# Factors affecting microbial protein synthesis in the rumen with emphasis on diets containing forages

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## Introduction

Microbial protein contributes about two-thirds of the amino acids absorbed by ruminants. Although it is characterised by a relatively high proportion of non-protein nitrogen (25%, AFRC 1992) it has an invaluable role in the nutrition of ruminant animals. The amino acid composition of microbial true protein is similar to that of protein in the main animal products, i.e. milk, lamb and beef (ØRSKOV 1992). Compared with oil seed meals and legume grains (DLG 1976) microbial protein contains a higher proportion of methionine and lysine (STORM and ØRSKOV 1983). In fact, after the ban of feedstuffs of animal origin in ruminant diets, there are no protein sources which would meet animal requirements better than microbial protein.

Although extensive research work has been carried out during the last few decades with the aim of improving the prediction of microbial protein synthesis in the rumen, the level of reliability of currently used models is still low. The aim of this paper is to review the limited work on this field in Slovenia and to discuss some factors which could affect microbial protein synthesis in diets containing forages.

## Factors affecting microbial protein synthesis in the rumen

### The supply of fermentable energy

Energy supply is usually the first limiting factor for microbial growth in the rumen. To estimate the microbial protein yield, modern European protein systems use information which is directly or indirectly used in estimating the energy supply to the animal. The microbial protein yield can be estimated on the basis of metabolizable energy (ME), net energy for lactation (NEL), fermentable ME, digestible carbohydrates (DCHO) or fermentable organic matter (FOM) (INRA 1988, AFRC 1992, DACCORD 1994, TAMMINGA et al. 1994, MADSEN et al. 1995, VERBIC and BABNIK 1997, GfE 2001). In all these systems a constant microbial yield per unit of ME, NEL, fermentable ME, DCHO or FOM is proposed. Some of the above-mentioned systems already take into account the fact that not all the nutrients available for ruminant animals can be utilised by the rumen microbes. In other systems this fact is neglected. In experiments the efficiency of microbial protein synthesis often deviates markedly from values proposed in protein systems. This confirms the possibility that several further factors affect microbial growth in the rumen, apart from those which are nowadays taken into account. We need to be aware that minor changes already made to the diet, feeding regime or environment (ambient temperature) can alter microbial flora and fauna. As a consequence the microbial protein yield in the rumen can be changed as well.

The maximum potential of rumen microbes to produce microbial protein can be explored only by the provision of high-quality forage. The importance of forage quality was clearly pointed out by an experiment in which forage digestibility and microbial protein synthesis was monitored during the ageing of grass clover sward. From the last week of April until the first week of June organic matter digestibility decreased from 82% to 60%. During the same period the microbial protein yield decreased from approximately 130 to 90 g per kg of dry matter intake (*Graph 1*). It can be estimated roughly that, along with a decrease in the NEL concentration in forage for 1 MJ, the microbial protein yield decreased by about 16 g. The problem of low microbial protein yield in diets containing low quality forages can not simply be solved by supplementing diets with high amounts of concentrates. It has been shown that in diets containing high levels of concentrates the efficiency of microbial protein synthesis in the rumen is lower than in well-balanced forage-based diets (ARC 1984).

The preservation of forages as silages can induce a reduction of microbial protein synthesis in the rumen. A certain number of the fermentation end products to be found in silages do not contribute any energy for microbial growth while the utilization of others is limited. The theoretical assumption performed by CHAMBERLAIN (1987) suggested that, in the case of the most favourable fermentation pathway in the silo, i.e. homolactic fermentation, rumen microbes can reach only one-quarter of the energy that would be available in the case of direct fermentation of hexose in the rumen. The problem of low-energy supply to rumen microbes can be expected first of all in extensive fermented direct cut silages. In a direct comparison of silages and hay from the same parental grass, it was established that microbial protein yield in highly wilted silage and hay was about 15% higher than in direct cut and moderately wilted silage (*Table 1*). It has been shown that, even in the case of ensiling highly wilted Italian ryegrass, microbial protein yield has been reduced by about 10% due to ensiling (*Table 1*).

As mentioned above, some protein systems try to overcome discrepancies between energy utilisation in microbes and animals by taking into account the fact that only fermentable organic matter can be utilised by rumen microbes. The term "efficiency of microbial protein synthesis" is used when microbial protein synthesis is expressed per unit of fermentable organic matter. Regarding the accepted concept, constant efficiency in microbial protein synthesis might have been expected. However, the efficiency of microbial protein synthesis varied widely between forages (*Table 1, Graph 1*). In

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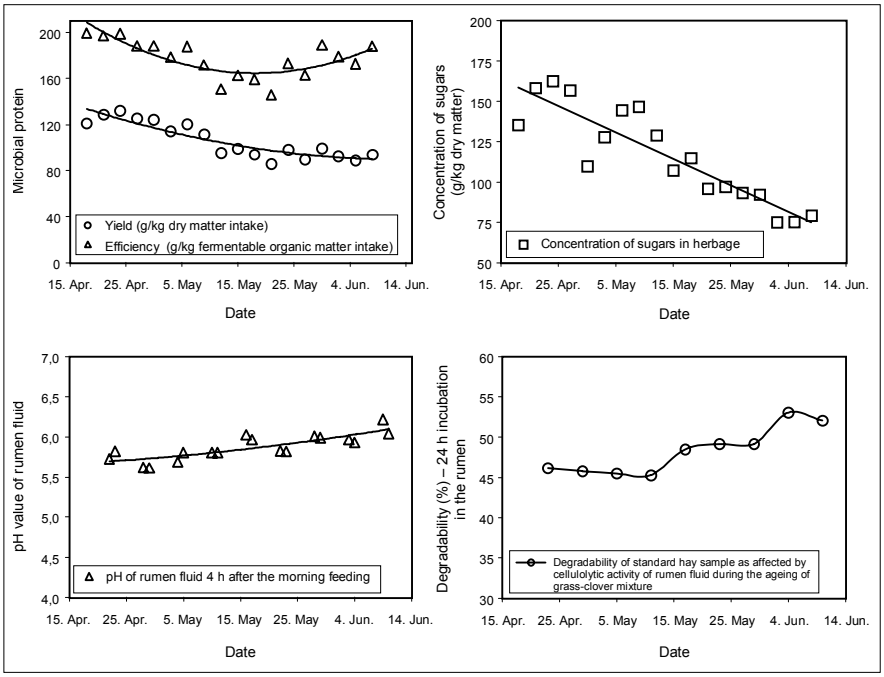
*Table 1:* **Efficiency of microbial protein synthesis in various forages** (VERBIC and BABNIK 1998, VERBIC et al. 1999a, VERBIC and BABNIK, unpublished results)

| | Dry matter (DM) | Microbial protein yield | Efficiency of microbial protein synthesis |
|---|---|---|---|
| | g/kg | g MP/kg DM | g MP/kg FOM |
| **Forage from permanent grasslands: first cut** | | | |
| Direct cut silage | 213 | 69,3 | 139 |
| Formic acid treated silage | 236 | 70,8 | 132 |
| Wilted silage | 432 | 68,1 | 115 |
| Highly wilted silage | 521 | 78,9 | 133 |
| Hay | 893 | 79,8 | 126 |
| **Italian ryegrass: third cut** | | | |
| Green forage (frozen) | 218 | 82,8 | 162 |
| Silage | 477 | 73,6 | 158 |
| **Maize silage** | | | |
| Flint type hybrid | 369 | 113,8 | 217 |
| Dent type hybrid | 374 | 99,4 | 165 |




*Graph 1:* **Microbial protein synthesis, concentration of sugars, rumen pH value and cellulolytic activity of the rumen fluid as expressed by dry matter degradability of a standard hay sample in the rumen during the ageing of grass-clover herbage** (VERBIC et al. 1999b, VERBIC et al. 2002a).

grass silages it varied from 115 to 158, in hay it was 126, in maize silages it varied from 165 to 217 and in green forage from 145 to 199 g of microbial protein per kg of fermentable organic matter. It is evident that microbial protein synthesis in the rumen also depends on other factors which can not simply be explained by the concentration of fermentable organic matter.

One of the possible explanations for the wide variability in the efficiency of microbial protein synthesis in forage-based diets can be associated with the role of specific compounds which may stimulate microbial growth. CHAMBERLAIN et al. (1993) reported higher microbial protein yield when grass silage based diets were supplemented by sucrose, fructose, lactose or xylose in comparison to the addition of starch. If the assumption of beneficial effect of sugars is true, it can at least partially explain the variability in efficiency of microbial protein synthesis between forages. In experiments in which the effect of the ageing of a grass-clover mixture on the efficiency of microbial protein synthesis was measured, it has been shown that a decline in efficiency of microbial protein synthesis during the early maturity stages coincided with a decline in the concentration of sugars (*Graph 1*). An increase in microbial protein synthesis in mature grass (*Graph 1*) still remains to be explained.

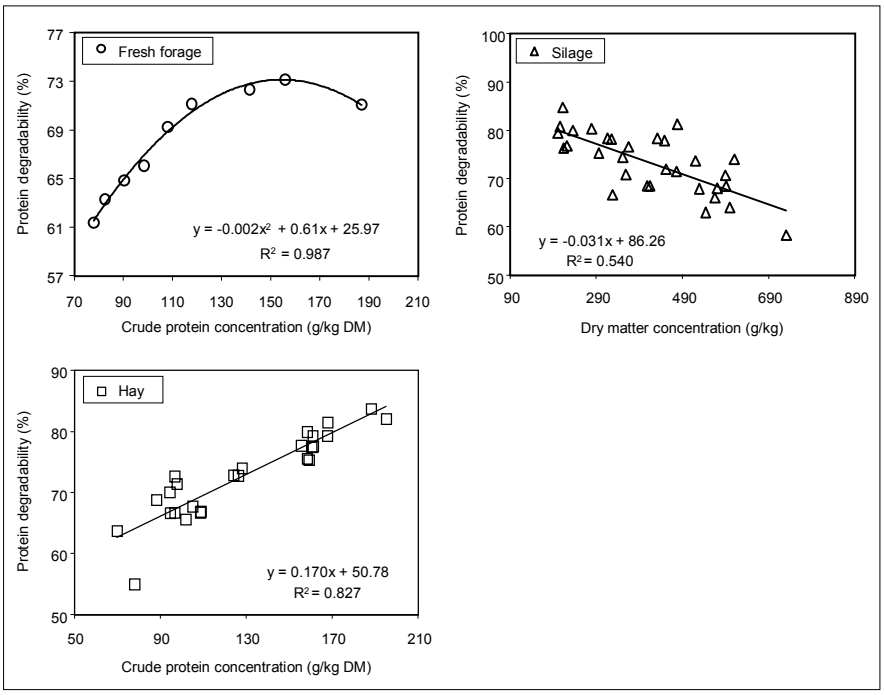
Considerable progress in the selection of grass for high sugar concentration has recently been achieved. It has been shown that a variety of such grass can at certain times of the year contain up to 350 g of water soluble sugars per kg of dry matter. When this variety was fed to dairy cows the milk production and efficiency in the use of grass protein increased (MILLER et al. 2001).

## Rumen environment

An important factor which may alter the microbial protein yield in the rumen is pH value. Low pH value can be deleterious to rumen microbes, and especially sensitive are protozoa. A low pH value is also expected to reduce the digestibility of fibrous plant tissues. Due to low pH value, energy within the rumen is diverted to non-growth functions, i.e. maintaining neutral pH in bacterial cells (STROBEL and RUSSEL 1986). Although forage-based diets are generally not considered to promote rumen acidity, the rumen pH value in diets containing solely immature grass may be well below the optimum. In a fresh forage experiment (*Graph 1*) we can speculate that the increased efficiency of microbial protein synthesis in mature grass could be due to the improved rumen environment. With advancing maturity, the pH value of rumen fluid increased. An increase in the rumen pH value was accompanied by a pronounced rise in the cellulolytic activity of the rumen fluid (*Graph 1*). The degradability of a standard hay sample, which was incubated in the rumen every six days of experiment (24 h incubation), was relatively constant (45%) until the middle of May and gradually increased to 52% thereafter. Another example in which rumen environment played an important role in the determination of microbial protein yield can be found in maize silage diets. Silage made from the flint type hybrid supported a higher microbial protein production than that from the dent type hybrid (*Table 1*).

This occurred despite similar concentrations of rumen degradable starch and despite the fact that the degradability of non-grain fraction *per se* was higher in dent type hybrid than in flint. Again, it was clearly shown that a higher efficiency of microbial protein synthesis in silage made from flint type hybrid was related to a higher rumen pH value (6.33 *vs.* 6.21) and better conditions for cellulolysis which were expressed through the higher effective degradability of insoluble non-starch carbohydrate fraction of maize silage (35.5 *vs*. 31.7 %, VERBIC and BABNIK, unpublished results).

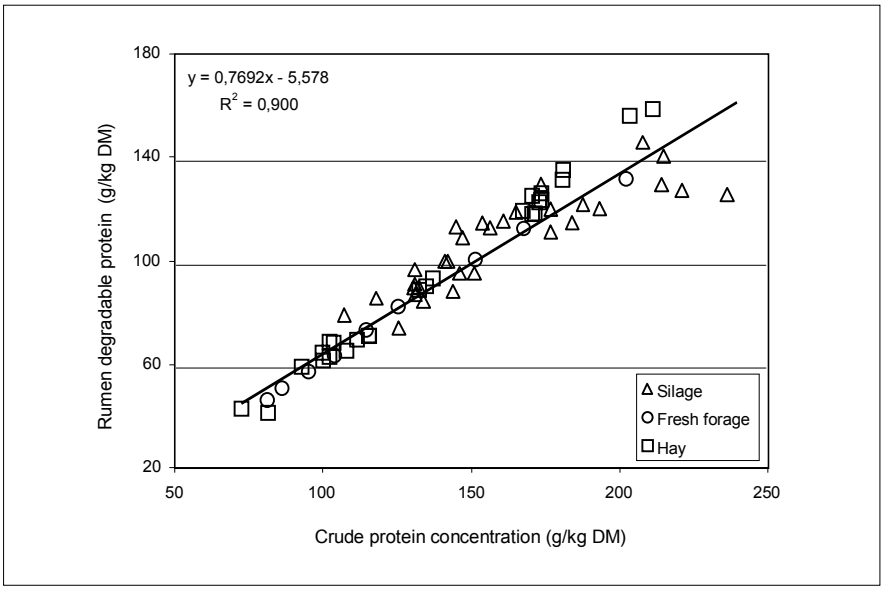
In conclusion, it should be pointed out that a low rumen pH value may inhibit microbial protein yield by inhibiting the degradation of fibrous material as well as by diverting the available energy to non-growth functions.




*Graph 2:* **Most pronounced relationship between forage composition and protein degradability in green forage, silage and hay** (BABNIK and VERBIC 1996, VERBIC et al. 1999a, VERBIC et al. 2002a, VERBIC et al. 2002b)

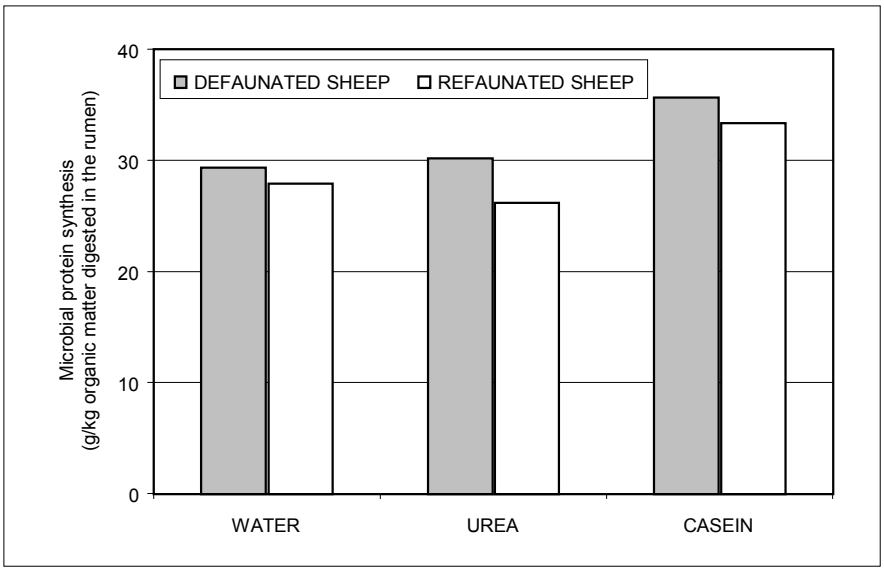
## The supply of nitrogen compounds

Protein degradation in the rumen is one of the main reasons for the inefficient utilisation of protein in ruminants. On the other hand, nitrogen compounds which are released during the protein degradation are crucial for microbial growth in the rumen. In modern protein systems it is required that the needs of rumen microbes for nitrogen compounds are fully covered either by degradable dietary protein or by metabolic nitrogen, which arise from the oxidation of amino acids in animal tissues and which can be recycled into the rumen. In some systems it is proposed that the capture of rumen degradable protein is not complete (INRA 1988, AFRC 1992) and therefore a surplus of rumen degradable protein is required.



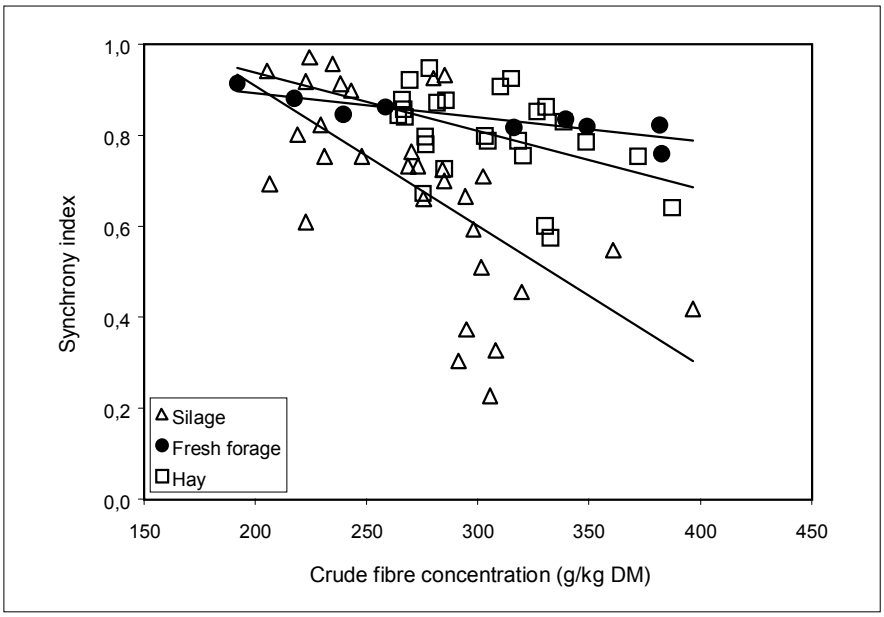

*Graph 3:* **Relationship between crude protein concentration and the supply of rumen microbes by rumen degradable protein in green forage, grass silage and hay** (BABNIK and VERBIC 1996, VERBIC et al. 2002a, VERBIC et al. 2002b)

As already discussed, the efficiency of microbial protein synthesis is highly variable. Therefore, in practice, the minimal requirements for rumen degradable protein can be under- or overestimated. On the other hand, a wide variation was also observed in the protein degradability of forages. It was found that in fresh forage and hay, protein degradability was closely related to protein concentration. Protein degradability with increasing protein concentration in forage increased (*Graph 2*). This means that with increasing protein concentration in fresh forages or hay the supply of rumen degradable nitrogen increased to a greater extent than can be expected from protein concentration *per se*. It was established that in grass silages protein degradability mainly depends on dry matter concentration. For each g of increased DM concentration protein degradability decreased by about 0.031 %. Despite the pronounced effect of forage preservation methods on protein degradability (VERBIC et al. 1999a) it can generally be claimed that forages of similar protein concentration provide similar amounts of rumen degradable protein (*Graph 3*). There are a number of reports of increased microbial protein yields in response to the addition of N in the form of amino acids or peptides. Increased microbial growth can be expected first of all in diets containing a high level of starch (RUSSEL et al. 1992). However, recently OH et al. (1999) reported that




*Graph 4:* **Effect of intraruminal infusion of ammonia or casein on the efficiency of microbial synthesis in the rumen of defaunated sheep which were given ammonial treated straw** (VERBIC and CHEN 1989)

the response of microbial protein synthesis to the inclusion of casein as opposed to urea was even greater at low level of starch. VERBIC and CHEN (1990) for instance infused urea or casein into the rumen of sheep which were given ammonia-treated straw (*Graph 4*). In comparison with urea, casein improved the efficiency of microbial protein synthesis in the rumen. That happened in sheep with a normal rumen population as well as in defaunated sheep. Considerable variation in the concentration of available peptides and free amino acids can be expected in forages. Plant protein is broken down into peptides and free amino acids by the action of plant proteases (KEMBLE 1956) while the breakdown of amino acids to ammonia and other forms of non-protein compounds is mainly caused by the action of clostridia in the silo (OHSHIMA and McDONALD 1978). Therefore, concentrations of peptides and amino acids are expected to be higher in fresh forage and hay than in silage. It is considered that free amino acids and peptides are quickly degraded in the rumen and it is questionable whether they can really support microbial growth. A considerably better source of free amino acids and peptides may be found in slowly degradable protein fraction which comprise in green forage 74% (52–84%), in hay 46% (27–55%) and in silage 35% (16–56%) of total protein (VERBIC and BABNIK, unpublished results). Due to the specific needs of rumen microbes for amino acids and peptides, it can be expected that by synchronising the availability of fermentable energy and degradable protein in the rumen, the efficiency of microbial protein production can be increased. Typical synchrony indexes, which described synchrony of crude protein and dry matter degradation in the rumen, are presented in *Graph 5*. It is evident that with advancing maturity the synchronicity of forages decreased. Relative to silages (mean $I_S$=0.69), synchrony indexes ($I_S$) were generally higher in fresh forages (mean $I_S$=0.84) and hay (mean $I_S$=0.80).




*Graph 5:* **Relationship between crude fibre concentration and synchrony index which describes the synchrony of crude protein and dry matter degradation in the rumen for green forage, grass silage and hay** (VERBIC and BABNIK, unpublished results).

## Rumen outflow rate

One of the factors which affect the efficiency of microbial protein synthesis is rumen outflow rate. Faster outflow rate is expected to reduce the maintenance costs of microbes because they spend less time within the rumen. From a theoretical point of view, it would be expected that the maximum microbial yield would occur when the dilution rate was equal to the multiplication rate of bacteria (ØRSKOV 1992). The theory has been confirmed experimentally (HARRISON et al. 1975, KENNEDY and MILLIGAN 1978, DEWHURST and WEBSTER 1992, MURPHY et al. 1994) and there are some protein systems which already takes it into account. In AFRC (1992) for instance, it is supposed that the efficiency of microbial protein synthesis can be increased by about 20% if rumen outflow rate is increased from 0.02 to 0.08 $h^{-1}$. Rumen outflow rate is a function of dry matter intake and therefore it can be assumed that the efficiency of microbial protein synthesis in the rumen can be increased by an increase in dry matter intake. One of the most important factors which limits intake of low quality roughages is their

slow rate of degradation in the rumen. High quality roughages are therefore expected not only to increase microbial protein yield by providing high amounts of fermentable substrate but also by increasing the level of intake. Studies at the Federal Research Institute for Agriculture in Alpine Regions Gumpenstein (GRUBER et al. 2001) indicated that dry matter intake in dairy cows increased by 0.2 to 2.2 kg for each MJ increase in NEL concentration in forages. The lowest response was observed in diets consisting of green forages and the highest in diets consisted of grass silages and hay. Dry matter intake can also be affected by forage conservation. Relative to conserved forages, higher dry matter intakes can be expected in green forage (GRUBER et al. 2001).

All measurements of microbial protein yields, presented in *Table 1*, were undertaken in a restricted feeding regime which allowed similar passage rates. Variation in rumen solid outflow rates was small (from 0.035 to 0.048 $h^{-1}$) and is therefore not considered to have an important effect on the results. In green forage experiment (*Graph 1*) rumen outflow rates were not measured. However, dry matter intakes were similar to other experiments and were therefore also probably outflow rates. Variability in the microbial protein yields which are presented in this paper do not comprise the effect of possible differences in outflow rates, which may be induced by variability in dry matter intakes. It can be speculated that real differences between highly wilted silages and direct cut silages as well as differences between highly digestible forages and forages harvested at late maturity stages would be even greater.

## Conclusions

The efficiency of microbial protein synthesis varied widely between forages. This confirms that several factors other than the supply of fermentable energy and rumen degradable protein affect microbial growth in the rumen. Variability in microbial protein yield can be explained at least in part by rumen pH value and resulting cellulolytic activity of the rumen fluid. An important factor which affects microbial protein synthesis in the rumen could be the supply of rumen microbes by free amino acids and peptides. No negligible differences between forages were observed in the fraction of slowly degradable protein and in the synchronicity of crude protein and dry matter degradation in the rumen. It seems that the efficiency of microbial protein synthesis in fresh forage is comparable to those in maize silage but considerably higher than in grass silages or hay.

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