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# ASSESSING THE EFFECT, ON ANIMAL MODEL, OF MIXTURE OF FOOD ADDITIVES, ON THE WATER BALANCE

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#### ABSTRACT

**Purpose.** The purpose of this study was to determine, on the animal model, the effect of modification of diet composition and administration of selected food additives on water balance in the body.

Material and methods. The study was conducted with 48 males and 48 females (separately for each sex) of Wistar strain rats divided into four groups. For drinking, the animals from groups I and III were receiving water, whereas the animals from groups II and IV were administered 5 ml of a solution of selected food additives (potassium nitrate - E 252, sodium nitrite - E 250, benzoic acid - E 210, sorbic acid - E 200, and monosodium glutamate - E 621). Doses of the administered food additives were computed taking into account the average intake by men, expressed per body mass unit. Having drunk the solution, the animals were provided water for drinking.

Results. The mixture of selected food additives applied in the experiment was found to facilitate water retention in the body both in the case of both male and female rats, and differences observed between the volume of ingested fluids and the volume of excreted urine were statistically significant in the animals fed the basal diet. The type of feed mixture provided to the animals affected the site of water retention – in the case of animals receiving the basal diet analyses demonstrated a significant increase in water content in the liver tissue, whereas in the animals fed the modified diet water was observed to accumulate in the vascular bed.

Conclusion. Taking into account the fact of water retention in the vascular bed, the effects of food additives intake may be more adverse in the case of females.

Key words: food additives, rat

#### INTRODUCTION

Contemporarily, the production of foods without additives is inconceivable both from the theoretical and practical point of view [Duda 2003]. They are applied not only to increase the product's quality and sensory attractiveness but also to assure the proper course of technological and storage processes.

The advance of the food industry and modern processing, as well as increasing awareness of the society have caused that consumers' expectations of food are subject to continuous changes. For it is commonly

believed that only natural products are good and safe, it would be best if food could be convenient in use but low-processed, with a long shelf-life but without preservatives, aromatic but without flavour-enhancing substances. Consumers are afraid of food additives as they are associated with "chemical synthesis". In addition, the opinion on "food chemization" and its negative effect on the body is fed by the non-specialist press which often publishes hostile articles many of which contain untrue information on food additives

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[Krygier 2003, Ozimek 2006]. In fact, however, without specified food additives it would be impossible to achieve many traits of a product that are mostly desired by consumers [Zawirska-Wojtasiak 2005]. Anxieties related to the potential detrimental effects of food products containing additives are, nevertheless, understandable. In spite of the fact that all substances permitted for use in food products, on strictly specified terms, are thoroughly examined and should not raise any doubts [Krygier 2005], scientific works increasingly often report on their adverse effects on a human body [Zagórecka et al. 2000]. In contrast, still sparse data is available on the synergistic effect of a mixture of various food additives on body functions and main metabolic pathways [Sadowska and Kuchlewska 2011].

Since many of the food additives most commonly used in food technology and consumed by man are sodium salts, the objective of this study was to determine, on an animal model, the effect of modification of diet composition and administration of selected food additives on water balance in the body.

## MATERIAL AND METHODS

The experiment was conducted at the viviarium of the Chair of Human Nutrition Physiology at the West Pomeranian Technological University in Szczecin on 48 males and 48 females (separately for each sex) of Wistar strain rats at the age of 5-7 months. The experimental procedure was approved by the Local Ethical Commission (approval no. 31/2006).

After one-week conditioning at the vivarium's conditions (temp.  $21 \pm 1^{\circ}$ C, light/dark cycle 12 h/12 h), the animals were weighed and divided into 4 feeding groups (12 rats each, of initial body weight (g): male - I: 445.6 ±37.3, II: 446.7 ±38.0, III: 445.1 ±40.6, IV: 441.1  $\pm$ 49.4; female – I: 233.9  $\pm$ 14.1, II: 234.0  $\pm$ 13.6; III: 233.7  $\pm$ 14.7, IV: 235.6  $\pm$ 18.6). The rats were fed ad libitum with pelleted feed mixtures produced from the same components, apart from the differentiating ones, by the Feed and Concentrates Production Plant in Kcynia, after implementation of the procedure no. 5.14.5. "Cleansing of equipment and devices". Groups I and II were receiving a basal feed mixture containing, among other things, whole grains of wheat and maize, whereas groups III and IV were administered a modified feed mixture in which, compared to the

basal mixture, 83.5% of wheat grain were substituted with wheat flour (type 500), and 50% of maize – with saccharose, which in a certain way imitated the human nutrition manner. Contents of the other diet constituents were the same in both feed mixtures (Table 1). Diet composition was formulated based on recommendations for AIN-93 mixture [Reeves et al. 1993].

**Table 1.** Ingredients contained in feedstuffs applied in the experiment

Component	Basic feed, %	Modified feed, %		
Wheat	36.4	6.0		
Corn grain	20.0	10.0		
Wheat bran	20.0	20.0		
Soya-bean grain	17.0	17.0		
Dry whey	3.0	3.0		
Phosphate 2-CA <sup>1</sup>	0.8	0.8		
Fodder chalk <sup>2</sup>	1.5	1.5		
Fodder salt <sup>3</sup>	0.3	0.3		
Premix LRM <sup>4</sup>	1.0	1.0		
Wheat flour (type 500)	_	30.4		
Saccharose	_	10.0		

<sup>1</sup>CaHPO<sub>4</sub>.

<sup>2</sup>Mainly CaCO<sub>3</sub>.

<sup>3</sup>Mainly NaCl.

<sup>4</sup>Vitamin and mineral Premix LRM contents per kg: IU – A 1 500 000, vit.  $D_3$  100 000; mg – vit. E 8000, vit. K 300, vit. B<sub>1</sub> 1200, vit. B<sub>2</sub> 1200, vit. B<sub>6</sub> 1000, vit. B<sub>12</sub> 8, Se 100, Fe 16 000, Mn 4500, Zn 6000, Cu 1300, J 100, Co 200.

In order to determine the exact chemical composition of feed mixtures applied in the experiment, they were subjected to proximate chemical analyses. Following respective standards, 4 weighted portions were collected from the prepared samples in order to determine contents of: dry matter, crude fat, total protein and total ash. The relative content of carbohydrates was computed from the difference between dry matter content and total content of protein, fat and ash. Results achieved enabled calculating the energetic value of the feed mixtures. Contents of sodium and potassium were assayed with the method of atomic absorption spectrometry, according to the Polish Standard PN-EN ISO 6869:2002 without pt. 8.1-8.4, and Polish Standard PN-EN 14084:2004 without pt. 6.2 (Table 2).

**Table 2.** Chemical composition of feedstuffs applied in the experiment

Component	Basic feed	Modified feed	
Total protein, %	19.2	18.5	
Crude fat, %	2.81	2.33	
Carbohydrates, %	63.8	65.5	
Dry matter, %	91.9	92.3	
Total ash, %	6.09	6.00	
Sodium, %	0.113	0.106	
Potassium, %	0.976	0.896	
Brutto energy			
kcal/g	3.99	3.98	
kJ/g	16.7	16.7	
Metabolic energy			
kcal/g	3.57	3.56	
kJ/g	14.9	14.9	

For drinking, the animals from groups I and III were receiving tap water, whereas the animals from groups II and IV were administered, in the period of enhanced activity, 5 ml of an aqueous solution of selected food additives: sodium nitrite (E 250) in a dose of 0.07 mg/kg body weight, potassium nitrate (E 252) in a dose of 5.07 mg/kg body weight, benzoic acid (E 210) in a dose of 1.39 mg/kg body weight, sorbic acid (E 200) in a dose of 0.51 mg/kg body weight, and monosodium glutamate (E 621) in a dose of 17.65 mg/kg body weight. The doses corresponded to the mean intake of the additives by humans and met: 11.7% of ADI for sodium nitrite, 137.0% of ADI for potassium nitrate, 27.8% of ADI for benzoic acid, and 2.0% of ADI for sorbic acid [Rutkowski et al. 1997].

Every week, after rats weighing, the doses of food additives were updated for they were calculated per body weight unit.

Having drunk the solution of additives, the animals were provided pure, settled tap water for drinking.

Throughout the experimental period, water and feed intakes by animals were controlled every day, whereas their body weights were measured once a week, always in the same period of day. The rats were weighed exact to 1 g. The total body weight gains were computed from the difference between the final and the initial body weight of the animals.

In the sixth week of the experiment, the animals were placed in 3701M081 type metabolic cages by Tecniplast Italy, and after 48-h conditioning water intake and urine excretion were determined for the period of 24 h.

After 7 weeks of the experiment, the animals were anaesthetized with Ketanest. Their blood was sampled and analysed for the hematocrit value. Afterwards, their livers, as well as scapular and thigh muscles (*m. quadriceps femoris, m. biceps femoris, m. semimembranosus, m. adductor femoris*) were collected for analyses.

The hematocrit value was determined with the microhematocrit method by centrifugation of full blood, at the rate of 12,000 rpm for 5 min, in heparinized capillaries by "Polamed" company, using an MPW 52 centrifuge no. 264.

Muscle tissue analysis was conducted for scapular and thigh muscles, taking into account the division according to sex of the animals and feeding groups. In order to obtain a homogenous sample, the collected muscle tissue was threefold ground and homogenized. Thus the prepared sample was determined for:

- content of dry matter with the method of samples drying to a constant weight [PN-ISO 1442:2000]
- content of total ash with the method of ashing to a constant weight [PN-ISO 936:2000]
- content of water computed from a difference between sample mass before and after drying to a constant weight.

All assays were conducted in three replications.

The composition of liver tissue was determined according to the same methodology as in muscle tissue analysis.

Once normal distribution of the results was stated, they were subjected to a statistical analysis with a Statistica<sup>®</sup> 8 software by StatSoft, for Windows, using the Duncan's test.

## **RESULTS AND DISCUSSION**

In analysing the effect of food additives applied in the experiment on feed intake, it was established that they modified the intake both in male and female rats.

In the case of males, this effect was manifested by an increased intake of feed mixture, irrespective of its type, when expressed in both absolute values and per body weight unit. In the case of female rats, the impact of the food additives was manifested in an increased total feed intake in the females fed the basal diet and in a decreased total feed intake in the groups fed the modified feed mixture. However, when analysing feed intake per 100 g of body weight, an increase was observed – likewise in the male rats – in its value in the groups receiving food additives, irrespective of the type of feed mixture (Table 3).

One of the many factors regulating the intake of feed by animals is its energetic value. As shown by Cole and Chadd [1989] in their study with miniature pigs, in the ad libitum feeding system the intake of feed is increasing with a decreasing energetic value. Taking into account, however, that the feed mixtures applied in the reported experiment were isocaloric and the food additives added to water did not change their energetic value, this factor could have no impact in this respect.

**Table 3.** Effect of a mixture of selected food additives and diet composition on intake of feedstuff, sodium and potassium ions, and body gain at rats ( $\bar{x} \pm SD$ , n = 96)

Examined trait	Sex	Basic feed (a)	Basic feed + food additives (b) (c)		Modified feed + food additives (d)	Statistically significant	
Feed consump-	male	1 348 ±29.4	1 353 ±48.6	1 224 ±50.3	1 245 ±72.9	a – c**, d*; b – c, d**	
tion, g	female	831 ±47.7	892 ±62.9	859 ±43.0	$840\pm\!\!55.5$	a – b**; b – d*	
Feed consump-	male	276 ±13.8	278 ±16.0	254 ±15.9	265 ±20.5	a-c**; b-c**	
tion, g/100 g body mass	female	312 ±15.4	331 ±16.6	323 ±19.2	333 ±21.9	a – b*, d**	
Total sodium	male	$1.524 \pm 0.033$	$1.591 \pm 0.055$	$1.298 \pm 0.053$	$1.381 \pm 0.077$	a – c, d**; b – c, d**	
consumption, g	female	$0.940 \pm 0.054$	$1.043 \pm 0.071$	$0.911 \pm 0.046$	$0.926 \pm 0.059$	b – a, c, d**	
Sodium con-	male	0.313 ±0.017	0.328 ±0.019	$0.270 \pm 0.017$	$0.294 \pm 0.023$	a – c**, d*; b – c, d**	
sumption, g/100 g body mass	female	0.353 ±0.017	$0.387 \pm 0.019$	0.343 ±0.020	$0.367 \pm 0.024$	b-a, c**, d*; c-d**	
Total potassium	male	$13.163 \pm 0.287$	$13.263 \pm 0.475$	$10.973 \pm 0.451$	$11.205 \pm 0.653$	a – c, d**; b – c, d**	
consumption, g	female	8.117 ±0.465	8.738 ±0.615	$7.697 \pm 0.385$	$7.560 \pm 0.497$	a – b, d**, c*; b –c, d**	
Potassium con-	male	2.701 ±0.143	2.731 ±0.156	2.280 ±0.143	$2.385 \pm 0.184$	a-c, d**; b-c, d**	
sumption, g/100 g body mass	female	3.051 ±0.150	3.243 ±0.164	2.896 ±0.172	$2.996 \pm 0.196$	a – c*; b – a, c, d**	
Body weight	male	$37.2 \pm 12.84$	$40.6 \pm 9.17$	$36.5 \pm 8.75$	$31.4 \pm 10.92$	-	
gain, g	female	13.5 ±7.89	$14.8 \pm 6.07$	$12.0 \pm 7.56$	$6.84 \pm 1.57$	d – a, b, c**	
Body weight	male	2.74 ±0.493	$2.99 \pm 0.720$	2.98 ±0.746	$2.52\pm\!\!0.837$	_	
gain, g/100 g feed	female	1.61 ±0.365	1.65 ±0.383	1.39 ±0.574	$0.81 \pm 0.188$	d – a, b, c**; b – c*	

\*,\*\*Statistically significant difference  $p \le 0.05, 0.01$ .

It is also hardly likely that the increased feed intake might result from the change in feed mixture taste perceived by the animals, which could be mainly attributed to the addition of monosodium glutamate (MSG), because the animals were administered food additives in the form of an aqueous solution.

Literature data about the modifying effect of MSG on feed intake refer to its application as an ingredient of dishes, whilst sparse information is available on MSG impact on feed intake once administered in a solution [Bellisle 1999, Okiyama and Beauchamp 1998, Prescott 2004].

Rolls et al. [1996], who in a study with macaques were examining the activity of brain regions responsible for food intake, demonstrated that MSG may indeed affect the food intake. In turn, a human trial showed that the addition of MSG to food did not always affect the intake of the offered meal [Galiński et al. 2005].

The observed higher total intake of feed by male rats of all groups, compared to the females, may be explained by differences in their body weight which determines energetic and metabolic demands of the body. In contrast, when analysing feed intake per body weight unit, its higher value was observed in the females. This was, however, not reflected in their body weight gains, both in absolute values and when expressed per feed intake unit (Table 3).

The food additives applied in the experiment facilitated greater body weight gains only in the case of animals fed the basal diet, whereas in these fed the modified diet they caused reduced body weight gains, both when expressed in absolute values and per 100 g of consumed feed (Table 3).

The observed effect is difficult to explain, but it enables speculating that as a result of additives administration the components of the modified diet were either not fully utilized or were utilized in the way which made the body weight gains inadequate to their energetic value. It seems that this could be due to the nitrate and nitrite applied in the experiment [Bilczuk 1980].

Many studies have already shown that depleted of components taking part in bodily metabolism, facilitate – especially in animals – lower body weight gains [Dorup and Clausen 1991, Eder et al. 1999, Waldhauser et al. 1999]. It seems crucial, therefore, to draw attention to the fact that various food additives, being constituents of mainly processed and purified foods, may enhance the development of the so-called concealed deficiencies, thereby modifying body functions.

It is common knowledge that body weight and its gains are determined, most of all, by adipose tissue and water content in the body.

Water is the main, in terms of quantity, body constituent and depending on age, gender, and fatty tissue content may constitute from 45 to 80% of body weight. Water transfer proceeds continuously between the body and the environment, and in the body itself water is produced in the course of metabolic processes. The maintenance of a constant water volume in the body and its stable distribution in particular water compartments is controlled by homeostatic mechanisms.

Body fluids are solutions of various organic and inorganic substances, and differences in their composition are mostly determined by the presence of proteins in blood serum and diverse electrolytic composition of the intra- and extracellular fluid.

The most important cation of the extracellular fluid in a body of man and animals is a sodium ion. It takes part in, among other things, maintenance of the acidic-base balance, production of voltage between cell nucleus and the area outside the cell membrane, maintenance of extracellular fluid volume and oncotic pressure. The concentration of the sodium ion in the body is controlled by kidneys. As reported by Knypl [2002], a strict dependency exists between sodium metabolism, volume of the extracellular fluid and aldosterone-controlled sodium retention in kidneys. Sodium ions are filtrated in kidneys in high quantities, but under normal conditions, from 96 to over 99% of Na<sup>+</sup> are subject to reversible resorption from the filtrate. These cations are the main factor determining the volume of the extracellular fluid, because they occur in the highest quantity in this fluid and because sodium salts constitute over 90% of osmotically-active substances, dissolved in the serum and the interstitial fluid.

In the conducted experiment, two of the applied food additives were containing sodium ions, these included sodium nitrite and monosodium glutamate.

Apart from the sodium ion, other ions are as well responsible for the proper water-electrolyte balance, including e.g. ions of potassium – which was a constituent of potassium nitrate applied in the study. Potassium is one of the key intracellular ions. It determines the molar concentration of intracellular fluid and size of intracellular water compartment. Changes in serum concentration of potassium affect, most of all, functions of the cardiovascular system, but also the proper functioning of nervous and muscular systems, for it plays a significant role in stimuli conduction. The intracellular concentration of potassium serves many metabolically-important functions. Potassium takes part in the regulation of pH and osmotic pressure of a cell, is indispensable for protein synthesis and participates in carbohydrate metabolism.

Kidneys' capability for potassium retention in the body – unlike that for sodium – is far lesser. Under normal conditions, ca. 90% of absorbed potassium are excreted with urine, whereas the other part – through gastrointestinal tract.

Potassium contained in the intracellular fluid usually occurs in the free form as a cation. It is also, though to a lesser extent, bound with proteins, glycogen or phosphates. Enhanced synthesis of glycogen and protein facilitates potassium accumulation within cells, yet mostly in the osmotically-inactive form.

The water-electrolytic balance in the body is maintained owing to the action of regulatory enzymes, modifying the uptake and excretion of water and electrolytes, on condition of a lack of extreme effects of osmotically-active compounds on the body (Table 4).

In the conducted experiment, it was established that the applied food additives caused a significantly higher daily intake of fluids by the examined animals, yet the daily excretion of urine by both males and females did not reflect that intake. This indicates that the additives were facilitating water retention in the body. The differences evoked by the additives between the fluids intake and the volume of excreted urine were statistically significant in the animals fed the basal diet. In the animals receiving the modified diet, only ascending tendency could be observed. However, when comparing the quantity of water excreted with urine, which in the animals fed the basal diet constituted 55.4% and 43.9% of daily fluid intake in males and females, respectively, and in the animals administered the modified diet - 56.5% and 49.2% respectively, it may be concluded that the percentage of water retained in the body was comparable (Table 5).

Sex	Animals fed the basal diet	Animals fed the modified diet	
male	62.7	61.3	
female	35.0	35.6	
male	57.7	49.8	
female	32.1	33.6	
male	4.1	4.6	
female	3.5	4.0	
male	0.44	0.44	
female	0.37	0.44	
	male female male female female female male	male 62.7 female 35.0 male 57.7 female 32.1 male 4.1 female 3.5 male 0.44	

**Table 4.** Intake of Na<sup>+</sup> i K<sup>+</sup> with food additives and their percentage in overall consumption of these ions from the feedstuff (n = 96)

It was demonstrated, however, that the sites of water retention upon the administration of a food additives mixture were different and depended on the type of feed mixture provided to the animals. In the case of the animals fed the modified diet water was accumulated in the vascular bed, which was indicated by a significant decrease in the hematocrit value (Table 5). This fact may point to the possibility of exceeding kidney's capability for sodium ion accumulation in its parenchyma and may facilitate the development of arterial hypertension.

Water retention may as well be affected by the high concentration of glucose observed in blood of animals fed the modified diet [Kuchlewska 2010]. Despite lesser significance of this component in the physiological mechanism of the maintenance of a stable volume and distribution of fluids, it might have influenced the observed phenomenon.

In the animals receiving the basal diet, the hematocrit value was observed to decrease insignificantly under the influence of the food additives only in the case of females. In turn, both the males and females were characterized by a significant increase of water content in the liver tissue (Table 5). Taking into **Table 5.** Effect of a mixture of selected food additives and diet composition on selected indicators of the water balance at rats ( $\overline{x} \pm SD$ , n = 96)

Examined trait	Sex	Basic feed (a)	Basic feed + food additives (b)	Modified feed (c)	Modified feed + food additives (d)	Statistically significant
Daily intake of fluids, ml	male	22.0 ±2.71	28.0 ±5.79	26.5 ±3.39	31.5 ±4.48	a – b, d**; c – d*
	female	$21.2 \pm 1.11$	$28.9 \pm \!$	29.1 ±4.57	32.7 ±3.22	a – b, c, d**; d – b, c*
Daily excretion of urine, ml	male	$13.2 \pm 2.86$	15.5 ±4.56	15.3 ±3.16	$17.8 \pm 2.67$	a – d*
	female	$11.2 \pm 3.50$	$12.7 \pm 3.63$	$14.0\pm3.10$	16.1 ±2.52	a – d**; b – d*
The differences between the	male	$8.8 \pm 2.76$	12.6 ±2.53	$11.2 \pm 2.32$	13.6±3.13	a – b, c*, d**
fluids intake and the volume of excreted urine, ml	female	$10.0 \pm 3.87$	16.2 ±4.30	15.1 ±3.35	16.6 ±3.48	a – b, c, d**
Hematocrit value, %	male	41.6±1.63	42.4 ±1.32	43.3 ±1.93	41.3 ±1.25	a – c*; c – d**
	female	41.1 ±1.18	$40.3 \pm 1.98$	41.1 ±1.70	39.1 ±1.64	$c - d^{**}$
Water content in the muscle	male	$72.7 \pm 0.61$	$72.2 \pm 0.50$	$72.5 \pm 0.54$	$72.4\pm\!\!0.46$	-
tissue, %	female	$75.4 \pm 0.47$	$75.7\pm\!\!0.84$	$75.5 \pm 0.73$	$75.9 \pm 0.68$	_
Water content in the liver	male	$68.5\pm\!\!0.45$	$69.0\pm\!0.33$	$68.7\pm\!\!0.50$	69.1 ±0.31	a – b*, d**
tissue, %	female	$68.2\pm\!\!1.26$	$68.6 \pm 1.05$	$68.9\pm\!\!0.60$	$69.0\pm\!\!0.75$	a – b*
Dry matter content	male	$27.3 \pm 0.61$	$27.8 \pm 0.50$	$27.5\pm\!\!0.45$	$27.6\pm\!\!0.45$	_
in the muscle tissue, %	female	$24.6\pm\!\!0.46$	$24.3 \pm 0.84$	$24.5\pm\!\!0.73$	$24.1\pm\!\!0.68$	_
Dry matter content	male	$31.5\pm\!\!0.45$	$31.1 \pm 0.32$	$31.3\pm\!\!0.50$	$30.7\pm0.31$	a – b*, d**
in the liver tissue, %	female	31.8 ±1.25	$31.4 \pm 1.05$	$31.0\pm0.60$	$30.9\pm0.75$	_
Ash content in the muscle tissue, %	male	1.168 ±0.046	$1.139 \pm 0.057$	$1.254 \pm 0.086$	1.319 ±0.032	$a - c, d^{**}; b - c, d^{**}; c - d^{*}$
	female	$1.364 \pm 0.102$	$1.328 \pm 0.022$	$1.056 \pm 0.027$	1.131 ±0.159	a – c, d**; b – c, d**
Ash content in the liver	male	$1.554 \pm 0.049$	$1.649 \pm 0.062$	$1.519 \pm 0.046$	$1.502 \pm 0.042$	a – b**, d*; b – a, c, d**
tissue, %	female	$1.738 \pm 0.086$	$1.736 \pm 0.059$	$1.906 \pm 0.054$	$1.944 \pm 0.059$	a – c, d**; b – c, d**

\*,\*\*Statistically significant difference  $p \le 0.05, 0.01$ .

consideration mechanisms regulating water compartments, it seems that this effect could rather result from enhanced accumulation of glycogen in the liver whose water binding capability is known to some extent. It also indicated by the fact of no changes in water content of the muscle tissue. In contrast, when comparing the percentage of water retained in the body, being comparable to that noted in the groups on the modified diet, it may be speculated that part of water could be retained in fatty tissue, which could be indicated by increased body weight gains noted both in males and females.

Taking into account the total uptake of sodium and potassium ions from food additives by the animals, all of the observed changes could be due to their action, because results of most recent investigations show that an increased potassium intake and absorption in renal tubules are accompanied by on the one hand

	Statistically significant and interaction							
Examined trait	Additives (A)	Diet (D)	Sex (S)	AxD	AxS	DxS	AxDxS	
Daily intake of fluids, ml	**	**	**	_	_	*	_	
Daily excretion of urine, ml	*	*	*	_	_	-	_	
Differences between the fluids intake and the volume of excreted urine, ml	**	**	**	-	-	-	_	
Hematocrit value, %	*	_	**	*	*	_	_	
Water content in the muscle tissue, %	_	*	**	_	_	-	_	
Water content in the liver tissue, %	-	_	_	_	*	_	_	
Dry matter content in the muscle tissue, %	_	_	_	_	_	_	_	
Dry matter content in the liver tissue, %	*	_	_	*	_	_	_	
Ash content in the muscle tissue, %	-	*	_	*	_	_	_	
Ash content in the liver tissue, %	*	*	_	*	-	_	_	

**Table 6.** Statistical analysis of the impact of the food additives and dietary composition on selected parameters of the water

 balance in examined rats

\*,\*\*Statistically significant difference  $p \le 0.05, 0.01$ .

diminished sodium absorption, and on the other hand
by reduced calcium excretion [Morris et al. 2006].

It ought to be emphasized that the observed effects, evoked by the applied food additives, could not be affected by different uptake of sodium and potassium ions by the animals, because their quantities were systematically adjusted to changes in their body weight of the rats.

When generally analysing the percentage of water excreted by the animals, it may be concluded that also diet modification itself enhanced water retention in the body. This effect could be due to the observed in earlier studies upon diet modification [Podlaszewska et al. 2009], decrease in the concentration of corticosterone which by inhibiting the release of vasopressin facilitates increased filtration and diuresis. A decrease in its concentration could, therefore, result in water retention in the body.

In the conducted experiment, changes in water balance of the body were significantly affected not only by the food additives and diet composition, but also by the sex of the animals examined (Table 6). The daily excretion of urine was higher in the males, which was due, among other things, to their higher body weight, whereas the females were characterized by significantly higher water retention in the body as a result of both food additives and diet modification (Table 5). It may not be excluded, however, that this change was affected by estrogens which by making the epithelium of distal tubules in a kidney more susceptible to the action of vasopressin are influencing water retention in the body.

# CONCLUSIONS

The analysis of results achieved enabled concluding that:

1. The mixture of selected food additives applied in the experiment facilitated water retention in the body in the case of both male and female rats.

2. The differences observed between the intake of fluids and quantity of excreted urine were statistically significant in the animals fed the basal diet.

3. The type of feed mixture administered to the animals affected different sites of water retention in the body - in the animals on the basal diet a significant increase of water content was observed in liver tissues, whereas in these receiving the modified diet water was accumulated in the vascular bed. 4. Taking into account the fact of water retention in the vascular bed, the effects of food additives consumption may be more adverse in the females.

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# OCENA, NA MODELU ZWIERZĘCYM, WPŁYWU MIESZANINY DODATKÓW DO ŻYWNOŚCI NA GOSPODARKĘ WODNĄ

## STRESZCZENIE

**Cel.** Celem badań było określenie, na modelu zwierzęcym, wpływu zmiany składu diety i wybranych składników dodatkowych na gospodarkę wodną ustroju.

**Materiał i metody.** Badania przeprowadzono na 48 samcach i 48 samicach (osobno dla każdej płci) szczura szczepu Wistar, podzielonych na cztery grupy. Do picia zwierzęta grupy I i III otrzymywały wodę. Zwierzętom z grupy II i IV podawano 5 ml roztworu wybranych dodatków do żywności (azotanu potasu – E 252, azotynu sodu – E 250, kwasu benzoesowego – E 210, kwasu sorbowego – E 200 i glutaminianu sodu – E 621). Ilość podawanych dodatków wyliczono, biorąc pod uwagę średnie spożycie wśród ludzi, w przeliczeniu na jednostkę masy ciała. Po wypiciu roztworu zwierzęta dopajano czystą wodą.

**Wyniki.** Stwierdzono, że zastosowana w doświadczeniu mieszanina wybranych dodatków do żywności sprzyjała zatrzymaniu wody w organizmie badanych samców i samic. Obserwowane różnice pomiędzy ilością spożytych płynów a ilością wydalonego moczu były statystycznie istotne u zwierząt żywionych paszą podstawową. W zależności od rodzaju spożywanej paszy odmienne było miejsce, w którym była zatrzymywana woda – u zwierząt żywionych paszą podstawową stwierdzono istotny wzrost zawartości wody w tkance wątrobowej, u karmionych paszą zmodyfikowaną woda gromadziła się w łożysku naczyniowym.

**Wnioski.** Biorąc pod uwagę fakt zatrzymywania wody w łożysku naczyniowym, skutki spożywania dodatków do żywności mogą być bardziej negatywne u samic.

Słowa kluczowe: dodatki do żywności, gospodarka wodna, szczury

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