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Meat by-product is a source of tissue-specific bioactive proteins and peptides against cardio-vascular diseases

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Abstract

The aim of the study was to investigate the functional properties of extracts and utrafiltrates prepared from pig aorta tissues using Guinea pigs with an experimental atherosclerosis model to determine the possibility of their implementation against cardiovascular disorders. Serum total cholesterol content, atherogenic index (AI), low density lipoproteins (LDL) and residual cholesterol (VLDL, IDL) reduction was noted (44.1%, 43.7%, 43.7% and 79.5%, respectively) in animals treated with low-molecular weight ultrafiltrate (LMU). Serum C-reactive protein (CRP) and Vascular endothelial growth factor (VEGF) concentrations declined by 34.0% and 58.3% on average, respectively in animals treated with LMU.

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Keywords: livestock by-products; bioactive molecules; tissue specificity; inflammation markers

1. Introduction

Nowadays, high technology is an important aspect of the food industry. The implementation of modern technologies and biological research results is an essential part of functional food development. Latest trends in healthy eating have led to a demand for a special line of products demands — functional foods. Bakery, dairy and meat industries are not only developing technology resulting in product with bio-corrective properties, but they are

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also actively introducing received innovations. Various methods of intravital raw material modifications, genetic technologies and feed variations, plus food processing changes are used in the meat industry[2,3,5,10,11]. The proteomic approach is also being actively introduced. Meat is a functional system[2] including protein, conjugated linoleic acid (CLA), minerals (iron, zinc and selenium), vitamins (B, E), glutathione, ubiquinone, lipoic acid etc.[2,6,14]. Moreover, the meat proteome is a source of bioactive sequences possessing hypotensive, antioxidant, opioid, immunomodulatory, prebiotic, mineral-binding, cholesterol-lowering or antimicrobial activity[1,3,6,10,14]. However, it should be noted that identification and the biological action of tissue-specific biological substances contained in meat by-products are still underdeveloped area.

Atherosclerosis is a complex disease including lipid infiltration and general vascular wall inflammation[7,13]. For correction of this pathological condition not only must traditional serum lipid profile (cholesterol (LDL), triglycerides (TG), low density lipoprotein cholesterol (LDL), high density lipoproteins (HDL), atherogenic index (AI), the residual cholesterol) be evaluated, but markers of inflammation (CRP and VEGF) assessment should also be determined. The lipid-lowering, anti-inflammatory and antiatherosclerotic properties corresponding to native pig aorta extract and its ultrafiltrates *in vivo* are presented in this study.

2. Materials and methods

Aorta tissues were homogenized in a grinder KENWOOD (UK) with a hole diameter of 3-5 mm, re-frozen and then homogenized in a cutter KG Wetter 258/1336 (Germany) with the addition of distilled water in the ratio (4:1) and knife shaft speed of 2000 rpm. Then, homogenates were reconstituted in 0.9% NaCl solution, and extracted during 24 h, stirrer speed of 500 rpm. Extract separation was carried out by centrifugation for 7-10 minutes at 3,000-3,500 rpm; centrifuge CM-6M (ELMI, Latvia). Supernatants were collected and ultrafiltered through PES membrane (MWCO 30kDa) by tangential filtration on the VivaFlow 200 system (Sartorius, Germany). Native extract, high-molecular weight ultrafiltrates (Mw>30kDa, HMU) were reconstituted in 0.9% NaCl solution to protein concentration 2.45g/l, low-molecular weight ultrafiltrates (Mw<30kDa, LMU) were lyophilized in INEY-4 (IPB RAN, Russia) to protein concentration 2.45g/l.

Fifty male Guinea pigs (580 ± 20 g) approximately 1 year old were kept in conventional standard conditions with an unlimited access to drinking water and feed. Animals were randomly divided in 5 groups: intact (n = 10); control (n = 10) and 1-3 experimental groups ($n_{i=1-3}$ = 10). Hyperlipidemia and atherosclerosis were modeled in control and experimental animals by *per os* administration of cholesterol (0.5 g/kg b.w.) reconstituted in animal fat and vitamin D2 (35000 U/kg b.w.) during 21 days. After modeling, experimental animals were treated with extract, HMU and LMU (8 mg of protein/kg b.w.) *per os*: Then, 1 group animals were administered extract, 2 group – HMU, 3 group – LMU, while control group animals were administered 0.9% NaCl. At the end of the modeling, on the 14th day, Guinea pigs were euthanized in VETtech camera according to animal welfare rules, and blood samples for hematologic, biochemical and immunoassays investigations were taken. Biochemical investigations were carried out on a semiautomatic analyzer BioChem FC-360 (HTI, USA) according to instructions applied to measurement kits (HTI, USA); immunoassays were carried out on a microplate reader ImmunoChem 2100 (HTI, USA) with microplate thermoshaker ImmunoChem 2200 (HTI, USA) and microplate washer ImmunoChem 2600 (HTI, USA) according to the ELISA test (Cusabio, China).

3. Results and discussion

The most intensive cholesterol reduction, of 44.1%, was observed in Guinea pigs treated with LMU due to LDL level decrease by 43.7% in comparison with control animals on the 14th day. Residual cholesterol was significantly reduced by 53.8%, 66.7% and 79.5% in 1st, 2nd and 3rd experimental groups in comparison with control animals on the 14th day. This corresponds to lipid metabolism acceleration (Fig. 1). Serum AI declined by 24.0%, 34.4% and 43.7% in 1st, 2nd and 3rd experimental groups on the 14th day in comparison with control (Fig. 2).