A comparison of effects of body weight and feed intake on digestion in broiler cockerels with effects of tannins

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The effects of body size and feed intake on N digestibility, pancreas and liver weight, and digestive enzyme activities in male broiler chicks were compared with those induced by dietary tannins. Four groups (SSM, *ad lib.*, pair-fed and young) of sixteen birds each (2 weeks old) were used as experimental animals. They were fed on experimental diets for 4 weeks, except the young group which were fed from age 15 d to 24 d only. Two isonitrogenous and isoenergetic diets with (SSM) or without salseed (*Shorea robusta*) meal (CONTROL) were used. SSM diet was fed *ad lib.* to SSM group and control diet was fed *ad lib.* to *ad lib.* and young birds and to pair-fed birds at same intake level as SSM birds. Birds fed *ad lib.* utilized their diet more efficiently than the SSM and pair-fed birds. Digestibility of N, both apparent and ileal, was substantially lower with SSM diet than with the control diet. Each of the treatments induced enlargement of the pancreas (g pancreas/kg live weight) when compared with *ad lib.* birds. There was no difference between the relative liver weights of SSM and *ad lib.* birds; however, pair-fed and young birds had comparatively bigger livers. In pair-fed birds the trypsinogen activity of pancreatic tissue (U/g pancreatic tissue) was significantly depressed but there was a significant elevation in trypsinogen (U/kg live weight) activity in SSM birds; again pair-fed birds exhibited the lowest value for this variable. Compared with control birds all the other treatments resulted in significant depression of α-amylase (*EC* 3.2.1.1) activity in pancreatic tissue (U/g) and jejunal digesta (U/g), but because of pancreatic enlargement α-amylase activity per kg live weight was significantly lower only in SSM birds. The activity of trypsin (*EC* 3.4.21.4) in jejunal digesta was very low in SSM birds, but it was slightly higher in the young birds. Dipeptidase (*EC* 3.4.13.11) and disaccharidases in duodenal and jejunal mucosa were markedly depressed by the diet containing salseed meal, with the exception of maltase (*EC* 3.2.1.20) which was unaffected in jejunal mucosa. Enterokinase (*EC* 3.4.21.9) activity was not inhibited by the presence of tannins in the diet, rather it increased in the duodenal mucosa of SSM birds.

Tannins: Digestive enzymes: Pancreas: Feed intake: Live weight: Salseed meal

The antinutritional properties of tannins are well documented from numerous *in vitro* (Schaffert *et al.* 1974; Ramachandra *et al.* 1977; Chibber *et al.* 1980; Horigome *et al.* 1988; Garrido *et al.* 1989), and *in vivo* (Cousins *et al.* 1981; Horigome *et al.* 1988; Ahmed *et al.* 1991) studies, which showed reduced digestibility of protein in diets containing tannins. Many experiments have shown that tannins in the diet result in reduced weight gain and poor feed efficiencies in chicks (Armstrong *et al.* 1974; Ahmed *et al.* 1991), rats (Featherston & Rogler, 1975; Elkin *et al.* 1990; Mole *et al.* 1990) and pigs (Pathak & Ranjahan, 1973; Cousins *et al.* 1981; Mitaru *et al.* 1984).

Tannins may form stable complexes with dietary protein thereby reducing its digestibility (Oh *et al.* 1980; Hagerman & Klucher, 1986; Marquardt, 1989). They may also form tannin–enzyme complexes with digestive enzymes (Griffiths, 1979) and inhibit enzyme

activity. Condensed tannins have been shown to inhibit *in vitro* activity of digestive enzymes including trypsin (*EC* 3.4.21.4), α-amylase (*EC* 3.2.1.1) and lipase (*EC* 3.1.1.3) (Tamir & Alumot, 1969; Griffiths, 1981; Singh, 1984). Results from *in vivo* studies showed lower activities of trypsin and α-amylase in the digesta from various parts of the digestive tract of animals fed on tannin-containing diets (Horigome *et al.* 1988; Ahmed *et al.* 1991; Longstaff & McNab, 1991), but increased activities of lipase in digesta have been reported (Griffiths & Moseley, 1980; Horigome *et al.* 1988; Longstaff & McNab, 1991).

In a recent study Ahmed *et al.* (1991) found that N digestibility and feed intake declined as the proportion of salseed (*Shorea robusta*) meal tannin in the diet of male broiler chicks was increased. A significant increase in the relative weight of the pancreas has been reported in birds fed on diets containing tannin (Ahmed, 1991; Ahmed *et al.* 1991) and trypsin inhibitor (TI) (Gertler & Nitsan, 1970; Rubio *et al.* 1989). Thus, pancreatic hypertrophy in birds has been associated with the presence of tannin or TI in the diet. However, birds fed on tannin-containing diets eat less feed and are lighter in weight than normally-fed birds. Because it is not known whether birds fed on tannin-free diets at a lower feed intake or fed to a lighter body weight would have an enlarged pancreas, the contribution of the pancreas may be overestimated. The present study was planned to ascertain whether restriction of feed intake to the same level as that of birds receiving tannin, or the use of younger birds with smaller body weights, would show effects, similar to those associated with dietary tannins, on pancreas weight and on activities of enzymes in the pancreas and the small intestine. The experiment was also used to examine effects on the digestibility of N and live-weight gain. To provide points of reference to the effects of dietary tannins, groups of birds were fed *ad lib.* on either a tannin-containing diet (SSM) or a tannin-free diet (CONTROL). The effect of lower feed intake was studied by pair-feeding a group of birds (pair-fed) with the tannin-free diet at the same level as the SSM birds. The effect of body weight was studied in a group of birds (young) by feeding them *ad lib.* on the control diet until they achieved approximately the same body weight as the SSM group. For the present experiment salseed meal from south-east India (Grosvenor Grain and Feed Co. Ltd, Liverpool), the residue of sal tree fruit after the extraction of oil, was used as a source of tannin.

MATERIALS AND METHODS

Animals

Sixty 1-d-old male broiler (Cobb) chicks were purchased from a commercial hatchery and were reared in a group for 2 weeks, under the same conditions of space, light, temperature and humidity. They were fed on a commercial broiler starter mash up to 2 weeks of age. At the end of this adaptation period the birds were weighed individually and forty-eight birds of middle weight-range (335–380 g) were selected and transferred to individual battery cages to be used as experimental animals. Each cage was supplied with an individual drinker, a feeder and a tray for excreta collection. The caged birds were allocated to three groups (SSM, *ad lib.* and pair-fed), sixteen birds in each group and were fed on the experimental diets.

At the end of week 3 another group of twenty broiler chicks of the same breed and sex were obtained from the same hatchery and were reared under the same management conditions for 2 weeks. At the end of the second week these chicks were weighed and a group of sixteen chicks (young) having approximately the same weight as those of the first forty-eight chicks were randomly allocated to individual battery cages and fed on the experimental diet.

Table 1. *Composition of the experimental diets*

Diet...	SSM	CONTROL
Ingredients (g/kg)		
Salseed meal	507·5	—
Barley	—	507·5
Maize	171·0	199·0
Soya-bean meal	200·0	200·0
Fish meal	83·0	61·5
Vegetable oil	38·5	32·0
Chemical composition (calculated)		
Crude protein (N × 6·25; g/kg)	204·9	204·9
Metabolizable energy (MJ/kg)	12·2	12·2
Tannins (g/kg)	41·0	—

Diets, groups and their feeding plan

Two isonitrogenous (32·8 mg N/g dry matter (DM)) and isoenergetic (12·2 MJ/kg DM) rations (Table 1) containing 0·5 g Cr_2O_3/kg diet as an indigestible marker were formulated, one containing salseed meal (SSM) and the other containing barley (CONTROL) as a major energy source. The salseed meal used in the SSM diet was analysed for condensed tannin (12·4 g/kg) using the vanillin reaction (Price *et al.* 1978) and for hydrolysable tannin (68·3 g/kg) using dye-labelled protein (Asquith & Butler, 1985). The groups and their feeding plan after the adaptation period were as follows: (1) SSM, fed *ad lib.* on SSM diet during weeks 3, 5 and 6; (2) *ad lib.*, fed *ad lib.* on CONTROL diet during weeks 3, 5 and 6; (3) pair-fed, fed on CONTROL diet at same intake as SSM birds during weeks 3, 5, and 6; (4) young, fed *ad lib.* on CONTROL diet to age 25 d.

Each bird in the pair-fed groups was matched with one of similar body weight in the SSM group for the purpose of determining the amounts of feed offered. The quantity of feed offered each day to each pair-fed bird was the same as the quantity consumed by its pair in the SSM group. The birds in the young group were started on the trial 3 weeks after the other birds and were fed *ad lib.* until the group mean live weight was the same as that of the SSM group at slaughter.

Nitrogen digestibility and retention

Digestibility of N was determined only for birds in the SSM, *ad lib.* and pair-fed groups. For the first 7 d of the experimental period (week 3) no excreta collection was made while the Cr_2O_3 passage was becoming stabilized (Kotb & Luckly, 1972) in faeces. During the digestibility collection period (week 4) the amount of feed offered to all groups was the same. The amount offered was limited to the amount which the SSM birds would consume. Excreta collections, which started on the 8th experimental day, were made six times daily over twelve consecutive hours, for a period of 7 d. The times of the 12 h collection periods were changed, starting 2 h later each day, so that the collections were representative of fresh excreta from the equivalent of four full days. Excreta was immediately frozen after each 2 h collection. At the end of the 7 d collection period the excreta was freeze-dried, ground and stored in air-tight plastic containers at 4° until they were analysed for total N, Cr_2O_3, uric acid and NH_3-N as described by Ahmed *et al.* (1991). N digestibility and retention were calculated by the method of Terpstra & De Hart (1974). Ileal digestibility of N was determined in the digesta collected from the terminal ileum when the birds were killed.

Collection of digesta, mucosa and pancreas samples

At the end of the experiment each bird was anaesthetized and killed by cervical dislocation and the digestive tract and pancreas were excised. Each pancreas was weighed, immediately frozen in liquid N_2 and stored at $-70°$. Sections (150 mm) of distal duodenum, jejunum and terminal ileum were taken. Digesta were collected from the ileal and jejunal sections and stored at $-20°$. Remaining digesta from the duodenal section and digesta from a separate jejunal section were washed out carefully with normal saline (9 g NaCl/l) solution. The sections were then cut open on an ice-cooled sheet of glass and the mucosa was scraped from the gut section with a microscope slide and stored at $-20°$.

Determination of enzyme activities

Jejunal digesta (0·8–1·0 g) was homogenized in 15 ml buffer solution (0·04 M-Tris, 0·01 M-$CaCl_2$, pH 8·1) and the homogenates were centrifuged at 35000 ***g*** for 20 min at 4°. Supernatant fractions were assayed for trypsin activity (Liu & Markakis, 1989) using sodium benzoyl-DL-arginine *p*-nitroanilide as substrate, and for α-amylase using maltotetraose as the substrate (clinical analyzer MA-KIT, Art. 07 1094 6, Roche).

Each pancreas was thawed, sliced into smaller pieces and homogenized in 30 ml buffer solution (Tris 50 mM, KCl 0·154 M, pH 7·5), centrifuged at 35000 ***g*** for 20 min at 4°. Trypsinogen in a portion of the supernatant fraction was activated by incubating with enterokinase (*EC* 3.4.21.9) at 37° for 1 h (Gertler & Nitsan, 1970) and then activity of trypsin was determined. α-amylase activity was determined in a second portion of supernatant fraction.

Mucosal samples were thawed and approximately 1 g mucosa was accurately weighed and homogenized in 25 ml ice-cold glycerol (140 ml/l). The homogenates were centrifuged at 35000 ***g*** for 20 min at 4°. The supernatant fraction was diluted with the homogenizing solution and analysed for dipeptidase (*EC* 3.4.13.11) activity by the method based on Nicholson & Kim (1975), modified by Collington (1990). Activities of the disaccharidases sucrase (*EC* 3.2.1.48) and maltase (*EC* 3.2.1.20) were determined by the methods described by Dahlqvist (1968), terminating the reaction in a boiling water-bath and substituting ABTS for *o*-dianisidine as the chromogen. Enterokinase (*EC* 3.4.21.9) activity was determined by the method used by Ahmed (1991). Protein contents in the mucosal supernatant fraction were determined by a protein–dye-binding method (Bradford, 1976).

Statistical analysis

Analysis of variance was carried out to detect differences between treatments and the means were compared using Duncan's multiple-range test.

RESULTS

In the following commentary on the results of the experiment the mean values for the *ad lib.* birds have been taken as reference points for comparison of the other treatments. Not unexpectedly, the pair-fed birds, whose feed allocation was restricted, grew more slowly (Table 2). However, the SSM birds which consumed the same weight of feed grew still more slowly. *Ad lib.* birds utilized their diet more efficiently than both the SSM and the pair-fed birds. Digestibility of N, both apparent and ileal, was substantially lower with the SSM diet than that of the CONTROL diet. Whilst there was no difference between the relative liver weights of SSM and *ad lib.* birds, those whose diets were restricted (pair-fed) and those which were younger (young) had comparatively bigger livers (Table 3). Each of the

Table 2. *Final live weight, live-weight gain, feed conversion efficiency, apparent and ileal N digestibility and N retention in birds fed* ad lib. *on a tannin-containing diet or on a tannin-free diet under different feeding regimens**

(Mean values with their standard errors)

Diets...	SSM		CONTROL					
Groups...	SSM		*ad lib.*		Pair-fed		Young	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Period of feeding (d)	28	—	28	—	28	—	10	—
Final live wt (g)	850	—	1903	—	1265	—	859	—
Live-wt gain (g in 28 d)	496[a]	36	1549[c]	21	911[b]	41	—	—
Feed conversion efficiency (g gain/g feed intake)	0·25[a]	0·01	0·51[c]	0·007	0·47[b]	0·008	—	—
Apparent digestibility of N	0·43[a]	0·09	0·78[b]	0·04	0·78[b]	0·07	—	—
N retention (g/kg)	27·25[a]	0·63	60·09[b]	0·98	60·33[b]	1·23	—	—
Ileal digestibility of N	0·50[a]	0·20	0·84[b]	0·08	0·84[b]	0·61	0·84[b]	0·08

[a, b, c] Values within the same row with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and treatment groups, see Table 1 and p. 703.

Table 3. *Relative liver weight, relative pancreas weight and enzyme activities (Units* $\times 10^{-2}$*) in the pancreatic tissue of birds fed* ad lib. *on a tannin-containing diet or on a tannin-free diet under different feeding regimens**

(Mean values with their standard errors)

Diets...	SSM		CONTROL					
Groups...	SSM		*ad lib.*		Pair-fed		Young	
	Mean	SE	Mean	SE	Mean	se	Mean	SE
Liver wt (g/kg live wt)	21·7[a]	0·57	21·1[a]	0·67	24·4[b]	0·86	27·4[c]	1·0
Pancreas wt (g/kg live wt)	3·38[a]	0·16	1·99[d]	0·06	2·30[c]	0·06	2·92[b]	0·05
Trypsinogen (*EC* 3.4.21.4)								
Units/g pancreatic tissue	169[a]	15	165[a]	16	119[b]	13	148[ab]	14
Units/kg live wt	521[a]	56	307[bc]	28	256[c]	29	427[ab]	42
α-Amylase (*EC* 3.2.1.1)								
Units/g pancreatic tissue	61[a]	6	177[c]	9	138[b]	11	133[b]	9
Units/kg live wt	183[a]	17	330[bc]	19	301[b]	28	385[c]	30

[a, b, c] Values within the same row with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and treatment groups, see Table 1 and p. 703.

treatments induced enlargement of the pancreas compared with the *ad lib.* birds. Only in pair-fed birds was trypsinogen activity of pancreatic tissue (U/g pancreatic tissue) significantly depressed. However, when both the weight of the pancreas and the body weight of the bird were taken into consideration there was a significant elevation in trypsinogen (U/kg live-weight) activity in SSM birds; pair-fed birds exhibited the lowest value for this variable. Each of the treatments resulted in significant depression of α-amylase activity in pancreatic tissue (U/g pancreatic tissue), but when pancreas weight and

Table 4. *Enzyme activities in the jejunal digesta of birds fed* ad lib. *on a tannin-containing diet or on a tannin-free diet under different feeding regimens**

(Mean values with their standard errors)

Diets...	SSM		CONTROL					
Groups...	SSM		*ad lib.*		Pair-fed		Young	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Trypsin (*EC* 3.4.21.4)								
Units/g wet chyme	1525[a]	107	2853[b]	138	2550[b]	143	3471[c]	215
Units/g dry chyme	3436[a]	285	7265[bc]	571	6174[b]	309	8681[c]	430
α-Amylase (*EC* 3.2.1.1)								
Units/g wet chyme	66[a]	9	546[c]	31	440[b]	42	373[b]	35
Units/g dry chyme	148[a]	20	1373[c]	98	1074[b]	102	939[b]	85

[a, b, c] Values within the same row with unlike superscript letters were significantly different ($P < 0.05$).
* For details of diets and treatment groups, see Table 1 and p. 703.

Table 5. *Enzyme activities (Units/g mucosa) in the duodenal mucosa of birds fed* ad lib. *on a tannin-containing diet or on a tannin-free diet under different feeding regimens**

(Mean values with their standard errors)

Diets...	SSM		CONTROL					
Groups...	SSM		*ad lib.*		Pair-fed		Young	
	Mean	SE	Mean	SE	Mean	se	Mean	SE
Dipeptidase (*EC* 3.4.13.11)	111[a]	6	275[c]	12	233[b]	11	221[b]	13
Sucrase (*EC* 3.2.1.48)	0·23[a]	0·01	0·41[c]	0·02	0·37[bc]	0·01	0·33[b]	0·02
Maltase (*EC* 3.2.1.20)	16·2[a]	0·60	22·9[c]	1·62	20·5[bc]	0·87	18·8[ab]	1·32
Enterokinase (*EC* 3.4.21.9)	572[a]	17	432[b]	21	378[c]	15	325[d]	17

[a, b, c, d] Values within the same row with unlike superscript letters were significantly different ($P < 0.05$).
* For details of diets and treatment groups, see Table 1 and p. 703.

body weight were taken into consideration the total pancreatic α-amylase activity was significantly lower only in SSM birds.

In contrast to the levels of pancreatic trypsinogen, which exhibited an increased activity in response to the SSM diet, the activity of trypsin in jejunal digesta was severely depressed in SSM birds (Table 4). In the young birds trypsin activity in digesta was slightly higher. On the other hand the activities of α-amylase in the digesta followed a trend similar to that in the pancreatic tissue.

Dipeptidase and the disaccharidases in duodenal mucosa (Table 5) were markedly depressed in SSM birds but only slightly lower in pair-fed and young birds. In the jejunal mucosa (Table 6), with the exception of maltase which was unaffected, the enzymes showed a similar trend but differences between treatments were smaller. Comparing the activities of the mucosal enzymes of the duodenum and the jejunum, dipeptidase was higher in the duodenum whilst the disaccharidases were present at higher activities in the jejunum. Enterokinase activity was not inhibited by the presence of tannins in the diet, instead it increased significantly in the duodenal mucosa.

Table 6. *Enzyme activities (Units/g mucosa) in the jejunal mucosa of birds fed* ad lib. *on a tannin-containing diet or on a tannin-free diet under different feeding regimens**

(Mean values with their standard errors)

Diets...	SSM		CONTROL					
Groups...	SSM		*ad lib.*		Pair-fed		Young	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Dipeptidase (*EC* 3.4.13.11)	81[a]	5	187[c]	17	134[b]	11	133[b]	10
Sucrase (*EC* 3.2.1.48)	0·60[a]	0·03	0·73[b]	0·04	0·69[a b]	0·04	0·66[a b]	0·04
Maltase (*EC* 3.2.1.20)	44·3[a]	2·7	46·5[a]	2·7	46·0[a]	2·8	44·0[a]	3·0
Enterokinase (*EC* 3.4.21.9)	220[a]	8	233[a]	8	197[b]	8	151[c]	5

[a, b, c] Values within the same row with unlike superscript letters were significantly different ($P < 0·05$).

* For details of diets and treatment groups, see Table 1 and p. 703.

DISCUSSION

Pancreatic hypertrophy in birds fed on diets containing TI (Levison *et al.* 1979), lectin (Abbey *et al.* 1979; Grant *et al.* 1987) or tannin (Ahmed *et al.* 1991) has been tacitly ascribed in its entirety to the direct effect of the antinutritional substance. However, the results of the present experiment show that merely limiting the feed intake of birds fed on a tannin-free diet can result in a higher relative pancreas weight compared with those which are fed *ad lib.* on the same diet. Also in younger, lighter birds which were fed *ad lib.* on tannin-free diet the pancreas constituted a higher proportion of live weight. The number of treatments applied in the present experiment is not sufficient to justify an unequivocal claim of a relationship between live weight and relative pancreas weight. Nevertheless, the fact that the regression of relative pancreas weight (x) *v.* live weight (y) for the birds fed on the tannin-free diet ($x = 3·37–0·677y$; R^2 0·678; $P < 0·001$) was statistically significant does give strong support for the notion that part of the pancreatic hypertrophy associated with dietary tannin is due to the smaller size of the bird. In the present experiment the lower live weights of birds fed on the tannin-free diet were obtained by two different means, namely limitation of feed intake and age; this introduced unnecessarily an additional variable, age. With hindsight it can be seen that a better approach would have been to control live weight by imposing several different limits on feed intake rather than by using birds of different ages. The clearly higher relative weight of the pancreas in the birds fed on the tannin-containing diet compared with all the other groups demonstrates that, in addition to any indirect effect of tannin by way of live-weight limitation, tannin also exerts a separate effect on the pancreas. The means which were used to limit live weights of the birds fed on the tannin-free diet also lowered the contents of trypsinogen and α-amylase in the pancreatic tissue; in the case of trypsinogen this effect is the opposite of the effect of the tannin on trypsinogen. The depression of pancreatic tissue α-amylase activity in the birds fed on the SSM diet evident in the present experiment was not seen in that of Ahmed *et al.* (1991), but Ahmed (1991) did obtain similar responses in later experiments.

The activities of trypsin and α-amylase in the jejunum of the groups fed on tannin-free diets follow reasonably closely the pattern of the trypsinogen and α-amylase in the pancreas when expressed on a live-weight basis. In the birds fed on the tannin-containing diet the depression in the activities of both of these enzymes is severe, far exceeding the effects of lighter body weight associated with feed restriction or age. The effect of the tannin-containing diet on the activities of trypsin and α-amylase in the jejunum is more than might

be expected from the amounts of enzyme or zymogen in the pancreas. Ahmed (1991) has shown that addition of polyvinyl-polypyrrolidone, which is known to bind strongly with tannin, to jejunal digesta of birds fed on tannin-containing diets increases the activities of trypsin and α-amylase. These findings suggest that the major effect of the tannin on these enzymes is through direct tannin–enzyme interaction in the intestine (Griffiths, 1979). A further influence on the activity of trypsin in the intestine is the activity of enterokinase. However, since the enterokinase appears to have been higher in the birds fed on the tannin-containing diet, it is unlikely that the lower tryptic activity of intestinal digesta results from depressed capacity for activation of trypsinogen.

Although the results of the present experiment strongly implicate inactivation of intestinal digestive enzymes in the reduction of protein digestion by the presence of tannins in the diets, the relative contribution of tannin–enzyme and tannin–dietary protein interaction will only be decided by studies which identify the contribution of endogenous protein to faecal N (de Lange *et al.* 1989, 1990).

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