Quantification of cellulolytic bacteria using *in vitro* culture containing treated or untreated cottonseed hulls determined by real-time polymerase chain reaction

A Faramarzi Garmroodi, M Danesh Mesgaran, A R Vakili, A R Heravi Moussavi, A Tahmasbi, H Jahani-Azizabadi
Department of Animal Science, Excellence Center for Animal Science, Ferdowsi University of Mashhad, Mashhad, Islamic Republic of Iran
Email: ali_faramarzi_g@yahoo.com

Introduction Treatment of lignocellulosic substances with an alkali solution removes lignin and decreases the crystallinity of cellulose, thereby increasing the biodegradation of cell walls by fibrolytic micro organisms located in the rumen (Gould, 1984; Krause, 2003). Major fibrolytic bacteria are the gram-negative *Fibrobacter succinogenes*, and two species of gram-positive bacteria, *Ruminococcus albus* and *Ruminococcus flavefaciens* (Krause, 2003). The objective of the present experiment was to quantify the cellulolytic bacteria population using *in vitro* culture containing sodium hydroxide treated or untreated cottonseed hulls (CH) determined by real-time polymerase chain reaction (RT-PCR).

Material and methods Cottonseed hulls were used as untreated or treated with NaOH as 20 g/kg DM [a 20% solution of NaOH was sprayed on CH and kept for 48 h (CH2S48) at room temperature]. Samples were incubated in medium prepared as described by Arroquy *et. al.* (2005). Forty-five ml of medium was supplied into a 100 ml bottle containing 0.45 g of the feed sample (3 replicates). Then, each bottle was inoculated under carbon dioxide with 5 ml of isolated rumen bacteria. Rumen fluid was obtained from three sheep (49.5±2.5 kg body weight) fitted by rumen fistulae, before the morning feeding. The animals fed 1 kg/d DM of lucerne hay and 0.3 kg/d DM of concentrate (165 g CP/kg DM). Rumen fluid was immediately strained through four layers of cheesecloth. Then, the rumen fluid was centrifuged (10 min, 3000 rpm) and a solution of cycloheximide was added to protozoa free supernatant. The bottles were incubated for 96 h at 38.6° C. After the incubation, 1 ml of each bottle was sampled for DNA extraction. The extraction was done using Bioneer Accuprep Genomic DNA Extraction Kit. The 16s rRNA gene-targeted primers sets used in the present study were forward: 5′-GTGSTGCAYGGYTGTCGTCA-3′, 5′-GTTCGGAATTACTGGGCGTAAA- 3′, 5′-CCCTAAAAGCAGTCTTAAGTTCG- 3′ and 5′-CGAACGGAGATAATTTGAGTTTACTTAGG- 3′ for total bacteria, *Fibrobacter succinigenes, Ruminococcus albus and Ruminococcus flavefaciens,* respectively, and reverse: 5′-ACGTCRTCCMCACCTTCCTC- 3′, 5′-CGCCTGCCCCTGAACTATC- 3′, 5′-CCTCCTTGCGGTTAGAACA- 3′, 5′-CGGTCTCTGTATGTTATGAGGTATTACC- 3′ for total bacteria, *Fibrobacter succinogenes, Ruminococcus albus and Ruminococcus flavefaciens,* respectively. Then, quantification of cellulolytic bacteria was carried out using RT-PCR (2 replicates for each primer). Bacterial rDNA concentrations were measured relative to total bacteria amplification (ΔΔCt). Data were analyzed using the GLM procedure of SAS 9.1 and the means were compared by the Tukey test ($P< 0.05$).

Results Quantity of the major species of cellulolytic bacteria existing in the *in vitro* culture relative to total bacteria population is shown in Table 1. Chemical treatment had no significant effect on the quantity of cellulolytic bacteria under present experimental condition.

Table 1 Quantity of the major species of cellulolytic bacteria existing in the *in vitro* culture relative to total bacteria population

	Bacteria		
Items	*Fibrobacter succinogenes* × (10^{-4})	*Ruminococcus flavefaciens* × (10^{-7})	*Ruminococcus albus* × (10^{-4})
Untreated CH	12	950	17
NaOH-treated CH	13	560	14
s.e.m	0.002	0.003	0.004
P	> 0.05	> 0.05	> 0.05

Conclusions Results of the present study indicate that the *in vitro* relative quantity of the major species of cellulolytic bacteria was not influenced by sodium hydroxide treatment of CH. Therefore, it was concluded that the treatment of CH with NaOH solution, as done in the present study might not alter the fibrolytic bacteria population. It was previously indicated that the digestibility of fibrous materials is generally related to rumen bacterial populations which are capable of producing wide range of fibrolytic enzymes (Krause, 2003). Therefore, it is not reasonable to get significant difference in digestibility of fibrous materials when treated with NaOH as obtained by Petersen *et al*. (1981), who reported no significant differences in OM digestibility of the roughages treated with NaOH at 4% of DM.

References

Arroquy, J. I., Cochran, R. C., Nagaraja, T. G., Titgemeyer, E. C., and Johnson, D. E. 2005. Animal Feed Science and Technology. 120, 93-106.
Gould, J. M. 1984. Biotechnology and Bioengineering. 26, 46.
Krause, D. O., Denman, S. E., Mackie, R. I., Morrison, M., Rae, A. L., Attwood, G. T., and Mcsweeney, C. S. 2003. FEMS Microbial Review. 27, 663-693.
Petersen, M. K., Thomas, V. M., and Roffler, R. E. 1981. Journal of Animal Science. 52, 398-405.

https://doi.org/10.1017/S2040470010003857 Published online by Cambridge University Press