Meat product based on porcine hearts and aortas ameliorates serum lipid profile and inflammation in hyperlipidemic rats

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Abstract. The biological effect of porcine hearts and aortas in a hyperlipidemic rat model was confirmed. Porcine heart and aorta mixture in a 3:1 ratio was blended, canned and sterilized at 115°C and 0.23 Mpa for 40 min. Administration of experimental meat product to the animal model decreased total cholesterol, triglycerides and cholesterol low density lipoproteins by 31.8% (P<0.05), 28.2%, and 21.6% (P<0.05), respectively, compared to those of hyperlipidemic control rats, as well significantly reducing the serum atherogenic index by 41.3% (P<0.05) in rats fed the experimental meat product compared with hyperlipidemic control rats. Normalization of white blood cell populations was also detected. Monocyte and granulocyte counts in blood of rats fed the meat product decreased by 71.1% (P<0.05) and 57.6% (P<0.05) compared to those of the hyperlipidemic control animals. The granulocyte/leucocyte ratio was also reduced by an average of 38.6% (P<0.05) in rats fed the meat product compared with hyperlipidemic control rats. The data confirmed the hypolipidemic action of the sterilized meat product. Normalization of white blood cell populations led us to hypothesize an anti-inflammatory action of the new meat product, which, therefore, could be recommended as a part of maintenance therapy for people with lipid disorders or atherosclerosis.

1 Introduction
Novel technologies are actively sought for implementation in the food industry, and special attention is being paid to the development of functional and specialized products. An abundance of publications highlights the results of studies aimed at recipe modification by adding essential nutrients as well as ingredients of vegetable or animal origin [1-3]. Peptides that naturally occur in raw meats or that form during enzymatic hydrolysis or technical processing have become an area of intensive research, especially in the last decade. In numerous studies, it was shown that these peptides have characteristic antioxidant, hypotensive and anticaner, etc. action [4-8].

Modern scientific approaches, particularly proteomics, have enabled confirmation that the proteome of each organ or tissue contains constitutive structural and functional proteins, characterized by specific proteins and peptides, which are involved in organ/tissue function and support the normal physiological state [9]. In this regard, the study of meat by-products as sources of bioactive sequences promoting normalization of metabolic disturbance is a challenging scientific task, as is development of specialized and functional products utilizing bioactive substances. Previously, a significant decrease of total cholesterol, triglycerides and atherogenic fractions of lipoproteins was shown in hyperlipidemic rats which received native tissues of cattle and pigs hearts and the aortas [10], while diets with porcine
hearts and aortas demonstrated the greatest efficiency. Proteomic analysis revealed several specific proteins, including apolipoprotein A-1, peroxiredoxin-1, galectin-1, and heat shock proteins in porcine aortas. Fatty acid-binding protein was detected in heart. It was also found that these bioactive substances are destroyed during sterilization process, except fatty acid-binding protein [11]. Nevertheless, we can assume that tissue-specific proteins can decompose into some peptides which can also possess biological action similar to that of native tissues. Therefore, the aim of this study was to assess the influence of the biological effect of meat product produced from porcine hearts and aortas on rat serum lipid profile and white blood cell count.

2 Materials and Methods
Meat product was produced using a ZAO Yoshkar-Olinskiy Myasokombinat. Porcine hearts were chopped into pieces of 2-3 mm and then salted for 12 h. Aortas were chopped into pieces of 2-3 mm and minced in cutter at 3000 rpm for 2-3 min. Heart emulsion was quantitatively added in a 3:1 ratio to minced aortas and the mixture was then additionally homogenized at 3000 rpm for 6-8 min. Homogenate was packed in cans and sterilized at 115°C, 0.23 MPa for 40 min to produce the meat product.

Thirty male Wistar rats (380±20 g) approximately 1 year old were housed in conventional standard conditions; water and feed were available ad libitum. Animals were randomly divided into 3 groups: group 1 – negative control (n=10); group 2 – hyperlipidemic control (n=10), and; group 3 – experimental (hyperlipidemic+experimental diet; n=10). Group 1 (negative control) were fed standard chow (Labkorm, Russia) ad libitum during the study. A rat model of alimentary hyperlipidemia was developed in group 2 and 3 rats by adding cholesterol (2.0-10.0%) and fat (10.0-25.5%) to the standard diet, and each animal received per os vitamin D2 injection (35,000 IU/kg b.w.). After modeling, rats in group 2 (hyperlipidemic control) were fed with standard chow, while group 3 rats received meat product (8 g/kg b.w.) mixed with standard chow. All diets were equally balanced according main nutrients: protein, fat, minerals, etc., and were fed to the rats for 42 days.

Forty-two days after rats commenced the experimental diet, they were euthanized in a VETtech chamber according to the animal welfare rules, and blood samples for biochemical and flow cytometry analysis were taken. Total cholesterol (TC), triglyceride (TG), cholesterol low-density lipoproteins (CL LDL) and cholesterol high-density lipoproteins (CL HDL) were measured in rat serum on an automatic analyzer BioChem FC-360 (HTI, USA) according to the manufacturer’s instructions for measurement kits (HTI, USA). Atherogenic index was calculated as AI = (TC-CL HDL)/ CL HDL. White blood cell (WBC), lymphocytes (LYM), granulocytes (GRA) and monocytes (MON) in blood were measured on the Guava EasyCyte cytometer (Merck Millipore, Germany).

STATISTICA 10.0 software was used. Significant differences were tested using two-way analysis of variance (ANOVA), followed by Newman-Keul’s test. Differences with P-values less than 0.05 were considered as statistically significant.

3 Results and Discussion
It was shown that long-term consumption of a cholesterol- and animal fat-rich diet led to increases of TC, TG and atherogenic fractions of lipoproteins in rat serum. On day 42, rat serum TC, TG and CL LDL in the group 2 hyperlipidemic rats exceeded those in group 1 by 35.8% (P<0.05), 17.0% and 15.5%, respectively. In serum of group 3 rats (fed the experimental meat product), TC, TG and CL LDL decreased by 31.8% (P<0.05), 28.2%, and 21.6% (P<0.05), respectively, compared to those of the hyperlipidemic control rats (table 1).
Table 1. Rat serum lipid profile.

| Group 1 | Group 2          | Group 3          |
|---------|------------------|------------------|
| CL, mmol/L | 2.18±0.12        | 2.96±0.08*       | 2.02±0.19#       |
| TG, mmol/L | 1.76±0.27        | 2.06±0.33        | 1.48±0.18        |
| CL LDL, mmol/L | 0.84±0.05        | 0.97±0.02        | 0.76±0.11#       |
| CL HDL, mmol/L | 0.85±0.03        | 0.88±0.06        | 0.81±0.03        |
| AI      | 1.58±0.09        | 2.52±0.12*       | 1.48±0.22#       |

*Significantly different compared with group 1.
#Significantly different compared with group 2.

Group 1 = negative control rats.
Group 2 = hyperlipidemic control rats.
Group 3 = hyperlipidemic rats receiving meat product.

Redistribution of lipoproteins fractions resulted in a significant increase of serum AI in group 2 rats compared to that of negative control rats by 59.5% (P<0.05), while in group 3 rats on the experimental diet, AI was lower than in hyperlipidemic control rats by 41.3% (P<0.05) (table 1).

Not only serum lipid profile, but also hematologic parameters have prognostic importance in cardiovascular disease. Blood is a carrier of metabolic products from and to the organs and tissues and is affected by the clinical status of the tissue environment [12]. Thus, the relation between atherosclerosis progression and hematologic parameters is not well defined, but it was determined that progression rate of coronary atherosclerosis was shown to be significantly higher in patients with high GRA/LYM ratios [13-15], as well as in those with increased MON counts [15,16] which is of great importance taking into consideration that monocytes are crucial cells in the genesis of atherosclerotic lesions, as they stick to endothelium, which results in cardiovascular disease [17].

The WBC count increased by 24.1% in blood of control group 2 rats, mainly due to MON and GRA counts, which increased by 2.1 (P<0.05) and 2.4 times (P<0.05), respectively, compared with negative control rats. MON and GRA counts in blood of group 3 rats, treated with experimental meat product, decreased by 71.1% (P<0.05) and 57.6% (P<0.05) compared to hyperlipidemic control animals (group 2) (figure 1).

Figure 1. WBC, differentiated into LYM, GRA and MON in the three groups of rats. A – group 1 (negative control), B – group 2 (hyperlipidemic control), C – group 3 (experimental meat product).

The mean GRA/LYM ratio in group 2 rats was 0.57±0.03, which was 2.5 times (P<0.05) greater than that of the negative control rats (group 1), while in blood of animals treated with the meat product (group 3) for 42 days, the GRA/LYM ratio was 0.35±0.03; this was 38.6% lower than that of the hyperlipidemic control rats (figure 2).
The results of this study confirm the hypolipidemic action of the prepared porcine meat product, despite the fact we previously demonstrated decomposition of functional proteins during sterilization. Moreover, normalization of WBC populations was also detected, showing hematopoietic changes due to the anti-inflammatory action of the experimental meat product made from porcine harts and aortas. The developed meat product could be recommended as a part of maintenance treatment for people with lipid disorders or atherosclerosis.

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