# Influence of nitrogen fertilization on the crude protein fractions of grassland forage

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# Abstract

Fertilization of permanent grassland ensures satisfactory dry matter DM yields in the long-term. But intensive fertilization may also influence crude protein (CP) quality in forages, which may decide about N-use efficiency in feeding ruminants. In a permanent grassland experiment (cultivated since 1966 in Gumpenstein, Austria) five treatments (1: control – no fertilization; 2: P and K; 3: P, K, 80 kg N; 4: as 2 but plus 120 kg N; 5: as 2 but plus 180 kg N; r=4 each) were sampled in the first cut 2014. Fresh forage samples were separated into grass, legumes and herbs, dried (58 °C) and milled (1 mm). Protein fractions were analysed according to CNCPS (Fraction A, B and C) in bulk samples. Legume proportion was highest at treatment 2, whereas grasses benefited most from increasing N fertilization with highest proportion at treatment 5. In all treatments CP concentrations were highest in legumes, as expected. CP fraction A increased in the bulk sample proportionally to the N fertilization level. Concluding, grasses seem to dominate the forage production in all treatments. However, high N fertilization may reduce CP quality in permanent grassland with well-defined botanical composition.

Keywords: permanent grassland, crude protein fractionation, long-term experiment

## Introduction

The contribution of grassland for the protein supply of ruminants becomes more and more important. In this context, not only the crude protein (CP) content in grassland forage is relevant, but also the CP fractionation which provides detailed information about the CP quality for ruminants. CP quality in grassland also implies in a contribution of a more efficient N use as a high N-excretion causes problems for the N balance at the farm.

For permanent grassland regions, decisive strategies to obtain high N yields per hectare involve the optimum botanical composition and the balance between the ideal cutting frequency, nutritive value and dry matter (DM) yield (Pötsch, 1998). One important aspect in intensively used permanent grassland, with up to 5-6 cuts year<sup>-1</sup>, is the forage containing high proportions of proteins with a fast degradation rate. A low proportion of easy fermentable carbohydrates like water soluble carbohydrates, combined with a high proportion of fast degraded protein results in high production of ammonia in the rumen, and the surplus N is consequently excreted in urine. Improving the protein quality of permanent grassland, forage legumes determined the feed quality of binary mixtures with perennial ryegrass (Gierus *et al.*, 2011). Although N fertilization suppresses legumes facilitating the dominance of grasses, CP quality may decrease. The objective of the present study was to determine the CP fractionation in permanent grassland plots with different levels of N fertilization.

## Materials and methods

The samples originated from the first cut in 2014 of a long-term grassland experiment, established already in 1966 at Gumpenstein, Austria. Consequently, the botanical composition of the experimental plots is very well adapted to the fertilization strategy. In total five treatments, each with four replicates, were

Table 1. Overvie	w of results for t	he first cut 2014.
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	Fertilization level					SEM	P-value
	1	2	3	4	5		
DM yield, Mg ha <sup>-1</sup>	0.74 <sup>d</sup>	1.90 <sup>c</sup>	3.40 <sup>b</sup>	3.59 <sup>b</sup>	4.57 <sup>a</sup>	321	<0.01
N yield, kg ha <sup>-1</sup>	12.3 <sup>c</sup>	33.6 <sup>b</sup>	52.3ª	51.3 <sup>ab</sup>	68.4 <sup>a</sup>	4.7	<0.01
ADF, g kg <sup>-1</sup> DM	303.4 <sup>c</sup>	332.9 <sup>bc</sup>	363.9 <sup>ab</sup>	369.5 <sup>a</sup>	388.6 <sup>a</sup>	7.9	<0.01
Crude protein, g kg <sup>-1</sup> DM <sup>1</sup>	101.3	101.8	96.1	89.5	93.7	1.8	0.23
fraction A, g kg <sup>-1</sup> CP	122.6 <sup>b</sup>	123.3 <sup>b</sup>	162.1 <sup>ab</sup>	155.0 <sup>ab</sup>	169.8 <sup>a</sup>	6.4	0.03
fraction B, g kg <sup>-1</sup> CP	866.3ª	866.0 <sup>a</sup>	826.5 <sup>ab</sup>	832.3 <sup>ab</sup>	819.5 <sup>b</sup>	6.4	0.03
fraction C, g kg <sup>-1</sup> CP	11.0	10.7	11.4	12.4	10.7	0.3	0.37

<sup>1</sup> Crude protein fractionation according to Licitra *et al.* (1996).

selected and evaluated: Treatment 1: control, no fertilization; Treatment 2: P and K dynamic, i.e. the P and K fertilization was adjusted to the DM yield of the year before; Treatment 3: P and K dynamic plus 80 kg N ha<sup>-1</sup>; Treatment 4: P and K dynamic plus 120 kg N ha<sup>-1</sup>; Treatment 5: P and K dynamic plus 180 kg N ha<sup>-1</sup>. Fertilization was realized with  $NH_4NO_3$  (27% N), phosphate (25%  $P_2O_5$ ) and potassium  $(40\% \text{ K}_2\text{O})$ . At the harvest date of the first cut, the botanical composition as well as the development stage was estimated visually. Swards were cut to 5 cm height and DM yield was determined after drying. A bulk sample was collected, as well as a representative manual separation in grasses, legumes, and herbs was performed. All samples were dried in hot-air cabinet at 58°C, milled in a Cyclotech mill to pass a 1 mm sieve and stored for further analyses. The CP fractionation was performed for the bulk sample. Fraction A was determined with a 10% tungstic acid solution (Licitra et al., 1996) and N measured in the residue after filtration. The fraction A was calculated as difference between total N content and N content in residue. For determination of Fraction C, acid detergent fibre (ADF) was determined using a semiautomatic apparatus (Fibertech, Gerhardt, Germany) and N determined in ADF residue. The Fraction B was estimated by difference between total crude protein and Fraction A and C on DM basis. Data were statistically analyzed using the GLM procedure of SAS as a completely randomized block design, with the least significant difference procedure for mean comparison and probabilities being adjusted by Tukey-Kramer test.

#### **Results and discussion**

DM yield clearly corresponded to the expectations with regard to fertilization intensity (Table 1). N yields increased with the fertilization level, although they did not differ among treatments 3-5, probably as a consequence of dilution effects. The highest level was obtained in treatment 5 with 68 kg N-yield ha<sup>-1</sup> (Table 1), which corresponded to the highest DM yield. The dominant grasses were *Dactylis glomerata* and *Trisetum flavescens* in treatments 2-5. For legumes, *Trifolium pratense* and *Trifolium repens* were mostly observed. *Leontodon hispidus* dominated among herbs. The botanical composition of the sward in the first cut confirmed the clear differentiation between grasses, legumes and herbs in dependence of the fertilization level, which was expected after almost 50 years fertilizer application (Figure 1). The proportion of legumes was highest in treatment 2 and declined with higher N-fertilization level, achieving less than 10% (on DM basis) in the other treatments. Legumes benefited from P and K fertilization mostly. The highest proportion for grasses, with values ranging up to 80%, was observed in treatment 5.

With regard to the CP fractionation, the proportion of fraction A (NPN-fraction) increased with N-fertilization level, achieving high values in treatment 5 (Table 1). Consequently, low content of fraction B was also observed in the same treatment. As fraction C was not affected by fertilization level,

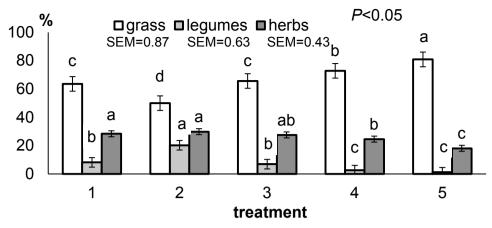


Figure 1. Botanical composition of experimental plot as averages (1993-2014).

the lower fraction B corresponded to the higher fraction A. Maybe the additional N uptake by the plant community in treatment 5 (mostly grasses) could not be converted into protein, and this N was retained as non-protein N in plant tissue. The CP quality suggests that lower N use efficiency in ruminant feeding is likely (Peyraud and Astigarraga, 1998).

As a long term fertilization trial, the cutting frequency is one of the major concerns. In practice, a higher fertilization level implies a higher cutting frequency. This was not performed in this experimental set up to avoid confounding effects over the years.

#### Conclusions

The knowledge about the botanical composition after almost 50 years of trial set up supports the observation that grasses are dominating with increasing level of N-fertilization. At the same time, the CP quality decreased. Although higher DM yields are possible with the highest fertilization level, the CP quality suggests that lower N use efficiency in ruminant feeding is likely. Moderate N fertilization supports higher legume proportion and adequate CP quality.

### References

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