Effect of silver nanoparticles on product loss reduction under technological stress

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Abstract. Silver nanoparticles, which until recently were used as an antibacterial agent, are of great interest. In our study, we tested them as an effective anti-stress agent for animals subjected to short-term stressors – transport and pre-slaughter. Intramuscular administration of silver nanoparticles at a dose of 0.01 and 0.05 mg/kg body weight for seven days before technological stress had a positive effect on the adaptive qualities of animals. In the control group, weight loss in 18-month-old bulls weighing 481.6 kg were 2.0% (P < 0.05) and 1.8% (P < 0.05) higher, respectively. At the same time, during transport and pre-slaughter the loss of body weight in animals of the experimental groups was 19.3% and 13.6% lower compared to the control group. The best results were obtained for bulls, which were intramuscularly injected with emulsion of silver nanoparticles at a dose of 0.01 mg/kg body weight.

1. Introduction

Various nanoparticles are increasingly used in livestock farming in the form of feed additives, growth enhancers, and antibacterial and anti-stress agents. One of these are silver nanoparticles that have been poorly studied as yet [1, 2].

Cattle are constantly exposed to various types of stress throughout the growing period. The strongest and most complex are transport and pre-slaughter, which are accompanied by the psycho-physiological effect of environmental factors and the increased intensity of oxidative processes that cause weight loss and affect meat quality. Microelements such as silver, which are involved in the formation of nervous tissue and maintaining its functions, can be used as alternative sedative medication to eliminate the adverse effects of technological stress. Silver nanoparticles target endothelial cells that form the blood-brain barrier, neurons and glial cells, and finally lead to death of cells associated with oxidative stress.

Silver nanoparticles are widely used in medicine, physics, materials science and chemistry. They can be referred to the category of strong antibacterial agents that act as growth regulators, and a limited dose of silver nanoparticles enhances immune function. Silver nanoparticles are of great interest due to their ability to act as antibacterial agents in the solid state. Silver salts are used to inhibit the growth of various bacteria in the animal’s body.

Technological stress is the main problem. The response of animals to stress triggers a complex interaction between neurons and hormones. The results of this interaction are manifested in changes in the physical (external changes), hematological, biochemical, and hormonal parameters (internal changes) [3,4].

Stress can be defined as a biological response caused by a threat to the homeostasis of the body.
Stress can also be defined as adverse effects of the environment or the control system that causes changes in the physiology and behavior of the animal to help the body cope with the stimulus. Nanoparticles contribute to the absorption of substance and thus can be used for delivery of medications. Low concentration of silver nanoparticles significantly improves growth rates, immunological and antioxidant status and reduces other cellular stresses.

A number of studies have been conducted to reduce weight loss caused by technological stress, but this issue is still relevant [5, 6].

The study aims to investigate the effect of silver nanoparticles on reduction of weight loss caused by technological stress.

2. Materials and methods

The study was carried out on 18-month-old bulls of black-and-white breed weighing from 481.6 to 491.1 kg in JSC Agricultural Company Nur, Sterlibashevsky district, Republic of Bashkortostan. Three groups of animals were involved in the study: one control and two experimental groups. Emulsion (pH 9.5, redox potential Eh = –450 mV) with silver nanoparticles was intramuscularly administered to experimental animals at a dose of 0.01 and 0.05 mg/kg body weight for seven days before exposure to stress. The body weight was determined by weighing.

Samples of suspensions of silver nanomaterials prepared at a concentration of 0.1 M were subjected to sonication for 30 minutes. To assess the effect of various doses of nanomaterials, 10 double serial dilutions were prepared from the resulting suspensions.

The bioluminescence inhibition test was employed for the initial assessment of silver nanoparticles. As a test object for exposure, we used luminescent strain of Echerichiacoli K12 TG1 to constitutively express luxCDABE genes of the natural marine microorganism Photobacterium leionguthi 54D10, which was produced by Immunotech (Russia, Moscow) in a lyophilized state under the commercial name Ecolum. Immediately before the experiment, this preparation was restored by the addition of chilled distilled water. The bacteria suspension was kept at +2.4 °C for 30 minutes with subsequent temperature increase to 15...25 °C.

The bioluminescence inhibition test was carried out through the introduction of the test substance and suspension of luminescent bacteria at 1:1 ratio into the cells of 96-well opaque plastic tablets. After that, the tablets were placed in a measuring unit of a multifunctional reader (luminometer), which dynamically recorded the luminescence intensity in the resulting mixtures during 180 min with an interval of 5 min. The effect of nanomaterials on the intensity of bioluminescence (I) was assessed using the formula, where \( I_k \) and \( I_0 \) are luminescence intensity of the control and experimental samples at the 0th and nth minutes of measurement. To assess the toxicity degree of the nanomaterials studied, we calculated EC50 for 3 measurement points (60, 120 and 180 min) that cause a 50% luminescence quenching.

Silver nanoparticles were up to 100 nm in size (chemical and phase composition was 99.99% of metallic silver and up to 0.01% of adsorbed gases – CH₄, CO₂, Ar, N₂, the method employed was electric explosion in an argon atmosphere, specific surface area \( S_s = 6.5 \text{ m}^2/\text{g} \) ). To prepare the suspension, silver nanoparticles, depending on the body weight of the animal, were mixed with catholyte and subjected to dispersion in an ultrasonic disperser of UZDN-2T in a 0.5 A, 44 kHz mode. The emulsion was administered to test animals into the femoral muscles.

The animals were weighed on a VSP4-ZhSO livestock electronic scale; the emulsion with silver nanoparticles was administered using a veterinary syringe.

3. Results

The results of the study showed the dependence of the recorded effects on the form of substance and concentrations used.

Silver nanoparticles 100 nm in size caused the complete luminescence quenching in the concentration range from 0.1 to 0.025 M during first 5–10 contacts and at a dose of 13.48 mg after 145 minutes of contact with the cell (Fig. 1).
Figur 1. Dynamics of luminescence of *E.coli* K12 TG1 with cloned *lux CDABE* genes of *P. leiongnathi* 54D10 when in contact with 100 nm silver nanoparticles at concentrations of 10786.8 (1); 5393.4 (2); 2696.7 (3); 1348.4 (4); 371.1 (5); 168.7 (6); 84.2 (8); 42.2 (9); 21.0 (10); c – control.

Dilutions of concentrations ranged from 21.0 to 617.2 mg showed a non-toxic effect.

Table 1. Values of the toxicological parameter EC50 (M) when the test organism *E. coli* K12 TG1 with cloned *lux CDABE* genes of *P. leiongnathi* 54D10 is in contact with silver nanoparticles

| Nanoparticles | Contact duration, min |
|---------------|-----------------------|
| Ag 100        | 60 1348.4  >6741.8  >6741.8 |

The results of the dynamics of luminescence of bacteria and the values of the toxicological parameter show that a 60-minute contact with silver nanoparticles exhibit a 50% toxic effect after administration of the preparation at a dose of 1348.4 mg. An increase in contact duration up to 120 and 180 minutes leads to the same toxic effect at a dose of more than 6741.8 mg.

In terms of one animal, the dose for group I (0.01 mg/kg body weight) is -4.91 mg and that for group II (0.05 mg/kg body weight) is -24.5 mg.

The most important problem in beef production is preservation of farmed products during marketing, when losses can be significant [7.8]. Therefore, we studied the effect of some antistressors on the reduction of weight loss in young cattle during transport and pre-slaughter (Table 2).

Table 2. Reduction of weight loss during transport and pre-slaughter when using silver nanoparticles

| Indicator                        | Group | control | I    | II   |
|----------------------------------|-------|---------|------|------|
| Final body weight, kg            |       | 481.6±1.77 | 491.1±1.84 | 490.1±2.05 |
| Body weight after transport, kg  |       | 462.3±1.63 | 476.4±1.91 | 474.1±2.35 |
| Loss in weight during transport  | kg    | 19.3     | 14.7  | 16.0 |
| %                               |       | 4.2      | 3.1   | 3.4  |
| Body weight after fasting        | kg    | 444.8±1.81 | 461.4±1.97 | 458.3±2.08 |
| %                               |       | 17.5     | 15.0  | 15.8 |
| Weight loss after fasting        | kg    | 36.8     | 29.7  | 31.8 |
| %                               |       | 8.1      | 6.4   | 6.9  |
| Total loss                       | kg    | -        | 7.1   | 5.0  |
| Reduction of weight loss         | %     | -        | 1.7   | 1.2  |
Bulls of basic variant lost 19.3 kg during transport, while the animals intramuscularly injected with catholyte with silver nanoparticles lost 14.7 and 16.0 kg, respectively, that is 23.8 and 17.1% less than that in the bulls of basic variant. Among the animals injected with the tested preparations, Group I can be distinguished by the smallest weight loss during transport, which is 8.1% less than that in Group II.

Pre-slaughter animals showed a decrease in body weight. Losses during the period of fasting ranged from 15.0 to 17.5 kg. Moreover, these values in the experimental groups were 14.3 and 9.7% lower as compared to the control one.

During the period of transport and pre-slaughter, the total weight loss in bulls of the control group was 36.8 kg, whereas in the experimental groups these values were 29.7 and 31.8 kg, respectively. At the same time, the bulls injected with the tested preparations before technological stresses retained 23.9 and 15.7% more of body weight.

4. Discussion
Being an excellent antibacterial agent, silver has a beneficial effect both on the physiological functions of the body and on the antioxidant defense system to decrease oxidative stress [9–11].

At the initial stage, we studied a toxic effect of silver nanoparticles using the bioluminescence suppression test. The data obtained were consistent with those of other researchers [1] and showed that a 50% toxic effect can be observed at high doses of silver nanoparticles.

Meat processing plant was located 150 km away. Bulls of the control group were more subjected to stress; weight loss during transport was 19.3 kg. The estimated weight losses in young cattle intramuscularly injected with emulsion-type catholyte with silver nanoparticles were 23.8 and 17.1 % lower. The best results were obtained when using the preparation at a dose of 0.01 mg/kg body weight. In this case, animals were characterized by minimum weight loss during transport (8.1%) compared to that at a dose of 0.05 mg/kg body weight. In addition, weight loss in these groups was lower after fasting [7].

Intramuscular administration of emulsion with silver nanoparticles contributed to greater preservation of body weight by 23.9 and 15.7%.

5. Conclusion
Intramuscular administration of silver emulsion at a dose of 0.01 mg/kg body weight had the greatest effect on the reduction of production losses. This preparation contributed to 1.7% reduction of the loss of meat products as compared to the initial level.

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