Bacterial DNA quantification and ^{15}N as methods to estimate microbial growth: a comparative study

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Take home message Microbial growth in the rumen could be estimated by quantification of total bacterial DNA.

Introduction ^{15}N is an external marker widely used for estimating microbial protein synthesis (MPS) in ruminants. Bacterial DNA (BDNA) has been also proposed as a potential internal marker to assess MPS, but studies comparing both procedures are limited. The objective of this study was to compare values of microbial growth in Rusitec fermenters determined using either ^{15}N or total BDNA concentration, and to assess if both procedures detect similar differences between diets in solid, liquid and total digesta.

Materials & methods Two independent Rusitec trials were carried out to assess microbial growth and rumen fermentation of four different diets containing 50:50 forage:concentrate. In trial 1, the concentrate was formulated either with maize (M) or with maize and citrus pulp (CP), and in trial 2 the forage in the diets contained either barley straw (BS) or olive cake (OC). In each trial, four Rusitec fermenters were used in a cross-over design in two 14-day incubation periods (giving four replicate fermenters per diet). From day 10 to 14, a solution of $^{15}NH_4Cl$ was added to the artificial saliva following the procedure described by Martínez *et al*. (2010). On days 13 and 14, samples of solid and liquid digesta were taken to estimate MPS in each digesta phase and for measuring BDNA by qPCR. Data were analysed as a mixed model, with diet, incubation period and their interaction as fixed effects and fermenter as a random effect. The relationship between values of MPS determined by ^{15}N and BDNA concentrations was assessed by Pearson's correlation coefficient.

Results & discussion In trial 1, there were no differences ($p > 0.05$) between M and CP diets in MPS estimated by ^{15}N in any digesta phase (solid, liquid and total; Table 1). Similarly, no differences between diets were found ($p > 0.05$) in BDNA concentrations. In trial 2, MPS was greater for BS diet compared with OC diet in the solid phase, but the opposite was detected in the liquid phase and there were no differences ($p > 0.05$) between diets in total MPS values. In contrast, BDNA concentrations were similar ($p > 0.05$) for both diets in both digesta phases. When using data from both trials (n=16), there was a positive and significant relationship between the MPS values and the amount of BDNA in each fermenter both in the liquid phase ($r=0.762$; $p < 0.001$) and in total digesta ($r=0.409$; $p > 0.05$), but no correlation ($p > 0.05$) was detected in the solid contents. These results are in agreement with those observed by Mateos *et al.* (2017) in Rusitec fermenters fed different diets.

Table 1 Microbial protein synthesis (MPS) estimated using ^{15}N as a marker and bacterial DNA concentrations (BDNA) in Rusitec fermenters fed diets containing maize (M) or citrus pulp (CP) in trial 1, and barley straw (BS) or olive cake (OC) in trial 2.

Trial 1						Trial 2					
	Digesta phase	M	CP	SEM	p =		Digesta phase	BS	OC	SEM	p =
MPS, mg microbial N/d	Solid	180	200	7.71	0.14	MPS, mg microbial N/d	Solid	199	173	6.89	0.05
	Liquid	137	116	7.92	0.15		Liquid	107	119	2.65	0.03
	Total	317	316	6.45	0.92		Total	306	292	5.87	0.16
BDNA, mg DNA/d	Solid	2.51	3.14	0.23	0.13	BDNA, mg DNA/d	Solid	4.25	3.61	0.99	0.67
	Liquid	3.11	2.30	0.47	0.29		Liquid	2.11	1.06	0.40	0.14
	Total	5.62	5.44	0.34	0.73		Total	6.36	4.67	0.96	0.28

Conclusion Both methods detected similar differences between diets in total microbial growth but differed when considering microbial growth either in solid or liquid phase of the fermenters. More studies are warranted to confirm BDNA quantification as a routine method for estimating microbial growth in the rumen.

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References

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