COBISS: 1.01
Agris category code: L51

EFFECTS OF DIETARY PECTIN ON PROTEIN DIGESTION AND METABOLISM IN GROWING RATS

Tatjana PIRMAN [1], Philippe PATUREAU MIRAND [2], Andrej OREŠNIK [3], Janez SALOBIR [3]

Delo je prispelo 02. julija 2009, sprejeto 17. novembra 2009.
Received July 02, 2009; accepted November 17, 2009.

Effects of dietary pectin on protein digestion and metabolism in growing rats

In an attempt to clarify the relationships between the digestive and metabolic effects of additional feeding of citrus pectin, the intestinal contents, tissues weights, nitrogen excretion and retention were studied in laboratory rats. Twenty-four growing male Wistar rats (98.8 g ± 5.3 g of body weight) were fed *ad libitum* for 20 days with balanced diets containing casein as the source of protein. In the experimental diet 96 g of wheat starch was replaced by 80 g of citrus pectin and 16 g of vegetable oil. Apparent digestibility and apparent protein biological value were calculated, the weights of digestive tissues and digestive organ content weights were also determined. All tissues of different parts of the digestive tract were heavier in the pectin fed group, and small intestine and caecum were statistically significant different as compared to control group. The contents of the small intestine and caecum were significant heavier in the pectin group. Pectin significantly lowered dry matter intake and growth rate and significantly increased faecal excretion of nitrogen and significantly decreased urinary nitrogen excretion. The consequence of alteration in the nitrogen excretion route was significantly lower apparent protein digestibility and apparent net protein utilisation, but apparent protein biological value was unchanged.

Key words: proteins / digestibility / metabolism / pectin / small intestine / large intestine / laboratory rats

Vpliv pektina na prebavo beljakovin in metabolizem pri rastočih podganah

Da bi poskusili razložiti povezavo in vpliv krmljenja pektina iz limonine lupine na prebavo in presnovo, smo izmerili maso vsebine prebavil, maso tkiv prebavil ter izločen in absorbiran dušik pri laboratorijskih podganah. Štiriindvajset rastočih laboratorijskih podgan moškega spola seva Wistar (s povprečno telesno maso 98,8 g ± 5,3 g) smo 20 dni krmili *ad libitum* z uravnoteženo krmo, ki je vsebovala kazein kot vir beljakovin. V poskusni krmi smo 96 g pšeničnega škroba zamenjali z 80 g pektina iz limonine lupine in 16 g mešanice rastlinskih olj. Izračunali smo navidezno prebavljivost beljakovin in navidezno biološko vrednost beljakovin ter določili maso posameznih tkiv in vsebine prebavil. V poskusni skupini, ki je imela v krmi pektin, smo izmerili večjo maso tkiv posameznih delov prebavil v primerjavi s kontrolno skupino, s statistično značilnimi razlikami pri tankem in slepem črevesju. Masa vsebine tankega in debelega črevesja je bila statistično značilno večja pri skupini s pektinom. V skupini s pektinom v krmi so živali zaužile značilno manj suhe snovi krme in imele manjše priraste, značilno več izločenega dušika v blatu, pa tudi značilno manj izločenega dušika preko seča. Posledica razlik v izločanju dušika preko blata ali seča je značilno manjša navidezna prebavljivost in navidezna neto izkoristljivost beljakovin, medtem, ko je navidezna biološka vrednost beljakovin ostala nespremenjena.

Ključne beseda: beljakovine / prebavljivost / presnova / pektin / tanko črevo / debelo črevo / laboratorijske podgane

1 Univ. of Ljubljana, Biotehnical Fac., Dept. of Animal Science, Groblje 3, SI-1230 Domžale, Slovenia, e-mail: tatjana.pirman@bfro.uni-lj.si
2 INRA, Unité Nutrition Humaine, INRA Clermont-Ferrand – Theix, F-63122 Theix, France
3 Same address as 1, Prof., Ph.D.

1 INTRODUCTION

Pectin is a component of many feedstuffs. Especially citrus and sugar beet pulp are rich in pectin and to a limited degree it is present in grains and legumes. Pectin improves the stability and tight connection of plant cells, their osmolality and water content, and together with hemicellulose, cellulose and lignin it reinforces the cell matrix.

The name pectin is derived from the Greek word "pectos", which means gelatinizing or swelling and is related to the main physical and chemical character of this molecule in establishing gels. Most authors point out the resistance of pectin to the digestive enzymes of mammals and include it among the non-starch polymers (Eastwood, 1992). A logical approach is the classification of pectin as part of dietary fibre (Trowell *et al.*, 1976; Eastwood, 1978; Schneemann, 1986; McDougall *et al.*, 1996), but only a small part of pectin belongs to the cell wall. The rest is part of the cell cytoplasm and most fractions of pectin are soluble. After isolation, pectin can be used as a special dietetic feed composition.

A large number of investigations have been carried out to study the effect of dietary fibre on the digestibility of nutrients. Most authors report that the fibre content of diet can impair the apparent digestibility of nutrients. In particular, the effect of dietary fibre differs with the source and nature of the fibre and relates to its chemical composition as well as to its physical and chemical properties. The effect of the viscous nature of dietary fibre, like pectin, on digestibility is contradictory. Already Murray *et al.* (1977) reported a decrease in the apparent digestibility of nitrogen in pigs after feeding gel-forming polysaccharides (methyl-cellulose or pectin), whereas the replacement of starch by cellulose caused no decrease. Other authors observed no effect of gel-forming polysaccharides on nitrogen digestion in rats (Larsen *et al.*, 1994) and in pigs (Li *et al.*, 1994). Soluble polysaccharides have been found generally to be without significant effect on the apparent digestibility of nutrients in pigs (Huisman *et al.*, 1985). For other authors, not only the amount of nitrogen excreted in faeces, but also the nitrogen excreted in urine was found to be affected by the type and fermentability of carbohydrate. Pastuszewska *et al.* (2000a) found that faecal nitrogen excretion in rats was increased by all carbohydrates (potato starch, pectin or cellulose) when substituted for cereal starch, but only pectin decreased urinary nitrogen excretion. Carbohydrates significantly altered the routes of nitrogen excretion in protein-free diets too (Pastuszewska *et al.*, 2000b).

The present study was designed to examine the effect of pectin on protein utilisation and on the development of the gastrointestinal tract to obtain a better picture of the nutritive functions of pectin in growing rats.

2 MATERIAL AND METHODS

2.1 DIETS

Two diets were prepared, a control diet and a pectin diet in which a fraction of the wheat starch was replaced by pectin from citrus peel (Fluka ref. No. 76280, degree of esterification 63–66%, MW 30 000–100 000). The diets contained different amounts of an oil mixture to adjust the diets' energy concentrations. The protein source in the diets was casein purchased from Union des Caséineries de Charente Maritime (La Rochelle, France) and the diets were calculated to contain 110 g of crude protein. Diets (Table 1) were designed to meet the nutritional requirements of growing rats (NRC, 1995). Weende analysis, mineral content and dietary fibre analysis (Lee *et al.*, 1992) of the diets were performed.

2.2 ANIMALS AND EXPERIMENTAL PROCEDURE

All procedures were performed according to current legislation on animal experimentation in Slovenia. Permission for the experiment was granted by the Veterinary Administration of the Republic of Slovenia (VURS) under the number 323-02-215/2004/2. Twenty four male Wistar rats (98.8 g ± 5.3 body weight) reared in the Lek laboratory animal unit (Ljubljana, Slovenia) were housed in cages placed in a room kept at about 21 °C and 60% humidity (checked and recorded each day), with light automatically regulated on a 12-hour light/dark cycle starting at 7.00 a.m. After a 4 days adaptation period, in which the rats received a control diet, rats were separated into two equal groups (n = 12), with average body weight 120.1 ± 5.5 g and 120.1 ± 5.2 g in the control and pectin groups, respectively. Animals were individually housed in metabolic cages which permitted the collection of urine and faeces separately during the experiment, and had free access to drinking water. They received *ad libitum* control or pectin semi-synthetic diet during an 18 or 20-day period.

On the 6th day of the experimental period the 5-day balance study began (Orešnik and Cvirn, 1984; Orešnik *et al.*, 1982; Stekar *et al.*, 1984). Each day animals received a new weighed daily meal and the residue from the day before was weighed. Body weights were recorded on the first day of the balance experiment, on the third day and on the last day. Urine was collected in a bottle after filtra-

Table 1: *Diets (g/kg)*
Preglednica 1: *Krma (g/kg)*

	Control Kontrola	Pectin Pektin
Casein / Kazein	120	120
Wheat starch / Pšenični škrob	616	520
Premix / Premiks [1]	40	40
Sugar (sucrose) / Sladkor (saharoza)	50	50
Mixture of vegetable oils Mešanica rastlinskih olj [2]	54	70
Agar-agar / Agar-agar	40	40
Pectin / Pektin	0	80
Mineral mixture Mešanica rudninskih snovi [3]	70	70
Vitamin mixture Mešanica vitaminov[4]	10	10
Sum / Vsota	1000	1000

[1] Mixture of wheat starch and L-cystine 55 mg/g (2.2 g of cystine added in each diet) / Mešanica pšeničnega škroba in L-cistina 55 mg/g (2,2 g cistina dodanega vsaki krmi)
[2] Rape oil, groundnut oil and sunflower oil (50:45:5) / Repično olje, arašidovo olje in sončnično olje (50:45:5)
[3] Minerals UAR 205 b (UAR, Villemoisson-sur-Orge, France) / Rudninske snovi UAR 205 b
[4] Vitamins UAR 2.00 / Vitamini UAR 2,00

tion. This bottle contained 10 ml of 6 M HCl for each cage to stop all reactions in the urine and to prevent losses of nitrogen. The faeces samples were collected in a different vessel. On the last day (5^{th}) of the balance study, the urine was transferred to a prepared plastic bottle, weighed and stored at −20 °C until analyses were performed. Faeces samples were also stored in prepared plastic bottle, weighed and frozen.

Before taking an aliquot of the sample for analysis, faeces were homogenised in a ceramic holder. Urine was homogenised by shaking to prevent stratification. In diets, faeces and urine nitrogen was determined by the Kjeldahl method. Dietary crude protein (CP) was evaluated as N × 6.25. Dry matter and crude ash were determined in diets and faeces. Based on the amount of N intake, N in faeces and N in urine, the following indices of N utilisation were calculated: nitrogen apparent digestibility = (N intake − N in faeces) / N intake; apparent protein biological value = (N intake − N in faeces − N in urine) / (N intake − N in faeces) and apparent net protein utilisation (NPU) = (N intake − N in faeces − N in urine) / N intake; protein efficiency ratio (PER) = growth rate / CP intake. Digestibility of organic matter (OM) = OM intake − OM in faeces / OM intake and dry matter efficiency: growth rate / dry matter intake × 100 were also calculated.

2.3 SAMPLING OF TISSUES

Half of the animals in each group were sacrificed on the 18^{th} day of the experiment and the other half on the 20^{th} day in the morning between 9 and 11 o'clock. Rats were anaesthetized with an abdominal injection of Pentothal (0.1 ml/100 g of body weight, Sigma, Saint Louis, Mo., USA). The digestive tract was quickly removed. Each part of the digestive tract (stomach, small intestine, colon and caecum) was weighed with its content. The digestive content of the small intestine was collected. The small intestine wall was rinsed with a cold 2% TCA solution, wiped and weighed. Stomach, colon and caecum treatment was similar to treatment of the small intestine.

2.4 DATA ANALYSIS

Data were analysed by the General Linear Models (GLM) procedure (SAS/STAT, 2000), taking into consideration the diet as the main effect, and in the case of the relative weight of intestinal tissues and gastrointestinal content also the day of slaughter. Data are expressed as least square means (LSM) ± standard deviation (SD). Significance was considered established at $P < 0.05$.

Table 2: *Relative weight of digestive tissues (g per 100 g of the body weight) (average ± SD)*
Preglednica 2: *Relativna masa tkiv prebavnega trakta (g / 100 g telesne mase) (povprečje ± SD)*

	Control / Kontrola (12)	Pectin / Pektin (12)	P-value / p-vrednost *
Stomach / Želodec	0.63 ± 0.14	0.66 ± 0.06	0.5410
Small intestine / Tanko črevo	2.94 ± 0.29	3.63 ± 0.84	**0.0151**
Caecum / Slepo črevo	0.41 ± 0.07	0.65 ± 0.10	**< 0.0001**
Colon / Kolon	0.63 ± 0.07	0.70 ± 0.11	0.0656
Whole intestine / Celotno črevesje	3.97 ± 0.33	4.97 ± 0.88	**0.0016**
Digestive tract / Prebavni trakt	4.60 ± 0.42	5.63 ± 0.88	**0.0016**

* Diet and day of slaughtering as two main effects / Krma in dan žrtvovanja kot dva glavna vpliva

Table 3: *Weights (g) of the gastrointestinal content (average ± SD)*
Preglednica 3: *Mase (g) vsebine prebavil (povprečje ± SD)*

	Control Kontrola (12)	Pectin Pektin (12)	P-value p-vrednost *
Content of stomach / Vsebina želodca	4.40 ± 5.71	3.06 ± 3.08	0.1634
Content of small intestine / Vsebina tankega črevesja	1.42 ± 0.78	3.34 ± 2.57	**0.0069**
Content of caecum / Vsebina slepega črevesja	2.36 ± 0.72	3.91 ± 0.72	**< 0.0001**
Content of colon / Vsebina kolona	1.44 ± 0.58	1.60 ± 0.50	0.4928
Content of whole intestine / Vsebina celotnega črevesja	5.23 ± 1.40	8.85 ± 3.07	**0.0002**
Content of digestive tract / Vsebina prebavnega trakta	9.70 ± 6.21	11.91 ± 5.69	0.1028

* Diet and day of slaughtering as two main effects / Krma in dan žrtvovanja kot dva glavna vpliva

3 RESULTS

In Table 2 the weights of different organs of the digestive tract are expressed as relative weights (g/100 g of body weight) to minimise the body size effect. The average relative weights of the small intestine and caecum were significantly ($P < 0.05$) higher in the pectin group as compared to the control. The relative weight of the stomach was similar in both groups. The average colon relative weight was higher in the pectin group than in the control, but the difference was not significant. The average relative weight of the whole intestine or whole digestive tract was also significantly ($P < 0.05$) higher in the pectin group.

Similar results were found for the weights of the digestive contents. There was significantly ($P < 0.05$) more digestive contents in the small intestine, caecum and whole intestine in the pectin fed rats compared to the control group (Table 3).

After the 5-day pre-experimental period, the average body weight of animals in the pectin group was significantly ($P < 0.05$) lower as compared to the control group. Since the dry matter intake in pectin group was lower all the time of experimental period, the difference become significant already at the beginning of the balance experiment (15.9 g ± 11.04 g) and increased (30.7 g ± 13.98 g) until the end of the balance experiment (Table 4). The result was significantly lower growth rate in pectin group as compared to control group. The problem of different body weight could be minimise, if animals of the control group have some lower body weight at the beginning of experimental period, but in this case the age of animals will be different and the difference in the growth rate even bigger. The differences in the dry matter efficiency and PER value were also significantly lower in the pectin group than in the control group.

Since the dry matter intake was lower in the pectin group, the nitrogen intake was also significantly ($P < 0.05$) lower than in the control group (Table 5). The amount of faeces excreted per day, the amount of N excreted in faeces per day and the amount of urine excreted per day were significantly ($P < 0.05$) higher in the pectin group as compared to the control group. On the other hand, the nitrogen excreted in urine was significantly lower in

Table 4: *Body weight, dry matter intake and growth rate (average ± SD) in 5 days balance measurements*
Preglednica 4: *Telesna masa, zaužita suha snov in prirast (povprečje ± SD) v 5 dneh bilančnih meritev*

	Control Kontrola (6)	Pectin Pektin (6)	P-value p-vrednost
Initial body weight (g) / Telesna masa ob začetku poskusa (g)	149.3 ± 7.11	133.4 ± 6.27	0.0021
Final body weight (g) / Telesna masa ob koncu poskusa (g)	179.2 ± 8.10	148.5 ± 7.22	**< 0.0001**
Dry matter intake (g DM/day) / Zaužita suha snov (gSS/dan)	17.0 ± 0.70	13.9 ± 0.46	**< 0.0001**
Growth rate (g/day) / Prirast (g/dan)	6.0 ± 0.51	3.0 ± 0.40	< 0.0001
DMI/average body weight (g/g) / ZSS/povprečno telesno maso (g/g)	0.103 ± 0.004	0.099 ± 0.005	0.0734
Dry matter efficiency (%) / Izkoristek suhe snovi krme (%)	35.22 ± 2.34	21.75 ± 2.32	**< 0.0001**
PER (g growth/g CP) / PER (g prirasta/g SB)	3.0 ± 0.20	1.6 ± 0.20	**< 0.0001**

DMI – dry matter intake / ZSS – zaužita suha snov; PER – protein efficiency ratio / učinkovitost beljakovin za prirast; CP – crude protein / SB – surove beljakovine

Table 5: *Balance experiment, digestibility, protein biological value and apparent net protein utilisation (average ± SD)*
Preglednica 5: *Bilančni poskus, prebavljivost, biološka vrednost beljakovin in navidezna neto izkoristljivost beljakovin (povprečje ± SD)*

	Control Kontrola (6)	Pectin Pektin (6)	P-value p-vrednost
N intake (mg/day) Zaužiti N (mg/dan)	323 ± 13	259 ± 9	< **0.0001**
N intake/average body weight (mg/g) Zaužiti N/povprečno telesno maso (mg/g)	2.0 ± 0.1	1.8 ± 0.1	**0.0188**
Excreted faeces (g fresh mass/day) Izločeno blato (g svežega blata/dan)	2.4 ± 0.2	3.1 ± 0.6	**0.0016**
N in faeces (mg/day) N v blatu (mg/dan)	28.0 ± 3.2	44.1 ± 5.9	**0.0002**
N in faeces/average body weight (mg/g) N v blatu/povprečno telesno maso (mg/g)	0.171 ± 0,023	0.314 ± 0.050	< **0.0001**
Excreted urine (g/day) Izločen seč (g/dan)	19.5 ± 2.9	24.4 ± 2.7	**0.0120**
N in urine (mg/day) N v seču (mg/dan)	86.8 ± 9.3	66.2 ± 7.1	**0.0015**
N in urine/average body weight (mg/g) N v seču/povprečno telesno maso (mg/g)	0.529 ±0.053	0.471 ± 0.057	0.0980
N balance (mg/day) Bilanca N (mg/dan)	208 ± 10	149 ± 13	< **0.0001**
N balance/average body weight (mg/g) Bilanca N/povprečno telesno maso (mg/g)	1.27 ±0.05	1.06 ± 0.08	**0.0003**
Apparent digestibility of protein (%) Navidezna prebavljivost beljakovin (%)	91 ± 1	83 ± 2	< **0.0001**
Digestibility of organic matter (%) Prebavljivost organske snovi (%)	95 ± 1	92 ± 1	< **0.0001**
Apparent protein biological value (%) Navidezna biološka vrednost beljakovin (%)	71 ± 2	69 ± 4	0.4519
Apparent net protein utilisation (%) Navidezna neto izkoristljivost beljakovin (%)	64 ± 2	57 ± 4	**0.0047**

N – nitrogen / dušik

the pectin group. Consequently, the nitrogen balance was significantly decreased in the pectin group (on average only 71.5% of the value in the control group). Results expressed per 1 g of average body weight show significant increase excretion of N in faeces (for 84%) and decrease consumed N (for 10%) and N balance (for 16%) in pectin group, but no significant differences in excretion of N through urine.

The apparent protein digestibility, digestibility of dry matter and organic matter decreased significantly ($P < 0.0001$) as a result of pectin addition to the diet (Table 5). On the contrary, the apparent biological value of protein was not affected by pectin, the average values were not different in the two groups ($P = 0.4519$), but apparent net protein utilisation was also significantly decreased in the pectin group as compared to the control.

4 DISCUSSION

It is well recognized that dietary non-starch polysaccharides, especially soluble ones, such as pectin, can decrease the apparent digestibility of whole protein or of amino acids in pigs (de Lange *et al.*, 1989; Mosenthin *et al.*, 1994; Zhu *et al.*, 2005; Libao-Mercado *et al.*, 2006). Such effects are likely to be related to endogenous nitrogen losses, which can be seen in increased secretion and impaired reabsorption in the lower part of the gastrointestinal tract in pigs (Grela *et al.*, 1998) and in rats (Larsen *et al.*, 1993), and in stimulation of the rate of microbial fermentation in the gut of monogastric animals (Eggum, 1995; Schulze *et al.*, 1995; McCullough *et al.*, 1998). The present study demonstrates that the course of

digestion and also the enlargement of the intestinal tissues are influenced by pectin.

It has been found that the addition of pectin to the diet significantly increases endogenous nitrogen flow in rats (Pastuszewska *et al.*, 2000b) and in pigs (de Lange *et al.*, 1989; Libao-Mercado *et al.*, 2006) which includes digestive gland secretions, desquamated cells from active replacement of the gastrointestinal mucosal lining, and secretion of plasma components (urea and a small amount of plasma protein) (Shah *et al.*, 1982). Indeed pectin has been shown to stimulate enterocyte turnover (Fukunaga *et al.*, 2003; Chun *et al.*, 1989), and may increase mucin secretions because of stimulation by the increase in caecal short chain fatty acid production (Barcelo *et al.*, 2000) in laboratory rats.

The effect of pectin on protein flow (of dietary and endogenous origin) could also be the result of an interference with luminal protein digestion either of dietary or of endogenous origin, as shown for other dietary fibers that enhance endogenous nitrogen secretion (Schulze *et al.*, 1995). Endogenous amino acids may not be available for absorption because of the physical and chemical adsorptive properties of pectin (Souffrant, 2001). El Kossori *et al.* (2000) suggested that protein hydrolysis could be prevented by interactions between protein or enzyme and fiber without modification of the viscosity, and would depend on the kind of fiber. Animals producing more mucus could have a slower absorption rate, because it has been postulated that mucus contributes to the apparent thickness of the unstirred layer and affords protection to the mucosal surface (Nimmerfall and Rosenthaler, 1980). However, increased luminal mucin did not disturb glucose or ovalbumin absorption (Morita *et al.*, 2006).

Undigested endogenous or dietary nitrogenous compounds can be transformed by microorganisms in the large intestine. In the intestinal tract of pigs, microbes degrade up to 90% of pectin by fermentation (Drochner *et al.*, 2004). Such microbes use pectin as an energy source and also use most of the luminal nitrogen, which is consequently excreted in faeces. However, this phenomena cannot be responsible for the enlargement of excreted nitrogen in the faeces unless the increased microbial fermentation, evidenced by the increase in the production of short chain fatty acids (SCFA) in the large intestine (Pirman *et al.*, 2007), induces a stimulation of endogenous nitrogen secretions. Beside that, SCFAs have some other important roles in the intestinal lumen. Research on rats showed that an increased concentration of SCFA in the intestinal lumen, because of the ingestion of non-starch polysaccharides or non-digestible oligosaccharides decreased the pH value in the large intestine, leading to the conversion of NH_3 to NH_4^+. This form of ammonia cannot diffuse through the intestinal wall (Younes *et al.*, 1995), leading to the change of the nitrogen metabolism, since the more nitrogen is excreted by faeces, the less nitrogen is transferred to urea in the liver and consequently less nitrogen is excreted by urine (Mosenthin *et al.*, 1992). On the other hand, where microbial fermentation is very intensive (because of the presence of soluble dietary fiber, like pectin), the utilization of urea by microflora in the intestinal lumen is increased and again nitrogen is excreted through faeces (microbial mass) and less nitrogen is excreted through urine.

In the present study, the enlarged faecal nitrogen loss seems to be of endogenous origin. Indeed, faecal nitrogen loss was increased by 57% by pectin feeding, which is very similar to the increase described for endogenous losses (53%) in rats fed a pectin nitrogen–free diet when compared with a control nitrogen-free diet (Pastuszewska *et al.*, 2000b). This conclusion suggests that the increase in nitrogen faecal loss induced by pectin feeding is unlikely to result from an increase in faecal loss of dietary nitrogen. Consequently most dietary amino acids in our experiment would have been absorbed as with the control diet.

All the carbohydrates (potato starch, pectin, cellulose and tannic acid) used in the study on laboratory rats of Pastuszewska *et al.* (2000a) tended to decrease the blood urea nitrogen concentration as compared to the control (wheat starch as carbohydrate), and nearly 20% nitrogen excreted by urine in a control group (319 mg vs. 254 mg in urine of pectin group) was obviously metabolized in the large intestine (72 mg vs. 110 mg in faeces of pectin group). If such a proportion was applied in the present study, on average 16.54 mg of endogenous urea nitrogen (blood urea nitrogen excreted by faeces) would be redirected from urinary excretion to fecal excretion. This value is close to the difference between nitrogen faecal excretion in the pectin (28.00 mg/d) and control rats (44.05 mg/d). Nitrogen originating from endogenous urea may be a major component of the increase in nitrogen faecal losses by pectin feeding. Another consequence of this exchange is that the assessments of protein nutritional value need to be corrected to take into account this exchange between urine and faecal nitrogen excretion. In this case, the corrected nitrogen digestibility becomes 91.31% and 89.37% for the control and the pectin diets, respectively, while the corrected biological value becomes 70.58% and 64.21% in the control and pectin diets, respectively. Consequently the main difference in protein and diet nutritional value between the control and the pectin diets seems to be not at absorption level but rather in differences in urea excretion pathways and possibly in amino acid metabolism.

This could be a consequence of an increase in the requirements for a specific nutrient (energy, amino ac-

ids like threonine) due to the high protein turnover in intestinal tissues observed in pectin fed rats, which may impair amino acid utilization in other organs (Pirman *et al.*, 2007). Indeed the weights of intestinal tissues in rats were increased as a result of addition of pectin to the diet, as it was found in a previous study (Pirman *et al.*, 2007). When different fibers were compared, the greatest enlargement of caecal digests and tissue was observed after addition of pectin to the diet (Pastuszewska *et al.*, 2000a). This intestinal hypertrophy corresponded to hyperplasia in the small intestine (Brown *et al.*, 1979), to an increase in villous cell exfoliation and crypt cell proliferation (Jacobs 1983), and to alteration of gut morphology by increasing the number of goblet cells and mucus production (Cassidy *et al.*, 1981) in laboratory rats. In our previous study on addition of pectin to the diet of the laboratory rats (Pirman *et al.*, 2007) the villous height in the small intestine and crypt depth in small intestine, caecum and colon were significantly increased as compared to the control diet. In a previous study, the strong stimulation of intestinal protein turnover corresponded to a slight decrease in muscle protein turnover (Pirman *et al.*, 2008). In the study of Zhu *et al.* (2005) on pigs the utilization of threonine in whole body protein deposition was linearly decreased with the dietary pectin level, but not of lysine. This was connected to the high amount of threonine in mucoproteins, so the influence of pectin on digestive physiology operates through amino acid and nitrogen utilization at the whole body level.

5 CONCLUSIONS

Pectin is an important factor affecting the proportions of faecal and urinary nitrogen excretion and ultimately both apparent protein digestibility and corrected apparent protein biological value. This effect is related to the fermentability of pectin, especially in the large intestine. Furthermore, the study confirmed the effects of pectin on digestive physiology, namely increased urea excretion from blood to intestine and reduced urea excretion by urine. Both consequences (in digestive tract and in kidney function) of the presence of pectin in the diet are of benefit for health status in animals and men.

6 POVZETEK

Pektin spada v skupino topne vlaknine in ga najdemo v mnogih in različnih vrstah hrane in krme. V literaturi najdemo različne in nasprotujoče učinke na prebavljivost hranljivih snovi pri dodajanju topne vlaknine v obrok. V večini primerov povečajo izločanje dušika preko blata, a le redko zmanjšajo izločanje dušika preko seča. Z našo raziskavo smo želeli ugotoviti vpliv pektina na izkoristljivost beljakovin in na razvoj posameznih delov prebavil, s tem pa bi dobili boljšo predstavo o prehranski vlogi pektina. Pripravili smo dve krmi, v katerih je bil kazein kot vir beljakovin. V poskusni krmi smo del pšeničnega škroba zamenjali s pektinom iz limonine lupine in mešanico rastlinskih olj, da sta bili krmi izoenergijski. 24 rastočih laboratorijskih podgan moškega spola smo po 4 dnevih predposkusa razdelili v dve homogeni skupini (telesna masa 120,1 g ± 5,5 g in 120,1 g ± 5,2 g za kontrolno in pektinsko skupino) in jih 20 dni *ad libitum* krmili z eno od pripravljenih krmnih mešanic in merili zauživanje krme in priraste. V času poskusa smo 5 dni zaporedoma ločeno zbirali blato in seč (bilančni poskus). Na koncu poskusa smo živali žrtvovali, odvzeli tkiva prebavil in jih stehtali polne in prazne. Izračunali smo navidezno prebavljivost beljakovin in organske snovi, navidezno biološko vrednost beljakovin in navidezno neto izkoristljivost beljakovin. Pektin vpliva na povečanje mase tkiv prebavil in vsebine prebavil, kar v največji meri velja za slepo črevo. Pektin je tudi pomembno vplival na porazdelitev izločenega dušika preko blata oz. preko seča, kar ima vpliv na navidezno prebavljivost beljakovin in korigirano biološko vrednost beljakovin. To je povezano s fermentabilnostjo pektina. Naši rezultati potrjujejo vpliv pektina na fiziologijo prebave, saj povečuje izločanje sečnine (dušika) preko krvnega obtoka nazaj v prebavila in zmanjšuje izločanje le-te preko seča. Oboje ima ugoden vpliv na zdravstveno stanje živali in ljudi.

7 ACKNOWLEDGMENTS

The authors are grateful to Christian Lafarge for diet preparation. Assistance in the laboratory provided by Marko Kodra is gratefully acknowledged.

8 REFERENCES

Barcelo A., Claustre J., Moro F., Chayvialle J.A., Cuber J. C., Plaisancié P. 2000. Mucin secretion is modulated by luminal factors in the isolated vascularly perfused rat colon. Gut, 46: 218–284

Brown R.C., Kelleher J., Losowsky M.S. 1979. The effect of pectin on the structure and function of the rat small intestine. British Journal of Nutrition, 42, 3: 357–365

Cassidy M.M., Lightfoot F.G., Gray L.E., Story J.A., Kritchevsky D., Vahouny G.V. 1981. Effect of chronic intake of dietary fibers on the ultrastructural topography of rat jejunum and colon: a scanning electron microscopy study. American Journal of Clinical Nutrition, 34, 2: 218–228

Chun W., Bamba T., Hosoda S. 1989. Effect of pectin, a soluble dietary fiber, on functional and morphological parameters of the small-intestine in rats. Digestion, 42, 1: 22–29

de Lange C.F.M., Sauer W.C., Mosenthin R., Souffrant W.B. 1989. The effect of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and faeces in pigs. Journal of Animal Science, 67, 3: 746–754

Drochner W., Kerler A., Zacharia B. 2004. Pectin in pig nutrition, a comparative review. Journal of Animal Physiology and Animal Nutrition, 88, 11–12: 367–380

Eastwood M.A. 1978. Fiber in gastrointestinal-tract. American Journal of Clinical Nutrition, 31, 10: S30–S32

Eastwood M.A. 1992. The physiological effect of dietary fiber – an update. Annual Review of Nutrition, 12, 1: 19–35

Eggum B.O. 1995. The influence of dietary fibre on protein digestion and utilization in monogastric. Archives of animal nutrition, 48, 1–2: 89–95

El Kossori R.L., Sanchez C., El Boustani E.S., Noëlle Maucourt M., Sauvaire Y., Méjean L., Villaume C. 2000. Comparison of effects of prickly pear (Opuntia ficus indica sp) fruit, arabic gum, carrageenan, alginic acid, locust bean gum and citrus pectin on viscosity and *in vitro* digestibility of casein. Journal of the Science of Food and Agriculture, 80, 3: 359–364

Fukunaga T., Sasaki M., Araki Y., Okamota T., Yasuoka T., Tsujikawa T., Fujiyama Y., Bamba T. 2003. Efects of the soluble fibre pectin on intestinal cell proliferatzion, fecal short chain fatty acid production and microbial polulation. Digestion, 67, 1–2: 42–49

Grala W.M., Verstegen W.A., Jansman A.J., Huisman J., Wasilewko J. 1998. Nitrogen utilization in pigs fed diets with soybean and rapeseed products leading to different ileal endogenous nitrogen losses. Journal of Animal Science, 76, 2: 569–577

Huisman J., Den Hartog L.A., Boer H., Van Weerden E.J., Thielen W.J.G. 1985. The effect of various carbohydrate sources on the ileal and faecal digestibility of protein and amino acids in pigs. In: Proceedings of the 3rd International Seminar on Digestive Physiology in the Pig. Copenhagen, Denmark.

Jacobs L.R. 1983. Effects of dietary fiber on mucosal growth and cell proliferation in the small intestine of the rat: a comparison of oat bran, pectin and guar with total fiber deprivation. American Journal of Clinical Nutrition, 37, 6: 954–960

Larsen F.M., Moughan P.J., Wilson M.N. 1993. Dietary fibre viscosity an endogenous protein excretion at the terminal ileum in growing rats. Journal of Nutrition, 123, 11: 1898–1904

Larsen F.M., Wilson M.N., Moughan P.J. 1994. Dietary fibre viscosity and amino acid digestibility, proteolytic digestive enzyme activity and digestive organ weights in growing rats. Journal of Nutrition, 124, 6: 833–841

Lee S.C., Prosky L., Devries J.W. 1992. Determination of total, soluble and insoluble dietary fiber in foods – Enzymatic-Gravimetric method, MES-TRIS Buffer: Collaborative study. J AOAC International, 75, 3: 395–416

Libao-Mercado A.J., Yin Y., van Eys J., de Lange C.F.M. 2006. True ileal amino acid digestibility and endogenous ileal amino acid losses in growing pigs fed wheat shorts- or casein-based diets. Journal of Animal Science, 84, 6: 1351–1361

Li S., Sauer W.C., Hardin R.T. 1994. Effect of dietary fibre level on amino acid digestibility in young pigs. Canadian Journal of Animal Science, 74, 2: 327–333

McCullough J.S., Ratcliffe B., Mandir N., Carr K.E., Goodlad R.A. 1998. Dietary fibre and intestinal microflora: Effects on intestinal morphometry and crypt branching. Gut, 42, 6: 799–806

McDougall G.J., Morrison I.M., Stewart D., Hillman J.R. 1996. Plant cell walls as dietary fibre: Range, structure, processing and function. Journal of the Science of Food and Agriculture, 70, 2: 133–150

Morita T., Tanabe H., Ito H., Yuto S., Matsubara T., Matsuda T., Sugiyama K., Kiriyama S. 2006. Increased luminal mucin does not disturb glucose or ovalbumin absorption in rats fed insoluble dietary fiber. Journal of Nutrition, 136, 10: 2486–2491

Mosenthin R., Sauer W. C., Ahrens F. 1994. Dietary pectin's effect on ileal and fecal amino acid digestibility and exocrine pancreatic secretion in growing pigs. J Nutr 124, 8: 1222–1229

Mosenthin R., Sauer W.C., Henkel H., Ahrens F., de Lange C.F.M. 1992. Tracer studies of urea kinetics in growing pigs. 2. The effect of starch infusion at the distal ileum on urea recycling and bacterial nitrogen-excretion. Journal of Animal Science, 70, 11: 3467–3472

Murray A.G., Fuller M.F., Pirie A.R. 1977. The effect of fibre in the form of various polysaccharides on the apparent digestibility of protein in the pigs. Animal Production, 24, 2: 139

Nimmerfall F., Rosenthaler J. 1980. Significance of the goblet cell mucin layer, the outermost barrier to passage through the gut wall. Biochemical and Biophysical research communications, 94, 3: 960–966

NRC. 1995. Nutrition Requirements of Laboratory Animals. Chapter 2, Nutrient requirements of the laboratory rat. Washington, The National Academy of Science: 11–79

Orešnik A., Cvirn M. 1984. The nutritive value of buckwheat and barley produced in Yugoslavia. Zb. Bioteh. Fak. Univ. Edvarda Kardelja v Ljubljani, Kmet. Živin., 44: 113–120

Orešnik A., Golob A., Žgajnar J. 1982. Nutritive value of leaf protein produced from different grass and legume forages. Zb. Bioteh. Fak. Univ. Edvarda Kardelja v Ljubljani, Kmet. Živin., 40: 107–119

Pastuszewska B., Kowalczyk J., Ochtabińska A. 2000a. Dietary carbohydrates affect caecal fermentation and modify nitrogen excretion patterns in rats II. Studies with diets differing in protein quality. Archives of Animal Nutrition, 53, 4: 335–352

Pastuszewska B., Kowalczyk J., Ochtabińska A. 2000b. Dietary carbohydrates affect caecal fermentation and modify nitrogen excretion patterns in rats I. Studies with protein-free diets. Archives of Animal Nutrition, 53, 3: 207–225

Pirman T., Ribeyre M.C., Mosoni L., Rémond D., Vrecl M., Salobir J., Patureau Mirand P. 2007. Dietary pectin stimulates protein metabolism in the digestive tract. Nutrition, 23, 1: 69–75

Pirman T., Mosoni L., Rémond D., Ribeyre M.C., Buffiere C., Salobir J., Patureau Mirand P. 2008. Differential response

of protein metabolism in splsnchnic organs and muscle to pectin feeding. British Journal of Nutrition, 100, 2: 306–311

SAS/STAT® Users's Guide. 2000. Version 8, vol. 2. Cary SAS Institute: 1162 p.

Schneemann B.O. 1986. Dietary fiber: Physical and chemical properties, methods of analysis, and physiological effects. Food Technology, 40, 2: 104–115

Schulze H., van Leeuwen P., Verstegen M.W.A., van den Berg J.W.O. 1995. Dietary level and source of neutral-detergent fiber and ileal endogenous nitrogen flow in pigs. Journal of Animal Science, 73, 2: 441–448

Shah N., Atallah M.T., Mahomy R.R., Pellet P.L. 1982. Effect of dietary fiber components on fecal nitrogen excretion and protein utilization in growing rats. Journal of Nutrition, 112, 4: 685–666.

Souffrant W.B. 2001. Effect of dietary fibre on ileal digestibility and endogenous nitrogen losses in the pig. Animal Feed Science and Technology, 90, 1–2: 93–102

Stekar J., Orešnik A., Ževart-Stumberger I. 1984. The biological value of proteins from three maize cultivars. Zb. Bioteh. Fak. Univ. Edvarda Kardelja v Ljubljani, Kmet. Živin., 44: 81–111

Trowell H., Southgate D.A.T., Wolever T.M.S., Leeds A.R., Gossul M.A., Jenkins D.J.A. 1976. Dietary fiber redefined. Lancet, 1, 7966: 976

Younes H., Garleb K., Behr S., Remesy C., Demigne C. 1995. Fermentable fibres or oligosaccharides reduce urinary nitrogen-excretion by increasing urea disposal in the rat cecum. Journal of Nutrition, 125, 4: 1010–1016

Zhu C.L., Rademacher M., de Lange C.F.M. 2005. Increasing dietary pectin level reduces utilization of digestible threonine intake, but not lysine intake, for body protein deposition in growing pigs. Journal of Animal Science, 83, 5: 1044–1053