Relation between different carbohydrates and microbial synthesis of protein

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1. Introduction

Ruminant animal production gives an important contribution to the world's human food supply. This is mainly because 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 relationship between ruminant animals and micro-organisms inhabiting 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 carbohydrates 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 intermediary metabolism of ruminants receives protein from two sources viz. dietary protein which escapes microbial degradation 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 anaerobic 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 \( \text{(CO}_2\text{)} \), ammonia \( \text{(NH}_3\text{)} \), 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 anaerobiosis 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 aerobic 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**

- **Structural carbohydrates**
  - Fibre polysaccharides
  - Matrix polysaccharides
  - Storage polysaccharides
- **Non-structural carbohydrates**
  - Metabolic intermediates
  - Polysaccharides
  - Free sugars
  - Celluloses
  - Hemicelluloses
  - Pectic substances
  - Starches
  - Fructosans
  - Saccharose

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 encrustation 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-
inates whereas in celluloses and hemicelluloses β-configurations are more important.

Unlike structural carbohydrates degradation of non-structural carbohydrates does not require a lag period. Degradation 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 partitioning 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. Differences in the extent of digestion of the different carbohydrates were however reported. These findings can be summarized as follows:

Within the structural carbohydrates total apparent digestion of cellulose and hemicellulose varies, but of both digestion is mainly restricted to the forestomachs, particularly 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 apparent 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-
Carbohydrates 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) significant amounts also appear to escape degradation in the forestomachs. This may be because water soluble polysaccharides 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 digested in the forestomachs.

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

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

<table>
<thead>
<tr>
<th>Carbohydrate source</th>
<th>Total apparent digestion (% of intake)</th>
<th>Digestion in forestomachs (% of total apparent digestion)</th>
<th>Reference(s)</th>
</tr>
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<tbody>
<tr>
<td>cellulose</td>
<td>62-86</td>
<td>95-103</td>
<td>(19)</td>
</tr>
<tr>
<td>hemicellulose</td>
<td>53-81</td>
<td>97-102</td>
<td>(19)</td>
</tr>
<tr>
<td>pectic substances(^1)</td>
<td>95-100</td>
<td>95-100</td>
<td>(11)</td>
</tr>
<tr>
<td>starch(^2)</td>
<td>90-100</td>
<td>75-100</td>
<td>(19,37)</td>
</tr>
<tr>
<td>fructosans</td>
<td>65-90</td>
<td>65-75</td>
<td>(19)</td>
</tr>
<tr>
<td>saccharose</td>
<td>90-100</td>
<td>80-95</td>
<td>(19)</td>
</tr>
</tbody>
</table>

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

\(^1\): In sheep \quad \(^2\): 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 multiple regression analysis. Although not statistically significant, 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 intestines 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 compared with sheep, dairy cows are generally fed more digestible 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 carbon dioxide 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. Celllobiosephosphorylase, sucrosephosphorylase and maltosephosphorylase were shown to be present.
in rumen micro-organisms (Prins, 1977). These enzymes catalyze the general reaction:

\[
d\text{disaccharide} + P_i \rightarrow \text{monosaccharide} + \text{monosaccharide-phosphate}
\]

The importance of these reactions in the degradation of polysaccharides in the rumen is however uncertain (Prins, 1977).

Various metabolic pathways for the degradation of monosaccharides are known to occur in the rumen, but the Embden-Meyerhof pathway (EMP) predominates (Prins, 1977). This pathway 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 degradation of disaccharides to monosaccharides, the resulting hexosephosphate requires only 1 mole of ATP for activation. After activation of hexose in the degradative pathway of hexose 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 monosaccharides, the pentose phosphate cycle, may become of significance if the dietary carbohydrates contain substantial amounts of pentoses. The pentoses are converted to the glycolytic intermediates 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, CO₂ and H₂ (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 (ΔG°) of at least 7.6 kcal/mole (Thauer et al., 1977). In figure 1 a survey is given of the ΔG° (derived from Thauer et al., 1977) of the various steps in the pathways of conversion of pyruvate to VFA.

Comparing the ΔG° 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° was observed, which may prevent ATP

![Figure 1](chart.png)

Figure 1. The change in standard free energy (ΔG° 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 acetyl-CoA and butyryl-CoA via acetyl-P and butyryl-P respectively, yields 1 mole of ATP. It should be noted that because of the formation of acetoacetyl-CoA from 2 moles of acetyl-CoA 1 potential site of ATP-generation is lost. There is however some evidence that the reduction of crotonyl-CoA to butyryl-CoA 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 dicarboxylic pathway the reduction of fumarate to succinate causes a $\Delta G^\circ$ 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 certain 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, lactyl-CoA is activated to phospholactate, which in turn is converted to acryly-CoA. 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 reduction-equivalents is used for both. This "pool" of reduction equivalents is very important in the regulation of the fermentation pattern.

In the conversion of carbohydrates to pyruvate the reduction 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 $H_2$:

\[
\text{NADH} + H^+ \rightarrow \text{NAD}^+ + H_2
\]
The equilibrium of this reaction lies very much to the left and the reaction will only proceed if the partial pressure of $H_2$ ($P_{H_2}$) is extremely low. This low $P_{H_2}$ 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 $P_{H_2}$ low enough the fermentation of carbohydrates will proceed, mainly to acetate. If however the $P_{H_2}$ 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.
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 \( \text{CH}_4 \), yielding 1 mole of ATP per surplus of 8 reduction equivalents.
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 stoichiometrically as:

\[
0.5a \text{C}_6\text{H}_{12}\text{O}_6 + a \text{H}_2\text{O} \rightarrow a \text{C}_2\text{H}_4\text{O}_2 + a \text{CO}_2 + 4a [\text{H}]
\]
\[
0.5b \text{C}_6\text{H}_{12}\text{O}_6 + 2b [\text{H}] \rightarrow b \text{C}_3\text{H}_6\text{O}_2 + b \text{H}_2\text{O}
\]
\[
c \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow c \text{C}_4\text{H}_8\text{O}_2 + 2c \text{CO}_2 + 4c [\text{H}]
\]
\[
4a-2b+4c [\text{H}]+ \frac{2a-b+2c}{4} \text{CO}_2 \rightarrow \frac{2a-b+2c}{2} \text{CH}_4 + \frac{2a-b+2c}{2} \text{H}_2\text{O}
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\]
Table 3. ATP-yield per mole of hexose converted to volatile fatty acids in various molar proportions in the rumen of dairy cows.

<table>
<thead>
<tr>
<th>HAc :</th>
<th>HP :</th>
<th>HB</th>
<th>Max.</th>
<th>Min.</th>
</tr>
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<tr>
<td>0.50</td>
<td>0.35</td>
<td>0.15</td>
<td>5.2</td>
<td>4.2</td>
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<td>0.60</td>
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<td>0.15</td>
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<td>0.15</td>
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<td>0.15</td>
<td>0.25</td>
<td>5.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

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 calculations 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 fermentation pattern shifts from an acetate based fermentation to a propionate or butyrate based fermentation. The main differences appear as a result of the involvement of phosphorylases 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 considerable 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 proportions 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 theoretical 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 and microbial protein production. In sheep this could be confirmed 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 consisting 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 animals 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-

<table>
<thead>
<tr>
<th>macromolecule</th>
<th>ATP/100 g¹</th>
<th>% in DM</th>
<th>ATP % in DM</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N x 6.25</td>
<td>4.5</td>
<td>35</td>
<td>1.58</td>
<td>65</td>
</tr>
<tr>
<td>carbohydrates</td>
<td>1.2</td>
<td>35</td>
<td>0.42</td>
<td>5</td>
</tr>
<tr>
<td>residue</td>
<td>0.1</td>
<td>30</td>
<td>0.03</td>
<td>30</td>
</tr>
<tr>
<td>total ATP</td>
<td></td>
<td></td>
<td></td>
<td>2.03</td>
</tr>
<tr>
<td>total ATP/100 g N x 6.25</td>
<td></td>
<td></td>
<td>5.80(125)</td>
<td>4.65(100)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>92.2</td>
<td>95.8</td>
<td>230.9</td>
<td></td>
</tr>
<tr>
<td>Duodenal N (% intake)</td>
<td>133</td>
<td>133</td>
<td>113</td>
<td>126</td>
</tr>
<tr>
<td>B</td>
<td>96.0</td>
<td>89.1</td>
<td>229.1</td>
<td></td>
</tr>
<tr>
<td>Duodenal N (% of intake)</td>
<td>157</td>
<td>179</td>
<td>148</td>
<td>161</td>
</tr>
<tr>
<td>Duodenal N (Ratio B/A)</td>
<td>1.18</td>
<td>1.35</td>
<td>1.31</td>
<td>1.28</td>
</tr>
</tbody>
</table>

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

animals fed roughage diets with diets mainly based on concentrates 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 content the effect on microbial protein production was studied (Stern et al., 1978). Increasing the ratio non-structural carbohydrates to structural carbohydrates increased the amount of microbial N produced per 100 g of dry matter fermented (table 6).
Table 6. Effect of carbohydrates on ruminal microbial protein synthesis (Stern et al., 1978).

<table>
<thead>
<tr>
<th>Non-structural carbohydrates (%/DM)</th>
<th>Microbial N (g/100 g DM as % of the fermented) highest value</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>3.12</td>
</tr>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>33</td>
<td>2.80</td>
</tr>
<tr>
<td></td>
<td>90</td>
</tr>
<tr>
<td>20</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td>77</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Main dietary carbohydrate source</th>
<th>Microbial N (g/MJ as % of the fermented) highest value</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>7.6</td>
</tr>
<tr>
<td>starch</td>
<td>6.3</td>
</tr>
<tr>
<td>paper</td>
<td>5.5</td>
</tr>
<tr>
<td>starch+paper</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

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...
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).

<table>
<thead>
<tr>
<th>Dietary supplement</th>
<th>Microbial N (g/MJ as % of the fermented) highest value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>315</td>
</tr>
<tr>
<td>Sucrose</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>228</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Main dietary carbohydrate source</th>
<th>Duodenal-N as % of the highest value N-ingested</th>
</tr>
</thead>
<tbody>
<tr>
<td>straw</td>
<td>0.92</td>
</tr>
<tr>
<td>barley</td>
<td>0.82</td>
</tr>
<tr>
<td>molasses</td>
<td>0.69</td>
</tr>
</tbody>
</table>

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.

![Graph](image)

**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 \(\frac{D_{CF,S}}{D_{NfE,S}}\).
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 become 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 protein 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 microbiëel 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 koolhydraten). 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 weggewerken van de laatsten verschuift de verhouding waarin vluchtige vetzuren worden gevormd van veelacetaat naar meer propionzuur en/of melkzuur. 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 efficiëntie van microbiële eiwitproductie hangt samen met de groeisnelheid, de mate van recycling en de samenstelling van de microbiële groei.

De groeisnelheid hangt sterk af van de snelheid waarmede ATP uit de afbraak van koolhydraten kan worden vrijgemaakt. 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 afsterfing van een deel der micro-organismen plaats, waardoor reeds geproduceerd eiwit weer wordt vernietigd en de netto eiwitproduktie afneemt. Deze "recycling" verlaagt de energetische efficiëntie van microbiële eiwitproduktie. Het met korte tussenpozen verstrekkken van kleine hoeveelheden niet-structurele koolhydraten lijkt deze nadelen grotendeels te kunnen opheffen.

Wanneer structurele koolhydraten als substraat beschikbaar 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 variëren, vooral wat het gehalte aan eiwit en koolhydraten betreft. Koolhydraten kunnen (tijdelijk) in aanzienlijke hoeveelheden in microbiële cellen worden opgeslagen, vooral na het verstrekken 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 microbiëel eiwit die per 100 g in de voormagen afgebroken organische 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, maximaal is, maar een nauwkeurige vaststelling van dit optimum is tot nu toe niet mogelijk gebleken.
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