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## THE EFFECT OF THREE SOURCES OF FIBRE ON THE DIGESTIBILITY OF NUTRIENTS IN GROWING RATS

## DJELOVANJE TRIJU IZVORA VLAKNA NA PROBAVLJIVOST HRANJIVIH TVARI U ŠTAKORA U RASTU

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### SUMMARY

In an experimental study on laboratory rats we examined the digestibility of crude protein, dry matter and organic matter after the addition of different sources of fibre in the diet. A control diet was prepared to meet the nutritional requirements of growing rats. In the experimental diets a fraction of starch was replaced with a source of fibre. The apple pectin (Pectin classic AU 701 Herbstreith&Fox, degree of esterification 36-44 %), the guar gum (Fluka 09999) and the wheat bran were used. The protein source in all diets was casein. 20 young male Wistar laboratory rats (five per group) were housed in individual balance cages and fed *ad libitum* either a control diet, or one of the experimental diets with pectin, guar gum or wheat bran diet. During the experiment the amount of diet consumed and the weight of the animals were registered. At five days intervals urine and the faeces were collected and nitrogen, dry matter and crude ash contents were determined in the collected samples. The apparent and true digestibility of protein were significantly ( $P > 0.05$ ) decreased in the experimental diets as compared to the control. The true digestibility in the control diet was 92.60 %. Among the experimental diets the best value was found for wheat bran (87.93 %), and significantly ( $P > 0.05$ ) lower in guar gum (84.98 %) and in pectin (84.35 %). Biological value of protein in all groups was similar, between 71.44 % and 74.75 %. The results for the digestibility of dry matter and organic matter were similar to those for crude protein, being significantly ( $P > 0.05$ ) lower in the experimental groups as compared to the control, but in this case the wheat bran group had significantly ( $P < 0.05$ ) the smallest value. Guar gum and pectin as the source of soluble fibre had similar effects on the digestibility of nutrients, but on the other hand, wheat bran, which contains more insoluble than soluble fibre, caused some different reactions.

**Key words:** nutrition, laboratory rats, dietary fibre, digestibility, protein biological value

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## INTRODUCTION

The digestibility of nutrients is directly related to dietary fibre in the diet. Not only is the amount of dietary fibre in the diet important, but also the nature of the dietary fibre. Dietary fibre may be divided into insoluble dietary fibre, which is not viscose and poorly fermentable, and soluble dietary fibre, which forms gels, is viscose and mainly fermentable. The chemical and physical characteristics of dietary fibre have an influence on what in the digestive tract. Dietary fibre, in varying degrees, appears to reduce or delay the absorption of protein, fat and carbohydrates, as well as certain minerals and vitamins. The digestion and absorption of nutrients in the small intestine are influenced by the consumption of dietary fibre and different sources of dietary fibre have different effects on small intestine nutrient absorption. It has been demonstrated that soluble dietary fibre increases the time of intestinal transit (Brow *et al.*, 1988), delays gastric emptying (Rainbird and Low, 1986) and glucose absorption (Todd *et al.*, 1990) and can alter lipid assimilation (Pasquier *et al.*, 1996). Insoluble dietary fibre decreases intestinal transit time and increases faecal mass (Schneeman, 1990).

Guar gum prolongs transit time (Jenkins *et al.*, 1978) but pectin and wheat bran do not significantly influence transit time (Jenkins *et al.*, 1978; Bond and Levitt, 1978). Dietary fibre exerts an influence on both secretory and brush-border enzymes, but this appears to be only a part of the story. Changes in intestinal morphology and site of absorption also appear to be active (Dreher, 1987). One of the consequences of fibre intake that has been associated with viscosity is a decrease in protein digestibility and an increase in nitrogen faecal excretion (Stephen, 1987). This effect has been observed when large grain, wheat bran, fruit fibre, legumes and pectin are used as the source of dietary fibre (Kelsay, 1978; Cummings *et al.*, 1978)

Pectin is a vegetable polysaccharide and belongs to the class of soluble dietary fibre. Guar gum is also part of the soluble dietary fibre group. It is a highly fermentable and viscose polysaccharide. Wheat bran contains a high amount of insoluble lignified cell wall, which has little influence on digestibility and absorption in the small and in large intestine, and is resistant to microbial fermentation. In fact wheat bran is one of the sources which increases the amount of excreted faeces and transit time very efficiently.

The present study was designed to evaluate the effect of three different sources of dietary fibre (apple pectin, guar gum and wheat bran) on the digestibility of different nutrients, as well as on the biological value of casein protein and net protein utilisation.

## MATERIAL AND METHODS

Diets were prepared in order to meet rat requirements (NRC, 1995). As a source of protein casein was added, sunflower oil and linseed oil were added as fats, and cellulose for basic crude fibre needs. Diets were supplemented with macro-minerals, micro-minerals and vitamins. In the experimental diets a fraction of wheat starch was replaced with apple pectin (Pectin classic AU 701 Herbstreith&Fox, degree of esterification 36-44 %), guar gum (Fluka 09999) or wheat bran. Pectin and guar gum were added as sources of soluble fibre and wheat bran as insoluble fibre. The composition of the diets is presented in Table 1. In the chemical laboratory of the Chair of Nutrition Weende analysis and dietary fibre analysis (Lee *et al.*, 1992) of the diet samples were performed.

In the animal experiment 20 young growing laboratory Wistar male rats were used. Animals were 30 days old with an average body mass of 98.7 g  $\pm$  8.9 g. They were divided into 4 homogeneous groups each with 5 rats. Animals were individually housed in balance cages, which permitted the collection of urine and faeces separately during the experiment. Room temperature was practically constant, about 21°C, with moisture about 60 % and lighting was regulated automatically with a 12 hours light / dark regime. The experiment lasted for 10 days. Animals were acclimatized for 5 days in pre-experimental period to adapt to the conditions in the experimental room, to the cages and diets. All the time of the experiment the animals had free access to water and diet. Every day each animal received a weighed amount of diet and any residue from the day before was weighed. Animals were weighed at the beginning of the experiment, on the first day of the balance study and on the last day of the experiment in order to record data on the diet consumed and growth rate.

On the 5<sup>th</sup> day of the experiment the balance study was performed, following the mode of operation developed at the Department of Nutrition

(Orešnik *et al.*, 1981, 1982; Stekar *et al.*, 1984; Orešnik and Cvirn, 1984). Before taking an aliquot of the sample, faeces was homogenised in a ceramic holder. Urine was homogenised by shaking to prevent stratification. In faeces and urine nitrogen was determined by the Kjeldahl method ( $N \times 6.25$ ). Dry matter and crude ash were determined only in faeces. From these data true protein digestibility, biological value of protein, net protein utilisation, protein efficiency ratio and digestibility of dry matter and organic matter were calculated.

Data were analysed by the GLM procedure (SAS/STAT, 2000), taking into consideration the diet as the only main effect. Data are expressed as least square means (LSM)  $\pm$  standard deviation. If not stated otherwise, a least significant difference of 0.05 was used to separate treatment means.

## RESULTS AND DISCUSSION

In Table 1 results of Weende analyses and determination of dietary fibre in the diets are presented. The dietary fibre content in the experimental diets was higher than in the control diet. Among the experimental diets the soluble dietary fibre content in the guar gum and pectin diets was higher than in the wheat bran diet, but the insoluble dietary fibre content was higher in the latter than in the other diets, as was expected. The guar gum and pectin were added as sources of soluble dietary fibre and wheat bran as a source of insoluble dietary fibre. Of the total dietary fibre in wheat bran less than 10 % was found to be soluble dietary fibre (determined in the chemical laboratory of the Department of Nutrition).

**Table 1. Composition of diets (g/kg)**

**Tablica 1. Sastav obroka (g/kg)**

Composition (g/kg)	Control	Guar gum	Pectin	Wheat bran
Casein <sup>1</sup>	120	120	120	120
Guar gum <sup>2</sup>		70		
Apple pectin <sup>3</sup> (36-44 % DE)			70	
Wheat bran				155
Wheat starch	695	625	625	540
Sunflower oil	30	30	30	30
Linseed oil	80	80	80	80
CaCO <sub>3</sub>	7	7	7	7
NaCl	2	2	2	2
CaPO <sub>3</sub>	10	10	10	10
KCl	6	6	6	6
Cellulose	45	45	45	45
Premix	5	5	5	5
Determined composition (g/kg dry matter)				
Dry matter (g/kg)	921.44	924.02	921.97	917.29
Crude protein	115.85	116.07	118.72	141.91
Crude fat	109.56	123.22	121.82	116.05
Crude ash	29.68	26.76	33.24	40.13
Dietary fibre	141.13	177.85	207.75	183.32
Insoluble fibre	100.73	100.59	101.58	157.85
Soluble fibre	40.39	77.26	106.17	25.48

<sup>1</sup> Casein (Casein bovine milk) – Fluka (22078)

<sup>2</sup> Guar gum (Gum guar) – Fluka (09999)

<sup>3</sup> Apple pectin – Classic AU 701 (Apple pectin, pure pectin, unstandardized); 36 – 44 % degree of esterification; Herbreith&Fox.

**Table 2. Body mass, diet consumed and growth rate (average  $\pm$  standard deviation)****Tablica 2. Tjelesna masa, konzumirani obroci i stopa rasta (prosječno  $\pm$  standardno odstupanje)**

	Control (5)	Guar gum (5)	Pectin (5)	Wheat bran (5)
Initial body mass (g)	100.3 $\pm$ 9.3 <sup>a</sup>	95.1 $\pm$ 9.6 <sup>a</sup>	94.8 $\pm$ 6.5 <sup>a</sup>	104.6 $\pm$ 8.7 <sup>a</sup>
Final body mass (g)	112.4 $\pm$ 8.3 <sup>a</sup>	105.8 $\pm$ 9.4 <sup>a</sup>	105.2 $\pm$ 9.5 <sup>a</sup>	126.3 $\pm$ 7.7 <sup>b</sup>
Average diet consumed (g/day)	11.0 $\pm$ 0.6 <sup>a</sup>	9.3 $\pm$ 1.5 <sup>b</sup>	9.8 $\pm$ 1.7 <sup>ab</sup>	13.8 $\pm$ 0.7 <sup>c</sup>
Average growth rate (g/day)	2.43 $\pm$ 0.47 <sup>a</sup>	2.14 $\pm$ 0.40 <sup>a</sup>	2.08 $\pm$ 0.73 <sup>a</sup>	4.34 $\pm$ 0.34 <sup>b</sup>
Efficiency of diet consumed (%)	21.92 $\pm$ 3.26 <sup>a</sup>	23.20 $\pm$ 3.26 <sup>a</sup>	20.82 $\pm$ 4.89 <sup>a</sup>	31.48 $\pm$ 1.69 <sup>b</sup>

a, b, c values with the same subscript are not significantly different ( $P < 0.05$ )

The average initial body mass of laboratory rats was similar in all four groups, but at the end of the experiment the final body mass was significantly increased in the wheat bran group (Table 2). The same was found for diet consumption and average growth rate, where animals in the wheat bran group had significantly ( $P < 0.05$ ) higher values than the other three groups. Diet consumption in the guar gum and pectin groups was lower than in the control group, the difference in the guar gum group being significant ( $P < 0.05$ ). Soluble dietary fibre appears to be more effective in controlling hunger (regulation of consumption) than wheat bran. Krotkiewski (1984) fed either 10 g of guar gum or wheat bran and guar gum supplementation produced lower hunger ratings than the wheat bran supplementation. From our results it seems that rats on pectin or guar gum (soluble fibre) ate less diet. But the efficiency of the diet consumed was significantly ( $P < 0.05$ ) greater in the wheat bran diet as compared to the control. The other two experimental groups had the same diet efficiency as the control group.

A statistically significant increase of faecal mass produced as a consequence of dietary fibre is clearly observable (Table 3). This increase was more pronounced in the wheat bran group. In this group the faecal mass was twice as large as the faecal mass in the control group. Rats fed 10 % of wheat or rice bran dietetic fibre for 21 days showed an increase of faecal mass of 258 and 217 %, respectively (Gestel *et al.*, 1994). Pacheco-Delahaye (1999) also showed that faecal excretion increased more in a group of rats fed oil palm fat-free flour than in a group based on cellulose, and more in a diet with 10 % of insoluble dietary fibre than in a 5 % diet.

Linear correlations between the intake of dietetic fibre and faecal mass have been reported (Cummings *et al.*, 1992), though this increment can depend on the type of diet and the origin of the dietetic fibre (Edwards and Eastwood, 1995). The faecal mass increase could be due to several factors, such as an increase of water in the faeces (Robertson and Eastwood, 1981; Pacheco-Delahaye, 1999), the presence of insoluble dietary fibre in faeces (Pacheco-Delahaye, 1999) and an increase of bacterial mass (Cummings *et al.*, 1978; Stephen, 1987).

The nitrogen content excreted in faeces increased in all groups with added dietary fibre, more in the wheat bran (insoluble dietary fibre) group than in the two other groups with soluble dietary fibre (Table 3), but the protein digestibility was higher in the wheat bran group than in the guar gum and pectin groups. In all three experimental groups the apparent and true protein digestibility significantly ( $P < 0.05$ ) decreased as compared to the control group. The decrease was more pronounced in the guar gum and pectin groups than in the wheat bran group. The protein biological value and net protein utilisation were similar in all groups. The PER value was the highest in the wheat bran group and the smallest in the pectin group, with a significant ( $P < 0.05$ ) difference between these two groups. Some researchers ascribed the lower nitrogen digestibility to the nitrogen indigestibility of dietetic fibre (Van Soest, 1985), while others interpret the findings in terms of the increase of bacterial nitrogen in faeces (Stephen, 1987; Stephen and Cummings, 1980). Other studies suggest that fibre blocks the access of digestive enzymes to absorbable compounds (Onning and Asp, 1995).

**Table 3. Faecal mass, N in faeces, protein digestibility, biological value of protein and protein efficiency ratio (PER) (average ± standard deviation)**

**Tablica 3. Masa fecesa, N u fecesu, probavljivost bjelančevina, biološka vrijednost bjelančevina i omjer djelotvornosti bjelančevina (PER) (prosječno ± standardno odstupanje)**

	Control (5)	Guar gum (5)	Pectin (5)	Wheat bran (5)
Faecal mass (g/day)	1.21 ± 0.13 <sup>a</sup>	1.70 ± 0.38 <sup>b</sup>	1.90 ± 0.44 <sup>b</sup>	2.49 ± 0.57 <sup>c</sup>
N in faeces (mg/day)	25.64 ± 3.22 <sup>a</sup>	35.21 ± 7.29 <sup>b</sup>	38.25 ± 8.02 <sup>b</sup>	46.99 ± 2.31 <sup>c</sup>
Apparent protein digestibility (%)	86.42 ± 1.38 <sup>a</sup>	77.86 ± 2.23 <sup>b</sup>	77.78 ± 1.94 <sup>b</sup>	83.62 ± 0.82 <sup>c</sup>
True protein digestibility (%)	92.60 ± 1.42 <sup>a</sup>	84.98 ± 2.31 <sup>b</sup>	84.35 ± 2.21 <sup>b</sup>	87.93 ± 0.85 <sup>c</sup>
Biological value of protein (BV) (%)	74.50 ± 5.97 <sup>a</sup>	71.44 ± 10.44 <sup>a</sup>	74.75 ± 8.89 <sup>a</sup>	72.36 ± 2.62 <sup>a</sup>
Net protein utilisation (NPU) (%)	69.04 ± 6.48 <sup>a</sup>	60.80 ± 9.93 <sup>a</sup>	63.56 ± 6.03 <sup>a</sup>	63.63 ± 2.41 <sup>a</sup>
PER (g mass increase/g CP)	2.05 ± 0.31 <sup>ab</sup>	2.16 ± 0.30 <sup>ab</sup>	1.90 ± 0.45 <sup>a</sup>	2.42 ± 0.13 <sup>b</sup>

a, b, c values with the same subscript are not significantly different (P < 0.05)

Fibre is able to alter the digestion rate by various mechanisms, which depend on the chemical composition of the fibre and its physical properties, such as viscosity (Stasse-Wolthuis, 1981; Topping *et al.*, 1988). Dietary fibre could decrease casein hydrolysis by different mechanisms, including a direct effect, reducing the enzyme-substrate binding (Schneeman and Gallahar, 1985), by altering the enzymatic conformation (Roehring, 1988) or in the

case of non-purified fibre sources, by interaction with enzyme inhibitors (Dunaif and Schneeman, 1981). Viscosity can reduce digestibility by modulation of the movement and contact of digesta and enzymes (Shah *et al.*, 1986). Lamghari El Cossori *et al.* (2000) thought that the decrease in N release caused by soluble dietary fibre was due to the interaction of the fibre with enzymes or protein rather than to the viscosity.

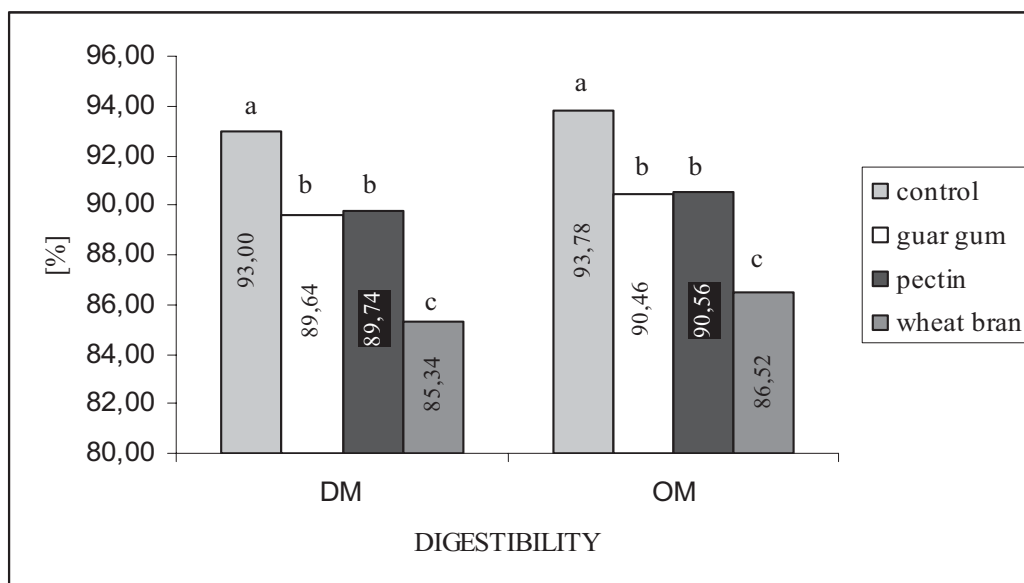


Figure 1. Digestibility of dry matter and organic matter  
Slika 1. Probavljivost suhe tvari i organske tvari

In Figure 1 the results for digestibility of dry matter and organic matter are presented. In both cases the highest digestibility was found in the control group, and decreased significantly ( $P < 0.05$ ) in the guar gum and pectin groups. In the wheat bran group the values were significantly ( $P < 0.05$ ) the smallest. Some investigations have been carried out to study the effect of dietary fibre on the digestibility of dietary nutrients. Recent investigations have shown that the effect of dietary fibre differs with the source and nature of the fibre and is related to its chemical composition, as well as to its physico-chemical properties (Souffrant, 2001).

Our results on the influence of fibre source on nutrient digestibility explain the similar response of guar gum and pectin. Wheat bran showed some differences compared to the guar gum and pectin groups. The digestibility was significantly ( $P < 0.05$ ) decreased in all three experimental groups as compared to the control group. In wheat bran the protein digestibility was intermediate, with a significant ( $P < 0.05$ ) difference from the other groups, but the digestibility of the dry matter and organic matter was significantly ( $P < 0.05$ ) the smallest.

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## SAŽETAK

U pokusima istraživanja provedenog na laboratorijskim štakorima ispitali smo probavljivost sirovih bjelančevina, suhe tvari i organske tvari nakon dodavanja raznih izvora vlakna u obroke. Kontrolni obrok je pripremljen tako da se udovolji potrebama štakora u rastu. U pokusnim obrocima frakcija škroba zamijenjena je izvorom vlakna. Upotrijebljeni su jabučni pektin (Klasični pektin AU 701 Herbstreith Fox, stupanj esterifikacije 36-44%), guar gum (Fluka 09999) i pšenične posije. U svim je obrocima kazein bio izvor bjelančevina. Dvadeset mladih mužjaka laboratorijskog štakora Wistar smješteno je (po pet u skupini) u pojedinačne kaveze i hranjeno *ad lib.* kontrolnim obrokom ili jednim od pokusnih obroka s pektinom, guar gum-om ili pšeničnim posijama. Za vrijeme pokusa bilježena je količina konzumiranog obroka i težina životinja. U razmacima od pet dana sakupljani su mokraća i feces te određivan sadržaj dušika, suhe tvari i sirovog pepela u sakupljenim uzorcima. Prividna i prava probavljivost bjelančevina znatno se smanjila ( $P>0.05$ ) u pokusnim obrocima u usporedbi s kontrolnim obrokom. Prava probavljivost u kontrolnom obroku bila je 92.60%. U pokusnim obrocima

najbolja je vrijednost ustanovljena za pšenične posije (87.93%) i znatno niža ( $P>0.05$ ) za guar gum (84.98%) i pektin (84.35%). Biološka vrijednost bjelančevina u svim je skupinama bila slična, između 71.44% i 74.75%.

Rezultati za probavljivost suhe tvari i organske tvari bili su slični rezultatima za sirove bjelančevine, znači značajno niži ( $P<0.05$ ) u pokusnim skupinama u usporedbi s kontrolnom skupinom, ali u tom je slučaju skupina s pšeničnim posijama imala značajno najnižu ( $P<0.05$ ) vrijednost. Guar gum i pektin kao izvor topivog vlakna imali su sličan učinak na probavljivost hranjivih tvari, ali s druge strane pšenične posije, što sadrže više netopivog nego topivog vlakna, prouzročile su nešto drugačije reakcije.

Ključne riječi: hranidba, laboratorijski štakori, hranjivo vlakno, probavljivost, biološka vrijednost bjelančevina

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