AN EFFECT OF GAS STUNNING REGIMES ON CHANGES IN POST-MORTEM CHARACTERISTICS IN THE CONDITIONS OF A MODEL EXPERIMENT

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Abstract –A model experiment on the laboratory rats was carried out using a CO_2 chamber for euthanasia and varying the flow rate of carbon dioxide (0.3 l/min. and 1.5 l/ min) within the framework of the study on animal stress under exposure to gas mixtures. After stunning, the animals were weighed and decapitated; the bleeding time was measured and serum biochemical analysis was carried out. The comparative weight of internal organs (heart, lung, liver, kidney, adrenal gland, spleen, brain) was measured. The blood levels of triglycerides, glucose and creatinine, and the activity of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) were determined. It was established that accelerated supply of carbon dioxide (1.5 l/min) allowed reducing exposure time until loss of sensitivity in animals on average by 378 s, ensured more complete bleeding and less damage of the internal organs as well as less stress level, which was evident from the activity of lactate dehydrogenase and creatine phosphokinase, as well as the blood level of creatinine.

Key Words – bleeding, carbon dioxide, stress.

I. INTRODUCTION

Animal stunning directly affects a level of stress, a degree of animal bleeding and the development of autolytic processes and, therefore, determines to a large extent, meat quality characteristics and product shelf life. A method of animal stunning using gas mixtures under correctly selected technological regimes allows avoiding stress when pigs are driven forward in groups, bones fractures, hemorrhages and risks associated with employees' safety, and fully corresponds to the principles of farm animal welfare stated in the Council Directive 98/58/EC.

The use of carbon dioxide is one of the most common methods for anesthesia both of laboratory and farm animals. Currently, the Institute conducts researches on an influence of different gas mixtures parameters for stunning laboratory animals in the conditions of a model experiment with the aim of the following use of the obtained results while determining optimal regimes of gas anesthesia for farm animals.

II. MATERIALS AND METHODS

The experimental researches were carried out on adult white male Wistar rats (body weight of 365±30g) obtained from the Federal State Institution of Science "Scientific Center for Biomedical Technology of the Federal Medical and Biological Agency" and kept in cages "TECNIPLAST" type IV S in the standard vivarium conditions with identical levels of temperature (20±2 °C), humidity (48±2 %) and lighting (from 6:00 to 18:00) upon free access to water and food. The rats were fed with full-ration combined feed from purified and comminuted cereal raw material and animal raw material.

Keeping, feeding and handling of animals and removing them from the experiment were carried out in accordance with the requirements of Guide for the Care and Use of Laboratory Animals [1].

Twelve hours prior to the experiment, the animals were subjected to food deprivation. The animals were exposed to carbon dioxide in a chamber for euthanasia ("VetTech", UK). Stunning of group 1 was carried out at the flow rate of carbon dioxide recommended by the manufacturer of the chamber (0.3 l/min); stunning of group 2 was performed at a rate of 1.5 l/min. After loss of sensitivity, the animals were decapitated using a guillotine ("Open Science", Russia) and the blood was taken for biochemical analysis. Duration of bleeding was recorded with a mechanical stopwatch.

A condition of the internal organs was assessed under a microscope Stemi2000-C (Carl Zeiss, USA) with determination of relative weights of the liver, kidney, spleen, heart, lungs, adrenal gland and brain by weighing on electronic scales (Acculab Vicon, USA) with an accuracy of ±0.001 g.

Serum for biochemical analysis was prepared as follows: the collected blood was settled in glass tubes for 20 min. until formation of a clot, serum was separated on a centrifuge CM-6M (ELMI, Latvia) at 3500 rpm for 8 min. Serum was analyzed for triglycerides, glucose, total lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and creatinine using the automatic analyzer BioChem FC-360 (HTI, USA) in accordance with the methodologies accompanying the reagents (HTI, USA).

The software package STATISTICA 10 was used for statistical data processing of all indicators. In the software package STATISTICA 10, the results were presented as a weighted average ± standard deviation. Statistical significance was calculated using one-way ANOVA with Duncan's test. The probability of 0.1 was chosen as the significance level.

III. RESULTS AND DISCUSSION

The time to loss of sensitivity differed significantly between animals: it was 548±27 s for group 1 and 170±24 s for group 2. The results of monitoring of the animal carcass bleeding time suggest faster bleeding of the animals from group 1 (73.0±7.4 s) compared to the animals from group 2 (92.0±7.0 s). Long time to loss of sensitivity in the animals from group 1 indicates that the equipment manufacturer has inaccurately selected a regime of gas supply into the stunning chamber as a necessary gas concentration is achieved over a long period of time, which can be negatively reflected in post mortem indicators of animals [2].

A visual assessment of the internal organs showed that the lungs and heart of animals from group 1 had a high level of blood filling, the heart coronary vessels were filled with blood and swollen, the lungs had a marbled pattern and punctate hemorrhages; the alveoli were enlarged. The heart and lungs of animals from group 2 had the medium degree of blood filling, the alveoli were also enlarged, hemorrhages in the lungs had a sporadic character. The cerebral blood vessels of the animals from group 1 were injected. Therefore, the internal organs of the animals from group 2 had fewer lesions: less blood filling of the heart and lungs, cerebral blood vessels were without visible changes, which suggests more complete bleeding. This correlates with the data of Muzhikyan A.A. and others [3], who studied changes in the internal organs of laboratory animals on pathoanatomical examinations.

It was established that the relative weights of the heart, liver, kidney, adrenal gland, spleen and brain in both groups of animals were not significantly different. A statistically significant increase ($P \leq 0.05$) in the relative weight of the lungs in the animals from group 1 (by 19.4%) was associated with a high degree of blood filling (Table 1).

Table 1 The relative weight of the internal organs, %

Organ	Group 1	Group 2
Heart	0.38±0.02	0.37±0.02
Lungs	0.72±0.04	0.58±0.03
Liver	2.80±0.09	2.93±0.11
Kidney	0.32±0.01	0.32±0.01
Adrenal gland	0.02±0.00	0.02±0.00
Spleen	0.22±0.02	0.24±0.02
Brain	0.57±0.02	0.59±0.02

The results of the biochemical blood analysis demonstrated a two-fold decrease in triglycerides in group 2 (0.47±0.06, mMol/l) compared to group 1 (0.93±0.03, mMol/l) ($P < 0.1$).

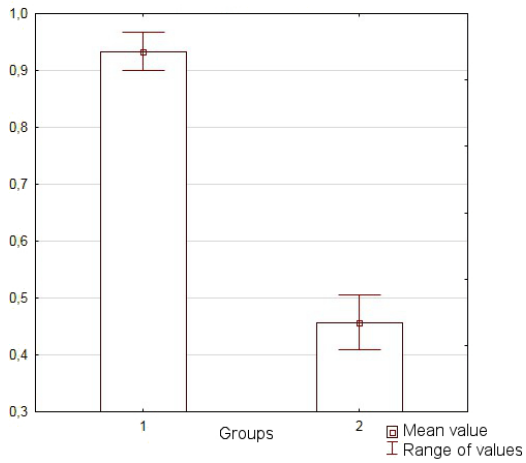

Figure 1. The content of triglycerides in the blood of the animals from both groups, mMol/l

The glucose level was 7.54±2.22 mMol/l in group 1 and 5.86±0.83 mMol/l in group 2; however, no statistically significant differences in the values were established. It is necessary to note that the study on an effect of a complex of factors on the glucose and cortisol levels in beef (Chulayo, A.Y. et. al.) did not reveal differences in the glucose level depending on the method of stunning [4], which can suggest an insignificant effect of a stunning method on the glucose level. This is also confirmed by the investigations carried out on pigs [5], where the glucose level depended largely on the transportation regimes rather than the regimes of CO_2 stunning.

The LDH activity in group 2 (418.17±40.50, mMol/l) was lower by 22.7 % ($P < 0.1$) compared to group 1 (541.08±47.73, mMol/l) ($P<0.1$) (Fig. 2).

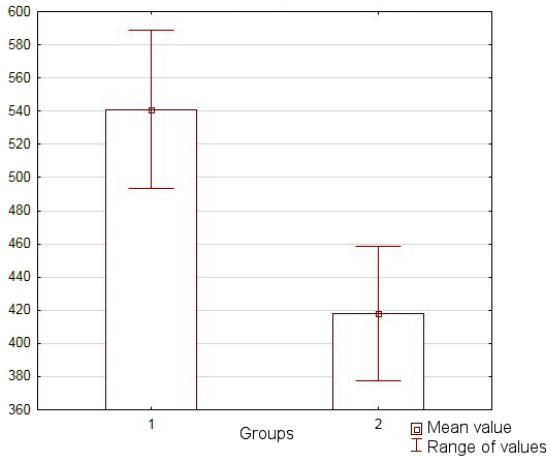

Figure 2. The activity of lactate dehydrogenase (LDH) in the animal blood of both groups, U/L

The activity of creatine phosphokinase (CPK) in group 2 (2707.38 ± 631.70, U/L) was also lower (by 39.2 %) than in group 1 (4452.66±891.21, U/L) ($P < 0.1$) (Fig.3).

The creatinine content in group 2 (95.20±1.69, µmol/l) was also lower (by 15.9 %) than in group 1 (112.50±3.67, µmol/l) ($P < 0.1$) (Fig. 4).

Low triglyceride and glucose levels are associated with food deprivation in animals; however, release of triglycerides in group 1 can be a consequence of activation of lipid peroxidation.

An insignificant increase in the LDH activity in group 1 can be caused by the physiological stress, disorders of the cardiac rhythm and hemolysis.

A significant decrease in CPK activity (four times in group 1 and almost 7 times in group 2) and creatinine in blood (1.6 and 1.4 times, respectively) relative to the norm [6] indicates a high level of stress in animals.

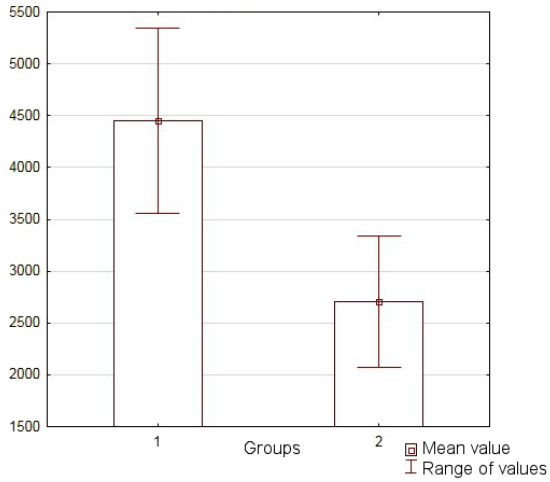

Figure 3. The activity of creatine phosphokinase (CPK) in the animal blood of both groups, U/L

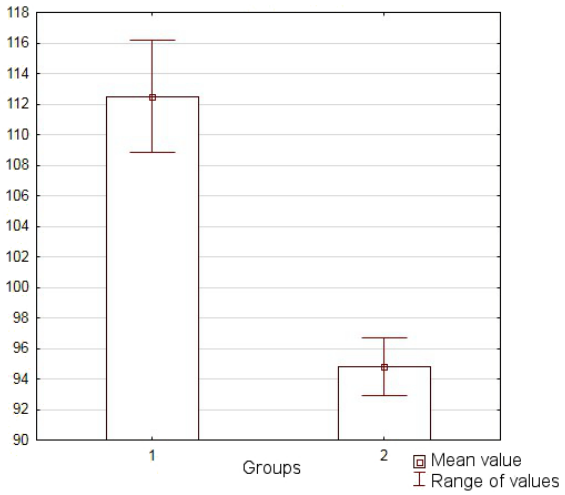

Figure 4. The creatinine level in the animal blood of both groups, µmol/l

IV. CONCLUSION

Accelerated CO_2 supply applied to group 2, led to faster loss of sensitivity in animals and ensured

less damage of the internal organs and their lower blood filling due to more complete bleeding.
On accelerated CO_2 supply, the lower level of stress in animals was observed, which was evident from the activity of lactate dehydrogenase and creatine phosphokinase, as well as the creatinine level in the animal blood.

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