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The effect of concentration of tannin-rich bean hulls (*Vicia faba* L.) on activities of lipase (*EC* 3.1.1.3) and α-amylase (*EC* 3.2.1.1) in digesta and pancreas and on the digestion of lipid and starch by young chicks

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The effect of different concentrations of tannin-rich field-bean (*Vicia faba* L.) hulls at 0, 20, 50, 150 and 300 g/kg dietary inclusion on the activities of lipase (*EC* 3.1.1.3) and α-amylase (*EC* 3.2.1.1) in digesta and pancreas and on the digestion of lipid and starch was studied in 2–3-week-old male broiler chicks. Low dietary concentrations of tannins (20 and 50 g hulls/kg) enhanced the activity of lipase in digesta from both the jejunum and ileum, the 20 g hulls/kg effecting the greatest enhancement, but no stimulatory effect on the activity of digesta α-amylase was observed. High dietary concentrations of tannins (150 and 300 g hulls/kg) inhibited both lipase and α-amylase activities in digesta from both the jejunum and ileum, the 30 g hulls/kg causing the most inhibition. Tannins did not increase the activities of lipase or α-amylase in pancreatic homogenates, but at high concentrations (150 and 300 g hulls/kg) they lowered slightly the pancreatic activity of α-amylase. Nutrient digestion was less influenced by the concentration of tannins than digesta enzyme activities.

Tannins: Digestive enzymes: Nutrient digestion: Chick

There appears to be general agreement, albeit from the few studies undertaken in vivo, that condensed tannins decrease the digestibility of dietary protein (Rostagno *et al.* 1973; Lindgren, 1975; Marquardt *et al.* 1977; Martin-Tanguy *et al.* 1977; Longstaff & McNab, 1991) and starch (Hibberd *et al.* 1985; Longstaff & McNab, 1991). The most likely explanation for this effect has been claimed to be a reduction in the activities of trypsin (*EC* 3.4.21.4) and α-amylase (*EC* 3.2.1.1) in the digestive tract (Griffiths & Moseley, 1980; Horigome *et al.* 1988; Longstaff & McNab, 1991). Contradictory evidence, however, has appeared concerning the effect of condensed tannins on lipid digestion and digesta lipase activity. Marquardt *et al.* (1977) reported that fat retention increased when young chicks were fed on a diet containing extracts of semi-purified condensed tannins from faba bean (*Vicia faba* L.) hulls, and both Griffiths & Moseley (1980) and Horigome *et al.* (1988) reported that the activity of lipase (*EC* 3.1.1.3) increased in digesta from rats fed on a diet containing tannin-rich field-bean hulls and condensed tannin extracts from the leaves in fodder plants respectively. Longstaff & McNab (1991), however, found decreased lipid digestion as well as decreased digesta lipase activity in young chicks fed on diets containing high concentrations of tannin-rich field bean hulls.

The present study was undertaken to investigate the conflicting reports concerning the ambivalence of condensed tannins towards lipase activity in vivo and on lipid digestion by asking the question whether tannins at low dietary concentrations have the ability to

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enhance lipase activity and at higher concentrations to inhibit it. Consequently, diets containing 0, 20, 50, 150 and 300 g hulls from the tannin-rich field bean (var. Minica)/kg were fed for 4 d to male broiler chicks of 2–3 weeks of age. Activities of lipase and α-amylase in both the digesta and pancreas were measured and lipid and starch digestibility coefficients calculated from measurement of these nutrients in diets and excreta.

MATERIALS AND METHODS

Diets

The composition of the control diet is shown in Table 1. It was compounded using a broiler diet composed mainly of maize and soya-bean meal, which had already been mixed in the mill, to which was added soya-bean oil and titanium dioxide. In total, 5·210 kg control diet (five times the amount shown in Table 1) was made up to allow enough feedingstuff for one control diet and four other diets substituted by varying amounts of hulls. Soya-bean oil was used to increase the dietary lipid content in order to try and highlight any effects on lipid digestion. Titanium dioxide was included as an inert marker to allow nutrient digestibility coefficients to be calculated. Hulls were mechanically removed from the coloured flowered variety of field bean, Minica, with a modified laboratory pearl-barley de-huller. Hulls were blown free from cotyledons using an air gun and any remaining cotyledons removed by hand. Pure hulls were ground through a 1 mm sieve. Batches of 1 kg of each of the five experimental diets were made up by addition of 0, 20, 50, 150 and 300 g hulls to 1000, 980, 950, 850 and 700 g control diet respectively. The hull tannins which were extractable in aqueous acetone were measured using the vanillin–glacial acetic acid method (Butler *et al.* 1982). Hulls were found to contain 21·4 g tannins/kg when catechin was used as the standard, and 96·5 g tannins/kg when a purified tannin extract from bean hulls was used as the standard. An aqueous acetone extract from 300 g hulls provided 30 g freeze-dried crude tannins which was subsequently shown to be composed largely of high-molecular-weight condensed tannins (M. A. Longstaff, unpublished results). The crude tannin was further purified by means of Sephadex LH20 column chromatography and used as a standard.

Birds

(a) *Allocation of diets, feeding protocol and excreta collection.* Fifty 2-week-old male broiler chicks were selected as the middle weight range from a batch of eighty 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 with 23 h light/d. Each of the five diets was tube-fed to ten chicks because a preliminary experiment had established that chicks would not voluntarily consume diets containing the higher concentrations of tannin-rich hulls. On day 1 following 16 h starvation, chicks were tube-fed 10 g of the appropriate diet at approximately 08.30 hours and again at approximately 15.30 hours. The same feeding protocol was carried out on days 2 and 3. During the afternoon of day 2, clean trays were placed under each cage and the excreta voided over the subsequent 48 h was collected, frozen at −20° and freeze-dried and kept for nutrient digestibility measurements. Throughout the experiments chicks were allowed free access to water. On day 4, chicks were given 10 g of the appropriate diet at approximately 08.30 hours.

(b) *Collection of digesta and pancreas.* Chicks were killed by intravenous injection of sodium pentobarbitone starting at 14.00 hours on day 4. The pancreas was removed from each bird and placed in vials which were immediately plunged into a Dewar flask containing liquid nitrogen. Digestive tracts were removed and the contents of the jejunum

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

Maize	743·0
Soya-bean meal	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, thiamine 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.

and ileum (digesta) were gently squeezed out into separate vials, mixed thoroughly and kept frozen in liquid N_2 during the period of collection. Thereafter, pancreas and digesta were transferred to a freezer at $-20°$ until required for enzyme activity measurements.

Chemical analysis

Starch content of diets and excreta. Starch was quantified from the amount of glucose released after its gelatinization and enzymic hydrolysis by amyloglucosidase (*EC* 3.2.1.3; Sigma Chemical Co.). The starch in the diets (50 mg) and excreta (100 mg) was gelatinized in 9 ml 0·2 M-sodium acetate buffer (pH 4·5) for 4 h at 100°. After cooling to below 50°, solutions were incubated after the addition of a further 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. Glucose was detected after its reaction with *p*-hydroxybenzoic acid hydrazide (PAHBAH) according to the method of Lever (1972) using a Technicon AutoAnalyser.

Lipid content of diets and excreta. Diets and excreta samples (2·5 g) were boiled for 1 h in 100 ml 3 M-hydrochloric acid and filtered. The precipitate and filter paper were dried and extracted with 100 ml light petroleum (b.p. 40–60°) according to the EEC procedure B (European Communities, 1971).

Titanium dioxide content of diets and excreta. Titanium dioxide was measured in diets and excreta according to the method of Peddie *et al.* (1982) based on that of Leone (1973).

Enzyme analysis

Sample preparation. Samples (400 mg) of frozen digesta were weighed into test-tubes which were kept on ice, and ice-cold physiological saline (9 g sodium chloride/l; 4 or 8 ml) was added with thorough mixing. Tubes were left at least 1 h at 5°. After centrifugation at 1500 ***g*** for 10 min at 5°, portions of supernatant fractions were removed for the appropriate enzyme analysis.

Samples (200 mg) of frozen pancreas were weighed into vials kept on ice and, thereafter, quantitatively transferred to a Potter Elvehjelm glass homogenizer containing a small quantity of ice cold physiological saline and homogenized at maximum speed for 1 min. Homogenates were quantitatively rinsed into 20 ml volumetric flasks, kept at 5° for at least 1 h, and appropriate portions were taken for enzyme analysis.

Determination of lipase activity. Lipase activity was determined using the diagnostic kit no. 800 (Sigma Chemical Co.). In this titrimetric method the amount of standard sodium

hydroxide required to neutralize fatty acids released from olive oil triacylglycerols is directly 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 at 37°. The reaction was stopped by the addition of alcohol (950 ml/l) and titration performed with 0·05 M-sodium hydroxide solution.

Determination of α-amylase activity. Gelatinized maize starch was used as the substrate to assess α-amylase activity, and the amounts of glucose released after incubation with portions of digesta and pancreas were presumed to be directly proportional to the activity of α-amylase. Digesta supernatant fraction (1 ml) and pancreas homogenates (0·2 ml) were incubated with 2 ml 0·02 M-phosphate buffer (pH 5·9) with or without gelatinized maize starch (10 g/l buffer) for 10 min at 37°. The reaction was stopped by placing tubes in a boiling water-bath for 7 min. The glucose released was measured colorimetrically by the method of Lever (1972) as described previously for starch determination.

Statistical analysis

Analyses of variance between and within birds were performed on the lipase and α-amylase activities in the digesta, assuming constant variance. Missing values were estimated from the data by minimizing the residual variance within birds. Standard errors presented have been adjusted for these missing values and only average standard errors are given.

Analyses of variance between birds were performed on the lipase and α-amylase activities in the pancreas, again assuming constant variance.

For digestibilities of lipid and starch near 100 it is to be expected that the distributions of the observations will be very skewed with variance increasing as the mean digestibility decreases. Weighted analyses of variance between birds were performed using the inverse of the sample variances as weights. Satterthwaite's approximation to Student's t distributions (Snedecor & Cochran, 1976), involving reduced degrees of freedom depending on the degree of heterogeneity of variances, was used to determine the statistical significance of effects. Again, average standard errors were presented with a range of approximate degrees of freedom.

A similar approach was used for metabolizable energy (ME) as this has an upper limit equal to the gross energy of the feed rather than 100.

RESULTS

The influence of the concentration of tannin-rich hulls on lipase and α-amylase activities in digesta removed from jejunum and ileum is shown in Table 2. Ileal activities paralleled those in the jejunum, albeit at a much lower level of activity in the case of lipase. There was a highly significant linear response to hulls by both lipase and α-amylase ($P < 0·001$). The quadratic component of variation with hull concentration was insensitive to the apparent initial rapid increase in lipase activity when birds were fed on the lowest level of hulls (20 g/kg). However, the difference between means for lipase activity of birds fed at 0 and 20 g hulls/kg for values averaged over the jejunum and ileum regions was 16·0 (SE 7·13) with 42 df and was significant at $P < 0·05$. No rapid increase at low dietary concentration of hulls was observed for α-amylase.

The influence of the concentration of tannin-rich hulls on lipase and α-amylase activities in pancreatic homogenates is shown in Table 3. No significant differences were found in lipase activities from chicks fed on diets containing increasing amounts of hulls. The activity of α-amylase, however, was slightly lower in pancreatic homogenates from chicks fed on the diets containing the two higher levels of hulls.

The ME values of the five experimental diets and the digestibilities of their lipid and

Table 2. *Influence of concentration of tannin-rich hulls on activities of lipase* (EC *3.1.1.3*) *and α-amylase* (EC *3.2.1.1*) *in digesta in 2–3-week-old male broiler chicks**

	Digesta enzyme activities			
	Lipase (Sigma-Tietz units†/g digesta)		α-Amylase (mg maltose/g digesta)	
Hulls (g/kg)	Jejunum	Ileum	Jejunum	Ileum
0	62·2	36·8	264·7	276·4
20	81·6	49·4	298·0	279·7
50	72·2	43·3	256·3	285·7
150	53·6	36·0	225·8	208·1
300	47·6	26·7	50·6	84·9
Average SED (horizontal means)		8·4		28·3
df		34		30
Average SED (vertical means)		10·1		54·1
df		39		30
Statistical significance of effects (*P*)				
Regions	< 0·001		NS	
Diets	< 0·001		< 0·001	
Linear	< 0·001		< 0·001	
Quadratic	NS		NS	
Diet *v.* region	NS		NS	

NS, not significant; SED, standard error of difference.
* For details of diets and procedures, see pp. 140–142.
† Lipase diagnostic kit no. 800, Sigma Chemical Co Ltd.

Table 3. *Influence of concentration of tannin-rich hulls on activities of lipase* (EC *3.1.1.3*) *and α-amylase* (EC *3.2.1.1*) *in the pancreas in 2–3-week-old male broiler chicks**

Hulls (g/kg)	Lipase (Sigma-Tietz units†/g pancreas)	α-Amylase (mg maltose/g pancreas)
0	1926	5374
2	1735	5304
50	1882	5688
150	1741	5071
300	1858	5010
Average SED	102	222
df	42	43
Statistical significance of effects (*P*)		
Diet	NS	< 0·05
Linear	NS	< 0·05
Quadratic	NS	NS

NS, not significant; SED, standard error of difference.
* For details of diets and procedures, see pp. 140–142.
† Lipase diagnostic kit no. 800, Sigma Chemical Co. Ltd.

Table 4. *Influence of concentration of tannin-rich hulls on the metabolizable energy (ME) of diets and on their lipid and starch digestibilities in 2–3-week-old male broiler chicks**

Hulls (g/kg)	ME (kg/g)	Lipid digestibility	Starch digestibility
0	13·68	0·876	0·993
20	13·81	0·892	0·990
50	13·15	0·887	0·991
150	12·64	0·899	0·985
300	10·31	0·877	0·956
Average SED	0·415	0·1246	0·005
Range of df	9–16	9–17	9–16
Statistical significance of effects (*P*)			
Diets	< 0·001	< 0·05	< 0·001
Linear	< 0·001	< 0·05	< 0·001
Quadratic	NS	< 0·05	< 0·05

NS, not significant; SED, standard error of difference.
* For details of diets and procedures, see pp. 140–142.

starch contents are shown in Table 4. There was a highly significant linear decrease in ME and starch digestibility with increase in dietary hull concentration ($P < 0·001$). Lipid digestion showed both a linear and quadratic component of variation at a much lower level of significance ($P < 0·05$) which tended to suggest a rise in lipid digestion at low dietary hull concentration and an inhibition of lipid digestion at higher dietary hull concentration. The significant quadratic component for starch suggests that digestibilities decreased slowly at first, then more rapidly the higher the hull concentration became.

DISCUSSION

Low dietary concentrations of tannins (20 and 50 g field-bean hulls/kg) appeared to increase the activity of lipase in chick digesta taken from both the jejunum and ileum. When Griffiths & Moseley (1980) examined enzyme activities in rat digesta removed from the entire tract they reported an increased activity when rats were fed on diets containing 100 g tannin-rich field-bean hulls/kg. Horigome *et al.* (1988), after dividing the tract into upper, middle and lower regions, reported an increase lipase activity in digesta from only the middle region after rats were fed on diets containing condensed tannin extracts from leaves of fodder plants. Increased lipase activity might also explain the increased lipid digestion found by Marquardt *et al.* (1977) when condensed tannin extracts from sorghum were fed to chicks. Higher concentrations of tannins in the present study (150 and 300 g hulls/kg) inhibited lipase activity in both jejunum and ileum, the diet containing 300 g hulls/kg causing the most inhibition.

Low dietary concentration of tannins (from 20 and 50 g field-bean hulls/kg) did not appear to affect digesta α-amylase activities either in the jejunum or ileum, but higher tannin concentrations (from 150 and 300 g field-bean hulls/kg) inhibited α-amylase activity, 300 g hulls/kg causing the most inhibition.

What is puzzling in the present study is the discrepancy between the large reduction in enzyme activities compared with the much smaller reductions in nutrient digestibilities with increasing hull concentrations. While enzyme activities may not be the limiting factor in digestion–absorption, the fact that they are required at an initial stage in the process would seem to imply a crucial role. However, in the present study enzymic breakdown of digesta may have taken place over a longer period of time, and possibly extending further down the digestive tract, such that digestion was not so severely impaired overall.

In a previous study (Longstaff & McNab, 1991) when hulls from the same variety of field beans were fed at 400 g/kg dietary inclusion, inhibitions of lipase and α-amylase activities were found similar to those seen in the present study, but considerably larger reductions in lipid and starch digestion resulted, i.e. decreases from 0·87 to 0·66 for lipid and from 0·98 to 0·54 for starch respectively. It is difficult to believe that the 10% extra hulls in the previous study was entirely responsible for such large differences in nutrient digestion, for it would have to be concluded that these extra hulls were directing their anti-nutritional activity on substrates only. At present, therefore, no convincing explanation can be put forward for the better nutrient digestion found in the present study.

Enzyme activities were not elevated in pancreatic homogenates when chicks were fed on diets containing tannin-rich hulls; on the contrary, α-amylase activity was slightly depressed in pancreatic homogenates from chicks fed on the diets containing the two higher concentrations of tannins (150 and 300 g hulls/kg); this is in agreement with previous results (Longstaff & McNab, 1991). Diets containing trypsin inhibitors have also been shown to cause a slight reduction in α-amylase activity in the pancreas of rats and chicks (Kakade *et al.* 1967; Konijin *et al.* 1970).

Increasing the hull content of the diets caused a decrease in their ME values. A large part of this decrease in ME is obviously explained by a substitution of highly-digestible material by poorly-digestible hulls which are composed mainly of cell-wall polysaccharides. Consequently, the observation that the ME value of the diet containing 20 g hulls/kg was slightly higher than that of the control diet which contained 20 g/kg more highly-digestible ingredients, tends to support the observation that lipase activity and lipid digestion were enhanced at this low dietary concentration of tannins.

Whether tannins actually activate lipase *per se*, or whether they render intestinal conditions more favourable for lipase to work, requires investigation. There have been no reports of lipase activation in vitro by tannins; on the contrary, tannins have been shown to inhibit lipase activity in vitro (Griffiths, 1979; Horigome *et al.* 1988). Other enzymes, however, have been shown to be activated by tannins. Mole & Waterman (1985) observed that tannins can both stimulate and inhibit tryptic hydrolysis in vitro, depending on the tannin:substrate ratio present. At low levels of tannin and excess substrate, tryptic hydrolysis was enhanced. The authors suggested that tannins were causing conformational changes in the substrate which allowed trypsin better access to it. The stimulatory effects of tannins from oak (*Quercus incana*) leaves on the enzyme glutamine synthetase (*EC* 6.3.1.2) in bovine rumen was reported by Makkar *et al.* (1988), and there are many instances where tannins have shown no adverse digestive effects with insect herbivores and, indeed, hydrolysable tannins may even act as phagostimulants (Bernays, 1981). Increased N retention from tannin-containing diets in sheep has been explained (Jones & Mangan, 1977) on the basis of the formation of soluble tannin–protein complexes, making protein more available for tryptic digestion.

On the other hand tannins, because of their unique astringent property causing dryness in the mouth and in the digestive tract, may act by reducing the viscosity of the digesta. There have been several reports of the adverse effects of viscous polysaccharides such as β-glucans and rye pentosans on nutrient digestion (Antoniou & Marquardt, 1981; Classen *et al.* 1985; Broz & Frigg, 1986) and in particular on their interference with lipid digestion (Graham *et al.* 1986) and lipase activity (Schneeman, 1978; Isakssen *et al.* 1982).

In conclusion, these results have shown that low dietary concentrations of tannins can enhance the activity of lipase in the digestive tract of the chicks. High dietary concentrations of tannins can reduce the activities of digesta lipase and α-amylase and decrease starch digestion, though this does not invariably result in severe nutrient indigestion. Such contrary effects of tannins on digestion may be the result of a complex array of interactions

between tannins and substrates, and between tannins and enzymes, resulting in the formation of soluble and insoluble complexes, all of which may be greatly influenced by conditions within the digestive tract. Future experiments will explore the mechanism by which lipase is enhanced at low dietary tannin concentrations and may throw some light on the unpredictable duplicity of tannins towards digestion.

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