In vitro rumen fungi quantification in medium containing sodium hydroxide or formaldehyde treated sunflower meal using quantitative competitive PCR assay

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Introduction The rumen anaerobic fungi are primary colonizers of fibrous plant materials that degrade lignin-containing plant cell walls. With the advancement of molecular enumeration methods, in particular 18S rDNA gene probing methods, researchers were able to monitor fungal species within the rumen (Stahl *et al*., 1998). Quantitative competitive PCR (QC-PCR) techniques play an important role in nucleic acid quantification because of their significant lower cost of equipment and consumables (Franz *et al*., 2001). The aim of this study was to use the QC-PCR assay to evaluate the relative quantitative comparison of anaerobic fungi population in medium containing sunflower meal, either untreated or treated with formaldehyde (3 and 6 g/kg DM) or sodium hydroxide (40 g/kg DM).

Materials and methods Rumen fungi were isolated from pre-incubated wheat straw in the rumen of rumenal fistulated sheep and then method of Joblin (1981) was used to grown under anaerobic conditions at 39 ºC for 3 days (Joblin, 1981). These isolates were used (1:9) as a source of fungi inoculum. Serum bottles containing fungi culture medium, 1 g of sunflower meal as untreated (USM) or treated with 3 (F1SM) and 6 g (F2SM) formaldehyde/kg DM or 40 g NaOH/kg DM (SHSM) and 1 ml antibiotic solution were used to culture the isolated fungi at 39 ºC in an incubator. To preparing fungi pure culture, subculturing was done three times and the fungi were identified using rumen anaerobic fungi primer (GAF) in pure culture. Total genomic DNA was isolated from pure culture samples using Guanidine Thiocyanate-Silica Gel method. A universal PCR primer pair GAF (F): 5'-GAG GAA GTA AAA GTC GTT AAC AAG GTT TG-3' and GAF(R): 5'-GAA ATT CAC AAA GGG TAG GAT GAT TT-3' was used to amplify a specific region of 18S rDNA from anaerobic rumen fungi. Standard control DNA was constructed to use in the QC-PCR and was shown to amplify under the same reaction condition and the same amplification efficiency as the target DNA. The PCR was performed in a final volume of 25 µl sealed in a capillary tip, and thermocycling was carried out in a model 2000 (Biometra). The PCR amplification condition was as follows: denaturation at 94 ˚C for 4 min followed by 35 cycles of 94 ˚C for 30 s; 56 ˚C for 30 s; and 72 ˚C for 1 min followed by a final extension at 72 ˚C for 5 min. The PCR products were separated by electrophoresis on agarose gels, stained with ethidium bromide, and visualized by UV transillumination. The relative intensities of PCR products were used to compare fungal biomass under different samples. The signal intensity was quantified by ImageJ 1.29x and expressed in arbitrary units. The data was analyzed using the GLM procedure of SAS (2001) for a completely randomized design.

Results The competitive PCR reaction for DNA extracted from fungi media is given in Figure 1. The result of QC-PCR (Figure 2) showed that the growth of rumen fungi in the medium containing sunflower meal treated with NaOH was greater than the other treatments (P< 0.05). Formaldehyde decreased the growth of rumen fungi.

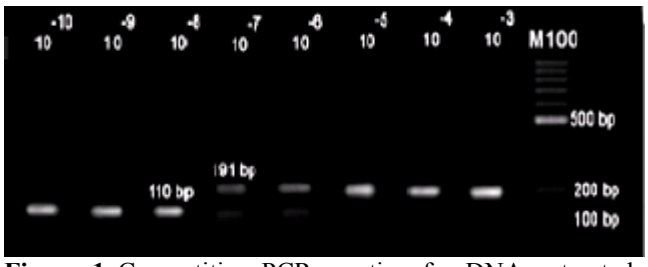

Figure 1 Competitive PCR reaction for DNA extracted from fungi medium

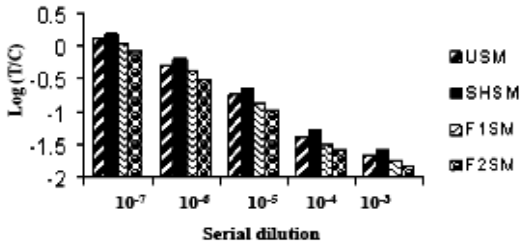

Figure 2 Alteration in rumen fungi population in medium containing: untreated sunflower meal (USM); 40 g NaOH /kg DM treated sunflower meal (SHSM); 3 g formaldehyde /kg DM treated sunflower meal (F1SM); 6 g formaldehyde /kg DM treated sunflower meal (F2SM), log ratio of intensities of amplified target DNA to standard (Log (T/C))

Conclusions The method of QC-PCR for enumerating anaerobic rumen fungi in the present study demonstrated that the treatment of sunflower meal with NaOH increased fungal growth, while formaldehyde decreased it. Increasing rumen fungi populations and aviclase enzyme and following digestibility with NaOH was reported by Chen *et al*. (2007). Sodium hydroxide might hydrolyze the ester bonds between lignin and hemicellulose and expose the cellulose to microbial attachment and improve digestibility (Goto *et al*., 1993). The data obtained in the present study indicated the ability of QC-PCR to determine the quantity of rumen fungi using *in vitro* culture.

References

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