



## ***In vitro* gas production of high forage diets incubated with two live yeast (Biosaf SC47 and Procreatin 7)**

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The effect of yeast cultures in animal production has been well documented (Chaucheyras-Durand and Durand 2010) and the relationships between nutritional characteristics and *in vitro* gas production for gramineous and leguminous forages have been established (Zhong *et al.* 2016). However, the interaction with different kinds of probiotics is not clear. For that reason, the aim of the present study was to determine the effects of two yeast cultures dosed at the same CFU per gram of substrate on *in vitro* digestibility, methane and CO<sub>2</sub> productions of high forage diets.

Treatments were arranged in a 2 × 3 factorial design with two high forage diets (alfalfa and oat hay) prepared adding a proportion of 80% forage with 20% sorghum grain for the incubations and two types of probiotics (Procreatin 7<sup>®</sup> and Biosaf SC47<sup>®</sup>, both marketed by Societe Industrielle Lesaffre, France), tested at 3 inclusion levels (0, 1.5 and 3.0 × 10<sup>7</sup> CFU/g DM). Feeds were analysed for proximate composition (AOAC 1996).

The *in vitro* kinetics of gas production was determined following the technique of Menke and Steingass (1988), using 0.5 g sample of oat or alfalfa diets. Previously, each mixture was prepared with each probiotic to obtain zero, 1.5 and 3.0 × 10<sup>7</sup> CFU/g of DM. Yeast concentrations were estimated to represent doses of 0, 1 and 2 g/day per ruminant 25 kg/BW. Rumen liquor was obtained from male and female sheep (34±1.6 kg BW) fed with a 50:50 concentrate: forage ratio. Rumen liquor was flushed with CO<sub>2</sub> before use as inoculum. Three incubation runs were performed in different weeks. Bottles were inoculated within each incubation run. For each run, 3 substrate-negative controls were also included. Gas production volumes were measured using a manometer, after 2, 4, 6, 8, 10, 14, 18, 24, 30, 36,

42, 48, 60 and 72 h of inoculation. Total gas (V<sub>max</sub>) values were corrected for the blank incubation. In each measurement, the CO<sub>2</sub> was estimated (Statham and Williams 1983). Methane (CH<sub>4</sub>) production was calculated from the methane concentration in each gas sample and the corresponding total gas production.

The N-NH<sub>3</sub> concentrations were determined according to Searle (1984). After incubation, the contents of each serum bottle were filtered and fermentation residues were used to estimate DM digestibility (DMD).

Gas production kinetic parameters were analysed according to a completely randomized design, testing linear and quadratic effects of yeast levels. The means were compared by Tukey's test (Steel *et al.* 1997).

The chemical composition of ingredients and diets are shown in Table 1.

The DMD of the oat diet was higher than alfalfa diet; consequently, V<sub>max</sub> was greater in the oat diet (Fig. 1). The reduction in the lag phase in oat is most likely because the

Table 1. Nutritional composition of the high forage diets

Item (%)	Alfalfa hay	Oat hay	Sorghum grain	Alfalfa sorghum	SD	Oat sorghum	SD
CP	18.49	10.3	10.55	18.22	0.249	10.51	0.094
NDF	46.69	46.29	21.11	41.57	0.614	45.35	0.501
ADF	8.47	8.45	7.30	8.23	0.08	8.41	0.121
NFC	22.44	68.88	34.52	62.17	0.80	55.06	0.563
Ash	3.90	3.55	2.85	3.69	0.05	3.58	0.033

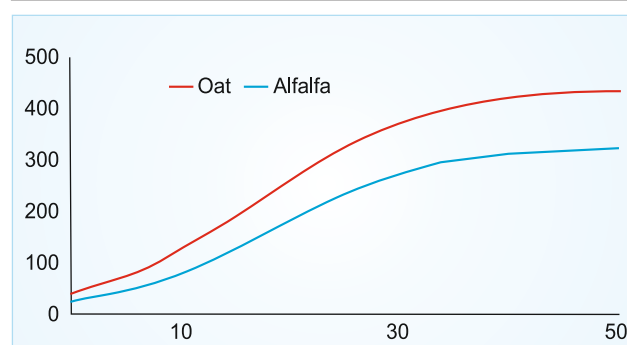


Fig. 1. *In vitro* gas production for high forage diets.

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Table 2. Main effects of high forage diets and commercial probiotics on ruminal and gas production parameters

Parameter	a	L	DDM (%)	CO <sub>2</sub> *	CH <sub>4</sub> *	N-NH <sub>3</sub> **
Alfalfa	324.99	3.68	68.49	96.17	52.33	8.39
Oat	442.68	1.78	73.72	78.04	40	5.73
SE	3.702	0.06	2.61	3.35	3.45	0.457
<i>Biosaf</i> (mg/kg)						
0	375	2.66	71.1	95.6	44.2	6.3
20	388	2.58	73.1	85.4	66.7	7
40	399	2.89	74.41	103.7	47.66	7.11
<i>Procreatin</i> (mg/kg)						
20	378	2.8	78.6	94.9	45.7	6.8
40	385	2.93	75.2	98	48.7	8.15
SE	26.1	0.47	2.38	5.21	5.51	1.07
Forage	0.0001	0.0001	0.022	0.0002	0.007	0.0002
Biosaf L <sup>1</sup>	0.008	0.52	0.56	0.01	0.009	0.43
Q <sup>2</sup>	0.84	0.74	0.69	0.14	0.65	0.73
Procreatin L <sup>1</sup>	0.21	0.03	0.03	0.64	0.66	0.08
Q <sup>2</sup>	0.76	0.41	0.32	0.92	0.56	0.6

a, V<sub>max</sub> (ml/g DM); L, Lag (h); \*ml/g DMD; \*\*mg/100 ml  
<sup>1</sup>Linear; <sup>2</sup>Quadratic.

gramineous forages are more quickly fermented (Zhong *et al.* 2016). The higher production of greenhouse gases in alfalfa diet results from a greater total activity of ruminal bacteria and methanogenic archaea associated with a lower concentration of ruminal fermentable carbohydrates (Mohammadzadeh *et al.* 2014). This reduction, affected bacterial production and increased the ammonia-N concentrations (Brito *et al.* 2014).

Table 2 shows the effects of the probiotics on *in vitro* gas production parameters. There was a linear effect (P<0.008) on V<sub>max</sub> with Biosaf®, while Procreatin® did not modify this parameter (P<0.21). The higher gas production with Biosaf was associated with an increased production of greenhouse gases according to the added probiotic quantity (AlZahal *et al.* 2014). Elghandour *et al.* (2016) reported a similar effect with the addition of *S. cerevisiae* to the diet and showed that yeast increased the methane production; this effect was attributed to the ability of the yeast to shift H<sub>2</sub> utilization from methanogenesis to reductive acetogenesis through the homoacetogenic bacteria that can produce acetate from CO<sub>2</sub> and H<sub>2</sub>.

Lag time decreased in response to Procreatin (linear, P<0.03), confirming that yeast strains may act differently in the rumen. Other studies also reported that the addition of yeast cultures decreased the lag time (Elghandour *et al.* 2016).

The reduction in the lag time results in increased digestibility (Tang *et al.* 2008), confirming that yeast strains may act differently in the rumen. Several studies have shown that probiotics based on *S. cerevisiae* increase the number of cellulolytic bacteria (Pinloche *et al.* 2013); however, the fact that Procreatin did not stimulate gas production suggests that its mechanism of action may be different in some microbial communities.

Total CO<sub>2</sub> and methane concentrations were higher with the addition of Biosaf SC 47 (linear; P<0.01 and P<0.009, respectively); however in other studies, the total enteric CH<sub>4</sub> production expressed as grams/day was reduced by yeast dose feed (Hernández *et al.* 2017).

Ammonia-N production tended (P<0.08) to increase with Procreatin 7 addition, but not with addition of Biosaf SC47. In contrast, other studies found that the addition of yeast at a suitable level was beneficial for a more efficient use of the N source for microbial protein synthesis (Mao *et al.* 2013). Hristov *et al.* (2010) also found an increment in the overall use of ammonia-N and increased microbial protein synthesis rates with the addition of yeast; however, ammonia-N concentrations in this study were low and indicate inefficiency of the system.

## SUMMARY

The aim of this experiment was to determine the effect of two probiotics administered at the same doses at two high forage diets (alfalfa or oat hay), using the *in vitro* gas production kinetics recording dry matter digestibility, CH<sub>4</sub> and CO<sub>2</sub> production. Treatments were arranged as a 2 × 2 factorial experiment with 2 sources of forages and 2 types of probiotics, tested at 3 inclusion levels (0, 1.5 and 3.0 × 10<sup>7</sup> CFU/g). Ruminal and gas production parameters, including *in vitro* DM digestibility were complete different with oats than alfalfa diets. Procreatin increased *in vitro* DM digestibility and reduced the lag time linearly with dose. Also proteolysis tended to be increased linearly (P<0.08) with procreatin. Whereas a linear effect was observed in V<sub>max</sub> with Biosaf, this effect resulted in greater productions of CH<sub>4</sub> and CO<sub>2</sub>. Consequently, Biosaf resulted in greater productions of greenhouse gases than Procreatin.

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