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HYPOLIPIDEMIC ACTION OF THE MEAT PRODUCT: *IN VIVO* STUDY

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ABSTRACT

Results of meat product influence on the serum lipid profile of hyperlipidemic rats are presented. Meat product for specialized nutrition content porcine aortas and hearts in ratio 1:3. Thirty male Wistar rats (380 ±20 g) aged approximately 1 year were kept in conventional standard conditions; water and feed were available ad libitum. Animals were randomly divided in 3 groups: group 1 – negative control (n=10); group 2 – positive control (n=10) and group 3 – experimental animals (n=10). Animals in group 2 and 3 were modeled an alimentary hyperlipidemia by adding cholesterol, fat and vitamin D2 into diet. After modeling, rats in group 2 were fed with standard chow, in group 3 – meat product (8g/kg b.w.) with standard chow. On the 42nd day serum lipid profile was investigated and immunoassay was carried out. It was found that the developed meat product given to the hyperlipidemic rats led to a decrease in the concentration of cholesterol, triglycerides and atherogenic fractions of lipoproteins by 31.8% (p <0.05), 28.2% and 2.4 times (p <0.05), respectively. Estimation of the concentration changes in apolipoproteins, forming lipoprotein particles, allowed to indirectly determining the main lipoprotein reduction that contributed to the total decrease in the atherogenic index of serum, which reached 41.3% (p <0.05). Previous proteomic study revealed the presence of a number of specific proteins and peptides in tissues of porcine aortas and heart. The hypothesis that tissue-specific proteins could decomposed into active peptides with antiatherogenic action is considered.

Keywords: meat product; lipids; hyperlipidemia; apolipoproteins; cholesterol

INTRODUCTION

Modern technologies are actively implemented in the food industry, among which a special place is occupied by the functional and specialized products. Major number of publications highlights the results of studies aimed at the modification of the recipe by adding essential nutrients as well as ingredients of vegetable and animal origin in order to achieve a certain biological effect **(Hui, 2012; Weiss et al., 2010)**.

Meat is a functional system including protein, conjugated linoleic acid (CLA), minerals (iron, zinc and selenium), vitamins (B, E), glutathione, ubiquinone, lipoic acid etc. **(Arihara, 2006; Arihara and Ohata, 2011)**. Nowadays, the functional properties of meat are associated with biologically active peptides, such as L-carnitine, carnosine, anserine, creatine, taurine etc. **(Arihara, 2006; Udenigwe and Howard, 2013; Lafarga and Hayes, 2014)**. Moreover, meat proteome is a source of bioactive sequences possessed hypotensive, antioxidant, opioid, immunomodulatory, prebiotic, mineral-binding, cholesterol-lowering and antimicrobial activity **(Bauchart et al., 2006; Ahhmed and Muguruma, 2010; Toldrá et al., 2012; Udenigwe and Howard, 2013; Lafarga and Hayes, 2014)**.

During the last decade a special emphasis is paid on peptides, native contained in raw materials or formed during the enzymatic hydrolysis or food processing **(Mine and Shahidi, 2006)**. Moreover, advanced methodological approaches, particularly proteomics, confirmed that proteome and peptidome of any tissue are formed by not only constitutive structural and functional proteins and peptides, but also are characterised by a number of specific molecules involved in maitance of normal physiological condition **(Fagerberg et al., 2014)**.

In this regard, the study of by-products as sources of bioactive sequences involved in normalization of the metabolic disorders is very relevant to the food industry as well as development specialized and functional products on their basis.

Scientific hypothesis

Previously, authors revealed that introduction of native tissues of cattle and porcine hearts and the aortas into the diet of hyperlipidemic rats led to a significant decrease of total cholesterol, triglycerides and atherogenic fractions of lipoproteins in serum **(Chernukha et al., 2014)**. Porcine tissues possessed the greatest efficiency.

Proteomic study revealed the presence a number of specific proteins in tissues of porcine aortas: apolipoprotein A-1 involved in the formation of high density lipoproteins, peroxiredoxin-1 (in mixture with transgelin) involved in the

suppression of oxidative stress, galectin-1 induced apoptosis of T-lymphocytes, a number of heat shock proteins as well as about 22 tissue-specific peptides with unknown function.

Fatty acid-binding protein and about 6 tissue-specific peptides with unknown function were detected in tissues of porcine heart. However, it was found that these bioactive substances are decomposed after sterilization process of the product, except fatty acid-binding protein and several peptides **(Chernukha et al., 2016)**.

Therefore we hypothesized that tissue-specific proteins could decomposed into active peptides with similar biological action. This fact could make it possible to create a functional meat product based on porcine heart and aortas.

MATERIAL AND METHODOLOGY

Meat product production

Meat product for specialized nutrition was produced on ZAO "Yoshkar-Olinskiy Myasokombinat". Porcine hearts were chopped with a particle size of 2-3 mm and salted for 12 h. Porcine aortas were chopped with a particle size of 2-3 mm and homogenized in cutter at 3000rpm for 2-3 min. Minced hearts with the juice were quantitatively transferred in the cutter and homogenized at 3000rpm for 6-8 min (ratio of aorta to hearts 1:3). Obtained mince was packed in cans of lamister and sterilized at 115 °C, a pressure of 0.23 MPa for 40 min. Meat product contained 17.53 ±0.95% protein, 3.82 ±0.13% fat, 0.305 ±0.015% sodium chloride, and 2.35 ±0.25% starch.

Animal experiments

Thirty male Wistar rats (380 ±20 g) aged approximately 1 year were kept in conventional standard conditions; water and feed were available ad libitum. Animals were randomly divided in 3 groups: group 1 – negative control (n=10); group 2 – positive control (n=10) and group 3 – experimental animals (n=10). Animals in group 1 (negative control) got a standard chow (Labkorm, Russia) ad libitum during the experiment. Rat model of alimentary hyperlipidemia was developed by adding cholesterol (2.0-10.0%) and fat (10.0 – 25.5%) to the standard diet and vitamin D2 injection per os (35,000 IU/kg b.w.). After modeling, rats in group 2 (positive control) were fed with standard chow, in group 3 – meat product (8g/kg b.w.) with standard chow.

On the 42nd day rats were euthanized in VETtech camera according to the rules of the animal welfare, blood samples biochemical investigations were taken.

Biochemical analysis

Biochemical investigations were carried out on automatic analyzer BioChem FC-360 (HTI, USA) according to instructions applied to measurement kits (HTI, USA). Total cholesterol (TCL), triglyceride (TG), cholesterol low-density lipoproteins (CL LDL) and cholesterol high-density lipoproteins (CL HDL) levels were measured in rat serum. Cholesterol non-LDL and non-HDL was calculated as the difference between TCL and CL LDL and HDL. Atherogenic index (AI) = (TCL - CL HDL)/ CL HDL.

Immunoassay

Evaluation of apolipoprotein A-1 (Apo A-1), apolipoprotein B-100 (Apo B-100), and apolipoprotein E (Apo E) levels in the serum were measured on ImmunoChem 2100 (HTI, USA) using the respective commercial ELISA kits, according to the respective instructions (Cloud-Clone Corp., China).

Statisic analysis

STATISTICA 10.0 software was used in this study for the statistical analyses. Significant differences were tested by using two-way analysis of variance (ANOVA), followed by Duncan's test. Differences with *p*-values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Long-term consumption of a diet enriched with cholesterol and animal fats led to the increase of TCL and of atherogenic fractions of lipoproteins and TG in rat serum. On the 42nd day after the cancellation of proatherogenic diet the concentration of TCL and TG in rat serum of group 2 exceeded the group 1 level by 35.8% (p <0.05) and 17.0% (p <0.05). Redistribution of lipoprotein fractions was also noticed: CL LDL cholesterol increased by 15.5% (p <0.05) as well as CL non-LDL and non-LDL by 2.3 fold (p <0.05) (Table 1).

Introduction of meat product into rat diet (group 3) led to a decrease in the serum concentration of TCL (31.8%, p <0.05) and TG (by 28.2%, p <0.05) compared with group 2. Redistribution of lipoprotein fractions was also noticed: CL LDL cholesterol decreased by 21.6% (p <0.05) as well as CL non-LDL and non-LDL by 2.4 fold (p <0.05) (table 1).

Elevation of athergenic lipoproteins rate in group 2 resulted in a significant increase of AI by 59.5% compared to group 1, while in group 3 it reduced by 41.3% compared with group 2 (table.1).

Apolipoprotein levels directly correlate with the concentration of lipoproteins circulating in the blood. Apo A-I is the major protein of HDL and provide HDL binding with receptor, but also detected in chylomicrons (minor). Apo B-100 is the main ligand of very low and middle density lipoproteins (VLDL, MDL) and LDL to corresponding receptor. Apo E is the protein part of chylomicrons, VLDL, MDL and HDL (minor) and also provides ligand-specific regulation of lipoprotein-receptor binding process responsible for cholesterol and cholesterol ether transfer from blood to internal organs and tissues, mostly in liver **(Olofsson et al., 2007; Camejo et al., 2014)**.

It is interesting to note that a significant increase of Apo A-I by 36.5% (p <0.05) and 38.0% (p <0.05) was revealed in group 2 compared with group 1 and 3, while CL HDL between all groups was not significantly changed. On the contrary, changes in the content of Apo B-100 were not observed. However, the concentration of CL LDL in group 3 was lower by 21.6% (p <0.05) than in group 2.

On the other hand, the content of APO E in group 2 was significantly increased by 53.2% (p <0.05), while in group 3 decreased to group 1 level (Table 1). These changes correlated with a decrease in the concentration of CL non-LDL and non-LDL, characterizing a content of atherogenic lipoproteins such as chylomicrons, VLDL and MDL.

Table 1 Serum lipid profile and apolopoprotein concentration in rat serum.

Group	AI (relative units ±SD)	TG (mmol.L^{-1} ±SD)	CL (mmol.L^{-1} ±SD)			
			Total	LDL	HDL	non-LDL and non-HDL
Group 1	1.58 ±0.09	1.76 ±0.27	2.18 ±0.12	0.84 ±0.05	0.85 ±0.03	0.49 ±0.06
Group 2	2.52 ±0.12*	2.06 ±0.33	2.96 ±0.08*	0.97 ±0.02	0.88 ±0.06	1.12 ±0.03*
Group 3	1.48 ±0.22$^{\#}$	1.48 ±0.18	2.02 ±0.19$^{\#}$	0.76 ±0.11$^{\#}$	0.81 ±0.03	0.47 ±0.14$^{\#}$

Apolipoproteins

Group	Apo A-I (mg.mL^{-1} ±SD)	Apo B-100 (mg.mL^{-1} ±SD)	Apo E (µg.mL^{-1} ±SD)
Group 1	0.444 ±0.016	2.506 ±0.207	21.449 ±0.795
Group 2	0.586 ±0.020*	2.504 ±0.200	32.851 ±1.701*
Group 3	0.424 ±0.027$^{\#}$	2.632 ±0.177	25.326 ±3.644$^{\#}$

Note: *Significant different while compared with group 1, $^{\#}$Significant different while compared with group 2.

Thus, despite of CL LDL reduction in group 3, there were no significant differences in the change of Apo B-100 concentration between the experimental and control groups. Therefore this observation may be linked to an increase of MDL and VLDL, whereas the increased content of Apo A-I in the serum of positive control animals may be correlated not only with increase of HDL, but also with elevated chylomicron content. The hypothesis is confirmed by the increase of Apo E in the serum of positive control rats. Apo E content is correlated with the level of chylomicrons, MDL and VLDL summarized in CL non-LDL and non-LDL, and TG, which were in positive control group by 2.3 times (p <0.05) and 28.2% higher than in group 1.

According to scientific hypothesis mentioned above, identified tissue-specific proteins could be decomposed into active peptides with similar biological action or observed hypolipidemic effect could be linked with saved after sterilization tissue-specific peptides (**Chernukha et al., 2016**). More of them were identified in porcine aorta.

In two reference studies, authors investigated the influence of fractions containing target proteins and peptides in animal experiments. It was revealed that *per os* injection of low-molecular ultrafiltrate (Mw<30kDa) during only 14 days to hyperlipidemic Guinea pigs lead to total cholesterol content and atherogenic index reduction by 44.1% and 43.7%, respectively, due to CL LDL and CL non-LDL and non-LDL levels decrease by 43.7% and 79.5%, respectively. Moreover, such inflammatory markers as serum C-reactive protein (CRP) and vascular endothelial growth factor (VEGF) was also reduced by 34.0% and 58.3% on average, respectively **(Chernukha et al., 2015)**. CRP is knewn as an acute phase protein involved in general atherosclerosis inflammation by attracting monocytes into atherosclerotic plaques zone by binding to a specific receptor **(Seidova 2005; Huang et al., 2014)**. VEGF is a key regulator of the formation of microvessels and also known as a factor of endothelium disfunction **(Mangilova, 2012; Swirski and Robbins, 2013)**.

In second reference study authors revealed that *per os* injection of peptide ultrafiltrate (Mw<5kDa) during only 14 days to hyperlipidemic rats mainly contributed to normalization of inflammation and demonstrated regulatory activity, while that *per os* injection of ultrafiltrate containing middle weight proteins (Mw=5-30kDa) lead to total cholesterol content and atherogenic index reduction by 30.1% and 40.7%, respectively, due to CL non-LDL and non-LDL levels decrease by 66.9% **(Kotenkova, 2017)**.

Thus, in both two reference studies hypolipidemic effect was observed after 14 days of per os injection of fractions containing target substanses, while meat product introduction into diet of rats with hyperlipidemia lead to noticeable serum lipids reduction only on 42nd day. This fact could be explained by target protein breakdown into peptides with less residual biological activity. On the other hand, numerous publications also confirmed structural proteins as a good sourse of bioactive peptides, including peptides with lipid-lowering action **(Bauchart et al., 2006; Ahhmed and Muguruma, 2010; Toldrá et al., 2012; Udenigwe and Howard, 2013; Lafarga and Hayes, 2014)**. In this regard, heart tissue is enriced with muscle tissue proteins, while aorta – collagen and elastine, which are a source of GLY-PRO peptides with hypolipidemic action **(Lyapina et al., 2015)**. Presumably, active peptides could be generated both during meat product processing and digestion processes.

CONCLUSION

Developed meat product contributed to serum lipid level reduction in hyperlipidemic rats, mainly due to the decrease of chylomicrons and VLDL content, which is resulted in AI reduction by 41.3% (p <0.05). Despite on the decomposition of functional protein and peptides compounds after heat treatment process, a pronounced lipid-lowering effect of the developed product was noted. This effect can be linked with protein breakdown during meat product processing and digestion processes which occur on peptides with less residual biological activity.

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