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REGULAR ARTICLES

Effects of supplementing different levels of sun-dried groundnut foliage on intake, apparent digestibility and nitrogen metabolism in cattle offered a basal diet of a mixture of rice straw and para grass

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Abstract Eight male Cambodian yellow cattle, initial body weight (BW) 136 ± 11.9 (±SD) kg; age 20 ± 2.5 (±SD) months, were randomly allocated to treatments in a double 4 × 4 Latin square design to investigate the effects of different levels of crude protein (CP) from sun-dried groundnut (*Arachis hypogaea* L.) foliage (DGF) on feed intake, apparent digestibility and nitrogen metabolism. All animals were fed a basal diet of rice straw ad libitum and para grass (*Brachiaria mutica*) at 1% of BW. The DGF was offered as a supplement at 0, 1, 2 and 3 g CP/kg BW, denoted DGF0, DGF1, DGF2 and DGF3, respectively. The results showed that the intake of DGF contributed 0, 25, 34 and 42% of total dry matter (DM) intake. Rice straw intake decreased when DGF intake increased. Total intake of DM, organic matter (OM), digestible OM, ash, neutral detergent fibre and acid detergent fibre increased with increased level of DGF inclusion but did not differ for the two highest DGF levels. Total DM intake as proportion of BW increased from 2.3% in DGF0 to 2.8% in DGF3. Crude protein digestibility and nitrogen retention improved as DGF intake increased. Daily weight gain of DGF1 cattle was higher than DGF0 but was not further improved at the higher levels of DGF inclusion. Microbial protein synthesis and efficiency of microbial protein production in DGF1 did not differ from DGF0 but both measures were higher in DGF2 and DGF3. The responses were diminished at higher DGF inclusion so it is suggested that DGF supplementation at 1 g CP/kg BW may be the optimal level.

Keywords Cambodian yellow cattle · Daily weight gain · Microbial crude protein · Nitrogen retention

Introduction

Seasonal fluctuations in both quantity and quality of feed supply limit ruminant production in Cambodia and other countries in the region. The feed supply for smallholder ruminant production relies mainly on rice straw which does not provide sufficient nutrients due to its low nitrogen (N) content and low digestibility both of which lower feed intake (Van Soest 2006). This means that the animals are frequently undernourished (Young et al. 2014). In a recent survey in Cambodia the need to enhance cattle production by increasing the utilisation of forages and crop residues was emphasised (Samkol et al. 2015).

Supplementing diets based on low quality roughage with forage legumes and grasses that contain relatively high crude protein (CP) levels improves ruminant productivity (Sath et al. 2012; Pen et al. 2013). However, a supplement of grass alone may not always provide sufficient nutrients, including ruminal degradable nitrogen (RDN), for optimal microbial cell synthesis. A supplement containing forage legumes may therefore be required to optimise animal performance (Phelan et al. 2015). Pen et al. (2013) showed that supplementing cattle given basal diets of rice straw and grass with the forage legume, *Stylosanthes guianensis* CIAT 184 doubled their intake of rice straw and total N, and increased their total dry matter (DM) intake. These workers also found that microbial crude protein

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(MCP) synthesis and efficiency of MCP production (EMCP) were improved when forage legume was included in the diets.

Groundnut (*Arachis hypogaea* L.) is an annual cash crop mostly grown by smallholder farmers (Idoko and Sabo 2014). The crop is important for human nutrition due to its high protein and energy content, but the foliage also provides excellent hay with high protein content for livestock feeding (Taru et al. 2010). When used as a supplement with low digestibility roughage, the foliage provides protein which is beneficial for the rumen environment (Detmann et al. 2009). The aim of the study was to evaluate the effects of different levels of DGF inclusion in a basal diet based on a mixture of rice straw and para grass (*Brachiaria mutica*) on feed intake, apparent digestibility, N balance and MCP production in growing Cambodian yellow cattle.

Materials and methods

Description of the experimental site

The experiment was carried out from November, 2015 to March, 2016 at the research farm of the Centre for Livestock and Agriculture Development (CelAgrid). The farm is situated at a latitude of 104° 53′ E and longitude 11° 26′ N. The site is subject to the annual monsoon cycle. The wet season extends from May to October with annual rainfall of 800–1400 mm. The minimum and maximum temperature and humidity during the study were 22–35 °C and 44–96%, respectively.

Animals and experimental procedure

Eight male Cambodian yellow cattle (*Bos indicus*), aged 20 ± 2.5 (±SD) months with an initial BW of 136 ± 11.9 (±SD) kg were randomly allocated to a double 4 × 4 Latin square design with four dietary treatments and four periods. The cattle were housed individually in metabolism crates allowing total urine and faeces collection. The total duration of the experiment was 105 days; each period consisted of 21 days, including 14 days for adaptation and another 7 days during which feed refusals, and faeces and urine excretion were determined. Between each experimental period, the animals were allowed to graze freely at the research farm for 7 days. Before the start of the experiment, the cattle were dewormed against internal and external parasites using Ivermectin (2 ml/100 kg BW) and vaccinated against foot and mouth disease and pasteurellosis.

Feeds and feeding

All cattle were offered rice straw ad libitum, the amount offered at the start was about 3% of BW and adjusted according to intake from the previous day (plus roughly 10% on DM basis). All animals were also offered para grass at the level of 1% of BW. The four experimental treatments were supplementation of DGF at 0, 1, 2 and 3 g CP/kg BW, denoted DGF0, DGF1, DGF2 and DGF3, respectively. Fresh water was always freely available during the whole period.

The rice straw was mechanically harvested from a local short-season rice crop variety that was grown for about 3 months during the dry season, dried for 3–4 days and stored in a shed for 1–2 months until used. The para grass was grown as a monoculture, harvested daily and the next day manually chopped to 30–40 cm lengths prior to feeding. Groundnut foliage was collected after the groundnuts were harvested, sun-dried for 3–4 days, then mechanically chopped into 5–10 cm lengths before storage in hay bags under a roof until it was fed. The chemical composition of the rice straw, para grass and DGF is shown in Table 1.

The supplemental DGF was fed at 09:00 h; then, the rice straw and para grass were offered at 17:00 and 20:00 h. The feed refusals were collected and weighed in the morning before the first feeding at 9:00 h.

Sampling procedure and measurements

The cattle were weighed in the morning before feeding on two consecutive days at the beginning and the end of each experimental period, and the BW of the second day when feed and water intake was re-established after the grazing period were used for initial and final BW per period. The amounts of feed offered and refused and excretion of faeces and urine were recorded daily during the data collection period. A subsample of feed offered, feed refused and faeces (about 10% of daily output) of each animal were collected daily and kept in plastic bags at −20 °C. The samples of each period were pooled and then divided into two before analysis: one for DM analysis and one that was dried at 60 °C for 48 h for further analyses.

Urine was collected in a plastic bucket containing 250 ml of 10% H_2SO_4 in order to keep pH below 3.0 to prevent bacterial destruction of purine derivatives (PD). The daily urine output was immediately recorded and 50 ml stored at −20 °C for 7 days before total N analysis. The remaining urine was diluted with tap water to a final weight of 20 kg. Thus the final volume of diluted urine was the same for all experimental animals (IAEA 1997). Subsamples of 100 ml were stored at −20 °C for 7 days prior to analysis of allantoin.

Chemical analyses

Samples of feed offered, feed refusals and faeces were dried in a fan forced oven at 105 °C for 16 h (AOAC 1990, ID 967.03). Ash content of feed components was determined by combustion in a muffle furnace at 600 °C for 2 h (AOAC 1990, ID 942.05) and organic matter (OM) was obtained as [100-% ash]. Total N concentration in feed, faeces and urine were

Table 1 Chemical composition (g/kg dry matter) of the feeds used in the experiment, mean values and range (minimum-maximum), dry matter content was based on four observations, while the content of the compounds were based on two observations

Items	Rice straw	Para grass	DGF[a]
Dry matter, g/kg	938 (930–943)	220 (197–234)	920 (906–928)
Ash	122 (112–132)	116 (103–129)	100 (97–103)
Organic matter	878 (868–888)	884 (871–897)	900 (897–903)
Crude protein (N × 6.25)	55.8 (53.9–57.8)	135 (124–148)	126 (124–129)
Neutral detergent fibre	708 (703–714)	694 (683–704)	539 (531–546)
Acid detergent fibre	453 (444–468)	442 (432–452)	465 (463–468)

[a] Dried groundnut foliage

performed by the Kjeldahl procedure (AOAC 1990, ID 981.10). Crude protein content was calculated as N × 6.25. The analysis of neutral detergent fibre (NDF) and acid detergent fibre (ADF) was determined using the procedure of Goering and Van Soest (1970). Allantoin concentration in urine samples was determined on a spectrophotometer using the colorimetric method as described by Young and Conway (1942).

Calculations

The PD and MCP were calculated based on the relationship derived by IAEA (1997):

> The rate of excretion of urinary PD was obtained from allantoin excretion rate which was assumed to be 85% of total PD excretion.
> The amount of microbial purine absorbed (X mmol/day) corresponding to PD excreted (Y mmol/day) was calculated as:

$Y = 0.85\,X + 0.385\ \mathrm{BW}^{0.75}$; where, $\mathrm{BW}^{0.75}$ is the metabolic BW at the end of each experimental period (kg), 0.85 is the recovery of absorbed purines as PD of total excretion after correction for the utilisation of microbial purines by the animals and 0.385 is the endogenous contribution taken as a constant at 0.385 mmol/kg $\mathrm{BW}^{0.75}$ per day.

> The amount of MCP supplied to the animal was calculated as:

MCP (g/day) = $[(X\ (\mathrm{mmol/day}) \times 70)/(0.116 \times 0.83 \times 1000)] \times 6.25$; where, 70 is N content of purines (70 mg N/mmol), 0.116 is ratio of purine N/total N in mixed rumen microbes and 0.83 is the estimated digestibility of microbial purines.

Statistical analyses

Data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure (MINITAB 16.1.1; Minitab 2010) with the model: $Y_{\mathrm{ijkl}} = \mu + T_{\mathrm{i}} + P_{\mathrm{j}} + A_{\mathrm{k}} + B_{\mathrm{l}} + e_{\mathrm{ijkl}}$, where Y_{ijkl} refers to observations; μ, overall mean; T_{i}, the fixed effect of treatment (i = 0, 1, 2 and 3 g CP/kg BW DGF); P_{j}, the fixed effect of period (j = 1, 2, 3 and 4); A_{k}, the random effect of animal (k = 1, 2, 3 and 4); B_{l}, the random effect of block (l = 1 and 2) and e_{ijkl}, the residual error. One animal that became ill during the experiment was excluded from the data set. Results are presented as least square means (LSM) with the standard error of the means (SEM). The treatment LSMs were compared using Tukey's pairwise comparison procedures and were considered to differ significantly at the probability level of $P < 0.05$ and to represent a tendency if $0.05 < P < 0.10$.

Results

Feed and nutrient intakes

The intake of DGF by cattle in the DGF1, DGF2 and DGF3 treatments was lower than planned, reaching 86.0, 60.5 and 52.0% of the allotted amounts, respectively. The DGF contributed 0, 25, 34 and 42% of the total DM intake. The animals consumed virtually all of their allowance of para grass in all dietary treatments. However, rice straw intake decreased ($P < 0.001$) as DGF intake increased (Table 2). Total intake of DM, OM, digestible OM, Ash, NDF and ADF increased with increasing intake of DGF, but did not differ significantly between the two highest levels of DGF inclusion.

Apparent digestibility and nitrogen balance

Inclusion of DGF in the diets increased ($P < 0.001$) CP digestibility and tended ($P = 0.062$) to increase DM digestibility (Table 3). There were no effects of dietary treatments on the apparent digestibility of OM, NDF or ADF.

Total N intake increased with increased intake of N from the DGF inclusion, contributing 0, 30.5, 41.8 and 50.2% of the total N supply in the dietary treatments DGF0, DGF1, DGF2 and DGF3, respectively (Table 4). A higher DGF intake was also associated with increased urinary and faecal N losses. Nitrogen retention and N utilisation (calculated as the percentage of N intake retained) increased with increased

Table 2 Feed and nutrient intakes of Cambodian yellow cattle offered increasing levels of dried groundnut foliage (DGF) to supplement a basal diet of rice straw and para grass

Items	Dietary treatment[a]				SEM	*P* value
	DGF0	DGF1	DGF2	DGF3		
Dry matter (DM) intake						
Rice straw intake						
g DM/day	2,070a	1,450b	1,260c	1,000d	51	<0.001
in % BW	1.35a	0.94b	0.80c	0.64d	0.0327	<0.001
DGF intake						
g DM/day	0.00d	994c	1,480b	1,810a	53	<0.001
in % BW	0.00d	0.63c	0.92b	1.16a	0.0294	<0.001
Total intake						
g DM/day	3,650c	3,980b	4,340a	4,330a	72	<0.001
in % BW	2.35c	2.57b	2.71a	2.78a	0.0230	<0.001
Nutrient intake, g/day						
Organic matter	3,270c	3,600b	3,840a	3,860a	40	<0.001
Digestible organic matter[b]	1,860c	2,070b	2,240a	2,260a	40	<0.001
Ash	445c	465b	504a	509a	5.06	<0.001
Neutral detergent fibre	2,600c	2,700b	2,810a	2,780ab	29	<0.001
Acid detergent fibre	1,690c	1,850b	1,980a	1,990a	20	<0.001

Means within rows with different letters are significantly different ($P < 0.05$)

SEM standard error of the mean, *BW* body weight (kg) = (initial BW + final BW) / 2

[a] DGF0, DGF1, DGF2 and DGF3 denotes the level of supplementation of DGF by: 0, 1, 2 and 3 g crude protein/kg body weight, respectively

[b] Calculated as (organic matter intake × digestibility of organic matter) / 100

levels of DGF, but N utilisation did not differ among the diets supplemented with DGF.

Microbial crude protein production and daily weight gain

Supplementing the diets with DGF to the level of DGF2 or DGF3 increased ($P < 0.01$) urinary allantoin excretion, microbial protein outflow from the rumen and efficiency of microbial crude protein supply (EMCPS) (Table 5). However, daily weight gain was increased ($P < 0.05$) by the presence of DGF in the diet but did not differ between diets containing DGF.

Table 3 Apparent digestibility (%) of Cambodian yellow cattle offered increasing levels of dried groundnut foliage (DGF) to supplement a basal diet of rice straw and para grass

Items	Dietary treatment[a]				SEM	*P* value
	DGF0	DGF1	DGF2	DGF3		
Dry matter	53.8	54.5	55.5	56.0	0.659	0.062
Organic matter	56.7	57.2	58.1	58.3	0.618	0.177
Crude protein	57.3c	61.2b	62.3ab	63.6a	0.586	<0.001
Neutral detergent fibre	52.5	53.5	53.9	54.1	0.686	0.313
Acid detergent fibre	41.6	42.8	43.0	44.6	0.798	0.111

Means within rows with different letters are significantly different ($P < 0.05$)

SEM standard error of the mean

[a] DGF0, DGF1, DGF2 and DGF3 denotes the level of supplementation of DGF by: 0, 1, 2 and 3 g crude protein/kg body weight, respectively

Discussion

Inclusion of DGF in a basal diet of rice straw and para grass increased total DM intake in Cambodian yellow cattle. This is in line with previous studies showing increased DM intake as a result of protein supplementation of diets with poor quality roughage such as rice straw (Pen et al. 2013; Phesatcha and Wanapat 2017).

Although DGF intake increased, the amounts of feed refused also increased. Indeed, the animals refused about 50% of the DGF DM offered at the highest DGF inclusion level. It is likely that, as the availability of DGF increased, the animals selected more leaves and other components with high nutrient value and refused the less digestible components. A DGF offer supplying greater than 2 g CP/kg BW did not give any further improvement in total DM intake because the intake of rice straw DM was equally reduced. The intake of NDF was higher in DGF-supplemented animals, even though DGF had a lower NDF content, because total feed intake increased. It

Table 4 Nitrogen retention of Cambodian yellow cattle offered increasing levels of dried groundnut foliage (DGF) to supplement a basal diet of rice straw and para grass

Items	Dietary treatment[a]				SEM	*P* value
	DGF0	DGF1	DGF2	DGF3		
Total nitrogen (N) intake						
g/day	55.1d	70.4c	81.5b	86.3a	0.740	<0.001
N DGF						
g/day	0.00d	21.5c	34.1b	42.3a	0.888	<0.001
g/kg total N intake	0.00d	312c	420b	493a	8.62	<0.001
g CP/kg BW	0.00d	0.89c	1.39b	1.78a	0.0357	<0.001
Urinary N						
g/day	11.6c	13.8b	16.7a	17.8a	0.429	<0.001
g/kg total N intake	208	196	202	209	5.29	0.305
Faecal N						
g/day	23.5c	27.2b	30.5a	31.3a	0.412	<0.001
g/kg total N intake	427a	388b	376bc	363c	5.86	<0.001
Total N excretion						
g/day	35.1c	41.0b	47.2a	49.1a	0.623	<0.001
g/kg total N intake	635a	584b	578b	573b	7.38	<0.001
N retention						
g/day	20.0d	29.4c	34.4b	37.2a	0.709	<0.001
N utilisation[b]	36.5b	41.6a	42.1a	42.7a	0.738	<0.001

Means within rows with different letters are significantly different ($P < 0.05$)

CP calculated as N × 6.25

SEM standard error of the mean, *CP/kg BW* crude protein/kg body weight

[a] DGF0, DGF1, DGF2 and DGF3 denotes the level of supplementation of DGF by: 0, 1, 2 and 3 g crude protein/kg body weight, respectively

[b] Calculated as the percentage of N intake retained in the animal

may be assumed that the increased N supply improved rumen microbial fibre degradation which would reduce rumen fill and allow a higher DM intake. The NDF intake in the present study is in line with previous studies (Sath et al. 2012; Khan et al. 2013). It is well known that high NDF contents may limit voluntary DM intake and reduce rumen DM digestibility (Teixeira et al. 2014). Thus, it is most probable that NDF also limited DM intake in the present study. In contrast to dried

Table 5 Microbial crude protein supply and daily weight gain of Cambodian yellow cattle offered increasing levels of dried groundnut foliage (DGF) to supplement a basal diet of rice straw and para grass

Items	Dietary treatment[a]				SEM	*P* value
	DGF0	DGF1	DGF2	DGF3		
Urinary excretion (mmol/day)						
Allantoin	19.9b	27.7ab	34.0a	35.1a	2.43	0.001
MCP						
Supply, g/day	89.0b	138ab	178a	184a	15.3	0.001
Supply, g/kg BW	0.56b	0.89ab	1.12a	1.19a	0.102	0.002
EMCPS						
g CP/kg DOMI	48.8b	68.1ab	79.7a	82.2a	7.38	0.018
Daily gain, g/day	297b	487a	469a	502a	42.9	0.010

Means within rows with different letters are significantly different ($P < 0.05$)

SEM standard error of the mean, *MCP* microbial crude protein, *EMCPS* efficiency of microbial crude protein supply, *BW* body weight (kg) = (initial BW + final BW) / 2, *DOMI* digestible organic matter (OM) intake calculated as (OM intake × digestibility of OM) / 100

[a] DGF0, DGF1, DGF2 and DGF3 denotes the level of supplementation of DGF by: 0, 1, 2 and 3 g crude protein/kg body weight, respectively

cassava foliage, the apparent digestibility of DM, OM and fibre fractions were not negatively affected by DGF (Sath et al. 2012). Cassava foliage has higher concentrations of digestibility supressing anti-nutritional compounds such as tannins than DGF (Thang et al. 2010).

The CP concentration of DGF shows a large variation, presumably mainly related to stage of maturity at harvest. However, the harvesting technique also has impact on the field losses of nutrients. Hill (2002) reported that when groundnut forage was mechanically harvested, there was a loss of leaves which are highly nutritious. The groundnut used in our experiment was harvested manually, thus the loss of leaves was minimised and the CP content of DGF in the present study (126 g/kg DM) is at the top end of reported range of Chakeredza et al. (2002); Khan et al. (2013), but falls in the range value of Göhl (1982) which is 99–262 g/kg DM. In the present study, concentrations of NDF and ADF were higher than those reported by Chakeredza et al. (2002) and Khan et al. (2013). This was probably because the groundnuts were grown with the purpose of nut production and so, at the time of harvest, the foliage was at a mature stage with a higher proportion of cell walls.

The weight gain of the cattle was improved by inclusion of DGF in the diet. This improvement reflects both a true BW gain and changes in fill of the gastrointestinal tract (GIT) in response to the increased feed intake. This result was similar to that found by Sath et al. (2012) when dried cassava foliage was included as a supplement in the same basal diet. However, supplementation with DGF at levels greater than 2 g CP/kg BW did not further improve weight gain which could be due to the low energy concentration and inappropriate energy:protein ratio in the diets.

Apparent N retention was improved by increasing DGF supply, but not reflected by a corresponding increase in weight gain. It is possible that N retention was overestimated due to rapid loss of N in the hot climate (Pen et al. 2013).

Urinary allantoin excretion may be used as a predictor of microbial protein synthesis (IAEA 1997; Südekum et al. 2006). The allantoin excretion increased with increasing DGF supply although the response appeared to be diminishing at higher DGF inclusions. Protein synthetised in the rumen by microbes is the main source of protein used for growth of cattle, especially if they have a relatively low growth rate as in the present study (Rodríguez et al. 2007). Taken together, the present results indicate that DGF supplementation improved rumen available N, EMCPS, microbial protein outflow from the rumen and N utilisation for tissue growth. The responses increased numerically as the level of DGF increased, but there was no statistical increase in daily weight gain at DGF inclusion levels greater than 1 g CP/kg BW. The result was in agreement with the finding of Pen et al. (2013) who used the forage legume, stylosanthes as a feed supplement with a basal diet of rice straw and tropical grass. It is likely that the improvement in weight gain was mainly due to increased availability of digestible OM, peptides, amino acids, and ammonia from the feed supplement which enhanced rumen microbial synthesis (Khandaker et al. 2012).

In conclusion, supplementation with DGF increased DM intake, apparent digestibility of crude protein and microbial protein synthesis in cattle fed a diet based on rice straw and para grass. Furthermore, N retention and daily BW gain were improved by DGF. The responses were generally declining at higher DGF inclusion as the feed refusals increased. Thus based on the present results, it is suggested that the optimal level of inclusion of DGF is 1 g CP/kg BW.

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Compliance with ethical standards

Ethical guidelines All procedures for the care and use of the animals were followed through the international, national and/or institutional guidelines.

Conflict of interest The authors declare that there are no conflict of interest.

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