

## Effect of dried pumpkin (*Cucurbita maxima* D.) supplementation on growth performance, serum biochemistry and parameters of antioxidant status of rats

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**Abstract:** *Effect of dried pumpkin (Cucurbita maxima D.) supplementation on growth performance, serum biochemistry and parameters antioxidant status of rats.* The aim of the studies was to determine the effect of dried pumpkin, used in the diets for rats on parameters of growth, nutrient metabolism and antioxidant status of the animals. The experiment was carried out for 7 weeks with 30 growing male Wistar rats. The animals were classified into three groups, 10 individuals in each group, with the initial body weight of 108 g. The control group (G-0) was fed the semi-synthetic mixture without dried pumpkin additive whereas the experimental groups received the mixture with 5% (G-5) and 10% (G-10) additive of the dried pumpkin, Ambar variety. The dry substance was obtained from disintegrated fruits, deprived of seed nests, dried at temperature of 60°C. During the experiment weight gains and feed intake were controlled. After termination of the experiment, the rats were killed by anaesthesia; the blood samples were collected and biochemical indices and indicators of antioxidant status were determined. The dietary treatments had no effects on animal growth and feed utilization. In the animals receiving dried pumpkin in their diets (G-5, G-10) significantly lower level of glucose concentration in serum was found. In group G-0, the higher concentration of triacylglycerols in relation to group G-10 was recorded. Also, the concentration of total cholesterol in group G-0 was higher in comparison to groups G-5 and G-10. In group G-0, VLDL concentration was also higher in relation

to group G-10. In group G-10 compared to groups G-5 and G-0, the higher activity of glutathione peroxidase (GPx) was recorded. Total antioxidant status (TAS) was higher in group G-10 in comparison to groups G-0 and G-5. The effect of the administered diet on indicators of the degree of lipid oxidation was also found. In group G-10 compared to group G-0, thiobarbituric acid reactive substances (TBARS) concentration was lower.

*Key words:* rats, dried pumpkin, serum biochemistry, antioxidant status

### INTRODUCTION

New varieties of pumpkin in relation to the traditional ones are characterized by higher dry matter content, reaching up to ca. 20% and relatively high participation of carotenoids (Daničlenko 2000, Murkovic et al. 2002, Korzeniewska et al. 2004). Fruits of pumpkin contain a lot of cellulose and pectins, and, also starch, glucose, fructose and saccharose. They are also rich in minerals – mainly potassium, calcium, phosphorus and magnesium and vitamins A, E and C and vitamins from B group (Daničlenko 2000, Korzeniewska et al. 2004, Nawirska et al. 2008).

The higher dry matter content in pumpkin fruits enables their easier drying and makes the process more effective. It increases the possibilities of utilizing the dried pumpkin in production of mixtures and feed for farm animals and pets.

High content of carotenoids in the pumpkin fruits is very important from the viewpoint of dietetic value of the discussed fruits. Nawirska-Olszańska (2011) informs that the content of carotenoids in the flesh of the pumpkin varies, in average, 2–10 mg/100 g DM. In Ambar variety, the level of carotenoids amounted to 42.41 mg/100 g DM, including 24.24 mg of beta-carotene in 100 g/DM. Owing to its polyene structure, carotenoids absorb light and neutralize free radicals, i.e. singlet oxygen and organic radicals. Carotenoids reveal also stimulating effect on immunological system (Caili et al. 2006, Krzysik et al. 2007, Yang et al. 2007).

A high participation of pumpkin in the diet *via* the contained polysaccharides, including a high level of pectins, affects the increase of insulin level in the blood circulation, a phenomenon resulting in lowering the glucose content. The effect of structural carbohydrates, as present in the pumpkin, on decrease of the cholesterol level and that of triglycerides in blood, was also recorded (Zhang 2004, Kim 2005, Wikiera et al. 2014).

Owing to its chemical composition, dry pumpkin may be a valuable component of feed rations and mixtures, prepared for animals, including pets. The application of dried pumpkin in the diet should positively affect the utilization of diet's nutrients and enhance the antioxidative potential of the organism.

The aim of the study was the examination of the influence of dried pumpkin

applied in the diet for rats on the growth of animals, indicators of metabolic changes as well as the oxidative stress parameters of the organism.

## MATERIAL AND METHODS

### Animals and nutrition

The study was carried out following the procedures approved by the local ethical commission for experiments with animals. The experiment was conducted with 30 growing Wistar rats with the initial body weight of 106 ± 8 g. The animals were randomly assigned to three experimental groups, 10 rats each. All were fed semi-purified AIN-93G-based diets (Reeves 1997). Rats from control group (G-0) were fed AIN-93G-based diets without pumpkin.

The experimental groups received the mixture, containing 5% (G-5) and 10% (G-10) of dried pumpkin, Amber variety. The material destined for drying was obtained from ripened fruits, deprived of seed nests. The disintegrated pumpkins were dried at temperature of 60°C and then, added to experimental mixtures. All the mixtures were equalized in respect of crude protein, crude fat and crude fiber content. The additional quantity of water-soluble carbohydrates and of bioactive compounds (mainly carotenoids), assimilated by the animals in the diets with dehydrated pumpkin was the differentiating factor.

The composition and nutritive value of the mixtures is given in Table 1.

The rats were housed in individual cages with 12-hour light–dark cycle, temperature of 22°C and humidity of 50–60%. The experiment lasted for

TABLE 1. Composition and nutritive value of experimental diets

Item	Group		
	C-0	G-5	G-10
	g/kg diet		
Dried pumpkin*	–	50	100
Casein	197	190	183
Starch	674	637	600
Cellulose	40	35	30
Rapeseed oil	40	39	38
**AIN-93G Mineral Mix	35	35	35
***AIN-93-VX Vitamin Mix	10	10	10
Choline chloride	2	2	2
Methionine	2	2	2
In kg of mixture			
Dry matter (g)	904	911	906
Crude fiber (g)	40	40	40
Crude protein (g)	161	162	162
Crude fat (g)	40	40	40
Pectin (g)	–	17	36
Beta-carotene (mg)	–	18	34

C-0 control diet; G-5, diet with 5% dried pumpkin; G-10, diet with 10% dried pumpkin.

\*Chemical composition of 1 kg of dried pumpkin: dry matter – 910 g, crude protein – 102 g, crude fat – 18 g, crude fiber – 101 g, pectin – 374 g, beta-carotene – 341 mg, luteine – 76 mg, total polyphenol content (TPC) – 3476 mg; \*\*AIN-93G Mineral Mix (No 94046); \*\*\*AIN-93-VX Vitamin Mix (No 94047).

7 weeks. The animals had a free access to feed and water. During the experiment feed intake was controlled every day and weight gains once per week.

centrifuged (1,500 rpm for 10 min). The samples were frozen ( $-70^{\circ}\text{C}$ ) and stored until analyzed.

### Collection and preparation of blood

The rats were subject to fasting for 12 h before the experiment termination. After anesthesia (overdose of ketamine – 50 mg/kg of body weight) the blood samples were collected from the left cardiac ventricle to plastic test tubes without and with an anticoagulant. To obtain serum and plasma blood was

### Analyses

Analyses of three samples of dry pumpkin and three respective diets were performed. On this basis, the average content of the ingredients was determined. The chemical composition of the dried pumpkin and diets was determined according to AOAC methods (2005). The pectin were determined according to Morris method (Pijanowski et al. 1993).

The analysis of carotenoids separation and contents was made by applying the HPLC system (Dionex) equipped with a CoulArray electrochemical detector (ESA Inc). The separation was conducted on a Hypersil BDS 150 × 4.6 mm, 5 µm column (Sigma-Aldrich) at a mobile phase flow rate of 1.2 ml/min. The mobile phase consisted of a methanol and isopropanol mixture (98 : 2). The conditions of electrochemical detection were: four electrodes with potentials 400, 500, 600, and 750 mV. The chromatograms were processed by identifying the pigments on the basis of standards and areas of chromatographic peaks, taking into account their retention times as well as the ratio of the peak area for the dominating electrode to that of neighbouring electrodes. The TPC was determined according to Fisk et al. (2006), applying Folin–Ciocalteu's reagent, using gallic acid (GA) as a standard for calibration curve. The results were read at 765 nm after 1 h in a Tecan Infinite M200 analyser.

Glucose, total protein, albumin, urea, ALT, AST, ALP, total cholesterol, VLDL cholesterol, HDL cholesterol and triacylglycerides (TAG) were determined in blood serum by the spectrometric method using VITROS analyzer in a system EKTAchem DT-60-II with module, DT, DTE, DTSC, using sets of slides of Johnson & Johnson Clinical Diagnostics.

Glutathione peroxidase (GPx) activity was measured in blood by the modified Kraus and Gather (1980) method. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is converted to the reduced form with a contaminant oxidation of NADPH to NADP<sup>+</sup>. The absorbance was measured at wavelength 340 nm (Rancel RS

505, Randox, Crumlin, UK). The activity of GPx was expressed in U/ml blood, with 1 U corresponding to oxidation of 1 µmol NADPH/min.

The total antioxidative activity of blood plasma (TAS) was determined by the colorimetric method using kits by Randox Laboratories Ltd. and expressed in mmol/l. The results were read out with a Cobra Mira biochemical analyzer (Roche) at a wavelength of 600 nm (Smart et al. 1996).

Malondialdehyde (MDA), the most abundant product of all lipid peroxidation products, was measured in serum using thiobarbituric acid (TBA) according to the Uchiyama and Mihara (1978) technique. Absorbance was measured at a wavelength of 535 nm with a Tecan Infinite M200 analyser (Tecan Group Ltd., Switzerland). The results represent the concentration of thiobarbituric acid reactive substances (TBARS) in the samples.

### Statistical analysis

The obtained results were elaborated statistically using a one-way analysis of variance with the least square method. Account was taken of the effect of feeding on the analyzed parameters. The tables contain mean values of parameters and standard errors of the means. Computations were made with Statgraphics 6.0 Plus Statistical Package.

## RESULTS AND DISCUSSION

No significant differences in the growth indicators between animals from various groups were reported. With animals on diets with dry pumpkin, there was only a slight tendency towards a higher final

mass and a higher daily gain. This tendency was particularly visible with the group on a diet with 10% dry pumpkin content – group G-10 (Table 2).

In case of the animals, receiving dried pumpkin in their diets (G-5, G-10), significantly lower concentration of serum glucose as compared to the control group (G-0) ( $P \leq 0.01$ ) was found. The application of dried pumpkin in the diets of the rats had an influence on indicators of

lipid changes. In the control group, the higher concentration of triacylglycerols in relation to group G-10 ( $P \leq 0.05$ ) was recorded. Also, the concentration of total cholesterol in control group (G-0) was significantly higher in comparison to group G-5 ( $P \leq 0.05$ ) and G-10 ( $P \leq 0.01$ ). In the control group, VLDL cholesterol concentration was also significantly higher ( $P \leq 0.01$ ) in relation to group G-10 (Table 3).

TABLE 2. Parameters of growth performance of rats fed the diets containing dried pumpkin

Item	Group			SE	P-value
	G-0	G-5	G-10		
Initial body weight (g)	105.8	106.5	106.2	6.079	NS
Final body weight (g)	283.3	286.7	291.5	9.361	NS
Average daily gain (g)	4.27	4.30	4.41	0.173	NS
Feed conversion ratio (g/g)	4.23	4.42	4.29	0.152	NS

C-0 – control diet; G-5 – diet with 5% dried pumpkin; G-10 – diet with 10% dried pumpkin.

TABLE 3. Biochemical parameters in serum of rats fed the diet containing dried pumpkin

Item	Group			SE	P-value
	G-0	G-5	G-10		
Glucose (mmol/l)	9.81 <sup>B</sup>	8.09 <sup>A</sup>	7.84 <sup>A</sup>	0.388	$\leq 0.01$
Albumin (g/l)	33.72	31.94	31.35	0.854	NS
Total protein (g/l)	59.81	56.10	56.02	1.339	NS
Urea (g/l)	4.66	5.07	5.16	0.210	NS
ALT (U/l)	9.90	10.41	6.78	1.173	NS
AST (U/l)	47.04	48.74	48.36	3.998	NS
ALP (U/l)	219.34	190.02	187.84	15.406	NS
Triacylglycerols (TAG) (mmol/l)	1.77 <sup>b</sup>	1.53 <sup>ab</sup>	1.44 <sup>a</sup>	0.104	$\leq 0.05$
Total cholesterol (mmol/l)	1.94 <sup>Aa</sup>	1.71 <sup>b</sup>	1.66 <sup>abB</sup>	0.062	$\leq 0.01$
HDL cholesterol (mmol/l)	1.13	1.16	1.14	0.056	NS
VLDL cholesterol (mmol/l)	0.92 <sup>a</sup>	0.77 <sup>ab</sup>	0.72 <sup>b</sup>	0.055	$\leq 0.05$

C-0 – control diet; G-5 – diet with 5% dried pumpkin; G-10 – diet with 10% dried pumpkin.

AB – differences between the selected rows ( $P \leq 0.01$ ); ab – differences between the selected rows ( $P \leq 0.05$ ).

The results, obtained in our studies have been also confirmed in the studies of other authors. Hypoglycemic properties of pumpkin pulp in the studies on rabbits and rats were confirmed by Fu et al. (2006). The decrease of the level of glucose, total cholesterol and triglycerides in blood serum of the rabbits, receiving dried pumpkin in their diets was also found by Zhang (1998). Yoshinari et al. (2009) employed 1% of lyophilisate obtained from pumpkin paste concentrate in the diets for adult Wistar and GK (Goto-Kakizaki) rats and found, i.a, lower level of glucose and total cholesterol in the group, receiving pumpkin lyophilisate.

Dried pumpkin is a rich source of water-soluble carbohydrates and pectin. The intake of the mentioned compounds by the animals, receiving dried pumpkin in their diets may be related to the lowered level of glucose and total cholesterol, triglycerides and VLDL in serum as compared to the control group (Table 1, Zhang 2004). The discussed compounds – *via* decrease of gastric and intestinal mobility – decrease the contact of glucose and mucous membrane and its absorption is worsened (Wikiera et al. 2014). Pectin and water-soluble carbohydrates cause also the increase in thickness and viscos-

ity of mucus, covering intestinal mucous membrane what limits also absorption of glucose and affect lowering of its level in blood (Kim 2005).

The ability of creating gel layer in gastrointestinal tract limits also the process of lipolysis and deestrication of cholesterol; it decreases also absorption of the resulting products (Gulfi et al. 2006). Pectin in the diets increase also excretion of bile acids in feces what, in consequence, leads to decrease of cholesterol concentration in blood (Wikiera et al. 2014).

In group G-10 as compared to group G-5 and the control group (G-0) significantly higher activity of glutathione peroxidase was found ( $P \leq 0.01$ ). The reductase was recorded on the same level in all groups. The diet with the highest content of dried pumpkin increased the total antioxidative potential of the body. In group G-10, TAS was highly significantly higher ( $P \leq 0.01$ ) as compared to the group G-0 and group G-5. Also, the effect of administered diet on indicators of the degree of lipid oxidation was found. In group G-10 compared to control group, significantly lower concentration of compounds, reacting with thiobarbituric acid (TBARS) was recorded (Table 4).

TABLE 4. Parameters of the antioxidant status in blood serum and plasma of rats fed the diet containing dehydrated pumpkin

Item	Group			SE	P-value
	G-0	G-5	G-10		
Glutathione peroxidase – GPx (U/ml)	0.65 <sup>B</sup>	0.64 <sup>B</sup>	0.74 <sup>A</sup>	0.018	$\leq 0.01$
Glutathione reductase – GR (U/ml)	23.13	23.34	24.88	1.206	NS
TAS (mmol/l)	1.24 <sup>B</sup>	1.26 <sup>B</sup>	1.35 <sup>A</sup>	0.022	$\leq 0.01$
TBARS (nmol/g)	0.41 <sup>a</sup>	0.36 <sup>ab</sup>	0.34 <sup>b</sup>	0.018	$\leq 0.05$

C-0 – control diet; G-5 – diet with 5% dried pumpkin; G-10 – diet with 10% dried pumpkin; AB – differences between the selected rows ( $P \leq 0.01$ ); ab – differences between the selected rows ( $P \leq 0.05$ ).

Dried pumpkin, as employed in diets G-5 and G-10 is a rich source of carotenoids, including beta-carotene (Table 1). Owing to its structure and effect, beta-carotene is one of the most important natural antioxidants for biological systems (Caili et al. 2006, Krzysik et al. 2007). Therefore, its presence in the diet and its intake by the animals increase also antioxidant potential of animal. It is also reflected in the results of our studies where in the rats, receiving dried pumpkin, TAS was significantly higher as compared to the control group. The highest TAS was found in the animals, receiving highest content of dried pumpkin in their diets. It should be mentioned that apart from beta-carotene, dried pumpkin supplied also other carotenoids and compounds with antioxidative effect, e.g. flavonoids (Table 1).

In the studies, a positive effect of beta-carotene on the increase of antioxidative enzymes' activity was found (Zamora et al. 1991, Iyama et al. 1996). The higher level of GPx in group G-10 as compared to the remaining groups may be, therefore, the effect of higher intake of beta-carotene and other carotenoids in the diet. In the studies of Dang (2004), the stimulation of activity of antioxidative enzymes in the mice which received pumpkin extract, was recorded.

Owing to their antioxidative activity, beta-carotene and other bioactive compounds may limit the degree of oxidation of lipids in animal bodies. The effect of beta-carotene includes, i.a, inhibition of lipid oxidation in liposomes. It results in lower TBARS concentration in liver or serum. Such effect was found in our studies in the rats, receiving dehydrated pumpkin in the diets. The limitation of

the degree of lipid oxidation in case of introducing antioxidants to the diet was also recorded by other researchers in the studies, conducted on humans and animals (Furusho et al. 2002, Actis-Goretta et al. 2004).

## CONCLUSION

The application of dried pumpkin in the diets for rats had an influence on decrease of the level of glucose, triacylglycerols, total cholesterol and VLDL cholesterol in blood serum of the rats. The application of the mentioned dried substance in the diets increased also antioxidative potential of the rats. The results of the studies indicate that the dried pumpkin may be interesting and valuable component of feed for animals, including feed with a dietetic effect.

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**Streszczenie:** *Wpływ suszu z dyni (Cucurbita maxima D.) zastosowanego w dietach na wzrost, wskaźniki biochemiczne oraz wskaźniki statusu antyoksydacyjnego szczurów.* Celem badań było określenie wpływu suszu z dyni zastosowanego w dietach dla szczurów na wzrost zwierząt, wskaźniki przemian metabolicznych oraz status antyoksydacyjny organizmu. Doświadczenie przeprowadzono przez 7 tygodni na 30 rosnących szczurach samcach Wistar. Zwierzęta zostały podzielone na trzy grupy po 10 osobników o początkowej masie ciała 108 g. Grupa kontrolna (G-0) była żywiona mieszkanką półsyntetyczną bez dodatku suszu z dyni, a grupy doświadczalne otrzymywały mieszankę z udziałem 5% (G-5) i 10% (G-10) suszu z dyni ambar. Susz uzyskano z rozdrobnionych owoców, pozbawionych gniazd nasiennych, suszonych w temperaturze 60°C. W trakcie trwania badań kontrolowano przyrosty masy ciała zwierząt oraz pobranie mieszanek. Po zakończeniu doświadczenia szczury poddano eutanazji i pobrano od nich krew, w której oznaczono wskaźniki biochemiczne w surowicy oraz wskaźniki potencjału antyoksydacyjnego. Nie stwierdzono wpływu diety na wzrost zwierząt i wykorzystanie paszy. U szczurów otrzymujących w dietach susz z dyni stwierdzono niższe stężenie glukozy w surowicy w porównaniu do zwierząt z grupy kontrolnej (G-0). W grupie G-0 występowało wyższe stężenie trójglicerydów w stosunku do grupy G-10. Stężenie cholesterolu całkowitego w grupie G-0

było także wyższe w stosunku do grup G-5 i G-10. W grupie G-0 wyższe było także stężenie cholesterolu VLDL w stosunku do grupy G-10. W grupie G-10 w porównaniu do grup G-5 oraz G-0 stwierdzono istotnie wyższe stężenie peroksydazy glutationowej (GPx). W grupie G-10 TAS był na wyższym poziomie w porównaniu do grup G-0 oraz G-5. Stwierdzono również wpływ podawanej diety na wskaźniki stopnia utleniania lipidów. W grupie G-10 w porównaniu z grupą kontrolną stwierdzono niższe stężenie kwasu tiobarbiturowego (TBARS).

*Słowa kluczowe:* szczury, susz z dyni, wskaźniki biochemiczne krwi, status antyoksydacyjny

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