Nutritive value of unconventional fibrous ingredients fed to Guinea pigs in the Democratic Republic of Congo

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Abstract The energy and protein value for Guinea pigs (GP) of 9 forages (7 dicots and 2 grasses) and 5 hay-based diets was determined. The apparent faecal digestibility of dry matter, organic matter, crude protein and energy was measured on GP housed in metabolic cages. The forages and the diets were digested *in vitro* using pepsin and pancreatin hydrolysis and gas fermentation test to simulate stomach, small intestine and large intestine, respectively. Most of the dicots had high digestible crude protein content (152-201 g/kg DM) and the 2 grasses showed lower values (80-85 g/kg DM). Digestible energy content of the forages ranged between 5.79 to 13.08 MJ/kg DM. None of the forage species or hay-based diets provided sufficient energy to supply the 11.7 MJ/kg metabolic energy requirements. The influence of intestinal fermentation on energy and protein values was highlighted by correlations ($P<0.05$) between in *vivo* and *in vitro* data, including gas fermentation. It is the first time that such relationships are reported in single-stomach animals.

Keywords Guinea pig · nutritive value · forage · in vitro method

Abbreviations

ADF acid detergent fibre

ADL acid detergent lignin

CP crude protein

DCP digestible crude protein

DE digestible energy

DM dry matter

GP Guinea pig

IVCPD in vitro crude protein digestibility

IVDMD in vitro dry matter digestibility

NDF neutral detergent fibre

T/2 half-time to asymptote

Introduction

In Kinshasa, Guinea pigs (*Cavia porcellus*) (GP) are raised by periurban families as a strategy to cope with food insecurity (Nkidiaka 2004). Besides poor breeding management which leads to low performances and probable high levels of consanguinity, farmers feed their animals local herbs harvested from their backyards, along the roads or nearby rivers. As shown in a previous study (Bindelle et al. 2007c), the choice of forage species fed relies more on forage availability and on its palatability to GP rather than on animal requirements and plant nutritive value.

Currently, the growth requirements for GP raised for meat production have not yet been determined, and recommendations for laboratory animals are used. For growing GP, ME content should be at least 11.7 MJ kg^{-1} (NRC, 1995) while CP could vary from 13 to 17 % of the diet (Chauca de Zaldívar 1997) depending on the DM intake. This wide range of variation stresses the high versatility of the GP's digestion system. It can be fed either concentrates or forages as it can rely either on starch digestion or on fermentation of non-starch polysaccharides in its large hindgut as the main energy source. Fermentation contributes very significantly to nutrient supply through direct absorption of bacterial metabolites and short-chain fatty acids or ingestion of faeces during coprophagy, especially when high fibre diets are provided. Depending on the ingredient, Chauca de Zaldívar (1997) indicates a decrease in DM digestibility ranging from 5 to 30 % when coprophagy is hindered. Studies on the nutrient value of forages for GP in South America have been conducted (Chauca de Zaldívar 1997; Clemente et al. 2003; Mattos et al. 2003). However, no research has been carried out to determine nutritive value of forages for GP in Africa.

This study was designed to determine the energy and protein value of local fresh forages available in periurban areas of Kinshasa and hay-based diets through *in vivo*

digestibility trials. The contribution of intestinal fermentation to energy and protein supply was also investigated through *in vitro* pepsin-pancreatin digestibility and gas fermentation tests.

Materials and methods

Study area

The *in vivo* experiments were performed between April and December 2007 at the Institut supérieur agro-vétérinaire (ISAV) experimental station in Kimwenza, Province of Kinshasa, Democratic Republic of Congo (4°27'S, 15°17'E). This area is characterised by a hilly relief (altitude 500 m) and a humid tropical climate with a mean annual rainfall of 1,374 mm, a dry season of 120 days (from end May to mid September) and a mean annual temperature of 23.3°C (Compère 1970).

Total tract *in vivo* digestibility

Animals, plant material and diets

A group of 40 male Guinea pigs (0.510 ± 0.107 kg) from the herd of the ISAV was used for the *in vivo* digestibility measurements. Nine forages were studied: *Amaranthus hybridus* (Amaranthaceae, Amaranth), *Commelina diffusa* (Commelinaceae, Dayflower), *Desmodium intortum* (Fabaceae, Greenleaf desmodium), *Euphorbia heterophylla* (Euphorbiaceae, Milk weed), *Panicum maximum* (Poaceae, Guinea grass), *Psophocarpus scandens* (Fabaceae, Winged bean leaves), *Synedrella nodiflora*

(Asteraceae, Nodeweed), *Talinum triangulare* (Portulacaceae, Waterleaf) and *Trypsacum laxum* (Poaceae, Guatemala grass).

The species were chosen according to their crude protein content (Table 1), their favourable palatability for Guinea pigs (Bindelle et al. 2007c) and to their availability in the local farming areas. All the forages were harvested during the vegetative growth phase, except for *D. intortum*, *E. heterophylla* and *T. triangulare* which were harvested during fructification.

Five *Panicum maximum* and *Desmodium intortum* sun-dried hay-based diets were also investigated (Table 2). These diets were investigated as a first attempt to cope with the low fresh forage availability during the dry season. The diets were designed to contain variable levels of crude protein and digestible energy.

Methodology

The methodology used for *in vivo* digestibility trials was adapted from the European reference method used in rabbits (Perez et al. 1995). The experimental design was completely randomized with change-over of Guinea pig between two periods of measurements for the same forage or diet. Six animals were allocated to one forage or one diet. After 7 days of adaptation to the forage or the diet, the animals were placed in individual metabolism cages designed to collect urine and feces separately. During the 5 d-collection period, the animals were fed *ad libitum* the forages or the diets in two meals (08:00 and 15:00). The amount of distributed forage was adjusted for each animal in order to reach a refusal ratio of 0.2 expressed in DM. The animals had free access to vitamin C-enriched water. Feces and refusals were totally collected every day, weighed and kept at -18°C. At the end of the experimental period, the animals were randomly

assigned to another experimental forage or diet and the procedure was repeated for a second period. An animal could be on the same diet or forage for 2 periods.

Chemical analyses

Distributed forages and diets, refusals and feces were oven dried at 60°C, ground to pass through a 1 mm-mesh screen by means of a Cyclotec 1093 Sample Mill (FOSS Electric A/S, Hilleroed, Denmark) and analyzed for their content of DM by drying at 105 ºC for 24 h (AOAC, 1990; 967.03 method), ash by burning at 550 ºC for 8 h (AOAC, 1990; 923.03 method), and crude protein using the Kjeldahl method (crude protein = N × 6.25; AOAC, 1990; 981.10 method). Gross energy was measured by means of an adiabatic oxygen bomb calorimeter (1241 Adiabatic Calorimeter, PARR Instrument Co., Illinois, USA). The forages and the diets were also analyzed for NDF using Termamyl (Novo Nordisk, Bagsværd, Denmark), ADF and ADL using the Fibercap system (Foss Electric, Bagsvaerd, Denmark) (Van Soest et al. 1991).

Calculations and statistical analyses

Apparent fecal digestibility was calculated by the difference between the amount of nutrients ingested and excreted. The individual Guinea pig was considered as the experimental unit. The influence of the forage or the diets on the digestibility coefficients was analyzed using the GLM procedure of the SAS 8.02 software (SAS Institute, 1999) and classification of means was realized with the Student-Newman-Keuls method.

In vitro digestion and fermentation

In order to simulate the digestion of the forages in the stomach and the small intestine of the GP as well as the fermentation occurring in the large intestine, a two step *in vitro* method including enzymatic hydrolysis followed by a gas fermentation test was used. *In vitro* pepsin and pancreatin digestion of the distributed forage samples was performed according to Boisen and Fernández (1997). Briefly, samples of 2 g were hydrolysed at 39°C with porcine pepsin (Merck n°7190) for 2 h at pH 2 and with pancreatin (Sigma P-1750) for 4 h at pH 6.8. After hydrolysis, the residues were collected by filtration on a Nylon cloth (42 µm), washed with 96 % ethanol and acetone, dried at 60°C and weighed. The enzymatic hydrolysis was performed 6 times (2 replicates × 3 periods). The hydrolysis residues from the different replicates and periods were pooled for subsequent *in vitro* fermentation.

The fermentation of the residues was carried out according to the procedure of Bindelle et al. (2007a) except that the inoculum was obtained from GP faeces. After collection, the faeces were kept in liquid nitrogen until use (Stanco et al. 2003). The inoculum was prepared by mixing faeces (50 g/l) with a buffer solution composed of salts and minerals (Menke and Steingass 1988). The fermentation at 38°C started with the introduction of 200 mg of one of the hydrolysed substrates and 30 ml of the inoculum into 100 ml-glass syringes.

The volume of released gas was recorded after 2, 5, 8, 12, 16, 20, 24, 30, 36, 48, 72 and 144 hours of incubation. Three syringes containing only inoculum (blanks) were systematically included in each run. The experimental scheme was as follows: (3 replicates × 14 substrates + 3 blanks) × 2 periods.

Calculations and statistical analysis

The *in vitro* dry matter disappearance (IVDMD) and crude protein disappearance (IVCPD) during the pepsin-pancreatin hydrolysis was calculated as follows:

$$dDM = \frac{X - Y}{X}$$

where X is the weight of the sample before hydrolysis and Y the weight of the residue. Gas accumulation curves were modelled according to France et al. (1993):

$$G = 0, \qquad \text{if } 0 < t < L$$

$$= G_f \left(1 - \exp\left\{-\left\langle b(t-L) + c\left(\sqrt{t} - \sqrt{L}\right)\right\rangle\right\}\right), \qquad \text{if } t \geq L$$

where G denotes the gas accumulation, G_f (ml/g hydrolyzed residue) the maximum gas volume for t = ∞ and L (h) the lag time before the fermentation starts. The constants b (1/h) and c (1/$\sqrt{}$h) determine the fractional rate of degradation of the substrate μ (1/h), which is postulated to vary with time as follows:

$$\mu = b + \frac{c}{2\sqrt{t}}, \qquad \text{if } t \geq L$$

The kinetics characteristics (G_f, L, $\mu_{t=T/2}$ and $T/2$) were compared in the statistical analysis (Figure 1). The half-time to asymptotic gas production when $G = G_f / 2$ is symbolized by $T/2$. At this time, the rate of gas production is in a linear phase, near its maximum. The syringes that suffered an accidental leakage of gas due to broken clips were discarded.

Statistical analyses of the volume of gas production and the kinetics parameters were performed using the GLM procedure of the SAS 8.02 software (SAS Institute, 1999) and classification of means was realized with the Student-Newman-Keuls method. Correlations and regressions were calculated according to the CORR and the REG procedures of the SAS 8.02 software (SAS Institute, 1999).

Results

Total tract *in vivo* digestibility

A. hybridus showed the highest digestibility coefficient and nutritive value in terms of digestible energy (DE) and crude protein (DCP) content followed by *E. heterophylla* and *D. intortum*. The low digestibility of *P. scandens* was counterbalanced by its high CP content (276 g/kg DM) to yield comparable DCP values to *A. hybridus*, *E. heterophylla* and *D. intortum* (179–201 g/kg DM) ($P>0.05$). However, DE content of *P. scandens* was as low as *P. maximum* and *T. laxum* (8.55-9.80 MJ/kg DM), which showed the lowest digestibilities, DE and DCP of all forage species ($P<0.05$), except *C. diffusa*. *C. diffusa* yielded low digestibility coefficients for all nutrients and low DE and DCP ($P<0.05$).

Crude protein content of the hay-based diets varied between 129 and 172 g/kg DM. NDF and ADF contents ranged from 423 to 516 and 239 to 311 g/kg DM, respectively, as a consequence of the variable concentration of *P. maximum*, *D. intortum* hays and wheat bran. Finally, as expected, the diets contained variable levels of DE and DCP ($P<0.05$), ranging from 7.61 to 11.45 MJ/kg DM and from 84 to 126 g/kg DM, respectively.

In vitro digestibility and fermentation

Pepsin-pancreatin *in vitro* digestibility of the DM (IVDMD) (Table 4) ranged between 0.301 and 0.596, depending on the forage ($P<0.001$), while *in vitro* digestibility of the CP (IVCPD) varied from 0.478 to 0.884. With lag time and half-time to asymptote values among the lowest (ranging from 10 to 13 h and 29 to 33 h, respectively) ($P<0.05$) and final gas volume among the highest (192 to 229 ml/g DM) ($P<0.05$), *A. hybridus*, *E.*

heterophylla and *D. intortum* were the most fermentable substrates. *T. triangulare* was highly fermentable as well, but its fermentation began later (14 h) and proceeded at lower rates (0.043 1/h), although these were not significantly different from the lag time and fermentation rates of the previous substrates. *S. nodiflora* had the fastest fermentation ($P<0.05$), highest fractional rate of degradation (0.080 1/h) and lowest half-time to asymptote (29 h). However, the final gas volume was low (135 ml/g DM) and fermentation started late - the lag time reached 21 h. With the lowest fractional rate of degradation (0.039 1/h) ($P<0.05$) and a final gas volume among the lowest (123 ml/g DM) ($P<0.05$) of the species examined, *P. scandens* was the poorest fermentable substrate.

Composition of the diets influenced their pepsin-pancreatin hydrolysis ($P<0.001$) with IVDMD ranging from 0.412 to 0.510 and IVCPD from 0.683 to 0.829. However, final gas production was not affected by the diet ($P>0.05$), while kinetics were affected ($P<0.01$). Diet 1 and Diet 5 showed the extreme values: Diet 1 yielded the highest lag time (21 h), but also the highest fractional rate of degradation (0.045 1/h), while the fermentation of the Diet 5 started the earliest (11 h) but had the lowest fractional rate of degradation (0.021 1/h) ($P<0.05$).

Relationships between chemical composition, *in vitro* and *in vivo* digestibility

Due to its poor protein digestibility and digestible crude protein values, *C. diffusa* was removed from the database for the calculation of the Pearson's correlation coefficients between *in vivo* measurements and chemical composition and *in vitro* digestibilities and gas fermentation test. CP content of forages and diets was positively correlated to all *in vivo* digestibility parameters as well as their digestible energy and crude protein content ($r>0.593$, $P<0.05$) (Table 5). Conversely, NDF, hemicellulose and ADF content was

(P<0.05), or tended to be (P<0.10), negatively correlated to digestibility of all nutrients, DE and DCP (r<-0.515).

In vivo digestibilities of all nutrients, DE and DCP content of the forages and the diets were correlated to IVDMD during pepsin-pancreatin hydrolysis (r>0.618, P<0.05). Lag time of *in vitro* fermentation of the hydrolyzed residues was negatively correlated to crude protein digestibility and to digestible energy content. Half-time to asymptote was negatively correlated to *in vivo* digestibility coefficients of all nutrients and to DCP of the forages and the diets (r<-0.639, P<0.05). Finally, final gas volume was correlated to DM, OM and energy digestibilities (r>0.710, P<0.05) and tended to be correlated to crude protein digestibility and DE content (r>0.487, P<0.10).

Discussion

The aim of this experiment was to determine the energy and protein values of forages available in periurban Kinshasa for feeding Guinea pigs. Except *C. diffusa* and *T. triangulare*, all dicots had high DCP content, meeting the recommendations (NRC, 1995) and, as expected, the 2 grasses, *P. maximum* and *T. laxum* showed lower values.
DE content of all forages except *C. diffusa, P. maximum* and *P. scandens* were higher than that reported for corn husk (9.97 MJ/kg DM; Chauca de Zaldívar 1997). *A. hybridus, E. heterophylla, D. intortum* and *S. nodiflora* also had higher DE content compared to alfalfa (10.38 MJ/kg DM; Chauca de Zaldívar 1997). However, none of the species or hay-based diet were able to provide the 11.7 MJ/kg metabolic energy requirements (NRC 1995). This stresses the importance of considering the voluntary intake of the animals which can vary significantly. Therefore, according to previous results on the palatability of these species (Bindelle et al. 2007c) and considering the DE contents measured here, *A. hybridus, E. heterophylla*, *D. intortum* are the best

forages among those that were investigated to feed GP in periurban Kinshasa. Due to the low dietary fibre content which has been shown to decrease intakes (Chauca de Zaldívar 1997), and due to their low NDF content, *A. hybridus* and *E. heterophylla* should be combined with high NDF containing species such as *T. laxum* or *P. maximum*, since these grasses have a favourable combination of high NDF content, high fibre fermentability, and high palatability. Interestingly, in this study, the high tannin content of *D. intortum* did not negatively influence the IVDMD, IVCPD, or fermentation kinetics compared to the other forage species. In the *in vitro* model of the gastro-intestinal tract of the GP, only the fibrous residue undergoes fermentation, which is consistent with the observation of Mbugua et al. (2008) who noted that there was no depressing effect of the tannins when, instead of the whole plant, isolated NDF of *D. intortum* was fermented by rumen bacteria.

Nevertheless, if the growth performances must be maximized using improved breeds, it is needed to complement the forages with high quality energy source such as corn which DE content must be higher than that reported for barley (15.58 MJ/kgDM; Chauca de Zaldívar 1997) or fullfat grains. Besides the problem of vitamin C supply, the drying of forages to make hays for this experiment markedly reduced the nutritive value of the diets compared to the fresh forages. Even for the diets with significant amounts of palm oil, the DE content of the diets containing high levels of *P. maximum* remained below the best forages. Further investigation on this specific topic is therefore still required to find valuable alternatives to fresh forages during the dry season.

The poor in vivo digestibility of *Commelina diffusa* was consistent with its poor palatability reported by Bindelle et al. (2007c). Although rats (Clark 1982) and chimpanzees (Huffman and Wrangham 1994) were reported to eat *C. diffusa* in the wild, and its potential as a ruminant feedstuff was recently investigated *in vitro* (Lanyasunya et al. 2006), this species must be advised against for feeding GP as it

probably contains some anti-nutritional and antimicrobial factors (Mensah et al. 2006) which may depress voluntary intake and digestion processes.

In vitro digestibility results indicate that the energy supply to GP fed the investigated tropical forages or forage-based diets relies on digestion processes occurring in the stomach and the small intestine, and on the direct or indirect absorption of intestinal fermentation products, SCFA and microbial cells. This role of intestinal fermentation was clearly pointed out by the relationships between DM, OM and energy digestibility and gas production parameters. The absence of relationship between IVCPD and *in vivo* CP digestibility or DCP content is surprising but is probably due to coprophagy which is not considered in the *in vitro* simulation of the upper tract digestion using pepsin and pancreatin. Hindgut fermentation strongly affects the protein value of fibrous ingredients in coprophagic animals like GP (Chauca de Zaldívar 1997), rats or rabbits, contrary to pigs or other single stomach animals that don't use coprophagy as a feeding strategy to spare protein. Protein digestibility of alfalfa in rabbits is approximately 50 % when coprophagy is hindered while it surpasses 75 % when coprophagy is allowed (Irlbeck 2001). In this study, the influence of fermentation on the protein value of the forages and the diets is confirmed by the negative relationship between *in vitro* half-time to asymptote (T/2) and *in vivo* crude protein digestibility and digestible crude protein content of the forages and the diets. Ingredients that are quickly fermented induce high intestinal bacterial growth (Bindelle et al. 2007b). Unlike the pig where this improved bacterial growth induces a shift of N excretion from urine to faeces but doesn't affect N retention (Bindelle et al. 2009), in GP, it probably increases microbial protein and amino acid recycling during coprophagy as well. The combined influence of upper digestive tract digestion and bacterial fermentation on the protein value of the investigated forages and diets was finally highlighted by a regression equation predicting DCP (g/kgDM) content from IVCPD (-)

and T/2 (h) (DCP=137 – 4.36 T/2 + 239 IVCPD, R^2=0.720, P<0.001, S=24.7), with IVCPD showing no correlation to T/2 (r=0.209, P=0.494).

Furthermore, it can also be noted that the combination of *in vitro* pepsin and pancreatin enzymatic hydrolysis and the gas fermentation test is a useful tool for investigating digestibility of fibrous ingredients in GP. It is also the first time that significant relationships between *in vivo* and *in vitro* digestibility including gas fermentation parameters are reported in single-stomach animals.

It can be concluded from the DE and DCP contents measured in this *in vivo* study that in periurban Kinshasa, *P. maximum* or *T. laxum* should be fed to GP in combination with *A. hybridus*, *E. heterophylla* or *D. intortum*. However, this recommendation should be confirmed through growth experiments in order to verify the need to combine these forages to an additional energy source to reach the optimal GP growth rates. Further investigation on the true protein value of these resources for coprophagic animals is also needed in order to find the right combination of forage species to properly balance the amino acid profile of the diets.

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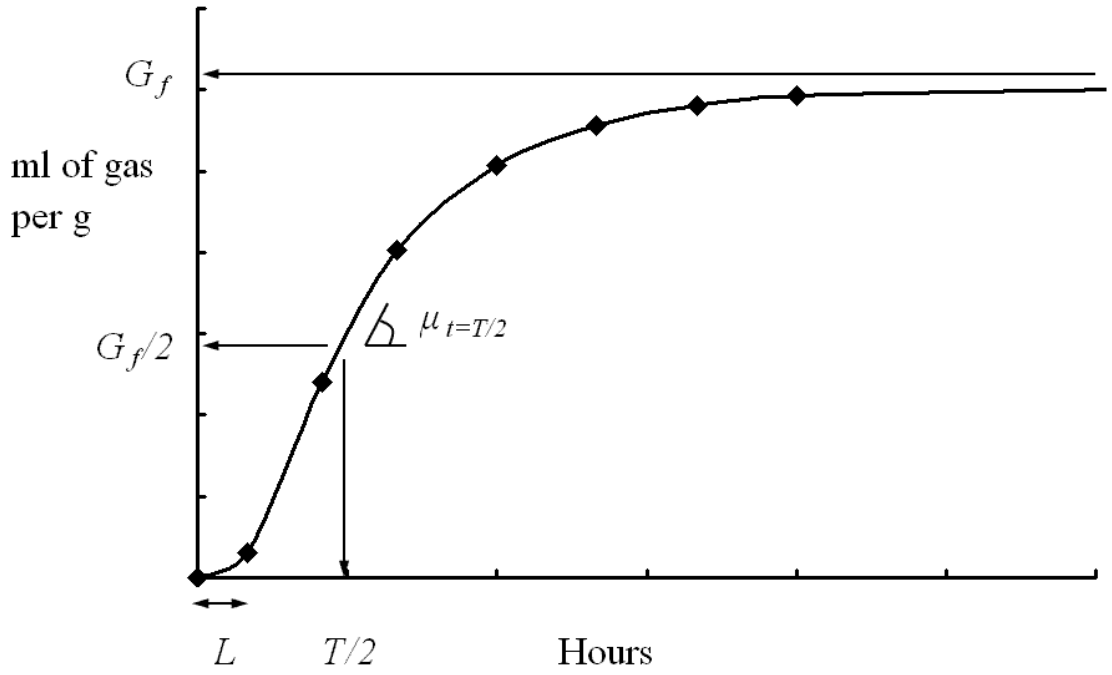

Fig. 1 Representation of the kinetic parameters of the gas accumulation curves modelled according to France et al. (1993), where G_f (ml/g of substrate) denotes the maximum gas volume for t = ∞, L (h) denotes the lag time before fermentation starts, $T/2$ denotes the half-time to asymptotic gas production when $G = G_f/2$, and $\mu_{t=T/2}$ (1/h) denotes the fractional rate of degradation of the substrate

Table 1 Analysis of the forages (g/kgDM)

	Amaranthus hybridus	*Commelina diffusa*	*Desmodium intortum*	*Euphorbia heterophylla*	*Panicum maximum*	*Psophocarpus scandens*	*Synedrella nodiflora*	*Talinum triangulare*	*Trypsacum laxum*
Dry matter	186	110	210	175	277	215	159	89	198
(g/kg, as fed basis)									
Gross energy (MJ/kg DM)	16.33	15.26	17.03	16.71	17.31	17.36	16.75	15.10	18.23
Ash	203	227	97	81	110	96	160	130	112
Crude protein	240	132	234	252	148	276	204	152	150
NDF	249	311	348	275	721	396	395	310	651
ADF	159	231	245	177	374	217	369	268	411
ADL	69	48	52	53	61	70	124	107	65

Table 2 Composition and analysis of the hay-based diets (g/kg, as fed basis)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Composition					
Desmodium intortum hay	-	428	5	332	159
Panicum maximum hay	800	531	558	333	519
Commercial rabbit concentrate[1]	200	-	4	195	61
Wheat bran	-	410	351	12	44
Okara	-	-	8	126	158
Palm oil	-	-	74	9	59
Analyzed					
Dry matter	914	939	930	919	917
Gross energy (MJ/kg DM)	17.57	17.39	19.49	18.57	20.11
Ash	89	122	72	112	83
Crude protein	133	160	129	172	161
NDF	512	516	474	423	431
ADF	306	307	239	239	257
ADL	57	80	41	54	40

[1] Midema (Matadi, Democratic Republic of Congo)

Table 3 *In vivo* apparent faecal digestibility and digestible energy and crude protein content of the forages and the diets

Diets	N[1]	dDM (-)	dOM (-)	dE (-)	dCP (-)	DE (MJ/kg DM)	DCP (g/kg DM)
Forages							
Amaranthus hybridus	12	0.767 a[2]	0.800 a	0.794 a	0.805 a	13.08 a	193 a
Commelina diffusa	8	0.434 d	0.433 d	0.380 e	0.066[3]	5.79 e	9[1]
Desmodium intortum	8	0.706 a	0.710 a	0.652 bc	0.807 a	11.10 abc	189 a
Euphorbia heterophylla	7	0.772 a	0.809 a	0.729 ab	0.798 a	12.17 ab	201 a
Panicum maximum	19	0.495 cd	0.468 cd	0.494 d	0.541 c	8.55 d	80 c
Psophocarpus scandens	7	0.596 bc	0.598 b	0.554 cd	0.648 bc	9.62 cd	179 ab
Synedrella nodiflora	12	0.678 ab	0.763 a	0.709 ab	0.742 ab	11.74 abc	152 b
Talinum triangulare	6	0.730 a	0.738 a	0.667 b	0.606 c	10.07 bdc	91 c
Trypsacum laxum	12	0.534 cd	0.557 bc	0.540 cd	0.567 c	9.80 cd	85 c
SEM[4]		0.015	0.018	0.016	0.017	0.275	6.33
Hay-based diets							
Diet 1	8	0.445 bc	0.442 b	0.433 b	0.627 bc	7.61 c	91 b
Diet 2	7	0.422 c	0.424 c	0.402 c	0.579 c	6.99 c	100 b
Diet 3	8	0.481 abc	0.496 ab	0.498 ab	0.590 c	9.71 b	84 b
Diet 4	6	0.520 a	0.496 ab	0.576 a	0.676 ab	10.69 ab	126 a
Diet 5	8	0.533 a	0.541 a	0.569 a	0.672 b	11.45 a	118 a
SEM		0.012	0.012	0.015	0.015	0.313	5.05

[1] N, number of replicates

[2] in a column for forages or diets separately, means followed by a different letter differ at a significance level of 0.05

[3] not included in the statistical analysis

[4] SEM, standard error of means

Table 4 *In vitro* digestibility and gas production parameters of the forages and the diets

Diets	N[1]	IVDMD (-)	IVCPD (-)	N	Lag time (h)	Half-time to asymptote (h)	Fractional rate of degradation (1/h)	Final volume (ml/g DM)
Forages								
Amaranthus hybridus	6	0.590 a[2]	0.792	6	11 c	29 d	0.049 b	192 bc
Commelina diffusa	6	0.452 e	0.597	4	20 b	37 bcd	0.060 ab	171 d
Desmodium intortum	6	0.559 b	0.747	6	13 c	33 cd	0.053 b	194 bc
Euphorbia heterophylla	6	0.596 a	0.884	6	10 c	32 cd	0.051 b	229 a
Panicum maximum	6	0.307 g	0.669	6	27 a	46 a	0.047 b	140 e
Psophocarpus scandens	6	0.494 d	0.736	6	14 c	42 ab	0.039 c	123 e
Synedrella nodiflora	5	0.423 f	0.554	3	21 b	29 d	0.080 a	135 e
Talinum triangulare	6	0.523 c	0.574	4	14 c	40 abc	0.043 bc	204 b
Trypsacum laxum	6	0.301 g	0.478	5	21 b	40 abc	0.049 b	181 cd
SEM[3]		0.015	-		0.93	1.03	0.00193	5.36
Hay-based diets								
Diet 1	6	0.412 d	0.756	6	21 a	47 b	0.045 a	107
Diet 2	6	0.429 c	0.790	6	18 a	46 b	0.041 a	105
Diet 3	6	0.476 b	0.683	6	12 b	44 b	0.032 a	108
Diet 4	6	0.510 a	0.829	6	14 b	47 b	0.035 a	97
Diet 5	6	0.483 b	0.824	5	11 b	57 a	0.021b	113
SEM		0.006	-		0.75	1.13	0.00168	3.13

[1] N, number of replicates

[2] in a column for forages or diets separately, means followed by a different letter differ at a significance level of 0.05

[3] SEM, standard error of means

Table 5 Pearson's correlation coefficient between *in vivo* apparent faecal digestibility and digestible energy and crude protein content and the *in vitro* enzymatic digestibility and gas fermentation parameters of the forages and the diets (N=13)

	dDM (-)	dOM (-)	dE (-)	dCP (-)	DE (MJ/kg DM)	DCP (g/kg DM)
Proximate composition						
Gross energy (MJ/kg DM)	-0.716**	-0.680*	-0.551†	NS	NS	NS
Ash (g/kg DM)	NS	NS	0.487†	NS	NS	NS
CP (g/kg DM)	0.630*	0.618*	0.593*	0.753**	0.559*	0.958***
NDF (g/kg DM)	-0.839***	-0.836***	-0.814***	-0.809***	-0.716**	-0.776**
Hemicellulose (g/kg DM)	-0.826***	-0.866***	-0.802**	-0.716**	-0.646*	-0.610*
ADF (g/kg DM)	-0.569*	-0.515†	-0.550†	-0.647*	-0.557*	-0.712**
ADL (g/kg DM)	NS	NS	NS	NS	NS	NS
Pepsin-pancreatin hydrolysis						
IVDMD (-)	0.651*	0.621*	0.637*	0.768**	0.618*	0.729**
IVCPD (-)	NS	NS	NS	NS	NS	NS
Gas fermentation parameters						
Lag time (h)	NS	NS	NS	-0.566*	-0.583*	-0.544†
Half-time to asymptote (h)	-0.782**	-0.814***	-0.732**	-0.642*	-0.527†	-0.639*
Fractional rate of degradation (1/h)	NS	-0.534†	NS	NS	NS	NS
Final volume (ml/g DM)	0.845***	0.800***	0.710**	0.487†	0.529†	NS

†, P<0.100; *, P<0.050; **, P<0.010; ***, P<0.001