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► **To cite this version:**

Véronique Julliand, G Laillet. Development of an in vitro technique to measure the cellulolytic activity of donkey caecal contents. *Annales de zootechnie*, 1995, 44 (Suppl1), pp.183-183. hal-00889345

HAL Id: hal-00889345

<https://hal.science/hal-00889345v1>

Submitted on 11 May 2020

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Development of an *in vitro* technique to measure the cellulolytic activity of donkey caecal contents

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We modified Tisserand and Zelter's ruminant technique (1965, Ann Biol Anim Bioch Biophys, 5 (1), 101-111) to measure cellulolytic activity in the caecum of donkey.

Three caecally-fistulated donkeys were fed a maintenance diet (30 % concentrate (16 % CP) + 70 % lucerne-cocksfoot-hay (15 % CP)) twice a day. Caecal samples were collected before the first meal from each donkey, mixed together and given 4 different treatments (T) : filtered through 6 layers of muslin (M) as indicated in the Tisserand and Zelter's technique in T1 and T2, or through a nylon (N) (blutex T120 - pores of 48 µm) in T3 and T4 in attempt to pick up more micro-organisms. In T2 and T4 fibres were collected in 200 ml of the Tisserand and Zelter's buffer and mixed in a stomacher for 10 min in to detach the fixed micro-organisms. The fibres + buffer were then filtered through muslin (T2) or nylon (T4).

20 ml of the first filtrate were inoculated in test-tubes and complemented with 20 ml of buffer in T1 and T3 or 20 ml of the second filtrate in T2 and T4. Each test-tube contained 0.5 g of filter-paper (FP) cut into narrow strips and was placed under continuous CO₂ current. After 24 h and 48 h of incubation at 39 °C for the 4 treatments, 6 tubes were removed. The pH was measured and the FP was collected and

dried at 80°C for 48 h. The disappearance of dry matter (dDM) was calculated.

The addition of micro-organisms detached from fibers by the stomacher decreased (P<0.05) the pH at 24 h (T2/T1 and T4/T3) and 48 h (T2/T1). The global effect of the stomacher was highly significant at 24 h (P<0.01) and significant at 48 h (P<0.05). The treatment with the stomacher homogenized the inoculates which resulted in a decrease in the coefficient of variation of dDM which was significant in T2 at 24 h (P<0.01).

The nylon was more permeable to free or detached micro-organisms than the muslin and allowed a significant greater degradation of FP after 24 (P<0.01) and 48 h (P<0.05) of incubation. The filtration material had no significant effect on pH.

There was no interaction between the 2 effects (filtration material/mixing machine) on pH nor on dDM at 24 h and 48 h.

In conclusion, the modification of the following parameters allowed to measure and increase the *in vitro* cellulolytic activity of caecal contents of donkey : filtration through a nylon, treatment with stomacher. More experiments have to be done to confirm these adjustments.

Time		pH			dDM (%)	
		0 h	24 h	48 h	24 h	48 h
T1 : M	Mean	6.65	7.14	6.63	0.0	26.5
	CV (%)	2.26	7.42	7.09		93.6
T2 : M + STO	Mean	6.65	6.47	5.92	24.7	54.4
	CV (%)	2.26	1.54	1.69	23.5	13.6
T3 : N	Mean	6.80	6.63	6.10	22.0	56.7
	CV (%)	1.47	2.11	6.56	59.5	33.3
T4 : N + STO	Mean	6.80	6.39	5.89	36.7	62.0
	CV (%)	1.47	1.88	2.21	18.8	8.7