# Polyphenols as Dietary Fiber Associated Compounds. Comparative Study on *in Vivo* and *in Vitro* Properties

Laura Bravo,*,†,‡ Rocio Abia,§ and Fulgencio Saura-Calixto†

Instituto de Nutrición y Bromatología, CSIC-UCM, Facultad de Farmacia, Ciudad Universitaria, 28040 Madrid, Spain, and Gastrointestinal Unit, Department of Medicine, Western General Hospital, Edinburgh EH4 2XU, U.K.

A comparative study between dietary fiber (DF) and polyphenols (PP) in terms of degradability and physiological properties was performed. Eight groups of Wistar rats were fed either a control diet free of DF and PP or diets containing DF constituents [cellulose (C), pectins (P), and lignin (L)] or PP, both soluble [catechin (CA) and tannic acid (TA)] and insoluble [condensed tannins (CT)]. A significant increase in the total stool output and in the water and fat content of feces was observed. Protein digestibility was significantly reduced. Intestinal degradation of the soluble compounds (P, CA, and TA) was almost complete. C was partially digested; L and CT were highly resistant. *In vitro* fermentation assays were performed, showing the different susceptibilities of the DF constituents and the polyphenolic compounds to fermentation and the inhibitory effect of TA and L on colonic microflora.

**Keywords:** *Dietary fiber, polyphenols, catechin, tannic acid, condensed tannins, feces composition, intestinal degradation, colonic fermentation*

## INTRODUCTION

Dietary fiber (DF) is defined as the nonstarch polysaccharides and lignin that are not digested or absorbed in the human small intestine (Asp, 1987). This definition does not consider other plant materials such as polyphenols, resistant protein, or resistant starch, which are resistant to digestion as well (Saura-Calixto et al., 1991).

Polyphenolic compounds in plants are a complex group of substances with a wide range of molecular mass and are found either free or bound to protein or dietary fiber. Soluble or extractable polyphenols (EPP) are low or intermediate molecular mass phenolics that are extracted easily using different solvents (water, methanol, aqueous acetone, etc.), while nonextractable polyphenols (NEPP) are mainly condensed tannins (CT) of high molecular mass (over 5000), some of which are in the free form and some are bound to protein and fiber (Saura-Calixto et al., 1991; Terrill et al., 1992).

Polyphenols (PP) are present in almost all plant organs: leaves, stems, roots, flowers, etc., and they are common in most plant foods (fruits, legumes, cereals, beverages, etc.).

PP have different effects in the intestine depending on their solubilities. EPP appear to be absorbed from the digestive tract and produce systemic effects, such as reduction of the metabolic utilization of absorbed amino acids and elevated plasma levels of growth hormone (Martin-Tanguy et al., 1976; Barry et al., 1986). NEPP are not absorbed in the intestine and are recovered quantitatively in feces (Bravo et al., 1992, 1993).

In previous *in vitro* experiments, we have noticed the presence of PP in fiber residues of different foods obtained after treatment with the enzymes used in the analysis of DF (Saura-Calixto, 1987, 1988; Goñi et al., 1989; Saura-Calixto et al., 1991). The aim of the present work was to perform a comparative study of PP and DF constituents in terms of degradability and physiological properties. *In vitro* fermentation studies and *in vivo* experiments using rats have been carried out.

## MATERIALS AND METHODS

**Materials.** DF constituents were cellulose (AVICEL, FMC International, Little Island, Cork, Ireland), lignin (ENCESA, Miranda de Ebro, Burgos, Spain), and apple pectins (P-2157, Sigma Chemical Co., St. Louis, MO). EPP included a flavan-3-ol phenolic [catechin (C-1251)] and tannic acid (T-0125), a hydrolyzable tannin composed of glucose esterified with six to nine gallic acid units (Sigma). Carob pod (*Ceratonia siliqua*) powder supplied by Nestlé Ltd. (Vers-chez-les-Blancs, Laussane, Switzerland) was used as a source of NEPP. Subsequent extraction of soluble materials was performed in the laboratory to obtain a carob pod concentrate richer in NEPP than the original matter. The powder was washed twice with distilled water (60 °C, 30 min), once with methanol/water 50:50 (v/v) (room temperature, 60 min), and once with acetone/water 70:30 (v/v) (room temperature, 60 min). The residue was dried at 60 °C overnight and milled to a particle size of less than 0.5 mm. The resulting fraction was a concentrate rich in highly polymerized condensed tannins (53.2%, dry weight basis).

Enzymes (heat-stable α-amylase A-3306, protease P-3910, and amyloglucosidase A-9913) as well as propionic (P-1386), butyric (B-2503), and 4-methyl-*n*-valeric (M-7396) acids were purchased from Sigma. Reference sugars, galacturonic acid, and acetic acid were obtained from Merck (Darmstadt, Germany). All of the reagents used were of analytical quality.

**Procedure.** *In Vivo Experiment.* Male Wistar rats were randomly divided into groups of eight and placed into individual metabolism cages in a room kept at 22 (±1) °C. Animals were fed different semisynthetic diets prepared in the laboratory (Table 1). In the different experimental diets, a fraction of wheat starch of the basal diet was substituted by an equivalent weight of the DF constituents and the polyphenolic compounds. Since lignin is quantitatively a minor component in the human diet, the amount of this compound was at half the level of the other DF constituents, cellulose and pectins, which are more common in our diet. Polyphenolic compounds were present in the test diets in greater amounts than those in a normal diet, with an estimated daily intake of approximately 1 g (Kühnau, 1976). These levels are far from the toxicity limits for these compounds, up to 5% for EPP and higher for NEPP (Singleton, 1981).

* Author to whom correspondence should be addressed.
† Ciudad Universitaria.
‡ Present address: Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH, U.K. [telephone (44) (223) 415695; fax (44) (223) 413763].
§ Western General Hospital.

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**Table 1. Composition of Diets**

| diet | ingredient | g/kg of dry matter |
|---|---|---|
| basal diet | casein | 144 |
| | DL-methionine | 2.9 |
| | sucrose | 96 |
| | oil[a] | 48 |
| | vitamin mixture[b] | 1.2 |
| | mineral mixture[c] | 36.2 |
| | wheat starch | 671.7 |
| experimental diets[d] (similar to basal diet except for wheat starch amount, plus one of the constituents in the second column) | cellulose | 96 |
| | lignin | 48 |
| | pectins | 96 |
| | cathechin | 19.2 |
| | tannic acid | 19.2 |
| | carob pod concentrate[e] | 96 |
| | CT-control, containing cellulose | 38.4 |

[a] Olive and sunflower oil. [b] Vitamin mixture (mg/kg of diet): pteroylmonoglutamic acid, 1.11; niacin, 22.22; calcium pantothenate, 8.88; riboflavin, 3.33; thiamin, 4.44; pyridoxine 6.66; cyanocobalamin, 0.055; choline, 1111.11; retinol, 8.88; cholecalciferol, 11.11; menadione, 0.055; tocopherol, 33.33. [c] Mineral mixture (g/kg of diet): $CaCO_3$, 10.0; $KHCO_3$, 6.1; $NaH_2PO_4$, 2.26; $KH_2PO_4$, 8.2; $CaHPO_4$, 6.8; $MgSO_4 \cdot 7H_2O$, 2.25; $MgCO_3$, 0.77; $FeSO_4 \cdot 7H_2O$, 0.199; $MnSO_4 \cdot H_2O$, 0.17; $ZnCO_3$, 0.026; NaF, 0.0002; KI, 0.0002; $Na_2CrO_4$, 0.001; $Na_2SeO_3 \cdot 5H_2O$, 0.0002; $CuSO_4 \cdot 5H_2O$, 0.025. [d] A fraction of wheat starch in the basal diet was substituted by an equivalent weight of the polyphenols and DF constituents. [e] The condensed tannins (CT) content of the carob pod concentrate was 53.2% (dry matter). The CT content of the diet was 51 g/kg.

Carob pods contain insoluble dietary fiber (IDF), consisting mainly of cellulosic polysaccharides (Saura-Calixto, 1988). The analysis of the carob pod concentrate used as a source of condensed tannins (NEPP) showed an IDF content of 35% (dry matter). A diet containing an equivalent amount of cellulose (4% dry matter) was used as a control for the group fed condensed tannins (CT). The basal diet free of DF and PP was fed to a control group. Food and water were available *ad libitum*.

After 4 days of adaptation to diet and cages, body weight, food intake, and fecal output were monitored throughout the 3-week experimental period. Urine and feces were collected daily during the third experimental week. Urine was collected in 0.06 mM hydrochloric acid and analyzed for nitrogen content. Feces were collected and weighed daily, freeze-dried, weighed again, pooled weekly, milled to a particle size of less than 0.5 mm, and analyzed for dietary fiber, polyphenols, fat, and nitrogen.

*In Vitro Fermentation Assays.* DF constituents and polyphenols were fermented by a closed batch culture technique under strict anaerobic conditions, using oxygen-free carbon dioxide as described by Adiotomre et al. (1990).

Rat cecal contents were used as inoculum. Approximately 12 g of fresh rat cecal content was removed from three rats previously fed a commercial high-fiber diet [Diet CRM(X), Labsure LTD, Croydon, England] and homogenized in sterile anaerobic medium to give 8 g of rat cecum content/100 mL of medium. The slurry was mixed at 37 °C for 45 min to break up the cecal fiber matrix and then filtered to remove large fiber particles. The filtrate was retained for inoculation.

Thirty milliliters of basal medium [made up of tryptone (2.5 g/L) and minerals $Na_2HPO_4$ 1.4 g/L, $KH_2PO_4$ 1.6 g/L, $MgSO_4 \cdot 4H_2O$ 0.2 g/L, $(NH_4)HCO_3$ 1 g/L, $NaHCO_3$ 8.7 g/L, $CaCl_2 \cdot 2H_2O$ 16.5 mg/L, $MnCl_2 \cdot 4H_2O$ 12.5 mg/L, $CoCL_2 \cdot 6H_2O$ 1.25 mg/L, and $FeCl_3 \cdot 6H_2O$ 1 mg/L] and 1 mL of reducing solution (cysteine hydrochloride 6.2 g, NaOH 1.6 g, and $Na_2S \cdot 9H_2O$ 6.2 g) were added to 200 mg of each sample, followed by 10 mL of inoculum. The bottles were flushed with carbon dioxide and maintained in an incubator at 37 °C under anaerobic conditions. The production of short-chain fatty acids (SCFA) was analyzed after 24 h of fermentation. A control free of DF and PP was used. Four fermentation assays were performed.

*Analytical Methods.* Cellulose and pectins were measured in the residues obtained after the feces were treated with heat-stable α-amylase (EC 3.2.1.1), protease, and amyloglucosidase (EC 3.2.1.3) (Prosky et al., 1988). Neutral sugars were quantified by GLC as alditol acetates (Englyst and Cummings, 1988). An HP-5890 A (Hewlett-Packard, Avondale, PA) chromatograph fitted with flame ionization detector and autoinjector and connected to an HP-3390 A computing integrator was used. An SP-2330 capillary column, 30 m × 0.32 mm i.d. (Supelco, Bellefonte, PA, catalog no. 2-4073) was used with the following conditions: injector temperature, 270 °C; oven temperature, 240 °C; detector temperature, 250 °C; carrier gas (nitrogen) flow rate, 3 mL/min. Uronic acids were determined by spectrophotometry (Scott, 1979) (Perkin-Elmer Lambda 2, Überlingen, Germany). Lignin was quantified spectrophotometrically (Morrison, 1972) after solubilizing in HCl/triethylene glycol.

Condensed tannins were determined gravimetrically after acid hydrolysis of IDF residues (12 M $H_2SO_4$, 30 °C, 1 h, and dilution to 1 M $H_2SO_4$, 100 °C, 90 min) correcting their protein content (N × 6.25) (Bravo et al., 1993). Catechin and tannic acid in feces were measured in the solutions obtained after extraction with methanol/water (50:50 v/v) and acetone/water (70:30 v/v) by assaying with Folin–Ciocalteu reagent (Montreau, 1972). The content of these PP was also determined in the samples of the fermentation solutions.

Short-chain fatty acids (acetic, propionic, and butyric acids) were determined by a modified Spiller et al. (1980) method, using 4-methyl-*n*-valeric acid as internal standard. A gas chromatograph equipped with flame ionization detector (Carlo-Erba 4200, Milan, Italy) was used. SCFA were separated on a 2-m glass column of 2 mm i.d., packed with 10% SP 1200/1% $H_3PO_4$ on 80–100-mesh Chromosorb W-AW. The injector, detector, and oven temperatures were 120, 80, and 200 °C, respectively. The temperature program was as follows: initial temperature, 120 °C, final temperature, 180 °C; increase rate of temperature, 15 °C/min; initial time, 0; final time, 5 min.

Total nitrogen and fat determinations were done for diets and feces. Urine was analyzed for its nitrogen content. Total nitrogen was measured by the Kjeldahl method (Tecator Kjeltec equipment, Höganäs, Sweden), and crude protein was calculated as N × 6.25. Fat was determined gravimetrically after extraction with petroleum ether with Soxhlet equipment (Kilab, Assens-Llofriu, S. A., Barcelona, Spain).

Data were analyzed by one-way analysis of variance. The homogeneity of variances was checked by Cochran's test. In those treatments with variance heterogeneity, Student's *t* test was used to evaluate differences between group means. The level of significance was $P < 0.05$.

## RESULTS

***In Vivo* Experiment.** The group fed the basal diet free of DF and PP was used as a control. Another group, fed the cellulose-containing diet, was included as a specific control for the condensed tannins (CT) fed group. Since the carob pod used as a source of CT contained appreciable amounts of cellulose, some of the effects observed in this group might be derived from the presence of the IDF. Comparison of the results in this CT group with the control containing the same amount of fiber could provide more accurate information about the effects of CT.

*Growth Rate and Food Intake.* Table 2 shows the weight gain and food intake during the 3-week experimental period.

Animals that were fed the cellulose and lignin diets showed a higher food intake than those in the control group, although their ponderal growth was not affected. The CT fed group showed a high food intake, but it was not significantly different from its control group, and the growth rate was not affected. Similarly, the addition of the extractable polyphenols (EPP) and pectins did not show any effect on either the growth rate or the mean food intake of the animals during the experimental period.

*Stool Weight and Water Excretion.* Of the soluble materials, only catechin did not affect the dry stool weight or the water content of feces (Table 3). Rats fed diets containing tannic acid (TA) and pectins showed a significantly higher fecal output and water excretion than the control group. Rats fed diets containing insoluble

**Table 2. Effect of Dietary Fiber (DF) Constituents and Polyphenols (PP) on Weight Gain and Food Intake during the 3-Week Experimental Period[a]**

| | DF,PP-free control | CT-control | soluble compounds | | | insoluble compounds | | |
|---|---|---|---|---|---|---|---|---|
| | | | pectins | catechin | tannic acid | cellulose | lignin | CT[b] |
| wt gain (g/21 days) | 97.61 ± 13.53 | 100.92 ± 12.40 | 91.13 ± 8.60 | 106.22 ± 12.08 | 99.32 ± 10.95 | 103.60 ± 14.62 | 111.45 ± 12.92 | 99.96 ± 12.31 |
| food intake (g of DM[c]/ 21 days) | 246.59 ± 16.69[a] | 271.22 ± 19.60[a] | 254.58 ± 10.98[a] | 276.17 ± 19.54[a] | 276.09 ± 13.87[a] | 303.14 ± 18.70[b] | 298.96 ± 21.29[b] | 296.23 ± 18.64[ab] |

[a] Mean values ± standard deviations ($n$ = 8 animals). Mean values within a row with different superscript letters are significantly different ($P < 0.05$). [b] Condensed tannins. [c] Dry matter.

**Table 3. Effects of Dietary Fiber (DF) Constituents and Polyphenols (PP) on Stool Output and Fat and Protein Digestibilities during the Third Experimental Week (Apparent *in Vivo* Digestibility of DF and PP)[a]**

| physiological effect | DF,PP-free control | CT-control | soluble compounds | | | insoluble compounds | | |
|---|---|---|---|---|---|---|---|---|
| | | | pectins | catechin | tannic acid | cellulose | lignin | CT[b] |
| dry stool wt (g/7 days) | 2.80 ± 0.28[a] | 5.62 ± 0.56[c] | 5.34 ± 0.43[c] | 2.42 ± 0.23[a] | 3.51 ± 0.44[b] | 11.84 ± 1.44[d] | 8.59 ± 0.40[e] | 13.76 ± 1.46[f] |
| water excretion (g/7 days) | 0.96 ± 0.09[a,b] | 1.13 ± 0.34[b,c] | 1.71 ± 0.14[d] | 0.64 ± 0.07[a] | 1.26 ± 0.44[c] | 2.25 ± 0.43[f] | 2.11 ± 0.40[f] | 4.17 ± 0.62[g] |
| fat excretion (% of intake) | 1.42 ± 0.16[a] | 1.25 ± 0.07[b] | 4.38 ± 0.24[c] | 3.46 ± 0.40[d] | 2.92 ± 0.20[e] | 2.22 ± 0.15[f] | 1.46 ± 0.07[a] | 2.74 ± 0.08[g] |
| protein excretion (g/7 days) | 0.96 ± 0.10[a] | 0.96 ± 0.10[a] | 1.81 ± 0.15[b] | 0.88 ± 0.08[a] | 1.69 ± 0.21[b] | 1.28 ± 0.15[c] | 1.90 ± 0.09[b] | 3.05 ± 0.32[d] |
| ADC[c] | 93.66 ± 0.71[a] | 93.61 ± 0.41[a] | 88.73 ± 0.63[b] | 94.48 ± 1.05[a] | 89.23 ± 0.57[b] | 93.01 ± 0.48[a] | 88.58 ± 0.69[b] | 82.62 ± 0.50[c] |
| PER[d] | 2.75 ± 0.65[a] | 2.07 ± 0.50[a,b] | 2.39 ± 0.38[a,b] | 2.08 ± 0.59[a,b] | 2.43 ± 0.40[a,b] | 2.27 ± 0.45[a,b] | 2.48 ± 0.27[a,b] | 1.92 ± 0.22[b] |
| digestibility[e] (% of intake) | | 34.45 ± 2.46 | 97.66 ± 0.05 | 96.87 ± 0.54 | 95.44 ± 0.28 | 37.87 ± 4.61 | 1.24 ± 0.26 | 2.29 ± 1.27 |

[a] Mean values ± standard deviations ($n$ = 8 animals). Mean values within a row with different superscript letters are significantly different ($P < 0.05$). [b] Condensed tannins. [c] ADC, apparent digestibility coefficient: (ingested − fecal) × ingested$^{-1}$ × 100. [d] PER, protein efficiency ratio: weight gain (g) × protein ingestion$^{-1}$ (g$^{-1}$). [e] Apparent digestibility: (ingested − fecal) × ingested$^{-1}$ × 100.

materials showed a significantly higher dry stool weight and fecal water excretion than their respective controls (Table 3), the highest values corresponding to the CT fed group.

When water excretion is expressed as percentage of the dry matter, the results seem to indicate that there are no differences between the test and control groups in terms of water holding capacity (WHC) (% dry matter excretion: 75.7% DF,PP-free control; 83.3% CT-control; 75.7% pectins; 79.1% catechin; 73.6% tannic acid; 84.0% cellulose; 80.3% lignin; and 76.7% CT). Nevertheless, significant differences in the net amount of water excreted in the feces can be observed, suggesting that the studied compounds have important effects on the overall fecal bulk.

*Protein and Fat Excretions.* The presence of catechin in the diet did not affect protein excretion as compared with the control (Table 3).The apparent digestibility coefficient of the protein (ADC) and the protein efficiency ratio (PER), indicators of the nutritional utilization of the dietary protein, were not affected either.

In contrast, all of the DF constituents as well as TA and CT were associated with a higher fecal nitrogen excretion, which was correlated to a reduction of the ADC, even though the PER was not affected.

Except for lignin, all of the studied compounds caused a significant increase in fat excretion, expressed as a percentage of ingested fat (Table 3). Pectins were associated with the greatest fecal fat elimination, which was increased by 208% compared with the DF and PP-free control group.

*Degradation of DF and PP.* The apparent digestibilities of the studied DF constituents and PP are shown in Table 3. These results are based on the fecal recovery of the different compounds and are expressed as a percentage of the amount ingested.

Soluble compounds were almost completely degraded in the gastrointestinal tract. Only 2% of the ingested pectins were recovered in feces. Similarly, TA and catechin were highly digestible, with a fecal recovery up to 5% of the amount ingested.

With regard to the insoluble materials, lignin and CT were almost totally resistant to degradation with more than 98% of the ingested compounds being recovered in feces. Up to 60% of the ingested cellulose was excreted in feces. This level of degradation was found in the experimental diet containing 10% of cellulose and in the diet used as a control for the CT group, which contained 4% cellulose.

**Table 4. Short-Chain Fatty Acid (SCFA) Production in the Presence of Dietary Fiber (DF) Constituents and Polyphenols (PP) after 24 h of *in Vitro* Fermentation[a]**

| | acetic acid (mmol/L) | propionic acid (mmol/L) | butyric acid (mmol/L) | total (mmol/L) |
|---|---|---|---|---|
| control | 15.94 ± 1.13[a] | 5.01 ± 0.46[a] | 4.85 ± 0.49[a] | 25.80 ± 1.31[a] |
| pectins | 50.41 ± 7.35[b] | 8.57 ± 0.66[b] | 8.62 ± 0.73[b] | 67.60 ± 7.42[b] |
| catechin | 14.64 ± 0.50[a] | 5.09 ± 0.33[a] | 5.44 ± 0.30[a] | 25.17 ± 0.58[a] |
| tannic acid | 8.61 ± 0.33[c] | 3.12 ± 0.09[c] | 4.01 ± 0.14[c] | 15.74 ± 0.37[c] |
| cellulose | 15.13 ± 0.78[a] | 4.61 ± 1.01[a] | 4.67 ± 0.40[a] | 24.41 ± 1.34[a] |
| lignin | 15.54 ± 0.15[a] | 3.47 ± 0.01[c] | 3.27 ± 0.08[c] | 22.28 ± 0.17[a,d] |
| CT[b] | 16.70 ± 0.50[a] | 5.28 ± 0.14[a] | 5.02 ± 0.25[a] | 27.00 ± 0.58[a] |

[a] Mean values ± standard deviations ($n$ = 4). Mean values with different superscript letters within a column are significantly different ($P < 0.05$). [b] Condensed tannins.

***In Vitro* Fermentation Experiment.** Table 4 shows the short-chain fatty acid (SCFA) production after 24 h of fermentation in the presence of the different substrates.

Pectins caused a significant increase in the production of all SCFA (acetic, propionic, and butyric acids), while catechin did not affect their levels of production. TA depressed the formation of all the volatile fatty acids.

Only 40–60% of the added catechin and between 75 and 95% of the added TA were recovered after fermentation, reflecting substantial degradation of these EPP during the fermentative process.

Concerning the insoluble compounds, neither cellulose nor CT affected the SCFA yield. Lignin caused a

significant inhibition of the production of propionic acid and butyric acid, but acetic acid levels were not affected (Table 4).

## DISCUSSION

The comparison between DF and PP was performed by taking into account the solubility of the assayed compounds. Pectins, the soluble dietary fiber constituent (SDF), were compared with the soluble, extractable polyphenols (EPP), and the insoluble dietary fiber (IDF) constituents, cellulose and lignin, were compared with the condensed tannins (NEPP).

The rat model used in the *in vivo* experiment has been found to correlate with human studies in terms of fermentation and bulking capacity of DF (Nyman et al., 1988).

Polyphenols have often been reported to have depressing effects on growth rate and voluntary food intake in short-term (Tamir and Alumot, 1970; Shahkhalili et al., 1990; Longstaff and McNab, 1991b) and long-term experiments (Würsch, 1979; Barry, 1985). These compounds have been reported to have no effect (Tamir and Alumot, 1970; Moulay et al., 1988; Alzueta et al., 1992), which is in agreement with our results.

Concerning DF, pectins did not affect weight gain or food intake, although the IDF constituents increased the dry matter intake but without affecting weight gain. In the CT-control group, fed a diet containing 4% cellulose, a small but not significant increase in food intake was observed (Table 2), suggesting that low levels of IDF in the diet do not affect mean food intake. These results are in agreement with those found by Park and Harrold (1983), who reported a linear increase in food intake when fiber levels were increased, although they did not find any difference in the growth rate.

Dry stool weight and water and protein excretions were not affected by the presence of catechin in the diet (Table 3). Pectins and TA were associated with increased fecal output and thus increased water, protein, and fat output as compared with the control group. The increased fecal protein excretion was correlated to a reduction of the apparent digestibility coefficient (ADC) of the protein in these groups, although the protein efficiency ratio (PER) was not significantly different from that of the control. None of these coefficients, indicators of the nutritional utilization of dietary protein, was affected in the catechin-fed group (Table 3).

Increased fecal weight and protein excretion were found in rats fed diets containing TA (Moulay et al., 1988; Nyman and Björck, 1989). The greater output of dry stool in this group is partly due to increased protein excretion. This higher level of excretion has been suggested to be of endogenous origin (Mitjavila et al., 1977). PP can also inhibit the intestinal absorption of dietary amino acids, increasing their fecal excretion (Santidrián and Marzo, 1989). Another possible mechanism to explain the high level of fecal N in the TA-fed animals is the formation of indigestible tannin–protein complexes that are excreted in feces. Any of these mechanisms may be responsible for the observed increase in N excretion.

Pectins, water-soluble fiber, are substrates for fermentation by colonic microflora, which can retain water and thus contribute to fecal bulk and water excretion. The fecal bacterial mass may also contribute to the endogenous N excretion. Since the bacterial mass may account for up to 55% of the total stool weight (Stephen and Cummings, 1980), an increased supply of fermentable materials in the diet may result in an increased fecal weight and a higher protein content of feces. A high level of protein excretion was observed in the groups fed the IDF constituents.

Some other mechanisms have been proposed to explain the effect of fiber on protein digestibility. A decrease in the activity of proteolytic enzymes, such as trypsin or chymotrypsin, caused by DF may result in incomplete protein digestion (Schneeman, 1990). This effect has been attributed mainly to viscous fibers such as pectins, which hinder access of the enzyme to its substrate (Stephen, 1987). Similarly, increased colonic volume, owing to the addition of fiber materials, soluble or insoluble, could interfere with the absorption of dietary protein and its degradation products (Bender et al., 1979). Increased fecal N may be due to an enhanced elimination of endogenous N as a consequence of stimulated mucosal cell proliferation in the presence of fiber (Fairweather-Tait et al., 1983).

The insoluble compounds showed a high fecal bulking capacity, with a significant increase in fecal weight and water, protein, and fat excretion. The ADC was lower in the lignin-fed and CT-fed groups, although the PER was not significantly different from the controls (Table 3). Tannins caused a significantly higher urinary N excretion than the CT-control. This effect was observed also in the lignin group (data not shown).

Fecal bulking capacity is more pronounced with the IDF materials, which resist intestinal degradation, leaving a substantial amount of water-binding fiber in the colon (Nyman and Asp, 1982, 1985). SDF constituents have a greater *in vitro* water holding capacity (WHC) than insoluble fibers, although *in vivo* this effect is minimized by the fermentative breakdown of the soluble fiber. Therefore, the nonfermentable fibers have a higher WHC (Stephen and Cummings, 1979; Robertson and Eastwood, 1981), which is in agreement with our results (Table 3).

The greatest stool weight and excretion of water, fat, and protein corresponded to the CT-fed group. Although the greater fecal excretions may be partially due to the presence of IDF in the carob pod concentrate, the cellulose-containing control group contained a similar fiber level. Consequently, the higher levels of fecal excretion in the CT group can be ascribed to NEPP. NEPP such as CT present a high fecal bulking capacity, comparable with that of the IDF constituents and the highest WHC of the tested compounds. Both fecal bulking and water holding capacities are related to the prevention and treatment of different intestinal disorders such as constipation and diverticular disease (Mendeloff, 1987; Eastwood, 1990), and CT potentially have excellent properties as intestinal regulators.

CT showed the highest level of fecal protein excretion, with a significant reduction of the ADC of the protein compared with the CT-control (Table 3). The best known property of CT is their ability to bind protein and form tannin–protein complexes that are insoluble at physiological pH and resist enzymatic hydrolysis. These indigestible complexes pass through the intestine and are excreted in feces (Tamir and Alumot, 1970; Alzueta et al., 1992; Bravo et al., 1992, 1993). Singleton (1981) suggested that the minimum molecular mass for an effective protein precipitation is 350 Da, which would be achieved by a dimeric catechin. This may explain the observed lack of effect on fecal N excretion of the monomeric catechin (molecular mass 298.3 Da) used in this study.

The effects of tannins on digestive enzymes may explain the high level of protein excretion. Tannins can bind and inactivate digestive enzymes (Tamir and Alumot, 1969; Horigome et al., 1988), an effect that induces a reduction of the intestinal digestion of dietary protein and of other nutrients. An increased endogenous N excretion may occur as a consequence of the infestion of tannins (Shahkhalili et al., 1990). This endogenous N may come from increased mucosal cell proliferation (Mitjavila et al., 1977), from

digestive secretions (Butler, 1989), or from proline-rich salivary proteins that are produced by the parotid glands as a mechanism of defense against the ingestion of tannins (Mehanso et al., 1983).

The PER in the CT-fed group was significantly lower than in the DF,PP-free control. Nevertheless, when it is compared with its corresponding control, this difference is not statistically significant, suggesting that the combined effect of IDF and CT on protein digestibility could be nutritionally adverse. As to the other tested compounds, none of them modified the PER, suggesting that the metabolic utilization of the dietary protein was not significantly affected by the DF constituents or by the polyphenolic compounds.

Soluble and insoluble compounds showed a similar effect on fat excretion. Except for lignin, all of the compounds caused a significant increase in the fat content of feces (Table 3). Although lignin is the fiber constituent that presents the highest *in vitro* capacity to retain bile acids (Story and Lord, 1987), it has been reported to have no influence on *in vivo* lipid metabolism (Pomare et al., 1987), which is in agreement with our results. Pectins were associated with the highest fecal fat excretion, which was increased by 208% compared with the control group. These compounds have been reported to be effective hypocholesterolemic agents (Judd and Truswell, 1985; Kritchevsky and Story, 1986). The effect of fiber on lipid metabolism is related to a reduction in the intestinal absorption of bile acids and dietary lipids as a result of the increased volume of the intestinal content and the formation of a gel matrix in the presence of viscous polysaccharides such as gums and pectins. Interference with the intestinal absorption of lipids occurs, resulting in increased fat excretion. On the other hand, the adsorption of bile acids by different fiber constituents enhances fecal excretion of bile acids (Vahouny and Cassidy, 1986; Miettinen, 1987).

Comparing TA and catechin with pectins in terms of their effect on fat excretion, and taking into account that the dietary levels of these EPP were lower (2%, dry weight basis) than those of pectins (10%, dry weight basis), it might be assumed that EPP could have an effect on fat metabolism similar to or even more important than that of SDF constituents.

Contradictory results on the effect of EPP on fat excretion have been reported in the literature. Moulay et al. (1988) did not find any effect of TA on fat excretion in guinea pigs. Nyman and Björck (1989) reported a decrease in the apparent digestibility of lipids in rats fed TA, while catechin did not show any effect. Régérat et al. (1992) observed an increase in total bile acid excretion and a significant reduction of plasma cholesterol levels in rats fed Quebracho tannins (water-soluble catechic tannins).

Little is known about the mechanisms of action of polyphenolic compounds on lipid metabolism. Würsch (1979) reported that CT can bind biliary acids *in vitro*. *In vivo*, this binding capacity resulted in a decreased absorption of bile acids and cholesterol, although blood cholesterol levels were not affected in rats. As we have mentioned above, CT may inhibit the action of digestive enzymes. A reduction of lipase activity could result in higher levels of fat excretion, although lipase is little affected by CT (Longstaff and McNab, 1991b; Yuste et al., 1992), and even an enhancing effect on its activity has been reported (Longstaff and McNab, 1991a).

One of the main differences between soluble and insoluble dietary constituents is their susceptibility to intestinal degradation. Soluble compounds, both pectins and EPP, were associated with a high apparent digestibility. Pectins, as a fermentable material, were largely degraded by the colonic microflora, with only 2% of the ingested polysaccharide being recovered in feces. Similarly, up to 5% of the ingested catechin and TA was digested and/or absorbed in the gastrointestinal tract (Table 3). Little is known about the metabolism of these PP. Catechin seems to be absorbed in the intestine and metabolized, undergoing oxidation of the aromatic ring and conjugation with glucuronic acid, glycine, etc. Conjugates would be excreted via the urine or in the bile; in the latter case, they may pass back to the intestine and undergo bacterial degradation (Kühnau, 1976).

With regard to insoluble materials, cellulose was partially degraded—up to 60% of the ingested polysaccharide was excreted in feces (Table 3). Human studies have shown up to 50% of ingested cellulose to be degraded, depending on the fiber source (Cummings, 1984). Only trace amounts of lignin and CT were degraded in the intestinal tract, with a high fecal recovery of both compounds—close to 98% of the ingested amount. These results indicate that NEPP are as resistant to degradation as lignin (which is also a highly polymerized phenol) and even more resistant than other IDF constituents such as cellulose.

***In Vitro* Fermentation.** *In vitro* fermentation assays were performed to study the susceptibility of DF constituents and PP to bacterial degradation. The *in vitro* fermentation system has been successfully used for fermentation studies of DF, resistant starch, and oligosaccharides (Adiotomre et al., 1990; Edwards et al., 1992).

Pectins, as a fermentable substrate, caused a large increase in the production of all the short-chain fatty acids (SCFA), as can be seen in Table 4.

Catechin did not show any effect on the levels of SCFA as compared with the control, while TA had an important inhibitory effect on the fermentative microflora, decreasing the amounts of all the SCFA formed. It has been reported that certain phenolic compounds may inhibit the ruminal (Chesson et al., 1982) and the gut microflora (Eberly et al., 1983). TA has been shown to depress the hemicellulose fermentability *in vivo* (Barry et al., 1986), and an inhibitory effect on the activity of cellulase and other digestive enzymes *in vitro* has been reported (Björck and Nyman, 1987). Nyman and Björck (1989) observed that catechin did not modify the activity of microbial enzymes *in vivo*, suggesting that it might be inactivated in the hind gut. This is in agreement with our results obtained with the EPP.

Concerning the insoluble materials, cellulose and CT did not affect the SCFA yields. As has been shown in the *in vivo* experiment, CT did not suffer intestinal degradation. The lack of effect of these NEPP on SCFA production seems to confirm their resistance to bacterial hydrolysis.

Cellulose was partially degraded during its passage through the intestinal tract, as shown in the *in vivo* experiment, but the *in vitro* results suggest that this polysaccharide would be resistant to fermentation, since it did not affect the SCFA production. Adiotomre et al. (1990), using (carboxymethyl)cellulose as fermentation substrate, observed a large increase of the SCFA production using the same *in vitro* fermentation system. The levels of acetic, propionic, and butyric acids were increased by 122, 83, and 56%, respectively, as compared with a fiber-free control, clearly indicating that cellulose may be fermented extensively by the colonic bacteria, which concurs with our *in vivo* results. The differences between the *in vitro* results and those reported by Adiotomre et al. (1990) and in our rat experiment might be due to the type of cellulose used in this study, which is a microcrystalline cellulose that might be resistant to *in vitro* fermentation but susceptible to *in vivo* degradation.

**Table 5. General Comparison of the Degradability and Physiological Effects of Dietary Fiber (DF) Constituents and Polyphenols (PP)**

| | soluble | | insoluble | |
|---|---|---|---|---|
| | SDF[a,b] | EPP[b,c] | IDF[b,d] | NEPP[b,e] |
| food intake | ↔ | ↔ | ↑ | ↔ |
| growth rate | ↔ | ↔ | ↔ | ↔ |
| fecal bulking | ↑ | ↕ | ↑↑↑ | ↑↑↑ |
| water excretion | ↑ | ↕ | ↑ | ↑↑ |
| fat excretion | ↑↑ | ↑↑ | ↑ | ↑↑ |
| protein digestibility | ↓ | ↕ | ↓ | ↓↓ |
| degradability | | | | |
| *in vivo* | ↑↑↑ | ↑↑↑ | ↓↓ | ↓↓ |
| *in vitro* | | | | |
| SCFA production | ↑↑ | ↕ | ↕ | ↔ |

[a] Soluble dietary fiber. [b] ↑, increase; ↓, decrease; ↔, no effect; ↕, variable depending on the assayed compound. [c] Extractable polyphenols. [d] Insoluble dietary fiber. [e] Nonextractable polyphenols.

When lignin was added to the fermentation system, a significant inhibition of the production of propionic acid and butyric acid was observed (Table 3). The *in vivo* digestibility of a plant material is inversely correlated to its degree of lignification, due to the protective effect of lignin against bacterial degradation. The observed inhibition of the SCFA production suggests lignin has a depressive effect on bacterial growth, which might also account for the reduced digestibility of lignified tissues. A growth-depressing effect on ruminal bacteria by cinnamic acid, a monomeric constituent of lignin, has been reported (Akin, 1982; Chesson et al., 1982).

SCFA are the main end products of fermentation of polysaccharide substrates. If the end products of fermentation of the polyphenolic compounds are other than SCFA, the observed results would give information only about the potentially toxic effect of PP on colonic flora, such as the case of TA.

When catechin and TA were determined in samples of the fermentation medium, only 40–60% of the added catechin and between 75 and 95% of the TA were recovered after fermentation. Substantial degradation seemed to occur, mainly of the catechic structure. These results suggest that EPP are quite resistant to bacterial degradation. However, the Folin–Ciocalteu method used in the determination of these PP is unspecific for phenolic compounds and will not detect any structural modification that occurred during the fermentation process.

Less than 5% of the ingested EPP was found in feces in the rat experiment, indicating that extensive absorption of catechin and TA derivatives takes place in the intestinal tract.

As a summary, Table 5 shows a general comparison between DF and PP in relation to their *in vivo* and *in vitro* degradabilities, as well as their influence on some physiological effects, considering their solubility properties.

Concerning the soluble compounds, although EPP and SDF seem to exert different effects, this difference is entirely due to the inclusion of catechin in this comparison. As has been observed, TA and pectins showed almost the same behavior, except for their effects upon the *in vitro* fermentation. Catechin, due to its monomeric nature, is not comparable with SDF or with other intermediate molecular weight polyphenols such as tannic acid.

TA caused extensive inhibition of SCFA production, displaying a toxic effect on the cecal microflora. Likewise, lignin, an IDF constituent, showed an inhibitory effect on colonic bacteria, suggesting that a mixed culture of cecal microorganisms may be affected to different extents by certain phenolic compounds.

In the light of these results, it is worth noting that some of the effects associated with the intake of DF in plants containing appreciable amounts of polyphenolic compounds may be due to the presence of these PP. The similarities between the properties of DF constituents and PP, along with the analytical evidence of the presence of PP in DF residues, suggest that these compounds might be considered DF constituents.

Irrespective of this suggestion, further research on the physiological effects of PP should be performed. As has been mentioned, the best known property of polyphenolic compounds is their effect on protein digestibility. Nevertheless, their influence on fecal bulking and lipid metabolism is not understood and is of potential interest from a nutritional point of view.

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