EVALUATION OF THE BIOLOGICAL EFFECTIVENESS OF SAUSAGES WITH MODELLED AMINO ACID AND FATTY ACID COMPOSITIONS

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SUMMARY: From the point of the latest requirements of physiology, the discrepancy between the medico-biological properties of the traditionally manufactured sausages and the steadily increasing number of patients suffering from hyper-lipemia (or its after-effects) necessitated the formulation of meat products with modelled amino acid and fatty acid compositions designed for nutrition of strictly differentiated groups of people.

The objective of the present work was to ascertain whether sausages with modelled amino acid and fatty acid compositions had better biological effectiveness than the same sausages

manufactured by traditional methods. To solve the issue, we carried out a biological experiment with white Wistar male rats as test animals that were fed according to a modified method of split feeding with test and control samples.

The results obtained for the coefficient of nutritive effectiveness were 0.344 and 0.316 for the test samples and were significantly higher than that for the control, 0.207. Similar were the results for the coefficient of protein effectiveness PER (1.73 and 1.71 for the test samples, and 1.51 for the control).

There were observed better characteristics for the lipid metabolism in the rats fed on test samples. Thus for instance, the amount of cholesterol was 2.26 and 2.79 mmol/l for the studied sausage samples, and 3.72 mmol/l for the control, while the amount of triglycerides was 0.61 and 0.68 mmol/1, and 0.72 mmol/l for the control.

It was concluded that the studied sausages possessed higher nutritive effectiveness, better balanced amino acid composition of the proteins resulting in better coefficient of pro-tein effectiveness, and had better characteristics for lipid metabolism compared to those of the control sample. These characteristics indicated that the studied sausages could be used as part of prophylactic diets aimed at restricting the risk of hyperlipemia.

INTRODUCTION: The need of formulating new foodstuffs for dietary and prophylactic nutrition is called forth by the immense market shortage of these products. On the other hand, the contemporary level of the science of nutrition, and the constant changes in the food industry, not only make possible, but also oblige us to apply strict scientific approaches and to look for ways of receiving foodstuffs with programmed quality that respond to the specific requirements in relation to a particular disease (Rogov et al., 1988).

A step in this direction are two experimental sausages that are the subject of the present study. They are designated as dietary and prophylactic food in cases generally referred as hyperlipemia; their consumption helps the introduction of complete protein, and at the same time reduces the risk of atherosclerosis, cardiac ischemia, and obesity.

There are a few changes made in the new meat products compared to the traditional varieties. The fat content is reduced and the amount of animal fats is restricted to a minimum. A part of these are substituted by vegetable fats so the sausages have the desired organoleptic properties and balanced fatty acid composition. The amino acid composition is also balanced and responds to the changes in the amino acid and fatty acid metabolism of patients with atherosclerosis, cardiac ischemia, and people endangered by these diseases (Table 1).

The objective of the present work is to follow experimentally the characteristics of the biological value of protein, the nutritive effectiveness of both products, and the effect of their consumption on the lipid metabolism as well as to find out how much has been achieved of the goal to model the compositions of such products.

MATERIALS AND METHODS: The experiments were carried out on white Wistar male rats with average body weight of 56.2-1.5 g (Pokrovekii, 1975) that were divided into 4 groups, each group consisted of 12 animals placed in individual metabolic pens (Stoinev et al., 1978). The animals of group I received test sausage, group II - test frankfurters, group III - control sample (traditional sausage). Group IV was kept on proteinfree diet. Because of the fact that the study of whole indivisible products is not standardized, we used a modification of the method of "split feeding" of Jacquot and Perret (Jacquot, Perret, 1972; Payne, 1972). The test animals sausage for consumption ad libitum for two hours daily and that was their only source of proteins and fats. During the rest of the day they received protein-free food.

In the course of 15 days, the following factors were followed: daily consumption (total amount of proteins, fats, carbohydrates and energy) was measured every other day; body weight was also taken down every other day; blood samples: cholesterol, HDL-cholesterol, triglycerides, and lipidogram, were taken on the 15th day after killing the animals.

cholesterol, HDL-cholesterol, triglycerides, and lipidogram, were taken on the 15th day after killing the animals. It was thus possible to determine the following factors: 1) Daily consumption of food, nutritive substances and energy, and dynamics of the average daily consumption; 2) Average daily growth of body weight, and growth dynamics; 3) The first two factors were used to calculate the biological value of proteins. Since the feeding was not carried out in accordance to the standard methods of evaluation of protein effectiveness (because of the above mentioned necessity of "split feeding"), the factors were relative but still used for comparing the three types of protein diets in the present experiment; 4) The nutritive effectiveness on the basis of the first two factors; 5) Lipid metabolism: cholesterol, HDL-cholesterol, triglycerides, and lipidogram.

## **RESULTS AND DISCUSSION:**

1. Body weight growth dynamics (Table 2). The body weight of all animals fed on sausage increased steadily. The fastest rate was registered with the test sausa-ge, then followed the test frankfurters, and the slowest growth rate was with the control sausage. The differences between the test sausages and the control were statistically significant while the differences between the test groups were insignificant. All sausages provided satisfactory daily growth of body weight that was entirely normal for meat diet.

2. Dynamics of the consumption of food, nutritive substances and energy (Table 3).

The average daily consumption of food was satisfactory, and sausage consumption was even higher in view of the very short period (2 hours daily) when the animals were admitted to food. The test sausage consumption only (8.08-1.84 g/24 h) was significantly higher than the consumption of the control sausage (5.41-1.35 g/24 h) (p<0.01). The results from the regression equations expressing the dynamics of the sausage consumption were analogous.

The daily protein consumption differed very much in the three groups. For the test sausage it was 1.49-0.34 g/24 h, for the test frankfurters 1.42-0.16 g/24 h, and these were si-gnificantly higher than for the control (0.74-0.18 g/24 h). It was obvious that under the conditions of split feeding when protein was not available to the animals throughout the whole day and because of the comparatively lower protein content of the control sausage, the animals did not receive the necessary proteins regardless the ability of their bodies to regulate the protein admission.

The daily consumption of fats was not so much different, and that of carbohydrates was almost the same for the three. test groups. The daily energy admission was also similar, how ever, it could be noted here that the energy received at the expense of the sausage was the highest in the control group, and the energy received from protein consumption was significantly higher with the experimental sausages. The explanation lied not so much in the differences in relation to the consumption but in the greated differences in the fat contents of the sausages, and the highest was in the control sausage.

3. Protein effectiveness (Tables 7 and 8).

PER was high and characterized the proteins in the three sausages as high-grade that was the normal result for proteins of animal origin. The lower values for the control sausage (1.51-0.39) were probably due to the presence of proteins of non-muscular origin. Similar were the results for NPR: higher values were observed for the test sausages. Again we should repeat the proviso about the relativity of the protein effectiveness factors in this type of feeding, and the fact that they were used only for comparison between proteins in the present experiments, we could hold that the test sausages had high-grade proteins.

4. Nutritive effectiveness (Table 6).

The nutritive effectiveness of the three diets was high. The differences, however, between the test and control sausages were significant. Beside that, having in mind the differ-ences between the fat and energy levels that were in favour of the control sausage, the results for the nutritive effectiveness testified to the very good quality of the proteins in the test sausages.

5. Lipid metabolism (Table 9).

The lipid metabolism factors were determined by clinical methods that were applied to analyse the lipid metabolism and the risk for cardiac ischemia. They were able to define the momentary body state as well as the treatment effect and the prophylaris of that socially important disease.

Prophylaxis of that socially important disease. The level of serum cholesterol - 2/3 bound with \$\vert -lipoproteins (LDL-cholesterol), and 1/3 bound with \$\vert -lipoproteins (HDL-cholesterol) is the main factor for cardiac ischemia. In the present experiment, the highest level of serum cholesterol was observed in the animal group fed on the control sausage - 3.72-0.44 mmol/1. The values were higher enough than those for the remaining sausages. A more interesting result was observed when comparing the cholesterol of the experimental groups to the initial values of each group during the period when the animals had received the standard food for rats that was almost fat-free. There were no significant differences between them. The fact that, regarding the fat content, the test sausages were rather close to the control while, regarding serum cholesterol, they were closer to the fat-free diet, was an explicit argument in favour of the quality of the fats in the test sausages. This was in support of the decision to substitute animal proteins with vegetable ones and so change the fat argument animal protein with vegetable ones and so change the fat argument animal protein with vegetable ones and so change the fat argument animal protein with vegetable ones and so change the fat animal a significantly lower level of serum cholesterol - the basic risk factor for cardiac ischemia.

basic risk factor for cardiac ischemia. The serum level of HDL-cholesterol has a great importance because of its supposed antianterogenic role. Thus, the higher the HDL-cholesterol levels, when the cholesterol:HDL-cholesterol ratio is in favour of the latter, the lesser the risk of cardiac ischemia. This factor also classified the test sausages higher than the control and closer to the values of the fat-free diet.

Triglycerides that are found in the body mainly originate from fats received with food i.e. they are indicators for the exogenic source of fats. Triglycerides are the basic form under which lipids are deposited in the fat depots. In this respect, they are a risk factor and a sygnal for wrong nutrition habits with prevailance of improper fats. The triglycerides in our experiment were found in the highest levels in the animal group fed on control sausage (0.72-0.13mmol/1), and the lowest levels were found in the group with standard diet for rats (initial values). The studied test sausages stood between them. This was, therrfore, a very good result for sausases, asn we again confirmed the statement about the suitable kind of fats in the approbated sausages.

CONCLUSIONS: 1. The test sausage and the test frankfurters have high protein effectiveness coefficients that charcterize them as high-grade protein products. They satisfy the needs for nutritive matter and ensure steady growth of body weight. field. The nutritive effectiveness of both sausages is signi-

ficantly higher than that of the control. 3. From the results for the lipid metabolism we conclude that both test sausages differ strongly from the control. They have lower cholesterol levels and higher HDL-cholesterol percentage as well as lower levels of triglycerides. They have significantly lower atherogenic effect and can be recommended for dietary nutrition or prophylaxis of hyperlipidemia. This is due to a lesser extent to the reduced fat content in the sausages, and more to their good fatty acid composition. 4. The experimental studies on both sausages indicate that

4. The experimental studies on both sausages indicate that the objectives set forth before their formulation have been fulfilled. They can be offered for clinical tests in medicinal or prophylactic nutrition of hyperlipoidemias and cardiac ischemia risk groups.

Table 1. Chemical composition of the test sausages.

Product	Water	Total	Fats	Salt	Ash
	(%)	(%)	(%)	(%)	(%)
1. Sausage	67.2	18.5	11.7	1.5	1.1
2. Frankfu	rter66.75	19.75	10.5	1.0	2.0
3. Control	. 67.5	13.7	14.9	2.0	1.9

Table 2. Test animal body weight growth.

Product	Ave. Daily Growth	Growth Dynamics (g/d)
1. Test sausage	$2.56 \div 0.77$ n = 11	y= 60.90 + 3.05 . x Sy= 1.25; r=0.997
2. Test frankfurters	$2.46 \stackrel{+}{=} 0.42$ n = 10	y= 63.65 + 2.99 . x Sy= 1.39; r=0.996
3. Control	$1.17 \stackrel{+}{=} 0.51$ n = 11	y= 59.45 + 1.46 . x Sy= 2.53; r=0.951

n = number of tests

y = test animal body weight

x = day

Sy= standard mean square deviation

 $\mathbf{r}$  = correlation coefficient

Table 3. Test consumption of sausages and protein-free food

Product	Daily consumption of food (g)	Sausage consump- tion dynamics (g/d)	Protein-free consumption dynamics (g/d)
<ol> <li>Test Sausage</li> <li>Test Frankftrs</li> <li>Control</li> </ol>	$8.04^{\pm}1.84$ n=11 $7.16^{\pm}0.79$ n=10 $5.41^{\pm}1.35$ n=11	y=-3.36+3.61.x Sy=3.74; r=0.997 y= 0.59+7.44.x Sy=2.55; r=0.998 y=-0.89+5.6.x Sy=4.01; r=0.991	y=3.14+6.99.x Sy=1.40;r=0.999 y=3.98+8.31.x Sy=2.55;r=0.998 y=2.63+7.93.x Sy=1.69;r=0.999

Product	Daily consumption of sausage prote- ins (g)	Daily consumption of sausage fats and protein-free food (g)	Daily consumption of carbohydrates (from protein- free food) (g)
1. Test Sausag	1.49 <sup>+</sup> 0.34	0.94-0.21	5.83-1.28
2. Test Frankf	1.42 <sup>±</sup> 0.16 trs	0.75-0.08	6.09±0.67
3. Contr	ol 0.74 <sup>+</sup> 0.18	0.80+0.20	6.47-1.38

Table 4. Daily consumption of nutritive matter.

Table 5. Received energy through consumption of nutritive matter from sausages and protein-free food.

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Da En Product (1	aily hergy kJ)	Daily Energy from Sausage (kJ)	Daily Energy from Proteins (kJ)	Daily Energy from Fats (kJ)	Daily Energy from Car- bohydra- tes (kJ)	Energy Balanced Sausage Consmpt. (kJ/kJ)
1.Test 10 Saus.	62.3 <del>*</del> 33.26	62.21 <sup>±</sup> 14.2	25.54 <sup>±</sup> 14.2	36.07 <sup>±</sup> 8.37	100.11+	0.05+
2.Test 1	58.1 <sup>±</sup> 17.37	53.7 <del>*</del> 5.89	24.34 <sup>+</sup> . 2.67	29.29 <sup>±</sup> 3.22	104.47-	0.04+ 0.005
3.Con-15 trol	55.08± 25.46	44.11 <sup>±</sup> 10.98	12.71 <sup>±</sup> 3.16	31.39 <sup>±</sup> 7.81	110.97 <sup>±</sup> 23.76	0.04+

Table 6. Nutritive effectiveness characteristics.

Product	Nutritive Effective- ness of Sausage	Theoretical Nutritive Effectiveness of Sausage (g-sausage/g-growth)		
1. Test Sausage	0.316 ± 0.05 y Sy	= -0.31 + 0.356.x; = 0.43 $r = 0.848$		
Frankftrs	0.244 - 0.05 y Sy	= 0.36 $r = 0.5937$		
3. Control	0.207 ± 0.054 y Sy	= 0.41 + 0.315.x; = 0.30 r = 0.801		

Table	7.	Protein	effectiveness	of	the	studied	sausages.
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Product	PER	EPER	Theoretical PER (g-protein/g-growth)		
1. Test Sausage	1.71 - 0.29	0.011 - 0.002	y = 0.25 + 1.93.x Sy = 0.43; r = 0.8484		
2. Test Frankfrts	1.73 - 0.24	0.011 - 0.002	y = 0.17 + 1.61.x Sy = 0.36; r = 0.5937		
3. Control	1.51 ± 0.39	0.009 - 0.002	y = 0.708 + 1.53.x Sy = 0.20; r = 0.9239		

Table 8. Relative protein effectiveness of the studied sausages.

Product	NPR	ENPR	Theoretical NPR		
1. Test Sausage	2.10 - 0.29	0.013 - 0.003	y = -0.57 + 2.09.x Sy = 0.339; r = 0.98		
2. Test Frankfrts	2.13 ± 0.25	5 0.013 <sup>±</sup> 0.02	y = -0.57 + 2.13.x Sy = 0.28; r = 0.9841		
3. Control	2.32 ± 0.24	4 0.009 ± 0.003	y = -0.60 + 2.40 Sy = 0.16; r = 0.9856		

Table 9. Blood lipid characteristics in test animal groups.

Animal Group	VLDL- Lipopro- teins	LDL- Lipopro- teins	HDL- Lipopro- teins	Trigly- cerides	Chole- sterol (total)	HDL- Chole- sterol
Standard	20.22 <sup>+</sup>	33.88 <sup>+</sup>	45.89 <sup>+</sup>	0.41 <sup>±</sup>	2.78+	1.92 <sup>±</sup>
Food	1.86	2.09	3.26	0.099		0.28
Test	26.25 <sup>+</sup>	36.83+	31.92 <sup>+</sup>	0.68+	2.79 <sup>±</sup>	1.66+
Sausage	3.65	2.12	3.75		0.28	0.28
Test	24.58 <mark>+</mark>	36.25 <sup>+</sup>	39.17 <sup>+</sup>	0.61+	2.65+	1.57-
Frankfrts	4.14	3.19	2.59	0.17	0.47	0.177
Control	21.58 <sup>±</sup>	43.5 <del>*</del>	34.92 <sup>+</sup>	0.72+0.13	3.72 <sup>+</sup>	2.02+
Sausage	5.25	4.62	1.98		0.44	0.26

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