Determining the feeding value and digestibility of the leaf mass of alfalfa (Medicago sativa) and various types of clover

H. Sommer and A. Sundrum

Department of Animal Health and Animal Nutrition, Faculty of Organic Agricultural Sciences, University of Kassel, Nordbahnhofstr. 1a, D-37213 Witzenhausen, Germany, corresponding author: hendriksommer@uni-kassel.de

Abstract: Improving farmland biodiversity and reducing greenhouse gas emissions are main objectives of the Common Agricultural Policy (CAP) from 2014-2020. To develop strategies for the use ofalternative protein sources of home-grown origin for monogastric animals matches to a high degree with these objectives. Separation of the leaf mass from the stem is expected to increase the concentration of crude protein and therewith the feeding value of alfalfa and selected clover species for pigs. To assess the potential of concentrating crude protein, the leaf mass yields and the nutritive value of leaf mass from different varieties and cutting dates were analysed for nutrient values and *in vitro* digestibility. In the year 2012 plant material of alfalfa, red clover, white clover, crimson clover and Persian clover was sampled from three cutting dates in three different locations; 90 samples have been examined. Nutrient content of leaf mass was determined by Weender and Van Soest analysis, the amino acid contents by chromatographic measurement. The highest content of crude protein in the leaf mass has been observed in Persian clover with 28.3 g XP 100 g DM^{-1}, followed by alfalfa with 28.0 g XP 100 g DM^{-1}. The concentration of leaf crude protein of red clover was 27.0 g XP 100 g DM^{-1}, of white clover 26.3 g XP 100 g DM^{-1} and of Crimson clover 25.9 XP 100 g DM^{-1}. The concentration of digestible crude protein in the leaf amounts to 22.3 g dXP g DM^{-1} in Persian clover, succeeded by red and white clover to 20.3 g dXP g DM^{-1}. The crude protein yield of leaf mass per ha and year of alfalfa (17 dt ha^{-1}), red clover (15.2 dt ha^{-1}) and white clover (12.6 dt ha^{-1}) was higher than for example the crude protein yield of faba beans (8-10 dt ha^{-1}). Separation of the leaf mass from the stems makes a relevant source of home-grown protein accessible to compensate the lack of high quality protein feed in the nutrition of pigs.

Keywords: protein crop, greening, protein digestibility, pig nutrition

Introduction

One objective of the Common Agricultural Policy (CAP) from 2014-2020 is to strengthen farmland biodiversity and to reduce greenhouse gas emissions. Looking for alternative sources for the supply of monogastric animals with high quality protein feed of home-grown origin seems to be an effective measure to achieve the CAP objectives. Fodder legumes have the potential to replace expensive feed components in relevant order (Werner and Sundrum 2009). In addition, they can stabilise the intestinal flora and inhibit pathogens and thereby promote animal health (Urdl et al. 2009). However, in conjunction with fluctuations in yields and imprecise calculable feed value, the economic benefit remains doubtful.

Increasing the feed value of fodder legumes for pig production

The feed value of fodder legumes like alfalfa is determined by the date of cutting. For the use in pig nutrition the content of crude protein is the most important factor. Willner and Jänike (2005) found a crude protein content of pure stand alfalfa of 159 g kg dry matter $[DM]^{-1}$ at the 1st cut,

213 g kg DM^{-1} at the 2nd cut and 239 g kg DM^{-1} at the 3rd cut. The results are comparable to the findings in other studies (e.g. Loges and Taube 1999). Compared to faba beans, alfalfa contains less crude protein. Furthermore, the digestibility of organic matter limits the use of fodder legumes. Therefore, they can only be added to grain legumes but not replace them. Some efforts are concentrated on the optimisation of the feed value by increasing the digestibility of the stems (Jung and Allen 1995), because the lignin content in the stem affects the digestibility of the whole plant (Buxton and Russel 1988). Jung et al. (1997) concluded that the leaf mass of alfalfa is rich in protein content with a low level of fibre content, and has potentially a high digestibility, especially of crude protein. Other studies focused on influencing the proportion of leaf and stem in favour of the leaf. Using the leaf mass separately is another approach to exploit fodder legumes as a protein source. Therefore, the potential effectiveness of using leaf mass in pig nutrition needs to be clarified.

Aims of this study

The current research project is designed to investigate the conditions under which native clover-like legumes can contribute to cover the need of protein in the diet of pigs. It is mentioned that the separation of the leaf mass of alfalfa (Popovics et al. 2001) and other clover species selected from the stem will increase the concentration of crude protein content. The hypothesis is that this leads to higher contents of essential amino acids of the leaf mass compared to the whole plant of clover-like legumes in the diet of pigs. Therefore it is necessary to assess the digestibility of these resources, determining the feeding value and protein quality. Using different varieties and cutting dates, leaf mass yields and nutritive values were determined by nutrient analysis and *in vitro* determination of digestibility.

Material and Methods

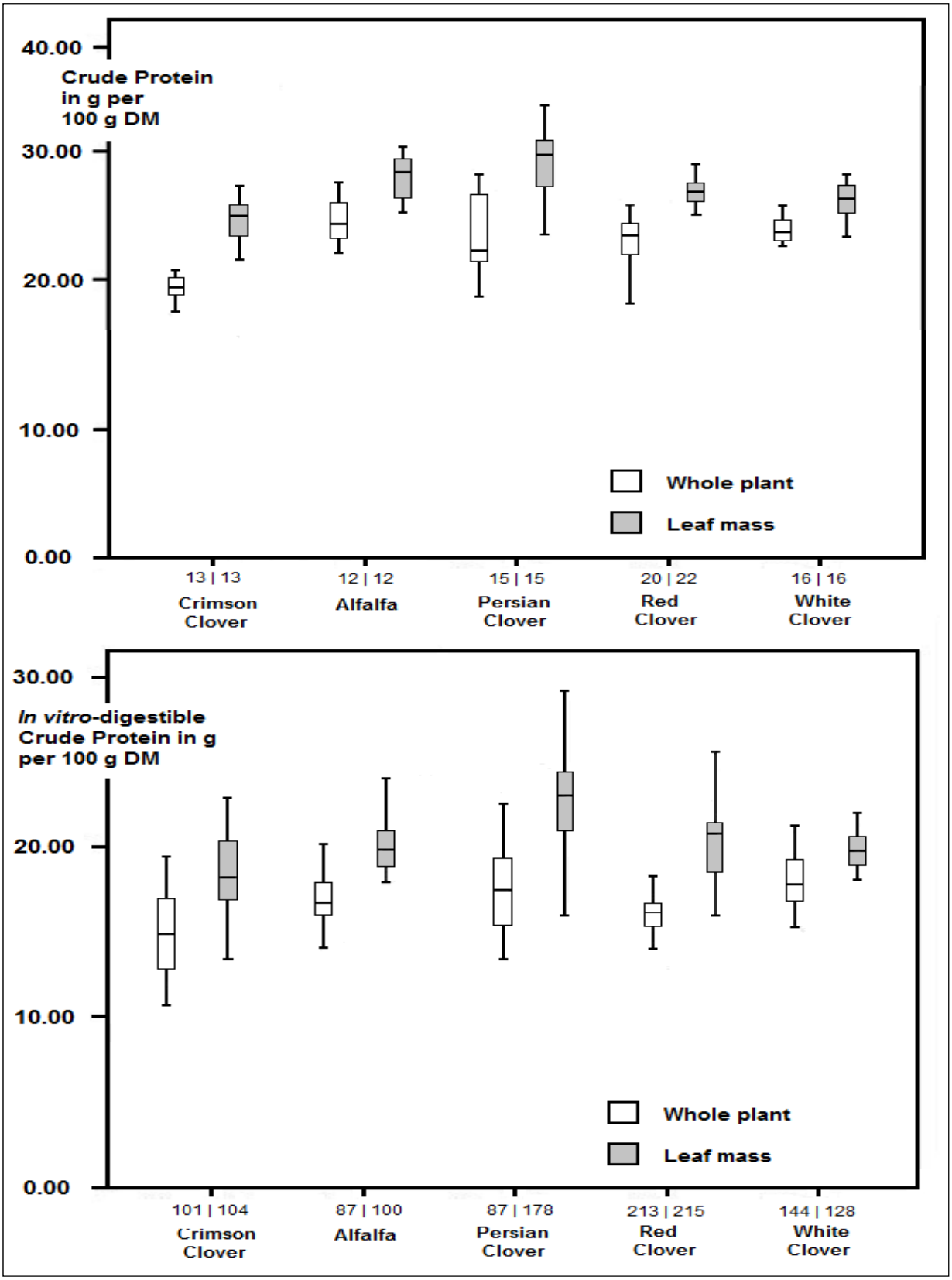
Plant material of alfalfa (*Medicago sativa)*, red clover (*Trifolium pratense*), white clover (*Trifolium repens*), crimson clover (*Trifolium incarnatum*) and Persian clover (*Trifolium resupinatum*) has been sampled. In total for the year 2012 with 3 cutting dates in three different locations, 90 samples have been examined. After harvesting, the samples were dried in a drying oven and then the leaf fraction was separated. The nutrient content of leaf mass was determined by Weender analysis for dry matter, crude protein, crude fat, crude fibre and crude ash. The content of acid detergent fiber [ADF] and neutral detergent fibre [NDF] were determined by Van Soest analysis. The amino acid contents were analysed by chromatographic measurement. The digestibility of crude protein of the leaf mass was determined by making use of the *in vitro* method according to Sappok et al. (2009).

Results

In vitro digestibility of crude protein

Results showed that the leaf mass [LM] of all investigated species contained a significantly higher crude protein content compared to the whole plant [WP] ($p<0.05$). The highest content of LF crude protein was found in Persian clover with 28.3 g crude protein [XP] 100 g DM^{-1}, compared to 23.6 g XP 100 g DM^{-1} in the whole plant, followed by alfalfa LF with 28.0 g XP 100 g DM^{-1} (WP: 24.8 g XP 100 g DM^{-1}). Crude protein in the leaf mass of red clover averaged 27.0 g XP 100 g DM^{-1} (WP: 23.5 g XP 100 g DM^{-1}), of white clover 26.3 g XP 100 g DM^{-1} (WP: 24.1 g XP 100 g DM^{-1}) and of Crimson clover 25.9 XP 100 g DM^{-1} (WP: 24.1 g XP 100 g DM^{-1}). Comparing the i*n vitro*–digestibility of crude protein, and especially of lysine, of the whole plant and the leaf mass, only small differences were found.

Figure 1: Box plot comparison of the crude protein content (figure above) and of the content of *in vitro*-digestible crude protein in the leaf mass and the whole plant of various clover like legumes. A box represents the range of values in which the mean 50% of the results are located. Bar indicates Median. Wiskers = 1.5 * IQR. Numbers (e.g. 101|104) = n.

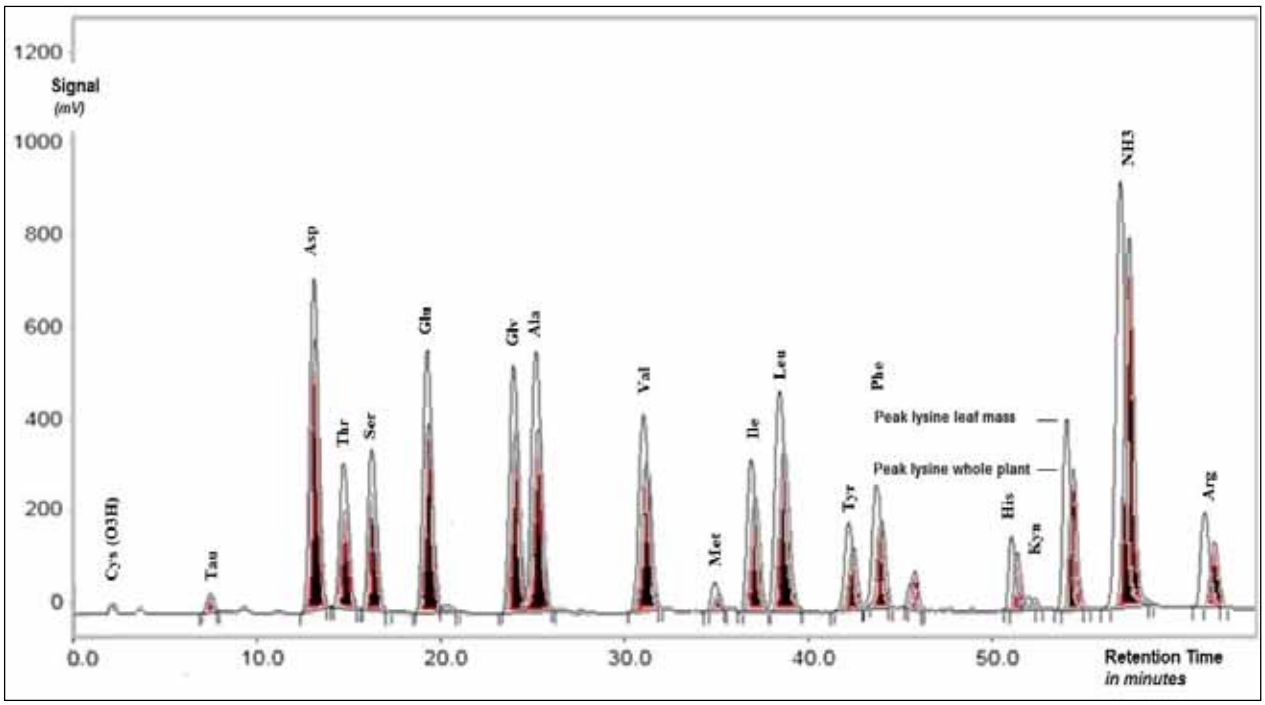


In Persian clover (79% LM - 74% WP), in alfalfa (74% LM - 70% WP) and in red clover (75% LM - 73% WP), the mean *in vitro*–digestibility of crude protein in leaf mass was higher than in the whole plant. The analysed *in vitro*–digestibility of crude protein in the leaf mass and whole plant of white clover was almost identical (77%), in crimson clover in the whole plant it was with 75% two percent higher than in the leaf mass.

In vitro digestibility of amino acids

In general, the concentration of amino acids in the leaf mass was higher than in the corresponding whole plant. In Persian clover (81% LM - 80% WP), in white clover (80% LM - 79% WP) and in alfalfa (79% LM - 76% WP), the mean *in vitro*–digestibility of lysine in leaf mass was higher than in the whole plant. The analysed *in vitro*–digestibility of lysine in the leaf mass and whole plant of crimson clover is almost identical (80%), in red clover it was in the whole plant with 82% two percent higher than in the leaf mass. Figure 2 shows as example two superimposed chromatograms, one of the leaf mass of red clover, one of the corresponding whole plant, displayed in black coloration. The peaks of the leaf mass material turned out to be higher than that of the whole-plant material. The lysine content [LYS kg DM^{-1}] in the leaf mass of alfalfa revealed 17.7 g 1000 g DM^{-1} compared to 15.3 g LYS kg DM^{-1} in the whole plant. The concentration of lysine in the leaf mass of red clover accounted for 15.3 g LYS 1000 g DM^{-1} (WP: 12.6 g LYS 1000 g DM^{-1}), of white clover 14.7 g LYS 1000 g DM^{-1} (WP: 12.7 g LYS 1000 g DM^{-1}), of Persian clover 13.8 g LYS 1000 g DM^{-1} (WP: 10.9 g LYS 1000 g DM^{-1}) and of crimson clover 13.3 LYS 1000 g DM^{-1} (WP: 10.4 g LYS 1000 g DM^{-1}).

Figure 2: Superimposed chromatograms of the amino acid analysis of leaf mass (transparent peaks) and the whole plant (black peaks) of red clover



Crude protein yields

The cultivation of clover-like legume for the production of leaf mass leads to higher leaf mass crude protein yields compared to faba beans and peas. The cultivation of faba beans leads to a crude protein yield of 8-10 decitonne [dt] ha^{-1}, depending on location and weather influence. The cultivation of peas leads to lower crude protein yields in the range of 2.2 to 7.8 dt ha^{-1}. The crude protein yield from leaf mass of crimson clover and Persian clover are at the level of field bean. The yields of digestible crude protein are higher than that of faba bean and pea in the clover-like legumes (see Table 1). Although the faba bean and pea have some higher digestibility of about

80%, the examined plant samples are due to the higher content of protein in the leaf mass rich in digestible crude protein.

Table 1: Digestible crude protein yield (mean, dt ha^{-1}) of the leaf mass of the studied species differentiated into cuts and accumulated compared to the range of faba bean and pea

	Alfalfa *dt ha^{-1}*	**Red clover** *dt ha^{-1}*	**White clover** *dt ha^{-1}*	**Crimson clover** *dt ha^{-1}*	**Persian clover** *dt ha^{-1}*	**Faba** *dt ha^{-2}*	**Pea** *dt ha^{-3}*
1. Cut	4.1	4.4	3.7	2.8	4.9		
2. Cut	4.4	4.1	3.1	1.8	2.4		
3. Cut	4.1	3.0	2.9	1.5	2.1		
∑	**12.6**	**11.4**	**9.7**	**6.2**	**9.3**	**6.8**	**3.1**

Discussion

In vitro feed value of leaf mass

The present results on the crude protein content of the whole plant are comparable to the known values (see e.g. Armentano et al. 1997). Information about the leaf mass is not widely known. Popovic et al. (2001) showed that separating the leaf mass of alfalfa from the stem lead to average crude protein content of 164.5 g kg DM^{-1} in the stem and 346.8 g kg DM^{-1} in the leaf mass. Although in the present study the concentration did not exceed 300 g kg DM^{-1}, the protein content in the leaf mass of the investigated varieties are comparable to faba beans and surpassthose of peas. In the present study, the observed lysine contents correspond to the results of several investigations (e.g. Nonn 1990, Guo et al., 2008). Nevertheless, only the results of alfalfa leaf mass exceed an average lysine content of faba bean (13.0 – 15.0 g kg DM^{-1}, Nalle et al. 2010) and of pea (5.97 g kg DM^{-1}, Palander et al. 2006). But the lysine content of soy bean derivates (> 20.0 g kg DM^{-1}) could not be achieved.

The aim of the *in vitro*-method to analyse the *in vitro*-digestibility of crude protein was to estimate the portion of digestible crude protein. According to Weißbach (1993), the content of digestible crude protein of Persian clover reaches 170 g kg DM^{-1}, followed by alfalfa with 150 g kg DM^{-1} and red clover with 120 g kg DM^{-1}. The present results clearly exceed these values as well as the crude protein digestibility. However, it needs to be proved if results of *in vivo*- and *in vitro*-digestibility are comparable. Perhaps the standardised activity of the used enzymes (see Boisen and Fernandez (1997) and Sappok et al. (2009)) favours the *in vitro*-method, and *in vivo* the content of digestible crude protein in reality might be lower. Another question asks what could be made of the stems. Further investigations are necessary to evaluate their feed value.

Greening the CAP

To successfully implement Greening, a high level of acceptance by agricultural managers of farms is required. On the other hand, the current high and volatile prices for soybean meal will lead to an increased demand for regional protein sources. The obtained leaf protein yield is higher than the yield of protein from beans or peas (Sommer 2010). The division on several cuts also leads to a better stability of the yield. Furthermore, the investigated sources meet the protein requirements in the diet of pigs. Using fodder legumes in pig nutrition increases the protein earn out and potentially the efficiency not only of organic farms, but of conventional farms as well. They have the potential to replace expensive feed components in relevant order. The current study also offers results for a more region specific biodiversity in clover-type production. If the individual farm location is not suitable for the cultivation of alfalfa, red or white clover can be an alternative. If farm managers realise and use this potential, it will lead to a rising clover-type production in general.

Conclusions

The results indicate that the separation of the leaf mass from the stems offers a relevant home-grown source for essential amino acids. Using the leaf mass of clover like legumes can be an alternative particularly in organic monogastric feeding. However feeding studies are necessary to validate how close these results of the *in vitro*-digestibility of crude protein and amino acids correspond with the *in vivo* digestibility.

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