Yeasts and Moulds in the Bovine Rumen

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SUMMARY

Only small numbers of fungi were found in the rumen fluid of cattle cultured on agar plates at 39 °C, the counts being up to 3500 yeast colonies/ml, with a similar number of mould colonies. However, considerably larger numbers of yeast colonies appeared on plates incubated at 25 °C. Nine species of yeasts were isolated belonging to *Candida* (including corresponding species of *Pichia*), *Trichosporon*, *Torulopsis*, *Kluyveromyces*, *Saccharomycopsis*, and *Hansenula*. The predominating species were *Candida krusei*, *Trichosporon cutaneum* and *Trichosporon capitatum*. The most common moulds were members of the Mucoraceae, of which *Absidia corymbifera*, *A. ramosa*, and *Mucor pusillus* were identified. *Aspergillus fumigatus* was isolated frequently. The fungal content of rumen fluid seemed to be dependent on the diet of the animal, and no particular fungal flora could be associated with the rumen *per se*. The predominating *Candida krusei*, and also the rarely-isolated species *Torulopsis pintolopesii* and *Kluyveromyces bulgaricus*, could reproduce under anaerobic conditions *in vitro*, but another commonly occurring yeast, *Trichosporon capitatum*, was unable to grow under the same conditions. The majority of yeast cells were obviously destroyed during passage through the alimentary tract, whereas large quantities of moulds could be excreted in a viable state.

INTRODUCTION

Yeasts were observed in the rumen contents of ruminants by Klein & Müller (1941) and by Rolle & Kolb (1955). Ingram & McGaughey (1948) and van der Westhuisen, Oxford & Quin (1950) isolated 'Candida-like organisms' and 'yeast-like organisms', respectively, from the rumen of sheep. Clarke (1960), Clarke & di Menna (1961) and Munch-Petersen (1963) have given information on the yeast flora in the rumens of cows and sheep. They isolated species of *Candida* (*albicans*, *tropicalis*, *krusei*, *rugosa*, *parapsilosis*) and *Trichosporon* (*cutaneum*, *sericeum*) by plating at 39 °C.

Clarke & di Menna (1961) found viable counts of from 80 to 13000 yeast colonies/g rumen content of cows, and Munch-Petersen (1963) reported a similar variation in the numbers of yeasts, generally finding about 1000 colonies/ml rumen content of sheep. Smith (1965) found an average of about 300 yeast cells/g rumen content of oxen.

As regards moulds in the rumen, van der Westhuisen, Oxford & Quin (1950) isolated *Mucor* sp. from the rumen contents of sheep, and Holtenius & Nielsen (1957) found that Mucoraceae were predominant. They isolated *Absidia ramosa* and in addition *Geotrichum candidum* and a *Fusarium* strain, and reported (though with some uncertainty) that the number of moulds was 20 to 100/ml rumen fluid. Sivers (1962*a, b*) stated that the total number of fungi varied from several hundred to 20000 colonies/ml rumen fluid of cattle, and that species of *Absidia* (*corymbifera*, *ramosa*), *Mucor* (*pusillus*, *circinelloides*, *hiemalis*), and *Rhizopus* (*oryzae*) were the moulds most frequently found. Bilai & Pidoplichko (1962)

also reported that the most common moulds in the rumen fluid of cows were species of *Absidia* and *Mucor*. Brewer & Taylor (1969) counted about 100 to 500 fungal colonies/ml rumen fluid of sheep and isolated *Aspergillus fumigatus*. Brewer, Duncan & Taylor (1972) isolated *Mucor rouxii* and counted about 100 to 1000 colonies/ml rumen fluid of sheep.

Investigations dealing with yeasts in the lower alimentary tract can be mentioned here for purposes of comparison (Parle, 1957; van Uden & Carmo Sousa, 1957*a*; van Uden, Carmo Sousa & Farinha, 1958; Soneda, 1959; Batista, de Vasconcelos, Fishman & Staib, 1961; Saëz, 1963; Smith, 1965). The species of *Candida* and *Trichosporon* isolated were similar to those found in the rumen contents. In addition, other yeasts, both sporogenous and asporogenous, were found. Varying numbers of yeasts were found in samples of faeces from various ruminants in zoological gardens, up to many thousands per gram being counted in cultures incubated at 30 °C (Soneda, 1959). Averages of about 250 yeasts/g were counted in the caecal contents of oxen, and <50 yeasts/g faeces of oxen and sheep (Smith, 1965). The number of yeasts in the intestinal tracts of cattle, sheep and goats was reported to be <1000/g (Mehnert, 1965).

The moulds found in the intestinal tract and faeces of cattle contained types similar to those known from the rumen contents, species of *Aspergillus*, *Absidia*, *Mucor*, *Rhizopus*, *Geotrichum* (van Uden & Carmo Sousa, 1957*b*; Batista, de Vasconcelos, Fischman & Staib, 1961).

The main purpose of the present work was to examine the yeasts in the rumen of cattle in order to establish their numbers and taxonomic positions, their dependence on various feeding-stuffs, and their growth under anaerobic conditions; however, the moulds which developed under the same cultural conditions were also included. The study forms part of a project on rumen microbiology under the auspices of the Danish Agricultural and Veterinary Research Council. A report on the bacterial flora in the rumen of cows on similar diets is in the press (Wolstrup, Jensen & Jensen, 1974).

METHODS

The yeast content in 49 samples of rumen fluid of cattle was examined, and moulds which appeared on the same plates as the yeasts were also included.

The rumen fluid samples were taken from ten fistulated animals as described by Hungate (1950), and from two non-fistulated cows by a stomach tube. Usually two or three samples were taken from each animal at varying intervals, though 16 samples were taken from one cow. Samples were taken 4 h after the morning feed, except in the case of cows fed on grass where they were taken just before the evening milking. The feeds used are shown in Table 1.

In order to find a possible correlation between the fungi content in the rumen fluid and in the feeds, 16 samples of feed material or feed components were examined. Six faeces samples were included to ascertain whether viable fungi were excreted.

The samples of rumen fluid and the suspensions of feed material and faeces were treated in a Waring blender for 2 min. Immediately after mixing, aerobic pour plates were made from dilutions 1:10 and 1:100, and from higher dilutions when necessary. The agar medium consisted of 10 g glucose, 2 g peptone (Difco), 2 g yeast extract (Difco), 5 g ox gall, 20 g agar and 1 l distilled water. Immediately before use, 30 mg streptomycin sulphate and 30 mg aureomycin were added per l (cf. dextrose-peptone-yeast extract agar described by Papavizas & Davey, 1959). Five plates were prepared from each dilution, and after incubation for 3 to 4 days at 39 or 25 °C, the fungal colonies were counted and the

Table 1. *Animal feeds*

	Feed	No. of animals*	No. of samples
I	Grass	2	4
II	Hay	2	7
III	Rolled oats	2	4
IV	Grass pellets	2	5
V	Grass pellets + dried beet pulp with molasses + concentrate pellets	1	16
VI	Grass silage†	1	2
VII	Grass silage + concentrates†	1	2
VIII	Beet-top silage + grass + concentrates	3	6
IX	Beets + straw + molasses + concentrates	1	3

* One of the ten cows was given feeds I and VIII, and two heifers given feeds III and IV. The animals were on each feed for four weeks before the first sampling.

† The cows on feeds VI and VII did not thrive, and thus the conditions in the rumen were possibly abnormal.

numbers were recorded as the average per millilitre or gram of material. Finally, colonies were transferred to malt-extract agar slopes.

In addition, several samples of rumen fluid were examined for yeasts by means of enrichment cultures, 5 ml (dilution 1:10) being transferred to 50 ml of the above medium without agar. Five cultures were prepared from each sample and incubated at 39 °C for 7 to 8 days. Plating was made on to the agar medium and, after incubation at 39 °C, yeast colonies were transferred to malt-extract agar slopes.

In the experiments on anaerobic growth of some of the yeasts isolated, the method of Hungate (1950) for cultivation of strictly anaerobic bacteria was used. A medium consisting of 20 g glucose, 20 g peptone (Difco), 5 g yeast extract (Difco) and 1 l distilled water was filled into tubes (10 ml/tube) and immediately before inoculation, 0.5 ml of a solution containing NaHCO₃ (10 %, w/v), cysteine HCl (0.5 %, w/v) and Na₂S.9H₂O (0.5 %, w/v) was added to each tube. The tubes were inoculated with 0.1 ml yeast suspension (one loopful of a young agar culture suspended in 10 ml distilled water) and were incubated at 39 °C. Both the preparation and the inoculation processes were carried out under strictly anaerobic conditions in an oxygen-free atmosphere. Immediately after inoculation and at intervals during the incubation period, pour-plates were made on malt-extract agar, and the colonies were counted after incubation at 39 °C.

RESULTS

Yeasts and moulds in rumen fluid

Yeasts. Table 2 shows that only small numbers of yeasts were found in rumen fluid, when counting was on agar plates incubated at 39 °C. Half the samples contained no, or only a few, yeasts. Rumen fluid of animals given feeds I to IV rarely contained more than 100 yeasts/ml, while some samples from cows on feeds V to IX contained up to 3500/ml.

The numbers could vary considerably in samples taken at different times from the same animal. The greatest number of samples was taken from a cow on feed V, and most of these contained no, or only a few, yeasts, but greater numbers (up to 3000/ml) were sometimes found. The numbers of yeasts in the rumen fluids of a fistulated cow and two non-fistulated cows given the same diet were about the same, as shown in Table 3. Two samples, with a 9 day interval, were taken from each cow and the cultures were incubated at 39 °C.

Table 2. *Numbers of yeasts in rumen fluid; incubation 39 °C*

Feeds	No. of samples in each of the following ranges:*				
	0-10	11-100	101-500	501-1000	1001-3500
I	3	0	1	0	0
II	5	2	0	0	0
III	4	0	0	0	0
IV	3	2	0	0	0
V	10	1	2	1	2
VI	0	0	1	1	0
VII	0	1	0	1	0
VIII	0	1	0	3	2
IX	0	1	2	0	0

* The ranges are expressed as the numbers of yeasts/ml rumen fluid.

Table 3. *Numbers of yeasts in rumen fluid of fistulated and non-fistulated cows given feed VIII; incubation 39 °C*

	No. of yeasts/ml rumen fluid	
	Sample 1*	Sample 2*
Fistulated cow	1000	600
Non-fistulated cow	3500	50
Non-fistulated cow	600	2000

* Sample 1 was taken on 18 July 1972, and sample 2 on 27 July 1972.

Table 4. *Numbers of yeasts in rumen fluid; incubation 25 and 39 °C*

Feed	No. of yeasts/ml rumen fluid incubated at:	
	25 °C	39 °C
IV	60	0
	100	20
	500	80
V	1000	7
	> 1000	0
	2000	200
	4000	1500
	22000	0
VIII	100	50
	7000	600
	10000	2000
	16000	600
	20000	3500
	130000	1000

Samples were taken on different days.

Table 4 shows that considerably more yeast colonies developed on plates incubated at 25 °C than on corresponding plates at 39 °C.

From samples of the rumen fluids of animals on various diets, nine species belonging to *Trichosporon*, *Candida* (including corresponding species of *Pichia*), *Torulopsis*, *Khuyveromyces*, *Saccharomycopsis*, and *Hansenula* were isolated after incubation of cultures at 39 °C. Table 5 shows that *Candida krusei*, *Trichosporon cutaneum*, and *Trichosporon capitatum*

Table 5. Yeasts isolated from rumen fluid*; incubation 39 °C

Species	Feed							
	I	II	IV	V	VI	VII	VIII	IX
	No. of isolates							
<i>Trichosporon cutaneum</i> (de Beurm., Gougerot et Vaucher) Ota	0	0	5	5	0	0	4	0
<i>Trichosporon capitatum</i> Diddens et Lodder	0	6	0	2	0	1	0	3
<i>Candida krusei</i> (Cast.) Berkhout (<i>Pichia</i> <i>kudriavzevii</i> Boidin, Pignal et Besson)	6	0	0	0	4	3	7	6
<i>Candida valida</i> (Leberle) van Uden et Buckley (<i>Pichia membranaefaciens</i> Hansen)	4	0	0	2	0	0	0	0
<i>Candida ingens</i> van der Walt et van Kerken (?)	0	0	0	0	0	0	0	1
<i>Torulopsis pintolopesii</i> van Uden	0	0	0	1	0	0	0	0
<i>Kluyveromyces bulgaricus</i> (Santa Maria) van der Walt	0	3	0	0	1	1	0	0
<i>Saccharomyopsis lipolytica</i> (Wickerham <i>et al.</i>) Yarrow	0	0	0	0	0	0	0	1
<i>Hansenula fabianii</i> Wickerham	0	0	0	0	0	0	0	1

* *C. ingens* (?), *S. lipolytica* and *H. fabianii* were identified at Centraalbureau voor Schimmelcultures, Delft, Holland, where some of the other species determinations were also revised.

were the species found most frequently. These yeasts together represented 52 of the total of 67 isolates, and one or two of them were found in the rumen fluids of animals on all feeds. The remaining 15 isolates belonged to the other six species and, with the exception of *Candida valida* and *Kluyveromyces bulgaricus*, only a single isolate of each species was found.

Usually only one or sometimes two species, either *Candida krusei*, *Trichosporon cutaneum* or *T. capitatum*, appeared on platings of rumen fluid, and other yeasts were isolated only rarely. In enrichment cultures, *C. krusei* also developed, whereas *Trichosporon* spp. were found less frequently. On the other hand, *C. valida* and *Kluyveromyces bulgaricus* were isolated almost exclusively from enrichment cultures. Table 6 shows the yeasts isolated, partly by plating and partly by enrichment, from samples of rumen fluid incubated at 39 °C.

Moulds. The numbers of mould colonies per millilitre of rumen fluid counted after incubation at 39 °C are shown in Table 7. Samples from animals on feeds I, IV, V and VIII contained small numbers of moulds, whereas those from animals on feeds II, III, VI, VII and IX could contain greater quantities. The moulds were primarily members of the Mucoraceae. *Aspergillus* was also common, while other moulds were found only rarely and then in insignificant numbers. Three species of Mucoraceae were identified, *Absidia corymbifera*, *A. ramosa* and *Mucor pusillus*, together with one species of *Aspergillus*, *A. fumigatus*. Table 8 shows that *Absidia* spp. and *Aspergillus fumigatus* were isolated from the rumen fluids of animals on all the diets, whereas *M. pusillus* was found in one sample only.

Table 6. *Yeasts isolated from rumen fluid by plating and enrichment; incubation 39 °C*

Species	No. of isolates	
	Platings	Enrichment
<i>Trichosporon cutaneum</i>	7	0
<i>Trichosporon capitatum</i>	9	2
<i>Candida krusei</i>	9	8
(<i>Pichia kudriavzevii</i>)		
<i>Candida valida</i>	1	5
(<i>Pichia membranaefaciens</i>)		
<i>Candida ingens</i> (?)	1	0
<i>Kluyveromyces bulgaricus</i>	0	5
<i>Saccharomycopsis lipolytica</i>	1	0
<i>Hansenula fabianii</i>	0	1

Table 7. *Numbers of moulds in rumen fluid; incubation 39 °C*

Feed	No. of samples in each of the following ranges:*				
	0-10	11-200	201-500	501-1000	1001-4000
I	2	2	0	0	0
II	0	2	3	1	1
III	0	0	0	1	3
IV	5	0	0	0	0
V	12	4	0	0	0
VI	0	0	2	0	0
VII	0	1	0	1	0
VIII	5	1	0	0	0
IX	0	0	2	0	1

* The ranges are expressed as the numbers of moulds/ml rumen fluid.

Table 8. *Moulds isolated from rumen fluid; incubation 39 °C*

Species	Feed						
	I	II	III	V	VI	VII	IX
	No. of isolates						
<i>Absidia corymbifera</i> (Cohn) Saccardo et Trotter	2	1	1	1	1	2	2
<i>Absidia ramosa</i> (Lindt) Lendner	1	2	2	1	0	0	1
<i>Mucor pusillus</i> Lindt	0	0	0	1	0	0	0
<i>Aspergillus fumigatus</i> Fresenius	4	3	11	2	5	4	5

The strains of Mucoraceae were identified at Centraalbureau voor Schimmelcultures, Baarn, Holland, and the isolates of *Aspergillus* by Dr P. Krogh, Department of Bacteriology and Hygiene, Royal Veterinary and Agricultural University, Copenhagen, Denmark. Although the number of *Aspergillus* identified was larger than that of Mucoraceae, the latter were the most common moulds in the rumen.

Yeasts and moulds in feeds. Most of the feeds contained no, or only a few, yeasts when cultured at 39 °C (Table 9). However, larger numbers of yeasts were found in beets and concentrates (feed IX) and in beet-top silage (feed VIII).

The yeasts isolated from the feeds consisted of *Candida krusei* (concentrates, concentrate pellets, beet-top silage), *Trichosporon* sp. (concentrates), *Kluyveromyces bulgaricus* (beets), *Candida valida* (beets), and *C. guilliermondii* (concentrates, grass).

Table 9. *Numbers of yeasts and moulds in feeds; incubation 39 °C*

	Feed or feed component	No./g feed	
		Yeasts	Moulds
I	Grass	0	10
	Grass	10	35
III	Oats	0	7000
IV	Grass pellets	0	20
V	Grass pellets	0	Few
	Dried beet pulp with molasses	0	Few
	Concentrate pellets	0	0
	Concentrate pellets	40	130
VI	Grass silage	0	0
VIII	Beet-top silage	29000	20
	Concentrates	130	300
IX	Beets	450	60
	Straw	0	9000
	Straw	0	25000
	Molasses	0	20
	Concentrates	400	2000

Table 10. *Numbers of yeasts and moulds in faeces and simultaneous samples of rumen fluid; incubation 39 °C*

Feed	No./g faeces		No./ml rumen fluid	
	Yeasts	Moulds	Yeasts	Moulds
I	30	130	150	15
II	0	> 1000	0	1000
III	0	4000	0	1500
V	10	30	1500	30
VI	40	> 1000	500	300
VII	10	> 1000	1000	200

Considerable quantities of moulds were present in oats (feed III) and straw and concentrates (feed IX) incubated at 39 °C, whereas other feeds examined contained no, or only small, numbers of moulds (Table 9). The predominating moulds belonged to *Mucorales* and *Aspergillus*.

Yeasts and moulds in faeces. Samples of faeces just dropped, and rumen fluid taken simultaneously from the animals, were examined in cultures incubated at 39 °C. Table 10 shows that the faeces samples contained only a few yeasts, even when a comparatively large number of yeasts was found in the rumen fluid samples. *Candida krusei* and *Trichosporon capitatum* were isolated from faeces.

A small number of moulds was found in the faeces of cows on feeds I and V, and simultaneously only insignificant quantities occurred in the samples of rumen fluid. A great many moulds were found in the faeces of animals on feeds II, III, VI and VII, and there were relatively large numbers in the rumen fluid of the same animals. The predominating moulds were members of *Mucorales*, though *Aspergillus* was quite common.

Anaerobic growth of yeasts isolated from rumen fluid. Two strains of each of the predominating species, *Trichosporon capitatum* and *Candida krusei*, were used, as well as one strain of *Torulopsis pintolopesii* and one of *Kluyveromyces bulgaricus*. *Torulopsis pintolopesii*

Table 11. *Anaerobic growth of yeasts isolated from rumen fluid at 39 °C*

For methods and media see text.

Incubation period (days)	No. of yeasts $\times 10^{-3}$					
	<i>Trichosporon capitatum</i>		<i>Candida krusei</i>		<i>Torulopsis</i>	<i>Kluyvero-</i>
	Strain 1	Strain 2	Strain 1	Strain 2	<i>pintolopesii</i>	<i>myces</i> <i>bulgaricus</i>
0	4	57	4	200	20	100
3	8	22	42	11000	61	4000
6	8	27	NT	NT	35	3000
10	4	25	8000	4000	600	5000

NT, not tested.

was examined since it belongs to the obligate saprophytes found in warm-blooded animals (van Uden *et al.* 1958) and *K. bulgaricus* was examined because this and other species of *Kluyveromyces* have been isolated from milk products (van der Walt, 1970). Table 11 shows that no growth of *Trichosporon capitatum* took place. Either the yeast number remained constant during the experimental period of ten days, or it became reduced. On the other hand, distinct reproduction was found in *Candida krusei*, *Torulopsis pintolopesii* and *Kluyveromyces bulgaricus*.

DISCUSSION

The predominating yeasts in rumen fluid in the present investigation, *Candida krusei* and *Trichosporon* spp., were also among the yeasts isolated by Clarke & di Menna (1961) and Munch-Petersen (1963). The considerable variation in yeast numbers found by those authors was also observed in the present study, though the maximum number was smaller.

While some of the yeasts isolated, such as the commonly occurring *Candida krusei*, were able to reproduce under anaerobic conditions at 39 °C *in vitro*, no growth of the frequently occurring *Trichosporon capitatum* could be found under the same conditions. Krogh (1961) reported that vigorous growth of *C. krusei* and *C. tropicalis* took place in the rumen of sheep fed on hay with excess of sucrose, and Clarke & di Menna (1961) interpreted the yeasts isolated from rumen fluid of cows after incubation of cultures at 39 °C as true rumen inhabitants since they were not found in the fodder of the animals. On the other hand, Rolle & Kolb (1955) presumed that yeasts were introduced into the rumen with the fodder, and that they could be considered as occasional transients. Munch-Petersen (1963) did not find any growth of sheep-rumen yeasts on agar plates incubated anaerobically at 39 °C, and yeasts were reported by Mehnert (1965) and Gedek (1968) as being unable to reproduce in the forestomachs of ruminants.

It is probable that the reproduction of yeasts in the rumen is rather limited and the majority of the yeasts are introduced with the fodder. This is supported by the observation that *Trichosporon capitatum* was frequently present in great numbers in rumen fluid although it was unable to grow under anaerobic conditions *in vitro*, and by the finding of a great many yeasts in the rumen which were unable to grow on agar plates at 39 °C. The considerable variation in the number of yeasts occurring in rumen fluid perhaps indicates the lack of any particular yeast flora of the rumen.

Clarke & di Menna (1961) and Munch-Petersen (1963) did not find yeasts in fodder cultured at 39 °C. However, this corresponds to the finding of no, or only small quantities of, yeasts in the majority of samples of rumen fluid of animals on feeds I to V. Many

yeasts were found in beet-top silage (feed VIII) and in beets and concentrates (feed IX), and most of the samples of the rumen fluid of cows on these diets contained relatively large numbers of yeasts. However, in spite of the presence of quite large numbers of yeasts in the rumen of cows on feeds VI and VII no yeasts were found in grass silage. The cows fed on grass silage did not thrive and thus the conditions in the rumen were possibly abnormal. Yeasts were found in remnants of food and water from feeding and drinking troughs and this could contribute to the introduction of yeasts into the rumen.

Clarke & di Menna (1961) reported a difference in the yeast flora dependent on the diet. The rumen fluid of cows fed on clover hay contained only non-fermenting species, while fermenting species were also present in the rumen fluid of cows fed with fresh clover. In the present study, mostly non-fermenting species were isolated from the rumen fluid of animals fed on dried fodder (feeds II, IV, and V), while fermenting species seemed to predominate in the rumen fluid of cows fed on fresh material (feeds I, and VI to IX).

The most common members of Mucoraceae in rumen fluid were species of *Absidia*, which were also among the moulds found most frequently in previous investigations (Bilal & Pidoplichko, 1962; Sivers, 1962*b*). In addition, *Aspergillus fumigatus* was found frequently; this species has also been isolated previously from rumen fluid (Brewer & Taylor, 1969).

Considerable quantities of moulds were found in oats (feed III) and straw and concentrates (feed IX), and the rumen fluid of animals on these diets also contained a large number of moulds. Only a few moulds were found in other feeds examined, and the rumen fluid of animals on those diets contained only a few moulds. However, in the rumen fluid of cows fed on grass silage (feeds VI and VII) rather large quantities of moulds were found although there were no moulds in the feed material.

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