The nutritive value of rumen micro-organisms in ruminants

1. Large-scale isolation and chemical composition of rumen micro-organisms

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1. A method is described whereby a large quantity of rumen microbial dry matter of high purity was isolated from whole rumen contents obtained from abattoirs, by means of a continuous process of one filtration through four sieves followed by three differential centrifugations.

2. The contents of ash, carbohydrate, lipid, nitrogen, RNA, DNA and individual amino acids of the three centrifugal fractions are given and compared with values summarized from more than sixty published reports on the chemical composition of rumen micro-organisms isolated from both whole rumen contents and pure cultures.

3. The amino acid composition of isolated rumen micro-organisms, in particular that of the bacteria, was found to be remarkably constant.

Amino acids (AA), nucleic acids (NA) and a wide variety of other organic compounds are synthesized from dietary and recycled endogenous constituents by a dense rumen microbial population, which usually includes a mixture of many species of bacteria, protozoa and possibly fungi (Bryant, 1975; Hungate, 1975; Bauchop, 1979). The extent of degradation of feed and conversion to microbial matter depends primarily on the degradability of the feed, its available energy content and the time it is retained within the rumen. For a wide range of normal diets, it has been shown that 60–85% of the protein passing to the small intestine is of microbial origin (Smith, 1975; Agricultural Research Council, 1980).

Although bacteria usually represent the major part of the microbial biomass in the rumen, protozoa may also form a considerable proportion under certain feeding regimens (Eadie *et al.* 1970; Harrison *et al.* 1979). However, differential retention of the larger protozoa in the rumen is considered to reduce their actual contribution to the dry matter (DM) leaving the rumen, to a value of less than 0·1 (Leng *et al.* 1980) or 0·2 (Weller & Pilgrim, 1974) of their proportion.

It has been demonstrated previously that the AA compositions of mixed microbial proportions isolated from whole rumen contents of animals given a very wide variety of diets are rather similar (Weller, 1957; Bergen *et al.* 1968; Burris *et al.* 1974; Czerkawski, 1976). Nevertheless, the extent of this similarity and the effects of nutrient supply, the type of organism and the isolation techniques on the AA composition of the biomass have not been adequately assessed. Furthermore, few estimates have been made of the full chemical composition of rumen micro-organisms (RM).

Most published methods for the isolation of individual fractions of whole rumen contents have included filtrational, gravimetric and differential centrifugation techniques, followed by a variety of drying methods as discussed by Storm (1982).

The present paper describes a large-scale isolation procedure whereby up to 1 kg microbial DM was harvested daily. The chemical composition of the material prepared by the method was compared with published values for the chemical composition of RM obtained from both whole rumen contents or pure cultures.
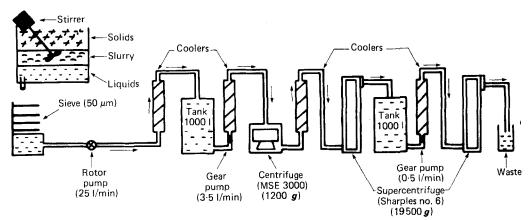
Fig. 1. Flow diagram of the large-scale isolation process, whereby whole rumen contents were continually separated, according to particle size and relative density (see p. 465 for further details).

MATERIALS AND METHODS
Preliminary considerations

Source of microbial raw material. It was estimated that at least 50 kg of microbial DM would be needed to study the utilization of rumen microbial nitrogen (RM-N) in growing lambs. In order to obtain sufficient raw material from which to isolate such a large quantity, whole rumen contents from cattle and sheep were collected at the local abattoir. Before slaughter these animals had usually been kept overnight in the lairage, having access only to straw and water. This period of feed restriction usually resulted in the rumen contents having a relatively low DM concentration. To enable large volumes to be processed, a continuous isolation process was developed.

Rumen contents were transferred, within 10 min of slaughter, to a stainless-steel tank. To help to ensure that most of the various micro-organisms present in the initial material were included in the liquid suspension used in the isolation process, the whole rumen contents were agitated using a stirrer. The agitated rumen contents were then allowed to settle for approximately 5 min, after which the liquid fraction was removed from the middle layers of the tank so that no sand or silt (bottom layer) and no long fibres (top layer) were included (Fig. 1).

Pilot plant

Initially a pilot plant was developed in which the liquid suspensions obtained from the abattoir were simply fractionated continuously according to particle size and relative density by a series of four filtrations, followed by three differential centrifugations.

The two main objectives, namely that of obtaining the least contaminations of dietary material and the maximum quantity of micro-organisms, were difficult to reconcile. However, microscopic examination and chemical analyses of samples of the filtrates, sediments and supernatant fractions from each of the stages of centrifugation, enabled a choice of the optimal filter pore size, centrifugal force, retention time and temperature to be made, so that most protozoal and dietary residues were included in the first centrifugal sediment, and virtually all remaining microbes in the second and third sediments.

Large-scale production plant

Based on the experience and information obtained from the pilot plant (which gave 50–80 g microbial DM daily), a large-scale production plant was set up in the basement of a local abattoir.

The layout of the final process is demonstrated in Fig. 1. Examples of the gross effect of the filtration and centrifugation steps on the species composition of the liquid as it passed through the system is illustrated in Plate 1(*a–d*) illustrating the various filtrates and supernatants. Details of the procedure are given by Storm (1982).

The combined filtrations removed most plant fragments and allowed only protozoa, some very small dietary particles, and the bacteria (see Plate 1(*a*)) to pass through to the first cooler (14°) and subsequently to the plastic holding tank (tank no 1). Rumen fluid was removed from this holding tank simultaneously and passed through the second cooler (8°) before it was centrifuged at 1200 *g* for 4 min of mean residence time in an MSE 3000 continuous-flow basket centrifuge (MSE Ltd, London). This first centrifugal treatment was considered to remove most protozoa and most of the remaining dietary matter (fraction 1) from the filtered rumen fluid, since the resultant supernatant fraction was found to consist mainly of suspended bacteria (Plate 1(*b*)). The supernatant fraction was passed through the third cooler (maintained at 8°) and was then re-centrifuged at 19500 *g* for 8 min of mean residence time in a Sharples no. 6 industrial (6000 ml) supercentrifuge (Penwalt, Camberley, Surrey). This second centrifugal treatment removed the bulk of the remaining micro-organisms (fraction 2) as judged by microscopy of the supernatant fraction flowing into holding tank no. 2 (Plate 1(*c*)). The rumen fluid was again cooled (8°) and subsequently recentrifuged at 19500 *g* for 15 min (flow-rate of 0·4 l/min).This last centrifugal treatment harvested virtually all the remaining organisms (fraction 3) as judged by microscopy of the discharged supernatant fraction (Plate 1(*d*)). The production capacity of the large-scale isolation plant was 1 kg microbial DM/d. The chemical compositions of the three fractions are given in Table 1.

Freeze-drying, preparation and storage

The various sediments were immediately frozen at −20° and freeze-dried within 3 d to 950–960 gDM/kg. The material was then stored in individual air-tight polyethylene bags at −20°. The daily harvests of each fraction were mixed on a 2-monthly basis. These bulked fractions were then sampled and analysed. At the end of the entire production period the total amounts of fractions 2 and 3 were combined, sampled and stored at −20°. Fraction 1 was kept separate.

Chemical analysis

The recommendations of the Association of the Official Analytical Chemists (AOAC, 1965, 1970, 1975) were followed for sample preparation (method 7002, AOAC, 1975), DM (method 22003, AOAC, 1975), ash (method 70101, AOAC, 1975), fibre (method 7055, AOAC, 1975) and N (method 2041, AOAC, 1965, as modified by Davidson *et al.* 1970). Carbohydrate was determined by the anthrone method of Clegg (1956). Lipid was determined by the technique of Atkinson *et al.* (1972). DNA was estimated by the colorimetric method of Burton (1956) using calf thymus DNA as standard and RNA by a modification of the Schmidt-Thannhauser procedure as described by Munro & Fleck (1966). The AA composition including diaminopimelic acid (DAPA) was determined by ion-exchange column chromatography of samples hydrolysed in 6 M-hydrochloric acid at 137° for 18 h. Cystine and methionine were estimated separately after oxidation to cysteic acid and methionine-sulphone (Moore, 1963). Tryptophan was also determined separately using a colorimetric method involving alkaline hydrolysis with barium hydroxide (Mathieson, 1974).

RESULTS

Isolation process

The painstaking filtration used at the beginning of the centrifugal process was important because it increased the N content of all the fractions obtained subsequently by centrifugation.

Table 1. *The mean chemical compositions with their pooled standard errors of individual and combined fractions of rumen micro-organisms (RM) obtained in the present study*

Centrifugal fractions...	1	2	3	2+3	SE	Published values for rumen bacteria
Proximate analysis (g/kg DM)						
Crude fibre	17·1	9·4	4·2	5·8	1·93	—
Moisture	76·2	62·0	49·5	55·9	2·33	—
Ash	143·2	116·4	98·3	104·3	4·02	168·5
Lipid	—	—	—	92·1	6·65	101·0
Carbohydrate	—	—	—	93·2	7·05	155·2
Nitrogen	84·8	99·7	103·2	102·4	1·59	77·7
Composition of N (g/kg N)						
RNA-N	96·4	112·0	114·1	113·2	7·45	100·1
DNA-N	33·4	40·6	42·9	41·3	9·20	51·7
Amino acid (AA)-N	799·3	810·3	806·1	808·6	5·06	825·0
AA composition (g/kg AA)						
Arginine	46·0	46·2	46·4	49·3	0·99	52·2
Histidine	17·5	18·3	18·0	17·0	0·24	20·7
Isoleucine	55·9	52·7	54·4	54·4	1·13	56·5
Leucine	73·9	71·0	70·0	74·4	1·35	75·7
Lysine	79·6	77·4	81·3	81·2	2·01	85·4
Methionine	—	—	—	24·7	0·82	24·2
Cystine	—	—	—	10·0	0·44	11·9
Phenylalanine	55·1	53·2	52·1	54·5	1·00	49·0
Tyrosine	50·2	49·6	52·9	44·8	1·14	43·8
Threonine	51·6	53·7	53·0	52·0	0·76	53·6
Valine	57·6	60·2	57·4	53·4	0·80	59·8
Tryptophan	—	—	—	16·4	1·35	13·4
Total essential (E) and semi-essential AA	—	—	—	532·1	—	546·2
Alanine	65·0	73·8	67·9	69·8	1·30	71·4
Aspartic acid	121·6	124·0	118·6	119·6	1·39	112·3
Glutamic acid	134·5	127·8	131·4	133·3	2·03	125·6
Glycine	49·7	51·8	49·3	51·6	0·49	55·3
Proline	35·4	34·4	45·8	38·2	2·53	34·6
Serine	45·1	43·7	41·1	43·6	0·84	40·6
DAPA	10·2	10·4	10·5	11·6	0·48	10·2
Total non-EAA	—	—	—	467·7	—	450·0

DAPA, diaminopimelic acid; DM, dry matter.

It also ensured trouble-free flow through metering pumps, coolers and centrifuges. The initial filtration was by far the most laborious part of the entire isolation process. Although this filtration could fairly easily be mechanized it could not be omitted without seriously affecting the nature of the final products.

Centrifugational sedimentation of RM. The rate of sedimentation of particles out of a given suspension by centrifugation is generally a function of centrifugal force, retention time and temperature (Thomson & Foster, 1969). In our experience, forces above 15000 g, temperature and retention time were particularly important when dealing with these mixtures of water, bacteria, protozoa and partly-hydrolysed dietary particles, since only by varying these factors could yield be increased.

Chemical composition

The three fractions obtained by centrifugation at 1200 *g* for 4 min, 19 500 *g* for 8 min, and 19 500 *g* for 14 min, and the two latter fractions combined are hereafter referred to as fractions 1, 2, 3 and 2 + 3 respectively. The mean chemical compositions of these fractions, expressed on a DM basis, are presented in Table 1, together with their standard errors for each component, pooled over all fractions. Each value generally represents the mean of four determinations carried out in duplicate.

Nucleic acids. The NA content of the microbial isolates given in Table 1 is expressed as the constituent RNA-N and DNA-N relative to the total N estimated. There were no real differences between fractions 2 and 3, both of which had somewhat higher DNA and RNA concentrations than fraction 1.

AA. The concentrations of individual AA in the different fractions, expressed as g AA/kg AA estimated, are presented in Table 1. The determinations were carried out on samples taken approximately every second month during the period of isolation. However, cystine, methionine and tryptophan were estimated for the combined fractions only.

DISCUSSION

Species composition of microbial isolates. In most of the isolation techniques described in the literature, a preliminary filtration (squeezing) of whole rumen content through a number of layers of muslin, cheesecloth or gauze of undefined pore size, is used to obtain a liquid suspension of the RM. However, it has been demonstrated that a considerable proportion of the RM adheres quite strongly to the solid fibrous fraction of whole rumen contents (Cheng & Costerton, 1980), and that mechanical dislodging is necessary if a truly-representative sample of microbes is to be extracted (Hoogenraad & Hird, 1970). The procedure used here was designed to attempt to get a representative sample of the organisms from whole rumen contents, though it is accepted that a considerable portion of microbes must have been left in the fibrous residue.

Chemical composition

Mean published estimates of chemical composition of rumen bacteria (RB) isolated from a very wide variety of environmental conditions were taken from a recent survey of the literature on the chemical composition of RM (Storm, 1982). These mean values for RB are shown in Table 1 for twenty-nine estimates of pure cultures. In this survey, Storm (1982) also noted relatively little difference in AA composition whether the RB was obtained from purified or concentrate diets.

In Table 2 values for the composition of AA have been expressed in different ways.

Ash. The ash content of fraction 1 was slightly higher than that of fractions 2 and 3. Published values for ash have been highly variable, ranging from 50 g/kg (Hoogenraad & Hird, 1970) to almost 300 g/kg (Smith & McAllan, 1973). Smith & McAllan (1974) estimated that sodium, potassium, calcium, magnesium and phosphorus together accounted for only approximately 40% of the estimated ash in their samples. Since during separation they applied a force of only 200 *g* for 5 min to eliminate dietary and protozoal contaminants, it is probable that some non-microbial minerals may have been included in their isolate.

The average value of 104·3 g ash/kg DM for the bulked fraction, 2 + 3, in the present study is slightly lower than that of bacterial estimates taken from the literature.

Carbohydrate. It is well established (Jouany & Thivend, 1972; Bryant, 1975) that the polysaccharide content of RM increases during periods of energy surplus and, for pure cultures, that polysaccharides can account for as much as 150–200 g/kg DM (Hungate,

Table 2. *The amino acid (AA) composition of the freeze-dried preparations of rumen micro-organisms*

AA	g AA/ kg AA	g AA–N/ kg AA–N*†	mmol AA/ mol AA‡	g AA/kg true protein§
Arginine	49·3	117·8	36·2	57·4
Histidine	17·0	34·3	14·1	19·8
Isoleucine	54·4	43·2	53·1	63·2
Leucine	74·4	58·9	72·6	86·6
Lysine	81·2	115·5	71·0	94·5
Methionine	24·7	17·1	21·3	28·7
Cystine	10·0	8·9	10·6	11·6
Phenylalanine	54·5	34·3	42·2	63·4
Tyrosine	44·8	26·1	31·6	52·1
Threonine	52·0	45·5	55·9	60·5
Valine	53·4	47·7	58·4	62·1
Tryptophan	16·4	16·4	10·2	19·1
Total essential (E) and semi-essential AA	532·1	565·7	477·2	619·0
Alanine	69·8	82·0	100·2	81·2
Aspartic acid	119·6	93·9	115·1	139·1
Glutamic acid	133·3	94·7	116·0	155·1
Glycine	51·6	71·6	87·9	60·0
Proline	38·2	34·3	42·5	44·4
Serine	43·6	44·0	53·1	50·7
DAPA	11·6	12·7	7·7	13·5
Total non-EAA	467·7	433·2	522·5	544·0
Total	999·8	999·8	999·7	1163·0

DAPA, diaminopimelic acid.
* The AA–N constitutes 0·8086 of total microbial N.
† Values obtained by multiplying by the N concentrations of the individual AA.
‡ Values obtained by dividing by the molecular weight of the individual AA.
§ Values multiplied by 1·1636 to give g AA/kg true protein.

1975; Hobson, 1969a). The 12–16 h fast imposed on the donor animals is the most likely reason (McAllan & Smith, 1977) why the carbohydrate concentration of our microbial isolates is considerably lower than those summarized in the literature. As indicated by the high standard error, published carbohydrate values are highly variable. Czerkawski (1976) found that most of the variation in composition of RM was removed by correcting to a carbohydrate-free basis.

The extent to which digestibility and other nutritional properties of RM are influenced by the carbohydrate content is not well established but microbial carbohydrate may contribute significantly to the glucose supply of ruminants (Lindsay, 1980; Ørskov, 1980).

Lipid. The lipid content of fraction 2+3 was 92·1 g/kg DM. This value is very similar to those reported in the literature (Garton & Oxford, 1955; Czerkawski, 1976; Kurilov *et al.* 1976).

The lipid content of RM generally appeared to be less variable than most other main microbial components, possibly due to its more functional role and, therefore, presumably its lesser dependence on type of substrate (Thomson & Foster, 1969).

N. The N concentration of fraction 1 was considerably lower than that of fractions 2 and 3. The N contents of pure cultures grown on energy-rich media, where no substrate contamination of subsequent centrifugal isolates was possible, were approximately

100–125 g/kg DM (Hungate, 1966; Hobson, 1969a). It therefore seems possible that a considerable proportion of the differences in N concentrations between our values and those in the literature may be due not only to variable carbohydrate content (Smith & McAllan, 1974) but also to dietary contamination. The N content itself may be used as a tentative indicator of purity (the higher the N concentration the purer the isolate).

NA. Our estimates of both DNA and RNA agree closely with the mean values obtained from the RB and other estimates from the literature of Storm (1982).

AA. Where the AA profiles determined in the present study or those obtained from the literature were incomplete (i.e. when not all nineteen individual AA were determined) an average nominal value for the missing AA was taken from the complete profiles available. The total AA-N comprised 0·81 as a proportion of the total N. This value compares well with that summarized from the literature from RB (Table 1) for other values summarized by Storm (1982) and also that summarized by the Agricultural Research Council (1980).

The value for DAPA is quite similar to the mean value quoted from the literature (Storm, 1982). It is higher than those reported by Hutton *et al.* (1971) but similar to those quoted by Harrison *et al.* (1979). If the DAPA content had been very much different from other values one could have suspected a higher cell wall content in the isolates used. There is, however, no reason to suspect this from the DAPA content of the material isolated here. It should also be pointed out that the DAPA was calculated from the AA chromatograms while in other work the DAPA was determined directly and presumably with greater accuracy, e.g. Mason & Palmer (1971).

Small differences between certain individual AA have been reported (Syvaoja & Kreula, 1979; Purser & Buchler, 1966; Hobson, 1969b), but such differences were between isolated pure cultures only and were not regarded by the respective investigators as being significant for mixed populations.

Nevertheless, when all the published values from the large number of independent reports on the amino acid composition of RB isolated from both pure cultures and whole rumen contents were compared, the variation found among different estimates was still remarkably small (Storm, 1982). Indeed, when a similar examination was made of the published information available on the AA composition of whole-egg protein (six independent reports) or of casein (seven independent reports), both of which are considered to be reference proteins (Food and Agriculture Organization, 1970), the coefficients of variation for the individual amino acids were of the same order. This comparison indicates that variation due to the errors associated with AA determinations are probably greater than that between individual microbial preparations.

The value of RM to the host ruminant animal (lambs) will be reported in subsequent papers.

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EXPLANATION OF PLATE

Plate 1. Micrographs ($\times$ 1500) of rumen fluid after various isolation treatments. (*a*) Rumen fluid after filtration through a 50 μm sieve, showing a mixture of bacteria, protozoa and dietary matter; (*b*) supernatant fraction after extraction of fraction no. 1 by centrifugation at 1200 *g* for 4 min, showing a large number of bacteria but virtually no dietary matter and no protozoa; (*c*) supernatant fraction after extraction of fraction no. 2 by centrifugation at 19500 *g* for 8 min, showing a greatly reduced number of bacteria; (*d*) supernatant fraction after extraction of fraction no. 2 by centrifugation at 19500 *g* for 15 min, showing only very few organisms.
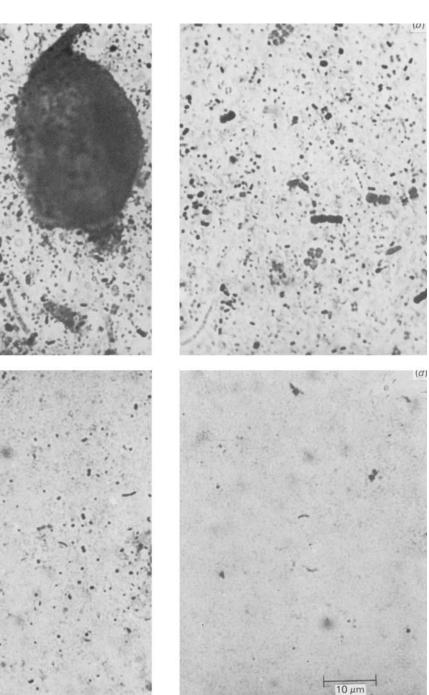
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