Contribution

CALCIUM AND PHOSPHORUS CONCENTRATION IN RABBITS WITH *STAPHYLOCOCCUS AUREUS* INFECTION

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ABSTRACT

The present study was conducted to examine the changes in calcium and phosphorus levels during *Staphylococcus aureus (S. aureus)* infection in rabbits. The experimental procedures were carried out with 12 male New Zealand white healthy rabbits, divided into two equal groups - the experimental group (n=6, rabbits infected with *S.aureus*) and the control group (n=6, uninfected animals). Blood samples were collected at 0 hours before infection, as well on 6, 24, 48, 72 hours and 7, 14 and 21 days after the infection. The amount of calcium in infected rabbits decreased significantly by the 48-th hour ($p<0,01$) after infection with bacterial suspension. A significant increase of phosphorus in the experimental group was observed at 24 hours and on the 7-th day after infection.

Key words: *Staphylococcus aureus*, rabbits, calcium, phosphorus, Ca, P

INTRODUCTION

Staphylococcal infection in rabbits is a contagious, infectious disease that affects animals of different ages. It is characterized by the development of suppurative dermatitis, skin, subcutaneous or multisystem abscesses, mastitis, pyometra, pododermatitis, septicemia, etc. (1-4). The etiological agent is *Staphylococcus aureus*. This microorganism is capable of causing a wide range of diseases in both animals and humans (5).

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The disease is common in young and growing animals. Staphylococci are capable of infecting small skin lesions, followed by an invasion of the subcutaneous tissue (6). These can result from poor quality cage wire floors or walls, fights between animals during regrouping. Other possible routes of infection may be the navel in new-born rabbits (2). In infants, the most typical lesion is exudative dermatitis (1, 7). In adolescent rabbits, abscesses present as spherical, swollen nodules of variable size. They are movable; if they are ruptured they release copious, thick, white pus. Subcutaneous tissue around abscesses is cyanotic due to local circulatory disturbance, which may be followed by necrosis and skin ulcers (8). Infection caused by *S. aureus* in rabbits can be characterized by fatal septicemia or

purulent inflammation that can occur in virtually any organ or site (9).

Two types of *S. aureus* infections can be distinguished in rabbits. The first type is caused by so-called low virulence (LV) strains and is of minor economic importance as the infection remains limited to a small number of animals. The second type of infection, caused by high virulence (HV) strains, leads to chronic problems and a decline in production as the infection spreads rapidly on the farm (10). The pathogenicity of *S. aureus* results mainly from the ability to form a biofilm and produce extracellular protein toxins such as exfoliative toxins, leukotoxins, and superantigens (11).

The consequences of staphylococcal infection are metabolic disturbances followed by weight loss, cachexia and death (12) and disorders in the metabolism of basic minerals such as calcium and phosphorus. These disorders in the metabolism of calcium and phosphorus have particularly adverse effects during the period of increased growth and building of the bone system in adolescent rabbits. In addition to being a building block of bones and teeth, calcium circulates in body fluids and blood, helping the body perform a variety of functions - blood clotting, milk production, nerve impulse conduction, muscle contractions, heart contraction (or regulation of heart activity), keeping of intact cell membranes, metabolism of enzymes and hormones, intestinal movement and transformation of light into electrical impulses in the retina (4, 13). Other important functions of calcium are decreasing blood pressure and cholesterol levels, as well as improving sleep (4, 13). If there is a lack of calcium in the animal's body, hypocalcemia occurs. The animal exhibits neurologic signs such as tetany and tingling, cataracts, mental changes, and cardiovascular changes with an abnormal ECG. Hypocalcemia can occur as a result of the following etiological factors: hypoparathyroidism or pseudohypopara-thyroidism, vitamin D deficiency and kidney disease (14).

Animals obtain the necessary calcium and phosphorus from their food. Their absorption depends on the mineral source, intestinal pH, and the concentration of activated vitamin D in the extracellular space (5, 15). Phosphorus is also an essential element in the composition of bones and teeth, but it is also present in lipids (fats and oils), proteins, and DNA. It is needed for the development of cells and molecules that store energy, such as ATP (adenosine triphosphate) (3, 16). Phosphorus deficiency shows reduced appetite, desire to eat unusual foods, reduced lactation, skeletal problems (joint stiffness and lameness) (3, 15).

However, data on the impact of staphylococcal infection on the metabolism of macro- and micronutrients remain limited. In this context, the aim of this study was to evaluate the effect of experimental infection with *S. aureus* on calcium and phosphorus concentrations in the blood serum of rabbits.

MATERIALS AND METHODS

- Experimental design

The experiment was approved by the Bulgarian Food Safety Agency (Approval protocol №158/2016). We used 12 male, 3 months old New Zealand white rabbits. The animals are housed in metal cages, which are placed in a room with a temperature (20-22°C). The rabbits were fed the appropriate pelleted feed and had free access to water. The animals were divided into two groups. Six of them were injected with *S. aureus* (experimental group) and six were injected with saline (control group). Rabbits were injected intradermally with 100 μL of the bacterial suspension of the *S.aureus* field strain (density: 8x108 cfu/mL), as described by Wills et al. (17). Blood samples were taken from each rabbit from v. auricularis externa in sterile heparinized tubes as follows: 0 hour before infection and 6, 24, 48, 72 and days 7, 14 and 21 after *S. aureus* infection. The samples were centrifuged immediately (1500 g, 10 min, 4°C) and plasma was obtained. After receiving the plasmas, we decanted them and stored them at -20°C until analysis.

- Methods for Ca- and P – determination in blood serum

Determination of calcium (Ca, mg/dl) and phosphorus (P, mg/dl) concentrations was performed by Olympus Biochemistry Analyst (Spain) at the Faculty of Veterinary Medicine of

the Autonomous University of Catalonia, Barcelona, Spain. During the abscess fistulation, swab samples were taken of 6 rabbits from the experimental group. The material was seeded on blood agar with 8-10% sheep blood (BUL-BIO NCIPD, Sofia, Bulgaria). The cultures were incubated for 24 hours at 37°C.

- Statistical analysis

The data obtained from the experiment were statistically analyzed by 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

As shown in Figure 1, the amount of calcium in infected rabbits decreased significantly by the 72nd hour, ranging between 11,72±1,03 and 11,83±1,27 mg/dl after infection with bacterial suspension. The lowest levels of calcium were observed on the 48th hour after the infection (10,67±0,96 mg/dl, $p<0,01$). On days 7 and 14, calcium levels began to rise slightly, with no statistical significance. As seen in **Figure 1**, the calcium levels of the control and experimental groups at day 21 did not differ.

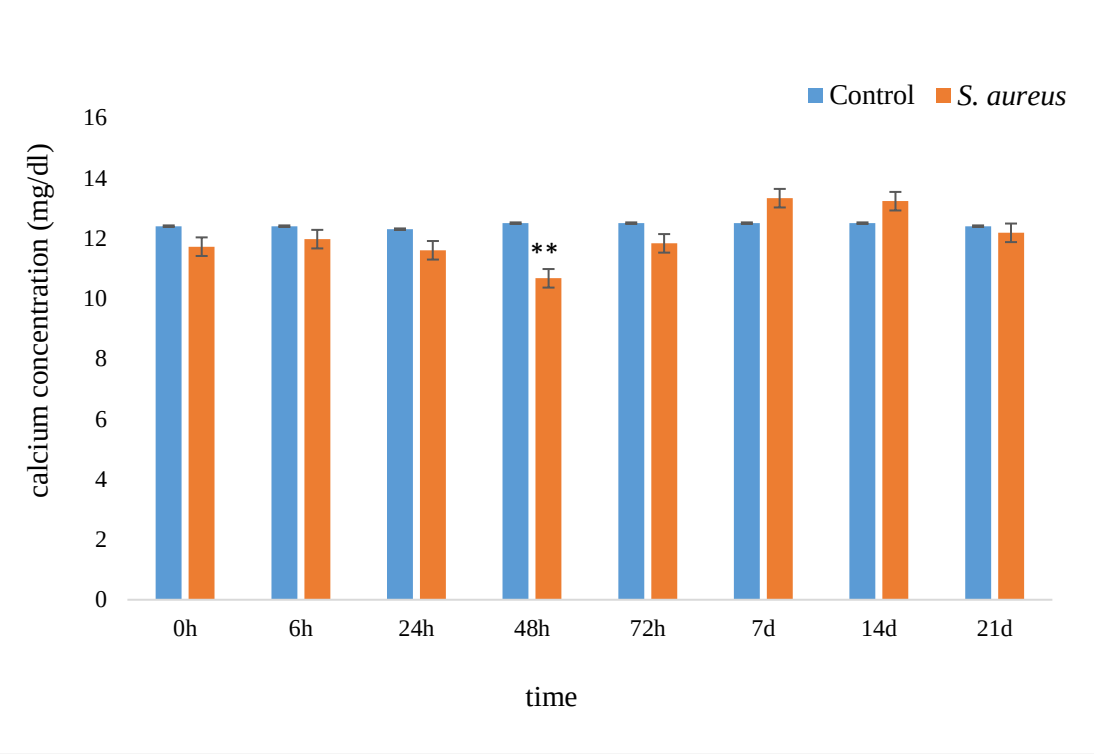

Figure 1. Calcium content in control and infected groups of rabbits
**Significance of differences between groups $p< 0,01$

As seen from the **Figure 2** the amount of phosphorus is higher in the experimental group than in the control one. At the 6th hour after infection of the animals, the phosphorus levels began to rise, reaching values of 5,1±0,66 (p=0,0501), which is consider to be not quite statistically significant. A significant increase of phosphorus in the experimental group was observed on the 7th day after infection, reaching values of 5,65±0,47 mg/dL ($p<0,01$).

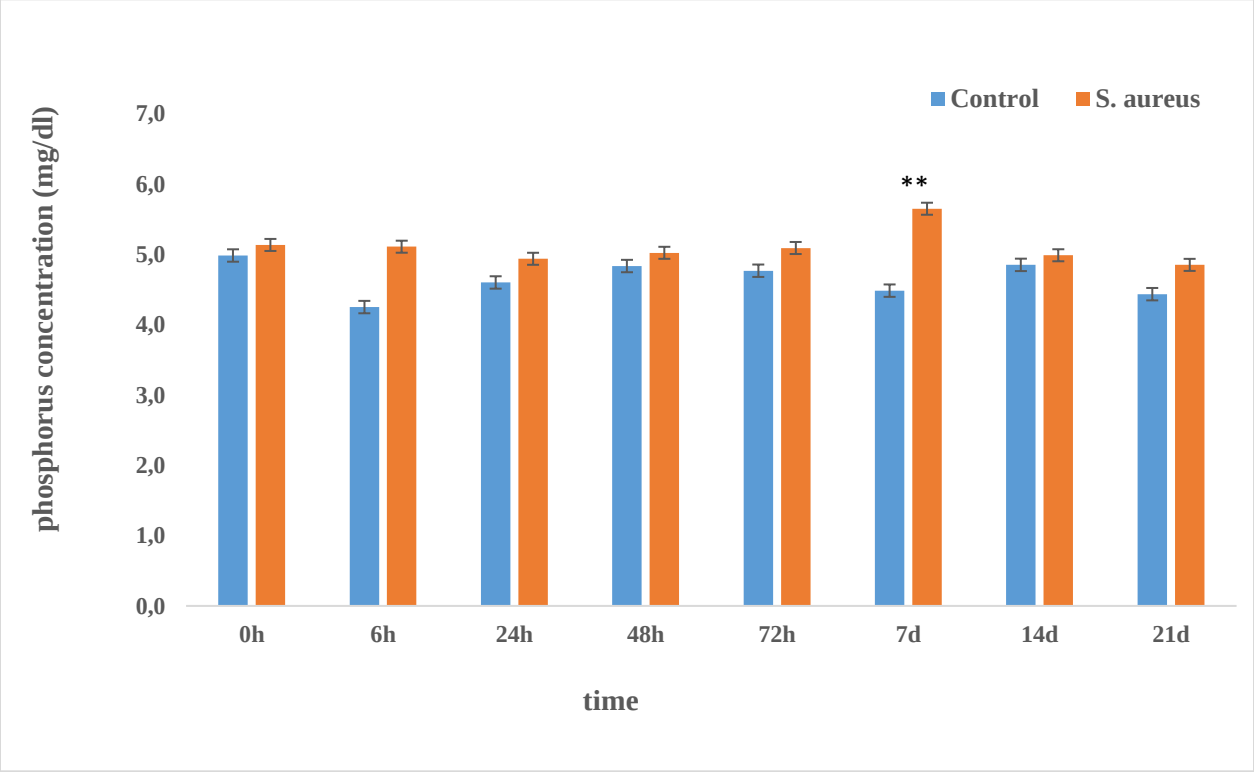

Figure 2. Phosphorus content in control and infected groups of rabbits
**Significance of differences between groups p< 0,01

DISCUSSION

In our work blood calcium and phosphorus levels were monitoring in rabbits with experimentally induced *S. aureus* infection. The presence of abscesses indicated a successfully induced staphylococcal infection in the trial animals, with the development of abscesses occurring within 48-96 hours after inoculation, which is consistent with the experiments performed and the results are shown by Wills et al. (17). In the present investigation it was observed that Staphylococcal infection leads to a reduction in calcium levels, compared to controls. This observation is confirmed by Da Silva et al. (18), which found a significant reduction in calcium levels (at 35, 50, 80 and 118 days) in rabbits with the trypanosomal invasion. They establish that calcium is needed for the growth of trypanosomes and therefore its amount in the blood decreases with parasitic invasion. Harcourt-Brown (19) considers that decreased levels of calcium and vitamin D influence progressive syndrome of acquired dental disease (PSADD) in rabbits, along with neoplasia, incorrect diet, congenital prognathic abnormalities, or trauma. In support of these findings, Gundasheva and Georgieva (20) observed a significant reduction in calcium values on days 2 and 4 in horses that trained.

According to, the results obtained in our research *S. aureus* infection leads to significant elevation of phosphorus concentration. Our dates support the findings of Parker, which observed an increase in phosphorus levels in acute pathological conditions and associated them with extrahepatic infections (21).On contrary decreased phosphorus concentration was observed by Da Silva after the trypanosomal invasion in rabbits, which is most likely due to the hyporexia of rabbits (18). Similar studies were done by H. Sandstedt et al. (22) in cows and reported that inoculation of *E. coli* in healthy cows resulted in significant reductions in calcium and phosphorus levels (22). On the other hand, the reduced phosphorus levels may be due to insufficient phosphorus in diet for a long time. This condition can be seen in grazing animals in dry areas with low phosphorus content in the soil

or in sick animals that are anorectic for a long time with the loss of weight and the appearance of skin wounds. Decreased phosphorus levels may be due to chronic renal tubular disease, which is caused by impaired renal phosphorus reabsorption. Other diseases that affect the amount of phosphorus are primary or secondary hyperparathyroidism, causing increased renal phosphorus excretion (23).

In conclusion, experimentally induced *S. aureus* infection in rabbits showed decreased calcium content and increased phosphorus concentration. Variations in calcium levels began 48 hours after inoculation with a decrease in calcium concentration. Changes in phosphorus levels started at 6 hours in the early post-inoculation period with an increase in its concentration.

REFERENCES

1. Okerman, L., Devriese, L. A., Maertens, L., Okerman, F. and Godard, C., Cutaneous Staphylococcosis in rabbits. *Vet Rec.*, 114(13), 313-315, 1984.
2. Hagen Jr. K. W. Disseminated staphylococcic infection in young domestic rabbits. *AVMA*, 142, 1421-1422, 1963.
3. Segura, P., Martinez, J., Peris, B., Selva, L., Viana, D., Penades, J. R. and Corpa, J. M., Staphylococcal infections in rabbit does on two industrial farms. *Vet Rec.*, 160(25), 869-872, 2007.
4. Vancraeynest, D., Hermans, K. and Haesebrouck, F., Genotypic and phenotypic screening of high and low virulence Staphylococcus aureus isolates from rabbits for biofilm formation and MSCRAMMs. *Vet microbiol.*, 103(3-4), 241-247, 2004.
5. Cucarella, C., Tormo, M. A., Ubeda, C., Trotonda, M. P., Monzón, M., Peris, C. and Penadés, J. R., Role of biofilm-associated protein bap in the pathogenesis of bovine Staphylococcus aureus. *Infect immun.*, 72(4), 2177-2185, 2004.
6. Hermans, K., Devriese, L. A. and Haesebrouck, F., Staphylococcus. Pathogenesis of bacterial infections in animals, 75-89, 2010.
7. Holliman, A. and Girvan, G. A., Staphylococcosis in a commercial rabbitry. *Vet rec.*, 119(8), 187, 1986.
8. Corpa, J. M., Hermans, K. and Haesebrouck, E., Main pathologies associated with Staphylococcus aureus infections in rabbits: a review. *WRS*, 17(3), 115-125, 2009.
9. Flatt R.E., Weisbroth S.H., Flatt R.E., Kraus A.L. (ed), The biology of the laboratory rabbit. Academic press, New York, USA, 1974.
10. Devriese, L. A., Hendrickx, W., Godard, C., Okerman, L. and Haesebrouck, F., A new pathogenic Staphylococcus aureus type in commercial rabbits. *J. Vet. Med. B*, 43(1-10), 313-315, 1996.
11. Vancraeynest, D., Hermans, K. and Haesebrouck, F.). Prevalence of genes encoding exfoliative toxins, leucotoxins and superantigens among high and low virulence rabbit Staphylococcus aureus strains. *Vet. Microbiol*., 117(2-4), 211-218, 2006.
12. Marcato, P. S. and Rosmini, R. Pathology of the Rabbit and Hare. A Color Atlas and Compendium, Società Editrice Esculapio, Bologna, 1986.
13. Govind Pandey, Role of calcium in human and animal health. *Jigyasa* (ISSN: 2231-2978), 5: 61-65, 2011.
14. Gaw, A., Cowan, R. A., O'Reilly, D. St. J., Stewart, M. J., Shepherd, J. Clinical biochemistry, 64-65, 1995
15. Grünberg, W., Dystrophies Associated with Calcium, Phosphorus, and Vitamin D in Animals. *MSD manual*, 2020.
16. Sultenfuss, J. H. and Doyle W. J., Phosphorus in Animal Nutrition, Better Crops/Vol. 83, No. 1 1999.
17. Wills Q. F., Kerrigan C., Soothill J. S., Experimental bacteriophage protection against Staphylococcus aureus abscesses in a rabbit model. *Antimicrob. Agents Chemother*., 49, 1220-1221, 2005
18. 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. and Monteiro, S. G., Experimental infection by Trypanosoma evansi in rabbits: Levels of sodium, potassium, calcium and phosphorus in serum. *Acta Sci. Vet.*, 39, 2, 959-967, 2011.
19. Harcourt-Brown F., Diseases Related to Calcium Metabolism in Rabbits. *WSAVA gongress*, 2010

20. Gundasheva, D. and Georgieva, T.M., 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. *TJS,* 10, 1, 348-352, 2012.
21. Parker, S.G., Transient hyperphosphatasaemia in association with acute infection in adults. *Postgrad Med. J.*, 67, 789, 638–642, 1991.
22. Sandstedt, H., Larsson, L. and Kvart C., Effect of E. coli ectotoxin on calcium and phosphate concentration in serum of dairy cows, *Nord Vet Med.* 36(11):406-7, 1984
23. Grünberg, W., Hypophosphatemia in animals, *MSD manual,* 2020.