

Effects of dietary fibre and tannins from apple pulp on the composition of faeces in rats

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The present study was undertaken to explore the effect of apple pulp on weight and composition of faeces. This material is rich in dietary fibre (DF; 620 g dry matter/kg) and contains appreciable amounts of polyphenols. Recent reports indicate that both condensed tannins (CT) and soluble polyphenols form cross-links with protein and inhibit digestive enzymes, affecting the protein digestibility, and may produce a stimulation of endogenous nitrogen excretion. Two groups of male Wistar rats were fed on either a control diet free of DF or a diet containing 100 g apple pulp DF/kg during 7 d after a 4 d adaptation period. Body-weight and food intake were monitored daily and faeces and urine were collected once daily. DF, water content and polyphenolic compounds were measured in faeces, and N content in both faeces and urine. Faecal weight increased in the fibre group by 280 and 240% when compared with wet and dry faecal weights of animals fed on the fibre-free diet. Soluble dietary fibre (SDF) excreted in faeces was 10.9% of the SDF ingested, which suggested a low resistance to fermentation of this fraction. Of the insoluble DF, 43% of the ingested fibre was fermented. Polyphenols were degraded in the intestinal tract. Of the ingested CT, 68.6% was recovered in faeces, while the soluble polyphenols were extensively degraded (85.7% of that ingested). On the other hand, a higher faecal N excretion was observed for the fibre-fed group, suggesting a decrease in the digestibility of the dietary protein and lower apparent digestibility and N balance indices.

Apple pulp: Dietary fibre: Faeces composition: Polyphenols

The relative amounts of insoluble and soluble fractions in dietary fibre (DF) are important due to their physiological effects. Water-insoluble fibre appears to be less available for fermentation in the large bowel than water-soluble fibre. The bulking effect is more pronounced with insoluble DF (IDF) that is resistant to degradation, and leaves a substantial amount of water-binding fibre in the colon. On the other hand, water-soluble types of DF (SDF) have significant effects on bile acids, cholesterol and glucose metabolism (Nyman & Asp, 1985).

Cereals are high in insoluble fibre and their contribution to the DF intake in European countries is 35–60%, while fruit only contributes 7–15% (Bingham, 1985, 1987). Several authors have recommended an increase in the consumption of fruit and vegetables since these products are richer in soluble and fermentable fibres than cereals (Spiller, 1986; Eastwood, 1987).

Apple pulp is a typical source of DF (Goñi *et al.* 1989; Fibre-90, 1990). It contains condensed tannins (CT)-proanthocyanidin polymers and soluble polyphenols, both of which form effective cross-links with protein and inhibit digestive enzymes, thereby affecting protein digestibility (Kumar & Singh, 1984; Oh *et al.* 1985). In vitro these two effects induce significant amounts of non-digested protein and CT in the dietary fibre residues of carob

* For reprints.

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

Ingredients	Fibre-free diet	Fibre diet
Casein + 6 g DL-methionine	150	150
Sucrose	100	100
Olive and sunflower oil	50	50
Mineral mixture*	38.7	38.7
Vitamin mixture†	11.9	11.9
Wheat starch	620	520
Apple-pulp fibre	—	100

* Contained (g/kg diet): CaCO_3 10.0, KHCO_3 6.1, NaH_2PO_4 2.26, KH_2PO_4 8.2, CaHPO_4 6.8, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 2.25, MgCO_3 0.77, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.199, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 0.17, ZnCO_3 0.026, NaF 0.0002, KI 0.0002, Na_2CrO_4 0.001, $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$ 0.0002, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.025.

† Contained (mg/kg diet): pteroylmonoglutamic acid 1.11, niacin 22.22, calcium pantothenate 8.88, riboflavin 3.33, thiamin 4.44, pyridoxin 6.66, cyanocobalamine 0.005, choline 1111.11, retinol 8.88, cholecalciferol 11.11, menadione 0.055, α -tocopherol 33.33 (Nyman & Asp, 1982).

Pods, grapes and apple pulp after enzymic treatments with heat-stable amylase (*EC* 3.2.1.1), protease and amyloglucosidase (*EC* 3.2.1.3) (Saura-Calixto, 1988; Goñi *et al.* 1989; Saura-Calixto *et al.* 1991). Nevertheless, the increase in the excretion of faecal nitrogen may also be due to the stimulation of endogenous N production by the polyphenolic compounds (Butler, 1989; Shakhhalili *et al.* 1990). Foods containing CT have been reported to depress the activity of digestive enzymes and increase N excretion in rats (Griffiths & Moseley, 1980; Eggum *et al.* 1983; Horigome *et al.* 1988; Shakhhalili *et al.* 1990).

On the other hand, DF may also affect faecal protein excretion, decreasing dietary protein digestibility (Gallaher & Schneeman, 1986) and affecting endogenous N metabolism (Stephen & Cummings, 1980; Fairweather-Tait *et al.* 1983). The effects of apple-pulp DF on the weight and composition of faeces in rats is reported in the present paper, which also considers the fermentation of fibre constituents as well as the presence of faecal CT and protein. In terms of faecal bulking capacity and fermentability of fibre, the rat model has been shown to correlate with human studies (Nyman *et al.* 1986).

MATERIALS AND METHODS

Animals and diets

Male Wistar rats weighing 62 (SD) 1 g were randomly divided into two groups of ten. The animals were put into individual metabolism cages and kept on a normal 12 h light–dark cycle with constant air recirculation. Room temperature was kept between 21 and 22°. Both groups received a powdered semi-synthetic diet prepared in the laboratory. Diet composition is given in Table 1. Both diets were similar, but the starch in the fibre-free basal diet was replaced with apple pulp to supply DF. The fibre source was a concentrate of apple pulp containing peels and seeds obtained after ethanolic washing with ethanol (960 ml/l). The DF composition of apple pulp is described in Table 2. Food and water were available *ad lib*. Body-weight and food intake were monitored daily.

After 4 d adaptation to diet and cages, urine and faeces were collected daily during a 7 d experimental period. Urine was collected in chlorhydric acid (5 ml/l) and analysed for N. Faeces were removed once daily, weighed and frozen at -20° , then lyophilized, weighed

Table 2. *Dietary fibre (DF) content of the apple-pulp fibre (g/kg dry matter)*
(Mean values and standard deviations for three determinations)

	Insoluble DF		Soluble DF		Total DF*
	Mean	SD	Mean	SD	
Neutral sugars	317.2	0.8	30.5	0.5	347.7
Uronic sugars	74.7	2.9	108.9	1.5	183.6
Klason lignin	90.2	5.8	—	—	90.2
Total	482.1		139.4		621.5

* Total DF calculated as insoluble DF plus soluble DF.

again and milled to a particle size less than 0.5 mm. N, DF, uronic acids, neutral sugars and soluble and condensed polyphenol analyses were performed on the mixed samples from the whole experimental period.

Analytical methods

DF. The soluble and insoluble DF fractions in food and faeces were measured following the Association of Official Analytical Chemists' method (Prosky *et al.* 1988).

A 1 g sample was successively treated with heat-stable α -amylase (100°, pH 6, 30 min), protease (60°, pH 7.5, 30 min) and amyloglucosidase (60°, pH 4.5, 30 min). Then it was filtered and washed with distilled water, ethanol (960 ml/l) and acetone. The residue corresponded to IDF. Filtrate and washing water were saved and dialysed with distilled water for 48 h at room temperature before freeze-drying and weighing. This fraction corresponded to SDF.

After acid-hydrolysis of the IDF residue (12 M-sulphuric acid, 30°, 1 h and dilution to 1 M- H_2SO_4 , 100°, 1.5 h), neutral sugars and uronic acids were assayed in the filtrate. The SDF residue was solubilized with distilled water and also assayed for neutral sugars and uronic acids.

After the previously described acid treatment of the IDF residue, lignin in the form of Klason lignin (KL) was determined gravimetrically.

The spectrophotometric analysis of the neutral sugars used anthrone–thiourea as reagent and glucose as standard (Southgate, 1976). Uronic acids determination was performed by the Scott (1979) method with galacturonic acid as standard and 3,5-dimethylphenol as reagent.

Polyphenolic compounds. The CT content was spectrophotometrically assayed in food and faeces by reading absorbance at 550 nm of the anthocyanidin solutions obtained after treating the samples with hydrochloric acid (50 ml/l)–butanol for 3 h, at 100° (Reed *et al.* 1982). Carob-pod CT, supplied by Nestlé SA, were used as standard.

Soluble polyphenols were assayed as flavonols after extraction with distilled water (60°, 30 min, twice) according to the Swain method (Swain & Hillis, 1959), using vanillin as reagent and D-catechin as standard.

N determination. Diets, urine and faeces were analysed for total N by the Kjeldahl method, using concentrated H_2SO_4 at 350°, with selenium as catalyst (Tecator Kjeltac equipment). Crude protein was calculated as $N \times 6.25$.

Calculation of digestibility and biological value. From the analysis of the N content in faeces and urine, the digestive efficiency index (apparent digestibility coefficient) and metabolic efficiency index (N balance) were calculated according to the Thomas–Mitchell equations (Mitchell, 1923–24).

Table 3. *Effect of apple-pulp dietary fibre on growth and food intake of rats**
(Mean values and standard deviations)

Dietary group	Initial wt (g)		Wt increase (g)		Total food intake (g dry matter)	
	Mean	SD	Mean	SD	Mean	SD
Fibre-free	63.54	2.33	48.30	3.26	78.24	4.65
Fibre	62.98	1.26	46.52	4.36	83.86	5.21

* For details of diets and procedures, see Tables 1 and 2 and pp. 464–465.

Table 4. *Effect of apple-pulp dietary fibre on stool weight and water content of faeces of rats†*
(Mean values and standard deviations)

Dietary group	Total stool wt (g/7 d)		Total dry stool wt (g/7 d)		Water content of faeces			
	Mean	SD	Mean	SD	g/kg		g/7 d	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fibre-free	2.81	0.54	2.11	0.30	225.7	45.8	0.70	0.24
Fibre	10.71***	0.90	7.32***	0.25	317.1*	56.3	3.39**	0.99

Mean values were significantly different from those for fibre-free diet: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

† For details of diets and procedures, see Tables 1 and 2 and pp. 464–465.

Statistics. The results were tested for significance using the two-tailed Student's *t* test (Snedecor & Cochran, 1987).

RESULTS

The addition of DF affected neither the ponderal growth nor the mean food intake of the animals during the experimental period, as shown in Table 3. The weight increase was similar in both groups (48.3 and 46.5 g/7 d in the control and test groups respectively), and no significant differences were found in the total food intake when dietary fibre was added to the basal diet.

Faecal bulking

The addition of apple pulp as DF caused a significant increase in both wet and dry faecal weights, as shown in Table 4. In the fibre-fed rats, the weight of faeces increased by 280 and 240% respectively compared with the wet and dry faecal weights of the animals fed on the fibre-free control diet. The water content of faeces was significantly higher in the rats fed on the apple-pulp fibre (3.39 g/7 d) than in those fed on the fibre-free diet (0.70 g/7 d).

Fermentation of DF constituents and polyphenol degradation

DF and polyphenol intake and excretion are detailed in Table 5. The small but detectable amounts of DF that appeared in the control diet probably came from indigestible starch or, to a lesser degree, from impurities of the elements used to prepare the diet (Nyman & Asp, 1982). The phenolic compounds present in the fibre-containing diet are constituents

Table 5. *Intake and faecal excretion of apple-pulp dietary fibre and polyphenols (g/7 d) by rats**

(Mean values and standard deviations)

Dietary group...	Intake				Excretion			
	Fibre-free		Fibre		Fibre-free		Fibre	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TDF†	0.93	—	6.90	—	0.07	—	3.05	—
SDF:								
Sugars	0.52	0.03	1.06	0.07	0.04	0.01	0.13	0.02
Uronic acids	0.08	0.00	0.86	0.05	0.003	0.00	0.08	0.03
IDF:								
Sugars	0.25	0.01	3.05	0.19	0.04	0.01	1.15	0.15
Uronic acids	0.08	0.00	0.40	0.03	0.002	0.00	0.14	0.02
Klason lignin	—	—	1.53	0.10	—	—	1.55	0.05
Condensed tannins	—	—	0.51	0.03	—	—	0.35	0.06
Soluble polyphenols	—	—	0.014	0.001	—	—	0.002	0.001

TDF, total dietary fibre; SDF, soluble dietary fibre; IDF, insoluble dietary fibre.

* For details of diets and procedures, see Tables 1 and 2 and pp. 464–465.

† TDF calculated as IDF + SDF.

Table 6. *Degradation of dietary undigestible compounds in rats fed on apple-pulp-fibre diet**

Indigestible compounds	Intake (g/7 d)	Faecal excretion	
		g/7 d	% of intake
Soluble dietary fibre†	1.92	0.21	10.94
Insoluble dietary fibre‡	4.98	2.85	57.23
Total dietary fibre§	6.90	3.06	44.35
Condensed tannins	0.51	0.35	68.63
Soluble polyphenols	0.014	0.002	14.29

* For details of diets and procedures, see Tables 1 and 2 and pp. 464–465.

† Defined as sugars + uronic acids.

‡ Defined as sugars + uronic acids + Klason lignin.

§ Calculated as IDF + SDF.

of the apple-pulp fibre. No phenolics were found in the control diet. The addition of apple-pulp fibre to the diet provided 6.9 g total dietary fibre (TDF) and 0.52 g polyphenolic compounds during the experimental period.

As can be seen from Table 5, both DF and polyphenols were extensively degraded in the intestinal tract. Except for KL, the neutral and acidic polysaccharides (expressed as sugars and uronic acids respectively) of both IDF and SDF fractions were thoroughly fermented. An appreciable degradation of CT and soluble polyphenols was also observed.

As shown in Table 6, SDF excreted in faeces was 10.9% of the ingested SDF, indicating low resistance to fermentation by this fraction. Both sugars and uronic acids showed similar degree of fermentability (87.74 and 90.70% respectively; Table 5).

For IDF, 43% of the ingested fibre was fermented; this was similar to the individual values for sugars and uronic acids in the ingested IDF (Table 5). The KL fraction of the

IDF residue was totally resistant to bacterial degradation, which is in agreement with findings of earlier studies in rats (Nyman & Asp, 1982).

CT were partially degraded in the intestinal tract, since only 68.6% of the ingested CT was recovered in faeces (Table 6). A high percentage of the soluble polyphenols was degraded (85.7 of that ingested).

Protein excretion

Protein intake, faecal and urinary N excretion, and apparent protein digestibility and N balance indices are given in Table 7. Protein intake did not differ significantly between the control and the fibre-fed animals. Faecal N excretion was significantly increased when dietary fibre was added to the diet, and, consequently, the apparent digestibility index was lower than in the control group. The urinary N excretion was not affected by the presence of DF in the diet.

DISCUSSION

The addition of DF to the diet did not affect either the ponderal growth or the food intake of the animals during the experimental period. In longer experiments (7 weeks), Park & Harrold (1983) reported a linear increase in the food intake when fibre levels were increased and the energy intake remained the same, although no differences in the growth rate of the rats were found. In contrast, Nomani *et al.* (1979) reported a higher weight gain in rats fed on different fibre diets with a high energy level than in those fed on a non-fibre diet. Nevertheless, weight gain was not significantly different when the energy level was lower.

The presence of CT in the test diet (6 g/kg dry matter) did not influence the growth rate or the food intake in our experimental conditions. A decrease in the growth rate of immature rats has been reported with higher levels of dietary CT, although food intake did not decrease (Würsch, 1979; Butler, 1989).

Faecal wet and dry weights were significantly increased when DF was added to the diet. These results were very similar to those obtained by Nyman *et al.* (1990) with vegetables with high fibre contents (carrots, rutabagas, peas, Brussel sprouts and green beans) under similar experimental conditions, as well as with purified pectins and sugar-beet fibre (Nyman & Asp, 1982).

As is well known, the capacity of fibre to increase faecal bulking is more pronounced with DF that is resistant to degradation in the intestine, leaving a substantial amount of water-binding fibre, than with easily fermented DF (Nyman & Asp, 1982). However, fermentable fibre materials such as fruits and vegetables provide a carbohydrate substrate in the large intestine which supplies the energy for the growth of colonic microflora (Stephen & Cummings, 1980). Therefore, increasing the supply of these materials in the diet stimulates microbial growth and results in an increased faecal bacterial mass, which is able to retain water and contributes to the faecal bulking by itself.

These facts might explain the high dry stool weight in the rats fed on the apple-pulp DF (1.53 g/d); larger than the values found when wheat bran was the DF source (1.06 g/d; Nyman & Asp, 1982). The reason might be that this apple-pulp fibre was rich in unfermentable DF (482 g/kg dry matter, Table 2), but also had a considerable amount of SDF (fermentable; 140 g dry matter/kg). Furthermore, the water content of faeces was, as expected, significantly higher in the rats fed on the apple-pulp diet than in those fed on the fibre-free diet. Both unfermentable and fermentable fibre fractions contributed to the amount of water held.

With regard to DF fermentation, SDF was highly fermented, since only 10.9% of the ingested SDF was recovered in faeces (Table 6), whereas IDF was notably more resistant, with only 42.8% of the ingested IDF being fermented. Approximately 90% of the soluble

Table 7. *Protein intake, nitrogen excretion and protein utilization indices in rats*
(Mean values and standard deviations)

Dietary group	g/kg DM		Protein intake				N excretion (mg/7 d)				Apparent digestibility coefficient		N balance (g/7 d)	
	mean	SD	g/7 d		Urine		Faeces		Mean	SD	Mean	SD	Mean	SD
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fibre-free	150.5	1.6	11.75	0.70	213.00	41.67	109.07	20.30	94.16	1.15	2.57	0.30		
Fibre	144.8	1.3	12.14	0.75	237.90	74.04	257.72***	20.84	86.64**	1.42	2.22	0.21		

Mean values were significantly different from those of the fibre-free group: ** $P < 0.01$; *** $P < 0.005$.
DM, dry matter.

pectins present in the SDF fraction were fermented. Insoluble pectic substances are less available to the bacterial mass because they are cell wall constituents and are associated with other fibre components; the fermentation of these substances in our samples was 65%.

The faecal recovery of total neutral sugars was similar to the results reported by Nyman *et al.* (1986) using apple-pulp fibre. The KL fraction of the IDF residue was totally resistant to bacterial degradation, which concurs with earlier studies in rats (Nyman & Asp, 1982). Woods & Gorbach (1986) reported that, while pectins were almost totally degraded (90–100%) and other fibre constituents were partially degraded, lignin was completely resistant to degradation by the human intestinal flora. This is in agreement with our results in rats. A good correlation has been found between the rat model and human studies (Nyman *et al.* 1986), showing that this experimental model is useful to predict fermentative breakdown and bulking capacity of apple-pulp DF in humans.

Of the phenolic compounds, CT seem to be partially degraded in the intestinal tract, since only 68.6% of the ingested tannins were recovered in faeces (Table 6). The soluble polyphenols were more extensively degraded than CT (85.9%). Compared with the fermentation of DF fractions, soluble polyphenols were degraded to the same extent as SDF, whereas CT were more resistant to degradation than IDF.

No information on faecal recovery of CT was available in the literature, and whether tannins can be absorbed through the intestinal wall or not is still an open question. Simple phenols, such as catechin, or low-molecular-weight polyphenols, such as tannic acid, are known to be degraded in the upper intestinal tract and utilized by the microflora in the hind-gut of rats (Nyman & Björck, 1989), but it is not known whether large tannin macromolecules can get through gastrointestinal tissues or not (Singleton, 1981).

Faecal N excretion was significantly increased when DF was added to the basal diet, while the small differences found in urinary N excretion were not significant. Hence, increased faecal N excretion was not compensated for by a decrease in urinary N excretion, which does not agree with previous findings in humans (Calloway & Krestch, 1978; Kelsay *et al.* 1978) as well as in animals (Nomani *et al.* 1979; Fairweather-Tait *et al.* 1983) using other DF sources. However, other authors did not find a full compensation for the increase in faecal N by the urinary N decrease (Gallaher & Schneeman, 1986).

In the present work, we have found that with the same protein intake, faecal N excretion was higher in the fibre-fed group. The increased faecal N excretion may be due to the influence of either DF or CT on the dietary protein utilization and on the endogenous N metabolism.

It has been shown that DF increases faecal N excretion when it is fed as a purified preparation (Greenberg, 1976; Cummings *et al.* 1979) or when it is derived from different sources (Cummings *et al.* 1976; Calloway & Kretsch, 1978; Kelsay *et al.* 1978; Anonymous, 1984). Several suggestions have been made to explain the manner in which fibre brings about this effect: (1) the protein of fibre itself is undigested and excreted in faeces (Saunders & Betschart, 1980); (2) the dietary protein is not completely absorbed because of the increased colonic volume due to the addition of fibre (Bender *et al.* 1979); (3) the fibre decreases the activity of proteolytic enzymes such as trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) (Schneeman, 1978; Stephen, 1987).

All these mechanisms involve a decrease in the dietary protein digestibility. However, the increased faecal N excretion may also be due to an increase in the endogenous N excretion. It is suggested that DF stimulates mucosal cell proliferation (Southgate, 1973; Fairweather-Tait *et al.* 1983) increasing the endogenous protein production, although most of the extra protein would be available for reabsorption (Fairweather-Tait *et al.* 1983). Sandaradura *et al.* (1986) reported an increased excretion of endogenous N resulting from increased mucosal cell turnover when high-fibre diets were fed to rats. Another explanation of the

increased faecal N output arises from the effect of fibre on faecal bacterial mass, which implies an increase in N excretion from the intestinal microflora (Stephen & Cummings, 1990).

The effect of polyphenols on the excretion of faecal N can also be explained by mechanisms that involve a decrease in the digestion and absorption of dietary protein, and by mechanisms that involve an increase in the excretion of endogenous N (Shahkhalili *et al.* 1990).

Three mechanisms for decreased dietary protein digestibility by tannins have been suggested (Hughes, 1989): (1) reduced protein digestion in the stomach by reducing acid production; (2) reduced protein bioavailability by precipitation of dietary protein bound to tannin; (3) reduced digestion in the small intestine caused by binding and inactivation of digestive enzymes.

Different authors (Eggum *et al.* 1983; Horigome *et al.* 1988) point out a significant increase in faecal N excretion as a consequence of the interaction between polyphenols and dietary protein. Our results (Table 5) show that a large amount of the ingested polyphenols are excreted in faeces, agreeing with *in vitro* experiments (Saura-Calixto, 1988; Goñi *et al.* 1989; Saura-Calixto *et al.* 1990, 1991) which show that, after treatment with digestive enzymes (α -amylase, protease and amyloglucosidase), a residue containing both polyphenols and proteins remains as the result of the formation of indigestible tannin-protein complexes (Aw & Sawson, 1985; Barroga *et al.* 1985) and of enzymic inhibition phenomena (Griffiths & Moseley, 1980).

The possible effect of polyphenol on the endogenous N excretion cannot be ignored. Comparison of the amino acid profile of faecal material and dietary protein shows large differences between rats fed on tannin diets (Butler, 1989) and those that were not fed such diets. It has also been reported that, sometimes, tannin consumption causes a greater total N excretion than the total amount of N ingested (Singleton, 1981). Therefore, it is clear that endogenous N is involved in the rise in N excretion due to tannin intake. Nevertheless, whether this N comes from an increased cellular turnover or from an increase in digestive secretions is not known. Mitjavila *et al.* (1977) reported an increased excretion of mucoprotein, sialic acid and glucosamine in the faeces. Leguminous diets, which contain appreciable amounts of polyphenols, have been shown to increase the rate of mucosal cell turnover in rats (Sandaradura *et al.* 1986).

Therefore, both compounds, DF and tannins, are involved in the increased faecal N excretion caused by the apple pulp. Further investigations are necessary in order to determine the mechanism by which this effect is produced as well as which compound produces such an effect and to what extent it carries it out.

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