POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

www.pan.olsztyn.pl/journal/
e-mail: joan@pan.olsztyn.pl

Pol. J. Food Nutr. Sci.
2007, Vol. 57, No. 3(A), pp. 11-14

INFLUENCE OF HIGH LEVEL OF GRAPEFRUIT PHENOLIC PREPARATION IN CASEIN DIETS ON THE SELECTED CAECAL PARAMETERS IN RATS

Sławomir Frejnagel, Jerzy Juśkiewicz

Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn

Key words: grapefruit extract, polyphenols, caecum, rats

The aim of the study was to characterise caecal indices in rats fed diets supplemented with high doses (0.4, 0.8 and 1.2%) of a grapefruit commercial phenolic extract. Addition of the preparation caused an increase of wet mass of ceacal digesta. Dry matter content decreased from 22% to 7.0% and pH increased from 7.11 to 8.0 in the control group and experimental groups, respectively. Increased pH was a consequence of the lower concentration of volatile fatty acids in rats fed the extract (77 μmol/g in control animals *versus* 8-13 μmol/g in extract treatments). However, glycolytic microbiological activity, measured indirectly through the measurement of its enzyme activity, decreased only in rats fed a diet containing the lowest level of the preparation.

INTRODUCTION

In plants we find a wide variety of phenolic compounds that demonstrate high biological activity *e.g.* antioxidant, antimutagenic, anticarcinogenic [Hollman, 2001]. Therefore, they are treated as an important supplement of functional food. However, polyphenols, particularly a high level of the condensed forms in diets, decrease the nutritive value of diets mainly through the depression of protein utilization [Tebib *et al.*, 1996; Zdunczyk *et al.*, 1996].

Polyphenols are known as natural chemical substances that can modulate microbiological activity *in vivo* and *in vitro* [Mai *et al.*, 2004; Rechner *et al.*, 2004; Tebib *et al.*, 1996; Levrat *et al.*, 1993]. The lack of specific enzymes in the upper part of the alimentary tract of rats and humans causes that most of intaken polyphenols reach the caecum or colon, thus modifying the microbiological ecosystem [Spencer, 2003; Keppler & Humpf, 2005]. However, results of *in vivo* experiments on caecum of rats fed diets containing high doses of polyphenols, especially grapefruit phenolics, still remain controversial and unknown.

The aim of the study was to determine the influence of high doses of commercial grapefruit polyphenolic extract on caecal parameters, including microbiological activity, in rats fed casein diets.

MATERIALS AND METHODS

A freeze-dried extract from hard parts of grapefruit (stone, peel, white coats) containing 52.8% of flavanoids, mainly flavanones, flavones and flavanols, was used in the study. Additionally, the extract tested contained 17.8% of glycerol, 4.4% of vitamin C and 25% of silicon dioxide (SiO_2). A commercial preparation of grapefruit flavanoids was obtained from CINTAMANI-POLAND company (Lodz, Poland).

Composition of diets was based on the recommendations of AIN-1993 [Reeves *et al.*, 1997]. The tested extract of polyphenols (for detailed composition see Zdunczyk *et al.* [2006]) was introduced to diets instead of maize starch at a dose of 0.4%, 0.8% and 1.2%.

The studies were conducted on 32 male young (about 35 days old) Wistar rats weighing 125-132 g divided into 4 groups. All animals were fed everyday fresh diet *ad libitum* with permanent access to distilled water. Rats were housed in individual plastic cages in a well-ventilated room with a constant temperature of 21-22°C and 12-h dark/light cycles.

After 4 weeks of the experiment, the rats were anaesthetised with 20% urethane and decapitated. Ceacums were dissected for further investigations. Dry matter, pH, ammonia content and activity of selected microbial enzymes and content of volatile fatty acids (VFA) were determined (measurement details, see Juśkiewicz *et al.* [2006]).

Experimental protocols were approved by the Local Ethic Council for Animal Experiments in Olsztyn, Poland.

RESULTS

Table 1 presents selected parameters of caecum. A high, dose-dependent increase in the mass of caecal digesta was recorded (from 2.8 g in a control group to 30 g in rats fed diets with the highest content of the preparation). A similar increase was observed in the case of the gut wall mass, which was accompanied by an elevated concentration of water. Dry matter content strongly decreased from 22% in the control group to

Author's address for correspondence: Sławomir Frejnagel, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, ul. Tuwima 10, 10-747 Olsztyn, Poland; e-mail: slawus@pan.olsztyn.pl

TABLE 1. Selected indices and glycolytic activity of caecal digesta.

Indices	Preparation level				S.E.M.[1]
	0%	0.4%	0.8%	1.2%	
Caecal digesta[2]	2.79[c]	20.11[b]	26.89[a]	29.69[a]	2.0
Mass of wall[2]	0.70[c]	2.67[b]	3.09[a]	2.68[b]	0.2
Dry matter (%)	22.06[a]	13.54[b]	7.07[c]	6.97[c]	1.1
pH	7.11[c]	7.98[b]	8.19[a]	8.14[a]	0.08
Ammonia (mg/g)	0.4[a]	0.16[b]	0.09[bc]	0.06[c]	0.03
Enzyme activity (U/g)					
α-Galactosidase	0.852[a]	0.662[b]	0.632[b]	0.737[ab]	0.02
β-Galactosidase	0.126[a]	0.074[b]	0.113[a]	0.135[a]	0.01
α-Glucosidase	0.304[a]	0.068[c]	0.246[b]	0.245[b]	0.02
β-Glucosidase	1.610[a]	0.311[b]	1.460[a]	1.418[a]	0.1
β-Glucuronidase	0.363[bc]	0.111[c]	0.384[ab]	0.445[a]	0.03

[1]- S.E.M.- standard errors of the mean; [2] – expressed in g/100 g of body mass; [3] - values in the same row with different superscripts are significantly different at $p \leq 0.05$

TABLE 2. Concentration of volatile fatty acids (VFA) in caecal digesta (μmol/g of fresh digesta).

Acids	Preparation level				S.E.M.
	0	0.4%	0.8%	1.2%	
Acetic	51.2[a]	7.1[b]	5.0[b]	6.7[b]	3.5
Propionic	13.1[a]	2.1[b]	0.9[c]	1.4[bc]	0.9
Isobutyric	1.4[a]	0.6[c]	0.6[c]	0.9	0.06
Butyric	8.4[a]	1.8[b]	0.7[c]	1.1[c]	0.5
Isovaleric	1.5[a]	0.6[c]	0.6[c]	0.9[b]	0.07
Valeric	2.0[a]	0.8[c]	0.8[c]	1.2[b]	0.09
Sum of VFA	77.7[a]	13.0[b]	8.6[b]	12.2[b]	5.2
Profile[1] C2	66[a]	54[b]	54[b]	59[ab]	1.4
Profile C3	17[a]	15[a]	12[b]	11[b]	0.6
Profile C4	11[b]	14[a]	10[c]	8[c]	0.5
Pool of acetic[2]	49.6	51.6	43.4	54.7	2.5
Pool of propionic	12.7[ab]	15.1[ab]	9.9[b]	18.5[a]	1.1
Pool of isobutyric	1.4[c]	4.3[b]	6.2[b]	11.6[a]	0.7
Pool of butyric	8.3[b]	13.6[a]	7.7[b]	14.3[a]	1.0
Pool of isovaleric	1.4[c]	4.4[b]	6.3[b]	11.8[a]	0.7
Pool of valeric	1.9[c]	5.8[b]	8.3[b]	15.5[a]	0.9
Sum of pools	75.5[b]	95[b]	81.9[b]	126.7[a]	5.0

[1] - profile – molar relation of acetic (C2) acid to propionic (C3) and butyric (C4) ones; [2] - pool – cecal VFA pool was calculated as [(VFA concentration × cecal digesta) / body mass] ×100; [3] - S.E.M.- standard errors of the mean; [4] - values in the same row with different superscripts are significantly different at $p \leq 0.05$

only 7% in cecal digesta of rats fed diets containing the highest levels of the extract (0.8% and 1.2%). In turn, an increase was recorded in pH, *i.e*. from 7.1 in a control group to 8.0 in the preparation treatments. Interestingly, the high levels (0.8 and 1.2%) of preparation in the diet caused only small changes in the activity of caecal microbial enzymes tested in comparison to control animals. In a contrary, 0.4% addition of the tested extract significantly reduced activity of these enzymes.

A highly significant decrease of VFA concentration was observed in the study (Table 2). A 6-8-fold lower concentration of these acids was recorded in the content of experimental groups in comparison to the control animals (77 μmol/g *versus* 8-13 μmol/g). However, the pool of VFA, expressed as the amount of acids per 100 g of body mass, was higher in the grapefruit groups.

DISCUSSION

Polyphenols possess different biological activity, positive (functional properties) and deleterious. Analyses of caecum show some negative properties of the extract, *e.g.* a drastic increase in the mass of caecal digesta and its pH in comparison to the control animals. However, also Zdunczyk *et al.* [2006] have observed a significant increase of water content and pH in caecal content in rats in the experiment with the use of 0.3% of the grapefruit extract in diets. Such a great increase of water in the caecum content was not recorded by other researchers [Tebib *et al.*, 1996; De Vos & De Schrijver, 2003] who fed animals diets containing polyphenols. A possible explanation of the laxative properties of the extract used in the experiment is a high amount of glycerol in the extract (17.8%) that is used as an absorber during the extraction procedure. The mentioned authors observed positive aspects of polyphenols in diets, *i.e.* an increased concentration of VFA, but also decreased pH of digesta. Levrat *et al.* [1993], who introduced 1% of Quebracho tannins to rat diets, have recorded the same tendency. It was not noted in our experiment. However, expressing VFA production as the amount of acids per 100 g of body weight, we observed its higher values in the grapefruit treatments. This can suggest that the production of VFA was even higher than in the control animals. Attention should be paid to increased concentrations of branched forms of VFA (isovaleric, isobutyric) in caecal digesta of rats fed extract tested. Alles *et al.* [1999] have connected this fact with higher fermentation of proteins and amino acids in the caecum or colon. However, in the present experiment ammonia concentration in caecum content was lower in animals fed the extract examined as compared to the control rats.

In our experiment a significant decrease of the activity of microbial enzymes was recorded only in the case of rats fed diets containing the lowest amount of the extract. Tebib *et al.* [1996] and Zdunczyk *et al.* [2006] have noted a decrease in the activity of caecal glycolytic enzymes in rats fed diets containing small doses of polyphenols. Unbeneficial seems to be the increased activity of β-glucuronidase in rats fed a diet with the highest content of the extract. This enzyme is thought to be a strong procarcinogenic factor in the caecum and colon [Goldin & Gorbach, 1976; Reddy *et al.*, 1992].

CONCLUSIONS

A high dose of a grapefruit polyphenols preparation in rat diets can strongly modify caecal parameters, especially through the increase of water content and pH in caecal digesta and a decrease of volatile fatty acids concentration.

ACKNOWLEDGEMENTS

The study was supported by the State Committee for Scientific Research, Grant No. 3P06T 046 24.

REFERENCES

1. Alles M.A., Hartemink R., Meyboom S., Harryvan J.L., Van Laere K.M.J., Nagengast F.M., Hautvast J.G.A.J., Effect of transgalactosaccharide on the composition of the human intestinal microflora and putative risk of markers for colon cancer. Am. J. Clin. Nutr., 1999, 69, 980-991.
2. de Vos S., de Schrijver R., Lipid metabolism, intestinal fermentation and mineral absorption in rats consuming black tea. Nutr. Res., 2003, 23, 527-537.
3. Goldin B.R., Gorbach S.L., The relationship between diet and rat fecal bacterial enzymes in colon cancer. J. Natl. Cancer Inst., 1976, 57, 371-375.
4. Hollman P.C.H., Evidence for health benefits of plant phenols: local or systemic effects? J. Sci. Food Agric., 2001, 81, 842-852.
5. Juśkiewicz J., Klewicki R., Zdunczyk Z., Consumption of galactosyl derivatives of polyols beneficially affects cecal fermentation and serum parameters in rats. Nutr. Res., 2006, 26, 531-536.
6. Keppler K., Humpf H-U., Metabolism of anthocyanins and their phenolic degradation products by the intestinal microflora. Bioorganic Med. Chem., 2005, 13, 5195-5205.
7. Levrat M.A., Texier O., Regerat F., Demigne C., Remesey C., Comparison of the effects of condensed tannin and pectin on cecal fermentations and lipid metabolism in the rat. Nutr. Res., 1993, 13, 427-433.
8. Mai V., Katki H.A., Harmsen H., Gallaher D., Schatzkin A., Baer D.J., Clevidence B., Effects of a controlled diet and black tea drinking on the fecal microflora composition and the fecal bile acid profile of human volunteers in a double-blinded randomized feeding study. J. Nutr., 2004, 134, 473-478.
9. Rechner A.R., Smith M.A., Kuhnle G., Gibson G.R., Debnam E.S., Srai S.K.S., Moore K.P., Rice-Evans C.A., Colonic metabolism of dietary polyphenols: influence of structure on microbial fermentation products. Free Rad. Biol. Med., 2004, 36, 212-225.
10. Reddy B.S., Engle A., Simi B., Goldman M., Effect of dietary fiber on colonic bacterial enzymes and bile acids in relation to colon cancer. Gastroenterology, 1992, 102, 1475-1482.
11. Reeves P., AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformation of the AIN-76A rodent diet. J Nutr., 1997, 123, 1939-1951.
12. Spencer J.P.E., Metabolism of tea flavanoids in the gastrointestinal tract. J. Nutr., 2003, 133, 3255S-3261S.
13. Tebib K., Besancon P., Rouanet J.-M., Effects of dietary grape seed tannins on rat cecal fermentation and colonic bacterial enzymes. Nutr. Res., 1996, 16, 105-110.
14. Zdunczyk Z., Frejnagel S., Krefft B., Effect of faba beans coat with different phenolics content on the use of protein by rats. Pol. J. Food Nutr. Sci., 1996, 46, 91-102.
15. Zdunczyk Z, Juskiewicz J., Estrella I., Cecal parameters of rats fed diets containing grapefruit polyphenols and inulin as single supplements or in a combination. Nutrition, 2006, 22, 898-904.

WPŁYW WYSOKIEGO DODATKU EKSTRAKTU ZWIĄZKÓW FENOLOWYCH Z GREJPFRUTA W DIETACH KAZEINOWYCH NA WYBRANE PARAMETRY JELITA ŚLEPEGO U SZCZURÓW

Sławomir Frejnagel, Jerzy Juśkiewicz

Instytut Rozrodu Zwierząt i Badań Żywności PAN, Olsztyn

Celem pracy była charakterystyka metabolizmu jelita ślepego u szczurów żywionych dietami kazeinowymi uzupełnionymi ekstraktem ekstraktu związków fenolowych z grejpfruta w ilości 0,4; 0,8 oraz 1,2%. Dodatek badanego ekstraktu spowodował wzrost zawartości jelita ślepego. Zawartość suchej masy w treści obniżyła się z 22% w grupie kontrolnej do 7% u zwierząt żywionych dietami z udziałem ekstraktu fenoli, podczas gdy pH podniosło się z 7,11 do 8,0. Wzrost pH był konsekwencją spadku koncentracji lotnych kwasów tłuszczowych (77 μmol/L/g *versus* 8-13 μmol/L/g w grupach grejpfrutowych).