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Analytical approach and effects of condensed tannins in carob pods (*Ceratonia siliqua*) on feed intake, digestive and metabolic responses of kids

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Abstract

There are contradicting data in the literature regarding condensed tannins content in carobs. In this study, urea (0.5 g/ml) under reflux conditions increased dramatically the yield of condensed tannins to 17% of dry matter, as compared to 3.5% in acidic methanol extraction. This shows that carob pods are rich source of nonextractable condensed tannins. The effect of PEG in diet rich in carob pods on voluntary feed intake, digestibility and growth in weaning kids was studied during 15 days of balance trials in 24 kids distributed into three dietary treatments. The corn grain and wheat bran components in the control diet (diet 1) were replaced by carob in treatments 2 and 3; treatment 3 was supplemented by 3.3% of PEG of molecular weight-4000. Food intake, diet utilization and growth rate of weaning kids fed a diet containing 52% carob pods were decreased considerably in comparison to kids fed the control commercial diet. Supplementing the kids fed the carob-based diet with PEG increased feed intake, crude protein digestibility and growth to the rate obtained with the control diet. The nutritional experiment highlights the following aspects relevant to all mammals consuming carob pods rich diets: (i) tannins increased the variability in feed intake between days, (ii) hypocholesterolemic effect of carob pods is related to lipid binding capacity of nonextractable condensed tannins in the digestive tract, (iii) supplementing high level of carob pods to animals with normal blood cholesterol level may induce hypocholesterolemia.

Keywords: Condensed tannins; PEG; Carob pods; Extraction; Voluntary feed intake; Digestion; Hypocholesterolemia; Kids

1. Introduction

Carob (*Ceratonia siliqua* L.) is a multipurpose tree common in Mediterranean countries for centuries. The

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pods contain high level of sugars (40–60%), but low protein (3–4%) and lipids (0.4–0.8%) (Marakis, 1996). Apart from non-structural carbohydrates, the pods contain high amounts of dietary fiber and polyphenols (Marakis, 1996). The ripe pods were consumed as food, especially in ancient times as a candy for children, or in emergency situations such as war. Nowadays, carobs are used in preparation of caffeine-free chocolate substitute, as anti-diarrheic and anti-emetic products and as feed for cattle (Calixto and Canellas, 1982).

In Israel, hilly marginal areas were planted with carob trees in order to use the fruits as a cheap source of feed for domestic animals. However, their inclusion in ruminants and poultry diets was eventually abundant. The presence of tannins in pods induced deleterious effects on digestion, growth and milk production (Volcani and Rodrig, 1961; Alumot et al., 1964), in consistency with more recent research showing that condensed tannins in pods negatively affected feed utilization and growth in lambs (Priolo et al., 2000, 2002).

The effects of tannins on animals are multifactorial. It was shown that the main negative effects of tannins on voluntary feed intake and feed utilization are related to the tendency of the hydroxyl components in tannins to form complexes with proteins and other organic constituents (see Silanikove et al., 2001 for a review). Tannins interact with food proteins, digestive tract enzymes, microbes, and oral and digestive tract mucosa. Polyethylene glycol (PEG) is a polymer containing large number of oxygen atoms capable of forming hydrogen bonds with hydroxyl groups of tannins (Jones and Mangan, 1977; Aharoni et al., 1998). PEG binds tannins irreversibly over a wide range of pH, replacing proteins in existing protein–tannin complexes, and reduces the formation of new protein–tannin complexes (Jones and Mangan, 1977; Makkar et al., 1995; Silanikove et al., 1996b). These properties of PEG allow to prevent the anti-nutritional effects of tannins (Silanikove et al., 2001). The purpose of many studies using PEG was to enhance the utilization of browse by livestock (Gilboa et al., 2000; Landau et al., 2000, 2002; Silanikove et al., 2001; Perevolotsky et al., 2005). PEG improved dramatically the growth and meat quality of lambs fed a diet based on carob pulp (Priolo et al., 2000, 2002).

According to Bravo (1998), carob pods are exceptionally rich source of nonextractable polyphenols, whereas Priolo et al. (2000, 2002) claimed that the pods have low content of condensed tannins (CT), but with exceptionally high biological activity (astringency). The green non-ripe pod is unpalatable due to strong astringency feeling in the mouth. It was shown that during ripening, the CT in pods undergoes substantial polymeration and condensation, explaining the loss of astringency in the ripe fruit (Tamir et al., 1971).

The aims of this study were the following:

1. To improve the method of extraction of tannins from carob pods, which would allow more complete recovery and accurate determination of the CT content.
2. To study the effect of PEG in diet rich in carob pods on voluntary feed intake, digestibility and growth in weaning kids.

2. Materials and methods

2.1. Animals and diets

Twenty four Anglo-Nubian kids were grown until weaning in a commercial farm, where they suckled colostrum from their dams during the first 3 days of life. From days 3 to 25 the kids received milk replacer three times a day and from days 25 to 45 twice a day. Concentrate and hay were available at all time. At the age of 45 days, kids were brought to the metabolic barn of the Agricultural Research Center, and after 4 days of adaptation to the new environment they were placed individually in metabolic cages in a barn protected from wind and rain. The kids were allotted randomly to three dietary treatments (control, carob, carob+PEG) designed to provide the nutritional demands of growing kids (Table 1). The corn grain and wheat bran components in the control diet were replaced by carobs in the other two treatments. Carob+PEG treatment was supplemented by 3.3% (DM basis) of PEG of molecular weight-4000. Metabolizable energy and crude protein contents of all diets were formulated to be similar by changes in oil and soybean meal proportions. The pods were incorporated in the diet after being dried at 55 °C for 72 h and

Table 1
Composition of the experimental diets (% as fed)[a]

	Treatments	
	Control	Carob based
Ingredients		
Corn	39.5	–
Wheat bran	20.9	–
Carobs	–	52.0
Soybean meal	26.0	36.0
Ground vetch hay	10.4	8.0
Soybean oil	2.0	2.8
Mineral mix	0.9	0.9
NH_3Cl	0.3	0.3
Chemical composition (% DM)		
Dry matter	90.5	87.5
Crude protein	21.7	22.1
NSC[b]	46.3	46.7
NDF	22.4	18.6
ADF	12.4	18.3
ADL	1.9	7.6
Ash	0.08	0.08

[a] Carob+PEG treatment was fed the same diet as carob-based treatment plus 3.3% (DM basis) PEG (mw 4000).
[b] Non-structural carbohydrates.

ground to coarse particles. The hay was chopped prior to mixing it with the diet. The feeds were given once a day ad lib, and the residues were weighed daily before the fresh food was given. The kids had free access to water.

2.2. Experimental procedure

The experiment was carried during the month of April under natural lighting (13:27 h of natural light) and ambient temperature (minimal 11.2, maximal 25.1 and average 18.2 °C) conditions. The experiment started after 7 days of adaptation of the kids to the metabolic cages and the experimental diets. During the 15 days of the experiment, the kids were weighed on alternate days eight times. Feces and urine of each kid were collected on days 12 to 14, and were kept at − 20 °C until analyzed. Urine was collected in bottles with 10% H_2SO_4, in order to prevent ammonia losses. During the collection days, the amount of drinking water was recorded. On day 15, rumen fluids were collected at 8.00 AM, before the morning feeding, and at 10.30 AM and 1.00 PM. After recording the pH of the rumen fluids, the samples were stored at − 20 °C. At the same time, blood samples were taken from the jugular vein into heparinised tubes, centrifuged and the plasma was separated and stored at − 20 °C.

2.3. Chemical analyses

Concentration in feeds and feces of dry matter, ash, non-structural carbohydrates (NSC), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL), crude protein (N × 6.25) and urine nitrogen (Kjeldahl method in a tecator Kjeltec auto analyzer 1030) was determined by routine procedures (Arieli et al., 2004). Amylase and trypsin activities in fecal samples, volatile fatty acids (VFA) and ammonia in rumen fluids and urea, total protein, β-hydroxy butyrate, total cholesterol, and the activities of gamma glutamyltranspeptidase (GGT) and aspartate aminotransferase (AST) in blood plasma were determined as described by Silanikove et al. (1994, 1996c).

Tannins analysis in diets and carob were carried out in triplicates. Condensed tannin was measured by the butanol/HCl method (Porter et al., 1986). Quebracho bark (Trask Chemical Corp., Georgia) purified on Shephadex LH-20 (Pharmacia) was used as a source for CT standard (Asquith and Butler, 1985). The CT were measured in feed samples extracted with 1% HC1 in methanol (Hagerman, 1988), or after the following extraction procedures: (1) Overnight extraction under constant agitation of 1 g DM of ground sample (to pass through 1 mm mesh) in 10 ml buffer citrate phosphate, pH 4.7; (2) Overnight extraction under constant agitation of 1 g DM of ground sample in 10 ml buffer citrate phosphate, pH 4.7+urea in concentration of 0.5 g/ml; (3) Extraction for 2 h under reflux conditions of 1 g DM of ground sample in 10 ml buffer citrate phosphate, pH 4.7; (4) Extraction for 2 h under reflux conditions of 1 g DM of ground sample in 10 ml buffer citrate phosphate, pH 4.7+urea in concentration of 0.5 g/ml. After the extraction the samples were centrifuged in 1000×*g* for 20 min and CT were determined in the supernatant.

2.4. Statistical analysis

Averages of body weight and gain, individual feed intake and feed efficiency, water intake, digestibility coefficients were compared by one-way analysis of

variances, with diet as main effect, using general linear methods of SAS (SAS, 2001). Differences between groups in the between day variation in feed intake were evaluated by two-way analysis of variance, with treatment, day in experiment and their interaction as main effects.

3. Results

The yield of CT obtained following extraction of carobs in acidic methanol was 5.0%. The yield of CT following extraction with buffer solution was 3.1%, whereas extraction with urea-buffer solution increased the yield to 4.3%. Under reflux conditions with buffer the yield of CT was 6.2%. The highest yield of CT, 17.2%, was obtained by reflux with urea-buffer solution (Fig. 1).

The carob pod-based diets and the control diet contained similar percentage of DM, protein, non-structural carbohydrates and cell wall (NDF) (Table 1). The level of ADF and ADL was high in the carob-based diets due to higher concentration of these components in the carob.

At the start of the experimental period, the kids weighed 11.4 kg, on average. At the end of the experimental period the average gain was 2.7 kg for the control and carobs–PEG supplemented groups while only 1.6 kg for the kids fed the carob diet without PEG ($P<0.05$) (Table 2). Feed intake was lower ($P<0.03$) in the control kids than in the group fed the carobs–PEG diet. The between-days variation in feed intake was significantly higher in kids fed the carobs diet than in kids fed the control and carobs–PEG diets (Fig. 2). Feed efficiency (feed per gain) did not differ between control and carobs–PEG diet but was significantly worse in the carob-fed kids ($P<0.002$). There was no difference in water intake by the three groups of kids (Table 2).

Digestibility of total dry matter and organic matter was higher in the control kids than in carob-fed counterparts ($P<0.02$ and $P<0.0001$, respectively; Table 3), and supplementation of PEG did not improve dry and organic matter digestibility in carob-fed kids. Crude protein digestibility was highest in the

Table 2
Body weight gain, feed intake and feed efficiency of kids fed a control diet or diets containing carobs with or without PEG for 14 days

	Treatments			S.E.M.	*P*
	Control	Carob	Carob+PEG		
Initial body weight, kg	10.9	11.2	12.0	1.03	0.12
Weight change, kg	2.7[a]	1.6[b]	2.7[a]	0.4	0.05
Feed intake, kg	7.90[b]	9.54[ab]	10.90[a]	1.99	0.03
Feed/gain	3.10[b]	6.67[a]	4.18[b]	1.59	0.002
Drinking water intake, kg	3.89	3.99	4.25	0.57	0.49

Mean values in the same line with different superscript are significantly different.

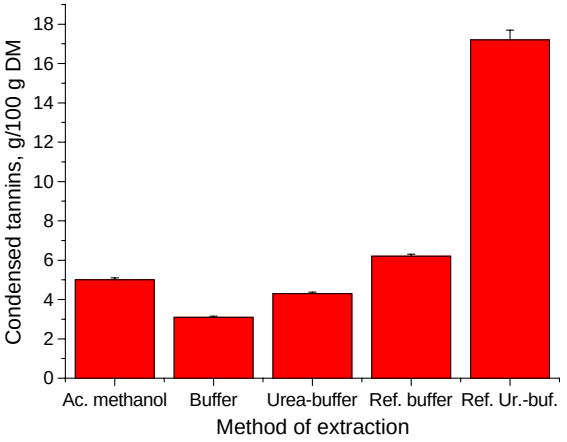

Fig. 1. Condensed tannins contents in carob pods following different methods of sample extraction. Ac. methanol=acidic methanol; Buffer=extraction in citrate buffer solution; Urea-buffer=extraction in citrate buffer-urea solution; Ref. buffer=extraction under reflux conditions in citrate buffer solution; Ref. Ur-buffer=extraction under reflux conditions in citrate buffer-urea solution.

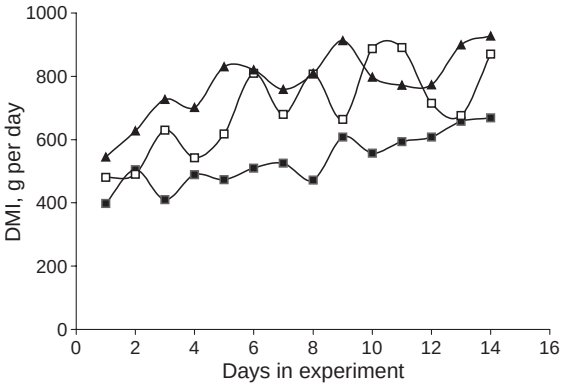

Fig. 2. Voluntary feed intake in the course of 14 days in kids fed the control (■), carob (□) or carob+PEG (▲) diets.

Table 3
Apparent digestibility of dry matter, organic matter, crude protein, non-structural carbohydrates (NSC) and cell wall fractions (NDF and ADF), nitrogen in urine and nitrogen retention in kids fed a control diet or diets containing carobs with or without PEG

Diet digestibility (%)	Treatments			S.E.M.	*P*
	Control	Carob	Carob+PEG		
Dry matter	82.2[a]	71.3[b]	75.1[b]	6.5	0.02
Organic matter	64.9[a]	58.0[b]	59.1[b]	1.29	0.0001
Crude protein	80.3[b]	79.8[b]	86.1[a]	2.76	0.0004
NSC[1]	94.1	94.0	95.5	2.51	0.86
NDF[2]	74.7[a]	55.5[b]	61.8[b]	8.73	0.002
ADF[3]	74.2[a]	60.8[b]	64.5[b]	7.91	0.014
Nitrogen in urine, g/3 days	8.99	8.99	9.74	2.42	0.74
Nitrogen retention (% of intake)	37.4	38.5	47.2	13.8	0.34

Mean values in the same line with different superscript are significantly different.
[1] Non-structural carbohydrates.
[2] Neutral detergent fiber.
[3] Acid detergent fiber.

carob–PEG fed kids ($P<0.0004$) and did not differ between the control and carob-without PEG groups. Nitrogen excretion in urine and overall nitrogen retention did not differ between groups. The digestibility of NDF and ADF was higher in the control group than in the kids fed the carobs with or without PEG ($P<0.002$ and $P<0.014$, respectively; Table 3).

Concentration of metabolites in rumen fluids is described in Table 4. The concentration of ammonia in the rumen fluids of carob–PEG kids was significantly higher than in the carob without PEG diet ($P<0.02$), whereas an intermediate value was found in the control kids. No difference between treatments in total VFA concentration in rumen fluids has been shown. However, butyrate was significantly higher in the carob–PEG group than in the control group, and caproate was highest in the carob-without PEG group.

Table 4
Metabolites in rumen fluid on day 15 of the experiment in kids fed a control diet or diets containing carobs with or without PEG

	Treatments			S.E.M.	*P*
	Control	Carob	Carob+PEG		
NH_3, mg/l	24.4[ab]	22.0[b]	27.7[a]	6.02	0.02
Total VFA, mM	41.3	43.3	45.9	10.3	0.68
Individual VFAs	Mol/100 mol				
Acetate	57.6	59.4	51.0	14.1	0.74
Propionate	23.0	18.9	22.9	5.9	0.28
Butyrate	15.0[b]	18.2[ab]	24.2[a]	6.1	0.015
Iso-caproate	2.4	1.4	1.1	1.0	0.11
Caproate	2.1[b]	3.1[a]	2.8[a]	1.0	0.07
Acetate/ Propionate	2.50	3.14	2.23		
pH	6.41	6.43	6.25	0.35	0.71

Mean values in the same line with different superscript are significantly different.

Enzymatic activities of trypsin and amylase in fecal samples were significantly lower in kids fed the carob-based diets than in those fed the control diet (Fig. 3).

Plasma urea concentration was higher in kids fed the carobs-based diets in comparison to controls (Table 5). The levels of GGT, AST, indicators of liver impairment did not differ between groups. Cholesterol concentration was significantly lower in the

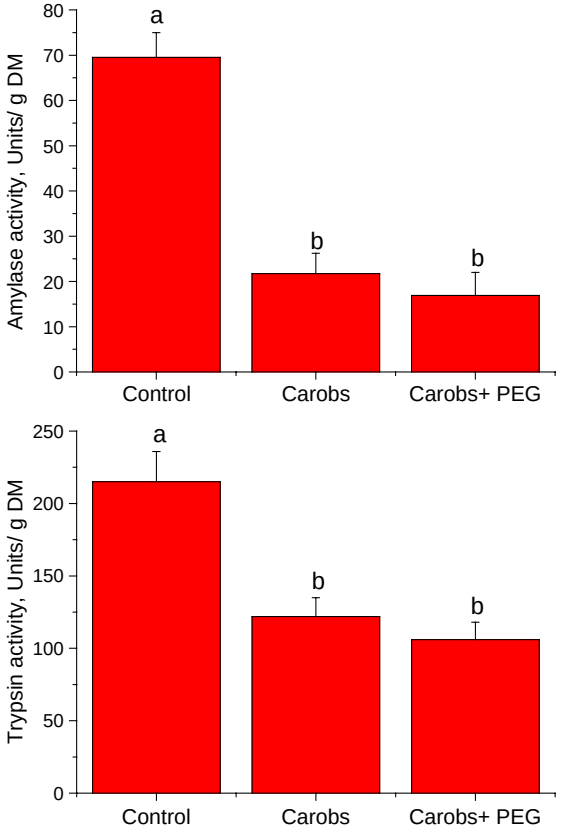

Fig. 3. Amylase (upper panel) and trypsin (lower panel) activities in fecal samples in kids fed the control and carob-based diets. Values marked by different superscript letter are significantly different ($P<0.03$, or higher).

Table 5
Metabolites in plasma on day 15 of the experiment in kids fed a control diet or diets containing carobs with or without PEG

	Control	Carob	Carob+PEG	S.E.M.	P
Urea, (mg/100 ml)	45.7[b]	54.3[a]	57.6[a]	6.10	0.0006
BHB[1] (mg/100 ml)	5.79	7.54	7.64	0.233	0.117
Protein (mg/100 ml)	6.20	6.02	6.08	0.314	0.376
AST[2] (unit/l)	106.8	107.9	101.7	10.9	0.505
GGT[3] (unit/l)	32.1	36.9	30.8	8.96	0.234
Cholesterol (mg/100 ml)	56.2[a]	37.8[b]	53.6[a]	13.1	0.003

Mean values in the same line with different superscript are significantly different.
[1] BHB=β-Hydroxy butyrate.
[2] AST=Aspartate aminotransferase.
[3] GGT=Gamma glutamyltranspeptidase.

carob-fed kids than in carob–PEG and control groups ($P<0.003$; Table 5).

4. Discussion

4.1. Tannin extractability yields in carobs

The yield of CT obtained by reflux in urea-buffer solution (Fig. 1) resembled the approximately 18% yield obtained by Bravo (1998), who used various enzymes followed by dialysis to isolate tannins from carobs. Urea was used at denaturizing level of proteins, a level expected to weaken the hydrogen bonds between tannins and proteins. The affinity of these intermolecular bindings is weaken at high temperatures (Charlton et al., 2002). The viscosity and size of proanthocyanidin polymers were significantly reduced by adding urea to tannin concentrated extracts (Sealyfisher and Pizzi, 1992; Kim et al., 1996). Thus, the effectiveness of urea-reflux procedure may be related to the breakdown of hydrophobic and hydrogen interactions between tannins and proteins and tannin and fiber components and prevention of phlobaphenes (tannins self-condensation products) formation. The CT yield obtained in these two studies exceeds considerably the 3.8% yield obtained when a method based on solvent-SDS extraction, supposed to extract all the tannins in plant samples (Terrill et al., 1992), was applied for carobs (Priolo et al., 2000, 2002). In fact, the yield obtained by the latter method was not higher than that obtained by a simpler one-stage extraction with acidic methanol (Guessous et al., 1989; Marakis, 1996; Fig. 1). The insolubility of CT in carobs is also consistent with the results of Silanikove et al. (1996b). The solvent-SDS method detected only 0.24–0.87% CT in canola hulls, as compared to a yield of 6% after six successive extractions with heated butanol/HCl mixture (Naczk et al., 2000). Thus, insolubility of CT in SDS occurs in several types of plants and is consistent with reports in which CT–protein complexes are detected as artificial NDF (Makkar et al., 1995; Silanikove et al., 1994, 1996a).

4.2. Productive response

The kids fed the diets that contained carob pods performed very poorly, compared to the kids on the control diets, whereas PEG supplementation increased the growth rate 1.7 fold to the level of kids fed the control diet (Table 2). In study with lambs (Priolo et al., 2000), PEG supplementation increased growth rate of lambs fed carob-based diet threefold. The poor performance of the kids was reflected with poorer feed efficiency as also found in lambs fed carobs (Priolo et al., 2000, 2002). However, the 16% lower digestible organic matter in kids fed the carob diet than those fed carob+PEG (calculated from Tables 2 and 3) does not explain the 40% lower growth rate in kids fed the carob diet, suggesting that additional factors may be involved. Whereas average coefficient of variation of daily food intake of kids fed the control and carob+PEG were 21.3% and 24.3%, respectively, that of the kids fed the carob was 73% higher (36.8%) (Fig. 2). Accordingly, the differences in daily feed intake may explain at least part of the low performance of the carob-fed kids. The fact that the erratic voluntary feed intake in kids fed the carobs disappeared in combination with PEG suggests the involvement of CT, and may have involved the interaction of tannins with gut epithelia (Silanikove et al., 2001). Hypocholesterolemia induced growth depression in chicks (Rogel and Vohra, 1983). Thus, additional factor may be cholesterol deficiency (see below) induced by eating carob-based diet without PEG supplementation.

In lambs, PEG supplementation improved dry matter, crude protein and NDF digestibility (Priolo et al., 2000), whereas in kids in the present study only crude protein digestibility was significantly improved. In comparison to the lamb study (Priolo et al., 2000), a diet richer in highly digestible non-structural carbohydrates was used, which may have masked the overall effect of tannins on OM and NDF digestibility. A higher level of PEG (approximately 20%) was used in the lamb study (Priolo et al., 2000), which likely counteracted more completely the negative effects of tannins. Additional factor, which may confound the interpretation of the digestibility data in the present experiment, hence, the comparison with the lamb study (Priolo et al., 2000) is the short duration of the balance trial in the present study.

Rumen ammonia concentration in the PEG supplemented kids was higher, as compared with the control

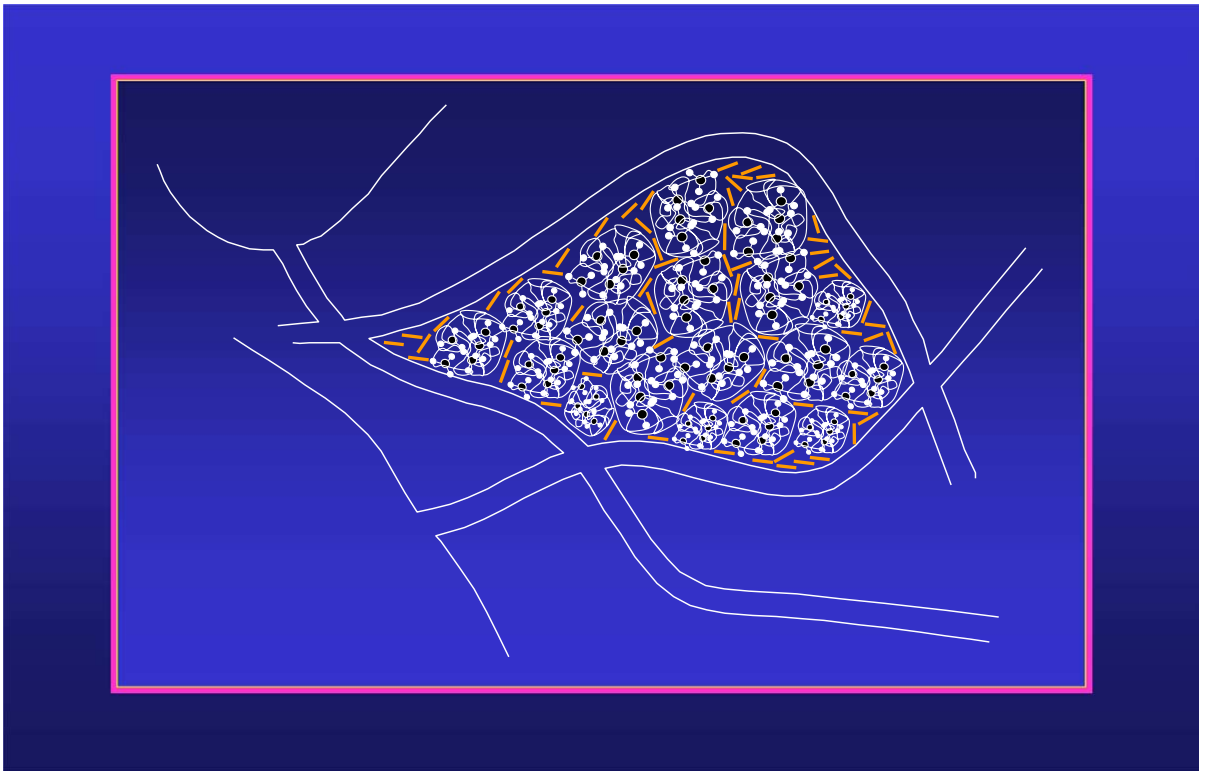

Fig. 4. A schematic model explaining trapping fat and cholesterol by carob pods fibers. The polysaccharide matrix of cell wall fibers is represented by the double strip. Multidentate CT is represented by circles. The protein molecule is represented as a coil, forming a shape of a ball. This ball is a product of the interaction between protein and CT. The rods represent trapped cholesterol and fat molecules. The polysaccharide matrix of plant cell wall swells under the hydrating conditions prevailing in the GIT. During mastication or commercial grinding, CT are released from cells vacuoles and are free to interact with polysaccharide matrix. Proteins originated from plant foods or microbial enzymes may diffuse into the lumen of the polysaccharide matrix and interact with CT trapped in the polysaccharide. The interaction between proteins, particularly rich in aromatic amino acids and CT, produces compact units in which the hydrophilic residues are inside and the hydrophobic residues on the surface of each unit (Jobstl et al., 2004). These units can trap hydrophobic cholesterol, free fatty acids and fragments of starch by hydrophobic interactions. This model is compatible with existing knowledge: (1) The formation of artificial NDF (i.e., SDS insoluble material) due to interaction of CT and proteins in cell walls (Makkar et al., 1995; Silanikove et al., 1996a,b; Aharoni et al., 1998). (2) The resistance of CT in carobs to extraction with conventional solvents (Makkar et al., 1995; Silanikove et al., 1996b) and SDS (present data). (3) The affinity between PEG and CT is higher than between proteins and CT. Thus, PEG treatment renders the formation of artificial NDF (Makkar et al., 1995; Silanikove et al., 1996a,b; Aharoni et al., 1998) and annuls the hypocholesterolemic effect (present data) because cholesterol and fat are release from their trap in the fiber. (4) The increase in the fermentation of sugars following PEG treatment (present data) may also be explained by release of starch from the fiber-sponge trap.

and carob unsupplemented kids, consistent with the results in lambs (Priolo et al., 2000). However, the higher amount of N available for microbial activity in the PEG group was not associated with higher VFA concentration in the rumen, in contrast with the lamb study (Priolo et al., 2000). Further support that CT in carobs affected the metabolism of microbial population in the rumen was the change in the profile of rumen VFA. The main change was the increase in butyric and caproic acids concentrations (Table 4). Butyrate formation is augmented as the result of highly fermentable sugars (Landau et al., 1992; Van Soest, 1994), which means that PEG not only increased the availability of N to microbes, but also increased the availability of relatively short-chain sugars.

The low amylase and trypsin activities in feces of kids fed the carobs-based diets (Fig. 2) are consistent with the previous results in sheep and goats fed the tannin-rich diets (Silanikove et al., 1994, 1996a), indicating that the negative effects of CT are distributed along the entire gastrointestinal tract. However, while in the previous studies (Silanikove et al., 1994, 1996a), PEG supplementation resulted in the increase of trypsin and amylase activity in fecal samples, this was not the case in the present study. The differences in enzymatic response to PEG supplementation may relate to the differences in the mode it is supplied. In the previous studies PEG was supplied 5 to 10 min prior to the major meal by mixing it with small amount of concentrate. In the present experiment, PEG was mixed with the diet, thus, irreversible complex between PEG and tannins was most likely formed during mastication.

4.3. Metabolic response

The kids in the present study consumed approximately 60 g/day of condensed tannins, an amount which did not induce apparent toxic syndromes, in consistent with the previous conclusions (Silanikove et al., 1996c). The most notable effect of tannins on the profile of plasma metabolites was the effect on total plasma cholesterol concentrations. The carob diet induced hypocholesterolemia in comparison to the control diet. Our results are in line with reports demonstrating that carob pulp preparation rich in insoluble fiber lowers total and LDL cholesterol in laboratory animals (Perez-Olleros et al., 1999) and human beings (Zunft et al., 2003). PEG supplementation annuls the hypocholesterolemic effect of carob, indicating that this effect was specific to the tannin component in carobs fibers. As CT in carobs are nondigestible, the hypocholesterolemic effect of tannins was caused most likely by elevated excretion of cholesterol out of the digestive tract, which is consistent with reports showing an increase of fat content in stool of animals fed the diet rich in nonextractable polyphenols (Bravo, 1998). In Fig. 4, a schematic model, which explains the cholesterol and fat lowering capacity of carob pods fibers is depicted. Trapping fat and cholesterol by this fiber-sponge results in sweeping up fat and cholesterol secreted into the digestive tract via the entero-hepatic circulation.

5. Conclusions

In the present study it was shown that a reflux treatment using urea-buffer solution exceeds considerably the yield of tannins obtained by the solvent-SDS method (Terrill et al., 1992). CT in carob exert their effect by reducing protein digestibility because of the interaction of food proteins with the nonextractable polyphenols. These properties of carob's CT also explain the hypocholesterolemic effect. PEG supplementation was proved as an effective mean to counteract the negative effects of tannins. It is suggested that a higher amount of PEG than that used in the present study is required to counteract the negative effect of dietary concentration of 9–10% tannins on OM digestibility.

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