Voluntary intake, chemical composition and *in vitro* digestibility of fresh forages fed to Guinea pigs in periurban rearing systems of Kinshasa (Democratic Republic of Congo)

J. Bindelle[1], Y. Ilunga[2], M. Delacollette[1], M. Muland Kayij[2], J. Umba di M'Balu[3], E. Kindele[3], A. Buldgen[1]

[1] *Gembloux Agricultural University, Department of Animal Husbandry, 2 Passage des Déportés, 5030 Gembloux, Belgium*

[2] *Centre agronomique et vétérinaire tropical, Gombe, Kinshasa, Democratic Republic of Congo*

[3] *Institut supérieur agro-vétérinaire, Kimwenza, Mont-Ngafula, Kinshasa, Democratic Republic of Congo*

Corresponding author :

Jérôme Bindelle

Gembloux Agricultural University, Department of Animal Husbandry, 2 Passage des Déportés, 5030 Gembloux, Belgium

Email : bindelle.j@fsagx.ac.be

Tel. + 32 81 62 21 33

Fax +32 81 62 21 15

Article is published in :
Trop Anim Health Prod (2007) 39:419–426
DOI 10.1007/s11250-007-9036-y

Abstract

Bindelle, J., Ilunga, Y., Delacollette, M., Muland Kayij, M., Umba di M'Balu, J., Kindele, E. and Buldgen, A. Voluntary intake, chemical composition and in vitro digestibility of fresh forages fed to Guinea pigs in periurban rearing systems of Kinshasa (Democratic Republic of Congo). *Tropical Animal Health and Production*.

The daily voluntary intake (DVI) of Guinea pigs (GP) fed 15 fresh forages used in periurban rearing systems of Kinshasa (Democratic Republic of Congo) was investigated. In order to determine the best forages combination for GP diet, DVI was compared to their nutritional value measured *in vitro* using 1) a pepsin-pancreatin hydrolysis, 2) an gas fermentation test on the hydrolysed residues with an inoculum prepared from GP faeces, and 3) the chemical composition of the offered feeds and the hydrolysis residues.

The forages ranking based on the DVI was correlated to the NDF content, but not to their nutritional values determined *in vitro*. According to their high DVI (from 4.23 to 7.75 g/kg liveweigth), and their valuable *in vitro* nutritional values (crude protein ranging from 261 to 279 g crude protein kg^{-1}DM, pepsin-pancreatin digestibilities of the dry matter from 0.55 to 0.59 and final gas production from 170 to 196 l kg^{-1}DM), *Desmodium intortum*, *Euphorbia heterophylla* or *Amaranthus hybridu*s, can be suggested to the farmers to complement the usual diet distributed to the GP based on *Panicum maximum*.

Keywords : Guinea pig, palatability, nutritive value, forage, urban agriculture

Abbreviations : ADF, acid detergent fibre; ADL acid detergent lignin; CF, crude fibre; CP, crude protein; DM, dry matter; DVI, daily voluntary intake; GP, Guinea pig; NDF, neutral detergent fibre; SCFA, short-chain fatty acids.

1. Introduction

The Guinea pig (*Cavia porcellus*) (GP) is a small herbivorous rodent originating from the central highlands of the Andes. Under intensified commercial systems it can be highly productive, but in tropical Africa, the rearing systems are characterised by a lack of management leading to low productivity (Hardouin et al., 1991). Like most of the unconventional livestock species, the actual contribution of GP to food security has been greatly ignored in the development policies. Nevertheless, the estimated stock of 32 millions GP raised for meat in the Andean region (Chauca de Zaldívar, 1997) is significant, compared, for example, to the 650 millions of rabbits reported over the world (FAO, 2006). In Africa, no data is available on the stock of GP and only limited research has been carried out in Cameroon to improve the traditional rearing and feeding systems (Kouonmenioc et al., 2000; Fonteh et al., 2005).

The growth requirements have not been determined yet and the recommendations for laboratory animals are used. Thanks to a large hindgut representing two thirds of its digestive tract, this monogastric animal has a very versatile digestion system and can be fed either on concentrates or on forages (Chauca de Zaldívar, 1997). Intestinal fermentation contributes to nutrient supply through direct absorption of bacterial metabolites and short-chain fatty acids or ingestion of faeces during coprophagy. However, like primates, GP are not able to produce enough C vitamin (NRC, 1995) and require to be fed with green plant materials or with C vitamin.

In Kinshasa, urban agriculture is a major strategy to face food insecurity (Trefon, 2000). GP are raised by 7 % of the people keeping farm animals at home, representing around 30 % of the families in the periurban area (Nkidiaka, 2004). They are fed with various grasses and herbaceous dicots harvested daily in the backyards, along the roads or

nearby the rivers. Until now, no objective information has been provided to the farmers for the feeding system, which is empirical and mainly depends on forage availability.

This study aimed to screen local forages available in urban and periurban areas of Kinshasa in order to evaluate their interest for feeding GP. For that purpose, we determined the palatability of a range of fresh forages and, their relation to their potential nutritive value, evaluated through their chemical composition, their *in vitro* pepsin-pancreatin digestibility and their fermentability using a gas-test method.

2. Materials and methods

Study area

The experiment was performed from October to December 2005 at the ISAV (Institut supérieur agro-vétérinaire) 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 1374 mm, a dry season of 120 days (from end May to mid September) and a mean annual temperature of 23.3°C (Compère, 1970).

Plant material and diet

Fifteen forages were studied : *Panicum maximum* (Poaceae, Guinea grass), *Aframomum alboviolaceum* (Zingiberaceae, Aframomum), *Desmodium intortum* (Fabaceae, Greenleaf desmodium), *Euphorbia heterophylla* (Euphorbiaceae, Milk weed), *Synedrella nodiflora* (Asteraceae, Nodeweed), *Talinum triangulare* (Portulacaceae, Waterleaf), *Amaranthus hybridus* (Amaranthaceae, Amaranth), *Ipomea batatas* (Convolvulaceae, Sweet potato leaves), *Psophocarpus scandens* (Fabaceae, Winged bean leaves), *Commelina diffusa* (Commelinaceae, Dayflower), *Urena lobata* (Malvaceae, Ceasar weed), *Pilea macrophylla* (Urticaceae, Artillery plant), *Sida acuta* (Malvaceae, Common wireweed), *Boerhavia erecta* (Nyctaginaceae, Erect spiderling) and *Asystasia gangetica* (Acanthaceae, Chinese violet).

The species were chosen according to their crude protein content, to the farmers habits to use them as feed ingredients (Table 2) or to their availability around the farms. All the forages were harvested during the vegetative growth phase, except for *D. intortum*, *E. heterophylla*, *T. triangulare* and *B. erecta* which were harvested during fructification.

All the forages were complemented by a mixture of 0.33 standard concentrate for rabbit (MIDEMA, Matadi, Democratic Republic of Congo) and 0.67 wheat bran. The chemical composition of the forages and the complement mixture are given in Table 1.

Animals

Sixteen male Guinea pigs (*Cavia porcellus*) of 184 ± 3 days from the herd of the Tropical Agronomic and Veterinarian Centre of Kinshasa (CAVTK, Kinshasa, Democratic Republic of Congo) weighing from 675 to 830 g were used. The animals were kept in 4 groups of similar total bodyweight during the entire experiment. Each of the 4 enclosures was equipped with 9 separate feeders. The animals had free access to fresh water.

Feeding procedure and experimental design

Forages were distributed over 2 periods, with 8 species per period. *D. intortum* was included in both periods. Each period included 7 days of adaptation to the forages and 10 days of data collection. Fresh forages were collected daily and distributed at 8:00 am and 3:00 pm *ad libitum* to the 4 groups of animals. Each group also received 185 g of the complement mixture at 3:00 pm. During the measurement phases, refusals were collected in the morning before the distribution. For each group, the forages and the complement mixture were randomly allocated to a feeder each day.

Animals were individually weighed on days 0, 7 and 17 of both periods.

Measurements and chemical analysis

The amounts of feeds and refusals were recorded for each group. Samples were taken and oven-dried at 60°C for dry matter determination. They were subsequently ground to pass a 1 mm-mesh screen in a Cyclotec 1093 Sample Mill (FOSS Electric A/S, Hilleroed, Denmark) and analysed for their content in dry matter (105 ºC for 24 h), ash (550 ºC for 8 h), nitrogen (Kjeldahl method, crude protein = 6.25 x N content), crude

fibre, NDF (using Na_2SO_3 and Termamyl, Novo Nordisk, Bagsværd, Denmark) and ADF and ADL, according to Van Soest et al. (1991), using the Fibercap system (Foss Electric, Bagsvaerd, Denmark).

In vitro digestion and fermentation

In order to simulate the digestion of the forages in the stomach and the small intestine of the GP and the fermentation occurring in the large intestine, a two step method including pepsin-pancreatin hydrolysis followed by a gas fermentation test was used.

In vitro pepsin-pancreatin digestion of the distributed forage samples was performed according to Boisen and Fernández (1997). Briefly, in this method, samples of 2 g were weighed in conical flasks and hydrolysed in a phosphate buffer solution by porcine pepsin (2000 FIP-U/g, Merck n°7190, pH 2, 120 min, 39 ± 0.5°C) and by pancreatin (Sigma P-1750, pH 6.8, 240 min, 39 ± 0.5°C)..Afterwards, the residues were collected by filtration on a Nylon cloth (42 µm), washed with 95 % ethanol and 99.5 % acetone, dried for 24 h at 60 ± 1°C and weighed. The enzymatic hydrolysis was performed 6 times (2 replicates x 3 periods).

The hydrolysis residues from the different replicates and periods were pooled for subsequent *in vitro* fermentation.

The fermentation of the residues was practised according to 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). Briefly, faeces (50 g l^{-1}) were mixed to a buffer solution composed of salts and minerals (Menke and Steingass, 1988). The fermentation at 37 °C started introducing 200 mg of one of the substrates and 30 ml of the inoculum into 100 ml-glass syringes.

The released gas volumes were recorded after 2, 5, 8, 12, 16, 20, 24, 30, 36, 48 and 72 hours incubation. Three syringes containing just inoculum (blanks) were systematically included in each run.

The experimental scheme was as follows : (3 replicates x 15 forages + 3 blanks) x 2 periods.

Calculations and statistical analysis

The daily voluntary intake (g g^{-1} liveweight x 100) of each forage species (*i*) was calculated daily for each group of animals (*a*) as follows :

$$\text{Intake}_i^a = \frac{\text{Offered DM}_i^a - \text{Refused DM}_i^a}{\text{LW}_a} \times 100 \quad (1)$$

where DM is the dry matter of the forage offered and refused daily (g) and LW is the mean liveweight of the group during the measurement phase (g).

The dry matter disappearance (*dDM*) of the forages during the pepsin-pancreatin hydrolysis was calculated as follows:

$$dDM = \frac{X - Y}{X} \quad (2)$$

where X is the weight of the sample before hydrolysis and Y the weight of the residue.

Gas accumulation curves were modelled using the mathematical monophasic model proposed by Groot et al. (1996) :

$$G = \frac{A}{1 + \frac{B^C}{t^C}}, \quad \text{if } t < 0 \quad (3)$$

where G (ml g^{-1}DM) denotes the gas accumulation to time, A (ml g^{-1}DM) the maximum gas volume for $t = \infty$, B (h) the time to half asymptote when $G = A/2$ and C a constant determining the slope of the inflexion point of the profile. R_M (ml g^{-1}DM h^{-1}) is the maximum rate of gas production, when the microbial population no longer limits the fermentation and t_{R_M} the time at which R_M is reached.

Statistical analysis of the daily voluntary intakes 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), with the following general linear model:

$$Y = \alpha + F_i + (P \times D)_{jk} + G_l + \varepsilon \quad (4)$$

where Y is the result, α the mean, F_i the effect of the forage (i = 1, ..., 15), P_j the effect of the period of distribution (j = 1, 2), D_j the effect of the day (k = 1, ..., 10), G_l the random effect of the group (l = 1, ..., 4) and ε the error term.

Statistical analysis of the gas volumes production and the kinetics parameters were performed using the following general linear model:

$$Y = \alpha + F_i + P_j + (P \times F)_{ij} + \varepsilon \quad (6)$$

where Y is the result, α the mean, F_i the effect of the forage (i = 1, ..., 15), P_j the effect of the period of incubation (j = 1, 2) and ε the error term.

Correlation was calculated according to the CORR procedure of the SAS 8.02 software (SAS inc., Cary, NC, USA).

3. Results

Voluntary intake

The daily voluntary intakes (DVI) of the forages are shown on Table 1. No influence of the period on *D. intortum* DVI was observed (P = 0.860).

The total DM ingestion (forages + complement mixture) reached 84.3 g/kg liveweight or 78.1 g/kg$^{0.75}$. The daily forage intake reach 179 g/kg liveweight on a fresh basis or 35.2 g DM/kg liveweight. High differences were observed according to the species (P < 0.001). *P. maximum* and *A. alboviolaceum* were highly palatable with DVI of nearly 10 g DM/kg liveweight. *D. intortum* and *E. heterophylla* with DVI approaching 7.5 g DM/kg liveweight were also appreciated by the GP. Other forages, from *S. nodiflora* to *P. scandens*, could be considered as well accepted since they represented from 8 to 17 % of the ingested forages. *U. lobata*, *C. diffusa* and *P. macrophylla* appeared to be moderately ingested by the GP, while *B. erecta*, *S. acuta* and *A. asystacia* were unpalatable.

Chemical composition, in vitro digestibility and fermentation

The chemical composition of the fresh forages and their pepsin-pancreatin hydrolysed residues are detailed in Table 2. Dry matter content of the fresh forages varied from 103 g kg^{-1} to 339 g kg^{-1}, crude protein (CP) content from139 g kg^{-1}DM to 297 g kg^{-1}DM. Ash content ranged between 33 and 246 g kg^{-1}DM, crude fibre from 171 to 299 g kg^{-1}DM, NDF from 270 to 617 g kg^{-1}DM and ADF from 176 to 346 g kg^{-1}DM.

Pepsin-pancreatin digestibilities and gas production kinetics of the hydrolysed forages modelled according to the monophasic model of Groot et al. (1996) are given in Table 3. The dDM ranged between 0.25 and 0.59, according to the forage (P<0.001), while dCP varied from 0.19 to 0.81. The pepsin pancreatin hydrolysis also induced an enrichment in NDF, ADF and crude fibre of all the substrates. Final gas production (*A*)

varied from 91 ml g^{-1}DM for *U. lobata* to 216 ml g^{-1}DM for *I. batatas* (P<0.001). These substrates gave also the extreme values for the maximum rate of fermentation (R_M) which ranged between 2.30 to 7.60 ml g^{-1}DM (P<0.001). Mid-fermentation times (B) and time at which R_M is reached (t_{R_M}) varied from 15.7 and 7.57 h to 38.5 and 35.1 h (P<0.001), respectively. For *A. alboviolaceum*, gas production was so low that the model could not be fitted to the recorded curves.

Correlation between the variables

DVI was correlated to the hemicellulose content of the forages (r = 0.706, P = 0.003). Hemicellulose was calculated as the difference between NDF and ADF. DVI was also correlated to the NDF (r = 0.682, P = 0.005) and hemicellulose contents (r = 0.558, P = 0.031) of the hydrolysed residues. No correlation linking DVI to *in vitro dDM* or the fermentation kinetics parameters was observed.

The *dDM* was negatively correlated to the NDF content of the substrates (r = -0.682, P = 0.005) and positively to ash and crude protein content (r = 0.600, P = 0.018 and r = 0.764, P =0.001 respectively). *A* and R_M were also correlated to *dDM* (r = 0.731, P = 0.003 and r = 0.697, P =0.006 respectively).

4. Discussion

The results show that daily voluntary intake (DVI) is the main factor influencing the farmers in their feed choice. The commonly-used forages are amongst those with high DVI. However, since palatability is extremely difficult to define in terms of biological processes involved in feed selection (Molyneux and Ralphs, 1992), some species show a strong discrepancy between daily voluntary intake (DVI) (Table 1) and nutritive value as measured through chemical composition, *in vitro* digestibility and fermentation parameters (Tables 2 and 3) : *P. maximum*, *A. alboviolaceum* with high DVI and *B. erecta* and *A. gagentica* with low DVI.

A large majority of the species covered the CP requirements of the growing GP. The low CP of *P. maximum* and *A. alboviolaceum* is however inconsistent with their high palatability, as are the low *in vitro* digestibility and fermentation values. As a Poacea, *P. maximum* does not contain any antinutritional factors and highly digestible regrowth of 2 to 3 leaves per tiller were used in this study as indicated by the high CP and dCP values, as compared to other studies using this grass (Guérin, 1999). It is very palatable to ruminants (Bogdan, 1977) and rabbits (Adehan et al., 1994). Furthermore, GP are used to be fed with this species in Kinshasa. On the contrary, the high palatability of *A. alboviolaceum* leaves is really surprising since the pepsin-pancreatin hydrolysis of DM and CP was very poor and since no gas production was recorded. Fruits of this species are appreciated by humans (Malaisse, 1997). However, the high NDF content, as illustrated by the negative correlation linking NDF to dDM, explain why low pepsin-pancreatin digestibility was recorded. The presence of diterpenoids with strong anti-bacterial activities such as the aframodial (Tane et al., 2006) are probably the cause of the poor *in vitro* fermentation.

D. intortum , *E. heterophylla*, and *A. hybridus* are valuable sources of protein (high CP and dCP) with good palatability. They can be recommended to complement a basal diet made of *P. maximum*. Their rapid (R_M) and extensive fermentability (A) can contribute significantly to energy supply via SCFA production (Rémésy et al., 1995) and to efficient intestinal bacterial growth (Bindelle et al., 2007b), improving the protein utilisation through coprophagy. In the case of *D. intortum*, the *in vitro* results are in contradiction with Bogdan (1977) who reports that this legume cannot secure high productivity in ruminants due to high tannin contents. Nevertheless, Ford (1978) recorded a discrepancy between low ruminal degradability and high pepsin CP degradability (0.70) that is consistent with the results of this study. For *A. hybridus*, Fayusi (2006) recorded lower growth rates in rats when the leaves of this species were used as sole protein source, as compared to casein, because its deficiency in lysine and threonine.

With high ash contents, *A. hybridus* and *T. triangulare* are also valuable ingredients in monogastric diets for mineral supplementation, excepted for Zn (Fasuyi, 2007). They can thus be recommended to the farmers. However, as already mentioned for *A. hybridus*, *T. triangulare* is deficient in lysine. Fresh leaves are, on the contrary, rich in ascorbic acid (3,54 g kg^{-1}DM or 365 mg kg^{-1} fresh leaves; Faboya, 1990), which represent approximately half of an orange content (Lim et al., 2007) and far above the recommended concentration in GP diet (230 mg kg^{-1}DM) (NRC, 1995).

Despite a moderate DVI, explained by the presence of lectins (Yagi et al., 1994), *P. scandens* leaves can also be recommended to GP producers. This species had the highest CP content and *Psophocarpus sp.* seed protein profile is very similar to that of soybean, *i.e.* high in lysine (7.5 g/16 g N) and threonine (4.5 g/16 g N) but low in

methionine (1.2 g/16 g N) (Černý et al., 1971). *I. batatas* protein is also deficient in methionine and cystine (1.1 and 1.6 g/16 g N, respectively) (Degras, 1998).

According to the results of this study, *C. diffusa, P. macrophylla* and *U. lobata* are not suitable as forages for GP and *B. erecta*, *S. acuta* and *A. asystacia* appeared to be obviously unpalatable to the GP and should therefore not be used by the farmers.

Tedding may improve the DVI of unpalatable fresh forages and modify the ranking of the substrates palatability. For example, using sun-dried forages, Adehan et al. (1994) observed on rabbits that *S. acuta* was nearly as palatable as *P. maximum* and *I. batatas* leaves. In sows, drying was also shown to increase voluntary intake of cocoyam and tree leaves (Leterme et al., 2005). This might be useful for forage conservation for dry season use. However, in the case of GP, fresh forages are important to supply C vitamin.

It can be concluded that the knowledge of the palatability of forages is important since it is not correlated to their nutritional values when used in fresh form. According to their high DVI and *in vitro* nutritional values, a combination of *Panicum maximum* with *Desmodium intortum*, *Euphorbia heterophylla* or *Amaranthus hybridu*s can be suggested to the farmers. This recommendation should however be confirmed through in vivo digestibility and growth experiments.

Acknowledgements

The authors gratefully acknowledge the Ministry of Agriculture of the Democratic Republic of Congo for his support, the personnel of the ISAV and the CAVTK (Kinshasa) for his expert technical assistance, Dr. Nicolas Gengler (FUSAGx, Gembloux) for his assistance in the elaboration of the statistical framework of this experiment, Dr. Pascal Leterme (PSC, Saskatoon) for critical review of this paper and Dr. Pululu (ISP, Kinshasa) for the expert identification of the plant materials. The research was financed by the Walloon Government (CAVTK Project, DGA, Namur) and the Belgian Co-operation (AMINEKIN project, CUD-CIUF, Brussels).

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Table 1. Daily voluntary intake (DVI) (g DM/ kg liveweight)

Forage	N[1]	DVI	
Rabbit concentrate-wheat bran mixture	80	49.12	restricted
Sum of forages ingestion	80	35.22	*ad libitum*
Ingestion per forage			
Panicum maximum	40	10.84	a[2]
Aframomum alboviolaceum	40	9.18	b
Desmodium intortum	80	7.75	c
Euphorbia heterophylla	40	7.18	cd
Synedrella nodiflora	40	5.94	d
Amaranthus hybridus	40	4.23	e
Ipomea batatas	40	3.80	ef
Talinum triangulare	40	3.28	fg
Psophorcapus scandens	40	2.78	gh
Urena lobata	40	2.26	gh
Commelina diffusa	40	1.98	hi
Pilea macrophylla	40	1.77	hij
Boerhavia erecta	40	0.86	ijk
Sida acuta	40	0.51	jk
Asystasia gangetica	40	0.32	k
Main effects	d.f.[3]	P-values	
Plant	14	< 0.001	
Period x day	19	< 0.001	
Variance parameter estimates			
Group		0.05	
Residual		4.18	

[1] N, number of observations.

[2] Means followed by different letters in the columns differ at a significance level of 0.05.

[3] d.f., degrees of freedom.

Table 2. Nutrient requirements for growth (NRC, 1995), farmers use to feed the Guinea pigs, dry matter content (g kg^{-1} fresh forage), proximate analysis and fibre content of the distributed and the pepsin-pancreatin hydrolysed forages offered to the Guinea pigs (g kg^{-1}DM)

		Distributed forages							Hydrolysed forages				
Feedstuffs	Farmers use	DM (g kg^{-1})	CP	CF	Ash	NDF	ADF	ADL	CP	CF	Ash	NDF	ADF
Nutrient requirements of GP		880	205	170	-	-	-	-					
Complement mixture		870	196	43	37	406	102	47	-	-	-	-	-
Forages													
Panicum maximum	Yes	234	180	299	121	617	346	36	84	414	51	879	528
Aframomum alboviolaceum	No	339	165	191	33	425	253	61	148	311	36	621	414
Desmodium intortum	Yes	208	272	206	130	356	211	33	113	356	46	662	392
Euphorbia heterophylla	Yes	192	279	171	144	270	176	59	130	278	16	484	281
Synedrella nodiflora	No	180	207	230	157	376	325	167	87	376	45	516	467
Amaranthus hybridus	Yes	146	261	162	200	356	189	45	132	235	111	444	305
Ipomea batatas	Yes	192	244	193	152	351	287	120	144	273	64	519	422
Talinum triangulare	Yes	103	211	225	246	347	277	76	172	297	154	471	347
Psophocarpus scandens	Yes	191	297	202	148	326	228	66	159	342	77	579	402
Urena lobata	No	298	139	222	91	428	305	118	152	332	118	507	375
Commelina diffusa	Yes	108	197	245	186	374	252	104	118	357	*95*	617	380
Pilea macrophylla	No	195	291	174	202	310	227	127	172	236	161	481	281
Boerhavia erecta	No	174	215	224	182	340	305	92	92	385	38	452	410
Sida acuta	No	251	223	215	132	359	285	124	89	364	70	519	366
Asystacia gagentica	No	105	216	212	195	346	271	167	97	306	126	484	326

Table 3. Dry matter (dDM) and crude protein (dCP) disappearance during pepsin-pancreatin hydrolysis and kinetics parameters of the gas production curves modelled according to Groot et al. (1996) for the hydrolysed forages incubated with Guinea pigs faeces (g kg^{-1} DM).

	Pepsin-pancreatin hydrolysis			Gas-test fermentation kinetics					
Forages	N[1]	dDM (-)	dCP (-)	N	A[2] (ml g^{-1}DM)	B[3] (h)	C[4] (-)	R_M[5] (ml g^{-1}DM h^{-1})	t_{RM}[6] (h)
Panicum maximum	6	0.32 f[7]	0.68	6	116 fg	36.2 b	4.34 ab	3.54 e	31.9 a
Aframomum alboviolaceum	6	0.37 e	0.43	6	17[8]	-	-	-	-
Desmodium intortum	6	0.55 ab	0.81	12	170 bcd	23.5 d	2.88 bcd	5.85 cd	17.3 de
Euphorbia heterophylla	6	0.55 ab	0.79	6	184 bc	23.4 d	2.88 bc	7.17 a	19.1 cde
Synedrella nodiflora	6	0.46 cd	0.77	6	177 bcd	21.8 d	2.66 cd	6.22 bc	15.8 e
Amaranthus hybridus	6	0.59 a	0.79	6	196 ab	26.9 c	2.67 cd	5.61 cd	19.7 cde
Ipomea batatas	6	0.54 ab	0.73	6	216 a	24.3 d	3.07 bcd	7.60 a	19.4 cde
Talinum triangulare	6	0.56 ab	0.64	6	170 bcd	27.3 c	3.31 bc	5.69 cd	22.4 c
Psophocarpus scandens	6	0.50 bc	0.73	6	107 g	28.1 c	3.32 bc	3.48 e	22.7 c
Urena lobata	6	0.25 g	0.19	5	91 g	35.6 b	3.44 bc	2.30 f	28.5 b
Commelina diffusa	6	0.44 d	0.66	5	147 ed	38.5 a	5.06 a	4.96 d	35.1 a
Pilea macrophylla	6	0.47 cd	0.69	6	135 ef	22.8 d	3.48 bc	5.54 cd	18.3 cde
Boerhavia erecta	6	0.50 bc	0.79	5	166 cd	21.8 d	2.67 cd	5.87 cd	15.8 e
Sida acuta	6	0.44 d	0.78	6	93 g	15.7 e	1.82 d	3.91 e	7.57 f
Asystacia gagentica	5	0.44 d	0.75	6	161 cd	24.3 d	3.92 bc	6.87 ab	20.9 cd
Source of variation	*d.f.*[9]	P-value		*d.f.*	P-values				
Plant	14	< 0.001		14	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Period		-		1	0.209	< 0.001	0.631	0.852	0.002
Plant x Period		-		14	0.565	0.120	0.526	0.380	0.652
Variance parameter estimates									
Residual		9.22^{E-4}			304	2.22	0.63	0.32	8.01

[1] N, number of observations

[2] A, final gas volume

[3] B, mid-fermentation time

[4] C, constant

[5] R_M, maximum rate of gas production

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

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

[8] Gas volume recorded after 72 h of incubation

[9] *d.f.*, degrees of freedom