

**Research Article****Effect of the use beef with a modified fatty acid composition on the development of atherosclerosis in experimental mice**

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**ABSTRACT**

This article presents the results of studying the effect of modified beef fat on plasma lipoproteins and the progression of atherosclerosis in experimental mice. An assessment is made of the possibility of using modified beef for the correction of the fatty acid profile of the blood of warm-blooded animals.

**Keywords:** polyunsaturated fatty acids, beef, atherosclerosis prevention, cattle breeding, lipid metabolism, in vivo experiments.

**INTRODUCTION**

The fatty acid composition of beef, at least partially, depends on the type of feed. Grass and clover contain a large amount of  $\alpha$ -linolenic acid, while grains are usually rich in linoleic acid. While most unsaturated fatty acids are biodegradable in the rumen, a significant amount of them avoid this process and end up in the tissues of the animal. These fatty acids can later be extended and desaturated (become unsaturated) to obtain a number of other fatty acids. In the case of  $\alpha$ -linolenic acid, this can lead to the formation of both EPA and DHA. The purpose of this study was to determine whether there is a difference in the fat-acid composition of herbal / clover-fed beef compared to animal feed (grain) fattening animals sufficient to affect plasma lipids and develop atherosclerosis on mouse models. For these purposes they used a model of mice with

artificially induced atherosclerosis, which makes it possible to model the profile of lipoproteins, as in humans. Such mice easily develop dialysis hyperlipidemia and atherosclerosis and are very sensitive to changes in the content of fatty acids in the diet.

**MATERIAL AND METHODS**

The beef used in this experiment was selected from 30 animals of the Kalmyk breed (age 22 weeks) contained on one of two diets; 1) fodder (grass / white clover during grazing, and grass silage in winter), 2) barley straw and animal feed (40:60 based on DM). After slaughter, animals were sampled from M. obliquus internus abdominis and frozen for transport to the laboratory. Samples were thawed, ground, freeze-dried, and ground to a powder. Samples of each dietary group were thoroughly mixed

before freeze-drying to ensure uniformity of collective samples.

Mice were randomly divided into two feeding groups (n = 10) with an average age for each group of 12 weeks. Both groups received a semi-synthetic diet containing 36% (w / w) of freeze-dried beef, either herbal (FB) or compound feed (CB) fattening, both diets also contained 0.25% cholesterol. Duration of feeding was 12 weeks, the animals were given fresh food every morning, and the remains were weighed, so daily food intake could be observed throughout the study. At the end of week 12, mice were collected for serum and frozen for subsequent analysis of lipids / lipoproteins. Liver to determine the composition of fatty acids and gene expression studies. The composition of fatty acids in diets and liver was determined using gas chromatography FAME. The heart and aorta were frozen, having previously separated the aorta at the point of connection with the heart. Every third part was sequentially stained

with hematoxylin and an isochrome diazo dye (oil-red O), and the staining area was determined by the latter to determine the degree of atherosclerosis. The aortic section, in which the first 3 tricuspid sheets appeared, was nominally designated, and atherosclerosis was identified in this and five more sections.

## RESULTS AND DISCUSSION

Table 1 shows the fatty acid composition of beef, and the total lipid composition of the liver of mice. Both sources of beef contained similar amounts of saturated fatty acids. The beef on FB was relatively rich in oleic acid,  $\alpha$ -linolenic acid, and EPA compared with CB beef diet, but contained significantly less linoleic acid. The livers of mice fed the FB diet of bovine contained significantly more  $\alpha$ -linolenic acid, EPA and DHA than animals fed the CB diet of the diet. The presence of a higher concentration of DHA indicates significant elongation and desaturation of  $\alpha$ -linolenic acid.

**Table 1.** Fatty acid composition of beef and liver of mice

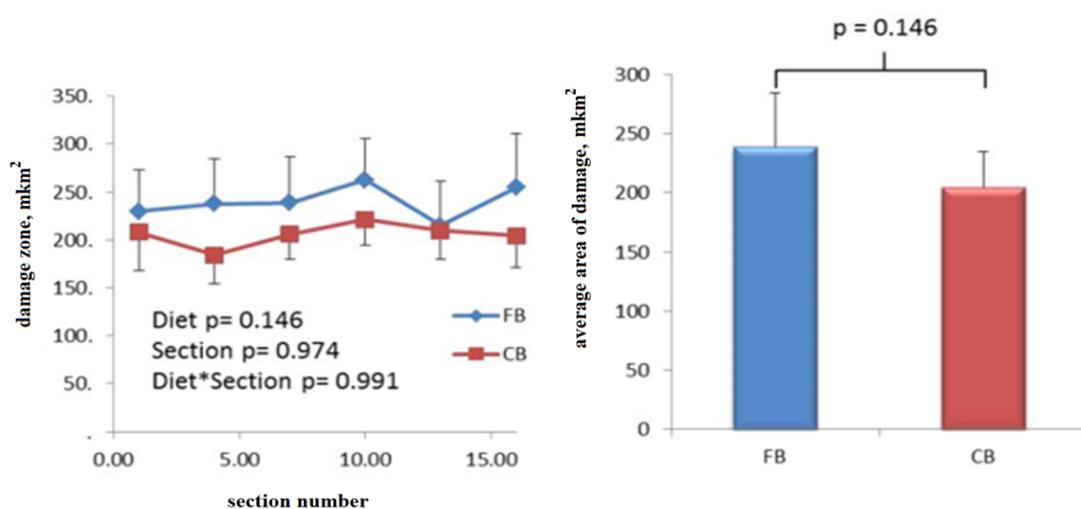
Total content of fatty acids,%	Beef (n = 6 replications)			Composition of the liver of mice		
	Grass feeding	Feed fattening	P	Grass feeding	Feed fattening	P
16: 0 palmitic	29.14 ± 0.75	30.71 ± 0.62	0.132	18.48 ± 0.41	16.47 ± 0.39	0.011
18: 0 stearic	13.37 ± 1.16	12.71 ± 0.56	0.605	7.29 ± 0.34	5.30 ± 0.32	<0.001
18: 1 n-9 oleic acid	38.09 ± 0.89	34.95 ± 0.76	0.021	46.34 ± 1.20	47.79 ± 1.02	0.143
18: 1 trans11	1.63 ± 0.11	2.29 ± 0.16	0.007	0.22 ± 0.10	0.58 ± 0.20	0.280
18: 2 n-6 linoleic	0.91 ± 0.08	2.55 ± 0.24	<0.001	6.45 ± 0.17	6.08 ± 0.18	0.148
CLA c9, t11	0.39 ± 0.03	0.24 ± 0.05	0.018	0.31 ± 0.07	0.09 ± 0.04	0.019
18: 3n-3 $\alpha$ -linolenic	0.74 ± 0.05	0.22 ± 0.06	<0.001	1.17 ± 0.05	0.98 ± 0.05	0.013
20: 4n-6 arachidonic	0.40 ± 0.05	1.03 ± 0.16	0.004	4.37 ± 0.28	4.54 ± 0.40	0.727
20: 5n-3 eicosapentaenoic	0.21 ± 0.03	0.06 ± 0.02	<0.001	0.24 ± 0.05	0.00 ± 0.00	<0.001
22: 5n-3 docosapentaenoic	0.39 ± 0.05	0.24 ± 0.03	0.022	0.39 ± 0.07	0.08 ± 0.03	0.005
22: 6n-3 docoheptaenoic	0.05 ± 0.01	0.03 ± 0.01	0.235	5.00 ± 0.27	2.52 ± 0.19	<0.001
The amount of saturated fatty acids	47.78 ± 1.06	48.70 ± 1.19	0.583	27.21 ± 0.66	22.60 ± 0.59	<0.001
Amount n-3	1.38 ± 0.12	0.55 ± 0.04	0.001	6.82 ± 0.23	3.57 ± 0.14	<0.001
Amount n-6	1.36 ± 0.13	3.96 ± 0.44	0.001	10.82 ± 0.42	10.62 ± 0.53	0.771

Table 2 presents plasma lipids and liver lipoproteins in mice consuming two types of beef. In animals fed with FB grass-fed beef, plasma triacylglycerol and HDL cholesterol levels were significantly lower than in animals fed with CB feed-fed beef.

In animals on both types of diets, the area of the lesion was consistent over the entire length of the aorta under study, and no significant effect of the diet on the lesion area was revealed.

**Table 2.** Plasma lipids and liver lipoproteins in mice, on two types of food

mMol/l	FB	CB	P
Total cholesterol	10.27 ± 0.62	11.28 ± 0.7	0.297
Cholesterol HDL (high density lipoprotein)	1.14 ± 0.06	1.41 ± 0.1	0.038
LDL cholesterol (low density lipoprotein)	9.13 ± 0.57	9.87 ± 0.67	0.496
LDL / HDL ratio	8.04 ± 0.37	7.22 ± 0.55	0.233
Total triacylglycerol	2.49 ± 0.22	3.16 ± 0.19	0.035



**Figure 1.** Damage area in mice feeding on different beef types

## CONCLUSION

Herb-fed beef had a significant difference in fatty acid composition than on a compound feed diet, the first one being enriched with n-3 PUFAs. This was reflected in the accumulation of n-3 PUFA in the liver of mice that consumed grass-fed FB, and further lengthening and desaturation of  $\alpha$ -linolenic acid was proved by increasing the concentration of DHA. Mice fed grass-fed FB bovine had low serum triglycerides and HDL cholesterol compared to those that received CB-fed formula-fed beef.

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