J. Gen. Appl. Microbiol., **61**, 31–33 (2015)
doi 10.2323/jgam.61.31

jGAM

Short Communication

Anaerobic degradation of tannins in *Acacia nilotica* pods by *Enterococcus faecalis* in co-culture with ruminal microbiota

(Received July 23, 2014; Accepted December 31, 2014)

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Key Words: *Acacia*; co-culture; degradation; fermentation; tannins; rumen

Tannins are water-soluble polyphenolic compounds present in plant tissues. The two classes of tannins—hydrolysable (HT) and condensed (CT)—are generally regarded as anti-nutritional factors for ruminants, as they form complexes with proteins, including enzymes (Goel et al., 2005a, b), resulting in a remarkable reduction in the biodegradation of the fibrolytic polymers in the rumen, feed intake and dry matter digestibility (McSweeney et al., 2001). Tannic acid (TA), a typical hydrolysable tannin, is even toxic to both ruminant and monogastrics, particularly when it is available in the diets of those animals in excessive amounts (Zhu et al., 1992). However, ruminants have developed an adaptation mechanism against tannins via a microbial ecosystem inhabiting in their gastrointestinal tract (Goel et al., 2005a).

Acacia nilotica pods are one of the highly proteinaceous, unconventional leguminous feed available in tropical countries which can be used as an energy source in a concentrate mixture for ruminants (Barman and Rai, 2005). However, the presence of tannins limits its use for feeding ruminants. Few *in vitro* studies have been conducted so far on the biodegradation of tannins using fungal treatments or mixed ruminal microbiota as reviewed by Goel et al. (2005a). The fungal treatments can only be used as prefeeding treatments which is a labor and cost intensive treatment. Therefore, this investigation has been carried out to study the biodegradation of tannins in *Acacia* pods using a previously-isolated tannin degrading bacterium *E. faecalis* (Goel et al., 2007) in co-culture with mixed ruminal microbial consortia. Rumen microbiota has been previously reported to degrade tannins; however, this is the first report to observe a synergistic activity of tannin degrading bacterium along with rumen microflora. The isolate could be used for the manipulation of rumen microflora to enhance tannin resistance in browsing on livestock and neutralizing the toxic effects of tannins.

The tannin rich extract from *Acacia* pods was prepared in acetone (400 mg of finely grounded pods in 20 ml of 70% acetone). Total phenols and tannins (tannic acid equivalent) were analyzed as per Makkar et al. (1993) and expressed on a dry matter (DM) basis. The CT (leucocyanidin equivalent) was estimated by the butanol-HCl method of Porter et al. (1986). The HT was calculated from the difference between the total tannin phenol and condensed tannin.

The Tilley and Terry (1963) procedure was used for *in vitro* incubation. The incubation mixture contained 500 mg of grounded *Acacia* pods 40 ml McDoughall's buffer (McDoughall, 1951) and 10 ml strained rumen liquor (SRL) and a culture inoculums of 10^6 cfu/ml of total incubation buffer. The incubations were done in triplicate according to the following treatments:

Treatment	Composition
Control:	McDoughall's buffer + SRL
T1:	McDoughall's buffer + *E. faecalis* (10^6 cfu/ml)
T2:	McDoughall's buffer + *E. faecalis* (10^6 cfu/ml) + SRL

Rumen fluid was collected from permanently fistulated cattle rumen with no prior history of the consumption of tannin rich forages. The rumen fluid was processed as described in Goel et al. (2008). All the flasks were flushed thoroughly with CO_2 gas, sealed and then placed in an anaerobic incubator at 39 ± 1°C for 24, 48 and 72 h. After the incubation, the fermentation broth was centrifuged at

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None of the authors of this manuscript has any financial or personal relationship with other people or organizations that could inappropriately influence their work.

Table 1. Degradation of tannins (% DM) in *Acacia* pods by *E. faecalis* in co-culture with mixed ruminal microflora at different time intervals.

Treatments	Tannin type	Time (h)			
		0	24	48	72
Control	TT	$19.6 \pm 0.20^{a,x}$	$10.4 \pm 0.38^{b,y}$	$8.6 \pm 0.20^{c,y}$	$8.4 \pm 0.10^{c,y}$
	HT	$18.5 \pm 0.22^{a,x}$	$9.5 \pm 0.37^{b,y}$	$7.9 \pm 0.23^{c,y}$	$7.8 \pm 0.07^{c,y}$
	CT	$1.1 \pm 0.10^{a,x}$	$0.9 \pm 0.04^{b,y}$	$0.7 \pm 0.03^{c,y}$	$0.6 \pm 0.04^{d,y}$
T1	TT	$19.7 \pm 0.18^{a,x}$	$12.5 \pm 0.10^{b,x}$	$10.2 \pm 0.10^{c,x}$	$9.7 \pm 0.15^{c,x}$
	HT	$18.7 \pm 0.20^{a,x}$	$11.6 \pm 0.14^{b,x}$	$9.4 \pm 0.14^{c,x}$	$9.0 \pm 0.19^{c,x}$
	CT	$1.0 \pm 0.01^{a,x}$	$0.9 \pm 0.04^{a,x}$	$0.9 \pm 0.04^{a,x}$	$0.9 \pm 0.01^{a,x}$
T2	TT	$19.5 \pm 0.31^{a,x}$	$9.3 \pm 0.10^{b,z}$	$7.6 \pm 0.38^{c,z}$	$6.3 \pm 0.45^{d,z}$
	HT	$18.3 \pm 0.41^{a,x}$	$8.4 \pm 0.09^{b,z}$	$6.9 \pm 0.37^{c,z}$	$5.9 \pm 0.50^{d,z}$
	CT	$1.2 \pm 0.17^{a,x}$	$0.8 \pm 0.01^{b,y}$	$0.7 \pm 0.02^{c,z}$	$0.4 \pm 0.05^{d,z}$

TT: Total Tannins; HT: Hydrolyzable Tannins (Tannic acid equivalent); CT: Condensed Tannins (Leucocyanidin equivalent); Control: Buffer + SRL; T1: Buffer + *E. faecalis* (10^6 cfu/ml); T2: Buffer + *E. faecalis* (10^6 cfu/ml) + SRL.

[a–d]Means in the same row bearing different superscripts differ significantly ($P < 0.01$).

[x–z]Means in the same column bearing different superscripts differ significantly ($P < 0.01$).

3,000 *g* for 15 min. The pellet was analysed for residual tannin content, *in vitro* dry matter digestibility (IVDMD) and crude protein content (AOAC, 1992). The supernatant was analysed for tannin degraded products using TLC (Goel et al., 2011), ammoniacal-N (Conway, 1962) and TVFA according to the method described by Barnett and Reid (1957).

The data was statistically analyzed using ANOVA according to the General Linear Models procedure of SYSTAT Version 6.0.1 (1996, SPSS Inc.). Significant differences among treatment means were compared by Fisher's Least Significant difference (Snedecor and Cochran, 1967).

The total tannin (TT), HT and CT in *Acacia* pods was shown to vary from 196–200 g/kg DM, 185–190 g/kg DM and 10–15 g/kg DM, respectively.

The synergistic activity of *E. faecalis* along with the rumen microbes was observed for the degradation of tannins (Table 1). A 57%, 59% and 42% reduction in TT, HT and CT, respectively, was obtained in control after 72 h of incubation. The *E. faecalis* as a monoculture was not able to degrade CT, whereas the co-culture activity resulted in a 65% reduction in CT content ($P < 0.01$). A possible reason for this synergistic activity could be the extracellular release of tannase by the isolate *E. faecalis*, which resulted in the liberation of glucose and gallic acid from tannin as end products. The monomer gallate is reported to be easily metabolizable to pyrogallol and resorcinol by other ruminal microflora (Bhat et al., 1998). A 66% and 56–65% reduction in CT was observed in the leaves of oak and *Sericea lespedeza* by fungal treatments (Gamble et al., 1996; Makkar et al., 1994).

Gallic acid, resorcinol and phloroglucinol were identified as degradation products of *Acacia* tannins in T2, whereas gallic acid, pyrogallol and resorcinol were observed in T1. The phloroglucinol was not identified by Goel et al. (2011) using the same isolate with tannic acid as a substrate, indicating that the isolate does not form phloroglucinol as an intermediate. Singh et al. (2001) also reported the conversion of HT to gallic acid, pyrogallol and resorcinol *in vitro* by mixed ruminal microflora.

Table 2. Effect of co-culture of *E. faecalis* with mixed ruminal microflora on rumen fermentation parameters of *Acacia* pods after 72 h of incubation.

Parameter	Control	T1	T2
IVDMD	56.9 ± 2.29^{b}	54.4 ± 1.76^{a}	65.7 ± 2.15^{c}
IVCPD	44.5 ± 0.88^{a}	43.5 ± 1.04^{a}	47.9 ± 1.16^{b}
Ammoniacal-N	13.5 ± 1.96^{a}	7.3 ± 0.84^{b}	15.2 ± 2.20^{a}
TVFA	8.3 ± 0.84^{a}	2.6 ± 0.37^{b}	9.1 ± 0.79^{a}

Control: Buffer + SRL; T1: Buffer + *E. faecalis* (10^6 cfu/ml); T2: Buffer + *E. faecalis* (10^6 cfu/ml) + SRL.

IVDMD, *in vitro* dry matter digestibility; IVCPD, *in vitro* crude protein digestibility; TVFA, total volatile fatty acids.

[a–c]Means in the same row bearing different superscripts differ significantly ($P < 0.01$).

The results for IVDMD, IVCPD, ammoniacal-N and TVFA production are presented in Table 2. The overall mean of IVDMD and IVCPD was highest in T2 compared with the control which clearly indicates that co-culture activity increased the digestibility of the feed and could have the potential to reduce the negative effects of tannins on feed digestibility as reported earlier both *in vitro* (Makkar et al., 1994; Patra et al., 2006) and *in vivo* (Animut et al., 2008; Grainger et al., 2009). The increase in IVCPD could be due to the degradation of CT which were able to bind most of the protein thereby limiting the availability of protein for the microbial attack. The results indicate that the isolate in complex microbial consortia was able to degrade tannins, especially the HT, along with cellulosic and hemicellulosic contents, thereby increasing the overall IVDMD and IVCPD. The T2 and control resulted in maximum ammoniacal-N and TVFA production. The increase in ammoniacal-N was expected in the treatment T2

as due to the degradation of tannins by the supplementation of *E. faecalis*, the bound protein fraction was available for the degradation by microbial consortia to release ammonia. The mixed effects of tannins on ruminal TVFA *in vivo* has been reported due to different doses and the source of tannin (Patra and Saxena, 2011).

The results of the present study indicated that *E. faecalis* has the potential to degrade tannins (HT) in *Acacia* pods as a co-culture with rumen microbiota. Further studies are warranted to confirm the co-culture activities of the isolate *in vivo* to check the viability and colonizing ability of DFM under a rumen ecosystem.

Acknowledgments

The authors wish to thank the Indian Council of Agriculture Research, New Delhi, and the National Dairy Research Institute, Karnal, India, for providing financial support and essential facilities required for the research work.

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