Nutritive value of tropical forage plants fed to pigs in the Western provinces of the Democratic Republic of the Congo

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Abstract

The nutritive value of 20 forage plants commonly used for feeding pigs in the Democratic Republic of the Congo was studied to determine chemical composition, protein amino acid profiles, mineral content, and *in vitro* digestibility using a two-steps method combining an enzymatic pepsin and pancreatin hydrolysis followed by a 72h gas-test fermentation. The highest protein contents (270-320 g/kg DM) were obtained for *Vigna unguiculata*, *Psophocarpus scandens*, *Leucaena leucocephala*, *Manihot esculenta*, and *Moringa oleifera*.

Abbreviations: AA,amino acid; ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; DE, digestible energy; DM, dry matter; DP, digestible protein; DRC, Democratic Republic of the Congo; EE, ether extract; IVDMD, *in vitro* dry matter digestibility, IVCPD, *in vitro* crude protein digestibility; IVED, *in vitro* gross energy digestibility; NDF, neutral detergent fibre; SCFA, short-chain fatty acid. [3]

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Grasses, *Acacia mangium*, and *Eichhornia crassipes*, showed the lowest crude protein (CP) and highest NDF contents. *Cajanus cajan* and *Trypsacum andersonii* had the most balanced amino acid profile, being deficient in Lysine and slightly deficient in Histidine, while *Megathyrsus maximus* displayed the highest number of essential amino acids deficiencies. High mineral contents were obtained from, in ascending order, with *Moringa oleifera, Vigna unguiculata, Eichhornia crassipes, Ipomea batatas* and *Amaranthus hybridus.* In vitro dry matter digestibility ranged from 0.25 to 0.52, in-vitro CP digestibility from 0.23 to 0.80, in vitro energy digestibility from 0.23 to 0.52. *M. esculenta, M. oleifera, I. batatas, Mucuna pruriens, V. unguiculata, P. scandens and A. hybridus* showed high digestibilities for all nutrients. Gas production during fermentation of the pepsin and pancreatin-indigestible fraction of the plants varied from 42 ml/g DM for *A. mangium* to 202 ml/g DM for *I. batatas* ($P<0.001$). Short-chain fatty acid production during fermentation varied from 157 to 405 mg/g of the pepsin and pancreatin indigestible fraction. It is concluded that some of these species are interesting sources of proteins and minerals with a good digestibility that might be used more economically than concentrate, especially in smallholder production systems, to improve pig feeding, mineral intake and intestinal health in pigs reared in the tropics.

Keywords: Fermentation, Forage, Nutritive value, Pigs, Short chain fatty acids

1. Introduction

In the tropics, pig production is only tolerated if pigs do not compete with humans for food (Leterme et al., 2006), especially in developing countries where monogastrics are in direct competition with humans for the resources required to produce concentrate feed. Because of the high and volatile prices of the latter (Braun, 2007; FAO, 2012), smallholders often replace the cereals and oilseed by-products in pig feeds with large amounts of cheap and unconventional fibre-rich ingredients such as crop residues, agro-industrial by-products, and grass and legume forage collected in the forest or in fallow fields near pigsties (Kumaresan et al., 2009; Phengsavanh et al., 2010). A recent survey realised in the Kinshasa and the Bas-Congo Provinces of the Democratic Republic of the Congo (DRC) (Kambashi et al., submitted) confirmed that less than 2% of the farmers use commercial feeds and the most abundant cereal resource, namely corn, is used as an ingredient in pig feed on less than 10% of the farms. Although the growth performances of forage-fed pigs is often lower than that of concentrate-fed and is negatively correlated with the inclusion rate of the forages (Phengsavanh and Lindberg, 2013; Régnier et al., 2013), farmers in Western DRC do not feed crop grains to their pigs because they consider it a waste of crops even in mixed farming systems producing both pigs and crops.

The use of forage resources as pig feeds does have several drawbacks including low digestibility of forage owing to their high content in fibre, the presence of anti-nutritive compounds and the lack of suitable conservation methods. However, compared to cereals, they have distinct advantages justifying their use by farmers: low cost, non-competitiveness with human food, high levels of protein, minerals and vitamins (reviewed by Martens et al., 2012). As feed is the most critical expense in pig rearing activity, it can be profitable to substitute a significant part of a concentrate-based diet with some forage ingredients (Kaensombath et al., 2013b). Unfortunately, the lack of information on the nutritive value of

most of the forage resources used in tropical areas in general and in Western DRC specifically can lead to unbalanced diets, low pigs growth and reproduction performances, low incomes for the farmers and less locally produced animal protein available on the market. The aim of this work is to assess using an *in vitro* model of the pigs gastro-intestinal tract, the nutritive value of the forage species the most commonly used by smallholder farmers in Western DRC in order to provide information that could guide them in the choice of forage resources for improved pig performances.

2. Materials and methods

2.1. Plant material

Samples of 20 forage species used as pig feed by farmers in the Kinshasa and Bas-Congo Provinces of the DRC and identified as the most commonly used during a survey of 319 pig smallholders (Kambashi et al. submitted) were gathered from the smallholders' farms (Table 1). For each species, 4 independent samples were collected on different farms. All forage samples were harvested during the vegetative growth phase before flowering and, depending on the species, whole plants or only leaves were sampled according to the farmers' common practices.

2.2 In vitro digestion and fermentation

Forage samples were oven-dried at 60°C and ground to pass through a 1 mm mesh screen in a Cyclotec 1093 Sample Mill (FOSS Electric A/S, Hilleroed, Denmark). The digestibility of their nutrients was assessed using the *in vitro* model developed by Bindelle et al. (2007a). Briefly, this method simulates the digestion in the pig gastro-intestinal tract in two steps. The stomach and small intestinal digestion are mimicked by an enzymatic hydrolysis with porcine pepsin (2h, 39°C, pH 2) and porcine pancreatin (4h, 39°C, pH 6.8), respectively. The indigestible residue recovered by filtration through a nylon cloth (42 µm),

after washing with ethanol and acetone, is subsequently fermented with faecal bacteria of sows in a carbonate-based buffer (72h, 39°C, pH 6.8) to simulate the fermentation processes occurring in the large intestine. The volume of gas produced during fermentation was modelled according to Groot et al. (1996). Four parameters describing the fermentation kinetics were calculated: final gas volume (A, ml g/DM)), mid-fermentation time (B, h), maximum rate of gas production ($R_{M,}$ ml g/DM) and time at which the maximum rate of gas production is reached ($tR_{M,}$ h). Fermentation broth collected after 72 h was centrifuged at 13 000 g for 15 min and the supernatants were sampled and frozen at -18°C until further short-chain fatty acid (SCFA) analysis.

For each of the 4 samples of each forage species, hydrolysis was performed between 4 to 6 times on 2-g samples to yield sufficient amounts of indigestible residues for the subsequent analyses and fermentation. In vitro fermentation was performed in quadruplicate on the pooled residues of each initial forage sample.

2.3. Chemical analysis

Forage ingredients and hydrolysis residues pooled by forage sample (N=4 per species) were analysed for their content in dry matter (DM) by drying at 105°C for 24 h (method 967.03; AOAC, 1990), ash by burning at 550°C for 8 h (method 923.03; AOAC, 1990), N according to the Kjeldahl method and calculating the crude protein (CP) content (N × 6.25; method 981.10; AOAC, 1990), and gross energy by means of an adiabatic oxygen bomb calorimeter (1241 Adiabatic Calorimeter, PARR Instrument Co., Illinois, USA). Forage ingredients were also analysed for their content in ether extract (EE) with the Soxhlet method by using diethyl ether (method 920.29; AOAC, 1990), in neutral detergent fibre (aNDFom) using thermostable amylase (Termamyl®, Novo Nordisk, Bagsværd, Denmark) and corrected for ash, in acid detergent fibre (ADFom) corrected for ash, in acid detergent lignin (ADL(sa)) according to Van Soest et al. (1991) using an ANKOM-Fiber Analyzer (ANKOM-

Technology, Fairport, NY), and in total amino acids (excluding methionine, cysteine and tryptophan) by HPLC after hydrolysis with a mixture of 6 mol HCl/l containing 1 g phenol/l at 110°C for 24 h and derivatization with AccQ-Fluor reagent Kit. DL-2-aminobutyric acid was used as internal standard. Ca, P, Mg, K, Cl, S, Se, Ni, Na, Fe, Mn, Cu and Zn contents of one sample per plant (N=1 per species) were analysed by atomic absorption spectrophotometry using a PerkinElmer AAS-800 (Wellesley, MA, USA).

The supernatants of the fermentation broth were analysed for SCFA contents after 72 h of fermentation with a Waters 2690 HPLC system (Waters, Milford, MA, USA) fitted with an HPX 87H column (Bio-Rad, Hercules, CA, USA) combined with an UV detector (210 nm, Waters, Milford, MA, USA).

2.4. Calculation and statistical analyses

The *in vitro* dry matter digestibility (IVDMD), crude protein digestibility (IVCPD) and gross energy digestibility (IVED) during the pepsin and pancreatin hydrolysis were calculated as follows: IVDMD = (X-Y)/X; where X is the weight of the sample before hydrolysis and Y the weight of the residue ; and $IVXD = 1 - \frac{Y \times (1 - IVDMD)}{X}$,where X is the nutrient content (CP, energy) in the sample before hydrolysis and Y the nutrient content in the residue after hydrolysis.

Potential contribution of fermentation in the large intestine to metabolic energy supply through SCFA was calculated according to Gaedeken et al. (1989) by multiplying the energy value of each SCFA (acetate 14.56 kJ/g, propionate 20.51 kJ/g, and butyrate 24.78 kJ/g) (Livesey and Elia, 1995) by the SCFA production during the fermentation of the hydrolysed forage ingredients.

Statistical analyses were performed by means of an analysis of variance and a classification of means by the Least Significant Difference method using the MIXED

procedure of the SAS 8.02 software (SAS inc., Cary, NC, USA). Correlation was calculated according to the PROC CORR procedure of the SAS 9.2 software (SAS inc., Cary, NC, USA). For all the analyses, the individual forage sample was considered as the experimental unit and the species was the effect that was tested (N = 4).

3. Results

3.1. Chemical composition

Crude protein contents of the forage species ranged from 88 to 324 g/kg DM and NDF content ranged from 279 to 688 g/kg DM (Table 1). The lowest CP values (88 to 147 g kg^{-1} DM) and the highest NDF contents (554 to 688 g/kg DM) were found in grasses (*M. maximus, P. purpureum, S. officinarum, U. ruziziensis, T. andersonii*) and *Eichhornia crassipes*. In contrast, the dicotyledons such as *A. hybridus, I. batatas, M. pruriens, V. unguiculata, P. scandens, L. Leucocephala, M. esculenta* and *M. oleifera* showed CP contents ranged from 225 to 326 g/kg DM and NDF content ranged from 208 to 395 g/kg DM. The AA profile differed between forages but all species were highly deficient in Lysine, with values ranging between 3.08 and 4.76 g/16g N against recommendations of 7.14 g/16g N (NRC, 2012), with grasses being the most deficient. The legume *C. cajan* and more surprisingly the grass *T. andersonii* had the most balanced protein profile being deficient in Lysine (4,76 and 3.04 g/16g N, respectively) and slightly deficient in Histidine (2.23 and 1.83 g/16g N, respectively). Conversely, *M. maximus* appeared to have the most unbalanced protein profile. In terms of total amount of total AA per gram of protein, the lowest value were obtained with *C. pubescens , M. pruriens, M. maximus* and *P. scandens* (59 to 63 g/16g N) while the highest values were found in *C. cajan, M. esculenta and, V. unguiculata* (77 to 80 g/16g N).

3.2. In vitro digestibility and fermentation

IVDMD ranged from 0.25 to 0.53, depending on the species (P<0.001), while IVCPD ranged from 0.23 to 0.81(P<0.001) and that of energy (IVED) ranged from 0.23 to 0.52 (P<0.001) (Table 2). *M. esculenta, M. oleifera, I. batatas, C. muconoides, V. unguiculata, P. scandens* and *A. hybridus* had the highest IVDMD, IVED and IVCPD values. Although it had a low IVDMD of 0.40, *P. phaseoloides* scored among the highest for IVCPD with 0.75. Gas production kinetics of the fibre-rich residues recovered after the pepsin and pancreatin hydrolysis showed that different forage species have different fermentabilities. Final gas production (A) varied from 42 ml/g DM for *A. mangium* to 202 ml/g DM for *I. batatas* (P<0.001). These two species also gave the extreme values for the maximum rate of fermentation (R_M) which ranged from 1.5 to 16.7 ml/h per g DM (P<0.001). Mid-fermentation times (B) and time at which R_M is reached (t_{RM}) ranged from 11.8 to 24.5 h and 8.4 to 18.7 h (P<0.001), respectively.

As a consequence of their lower CP content as well as their lower IVCPD and fermentability, all grasses (*M. maximus, P. purpureum, S. officinarum, U. ruziziensis*, and *T. andersonii*) as well as *A. mangium, C. cajan and E. crassipes*, ranked amongst the species with the lowest *in vitro* digestible protein (DP) values (40 to 92 g/kg DM). With DP ranging from 129 to 147 g/kg DM, *S. guianensis, C. muconoides, L. leucocephala, P. phaseoloides, I. batatas, M. pruriens,* and *C. pubescens* showed low DP values ranging from 129 to 147 g/kg DM in contrast to *A. hybridus, M. esculenta, P. scandens V. unguiculata* and *M. oleifera,* whose DP contents ranged from 176 to 261 g/kg DM. All grasses as well as *A. mangium and E. crassipes* had the poorest digestible energy (DE) contents with values as low as 5.7 MJ/kg. The species with the highest total energy, including the DE released from enzymatic hydrolysis and the contribution of SCFA from fermentation, were: *C. mucunoides*

(11.7 MJ/kg), *V. unguiculata* (12.3 MJ/kg), *M. Oleifera* (12.8 MJ/kg) and *M. esculenta* (13.0 MJ/kg)

3.3. Short chain fatty acids

Total SCFA production during the *in vitro* fermentation (Table 3) differed between forage species (P<0.001). These differences were consistent with those observed during fermentation kinetics as total SCFA production was correlated to maximum rate of gas production (r = 0.72, P<0.001) and final gas volume (r = 0.85, P<0.001). The fibre-rich residue of *V. unguiculata, I batatas, S. guianensis, A. hybridus, U. ruziziensis* and, *M. oleifera,* produced more SCFA (375 to 405 mg/g DM of enzymatically hydrolysed forage) than the other species (157 to 359 mg/g DM). *M. esculenta*, *E. crassipes*, *I. batatas* showed the highest acetate molar ratio (0.629 to 0.642) while grasses (*M. maximus*, *P. purpureum*, *T. andersonii*, *U. ruziziensis*) had the lowest acetate (0.581 to 0.589) and the highest propionate molar ratio (0.293 to 0.312). Although significant differences between forage species in butyrate and BCFA molar ratios were quite little in absolute value.

The NDF content affected IVDMD (r = -0.82, P<0.001) and IVED (r = -0.80, P<0.001). There was also a negative correlation (r = -0.71, P<0.001) between DP and NDF content for all forages.

3.4. Mineral content of forages

The contents of macro- and micro-minerals in the sampled forage species varied widely. Sulphur content ranged from 1.5 in *P. purpureum* to 20.9 g/kg DM in *M. oleifera.* Calcium content ranged from 3.6 in *P. purpureum* to 37.0 g/kg DM in *V. unguiculata* while phosphorus content ranged from 0.17 in *S. officinarum* to 6.0 g/kg DM in *A. hybridus.* Magnesium content ranged from 1.1 to 11.6 g/kg DM sodium and potassium content ranged from 0.2 to 3.8 g/kg DM and 7.0 to 62.9 g/kg DM, respectively. The highest macro mineral

209 contents were obtained from, in ascending order, *M. oleifera, V. unguiculata, E. crassipes, I.*
210 *batatas* and *A. hybridus.*

211 Levels of copper and nickel levels were low in all the forage plants compared to those
212 of other minerals. Cobalt and selenium levels were very low, and in some species below
213 detection levels. The iron levels were relatively high while phosphorus content was low in
214 almost all forage species compared to nutritional requirements (Table 4). Calcium-to-
215 phosphorus ratio was high in all plants. Among the studied plants, *A. hybridus* had the highest
216 macro- and micro-nutrient levels.

217 **Discussion**

218 Feeding is the most important component in the efficiency of pig production systems,
219 yet a recent survey (Kambashi et al., submitted) showed that in the Kinshasa and Bas-Congo
220 provinces of the DRC, smallholders feed their pigs with by-products and locally available
221 forage plants. The efficiency of such a system depends on the nutrients that are provided by
222 forage and the capacity with which these nutrients are assimilated and converted into meat.

223 The *in vitro* approach used in this research allowed to evaluate the potential nutritive
224 value of a large number of forage species providing an insight, not only on their chemical
225 composition, but also on their enzymatic digestibility and the fermentability of their
226 indigestible fraction in the large intestine. However, this methodology is not perfect as the
227 capacity of a feed ingredient to supply nutrients to an animal depends on both the quantity
228 that an animal will voluntary ingest and how much nutrients present will be digested and
229 metabolised by the animal. Intake was not assessed here and not all the features regarding
230 digestion are assessed using the *in vitro* method. For example, the impact of toxic compounds
231 in the plant and their consequences on the intake and the digestive processes (Acamovic and
232 Brooker, 2005, Martens et al. 2012) are not modelled in the *in vitro* method. Another example
233 is the interaction between feed ingredients and with digestive processes. Some forage species

234 are rich in fibre with high water-holding capacity. The swelling of such fibre in the upper tract
235 of the pig will impact the digestibility of the whole diet by reducing transit time and contact
236 between the feed particles and the digestive enzymes (Partanen et al., 2007; Régnier et al.,
237 2013). This effect cannot be evaluated in the chosen *in vitro* model either.

238 Considering chemical composition, except for *A. mangium* and *C. cajan,* all Fabaceae
239 have CP contents that meet the requirements for growing pigs (200 g/kg DM; NRC, 2012) and
240 yield high DP content. More specifically, the high protein content of *M. oleifera, M.*
241 *esculenta,* and the Fabaceae *L. leucocephala, P. scandens* and *V. unguiculata,* with 324, 280,
242 279, 277 and 272 g/kg DM, respectively, justifies their use in pig feeding since protein is the
243 most limiting factor in smallholder pig feeding systems in tropical areas (Leterme et al.,
244 2005). These protein-rich plants also have an interesting amino acid profile (Table 5).
245 However, none of them covers the essential amino acids requirement of growing pigs,
246 especially in Lysine which was 45 to 68 % of the Lysine requirement per g/16g N for growing
247 pigs (NRC, 2012). Therefore using the above-mentioned forage species to supplement
248 Lysine-deficient basal diets, such as brewers grains and wheat bran (Kambashi et al.,
249 submitted), requires to feed the animals above requirement levels for protein content or to
250 supplement forage-based diets with synthetic lysine. Moreover, the total AA contents
251 presented here do not consider digestibility of AA which can greatly vary between species
252 and could modify the ranking of the forages based on protein profile. As an example, the best
253 protein profile was found in *C. cajan*. However, the amino acid availability in this species is
254 expected to be limited by the low digestibility of the crude protein (0.33).

255 The high NDF and ADF contents of the grasses and *E. crassipes* explain their low *in*
256 *vitro* digestibility, as illustrated by the correlation linking NDF to IVDMD and IVED ($r = -0.71$ and -0.84 respectively, $P<0.001$) in this study and the more general observations by
258 Noblet and van Milgen (2004). The digestibility and fermentability of *A. mangium* and *C.*

cajan were low, probably because of the presence of plant secondary metabolites together with their high lignin contents (176g/kg DM of ADL, respectively). Clavero and Razz (2011) and Uwangbaoje (2012) found these plants to contain condensed tannins (4.8 and 38.7 mg/g DM) and phenols (29.1 and 2.2 mg/g). ,

In contrast to CP, the total digestible energy (DE) contents of the forages not meet the requirements for growing pigs (15.8 MJ/kg DM; NRC, 2012). *V. unguiculata* and *C. muconoides* present DE contents of 11.7 and 12.3 MJ/kg DM, respectively whereas *M. oleifera* and *M. esculenta* scored even better with 12.8 and 13.0 MJ/kg DM, respectively. In addition, to hydrolysed DE, the SCFA released through fermentation in the large intestine of the indigestible fibrous residue can supply up to 4 MJ/kg DM of additional metabolic energy that significantly increases the energy value of some forage species. Interestingly, grasses displayed high hemicellulose contents (calculated as the difference NDF-ADF) as opposed to many legumes which show lower NDF values but ADF values similar to grasses. This leads to distinct fermentation profile in grasses, yielding more propionate and less acetate than legumes and other dicots, and induces a significant contribution of hindgut fermentation to ME supply in the animal as a combination of (1) high indigestible feed particles reaching the intestine, (2) high fermentability of the fibrous matrix, and (3) the higher energy content of propionate as opposed to acetate (20.51 kJ/g vs. 14.56 kJ/g, respectively). Nevertheless, SCFA production are measured in the in vitro model after 72 h fermentation. It represents a long transit time in the large intestine that would be more consistent with sows than finishing pigs and growing pigs (Le Goff et al., 2002)

The high fermentability related to greater SCFA results in a decrease in pH, which in turn influences the composition of colonic microflora, decreases the solubility of bile acids and increases absorption of some minerals (Hijova and Chmelarova, 2007). Low pH values are also believed to prevent the overgrowth of pH-sensitive pathogenic bacteria. For example,

propionate or formate have been shown to kill *E. coli* or *Salmonella* under conditions of high acidity (pH 5) (Cherrington et al., 1991). Some *in vivo* studies support these findings, with greater SCFA production being related to lower numbers of potential pathogens (such as *Salmonella*) in swine (Pieper et al., 2012). Some species combining high DP and DE contents with high SCFA production can potentially contribute significantly to efficient nutrition together with the development of health-promoting bacteria in pig intestines by providing metabolizable energy (Bindelle et al., 2007b; Hijova and Chmelarova, 2007) and other metabolic end products for pig use, as well as nutrients for the colonic epithelium, modulators of colonic and intracellular pH, cell volume and other associated functions (Hijova and Chmelarova, 2007).

Nonetheless, attention must be paid to the maximum levels of forage incorporation in pig diets as some forage species may contain variable amounts of anti-nutritional or toxic factors such as tannins, as discussed earlier: HCN in *M. esculenta*, mimosine in *L. leucocephala*, and lectins in *I. batatas* and *P. scandens*. These compounds might reduce voluntary intake and *in vivo* digestibility (Régnier et al., 2012). However, with moderate inclusion rate of these forages, anti-nutritional effect is not significant. For example, the incorporation of 350 g/kg DM of *I. batatas* leaves or 150 g/kg DM of *M. esculenta* leaves or *L. leucocephala* in pig diets have shown ileal digestibility up to 74% (Phuc and Lindberg, 2000; An et al., 2004). However, the *in vitro* approach adopted in this research does not allow considering these issues and the presented results should be taken as a first orientation on the feeding value of one species. Obviously, one species scoring poorly on *in vitro* digestibility trials as performed here will be of little value as pig feed ingredient. Nevertheless, the opposite conclusion is not straightforward. A species scoring with high nutritive characteristics as evaluated *in vitro* might not necessarily be well consumed or digested *in vivo* possibly because of poor palatability, of the presence of plant secondary

309 metabolites displaying anti-nutritive or toxic attributes that are not always noticeable using an
310 *in vitro* approach.

311 The use of forage to supplement local feed resources can provide a better balanced diet
312 that improves growth performances keeping feeding costs under control (Lemke and Valle
313 Zárate, 2008) and in a sustainable way. For example, it has been reported that the inclusion of
314 ensiled *S. guianensis* in the diet of local pigs improved growth performance up to three times
315 compared to pigs fed ill-balanced diets based on locally available by-products (Kaensombath
316 et al., 2013b). However, the replacement of conventional sources of protein, such as soybean
317 meal, by protein-rich forage must be partial because Phengsavanh and Lindberg (2013)
318 reported that it reduces feed intake and growth performance. In another study, Kaensombath
319 and Lindberg (2013a) showed that when 50% of soybean protein was replaced with proteins
320 from ensiled *Colocasia esculenta*, growth performance and carcass traits of local and
321 improved pigs were not affected. Surprisingly, Men et al. (2006) report high cost
322 effectiveness of *E. crassipes*-based diets in Vietnam while this species scored really bad in
323 terms of nutritive value in the present investigation. This allows expecting even higher
324 efficiencies of feeding systems based on low cost forage with higher nutritive value than
325 water hyacinth.

326 Despite the expected high variability within species that was not assessed in this study
327 as only one sample per species was analysed, legumes seem to be a richer and better balanced
328 source of minerals than grasses. Yet variability among species, specifically with regard to the
329 bioavailability of minerals, must be considered as it ranges from 0.41 to 58% for P (Poulsen et
330 al., 2010), 3 to 27% for Fe (Kumari et al., 2004), 11 to 26% for Zn and 18 to 48% for Cu
331 (Agte et al., 2000). Due to the high calcium-to-phosphorus ratio, which decreases absorption
332 of phosphorus (Liu et al., 2000), as well as the low phosphorus content in most forages, these
333 species seem to perfectly supplement basal ingredients usually used by farmers, namely

brewers grains and wheat bran, which are deficient in Ca (2.1 and 1.4 g/kg, respectively) and rich in P (5.8 and 9.9 g/kg, respectively). The outstanding mineral content of *A. hybridus* deserves further attention, as according to NRC (2012), its Se level is quite high compared to the requirements (0.3 ppm) but is still below the toxicity level (5 ppm). Co, Cu and Ni were below toxicity levels in all forages, but with regard to this it must be noted that forage are rarely fed to pigs alone; but rather, mixed with other ingredients.

It can be concluded that among the investigated plants in this study, *A. hybridus, I. batatas, M. esculenta, M. oleifera, P. scandens* and *V. unguiculata* combine several interesting nutritive traits including moderate to high IVDMD, IVED, DCP, R_M, SCFA, Ca and low NDF contents. They represent potentially useful sources of proteins and minerals that might be used at low cost to improve pig feeding, mineral intake and intestinal health. Grasses as well as *A. mangium*, *E. crassipes* and *C. cajan* should be discouraged in pig diet because of their low nutritive value. Further studies are required to determine voluntary intake and *in vivo* nutritive value for the potentially useful species and their ideal inclusion level in pig diets for optimum performance in production environments with low quality basal diets.

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462 Table 1. Chemical composition (g/kg DM) and gross energy (MJ/kg DM) content of the forages (N = 4)

463

Species	Family	Name	Plant parts	OM[1]	CP[2]	GE[3]	aNDFom[4]	ADFom[5]	ADL(sa)[6]	EE[7]
Acacia mangium	Fabaceae	Lack wattle	leaves	970 ^{b8}	177 ^{efg}	21.7 ^{a}	505 ^{bcd}	344 ^{ab}	176 ^{a}	46.2 ^{bcd}
Amaranthus hybridus spp	Amaranthaceae	Smooth pigweed	whole plant	839 ^{h}	225 ^{d}	15.1 ^{h}	373 ^{ef}	208 ^{d}	22 ^{g}	21.3 ^{hi}
Cajanus cajan	Fabaceae	Pigeon pea	whole plant	951 ^{abcd}	217 ^{de}	22.5 ^{a}	545 ^{bc}	362 ^{ab}	176 ^{a}	60.6 ^{ab}
Calopogonium muconoides	Fabaceae	Wild ground nut	whole plant	914 ^{defg}	179 ^{efg}	19.6 ^{d}	489 ^{cd}	357 ^{ab}	70 ^{cde}	42.0 ^{cde}
Centrosema pubescens	Fabaceae	Centro	whole plant	936 ^{abcdef}	216 ^{de}	19.8 ^{cd}	543 ^{bc}	381 ^{a}	95 ^{bcd}	31.3 ^{defghi}
Eichhornia crassipes	Pontederiaceae,	Water hyacinth	whole plant	917 ^{cdefg}	138 ^{gh}	16.1 ^{h}	574 ^{b}	316 ^{ab}	31 ^{fg}	20.5 ^{i}
Ipomoea batatas	Convolvulaceae	Sweet potato	whole plant	899 ^{fg}	225 ^{d}	17.6 ^{fg}	389 ^{ef}	334 ^{ab}	99 ^{bc}	37.4 ^{cdefgh}
Leucaena leucocephala	Fabaceae	Leucena	leaves	927 ^{cdefg}	279 ^{ab}	20.5 ^{bc}	394 ^{ef}	213 ^{d}	96 ^{bcd}	49.7 ^{bc}
Manihot esculenta	Euphorbiaceae	Cassava	leaves	926 ^{cdefg}	280 ^{ab}	21.3 ^{ab}	313 ^{fg}	225 ^{cd}	86 ^{bcd}	68.0 ^{a}
Megathyrsus maximus	Poaceae	Guinea grass	whole plant	955 ^{abc}	147 ^{fgh}	18.8 ^{def}	688 ^{a}	397 ^{a}	47 ^{efg}	21.9 ^{ghi}
Moringa oleifera	Moringaceae	Moringa	leaves	888 ^{g}	324 ^{a}	19.4 ^{de}	279 ^{g}	183 ^{d}	31 ^{fg}	70.0 ^{a}
Mucuna pruriens	Fabaceae	Velvet bean	whole plant	933 ^{bcdef}	228 ^{cd}	19.1 ^{de}	499 ^{bcd}	395 ^{a}	109 ^{b}	40.0 ^{cdef}
Pennisetum purpureum	Poaceae	Elephant grass	whole plant	908 ^{efg}	110 ^{hi}	17.4 ^{g}	674 ^{a}	363 ^{ab}	43 ^{efg}	21.8 ^{ghi}
Psophocarpus scandens	Fabaceae	African winged-bean	whole plant	941 ^{abcde}	277 ^{b}	19.1 ^{de}	540 ^{bc}	345 ^{ab}	97 ^{bc}	28.2 ^{efghi}
Pueraria phaseoloides	Fabaceae	Tropical kudzu	whole plant	941 ^{abcde}	180 ^{efg}	19.4 ^{de}	519 ^{bc}	385 ^{a}	85 ^{bcd}	31.9 ^{defghi}
Saccharum officinarum	Poaceae	Sugarcane	leaves	977 ^{a}	88 ^{i}	18.8 ^{def}	685 ^{a}	389 ^{a}	48 ^{efg}	23.2 ^{fghi}
Stylosanthes guianensis	Fabaceae	Common stylo	whole plant	920 ^{cdefg}	194 ^{def}	18.2 ^{efg}	559 ^{bc}	396 ^{a}	77 ^{bcde}	30.4 ^{defghi}
Trypsacum andersonii	Poaceae	Guatemala grass	whole plant	935 ^{bcdef}	104 ^{hi}	18.9 ^{de}	678 ^{a}	372 ^{ab}	46 ^{efg}	22.4 ^{ghi}
Urochloa ruziziensis	Poaceae	Ruzi grass	whole plant	937 ^{abcdef}	101 ^{hi}	18.4 ^{defg}	672 ^{a}	358 ^{ab}	31 ^{fg}	20.8 ^{hi}
Vigna unguiculata	Fabaceae	Cowpea	whole plant	908 ^{efg}	272 ^{bc}	18.6 ^{defg}	422 ^{de}	302 ^{bc}	60 ^{def}	38.2 ^{cdefg}
SEM[9]				*4.46*	*8.34*	*0.21*	*15.26*	*9.27*	*5.55*	*2.11*
P values				*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*

464 [1]OM, organic matter

[2]CP, crude protein (N × 6.25)

[3]GE, gross energy

[4]aNDFom, neutral detergent fibre using thermostable amylase and corrected for ash content

[5]ADFom, acid detergent fibre corrected for ash content

[6]ADL(sa), acid detergent lignin

[7]EE, ether extract

[8]For one column, means followed by different letters differ ($P<0.05$)

[9]SEM, standard error of the means

Table 2. *In vitro* dry matter (IVDMD), energy (IVED) and crude protein (IVCPD) digestibility during pepsin-pancreatin hydrolysis and kinetic parameters of the gas production curves modelled according to Groot et al. (1996) for the hydrolysed forages incubated with pigs faeces (N = 4).

Scientific name	IVDMD (−)	IVED (−)	IVCPD (−)	DP g /kg DM	Hydrolysed DE[1] MJ /kg DM	Total DE[2] MJ /kg DM	A[3] (ml/g DM)	R_M[4] (ml/h per g DM)	tR_M[5] (h)
Acacia mangium	0.31 fgh6	0.26 gh	0.23 j	40 h	5.7 def	7.6 gh	42 k	1.5 i	11.6 d
Amaranthus hybridus	0.53 a	0.47 ab	0.78 a	176 cd	7.1 cd	10.2 cde	196 ab	14.7 b	10.2 de
Cajanus cajan	0.33 efg	0.32 efg	0.33 j	71 gh	7.2 bcd	9.2 efg	68 j	3.0 i	11.4 d
Calopogonium muconoides	0.44 bc	0.45 abc	0.74 abc	130 ef	8.9 ab	11.7 abc	134 fgh	9.1 e	9.9 de
Centrosema pubescens	0.37 def	0.37 cde	0.69 bcde	147 de	7.4 bcd	10.1 cde	116 hi	5.4 fgh	10.7 d
Eichhornia crassipes	0.33 efg	0.25 gh	0.51 hi	70 gh	3.9 f	6.8 h	116 hi	4.5 gh	13.8 c
Ipomoea batatas	0.47 ab	0.43 bcd	0.61 efg	137 de	7.6 bc	10.9 bcd	202 a	16.7 a	10.2 de
Leucaena leucocephala	0.39 cde	0.39 cde	0.47 i	130 ef	7.9 bc	10.3 cde	108 i	5.9 f	11.2 d
Manihot esculenta	0.45 bc	0.47 ab	0.64 cdefg	177 cd	10.1 a	13.0 a	164 cde	13.3 bc	10.5 de
Megathyrsus maximus	0.29 gh	0.28 fg	0.62 defg	92 fg	5.3 ef	9.2 efg	170 cd	6.3 f	16.5 b
Moringa oleifera	0.53 a	0.52 a	0.80 a	261 a	10.1 a	12.8 a	170 cd	13.5 bc	8.4 e
Mucuna pruriens	0.37 de	0.35 def	0.61 efg	138 de	6.8 cde	9.7 def	145 efg	8.2 e	10.0 de
Pennisetum purpureum	0.25 h	0.23 h	0.53 ghi	58 gh	3.9 f	8.2 fgh	164 cd	5.8 fg	17.6 ab
Psophocarpus scandens	0.43 bcd	0.42 bcd	0.69 bcde	192 bc	7.9 bc	11.1 bcd	152 def	8.2 e	11.4 d
Pueraria phaseoloides	0.40 cde	0.41 bcd	0.75 ab	134 e	7.9 bc	11.0 bcd	131 gh	8.8 e	11.0 d
Saccharum offinarum	0.30 gh	0.25 gh	0.58 fgh	48 h	5.0 ef	8.2 fgh	116 hi	4.4 h	16.0 b
Stylosanthes guianensis	0.33 efg	0.35 def	0.67 bcdef	129 ef	6.4 cde	10.6 bcde	170 cd	10.9 d	10.8 d
Trypsacum andersonii	0.27 gh	0.27 gh	0.50 hi	52 h	5.2 ef	9.4 def	153 de	5.4 fgh	18.7 a
Urochloa ruziziensis	0.30 fgh	0.28 fg	0.73 abcd	73 gh	5.2 ef	9.5 def	198 ab	8.0 e	15.6 bc
Vigna unguiculata	0.47 ab	0.48 ab	0.81 a	219 b	8.9 ab	12.3 ab	179 bc	12.4 c	11.6 d
SEM[7]	0.013	0.014	0.022	7.49	0.24	0.22	3.03	0.29	0.25
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[1] Digestible energy from enzymatically hydrolyzed fraction

[2] Value is the sum of the digested energy from the enzyme hydrolyzed fraction plus the contribution of SCFA from fermentation.

[3] A, final gas volume

[4] R_M, maximum rate of gas production

[5] t_{RM}, time at which the rate of gas production reaches R_M

[6] For one parameter, means followed by different letters in the columns differ at a significance level of 0.05.

[7] SEM, standard error of the means

Table 3. Short chain fatty acids (SCFA) production (mg/g DM) of the hydrolyzed forage ingredients during in vitro fermentation and potential contribution of SCFA to the metabolic energy supply from the initial ingredient to the pig (N=4).

Plants	SCFA (mg/g DM)	Acetate (mol//mol)	Propionate (mol//mol)	Butyrate (mol//mol)	BCFA (mol//mol)	Contribution of fermentation[c] to energy supply (MJ/kg DM)
Acacia mangium	157 ^{i1}	0.594 ^{fghi}	0.257 ^{fgh}	0.074 ^{ab}	0.021 ^{a}	1.68 ^{i}
Amaranthus hybridus	389 ^{ab}	0.624 ^{bc}	0.263 ^{efgh}	0.063 ^{cde}	0.014 ^{cdef}	2.97 ^{def}
Cajanus cajan	195 ^{h}	0.620 ^{bcde}	0.234 ^{i}	0.075 ^{a}	0.020 ^{a}	2.03 ^{h}
Calopogonium muconoides	321 ^{de}	0.605 ^{efg}	0.273 ^{de}	0.064 ^{cde}	0.016 ^{cde}	2.84 ^{ef}
Centrosema pubescens	267 ^{fg}	0.599 ^{fgh}	0.283 ^{bcd}	0.061 ^{cde}	0.017 ^{bc}	2.66 ^{fg}
Eichhornia crassipes	272 ^{fg}	0.631 ^{ab}	0.255 ^{gh}	0.060 ^{de}	0.016 ^{cde}	2.86 ^{ef}
Ipomoea batatas	401 ^{a}	0.629 ^{ab}	0.251 ^{h}	0.067 ^{bcd}	0.014 ^{def}	3.38 ^{c}
Leucaena leucocephala	262 ^{g}	0.608 ^{def}	0.252 ^{h}	0.068 ^{abcd}	0.019 ^{ab}	2.45 ^{g}
Manihot esculenta	342 ^{cd}	0.642 ^{a}	0.230 ^{i}	0.067 ^{abcde}	0.015 ^{cde}	2.92 ^{def}
Megathyrsus maximus	342 ^{cd}	0.583 ^{hi}	0.298 ^{ab}	0.066 ^{cde}	0.014 ^{cdef}	3.89 ^{b}
Moringa oleifera	375 ^{abc}	0.631 ^{ab}	0.231 ^{i}	0.069 ^{abc}	0.016 ^{bcd}	2.71 ^{fg}
Mucuna pruriens	299 ^{ef}	0.609 ^{cdef}	0.273 ^{def}	0.060 ^{de}	0.016 ^{cde}	2.96 ^{def}
Pennisetum purpureum	351 ^{cd}	0.589 ^{hi}	0.293 ^{bc}	0.067 ^{abcd}	0.013 ^{ef}	4.24 ^{a}
Psophocarpus scandens	347 ^{cd}	0.594 ^{fghi}	0.285 ^{bcd}	0.061 ^{cde}	0.016 ^{cde}	3.14 ^{cde}
Pueraria phaseoloides	320 ^{de}	0.607 ^{ef}	0.276 ^{cde}	0.061 ^{cde}	0.015 ^{cde}	3.07 ^{cde}
Saccharum officinarum	292 ^{efg}	0.581 ^{i}	0.297 ^{ab}	0.066 ^{bcd}	0.015 ^{cde}	3.27 ^{cd}
Stylosanthes guianensis	397 ^{a}	0.621 ^{bcde}	0.260 ^{efgh}	0.060 ^{cde}	0.015 ^{cdef}	4.18 ^{ab}
Trypsacum andersonii	359 ^{bc}	0.582 ^{hi}	0.312 ^{a}	0.062 ^{cde}	0.013 ^{ef}	4.30 ^{a}
Urochloa ruziziensis	389 ^{ab}	0.589 ^{ghi}	0.297 ^{ab}	0.070 ^{abc}	0.012 ^{f}	4.43 ^{a}
Vigna unguiculata	405 ^{a}	0.622 ^{bcd}	0.269 ^{defg}	0.058 ^{e}	0.014 ^{cdef}	3.39 ^{c}
SEM[2]	5.14	0.002	0.002	0.001	<0.001	0.05
P-value	P<0.001	P<0.001	P<0.001	P<0.01	P<0.001	P<0.001

[1]In each column; means followed by a different letter differ at a significance level of 0.05,

[2]SEM, standard error of means

491 Table 4. Mineral content of forage ingredients (N=1)

	Macro-minerals (%)							Micro-minerals (ppm)						
	S	Cl	Ca	P	Mg	K	Na	Mn	Zn	Fe	Cu	Se	Co	Ni
Requirements for growing pigs (%) (20-50 kg) (NRC, 2012)	N/A[1]	0.08	0.60	0.50	0.04	0.23	0.10	2	60	60	4	0.15	N/A	N/A
Acacia mangium	0.35	0.92	0.68	0.10	0.11	1.49	0.13	57	16	252	5.9	0.38	<0.1	2
Amaranthus hybridus	0.88	0.45	2.29	0.60	1.16	6.29	0.12	33	47	1345	10.4	1.53	0.4	74
Urochloa ruziziensis	0.26	0.57	0.62	0.16	0.21	2.08	0.03	75	54	244	7.3	0.05	<0.1	13
Cajanus cajan	0.18	0.04	0.74	0.13	0.18	0.81	0.03	93	23	755	8.2	0.05	<0.1	22
Calopogonium muconoides	0.38	0.34	1.74	0.14	0.33	0.77	0.03	44	33	665	5.8	0.08	0.2	14
Centrosema pubescens	0.47	0.46	1.58	0.16	0.31	1.19	0.04	65	38	625	9.8	0.09	0.3	18
Eichhornia crassipes	0.49	2.52	1.08	0.11	0.51	3.98	0.32	396	50	220	4.9	<0.01	<0.1	6
Ipomoea batatas	0.75	1.56	1.57	0.28	0.33	5.13	0.14	54	32	520	8	0.02	0.3	16
Leucaena leucocephala	0.51	0.59	2.42	0.10	0.22	1.48	0.02	40	23	294	6.2	0.87	0.7	3
Manihot esculenta	0.43	0.09	2.07	0.31	0.33	1.40	0.03	30	90	136	8.4	0.15	0.8	2
Moringa oleifera	2.09	0.07	2.83	0.26	0.27	1.59	0.02	21	20	182	6.8	0.19	0.5	3
Mucuna pruriens	0.24	0.08	2.63	0.16	0.25	1.39	0.03	136	53	183	3.7	<0.01	0.2	3
Megathyrsus maximus	0.36	0.85	0.74	0.21	0.34	2.38	0.05	61	49	385	11.3	<0.01	<0.1	23
Pennisetum purpureum	0.15	0.74	0.36	0.12	0.16	3.36	0.03	80	23	230	8.9	0.86	0.3	5

Psophocarpus scandens	0.67	0.39	1.45	0.27	0.21	2.44	0.02	43	42	206	11.8	0.01	0.4	2
Pueraria phaseoloides	0.27	0.13	1.05	0.21	0.25	1.43	0.03	95	32	145	8.8	0.05	0.2	5
Saccharum officinarum	0.61	0.29	0.46	0.07	0.12	0.70	0.05	23	24	218	3	0.02	0.2	9
Stylosanthes guianensis	0.52	0.49	2.19	0.38	0.41	1.14	0.03	64	73	308	12.8	0.17	<0.1	13
Trypsacum andersonii	0.32	0.25	0.40	0.15	0.22	1.54	0.03	51	20	437	8.8	0.07	0.9	20
Vigna unguiculata	0.49	0.33	3.70	0.27	0.48	2.11	0.03	27	71	375	11.5	0.02	0.1	1

492 [1]N/A, not available

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Table 5. Indispensable and total amino acids (AA) of forage ingredients (g/16 g total N) (N=4)

	Indispensable Amino Acids								
	Arg	His	Ile	Leu	Lys	Phe	Thr	Val	Σ AAs[1]
Acacia mangium	4.92 abcde2	2.22 ab	4.01 bcde	6.83 abc	4.53 ab	4.51 bcde	4.24 bcd	5.37 ab	70.6 abcd
Amaranthus hybridus spp	4.73 abcde	1.71 cd	3.96 bcdef	6.21 cdefg	4.03 bc	3.97 fg	3.90 bcde	4.81 bcdef	67.4 cdef
Urochloa ruziziensis	5.69 ab	2.66 a	3.77 bcdefg	6.57 abcdef	2.20 h	4.24 defg	5.84 a	5.17 bc	73.0 abcd
Cajanus cajan	5.59 a	2.23 ab	4.76 a	7.76 a	4.76 a	5.26 b	4.64 abcd	6.01 a	79.5 a
Calopogonium muconoides	4.53 abcde	2.00 bc	4.28 ab	6.89 abcd	3.83 bcdf	4.77 bcde	4.17 bcde	5.31 b	71.4 abcd
Centrosema pubescens	3.53 ef	1.55 cd	3.16 gh	5.01 g	3.31 cdeg	3.59 fg	3.13 e	4.24 ef	59.1 ef
Eichhornia crassipes	4.73 abcde	1.87 bcd	4.15 bcd	6.95 abc	4.09 b	4.61 bcde	4.33 bcde	5.20 b	74.0 abc
Ipomoea batatas	4.53 abcde	1.63 cd	3.90 bcdefg	6.66 abcdef	3.19 efg	4.30 defg	4.35 bcde	5.13 bcd	69.7 abcde
Leucaena leucocephala	4.97 abcde	1.93 bc	3.67 cdefg	6.24 bcdefg	3.97 bc	4.28 defg	4.22 bcde	4.76 bcdef	66.4 cdef
Manihot esculenta	5.39 abc	2.08 abc	4.32 abc	7.13 abc	4.33 ab	4.75 bcde	4.36 bcde	5.41 ab	78.1 ab
Moringa oleifera	5.68 a	1.89 bc	3.77 bcdefg	6.20 cdefg	3.78 bcde	6.60 a	4.18 bcde	4.82 bcdef	74.5 abc
Mucuna pruriens	3.75 def	1.69 cd	3.57 defgh	5.58 fg	3.22 eg	3.80 fg	3.63 cde	4.52 cdef	63.2 def
Megathyrsus maximus	2.95 f	1.35 d	3.05 h	5.15 g	3.08 g	3.56 g	3.45 e	4.36 ef	63.3 def
Pennisetum purpureum	4.41 abcde	1.83 bcd	3.80 bcdefg	6.79 abcde	3.65 bcdeg	4.83 bcde	4.65 abcd	4.99 bcde	73.1 abc
Psophocarpus scandens	3.98 cdef	1.92 bc	3.45 efgh	5.77 defg	3.33 deg	4.36 def	4.33 bcde	4.27 f	63.8 def
Pueraria phaseoloides	4.15 bcdef	1.91 bc	3.77 bcdefg	6.13 cdefg	3.41 cdeg	4.46 cdef	4.44 bcde	4.81 bcdef	69.4 bcd
Saccharum officinarum	4.37 abcde	1.83 bcd	3.73 bcdefg	6.49 bcdef	3.11 g	4.78 bcde	4.69 abc	5.08 bc	73.0 abc
Stylosanthes guianensis	5.24 abcd	2.07 abc	3.98 bcdef	6.63 abcdef	3.60 bcdeg	5.14 bcd	4.78 abcd	4.77 bcdef	72.6 abcd
Trypsacum andersonii	5.34 abc	1.83 bcd	4.02 bcde	7.36 ab	3.04 g	5.21 bc	4.91 ab	5.22 b	74.2 abc
Vigna unguiculata	5.12 abcd	1.93 bc	3.80 bcdefg	6.35 bcdef	3.33 deg	4.93 bcd	4.90 ab	4.87 bcdef	77.0 ab
SEM[3]	0.136	0.084	0.067	0.121	0.082	0.117	0.110	0.074	1.010
P value	0.022	0.049	0.001	0.004	<0.001	<0.001	0.049	0.001	<0.001

[1]Sum of total AA including essential and non-essential amino acids (except sulfur AA and tryptophan)

[2]In each column; means followed by a different letter differ at a significance level of 0.05

[3]SEM, standard error of the means

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