International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies

http://TuEngr.com

PAPER ID: 11A10N

BACTERIAL CONTAMINATION OF RABBITS INTERNAL ORGANS AND MEAT DURING STRESS

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ARTICLEINFO

Received 05 January 2020
Received in revised form 09 March 2020
Accepted 31 March 2020
Available online 21 April 2020

Keywords:
Intramuscular injection;
Organ contamination;
Bacterial translocation;
USP; Intestines; High temperatures stress;
Rabbit internal organs;
Microflora;
Enterobacteria;
Enterococci.

ABSTRACT

Bacterial translocation is defined as the passage of viable bacteria from the gastrointestinal tract (GIT) into extraintestinal sites, such as mesenteric lymph nodes complex, liver, spleen, kidneys, meat, and bloodstream. This article describes the studies' results of the contamination of internal organs and meat. To study the effect of heat stress on intestinal permeability in rabbits, a study was conducted under standard vivarium conditions using six male rabbits. All animals were exposed to high temperatures. Animals of the experimental group received an intramuscular injection of emulsion with ultrafine silver particles (USP) at the dose of 0.01 mg/kg, to reduce intestinal permeability, seven days before exposure to stress. The translocation of bacteria to the liver, lungs, and spleen was enterobacteria from 2.1 to 6.6 lg CFU, enterococci from 18.3 to 19.3 lg CFU; and to blood 8.6 and 13.2 lg CFU, respectively. Injection of USP at the dose of 0.01 mg/kg body weight led to a sharp decrease in translocation of bacteria to internal organs. Evaluation of meat productivity showed the superiority of the rabbits of the experimental group in terms of hot carcass weight by 7.8% ($p \leq 0.05$), slaughter weight by 6.2% ($p \leq 0.05$), slaughter yield by 0.5%. No enterococci or enterobacteria were found in the meat of rabbits that received USP injection.

Disciplinary: Animal Science, Biological Sciences.

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1. INTRODUCTION

Heat stress is a serious problem causing negative effects on animals, especially during the hot season (Altan et al., 2003). High temperature affects physiological parameters of animals causing numerous disorders of the immune system, as well as endocrine and electrolyte disorders which lead to decreased productivity (Quinteiro-Filho et al., 2012, Abdelqader and Al-Fataftah, 2014). Heat stress has a negative impact on the development and functions of the intestine (Abdelqader et al., 2017), especially on the integrity of intestinal epithelium (Pearce et al., 2013). Intestinal epithelium plays an important role in the digestion and absorption of nutrients and acts as a barrier between the internal and external environment. The intestinal mucosa is constantly exposed to a large load of antigenic molecules from digested food and microorganisms, resident, and invasive bacteria and viruses (Keita and Soderholm, 2010). The integrity of the intestinal barrier is essential for maintaining the health state and vital functions of living organisms. Stressors in animals cause colonization of intestinal pathogens which pass into the blood, lymph nodes, meat, and therefore impair the quality of the resulting animal products (Keita and Soderholm, 2010, Burkholder et al., 2008, Abdelqader and Al-Fataftah, 2016). Moreover, a stress-induced non-integrity of intestinal epithelium reduces the effectiveness of innate defense mechanisms in animals and may increase the possibility of intestinal inflammation (Burkholder et al., 2008).

Thus, studying the effects of heat stress on the translocation of microorganisms from the intestine to internal organs, blood, and meat in animals is of certain scientific and practical interest. This research aimed at studying the translocation of the intestinal microbiota of rabbits under the influence of heat stress. There were the following tasks: to determine the contamination of blood, meat, and internal organs during stress and to define the method of its reduction in rabbits under the influence of high temperature.

2. MATERIALS AND METHODS.

The object of research: Alaska rabbits which were purchased at a rabbit farm in the village of Chashkan, Sol-Iletsky district, Orenburg Region.

Animal keeping and experimental studies were performed in accordance with the instructions and recommendations of Russian Regulations, 1987 (Order of the USSR Ministry of Health No.755 as of December 08, 1977) and “Guide for the Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996)”. In the course of study, best efforts were made to minimize animal suffering and to reduce the number of test subjects.

Experiment design: Studies were performed using 6 male rabbits under standard vivarium conditions. Within 14 days, animals adapted to new conditions. After the preparation period, they were divided into two groups: control and experimental, 3 animal in each. Heat stress was created using a forced-air heater. Exposure duration was 3 hours at the temperature of +42°C and the humidity of 75%.

All rabbits were exposed to high temperatures, and the experimental group received intramuscular injections of emulsion with USP at the dose of 0.01 mg/kg seven days before the start of heat stress to reduce intestinal permeability.

Research material included the samples of meat, blood, liver, lungs, and spleen of animals. All manipulations were carried out in compliance with sterility rules.

Pure bacteria cultures were obtained by plating 0.1 mL of undiluted and diluted 10, 100, and

1,000 times fractions or homogenate in solid media: starvation agar, blood agar, 1.5% beef extract agar, bismuth sulfite agar, Endo agar, and Ploskirev medium. After 48-72 h of cultivation at the temperature of 25°C, grown colonies were transferred to nutrient agar slant and identified. Species identification of isolated strains was carried out using indicators (Berkeley et.al., 1994, Weyant et.al., 1996) and based on morphological, cultural and biochemical criteria in accordance with the recommendations (Abbott et.al., 2003, Buller, 2004). Morphological features of isolated strains were studied in two-day and seven-day cultures using transmission electron microscopy. In this regard, bacterial colonies were fixed with a 2.5% solution of glutaraldehyde in cacodylate buffer (pH 7.2) for 1-2 days, then additionally fixed with a 2% solution of osmium tetroxide for 1 hr. using the same buffer, dehydrated in alcohols and acetone, then the material was embedded into Araldite-epon mixture.

Equipment and tools: For studying microbiota, Petri dishes, microbial loop, and Mikromed 3 Lum luminescent microscope (Russia, St. Petersburg, OOO "Nablyudatelnye pribory") were used. Pathogenicity of isolated microbial cultures was determined by making a biological test on white mice by intraperitoneal infection with a suspension of daily culture in physiological saline at the dose of 0.5 mL and at the concentration of 1 billion microbial cells in 1 mL of studied culture. Observation was carried out for 10 days (Muradova, 2007; Muruyev, 1994; Netrusov, 2009; Sidorov, 1995). For defining quantitative characteristics of beneficial, conditionally pathogenic and pathogenic microorganisms, colonies of each type were counted on plate media according to the formula: M=10xNx10n

where: M - the number of living microbial cells in 1 g of preparation;

N - conversion factor when plating 0.1 mL of bacterial suspension;

10_n - dilution from which this type of microbe was isolated.

USP was up to 100 nm in size (chemical and phase composition – 99.99% metallic silver, up to 0.01% of adsorbed gases – CH_4, CO_2, Ar, N_2, obtaining method – electrical explosion in the argon atmosphere, specific surface – S_{sp} = 6.5 m^2/g). For preparing an injection suspension in the laboratory for nanotechnologies in agriculture, USP was mixed with the catholyte obtained in the AkvaLife device and then dispersed in UZDN-2T ultrasonic disperser in the mode of 0.5 A and 44 kHz.

The experimental group received intramuscular injections of emulsion (pH 9.5, redox potential Eh = -450 mV, according to Dvornikov, 2004) with USP at the dose of 0.01 mg/kg of live weight, once a day, within seven days before the start of exposure to stress factor [Azhmuldinov, 2019].

Statistical processing. Material obtained during this study was processed using Statistica 10.0 software package (Stat Soft Inc., USA); reliability was determined using Student's test.

3. RESULTS

Exposure to the stress factor results in developing bacterial translocation which is defined as the passage of viable opportunistic microorganisms through gastrointestinal mucosa into the bloodstream, liver, spleen, lungs, and meat (Table 1).

In rabbits of the control group exposed to heat stress, translocation of bacteria to liver, lungs and spleen was the following: *Enterobacter cloacae* from 2.1-6.6 lg CFU, and *Enterococcus faecalis* 18.3-19.3 lg CFU; their amount in blood was 8 6 lg CFU and 13.2 lg CFU, in meat 1.7 and 2.3 lg CFU, respectively.

Table 1: Bacterial translocation caused by heat stress (lg CFU).

Microorganisms	Liver	Lungs	Spleen	Blood	Meat
Control					
Enterobacter cloacae	6.6±0.25	4.0±0.26	2.1±0.28	8.6±0.25	1.7±0.23
Enterococcus faecalis	18.3±0.24	19.1±0.31	19.3±0.33	13.2±0.26	2.3±0.25
Experimental					
Microorganisms	Liver	Lungs	Spleen	Blood	Meat
Enterobacter cloacae	2.1±0.18	1.3±0.20	1.0±0.22	2.3±0.20	---
Enterococcus faecalis	3.0±0.21	2.2±0.19	2.2±0.19	1.1±0.22	---

In animals of the experimental group which received USP injections at the dose of 0.01 mg/kg 7 days before exposure to the stress factor, there was a sharp decrease in translocation of these bacterial cultures to internal organs: *Enterobacter cloacae* from 1.0 to 2.3 lg CFU, and *Enterococcus faecalis* – from 1.1 to 3.0 lg CFU. No enterococci or enterobacteria were found in the meat of rabbits received USP. This reaction occurred due to the antibacterial properties of silver that contributed to the destruction of pathogenic microflora and reduced the effects of stress on the animal organism.

Analysis of rabbit carcasses obtained after slaughter revealed good bleeding in all animals. After aging, a dry crust of a pale pink color was formed on the surface of carcasses. Fat had a yellowish-white color. Muscles were pale pink, dense, elastic, and when pressed with a finger, the resulting depression was quickly recovered.

Rabbits with intramuscular injections of USP showed better values of meat productivity (Table 2). Rabbits of the experimental group showed superiority in the terms of hot carcass weight by 7.8% ($p \leq 0.05$), slaughter weight by 6.2% ($p \leq 0.05$), slaughter yield y 0.5%. Thus, injections of USP antioxidants to rabbits under stress had a positive effect on the bacteriological parameters of slaughter products.

Table 2: Results of control slaughter of test animals (g).

Parameter	Group	
	Control	Experimental
Preslaughter weight	3,990±1.94	4,198±2.02*
Hot carcass weight	2,087±1.52	2,250±1.67*
Internal fat weight	238.0±0.27	218.3±0.31*
Slaughter weight	2,325.0±1.42	2,468.3±1.61*
Slaughter yield, %	58.27	58.80

Note: * $p \leq 0.05$ in relation to the control group

4. DISCUSSION

Exposure to high ambient temperatures can pose significant health problems for humans and animals, and the gastrointestinal tract is one of the main organs that is first of all subject to this negative effect (Tavares et. al., 2012). In addition to the absorption of nutrients, intestine serves as a barrier to the spread of bacteria and endotoxins in systemic organs and tissues. Bacterial translocation is the migration of bacteria or bacterial products from the intestinal lumen to mesenteric lymph nodes or other extraintestinal organs and sites. (Magnotti and Deitch, 2005, Wiest and Garcia-Tsao, 2005, Balzan et. al., 2007).

Factors contributing to bacterial translocation include different types of stress, excessive growth of gram-negative *Escherichia coli*, impaired immune defense of the organism, and damage to intestinal mucosa leading to increased intestinal permeability (Assimakopoulos SF et. al., 2005). These mechanisms can work together and contribute to synergistical systemic spread of indigenous bacteria that cause sepsis.

In our study of animals exposed to heat stress, the translocation of bacteria to liver, lungs and spleen was the following: enterobacteria – from 2.1 to 6.6 lg CFU, enterococci – from 13.2 to 19.3 lg CFU; their amount in blood was 8.6 lg CFU, and 13.2 lg CFU, respectively.

USP is the most well-known particles that are given special attention as antimicrobial agents that are effective in treating certain infectious diseases, burns, chronic ulcers, open wounds and inhibit the growth of bacteria, mold and harmful spores. USP can enter the organism with water, food, drugs and various additives (Atiyeh et. al., 2007). Some researchers have shown that silver ions released from consumed foods into the blood can accumulate in the body and have toxic effects, especially in the liver and kidneys. However, acute oral or transdermal doses of USP (2,000 mg/kg of body weight) in rats, guinea pigs, and rabbits did not lead to significant clinical signs, mortality, acute irritation or any irritation affecting eyes or skin (Sardari et. al., 2012).

This study aimed to evaluate the effect of USP on intestinal permeability in animals under stress. As we mentioned earlier, the physical barrier function of the mucous membrane seems to be of prime importance for preventing or limiting bacterial translocation, especially in animals with normal intestinal flora, while the immune system has a secondary or supporting role for the intestinal mucosa barrier (Magnotti, 2005). Injection of USP effectively inhibits the process of translocation of pathogenic microflora to internal organs (Assimakopoulos et. al., 2005).

Since normal functioning of the immune system is another important factor for good intestinal barrier function (Magnotti LJ, Deitch EA., 2005), USP can reduce bacterial translocation due to their antimicrobial activity and modulating effect on immunocompetent cells (Abuharfeil et. al., 1999, Watanabe et. al., 1998).

5. CONCLUSION

From this experimental study in rabbits, it has found that using intramuscular injection of USP at the dose of 0.01 mg/kg of body weight not only helps to reduce the stress state of animals but is also useful for lowering bacterial translocation.

6. AVAILABILITY OF DATA AND MATERIAL

Information of this study can be made available by contacting the corresponding author.

7. ACKNOWLEDGMENT

These studies were carried out in accordance with the research plan for 2019-2021. Federal State Budgetary Scientific Institution “Federal Research Center for Biological Systems and Agricultural Technologies of the Russian Academy of Sciences” (No. 0761-2019-0006).

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*Corresponding author (M.G. Titov). Email: titow.ru@mail.ru Volume 11 No.10 ISSN 2228-9860 eISSN 1906-9642 CODEN: ITJEA8 Paper ID:11A10N http://TUENGR.COM/V11A/11A10N.pdf DOI: 10.14456/ITJEMAST.2020.197

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Note: The original version of this article has been reviewed, accepted, and presented at the International Scientific and Practical Conference "From Inertia to Development: Scientific and Innovative Support for Agriculture" (IDSISA2020) at the Ural State Agrarian University, Ural, Russia, during 19-20 February 2020.

*Corresponding author (M.G. Titov). Email: titow.ru@mail.ru Volume 11 No.10 ISSN 2228-9860 eISSN 1906-9642 CODEN: ITJEA8
Paper ID:11A10N http://TUENGR.COM/V11A/11A10N.pdf DOI: 10.14456/ITJEMAST.2020.197