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Rumen bacterial and protozoal populations in cattle being relocated in tropical Queensland

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Abstract. Rumen microbial populations were measured in Brahman-cross steers that were relocated from spear grass (*Heteropogon contortus*) dominant pastures in northern Queensland to buffel grass (*Cenchrus ciliasis*) dominant pastures in central Queensland, to assess whether aspects of rumen function may contribute to the sometimes reported depressed growth rates following relocation.

Nine genera of ciliate protozoa (*Isotricha, Dasytricha, Entodinia, Epidinium, Diplodinium, Ostracodinium, Metadinium, Elytroplastron*, and *Eudiplodinium*) were recorded in the rumen fluid of the steers. In most steers all genera were present at any time and the generic mix persisted throughout the 10 months over which the study was conducted. Protozoal population composition fluctuated only slightly over the sampling period. *Entodinia* were predominant, occupying 50–70% of the population. Population density varied according to season, with the highest density (4–8×10^5/mL rumen fluid) occurring in the wet season.

Bacterial and protozoal populations were remarkably stable and little affected by relocation. Again, the major impact on population density was the season, with all carbohydrate (soluble sugar, starch, xylan, and cellulose) utilising bacterial subpopulations reaching the greatest density with the onset of the wet season.

Additional keywords: protozoa, bacteria, spear grass, buffel grass, seasonal variation, Brahman-cross cattle, relocation.

Introduction

Relocation of cattle, particularly male cattle, for finishing on better quality pasture or in feedlots is becoming of increasing importance in the northern Australian cattle industry as producers and pastoral companies capitalise on different markets. There has been continued feedback from the industry that relocation may be followed by poor growth rates of the cattle, which may persist for 12 months.

Many factors may be involved in this syndrome, including rate of physiological adaptation to environmental change, the impact of stress, readjustments to social order in the herd and behavioural factors, and modifications to the ecology of the rumen microbial populations and rumen function. Predictions of the role of the last of these factors in the syndrome are difficult due to a lack of general information on what microbial populations are present in the rumen of cattle grazing tropical pastures and how they vary with seasonal change. However, earlier studies on the transit and fasting of cattle in the United States indicate that this process can impact on the protozoal and bacterial populations of the rumen, at least in the short term (Galyean *et al*. 1981).

This paper reports the ruminal populations of protozoa and of major carbohydrate-utilising bacteria in the rumen fluid of yearling Brahman cross steers that were relocated from spear grass (*Heteropogon contortus*) dominant pastures in northern Queensland for growing out on better quality buffel grass (*Cenchrus ciliaris*) dominant pastures in central Queensland.

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These populations were monitored over a 10-month period encompassing the changes in pasture quality and availability (feeding value) from dry to wet season.

Materials and methods

Experimental design

Yearling Brahman-cross steers (120, 5/8 *Bos indicus* content) were allocated on fasted liveweight to 3 treatment groups of 40 animals. Allocation to groups was at Swan's Lagoon Beef Cattle Research Station in subcoastal northern Queensland (20°S, 147°E) early in the dry season (31 May). Treatment groups were as follows.

(*A*) Control. This group was immediately transferred to Brigalow Research Station, in central Queensland (25°S, 150°E), and grazed buffel grass pastures for 3 months whilst adjusting to the local environment.
(*B*) Relocated-unsupplemented. This group remained at Swan's Lagoon grazing spear grass pastures for 3 months and was then relocated to Brigalow Research Station in September 1994.
(*C*) Relocated-supplemented. This group was treated similarly to Group B except that a supplement was provided (see below) in an attempt to achieve growth rates of the steers similar to those of Group A steers at Brigalow Research Station.

With the arrival of Groups B and C at Brigalow Research Station, the 3 treatment groups were reallocated into mixed or segregated herds. This examined the effect on subsequent performance of having relocated cattle mixed with or segregated from local acclimatised cattle at the property of destination. Treatments were as follows.

(*i*) Mixed. There were 2 groups of 15 steers made up of 5 animals from each of the previous treatment groups Group D. The 5 steers were selected from Groups A, B, and C at random.
(*ii*) Segregated. There were 6 groups of 15 steers made up of 2 replicates from each of the previous treatment groups (as above), in a completely randomised design.

Climate, pastures, and supplements

The climate at both research stations is dry tropical with a summer predominate rainfall (wet season) from December to April followed by a dry season from May to November.

During the study period at Swan's Lagoon no rain fell, although the previous wet season had above average rainfall. Rainfall at Brigalow for 1994–95 was average (711 mm) with a seasonal break in late October 1994.

At Swan's Lagoon cattle grazed native (predominantly speargrass) pasture. McLennan *et al.* (1980) identified the principal grass species present as black spear grass (*Heteropogon contortus*) with contributions from giant spear grass (*H. triticeus*), golden beard grass (*Chrysopogon fallax*), *Bothriochloa petusa, B. bladhii*, and *Dicanthium* spp. Typically (Fordyce *et al.* 1992, 1993), the major nutritional characteristics from plucked samples of this pasture are: *in vitro* dry matter (DM) digestibility in the dry season 32·4–46·0% (average, 40·2%), and wet season 41·4–60·1% (average, 52·6%); and crude protein content in the dry season 2·9–6·8% DM (average 4·8%), and wet season 5·0–18·2% DM (average, 11·0%).

At Brigalow Research Station cattle grazed improved buffel grass pastures. Typically (S. R. McLennan, pers. comm.), the major nutritional characteristics from plucked green leaf samples of this pasture are: *in vitro* organic matter digestibility in the dry season 64·1–72·2% (average, 68·2%), and wet season 57·6–76·3% (average, 64·9%); and crude protein content in the dry season 8·8–12·6% DM (average 11·0%), and wet season 6·6–19·4% DM (average, 12·3%).

At Swan's Lagoon, the cattle in Group C were supplemented with cottonseed meal in an attempt to achieve similar growth rates to the cattle in Group A at Brigalow Research Station. Initially, intakes averaged 0·3 kg/steer·day but within 5 weeks increased to 1·5 kg/steer·day. The supplement was then changed to an *ad libitum* mixture (parts by weight) of molasses (100), urea (3), cottonseed meal (10), and dicalcium phosphate (1·6), and 50 g monensin/t supplement, for the rest of the period prior to relocation of the cattle to Brigalow Research Station. Intake of this supplement averaged 4·8 kg/steer·day. Supplementation ceased at relocation.

Rumen sampling

Rumen samples were collected from steers at 0, 7, 92, 99, 122, 174, and 320 days from allocation to treatment groups. At Day 0, samples were collected from 30 steers randomly selected from the 120 present at Swan's Lagoon. At Days 7 and 92, 10 steers per Group (A, B, and C) were sampled. Initial selection of animals to be sampled was random within Groups. After selection the same animals were sampled on Days 7 and 92. At Day 92, steers in Groups B and C were sampled prior to relocation. At Days 99, 122, 174, and 320, samples were collected from 8 steers in Groups A, B, and C and 12 from the mixed Group D. Steers were again selected at random, but were not necessarily the same as those sampled on Days 0–92.

Samples of crude rumen fluid (RF) were collected by stomach tube. Large plant material was removed by sieving the rumen contents through a single layer of nylon gauze. A 4-mL aliquot of the sample was added to 16 mL of formal saline for counting the protozoa. Samples for the determination of the culturable bacterial populations were stored using a modification of Teather's (1982) technique for the storage of anaerobic rumen bacteria. Basically, a second 4-mL aliquot of the sample was injected, using a 19-gauge needle and 10-mL syringe, through the septum into a serum bottle (Wheaton) containing 4 mL of an anaerobic solution of RF-based medium and glycerol (1 : 1). The preparation of the RF-based medium under anaerobic conditions has been described previously (Klieve *et al.* 1989). The sample and solution were well mixed and the serum bottle was immediately placed in an ice slurry. As soon as practical thereafter the samples were frozen at −20°C.

Protozoal population

Concentrations of protozoa were estimated using a counting chamber (Hawksley, Sussex, England) as described by McLennan (1992) (modified from Warner 1962). The protozoa were classified to the level of genera based on morphological characteristics and on the position of skeletal plates in the larger entodiniomorphid protozoans. The classification was based on that of Dehority (1993), and was facilitated by staining with Gram's iodine prior to using a combination of light and phase contrast microscopy (Olympus BH-2). At a magnification of 100× the microscopic examination and counting were combined in a single operation.

Culturable bacterial populations

Due to the large number of samples it was necessary to use 2 procedures to determine population size. Petri dishes (Leedle

et al. 1982) were used to count the bacteria utilising soluble sugars and starch, while roll-tubes (Hungate 1969) were used for the xylanolytic and cellulolytic bacteria. Both are standard microbiological procedures using the dilution procedures and solutions described by Holdeman *et al.* (1977) and Ogimoto and Imai (1981).

The medium used for enumeration was RF medium with glucose and cellobiose (Sigma chemicals), soluble starch (Univar), oatspelt xylan (Sigma), or ball-milled cellulose (Whatman) as the carbohydrate source to grow the respective populations of bacteria. The compositions of the selective media were based on RF medium as previously described (Klieve *et al.* 1989). To select soluble-sugar utilising bacteria the primary carbon and energy sources were glucose (0·2% w/v) and cellobiose (0·2% w/v). To select amylolytic, xylanolytic, and cellulolytic bacteria, soluble starch (0·4% w/v), oatspelt xylan (0·4% w/v), and ball-milled cellulose (0·2% w/v), respectively, replaced glucose and cellobiose in the medium. Ball-milled cellulose was prepared as previously reported by Ware *et al.* (1992), with the exception that the final ball-milled product was sterilised by autoclaving at 105°C for 45 min.

RF samples were thawed at room temperature and gently mixed and 0·5 mL was removed from the serum bottle using a 21-gauge needle and 1-mL syringe and transferred to a sealed Hungate tube containing 4·5 mL of dilution solution (Ogimoto and Imai 1981). A dilution series was then prepared by the serial transfer of 50-μL volumes from one tube to the next. Appropriate dilutions were then either spread on agar plates (50 μL) or injected into roll-tubes (0·5 mL). The dilution series and agar plate work were performed in an anaerobic chamber (Coy Laboratory Products) in an atmosphere of 95% CO_2 and 5% H_2. Agar plates were placed in anaerobic jars (BBL GasPak System), and the jars and roll-tubes were incubated outside the anaerobic chamber at 39°C. Soluble-sugar utilising, amylolytic, and xylanolytic colony-forming units (CFU) were enumerated after 24–48 h incubation, and cellulolytic CFU between Days 5 and 7 of incubation. Visual enumeration was assisted by the use of a binocular microscope at a magnification of ×10–40.

Statistical analyses

For data collected up to and including Day 92, the 3 treatment Groups (A, B, and C) were compared using ANOVA with the error term estimated from animal to animal variation within each treatment. From Day 92 onwards, the segregated treatments (cattle remaining in Groups A, B, and C) were compared using ANOVA for a completely randomised design; data for the mixed group were subjected to ANOVA to compare treatments after adjusting for paddock and animal block effects. Since there were only minor differences between treatments in the mixed group, only the overall means for Group D are presented. All statistical tests of significance were at $P = 0·05$.

Results

Protozoal populations

The ciliate protozoal populations were diverse, with 9 genera recorded: *Isotricha, Dasytricha, Entodinia, Epidinium, Diplodinium, Ostracodinium, Metadinium, Elytroplastron, Eudiplodinium.* In most steers all genera were present at any given time and the generic composition of the population persisted in all treatment groups throughout the experiment.

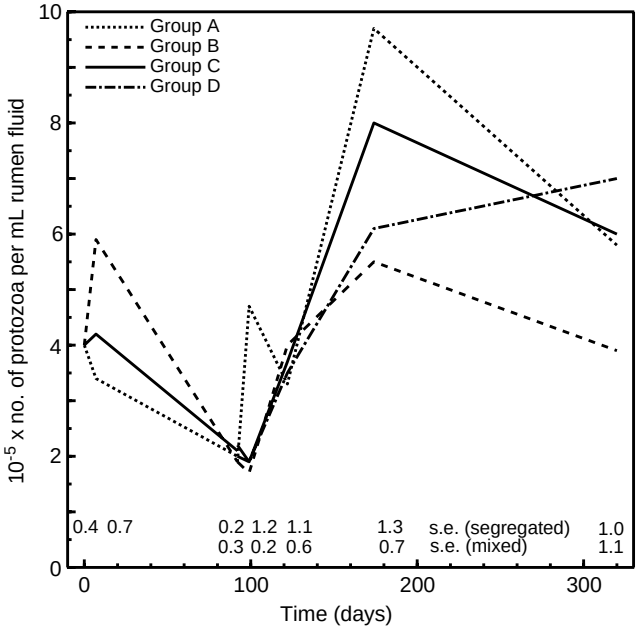

Fig. 1. Ciliate protozoal populations in the rumen fluid of steers sampled at Days 0, 7, 92, 99, 122, 174, and 320. Relocation of Group A (control) steers occurred after the Day 0 sampling, and that of Group B and C steers after the Day 92 sampling. Group D (mixed) was formed following relocation at Day 92. Sampling up to and including Day 122 was during the dry season and after Day 122 during the wet season. Standard errors of means are indicated at each sampling time.

Total protozoal concentrations in RF of steers are presented in Fig. 1. All treatment groups followed a similar trend with a decline in concentration from 4–6×10^5 to 1–2×10^5/mL RF during the dry season and an increase in concentration early in the wet season (Day 174). The density appears to have remained high (4–8×10^5/mL RF) during the wet season.

The percentage generic composition of the protozoal populations is shown in Table 1. Some significant treatment differences ($P < 0·05$) were observed prior to relocation (Day 92). On Day 7, the Group A steers had low percentages of *Isotricha, Dasytricha*, and *Diplodinium* and a high percentage of *Entodinium*. By Day 92, animals in both Groups A and C were low in *Dasytricha*, those in Group B were low in *Entodinium*, and those in Group C had low percentages of *Diplodinium, Ostracodinium*, and *Elytroplastron*. There were no significant differences between groups after relocation (Day 92).

Overall, the protozoal population composition fluctuated but did not vary greatly over the entire sampling period, including the relocation. *Entodinia* were always predominant (50–70% of the population).

One consistent deviation appeared at the Day 122 sampling, with the proportion of *Entodinia* declining from 70% at Day 99 to 51% at Day 122 (as averaged across

Table 1. Ciliate protozoan genera present in the rumen fluid of steers on tropical pastures

Within a genus and time period, means followed by the same letter are not significantly different at $P = 0{\cdot}05$

Treatment group	No. of steers sampled	Genus (% of total protozoal numbers)								
		Isotricha	*Dasytricha*	*Entodinium*	*Epidinium*	*Diplodinium*	*Ostracodinium*	*Metadinium*	*Elytroplastron*	*Eudiplodinium*
					Day 0					
Mean	30	7·1	21·4	53·0	2·7	4·3	5·1	3·7	1·4	1·3
s.e.		0·9	1·7	2·2	0·5	0·6	0·5	0·7	0·2	0·3
					Day 7					
A, control	10	1·0a	11·6a	74·6a	0·4	1·1a	7·3	1·4	0·3	2·3
B, unsuppl.	10	4·3b	25·1b	58·5b	0·6	2·5b	4·7	1·7	0·3	2·3
C, suppl.	10	6·5b	16·6ab	59·5b	1·3	4·3b	4·9	1·7	0·3	4·9
s.e.		1·0	3·1	3·3	0·4	0·7	1·0	0·4	0·1	1·3
					Day 92					
A	10	2·9	17·9a	63·5ab	0·6	3·4a	7·4a	1·3	0·7a	2·3
B	10	2·5	28·2b	54·1a	0·4	3·6a	6·1a	1·8	0·4ab	3·0
C	10	6·1	12·3a	74·5b	0·4	0·3b	1·4b	2·4	0·1b	2·5
s.e.		1·3	3·4	4·7	0·2	0·6	0·7	0·5	0·1	1·5
					Day 99					
A	8	2·3	22·1	67·7	0·2	3·3	2·3	0·4	0·1	1·7
B	8	0·8	12·8	77·0	0·3	2·5	3·2	0·6	0·0	2·8
C	8	1·9	11·8	72·0	1·5	0·1	6·8	1·3	0·0	4·5
s.e. (segregated)		0·9	3·2	5·8	0·1	1·1	1·0	0·2	0·0005	1·5
D	12	3·0	21·9	63·7	0·1	2·3	4·4	0·7	0·2	3·6
s.e. (mixed)		0·9	3·9	4·0	0·1	0·8	1·0	0·2	0·1	1·4
					Day 122					
A	8	5·4	26·7	51·8	0·7	3·7	6·4	1·2	0·2	4·0
B	8	3·5	24·4	58·7	0·4	4·5	5·0	1·6	0·1	1·7
C	8	3·9	34·7	48·0	1·1	0·3	6·4	2·0	0·1	3·4
s.e. (segregated)		1·5	8·6	6·5	0·5	1·4	1·7	0·2	0·1	1·1
D	12	8·1	31·2	44·8	0·8	2·1	6·5	2·3	0·5	3·7
s.e. (mixed)		1·8	3·8	4·5	0·3	0·9	0·9	0·8	0·2	1·2
					Day 174					
A	8	2·7	7·3	75·8	0·8	3·4	6·3	0·9	0·3	2·6
B	8	5·0	4·5	78·9	1·6	1·6	4·5	1·5	0·7	1·5
C	8	4·0	7·4	76·3	2·5	0·4	5·3	1·9	0·7	1·5
s.e. (segregated)		0·5	1·9	2·2	0·9	1·0	0·9	0·3	0·2	0·4
D	12	4·8	8·7	71·6	0·9	2·9	5·6	2·2	0·5	2·8
s.e. (mixed)		1·0	1·7	3·7	0·4	1·2	0·9	0·5	0·2	0·6
					Day 320					
A	8	6·9	13·4	65·4	0·6	3·5	5·3	1·8	0·2	3·1
B	8	4·6	11·1	71·3	3·1	1·4	4·5	1·3	0·7	2·1
C	8	8·4	13·8	64·8	2·2	1·7	4·9	1·3	0·3	2·6
s.e. (segregated)		4·2	1·9	8·9	1·5	0·6	0·6	0·9	0·3	1·2
D	12	8·0	18·6	63·5	0·5	2·7	3·7	0·5	0·2	2·3
s.e. (mixed)		1·4	2·4	4·6	0·2	0·5	0·8	0·2	0·1	0·6

all treatment groups). Concomitantly, the proportion of *Dasytricha* increased from 17% at Day 99 to 29% at Day 122. At the following sampling (Day 174) the proportion of *Entodinia* had re-established to 76%, the maximum reached for this genus, and the proportion of *Dasytricha* was 7%, the minimum reached for this genus.

Bacterial populations

In general, differences were not statistically significant, but differences or trends that were observed have been reported as they may prove significant in future studies with larger numbers of animals.

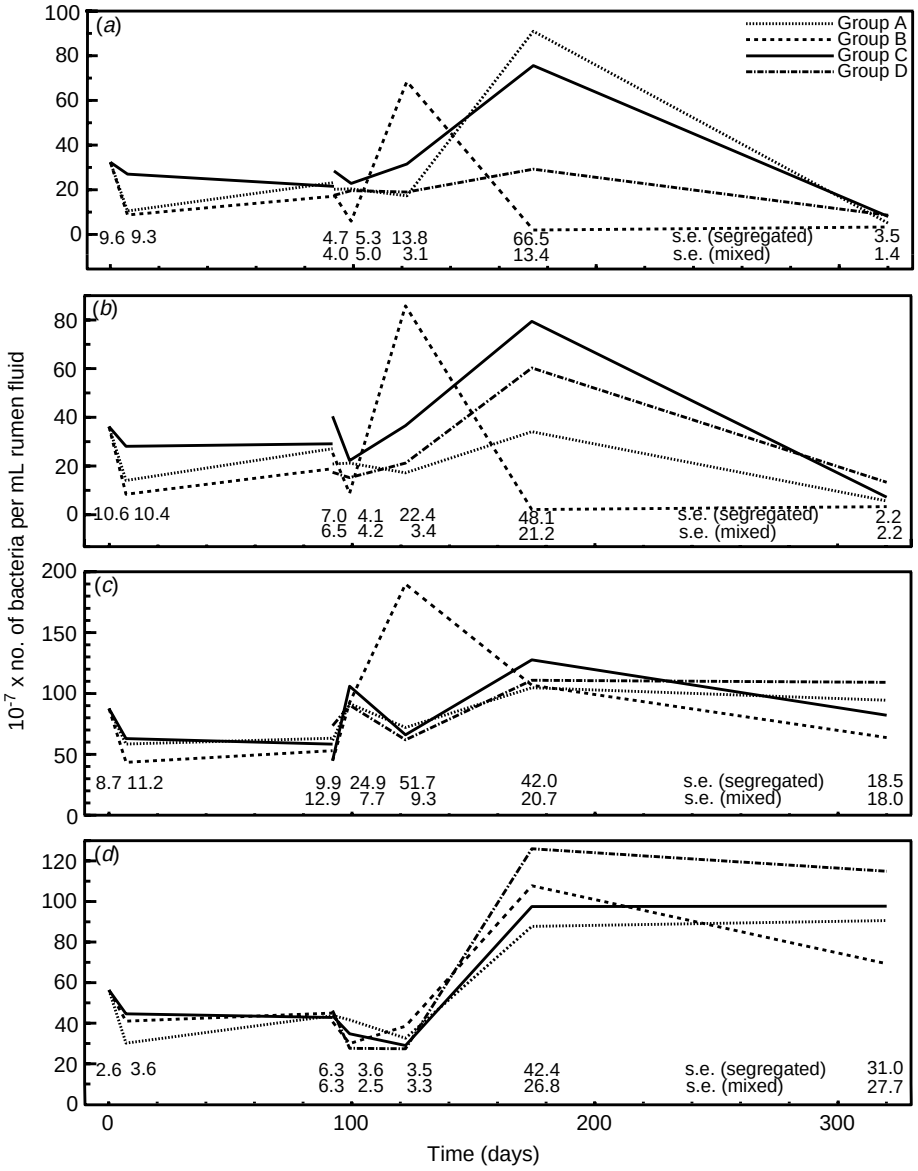

Fig. 2. Culturable carbohydrate-utilising populations of bacteria in the rumen fluid of steers. Carbohydrate-utilising subpopulations cultured were (*a*) soluble-sugar utilising, (*b*) amylolytic, (*c*) xylanolytic, and (*d*) cellulolytic. Samples were taken at Days 0, 7, 92, 99, 122, 174, and 320. Relocation of Group A steers occurred after the Day 0 sampling, and that of Group B and C steers after the Day 92 sampling. Group D (mixed) was formed following relocation at Day 92. Sampling up to and including Day 122 was during the dry season and after Day 122 during the wet season. Standard errors of means are indicated at each sampling time.

The populations of the major carbohydrate-utilising groups of bacteria are presented in Fig. 2. The populations appeared stable prior to and immediately following relocation of the cattle (Day 92). The soluble-sugar utilising, amylolytic, and xylanolytic populations increased early in the wet season (Day 174) and returned to pre-wet season numbers by the end of the wet (Day 320). The only noticeable treatment trend in these populations occurred in Group B. Soluble-sugar utilising, amylolytic, and xylanolytic CFU in Group B increased 1 month after relocation (Day 122) and prior to the onset of the wet season. Unlike the other Groups, the soluble-sugar utilising, amylolytic, and xylanolytic bacterial populations in Group B declined to previous levels by the first wet season sampling (Day 174) and appear to have remained at this lower level.

Populations of cellulolytic bacteria were extremely stable, with the only difference between groups occurring at Day 7, when the population in Group A was

less than that in Group C. Following the onset of the wet season, the population density increased to approximately double that maintained throughout the dry season. This population density appears to have been maintained throughout the wet season.

The mixing of steers from different treatment groups had no apparent impact on the microbial populations.

Discussion

The populations of ciliate protozoa present in the rumen of crossbred Brahman steers grazing tropical pastures was both diverse and remarkably stable despite the imposition of a number of treatments involving the movement of animals. The animals supported up to 9 genera of protozoa, and these genera were maintained throughout the experiment. Supplementation with molasses, urea, and cottonseed meal appear to have no effect on either total protozoal concentration or the generic composition of the population. It is noteworthy that after 3 months of separation by a distance of 1000 km (prior to relocation at Day 92), there were no differences in protozoal concentration and only minor differences in composition between steers grazing on buffel grass pastures at Brigalow Research Station and those grazing spear grass dominant pastures at Swans Lagoon.

The overall increase in numbers of protozoa observed through the wet season was expected with the improvement in the nutritive value of the feedbase (S. R. McLennan, unpublished data). This increase did not appear to have an impact on the generic composition of the protozoal population. A single noticeable perturbation to the population composition occurred with a decline in the proportion of *Entodinia* and a corresponding increase in *Dasytricha* toward the end of the dry season. This perturbation occurred in all treatment groups at the same time and was independent of a change in total protozoal concentration. Other genera appeared to be largely unaffected. The reason for this perturbation is unknown and must be due to factors that were not obvious in the work. We speculate that there is a link between the 2 genera such that when the *Entodinium* population is adversely affected *Dasytricha* is able to gain an advantage.

The bacterial populations, in common with the protozoa, were remarkably stable in terms of concentration and composition, and were unaffected by supplementation, geographical region, or pasture type. The soluble-sugar utilising, amylolytic, and xylanolytic populations generally increased in population density early in the wet season but did not maintain this higher population density to the end of the wet season as did the protozoa. An exception was in the Group B steers. Populations of bacteria (excluding the cellulolytic population) appear to have peaked in density earlier and before the onset of the wet season (late October). Why this occurred is unknown, although it is tempting to speculate on an association with the fact that these animals showed compensatory weight gain following relocation (Holroyd *et al.* 1994). However, a link between higher rumen bacterial density and compensatory gain may be entirely unrelated and more work would be required to substantiate an association.

The stability in microbial populations across treatment groups and following relocation is in agreement with previous studies (Galyean *et al.* 1981; Fluharty *et al.* 1994). Although short-term perturbations in microbial populations have been reported (Galyean *et al.* 1981), these were not evident in the current study from 7 days after relocation onwards.

The enumeration of bacteria in the present study was based on growth on media that selected for different carbohydrate-utilising populations. This is a classical culture-based approach to enumerating bacteria and there are limitations to it. These include the following: many bacteria are not cultured and thus these organisms will not have been enumerated; bacteria that are able to use components of the media other than the selective components may cause populations to be overestimated; and the different carbohydrate-utilising groups are not mutually exclusive and do overlap. However, the aim of this study was to observe changes during relocation and it was felt that if animal performance was affected as a result of rumen factors then this would be reflected in major perturbations in the culturable carbohydrate utilising populations and this could be observed using a classic culture-based technique as an initial step.

As relocation did not adversely impact on animal performance in the current study (R. G. Holyroyd unpublished data) it was not possible to determine whether this would be the case. In future, more sophisticated methods, such as the use of DNA probes for specific species and genera known to be of importance in rumen function and feed utilisation (e.g. the cellulolytic species), could be targeted to provide more detailed and accurate information. Such an approach may also challenge the stability observed using classical culture-based methods. Unlike culture-based methods, the DNA based techniques will allow strain variations and changes within populations to be visualised. Changes in populations may affect the efficiency of microbial protein synthesis and therefore the flow of microbial protein available for animal productivity. The present study has provided a record of shifts in the density of rumen bacterial populations that has not previously been available for cattle on tropical pastures in Australia.

In summary, this work has demonstrated that crossbred *Bos indicus* cattle on tropical grasslands support a diverse and stable population of ciliate protozoa in the rumen, and stable populations of the culturable carbohydrate-utilising bacteria. The density of these populations and the composition of the protozoal population appears to be little affected by transport, supplementation with molasses, urea, and cottonseed meal, or the pasture feed base, at least between spear grass and buffel grass. The major impact on rumen bacterial and protozoal concentrations appeared to be seasonal change, with marked increases in all the populations investigated occurring at the onset of the wet season. This would appear to correlate with increasing pasture quality that occurs at this time of year (Poppi *et al*. 1997).

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