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THE EFFECT OF CONSUMPTION OF BROILER CHICKEN MEAT WITH LIMITED PRE-SLAUGHTER STRESS ON THE NATURAL RESISTANCE IN PEOPLE

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Abstract. The data on some immunity indices in human blood after consumption of broiler chickens meat using natural immunomodulators of pre-slaughter stress are presented in the article. Spleen extract (1.4 ml of 70% alcohol solution per chicken) was added to the diet of broiler chickens of experimental groups by aerosol method. The 70% alcohol solution in the same volume and the same method was added to the diet of broiler chickens of control group five days before slaughter. The results obtained can be used in studies on natural resistance indices in farm animals, in order to improve the organism resistance, correction and to avoid pre-slaughter stress and improve product quality.

Key words: natural resistance, men, spleen extract, broiler chickens, pre-slaughter stress

INTRODUCTION

Ecological status of environment, lifestyle and nutrition, area of activity, physical exercises are not all factors that influence the immune system of the human body during its lifetime.

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Various kinds of stresses, oppressed state of organism, and usage of certain medicaments are deteriorative for the immune system. Herewith, in addition to an unbalanced nutrition, chemicals, physical factors, viruses and microorganisms, irrational storage, remaking, and packaging of food are mostly considered, while the stressful state of animals before their slaughtering is not taken into account.

Meat products derived from farm animals from stress status before slaughter can be inedible. Currently, attention, which is paid to the impact of food on the immune system of the human body, is not enough.

We used biologically active substances of natural origin with spleen extract, containing polyamines, as immunomodulators and anti stressors for broiler chickens. As it is known, the polyamines, including putrescine, spermidine, and spermine are small, aliphatic cations under physiological conditions. Polyamines (putrescine, spermidine and spermine) are aliphatic polycations, belonging to dietary amines, found in all living organisms (animals and plants, algae and fungi, bacteria and viruses). Fabrics eukaryotes contain spermine and spermidine in millimolar concentrations, while the putrescine is found in nanomol concentrations. Amount of polyamines in animal tissues depends on the type of fabric, its growth rate, age and other factors. The intracellular distribution of polyamines, their content in the meat of chickens, pigs, and neat was researched in many laboratories [Casero and Pegg 2011]. The greatest concentration of polyamines has been found in the pancreas, thymus, and other glands, as well as in the liver, i.e. in organs, where the intense protein synthesis is occurred [Rosinská and Lehotay 2014].

There are a lot of polyamines in the woman's breast milk (especially spermine) and thus, according to scientists' opinion, woman's milk has the antiallergic properties [Atiya Ali et al. 2013].

In previous exploirations (model research), the influence of ante-mortal condition on the cellular immunity and amount of cortisol in the blood and plasma of rats has been defined. Adding the spleen extract to the feed for five days before slaughter was accompanied by increase the number of T-lymphocytes, decrease the levels of cortisol in the blood and plasma of rats, which may indicate a reduction of stress before slaughter [Grabovskyi 2014]. Increasing cortisol level leads to oppression of the immune function [Buckingham et al. 1997].

The aim was to investigate the effect of ante-mortal condition on the concentration of the Adrenocorticotropic hormone (ACTH) in the blood plasma, polyamines – in blood and breast muscle of broiler chickens and influence the consumption of their meat on some indicators the immunity of people.

MATERIAL AND METHODS

First stage of research

The experiment was conducted on a one-month broiler chickens (hens) weighing 1.8–2.2 kg cross "Ross-308", which kept on chicken company standard diet of Velykyi Lubin town, Gorodotsky district, Lviv region. Two groups of one-month old broiler chickens (5 chicks each) were formed for the first research. Spleen alcohol extract was obtained by using ultrasound (first experimental group) and without ultrasound usage (second experimental group) was applied as biologically active substance in the pre-slaughter period (five days before slaughter). Extracts were applied on feed by an aerosol method (70% alcohol solution of 1.4 ml extract per chicken). The control 70% ethanol solution in the same volume was added to the feed of third group of broiler chickens. The feed intake was monitored daily. Slaughter of chickens was carried out in the morning. We also took blood and pectoral muscle for biochemical studies.

In the experiment, all bioethical standards under the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) have been observed.

The polyamines content in biomaterial was determined by HPLC pressure [Gerbaut 1991] on liquid chromatography Agilent 1200 (USA). The solvents and polyamines standards of Sigma Chemical Co. (USA) company were used in the paper. The Daisopak SP1205ODSRPS column (4.6 mm I.D. 250 mm; Daisoco Ltd) was used for the analysis.

The 20 µl aliquots for each sample concentration gradient water/acetonitrile from 50 to 100% within 20 min and pure acetonitrile 5 min at a flow rate of 1 ml $\cdot$ min^{-1} were analyzed. The polyamines level were determined at a wavelength of 342 nm radiation and emission of 512 nm.

The ACTH concentration (adrenocorticotropic hormone consisting of 39 amino acids) is determined by a set of DRG Intl ACTH ELISA. This two sites ELISA for determination of bioactive ACTH.

Glutathione concentration in the blood was determined after restoration of glutathione by changing optical activity caused the formation tionitro benzoate anion-dyanione in response DTNB (5,5'-dytiobis (2-nitro-benzoic acid) with reduced glutathione [Vlizlo et al. 2012].

Second stage of research

We recruited 10 healthy male medical students (20 years old, men) challenging the National Medical license examination. All experiments were conduc-

ted in accordance with the Declaration of Helsinki. The protocol and informed consent of this study were approved by the Institutional Review Board of Lviv University Hospital. The experimental procedures were fully explained to each subject and written informed consent was obtained. All subjects were in good physical health, taking no medication for at least three months prior to enrollment and during the experimental period, and had no history of psychiatric or somatic diseases. All subjects were non-smokers.

The experiment has lasted five days. Men ate the meat of the broiler chickens of the first and second groups, weighing 1.8–2.2 kg, twice: at the beginning and in the end of the experiment. The men's blood has been taken from the veins in the elbow before and after eating meat. Indicators of natural resistance were determined in whole blood. Stimulation index of neutrophils in peripheral blood was determined by the principle of which is captured in a quantitative definition of neutrophils latex beads. [Lapovets et al. 2014].

Mathematical treatment of the research results has been done statistically by using the software package Statistic 6.0 and Microsoft Excel. Probability of differences has been assessed by Student's t-test. We considered results as significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Analysis of blood biochemical parameters of broiler chickens under antemortal stress conditions showed the negative impact of his actions on the adaptive mechanisms of the organism, since the concentration of ACTH in plasma blood of the broiler chickens increased by 10% ($P \leq 0.05$) as compared to animals which received the extract spleen with usage of ultrasound (Table 1).

Table 1. Concentration of ACTH and glutamine in the plasma blood of broiler chickens before slaughtering (mean ±SD, n = 5)

Tabela 1. Koncentracja ACTH i glutaminu w surowicy krwi broilerów przed ubojem (średnia ±SD, n = 5)

Indicators – Wskaźniki	Groups – Grupy		
	I	II	Control – Kontrolna
ACTH, pg · ml^{-1}	3.328 ±0.51*	4.304 ±1.25**	7.895 ±2.25
Glutathione, nM · mg^{-1} – Glutathion, nM · mg^{-1}	24.002 ±0.81*	27.618 ±2.9	28.542 ±1.25

Note: statistically significant differences: *$P \leq 0.05$, **$P \leq 0.01$.

The clinical feature of stress in chickens is breathlessness and dropping the wings. There are two most important physiological responses to stress, including stimulation of the sympathetic nervous system and activation the connection hypothalamic-pituitary-adrenal's cortex. Stressors stimulate the hypothala-

mus that gives a signal the pituitary to secrete the adrenocorticotropic hormone (ACTH) in the blood, influencing the cortical layer of the adrenal glands that secrete corticoids [Sapolsky et al. 2000, Collin et al. 2007].

In our studies, the concentration of the ACTH was low as compared to the control group, where the chickens were under stress (Table 1). We found significantly higher levels of glutathione in the blood of broiler chickens of the control group, which may be a feature of oxidative stress or deceleration of transport of the amino acids into the cells of body tissues by reducing the activity of the glutamine/glutamate-gamma-amino butyric acid cycle.

In the process of analyzing the received data regarding the content of polyamines in the broiler chickens blood of the I experimental group before slaughtering, adding the spleen extract treated with ultrasound to the feed, we found that the total number of polyamines increased as compared to the control group by 39% ($P \leq 0.01$), spermidine concentration – by 34% ($P \leq 0.05$), and spermine – 40% ($P \leq 0.01$, Fig. 1).

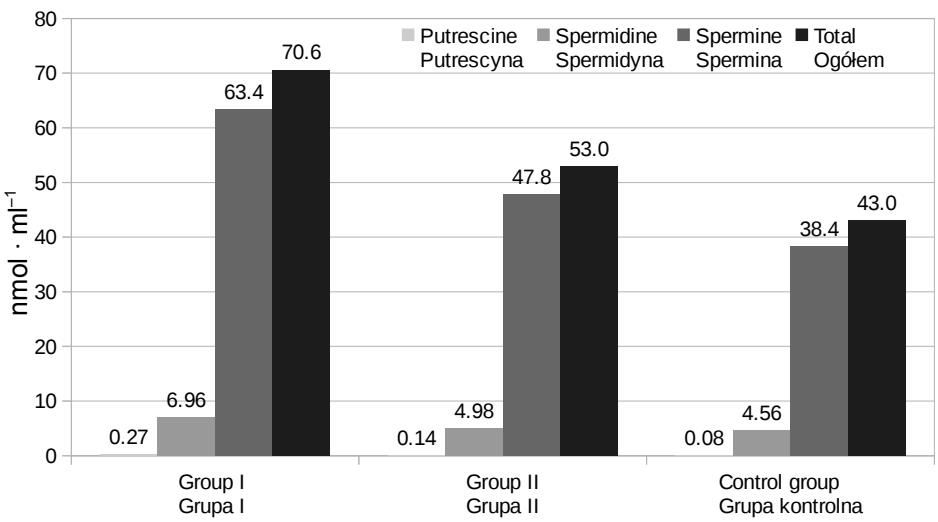

Fig. 1. Concentration of polyamines in the blood of broiler chickens, nmol · ml^{-1}; *P ≤ 0.05; **P ≤ 0.01

Rys. 1. Koncentracja poliaminów we krwi broilerów, nmol · ml^{-1}; *P ≤ 0,05; **P ≤ 0,01

The results are consistent with the authors' data [Soda et al. 2009], since the additional use of polyamines can influence on their concentration in the tissues and blood in particular.

The total amount of polyamines in the breast muscle was increased by 45% ($P \leq 0.05$) in the broiler chickens of the first experimental group (55.538 ±6.39

nmol · ml^{-1}), adding to the basic diet the spleen extract obtained by using the ultrasound, while in the second experimental group (44.77 ±4.61 nmol · ml^{-1}) of chickens – by 32% (P ≤ 0.05) as compared to the control group (Fig. 2).

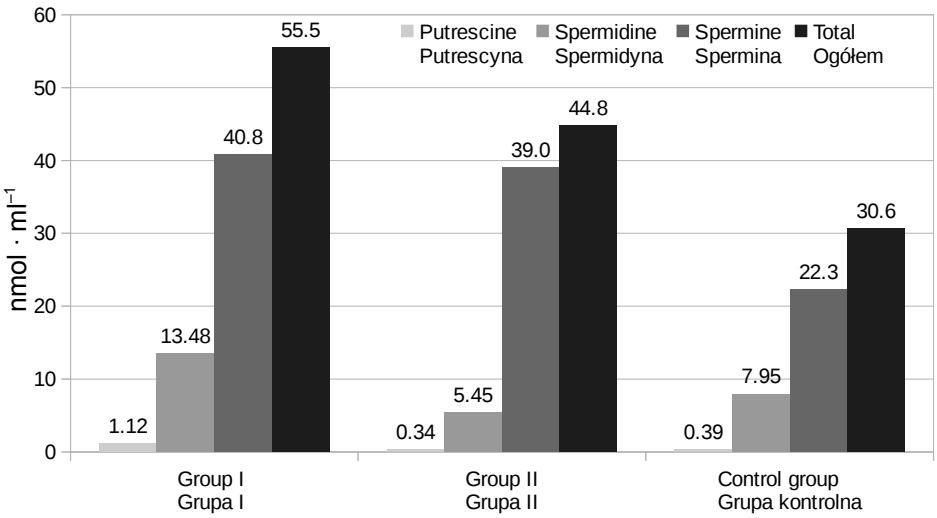

Fig. 2. Concentration of polyamines in breast muscle of broiler chickens, nmol · ml^{-1}; *P ≤ 0.05; **P ≤ 0.01

Rys. 2. Koncentracja poliaminów w mięśniach piersiowych broilerów, nmol · ml^{-1}; *P ≤ 0,05; **P ≤ 0,01

Putrescine content in breast muscle of chickens of the first experimental group was 1.12 ±0.42 nmol · ml^{-1} (P ≤ 0.01), which is significantly higher as compared to the same period in the chickens of the control group (0.39 ±0.45). We also found a significantly greater amount of spermine in the breast muscle of both experimental groups of the broiler chickens: by 45% (P ≤ 0.01) in the first experimental group (40.83 ±8.96 nmol · ml^{-1}) and by 43% (P ≤ 0.05) in the second experimental group (38.976 ±5.52 nmol · ml^{-1}) as compared to the control group. Spermidine concentration was significantly higher only for the first experimental group (13.484 ±6.71 nmol · ml^{-1}) in comparison with the control group of chickens (Fig. 2).

The index of stimulation of neutrophils (0.82) in the men's blood after consumption the meat of broiler chickens, which received spleen extract, obtained by using ultrasound, as anti stressors and immunomodulators, was increased. The mentioned index was reduced (–2.21) in men, who consumed the meat of broiler chickens under ante-mortal stress conditions as compared to the beginning of the experiment. We have not seen a significant difference in other indicators of natural resistance.

Stimulation index of neutrophils in men blood is shown in Fig. 3. As it is seen from the figure, the stimulation indices in men blood between the experimental and control groups were differed by 0.89 abs. number g-T[1], or 34.8%, although their values do not go beyond the physiological norms.

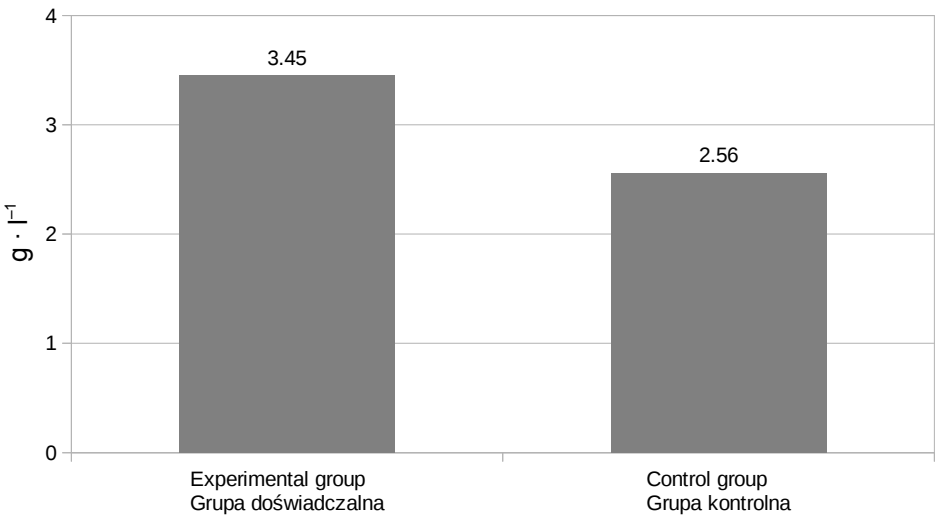

Fig. 3. The stimulation index of neutrophils, abs. number, $g \cdot l^{-1}$

Rys. 3. Wskaźnik stymuacji neutrofilów, liczba bezwzględna, $g \cdot l^{-1}$

It has been found that polyamines play an important role in controlling the innate immune response to bacterial infection. Systemic introduction of the lipopolysaccharide endotoxin increases the amount of mRNA and an activity the ornithine decarboxylase (ODC) in neurons and microglia of CNS that is blocked by an introduction of CDK inhibitors. Herewith, decrease in the amount of putrescine may prevent the startup synthesis of the brain tumor necrosis factor induced by lipopolysaccharide. As it is shown in the same figure, inhibition of the synthesis of polyamines helps to reduce the neurodegeneration and increase the survival of experimental animals. Maintenance the permanence in the exchange of polyamines in the central nervous system is very important for the correction of neurodegenerative processes, and for the homeostasis of the immune system [Soulet and Rivest 2003]. It is shown that the content of polyamines increases by many autoimmune diseases [Brooks 2012] and the polyamine hypothesis of the mechanisms of autoimmune diseases' development were proposed. The increased activity the enzymes of the polyamines metabolism and their quantitative value can lead to disruptions in the cellular DNA methylation, histone and other cellular processes associated with methylation [Hong 2010, Karouzakis et al. 2012].

Based on the results received on the broiler chickens, it can be concluded that the usage of immunomodulators, which contain the polyamines, reduces the negative impact of the ante-mortal stress and strengthens the immune system of chickens.

CONCLUSIONS

Additional input of polyamines derived from extract of spleen to the feed has been accompanied by an increase in the total number of polyamines and increase the concentration of some of them, especially putrescine, spermidine, and spermine in blood and breast muscle of broiler chickens.

Biologically active substances of natural origin can reverse the ante-mortal stress of chickens and, as a result, improve the quality of their meat. Consumption of meat of broiler chickens leads to increase the stimulation index of neutrophils in the blood of people and, thereby, increase the resistance of the organism.

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WPŁYW KONSUMPCJI MIĘSA OD KURCZĄT BROJLERÓW O OGRANICZONYM STRESIE PRZEDUBOJOWYM NA NATURALNĄ ODPORNOŚĆ U LUDZI

Streszczenie. Artykuł przedstawia dane dotyczące zawartości niektórych wskaźników odporności we krwi ludzi po spożyciu kurcząt brojlerów, u których zastosowano immunomodulatory naturalnego pochodzenia w celu redukcji stresu przedubojowego. Ekstrakt ze śledziony (70-procentowy roztwór alkoholu w objętości 1,4 ml na ptaka) dodano do diety brojlerów z grup doświadczalnych metodą aerozolową. Roztwór alkoholu 70-procentowego tej samej objętości tą samą metodą dodano do diety kurcząt brojlerów z grupy kontrolnej pięć dni przed ubojem. Wyniki, które uzyskano, można wykorzystać w badaniach wskaźników naturalnej odporności na zwierzętach gospodarskich w celu zwiększenia odporności organizmu, zmniejszenia stresu przedubojowego i poprawy jakości produktu.

Słowa kluczowe: odporność naturalna, człowiek, ekstrakt śledziony, brojlery, stres przedubojowy

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