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EFFECT OF STORAGE TIME AND TEMPERATURE ON RECOVERY OF *SYNERGISTES JONESII* FROM RUMEN FLUID AND FECES

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Abstract

Synergistes jonesii is a rumen bacterium that degrades 3,4-dihydroxypyridine (3,4 DHP), the toxic breakdown product of mimosine in leucaena (*Leucaena leucocephala*). Fecal culture is the most practical way to determine *S. jonesii* presence in zoological ruminants, particularly if feces can be collected from night penning facilities. Fresh rumen fluid and fecal or fecal slurry (sheep [*Ovis* spp.] only, 1:4 wt to vol. feces and culture media) from cattle (*Bos* spp.) and sheep, known to be colonized by *S. jonesii*, were subjected various storage times (0, 6, 12, and 24 h) and temperatures (5, 23, and 38^{o} C). Samples were inoculated into a culture medium that contained 3,4 DHP. In general, storage temperature had no affect on detection frequency. Regardless of animal species, detection of *S. jonesii* was higher (P=0.001) in rumen (97%) than in fecal (40%) samples and level of detection in rumen samples was relatively unaffected by storage time. Detection frequency was similar for both fecal sample types regardless of time (34% fecal vs. 29% fecal slurry). For all fecal samples, detection frequency generally exhibited a linear decline (P=0.01) with time. This study

showed that it will be important to collect fresh fecal samples

(<6-h old) from night penning facilities, and because detection levels were low in fecal material, fecal assay would be most accurate on a whole herd rather than an individual animal basis.

Keywords: *Leucaena leucocephala*, mimosine, 3,4-DHP, zoological ruminants

Introduction

Leucaena is a highly palatable, tropical browse legume that has proved useful in livestock production systems because of its high nutritive value, dry matter productivity, and persistence (Austin et al., 1995). The presence of a toxic nonprotein amino acid, mimosine, which is converted by ruminal microorganisms to the goitrogenic compound 3,4-DHP (Jones, 1979) limited its use to about 25% of the total diet. In the 1980's, researchers realized that 3,4-DHP could be detoxified when animals were colonized with a unique rumenal microorganism, *S. jonesii* (Allison et al., 1992). Inoculations with *S. jonesii* should allow leucaena to be safely used in zoological parks as a high quality feed and as a tool for positioning animals for viewing.

Rumen fluid and fecal cultures can be used to determine the presence of *S. jonesii* (Allison et al., 1990; Andrew et al., 2000). Fecal culture is the best method for use with zoological ruminants because of ease of collection, but collection of fresh samples may not be possible with zoological ruminants, which is a concern because *S. jonesii* is an obligate anaerobe. The objective of this study was to determine the effect of storage time and temperature on the viability of *S. jonesii* in rumen and fecal samples.

Material and Methods

Rumen fluid (10 ml) and fecal grab samples (6 - 10 g) were collected from two steers and four sheep known to have an established population of *S. jonesii*, and a combination of storage time

post collection (0, 6, 12, and 24 h) and storage temperature (5, 23, and 38^{o} C) was imposed. Rumen fluid was strained through cheese cloth at the 0 time, and at 0 time and all subsequent sample times, 0.5 ml of rumen fluid was injected into duplicate vials of Fe-1 media (Allison, 1991). Fecal samples were either directly injected into duplicate vials using a 12 ga. needle modified to allow about 60 mg of feces to be pressed into the lumen of the needle from the side or were mixed with media (1:4 wt to vol) under CO_2 at each sample time and then 0.5 ml of the fecal slurry was injected via an 18 ga. needle. At each sample time, different fecal pellets or subsamples were used. After the 0-time samples were taken, all material was stored under atmospheric conditions in sealed containers at the prescribed temperatures. At 16-wk post inoculation, visual inspections of the media were conducted (Allison, 1991) to determine if 3,4-DHP had been degraded. Data was analyzed as CRD with animals as replicates using PROC GLM (SAS, 1989) with repeated measures for time. Sample types were separated using the orthogonal contrasts of rumen vs. avg. fecal and fecal vs. fecal slurry.

Results and Discussion

Because steer feces was not diluted with media, species comparisons were made only between rumen and fecal samples. Neither species nor storage temperature nor their interactions affected detection frequency (Table 1). There was a difference between sample types (97% rumen vs. 40% fecal; P=0.001), but there was no interaction between sample type, species, and/or temperature. This difference is similar to what has been previously reported with rumen and fecal samples (Allison et al., 1990; Andrew et al., 2000). Time (P=0.01) and its interaction with species (P=0.01) and sample type (P=0.01) did affect detection frequency, which declined linearly (P<0.001) with storage time for sheep, but not for steers (Table 1). Storage time did not affect detection frequency of rumen samples, but detection frequency declined quadratically (P=0.07) with storage for fecal samples.

For sheep alone, detection frequency in rumen fluid was higher ($P<0.01$) at all storage times than in either fecal sample type (94% rumen vs. 31% avg. fecal). Detection frequency also did not differ due to fecal sample type (34% fecal vs. 29% fecal slurry, avg. of all times, $P>0.26$). This treatment comparison was made because we were concerned that current methods for handling fecal material, particularly that of most zoological ruminants which have fecal moisture content similar to sheep, may result in exposure of the fecal material to O_2 which could kill *S. jonesii*. Mixing fecal material with media under CO_2 was an attempt to reduce the amount of O_2 exposure. Similar detection frequencies with fecal or fecal slurry samples indicated that O_2 exposure, at least at the level found during needle filling, did not affect the viability of *S. jonesii*.

Temperature did not affect detection frequency, but there was a sample type by time by temperature interaction ($P= 0.04$). Across all temperatures, rumen and fecal slurry samples exhibited a linear ($P<0.05$) decline in detection frequency with time (Table 2). In contrast, detection frequency in fecal samples declined linearly at 5^o and 38^o C storage temperatures, but remained unchanged with storage time at 23^o C (Table 2). The reason for this temperature affect is unknown.

This study showed that temperature had relatively little effect on the detection frequency of *S. jonesii* from either rumen or fecal material, but detection frequency declined with storage time post collection, particularly for fecal material. This indicates efforts should be made to collect fecal material from night housing facilities that is as fresh as possible (<6 h old). Additionally, the detection frequency in feces from individual animals that were known positive for *S. jonesii* was quite variable ranging from 0 to 100% (data not reported). This suggests that there can be considerable variation in levels of *S. jonesii* excreted by individual animals. Because of this and the relatively lower detection frequencies in fecal material, fecal assays may be most useful for determining *S. jonesii* status at herd levels. As readily as this organism has been shown to move between commingled

individuals (Hammond et al., 1989), it is reasonable to assume that if at least one animal in a group has a positive fecal assay, all the animals will be positive.

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Table 1 - Effect of storage time on the percent (%) detection frequency of *Synergistes jonesii* in rumen and fecal samples from sheep and cattle, least square means.

	Sample type		
Storage Time, h	Rumen fluid	Fecal	Mean
Sheep			
0	100±0.06†	63±0.07	81±0.05
6	100±0.07	19±0.06	59±0.04
12	100±0.07	33±0.06	67±0.05
24	81±0.09	21±0.08	51±0.05
Cattle			
0	100±0.16	50±0.16	75±0.07
6	100±0.09	25±0.09	63±0.06
12	100±0.11	50±0.11	75±0.07
24	100±0.10	58±0.01	79±0.07
Mean	97±0.06	40±0.06	

†Mean ± std. error.

Table 2 - Effect of storage time and temperature on the percent (%) detection frequency of *Synergistes jonesii* in rumen, fecal, and fecal slurry (1:4 wt to volume culture media) samples from sheep, least square means.

	Sample type								
	Rumen			Fecal			Fecal Slurry		
Time (h)	5°C	23°C	38°C	5°C	23°C	38°C	5°C	23°C	38°C
0	-†	100±0.0‡	-	-	63±0.17	-	-	50±0.19	-
6	100±0.0	100±0.0	100±0.0	6±0.13	12±0.13	25±0.13	25±0.16	31±0.16	25±0.16
12	100±0.0	100±0.0	100±0.0	44±0.16	31±0.16	25±0.16	25±0.15	63±0.15	6±0.15
24	57±0.13	93±0.13	93±0.13	0±0.11	63±0.11	0±0.11	13±0.16	19±0.16	0±0.16

†Not available, but assumed to be same as 23°C for statistical analysis.

‡Mean ± std. error.