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Effects of feed intake level on efficiency of microbial protein synthesis and nitrogen balance in Boran steers consuming tropical poor-quality forage

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ABSTRACT

This study aimed at evaluating the effects of feed intake level on the efficiency of rumen microbial protein synthesis (EMPS), nitrogen (N) excretion, and N balance in twelve 18-months old Boran (*Bos indicus*) steers with initial average liveweight of 183 kg (standard deviation (SD) 15.2). The experiment followed a 4 × 4 complete Latin Square design with four dietary treatments tested in four periods. Each period ran for 5 weeks with 3 weeks of adaptation and 2 weeks of sample collection; separated by 2 weeks of re-feeding. Steers were fed at 100%, 80%, 60%, and 40% of their metabolisable energy requirement for maintenance (MER, referred to as MER100, MER80, MER60, and MER40, respectively). Steers receiving MER80, MER60, and MER40 were only fed Rhodes grass hay. MER100 steers were offered Rhodes grass hay at 80% of their MER and cottonseed meal and sugarcane molasses at each 10% of MER. Mean daily dry matter intake differed between treatments ($p < 0.001$) and ranged between 2.1 kg/animal (SD 0.13) in MER40 and 4.5 kg/animal (SD 0.31) in MER100. Urinary N excretion and N balance did not differ between MER80, MER60, and MER40. According to contrast test, declining feed intake level from MER80 to MER40 reduced duodenal microbial crude protein flow ($p < 0.001$), but did not alter the EMPS (g microbial N/kg digestible organic matter intake). Yet, if scaled to N intake, EMPS increased ($p < 0.049$), whereas total N and faecal N excretions decreased linearly with declining intake level ($p < 0.001$ for both variables). At similar grass hay intake, duodenal microbial crude protein flow was 41% higher in MER100 than in MER80 steers ($p < 0.002$). In cattle offered poor-quality tropical forage below their MER, the very low EMPS and thus microbial protein supply aggravate the negative effects of low dietary nutrient and energy intakes in periods of feed shortage.

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Maintenance requirement; nitrogen excretion; protein nutrition; purine derivatives; tropical ruminants

1. Introduction

In semi-arid to arid tropical environments, cattle are regularly exposed to periods of low availability and poor nutritional quality of feed resources, in particular during dry

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seasons and drought years. In addition, given current climate prediction, the occurrence of droughts is likely to increase with ongoing climate change (IPCC 2013). In such situations, microbial protein synthesised in the rumen is almost the sole source of amino acids for the animal. Differences in the efficiency of rumen microbial protein synthesis (EMPS; e.g. in g microbial nitrogen (N)/kg digestible organic matter (OM) intake) thus greatly determine protein supply and conversion in tropical cattle.

Few studies exist on the effect of low feed intake level on EMPS and they have shown inconsistent results. While EMPS decreased with declining feed intake of zebu cattle in some studies (Mo et al. 2004; Seresinhe et al. 2004; George et al. 2006), it was not affected by intake level in experiments with *Bos taurus* cattle by Doreau et al. (2004) or crossbred calves by Singh et al. (2007). Along this line, declining feed intake below the animals' energy requirements for maintenance increased N balance in cows in a study by Grimaud and Doreau (1995), but reduced N balances in sheep and crossbred calves in experiments of Atti et al. (2002) and Singh et al. (2008), respectively. Moreover, diets in all the above-mentioned studies comprised a mixture of roughage and concentrate feeds and were thus of moderate to high nutritional quality (crude protein (CP) ≥68 g/kg dry matter (DM); apparent total tract OM digestibility ≥56 g/100 g OM). However, diets of tropical ruminants in particular during dry seasons are characterised by, amongst others, considerably low CP concentrations than available in temperate diets, inferior nutrient digestibility, and slow rumen nutrient degradation, factors greatly influencing EMPS (Bach et al. 2005; Roman-Gracia et al. 2016). Hence, the effects of declining feed intake on EMPS and N balance may differ in tropical cattle fed poor-quality roughages only.

The present study therefore aimed at exploring how EMPS responds to decreasing feed intake level and a shift in diet composition of cattle fed poor-quality tropical grass hay, and how this response influences faecal and urinary N excretions and N balance. It was hypothesised that declining feed intake of cattle below energy requirements for maintenance would (i) decrease rumen microbial protein yield and EMPS (in g/kg digestible OM) due to lower energy and nutrient supply and a decrease in liquid and solid digesta passage rates at low intakes and (ii) reduce the animals' daily N balance and N excretion via faeces and urine, while shifting N excretion from faeces to urine, because of lower energy and N intakes and correspondingly reduced excretions of feed and microbial N via faeces.

2. Materials and methods

2.1. *Experimental design*

The feeding trial was carried out from July 2016 to January 2017 at the International Livestock Research Institute (ILRI), Nairobi, Kenya. All the experimental protocols involving animal handling and treatment were reviewed and approved by Institutional Animal Care and Use Committee of ILRI (permission of animal experiment: no. IACUC-RC2016-11). The experimental design was a 4 × 4 complete Latin Square design which consisted of four treatments tested in four periods. Each experimental period ran for five weeks with three weeks of adaptation and two weeks of sample collection (i.e. sampling period).

2.2. *Animals*

Twelve 18-month-old Boran steers with a mean initial liveweight (LW) of 183 kg (standard deviation (SD) 15.2 kg) were used. The steers were ear-tagged for identification and vaccinated against foot and mouth disease (FOTIVAX^{TM}, subcutaneous dose of 3 ml/animal, Kenya Veterinary Vaccines Production Institute, Nairobi, Kenya). Vaccination was repeated after 4 weeks. Additionally, the animals were treated once upon arrival against endoparasites and ectoparasites with albendazole (Albafas 10%, oral dose of 20 ml/animal, NORBROOK Kenya Ltd., Nairobi, Kenya) and acaricide (Bayticol® Pour-On, dose of 30 ml/animal, Bayer New Zealand Ltd, Glenfield, New Zealand). The LW of each animal was recorded once every week at 08:00 h just before morning feeding using a digital platform weighing scale (Gallagher weigh scale W210, FarmShop Australia, Kenmore-East Queensland, Australia).

Prior to the experiment, the steers were divided into four groups based on their initial LW. Of the four heaviest, medium, and lightest animals, one animal each was allocated randomly to one of the four groups. Each animal group was then randomly assigned to individual treatments in each of the four periods so that each group received every treatment once throughout the entire experiment. During the adaptation period, the steers were housed in individual unroofed pens (2.0 m × 3.0 m). During the sampling period, the steers were transferred to an indoor barn and housed in individual pens (1.0 m × 2.0 m) to ease urine and faeces collection. During the first week of the sampling periods, six animals were chosen randomly from each treatment group and used for respiratory chamber measurements of enteric methane emissions, whereas the other six animals were used for total urine and faeces collection. In the second week of the sampling period, the animals were swapped and subjected to the respective other measurements. After each sampling period, steers were allowed to regain LW during 2 weeks of refeeding period before being switched to the next treatment. During the refeeding period, a high-quality Rhodes grass hay was offered for *ad libitum* intake and a concentrate mixture of cottonseed meal (2 kg/animal) and sugarcane molasses (1 kg/animal; both as-fed basis) given once daily to all the steers.

2.3. *Feeds and feeding*

Experimental treatments comprised four feed intake levels that covered 100%, 80%, 60%, or 40% of the animals' metabolisable energy requirements for maintenance (MER), henceforth referred to as MER100, MER80, MER60, and MER40, respectively. The daily MER were estimated from the LW of each animal following recommendations by NRC (1989) for mature breeding bulls (i.e. 740 kJ/kg $LW^{0.75}$). Steers receiving treatments MER80, MER60, and MER40 were only fed Rhodes grass hay that was harvested at the seed stage. The MER100 animals were offered Rhodes grass hay at 80% of their MER as well as cottonseed meal and sugarcane molasses at each 10% of their MER. Cottonseed meal and sugarcane molasses were added to the diet MER100, because the grass hay was of very poor quality (Table 1), so that it was impossible to meet the animals' MER at maximum voluntary intake level. The amounts of feed offered were adjusted to the LW of the individual animals at the beginning of each experimental period. Prior to the experiment, all grass hay was chopped to a particle

Table 1. Ingredients and chemical composition of experimental diets[#] (arithmetic mean ± standard deviation).

	Grass hay (n = 8)	Cottonseed meal (n = 4)	Molasses (n = 2)*	MER100[§]
Dry matter (DM 5˜) [g/kg fresh matter]	915 ± 1.8	924 ± 0.3	699 ± 0.1	894
Crude ash [g/kg DM]	87 ± 0.5	53 ± 0.0	118 ± 0.7	86
Crude protein [g/kg DM]	33 ± 3.2	297 ± 0.5	26 ± 3.4	59
Ether extract [g/kg DM]	7.4 ± 0.31	77 ± 3.0	na[+]	na
Neutral detergent fibre [g/kg DM]	769 ± 1.1	494 ± 1.2	na	665
Acid detergent fibre [g/kg DM]	508 ± 1.0	362 ± 0.5	na	na
Metabolisable energy [MJ/kg DM][‡]	6.2 ± 0.26	8.3 ± 0.32	10.8 ± 0.32	6.9

[+]na, not available; *only two samples were analysed due to its liquid consistency; [‡] estimated from proximate nutrient concentrations and gas production during *in vitro* fermentation according to Menke and Steingass (1988); [§] calculated from the ingredient composition the diet of steers fed at 100% of their metabolisable energy requirements for maintenance (MER100). Steers at 80%, 60%, and 40% of their metabolisable energy requirements for maintenance received only grass hay; [#]mineral lick blocks contained [g/kg, as-fed basis]: 850 g sodium chloride, 21 g calcium carbonate, 13 g magnesium oxide, 11 g dicalcium phosphate, 6 g zinc sulphate, 4 g manganese, 3.5 g copper sulphate, 3 g iron sulphate, 500 mg cobalt carbonate, 300 mg calcium iodate, and 40 mg selenium yeast.

length of 4–5 cm and stored in bins until feeding. The grass hay was weighed individually and offered once daily at 09:30 h after removing and weighing the refusals from the previous day. Cottonseed meal and molasses were first weighed separately and then manually mixed. The mixture was then offered in a separate bucket once daily to MER100 animals after the grass hay was offered. Drinking water and mineral lick blocks were offered for *ad libitum* intake to all steers throughout the experiment.

2.4. *Sample collection, preparation, and analysis*

Samples of the offered grass hay (~100 g fresh matter) were collected directly from the bins on the first day of each sampling week (i.e. twice during each sampling period) and dried in a forced-air oven (Genlab oven, Genlab Ltd. Widnes, United Kingdom) at 50°C until constant weight. Samples of cottonseed meal (~100 g fresh matter) and sugarcane molasses (~70 g fresh matter) were collected once during each sampling period, because only one batch each was used for both sampling weeks. Throughout the experiment, there were no refusals of the concentrate mixture. The grass hay refused by each animal during the sampling periods was weighed and stored at room temperature until the end of each sampling week. Then, all refusals of each animal were homogenised, a sample of ~100 g fresh matter per animal taken, and dried as samples of offered feeds.

For six consecutive days during each sampling week, faeces excreted by individual animals were manually collected directly from the floor as soon as the steers defecated. Total faeces excreted by each animal over a 24-h-period (08:00 h to 08:00 h) was thoroughly mixed by hand, weighed (CTG6H table top scale, Citizen Scales Inc, New York, USA; 0.1 g accuracy), and a sample of ~300 g fresh matter was taken and dried in a forced-air oven (Genlab oven, Genlab Ltd. Widnes, United Kingdom) at 50°C until constant weight. A second sample of ~60 g fresh matter was taken from each animal and stored at −20°C until the end of the experiment. Then, frozen faecal samples were thawed overnight, homogenised, and pooled by animal and period. For the pool sample, the amount of faeces taken from each daily sample corresponded to the proportion of the faecal mass (dried at 50°C) on the respective day of the total faecal excretion of each animal during the sampling period. One aliquot (~100 g fresh matter) of each faecal pool sample was taken and directly analysed for N.

Total daily urine output was collected for five consecutive days during each sampling week using harnesses developed by the Department of Animal Nutrition and Rangeland Management in the Tropics and Subtropics of the University of Hohenheim, Germany. The harnesses were fitted to the animals and directed the urine via a hose into closed buckets. The buckets were prefilled with 100 ml of sulphuric acid (20%; v/v) to reduce urine pH to below 3 in order to prevent volatile ammonia losses and microbial degradation of purine derivatives (PD) (Chen and Gomes 1992). The buckets were emptied once daily, washed, and refilled with sulphuric acid. Total urine excreted by each animal over a 24-h-period (08:00 h to 08:00 h) was homogenised and urine volume measured and recorded. For PD analysis, 1% of the total daily urine volume of each animal was taken and diluted to 250 ml with distilled water. The mixture was homogenised and filtered through two layers of a surgical gauze to remove foreign materials (i.e. hair, feed) from urine. Then, 40 ml of the diluted filtrate were dispensed into a labelled falcon tube and frozen at −20°C until analysis. Additionally, a sample of ~100 ml of the remaining undiluted urine was taken and frozen at −20°C until the end of the experiment. Then, frozen undiluted urine samples were thawed overnight, homogenised and pooled by animal and period. For the pool sample, the amount of urine taken from each daily urine sample corresponded to the proportion of urine volume on the respective day of total urine excretion of each animal during the sampling period. An aliquot of ~100 ml of the pooled urine sample was taken and directly analysed for N (AOAC 1990; method no. 988.05).

Dried feed and faecal samples were ground to pass a 1-mm screen using a hammer mill (MF10 basic, IKA®Werke GmbH & Co. Kg, Staufen, Germany). The ground daily faecal samples were pooled by animal and period. For the pool sample, the amount of faeces taken from each daily sample corresponded to the proportion of the faecal mass (dried at 50°C) on the respective day of the total faecal excretion of each animal during the sampling period. An aliquot of ~100 g of the pooled dried material was then taken. The DM concentrations of feed and faecal samples were determined according to AOAC (1990; methods 967.03 and 924.05). Feed samples were analysed for neutral detergent fibre (using alpha-amylase, method no. 6.5.1) and acid detergent fibre (method no. 6.5.2) according to VDLUFA (2012) using semi-automatic fibre analyser (fibertec™analyser, Foss GmbH, Hamburg, Germany). Total N was analysed (AOAC 1990; method no. 988.05) and CP concentrations calculated (CP = N · 6.25). Ether extract concentrations of grass hay and cottonseed meal samples were analysed according to AOAC (1990; method no. 945.16). For estimation of metabolisable energy (ME) concentrations of grass hay, cottonseed meal, and sugarcane molasses, gas production during 24 h of *in vitro* incubation of samples was determined in triplicate during two incubation runs following procedures described by Menke and Steingass (1988). Incubations were repeated, if the differences between gas volumes for each sample within or between runs were greater than 5% of the overall mean.

For PD analysis, the diluted urine samples were thawed overnight at room temperature, homogenised, and further diluted with distilled water at 1:30. Urine samples from animals on days when considerable urine loss occurred were not included in PD analysis (10 incidents of 240 cases (4 periods × 12 animals × 5 days)). Urinary allantoin and uric acid concentrations [mmol/l] were determined by colorimetry method using double spectrophotometer (Shimadzu®UV-150-02, Scientific Instruments LLC, Manassas Park, USA) following the procedures of Chen and Gomes (1992). Xanthine and hypoxanthine concentrations were estimated from uric acid concentration after treating the urine samples with xanthine oxidase

(X1875-5UN, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Thus, the sum of xanthine and hypoxanthine is presented as "xanthine plus hypoxanthine" in the manuscript. Urinary allantoin, uric acid and xanthine plus hypoxanthine excretion [mmol/d] were calculated by multiplying the molar PD concentrations by the respective daily urine volume [l/d]. Total urinary PD excretion [mmol/d] was calculated as the sum of allantoin, uric acid, and xanthine plus hypoxanthine excretion. If not mentioned otherwise, all chemical analyses of feed, faecal, and urine samples were conducted in duplicate and repeated, if the difference between replicates was greater than 5 % of the mean.

2.5. Data processing and calculations

Daily feed intake was calculated as the difference between the amount of feed offered every day and the mean amount of feed refusals for each animal across the sampling week. Data on apparent total tract DM and OM digestibility and passage rate were taken from Ali et al. (2018). Digestible DM and OM intakes were calculated by multiplying DM and OM intakes with their respective apparent total tract digestibility. The N intake was calculated as the difference between the amount of N in the feed offered and feed refused by each individual animal across each sampling week. The N balance was calculated by subtracting the individual animal's faecal and urinary N excretions from total N intake across each sampling week. Daily LW change was calculated across the total experimental period.

The ME concentrations of grass hay and of cottonseed meal and sugarcane molasses were calculated following procedures described by Menke and Steingass (1988) using regression equations 16e for hay and 14b for concentrates.

Duodenal absorption of microbial PD in mmol/d was calculated based on the equation of Verbic et al. (1990) using a daily urinary excretion of endogenous PD of 0.243 mmol/kg $LW^{0.75}$ for zebu cattle as the mean of values determined in studies of Osuji et al. (1996), Liang et al. (1999), Pimpa et al. (2001), Ojeda et al. (2005), Bowen et al. (2006), Barbosa et al. (2011), Braga et al. (2012), and Prates et al. (2012). Duodenal microbial N flow [g/d] was then estimated from absorbed PD (i.e. based on the equation developed by Chen and Gomes (1992)).

2.6. Statistical analysis

A total of 48 observations (i.e. 4 periods × 4 treatments × 3 animals) were obtained for nutrient intake, urinary and faecal N excretion, N balance, urinary PD, concentrations and for all further calculated variables. All statistical analyses were carried out using SAS 9.4. Data were analysed the mixed model procedure using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \ a_k + \varepsilon_{ijk};$$

where Y_{ijk} is the observed response at a particular ijk case; μ is overall mean; α_i is the fixed effect of treatment i; β_j is the fixed effect of period j; γ_{ij} is the fixed effect of the interaction between treatment i and period j; a_k is the random effect of animal k; and ε_{ijk} is experimental error.

Least squares means and standard errors of the means for all measured and calculated variables were computed and differences between least squares means determined using Tukey test. Significance level was declared at $p < 0.05$, whereat p-values of 0.05 to <0.10 were considered as trend. The p-values of these pairwise comparisons are presented in the text. Linear and quadratic effects of decreasing feed intake level on measured and calculated variables for diets below maintenance (i.e. MER80, MER60, and MER40) were characterised using orthogonal polynomial contrasts.

3. Results

3.1. *Nutrient intake and liveweight change of steers*

As planned, the DM, OM, and ME intakes of steers differed ($p < 0.001$ for all variables) between treatments (Table 2). Similarly, daily intakes of OM, digestible DM, and ME as well as daily LW change of the steers differed between treatments ($p < 0.001$ for all variables). While MER100 animals gained LW, MER40, MER60, and MER80 steers lost LW, with greater LW losses for MER40 than for MER80 ($p < 0.001$) and MER60 ($p < 0.001$).

3.2. *Urinary PD excretion and efficiency of microbial protein synthesis*

Daily urine volume did not differ between treatments ($p = 0.13$). Urinary allantoin, uric acid, and total PD excretions were highest in MER100 steers compared to those subjected to the other treatments ($p < 0.001$ for all variables) and decreased linearly with decreasing feed intake level from MER80 to MER40 ($p < 0.001$; Table 3). There were no treatment effects on urinary xanthine plus hypoxanthine excretion ($p = 0.26$). Estimated duodenal microbial N flow was greatest in MER100 steers compared to those receiving the other treatments ($p < 0.001$) and decreased linearly with decreasing feed intake level from MER80 to MER40 ($p < 0.001$). The EMPS, expressed in g microbial N/kg digestible OM intake, was greatest in MER100 steers compared to those receiving MER60 ($p \leq 0.01$) or MER80 ($p \leq 0.02$), while being similar for MER100 and MER40 steers. Moreover, EMPS increased linearly with declining feed intake level from MER80 to MER40 when related to daily N intake (i.e. g microbial N/g N intake; $p = 0.049$). In contrast, the EMPS did not differ between MER80, MER60, and MER40.

3.3. *Nitrogen excretion and balance*

Mean daily N intake ranged between 11.1 g (SD 1.33) in MER40 and 41.5 g (SD 3.32) in MER100 and differed between treatments ($p < 0.001$, Table 4). Daily faecal N excretion was highest in MER100 steers ($p < 0.001$) compared to those subjected to the other treatments, and declined linearly with decreasing feed intake level from MER80 to MER40 ($p < 0.001$), mainly due to decreasing faecal DM excretion ($p < 0.001$; Table 4). Urinary N excretion did not differ between MER80, MER60, and MER40, but was lower in MER60 than in MER100 ($p = 0.008$) likely due to the smaller SD with MER60 (SD 0.98) compared to MER80 (SD 3.4) and MER40 (SD 2.4) treatments. The N balance was positive and highest in MER100 steers ($p < 0.001$). According to measured LW losses,

Table 2. Daily intakes of dry matter, organic matter, and metabolisable energy as well as liveweight (LW) change in Boran steers at four feed intake levels (in % of metabolisable energy requirements for maintenance; MER) (least squares means, standard errors of means (SEM); $n = 12$).

	Feed intake level [% of MER]					*p*-Value				
	100	80	60	40	SEM	Trt.[§†]	Prd.[*†]	Trt. × Prd.[†]	Linear[‡]	Quadratic[‡]
Dry matter [kg]	4.5[a]	3.7[b]	3.1[c]	2.1[d]	0.08	<0.001	<0.001	0.13	<0.001	0.019
Dry matter [g/kg $LW^{0.75}$]	81.6[a]	64.3[b]	56.6[c]	40.3[d]	0.94	<0.001	<0.001	0.003	<0.001	<0.001
Organic matter [g/kg $LW^{0.75}$]	74.5[a]	58.6[b]	51.8[c]	36.9[d]	0.87	<0.001	<0.001	0.003	<0.001	<0.001
Digestible dry matter [g/kg $LW^{0.75}$]	46.3[a]	36.3[b]	31.8[c]	21.9[d]	0.71	<0.001	<0.001	0.009	<0.001	0.004
Digestible organic matter [g/kg $LW^{0.75}$]	44.1[a]	35.0[b]	30.7[c]	21.2[d]	0.67	<0.001	<0.001	0.006	<0.001	0.003
Metabolisable energy [MJ/kg $LW^{0.75}$]	0.57[a]	0.42[b]	0.36[c]	0.25[d]	0.006	<0.001	<0.001	0.003	<0.001	0.002
LW change[#] [g/d]	195[a]	−67[b]	−321[c]	−706[d]	42.3	<0.001	<0.001	0.09	<0.001	0.12

[§] Trt., treatment effect; *Prd., period effect; [abcd]least squares means within the same row with different superscript are significantly different ($p < 0.05$); [†]*p*-values from analysis of variance for all four diets; [‡]*p*-values from contrast test for three diets differing in intake level (i.e. 80%, 60% and 40% of MER); [#]LW change was calculated across the total experimental period.

Table 3. Concentration and excretion of urinary purine derivatives (PD), duodenal microbial protein flow, and efficiency of rumen microbial protein synthesis (EMPS) in Boran steers at four feed intake levels (in % of metabolisable energy requirements for maintenance; MER) (least squares means, standard errors of means (SEM); $n = 12$).

	Feed intake level [% of MER]					*p*-Value				
	100	80	60	40	SEM	Trt.[§ †]	Prd.[* †]	Trt.× Prd.[†]	Linear[‡]	Quadratic[‡]
Urinary PD excretion [mmol/d]										
Allantoin	45.6^{a}	35.9^{b}	32.0^{c}	25.5^{d}	0.75	<0.001	<0.001	0.003	<0.001	0.15
Uric acid	5.36^{a}	3.87^{b}	3.04^{bc}	2.22^{c}	0.257	<0.001	0.016	0.29	<0.001	1.00
Xanthine plus hypoxanthine	1.84	1.70	1.74	1.61	0.092	0.39	<0.001	0.37	0.52	0.48
Total PD	52.8^{a}	41.6^{b}	36.8^{c}	29.3^{d}	0.85	<0.001	<0.001	0.010	<0.001	0.21
Allantoin: total PD ratio	0.86	0.86	0.87	0.87	0.009	0.77	<0.001	0.43	0.90	0.82
Total PD: urinary N ratio [mmol/mg N^{+}]	4.7^{a}	4.4^{a}	4.3^{a}	3.3^{b}	0.22	<0.001	0.029	0.21	0.002	0.08
Creatinine [mmol/kg liveweight$^{0.75}$ and d]	0.75	0.69	0.70	0.69	0.023	0.25	0.002	0.77	0.88	0.84
Total PD: creatinine ratio	1.28^{a}	1.08^{bA}	0.98^{bB}	0.84^{c}	0.028	<0.001	<0.001	0.16	<0.001	0.55
Duodenal microbial N flow										
Absorbed PD [mmol/d]	46.2^{a}	32.8^{b}	27.5^{c}	19.5^{d}	0.95	<0.001	<0.001	0.004	<0.001	0.14
Endogenous PD [mmol/d]	13.6^{ab}	13.8^{a}	13.4^{b}	12.7^{c}	0.32	<0.001	0.003	0.94	<0.001	0.08
Duodenal microbial N flow [g/d]	33.6^{a}	23.8^{b}	20.0^{c}	14.2^{d}	0.69	<0.001	<0.001	0.003	<0.001	0.14
EMPS [g N/kg digestible OM[#] intake]	13.7^{a}	11.9^{b}	11.8^{b}	12.7^{ab}	0.55	0.009	<0.001	0.24	0.15	0.32
[g N/MJ ME[◇] intake]	1.06	1.01	1.00	1.06	0.040	0.28	<0.001	0.07	0.20	0.38
[g N/g N intake]	0.81^{b}	1.19^{b}	1.21^{b}	1.28^{a}	0.041	<0.001	<0.001	0.07	0.049	0.52

[◇]ME, metabolisable energy; [+]N, nitrogen; [#]OM, organic matter; *Prd., period effect; [§] Trt., treatment effect; [abcd] least squares means within the same row with different superscripts are significantly different at $p < 0.05$; [A, B]least squares means within the same row with different uppercase superscript tended to differ at $0.05 \leq p < 0.10$; [†]*p*-values from analysis of variance for all four diets; [‡]*p*-values from contrast test for three diets differing in intake level (i.e. 80%, 60%, and 40% of MER).

N balances were negative in MER80, MER60, and MER40 animals without any differences between treatments.

3.4. Period and period by treatment interaction effects

The DM, OM, digestible DM, digestible OM, ME intakes, total urinary PD excretion, estimated duodenal microbial N flow, and faecal N excretion differed between periods and were greater in period 1 than in period 2 ($p \leq 0.003$ for all variables) and period 3 ($p \leq 0.007$ for all variables), and greater in period 4 than in period 2 ($p \leq 0.007$ for all variables) and period 3 ($p \leq 0.002$ for all variables), but were similar for periods 2 and 3. The N intake [g/d] and EMPS [g microbial N/kg digestible OM intake] was greater in period 4 compared to all other periods ($p \leq 0.005$). The N balance [g/d] was greatest in period 2 compared to the other periods ($p \leq 0.005$) with no differences between periods 1, 3, and 4.

There were effects of the interaction between period and treatment for DM, OM, digestible DM, and digestible OM intakes when expressed per kg of metabolic LW ($p \leq 0.009$ for all variables). Firstly, these interactions were due to the high amounts of hay refused by MER80 steers which led to similar intakes as MER60 steers in periods 2 and 3. Moreover, nutrient and ME intakes were higher in MER100 than MER80 animals in periods 2 ($p \leq 0.008$) and 3 ($p \leq 0.007$), but not in periods 1 and 4. Secondly, nutrient and ME intakes of MER80 steers differed between periods ($p \leq 0.011$) mainly because of the high amount of feed they refused in period 3, whereas intakes were similar across periods for all other treatment groups. Moreover, the estimated duodenal microbial N flow did not differ between MER40 and MER60 in periods 1, 2, and 3 except in period 4 ($p = 0.02$). Similarly, no difference was observed between MER60 and MER80 for periods 2, 3 and 4, except for period 1 ($p = 0.005$).

4. Discussion

4.1. Nutrient intake and chemical composition of feeds

Diets were designed to cover 40%, 60%, 80%, and 100% of the animals' MER. The ME concentrations in the grass hay offered to steers during the experiment (6.2 MJ/kg DM) were lower than values determined in grass hay samples before the trial and assumed in diet formulation (8.2 MJ/kg DM), maybe because, by the time the experiment had started, the grass hay had considerably matured. Hence, actual ME intakes of steers in the trial were lower than intended. Nevertheless, steers subjected to MER100 showed positive N balances (5.1 g N/d) and gained LW (204 g/d), suggesting (i) that ME intakes relative to MER of the animals in the present study were higher than the targeted 100%, 80%, 60%, and 40% of their MER and (ii) that recommendations by NRC (1989) for MER of matured breeding bulls may overestimate those of tropical growing steers. Considering measured LW gain of MER100 steers of 195 g/d during the experimental periods (Table 2) and ME requirements for LW gain of tropical cattle breeds of 24.3 kJ/g LW (Salah et al. 2014), the animals spent 4.7 MJ/d for body protein and fat deposition. At daily ME intakes of 31.5 MJ (i.e. 0.57 kJ/kg $LW^{0.75}$), this would leave 26.8 MJ (i.e.

Table 4. Nitrogen (N) intake and N excretion via urine and faeces in Boran steers at four feed intake levels (in % of metabolisable energy requirements for maintenance; MER) (least squares means, standard errors of means (SEM); $n = 12$).

	Feed intake level [% of MER]					*p*-Value				
	100	80	60	40	SEM	Trt. [§ †]	Prd. [* †]	Trt.× Prd.[†]	Linear[‡]	Quadratic[‡]
N intake [g/d]	41.5[a]	20.3[b]	16.5[c]	11.1[d]	0.45	<0.001	<0.001	0.20	<.001	0.14
N excretion										
Total N [g/d]	36.4[a]	26.3[b]	22.9[c]	19.3[d]	0.89	< 0.001	< 0.001	0.10	< 0.001	0.88
Urinary N [g/d]	11.4[aA]	9.9[ab]	8.6[b]	9.4[abB]	0.57	0.013	0.07	0.22	0.55	0.14
Faecal N [g/d]	25.0[a]	16.4[b]	14.3[c]	9.8[d]	0.59	< 0.001	< 0.001	0.23	< 0.001	0.10
Faecal N:urinary N ratio	2.2[a]	1.7[b]	1.7[b]	1.1[c]	0.09	< 0.001	0.24	0.49	< 0.001	0.024
N balance [g/d]	5.1[a]	−6.0[b]	−6.4[b]	−8.2[b]	0.75	< 0.001	< 0.001	0.26	0.05	0.48
Urine volume [l/d]	5.0	3.6	3.8	3.6	0.49	0.13	0.57	0.48	0.99	0.79
Faecal DM[+] excretion [kg/d]	2.0[a]	1.6[b]	1.4[c]	1.0[d]	0.047	< 0.001	0.52	0.37	< 0.001	0.08
Faecal OM[#] excretion [kg/d]	1.7[a]	1.3[b]	1.2[c]	0.8[d]	0.040	< 0.001	0.62	0.32	< 0.001	0.06

[+]DM, dry matter; [#]OM, organic matter; *Prd., period effect; [§]Trt., treatment effect; [abcd]least squares means within the same row with different superscripts are significantly different ($p < 0.05$); [A, B]least squares means within the same row with different uppercase superscripts tended to differ at $0.05 \leq p < 0.10$; [†]*p*-values from analysis of variance for all four diets; [‡]*p*-Values from contrast test for three diets differing in intake level (i.e. 80%, 60%, and 40% of MER).

0.48 MJ/kg $LW^{0.75}$) for maintenance purposes, which is close to MER reported by GfE (1995) and Jenet et al. (2004), but lower than estimates of daily MER of cattle by Salah et al. (2014; 0.63 MJ/kg $LW^{0.75}$). Accordingly, actual ME intakes of MER100, MER80, MER60, and MER40 steers were 118%, 88%, 75%, and 52% of their MER.

Yet, all animals received hay from the same batch that was chopped and mixed prior to the experiment, and chemical composition and ME concentrations of the grass hay were very similar across all periods (Table 1). Hence, irrespective of the discrepancy between targeted and actual ME intakes (both, absolute and relative terms), treatment effects discussed below can mainly, if not exclusively, be attributed to relative differences in the feed intake level (MER40 vs. MER60 vs. MER80) or in both, intake level and ingredient and chemical composition of the diets (MER100 vs. MER40, MER60, and MER80).

4.2. *Urinary PD excretion and efficiency of microbial protein synthesis*

Mean duodenal microbial N flow and EMPS observed in our study varied between 14.2 and 33.6 g microbial N/d and between 12.7 and 13.7 g microbial N/kg of digestible OM intake in MER40 and MER100, respectively. These values are within the range of values reported by Bowen et al. (2017) for steers grazing tropical grass pastures (18.9–77.7 g microbial N/d; 4.2–33.4 g microbial N/kg of digestible OM intake; recalculated from microbial CP flow by dividing by a factor of 6.25). They are also similar to results of Seresinhe et al. (2004) and Mo et al. (2004) for Sri Lankan Zebu cattle (8–22 g microbial N/d) and Chinese yellow steers (15–26 g microbial N/kg fermented OM intake, assuming a proportion of 65% of the digested OM to be fermented in the rumen; ARC 1990), respectively. However, EMPS determined in the present and above-mentioned studies are lower than values reported for temperate cattle systems (Doreau et al. 2004; Dickhoefer et al. 2015, 2016; Bowen et al. 2017). These differences are likely due to lower feed intake levels and poor nutritional quality of feedstuffs in tropical than in temperate cattle systems, resulting in slow fermentation rates and high abundances of solid-associated, fibrolytic microbes that in general have slower growth rates as compared to liquid-associated microbes (Russell et al. 1992). Duodenal microbial N flows determined in the present study were below the requirements for utilisable CP for maintenance of cattle defined by GfE (1995), suggesting that besides energy also protein supply was limiting animal growth. Further research is, however, needed to quantify duodenal microbial N flow and, more importantly, EMPS for animal genotypes and diets commonly found in the Tropics as the basis for accurate ruminant feed evaluation and improved diet formulation.

As expected, urinary PD excretion and estimated duodenal microbial N flow were greatest for MER100 and declined linearly as feed intake level decreased from MER80 to MER40, which is at least partly due to a lower substrate supply to rumen microbes. George et al. (2006) found a decline in urinary PD excretion when feed intake decreased from 120% to 60% of voluntary DM intake in crossbred bulls fed wheat straw and a concentrate mixture. Similarly, Singh et al. (2007) reported a reduced urinary PD excretion in 18-month-old crossbred calves when their feed intake decreased from 100% to 40% of their voluntary DM intake. Such reduced microbial protein supply to

ruminant animals fed below MER may aggravate the negative effects of low dietary nutrient and energy supply in periods of feed shortage. However, in contrast to our first hypothesis, EMPS [g N/kg digestible OM intake] was not affected by different levels of grass hay (i.e. MER40 to MER80), which contradicts the decline in EMPS with decreasing feed intake level in studies of Mo et al. (2004) and Seresinhe et al. (2004), as well as the positive linear relationship between feed intake and EMPS in ruminants fed above maintenance energy requirements determined by Djouvinov and Todorov (1994) and Gomes et al. (1994). Nevertheless, also Doreau et al. (2004) and Singh et al. (2007) did not find any difference in EMPS when feed intake decreased to 27% of the animals' energy requirement for maintenance or to 40% of their voluntary DM intake, respectively. The lack of effect of feed intake level (i.e. MER 80 vs. MER60 vs. MER40) on EMPS in the present study was likely related to prolonged digesta retention time in the rumen with decreasing feed intake as determined by Ali et al. (2018) within the frame of the same experiment. These prolonged rumen retention times might have increased the proportion of the digested feed nutrients that were fermented in the rumen and thus microbial protein yield per unit of digested OM (i.e. EMPS). Slower passage rates of liquid and solid digesta, however, also reduce microbial growth rates in the rumen (Clark et al. 1992; Dewhurst et al. 2000), which might have compensated any increase in EMPS due to prolonged rumen retention times and would thus explain the overall lack of feed intake effect on EMPS. In this line, EMPS (g microbial N/kg digestible OM intake) in the present study was negatively correlated with retention time of liquid digesta in the rumen [h] (results of Pearson correlation analysis: $r = -0.49$, $p < 0.001$ using passage rate data published by Ali et al. (2018)).

The accuracy of estimates of duodenal microbial N flow and EMPS from urinary PD excretion largely depends on the values assumed as contribution of endogenous to total PD excretion and as ratios of purine-N to total N in mixed rumen microbes (Ahnert et al. 2015; Dickhoefer et al. 2015, 2016). Urinary excretion of endogenous PD differs between cattle genotypes (Osuji et al. 1996; Bowen et al. 2006) and is affected by dietary factors and the animals' physiological state (Chen et al. 1992; Pérez et al. 1998). Feeding the animals below their MER might have altered their metabolic activity and body protein turnover and with this the excretion of endogenous PD. In the same line, differences in microbial growth rates (e.g. due to varying digesta passage or carbohydrate sources and fermentation rates) affect the purine-N to total N ratio in mixed rumen microbial mass (Cecava et al. 1990; Obispo and Dehority 1999; Rodríguez et al. 2000). A decline in microbial growth rate with decreasing feed intake level and digesta passage rate might have resulted in lower purine-N to total N ratios in rumen microbes. In this case, current values of duodenal microbial N flow and EMPS for lowest feed intake levels would overestimate actual rumen microbial protein synthesis when compared to those at higher feed intake levels. Hence, it cannot be fully excluded that there was no negative effect on EMPS and thus a more pronounced decrease in duodenal microbial N flow with declining feed intake level.

The EMPS (in both, g/kg OM or digestible OM intake) was lower in animals receiving grass hay only than in those supplemented with cottonseed meal and molasses (except MER40), suggesting that greater dietary concentrations and intakes of ME and N increased rumen fermentation, digesta passage (Ali et al. 2018), and thus EMPS. The EMPS in g microbial N per g of N intake even increased with declining feed intake level

and was lowest for MER100 steers, suggesting that absolute rumen-degradable protein supply was not the first factor limiting microbial growth. The rumen N balance is an indicator for the adequacy of N supply to rumen microbes and is calculated as the difference between the N intake and the duodenal flow of microbial and undegradable dietary N (GfE 1995). Rumen N balances would range from −9.7 g (MER40) to −15.0 g/d (MER80) according to measured N intakes, estimated duodenal flows of microbial N, and published rumen CP degradabilities of 46, 100, and 48 g/100 g CP for cottonseed meal, sugarcane molasses, and grass hay, respectively (Feedipedia: Animal feed resources information system 2012; Agroscope 2016; a value of rice straw was used for grass hay due to its poor nutritional value). Limited quantitative research exists on the urea-N recycling in tropical cattle. However, these values are lower than the amounts of urea-N entering the gut in Angus steers fed similar amounts of tropical grasses in a study by Archibeque et al. (2001) and reported by Marini et al. (2008). Hence, at very low feed intake levels such as in the present study, dietary and recycled N appears sufficient to cover N requirements of rumen microbes despite the very low CP concentrations of the grass hay.

4.3. *Nitrogen excretion and balance*

There was a strong correlation between DM intake and faecal DM excretion in the present study (results of Pearson correlation analysis: $r = 0.97$; $p < 0.001$). Accordingly, faecal N excretion [g/d] was highest for MER100 and declined linearly with decreasing feed intake from MER80 to MER40, which is in line with our second hypothesis that absolute faecal N excretion would decrease with declining feed intake level. Similarly, Peripolli et al. (2010) found a positive linear relationship between DM intake and faecal N excretion in sheep fed pasture-based diets. The greater faecal N excretion in MER100 than in MER80 steers and its decline with decreasing feed intake level are at least partly related to lower excretions of undigested feed N. Besides, lower endogenous N secretions and microbial protein synthesis associated to a reduced substrate supply might have contributed to the decline in faecal N excretion with declining feed intake. However, further research is needed to determine changes in the proportions of different N fractions in faeces and to better understand the effects of feed intake level and concentrate supplementation on feed CP degradation, rumen microbial growth, and manure quality.

In contrast to our expectations and despite the differences in the N intakes of steers, absolute urinary N excretion was similar across all treatments in the present study. Moreover, the proportion of total N excretion eliminated via urine even increased with declining feed intake level. Absolute urinary N excretion was much lower than values reported in the literature for Zebu cattle and their crossbreds with similar LW and DM intake levels fed a mixture of tropical roughage and concentrate feeds (Mo et al. 2004; Seresinhe et al. 2004; Singh et al. 2007). According to GfE (1995), endogenous N losses via urine in normally fed, European cattle breeds are quantified as endogenous urinary N [g/d] = 5.9206 · log LW [kg] – 6.76. Although these values might differ in Zebu cattle breeds, endogenous N losses via urine at measured animal LW as estimated using the previous equation would approximate 7.0, 7.1, 7.0, and 6.8 g/d in MER100, MER80, MER60, and MER40 steers, respectively, which is equivalent to 63%, 85%, 80%, and 74% of their measured daily urinary N excretion. The great proportion of endogenous N in urine therefore appears to limit the scope for reduction

in urinary excretion of dietary N with declining N intakes. Additionally, body protein mobilisation as indicated by the pronounced LW losses of the steers in response to ME intakes below MER most likely increased urinary excretions of endogenous N. Such shift in the proportions of different N fractions in urine thus explains the limited treatment effects on daily urinary excretion of total N in the present study.

Daily N balance [g/d] tended to linearly decrease from MER80 to MER60 and MER40 animals ($p = 0.08$) and was positively correlated with DM intake (results of Pearson correlation analysis: $r = 0.63$; $p < 0.001$), which is in line with the increase in LW loss of steers from MER80 to MER40 and our expectations that absolute N balance would decrease with declining feed intake level. Similarly, Doreau et al. (2004) observed a tendency of a lower absolute N balance when feed intake level decreased from 80% to 27% of the energy requirements for maintenance in non-lactating cows fed a high-forage diet. The negative effect of declining feed intake level on N balance is related to the lower N intake and greater endogenous N loss from body tissue mobilisation at low feed intake compared to high feed intake levels, which together exceeded the decline in faecal excretion of dietary and microbial N. On the contrary, feeding molasses and cottonseed meal to MER100 steers in the present study considerably increased their N balance and resulted in a positive N retention and LW gain when compared to animals fed grass hay only. Therefore, increasing feed intake level and dietary ME and CP concentrations is effective in improving both, dietary and microbial N flow and to thereby enhance amino acids supply and N retention in tropical ruminants.

4.4. *Period effects*

Period effects and interactions between period and treatment were found for several variables. Chemical composition and ME concentrations of the grass hay and cottonseed were very similar throughout the experiment. Moreover, the experimental animals were housed in a closed building during sampling, so that any effect of differences in ambient air temperature and humidity between periods appears small. However, feed offer was adjusted to the increasing LW of growing steers. Moreover, individual animals of treatment MER80 refused extraordinary great amounts of hay in periods 2 and 3. Hence, period effects were likely related to differences in daily nutrient and ME intakes of animals between periods.

5. Conclusions

Declining feed intake level in cattle offered tropical poor-quality forage below their MER linearly reduces rumen microbial protein yield, which may aggravate the negative effects of low dietary nutrient and energy supply in periods of feed shortage. The very low feed intake levels, N concentrations far below the assumed values for an adequate N supply to rumen microbes, do not negatively affect EMPS. Estimated duodenal microbial protein flow and EMPS observed in the present study are much lower than those reported for temperate ruminant diets. Further research is thus needed to quantify the EMPS for animal genotypes and diets commonly found in the Tropics and Subtropics and to identify factors determining rumen microbial protein synthesis in order to be able to improve protein nutrition and use efficiency in tropical ruminants.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Agroscope: Swiss feed database. 2016. [accessed 2016 Sept 5]. www.feedbase.ch.

Ahnert S, Dickhoefer U, Schulz A, Susenbeth A. 2015. Influence of ruminal quebracho tannin extract infusion on apparent nutrient digestibility, nitrogen balance, and urinary purine derivatives excretion in heifers. Livest Sci. 177:63–70.

Ali AIM, Wassie SE, Korir D, Goopy JP, Merbold L, Butterbach-Bahl K, Dickhoefer U, Schlecht E. 2018. Effect of feed intake levels on digesta passage and nutrient digestibility in Boran steers. In Society of Nutrition Physiology 2018. Proceedings of the Society of Nutrition Physiology 27, March 13–15., Göttingen. Frankfurt am Main: DLG-Verlag, Germany., p 42.

[AOAC] Association of Official Analytical Chemists. 1990. Official Methods of Analysis. 15th ed. Washington (DC, USA): Association of Official Analytical Chemists.

[ARC] Agricultural Research Council. 1990. The nutrient requirements of ruminant livestock. UK: Slough, Farnham Royal.

Archibeque SL, Burns JC, Huntington GB. 2001. Urea flux in beef steers: effects of forage species and nitrogen fertilization. J Anim Sci. 79:1937–1943.

Atti N, Kayouli C, Mahouachi M, Guesmi A, Doreau M. 2002. Effect of a drastic underfeeding on digestion in Barbary ewe. Anim Feed Sci Technol. 100:1–14.

Bach A, Calsamiglia S, Stern MD. 2005. Nitrogen metabolism in the rumen. J Dairy Sci. 88 (Suppl):E9–E21.

Barbosa AM, Valadares RFD, Valadares-Filho SC, Pina DS, Detmann E, Leão MI. 2011. Endogenous fraction and urinary recovery of purine derivatives obtained by different methods in Nellore cattle. J Anim Sci. 89:510–519.

Bowen MK, Poppi DP, McLennan SR. 2017. Efficiency of rumen microbial protein synthesis in cattle grazing tropical pastures as estimated by a novel technique. Anim Prod Sci. 57:1702–1712.

Bowen MK, Poppi DP, McLennan SR, Doogan VJ. 2006. A comparison of the excretion rate of endogenous purine derivatives in the urine of *Bos indicus* and *Bos taurus* steers. Austral J Agric Res. 57:173–177.

Braga JMS, Valadares RFD, Pellizzoni SG, Valadares-Filho SC, Prates LL, Costa E Silva LF. 2012. Estimation of endogenous contribution and urinary excretion of purine derivatives from the total digestible nutrient intake in Nellore heifers. R Bras Zootec. 41:1899–1906.

Cecava MJ, Merchen NR, Gay LC, Berger LL. 1990. Composition of ruminal bacteria harvested from steers as influenced by dietary energy level, feeding frequency, and isolation techniques. J Dairy Sci. 73:2480–2488.

Chen XB, Chen YK, Franklin MF, Ørskov ER, Shand WJ. 1992. The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep. J Anim Sci. 70:1534–1542.

Chen XB, Gomes MJ. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: an overview of the technical details. UK: University of Aberdeen.

Clark JH, Klusmeyer TH, Cameron MR. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J Dairy Sci. 75:2304–2323.

Dewhurst RJ, Davies DR, Merry RJ. 2000. Review article. Microbial protein supply from the rumen. Anim Feed Sci Technol. 85:1–21.

Dickhoefer U, Ahnert S, Schoof H, Moritz N, Susenbeth A. 2015. Response of urinary purine derivatives excretion to different levels of ruminal glucose infusion in heifers. Arch Anim Nutr. 69:128–142.

Dickhoefer U, Ahnert S, Susenbeth A. 2016. Effects of quebracho tannin extract on rumen fermentation and yield and composition of microbial mass in heifers. J Anim Sci. 94:1561–1575.

Djouvinov DS, Todorov NA. 1994. Influence of dry matter intake and passage rate on microbial protein synthesis in the rumen of sheep and its estimation by cannulation and a non-invasive method. Anim Feed Sci Technol. 48:Suppl:289–304.

Doreau M, Michalet-Doreau B, Bechet G. 2004. Effect of underfeeding on digestion in cows. Interaction with rumen degradable nitrogen supply. Livest Prod Sci. 88:33–41.

[GfE] Ausschuß für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie. 1995. Energie- und Nährstoffbedarf landwirtschaftlicher Nutztiere. Empfehlungen zur Energie- und Nährstoffversorgung der Mastrinder. Frankfurt am Main (Germany): DLG-Verlag.

Feedipedia: Animal feed resources information system. 2012. INRA, CIRAD, and FAO. [accessed 2017 Jun 15]. http:www.feedipedia.org/node/557.

George SK, Dipu MT, Mehra UR, Verma AK, Singh P. 2006. Influence of level of feed intake on concentration of purine derivatives in urinary spot samples and microbial nitrogen supply in crossbred Bulls. Asian Austral J Anim Sci. 19:1291–1297.

Gomes MJ, DeB HFD, Chen X, Nengomasha EM. 1994. The effect of starch supplementation of straw on microbial protein supply in sheep. Anim Feed Sci Technol. 49:277–286.

Grimaud P, Doreau M. 1995. Effect of extended underfeeding on digestion and nitrogen balance in non-lactating cows. J Anim Sci. 73:211–219.

[IPCC] Intergovernmental Panel on Climate Change. 2013. Climate change 2013: the physical science basis: contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge (UK): Cambridge University Press.

Jenet A, Fernandez-Rivera S, Tegegne A, Yimegnuhal A, Osuji PO, Kreuzer M. 2004. Growth and feed conversion of Boran (Bos indicus) and Holstein x Boran heifers during three physiological states receiving different levels of a tropical diet. Livest Prod Sci. 89:159–173.

Liang JB, Pimpa O, Abdullah N, Jelan ZA, Nolan JV. 1999. Estimation of rumen microbial protein production from urinary purine derivatives in zebu cattle and water buffalo: nuclear based technologies for estimating microbial protein supply in ruminant livestock. Vienna: International Atomic Energy Agency.

Marini JC, Fox DG, Murphy MR. 2008. Nitrogen transactions along the gastrointestinal tract of cattle: A meta-analytical approach. J Anim Sci. 86:660–679.

[NRC] National Research Council. 1989. Nutrient requirement of beef cattle. 7th ed. Washington (DC): National Academy Press.

Menke KH, Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim Res Dev. 28:7–55.

Mo F, Wang YX, Xing Z, Yang YF, Chen XB. 2004. The effect of different levels of feed intake on urinary excretion of purine derivatives in Chines yellow cattle: in estimation of microbial protein supply in ruminants using urinary purine derivatives (eds. Makkar HPS, Chen XB). Dordrecht (The Netherlands): Kluwer Academic Publishers; p. 103–108.

Obispo NE, Dehority BA. 1999. Feasibility of using total purines as marker for ruminal bacteria. J Anim Sci. 77:3084–3095.

Ojeda A, Parra O, Balcells J, Belenguer A. 2005. Urinary excretion of purine derivatives in Bos indicus × Bos taurus crossbred cattle. Br J Nutr. 93:821–828.

Osuji PO, Nsahlai IV, Khalili H. 1996. Effect of fasting on the urinary excretion of nitrogen and purine derivatives by zebu (*Bos indicus*) and crossbred (*Bos indicus* x *Bos taurus*) cattle. J Appl Anim Res. 10:39–47.

Pérez JF, Balcells J, Cebrian JA, Martín-Orue SM. 1998. Excretion endogenous and exogenous purine derivatives in sheep. Effect of Increases Concentrate Intake. Br J Nutr. 9:237–240.

Peripolli V, Êr P, Barcellos JOJ, Neto JB. 2010. Fecal nitrogen to estimate intake and digestibility in grazing ruminants. Anim Feed Sci Technol. 163:170–176.

Pimpa O, Liang JB, Jelan ZA, Abdullah N. 2001. Urinary excretion of duodenal purine derivatives in Kedah-Kelantan cattle. Anim Feed Sci Technol. 92:203–214.

Prates LL, Valadares RFD, Valadares-Filho SC, Detmann E, Santos SA, Braga JMS, Pellizzoni SG, Barbosa KS. 2012. Endogenous fraction and urinary recovery of purine derivatives in Nellore and Holstein heifers with abomasal purine infusion. Livest Sci. 150:179–186.

Rodríguez CA, González J, Alvir MR, Repetto JL, Centeno C, Lamarani F. 2000. Composition of bacteria harvested from the liquid and solid fractions of the rumen of sheep as influenced by feed intake. Br J Nutr. 84:369–376.

Roman-Gracia Y, White RR, Firkins J. 2016. Meta-analysis of postruminal microbial protein flows in dairy cattle. I. Derivation of Equations. J Dairy ScI. 99:7918–7931.

Russell JB, O'Connor JD, Fox DG, Van Soest PJ, Sniffen CJ. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J Anim Sci. 70:3551–3561.

Salah N, Sauvant D, Archimede H. 2014. Nutritional requirements of sheep, goats and cattle in warm climates. A meta-analysis. Animal. 8:1439–1447.

Seresinhe T, Pathirana KK, Jayasuriya MCN. 2004. Urinary excretion of purine derivatives as predictor of the nutritional status of local Zebu-cattle and crossbred- milking cows: in estimation of microbial protein supply in ruminants using urinary purine derivatives (eds. Makkar HPS, Chen XB). Dordrecht (The Netherlands): Kluwer Academic Publishers; p. 95–102.

Singh M, Sharma K, Dutta N, Singh P, Verma AK, Mehra UR. 2007. Estimation of rumen microbial protein supply using urinary purine derivatives excretion in crossbred calves fed at different levels of feed intake. Asian Austral J Anim Sci. 20:Suppl:1567–1574.

Singh P, Verma AK, Sahu DS, Mehra UR. 2008. Utilization of nutrients as influenced by different restriction levels of feed intake under sub-tropical conditions in crossbred calves. Livest Sci. 117:308–314.

[VDLUFA] Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten. 2012. Die chemische Untersuchung von Futtermitteln. VDLUFA-Methodenbuch. Darmstadt (Germany): VDLUFA-Verlag.

Verbic J, Chen XB, MacLeod NA, Ørskov ER. 1990. Excretion of purine derivatives by ruminants: effects of microbial nucleic acids infusion on purine derivative excretion by steers. J Agric Sci. 114:243–248.

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