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Prececal and cecal *in-vitro* digestibility of tropical legume grains for pig nutrition

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Introduction

In rural areas of many tropical countries, pig production is a livelihood for smallholder farmers, who use the pigs as sources of income and food. The benefit of this livelihood is sometimes limited by high costs or the absence of balanced commercial diets, which often results in preparing diets with ingredients low in protein but high in fibre (e.g. banana, harvest by-products) (Peters et al., 2006). Thus, we investigated the use of alternative local ingredients such as legume grains, which could be an interesting source of protein as well as starch, vitamins and minerals (D'Mello, 1995). The nutritional value of legume grains is highly dependent upon their antinutritional components (e.g. trypsin and α-amylase inhibitors), resistance of their proteins to breakdown and the limitation in sulphur-amino acids and tryptophan. Therefore, in the present work the nutritional characterisation of raw tropical legume grains (*Vigna unguiculata*, *Lablab purpureus*, *Canavalia brasiliensis*) was studied in terms of chemical composition, *in-vitro* protein and starch digestibility and *in-vitro* fibre fermentation.

Material and Methods

Legume grains were supplied by the Tropical Forages Program of the International Center for Tropical Agriculture (CIAT, Colombia): *Canavalia brasiliensis* (CB), *Lablab purpureus* (LP) and three varieties of *Vigna unguiculata*; 1088-4, 9611 and 625 (pink PVU, red RVU and white WVU colour hulls respectively). Extruded full-fat soybean (*Glycine max*, SB) was used as a control and was obtained from the regional market. Seeds were ground using a 0.5 mm mesh-screen and analyzed for protein (N x 6.25) (Kjeldahl method), starch, trypsin inhibitory activity (TIA), dry matter, ether extract, ash, neutral and acid detergent fibre (NDF and ADF), lignin and gross energy. The residues recovered after fermentation were analysed for DM and NDF.

***In-vitro* protein hydrolysis:** Samples were mixed with HCl (0.1 N, pH 2.0) and pepsin was added to the medium (1:67 enzyme:protein). Aliquots were taken after 0, 30 and 120 min of pepsin hydrolysis. Phosphate buffer saline (0.2 M, pH 8.0) was mixed (1:1) with the remaining incubation media and pancreatin was added (1:30 enzyme:protein). Aliquots were taken 20, 120 and 240 min after pancreatin addition (i.e. at times 140, 240 and 360 min after pepsin addition) (Montoya et al., 2008). Free amino groups (NH_2) were determined in aliquots using the o-phthaldialdehyde (OPA) method (Church *et al.* 1983). Each aliquot was immediately mixed with the OPA solution and the optical density measured 2 min later at 340 nm. The degree of

hydrolysis (DH) was calculated as the ratio between free amino (NH_2) groups and total amino groups in the starting material.

***In-vitro* enzymatic hydrolysis and fermentation:** The protocol for *in-vitro* prececal hydrolysis was carried out according to the method proposed by Bindelle et al. (2007). The indigestible residue obtained after enzymatic hydrolysis (pepsin 2h and pancreatin 4h) was used to determine the digestibility of DM and starch. The remaining residue was used for the fermentation at 37°C (simulation of large intestine fermentation; Bindelle et al., 2007). Short-chain fatty acids (SCFA), DM and fibre fermentation were determined in aliquots 72 h after incubation. Aliquots for SCFA determination were deproteinized and the concentration measured using a HPLC (CL-10A, Shimadzu) at 210 nm. A completely randomized design was used for all the statistical analyses. Analyses were performed using the Mixed Model procedure of SAS (SAS/STAT Version 9.1, SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Nutritional composition and *in-vitro* digestibilities: Protein and starch content were the main nutritional components in the tropical legume grains (Table 1). CB and SB had the highest protein content (291 and 367 g/kg DM respectively) and cowpea varieties the lowest (average 212 g/kg DM). The starch content ranged from 316-537 g/kg DM, being higher for cowpeas and lower for CB.

Table 1. Nutritional composition of different legume grains.

	Legume grain[a]					
	CB	LP	PVU	RVU	WVU	SB
Composition (g/kg DM)						
Dry matter (g/kg)	898	897	895	878	906	939
Crude protein (N x 6.25)	291	235	212	216	208	367
Ether extract	17	55	15	15	18	263
Ash	30	39	38	38	39	48
Starch	316	403	537	482	563	-
Neutral detergent fibre	275	234	210	260	143	117
Acid detergent fibre	174	131	52	75	22	68
Gross energy (MJ/kg DM)	15.9	17.8	16.0	15.7	16.5	19.9
Trypsin units inhibited (TUI/g DM)	14	26	21	22	22	7

[a] CB, *Canavalia brasiliensis*; LP, *Lablab purpureus*; PVU, RVU and WVU, *Vigna unguiculata* with pink, red and white coat respectively; SB, extruded full-fat soybean.

The kinetics of the *in-vitro* protein hydrolysis are shown in Figure 1. At 360 min SB and WVU presented the highest values (76 and 66% respectively) followed by RVU>PVU>CB>LP (Table 2). A high correlation between the hydrolysis values and TIA increased when CB was not taken into account ($r = 0.71$; $P < 0.05$). Pea samples with high TIA had lower protein digestibility values (Leterme et al., 1992). The low values for CB can be explained by its high ADF content. At 360 min there were also differences in starch digestibility with cowpeas being more digestible (especially PVU and WVU, 70-64% respectively) than CB (38%; $P < 0.001$) (Table 2). This can be explained by the lower fiber content of cowpeas vs. CB (210-143 vs. 275 g / kg DM respectively). In fact, a negative correlation was observed between the starch digestibility and NDF content ($r = -0.75$, $P < 0.05$). Additionally, it may be due to the amylose: amylopectin ratio, which influences starch hydrolysis, encouraging microbial degradation and profile of short-chain fatty acids (Tovar et al., 1992).

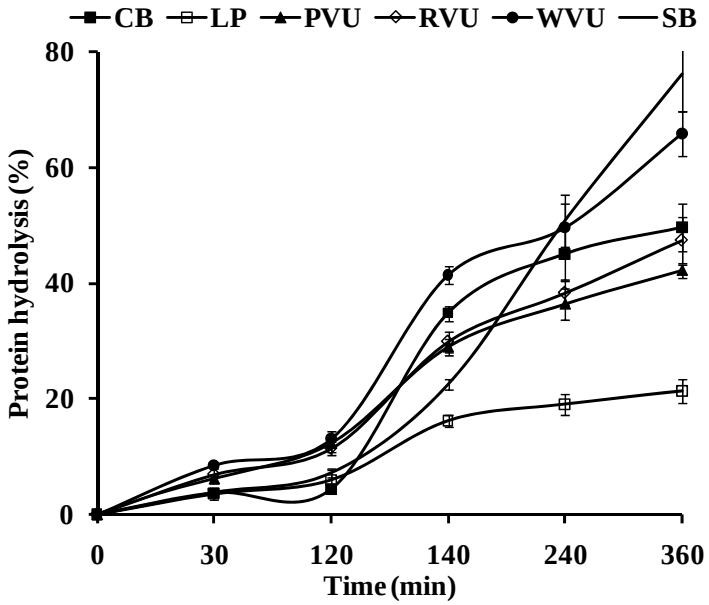

Figure 1: Kinetic of in-vitro protein hydrolysis of different legume grains following pepsin and pancreatin hydrolysis (120 min pepsin + 240 min pancreatin).

***In- vitro* fermentation:** The gas production obtained during 72 h of *in-vitro* fermentation varied according to the residue obtained after enzymatic hydrolysis. WVU and PVU had the highest gas production and SB and CB the lowest ($P < 0.0001$, Figure 2 and Table 2). This can be attributed to the differences in the resistant starch and NDF content available for the microbiota. WVU had a 1.4 to 1.8- fold higher content of resistant starch and 1.9 to 5.6-fold lower ADF content compared to the other grains. This could explain the different butyrate production levels (e.g. WVU vs. SB, 82 vs. 29 mg/g DM incubated, $P <0.001$) which were correlated to the resistant starch ($r = 0.70$, $P < 0.1$). Other variables of *in-vitro* fermentation were not influenced by the residue of legume grains (acetate, propionate, DM and NDF degradability).

Table 2. Protein and starch *in-vitro* digestibility after pepsin and pancreatin hydrolysis. Degradation of NDF and DM, and Sshort-chain fatty acids production during *in-vitro* fermentation of legume grains.

	Legume grain[a]							
	CB	LP	PVU	RVU	WVU	SB	SEM[b]	P[c]
***In-vitro* digestibility (%)**								
Protein	49.6b	21.3c	42.2b	47.4b	66.0a	76.0a	3.8	0.001
Starch	38.0c	51.7b	70.0a	53.0b	64.0a	-	3.5	0.001
***In-vitro* fermentation**								
Gas production (ml/g DM)	335d	389bc	408bc	427b	482a	382c	13	0.001
DM degradation (%)	81.2	79.6	88.2	86.6	88.8	82.5	2.6	0.348
NDF degradation (%)	84.8	86.9	90.3	91.0	88.8	87.8	2.1	0.565
Acetate (mg/g DM incubated)	110	153	185	153	169	124	30	0.241
Propionate (mg/g DM incubated)	74	84	116	94	109	68	14	0.085
Butyrate (mg/g DM incubated)	47ab	58ab	83a	69ab	82a	29b	17	0.015

[a] CB, *Canavalia brasiliensis*; LP, *Lablab purpureus*; PVU, RVU and WVU, *Vigna unguiculata* with pink, red and white coat, respectively; SB, extruded full-fat soybean. Values are means of 3 replicates.
[b] SEM: standard error of the mean.
[c] Values with different letters in the same row differ significantly ($P < 0.05$).

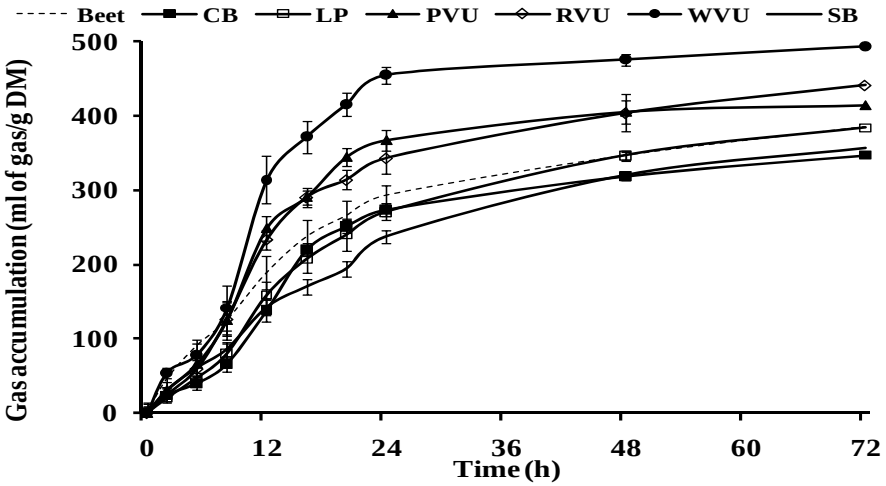

Figure 2: Kinetic of gas production during *in-vitro* cecal fermentation of SB and tropical grain legume residues. Beet was used as an internal control.

Conclusions

Differences in *in-vitro* digestibilities of protein (21-76%) and starch (38-70%) were observed for the different legume grains. These results can be partially explained by their different chemical compositions (e.g. fibre and TIA content). The fermentation of the indigestible residue also showed differences (total gas and butyrate production) among legumes grains, which may also be due to the chemical composition of these residues. In general, cowpeas were highly digested and fermented suggesting that they (mainly WVU) could be an interesting alternative to soybean in swine nutrition. In contrast, our results suggest that CB and LP should not be used in their raw forms. Further work is required to study these legumes following heat treatment as well as in *in-vivo* experiments.

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