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ScienceDirect
Livestock Science 113 (2008) 262–273
LIVESTOCK SCIENCE
www.elsevier.com/locate/livsci

# Variation in seed protein digestion of different pea (*Pisum sativum* L.) genotypes by cecectomized broiler chickens: 2. Relation between *in vivo* protein digestibility and pea seed characteristics, and identification of resistant pea polypeptides

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Received 12 October 2006; received in revised form 2 April 2007; accepted 4 April 2007

**Abstract**

Eight pea genotypes characterized for their major protein fractions were used to investigate the effect of seed protein composition variability on protein digestibility in poultry. These genotypes of various pea types, were also variable in other seed components. They showed variations in their carbohydrate (insoluble fibre compounds, soluble fibre, soluble carbohydrates) and trypsin inhibitor (TI) contents. To exclude the effect of tannins and of particle size, the seeds were dehulled and micro-ground. They were incorporated as the only protein source in isoproteinaceous diets with similar metabolisable energy content and fed to cecectomized chickens. The average amino acid digestibility (apparent and true) and endogenous amino acid excretion were related with pea diet characteristics (protein composition, carbohydrate composition and TI activity). This allowed to precise which of the diet characteristics affect protein digestibility and endogenous excretion. Average apparent digestibility of amino acids was negatively correlated with insoluble fibre components ($R=-0.71$ to $-0.72$; $p<0.05$) and TI activity ($R=-0.93$; $p<0.001$). Average endogenous losses of amino acids were positively correlated with soluble carbohydrate content ($R=0.77$; $p<0.05$) and TI activity ($R=0.84$; $p<0.01$). Average true digestibility of amino acids was positively correlated with the PA2 albumin level ($R=0.71$; $p<0.05$), and negatively with the legumin level ($R=-0.72$; $p<0.05$). Resistant peptides extracted from chicken excreta were analysed through electrophoresis and identified by immunodetection. Intensity of detected resistant peptides showed variation among genotypes. However, for the 8 pea genotypes, the pea proteins, which persisted at the end of the digestive tract, were mainly albumin PA1b and lectin. Other minor peptides were also detected: vicilin, albumin PA2 and legumin peptides which migrated at the same level as β-subunits.


*Keywords:* Broiler chickens; *Pisum sativum*; Pea seed characteristics; Trypsin inhibitor; Protein digestibility; Resistant pea proteins

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doi:10.1016/j.livsci.2007.04.005

## 1. Introduction

For feed manufacturers, it is important that raw material have high and reliable protein nutritional values. Variability in seed protein digestibility contributes to the limitation of inclusion of meal obtained from seeds in feeds. In peas, variations in protein digestibility have been observed both in poultry and in pigs (Conan and Carré, 1989; Igbasan et al., 1997; Grosjean et al., 1998, 1999; Gabriel et al., in press). There are several causes for this variability. Most seeds used in feeds are variable in composition. Some factors are known to have a negative effect on protein digestibility such as tannins (Grosjean et al., 1999), carbohydrates (Longstaff and McNab, 1991; Gdala et al., 1997), trypsin inhibitors (TI) (Huisman and Jansman, 1991) or particle size (Crévieu et al., 1997a). Variations in protein composition and structure are also involved. The composition of pea seed protein presents variability due to both genotype and environment (Burstin and Duc, 2006). Pea seed proteins have been classified in three main groups according to their solubility: water soluble albumins represent around 20–25% of seed proteins, salt-soluble globulins represent 55–65% and insoluble proteins represent around 15–20% (Guéguen, 1991). The albumin fraction contains very diverse proteins: the major ones, 11 kDa PA1 and 48–53 kDa PA2, and also lipoxygenase, glycosidases, TI and lectins. The globulin fraction includes two major storage protein groups encoded by multigene families and differing in their sedimentation coefficients: vicilins and convicilins (7S) and legumins (11S). Vicilins and convicilins are trimeres of 150 to 180 and 210 to 280 kDa respectively, composed of heterogeneous and differently matured polypeptides. Legumins are compact hexameres of 350 to 400 kDa associating acidic α-polypeptides and basic β-polypeptides. These proteins show various resistance to hydrolysis (Spencer et al., 1988; Crévieu et al., 1997b; Le Gall et al., 2005) as well as different amino acid composition, particularly with regard to sulphur-rich amino-acids which are rare in vicilins and high in albumins PA1 and PA2 (Gwiazda et al., 1980).

In a previous study, we assessed the variability of endogenous amino acid excretion as well as true amino acid digestibility using the $^{15}N$ dilution method, with seed meals from eight pea genotypes fed to cecectomized growing chickens (Gabriel et al., in press). Pea genotypes were chosen for their difference in protein composition. Thus they were of various types of peas (feed peas, garden peas and fodder peas) and therefore they also differed in their carbohydrate content and TI activity. To limit variation due to known factors such as tannins and particle size, seeds were dehulled and micro-ground. The objective of the present study was to identify factors involved in the variability of amino acid digestibility and endogenous losses. We analysed the chemical composition, in particular protein composition, of each pea seed meal, in order to relate this composition to *in vivo* digestibility and endogenous losses. Moreover, resistant pea proteins extracted from chicken excreta were separated through sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and identified by immunodetection.

## 2. Materials and methods

### 2.1. Characterization of seed composition for the different pea genotypes

Eight pea lines (cv 'Ballet', cv 'Caméor', China, E344, cv 'Finette', cv 'Préclamex', cv 'Sommette', VavD265) were field grown at INRA Dijon and harvested in the summer 2002. These genotypes have previously been described by Baranger et al. (2004). As two genotypes (VavD265 and E344) contained tannins, seeds from the 8 pea genotypes were dehulled (CREOL, Pessac, France). For each genotype, a seed sample was ground through a 0.5-mm screen and assayed for total nitrogen by the Kjeldahl procedure (ISO, 1997), and a factor of 6.25 was used to calculate the crude protein content. The pea samples were also assayed for amino acids (AFNOR, 1998a,b), starch (European Directive, 1999), soluble and insoluble fibre (AOAC, 1995), insoluble cell walls (AFNOR, 1998c), cellulose (AFNOR, 1993), soluble carbohydrates (European Directive, 1971), ash (AFNOR, 1977), tannins (INZO, 1999), TI (method of Kakade et al. (1974) modified by Valdebouze et al. (1980)) and dry matter (AFNOR, 1982). The protein composition was evaluated by fast protein liquid chromatography (FPLC) as described by Baniel et al. (1998). The relative quantity of each protein fraction was estimated by the ratio of the area below its corresponding peak on the chromatograms to the total area below the chromatogram curve.

### 2.2. Experimental design

Peas were used as the only protein source in experimental diets. The diets were formulated to be isoproteinaceous (19.5%) and to contain similar metabolisable energy (2950 to 3030 kcal/kg). Carbohydrates and antinutritional factors content in the diets were