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Original article

Effects of level of intake and nitrogen supplementation on digestion by cows in a tropical environment

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Abstract — The effects of severe underfeeding and N supplementation on nutrient digestion were studied in *Bos taurus* and *Bos indicus* cows. Eight non-lactating adult cows, four *Bos taurus* and four *Bos indicus* (body weight 173 and 234 kg, respectively) fitted with ruminal cannulas, were used in a 4 × 4 Latin square design within each genotype. They were fed a rice straw based-diet supplemented with cottonseed meal at four levels: HN–, high intake low N; LN–, low intake low N; HN+, high intake high N; LN+, low intake high N. The first diet, HN–, was formulated to supply 100% of net energy maintenance requirements. LN– corresponded to half of HN– and supplied 50 and 63% of the requirements in net energy and in digestible protein in the intestine, respectively. The two other diets, HN+ and LN+, were defined by providing the same amount of additional protein as the previous treatments. They supplied 110 and 60% of net energy requirements, and 165 and 100% of requirements in digestible protein in the intestine, respectively. The variation of digestibility was studied in relation to digesta kinetics and particle size. Apparent OM digestibility decreased with underfeeding, with no effect of protein supplementation (61.7, 53.3, 62.7 and 53.0% in *Bos taurus* and 59.6, 52.6, 62.1 and 55.7% in *Bos indicus*, respectively for HN–, LN–, HN+ and LN+). This lower digestibility at the low level of intake was observed despite a longer total tract particle retention time (72.2, 76.9, 68.2 and 82.0 h in *Bos taurus* and 74.9, 82.2, 67.4 and 85.7 h in *Bos indicus*, respectively for HN–, LN–, HN+ and LN+). Therefore, our data suggest that the retention time may not be a limiting factor for digestion at low intakes. Rumen microbial activity may thus decrease, although neither DM degradability measured in situ nor ruminal and faecal particle sizes varied with the level of intake. Protein supplementation did not avoid a drop of the digestibility when the level of intake decreased. The presence of a factor limiting microbial activity together with physical modifications of the rumen milieu may be the origin of this phenomenon, which does not support the adaptation of ruminants to severe undernutrition.

nitrogen supplementation / cattle / ruminal digestion / tropical climate / underfeeding

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Résumé — Effets du niveau d'ingestion et de la supplémentation azotée sur la digestion des bovins en climat tropical. Huit vaches adultes vides et fistulées du rumen, quatre *Bos taurus* et quatre *Bos indicus* (poids vifs respectifs de 173 et 234 kg), ont reçu selon un protocole en carré latin à l'intérieur de chacun des génotypes quatre régimes à base de paille de riz et de tourteau de coton à différents niveaux d'ingestion et d'azote : HN– ; LN– ; HN+ et LN+. Le régime de référence, HN– , couvrait 100 % des besoins énergétiques des animaux. LN– correspondait à la moitié des apports énergétiques et protéiques de HN– (respectivement 50 et 63 %). Les deux autres régimes (HN+ et LN+) ont été formulés pour apporter une même quantité supplémentaire d'azote aux deux régimes précédents. Ils couvrent ainsi respectivement 110 et 60 % des besoins énergétiques, et 165 et 100 % des besoins protéiques des animaux. Les variations de la digestibilité sont étudiées en relation avec le transit et la taille des particules. La baisse du niveau d'ingestion a entraîné une diminution de la digestibilité apparente de la matière organique, alors qu'une supplémentation azotée reste sans effet (61,7 ; 53,3 ; 62,7 et 53,0 % pour *Bos taurus* et 59,6 ; 52,6 ; 62,1 et 55,7 % pour *Bos indicus*, respectivement pour HN– ; LN– ; HN+ et LN+). Cette plus faible digestibilité à bas niveau d'ingestion est observée malgré un temps de rétention des particules plus long dans le tractus digestif (72,2 ; 76,9 ; 68,2 et 82,0 h pour *Bos taurus* et 74,9 ; 82,2 ; 67,4 et 85,7 h pour *Bos indicus*, respectivement pour HN–, LN–, HN+ and LN+). En conséquence, ces résultats suggèrent que le temps de rétention n'est pas forcément un facteur limitant de la digestion à faible niveau d'ingestion. L'activité microbienne dans le rumen serait donc réduite ; cependant, ni la dégradabilité in situ, ni la taille moyenne des particules ruminales et fécales n'ont varié avec le niveau d'ingestion. La supplémentation azotée n'a pas empêché cette chute de digestibilité avec le niveau d'ingestion. La présence d'un facteur limitant l'activité microbienne, combinée aux modifications physiques du milieu ruminal, peut être à l'origine de ce phénomène, qui va à l'encontre d'une adaptation des femelles des ruminants à une sous-alimentation drastique.

supplémentation azotée / bovins / digestion ruminale / climat tropical / sous-alimentation

1. INTRODUCTION

Cattle in sub-Saharan Africa frequently have to cope with long dry periods when both roughage and water are in a short supply. Furthermore, the dry season is associated with roughage generally of poor quality, and preserving grass as hay or silage is not usual in these countries. However, several strategies are available to enhance the supply of microbial protein to the small intestine such as the feeding of alkali-treated straws or through protein or non-protein nitrogen (N) supplementation: cottonseed meal, although relatively expensive, is becoming available for livestock feeding mostly in peri-urban areas. Consequently, the use of cottonseed meal as a feed supplement is being encouraged to create favourable conditions in the rumen by increasing the supply of nutrients, mainly energy and protein, thus resulting in more efficient fermentation and increasing the microbial protein supply to the lower gut [8].

In two previous experiments in which the animals underwent pronounced underfeeding, digestibility decreased with the level of intake; refeeding increased the digestibility back to initial levels [16, 17]. Most data in the literature report that increasing feeding levels above maintenance generally decreases digestibility [14, 30]. However, such results are sometimes observed when the level of feeding is below the maintenance requirements [6].

An experiment was conducted with animals fed rice straw and supplemented with cottonseed meal at high and low levels of intake and N supplementation, with the objectives of (1) confirming the drop of digestibility when the level of DM intake decreased at levels below maintenance intake, (2) determining whether a protein

supplementation could interact with the level of intake when animals were severely underfed, and (3) comparing the digestive events in two bovine genotypes common in the sub-Saharan Africa where the tsetse fly is present, Zebu *Bos indicus*, which has been shown to succumb to trypanosome infection, and trypanotolerant tropical *Bos taurus* [32].

2. MATERIALS AND METHODS

2.1. Animals, diets and experimental design

Eight non-lactating, non-pregnant adult cows, four Peuhl Zebus (*Bos indicus*) and four Baoule (*Bos taurus*), were penned in individual stalls within a tsetse fly-proof shed in a tropical environment. The experiment took place at the Centre International de Recherche-Développement sur l'Élevage en zone Subhumide (CIRDES) at Bobo-Dioulasso, in Burkina Faso. The annual mean rainfall is about 1 100 mm, with rains falling from June through October. Minimum and maximum temperatures range from 17 to 23 °C and 33 to 37 °C, respectively. This experiment was carried out during the dry season. Animals were fitted with permanent ruminal cannulas made of polyamide and polyvinyl chloride (Synthesia, Nogent sur Marne, France). Surgery took place more than 12 months before the initiation of the experiment and was performed under general anaesthesia (Xylazine, Bayer, Leverkusen, Germany). The cows received an antibiotic treatment (Streptopen, Avotec, Carros, France) for each of 5 d after surgery. The animals were accustomed to human contact and sampling via the ruminal cannula. Their body weight at the beginning of the experiment was 234 ± 43 kg and 173 ± 19 kg for *Bos indicus* and *Bos taurus*, respectively. The animals were managed according to the recommendations of the Canadian Council of Animal Care [18].

Throughout the experiment, the cows received four diets made of rice straw and cottonseed meal in limited amounts to achieve the desired levels of nutrition: (1) HN– (high intake, low N), was formulated to supply 100% of the requirements for net energy for lactation and 125% of the requirements of intestinally digestible protein, calculated according to the INRA [21] recommendations (300 kJ NE_l and 3.25 g of intestinally digestible protein per kg $BW^{0.75}$); (2) LN– (low intake low N), corresponded to half of the previous treatment (50% of NE_l and 63% of protein requirements). The ratio of forage: concentrate of both diets was 89:11. In diet HN–, protein supply was higher than the requirements to insure that enough non-protein nitrogen may be recycled into the rumen. In comparison with these diets for both levels of intake, an extra protein was supplied to the cows by adding an equal amount of cottonseed meal to both treatments HN– and LN–. High intake, high N (HN+) and low intake, high N (LN+) diets were thus established. Supply in net energy for lactation and intestinally digestible protein was 110 and 165%, respectively, for HN+, with a forage to concentrate ratio of 85:15, whereas the supply in net energy for lactation and intestinally digestible protein was 60 and 100%, respectively, for LN+, with a forage to concentrate ratio equal to 82:18. The supply of the net energy and digestible protein in the intestines by rice straw and cottonseed meal was estimated according to the INRA tables [21]. The composition of the diets and DMI are given in Table I.

Cottonseed meal was offered daily at 07.30 h. Chopped rice straw was fed twice a day, at 08.00 h after the concentrate was eaten and then at 15.00 h. The animals had continuous access to trace mineralised salt blocks (Oligosel; Akzo, Brussels, Belgium). The cows were given ad libitum access to water using individual 10 L-buckets, refilled

Table I. Chemical composition, net energy density, dry matter and net energy intake of diets given to *Bos taurus* and *Bos indicus* cows fed high or low levels of intake without or with N supplementation.

Item	LN– [a]	LN+ [a]	HN– [a]	HN+ [a]
Composition ($g \cdot kg^{-1}$)				
Rice straw	890	810	890	850
Cottonseed meal	110	190	110	150
Chemical composition ($g \cdot kg^{-1}$ DM)				
Organic matter	806	811	806	809
Crude protein	74	109	74	93
NDF	644	605	644	625
ADF	405	380	405	392
Ca	2.63	2.56	2.63	2.60
P	2.37	3.14	2.37	2.76
NE_l [b] ($MJ \cdot kg^{-1}$ DM)	3.72	3.93	3.72	3.83
Daily intake				
DMI ($kg \cdot d^{-1}$)				
Bos taurus	1.9	2.1	3.8	4.0
Bos indicus	2.4	2.6	4.8	5.1
NE_l ($kJ \cdot kg\ BW^{-0.75}$)				
Bos taurus	144	166	287	310
Bos indicus	144	168	288	311

[a] LN–: low intake, low nitrogen; LN+: low intake, high nitrogen; HN–: high intake, low nitrogen; HN+: high intake, high nitrogen.
[b] NE_l: net energy for lactation, according to INRA [21].

thrice a day. The experimental design was a duplicated Latin Square with one genotype in one square. The levels of DM intake in combination with N levels were tested with the HN–, LN–, HN+ and LN+ diets. Within the squares, the animals were allocated at random to the treatments. The duration of each of the four experimental periods was 35 d: each collection period was 12 d (d 1 to d 12), and began 23 d after the cows changed their diet.

2.2. Measurements and chemical analyses

The digestibility of the diets was measured after the faeces were collected for 6 d (d 1 to 6) during the collection periods when the personnel was present night and day. The feed samples were composed within each period; there were no feed refusals because the animals were fed in limited amounts below ad libitum intake. The

faeces were scooped in trash cans from the floor three times a day, at 07.00 h, 16.00 h, and 24.00 h. The output of the faeces was recorded at 07.00 h daily. After the faeces were homogenised and weighed, a sample of each daily faecal collection was dried to estimate the total faecal DM production. The DM concentrations of feeds and faeces were determined by drying at 80 °C for 48 h. The dried feeds and faeces were analysed for organic matter (OM) by ashing 5 h at 550 °C and for NDF and ADF (ash-free basis) according to AFNOR [1]. Hemicellulose was considered as the difference between NDF and ADF. The feeds and faeces were analysed for N with the Kjeldahl method [3].

The rice straw used in the experiment was mordanted with chromium and used during collection periods as a solids-flow marker to determine the retention time in the digestive tract [39]. After giving 50 g of chromium-mordanted rice straw on d 4 at 07.00 h, 26 samples of rectal contents were collected by hand 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 33, 37, 41, 48, 53, 57, 61, 72, 82, 96, 106, 120, 130, 144, 154, and 168 h later. Chromium was determined by atomic absorption spectrophotometry after double extraction using both nitric and perchloric acid [37]. The ruminal liquid dilution rate was measured using 40 g of polyethylene glycol (PEG) diluted in 150 mL of water and infused as a pulse dose into the rumen cannula on d 4 at 08.00 h. Eight samples were taken via the ruminal cannula 2, 3, 5, 8, 11, 14, 18 and 24 h after the infusion. On these samples, PEG was determined by turbidimetry [20].

Ruminal liquid was sampled via the ruminal cannula using a tube placed in the ventral sac, at 07.00 h (just before the meal), 09.00 h, and 12.00 h on d 1 and d 2. The pH was immediately measured using a combination electrode after filtration through a cheesecloth. A 10-mL sample of the filtrate was preserved with 1 mL of orthophosphoric acid for ammonia-N determination. Ammonia-N was analysed by microdiffusion [7].

At the end of each collection period (d 12), the rumen of the eight cows was manually emptied at 11.00 h, after the cows had entirely consumed their morning feed, and the contents were weighed; a representative 1-kg sample was dried for DM determination at 80 °C for 48 h. Another 1-kg sample was used to determine the mean particle size of the ruminal contents. The remaining contents were then reintroduced into the rumen. During the faeces collection, 1 kg of fresh faeces was sampled to determine particle size. Ruminal contents and fresh faeces samples were stored frozen (–20 °C) until analysis for particle size determination. After the samples were thawed and thoroughly mixed, DM was determined by drying at 80 °C for 48 h. Twenty grams of fresh ruminal contents and faeces were mixed with 200 mL of water. The particle sizes of the ruminal contents and faeces were then determined in duplicate using an analytical sieve shaker (Retsch AS200, Haan, Germany) under a current of water and fitted with six sieves (4 mm, 2 mm, 800 μm, 400 μm, 200 μm, and 100 μm pore sizes). The effluents from both ruminal and faecal contents were filtered through a 50 μm pore size filter to recover the very small particles, i.e. the fraction passing through a sieve pore size of 50 μm. The largest sieve was not used to determine faecal particle size. The remaining contents of each sieve were dried 48 h at 103 °C before they were weighed.

The in situ DM disappearance of the straw was determined using 5×10 cm dacron bags with heat-sealed edges (53-μm pore size; Ankom, Fairport, NY, USA). Triplicate (for each of four *Bos indicus*) or duplicate (for each of four *Bos taurus*) bags with approximately 3 g DM of straw ground through a 0.8-mm screen were placed into the rumen on d 4 at 09.00 h and incubated for 3, 6, 12, 24, 48, or 96 h. When removed from the rumen, the bags were

washed under tap water and then rinsed and dried at 80 °C for 48 h to determine the DM residues.

Blood samples were taken from the caudal vein on d 5 at 08.00 h. The samples were collected in a heparinised vacutainer (Becton Dickinson, Belliver, Plymouth, UK). After centrifugation (3000 × *g* at 4 °C for 15 min), the plasma was stored frozen (–20 °C) until analysis by enzymatic methods using a multianalyser (Elan, Merck-Clévenot, Nogent-sur-Marne, France). Commercial kits were used for glucose (Merckotest; Merck, Nogent-sur-Marne, France), urea (Boehringer Mannheim, Meylan, France), and non-esterified fatty acids (NEFA-C test, Wako, Biolyon, Lyon, France); 3-hydroxybutyrate was determined by an enzymatic method [5].

2.3. Calculations and statistical analyses

The ruminal liquid dilution rate was calculated by the exponential decrease of PEG concentrations. The ruminal and total tract particle retention time were calculated according to Dhanoa et al. [10] from the Cr excretion curve using the NLIN procedure of SAS [35] with the iterative Marquardt method. The rumen retention time was considered as the smallest rate constant of the model.

The kinetics of DM disappearance in situ were fitted to the exponential model:

$$D(t) = a + b\,(1 - e^{-c(t-l)})$$

where D(t) is the percentage of disappearance from the bag at time t of the "rapidly degradable" fraction (a), containing a soluble fraction, very small particles escaping the bag and the effective rapidly degradable fraction, and of the "slowly degradable" fraction (b); c is the rate constant of degradation of b, and l is the lag time. Degradability (D) was calculated as:

$$D = a + bc / (c + k)$$

where k is the particle passage rate from Cr-mordanted fibre. To estimate the efficiency of the microbial activity, independently of the variations in the passage rate, theoretical D was calculated with the mean value of k (i.e., 0.0261 per hour) in the experiment. To estimate ruminal degradation, the effective D was calculated using the actual value of k for each animal and each period.

Mean particle size was determined by the exponential equation of Fisher et al. [12]. Median particle size was determined by plotting the cumulative percentages undersize of retained particles on a probability scale against the logarithm of sizes of sieves [40].

Statistical analysis was performed by analysis of variance with the GLM procedures of SAS [35], according to the model:

$$Y_{ijklmn} = \mu + g_i + a(g)_j + p_k + i_l + n_m + in_{lm} + gi_{il} + gn_{im} + \varepsilon_{ijklmn}$$

where Y is the dependent variable, μ is the overall mean, g_i is the effect of genotype, *Bos taurus* or *Bos indicus* (i = 1 or 2), $a(g)_j$ is the effect of the animal nested within the genotype (j = 1 to 4), p_k the effect of the period (k = 1 to 4), i_l the effect of the level of intake (l = 1 or 2), n_m the effect of the concentration of dietary N (m = 1 or 2), in_{lm} is the interaction between the level of intake and concentration of dietary N, gi_{il} the interaction between the genotype and level of intake, gn_{im} the interaction between the genotype and concentration of dietary N, and ε_{ijklmn} is the error. For variables analysed at three sampling times (pH, and ammonia-N), the repeated option of GLM was used. When an interaction was shown between the time of sampling and treatment, the treatment effects were separately studied for each sampling time. Animal nested within genotype was used to test the effect of the genotype. The level of significance was declared at $P < 0.05$.

3. RESULTS

3.1. Digestibility

Digestibility of OM and fibre decreased when the level of intake was reduced ($P < 0.01$), in both *Bos taurus* and *Bos indicus*, with no interaction with genotype (Tab. II). No effect of concentration of dietary N was shown. An interaction was shown between the level of the intake and concentration of dietary N for apparent NDF and hemicellulose digestibility coefficients ($P < 0.05$); as N was increased, the decrease in apparent digestibility was more pronounced with the low level than the high level of intake.

3.2. Ruminal pool size, retention time of solid digesta and ruminal liquid turnover

The decrease in the level of the intake resulted in a decrease in ruminal pool sizes for all constituents ($P < 0.01$; Tab. III). A significant interaction between the genotype and the level of intake was observed, the increase in ruminal pools in the response to high intake being greater in *Bos indicus* compared with *Bos taurus* cows.

Table II. OM, fibre and N digestibility (%) in *Bos taurus* and *Bos indicus* cows fed high or low levels of intake without or with N supplementation.

	Diet[a]					
Item	LN–	LN+	HN–	HN+	SEM[b]	Significance[c]
OM						
Bos taurus	53.3	53.0	61.7	62.7	0.99	I**
Bos indicus	55.7	52.6	59.6	62.1		
NDF						
Bos taurus	59.7	56.9	66.5	65.8	0.97	I**, I × N*
Bos indicus	61.1	56.3	64.6	67.3		
ADF						
Bos taurus	58.8	56.9	66.1	66.3	1.08	I**
Bos indicus	59.8	56.2	63.9	64.1		
Hemicellulose						
Bos taurus	61.1	56.7	68.0	64.8	1.42	I**, I × N*
Bos indicus	63.2	56.6	65.8	72.5		
N						
Bos taurus	46.3	59.7	57.0	55.8	2.22	N**
Bos indicus	46.4	58.6	47.8	60.2		

[a] LN–: low intake, low nitrogen; LN+: low intake, high nitrogen; HN–: high intake, low nitrogen; HN+: high intake, high nitrogen.
[b] Standard error of treatment means, with $n = 8$.
[c] I = level of intake, N = level of nitrogen, I × N = intake x nitrogen interaction; *: $P < 0.05$, **: $P < 0.01$. No genotype × treatment interaction.

Table III. Ruminal passage kinetics in *Bos taurus* and *Bos indicus* cows fed high or low levels of intake without or with N supplementation.

	Diet[a]					
Item	LN–	LN+	HN–	HN+	SEM[b]	Significance[c]
Ruminal DM pool (kg)						
Bos taurus	3.3	3.3	3.8	4.3	0.16	G**, I**
Bos indicus	4.1	4.1	6.2	6.4		G × I**
Ruminal liquid pool[d] (kg)						
Bos taurus	23.3	24.1	24.2	25.0	0.94	G**, I**
Bos indicus	34.0	33.2	40.6	39.0		G × I*
Total pool (kg)						
Bos taurus	26.6	27.4	27.9	29.4	1.02	G**, I**
Bos indicus	38.1	37.3	46.8	45.4		G × I**
Ruminal DM (%)						
Bos taurus	12.4	11.9	13.5	14.6	0.41	I**
Bos indicus	10.8	11.1	13.3	14.1		
Ruminal liquid dilution rate (%·h^{-1})						
Bos taurus	7.9	8.6	11.2	11.4	0.07	I**
Bos indicus	8.2	7.6	10.3	11.2		
Mean ruminal particle retention time (h)						
Bos taurus	41.9	43.4	37.1	34.9	1.96	I**
Bos indicus	41.7	44.2	39.2	32.8		
Mean total tract particle retention time (h)						
Bos taurus	76.9	82.0	72.2	68.2	2.64	I**
Bos indicus	82.2	85.7	74.9	67.4		

[a] LN–: low intake, low nitrogen; LN+: low intake, high nitrogen; HN–: high intake, low nitrogen; HN+: high intake, high nitrogen.
[b] Standard error of treatment means, with $n = 8$.
[c] G = genotype, I = level of intake, G × I = genotype × intake interaction; *: $P < 0.05$, **: $P < 0.01$. No intake × nitrogen interaction.
[d] Ruminal liquid pool is the difference between total pool and ruminal DM pool.

Ruminal DM concentration was lower at the low level of intake ($P < 0.01$). Restricting DMI to achieve the desired energy level affected digesta kinetics ($P < 0.01$) in both *Bos taurus* and *Bos indicus*; ruminal dilution rates were slower and ruminal and total gastrointestinal tract particle retention times were higher when less DM was ingested. Digesta kinetics were not affected by N supplementation.

3.3. Ruminal and faecal particle size

Arithmetic mean and median size of ruminal particles were not affected by the level of intake; conversely, both values increased

with N supplementation ($P < 0.05$; Tab. IV). Mean faecal particle size was lower at low intake, but median particle size did not differ with intake; N supplementation had no effect on faecal particle size (Tab. IV).

3.4. In situ degradability

No difference was observed between the diets in lag time and values of rapidly (a), and slowly (b) degradable fractions of the straw (Tab. V). The fractional degradation rate (c) increased with the concentration of dietary N ($P < 0.05$). A similar effect was also found for the degradability when calculated from the mean particle passage rate, whereas it did not occur when the effective passage rate was used, owing to the higher retention time at the low level of intake.

3.5. Fermentation characteristics

Increasing intake and concentration of dietary N both decreased pH values ($P < 0.05$; Tab. VI), with no interaction between time and treatment. Mean ruminal ammonia-N concentrations did not differ with the treatment. However, there was a significant treatment-time interaction ($P < 0.05$). Ammonia-N concentrations after the morning meal (09.00 h and 12.00 h) were affected by the level of N intake ($P < 0.05$; Tab. VI).

Table IV. Ruminal and faecal particle size in *Bos taurus* and *Bos indicus* cows fed high or low levels of intake without or with N supplementation.

	Diet[a]					
Item	LN–	LN+	HN–	HN+	SEM[b]	Significance[c]
Ruminal						
Mean[d] (μm)						
Bos taurus	678	559	632	588	73.0	N*
Bos indicus	651	528	576	704		
Median[d] (μm)						
Bos taurus	233	313	181	260	24.6	N*
Bos indicus	253	290	242	259		
Faecal						
Mean[d] (μm)						
Bos taurus	142	168	224	269	22.6	I**
Bos indicus	174	232	236	229		
Median[d] (μm)						
Bos taurus	60	56	21	55	3.5	
Bos indicus	59	63	62	59		

[a] LN–: low intake, low nitrogen; LN+: low intake, high nitrogen; HN–: high intake, low nitrogen; HN+: high intake, high nitrogen.
[b] Standard error of treatment means, with $n = 8$.
[c] I = level of intake, N = level of nitrogen; *: $P < 0.05$, **: $P < 0.01$.
Neither genotype × treatment nor intake × nitrogen interaction.
[d] Mean is calculated according to [12]; Median is calculated according to [40].

Table V. In situ degradability and estimated ruminal DM digestibility in *Bos taurus* and *Bos indicus* cows fed high or low levels of intake without or with N supplementation.

	Diet[a]					
Item	LN–	LN+	HN–	HN+	SEM[b]	Significance[c]
a [d] (%)						
Bos taurus	16.1	15.6	16.3	16.4	0.410	
Bos indicus	15.9	16.3	17.3	16.2		
b [d] (%)						
Bos taurus	51.5	48.7	53.4	51.5	1.444	
Bos indicus	51.8	53.6	57.1	51.0		
c [d] (%·h^{-1})						
Bos taurus	2.04	2.54	1.94	2.28	0.017	N*
Bos indicus	1.97	2.28	1.57	2.45		
l [d] (h)						
Bos taurus	2.4	2.5	3.4	3.5	0.45	
Bos indicus	2.7	3.7	3.2	3.1		
Theoretical D (%)						
Bos taurus	38.3	39.5	38.8	40.2	0.56	N*
Bos indicus	37.1	40.3	38.6	40.6		
Effective D (%)[e]						
Bos taurus	39.2	41.0	38.2	38.9	0.79	
Bos indicus	38.0	42.1	38.7	38.7		

[a] LN–: low intake, low nitrogen; LN+: low intake, high nitrogen; HN–: high intake, low nitrogen; HN+: high intake, high nitrogen.
[b] Standard error of treatment means, with $n = 8$.
[c] N = level of nitrogen; *: $P < 0.05$.
Neither genotype × treatment nor intake × nitrogen interaction.
[d] a, b, c, and l are the terms of the exponential model $D(t) = a + b\,(1 - e^{-c(t-l)})$, where D(t) is the percentage of disappearance from a nylon bag placed in the rumen for the time t of the rapidly (a) and slowly (b) degradable components of rice straw, c the constant rate of degradation of b, and l the lag time.
[e] D is calculated as a + bc / (c + k), where k is the particle passage rate from Cr-mordanted fibre; theoretical D is calculated with a mean value of k, effective D is calculated with actual values of k.

3.6. Plasma metabolites

The level of intake affected only non-esterified fatty acid concentrations ($P < 0.05$), which were greater at the low level. Plasma urea increased ($P < 0.01$) at the high level of concentration of dietary N (Tab. VII). No difference among genotypes was observed for any metabolite (Tab. VII).

4. DISCUSSION

4.1. Effect of intake on digestion

Restricting DM intake to achieve a decrease from 100 to 50% of NE_1 maintenance requirements resulted in a decrease in digestibilities of OM and cell wall

Table VI. Ruminal pH and ammonia-N concentration in *Bos taurus* and *Bos indicus* cows fed high or low levels of energy intake without or with N supplementation.

	Diet[a]					
Item	LN–	LN+	HN–	HN+	SEM[b]	Significance[c]
pH[d]						
Bos taurus	6.66	6.61	6.66	6.41	0.12	I*, N*
Bos indicus	6.91	6.95	6.77	6.48		
Ammonia-N[e] ($mg \cdot L^{-1}$)						
07.00 h						
Bos taurus	40.5	67.4	53.1	52.9	3.70	
*Bos in*dicus	53.4	58.9	43.4	51.2		
09.00 h /12.00 h						
Bos taurus	56.0	78.0	68.8	76.9	3.64	N*
Bos indicus	62.4	76.7	66.3	69.5		

[a] LN–: low intake, low nitrogen; LN+: low intake, high nitrogen; HN–: high intake, low nitrogen; HN+: high intake, high nitrogen.
[b] Standard error of treatment means, with $n = 8$.
[c] I = level of intake, N = level of nitrogen; *: $P < 0.05$.
Neither genotype × treatment nor intake × nitrogen interaction.
[d] Mean values of pH at three times of sampling.
[e] Mean values of ammonia-N at 09.00 h and 12.00 h.

components. Apparent OM digestibility decreased by 6.2 units, compared to a 7.7 unit-decrease reported in a similar experiment in which energy intake was restricted from 120 to 60% of NE maintenance requirements [16]. In another experiment, Grimaud et al. [17] also reported a drop in apparent OM digestibility with Zebu cows fed at a level equal to one third of their maintenance requirements. Similarly, Atti et al. [4] observed with a low-quality forage a strong drop in digestibility in sheep underfed at 20% of their energy requirements. These results are inconsistent with most literature data [38]. Indeed, an increase in digestibility was expected when the energy intake decreased. However, Doreau et al. [11], from the literature, showed that results obtained at levels of intake above maintenance could not be transposed at lower levels, and they reported experiments where digestibility did not vary, or even decreased, with the reduction of feed intake at levels below maintenance requirements.

Ruminal digestion of a diet depends both on the time of contact between microbes and particles and on microbial activity. The modification in retention time of solid digesta generally induces a modification in digestibility [31], even if some authors reported no variation in ruminal retention time in underfed cattle [15] or in well-fed sheep [29] when a change in intake led to modification in digestibility. A longer retention time of dietary particles in the forestomachs increases the exposure of reticuloruminal digesta to a better attack by ruminal microorganisms. Such a longer retention time was observed in this experiment at the low level of intake, whereas a lower digestibility occurred at this level.

Table VII. Plasma metabolites in *Bos taurus* and *Bos indicus* cows fed high or low levels of intake without or with N supplementation.

	Diet[a]					
Item	LN–	LN+	HN–	HN+	SEM[b]	Significance[c]
Glucose ($g \cdot L^{-1}$)						
Bos taurus	0.59	0.61	0.60	0.62	0.009	
Bos indicus	0.61	0.59	0.62	0.64		
Non-esterified fatty acids (mM)						
Bos taurus	0.09	0.12	0.05	0.06	0.026	I*
Bos indicus	0.12	0.19	0.08	0.10		
3-hydroxybutyrate (mM)						
Bos taurus	0.27	0.24	0.29	0.28	0.012	
Bos indicus	0.29	0.29	0.31	0.30		
Urea ($g \cdot L^{-1}$)						
Bos taurus	0.14	0.21	0.17	0.25	0.022	N**
Bos indicus	0.15	0.21	0.16	0.26		

[a] LN–: low intake, low nitrogen; LN+: low intake, high nitrogen; HN–: high intake, low nitrogen; HN+: high intake, high nitrogen.
[b] Standard error of treatment means, with $n = 8$.
[c] I = level of intake, N = level of nitrogen; *: $P < 0.05$, **: $P < 0.01$.
Neither genotype × treatment nor intake × nitrogen interaction.

Luginbuhl et al. [28] studied whole tract digesta kinetics in steers fed hay at four levels of intake and did not observe any effect of intake level on digestibility, although these authors reported a linear decrease in the mean retention time when the intake level increased. Atti et al. [4] who fed Barbary ewes at a very low intake (20% of energy requirements) observed a very long ruminal stay, twice that than at maintenance, whereas digestibility strongly decreased. It is likely that, on the contrary to the results obtained above maintenance, ruminal particle retention time is not involved in modifications in digestive efficiency.

At levels of intake above maintenance, a decrease in feed intake results in a more efficient mastication, and Luginbuhl et al. [27], among others, reported a linear decrease of the size of ruminal and faecal particles as the level of DM intake decreased. In our experiment, underfeeding did not modify or decrease ruminal and faecal particle size. It seems that an insufficient reduction of particle size in the rumen cannot explain the decrease in ruminal digestibility at the low intake. The decrease in digestibility observed in the current trial with the reduction in intake could be a result of a reduction of microbial activity at the low level of intake. Microbial activity is the result both of the feed structure and of the intrinsic activity of bacteria and protozoa. Grimaud and Doreau [15] and Grimaud et al. [17] observed that a feed restriction led to a decrease in the ruminal protozoa population. The decrease in the liquid dilution rate observed in the present trial with the reduction of the level of intake could also involve a decrease in protozoa, because both

phenomena are highly correlated [26]. It is known that a decrease in the protozoa population lowers fibre ruminal digestion [22]. However, the decrease in the protozoa population is accompanied by a decrease in the digestible OM intake, so that the protozoa concentration is probably only partially involved in the reduction of the cellulolytic activity.

We observed no variation in the in situ DM degradability due to intake. In contrast, Kabré et al. [25] evidenced that reducing amounts of forage supplied to ewes results in an increase in NDF and ADF digestibility and in an increase in NDF degradability; this latter increases due to a higher enzymatic activity of cellulolytic bacteria [24]. In our experiment, the lack of variation in the theoretical degradability compared with the decrease in apparent digestibility could be due to a lack of sensitivity of the in situ method to appreciate differences in fibrolytic activity, when fibre degradation is impaired: the time spent by the particles in the rumen, the lack of mastication and the confinement of the feed in the bags may be responsible for the differences in the fibrolytic activity between the microbes present in the rumen content and in the bags [33]. In the case of a possible impaired microbial activity at a low intake, a reduced motility may have limited exchanges between bags and the ruminal milieu.

In addition to possible alterations in the bacterial populations, a lack of specific nutrients might have occurred which met the microorganisms maintenance requirements but limited their growth. Microbes require fermentable nitrogen, as ammonia or amino acid. The lack of variation of ruminal ammonia between high and low levels of intake is not in favour of a shortage in nitrogenous compounds for microbial growth. Other components such as phosphorus and sulphur can limit microbial growth. Their concentrations were not measured in the current trial, but it can be hypothesised that phosphorus is not a limiting factor, due to its high concentration in rice straw.

4.2. Interaction between the level of intake and nitrogen supplement on digestibility

An extra N supplement at each dry matter level resulted in slight modifications of the digestion parameters. Kabré et al. [23], who investigated the effects of fish meal supplementation on the digestibility of a medium-quality forage in sheep when energy supply was severely restricted, noted significantly higher OM and cell wall component digestibility. They hypothesized that fish meal provides additional specific nitrogenous nutrients, which promotes microbial activity. In our experiment, a trend for an increase in digestibility occurred with the supply of additional protein at a high level of DM intake. Ortigues et al. [34] noted that the effect of protein supplementation is noticeable with low quality forages, such as straws, when this supplementation provides N requirements for microbial growth. In the present trial, extra cottonseed meal limited but did not avoid a deficiency in fermentable N in the diet, estimated by the French PDI system [21], and microbial growth could have been limited due to an inadequate amount of readily available N. However, ammonia N concentration was in most cases above the minimal requirements for rumen bacteria [9].

4.3. Comparison between *Bos taurus* and *Bos indicus*

The decrease in digestibility when the level of DM intake decreased was observed with the same magnitude in both genotypes; this is in contrast with a previous experiment using the same animals, in the same environment with a similar diet, in which the decrease in digestibility due to a decrease in intake was of a higher magnitude for *Bos indicus* than for *Bos taurus* [16]. Comparisons between these

genotypes are not numerous, and the results differ. For animals fed on pasture, Forbes et al. [13] concluded, based on similarities in intake, digesta dynamics, and grazing behaviour, that *Bos taurus* heifers are likely to be as productive as *Bos indicus* heifers in different environmental conditions. Both genotypes have similar digestive efficiency for diets high in fibre and low in concentrates [36], which are the most common in sub-Saharan Africa. A higher DM degradability was registered in *Bos indicus* fed with a low N diet, with a higher ruminal ammonia-N concentration, than in *Bos taurus* [19]; endogenous N recycling efficiency was presumably better in *Bos indicus*. In our experiment, we did not find any difference in ruminal ammonia-N concentrations between genotypes, even for the diets low in N. Akinbamijo et al. [2], who studied the trypanosome infection in N'dama and Gobra Zebu, observed that in both genotypes, DM digestibility was not affected, whereas the infection reduced voluntary DM intake. In their experiment, there was a significant effect of genotype on different ruminal pool size measurements. The interaction between genotype and rumen pool size observed in our experiment may result in part from a larger difference in DM intake between high and low levels for *Bos indicus*.

In conclusion, this experiment confirmed the occurrence of a drop in digestibility when the level of intake decreased at levels below maintenance requirements. However, we could not specify the direct causes of this decrease. Experiments with more precise measurements have to be carried out to determine the causes of the disturbances of ruminal activity which appear in numerous cases at low intake. In the case of severe undernutrition, a decrease in the energy value of the ration, added to a strong limitation of the level of intake, leads to increase the energetic deficiency of the animal. The management of the animal in periods of feed shortage must integrate the fact that energy supply to animals can be limited not only by a low intake but also by a low digestibility. Despite the few opportunities in southern countries: use of alkali-treated straws, larger incorporation of local factory by-products, such as cottonseed or groundnut meals for example, must be encouraged to avoid tropical cattle to pass the dry season in too bad body conditions. Both genotypes (*Bos taurus* and *Bos indicus*) are sensitive to undernutrition, so that the choice between these genotypes will not be determined by the response of digestive events to underfeeding.

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