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AGRONOMIC FACTORS AFFECTING PRODUCTIVITY AND NUTRITIVE VALUE OF PERENNIAL FODDER CROPS: A REVIEW

J. HAKL*, J. ŠANTRŮČEK, M. PISARČIK, A. DINDOVÁ

Department of Forage Crops and Grassland Management, Czech University of Life Sciences Prague, Czech Republic

ABSTRACT

Perennial fodder crops such as legumes or grasses play an important role in ruminant nutrition all over the world. In the regions where area of permanent grassland is limited represent the very important protein and digestible fiber source. For successful forage production, high forage value of stand is generally required. This value consists of two parts: forage yield and quality. In spite of forage quality evaluating by a range of parameters, forage dry matter yield is only one-dimensional variable. It must be remembered yield is a key factor in forage production, especially for economic efficiency in relation to cost per hectare. Species selection, appropriate harvest frequency, level of fertilization and methods for estimate of qualitative traits were considered due to their practical impact. Changes in protein fractions and alternative forage usage were also included because they are associated with post-harvest forage utilization. It can be summarized that agronomic decisions before harvest have a high impact on both forage yield and quality.

Key words: forage quality; agronomic factors; fodder crops

INTRODUCTION

There is a range of factors influencing both forage yield and quality. The aim of this review is not list all of them but provide basic knowledge about these with the highest impact in the farming practice. It can be summarized that successful forage production of perennial fodder crops depends on achieved biomass yield and high forage quality. In this review, we would like to highlight the importance of agronomic decisions on harvested forage quality before start of conservation process. Although forage yield at the time of animal feeding is aloof interest, it contributed substantially to economic efficiency of animal production.

Suitable species/cultivars selection

Effective forage production cannot be naturally the same across different environments. In this regard, species or cultivars selection generally represents simple but highly effective tool for adaptation of feedstuff production to environment conditions. For example, in temperate zones, biennial or perennial legume or grass species are the most common whilst annual species can be in the first place in regions with intensive drought or frost period. For condition of the Europe, the most important traditional forage legume crops are lucerne (*Medicago sativa*) and red clover (*Trifolium pratense*). These two crops have complementary production responses to climatic conditions, where lucerne is high yielding in dry whilst red clover in wet conditions (Peterson *et al.*, 1992). In this zone, the important grass species are perennial and Italian ryegrass (*Lolium perenne* and *L. multiflorum*), meadow and tall fescue (*Festuca pratensis* and *F. arundinacea*), timothy (*Phleum pratense*) and orchard grass (*Dactylis glomerata*). There is a range of other grass species important for specific environments. Properties of all mentioned species are described in detail by Frame *et al.* (1997). Selection of proper species or composition of mixture of species remains the basic tool for successful start of forage production.

***Correspondence:** E-mail: hakl@af.czu.cz
Josef Hakl, Department of Forage Crops and Grassland Management, Czech University of Life Sciences Prague
Kamýcká 129, 165 21 Praha 6 – Suchdol, Czech Republic

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Breeding process is intended to improve the properties of the selected species important for human civilization. Yield improvement in forage crops during the last century has lagged far behind that of annual grain crops (Brummer, 1999) because breeders changed rather harvest index than total biomass production, which is an explanation for low yield progress of perennial forage crops. Lamb *et al.* (2006) concluded that evidence for changes in lucerne forage yield for cultivars released between 1940 and 1995 was environmentally dependent. In environments where conditions lead to plant stand losses, recently released cultivars with multiple disease resistance had a yield advantage over older cultivars, but in environments where no differences in plant density occurred, older cultivars yielded the same as the improved new cultivars.

Except for this slow but continuous improvement of yield or quality, there were also some milestones in this process for perennial forage crops. The following is an example with high practical impact on forage production. It has long been a goal for forage breeders to combine the stress tolerant characteristics of *Festuca* species with the earliness and high nutritive value of *Lolium* species. Some breeding programmes have been designed to transfer *Festuca* genes into *Lolium*, and as a result some *Festulolium* cultivars have been developed in Europe and in the USA (Humphreys *et al*., 2003). *Festulolium* provides specialized function and novel alternatives to existing grass species/cultivars that may lack resilience against abiotic or biotic stresses.

The following example represents a case of high perspective breeding method for improving forage quality. Lignin is defined as a complex organic compound that binds cellulose fibres and hardens and strengthens the cell walls of plants. This process accelerates as plants mature and gives structural support to the plants as they become taller. Regarding to animal nutrition, lignin is well-known as highly important anti-nutritive substance which is still essential for plant functions. Due to its negative effect during digestion, experiments with genetically reduced lignin synthesis have been made in various plant species. According to Shadle *et al*. (2007), analysis of lucerne forage quality parameters showed strong reductions of neutral- and acid-detergent fibre in the down-regulated lines, in parallel with large increases (up to 20 %) in dry matter forage digestibility. Reduction of hydroxycinnamoyltransferase (HCT) enzyme activity in these lines was from at least 15–50 %. The most severely down-regulated lines exhibited significant stunting, reduction of biomass yield and delayed flowering. Vascular structure was impaired in the most strongly down-regulated lines. Although manipulation of lignin biosynthesis can greatly improve forage digestibility, accompanying effects on plant development need to be better understood. In spite of these distresses, first low lignin lucerne cultivar was released for the commercial utilization by Aflorex Seed Company. In these "Hi-Gest" cultivars, content of lignin is reduced by 7 – 10 % by natural selection without declared negative impact to agronomic traits. The grower has two general harvest options available when growing lucerne with this new technology: (1) harvesting fields on a normal ~ 28 day cutting schedule to produce a high quality forage that has increased fibre digestibility and higher animal intake; (2) extend the peak harvest date by up to 7 days to ~35 days versus 28 days. This option utilizes the low lignin trait as a means of increasing yield without sacrificing forage quality. If a field is ready to be cut but rainy weather is forecast then harvest can be delayed up to 7 days to avoid rained-on forage. This flexibility at harvest time helps the producer minimize the effect of improper weather and reduced forage quality. Synthetic cultivars harvested at the later date would have lower forage quality due to its maturity and higher lignin content.

Harvest frequency

Optimal stand utilization is very important in terms of both yield and forage quality. It is well known that higher cut frequency improves nutritive value of harvested forage because of reduced stem weight proportion and its better digestibility in relation to lower lignification. However, it must be remembered that more intensive cut regime reduces stand yield and persistence. Regarding to yield, our results show that four-cut regime obtained significantly lower yield than three-cut in Central Europe region but this reduction represented only 4 –5 % (Hakl *et al*., 2011). For this environment, it seems that one cut over standard intensity of utilization only slightly reduces yield but provides high potential for improving forage quality. The adverse effect of intensity of lucerne harvesting on persistence and the following spring regrowth has been historically attributed to a reduction in the concentrations of organic reserves, especially total non-structural carbohydrates. For this purpose, it must be carefully distinguished between effect of number of cuts per year and their schedule over year. The regrowth interval between the last summer harvest and the autumn harvest is the major determinant of lucerne persistence and spring regrowth (Dhont *et al*., 2004). In Central Europe, this interval was traditionally expressed as number of days which should be at least 50 days. The accumulation of growing degree days > 5° after the last summer harvest has been proposed as a criterion to estimate the duration of this interval (Bélanger *et al*., 1992). For investigation in Europe conditions, field experiment was conducted in Central Bohemia in 2002–2004. In this experiment, the interval between summer and last autumn harvest was 40–50 days or 60–70 days, respectively. These intervals were expressed as cumulative growing degree-days (GDD) where GDD values ranged from 540 to 905 over three years period. The plants were sampled in each autumn with four replicates for each variant; the average depth of sampling was 150 mm. The weight of roots, amount of starch, and water soluble saccharides (WSC) per m^2 was determined. The total accumulation of root reserve saccharides was determined mainly by conditions over growing period in particular year. The length of the interval or cumulative GDD influenced only variation of this basic amount. It was documented by significant differences among evaluated years in dependence on weather condition and following stand development. Table 1 shows reduction of starch concentration and amount of all reserves at early harvest interval in 2002. GDD was very high at both intervals in 2003 which resulted in no significantly different amount of root reserves between intervals. In 2004, higher GDD value was obtained at early interval in spite of lower number of days which resulted in significantly higher concentration of starch. Total amount of root and root reserves was not affected by length of the interval. In Central Bohemia condition, the GDD around 600–700 ºC was preliminarily determined for maximal accumulation of root reserve saccharides. The GDD above this level did not significantly increase the root reserve accumulation.

Fertilization

Intensive agricultural cropping system requires large quantities of plant nutrients (Lloveras *et al*., 2012) which highlight importance of suitable fertilization management. It can be simply assumed that lack of nutrients in the soil significantly reduces forage yield. The impact of forage legumes fertilization has been traditionally focused on effects of direct application of phosphorus (P) and/or potassium (K) in various combinations (Macolino *et al*., 2013) whereas direct application of nitrogen (N) is not usually effective due to N fixation by legumes. Regarding to grass fertilization, Huhtanen and Broderick (2016) concluded that this step should be optimized according to crop DM yield with no benefits from increased N fertilization in nutritive value. In spite of intensive previous research about lucerne fertilization, there is a lack of long-term studies investigating indirect effect of organic and N fertilization on yield within applied crop rotation. At present, we can investigate differences in forage yield under different combination of mineral (6 treatments) and organic (3 treatments) fertilization in long-term experiment conducted since 1955 in Ruzyně (Hakl *et al*., 2016b). Long-term absence of fertilization provided average annual dry matter yield 8.64 $t.ha^{-1}$ (Figure 1). Indirect application of mere manure or slurry significantly increased yield to 9.68 and 9.37 $t.ha^{-1}$, respectively. The highest values of dry matter yield (DMY) over 10 $t.ha^{-1}$ were observed at treatments, where organic fertilizers were applied at N3P2K2 and N4P2K2 treatment, however the same value was also observed at application of manure under N1P1K1 treatment. These results reveal that not only direct but also indirect fertilization substantially influenced lucerne DMY (Hakl *et al*., 2016b). Effect of fertilization is generally more obvious for yield than forage quality and there are only few studies about effect of fertilization on nutritive value of perennial fodder legumes. According

Table 1: Effect of length of regrowth interval between the summer and autumn harvest on concentration and amount of starch and water soluble saccharides in lucerne roots over three year period (adapted from Hakl *et al*., 2008)

	2002			2003			2004		
	early	late	P	early	late	P	early	late	P
Interval:GDD	540	850		693	905		734	621	
Interval: days	43	72		42	67		54	63	
concentration ($g.kg\ DM^{-1}$)									
starch	104[a]	126[b]	0.0045	174[a]	153[b]	0.0022	199[a]	142[b]	0.0049
WSC	144	147	0.5766	193	188	0.3492	141	150	0.6663
amount ($g\ DM.m^{-2}$)									
starch	17[a]	33[b]	0.0004	22	22	0.9090	38	30	0.5263
WSC	23[a]	39[b]	0.0016	24	27	0.3782	27	31	0.4543
total	40[a]	72[b]	0.0008	46	49	0.5884	65	61	0.5841
root	158[a]	261[b]	0.0006	125	144	0.2686	190	212	0.6367

P = probability of F test, different letters document statistical differences in each column (Tukey HSD, α = 0.05).
GDD - growing degree-days, WSC - water soluble saccharides, DM – dry matter

to Lissbrant *et al.* (2009), low P and K soil fertility reduced fibre concentrations in the lucerne forage. This is in line our preliminary results from long-term experiment in Ruzyně, where variable fertilization resulted in different stand structure. The highest plant density was observed in control, slurry or manure treatments. Increasing rate of N reduced plant density but maintained stem density up to N3 level. Intensive fertilization also increased stand height which was in line with lower leaf weight ratio. These investigations suggest explanation for reduced forage nutritive value under higher nutrient supply described by Lissbrant *et al.* (2009). Further research is warranted to identify the influence by which long-term fertilization management affects lucerne yield components, nutrients content and digestibility within separate lucerne leaves and stems.

Forage quality prediction

Timing of the forage harvest is critical for obtaining optimal quality for animal production. For forage crops that serves as the primary fibre source in the diet, NDF is the principal forage quality variable of concern (Parsons *et al.*, 2006a). Some predictive equations can be used to estimate the forage quality, assisting the producers in decision making at harvest time. Parsons *et al.* (2006b) described an ideal method for estimating quality in the field as a harvest decision aid must be quick, simple, inexpensive, and consistent across all harvests during the season and across a wide range of environments. The most widely used of these are the predictive equations for lucerne quality (PEAQ). This method is based on the length of the tallest stem and the stage of the most mature stem in the sample (Hintz and Albrecht, 1991). These equations have been developed for many regions of the USA. Results indicated some bias in using the equations outside the state of development; however, the prediction errors have been sufficiently low to suggest the PEAQ equations are robust over a wide range of environments (Parsons *et al.*, 2006a). GDD are a temperature-derived index representing the amount of heat to which plants are exposed. It was used similarly to assess length of interval between harvests which was mentioned above. This method has been used with mixed success with the perennial types of forage (Sulc *et al.*, 1999). In the Czech Republic, these methods have not been tested for any perennial forage crops; therefore Hakl *et al.* (2010) tested their accuracy and suitability for lucerne prediction within the first cut period in Central

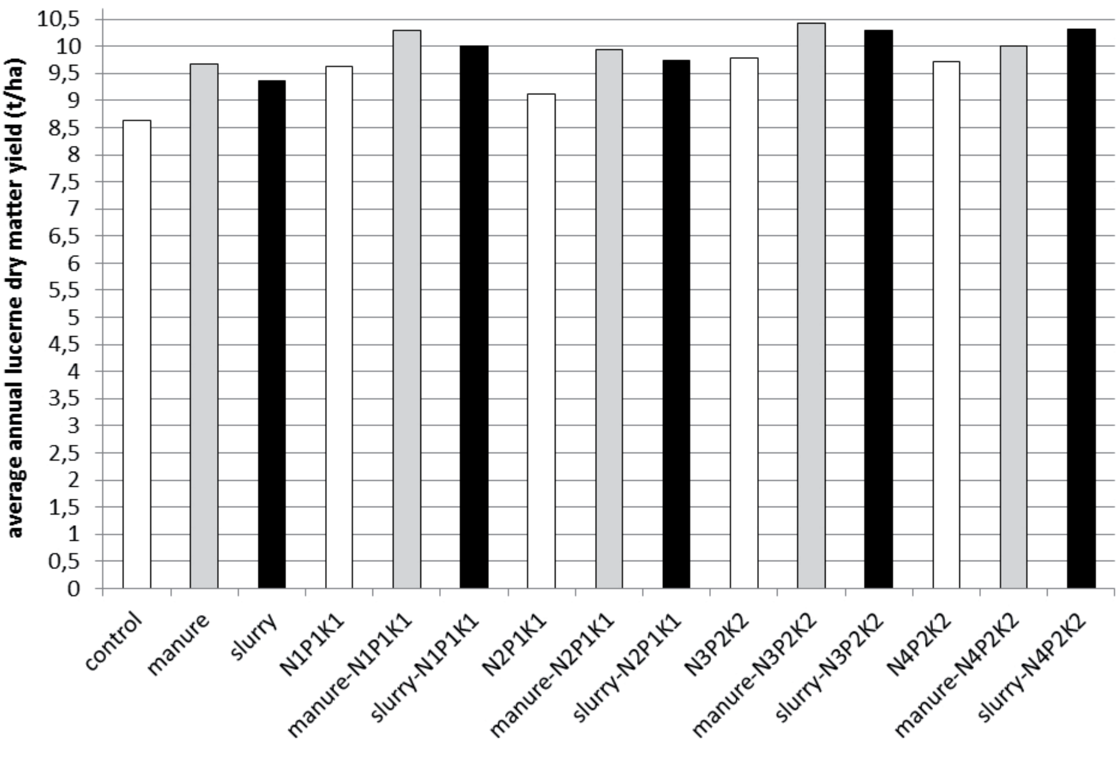

Fig. 1: Lucerne annual dry matter yield ($t.ha^{-1}$) after 60 years of various nutrient applications (adapted from Hakl *et al.*, 2016b)

Bohemia. Their results revealed higher accuracy for PEAQ in comparison with GDD. Suitability of PEAQ method was later reported by Anderzejewska *et al.* (2013) also for northern Europe. Further research has shown that the developmental stage was not suitable indicator for forage quality in year with untypical weather condition (Figure 2). The best solution was a combination with stem length with clear relation to crude fibre content whilst a lower relation was observed to crude protein content. For optimal lucerne quality, the term of first harvest should be in a bud stage when the stem length is to 60 – 65 cm (Hakl *et al*., 2012b). Recent investigations in this research area has shown that canopy reflectance (i.e., remotely sensed) data may allow rapid assessment of nutritive values, such as total N, neutral detergent fibre (NDF), and acid detergent fibre (ADF) of lucerne. The remote sensing based prediction equations explained from 78 to 83 % of the variation in measured total N, NDF, and ADF, correctly predicted about 78 % of the measured TDN/CP ratios. This technology could help improve profit margins by timing the cutting or harvesting of lucerne, in rapid assessment of nutritive values over large areas devoted to growing lucerne, and assessing nutritive quality in real time (Starks *et al.,* 2016).

Forage conservation

Forage conservation cannot be excluded from group of highly important factors affecting quality of feed for animals; however its impact is strongly limited in terms of forage quality before conservation. In this case, we properly cannot talk about increasing of quality but only about decreasing nutrients or quality losses during conservation process. Despite this limitation, the conservation of forage crops is one of the most risk-intensive processes undertaken by farm managers. From the time of harvest until it is used as feed, it is subject to significant losses both in quantity and quality. These losses occur during harvesting and field operations, and later during storage and handling of the product. To minimize the risk associated with forage conservation it is important to understand these processes, how they interact with one another, and how their effects can be mitigated through various management practices. These management tools were summarily described for example by Moore and Peterson (1995).

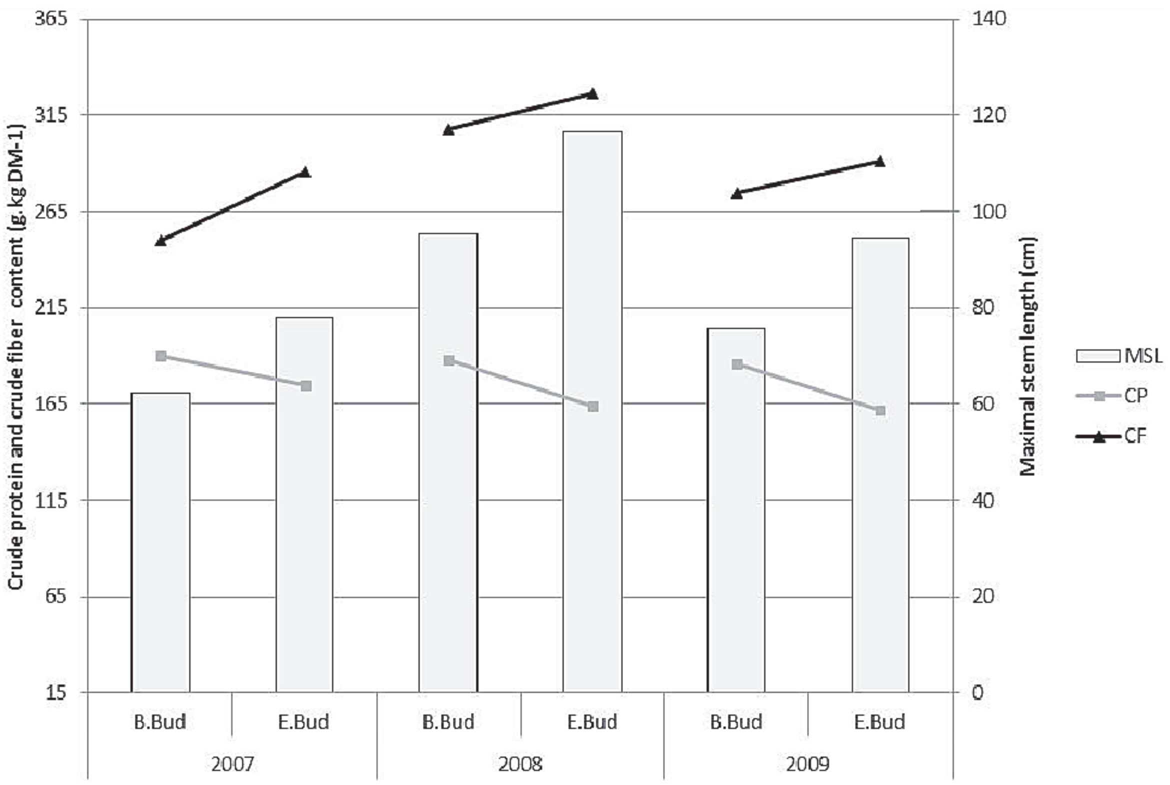

Fig. 2: Lucerne maximal stem length (cm), crude protein and fibre content (g.kg DM^{-1}) in the first cut (3-year period, site Červený Újezd, adapted from Hakl *et al*., 2012b)
MSL – Maximal stem length, CP – crude protein, CF – crude fiber

Protein utilization

Contrast to previous topics, this theme covers area about specific evaluation of legume forage quality in connection with animal utilization. According to our opinion, it is a very hot and highly important topic and therefore included in this review. Forage legumes such as lucerne or red clover represent a major protein source for ruminant nutrition in Europe (Krawutschke *et al.*, 2013). Protein degradability in forage legumes is of global importance because utilization efficiency of forage has economic and environmental consequences. Rumen protein degradation and the resulting imbalance between carbohydrate and protein supply leads to lower N-use efficiency by ruminants (Broderick, 1995). Increasing the amount of protein that escapes from the rumen could benefit ruminant nutrition and improve the economics of the dairy industry (Chen *et al.*, 2009). The most commonly studied factors affecting protein fractions include plant species and harvest maturity (Kirchhof *et al.*, 2010; Krawutschke *et al.*, 2013). Previous studies (e.g. Lemaire *et al.*, 2005) have shown that N concentration in forage is closely related to plant morphology in the lucerne stand. In spite of it, in almost all published studies that investigated CP fraction of legumes, information on stand traits was not presented (e.g., Kirchhof *et al.*, 2010; Krawutschke *et al.*, 2013). Therefore, we hypothesize that changes in plant morphology within a dense canopy could also be connected to variation in CP fractions. Within two year period, lucerne leaf and stem samples were taken in the three cuts and plant density, stem density, maximal stem length and leaf weight ratio were assessed. All dried stem and leaf samples were milled to pass through a 1 mm screen and analysed for CP fractions according to Licitra *et al.* (1996) where protein content was fractionated into A and B_1 (soluble fractions), B_2, and insoluble fractions B_3 and C. Recently published results of Hakl *et al.* (2016a) suggest that stand traits make an important contribution, accounting for about 75 % of CP fraction variability. Above all, maximal stem length is a variable that can be easily assessed for individual plants and has a strong negative correlation with leaf weight ratio, which is assessed as less easily than maximal stem length. The findings of this research indicate that plant morphology should be considered, particularly when evaluating the genetic variability of the CP fraction within legume species (Tremblay *et al.*, 2003) or measuring protein composition among lucerne cultivars (Chen *et al.*, 2009).

Alternative forage utilization

Traditional utilization of forage biomass is connected with ruminant nutrition. In many European countries, decrease of number of cattle units in connection with recently low milk production profitability make an issue with utilization of produced forage (e.g. Stypinsky *et al.,* 2009). This is a key problem for permanent grassland because grassland area cannot be reduced due to environmental impact in landscape. In the arable land, perennial fodder crops simply are not included in a crop rotation. However, absence of these crops together with lower production of organic fertilizers has negative impact on soil fertility and balance of organic matter. For this reason, researchers are looking for alternative utilization of these crops for various purposes. For example, there is a tendency for utilization of forage legumes as a protein source for monogastric animals, pharmacy or human nutrition. In spite of these minor possibilities, the major activity is energy production from forage biomass because generation of energy from biomass has a key role in current EU strategies to enhance energy security. At present, biogas production from energy crops in the arable land is mainly based on the anaerobic digestion of maize. Maize achieves the highest methane yield per hectare in comparison with cereal or sunflower (Amon *et al.,* 2007). On the other hand, it must be noted that maize cultivation is limited in some areas and can have some negative impact on environment as higher pesticide and fertilizers are required. Maize fields are, in general, relatively vulnerable to both water and wind erosion (Graebig *et al*., 2010).

Unlike maize, biogas production from lucerne or clover forage is not a common practice. Legume crops could also be a suitable source for biogas production and it is generally accepted that their cultivation significantly improves soil fertility in contrast to maize cultivation. According to Walla and Schneeberger (2006), lucerne grass mixture is a more efficient energy crop than silage maize on organic farms. Forage legume stands seem to be a suitable biomass source because of its persistency, high productivity, self-sufficiency of N_2 and positive impact on soil fertility. According to Amon *et al.* (2007), specific harvest and processing technologies are required when crops are used as a renewable energy source compared to growing them as a forage source for ruminants. The traditional harvest management for livestock feed recommend the cut term in the bud stage in relation to high quality of forage (Hakl *et al.,* 2010). In contrast, the suitable harvest management of lucerne in a biogas production system is unknown. It must be taken into account that a two cut management system produced more total forage than a three- or four- cut management system harvested at early bud (Lamb *et al.,* 2003). The impact of changes in lucerne biomass quantity and quality under different harvest management could be different for biogas production in comparison with animal utilization.

For clarifying these relationships, biogas production from lucerne biomass was tested over two years in a field plot experiment (Hakl *et al.*, 2012). Biomass was tested in 120 ml bottles in five replications for each variant. After basic homogenization and grinding of fresh matter, two grams of tested biomass and 80 ml of inoculum were dosed into fermentors. Active mesophile anaerobic sediment from biogas plant was used as the inoculum. Cultivation took place in thermo box at 40 °C for a period of 40 days. Production of biogas in laboratory tests of biomass was evaluated once a day, using gas-metric burette. In figure 3, values of substrate biogas yield were in wide range of 423 to 648 $L.kg^{-1}$ DM. When 10 % as average ash content in lucerne forage and 60 % methane content in biogas is considered, methane yield from 280 to 430 L $CH_4.kg^{-1}{}_{OM}$ could be obtained. This range corresponds with results published by Amon *et al*. (2007) about methane yield from other energy crops. The average methane yield 398 L $CH_4.kg^{-1}{}_{OM}$ was obtained from maize silage whilst from wheat it ranged between 140 and 343, from sunflower between 154 and 454, and from grassland between 128 and 392 L $CH_4.kg^{-1}{}_{OM}$. As was noted by Prochnow *et al.* (2009), the aim of energy crop for biogas production is to achieve the highest possible methane yield per hectare. Results show that area biogas yield from lucerne forage could be significantly increased by change in harvest management towards delayed cuts. It is in accordance with Lamb *et al*. (2003), that harvesting twice per season at a later maturity stage would be an effective management strategy for maximizing yield in a lucerne biomass energy production system. In our study with biogas production, the average increase of yield in late flower stage was relatively stable across the year and achieved approximately 50 and 35 % in the first and second cut, respectively. In spite of substrate biogas yield higher than 25 % in the bud stage in 2009, the higher area biogas yield was produced in late bloom stage. This results in increasing area biogas yield in spite of decrease in substrate biogas yield support idea, and requirements on the biomass quality are different when crops are anaerobically digested in biogas plants compared to being fed to cattle. The reason could be that the digester at the biogas plant offers more time to degrade the organic substance than the rumen does. Another important point could be a different micro-organism population in the digester (Amon *et al.,* 2007) or the fact that higher proportion of NDF in the forage does not result in lower dry matter intake in the case of biogas plant. In this experiment, lucerne reached lower methane yield per hectare in comparison with maize and probably would not play a dominant role in biogas production from crops growing on arable land. Nevertheless, the methane yield of lucerne seems to be higher or comparable with other crops as cereal or sunflower and lucerne cultivation could be a suitable supplement for biogas production due to lucerne's non-productive function with positive impact on soil fertility and reduction of soil erosion.

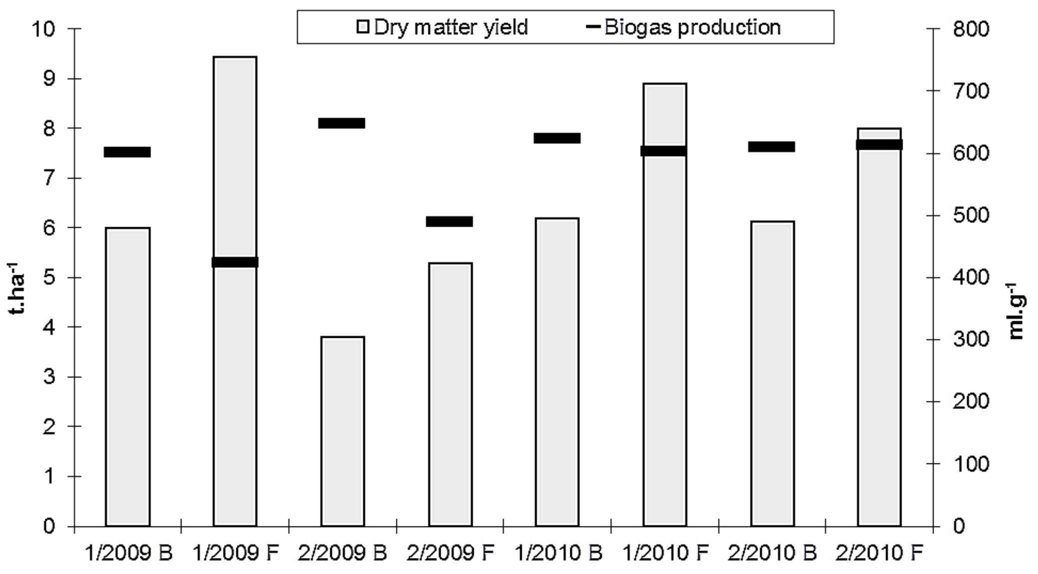

Fig. 3: Lucerne dry matter yield ($t.ha^{-1}$) and substrate biogas production (ml/g) in first (1) and second (2) cut at bud (B) and flower (F) stages in 2009 – 2010 (adapted from Hakl *et al*., 2012a)

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