

## *In vivo* and *in vitro* protein and amino acid digestibility of legume seeds in pig diets

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### ABSTRACT

The apparent ileal protein and amino acid digestibility was determined in legume seeds by *in vivo* and *in vitro* procedures. For both estimations 12 semisynthetic diets containing 3 varieties of faba bean, 3 varieties of pea and 6 varieties of different lupin species were used. For *in vivo* determinations cannulated pigs were used and *in vitro* analyses were performed as described by Boisen and Fernández (1995). The relationship between *in vitro* predicted and *in vivo* values of protein digestibility was close ( $r^2 = 0.90$ ). The highest correlation between *in vitro* predicted and *in vivo* values of amino acid digestibility was found for cystine and methionine ( $r^2 = 0.94$  and  $0.89$ , respectively). The lower correlation was obtained for lysine ( $r^2 = 0.76$ ) and poor correlation for threonine ( $r^2 = 0.43$ ). It was possible to predict with satisfactory precision the apparent ileal digestibility of protein, cystine and methionine in legume seeds using *in vitro* technique.

KEY WORDS: legumes, digestibility, protein, amino acids, *in vivo*, *in vitro*

### INTRODUCTION

Legume seeds are good source of protein in pig diets. However, legumes contain also antinutritional factors (e.g., tannins, trypsin inhibitors), which have negative effect on digestive processes (Huisman, 1990). In recent years several different *in vitro* methods have been developed to estimate protein and amino acid digestibility in pig feeds. The validation of these methods have been attempted by relationship between *in vitro* and *in vivo* results (e.g., Graham et al., 1989; Babin-szky et al., 1990; Cone and van der Poel., 1993; Boisen and Fernández, 1995).

The purpose of the present study was to compare *in vivo* (apparent ileal digestibility in pigs) and *in vitro* (predicted apparent ileal digestibility calculated from

*in vitro* results) values of protein and amino acid (lysine, methionine, cystine and threonine) digestibilities in faba bean, pea and lupin.

## MATERIAL AND METHODS

### *Feeds*

Twelve semisynthetic diets were used for the study: three diets with faba bean (Alen, Kamir, Tibo), three diets with pea (Kwestor, Sol, Vatra) and six diets with lupin (white: Bardo, Wat; yellow: Polonez, Radamez, Manru; narrowleaved: Emir). Diets with faba bean and pea contained legume seeds as a single source of protein. Diets with lupin contained also casein, which protein accounted for 70.6 % of total protein in diets with white lupin and 29.4 % of total protein in other diets. Protein level in the diets ranged from 184 to 211 g kg<sup>-1</sup>DM. Samples of diets used in trials with pigs were stored deep-frozen until *in vitro* assays were performed.

### *Methods*

The *in vivo* digestibility trials were performed with pigs within a body weight range from 30 to 60 kg as described by Buraczewska et al. (1999). The developed by Boisen and Fernández (1995) *in vitro* method was used for prediction digestibility of protein and amino acids at the ileal level. The pre-caecal digestion was simulated by two consecutive incubations corresponding to the digestion in the stomach and in the small intestine: with pepsin at pH 2.0 for 6 h and with pancreatin at pH 6.8 for 18 h at 39°C. The predicted apparent ileal digestibility of protein (pdN) and amino acids (pdAA) were calculated from *in vitro* values after correction for endogenous losses of protein and amino acids using regression equations. All *in vitro* data are mean values of two measurements made in different series.

## RESULTS AND DISCUSSION

Calculated values of predicted apparent ileal digestibility of protein from *in vitro* results (pdN) were higher than the corresponding *in vivo* values (dN) for faba bean, pea and white lupin-casein mixtures. For yellow and narrowleaved lupin-casein mixtures dN was higher than pdN (Table 1). As it is shown in Table 2, the relationship between pdN and dN for all samples was close ( $r^2 = 0.90$ ). Boisen and Fernández (1995) obtained poor correlation between pdN and dN ( $r^2 = 0.57$ ) for 48 feed mixtures, but high ( $r^2 = 0.92$ ) for 15 feedstuffs. Low relationship ( $r^2 = 0.23$ ) for 48 different feedstuffs and no linear relationship for 14 samples of pea ( $r^2 = 0.04$ )

TABLE 1

Apparent ileal digestibility values of protein and amino acids in legume seeds determined *in vivo* (d) and predicted (pd) with an *in vitro* method, %

	Protein		Lysine		Methione		Cystine		Threonine	
	d	pd	d	pd	d	pd	d	pd	d	pd
Faba bean										
Alen	70.3	78.1	81.5	85.9	61.6	79.1	56.3	76.1	66.4	75.8
Kamir	71.3	77.3	84.2	85.1	64.8	78.3	55.9	74.4	68.5	75.3
Tibo	68.3	75.9	78.4	84.2	55.1	77.3	51.7	73.7	66.7	73.5
Pea										
Kwestor	71.9	78.3	81.9	86.5	61.9	81.3	55.3	77.4	62.4	76.6
Sol	70.7	78.1	79.4	86.6	54.8	81.6	54.8	77.7	59.6	76.7
Vatra	69.9	72.6	80.2	83.0	56.4	77.2	51.9	73.8	60.9	81.9
Lupin+casein										
Bardo	86.4	87.0	91.9	90.3	92.8	89.4	74.5	83.7	83.5	84.0
Wat	86.3	86.9	89.3	90.2	92.5	89.4	77.8	83.7	81.1	84.1
Polonez	85.1	84.5	88.5	88.7	88.7	87.0	83.0	84.6	78.5	79.7
Radamez	85.6	84.1	89.3	88.3	87.7	86.1	84.6	85.1	79.5	78.8
Manru	85.1	84.9	89.4	88.9	88.3	87.9	82.4	85.0	79.1	80.5
Emir	87.4	85.2	93.0	89.1	92.9	87.1	78.5	83.3	84.2	81.7

between *in vitro* and *in vivo* data was found by Cone and van der Poel (1993) using similar *in vitro* technique.

The closest linear relationship between predicted *in vitro* and *in vivo* estimated values of amino acid digestibility (pdAA and dAA) was found for cystine and methionine ( $r^2 = 0.94$  and  $0.89$ , respectively). The lower correlation was obtained for lysine ( $r^2 = 0.76$ ) and poor correlation for threonine ( $r^2 = 0.43$ ). For faba bean and pea, all predicted *in vitro* results of lysine, methionine, cystine and threonine digestibilities were higher than the *in vivo* values. In contrast, for lupin-casein mixtures the *in vitro* and *in vivo* values were similar. Boisen and Fernández (1995)

TABLE 2

The relationship between *in vivo* (d) and predicted with *in vitro* method (pd) values of apparent ileal digestibility of protein and amino acid in legume seeds

	Equation	$r^2$	$p \leq$
Protein	$dN = -52.3 + 1.61 \text{ pdN}$	0.90	0.0001
Lysine	$dLys = -81.3 + 1.91 \text{ pdLys}$	0.76	0.001
Methionine	$dMet = -198.4 + 3.27 \text{ pdMet}$	0.89	0.0001
Cystine	$dCys = -159.0 + 2.83 \text{ pdCys}$	0.94	0.0001
Threonine	$dThr = -65.5 + 1.75 \text{ pdThr}$	0.43	0.05

found poor correlation for lysine, methionine and threonine ( $r^2 = 0.65, 0.55, 0.53$ , respectively) and no linear correlation for cystine ( $r^2 = 0.05$ ) for 48 feed mixtures.

## CONCLUSIONS

It seems possible to predict with satisfactory precision apparent ileal digestibility of protein, cystine and methionine in legume seeds using *in vitro* technique. However, the studies should be continued to improve *in vitro* method for better estimation of amino acids availability from feedstuffs containing different antinutritional factors.

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## STRESZCZENIE

### Strawność *in vivo* i *in vitro* białka i aminokwasów nasion roślin strączkowych w dietach dla świń

Pozorną jelitową strawność białka i aminokwasów w nasionach roślin strączkowych oznaczono metodą *in vivo* i *in vitro*. Do badań użyto 12 półsyntetycznych mieszanek, które zawierały 3 odmiany bobiku, 3 odmiany grochu i 6 odmian łubinów z dodatkiem kazeiny. Pozorną jelitową strawność *in vivo* białka i aminokwasów oznaczono na przetokowanych świiniach. Oznaczenia *in vitro* wykonano według metody Boisen i Fernández (1995). Korelacja pomiędzy oszacowaną *in vitro* i oznaczoną *in vivo* pozorną jelitową strawnością białka była wysoka ( $r^2 = 0.90$ ). Wysoką korelację pomiędzy przewidywaną *in vitro* i oznaczoną *in vivo* strawnością jelitową aminokwasów stwierdzono dla cystyny i metioniny (odpowiednio  $r^2 = 0.94$  i  $0.89$ ). Słabszą korelację uzyskano dla lizyny ( $r^2 = 0.76$ ), a najslabszą dla treoniny ( $r^2 = 0.43$ ). Badania są kontynuowane w celu uzyskania metody *in vitro*, która pozwoliłaby na dokładniejsze oszacowanie dostępności aminokwasów z pasz zawierających różne czynniki antyodżywcze.