Original Article

Metabolic Effects of a Hydrophobic Alginate Derivative and Tetrahydrolipstatin in Rats Fed a Diet Supplemented with Palm Fat and Cholesterol

(amidated alginate / tetrahydrolipstatin / cholesterol / rats / serum / liver)

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Abstract. The effects of octadecylamide of alginic acid (amidated alginate) and tetrahydrolipstatin on serum and hepatic cholesterol, and the faecal output of fat and sterols, were investigated in rats. Amidated alginate is a sorbent of lipids, tetrahydrolipstatin is an inhibitor of pancreatic lipase. Rats were fed diets containing cholesterol and palm fat at 10 and 70 g/kg, respectively. Palm fat was provided by coconut meal. Amidated alginate at 40 g/kg diet significantly decreased serum total cholesterol, low-density lipoprotein and hepatic cholesterol, and hepatic lipids and increased the faecal output of fat and coprostanol. Tetrahydrolipstatin at 300 mg/kg diet significantly decreased low-density lipoprotein cholesterol and hepatic lipids and increased the faecal output of fat. The intake of feed was not significantly influenced; however, the weight gains in rats fed amidated alginate were lower than in rats of the control group. Both amidated alginate and tetrahydrolipstatin modified the fatty acid profile in excreta lipids. Concentrations of saturated fatty acids were decreased and those of unsaturated fatty acids increased. Despite different modes of action, amidated alginate and tetrahydrolipstatin were equally efficient in removing the dietary fat from the body.

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Abbreviations: ALT – alanine aminotransferase, AST – aspartate aminotransferase, FA – fatty acids, HDL – high-density lipoproteins, HPLC – high-performance chromatography, LDL – low-density lipoproteins, PMP – 1-phenyl-3-methyl-5-pyrazolone, THL – tetrahydrolipstatin.

Introduction

Overweight is a risk factor for covid-19 and several chronic diseases (coronary heart disease, cancer, hypertension, type 2 diabetes). Classical treatments for overweight such as dieting, behavioural modification and exercise often fail; thus, there is the need for drugs and agents efficient in the treatment of overweight. New drugs are represented by inhibitors of food intake that reduce hunger perception, inhibitors of nutrient absorption, and drugs that increase energy expenditure (Alemany et al., 2003). Tetrahydrolipstatin (THL), commercially available as Xenical and Orlistat, is an inhibitor of pancreatic lipase with little or no activity against amylase, trypsin, chymotrypsin, and phospholipases (Guerciolini, 1997). Orlistat, a semisynthetic derivative of lipstatin, partially inhibits hydrolysis of triglycerides, and thus decreases subsequent absorption of monoglycerides and free fatty acids. THL inhibits hydrolysis of triglycerides, but does not interfere with the absorption of hydrolysis products if present in the feed (Porsgaard et al., 2003). Orlistat has been shown to decrease absorption of lipophilic micronutrients in rats (Cruz-Hernandez et al., 2010) and decreases absorption of vitamin E in healthy volunteers (Melia et al., 1996). The alternatives to drugs are herbal preparations that regulate lipid metabolism (Lee et al., 2016) and decrease weight gain (Mahmoud and Elnour, 2013). Soluble fibres (psyllium and gel-forming polysaccharides) increase intestinal viscosity and affect the process of digestion and absorption. Tsujita et al. (2003) and Edashige et al. (2008) showed that citrus pectin inhibited pancreatic lipase. Alginate, a copolymer of β-D-mannuronate and α-L-guluronate, also inhibited the activity of pan-

creatic lipase. The degree of inhibition depended on the mannuronate and guluronate ratio. High guluronate alginate inhibited lipase to a higher degree than high mannuronate alginate (Wilcox et al., 2014). The physiological effects of alginate and pectin are similar, as both polysaccharides are polymers of uronic acids. Converting hydrophilic pectin or alginate to their hydrophobic derivatives (amidated pectin and amidated alginate) significantly increased the concentration of fat in excreta and faecal cholesterol concentrations in rats (Marounek et al., 2007, 2017).

In rats fed high-fat diet containing corn oil, THL at 200 mg/kg significantly decreased the serum concentration of triglycerides, total and low-density lipoprotein (LDL) cholesterol, but increased the concentration of high-density lipoprotein (HDL) cholesterol (Mahmoud and Elnour, 2013). The authors, however, did not measure the faecal concentration of fat, cholesterol and daily faecal output of fat and sterols. In the present study, we assessed the effect of THL and amidated alginate on the faecal output of fat, sterols, and the fatty acid profile of faecal lipids in rats fed a diet supplemented with cholesterol and palm fat. We expect that this information may be useful for understanding the effects of agents suitable for the treatment of overweight. The alginate obtained from the supplier and amidated alginates were characterized in order to obtain information that may be important in the present experiment.

Material and Methods

Animals and diets

Twenty-one female Wistar rats (rats and rat diet ST-1 were obtained from Velaz Ltd., Lysolaje, Czech Republic), approximately 6-week-old, were used. The rats were housed individually in a temperature- and humidity-controlled room. The vivarium was maintained on a 12 h light : 12 h dark daily photoperiod cycle at a temperature of 22 ± 1 °C. All diets were supplemented with cholesterol at 10 g/kg, and with coconut meal at 124 g/kg. The coconut meal containing 56.5 % fat was purchased in a local shop with healthy food. Diets contained 10.9 % of fat, including cholesterol. After four weeks of adaptation of rats to the new environment, the rats were randomly divided into three groups of 7 rats each.

Table 1 presents composition of control and experimental diets. Diet No. 1 (control diet) was without additives. Diet No. 2 was supplemented with amidated alginate at 40 g/kg, at the expense of cellulose. A dose-response study was performed in which diets of rats were supplemented with amidated alginate at 0, 20 and 40 g/kg. The intake of feed and weight gain were not significantly affected (Marounek et al., 2019). The diet No. 2 was used in the present experiment. Diet No. 3 was supplemented with THL at 300 mg/kg. This dosing is the average of Orlistat concentrations used in the experiment of Cruz-Hernandez et al. (2010). Diets and water were available *ad libitum*. In this experiment, feed intake, initial and final body weights were measured. The study was approved by the Ethics Committee of the Institute of Animal Science and the Central Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic.

Table 1. Composition of control and experimental diets (g/kg)

Diet	**1**[a]	**2**	**3**
Cholesterol (g/kg)	10	10	10
Palm fat[b] **(g/kg)**	124	124	124
Amidated alginate (g/kg)	–	40	–
Tetrahydrolipstatin (mg/kg)	–	–	300
Cellulose (g/kg)	40	–	40
Diet ST-1[c] **(g/kg)**	866	866	866

[a] Control diet, [b] coconut meal containing 56.5% fat. [c] The rat diet ingredients were soybean meal, meat and bone meal, cereals, wheat bran, limestone, dicalcium phosphate, salt, and supplements vitamins, trace elements and amino acids. The diet ST-1 contained crude protein, fibre, fat, ash and cholesterol at 224, 45, 33, 43 and 0.29 g/kg, respectively.

The experiment duration was three weeks, and then, the rats were sacrificed by decapitation after anaesthesia by inhalation of isoflurane (Nicholas Piramal India Ltd., London, UK). The rats received 4 g of feed 4 h before they were sacrificed (Spielman et al., 2008).

Materials and reagents

The sodium salt of alginic acid, low-viscosity product A1112, from brown algae, was obtained in a dry powder form from Sigma-Aldrich (Prague, Czech Republic). THL (Orlistat), a certified reference material (PHR 1445), microcrystalline cellulose and *n*-octadecylamine were purchased from Sigma-Aldrich. *N,N*-dimethylformamide, ethanol, methanol, petroleum ether, hydrochloric acid, sulphuric acid, and acetone were purchased from P-Lab (Prague, Czech Republic). For analytical methods, we used formamide, sodium hydroxide, 3,5-dinitrosalicylic acid, potassium sodium tartrate, calcium acetate, and phenolphthalein purchased from P-Lab. D-mannuronic acid sodium and agent 1-phenyl-3-methyl-5-pyrazolone (PMP) were purchased from Sigma-Aldrich (Prague), L-guluronic acid sodium was obtained from Carbosynth Ltd. (Compton, UK). The Sylon HTP kit (hexamethyldisilazane-trimethylchlorosilane-pyridine 3 : 1 : 9) was purchased from Supelco (Supelco Inc., Bellefonte, PA). Isolithocholic acid, 12-ketolithocholic acid, norcholic acid, α-, β-, ω-muricholic acids, and β-sitostanol were purchased from Steraloids Inc. (Newport, RI). Other sterols were supplied by Sigma-Aldrich (Prague).

Preparation of amidated alginate

Alginic acid was prepared by washing the sodium alginate with acidified ethanol, pure ethanol and acetone, followed by air drying. Alginic acid was esterified with methanol in the presence of sulphuric acid. The solid

reaction product was filtered, washed with ethanol and acetone, and then dried in air. The methyl ester was amino-dealkoxylated with n-octadecylamine (Taubner et al., 2017).

Carboxyl group assay and determination of the degree of amidation

The content of all carboxyl groups $COOH_t$ (% m/m) in alginic acid and the content of free carboxyl groups $COOH_f$ (% m/m) in its methyl ester were determined by a titration method and calculated as described by Taubner et al. (2017). The degree of amidation (DA, mol. %) of the final products was calculated based on the carbon (*C*) and nitrogen (*N*) content (% m/m). See the previous report (Taubner et al., 2020) for details.

Molecular weight assay

The reaction with organic dinitro derivatives is based on the ability to reduce dinitrosalicylic acid by reducing polysaccharide groups (Lindsay, 1973). The colour intensity was measured using a spectrophotometer at a wavelength of 545 nm. Galacturonic acid was used for calibration. The degree of polymerization and the molecular weight were calculated from the absorbance of the polysaccharide sample.

Analysis of the mannuronic to galacturonic acid ratio in alginate

Alginate was hydrolysed using trifluoroacetic acid as described by Wang et al. (2016), with some modifications. Pre-column derivatization of uronic acids with 1-phenyl-3-methyl-5-pyrazolone and HPLC analysis were performed as described by Wu et al. (2014), with some modifications. The same procedure was used to treat monosaccharide standards and their equimolar mixture. An analytical C18-column (250 × 4.6 mm, 5 µm, ZORBAX, Agilent Company) was used at a temperature of 30 °C. Elution of the derivatized monosaccharides was performed with a mixture of 0.1 M phosphate buffer (pH 6.7) and acetonitrile. The UV absorbance of the effluent was monitored at 245 nm. Guluronate and mannuronate peaks were identified on the basis of the retention times of standards. The mannuronate to guluronate ratio was calculated from the ratio of peak areas corrected for the ratio of response factors.

Sampling

Mixed blood samples were collected from each rat at the time of euthanasia. The sera were separated by centrifugation and analysed the subsequent day. The livers were excised and kept at −40 °C until analysis. Faeces were collected during the last 5 days of the experiment, weighed, pooled, and stored frozen until analysis. Analyses of hepatic and faecal lipids (fat, fatty acids and sterols) were performed as described previously (Marounek et al., 2019). Faecal samples for analyses of sterols and fatty acid profile were freeze-dried. Serum cholesterol, triacylglycerols, bilirubin, aminotransferases AST and ALT were determined using kits of BioVendor Ltd. (Brno, Czech Republic).

Other analyses

The fatty acid (FA) profile of coconut meal, fat content in coconut meal, and analyses of ST-1 diet were performed as described by a previous study (Skřivan et al., 2018). Triacylglycerols in coconut meal were determined as described by Kobayashi et al. (2013). Lipids were dissolved in hexane, insoluble particles removed by centrifugation (Hermie Labor Technik 236 HK, Wehingen, Germany, 2 min, 24,000 *g*), and supernatant injected onto a Luna® 3 µm C18 (2)100 Å, LC column for separation of hydrophobic compounds (Phenomenex, Torrance, CA). A HPLC system Shimadzu (Kyoto, Japan) incorporating a guard column and evaporative light scattering detector was employed. The system consisted of the following components: a model LC-20AD pump, a model CTO-20AC column oven, a model ELSD-LT detector, a model CBM-20A HPLC communication interface, a model SIL-20AC auto injector, a model DGU-20A5 degasser. A gradient programme based on two solvents was used: the first solvent consisted of 98.9% hexane, 1% isopropanol and 0.1% acetic acid. The second solvent contained 99.9% isopropanol and 0.1% acetic acid. The method was calibrated with glyceryl tripalmitate dissolved in hexane.

Statistics

Data were analysed by one-way analysis of variance using the GLM procedure of SAS, version 8.2 (SAS Institute, Cary, NC). The results were expressed as the mean and standard deviation. Significant differences ($P < 0.05$) were identified using Tukey's test. The Pearson correlation coefficient was used as a measure of the dependence between pairs of observations.

Results

Average initial and final body weights of rats were 238.7 g and 272.3 g, respectively. Weight gains of rats fed the control diet, diet supplemented with amidated alginate, and THL-containing diet were 46 ± 14, 25 ± 10, and 30 ± 18 g/day, respectively. Body weights of rats and the feed intake did not differ significantly among the treatment groups; however, the weight gain of rats fed amidated alginate was significantly lower than that of control rats.

Characterization of alginate and amidated alginate

Table 2 presents characteristics of alginate, its methyl ester and amidated alginate prepared from alginate of low viscosity. The molecular weight of low-viscosity alginate was 58.9 kg/mol. After methylation and amidation, the molecular weight of the final product was decreased to 12.3 kg/mol. The average value of the man-

Table 2. Characterization of alginate, methyl ester of alginic acid, and amidated alginate

	Na-alginate		H-alginate
Content of uronic acids (% m/m)	72.2		74.5
Mannuronate/guluronate (m/m)		2.65	
Molecular weight (kg/mol)	58.9		43.9
Methyl ester of alginate			
Degree of methylation (mol. %)		66.7	
Molecular weight (kg/mol)		15.9	
Amidated alginate			
Degree of amidation (mol. %)		77.7	
Molecular weight (kg/mol)		12.3	

nuronate/guluronate ratio calculated from the results of three analyses carried out at different days was 2.65 ± 0.05.

Characterization of palm fat and coconut meal

In coconut meal, the main fatty acid was myristic acid followed by palmitic acid and capric acid. Oleic acid was the main unsaturated FA present in coconut meal (Table 3). Coconut meal contained 56.5% fat determined by extraction with petroleum ether. The HPLC analysis showed that coconut meal contained triacylglycerols expressed as tripalmitin at 623 mg/g.

Effects of amidated alginate and tetrahydrolipstatin on the serum and hepatic parameters and faecal output of fat and sterols in rats

Supplementation of the diet with amidated alginate and THL decreased serum total cholesterol, serum LDL cholesterol, cholesterol concentration in the liver tissue, and concentration of hepatic lipids. The concentration of serum bilirubin and activities of aminotransferases were not significantly affected (Table 4).

Table 3. Fatty acid profile of coconut meal (g per 100 g fatty acids determined)

Fatty acid		Coconut meal
Caproic	C 6:0	1.14
Caprylic	C 8:0	6.55
Capric	C 10:0	13.48
Lauric	C 12:0	8.62
Myristic	C 14:0	30.53
Palmitic	C 16:0	19.21
Stearic	C 18:0	7.33
Oleic	C 18:1c9	9.42
Elaidic	C 18:1t9	0.10
Linoleic	C 18:2	2.13

In total, 35 fatty acids were assayed.

The serum triacylglycerols correlated significantly with serum cholesterol ($r = 0.610$; $P = 0.003$) and non-significantly with total hepatic lipids ($r = 0.378$, $P = 0.091$). Serum cholesterol correlated significantly with hepatic cholesterol ($r = 0.804$, $P < 10^{-4}$). The daily faecal output of fat was significantly increased in rats fed diets supplemented with amidated alginate and THL. Amidated alginate significantly increased the faecal loss of coprostanol and total neutral sterols. The bile acid output, serum concentration of bilirubin, and activity of aminotransferases were not significantly influenced.

Amidated alginate and THL modified the fatty acid profile in excreta lipids. The concentration of saturated fatty acids was decreased and that of unsaturated fatty acids was increased (Table 5).

Discussion

In the present experiment, we used a diet supplemented with palm fat and cholesterol. Contrary to our previous experiments (Marounek et al., 2017, 2019), coconut meal containing 56.5% fat and triacylglycerols at 623 mg/g was used instead of palm fat AkoFeed Gigant 60. Dosing of amidated alginate (40 g/kg) has already been used previously (Marounek et al., 2019). Dosing of THL (Orlistat) was 3.75 mg/g fat, which was in the range of concentrations tested by Ackroff and Sclafani (1996). Both additives are safe. Neither amidated alginate nor THL negatively influenced parameters of hepatocellular health (serum bilirubin, ALT, AST). The EFSA panel "Food Additives Nutrient" concluded that there is no safety concern for the use of pectin and amidated pectin for the general population and that there is no need for "Acceptable daily intake" (Mortensen et al., 2017).

Alginate is a natural polysaccharide, the composition of which depends on the algae species. The molecular weight and the mannuronate to guluronate ratio play a significant role in the alginate's physicochemical properties (Lu et al., 2015). During the amidated alginate synthesis, acid hydrolysis conditions caused reduction of the average molecular weight. In the experiment of Sánchez-Machado et al. (2004), the mannuronate to gu-

Table 4. Effect of amidated alginate and tetrahydrolipstatin on the serum and hepatic parameters and faecal output of sterols in rats fed diets 1, 2, 3

Diet	1	2	3
Amidated alginate (g/kg)	–	40	–
Tetrahydrolipstatin (mg/kg)	–	–	300
Serum concentrations			
Total cholesterol (µmol/ml)	3.89 ± 0.29[a]	1.75 ± 0.21[b]	2.75 ± 0.90[ab]
LDL cholesterol (µmol/ml)	1.92 ± 0.20[a]	0.79 ± 0.11[b]	1.28 ± 0.54[c]
Triacylglycerols (µmol/ml)	2.67 ± 1.53	1.13 ± 0.61	1.71 ± 1.52
ALT (nkat/ml)	1.24 ± 0.32	1.43 ± 0.50	1.53 ± 0.72
AST (nkat/ml)	4.22 ± 1.26	3.82 ± 1.18	6.10 ± 3.26
Bilirubin (µmol/ml)	2.79 ± 0.20	2.71 ± 0.51	2.70 ± 0.32
Hepatic parameters			
Hepatic cholesterol (µmol/g)	37.14 ± 8.75[a]	7.00 ± 0.61[b]	23.17 ± 3.66[c]
Hepatic lipids (mg/g)	102.4 ± 15.7[a]	46.3 ± 5.1[b]	80.9 ± 4.9[c]
Daily faecal output			
Dry matter (g)	5.20 ± 0.72[ac]	5.98 ± 0.62[a]	4.60 ± 0.62[c]
Fat (g)	0.42 ± 0.07[a]	0.97 ± 0.11[b]	1.00 ± 0.16[b]
Cholesterol (µmol)	481 ± 84	569 ± 53	581 ± 80
Coprostanol (µmol)	166 ± 49[a]	245 ± 59[b]	146 ± 52[a]
Neutral sterols[d] (µmol)	750 ± 126[a]	908 ±67[b]	851 ± 140[ab]
Bile acids (µmol)	137 ± 22	104 ± 28	114 ± 30
Total sterols (µmol)	887 ± 132	1012 ± 89	965 ± 151

Seven rats per group. All diets contained cholesterol and coconut meal. [a–c] Values in the same row with different superscripts differ significantly ($P < 0.05$). [d] Including plant sterols

Table 5. Fatty acid profile[a] in excreta of rats fed control diet and diets supplemented with amidated alginate and tetrahydrolipstatin (g/kg)

Diet	1	2	3
Amidated alginate (g/kg)	–	40	–
Tetrahydrolipstatin (mg/kg)	–	–	300
Caproic	0	0	0.40 ± 0.12
Caprylic	1.38 ± 0.22[b]	0.57 ± 0.13[c]	7.64 ± 0.88[d]
Capric	1.88 ± 0.41[b]	0.67 ± 0.22[c]	6.84 ± 0.45[d]
Lauric	29.21 ± 1.67[b]	16.07 ± 5.80[c]	18.20 ± 1.83[c]
Myristic	20.75 ± 0.87[b]	14.56 ± 4.57[c]	23.31 ± 1.20[b]
Palmitic	16.36 ± 0.56[b]	22.10 ± 2.53[c]	14.08 ± 0.34[d]
Stearic	7.13 ± 1.38[b]	19.78 ± 7.17[c]	5.88 ± 0.68[b]
Oleic	4.82 ± 0.36[b]	5.63 ± 0.93[b]	8.64 ± 0.43[c]
Elaidic	1.16 ± 0.31[b]	2.72 ± 0.65[c]	0.70 ± 0.23[b]
Linoleic	7.02 ± 1.43[b]	6.39 ± 1.66[b]	9.59 ± 0.59[c]
Saturated FA	85.95 ± 1.89[b]	77.73 ± 2.68[c]	80.12 ± 1.35[c]
Monounsaturated FA	6.6 ± 0.65[b]	8.60 ± 1.07[c]	9.51 ± 0.55[c]
Polyunsaturated FA	7.37 ± 0.49[b]	13.67 ± 1.87[c]	10.23 ± 0.66[bc]

[a]g per 100 g of fatty acids determined. [b-d]Values in the same row with different superscripts differ significantly ($P < 0.05$).

luronate ratio of five seaweed species varied from 2.3 to 4.3. Our findings (2.6 and 2.8) were thus in this range.

Modified polysaccharides containing long hydrocarbon chains are efficient sorbents of lipids, as shown in experiments with amidated pectin (Marounek et al., 2013), amidated celluloses (Tůma et al., 2014), and amidated alginate (Marounek et al., 2017). In the present experiment, in rats fed amidated alginate, serum and hepatic cholesterol concentrations were decreased and faecal outputs of fat and coprostanol (metabolite of cholesterol) were increased. The loss of energy in the faeces decreased the weight gain in rats fed amidated alginate. The low concentration of hepatic cholesterol may be the reason of the low faecal output of bile acids in the rats. Bile acid synthesis in rat hepatocytes may be regulated by the availability of cholesterol for cholesterol 7α-hydroxylase (Davis et al., 1983).

Amidated alginate and THL increased the faecal loss of fat; the mode of action of both agents, however, was different. Amidated alginate is a non-specific sorbent of lipids and THL inhibits triacylglycerol hydrolysis, which is a prerequisite for lipid absorption. Both amidated alginate and THL decreased the concentration of saturated fatty acids in excreta of rats and increased the concentration of monounsaturated and polyunsaturated fatty acids. This may be related to the fact that unsaturated lipids are more easily incorporated into mixed micelles in the intestine. It is possible, however, that in amidated alginate-fed rats, also lipophilic vitamins may be partially lost in the faeces.

It follows from data in Table 4 that both amidated alginate and THL were equally efficient in the removal of dietary fat from the body. Hydrolysis of cholesterol esters is necessary for cholesterol absorption. Data on the effect of THL on the activity of intestinal cholesterol esterase are lacking in the literature.

References

Ackroff, K., Sclafani, A. (1996) Effects of the lipase inhibitor Orlistat on intake and preference for dietary fat in rats. *Am. J. Physiol. I* **271**, R48-R54.

Alemany, M., Remesar, X., Fernández-López, J. A. (2003) Drug strategies for the treatment of obesity. *IDrugs* **6**, 566-572.

Cruz-Hernandez, C., Oliveira, M., Pescia, G. J., Moulin, J., Masserey-Elmelegy, I., Dionisi, F., Destaillats, F. (2010) Lipase inhibitor orlistat decreases incorporation of eicosapentaenoic and docosahexaenoic acids in rat tissues. *Nutr. Res*. **30**, 134-140.

Davis, R. A., Hyde, P. M., Kuan, J. C. W., Malonemcneal, M., Archambaultschexnayder, J. (1983) Bile acid secretion by cultured rat hepatocytes – regulation by cholesterol availability. *J. Biol. Chem.* **258**, 3661-3667.

Edashige, Y., Murakami, N., Tsujita, T. (2008) Inhibitory effect of pectin from the segment membrane of citrus fruits on lipase activity. *J. Nutr. Sci. Vitaminol.* **54**, 409-415.

Guerciolini, R. (1997) Mode of action of orlistat. *Int. J. Obesity* **21**, S12-S23.

Kobayashi, N., Noel, E. A., Barnes, A., Rosenberg, J., DiRusso, C., Black, P., Oyler, G. A. (2013) Rapid detection and quantification of triacylglycerol by HPLC-ELSD in Chlamydomonas reinhardtii and Chlorella strains. *Lipids* **48**, 1035-1049.

Lee, Y. H., Jin, B., Lee, S. H., Song, M., Bae, H., Min, B. J., Park, J., Lee, D., Kim, H. (2016) Herbal formula HT048 attenuates diet-induced obesity by improving hepatic lipid metabolism and insulin resistance in obese rats. *Molecules* **21**, 1424.

Lindsay, H. (1973) A colorimetric estimation of reducing sugars in potatoes with 3,5-dinitrosalicylic acid. *Potato Res.* **16**, 176-179.

Lu, J., Yang, H., Hao, J., Wu, C. L., Liu, L., Xu, N. Y., Linhardt, R. J., Zhang, Z. Q. (2015) Impact of hydrolysis conditions on the detection of mannuronic to guluronic ratio in alginate and its derivatives. *Carbohyd. Polym.* **122**, 180-188.

Mahmoud, R. H., Elnour, W. A. (2013) Comparative evaluation of the efficacy of ginger and orlistat on obesity management, pancreatic lipase and liver peroxisomal catalase enzyme in male albino rats. *Eur. Rev. Med. Pharmacol. Sci.* **17**, 75-83.

Marounek, M., Volek, Z., Synytsya, A., Čopíková, J. (2007) Effect of pectin and amidated pectin on cholesterol homeostasis and cecal metabolism in rats fed a high-cholesterol diet. *Physiol. Res.* **56**, 433-442.

Marounek, M., Volek, Z., Dušková, D., Tůma, J., Taubner, T. (2013) Dose-response efficacy and long-term effect of the hypocholesterolemic effect of octadecylpectinamide in rats. *Carbohydr. Polym.* **97**, 772-775.

Marounek, M., Volek Z., Skřivanová E., Taubner, T., Pebriansyah, A., Dušková, D. (2017) Comparative study of the hypocholesterolemic and hypolipidemic activity of alginate and amidated alginate in rats. *Int. J. Biol. Macromol.* **105**, 620-624.

Marounek, M., Volek, Z., Taubner, T., Dušková, D., Čermák. L. (2019) Effect of amidated alginate on faecal lipids, serum and hepatic cholesterol in rats fed diets supplemented with fat and cholesterol. *Int. J. Biol. Macromol.* **122**, 499-502.

Melia, A. T., Koss-Twardy, S. G., Zhi, J. G. (1996) The effect of orlistat, an inhibitor of dietary fat absorption, on the absorption of vitamins A and E in healthy volunteers. *J. Clin. Pharmacol*. **36**, 647-653.

Mortensen, A., Aguilar, F., Crebelli, R., Di Domenico, A., Dosemund, B., Frutos, M. J., Galtier, P., Gott, D., Gundert-Remy, U., Lambre, C., Leblanc, J. C., Lindtner, O., Moldeus, P., Mosesso, P., Oskarsson, A., Parent-Massin, D., Stankovic, I., Waalkens-Berendsen, I., Wright, M., Younes, M., Tobback, P., Ioannidou, S., Tasiopoulou, S., Woutersen, R. A. (2017) Re-evaluation of pectin (E440i) and amidated pectin (ET440ii) as food additives. *EFSA Journal* **15**, 4866.

Porsgaard, T., Straarup, E. M., Mu, H. L., Høy, C. E. (2003) Effect of orlistat on fat absorption in rats: a comparison of normal rats and rats with diverted bile and pancreatic juice. *Lipids* **38**, 1039-1043.

Sánchez-Machado, D. I., López-Cervantes, J., López-Hernández, J., Paseiro-Losada, P., Simal-Lozano, J. (2004) Determination of the uronic acid composition of seaweed dietary fibre by HPLC. *Biomed. Chromatogr*. **18**, 90-97.

Skřivan, M., Marounek, M., Engelmaierová, M., Čermák, L., Vlčková, J., Skřivanová, E. (2018) Effect of dietary fat type on intestinal digestibility of fatty acids, fatty acid profiles of breast meat and abdominal fat, and mRNA expression of lipid-related genes in broiler chickens. *PLoS One* **13**, e0196035.

Spielman, J., Stangl, G. I., Eder, K. (2008) Dietary pea protein stimulates bile acid excretion and lowers hepatic cholesterol concentration in rats. *J. Anim. Physiol. Anim. Nutr. (Berl)* **92**, 683-693.

Taubner, T., Marounek, M., Synytsya, A. (2017) Preparation and characterisation of amidated derivatives of alginic acid. *Int. J. Biol. Macromol.* **103**, 202-207.

Taubner, T., Marounek, M., Synytsya, A. (2020) Preparation and characterization of hydrophobic and hydrophilic amidated derivatives of carboxymethyl chitosan and carboxymethyl beta-glucan. *Int. J. Biol. Macromol.* **163**, 1433-1443.

Tsujita, T., Sumiyoshi, M., Han, L.-K., Fujiwara, T., Tsujita, J., Okuda, H. (2003) Inhibition of lipase activities by citrus pectin. *J. Nutr. Sci. Vitaminol. (Tokyo)* **49**, 340-345.

Tůma, J., Volek, Z., Synytsya, A., Dušková, D., Marounek, M. (2014) Hydrophobically modified celluloses as novel cholesterol-lowering polymers. *BioRes.* **9**, 4266-4273.

Wang, Q. C., Zhao, X., Pu, J. H., Luan, X. H. (2016) Influences of acidic reaction and hydrolytic conditions on monosaccharide composition analysis of acidic, neutral and basic polysaccharides. *Carbohydr. Polym.* **143**, 296-300.

Wilcox, M. D., Brownlee, I. A., Richardson, J. C. Dettmar, P. W., Pearson, J. P. (2014) The modulation of pancreatic lipase activity by alginates. *Food Chem.* **146**, 479-484.

Wu, J., Zhao, X., Ren, L., Xue, Y. T., Li, C. X., Xu, G. L., Guan, H. S. (2014) Determination of M/G ratio of propylene glycol alginate sodium sulfate by HPLC with pre-column derivatization. *Carbohydr. Polym.* **104**, 23-28.