

Origins of variation in pea (*Pisum sativum* L.) protein digestibility in poultry

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Origins of variation in pea protein digestibility in the chicken were studied and an *in vitro* hydrolysis assay was evaluated to select varieties. Eight pea genotypes were selected for their difference in seed protein content and composition. These genotypes varied also in other seed components (carbohydrates, trypsin inhibitor (TI)). After dehulling and micro-grounding, the pea meals were incorporated as the only protein source in isoproteinaceous and isocaloric diets. Amino acid (AA) digestibility was studied in cecectomized chickens. Apparent digestibility (AD) was measured by balance method. True digestibility (TD) and endogenous losses (Endo) were determined after a meal with ¹⁵N-labelled peas. Data were subjected to analysis of variance, and compared using Student-Newman-Keuls test.

The 8 diets showed an average AD for all AA varying between 79.5 and 86.3%. This digestibility was negatively correlated with insoluble fibre content and TI activity. These differences in AD can be explained by the differences in Endo and in TD of pea proteins. Endo varied among genotypes between 3.6 and 5.4% of ingested AA. They were positively correlated with soluble carbohydrate content and TI activity. The TD varied among genotypes, between 84.4 and 90.2%. It was correlated with major protein fractions. These results emphasize the need for a characterization of the two components of AD, for a better understanding of the sources of digestibility variation, and a more effective selection of improved genotypes. This selection of genotype of high TD potential may be performed using an *in vitro* hydrolysis method involving first pepsin then trypsin and chymotrypsin.

Keywords: *Pisum sativum* L ; amino acids, digestibility, endogenous losses

Introduction

In Europe, leguminous seeds such as peas can be used as source of proteins to reduce the use of soyabean meal in feed. However, the variability of pea protein digestibility (Grosjean et al., 1999) reduces pea seed incorporation in diets. Various factors have an effect on protein digestion, such as tannins (Longstaff and McNab, 1991) or particle size (Créviu et al., 1997a). Moreover, variability of apparent protein digestibility (AD) may be due to variations in true digestibility (TD) or in intestinal endogenous protein losses (Endo) (digestive secretions, mucoproteins, desquamated epithelial cells, microbial proteins). Various factors could be involved in the variation of the Endo and the TD, such as the seed composition, in particular its protein composition.

In this work, we studied the protein digestibility in cecectomized chickens, of 8 dehulled pea genotypes with different protein composition, and also varying in other characteristics (carbohydrates, trypsin inhibitors (TI)). We determined the total Endo (basal and specific) by the ^{15}N dilution method. Moreover, we evaluated an *in vitro* protein hydrolysis procedure to find a tool to select genotypes with high digestibility potential.

Materials and methods

Eight pea genotypes were field grown at INRA Dijon, of which a part was labelled with ^{15}N . To limit the variation of protein digestion due to tannins and particle size, seeds were dehulled and micro-ground to break the cell walls. These pea meals were assayed for protein (Nx6.25), amino acids (AA), starch, insoluble cell walls, soluble carbohydrates, tannins, TI and dry matter (DM). The protein composition was evaluated by chromatography (Baniel et al., 1998). Peas were used as the only protein source in isoproteinaceous diets (19.5%) and were thus incorporated at variable rates (68 to 93%) according to their protein content. The diets were also isocaloric (2950 to 3030 kcal/kg). Labelled diets were formulated by substituting unlabelled seeds with labelled seeds; they also contained chromic oxide (Cr, 5g/kg) as an indigestible marker. These diets were pelleted in conditions minimizing biochemical changes of protein.

48 cecectomized male broiler chickens, housed in individual cages (6 blocks x 8 birds) were fed the 8 experimental pea diets. Each chicken was fed 2 different pea diets during 2 successive periods (Period 1: 18 to 25 d and Period 2: 25 to 32 d). Thus, each pea diet was consumed by 12 birds. Each experimental period consisted of 4 days adaptation, followed by 4 days of measurements composed of a digestive balance trial followed by a short-term feeding (1 h) with the ^{15}N labelled diets containing Cr, and hourly collections of excreta for 9 h. To determine the AD of AA, excreta of two animals collected during the digestive balance trial were pooled on basis of a similar AD of nitrogen. The coloured samples of the hourly collections after the ^{15}N labelled diet containing Cr were analyzed for total nitrogen and ^{15}N enrichment. The samples were then pooled for each bird, excluding those from early or late collections with the lowest enrichment values, and assayed for total nitrogen, 9 AA, their ^{15}N enrichments, Cr and uric acid. The total ^{15}N was quantified by an elemental analyzer (Carlo Erba, NA 1500) interfaced with an isotope ratio mass spectrometer (Micromass, Optima). The ^{15}N -labelled AA were determined by GC-combustion-isotope-ratio mass spectrometry.

The *in vitro* hydrolysis of proteins was performed with pepsin (3 h) followed by trypsin and chymotrypsin 15 min or 2 h. The enzymatic reaction was stopped by addition of trichloroacetic acid (TCA) and the TCA-soluble peptides (PM < 3 kDa) were quantified.

AD of AA was calculated from the digestive balance trial : $100 \times \{ (AA_i \times DM_i - AA_e \times DM_e) / AA_i \times DM_i \}$ where DM_i and DM_e were the amount of ingested and excreted DM, AA_i and AA_e the amounts of AA in g/kg DM, in the diet and excreta respectively. The Endo determined with ^{15}N -labelled diets were calculated for each of 9 AA, in percent of AA intake : $100 \times \{ (Cr_i / AA_i) / (Cr_e / AA_e) \times [1 - (AA_e^{15}\text{Nenr.}) / (AA_i^{15}\text{Nenr.})] \}$ where Cr_i and Cr_e were the amounts of Cr, in g/kg DM, in the diet and excreta respectively, $AA_e^{15}\text{Nenr.}$ and $AA_i^{15}\text{Nenr.}$ were the ^{15}N enrichment, in atom percent excess, for N from each AA in the excreta and diet respectively. Correction of AD of AA for total Endo gives the TD calculated as the digestibility of the ^{15}N of each AA from the labelled meal, in percent of AA intake: $100 \times \{ 1 - Cr_i / (AA_i \times AA_i^{15}\text{Nenr.}) / [Cr_e / (AA_e \times AA_e^{15}\text{Nenr.})] \}$. The results were presented for the all measured AA.

AD, TD and Endo were compared by analysis of variance with two factors (period, genotype). When no interaction between the period and the pea genotype were observed, the results were presented according to the period or the genotype. The means were then compared using the Student-Newman-Keuls test ($p \leq 0.05$). Correlation coefficients were calculated between AD, TD or Endo and pea diet characteristics or the results of *in vitro* hydrolysis.

Results and discussion

After dehulling, only two pea genotypes showed traces of tannins, VavD265 and E344, 0,16% and 0.02% of DM respectively, because of an incomplete dehulling. The TI activity ranged from low to intermediate values (1.9 to 6.8 TIU/mg). Protein content varied between 24.0 and 32.4%. Seed protein composition ranged from 10 to 14% for the albumin PA1, from 22 to 29% for PA2, from 15 to 20% for vicilin and 23 to 36% for legumin. Starch content varied between 45.5 and 54.2%, insoluble cell walls between 7.1 and 9.5%, and soluble carbohydrates (including mainly the α -galactosides, and sucrose) between 4.3 and 6.8%. As peas were incorporated in different amounts in the experimental diets, contents of the various components were calculated for each diet: The insoluble cell walls varied between 4.6 and 6.9%, soluble carbohydrates between 2.6 and 5.1% and TI content between 1.3 and 4.9 TIU/mg.

Average AD of AA, as well as Endo and TD showed no interaction between balance period and pea genotype (Table). There was no effect of period on AD and TD, while higher Endo was observed during Period 2. For AD, TD and Endo, an effect of pea genotype was observed.

Table . Amino acid digestibility (apparent, true) and endogenous losses of the 8 pea genotypes

		Apparent digestibility (%) ¹	Endogenous losses (%) ^{2,3}	True digestibility (%) ³
Period	Period 1	83.1	4.06 ^b	87.3
	Period 2	83.8	4.56 ^a	88.1
	SEM	0.73	0.132	0.49
Genotype	China	81.4 ^{ab}	5.11 ^a	84.4 ^{bc}
	Finette	86.0 ^a	3.99 ^b	90.2 ^a
	Sommette	83.8 ^{ab}	3.94 ^b	89.7 ^a
	Caméor	86.3 ^a	4.02 ^b	88.5 ^a
	VavD265	83.0 ^{ab}	3.64 ^b	87.2 ^{ab}
	Préclamex	85.8 ^a	4.28 ^b	89.4 ^a
	E344	81.9 ^{ab}	4.15 ^b	84.8 ^c
	Ballet	79.5 ^b	5.41 ^a	87.3 ^{abc}
	SEM	1.17	0.218	0.79
	Probability	Period	0.42	<0.01
Genotype		<0.01	<0.01	<0.01
Period x Genotype		0.56	0.57	0.84

¹ Average of 17 aa (Ala, Arg, Asp, Cys, Glu, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val) ; ² Expressed in percent of amino acid ingested by pea fed birds ; ³ Average of 9 aa (Ala, Asp, Ile, Leu, Lys, Phe, Pro, Thr, Val)

The average AD for all AA of the 8 dehulled pea genotypes varied between 79.5 and 86.3% (Table), and corresponded to the variations reported in the chicken, from 73 to 89% with whole seeds of pea without tannin (Grosjean et al., 1999). The AD was negatively correlated with the insoluble cell walls ($R=-0.71$, $p<0.05$), which confirms their negative effect on the digestion (Longstaff and McNab, 1991). A negative correlation was also observed between the AD and TI activity ($R=-0.93$, $p<0.001$), in agreement with the study of Wiseman et al. (2003). This negative effect seemed to be due to the increase in Endo. In practice in poultry feed, as peas are not dehulled, factors due to hull (fibre, tannin) can also intervene in variation in AD.

The average Endo ranged from 3.6 to 5.4% of ingested AA (Table), suggesting specific losses according to the pea genotype as observed previously in the pig (Hess, 1999). The Endo represented between 20 and 43% of excreted AA, thus a significant nutritional cost for the animal due to the energy and AA used to synthesise endogenous proteins from which they originated as observed in the pig (Lahaye et al., 2004). Average Endo of AA were positively correlated with soluble carbohydrate content ($R=0.77$; $p<0.05$) and TI activity ($R=0.84$; $p<0.01$). The stimulating effect of soluble carbohydrate mainly composed of α -galactosides may be due to their fermentation by the digestive flora due to the lack of α -galactosidase in the intestine of the chicken. The positive effect of TI could be explained, in part, by the stimulation of pancreatic secretion.

The average TD of AA varied between 84.4 and 90.2% (Table), and was positively correlated with the PA2 albumin level ($R=0.71$; $p<0.05$) and negatively with the legumin level ($R=-0.72$; $p<0.05$). The positive correlation with the PA2, suggested that this protein of tight and globular structure, detected in the digestive contents (Créviu et al., 1997b), would not be a major resistant peptide as it

has been observed in the pig (Le Gall et al., 2005). The negative correlation with the legumin was in agreement with the resistance to hydrolysis of the β -subunits of legumin, which could be due to the highly ordered structure of these polypeptides and to their high hydrophobicity (Créviu et al., 1997b). No correlation were observed with vicilin even though this protein fraction is very well hydrolysed (Créviu et al., 1997), nor with the PA1, even though this protein fraction is very resistant to hydrolysis (Gall et al., 2005). The lack of correlation could be due to the presence of peptides of different resistance in the peaks of chromatography. A finer characterization of pea protein composition could allow to refine the relations between protein composition and TD.

Various *in vitro* methods have been developed to predict *in vivo* protein digestibility. In this study, with the combined hydrolysis with pepsin (3 h), followed by trypsin and chymotrypsin (15 min), a positive correlation was observed with the average TD of 9 AA ($R=0.74$, $p<0.05$). A longer hydrolysis with trypsin and chymotrypsin (2 h) led to the disappearance of the correlation. This system of hydrolysis could thus be a tool to select pea genotypes with a high *in vivo* TD, but it requires optimization to maximize correlation with the *in vivo* digestibility.

In conclusion, the variations of AD of AA of various dehulled pea genotypes were due to differences in Endo and in TD. In this study, the Endo were positively correlated with soluble carbohydrates and TI activity, while the TD was correlated with some major protein fractions. This TD could be predicted by an *in vitro* method to select pea genotypes with good digestibilities.

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