Identification of Resistant Pea (*Pisum sativum* L.) Proteins in the Digestive Tract of Chickens

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This study was undertaken to determine if pea (*Pisum sativum* L.) protein structure could explain pea protein digestion. A nitrogen-free (NF) diet and two diets containing either whole ground peas or a globulin fraction purified from peas were fed to 3-week-old chickens. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was used to estimate the relative rates of degradation of proteins subfractions in the gastrointestinal contents of chicks. Proteins were quantified by image analysis of Coomassie blue stained bands. Convicilin disappeared already in the gizzard. Legumin α and vicilin were still present in gizzard but disappeared in jejunum. The polypeptides shown to persist until the end of digestive tract were albumin PA2, lectin, and polypeptides of MW in the range 19500−25000 originating presumably from legumin. An endogenous protein of about 57 000 was observed until terminal ileum. Apparent ileal protein digestibility was high and slightly lower for pea diet (89.5%) than for globulin diet (93.3%). Results suggested that, although some pea proteins appeared less susceptible to hydrolysis, they represented only a small amount at the terminal ileum.

Keywords: *Pea proteins; globulin; chick; digestion; electrophoresis*

INTRODUCTION

Peas are important sources of dietary protein for monogastric animals. Pea protein contents fall into the range 18−30% (Guéguen, 1991). Amino acid composition of pea proteins is characterized by a high lysine level. However, pea proteins are sometimes poorly digested and may exhibit a high variability. For example, protein apparent fecal digestibility of pea seeds may vary between 67 and 83% in poultry (Carré and Conan, 1989) and between 74 and 89% in pigs (Perez and Bourdon, 1992). Such a variability limits the incorporation of this raw material in feed.

The causes of variation of protein digestibility in legume seeds have been investigated and seemed to be multifactorial. Antinutritional factors such as protease inhibitors, lectins and tannins, have been supposed to be implicated. But studies performed on pea flours characterized by various trypsin inhibitor (TI) contents did not demonstrate evident correlation between TI content and digestibility of pea proteins in chickens (Carré and Conan, 1989) as well as in pigs (Perez and Bourdon, 1992) probably because of this multifactorial character. On the other hand, using semisynthetic diets, it was clearly shown that addition of TI reduced the N digestibility in piglets (Leguen et al., 1995). A low digestibility of nutrients can also be due to strong cellular cohesion in pea cotyledons.

Another factor could be protein structure itself as suggested in numerous *in vitro* studies with various legumes (Kakade, 1974; Deshpande and Damodaran, 1989; Nielsen et al., 1988) and recently with peas (Perrot, 1994). But few studies have been reported *in vivo*, particularly in monogastric animals (Aubry and Boucrot, 1986). Pea proteins contain two main groups of proteins, albumins (20−25%) and globulins (55−65%), the latter being composed of two major fractions, vicilin and legumin (Guéguen, 1991). Protein composition is highly variable, and proteins are characterized by different structure. The objective of this study was to follow pea proteins along the digestive tract in birds in order to know if some of them are resistant to hydrolysis *in vivo*.

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MATERIALS AND METHODS

Peas and Protein Source. Pea seeds (*Pisum sativum* L.) were provided by an industrial company (CAAR, Reims, France). Pea seeds were obtained from a spring variety (Messire) without tannin and with low trypsin inhibitor content (2.3 TIU/mg of DM). This characteristic was chosen in order to avoid the potential effect of these antinutritional factors. The pea seeds were ground using a hammer mill fitted firstly with a 4 mm screen, secondly with a 1 mm screen, and thirdly with a 0.5 mm screen. The analysis of particle size distribution was measured with a laser granulometer (AFNOR NF X 11-666, 1984). The mean diameters of pea particles were 30.9 μm.

Globulin fraction was obtained by selective extraction procedure of pea meal (Crévieu et al., 1996). Trypsin inhibitor activity of globulin fraction was very low (0.4 TIU/mg of DM).

Experimental Diets. The pea meal and globulin fraction were used as the only protein source in two experimental diets (pea and globulin diets, respectively). A nitrogen-free experimental diet (NF diet) was also used (Table 1). In each diet, polyethylene glycol (PEG 4000) was included as an indigestible marker, for determining protein digestibility.

Animal and Experimental Scheme. Twenty-one 1-d-old male broilers chickens (Ross) were put in metal cages with continuous lighting until 3 d, and 23 h light/d until 25 d. Temperature was 31 °C until 7 d, 28 °C until 14 d, 26 °C until 21 d, and 24 °C until 25 d. From hatching to day 20, chickens were fed a standard diet for growing birds and had free access to water.

At 17 d, they were divided into three groups of similar mean weight, with seven birds per group, and placed in individual cages. From 21 d, each group of birds received one of the

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Table 1. Composition of Experimental Diets (g kg^{-1})

diet ingredients	pea	globulin	N-free
pea	898.0		
globulin		200.0	
glucose			200.0
maize starch		584.3	584.3
cellulose		110.0	110.0
rapeseed oil	50.0	50.0	50.0
calcium carbonate	10.0	10.0	10.0
bicalcium phosphate	25.0	25.0	25.0
$MgCl_2$, $6H_2O$		4.2	4.2
KCl		3.8	3.8
sodium chloride	4.5	4.5	4.5
mineral mixture[a]	1.0	1.0	1.0
vitamin mixture[b]	5.0	1.7	1.7
L-lysine		1.0	
DL-methionine	1.0	3.0	
robenidine[c]	0.5	0.5	0.5
PEG 4000	5.0	5.0	5.0
total amino acids[d]	152.5	159.3	
protein (N × 5.42)	163.1	170.8	1.6

[a] The mineral mixture supplied (mg/kg of diet): Co, 0.33; Cu, 8.7; I, 1.2; Se, 0.2; Zn, 84, Fe, 44; Mn, 106. [b] The vitamin mixture contained oats (3.3 g/kg of diet) for pea meal and was free of oats for globulin and N-free diets; it supplied the following vitamins (per kg of diet): vitamin A, 10 000 IU; cholecalciferol, 1500 IU; vitamin E, 15 mg; butylated hydroxytoluene, 125 mg; menadione, 5 mg; thiamine, 0.5 mg; riboflavin, 4 mg; calcium pantothenate, 8 mg; niacin, 25 mg; pyridoxine, 1 mg; vitamin B_{12}, 8 μg; folic acid, 1 mg; biotin, 0.2 mg; choline, 0.75 g. [c] Robenz, supplied by Cyanamid, Rungis, France. [d] The amino acids measured are those presented in Table 5. Contents are expressed as deshydrated amino acids.

experimental diets. All diets were mixed with water in the ratio (60 g/40 g, diet/water) and given (36 g of dry matter) twice daily (at 9 and 16 h) by tube feeding. At 25 d, the mean weights (g ± SEM) of birds were 676.1 ± 16.30, 665.1 ± 15.45, and 551.2 ± 26.20 for pea, globulin, and NF diets, respectively.

At 25 d, 4.5 h after the test meal [means (g DM) ± SEM: 42.0 ± 0.64, 43.0 ± 0.91, 41.7 ± 2.11 for pea, globulin, and NF diets, respectively], the chicks were killed with an intracardiac injection of sodium pentobarbital (Sanofi, 1 mL/bird). The small intestine was exposed and divided into four segments: upper and lower jejunum and upper and lower ileum. Gizzard and each intestinal segment were washed out with about 10 mL of physiological saline solution (0.9% (w/v) NaCl) into a small plastic tube cooled in ice. Digesta were frozen immediately after collection and freeze-dried.

Analytical Methods. Nitrogen was determined in diets and intestinal samples by the Kjeldahl procedure (AFNOR, 1985) and by colorimetric assay (Reardon et al., 1966), respectively. Nitrogen to protein conversion factor was taken from that of peas (5.42; Mossé, 1990). PEG was measured in diet and digesta, using a turbidimetric analysis (Malawer and Powel, 1967).

Amino acids (AA) were determined in diets and terminal ileum, using an autoanalyzer (Biotronik, Amino Analyser LC 5000) after acid hydrolysis (HCl, 6 N, for 23 h at 115 °C). Cystine, methionine, and tryptophan were not determined.

Proteins of diets and individual digesta were extracted in boiling 1% SDS by continually stirring (350 rpm) for 20 min. The extracts were clarified by centrifugation at 16000*g* for 10 min and filtrated on 0.2 μm.

SDS extracts were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis in 10−25% gradient gel (Laemmli, 1970), with seven replicates for each diets. Samples were dissolved in buffer sample (62.5 mM Tris, pH 6.8, SDS 4%, glycerol 12%, β-mercaptoethanol 2%, bromophenol blue 0.01%) and heated to 100 °C for 3 min, for reduction of disulfide bonds. Samples were loaded onto the gel, with equivalent amounts of high molecular weight (MW > 15 000) nitrogenous compounds deposited for each track. These amounts were calculated from total nitrogen in the sample and the proportion of high molecular weight (MW > 15 000) determined by size exclusion chromatography (Crévieu et al., in press).

After electrophoresis of gastrointestinal contents of each bird (16 h, 8 mA), proteins (MW > 10 000) were fixed and stained (Crévieu et al., 1996).

For one bird, found representative after electrophoresis stained with Coomassie blue G250, electrophoresis of gastrointestinal contents was electroblotted on nitrocellulose membrane. Gel used for transfer was equilibrated for 15 min at 12 °C in a transfer buffer containing 25 mM Tris, 192 mM glycine, and 20% (vol/vol) methanol. Samples were blotted onto a nitrocellulose membrane (Sartorius AG, Germany) by using a constant intensity setting of 250 mA for 45 min. After transferring, the membrane was incubated for 1 h at 25 °C in blocking solution (1% bovine serum albumin, BSA, in phosphate-buffered saline, PBS). The membrane was incubated in solutions containing dilutions of primary antibodies in a solution of 1% BSA in PBS-Tween 20% (polyoxyethylene sorbitan monolaurate), PBS-T. Specific antivicilin, antilegumin, and antilectin sera were supplied by Dr. Guldager (Guldager, 1978), and serum anti-PA2 and antilectin was raised in rabbits as described by Perrot (1994). The membrane was incubated with the primary antibody for 1 h at ambient temperature. It was washed two times, 10 min per wash, in PBS-T. Bound primary antibodies were labeled with donkey anti-rabbit IgG horseradish peroxidase conjugated secondary antibodies (Amersham, Arlington Heights, IL) diluted 1:50 000 in 1% BSA in PBS-T (vol/vol) for 1 h at ambient temperature. The membrane was rinsed for 10 min in PBS-T and for 10 min in PBS, before detection. A chemiluminescent detection system was used as described by the supplier (Amersham) to detect labeled protein bands.

Gels with blue-stained proteins were used to quantify area of each polypeptide band. Image analysis (E1d software program, INRA Jouy-en-Josas) was used to obtain a profile for each track (Figures 4 and 5). Area of each protein peak relative to the area of total peaks was determined using the Borwin software program (JMBS developments).

Calculations and Statistical Analysis. Apparent protein digestibility (%) was calculated as

$$[1 - (P_{dig}/PEG_{dig})/(P_{diet}/PEG_{diet})] \times 100$$

with P_{dig} and PEG_{dig} being protein and PEG concentrations in digesta dry matter, respectively, and P_{diet} and PEG_{diet} being protein and PEG concentrations in diet dry matter, respectively. The same calculation was done for AA by substituting AA values for the *P* values in the equation. For dry matter digestibility, the calculation was as follows:

$$[1 - (1/PEG_{dig})/(1/PEG_{diet})] \times 100$$

Results were analyzed statistically by one-way analysis of variance followed by test of differences between means by Tukey's multiple-range test (Systat software program Wilkinson, Leland Systat Inc., Evanston, IL 60201).

RESULTS

Nitrogen and PEG content are reported in Table 2. The apparent protein digestibility in the proximal jejunum was similar for the two diets. But, in the ileum, the apparent protein digestibility was higher for globulin than for pea.

Amino acid compositions (AAC) of diets and ileum digesta for pea and globulin diets are shown in Table 3. AAC in ileum displayed higher relative amounts of Thr, Pro, Val, Ser, and Gly and lower amounts of Arg, Glu, and Lys than those found in both diets. A slight increase in hydrophobic AA was observed.

Electrophoretic patterns of protein bands showed different degrees of degradation of individual proteins (Figures 1−3). Convicilin (70 000) disappeared early in the gizzard, whereas vicilin (46 000 and 30000−35500) were still present. In the upper jejunum, no native vicilin was detectable, but a vicilin peptide of about 18000 appeared and was seen in the terminal ileum.

Table 2. Protein, PEG Contents, and Apparent Protein Digestibility Observed in Digesta of the Chicken after the Test Meal

	pea	globulin	N-free	SEM
	Gizzard			
protein (N × 5.42) content (g/kg of dry matter)	87[b]	131[b]	6[a]	16.0
	Upper Jejunum			
Protein (N × 5.42) content (g/kg of dry matter)	124[b]	124[b]	51[a]	9.4
PEG content (g/kg of dry matter)	10.3[a]	10.0[a]	9.0[a]	0.71
apparent nitrogen digestibility (%)	62.5[a]	61.9[a]		3.93
	Lower Ileum			
protein (N × 5.42) content (g/kg of dry matter)	59[b]	57[b]	16[a]	4.3
total amino acids content (g/kg of dry matter)	55[a]	48[a]		3.7
PEG content (g/kg of dry matter)	17.3[a]	25.2[b]	29.4[b]	1.69
apparent nitrogen digestibility (%)	89.5[a]	93.3[b]		0.73
apparent amino acids digestibility (%)	89.5[a]	94.0[b]		0.59
digestibility of dry matter (%)	67.8[a]	77.9[b]	81.2[b]	1.38

[a,b] Means in the same row with different superscript letter were significantly different ($p < 0.05$).

Table 3. Amino Acid Composition (Molar Percent) Determined in the Diet and the Terminal Ileum of Pea and Globulin Fed Birds

	diet		ileum		
	pea	globulin	pea	globulin	SEM
Asp	12.36	12.46	13.02[a]	12.86[a]	0.243
Thr	4.64	3.64	6.33[a]	7.58[b]	0.260
Ser	6.26	6.44	7.92[a]	7.80[a]	0.240
Glu	16.82	19.07	11.90[a]	12.98[b]	0.289
Gly	8.26	6.43	9.28[b]	8.47[a]	0.101
Ala	6.44	5.31	6.85[a]	6.81[a]	0.127
Val	5.13	4.93	6.49[a]	6.44[a]	0.128
Ile	4.54	4.75	5.10[a]	4.87[a]	0.194
Leu	7.73	8.92	8.23[b]	7.70[a]	0.129
Tyr	2.69	2.45	2.77[a]	2.77[a]	0.039
Phe	4.13	4.37	4.51[b]	4.24[a]	0.054
Lys	7.10	6.88	4.97[a]	5.16[a]	0.109
His	2.20	2.13	1.86[a]	1.90[a]	0.051
Arg	6.82	7.43	4.37[a]	4.41[a]	0.094
Pro	4.89	4.79	6.41[a]	6.02[a]	0.136

[a,b] Means in the same row with different superscript letter were significantly different ($p < 0.05$).

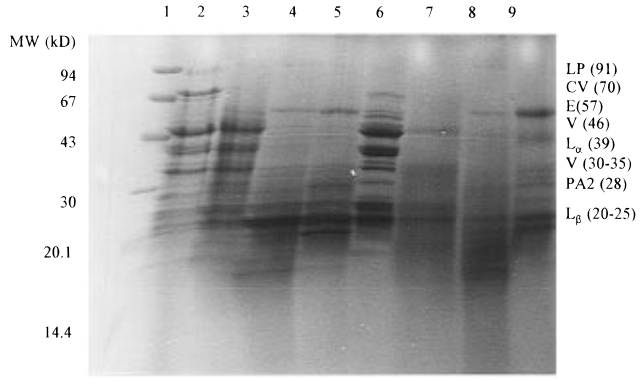

Figure 1. SDS−polyacrylamide gel electrophoresis in reducing conditions (gradient 10−25%) of pea proteins and proteolytic products from gastrointestinal digesta. Lane 1, polypeptide MW standards (Sigma): phosphorylase b (94 000), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), soybean trypsin inhibitor (20 100), α-lactalbumin (14 400). Lane 2, pea diet. Lanes 3−5, digesta of pea diet (gizzard, upper jejunum, lower ileum). Lane 6, globulin diet. Lanes 7−9, digesta of globulin diet. Legend: LP, lipoxygenase; CV, convicilin; V, vicilin; Lα and Lβ, legumin α and β, respectively; PA2, albumin; E, endogenous peptide.

The acidic polypeptide from legumin was present in the gizzard but not in the upper jejunum where it was already hydrolyzed in peptide of about 29 000. This latter peptide was not detectable in the terminal ileum. Polypeptides from legumin of MW in the range 19500−25000 presumably from legumin fractions according to the antibody detection (Figure 3) were found to be present until the end of digestive tract. Albumin PA2 (28 000) appeared until the terminal ileum. Lectin (β-subunit) that was not seen by blue Coomassie stain was detected by the antibody until the end of the digestive tract of pea-fed birds as a band at 18 500 (Figure 3). A single protein band of about 57 000 was observed in intestinal content of all animals, including N-free diet (Figure 2). This protein accumulated in digesta with resistant pea proteins. Other protein bands observed in the jejunum of N-free diet showed no overlapping with the main persistent pea proteins (Figures 1 and 2). Blots performed on digesta of birds fed the N-free diet were all negative.

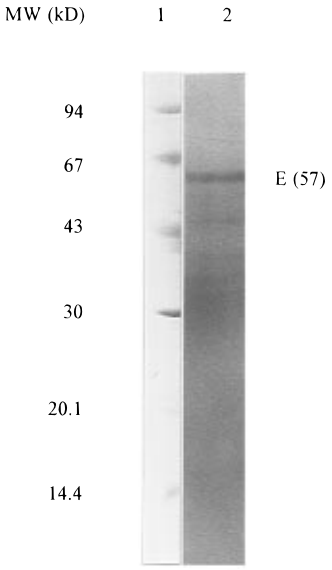

Figure 2. SDS−polyacrylamide gel electrophoresis in reducing conditions (gradient 10−25%) of digesta of a bird fed a N-free diet. Lane 1, polypeptide MW standards (see Figure 1); lane 2, upper jejunum of a bird fed a N-free diet.

Relative protein amounts for diets and ileal digesta were compared using quantification of blue stained protein bands (Figures 4 and 5; Table 4). In diets, globulin appeared to contain more legumin than vicilin when compared to pea proteins. The legumin/vicilin area ratio was 0.73 and 1.54 for pea and globulin diets, respectively. In the ileum, vicilin and legumin α disappeared. High increases were observed in the ileum compared to diets for the polypeptides of small MW (19500−25000), especially for the smallest (19 500) (Table 4). Albumin PA2 represented 8.9% of total proteins in ileum of pea fed birds against 5.1% in diet. Endogenous protein (57 000) was shown to represent 7%.

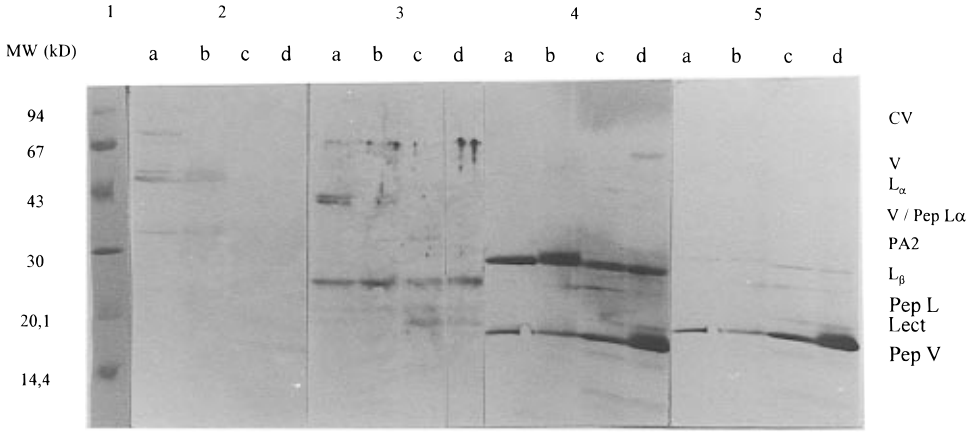

Figure 3. Electroblot analysis of digestion products immunoreactive against pea proteins antibodies in diet and gastrointestinal digesta of an animal consuming pea diet. Lane 1, polypeptide MW standards (see Figure 1). Lane 2, anti-vicilin antibody: (a) diet, (b) gizzard, (c) upper jejunum, (d) lower ileum. Lane 3, anti-legumin antibody. Lane 4, anti-albumin antibody. Lane 5, anti-lectin antibody. Legend: L, lipoxygenase; CV, convicilin; V, vicilin; Lα and Lβ, legumin α and β, respectively; PA2, albumin; Lect, lectin; Pep, peptide.

DISCUSSION

Globulin fraction used in this study was obtained without the precipitating step in order to keep their native structure as much as possible (Crévieu et al., 1996). However, a change in globulin composition was observed (Table 4).

A wide range of susceptibilities to hydrolysis of the major protein components was observed (Figures 1 and 3; Table 4). Convicilin immediately disappeared in the gizzard, which shows a high susceptibility to hydrolysis for this molecule, as already observed with *in vitro* pepsin and trypsin hydrolysis (Perrot, 1994) and in rumen (Spencer et al., 1988; Aufrère et al., 1994). Some polypeptides were detected in the gizzard but were degraded in the upper jejunum. That was the case for vicilin and acidic polypeptides of legumin. Vicilin, a 7S protein, was readily hydrolyzed in the intestine. Pea vicilin differs from phaseolin of *Phaseolus vulgaris*, another 7 S protein that showed high resistance to hydrolysis (Nielsen et al., 1988; Deshpande and Damodaran, 1989). In contrast with phaseolin, pea vicilin contains no disulfide bond (Croy et al., 1980) and is less glycosylated (Wright, 1987). Far-UV circular dichroism studies indicated a great number of β-turn regions in vicilin with a large proportion of lysine and arginine residues in these β-turn regions which provide susceptible bonds for trypsin to act upon (Deshpande and Damodaran, 1989). Contrary to phaseolin, vicilin has two large hydrophilic regions (residues 187−202 and 320−340) which have a high probability of occurring on the protein surface. The average hydrophilicity of vicilin (0.6057) was higher than that of phaseolin (0.3872). This may explain the higher susceptibility of vicilin to proteolysis (Nielsen et al., 1988). Its great susceptibility to hydrolysis could also be due to its high flexibility and readiness to undergo structural changes (Deshpande and Damodaran, 1989). This is in agreement with previous studies using *in vitro* trypsin hydrolysis (Deshpande and Damodaran, 1989; Perrot, 1994) and *in vivo* studies in rumen (Spencer et al., 1988; Aufrère et al., 1994). Legumin was still present in the gizzard. Legumin is known to be destabilized at low pH (Guéguen et al., 1988), which could facilitate its degradation. Retention in the gizzard might be too short for complete proteolysis. However polypeptide α of legumin disappeared at the beginning of the intestine. Degradation of this subunit was shown to be efficient *in vitro* with trypsin (Plumb et al., 1989; Perrot, 1994)

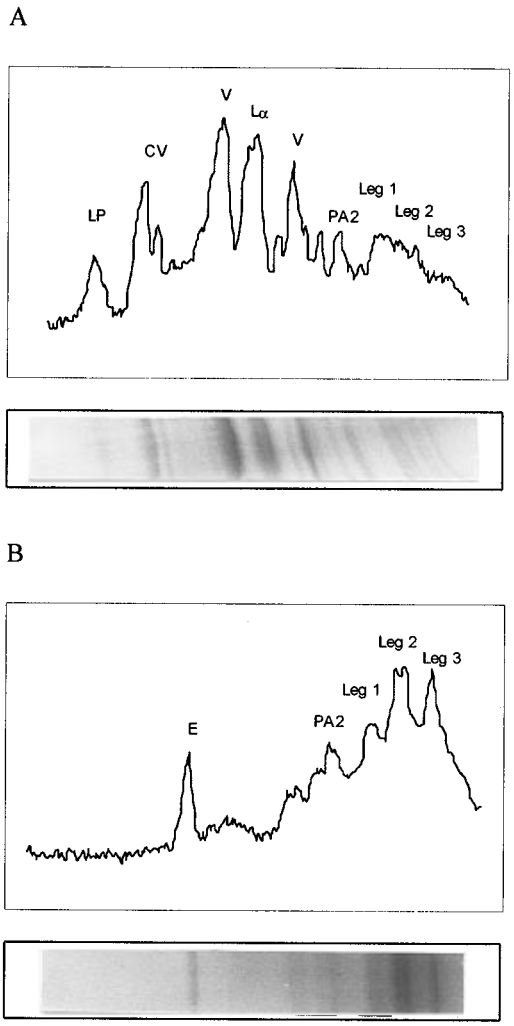

Figure 4. Coomassie blue stained gel and densitometric profile of (A) diet and (B) an ileal digesta, for pea diet. Legend: LP, lipoxygenase; CV, convicilin; V, vicilin; Lα and Lβ, legumin α and β, respectively; PA2, albumin; E, endogenous polypeptide. Legumin peak of low MW is divided in three peaks: Leg 1, Leg 2, Leg 3.

and in rumen as well (Spencer et al., 1988). This could be explained by the position of the acidic polypeptides on the surface of the protein (Guéguen et al., 1988) and thus its higher exposure to enzyme.

However some pea proteins persisted until terminal ileum, as shown by electrophoretic pattern. Polypep-

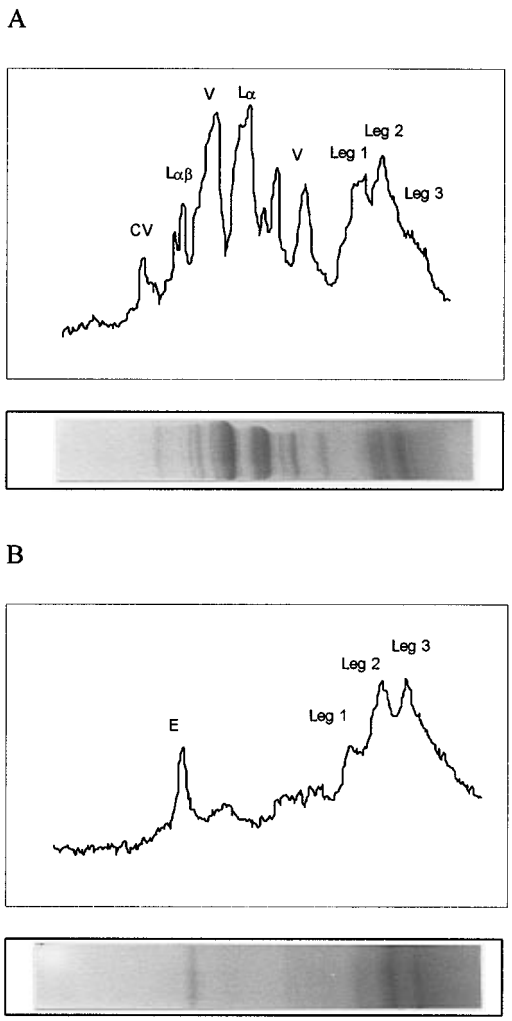

Figure 5. Coomassie blue stained gel and densitometric profile of (A) diet and (B) an ileal digesta, for globulin diet. Legend: CV, convicilin; V, vicilin; L$\alpha\beta$, legumin $\alpha\beta$; Lα and Lβ, legumin α and β, respectively; E, endogenous polypeptide. Legumin peak of low MW is divided in three peaks: Leg 1, Leg 2, Leg 3.

Table 4. Quantitative Analysis of SDS−Polyacrylamide Gel of Pea and Globulin Diets and the Ileum of Birds Fed with These Diets[a]

	pea			globulin		
protein bands	diet	ileum	SEM	diet	ileum	SEM
lipoxygenase (91 000)	4.0					
PA2 (28 000)	5.1^{A}	8.9^{B}	1.21			
convicilin (70 000)	13.1			2.8		
vicilin (46 000)	23.3			19.7		
Vic (30000−35500)	16.2			16.8		
legumin ($\alpha\beta$) (55 000)				6.2		
Leg α (39 000)	17.2			19.4		
Leg 1[b] (25 000)	9.4^{A}	15.6^{A}	2.37	13.5^{A}	12.5^{A}	1.31
Leg 2[b] (21 500)	6.8^{A}	25.0^{B}	1.85	15.6^{A}	21.4^{B}	1.22
Leg 3[b] (19 500)	5.0^{A}	25.9^{B}	1.56	6.0^{A}	32.9^{B}	2.04
endogen (57 000)		7.2			7.0	

[a] Surface of protein band expressed as percent of total protein surface (nonidentified area included in total area). Seven tracks were used for each group (pea diet; pea ileum; globulin diet; globulin ileum). [b] See legend on Figure 4 or 5. [A,B]Means inside a group (pea or globulin) with different superscripts were significantly different ($p < 0.05$).

tides of small MW (19500−25000) accumulated in the ileum. These peptides, probably originating from legumin, could be basic subunits and/or could arise from the cleavage of both acidic and basic subunits as observed in the *in vitro* peptic hydrolysate of soybean glycinin (Kella et al., 1986). However in the case of trypsin hydrolysis of pea legumin, no such overlap of peptides from acidic subunit with basic chains has been observed (Plumb et al., 1989). A resistance of basic polypeptides to enzyme hydrolysis could be explained by the highly ordered structure of β-polypeptide (Subirade et al., 1994) and high hydrophobicity (Lycett et al., 1984). The increase in hydrophobic AA in the ileum (Table 3) is in agreement with the accumulation of the latter polypeptides. Resistance of the β-subunit of legumins was also observed *in vitro* by tryptic digestion (Kamata and Shibasaki, 1978; Plumb et al., 1989) and in the rumen (Spencer et al., 1988). Although these later peptides arise probably from basic subunits, this should be ascertained in further study.

Among albumin, PA2 was shown to persist until the terminal ileum. This protein appeared relatively resistant to breakdown in the rumen (Spencer et al., 1988), during germination (Schroeder, 1984), and during *in vitro* trypsin hydrolysis (Gruen et al., 1987). This resistance could be due to a relatively high cysteine content (Schroeder, 1984) and consequently to the presence of disulfide bridges (Mahadevan et al., 1980). This leads to a tight and globular structure (Gruen et al., 1987).

Lectin was also shown to be not completely degraded and even seemed to resist more than other proteins (Figure 3). Binding of this protein to ligands may protect it from proteolytic breakdown (Pusztai et al., 1991). Its tightness and high content in β-sheet regions (Goldstein and Poretz, 1986) may contribute to its resistance. Lectin was also shown to resist in the rat intestine (Aubry and Boucrot, 1986).

This suggests that pea globulins are, on average, more digestible than pea albumins, which is in agreement with the digestibility values being higher for globulin than for pea diet (Table 2). Even if some protein fractions appeared resistant, the high apparent pea protein digestibilities (Table 2) showed that undegraded proteins represented only low amounts. Moreover, electrophoreses do not take account of low molecular weight peptides that represented high proportions of total nitrogen compounds: it was observed that the proportions of nitrogen compounds with low molecular weight (<15 000) reached 44, 75, and 59% for pea diet, terminal ileum of pea fed birds, and terminal ileum of NF fed birds, respectively (Crévieu et al., in press). For globulin, these proportions were 38 and 72% for diet and terminal ileum, respectively.

An endogenous polypeptide (57 000) appeared in the jejunum and was shown in the ileum (Figures 1 and 2). This endogenous protein seemed to account for only small amounts (Tables 2 and 4). It may be noticed that, among the four AA that displayed the highest relative increase from diet to ileum (Thr, Ser, Val, Pro; Table 3), three of them (Thr, Ser, Pro) are typical of endogenous proteins (Bielorai and Iosif, 1987).

This study shows that, among pea proteins, three fractions were less susceptible to hydrolysis until the end of the digestive tract of birds: albumin PA2, lectin, and polypeptides of MW 19500−25000 probably originating from legumin. This can probably be explained by structural features such as tight structure. However, as seen by high protein digestibility, it can be assumed that these polypeptides represented only small amounts of ingested dietary proteins, in the terminal ileum.

ABREVIATIONS USED

AA, amino acids; AAC, amino acids composition; BSA, bovine serum albumin; MW, molecular weight; PBS, phosphate-buffered saline; PBS-T, phosphate-buffered saline-tween; SDS, sodium dodecyl sulfate.

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