Can anaerobic fungi be manipulated in the sheep rumen to improve the utilisation of poor–quality feed?


Summary
It has been shown that anaerobic fungi, a normal component of the microbiota which digest feed in the rumen, play a vital role in stimulating intake when the feed is of low digestibility. Over the past decade a series of experiments were conducted on the role of the fungi in ruminant digestion and how these might be manipulated in the rumen to improve the intake and digestion of poor–quality feed. The fungal treatments that are the result of this research are directed towards improving sub–optimal ruminant production from poor quality pastures. Much of the poor–quality feed available in the latter stages of the dry seasons in both the tropical and mediterranean climate zones of Australia has a low content of sulphur. Early in the study of anaerobic fungi it was realised that they are more sensitive to a low dietary concentration of this element than the other rumen microbiota. Therefore, a search was initiated for a sulphur compound that had two essential properties: firstly, that it could satisfy the requirement of anaerobic fungi and, secondly, that it was not rapidly used by the bacteria and protozoa also present in the rumen unlike previously available sulphur–containing supplements. 3–Mercaptopropionic acid (MPA) met both of these criteria as a fungal–specific nutrient for use as a feed supplement. The most effective concentration was determined in pen trials with sheep where MPA increased voluntary feed intake by 19–25%. MPA has been trialed in grazing sheep in Western Australia where positive responses in liveweight gain were recorded. A second approach to the manipulation of anaerobic fungi has selected naturally–occurring strains of anaerobic fungi with superior fibre degrading properties which, when administered orally to sheep in pens, were found to increase feed intake by 5–12%. Other pen trials with MPA plus fungal administration in combination showed that their effects on feed intake were additive. More research is needed to refine the fungal cultures used as oral doses in sheep.

Keywords: anaerobic fungi, rumen, feed intake, ruminal digestion

Introduction
The differing rates of fibre degradation by a number of anaerobic fungal isolates from sheep and cattle (Gordon and Phillips 1989; Gordon 1990; Gordon et al. 1995) indicated that encouraging the growth of specific types of anaerobic fungi in the rumen may be beneficial to ruminants being given poor–quality feed. The extent of the contribution that the fungi can make to utilisation of poor–quality feed was evident when Gordon and Phillips (1993) showed that feed intake by sheep decreased by about one–third when anaerobic fungi were removed from the rumen by antibiotic treatment. When a single strain of a fungus from sheep, a Neocallimastix sp., was reintroduced into the rumen, feed intake was restored to slightly above the pre–treatment values, indicating that utilisation of poor–quality feed could be improved by dosing sheep with strains of ‘elite’ fungi with high capabilities for fibre–degradation. Subsequent studies found that some anaerobic fungi from herbivores other than sheep possessed fibre–degrading properties that were superior to the strains normally isolated from sheep. Some of these were able to colonise the fungus–free rumen of sheep but not the unaltered rumen, for example members of the polycentric genus Orpinomyces (Phillips and Gordon 1995a, and unpublished). Other strains were unable to colonise even the fungus–free sheep rumen, for example Piromyces sp. from kangaroos (Gordon and Phillips, unpublished). A number of other fungal strains not found in sheep were able to colonise and persist there for several months. The development of a DNA–based system to ‘track’ the introduced fungal strains was essential to the progress of this research (Brownlee et al. 1996). Subsequently a similar ‘tracking’ system was proposed by Brookman et al. (2000).

It was first realised at our laboratory in 1983 that the size of the ruminal population of anaerobic fungi was affected by the sulphur content of the diet and that very few fungi were present when the sulphur content was low (<1 g S/kg DM). It was also found that the addition of rumen–available sulphur (in the form of methionine) to a sulphur–poor ration permitted the
recovery of a functional population of anaerobic fungi (see Gordon and Phillips 1998 for a review of this early work). Therefore, a search was conducted for a sulphur compound that could be used as a nutrient by anaerobic fungi but was more slowly metabolised by the rumen bacteria and protozoa. A compound with these properties would be used at concentrations within the rumen well below those needed for a substance, such as methionine, that supplies S to all of the rumen microorganisms. Two compounds were identified as potentially useful fungal nutrients: 3–mercaptopropionic acid (MPA) and dimethyl disulphide (Phillips and Gordon 1991). The latter is a volatile liquid and thus potentially difficult to administer to animals. Therefore, MPA was further investigated for its potential as a fungal–specific nutrient.

In this paper we present the results of some experiments with sheep fed defined diets in pens, and a trial with sheep at pasture which demonstrated the efficacy of the fungal–specific nutrient and an oral dose of an elite fungal culture, both separately and in combination, on the feed intake and liveweight change of sheep in pens and on the liveweight of grazing sheep.

### Fungal cultures and microbial counts

Three strains of monocentric anaerobic fungi, *Piromyces* sp. CS15 (from a cow, *Bos taurus*), *Piromyces* sp. TZB2 (from a zebra, *Equus grevyi*) and *Neocallimastix* sp. TGB1 (from a gemsbok, *Oryx gazella*), were isolated from zoo specimens kept at pasture in Australia. The strains were maintained using methods previously described (Phillips and Gordon 1989). They were selected from a larger number of cultures isolated from these and other host animal species on the bases that they showed a high rate of cellulose degradation *in vitro* (Gordon 1990) and were able to persist in the rumen of sheep for at least one month (Phillips and Gordon 1995a). Fungal cultures were grown for 4 d at 39°C in straw medium (basal medium 10 containing 0.2% milled wheat straw and 10% centrifuged rumen fluid; Phillips and Gordon 1989) before 25 ml quantities of fresh culture were administered to sheep by mouth. The presence of any of these strains in rumen digesta from the dosed sheep was tested qualitatively by using specific oligonucleotide probes derived from the Internally Transcribed Spacer region (ITS) adjacent to the 18S ribosomal gene of the fungal chromosome (Brownlee et al. 1996). Counts of anaerobic fungi and bacteria in rumen contents were made in agar roll tubes of medium 10X either with or without antibiotics, respectively (Phillips and Gordon 1989).

### Pen trials

#### Dosing sheep with non–indigenous fungi

The first trial of dosing on feed intake was made when several cultures of anaerobic fungi showing a high rate of cellulose degradation *in vitro* were dosed by mouth into Merino crossbred wethers held in individual pens indoors. A chopped ration consisting of three parts by weight of barley straw to one part of lucerne hay (49% DM digestibility; 1.1 g S/kg DM) was fed *ad libitum* for 14 d, followed by a pretreatment period of 7 d when feed intakes were recorded. Two weeks after oral dosing with fungal culture, each to a group of eight sheep, and with one undosed control group of eight, intakes were again recorded for a period of 7 d.

Merino–cross wethers dosed with *Piromyces* sp. CS15 from cattle showed an increase of 5.9 ± 1.74% (mean ± SE, n = 8) in voluntary intake per kg0.75 of a straw–based diet over that observed for the control group (1.2 ± 1.21%). This greater intake (4.7 percentage points), which was not observed for several other fungal isolates from other host species, was significantly higher (*P*<0.05) than the control group.

A second trial was conducted with rumen–fistulated crossbred wethers which were fed *ad libitum* a poor–quality diet of the same composition (barley straw and lucerne hay; 45% DM digestibility) by means of interval feeders (3–hourly). After an initial period of three weeks followed by an eight–day period to determine DM intake and whole–tract digestibility, the sheep were allocated to two groups; one group of five became the control whereas the six members of the other were dosed (through the cannula) with *Piromyces* sp. CS15, previously found to be effective in sheep. After

<table>
<thead>
<tr>
<th>Measurement (and units)</th>
<th>Percent change from pre–dose period to post–dose feeding period</th>
<th>Percentage–point differential due to fungal dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM intake (g/kg0.75)</td>
<td>Control (n=5) 2.4 ± 4.92 CS15 dosed (n=6) 12.0</td>
<td></td>
</tr>
<tr>
<td>Digestible DM intake (g/kg0.75)</td>
<td>Control (n=5) 5.7 ± 5.71 CS15 dosed (n=6) 11.7</td>
<td></td>
</tr>
<tr>
<td>Liveweight (kg)</td>
<td>Control (n=5) –8.8 ± 1.46 CS15 dosed (n=6) 0.1</td>
<td></td>
</tr>
<tr>
<td>Wool growth (mm/week)</td>
<td>Control (n=5) 9.5 ± 1.36 CS15 dosed (n=6) 0.7</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Percentage change in feed intake, liveweight and wool growth by sheep after oral dosing with *Piromyces* sp. CS15, an anaerobic fungus from cattle (Gordon et al. 2000).
two months the same sheep were re-dosed with CS15, followed by a second period of 8 d to determine DM intake and digestibility. Samples of ruminal digesta were taken for analyses with the oligonucleotide probe C1 for the presence of the fungal strain CS15.

Feed intake in the second trial was 12 percentage points greater in sheep dosed with *Piromyces* sp. CS15 (Table 1); however, this difference was not significant, mostly due to the high between-sheep variability in the undosed control group. The oligonucleotide probe C1, a DNA sequence specific for strain CS15, showed that five of the six sheep dosed with strain CS15 still contained this fungus at the end of the digestion trial. Indeed, three of the sheep contained CS15 for the entire three months of the experiment, whereas two other sheep contained this strain for the last month. Nevertheless, the fungal dose resulted in a much greater intake of poor-quality feed, this being the first report of such an effect in adult sheep with a fully developed rumen microbial ecosystem (Gordon et al. 2000). Only small increases were recorded in liveweight change and wool growth due to the different duration of fungal persistence in each sheep over the full length of the experiment, implying that improved intakes of the poor-quality feed were unlikely to have been consistently maintained.

### Fungal–specific nutrient

The study was conducted with 13 crossbred wethers kept in metabolism cages and fed, *ad libitum*, a diet of alkali-treated wheat straw supplemented with urea and minerals (Weston et al. 1988) which contained 0.8g S/kg DM. There were three periods to the study: (i) adjustment to the diet for 14 days; (ii) seven sheep were supplemented with MPA for 21 days and six were unsupplemented; (iii) the treatments in period (ii) were crossed over. Comparisons were made for each animal between the unsupplemented and the supplemented periods. The MPA was infused directly into the rumen via a permanent cannula at the rate of 0.6 g/d (0.19 g S/d). Collections of feed refusals and faeces for the calculation of voluntary feed intake and whole tract digestibilities were made for the last 14 d of the treatment periods. Samples of rumen digesta were collected on the final day of each treatment period for determination of the numbers of fungal zoospores and total bacteria.

Table 2 shows that infusion of MPA (0.6 g/d) increased the fungal count in the rumen of individual sheep by around 15–fold (an increase in $\log_{10}$ of 1.2) whereas the bacterial count was increased to a much lesser extent (a log increase of 0.1). Also, this MPA treatment was accompanied by a substantial increase in feed intake. The large increase in fungal numbers compared to bacterial numbers when sheep were receiving MPA suggested that this compound was selectively boosting fungal numbers rather than contributing to the overall sulphur pool of the rumen. In this study, as previously (Gordon and Phillips 1993), a large increase in fungal numbers was associated with a substantial increase in feed intake (+25%), see Table 2. There was also an improvement in digestibility leading to a large increase in dry matter digestion (+37%). These results suggest that MPA supplementation may be a means of boosting fungal numbers and thus increasing the feed intake of sheep grazing poor quality pasture (Phillips et al. 2000).

### Fungal–specific nutrient plus fungi in combination

A pen trial was conducted to assess the combined effect for three weeks of MPA (0.6 g in a solution administered daily by mouth) and a single oral dose containing three strains of anaerobic fungi (*Piromyces* spp. CS15 from cattle, TZB2 from zebra, and *Neocallimastix* sp. TGB1 from gemsbok) as a mixture in a culture volume of 25 ml. The 29 Merino wethers were fed alkali-treated straw (45% DM digestibility) of low sulphur content (0.8 g S/kg DM) for a three-week period to establish baseline measurements of feed intake so that each sheep was used as its own control for the measurements made.

<table>
<thead>
<tr>
<th>Control</th>
<th>MPA</th>
<th>Increase per sheep (^a), (log 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungal zoospores (x 10^3/ml)</td>
<td>1.1± 0.4</td>
<td>5.9± 0.7</td>
</tr>
<tr>
<td>Bacteria (x 10^8/ml)</td>
<td>7.8± 1.0</td>
<td>9.3± 0.7</td>
</tr>
<tr>
<td>DM intake (g/d)</td>
<td>910± 66</td>
<td>1128± 71</td>
</tr>
<tr>
<td>DM digestibility (%)</td>
<td>47.4± 0.9</td>
<td>51.6± 0.9</td>
</tr>
<tr>
<td>DM digestion (g/d)</td>
<td>433± 34</td>
<td>581± 37</td>
</tr>
</tbody>
</table>

Data are mean ± SE for 13 Merino–cross wethers

\(^a\) Average increase due to MPA when each animal is used as its own control (± SE)

\(^b\) Means in rows with different superscripts are significantly different (P<0.05)
during the treatment period. The sheep were divided into three groups and received either the fungal dose or a drench of MPA; eleven sheep received both treatments. The animals were weighed at the commencement of the experiment, and again at the completion when samples of rumen digesta were collected from the 22 sheep that received a fungi dose. These samples were assayed for the presence of the three strains of non-indigenous fungi dosed into the sheep using oligonucleotide probes C1, Z1 and G1 which are specific for fungal isolates CS15, TZB2 and TGB1, respectively.

Feed intakes increased for all three treatment groups, the largest increase (22.5%) being observed for the combined fungal dose plus MPA treatment: 888 g/d compared with 726 g/d pre-treatment ($P<0.05$; see Table 3). The next largest increase was 19.9% for the MPA treatment but this was not significant ($P=0.2$) due to the large between sheep variability in this group. The intake of the fungal dosed group increased by the relatively small amount of 5.2% which was attributed to the very poor nature of the basal diet (alkali-treated wheat straw) and the low levels of sulphur, a critical nutrient for fungal growth. The combined treatment gave an increase above the value for MPA alone which indicated that the fungal dose could provide an additional boost to the MPA effect (Gordon 1999). The benefit of the combined treatment is shown by this group recording the least loss in liveweight ($–7$ g/d) for the period of the experiment.

At the end of the experiment, three DNA oligonucleotide probes (each specific for one of the fungal strains) were used to determine the presence of CS15, TZB2 and TGB1 in samples of ruminal digesta taken from the 22 sheep that received a fungal dose. TZB2 and TGB1 colonised only two and one sheep respectively, whereas CS15 persisted in nine dosed sheep (six of which also received MPA). It was concluded that CS15 was the most suitable fungal strain for further investigation.

### Field trial
A grazing trial with sheep was conducted with fine wool Merino wethers at the CSIRO field station, Yalanbee, Bakers Hill, Western Australia, which was designed to test the influence of MPA and fungal dose (with *Piromyces* CS15) separately and in combination. MPA was provided as the sodium salt in a commercial prototype lick block (2 kg/20 kg) and a ‘MegaPhos’ block (40 kg; Olsson’s, Morningside, Qld 4170) was always available to provide nitrogen (14% crude protein) and phosphorus (6%). The trial was conducted for four weeks on dry summer pasture (grass and clover during March and April) and no supplementary oaten hay was fed during the measurement period. The rainfall just prior to and during the trial was seasonally normal with 27 mm falling in March prior to the commencement of measurements and 4 mm being recorded during the trial.

Fifty wethers were randomly assigned to two groups of 25 and placed in separate paddocks of about 3 ha each. Samples of rumen digesta were taken by stomach tube from all sheep before any received the oral dose of anaerobic fungi. Twelve sheep from each of the two groups were randomly selected and were given by mouth 25 ml of fungal culture containing *Piromyces* sp. CS15 on two consecutive days. Each group of 25 sheep had free access to a ‘MegaPhos’ block and a salt block (only one of which was formulated to contain MPA; the other acted as the control). Weights of sheep and blocks were recorded at the commencement of the trial, then two weeks later when sheep and blocks were alternated between the two paddocks, and finally at the completion of the trial four weeks after its start. A second sample of rumen digesta was collected at the completion of the trial and was assayed for the presence of the dosed fungal strain using the specific oligonucleotide probe C1.

The live weight change over a one-month period for the MPA-treated sheep was a gain of 24 g/d

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Period A</th>
<th>Period B</th>
<th>Change in intake</th>
<th>Liveweight change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Pre-treatment)</td>
<td>Treatment</td>
<td>OM intake (g/d)</td>
<td>(B–A)/A</td>
</tr>
<tr>
<td>1 (11)</td>
<td>735±31</td>
<td>Fungal dose</td>
<td>771±32</td>
<td>5.2%</td>
</tr>
<tr>
<td>2 (11)</td>
<td>726±31</td>
<td>MPA+ fungi</td>
<td>888±37</td>
<td>22.5%</td>
</tr>
<tr>
<td>3 (7)</td>
<td>630±59</td>
<td>MPA</td>
<td>751±66</td>
<td>19.9%</td>
</tr>
</tbody>
</table>

Data are mean ± SE for the indicated number of sheep

Periods A and B were each of 7-d duration; Period B followed A by 17 d

<sup>a</sup> Measured between the end of Period A and the end of Period B (23 d)

<sup>a,b</sup> Means in rows with different letter superscripts are significantly different ($P<0.05$)

<sup>c,d</sup> Means in columns with different letter superscripts are significantly different ($P<0.05$)
(25 g/d for the 12 dosed with fungi and 23 g/d for the 13 that remained undosed), and a loss of 44 g/d for the untreated sheep over a four–week period (Table 4). In contrast, the fungal dose in sheep not receiving MPA resulted in a liveweight loss of 35 g/d, and sheep not receiving either fungal dose or MPA lost 52 g/d. Thus, MPA treatment had a more positive effect on live weight than fungal dose (Gordon 1999). However, there was no effect of either treatment on wool growth which was in the range 9.7–10.0 g/d over a single two–month period spanning the measurement period of the trial.

Salt block consumption was 7.4 g/d/head for the MPA group and 5.3 for the controls, and of the MegaPhos block was 9.1 and 26.6 g/d/head, respectively. Because the MPA–containing block was formulated for an anticipated daily consumption rate of 5 g/head, these sheep were eating about 50% more MPA than was originally intended.

Overall, this trial showed that sheep responded in liveweight gain to treatments of both MPA and fungal dose with *Piromyces* CS15. At the end of the trial, this fungal strain was detected in the rumen of 18 of the 24 sheep originally dosed with it.

### Conclusion

The results of several pen trials and a field trial presented here demonstrate that anaerobic fungi can be manipulated in the rumen of sheep to increase the supply of nutrients from poor–quality pasture. Depending on the conditions of the trials, the main production benefits observed from the use of the fungal treatments have been on liveweight. MPA was shown to be a fungal–specific nutrient that is very effective for sheep, either penned or at pasture, and it could have application in the cattle industry although this needs to be proven by further experimentation. Oral dosing with selected strains of anaerobic fungi, as the second approach to the manipulation of anaerobic fungi in the rumen, has been effective in increasing feed intake by penned sheep but less so when sheep were kept at pasture. The persistence of the strains of dosed fungi in sheep, as monitored qualitatively with specific DNA probes, was not consistent between the several strains of fungi that were tested. The best strain persisted for six to eight weeks in about 3 out of 4 sheep dosed with it while the two other fungal strains showed less persistence. There is a need for further research on the factors affecting the persistence of non–indigenous anaerobic fungi in the rumen.

The microbial environment and digestive processes of the cattle rumen are somewhat different from those of sheep, so the data obtained for sheep can be used only as a guide to the possible benefits for cattle. However, the approach of dosing of living cultures of certain elite strains of fibre–degrading anaerobic fungi is less well advanced than for sheep. Previously, an oral dose of *Neocallimastix* sp. led to increased weight gain in growing bulls (Theodorou et al. 1990). Sheep and cattle share some types of anaerobic fungi, but cattle contain several other types not found in sheep (Phillips and Gordon 1995b). Therefore, the development of a dose containing elite fungi for cattle would need to be achieved before there could be an integrated strategy to manipulate anaerobic fungi in cattle.

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### References


### Table 4

Influence of fungal nutrient in salt block (MPA 10 % w/w) and fungal dose with *Piromyces* sp. CS15 on liveweight change (g/d) of Merino wethers over a 28 day period on dry summer pasture in Western Australia (Gordon 1999).

<table>
<thead>
<tr>
<th></th>
<th>MPA treatment (no sulphur)</th>
<th>Control treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungal dosed</td>
<td>25±14 (12)</td>
<td>−35±10 (12)</td>
</tr>
<tr>
<td>Not fungal dosed</td>
<td>23±12 (13)</td>
<td>−52±17 (13)</td>
</tr>
<tr>
<td>Total (dosed and not dosed)</td>
<td>24±9 (25)</td>
<td>−44±10 (25)</td>
</tr>
</tbody>
</table>

Data are mean ± SE with the number of sheep in brackets

*a, b* Means with different letter superscripts are significantly different (P<0.05)


