The utilization of raw and autoclaved faba beans (*Vicia faba* L., var. minor) and faba bean fractions in diets for growing broiler chickens

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The effects of the inclusion of raw and autoclaved whole faba beans (*Vicia faba*; RFB and AFB respectively) or faba bean fractions (cotyledons and hulls) in diets for growing broiler chickens (0–4 weeks of age) on performance, intestinal physiology and jejunal histological structure have been studied in three experiments. Significant decreases in body-weight as well as lower food consumption and higher food intake:weight gain ratio were observed in those animals fed on diets containing 250, 350 and 500 RFB/kg in the diet. Birds fed on AFB diets (500 g/kg) had significantly greater body-weights than chicks fed on RFB or raw faba bean cotyledons (RC). Significant increases in the relative lengths of duodenum, jejunum, ileum and caeca, pancreas relative weight, and intestinal transit time of birds fed on diets containing 250, 350 and 500 g RFB/kg compared with control birds were observed. Including AFB (500 g/kg) in the diet significantly increased body-weight and significantly decreased pancreas weight compared with RFB (500 g/kg)-fed birds. The inclusion of RFB hulls had no effect on these variables. Dehulling or autoclaving of faba beans, or both, proved to have no significant effect on relative lengths of duodenum, jejunum, ileum and caeca, nor on caecal volatile fatty acid concentration in birds fed on 500 g faba beans/kg diet. Electron microscopy of the jejunal mucosa revealed discrete hyperplasia of polysomes and mitochondrial swelling in those animals fed on AFB (500 g/kg) or AC (426·4 g/kg). Pronounced strangulations were also observed along the microvilli, whose length was similar to that of control birds. The inclusion of RFB hulls, either autoclaved or raw, led to no ultrastructural changes in the enterocytes, as detected by electron microscopy. Birds fed on diets containing the cotyledons of RFB (RC, 426·4 g/kg) rather than whole RFB showed the same ultrastructural disorders as RFB (500 g/kg)-fed birds. The present study shows that factors other than those usually claimed, i.e. protease inhibitors, phytates, tannins and lectins, may be contributing to the low nutritional value of *V. faba* seeds for growing chickens.

Vicia faba: Growth: Broiler chickens

Livestock production in Europe has long relied on plant proteins contributing substantially to the diet. The nutritional importance of legume seeds has increased in the last years. The faba bean (*Vicia faba* L.) has been one of the most studied species of legumes and is receiving considerable interest as a potential indigenous source of plant protein for Western Europe.

Slow growth rates and low food intake of animals fed on diets containing high levels of faba beans have been repeatedly reported both in rats (Marquardt & Campbell, 1974; Abbey *et al.* 1979*a*; Moseley & Griffiths, 1979) and chickens (Marquardt & Campbell, 1974; Marquardt *et al.* 1974; Guillaume, 1977). The presence of toxic constituents, and the low sulphur-amino-acid content of faba bean proteins (Marquardt & Campbell, 1974, 1975; Boulter, 1982; Jeroch & Berger, 1983; Thacker & Bowland, 1985) have been claimed as the main reasons for the low nutritional value. The effects of the inclusion of raw faba beans (RFB) in diets for chicks on intestinal ultrastructure have only recently been reported in poultry (Rubio *et al.* 1989). Although lectin inactivation by heat treatment (Liener, 1986) and better growth responses in animals fed on autoclaved faba beans (Marquardt *et al.* 1974; Shannon & Clandinin, 1977) have been reported, no attempt has been made so far to study the ultrastructural characteristics of the small intestine in animals fed on autoclaved faba beans (AFB) or cotyledons (AC).

The aims of the present work were to study some of the physiological effects observed in chicks fed on diets containing RFB, as well as the physiological, histological and nutritional consequences of heat treatment, dehulling, or both, of the faba bean seeds.

MATERIALS AND METHODS

Animals and diets

Male broiler chickens of the Hubbard strain (1-d-old) were used in three experiments. They were distributed into groups of ten animals (two groups per treatment) in a fully randomized experimental design and were reared from 1-d-old to 4 weeks of age (Expts 1 and 2) and to 3 weeks of age (Expt 3) in electrically heated thermostatically controlled battery brooders with wire-screen floors. Room lighting was continuous, and food and water were offered *ad lib.* throughout the experiments. The composition of the faba beans used is shown in Table 1, and that of the diets in Tables 2 and 3. In Expt 1 (Table 2) the experimental diets contained 0 (control), 125 (A), 250 (B) and 500 (C) g RFB/kg diet. In Expt 2 (Table 2) the experimental diets contained 0 (control) and 350 (H) g RFB/kg. In Expt 3 (Table 3) test diets contained 500 g faba beans/kg (diets AC+H, RFB and AFB) or the amount of cotyledons (426·4 g/kg) corresponding to the same faba bean level of inclusion (diets AC and RC). A maize–soya-bean diet was used as control. The diets were isonitrogenous and isoenergetic in all cases. Metabolizable energy (ME) of RFB was estimated by using analysed values for these seeds and the following expression (Janssen *et al.* 1979):

$$\text{ME(MJ/kg)} = 0{\cdot}001\ (0{\cdot}6 \times \text{dry matter} + 10{\cdot}5 \times \%\ \text{crude protein}\ (\text{nitrogen} \times 6{\cdot}25) + 33{\cdot}1 \times \%\ \text{crude fat} + 14{\cdot}2 \times \%\ \text{N-free extract}).$$

A clean separation of cotyledon and hulls was effected by coarse milling the dried faba beans by a combined process of sieving and gravity separation in a forced-draught seed-cleaning device. Cotyledons and whole beans were autoclaved at 110° for 30 min. Straw and lucerne (*Medicago sativa*) were added to diets AC and RC (Table 3) in order to equalize their fibre content with the other diets. It is recognized that this introduces different sources of fibre, but equalizing was thought preferable to allowing large differences.

Sampling procedures

Chicks were weighed at 4 weeks of age in Expts 1 and 2 and at 3 weeks of age in Expt. 3. The amount of food remaining was recorded. Variables measured included growth rate, food consumption and food intake:weight gain ratio.

Immediately after the chicks had been killed by cervical dislocation, the pancreas and the small intestine were separated from the mesentery and removed by excising at the duodenal and ileo-caecal junctions. The weight of the pancreas and the lengths of the small intestinal sections were recorded. The small intestine was divided into duodenum, jejunum and ileum. The large loop between the gizzard and the end of the pancreas tail was considered as duodenum; the jejunum was defined as terminating at the yolk stalk and the ileum at the ileo-caecal junction. Caecal length was also recorded. Before these measurements were made, intestinal contents were removed with water from a syringe attached to the cut duodenal end.

Table 1. *Analysis of faba bean* (Vicia faba *L. minor cv. Prothabon 101*) *flour and faba bean fractions* (*g/kg dry matter*)

	Whole seed	Cotyledons	Hulls
Crude protein (nitrogen × 6·25)	310·7	355·8	61·0
Diethyl ether extract	9·0	9·7	4·0
Crude fibre	82·4	8·8	521·0
Ash	35·0	35·7	24·0
Hulls	147·1	—	—

Table 2. *Expts 1 and 2. Composition of diets*

	Expt 1				Expt 2
Diet ...	Control	A	B	C	H
Ingredients (g/kg air dry diet)					
Maize meal	569·3	547·0	525·8	381·8	476·8
Soya-bean meal	320·0	195·0	70·0	—	20·0
Faba bean (*Vicia faba*) meal	—	125·0	250·0	500·0	350·0
Maize gluten meal	34·4	67·8	99·7	51·3	96·0
Animal fat	40·0	27·0	14·6	2·8	2·8
Calcium carbonate	11·3	11·5	11·7	11·5	12·0
Bicalcium phosphate	18·0	18·1	18·1	17·7	17·7
Sodium chloride	2·8	2·8	2·8	2·8	2·8
DL-Methionine	1·6	1·9	2·3	4·0	2·9
L-Lysine	0·7	1·9	3·0	1·8	2·8
Premix*	2·0	2·0	2·0	2·0	2·0
Calculated composition (/kg diet)					
Protein (g)	212·4	212·4	211·3	212·1	212·5
Metabolizable energy (MJ)	13·0	13·0	13·0	13·0	13·0
Lysine (g)	11·2	11·2	11·2	11·2	11·2
Methionine + cystine (g)	8·7	8·7	8·7	8·7	8·7
Crude fibre (g)	36·4	35·3	34·2	42·3	—

* Vitamin and mineral supplement supplying (mg/kg diet): 3 retinol, 55 cholecalciferol, 5 tocopheryl acetate, 2 menadione, 2 thiamin, 5 riboflavin, 10 pantothenic acid, 30 nicotinic acid, 3 pyridoxine, 2 folic acid, 0·013 cyanocobalamin, 3 cobalt, 30 iron, 0·5 iodine, 70 manganese, 450 choline, 10 nitrovin, 125 ethoxyquin.

At 4 weeks of age, six animals per treatment in Expt 1 (three from each replicate) were randomly selected and housed in individual metabolism cages. Intestinal transit time was determined in these animals.

In Expt 3, samples of caecal contents for volatile fatty acids (VFA) determination were taken from eight birds immediately after killing. Caeca were removed and their contents placed into vessels containing 7 ml distilled water and analysed as described later.

Analytical methods

Caecal VFA concentrations. Four samples per treatment, each of them being the pooled caecal contents from two birds, were analysed for VFA. The samples were treated with 50% sulphuric acid (0·1 ml) (Sudo & Duke, 1980) and centrifuged at 15000 ***g*** immediately after collection and the supernatant fraction was prepared for gas–liquid chromatography (Chromatopac C-R3A, Shimadzu) with a column of 10% SP-1200/1% phosphoric acid in 80/100 Chromosorb W (AW Supelco Inc.) under the following conditions: column temperature 110°, sensitivity 2×10^{-10}, sample 1 μl. Internal standard solutions containing

Table 3. *Expt 3. Composition of diets*

Diet...	Control	AC+H	RFB	AFB	AC	RC
Ingredients (g/kg air-dry diet)						
Maize meal	552·4	390·3	390·3	390·3	451·9	451·9
Soya-bean meal	315·0	—	—	—	—	—
Whole faba bean (*Vicia faba*) meal	—	—	500	500	—	—
Faba bean cotyledon meal	—	426·4	—	—	426·4	426·4
Faba bean hulls	—	73·6	—	—	—	—
Lucerne (*Medicago sativa*) meal	20·0	—	—	—	20·0	20·0
Straw	—	—	—	—	22·0	22·0
Maize gluten meal	—	14·0	14·0	14·0	19·5	19·5
Meat meal	40·0	40·0	40·0	40·0	22·0	22·0
Sunflower oil	50·0	30·0	30·0	30·0	8·0	8·0
Calcium carbonate	—	—	—	—	4·5	4·5
Bicalcium phosphate	15·0	15·0	15·0	15·0	15·0	15·0
Sodium chloride	2·8	2·8	2·8	2·8	2·8	2·8
DL-methionine	2·3	4·3	4·3	4·3	4·3	4·3
L-lysine	—	1·1	1·1	1·1	1·1	1·1
Premix*	2·5	2·5	2·5	2·5	2·5	2·5
Calculated composition (/kg diet)						
Protein (g)	210·9		211·0			210·7
Metabolizable energy (MJ)	13·0		13·0			13·0
Methionine + cystine (g)	8·6		8·6			8·9
Lysine (g)	11·1		11·2			11·0
Crude fibre (g)	40·9		42·3			40·3

Control, maize–soya-bean diet; AC+H, autoclaved faba bean cotyledons plus raw faba bean hulls; RFB, raw whole faba bean; AFB autoclaved faba bean; AC, autoclaved faba bean cotyledons; RC, raw faba bean cotyledons.

* Vitamin and mineral supplement supplying (mg/kg diet): 3 retinol, 55 cholecalciferol, 5 tocopheryl acetate, 2 menadione, 2 thiamin, 5 riboflavin, 10 pantothenic acid, 30 nicotinic acid, 3 pyridoxine, 2 folic acid, 0·013 cyanocobalamin, 3 cobalt, 30 iron, 0·5 iodine, 70 manganese, 450 choline, 10 nitrovin, 125 ethoxyquin.

reagent grade acetic, propionic and butyric acids in various proportions were also prepared and used to calculate unknown concentrations of VFA in the samples.

Intestinal transit time. After a 14 h fast, animals were again offered food. The period of time from offering food to the first droppings, as detected by visual observation, was considered as intestinal transit time.

Lectin and haemagglutinating activity determination. Faba bean lectin was extracted and purified as described by Allen & Johnson (1976). The haemagglutinating activity was determined in AFB following the method of Lis & Sharon (1972).

Histological methods

Three birds from each treatment were randomly selected at 21 d of age. They were killed and the whole small intestine was removed. Jejunal samples were taken at the mid-point between the pancreas tail and yolk stalk and treated as described in Rubio *et al.* (1989). Formaldehyde (100 ml/l) and Bouin solution were used for light microscopy. Specimens for electron microscopy were fixed in glutaraldehyde solution in Milloning buffer and post-fixed in cold osmium tetroxide (10 g/l).

Statistical analysis

Performance values were analysed by analysis of variance. Multiple comparisons among means were made by Duncan's multiple range test (Steel & Torrie, 1960). Other values were compared using Student's *t* test.

RESULTS

Lectin concentration and haemagglutinin activity

Lectin extraction from *V. faba* seeds gave a yield of at least 1 g/kg dry matter. This is in agreement with the results reported by Matsumoto *et al.* (1983) and Allen *et al.* (1976). There was no detectable haemagglutinating activity in AFB.

Performance and physiology

Expts 1 and 2. Significant growth depression was found in birds given diets containing 250, 350 and 500 g RFB/kg compared with those fed on the control and 125 g RFB/kg diets (Table 4). Faba bean inclusion levels higher than 250 g/kg resulted in higher food intake: weight gain ratios and lower food intake values.

Birds fed on 125 g faba bean meal/kg diet showed no difference from control animals with respect to these production values. Significant increases in the relative lengths of duodenum, jejunum, ileum and caeca and in the relative weight of the pancreas from birds fed on the higher levels of RFB (350 and 500 g/kg) compared with those fed on the control and 125 g RFB/kg diets were recorded (Table 4). The intestinal transit time of animals fed on 500 g RFB/kg was significantly increased compared with those of control chicks and chicks fed on 125 and 250 g/kg (Table 4).

Expt 3. Significantly reduced body-weight was found in faba-bean-fed birds compared with control values. AFB-fed chicks (500 g/kg) exhibited significantly higher body-weights than those fed on the RFB diet (500 g/kg) and the RC diet (426·4 g/kg). Though not significant in all cases, RFB- and RC-fed chicks exhibited lower food intakes and higher food intake:weight gain ratios than control, AFB- and AC-fed birds (Table 5). Relative lengths of duodenum, ileum and caeca of the chicks on diets RFB, AFB, RC and AC were significantly increased compared with control birds; jejunum relative lengths were also significantly increased, except for AFB- and AC-fed animals. No significant differences in the intestinal segments were recorded among RFB-, AFB-, RC- and AC-fed groups of animals except for jejunum in RC-fed birds. The addition of the faba bean hulls to AC (diet AC+H) produced no significant effect on intestinal relative lengths, and dehulling of the seeds was also without effect (compare diets RC and AC with RFB and AFB, Table 5) with the only exception being the jejunum in RC-fed compared with RFB-fed birds. Autoclaving of the faba bean seeds (diet AFB) or cotyledons (diet AC+H) led to pancreas relative weights significantly lower than those of RFB- and RC-fed chicks, AC-fed chicks showing no significant difference when compared with either RFB- or control-diet-fed birds. Birds given diets AFB or AC+H were not significantly different from control birds in pancreas relative weight (Table 5). Significantly lower caecal concentrations of acetate and propionate were found compared with control chickens in those animals fed on diets containing RFB, AFB, RC or AC (Table 6).

Histology

The samples from the animals fed on diet AFB exhibited slightly shorter and narrower villi than control birds, and hyperplasia of enterocytes (Plate 1).

Electron microscopy of the mucosal surface of the jejunum of RFB- and RC-fed birds revealed extensive disruption and atrophy of microvilli and apical ribosomal hyperplasia (Plate 2), accompanied by mitochondrial swelling, a decreased number of multivesicular bodies, reduction of lysosomes and lipid drops and nuclear depolarization. The smooth endoplasmic reticulum was hypertrophied and vesiculated. The Golgi cisternae were frequently dilated and filled with not very dense substances. There were greatly increased numbers of goblet cells (Plate 3). The enterocytes of those birds fed on AFB, AC and

Table 4. *Expts. 1 and 2. Growth, relative lengths of small intestine sections and pancreas relative weight of chicks fed on diets containing different levels of raw faba beans* (Vicia faba) (*RFB*)

(Mean values with their pooled standard errors; no. of animals in parentheses)

Diet† (g RFB/kg)...	Expt 1 Control‡ Mean	Expt 1 A 125 Mean	Expt 1 B 250 Mean	Expt 1 C 500 Mean	Expt 1 SEM	Expt 2 Control Mean	Expt 2 H 350 Mean	Expt 2 SEM	Statistical significance of difference: $P <$
Body-wt (BW) (g)	705^{a} (19)	703^{a} (21)	637^{b} (18)	580^{c} (20)	12	713^{d} (19)	573^{e} (19)	17	0·01
Food intake (g/bird per d)	38·64^{a} (2)	38·80^{a} (2)	35·86^{b} (2)	34·80^{b} (2)	0·68	36·92^{a} (2)	32·33^{b} (2)	1·4	0·01
Food intake:wt gain ratio (g food intake/g gain)	1·48^{b} (2)	1·49^{b} (2)	1·52^{b} (2)	1·62^{a} (2)	0·02	1·45^{b} (2)	1·58^{a} (2)	0·04	0·01
Small intestine relative lengths (mm/kg BW)									
Duodenum	2·9^{a} (8)	2·7^{a} (8)	3·1^{ab} (8)	3·5^{b} (8)	0·08	2·8^{d} (8)	3·7^{e} (8)	0·13	0·001
Jejunum	5·8^{a} (8)	5·3^{a} (8)	6·7^{ab} (8)	7·3^{b} (8)	0·20	5·9^{d} (8)	8·1^{e} (8)	0·37	0·001
Ileum	5·9^{a} (8)	5·5^{a} (8)	6·8^{ab} (8)	8·1^{b} (8)	0·22	6·7^{d} (8)	8·5^{e} (8)	0·37	0·001
Caeca	1·3^{a} (8)	1·2^{a} (8)	1·5^{ab} (8)	1·8^{c} (8)	0·04	1·4^{d} (8)	1·7^{e} (8)	0·06	0·001
Pancreas relative wt (g/kg BW)	2·4^{a} (8)	2·7^{b} (8)	3·1^{c} (8)	3·5^{d} (8)	0·12	—	—		0·001
Intestinal transit time (min)	224^{a} (6)	219^{a} (6)	223^{a} (6)	264^{b} (6)	5·13	—	—		0·01

a, b, c, d, e Means in the same horizontal row with different superscript letters were significantly different.
† For details, see Table 2.
‡ Maize–soya-bean diet.

Table 5. *Expt 3. Growth, relative lengths of small intestine sections and relative pancreas weight of chicks fed on diets containing raw (RFB) or autoclaved (AFB) faba beans* (Vicia faba) *or faba bean fractions (raw (RC) or autoclaved (AC) cotyledons or hulls (H))*

(Mean values with their pooled standard errors; no. of animals in parentheses)

Diet…	Control	AC+H	RFB	AFB	AC	RC		Statistical significance of difference: $P <$
	Mean	Mean	Mean	Mean	Mean	Mean	SEM	
Body-wt (BW) (g)	474^{a} (15)	355^{bc} (14)	308^{c} (14)	387^{b} (13)	353^{bc} (15)	314^{c} (14)	10	0·01
Food intake (g/bird per d)	34^{a} (2)	28^{ab} (2)	22^{b} (2)	27^{ab} (2)	28^{ab} (2)	24^{b} (2)	1	0·01
Food intake:wt gain ratio (g food intake/g gain)	$1·49^{b}$ (2)	$1·63^{a}$ (2)	$1·53^{b}$ (2)	$1·47^{b}$ (2)	$1·65^{a}$ (2)	$1·63^{a}$ (2)	0·02	0·01
Small intestine relative lengths (mm/kg BW)								
Duodenum	$3·91^{a}$ (10)	$4·61^{b}$ (10)	$4·99^{b}$ (10)	$4·88^{b}$ (10)	$4·67^{b}$ (10)	$5·19^{b}$ (10)	0·11	0·05
Jejunum	$8·41^{a}$ (10)	$9·66^{ab}$ (10)	$10·09^{b}$ (10)	$9·89^{ab}$ (10)	$9·45^{ab}$ (10)	$11·78^{c}$ (10)	0·26	0·05
Ileum	$8·32^{a}$ (10)	$9·56^{b}$ (10)	$10·88^{bc}$ (10)	$10·65^{bc}$ (10)	$10·39^{bc}$ (10)	$11·50^{c}$ (10)	0·27	0·05
Caeca	$1·81^{a}$ (10)	$2·21^{b}$ (10)	$2·46^{b}$ (10)	$2·29^{b}$ (10)	$2·30^{b}$ (10)	$2·48^{b}$ (10)	0·05	0·05
Pancreas relative wt (g/kg BW)	$3·2^{a}$ (10)	$3·4^{a}$ (10)	$3·9^{bc}$ (10)	$3·1^{a}$ (10)	$3·5^{ab}$ (10)	$4·0^{c}$ (10)	0·08	0·01

[a, b, c] Means in the same horizontal row with different superscript letters were significantly different.

Table 6. *Expt 3. Caecal concentrations of volatile fatty acids (VFA) from chicks fed on raw (RFB) and autoclaved (AFB) faba beans* (Vicia faba) *and faba bean fractions (raw (RC) and autoclaved (AC) cotyledons and hulls (H))*

Diet	VFA concentrations (mmol/kg caecal content)		
	Acetate	Propionate	Butyrate
Control	70·11[a]	24·07[a]	5·13
AC+H	39·05[b]	2·53[b]	3·66
RFB	35·99[b]	1·09[b]	3·64
AFB	44·64[b]	0·99[b]	4·17
AC	43·31[b]	1·08[b]	4·63
RC	33·27[b]	1·63[b]	4·69
SEM	3·17	1·87	0·28

[a, b] Means in the same vertical column with different superscript letters were significantly different ($P < 0·01$).

AC+H showed a slight ribosomal hyperplasia and mitochondrial swelling (Plates 5 and 6). Pronounced strangulations were observed along the microvilli which, on the other hand, were similar in length to those of control birds (Plates 4, 5 and 6). The presence or absence of faba bean hulls in the diet (Plates 5 and 6) produced no histological changes as detected by electron microscopy.

DISCUSSION

The histological alterations observed in the birds fed on diets containing 500 g RFB/kg have been widely described and discussed elsewhere (Rubio *et al.* 1989). These effects are likely to be produced by faba bean lectins and the results reported here support this hypothesis. Thus, the slight shortening and narrowing of jejunal villi in those chicks fed on AFB and AC diets compared with control animals, and the gross atrophy of those structures observed in RC-fed birds, show that the factors involved are lectins since tannins, the other possible factor, are located almost totally in the hulls (Marquardt *et al.* 1974). In addition, Southon *et al.* (1984) have recently demonstrated a distinct narrowing of the villi and a higher proportion of DNA and protein in the jejunum which appear to be associated with zinc deficiency in the growing rat. We have found a significantly decreased plasma Zn content in chicks fed on diets containing either AFB or AC (Rubio & Brenes, 1988). The atrophy of microvilli and the swollen enterocytes, which were the major features observed in RFB (500 g/kg)- and RC (426·4 g/kg)-fed birds, were not detected in AFB (500 g/kg)- or AC (426·4 g/kg)-fed chicks. However, although the microvilli were similar in length to those of control chicks, pronounced strangulations could be observed along the microvilli of the animals fed on AFB- or AC-containing diets as well as a discrete hyperplasia of polysomes and swollen mitochondria. A significantly increased relative length of small intestine and caeca, intestinal distension and increased numbers of goblet cells were also found in chicks offered diets containing AFB or AC. These relatively minor disorders could indicate: (a) that the faba bean lectin activity has not been totally abolished by the heat treatment used, even though no agglutinating activity was observed, or (b) some substances other than lectins could be playing a role in this context, or both.

Among the anti-nutritive factors reported to be present in faba bean seeds, protease inhibitors, tannins, phytic acid and lectins are likely to be those of greatest importance for growing chickens. The presence of protease inhibitors in RFB seeds can be inferred from the significant increase in pancreas relative weight in chickens fed on the RFB-containing diet as this is a typical response to these anti-nutritive factors (Abbey *et al.* 1979*b*). In addition, the absence of pancreatic hypertrophy in those animals fed on both AFB- or AC-containing diets demonstrates the thermolabile nature of the substances responsible for these pancreatic effects. However, the most broadly accepted conclusion about trypsin and chymotrypsin inhibitors is that they have little effect on body-weight and food intake of birds fed on RFB, although they are likely to have a considerable effect on pancreatic physiology (Wilson *et al.* 1972*a*, *b*; Marquardt *et al.* 1974). The difference in body-weight between AFB- or AC-fed groups and control chicks obtained in the present study, with no significant differences in pancreas weight, offers more support for this conclusion.

Hulls proved to have no effect on pancreatic response (groups AC+H *v*. AC and RC *v*. RFB in Table 5) contrary to the report of Ward *et al.* (1977) who attributed 50% of the anti-protease activity of RFB to the hulls. The preceding comparison between groups of animals fed on diets with and without hulls shows the weak effect of faba bean tannins on the performance of growing broiler chicks. The white-flowered faba bean used in our experiments and the low condensed-tannin concentration determined in these seeds (2·49 (SE 0·05) mg/g; Rubio *et al.* 1989) are probably the main reasons for these results, which agree with those of Sjodin (cited by Ward *et al.* 1977) and Moseley & Griffiths (1979). The role that phytic acid might play in this context is not likely to be of great importance, taking into account that the phytate content of faba bean seeds is very similar to that determined in soya-bean seeds (Thompson, 1986; Rubio & Brenes, 1988). With respect to faba bean lectins, these substances should be considered as responsible for the main histological disorders observed in RFB-fed chicks as discussed previously. However, since the most relevant toxic factors are thermolabile and autoclaving of faba bean seeds proved to have only a slight advantageous effect on chick performance, these anti-nutritive substances are possibly not the factors with major responsibility for the low productive response in chickens fed on diets containing more than 250 g faba beans/kg.

The low digestibility of carbohydrates and proteins must be taken into account. High amounts of unavailable carbohydrates (Pritchard *et al.* 1973), non-digestible oligo-saccharides of the raffinose family (Mercier, 1979) and high amylose content (Lineback & Ke, 1975), as well as low protein digestibility (Huyghebaert *et al.* 1979), have been reported in faba beans. Reisenfeld *et al.* (1980) have observed that starch is mainly digested and absorbed as glucose in the jejunum of the chick. As this is the most histologically-damaged section in RFB-fed birds (Rubio *et al.* 1989), impaired digestion and absorption of nutrients has to be expected in these animals. The anti-enzymic effects of the main toxic factors present in faba beans (Griffiths, 1979; Liener, 1986; Thompson, 1986) must, therefore, be considered along with the low digestibility of the materials *per se* in RFB-fed birds. These circumstances favour the accumulation of undigested materials in the gut leading both to distension of the gastrointestinal tract and to a significant increase of the relative length of the small intestine as a response to increased work of the bowel to move its contents. The increased intestinal transit time found in animals fed on the highest concentrations of faba beans is also consistent with this idea. Heat treatment and the presence or absence of faba bean hulls in the diets did not produce any significant effect on intestinal relative length, proving that heat-stable cotyledonous substances are likely to be those mainly involved in the gut modifications seen in chicks fed on diets AFB or AC.

Inadequate nutrient absorption across the gut wall may be inferred from the observation of the histological lesions of the gut mucosa of those animals fed on diet RFB. Differences in the rate of absorption of glucose across the intestinal wall have been reported (Lasheras *et al.* 1980; Motilva *et al.* 1983) in rats fed on diets containing high levels of legume seeds. Sjolander *et al.* (1984) have demonstrated a selectivity in the absorption of given substances as a consequence of changes caused in the intestinal wall of rats fed on lectins.

VFA are the major end-products of fermentation in the digestive tract of the fowl, and the caeca are the main sites of their formation. The type and extent of fermentation depend on the microbial population and the nature and amount of substrate that escapes digestion in the upper part of the digestive tract and enters the caeca. Hence, it is possible that the extent of VFA production in the caeca may be modified under different dietary regimens (Annison *et al.* 1968). The presence of high percentages of faba beans in the diets used in the present study produced several digestive effects, such as increase in the length of small intestine sections and caeca, longer transit time and lower food consumption. All these disturbances are likely to be caused by the presence of a high content of unavailable materials, substances that might be responsible for the observed changes in the production of VFA in faba-bean-fed chicks. These effects agree partly with those reported by Ricke *et al.* (1982), who observed differences in the production of VFA in the intestine of chicks fed on diets containing variable amounts of lignin compared with those given diets containing another type of fibre. Caecal VFA values in our experiments suggested that different cell wall constituents affected gut microbial metabolism, giving rise to a different fermentation pattern in the birds fed on faba bean diets and changing the quality and proportion of VFA. Neither autoclaving and dehulling of the faba bean seeds nor the inclusion of hulls in the diet had any significant effect on the production of VFA, indicating that the factors responsible for the fermentation changes are located in the cotyledon and are not affected by autoclaving under the conditions of the present study.

In conclusion, factors other than those usually claimed as responsible for the low nutritional value of *V. faba* seeds for growing chickens, i.e. phytates, tannins, lectins and protease inhibitors, must be taken into account. The effects that undigestible materials could play in this context should be investigated in the future. The histological disorders in small intestinal mucosa reported to occur in chicks fed on diets containing levels higher than 250 g RFB/kg (Rubio *et al.* 1989) have to be attributed to faba bean lectins. On the other hand, at least 125 g RFB meal/kg can be added to commercial diets for growing chickens at the expense of soya-bean without any physiological or productive damage for the animals.

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EXPLANATION OF PLATES

Plate 1. Light micrograph of part of a section through the jejunum of a bird fed on a diet containing 500 g autoclaved faba beans (*Vicia faba*)/kg, showing shortening of the villi. Haematoxylin and eosin stain, × 120. Normal appearance of jejunal villi in control birds can be observed in Rubio *et al.* (1989).

Plate 2. Transmission electron micrograph of part of a section through the jejunum of a bird fed on a diet containing 427·4 g raw faba bean (*Vicia faba*) cotyledons/kg, showing atrophy and disruption of microvilli, hydropic degeneration and ribosomal apical hyperplasia.

Plate 3. Transmission electron micrograph of part of a section through the jejunum of a bird fed on a diet containing 500 g raw faba beans (*Vicia faba*)/kg, showing disruption of microvilli and swollen enterocytes (white 'holes') accompanied by pronounced goblet cell (→) hyperplasia.

Plate 4. Transmission electron micrograph of part of a section through the jejunum of a bird fed on a control diet (maize–soya-bean), showing normal length and distribution of the microvilli.

Plate 5. Transmission electron micrograph of part of a section through the jejunum of a bird fed on a diet containing 500 g autoclaved faba beans (*Vicia faba*)/kg, showing length of the microvilli similar to that of control birds with pronounced strangulations. There is slight mitochondrial swelling with ribosomal hyperplasia.

Plate 6. Transmission electron micrograph of part of a section through the jejunum of a bird fed on a diet containing 427·4 g autoclaved faba bean (*Vicia faba*) cotyledons/kg plus 73·6 g raw faba bean hulls/kg, showing pronounced strangulation in microvilli which are similar in length to those of control birds.

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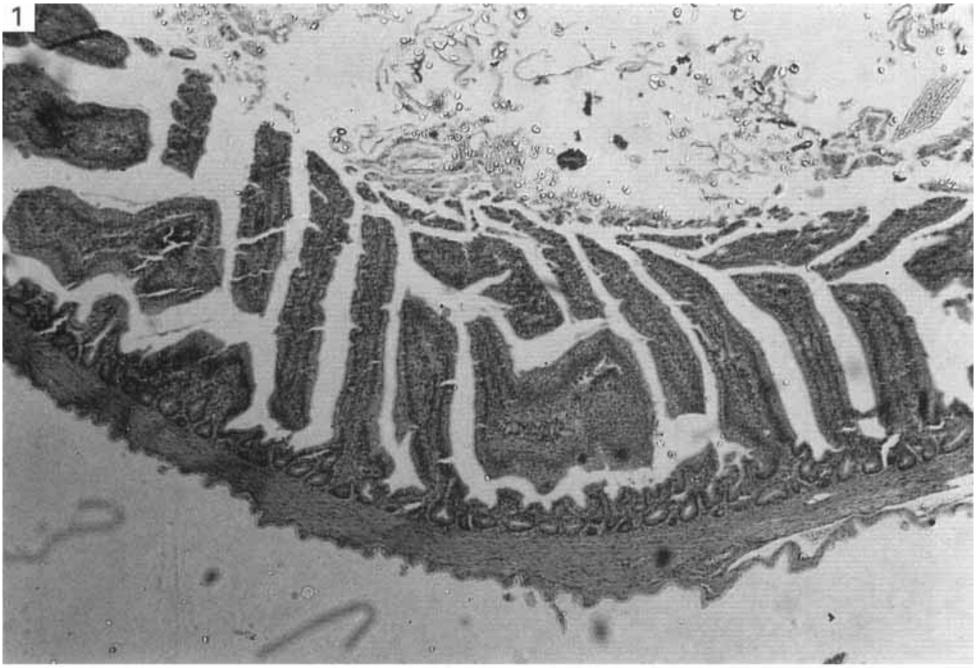

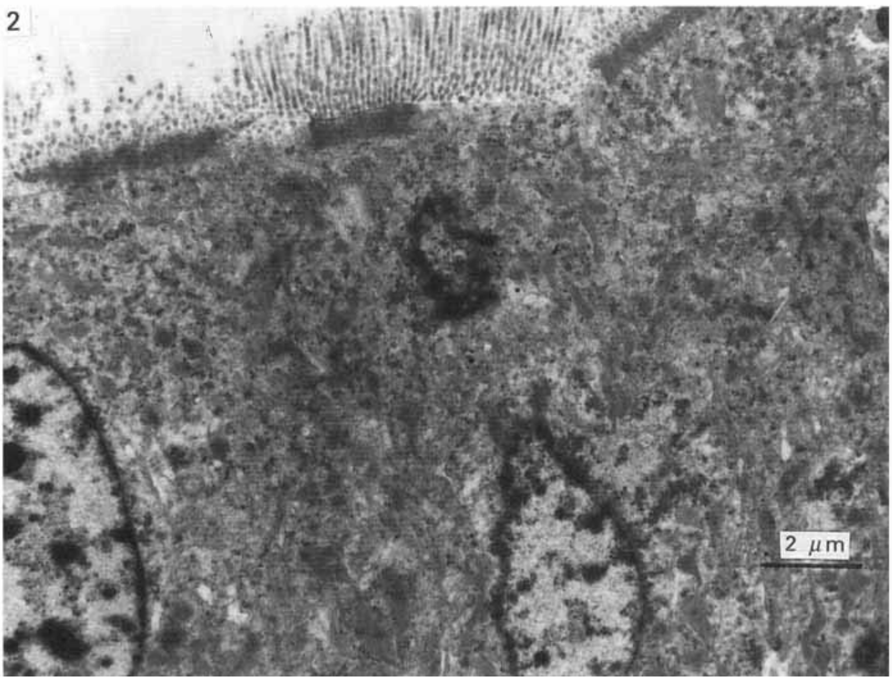

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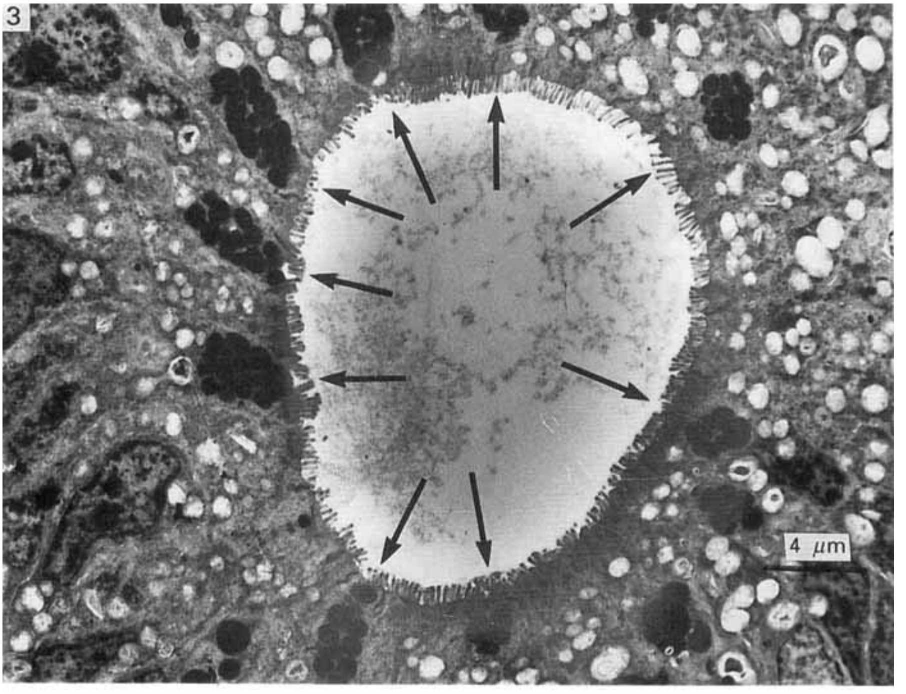
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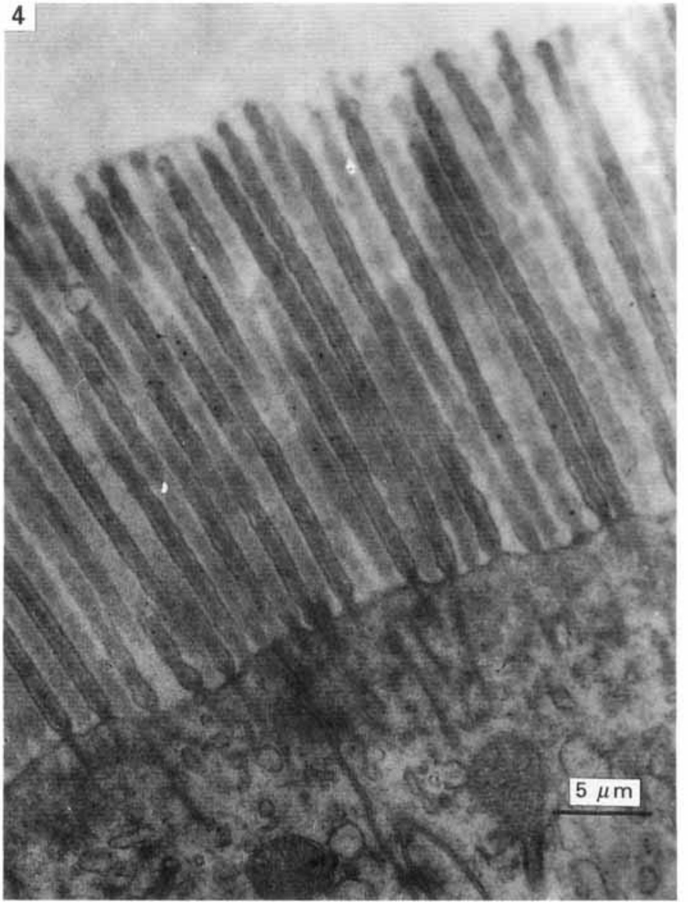
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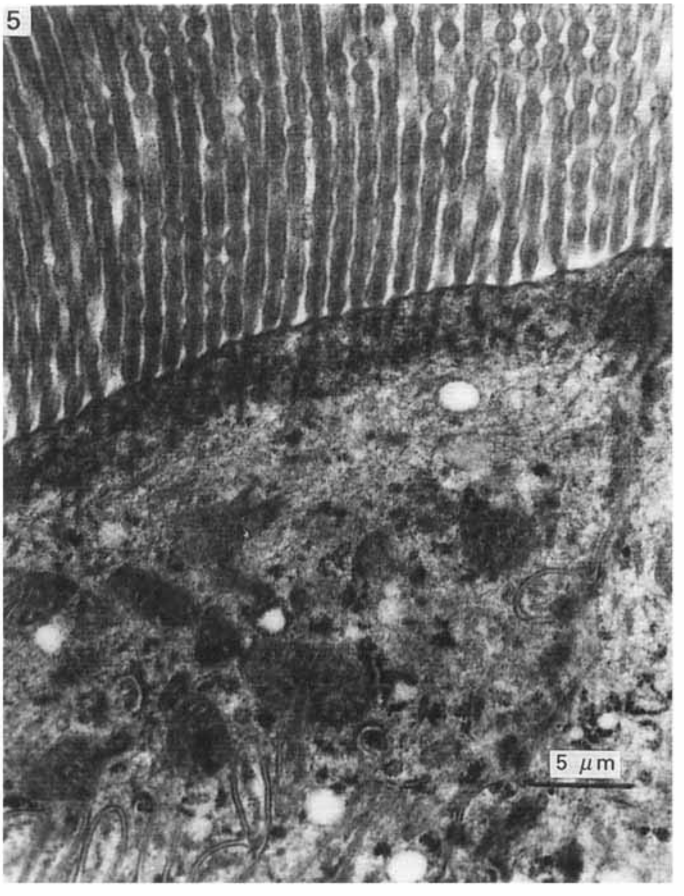
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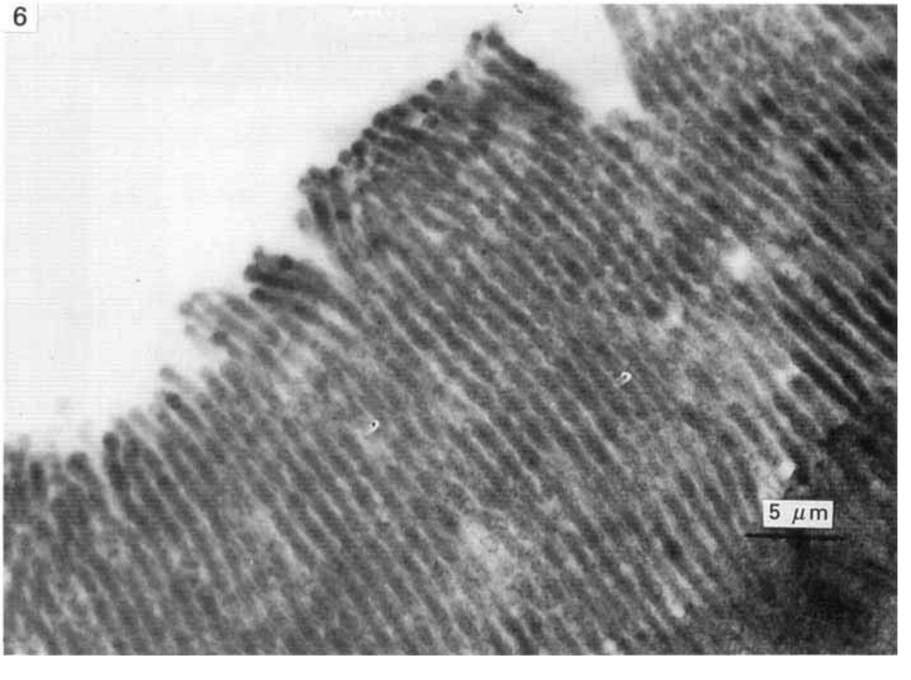
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