The inhibitory effects of hull polysaccharides and tannins of field beans (*Vicia faba* L.) on the digestion of amino acids, starch and lipid and on digestive enzyme activities in young chicks

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(*Received 27 September 1989 – Accepted 27 July 1990*)

The effects of polysaccharides and tannins present in the hulls of field beans (*Vicia faba* L.) on the digestion of amino acids, starch and lipid were studied in poultry. A control diet without hulls and the same diet substituted with 400 g hulls/kg diet from three different varieties of beans were fed to 3-week-old chicks for 4 d. Digestibility coefficients for amino acids, starch and lipid were calculated from measurements made of these nutrients in the diets and the freeze-dried excreta with the aid of titanium dioxide as a marker. Activities of trypsin (*EC* 3.4.21.4), α-amylase (*EC* 3.2.1.1), and lipase (*EC* 3.1.1.3) in digesta removed from the upper jejunum, sucrase (*EC* 3.2.1.48) in the gut mucosa from the upper jejunum, and α-amylase and lipase in the pancreas were measured. The hulls were analysed for their polysaccharide and tannin contents. Results showed that the hulls were mostly carbohydrate in composition, with cellulose the predominant polysaccharide. Tannins present in the hulls of two coloured-flowering varieties (Brunette and Minica) were of the condensed type. The diet with tannin-free hulls (white-flowering variety Medes) lowered slightly the digestion of amino acids, starch and lipid compared with the control diet. This effect was believed to be due to inhibition of digestive enzymes, possibly through their adsorption onto the hulls. Diets with tannin-rich hulls (varieties Brunette and Minica) caused a large reduction in the digestion of amino acids, starch and lipid compared with the control diet mainly due to inactivation of digestive enzymes by the formation of tannin–enzyme complexes in the digestive tract. Enzyme activities could be partially restored by the addition of polyvinylpyrrolidone to the digesta. Tannins inactivated trypsin the most, α-amylase to a lesser extent and lipase the least and as a consequence lowered the digestion of amino acids the most, starch to a lesser extent and lipid the least. Tannins did not induce an increased pancreatic production of digestive enzymes, nor did they affect activity of jejunum mucosal sucrase. Condensed tannins from Brunette and Minica hulls were partially extractable in methanol alone, but required acidic methanol for fuller extraction. The vanillin:anthocyanidin ratio suggested that tannins were polymerized to the same degree in the Brunette and Minica varieties, both in the methanol and acidic methanol extracts. Hulls from the variety Minica contained a greater amount of methanol-extractable tannins, the quantity of remaining tannins that required acidic methanol for extraction being the same for both varieties.

Nutrient availability: Dietary fibre: Tannins: *Vicia faba* L.: Chick

Non-starch polysaccharides and condensed tannins have been shown to interfere with nutrient digestion in the single-stomached animal and to promote the excretion of endogenous nitrogen. The role of condensed tannins as enzyme inhibitors through the formation of tannin–enzyme complexes is well documented from the numerous in vitro studies undertaken. Yet there is still a dearth of information concerning which particular nutrients are rendered indigestible by them in vivo. On the other hand, considerably more information is available from human and animal studies on the effects of various non-starch polysaccharides, particularly viscous polysaccharides, on lipid metabolism, glucose transport and nutrient availability, but the mechanisms involved are only beginning to be elucidated.

Viscous fibre polysaccharides, both ionic (pectins) and non-ionic (guar gum) are believed to affect lipid metabolism, lowering blood cholesterol (Mathe *et al.* 1977; Cerda *et al.* 1985) by promoting the excretion of bile acids through their adsorption onto the fibre matrix (Meittinen & Torpilla, 1977; Robertson *et al.* 1980). Sugar-beet pulp in pig diets has been shown to decrease the digestibility of ash, protein and fat in the ileum and decrease the faecal digestibility of fat (Graham *et al.* 1986). Decreased protein utilization occurred in rats (Arnal-Peyrot & Adrian, 1974) by feeding viscous polysaccharides as well as encouraging an increased endogenous N excretion (Shah *et al.* 1982). Low & Rainbird (1984) reported an increased excretion of nitrogenous material from the mucosa of the small intestine of pigs after feeding guar gum, and various types of non-starch polysaccharides have been shown to interfere with glucose absorption in the intestine (Jenkins *et al.* 1977; Johnson & Gee, 1981). The observations that non-starch polysaccharides can also depress enzyme activities implies that one of their many modes of action is that of enzyme inhibitors (Schneeman, 1978; Isaksson *et al.* 1982; Gagne & Acton, 1983).

Condensed tannins of sorghum (*Sorghum vulgare*) and faba bean (*Vicia faba* L.) have been shown to affect dry matter digestibility in diets of rats and chickens (Miller *et al.* 1972; Maxon *et al.* 1973; Nelson *et al.* 1975), and condensed tannins from horse and faba beans have been reported to cause a depression in feed consumption, growth, egg weight and N digestibility in chickens (Marquardt *et al.* 1977; Martin-Tanguy *et al.* 1977).

Tannins can form tannin–protein complexes (Oh *et al.* 1980; Hagerman & Klucher, 1986) rendering dietary proteins indigestible. More recently cell-wall carbohydrates have been shown to escape fermentation in the rumen (Barry *et al.* 1986) due to tannin–polysaccharide bonds in the plant (Gupta & Haslam, 1980; Beart *et al.* 1985; Reed *et al.* 1987). The finding that tannins can adsorb onto starch in vitro (Davis & Hoseney, 1979) suggests that the simple-stomached animal fed on a cereal-based diet may be particularly disadvantaged by diets containing tannins. The digestive enzymes, trypsin (*EC* 3.4.21.4), α-amylase (*EC* 3.2.1.1) and lipase (*EC* 3.1.1.3) have been shown to be inhibited in vitro by condensed tannins (Tamir & Almut, 1969; Griffiths, 1981; Singh, 1984). Studies in vivo with rats have shown that both α-amylase and trypsin activities in digesta removed from the tract were inhibited by tannins, while digesta lipase activity, on the other hand, was enhanced (Griffiths & Moseley, 1980; Horigome *et al.* 1988).

With such an abundance of information on the potential of condensed tannins to cause digestive havoc it is surprising that so few digestibility measurements have been carried out in vivo to quantify their antinutritional effect on amino acid or protein digestion (Rostagno *et al.* 1973; Marquardt *et al.* 1977; Mitaru *et al.* 1984; Hibberd *et al.* 1985; Hussein *et al.* 1989), and fewer still on starch digestion (Hibberd *et al.* 1985) and lipid digestion (Marquardt *et al.* 1977).

Field bean hulls are almost entirely non-starch polysaccharide in nature (M. Longstaff, unpublished results). The hulls from the coloured-flowering varieties contain condensed tannins, while those from the white-flowering varieties are devoid of them. Hence, in the present study the effect of non-starch polysaccharides alone or the combined effect of non-starch polysaccharides and condensed tannins on nutrient digestibility and enzyme activities was investigated.

MATERIALS AND METHODS

Diets

The composition of the control diet used in the present study is shown in Table 1. It was based on maize and soya-bean meal and supplemented with soya-bean oil to facilitate the study of lipid digestibility.

Three varieties of field beans, Medes (white-flowering with tannin-free hulls), Brunette and Minica (coloured-flowering with tannin-rich hulls) had their hulls removed using a modified pearl-barley dehuller. Any remaining pieces of cotyledon were removed by hand and the hulls were ground through a 0·12 mm sieve. The four experimental diets were composed as follows: 1, control (100); 2, control–Medes hulls (60:40, w/w); 3, control–Brunette hulls (60:40, w/w); 4, control–Minica hulls (60:40, w/w).

Although the high content of hulls rendered three of the diets nutritionally inadequate it was felt that because the experiment was of such short duration (4 d) deficiencies of essential minerals or vitamins would not manifest themselves and influence the interpretation of the results.

Birds

Allocation of diets, feeding protocol and excreta collection. Forty-eight 3-week-old male broiler chicks were selected as the middle weight-range from a batch of sixty which had been reared on a common starter diet in a tiered battery brooder. The chicks were housed in individual cages, equipped for the quantitative collection of excreta in a room with a controlled environment and with 23 h light/d. Each of the four diets described previously was tube-fed to twelve chicks. A preliminary experiment had established that chicks of this age would not voluntarily consume diets containing the tannin-rich hulls. On day 1, after 5 h starvation the chicks were tube-fed with the appropriate diet at 15.30 hours until the crop was considered full. On days 2 and 3 the chicks were fed at 08.30 and 15.30 hours and on day 4 at 08.30 hours. During the afternoon of day 2 clean trays were placed under each cage and the excreta voided during the subsequent 24 h were collected, frozen at −20°, freeze-dried and kept for nutrient digestibility studies.

Collection of digesta, mucosal scrapings and pancreas. Chicks were killed starting at 12.00 hours on day 4 and their pancreases and digestive tracts were removed. Each pancreas was weighed and divided into two approximately equal portions and kept frozen for the estimation of α-amylase and lipase activities. The upper part of the digestive tract from the end of the duodenum to Meckel's diverticulum (jejunum) was separated out, cut into two equal pieces and the contents from the proximal part squeezed out, mixed thoroughly and divided into three approximately equal portions and kept frozen for estimation of trypsin, α-amylase and lipase activities. The tract was then opened, any adhering digesta removed by washing, blotted dry and the mucosal surface removed by gentle scraping with a spatula and kept frozen for estimation of sucrase (*EC* 3.2.1.48) activity. The two caeca with their contents were removed and weighed. All material required for enzyme studies was immediately frozen in vials plunged into liquid N and transferred to a freezer at −20°, until required for analysis. Two chicks fed on the control diet died and samples of the digesta and excreta of one chick fed on the diet containing Medes hulls were rejected because of their abnormal green discolouration.

Chemical analysis

The hulls were analysed for non-starch polysaccharide and tannin content. Amino acid analyses were carried out on all diets and on the excreta from five chicks chosen at random

Table 1. *Composition of control diet (g)*

Maize	743·0
Soya	174·0
Maize oil	20·0
Pruteen	20·0
Limestone	15·0
Dicalcium	20·0
Vitamin mix*	2·5
Mineral mix†	2·5
Salt	3·0
Titanium dioxide	2·0
Soya bean oil	40·0
Total	1042·0

* Contained (mg): vitamin A 2·4, vitamin D_3 0·05, vitamin E 30, menadione 2, riboflavin 5, thiamin 2, nicotinic acid 28, pantothenic acid 10, biotin 0·15, pyridoxine 2.

† Contained (mg): zinc 50, copper 3·6, iodide 0·4, iron 80, manganese 100, selenium 0·15.

from each diet. Starch and lipid analyses were carried out on all diets and on the freeze-dried excreta from all available chicks (forty-five).

Hull carbohydrate determination. The carbohydrate composition of non-starch polysaccharides was determined after solubilization of 50 mg hulls in 0.25 ml 12 M-sulphuric acid for 1 h at 40°, followed by hydrolysis in 3 ml 1 M-sulphuric acid by dilution with 2·75 ml of a solution containing inositol (2 mg/ml) as an internal standard for 3 h at 100°. Sugar residues were quantified by gas–liquid chromatography after their derivatization to alditol acetates according to the method of Blakeney *et al.* (1983).

Hull tannin determinations. The tannin content expressed as catechin equivalents, was measured by the vanillin–acetic acid method of Butler *et al.* (1982). Hulls (50 mg) were weighed into screw-capped bottles and their tannins extracted into methanol (7·5 ml) or methanol containing 10 ml hydrochloric acid/l (7·5 ml) with continuous shaking for 40 min. After centrifugation at 1500 ***g***, portions (0·2 ml) were added to 5 ml glacial acetic acid with or without vanillin (0·5 g/100 ml). Solutions were mixed and heated for 15 min at 30°. The colour formed was read after 20 min at 510 nm. Catechin was used as a standard.

Anthocyanidin formation from methanol and acidic-methanol-extracted tannins from Brunette and Minica hulls was measured by the method of Porter *et al.* (1986). Portions (1 ml) of supernatant fractions containing tannins were hydrolysed in 6 ml *n*-butanol–HCl–Fe^{3+} solution for 1 h at 100°, and after cooling tubes to room temperature the magenta colour formed was read at 520 nm. A crude tannin preparation obtained from Brunette hulls was used as a standard to verify that the reaction was linear over the absorbance range measured. The vanillin:anthocyanidin ratio was calculated by dividing the absorbance given by a known amount of hulls from the vanillin reaction by that obtained from the anthocyanidin reaction by the equivalent amount of hulls. This ratio, as recommended by Butler (1982), was used to investigate the relative degree of polymerization of tannins from Brunette and Minica hulls and from those extracted by acidic methanol and methanol alone.

Amino acid determination in diets and excreta. Amino acid analysis was performed on a Rank Hilger chromaspek after acid hydrolysis, exactly according to the method of Blair *et al.* (1981).

Starch determination of diets and excreta. Starch was quantified from the amount of reducing sugars released after its enzymic hydrolysis by amyloglucosidase (*EC* 3.2.1.3). Diets (50 mg) and 100 mg excreta were gelatinized in 9 ml 0·2 M-sodium acetate buffer (pH 4·5) for 3 h at 100°. After cooling to below 50° tubes were incubated with 1 ml sodium

acetate buffer (pH 4·5) with or without amyloglucosidase (0·1 mg/ml, BDH) in a shaking water-bath at 50° for 16 h.

Reducing sugars were detected after their reaction with *p*-hydroxybenzoic acid hydrazide according to the method of Lever (1972).

Lipid determination of diets and excreta. Lipids were measured after extraction with acidic petroleum ether according to the European Communities (1971) procedure B.

Enzyme analysis

Sample preparation. Samples (400 mg) of frozen digesta were weighed into test tubes and ice-cold physiological saline (9 g sodium chloride/l; 4 or 8 ml) was added with mixing and left for 1 h at 4°. After centrifugation at 1500 ***g*** for 10 min, portions of the supernatant fraction were removed for analysis. When enzyme re-enactivation studies using polyvinylpyrrolidone (PVP) were being carried out, samples of digesta were pooled from pairs of chicks. Tannins have been shown to bind more strongly to PVP than to digestive enzymes (Griffiths & Moseley, 1980) and hence on the addition of PVP to digesta any previously formed tannin–enzyme complexes will be reversed. A procedure similar to that used by Griffiths & Moseley (1980) was followed. Because the control diet was well digested by birds, only enough digesta were available for re-enactivation studies of trypsin and lipase. Samples (200 mg) were weighed into test-tubes and 4 ml ice-cold physiological saline with or without PVP (50 g/l) were added with mixing and left at 4° for 1 h followed by centrifugation at 1500 ***g*** for 10 min. Suitable portions were taken for enzyme assays.

Samples of pancreas (200 mg) and jejunum mucosa (400 mg) were weighed into a Potter Elvehjelm glass homogenizer containing ice-cold physiological saline and homogenized for 1 min. Homogenates were then quantitatively rinsed into 20 ml volumetric flasks and appropriate portions taken for enzyme assay.

Determination of trypsin activity. Trypsin activity was determined according to the method of Erlanger *et al.* (1961) using the synthetic substrate *N*-benzoyl-DL-arginine-*p*-nitroaniline (BAPNA). Digesta supernatant fraction (1 ml) was incubated with 5 ml 0·05 M-Tris buffer (pH 8·2) containing 0·02 M-calcium chloride with or without BAPNA (0·435 g/l buffer) for 15 min at 30°. The reaction was stopped by the addition of 1 ml glacial acetic acid. The colour formed was read at 410 nm and *p*-nitroaniline was used as a standard.

Determination of α-amylase and sucrase activities. Gelatinized maize starch was used as the substrate for α-amylase, and glucose released was quantified colorimetrically. Sucrose was used as the substrate for sucrase and glucose plus fructose released was quantified colorimetrically. The colorimetric method employed was that of Lever (1972) for the detection of reducing sugars after their reaction with *p*-hydroxybenzoic acid hydrazide. α-Amylase activity was measured from the quantification of reducing sugars released after incubation of 1 ml supernatant fraction from digesta or pancreas homogenates with 2 ml 0·02 M-phosphate buffer (pH 5·9) with or without gelatinized maize starch (10 g/l buffer) for 10 or 5 min at 37°. The reaction was stopped by placing tubes in a boiling water-bath for 7 min. Sucrase activity was measured from the quantification of reducing sugars (glucose + fructose) released after incubation of 1 ml jejunum mucosal homogenate with 2 ml 0·2 M-sodium acetate buffer (pH 4·5) with or without sucrose (10 g/l buffer) for 2 h at 37°. The reaction was stopped by placing tubes in a boiling water-bath for 7 min.

Determination of lipase activity. Lipase activity was determined using the diagnostic kit no. 800 (Sigma Chemical Co.); the amount of sodium hydroxide required to neutralize the fatty acids released from olive oil triacylglycerols is proportional to lipase activity. Digesta supernatant fractions (1 ml) and pancreas homogenates (0·5 ml) were incubated with 3 ml olive oil triacylglycerols for 6 h or 3 h at 37°. The reaction was stopped by addition of alcohol (950 ml/l) and titration performed with 0·05 M-sodium hydroxide.

Table 2. *Carbohydrate composition of three varieties of faba bean* (Vicia faba *L.*) *hulls (g/kg dry matter)*

(Mean values and standard deviations)

Variety...	Medes		Brunette		Minica	
Sugar residue	Mean	SD	Mean	SD	Mean	SD
Rhamnose	7·8	0·2	5·1	0·4	5·3	0·3
Fucose	2·4	0·2	1·6	0·1	1·8	0·6
Arabinose	15·5	0·3	12·9	0·7	11·0	0·4
Xylose	143·7	5·1	80·8	2·4	86·6	1·2
Mannose	2·2	0·8	2·0	0·2	2·0	0·1
Galactose	21·3	0·9	18·3	1·1	18·7	1·2
Glucose^{H+C}	597·0	7·1	542·6	23·4	561·6	13·0
Uronic acids	171·2	4·8	138·4	6·8	139·6	21·6
Total	961·2		801·7		826·6	

H, hemicellulose; C, cellulose.

Statistical analysis

Analysis of variance was carried out and the statistical significance of differences between treatments was evaluated using Student's *t*-test with the appropriate standard errors of difference between treatment means. In Tables 4 and 5, due to the unequal variance associated with digestibility coefficients, Satterthwaite's approximation (Satterthwaite, 1946) was employed to generate standard errors of difference between treatment means with similarly low variance (control and Medes) and between means with similarly high variance (Brunette and Minica).

RESULTS

Carbohydrate composition of the hulls

Hulls from the three bean varieties were found to be mainly composed of non-starch polysaccharide material, the carbohydrate composition of which is shown in Table 2. A preliminary step for the extraction of starch was not carried out since previous examination of these hulls indicated that no starch was present. The high content of non-starch glucose which required 12 M-sulphuric acid for solubilization indicated that cellulose was the major polysaccharide. The presence of rhamnose and galactose together with substantial amounts of uronic acids suggested the occurrence of pectins, while the moderate amounts of xylose indicated that either acidic xylans or xyloglucans were present. There were no significant differences in polysaccharide composition between the two tannin-rich hulls (varieties Brunette and Minica). The concentrations of all sugar residues were less in these hulls than in the tannin-free hulls (variety Medes) due perhaps to their dilution by tannins. A greater decrease in polysaccharides containing xylose, however, was noted.

Tannin content of hulls and their degree of polymerization

The tannin content of hulls was expressed as catechin equivalents, and is shown in Table 3. Hulls from the Medes beans were devoid of tannins. Hulls from the Minica beans possessed more vanillin-reacting material than those from the Brunette beans and more vanillin-reacting material was extracted with acidic methanol than with methanol alone. The vanillin:anthocyanidin ratio was the same for all four extracts. Hence, the degree of polymerization of hull tannins from Brunette and Minica and those extracted in methanol

Table 3. *Tannin content of three varieties of faba bean* (Vicia faba *L.*) *hulls and relative degree of polymerization*

(Mean values and standard deviations)

	Catechin equivalents (%)				Degree of polymerization (V/A × 100)*			
	MEOH		MEOH–HCl (10 ml/l)		MEOH		MEOH–HCl (10 ml/l)	
Variety	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Medes	0	0	0	0	—	—	—	—
Brunette	0·45[a]	0·05	1·53[c]	0·16	136	8	131	17
Minica	1·23[b]	0·20	2·10[d]	0·24	134	13	141	10
df, SED	12, 0·31				12, 9			
Statistical significance of difference: *P*	< 0·001				NS			

MeOH, methanol; NS, not significant; SED, standard error of difference.

[a,b,c,d] Values with unlike superscript letters were significantly different: $P < 0{\cdot}001$.

* V/A, Absorbance of 100 μg hull extract at 510 nm in vanillin reaction divided by absorbance of an equal amount of hull extract at 520 nm in the anthocyanidin reaction. For details, see p. 202.

and acidic methanol was considered to be the same. It was concluded, therefore, that the hulls from the variety Minica possessed more methanol-extractable tannins than Brunette. By difference it can be seen that both varieties possessed the same amount of tannins requiring acidic methanol *per se* for extraction.

Apparent digestion of amino acids

The apparent digestion of amino acids from excreta is shown in Table 4. Chicks fed on the control diet digested amino acids best, while those fed on the diet containing the tannin-free hulls from the Medes beans digested slightly less amino acids. Chicks fed on the diets with tannin-rich hulls digested considerably less amino acids, the hulls from the variety containing the most tannins (Minica) depressing amino-acid digestion the most. By far the poorest digestibilities were obtained from the sulphur-amino acids, cystine and methionine.

Apparent digestion of starch and lipid

The apparent digestion of starch and lipid from the excreta is shown in Table 5. Chicks fed on the control diet digested starch and lipid best. Chicks fed on the tannin-free hulls from the Medes beans digested slightly, but significantly less starch ($P < 0{\cdot}001$) and lipid ($P < 0{\cdot}01$). Chicks fed on the tannin-rich hulls from the Brunette and Minica beans digested considerably less starch and lipid, the Minica hulls depressing starch and lipid digestion the most.

Digesta enzyme activities

The activities of digesta trypsin, α-amylase and lipase from chicks fed on the four experimental diets are shown in Table 6. Hull polysaccharides from the tannin-free variety Medes had no effect on the activity of α-amylase, while they slightly but not significantly lowered trypsin activity and slightly and significantly ($P < 0{\cdot}05$) lowered lipase activity. By far the greatest reduction of all three enzyme activities was observed by feeding the hulls from the tannin-containing varieties, Brunette and Minica, both exerting their inhibitory effects equally.

Table 4. *Influence of three varieties of faba bean* (Vicia faba *L.*), *hull polysaccharides and tannins on the digestibility of amino acids*
(Mean values and standard deviations)

Amino acid...	Digestion (%)									
	Lys		Met		Cys		Thr		Isoleu	
Treatment*	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control† (0 g/kg)	77·3	5·0	77·9	6·4	61·7	8·9	72·2	5·7	79·6	5·1
Medes (400 g/kg)	72·4	5·0	73·6	4·4	53·3	4·8	64·1	5·3	77·9	4·4
df	8		8		8		8		8	
SED	3·2		3·5		4·5		2·4		3·0	
Statistical significance of difference: *P*	NS		NS		NS		< 0·01		NS	
Brunette (400 g/kg)	33·9	6·0	6·8	18·2	−22·0	25·8	9·0	14·2	20·0	7·3
Minica (400 g/kg)	34·9	5·7	−13·7	25·8	−42·0	27·4	−6·3	16·2	12·6	6·8
df	8		8		8		8		8	
SED	3·7		14·1		16·8		4·7		4·5	
Statistical significance of difference: *P*	NS		NS		NS		NS		NS	
df	15		8		9		11		13	
SED‡	3·4		10·3		12·3		3·8		3·8	
Statistical significance of difference: *P*	< 0·001		< 0·001		< 0·001		< 0·001		< 0·001	

Amino acids...	Digestion (%)									
	Leu		Phe		Tyr		Val		Arg	
Treatment*	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control† (0 g/kg)	85·5	3·7	83·8	4·2	81·5	4·4	76·8	5·8	87·4	3·7
Medes (400 g/kg)	81·8	2·5	80·5	2·7	73·4	3·0	72·8	5·2	87·3	4·2
df	8		8		8		8		8	
SED	2·0		2·2		2·4		3·7		2·6	
Statistical significance of difference: *P*	NS		NS		< 0·01		NS		NS	
Brunette (400 g/kg)	28·9	6·6	33·1	5·5	24·6	9·0	11·1	8·7	45·9	12·1
Minica (400 g/kg)	19·4	3·3	24·7	4·3	18·0	5·5	0·8	8·2	29·2	4·2
df	8		8		8		8		7	
SED	3·3		3·1		4·7		5·4		6·4	
Statistical significance of difference: *P*	< 0·05		< 0·05		NS		NS		< 0·05	
df	13		14		11		13		9	
SED‡	2·7		2·7		3·8		4·5		5·2	
Statistical significance of difference: *P*	< 0·001		< 0·001		< 0·001		< 0·001		< 0·001	

NS, not significant; SED, standard error of difference.
* For details of dietary treatments, see p. 201; Medes, Brunette and Minica hulls were added at 400 g/kg control diet.
† For details of composition, see Table 1.
‡ SED between Control or Medes and Brunette or Minica calculated using Satterthwaite's approximation.
For details of statistical procedure, see Materials and Methods, p. 204.

Table 5. *Influence of three varieties of faba bean* (Vicia faba *L.*) *hull polysaccharides and tannins on the digestibility of starch and lipid*

(Mean values and standard deviations)

Nutrient...	Digestion (%)			
	Starch		Lipid	
Treatment*	Mean	SD	Mean	SD
Control† (0 g/kg)	97·7	1·1	87·4	5·2
Medes (400 g/kg)	91·2	3·3	80·9	3·6
df, SED	19, 1·1		19, 2·0	
Statistical significance of difference: *P*	< 0·001		< 0·01	
Brunette (400 g/kg)	74·8	11·1	76·4	7·4
Minica (400 g/kg)	53·8	9·8	65·5	19·4
df, SED	22, 43		22, 6·0	
Statistical significance of difference: *P*	< 0·001		NS	
df, SED‡	24, 3·1		26, 4·5	
Statistical difference of difference: *P*	< 0·001		< 0·001	

NS, not significant; SED, standard error of difference.

* For details of dietary treatments, see p. 201; Medes, Brunette and Minica hulls were added at 400 g/kg control diet.

† For details of composition, see Table 1.

‡ SED between Control or Medes and Brunette or Minica calculated using Satterthwaite's approximation. For details of statistical procedure, see Materials and Methods, p. 204.

Table 6. *Influence of three varieties of faba bean* (Vicia faba *L.*) *hull polysaccharides and tannins on enzyme activities in the digesta*

(Mean values and standard deviations)

Enzyme...	Digesta enzyme activities					
	Trypsin (*EC* 3.4.21.4) (*μ*g *N*-aniline/ g digesta)		α-Amylase (*EC* 3.2.1.1) (mg maltose/ g digesta)		Lipase (*EC* 3.1.1.3) (Sigma-Tietz units/g digesta)	
Treatment*	Mean	SD	Mean	SD	Mean	SD
Control† (0 g/kg)	1708[a]	590	66·8[a]	39·4	73·8[a]	19·0
Medes (400 g/kg)	1412[a]	337	75·4[a]	36·3	61·1[b]	12·4
Brunette (400 g/kg)	464[c]	400	25·3[c]	17·3	37·9[c]	10·6
Minica (400 g/kg)	313[c]	116	27·3[c]	17·3	36·8[c]	9·7
df, SED	40, 159		40, 12·3		41, 5·4	
Statistical significance of difference: *P*	< 0·001		< 0·001		< 0·001	

SED, standard error of difference.

[a,b,c,d] Values in vertical columns with unlike superscript letters were significantly different: $P < 0{\cdot}05$.

* For details of dietary treatments, see p. 201; Medes, Brunette and Minica hulls were added at 400 g/kg control diet.

† For details of composition, see Table 1.

The effect of PVP (50 g/l physiological saline) on the extracted activities of digesta enzymes is shown in Table 7. The presence of PVP had no effect on digesta enzyme activities from chicks fed on either the control diet or the diet containing the tannin-free hulls (Medes). Its presence, however, increased the activities of all three digesta enzymes from chicks fed on the tannin-rich hulls (Brunette and Minica). Complete restoration of activities to equal those from the chicks fed on the control diet or diet containing Medes hulls, however, did not occur. This may have been partly due to optimum conditions not having been achieved since further studies (results not shown) using a more concentrated solution of PVP (100 g/l physiological saline) and a longer extraction time (3 h) resulted in a further increase of enzyme activities.

Pancreatic and jejunum mucosal enzyme activities

None of the dietary treatment was found to affect pancreas weights which were 1·47 (SD 0·16), 1·44 (SD 0·28), 1·37 (SD 0·25) and 1·37 (SD 0·34) g for chicks fed on the control diet or control diet substituted with hulls from Medes, Brunette or Minica beans respectively. The activities of α-amylase and lipase in the pancreas and sucrase in jejunum mucosa are shown in Table 8. Hull polysaccharides from the tannin-free Medes variety had no effect on α-amylase nor on lipase activity in the pancreas. Tannin-rich hulls, however, significantly ($P < 0{\cdot}05$) lowered α-amylase activity, the hulls from the Minica bean depressing activity the most. Neither lipase activity in the pancreas nor sucrase activity in the mucosa of the jejunum was affected by any of the dietary treatments.

Caecal weights

The weights of caeca plus contents from chicks fed on the control diet, or control diet substituted with hulls from Medes, Brunette or Minica beans were 3·9 (SD 0·8), 4·3 (SD 1·0), 5·2 (SD 2·2), 5·5 (SD 1·6) g respectively. These results suggest that chicks fed on the tannin-rich hulls had more undigested material present at the terminal ileum and that some of this undigested material entered the caeca.

Reducing sugars in diets, digesta and excreta.

The procedure for the quantification of starch in the diet and excreta and for the determination of α-amylase activity in the digesta provided also a measurement of the reducing sugar content in the diet, excreta and digesta before the enzymic breakdown of starch in vitro. In Table 9 it can be seen that all four diets contained similarly small amounts of reducing sugars. Less were present in the digesta and excreta of chicks fed on hulls from the Medes variety of beans than those fed on the control diet and chicks fed on the tannin-rich hulls showed the least amounts of reducing sugars in digesta and excreta.

DISCUSSION

An indication of the nature and composition of bean hull polysaccharides may be deduced from the type and quantity of sugar residues that are liberated on acid-hydrolysis. Previous analysis of these hulls showed them to be uncontaminated with starch. The hulls were almost entirely polysaccharides in composition, mainly consisting of cellulose with smaller amounts of pectins and a xylan polysaccharide which may be either an acidic xylan or a xyloglucan. Aspinal *et al.* (1966) identified an acidic xylan composed of 1,4-linked xylose units branched with 1,2-linked glucuronic acid units in soya-bean hulls, while more recently Aspinal (1976) and Aspinal & Krishnamurthy (1976) isolated the polysaccharides of

Table 7. *The effect of addition of polyvinylpyrrolidone to digesta on enzyme activities*

(Mean values and standard deviations)

Enzyme...	Increase in enzyme activities (%)					
	Trypsin (*EC* 3.4.21.4) (μg *N*-aniline/ g digesta)		α-Amylase (*EC* 3.2.1.1) (mg maltose/ g digesta)		Lipase (*EC* 3.1.1.3) (Sigma-Tietz units/g digesta)	
Treatment*	Mean	SD	Mean	SD	Mean	SD
Control† (0 g/kg)	11	7	—	—	0	28
Medes (400 g/kg)	1	11	−4	4	−12	16
Brunette (400 g/kg)	80	31	111	30	39	14
Minica (400 g/kg)	127	70	77	46	37	49
df, SED	19, 23		4, 26		20, 16	
Statistical significance of difference: *P*	< 0·001		< 0·01		< 0·05	

SED, standard error of difference.

* For details of dietary treatments, see p. 201; Medes, Brunette and Minica hulls were added at 400 g/kg control diet.

† For details of composition, see Table 1.

Table 8. *Influence of three varieties of faba bean* (Vicia faba *L.*) *hull polysaccharides and tannins on enzyme activities in the pancreas and gut mucosa*

(Mean values and standard deviations)

Enzyme...	Enzyme activities in pancreas and gut mucosa					
	α-Amylase (*EC* 3.2.1.1) (mg maltose/ g pancreas)		Lipase (EC 3.1.1.3) (Sigma-Tietz units/g pancreas)		Sucrase (*EC* 3.2.1.48) (μg glucose/ g mucosa)	
Treatment*	Mean	SD	Mean	SD	Mean	SD
Control† (0 g/kg)	3298[ab]	810	1353	465	160	32
Medes (400 g/kg)	3442[a]	888	1259	362	146	30
Brunette (400 g/kg)	2675[b]	1008	1091	476	146	24
Minica (400 g/kg)	1503[c]	761	1172	461	155	131
df, SED	41, 357		41, 181		41, 12	
Statistical significance of difference: *P*	< 0·001		NS		NS	

NS, not significant; SED, standard error of difference.

[a,b,c] Values with unlike superscript letters were significantly different: $P < 0{\cdot}05$.

* For details of dietary treatments, see p. 201; Medes, Brunette and Minica hulls were added at 400 g/kg control diet.

† For details of composition, see Table 1.

rapeseed hulls, identifying cellulose and pectins but no acidic xylan and instead a xyloglucan which is more typical of primary cell-wall material. Because polysaccharides were present in such high concentrations in bean hulls it is more than likely that the antinutritional effect exhibited by the hulls from the white-flowering tannin-free variety, Medes, was due entirely to the nature of these polysaccharides. These findings are in agreement with Bailey *et al.* (1974) who fed the hulls of sweet lupin seeds to rats at 200, 400

Table 9. *Influence of three varieties of faba bean* (Vicia faba *L.*) *hull polysaccharides and tannins on the presence of reducing sugars in diets, digesta and excreta*

(Mean values and standard deviations)

	Reducing sugars (g/kg)					
	Diet (DM)		Digesta (wet wt)		Excreta (DM)	
Treatment*	Mean	SD	Mean	SD	Mean	SD
Control† (0 g/kg)	9·8	1·8	6·0[a]	1·9	13·0[a]	2·2
Medes (400 g/kg)	8·0	0·1	3·0[b]	1·5	7·1[b]	1·7
Brunette (400 g/kg)	7·5	2·5	1·7[c]	1·5	5·2[c]	1·8
Minica (400 g/kg)	7·3	0·6	0·7[c]	0·3	5·4[c]	1·4
df, SED	8, 1·3		41, 0·5		41, 7·0	
Statistical significance of difference: *P*	NS		< 0·001		< 0·001	

DM, dry matter; NS, not significant; SED, standard error of difference.

[a,b,c] Values with unlike superscript letters were significantly different: $P < 0{\cdot}05$.

* For details of dietary treatments, see p. 201; Medes, Brunette and Minica hulls were added at 400 g/kg control diet.

† For details of composition, see Table 1.

and 600 g/kg diet and found that food and protein digestion were depressed in direct proportion to the amount of hulls incorporated in the diet. The authors suggested that physical entrapment of protein in the excessive bulk of material in the gut prevented enzyme–substrate contact. The ability of polysaccharides, however, to act as adsorbant resins could well be augmenting the sheer bulk effect. The finding in the present study that digesta trypsin and lipase activities were depressed suggests that the hulls were acting not merely as inert diluents but playing a more specific role. Schneeman (1978) suggested that the availability of gut enzymes such as lipase, trypsin and chymotrypsin (*EC* 3.4.21.1) could be limited by their being adsorbed onto fibres such as solka fibre, xylan, cellulose acetate, wheat bran and rice bran. Gagne & Acton (1983) while studying the effect in vitro of karayagum, xylan, pectin, lignin and holocellulose on the digestion of casein believed that enzyme–fibre complexes may be formed in the gut, decreasing the availability of the enzymes for the substrates. Arnal-Peyrot & Adrian (1974) concluded that the gums and mucilages from baobal leaf (*Adansinia digitata*) fed to rats reduced nutrient digestibility in a double mechanism: (1) by retaining water into which water-soluble nutrients were trapped thus making them unavailable for absorption by the intestine and hence excreted, and (2) by inhibiting digestive enzymes. More recently Isaksson *et al.* (1982) attributed the decreased activities of trypsin, lipase, α-amylase and phospholipase (*EC* 3.1.1.4) in human duodenal juice when incubated with different fibres in vitro to viscosity and pH of the intestinal contents aiding enzyme adsorption onto the fibre matrix. They pointed out that viscosity in particular affected lipase activity the most. In the present study, trypsin and lipase digesta activities were depressed by hull polysaccharides, but no depression was observed with digesta α-amylase. However, the finding that there was a lower concentration of reducing sugars in both digesta and excreta from these chicks compared with those fed on the control diet (Table 9) suggests that when α-amylase was in the environment of the intestine it was less active. Reducing sugars detected in the digesta and excreta would most likely have arisen from the enzymic breakdown of starch in the intestine with the production of malto-dextrans, maltose and glucose.

When chicks were fed on the tannin-rich hulls, Brunette and Minica, the digestion of

amino acids, starch and lipid was severely depressed, amino acid digestion being affected the most, starch to a lesser extent and lipid the least. As expected depression of digesta enzyme activities paralleled nutrient indigestion, with trypsin activity being depressed the most, α-amylase to a lesser extent and lipase the least. The observation that the Minica hulls depressed the digestion of all three nutrients the most is in keeping with it possessing the highest concentration of tannins. What remains puzzling is that the digesta enzyme activities from chicks fed on these hulls were not correspondingly lower to explain this decreased nutrient digestion. However, the fact that tannins have been reported to interact not only with enzymes but also with substrates may be of relevance here. Davis & Hoseney (1979) suggested the presence of at least two fractions in condensed tannins from sorghum: an α-amylase-inhibiting fraction which was not adsorbed onto starch and an α-amylase-inhibiting fraction which was adsorbed onto starch. Hulls from the Minica variety may have been exerting their additional antinutritional potency on substrates rather than enzymes.

Results of chick digesta enzyme activities reported here are in part agreement with other reports of work with rats (Griffiths & Moseley, 1980; Horigome *et al.* 1988) in that condensed tannins were shown to inhibit the activities of trypsin and α-amylase, but they differ in their effect on lipase. In the present study condensed tannins decreased the activity of digesta lipase and consequently explained the lowered lipid digestion found. The previously mentioned authors witnessed an increase in the activity of lipase in rat digesta, in spite of the fact that they demonstrated tannin inhibition in vitro of commercial lipase. Sell & Rogler (1983) demonstrated a species difference between chicks and rats in dealing with tannins. They showed that there was a significant elevation in the enzyme UDP-glucuronyl transferase (*EC* 2.4.1.17) that detoxifies phenolic compounds in the liver of chicks but not of rats after feeding sorghum tannins, indicating that the microbial degradation of tannin oligomers or subsequent absorption of breakdown products may be different in different species. The findings of Marquardt *et al.* (1977), however, detract from a species difference being the only explanation for the dual behaviour of digesta lipase, for these authors found that both water extracts and a purified extract of condensed tannins from faba bean hulls when fed to young chicks increased the digestion of dietary lipid even though dry matter, crude fibre and amino acid digestion was decreased. Enzyme activities were not measured by them but an increase in the activity of digesta lipase would seem to be the most likely explanation for an increase in lipid digestion. Griffiths & Moseley (1980) suggested that increased digesta lipase activity in rats arose because tannins caused an increased production of all digestive enzymes by the pancreas while the selective inhibition of trypsin and α-amylase occurred in the gut. This theory sounds plausible in the light of the fact that results here show lipase to be the enzyme that tannins have the least affinity for and the overproduction of pancreatic enzyme has been shown to occur as a result of pancreatic enlargement, albeit due to antinutritional substances such as pectins and proteolytic trypsin inhibitors (Liener & Kakade, 1980; Grant *et al.* 1987). Nevertheless, neither Griffiths & Moseley (1980) nor Marquardt *et al.* (1977) found evidence of pancreatic enlargement when rats or chicks were fed on tannin-rich hulls over a period of 15 or 16 d and unfortunately these authors did not analyse pancreatic tissue for enzyme activities. In the present study no increase in pancreatic weight nor in pancreatic α-amylase and lipase activities in chicks fed on tannin-rich hulls over a period of 4 d was observed. On the contrary, tannins caused a significantly large decrease in pancreatic α-amylase activity, the reason for which is not yet clear.

The crucial factor determining whether lipase activity decreases or increases may be the quantity of tannin ingested. In the present study an excessive amount of hulls were ingested by chicks. At lower tannin concentrations and because affinity for lipase appears to be less

than for other digestive enzymes, tannins may indirectly aid its action by removing substances which tend to decrease lipase activity. The removal of trypsin itself thereby sparing lipase from enzymic digestion is a simple though unlikely explanation. Alternatively the astringent nature of tannins, i.e. their ability to reduce lubrication, could lead to dryness in the digestive tract and so counteract any adverse affects of viscosity on lipase activity. Whether tannins in low concentration actually stimulate lipase activity *per se* remains to be investigated. A stimulatory effect in vitro on trypsin hydrolysis by tannins has been reported by Mole & Waterman (1985).

Gut mucosal sucrase activity was not affected by tannins, at least not in the short time that chicks were exposed to them in the present study. Villanueva *et al.* (1987) showed that both maltase (*EC* 3.2.1.20) and sucrase activities of intestinal mucosa of rats fed on faba beans were depressed and the authors attributed this to bean polyphenolics.

The extremely low digestibilities of amino acids together with the negative digestibility coefficients obtained for the sulphur-containing amino acids may be due to tannins provoking an increased excretion of inactivated enzymes and glycoproteins of the gut wall mucosa. Sell *et al.* (1985) showed that high-tannin in contrast to low-tannin sorghums caused an increased total mucin excretion in rats.

In the present study it was observed that chicks fed on the diets containing tannin-rich hulls consumed much less, for such was the delay in crop emptying that it was not physically possible to insert as much food into the crops of these chicks. The average amount of food consumed over the 4 d was as follows: 116 (SD 7), 101 (SD 17), 85 (SD 18) and 83 (SD 16) g for chicks fed on the control diet or control diet substituted with hulls from the variety Medes, Brunette or Minica. Sheer bulk of indigestible material may have been one factor in delaying crop emptying since chicks fed on the tannin-free hulls consumed slightly less than the control-fed chicks. However, tannin-rich hulls affected crop emptying to a much greater extent. Reduced lubrication in the crop may have been responsible for it was observed that the digesta removed from chicks fed on the tannin-rich hulls was noticeably drier. It remains to be answered from further investigations whether food passage rate was affected throughout the entire tract.

The tannins of field bean hulls appear to be similar to those of group III sorghums in that they are partly extractable in methanol alone, while fuller extraction can be achieved with the use of acidic methanol. It has been suggested that tannins requiring acidic methanol for extraction may be covalently bound to cell wall polysaccharides in the plant (Price *et al.* 1978). Contradictory information has appeared in the literature concerning the degree of polymerization of methanol and acidic methanol extractable tannins from sorghum. Butler (1982) reported that tannins requiring acidic methanol for extraction were more highly polymerized, while Asquith *et al.* (1983) showed no difference in the degree of polymerization between the two populations. The tannins which are most highly polymerized while still remaining soluble appear to be the most antinutritional (Martin-Tanguy *et al.* 1977; Horigome *et al.* 1988). Since the tannins present in the two varieties of bean used in the present experiment were polymerized to the same degree, the additional potency of the hulls from the Minica variety was considered to be due to an increased amount of tannins present.

Future experiments will investigate the effect of tannin-rich and tannin-free hulls at much lower dietary concentrations on digestive enzyme activities for it is fully acknowledged that intubating such high concentrations of hulls may have created artificial conditions in the gut. In particular the effect of semi-purified tannin extracts as well as varying concentrations of tannin-rich hulls on digestive lipase activity will be studied.

Thanks go to Stephen Harper for the organization of the chicks and their tube feeding, to Kim Henderson and Anne Knox for collection of digesta and excreta, to Lindsey McNeil and John Downie for analytical services and to Martin Longstaff for his help with the laborious task of dehulling beans.

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Printed in Great Britain