Relation between different carbo-
hydrates and microbial synthesis
of protein

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1. Introduction

Ruminant animal production gives an important contri-
bution to the worlds human food supply. This is mainly be-
cause of their capability of converting carbohydrates in
the fibrous parts of plants into high quality human foods.
This conversion is possible due to the symbiotic relation-
ship between ruminant animals and micro-organisms inhabi-
ting their forestomachs. These micro-organisms can degrade
the fibrous or structural carbohydrates ingested by the
host animal, which are undigestible to the digestive system
of monogastrics (including men), to end products which can
be digested and utilized by ruminants and converted to milk,
meat and wool.

Not only fibrous carbohydrates are degraded by the
micro-organisms, so are other carbohydrates, proteins and
to a limited extent lipids. As a result of this degradation
the micro-organisms extract energy and "building stones"
from the food ingested by the host animal, which enables
them to grow. This microbial growth means a synthesis of
biomass containing protein, nucleic acids, lipids and carbo-
hydrates of which protein forms the major component. This
microbial protein can subsequently be used as a protein
source by the host animal and thus can compensate the loss
of dietary protein in the forestomachs resulting from
microbial degradation. It becomes evident that the inter-
mediary metabolism of ruminants receives protein from two
sources viz. dietary protein which escapes microbial degre-
dation in the forestomachs and microbial protein synthesized
therein. Both protein sources are digested in and absorbed
from the small intestine.

In the last decade much attention has been paid to ways
of increasing the intestinal protein supply in ruminants. So
far most attention was focussed on ways of decreasing the
degradation of dietary protein (Clark, 1975; Chalupa, 1978;
Tamminga, 1979) and less attention was given to means of
increasing the synthesis of microbial protein.

It is beyond doubt that the first limiting factor for
the synthesis of microbial protein is energy in adenosine-

tri-phosphate (ATP), resulting from the degradation of food ingested by the host animal. Carbohydrates form a major part of this substrate and in the following sections various aspects of the relation between dietary carbohydrates and microbial protein synthesis will be discussed.

2. Microbial_growth_in_the_forestomachs_and_its_requirements

Before discussing various aspects of the relation between carbohydrates and microbial growth it seems appropriate to discuss the principles of microbial growth under anaërobic conditions as it occurs in the forestomachs of ruminants. It was mentioned already that microbial growth implies the formation of biomass of which main components are proteins, nucleic acids, carbohydrates and lipids. The nutritional requirements of the microflora in the forestomachs of ruminants are very complex (Hungate, 1966) and include volatile fatty acids (VFA), carbon dioxide (CO_2), ammonia (NH_3), amino acids, minerals and vitamins. As a general rule it may be stated however that microbial growth is mainly determined by the availability of enough of a suitable substrate from which energy (ATP) and precursors for the synthesis of the various macromolecules can be extracted.

In energetic terms the degradation of the various dietary components, mainly carbohydrates, proteins and lipids, ingested by the host animal, means the release of a certain amount of energy, part of which is temporarily stored in the terminal pyrophosphate bonds of ATP. The energy stored in ATP can be used by the micro-organisms for various purposes such as motility, transport, the synthesis of macromolecules, etc. Generally this can be divided into two processes viz. maintenance and growth. The ratio in which energy is used for the two requirements mainly depends on the growth rate (Stouthamer and Bettenhaussen, 1973). The ATP required for maintenance per unit of microbial dry matter (e.g. per 100 g) is constant per unit of time. On the other hand, the ATP required for growth is expected to be fairly constant per unit of dry matter synthesized. At a

low rate of ATP generation most or all of the ATP will be required for maintenance. At a higher rate of ATP generation only part of it will be needed for maintenance and the surplus is available for growth. With an increasing rate of ATP generation the surplus of ATP available for growth will increase, resulting in a higher growth rate. Not only will the surplus of ATP increase in an absolute sense, but also a larger proportion of the total ATP generated will be available for growth, making the growth more efficient.

The energetic efficiency is often expressed as the amount of microbial dry matter synthesized per mole of ATP. For this the term Y_{ATP} was introduced (Bauchop and Elsden, 1960). This Y_{ATP} is much lower than the maximum possible yield at maximum growth rate. For the latter the term Y_{ATP}^{Max} was introduced and for this values of between 25 and 30 g of microbial dry matter were estimated (Stouthamer, 1973; Hespell and Bryant, 1979) in continuous cultures. Estimates for Y_{ATP} under practical conditions in the rumen in vivo usually vary between 10 and 15 (Hogan and Weston, 1970).

Estimating Y_{ATP} values in the rumen in vivo under practical conditions is very difficult (Tamminga, 1978). Besides, usually more interest exists in microbial protein synthesis than in microbial growth. It has therefore become common practice to express the efficiency of microbial protein production in the rumen as g of microbial N formed per 100 g of organic matter (OM) fermented, rather than per mole of ATP. Reported values usually range between 2,5 and 4,0 g of N per 100 g OM fermented (Armstrong, 1976; Kaufmann, 1977).

Because of anaërobiosis the amount of ATP which can be extracted from the degradation of organic matter in the forestomachs is limited and restricted to some 10 to 15% of what can be extracted under aërobic conditions. The main ATP-yielding substrate for microbial growth in the forestomachs are carbohydrates. This is not only because carbohydrates form the major component of the ingested feed, but also because on biochemical grounds fermentation of 100 g of carbohydrates is expected to yield more ATP than fermentation of 100 g of proteins or lipids (Tamminga, 1979).
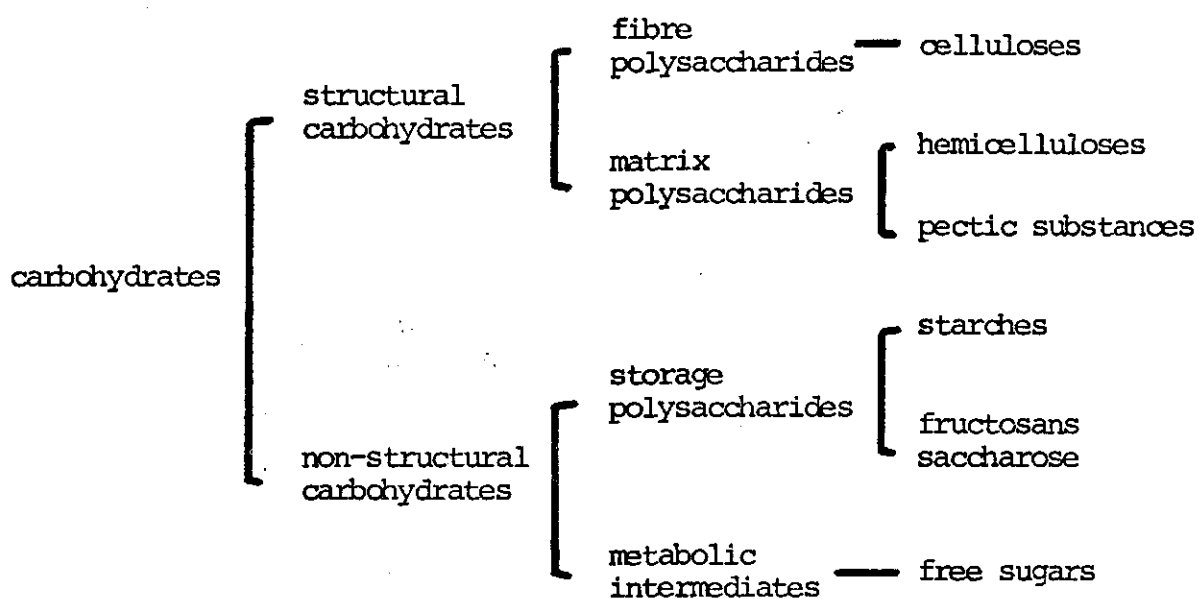
The necessary precursors for the synthesis of biomass are either intermediates or end products of the degradation of dietary carbohydrates, proteins and lipids (Van Es and Tamminga, 1978). Quantitatively protein synthesis is the most important anabolic process. The amino acids which are required as precursors for this process may originate from de novo synthesis, from hydrolyzed dietary protein or from hydrolyzed microbial protein. De novo synthesis is possible from intermediates and end products of the microbial degradation of dietary carbohydrates and proteins. Both a suitable N-source, from which ammonia can be released and preformed amino acids seem necessary. Very often sufficient dietary protein is degraded to generate the required amino acids and (after deamination of amino acids) ammonia. If, under certain conditions additional ammonia is needed, a non-protein-N source from which ammonia can be liberated, such as urea, is sufficient.

3. Availability of carbohydrates as a substrate for microbial growth and protein synthesis

3.1. Rate of degradation

Microbial growth and microbial protein synthesis are continuous processes of which the rate may vary. The energetic efficiency of microbial growth is influenced by its growth rate and thus factors influencing the latter are of interest. One of these is the rate with which ATP and precursors become available from the degradation of substrate. The rate of this degradation differs between different carbohydrates (Johnson, 1976) and depends on certain physical and to a lesser extent chemical properties. The dietary carbohydrates can be divided in various classes which differ in physical rather than in chemical properties. Such a division is shown in table 1.

Table 1. Distribution of dietary carbohydrates

```
                                        fibre
                                        polysaccharides ── celluloses
                    structural
                    carbohydrates
                                        matrix              hemicelluloses
                                        polysaccharides
carbohydrates                                               pectic substances


                                                            starches
                                        storage
                                        polysaccharides
                    non-structural                          fructosans
                    carbohydrates                           saccharose

                                        metabolic
                                        intermediates ── free sugars
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In the degradation of carbohydrates two parts can be distinguished. Firstly hydrolytic cleavage of the glycoside linkages in the polysaccharide chain occurs. This is followed by a further degradation of the resulting monosaccharides. The rate limiting part of the degradation of carbohydrates seems to be the hydrolytic cleavage.

Before the degradation of structural carbohydrates starts a lag period of several hours is usually observed (Mertens, 1978). This period might be required for the development of a slime layer, necessary for microbes to attach to dietary fibre (Cheng et al., 1978). Part of the structural carbohydrates is resistant against degradation as was demonstrated in kinetic studies by Waldo et al., (1972). This is caused by encrustration of structural carbohydrates with lignin and silica which act as physical barriers to the rumen micro-organisms. The remaining degradable part could be divided in two fractions of which one was degraded rather rapidly and the other more slowly (Waldo et al., 1972). Although functionally part of the structural carbohydrates, pectic substances are degraded much more rapidly than the other structural carbohydrates celluloses and hemicelluloses. This may be the result of a difference in chemical configuration. In pectic substances α-configurations predom-

inate whereas in celluloses and hemicelluloses β-configu-
rations are more important.

Unlike structural carbohydrates degradation of non-
structural carbohydrates does not require a lag period. Deg-
radation of storage carbohydrates is usually fast, but the
rate may differ from one source to another. Degradation of
storage carbohydrates may become retarded by a temporary
storage inside the cells of both bacteria and protozoa
(McAllan and Smith, 1974; Czerkawski, 1976).

The degradation of free sugars, which are mainly present
as metabolic intermediates hardly involves the cleavage of
glycosidic bonds, the rate limiting step in the degradation
of carbohydrates. It is therefore not surprising that free
sugars are degraded almost instantaneously.

3.2. Extent of degradation

Only a few detailed studies are available on the extent
of degradation in the forestomachs of ruminants for the
various classes of carbohydrates. Most studies on the par-
titioning of digestion of dietary components are restricted
to organic matter, energy and (crude) protein. From these
studies it appears that usually 60-70% of the total apparently
digestible organic matter is digested in the forestomachs. Dif-
ferences in the extent of digestion of the different carbo-
hydrates were however reported. These findings can be summar-
ized as follows:

Within the structural carbohydrates total apparent di-
gestion of cellulose and hemicellulose varies, but of both
digestion is mainly restricted to the forestomachs, particu-
larly in dairy cows (Van't Klooster and Gaillard, 1976).
Hemicellulose appears to be slightly more resistant against
degradation in the forestomachs than cellulose. In sheep the
proportion of total digestion of cellulose and hemicellulose
taking place in the (fore)stomachs tends to decrease with a
decreasing total apparant digestion (Ulyatt et al., 1975).
The digestion of pectic substances in the forestomachs in
sheep appeared to be virtually complete (Egan et al., 1975).

Within the non-structural carbohydrates, storage polysac-

charides usually show a high apparent digestion. Within these,
starches show differences depending on their origin. Digestion
of barley starch occurs almost completely in the forestomachs
but of corn starch and sorghum starch amounts of over 20% may
escape degradation in the forestomachs (Waldo, 1973). Of the
water soluble polysaccharides (fructosans, saccharose) sig-
nificant amounts also appear to escape degradation in the
forestomachs. This may be because water soluble polysaccha-
rides of microbial origin and mucus enter the small intestine
resulting in a relatively low apparent digestion (65-75% of
the total digestion) in the forestomachs (Van 't Klooster
and Gaillard, 1976). Free sugars are almost completely diges-
ted in the forestomachs.

Some results on site of digestion of different carbohy-
drates, mainly obtained in dairy cows are shown in table 2.

A crude estimate of the extent of degradation of struc-
tural and non-structural carbohydrates can also be obtained
by comparing the site of digestion of crude fibre (represen-
ting part of the structural carbohydrates) and N-free ex-
tracts (representing most of the non-structural carbohydrates)
respectively. In our research with dairy cows, fitted with

Carbohydrate source	Total apparent digestion (% of intake)	Digestion in forestomachs (% of total app.digestion)	Reference(s)
cellulose	62-86	95-103	(19)
hemicellulose	53-81	97-102	(19)
pectic substances[1]	95-100	95-100	(11)
starch[2]	90-100	75-100	(19,37)
fructosans saccharose	65- 90	65- 75	(19)
free sugars	90-100	80- 95	(19)

Table 2. Apparent digestion of carbohydrates in ruminants and
the proportion of this digestion occurring in the
(fore-)stomachs.

1): In sheep   , 2): Sheep data included

re-entrant cannulae in the small intestine such studies were
performed. A range of diets consisting of long roughage with
ground and pelleted mixed concentrates in various proportions
were fed at various levels of intake. Mean total apparent
digestion was found to be 0.70 (s_d=0.083) and 0.84 (s_d=0.40)
for crude fibre and N-free extracts respectively of
which proportions of 0.89 (s_d=0.097) and 0.77 (s_d=0.050) were
digested in the stomachs. The results were analyzed by mul-
tiple regression analysis. Although not statistically signif-
icant, a tendency was found that total apparent digestion of
both crude fibre and N-free extracts slightly decreased with
increasing dry matter intake, and that the digestion of crude
fibre tended to shift from the forestomachs towards the in-
testines with an increasing dry matter intake.

Comparing sheep and dairy cows some differences with
respect to site of digestion of carbohydrates seem to exist,
particularly for structural carbohydrates. In dairy cows the
contribution of the large intestine seems of minor importance,
whereas in sheep fed chopped forages proportions of up to 30%
of the digestion of structural carbohydrates taking place in
the large intestine were reported (Ulyatt et al., 1975). A
possible explanation of this apparent difference is that com-
pared with sheep, dairy cows are generally fed more digest-
ible diets.

4. ATP-yields of fermented carbohydrates in the forestomachs

In the fermentation of carbohydrates a number of stages
can be (rather arbitrarily) distinguished, such as:
- degradation of polysaccharides to monosaccharides
- conversion of monosaccharides to pyruvate
- conversion of pyruvate to volatile fatty acids
- reduction of carbondioxide to methane.

Degradation of polysaccharides to monosaccharides means
hydrolytic cleavage of glycosidic bonds in which no ATP
generation occurs. However, as a final step of degradation of
polysaccharides to monosaccharides, phosphorylytic cleavage
of the glycosidic linkages in disaccharides seems also to
occur in the rumen. Cellobiosephosphorylase, sucrosephos-
phorylase and maltosephosphorylase were shown to be present

in rumen micro-organisms (Prins, 1977). These enzymes cata-
lyze the general reaction:

$$\text{disaccharide} + P_i \longrightarrow \text{monosaccharide} + \text{monosaccharide-phosphate}$$
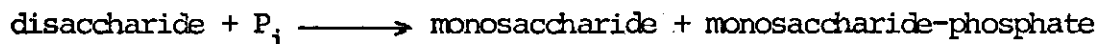
The importance of these reactions in the degradation of poly-
saccharides in the rumen is however uncertain (Prins, 1977).

Various metabolic pathways for the degradation of mono-
saccharides are known to occur in the rumen, but the Embden-
Meyerhof pathway (EMP) predominates (Prins, 1977). This path-
way leads to the formation of pyruvate. Activation of the
monosaccharides is necessary, requiring 2 moles of ATP per
mole of hexose. If phosphorylases are involved in the degra-
dation of disaccharides to monosaccharides, the resulting
hexosephosphate requires only 1 mole of ATP for activation.
After activation of hexose in the degradative pathway of hex-
ose to pyruvate, 4 moles of ATP are generated per mole of
hexose converted, resulting in a net yield of 2 moles of ATP
(2.5 moles if phosphorylases are operative) per mole of hexose
converted.

One of the other pathways of degradation of monosaccha-
rides, the pentose phosphate cycle, may become of significance
if the dietary carbohydrates contain substantial amounts of
pentoses. The pentoses are converted to the glycolytic inter-
mediates hexose-phosphate and triose-phosphate, then ultimately
to pyruvate. The conversion of 6 moles of pentose will yield
4 moles of hexose-phosphate and 2 moles of triose-phosphate,
resulting in a net yield of 10 moles of ATP. So, per hexose-
equivalent (a non-hydrolyzed monomer in polysaccharides with
a molecular weight of 162 and equivalent to 1.20 mole of
pentose), pentose based carbohydrates yield the same amount
of ATP than hexose based carbohydrates.

The main end products of the conversion of pyruvate are
acetate, propionate, butyrate and carbondioxide.

Pyruvate can be converted to either acetylCoA, CO_2 and
H_2 (1) or to acetylCoA and formate (2). In both cases acetate
can be liberated from acetylCoA, often after a conversion to
acetyl-phosphate.

Propionate can be formed by two pathways. One involves
succinate as an intermediate and is known as the "dicarboxylic

acid pathway". The other involves lactylCoA as an intermediate and is called the "direct reductive pathway" or the "acrylate pathway". Under normal conditions the dicarboxylic pathway predominates, but if high grain diets are fed the acrylate pathway may become important.

The formation of butyrate occurs usually by a reversal of β-oxidation from two moles of acetylCoA. From the resulting butyrylCoA, butyrate can be generated, often after conversion to butyryl-P.

The degradative pathways involved in the conversion of pyruvate to VFA comprises of a number of steps, some of which are associated with a free energy change large enough to enable the generation of ATP. The generation of ATP from ADP and P_i requires a free energy change under standard conditions $(\Delta G'_o)$ of at least 7.6 kcal/mole (Thauer et al., 1977). In figure 1 a survey is given of the $\Delta G'_o$ (derived from Thauer et al., 1977) of the various steps in the pathways of conversion of pyruvate to VFA.

Comparing the $\Delta G'_o$ required for the generation of ATP with these in figure 1 for the various steps, suggests the possibility for the generation of ATP at a number of sites. However, the actual conditions in the rumen or within a bacterial cell may differ markedly from the standard conditions under which ΔG_o was observed, which may prevent ATP
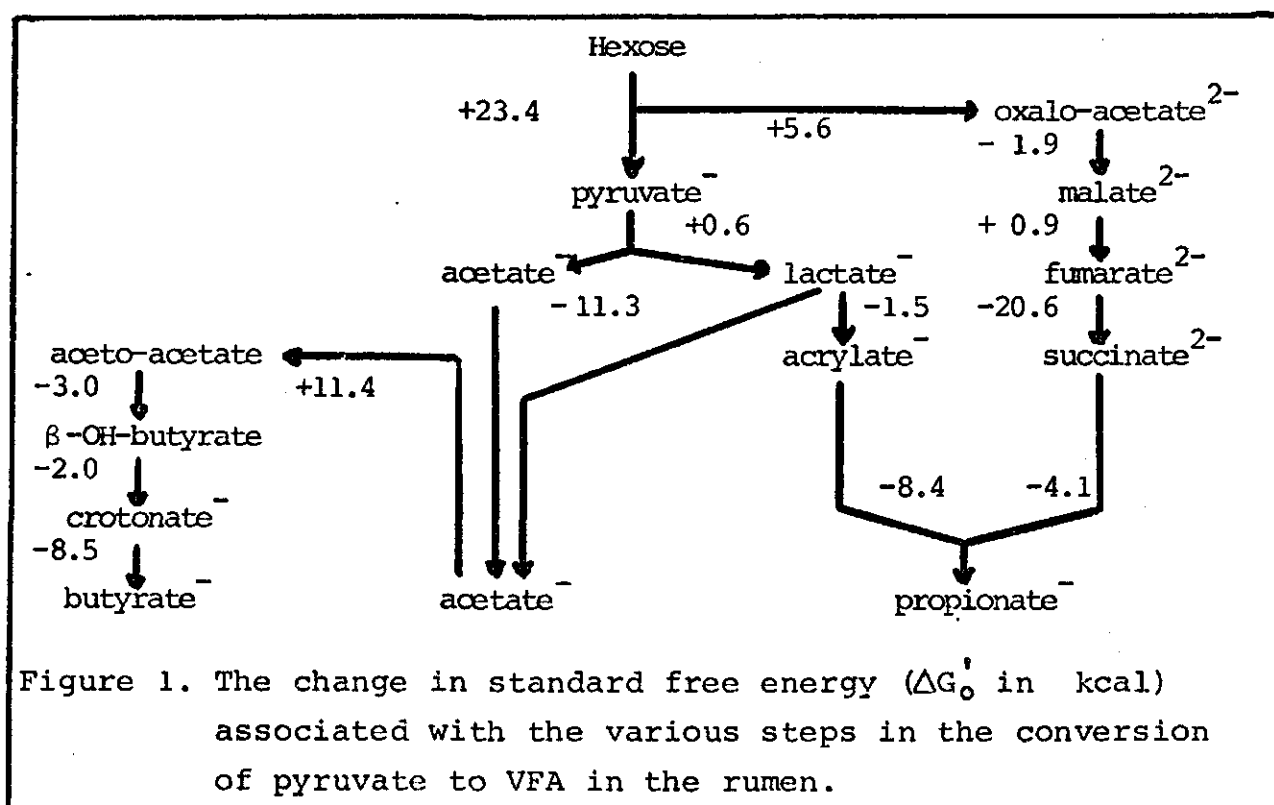

Figure 1. The change in standard free energy ($\Delta G'_o$ in kcal) associated with the various steps in the conversion of pyruvate to VFA in the rumen.

generation at certain steps.

It is beyond doubt that the formation of acetate and
butyrate from acetylCoA and butyrylCoA via acetyl-P and buty-
ryl-P respectively, yields 1 mole of ATP. It should be noted
that because of the formation of acetoacetylCoA from 2 moles
of acetylCoA 1 potential site of ATP-generation is lost.
There is however some evidence that the reduction of croton-
ylCoA to butyrylCoA may yield additional ATP in some strains
of rumen bacteria, but not in others (Prins, 1977).

With respect to the formation of propionate via the di-
carboxylic pathway the reduction of fumarate to succinate
causes a $\Delta G_o'$ large enough to permit ATP-generation, and
this seems to be the case in a number of strains of rumen
bacteria (Prins, 1977; Reddy and Peck, 1978). It has even
been assumed that this reduction yields 2 moles of ATP in cer-
tain strains (Stouthamer, 1976).

In the other propionate generating pathway the reduction
of acrylate to propionate may yield ATP, but in the conversion
of lactate to acrylate, lactylCoA is activated to phospholac-
tate, which in turn is converted to acrylyCoA. This activation
is at the expense of acetyl-P (which would normally result in
the formation of ATP), thus nullifying the ATP yield due to
the reduction of acrylate.

The conversion of carbohydrates to pyruvate and further
to VFA results in a surplus of reduction-equivalents, either
as NADH or as H_2. For matters of convenience the term reduc-
tion-equivalents is used for both. This "pool" of reduction
equivalents is very important in the regulation of the fer-
mentation pattern.

In the conversion of carbohydrates to pyruvate the re-
duction equivalents become present as NADH. In order to keep
the fermentation going, an oxidation of this compound back
to NAD^+ is required. This is possible by the generation of H_2:

$$NADH + H^+ \longrightarrow NAD^+ + H_2$$

The equilibrium of this reaction lies very much to the left and the reaction will only proceed if the partial pressure of H_2 (PH_2) is extremely low. This low PH_2 can be maintained in the rumen because the H_2 is captured by methanogenic bacteria and used for the reduction of CO_2 to CH_4. Although the exact mechanism of CH_4-formation is still rather obscure, it is generally believed that CH_4-formation yields 1 mole of ATP per mole of CH_4 generated (Demeijer, 1976; Thauer et al., 1977).

The degradation of pyruvate to acetylCoA also gives yield to the formation of H_2, without NAD^+ being required. This H_2-formation proceeds even at high concentrations of H_2 (Thauer, et al., 1977). If the methanogenic bacteria can keep the PH_2 low enough the fermentation of carbohydrates will proceed, mainly to acetate. If however the PH_2 becomes too high, for instance because the fermentation rate and therefore the rate of H_2-formation from the conversion of pyruvate to acetylCoA is too high, the liberation of H_2 from NADH will no longer proceed. To prevent accumulation of NADH, other electron acceptors have to be found and the fermentation is diverted from acetate towards butyrate and/or propionate. The formation of butyrate from acetylCoA means the consumption of reduction-equivalents of NADH. The formation of propionate also means the uptake of reduction equivalents. By diverting the pattern, fermentation can proceed.

Diverting the fermentation pattern away from the generation of CH_4, means the loss of the generation of 1 mole of ATP for each 8 reduction equivalents, which is usually not compensated in the diverted fermentation pattern. It therefore frequently means an energetically less favourable system and diversion is usually restricted to what is absolutely necessary. The result may be a mixed fermentation with for instance the production of acetate and enough butyrate to consume the reduction equivalents of NADH generated in the conversion of hexose to pyruvate (Thauer et al., 1977).

Figure 2 shows a simplified biochemical pathway of the degradation of polysaccharides and the sites where ATP is believed to be generated, or where ATP (or other energy rich compounds) is needed for activation.
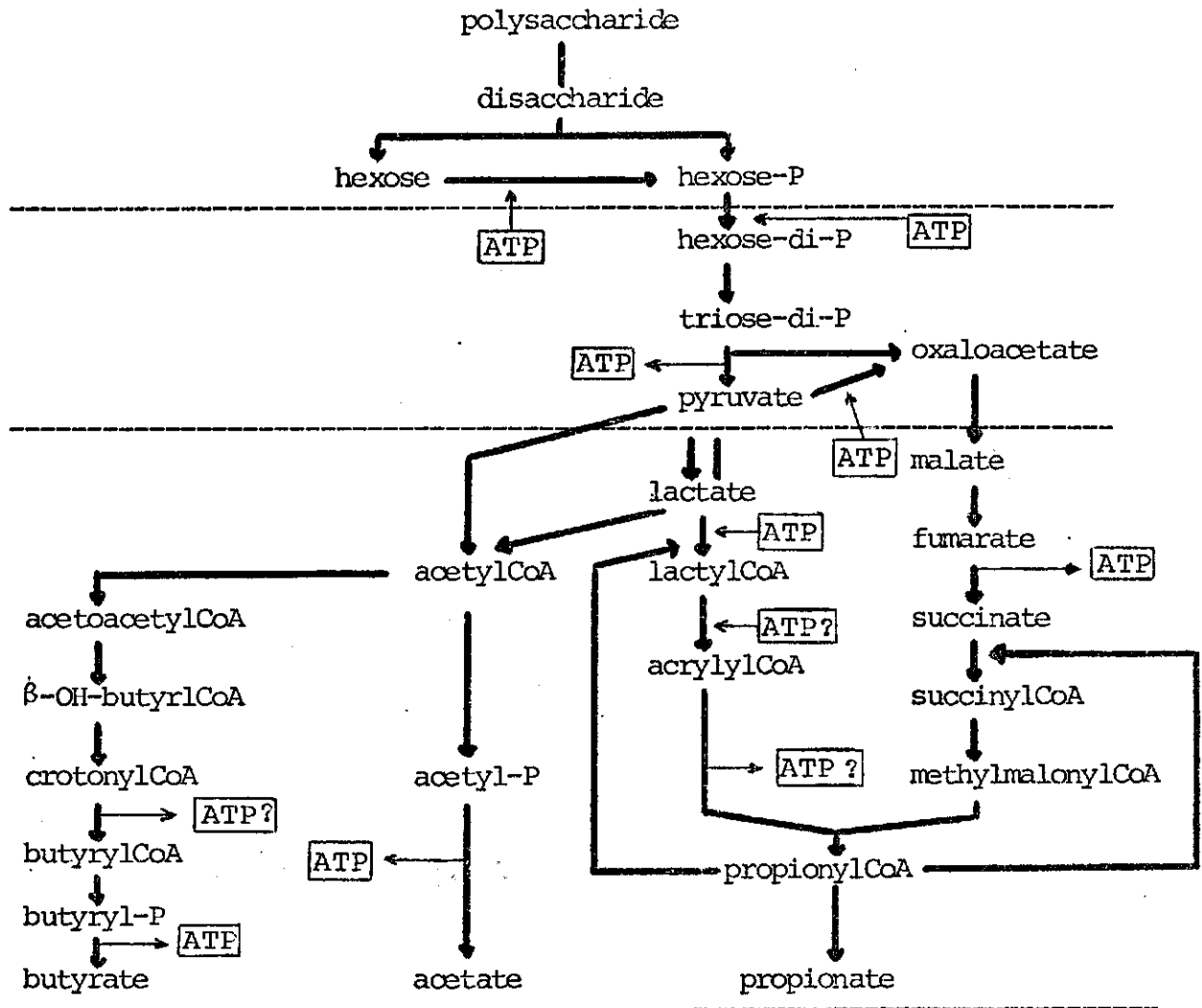
Fig. 2. Simplified pathway for the degradation of carbohydrates in the rumen and sites of ATP generation.

In table 2 a summary is given of the ATP yields per hexose-equivalent if acetate, butyrate, propionate or lactate are the only end product of fermentation respectively. It appears that differences in ATP yield do occur depending on whether phosphorylation is involved in the cleavage of glycoside bonds of disaccharides or not, depending on which pathway of propionate formation is used and depending on the surplus of reduction equivalents which can be used for the formation of CH_4, yielding 1 mole of ATP per surplus of 8 reduction equivalents.

acid	direct ATP-yield	[H]	total ATP-yield
acetate	$4^{1)}$-$4.5^{2)}$	+8	5 - 5.5
butyrate	3 -3.5	+4	4.0 - 5.0
propionate I$^{3)}$	3 -6.5	-4	3.0 - 6.0
propionate II$^{4)}$	2 -2.5	-4	1.5 - 2.0
lactate	2 -2.5	0	2 - 2.5

Table 2. ATP-yield per hexose equivalent converted to the
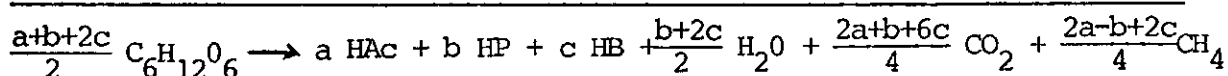various volatile fatty acids.
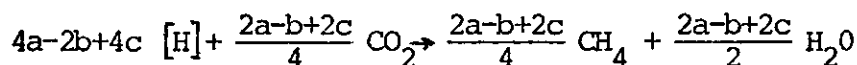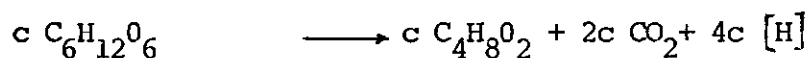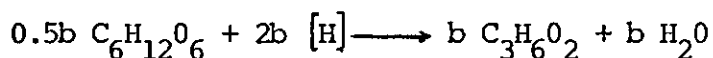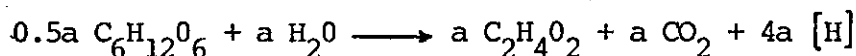1) no disaccharide-phosphorylases involved
2) disaccharide-phosphorylases involved
3) dicarboxylic acid pathway
4) direct reductive pathway.

Under certain conditions, particularly when concentrate
rich diets are fed, lactic acid may become the end product
of rumen fermentation in significant quantities. From the
schematic pathway in figure 2 it can easily be seen that the
ATP yield per hexose equivalent converted to lactate is low
compared with the conversion in volatile fatty acids, the
conversion to propionate via the acrylate pathway excepted.

The degradation of carbohydrates to volatile fatty
acids and fermentation gases can be represented stoichio-
metrically as:

$$0.5a\ C_6H_{12}O_6 + a\ H_2O \longrightarrow a\ C_2H_4O_2 + a\ CO_2 + 4a\ [H]$$

$$0.5b\ C_6H_{12}O_6 + 2b\ [H] \longrightarrow b\ C_3H_6O_2 + b\ H_2O$$

$$c\ C_6H_{12}O_6 \longrightarrow c\ C_4H_8O_2 + 2c\ CO_2 + 4c\ [H]$$

$$4a-2b+4c\ [H] + \frac{2a-b+2c}{4}\ CO_2 \longrightarrow \frac{2a-b+2c}{4}\ CH_4 + \frac{2a-b+2c}{2}\ H_2O$$

$$\frac{a+b+2c}{2}\ C_6H_{12}O_6 \longrightarrow a\ HAc + b\ HP + c\ HB + \frac{b+2c}{2}\ H_2O + \frac{2a+b+6c}{4}\ CO_2 + \frac{2a-b+2c}{4}CH_4$$

			ATP/mol of hexose	
HAc :	HP :	HB	Max.	Min.
0.50	0.35	0.15	5.2	4.2
0.60	0.25	0.15	5.2	3.8
0.70	0.15	0.15	5.3	3.5
0.60	0.15	0.25	5.0	4.0

Table 3. ATP-yield per mole of hexose converted to volatile
fatty acids in various molar proportions in the
rumen of dairy cows.

Based on these stoichiometric relationship of degradation of
hexose units to volatile fatty acids in the proportions a,
b and c for acetate, propionate and butyrate respectively,
ATP yields per mole of hexose fermented can be calculated
for various fermentation patterns. The results of such cal-
culations for a number of characteristic VFA ratios in rumen
fermentation of dairy cows are shown in table 3. From the
results it appears that relatively small differences in ATP
yield per hexose equivalent fermented occur if the fermen-
tation pattern shifts from an acetate based fermentation to
a propionate or butyrate based fermentation. The main dif-
ferences appear as a result of the involvement of phosphor-
ylases in the cleavage of glycosidic bonds and also depend
on which of the two propionate generating pathways is used.

If diets containing only roughages and therefore con-
siderable amounts of structural carbohydrates are fed, the
proportion of acetate in the rumen is usually high and often
exceeds 0.65. Propionate and butyrate are found in much
smaller proportions of between 0.15 and 0.20 or even lower
(see Church, 1970).

If on the other hand diets containing large quantities
of non-structural carbohydrates are fed the proportion of
propionate may rise considerably. Particularly extruded or
steam-flaked grains in the diet may cause very high propor-
tions of propionate (up to 0.40 or even higher). During the
changeover of a diet rich in structural carbohydrates to a
diet rich in non-structural carbohydrates temporarily high
proportions (up to 0.25) of butyrate may occur (Van Vuuren,

personal communication). Also high levels of lactic acid are possible and it is believed that propionate formation via the acrylate pathway becomes important. This suggests that feeding excessive amounts of concentrates to dairy cows may reduce the ATP-yield per hexose equivalent fermented in the rumen.

Diets containing large amounts of free sugars are not often fed. Exceptions may be diets containing large quantities of sugar-beets, sugar-beet pulp, sugar-cane, molasses or fresh young grass in spring. If such diets are fed high proportions of butyrate and propionate may be the result as was found if diets containing large amounts of sugar-beets (Kaufmann and Rohr, 1966), sugar-beet pulp (Bath and Rook, 1965), molasses (Marty and Preston, 1970) and to a lesser extent perennial ryegrass and Italian ryegrass while grazing (Bath and Rook, 1965) were fed.

It should be realised that high rates of fermentation result in high acid production rates, causing a decrease in ruminal pH which may also affect microbial growth.

5. Efficiency of utilisation of carbohydrates as an energy source for microbial growth and microbial protein production

In discussing the principles of microbial growth it was stated that the energetic efficiency of microbial growth depends on the growth rate. One of the factors determining growth rate is the rate of ATP-generation in the degradation of carbohydrates. This seems to place non-structural carbohydrates in a favourable position because of their fast degradation rate in the forestomachs (Johnson, 1976).

From the fermentation patterns discussed earlier it became evident that excessive amounts of non-structural carbohydrates are less efficient in generating ATP, thus nullifying to a certain extent the advantage of the high rate of degradation.

Besides the supply of substrate in ruminant feeding is often discontinuous. For instance, during the indoor period the supply of substrate is usually restricted to twice daily, particularly for non-structural carbohydrates. As a consequence the rate of microbial growth is often discontinuous.

Shortly after the intake of large amounts of non-structural carbohydrates by the host animal the microbial population in the rumen will start growing at a very high rate. This phase of (exponential) growth, sometimes preceded by a short lag phase, is followed by a period of maximum stationary growth, but passes soon into a so-called "death phase". During this latter period, in between the maximum growth rate and the supply of new substrate, energy (ATP) becomes limiting, possibly even for maintenance of the microbial population. The microbial population starts "consuming itself", micro-organisms die, disintegrate and are degraded. The resulting (small amounts of) ATP can be used to meet the maintenance requirement of the surviving part of the population. The final size of the latter depends on the length of the death phase i.e. the time between maximum growth and the next feeding. The result is a decrease in the net production of biomass and a lower energetic efficiency.

If the supply of non-structural carbohydrates is abundant, significant quantities of monomers are taken up by the micro-organisms and stored as polysaccharides. In both bacteria and protozoa values of up to 35% of polysaccharides in the dry matter were reported (McAllan and Smith, 1974; Czerkawski, 1976). If the energetic efficiency of microbial protein production is considered, as is often done in ruminant nutrition, any variation in the synthesis of compounds other than protein will affect its apparent energetic efficiency. This is illustrated in table 5 where the theoretic energetic efficiency of microbial protein synthesis is compared for microbial mass with different chemical composition.

From table 4 it becomes evident that after feeding diets rich in non-structural carbohydrates the microbial protein production may seem less efficient because part of the energy is required for the synthesis of compounds other than proteins.

Changing the feeding pattern by more frequent feeding, particularly of the non-structural carbohydrates containing part of the diet, will restrict the amount of substrate available at one time and will therefore restrict the maximum

size of the microbial population. It will also restrict the
death period and thus decrease the recycling of microbial
mass. This would enable a more efficient microbial growth

macromolecule	ATP/100 g[1]	% in DM	ATP	% in DM	ATP
N x 6.25	4.5	35	1.58	65	2.93
carbohydrates	1.2	35	0.42	5	0.06
residue	0.1	30	0.03	30	0.03
total ATP			2.03		3.02
.total ATP/100 g N x 6.25			5.80 (125)		4.65 (100)

1) Estimated from Hespell and Bryant, 1979.

Table 4. Total ATP required for the synthesis of 100 g crude
protein in microbial mass of differing chemical
composition.

and microbial protein production. In sheep this could be con-
firmed in vivo when the animals were fed a diet consisting of
grass nuts and mixtures of barley and sucrose. Feeding these
diets at 2 hourly intervals increased the energetic efficiency
of microbial protein production from 3.0 g microbial protein/
MJ of energy truly digested in the forestomachs in 4.6 g/MJ
(Al Attar et al., 1976).

In a recent experiment in our institute 3 dairy cows
were fed the concentrate part of the diet in 6 portions per
day rather than twice daily. The animals were fed diets con-
sisting of long hay (25% of the dry matter) and concentrates
(75% of the dry matter) largely (animal no.3) or entirely
(animals no.1 and 2) consisting of flaked maize. Crude protein
content of the diet was 10.8% in the dry matter for the ani-
mals 1 and 2 and 13.5% in the dry matter for animal 3.
Frequent feeding also increased the flow of N in the small
intestine as is shown in table 5. The difference in N-flow
was thought to be the result of a difference in microbial
protein production.

Feeding diets mainly based on long roughages will result
in a more continuous degradation of the carbohydrates, be-

cause structural carbohydrates form a major part. Comparing
the energetic efficiency of microbial protein production in

Treatment		1	2	3	Mean
A	Intake (gN/day)	92.2	95.8	230.9	
	Duodenal N (% intake)	133	133	113	126
B	Intake (gN/day)	96.0	89.1	229.1	
	Duodenal N (% of intake)	157	179	148	161
	Duodenal N (Ratio B/A)	1.18	1.35	1.31	1.28

Table 5. The effect on duodenal N-flow of feeding the con-
centrate part of the diet in 6 times per day
(treatment B) compared to 2 times per day (treat-
ment A) in dairy cows.

animals fed roughage diets with diets mainly based on concen-
trates showed a much better efficiency with roughage diets
(McMeniman et al., 1976). Feeding the roughage diets resulted
in 3.3 ± 0.1 g of microbial N synthesized per 100 g OM truly
fermented in the rumen; after feeding concentrates this figure
was only 2.2 ± 0.2 g. Since the concentrate part of the latter
diets consisted of maize or barley the possibility of N being
limiting for maximum microbial growth can however not entirely
be excluded.

Only a limited number of experiments in which the effect
of the dietary carbohydrate source upon microbial protein
production was studied, were reported in literature.

In a continuous culture system which was supplemented
with 3 substrates differing in non-structural carbohydrate con-
tent the effect on microbial protein production was studied
(Stern et al., 1978). Increasing the ratio non-structural car-
bohydrates to structural carbohydrates increased the amount of
microbial N produced per 100 g of dry matter fermented (table 6).

Non-structural carbohydrates (%/DM)	M i c r o b i a l N (g/100 g DM fermented)	as % of the highest value
49	3.12	100
33	2.80	90
20	2.40	77

Table 6. Effect of carbohydrates on ruminal microbial protein synthesis (Stern et al., 1978).

In another experiment sheep were fed at 2-hourly intervals a protein rich basal diet (soy been meal + dried grass) which was supplemented with wheat starch, paper or a mixture of both (Offer et al., 1978). The production of microbial amino acids was highest if the basal diet was supplemented with the mixture (table 7).

Main dietary carbohydrate source	M i c r o b i a l N (g/MJ) fermented	as % of the highest value
none	7.6	53
starch	6.3	44
paper	5.5	42
starch+paper	14.4	100

Table 7. Effect of carbohydrates on ruminal microbial protein synthesis (Offer et al., 1978).

Only few data are available on the effect of the presence of free sugars in the diet on the efficiency of microbial protein production. When the barley (315 g/day) in the experiments of Al Attar et al., (1976) was entirely replaced by sucrose (228 g/day), the efficiency of microbial protein

production was severely reduced, both with feeding once daily
and with feeding every 2 hours. If only part of the barley
was replaced no such effect was found (table 8).

Dietary supplement		Microbial N	as % of the
Barley	Sucrose	(g/MJ fermented)	highest value
315	0	4.60	100
210	76	4.25	92
105	152	4.55	99
0	228	1.90	41

Table 8. Effect of free sugars on ruminal microbial protein
synthesis (Al Attar et al., 1976).

Diets in which the main carbohydrate source was ground
barley straw, ground barley or molasses were fed once daily
to sheep (Oldham et al., 1977). The highest ratio between
duodenal N-flow and N intake was found after feeding the
barley straw containing diet and particularly after feeding
the molasses containing diet this ratio was severely reduced
(table 9).

Main dietary carbohydrate source	Duodenal-N N-ingested	as % of the highest value
straw	0.92	100
barley	0.82	89
molasses	0.69	75

Table 9. Effect of carbohydrates on the duodenal N flow in
sheep (Oldham et al., 1977).

If the carbohydrate source has an influence on the
efficiency of microbial protein production, the ratio between
structural and non-structural carbohydrates fermented in the
forestomachs becomes of interest. For this the ratio between
crude fibre and N free extracts may be used. In our research

with dairy cows equipped with re-entrant cannulae in the small intestine a range of diets with different ratios between long roughage and ground and pelleted concentrates were fed. This resulted in a ratio between crude fibre and NfE fermented in the forestomachs ranging from 0.15 to 0.70. The efficiency of microbial protein synthesis (expressed as microbial N per 100 g of CF+NfE fermented in the forestomachs and based on DAPA as a microbial marker) was related to this ratio and the results are shown in figure 3.

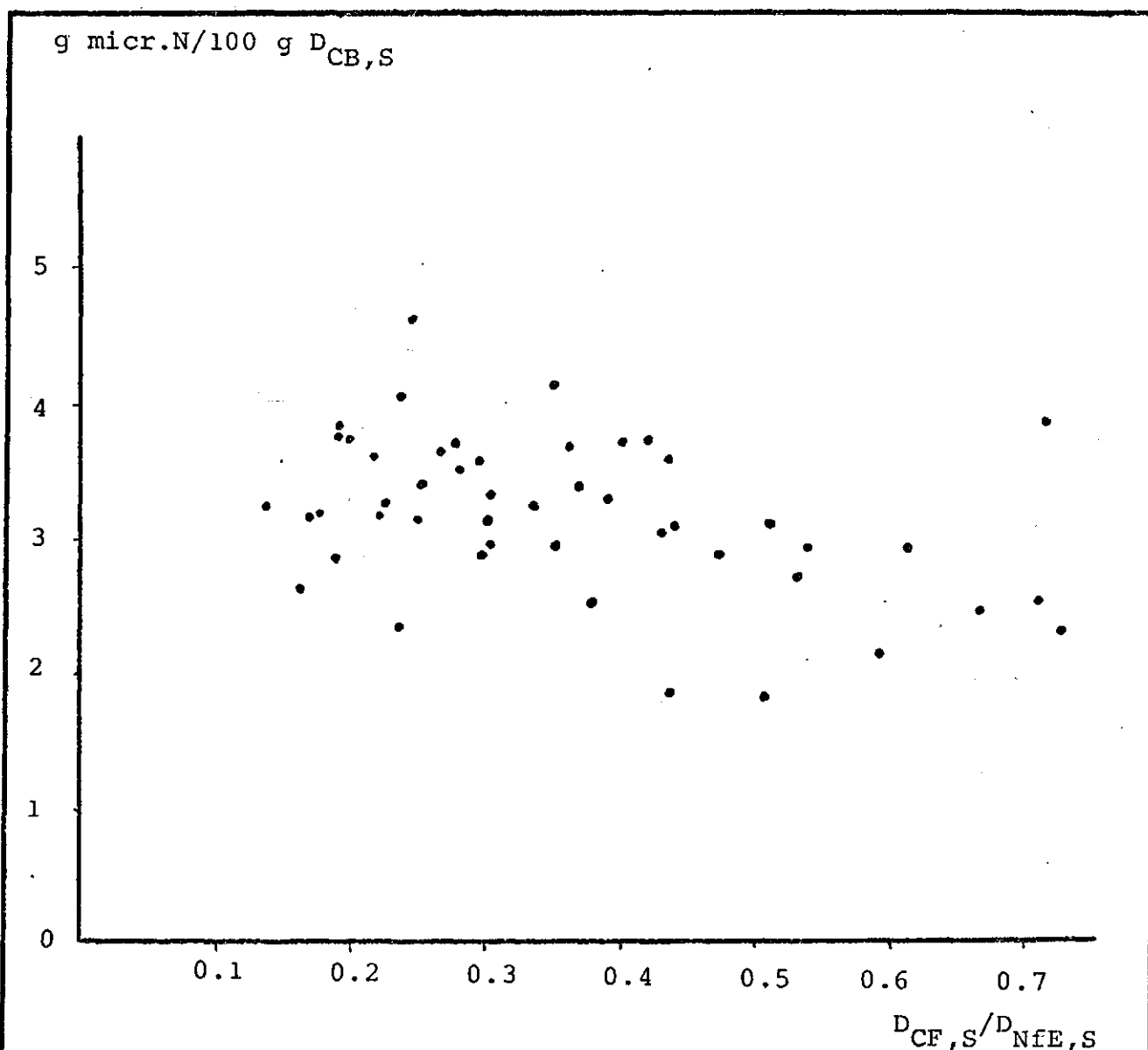g micr.N/100 g $D_{CB,S}$

$D_{CF,S}/D_{NfE,S}$

Figure 3. Relation between the efficiency of microbial N synthesis (g micr. N/100 g carbohydrates fermented in the stomachs) and the ratio between structural and non-structural carbohydrates fermented in the stomachs $(D_{CF,S}/D_{NfE,S})$.

The efficiency of microbial protein production shows a
wide variation, which is not surprising taking into account
all possible factors which may affect this efficiency. If the
results are looked at carefully there seems to be an optimum
ratio between crude fibre and NfE fermented in the forestomachs
of approximately 0.3 which enables maximum efficiency of
microbial protein production. At higher ratios the rate of
degradation and hence the rate of energy (ATP) supply may be-
come limiting for the microbial growth rate; at lower ratios
the pH in the rumen or the production of propionate via the
acrylate pathway or the production of lactate may reduce the
rate of ATP-generation and therefore the rate of growth. The
storage of polysaccharides inside the microbial cells may also
have an influence.

6. Conclusions

As a result of different fermentation patterns the ATP
yield of the degradation of different carbohydrates may differ.
Excessive amounts of non-structural carbohydrates or free
sugars are expected to reduce the ATP-yield.

The efficiency of utilisation of ATP for microbial pro-
tein production may differ with different carbohydrate
sources. This may be caused by differences in the composition
of microbial mass synthesized, in growth rate and in the extent
of recycling of microbial mass.

For achieving a maximum microbial protein production an
optimum ratio between dietary structural and non-structural
carbohydrates seems to exist, but the value of this ratio is
not yet clearly established.

Summary

Protein supply in ruminants depends on dietary protein escaping microbial degradation in the forestomachs and on microbial protein synthesized therein. This latter results from microbial growth which requires a substrate from which energy (ATP) and precursors can be extracted.

Energy for microbial growth mainly results from the conversion of carbohydrates into volatile fatty acids (VFA), fermentation gases and water. This conversion differs between structural and non-structural carbohydrates. Total digestion of structural carbohydrates (cellulose, hemicellulose) is usually lower than of non-structural carbohydrates (starch, fructosans), but a larger proportion is digested in the forestomachs. The rate of degradation of non-structural carbohydrates usually exceeds that of structural carbohydrates. This results in a higher rate of ATP generation and of the formation of "reduction-equivalents". To prevent accumulation of reduction equivalents the fermentation pattern is diverted from mainly acetate to more propionate and/or lactate. This shift often results in a decrease in pH and a decrease in ATP generated per 100 g of carbohydrates fermented.

The energetic efficiency of microbial protein production is influenced by microbial growth rate, the extent of recycling and the composition of microbial growth.

The growth rate largely depends on the rate of ATP generation from the degradation of carbohydrates. Non-structural carbohydrates have an advantage here. Their fast rate of degradation may however initially result in an excessive growth rate but later on in dying off of part of the population because of a shortage of ATP, resulting in a decrease of net growth. This recycling lowers the energetic efficiency of microbial protein production. This disadvantage can be overcome by supplying non-structural carbohydrates in smaller quantities with shorter intervals.

Structural carbohydrates give a lower rate of ATP-generation, resulting in a lower efficiency of growth, because a larger proportion of the energy is required for maintenance. This slow but continuous release not only prevents excessive

growth, but also the dying off of part of the microbial population. The final efficiency of microbial growth is therefore often as high or even higher as after the supply of non-structural carbohydrates.

The composition of microbial growth varies particularly with respect to protein and carbohydrate content. Substantial amounts of carbohydrates may be (temporarily) stored inside microbial cells, particularly if the supply of non-structural carbohydrates is high. The energy required for this storage is not available for protein production and the energetic efficiency of the latter apparently decreases.

From experiments with animals it appears that feeding concentrates more frequently in smaller quantities has a positive effect on the amount of microbial protein synthesized per 100 g organic matter fermented in the forestomachs. Large amounts of free sugars appear to have a negative influence on the microbial protein synthesis. Between structural and non-structural carbohydrates an optimal ratio seems to exist at which the microbial protein production in the forestomachs reaches a maximum efficiency but this optimum could not yet accurately be determined.

Samenvatting

Herkauwers zijn voor hun eiwitvoorziening afhankelijk van twee eiwitbronnen t.w. voereiwit dat niet-afgebroken de voormagen passeert en microbieel eiwit dat in diezelfde voormagen wordt gesynthetiseerd. Dit laatste is afhankelijk van microbiële groei in de voormagen, waarvan het wezen in het onderhavige artikel wordt uiteengezet met speciale aandacht voor de relatie met de verschillende koolhydraten in het rantsoen.

Microbiële groei hangt vooral samen met de beschikbaarheid van een substraat waaruit energie (ATP) en bouwstenen kunnen worden verkregen. Energie voor microbiële groei komt voornamelijk beschikbaar uit de omzetting van koolhydraten in vluchtige vetzuren (VFA), fermentatiegassen en water. De hoeveelheid die wordt afgebroken is afhankelijk van de aard

van de koolhydraten (structurele en niet-structurele kool-
hydraten). De totale vertering van structurele koolhydraten
(cellulose, hemicellulose) is doorgaans lager dan die van
niet-structurele (zetmeel, fructosanen, vrije suikers),
maar een groter deel van de vertering vindt plaats in de
voormagen.

De afbraak van niet-structurele koolhydraten in de
voormagen verloopt doorgaans sneller dan die van structurele
koolhydraten. Deze snelle afbraak resulteert niet alleen in
een snel beschikbaar komen van veel ATP, maar ook van veel
"reductie-equivalenten". Het wegwerken van de laatsten ver-
schuift de verhouding waarin vluchtige vetzuren worden ge-
vormd van veelazijnzuur naar meer propionzuur en/of melk-
zuur. Deze verschuiving gaat meestal enerzijds gepaard met
een verlaging van de pH en anderzijds met een verlaging van
de hoeveelheid ATP welke per 100 g afgebroken koolhydraten
wordt gevormd.

De energetische efficientie van microbiële eiwitpro-
ductie hangt samen met de groeisnelheid, de mate van re-
cycling en de samenstelling van de microbiële groei.

De groeisnelheid hangt sterk af van de snelheid waar-
mee ATP uit de afbraak van koolhydraten kan worden vrijge-
maakt. In dit opzicht zijn niet-structurele koolhydraten in
het voordeel. De afbraak ervan verloopt echter vaak zo snel,
dat na een aanvankelijke stormachtige groei er een tekort
optreedt aan substraat en daarmee aan ATP. De beschikbaar
komende ATP is geheel nodig voor onderhoud en resulteert
niet meer in groei. Vaak vindt zelfs afsterving van een deel
der micro-organismen plaats, waardoor reeds geproduceerd ei-
wit weer wordt vernietigd en de netto eiwitproduktie afneemt.
Deze "recycling" verlaagt de energetische efficientie van
microbiële eiwitproductie. Het met korte tussenpozen ver-
strekken van kleine hoeveelheden niet-structurele koolhy-
draten lijkt deze nadelen grotendeels te kunnen opheffen.

Wanneer structurele koolhydraten als substraat beschik-
baar zijn is de toevoer van ATP langzamer, resulterend in
een langzamer microbiële groei met een groter aandeel van de
energie nodig voor onderhoud. De geleidelijkheid van het
vrijkomen van ATP voorkomt echter een explosieve groei

en daarmee ook het daarna afsterven van een deel van de
micro-organismen, waardoor de uiteindelijke groei en eiwit-
productie minstens zo efficient zijn als bij niet structurele
koolhydraten.

De samenstelling van de microbiële groei kan varieren,
vooral wat het gehalte aan eiwit en koolhydraten betreft.
Koolhydraten kunnen (tijdelijk) in aanzienlijke hoeveelheden
in microbiële cellen worden opgeslagen, vooral na het ver-
strekken van veel niet-structurele koolhydraten. De hiervoor
nodige energie (ATP) is niet meer beschikbaar voor microbiële
eiwitsynthese en de energetische efficientie van de laatste
neemt schijnbaar af.

Uit dierproeven blijkt dat vaker voeren van vooral
krachtvoer een gunstig effect heeft op de hoeveelheid micro-
bieel eiwit die per 100 g in de voormagen afgebroken orga-
nische stof wordt geproduceerd. De aanwezigheid van veel
vrije suikers daarentegen heeft hierop een negatieve invloed.
Er lijkt een optimale verhouding tussen structurele en niet-
structurele koolhydraten te bestaan, waarbij de efficientie
waarmee in de voormagen microbiëel eiwit wordt gevormd, maxi-
maal is, maar een nauwkeurige vaststelling van dit optimum is
tot nu toe niet mogelijk gebleken.

Literature

1. Al Attar, A., R.A. Evans and R.F.E. Axford, 1976
   The effect of frequency of feeding and of dietary energy
   source upon microbial protein synthesis in the rumen of
   sheep
   Proc. Nutr. Soc. 35:109A

2. Armstrong, D.G., 1976
   Proteinverdauung und -absorption bei Monogastriden und
   Wiederkäuern.
   Ubers. Tierernährng. 4:1-24

3. Bath, I.H. and J.A.F. Rook, 1965
   The evaluation of cattle foods and diets in terms of the
   ruminal concentrations of volatile fatty acids
   J. Agric. Sci. (Camb.), 64:67-75

4. Bauchop, T. and S.R. Elsden, 1960
   The growth of micro-organisms in relation to their energy
   supply
   J. Gen. Microbiol. 23:457-469

5. Chalupa, W., 1978
   Digestion and absorption of nitrogenous compounds in
   ruminants
   Page 211-230 in Proc. 3rd Wrld Conf. on An. Feeding, Madrid

6. Clark, J.H., 1975
   Nitrogen metabolism in ruminants: protein solubility and
   rumen bypass of protein and amino acids
   Page 261 in M.Friedman (ed.), Protein nutritional quality
   of foods and feeds, vol.I, part II
   Marcel Dekker, New York

7. Cheng, K.J., D.E. Akin and J.W. Costerton, 1978
   Rumen bacteria: interaction with particulate dietary com-
   ponents and response to dietary variation
   Fed. Proc. 36:193-197

8. Church, D.C., 1970
   Digestive Physiology and Nutrition of Ruminants
   O.S.U. Book Stores, Corvallis, Oregon

9. Czerkawski, J.W., 1976
   Chemical composition of microbial mass in the rumen
   J. Sci. Fd. Agric., 27:621-632

10. Demeijer, D.I.,1976
    Een kwantitatieve studie van het metabolisme van pensmaag-
    inhoud
    Thesis State University Ghent, Belgium

11. Egan, A.R., D.J. Walker, C.J. Nader and G. Storer, 1975
    Comparative aspects of digestion of four roughages by
    sheep
    Aust. J. Agric. Res., 26:909-922

12. Es, A.J.H. van and S. Tamminga, 1978
Utilisation of nutrients for net protein synthesis
Page 171-182 in Proc. 3rd Wrld Conf. on An. Feeding, Madrid

13. Hespell, R.B. and M.P. Bryant, 1979
Efficiency of rumen microbial growth; Influence of some theoretical and experimental factors on Y_{ATP}
J. An. Sci. (in press)

14. Hogan, J.P. and R.H. Weston, 1970
Quantitative aspects of microbial protein synthesis in the rumen
Page 474 in A.T. Phillipson (ed.), Physiology of digestion and metabolism in the ruminant
Oriel Press, Newcastle upon Tyne

15. Hungate, R.E., 1966
The rumen and its microbes
Academic Press, New York

16. Johnson, R.R., 1976
Influence of carbohydrate solubility on NPN utilisation in the ruminant
J. An. Sci., 43:184-191

17. Kaufmann, W., 1977
Economic and other considerations governing decisions on the advisability of incorporating additional and new sources of protein and NPN into the diet of beef cattle
Page 101 in, Protein and non protein nitrogen for Ruminants
Pergamon Press, Oxford

18. Kaufmann, W. and K. Rohr, 1966
Ergebnisse gaschromatografischer Bestimmung der flüchtigen Fettsäuren im Pansen bei unterschiedlicher Fütterung
Z. Tierernärng, Tierphysiol. u. Futtermittelkde. 22:1-

19. Klooster, A.Th. van't and B.D.E. Gaillard, 1976
Digestion of carbohydrates in the intestines of dairy cows
Misc. Papers Landbouwhogeschool 12:79-94

20. Marty, R.J. and T.R. Preston, 1970
Molar proportions of the short chain fatty acids (VFA) produced in the rumen of cattle given high molasses diets
Rev. cubana Cienc. agric. 4:183-186

21. McAllan, A.B. and R.H. Smith, 1974
Carbohydrate metabolism in the ruminant. Bacterial carbohydrates formed in the rumen and their contribution to digesta entering the duodenum
Br. J. Nutr. 31:77-88

22. McMeniman, N.P., D. Ben Ghedalia and D.G. Armstrong, 1976
Nitrogen-energy interactions in rumen fermentation
Page 217 in K.N. Boorman et al., (eds.), Protein metabolism and nutrition
Butterworth, London

23. Mertens, D.R., 1977
    Dietary fibre components: relationship to the rate and
    extent of ruminant digestion
    Fed. Proc. 36:187-192

24. Offer, N.W., R.F.E. Axford and R.A. Evans, 1978
    The effect of dietary energy source on nitrogen metabolism
    in the rumen of sheep
    Br. J. Nutr. 40:35-44

25. Oldham, J.D., P.J. Buttery, H. Swan and D. Lewis, 1977
    Interactions between dietary carbohydrates and nitrogen
    and digestion in sheep
    J. Agric. Sci. (Camb.), 89:467-479

26. Prins, R., 1977
    Biochemical activities of gut micro-organisms
    Page 73 in R.T.J. Clarke and T. Bauchop (eds.), Microbial
    ecology of the gut
    Academic Press, London

27 Reddy, C.A. and H.D. Peck, 1978
    Electron transport phosphorylation coupled to fumarate
    reduction by H_2- and Mg^{2+} - dependant adenosine triphos-
    phatase activity in extracts of the rumen anaërobe Vibrio
    succinogenes
    J. Bacteriol., 134:982-991

28. Stern, M.D., H. Hoover, C.J. Sniffen, B.A. Crooker and
    P.H. Knowlton, 1978
    Effects of non-structural carbohydrates, urea and soluble
    protein levels on microbial protein synthesis in contin-
    uous culture of rumen contents.
    J. An. Sci., 47:944-956

29. Stouthamer, A.H., 1973
    A theoretical study of the amount of ATP required for
    synthesis of microbial cell material
    Antonie van Leeuwenhoek, 39:545-565

30. Stouthamer, A.H., 1976
    Yield studies in micro-organisms
    Meadowfield Press Ltd., Durham

31. Stouthamer, A.H. and C. Bettenhaussen, 1973
    Utilisation of energy for growth and maintenance in con-
    tinuous and batch cultures of micro-organisms
    Biochim. Biophys. Acta 301:53-70

32. Tamminga, S., 1978
    Measurement of microbial protein synthesis in the rumen
    Page 5.1 in D.F. Osbourn, D.E. Beever and D.J. Thomson
    (eds.), Ruminant digestion and feed evaluation
    Agricultural Research Council, London

33. Tamminga, S., 1979
    Protein degradation in the forestomachs of ruminants
    J. An. Sci., (in press)

34. Thauer, K., K. Jungermann and K. Decker, 1977
Energy conservation in chemotrophic anaerobic bacteria
Bact. Rev. 41:100-180

35. Ulyatt, M.J., D.W. Dellow, C.S. Reid and T. Bauchop, 1975
Structure and function of the large intestine of ruminants
Page 119 in I.W. McDonald and A.C.I. Warner (eds.),
Digestion and metabolism in the ruminant

36. Waldo, D.R., L.W. Smith and E.L. Cox, 1972
Model of cellulose disappearance from the rumen
J. Dairy Sci., 55:125-129

37. Waldo, D.R., 1973
Extent and partition of cereal grain starch digestion in
ruminants
J. An. Sci., 37:1062-1074