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Fibre and Protein Quality of Silages and their Effects on Ruminant Performance

BACK

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INTRODUCTION

Forage is the main feed for ruminant animals as their microbes in the rumen can utilize cell-wall components, which constitute a significant part of forage dry matter (DM). In forage cell walls, there are the structural carbohydrates cellulose and hemicellulose, which are linked to lignin, which is classified as a polymer of hydroxycinnamylalcohols (Jung and Allen, 1995). The degree of these cross-linkages and the amount of lignification cause variations in microbial degradation of cellulose and hemicellulose as lignin is indigestible (Jung and Allen, 1995). Some of the forage protein is present in the cell walls, of which a small part is linked to the cellulose-lignin matrix, and cannot be utilized by the ruminant animal (Sniffen et al., 1992). Additionally, pectin is part of the cell walls with a larger amount in legumes than in grasses. In nutrition, the cell-wall components are classified as fibre. Fibre is analysed as neutral detergent fibre (NDF), which contains hemicellulose, cellulose, lignin and cell-wall bound protein. However, if sodium sulphite is added to neutral detergent (ND) solution, much of the protein is removed (Mertens, 2002). Neither is pectin found in the NDF as pectin is solubilized in the ND solution (Jung and Allen, 1995). Cellulose, lignin and protein bound to these compounds constitute the acid detergent fibre (ADF), which is the remaining cell wall after treatment with an acid detergent (AD) solution (Van Soest et al., 1991). The lignin can be determined as the residue after treatment with 72% sulphuric acid and defined as acid detergent lignin (ADL; Van Soest et al., 1991).

Forages differ in nutrient composition and perennial forages can be classified in legumes and grasses. Grasses further can be divided into cool-season and warm-season grasses of which the cool-season grasses have lower NDF concentration and higher organic matter (OM) digestibility than the latter ones (Harrison et al., 2003). This paper focuses on cool-season grasses as they are the predominant grasses grown in north Europe, and their comparisons to legumes. At similar maturity stages, legumes contain less sugar and more protein than grasses but the crude protein (CP) concentration of grasses fertilized with high rates of nitrogen can be nearly as high as for legumes (Buxton and O´Kiely, 2003). As grasses and legumes mature, the CP and sugar concentrations decrease while the NDF concentration increases and digestibility of NDF decreases, resulting in a decreased OM digestibility (Kuoppala et al., 2009; Nadeau et al., 2000), which can affect intake and ruminant performance (Nadeau et al., 2015a; Alstrup et al., 2016). Whole crop cereals and maize, which are annual forages, contain moderate-to-high amounts of digestible starch from the grain at later stages of maturity. As whole crop maize and cereals develop, the awn-to-stalk ratio increases, resulting in increasing starch content while the NDF concentration decreases and the NDF of the stalks becomes less digestible, resulting in no or small changes in whole plant OM digestibility and metabolizable energy concentration. These forages have low CP concentrations (Hetta et al., 2012).

Nutrient composition of forages plays a role in the ensiling ability of the forage. Legumes, such as lucerne, have low sugar concentrations and high protein concentrations and buffering capacity, resulting in a slow acidification rate to reach a desirable pH to decrease proteolytic activities by enterobacteria and clostridia (Buxton and O'Kiely, 2003). Grasses contain more sugar and have a lower buffering capacity than legumes enhancing the growth of lactic acid bacteria to produce lactic acid and acetic acid, thereby decreasing pH faster to a level that decreases the risks for secondary fermentation (Pahlow et al., 2003). Whole crop cereals and maize have relatively thick stalks, which require to be chopped finely and packed thoroughly in the silo to decrease the risks for air infiltration during storage. Air infiltration enhances yeast growth in the silage, which increases the risks for aerobic instability upon opening of the silo and during feed-out (Weiss et al., 2016). The temperature rise of the silage indicates nutrient losses, which can cause reduced energy intake for the ruminant animal (Muck et al., 2003).

Depending on differences in nutrient composition and fermentation characteristics, various forages complement each other in rations for ruminants, where the main goal is to optimize protein and energy intakes, while providing sufficient amounts of structural fibre for an optimal rumen function to maintain a high feed efficiency of the ruminant animal. Quality of the protein and of the fibre fed is of major importance for a healthy rumen environment resulting in high feed efficiency of the ruminant animal. Therefore, this paper focuses on forage fibre content, mastication, degradation and utilization and on forage protein solubility, degradation and utilisation as affected by forage and animal characteristics.

STRUCTURAL FIBRE OF FORAGES

The content of NDF is the chemical fraction in forages, which has the largest effect on digestibility (Huhtanen et al., 2006), energy value, intake (Mertens, 2007), chewing activity (Nørgaard et al., 2010), rumen function (Mertens, 1997), the distribution of particle size in rumen content (Schulze et al. 2014b), and faeces characteristics (Jalali et al., 2015). The content of NDF depends on stage of maturity at harvest and type of forage. The legumes, such as lucerne (*Medicago sativa* L.) and red clover (*Trifolium* pratense L.), have in general lower NDF contents than grasses at similar stages of maturity (Table 1). The dietary characteristics of the NDF fraction includes the proportions of ADF, ADL, indigestible NDF (iNDF), digestible NDF (DNDF) and the rate of degradation of the DNDF fraction (k_d_DNDF). The cell walls generally are much more lignified in legumes compared to grasses. The lignification in term of the ADL/NDF ratio generally increases due to advanced maturity at delayed harvest, which leads to decreased digestibility of NDF and DM of the forage. The iNDF content is closely linked to the lignin content and the iNDF value is 2.5-3 times the ADL content in grass and lucerne. However, this relationship is affected by forage type and by stage of maturity at harvest (Huhtanen et al., 2006). The proportion of DNDF in NDF of grasses ranges typically between 70 and 90% of NDF, whereas the proportions of NDF in DM and of DNDF in NDF are much lower in lucerne of similar maturity stage and OM digestibility as illustrated by the values in Table 1. However, the low proportion of digestible NDF in lucerne is partly compensated by a high rate of degradation of DNDF (k_d_DNDF) of lucerne, which makes the digestibility of lucerne silage less limiting to increased intake compared to grasses. Red clover has a relatively high proportion of digestible NDF at early harvest compared to lucerne. White clover (*Trifolium repens* L.) has generally much higher content of cell solubles and lower NDF content compared to grasses, lucerne and red clover, which makes white clover highly digestible. For whole-crop maize, there is a strong negative correlation between the concentrations of NDF and starch ($r = -0.83$) as there is an increased proportion of the starch-rich cob while the proportion of the fibre-rich stalk is decreasing (Nadeau et al., 2010).

Table 1 Effect of harvest time (early, medium, late) on fibre characteristics and *in vivo* organic matter digestibility (OMD) in different forages.

	Grass silage[1]			Lucerne silage[2]		White clover silage[3]		Red clover silage[3]	
	Early	Medium	Late	Early	Late	Early	Late	Early	Late
NDF, % DM	44.9	57.8	63.4	37.9	44.6	19.3	28.7	36.0	45.0
ADF, % DM	26.7	35.7	38.3	30.1	35.1	18.7	27.9	24.7	39.8
ADL, % NDF	4.2	6.7	8.2	18	19	13	18	10	16
iNDF[4], % NDF	7.7	16	27	43	62	13	22	17	50
DNDF[5], % NDF	92	84	73	57	48	87	78	83	50
k_d_DNDF[6], %/h	6.4	4.7	4.4	6.4*	5.6*	6.9*	5.9*	4.5*	4.0*
OMD, %	80	73	64	70*	59*	78*	75*	72*	66*

[1] Jalali et al. 2012a
[2] Kornfelt et al. 2013b, cv. Pondus
[3] Kornfelt et al. 2013a, white clover cv. Klondike and red clover cv. Rajah
[4] Indigestible NDF *in situ*
[5] Digestible NDF=NDF-iNDF
[6] Rate of degradation of DNDF *in situ*
*Kornfelt, 2012

Chewing activity

The daily time spent eating and ruminating, which sums to total chewing time, is closely related to the intake of forage NDF (NDF_f; Table 2). The time spent eating and ruminating per kg NDF_f decreases at increasing BW from lamb, sheep, growing cattle to mature dairy cows and beef cows, which are considered to spend 50 minutes eating and 100 minutes ruminating per kg NDF intake according to the Nordic Chewing Index system (Nørgaard et al., 2011). However, the time spent ruminating per kg NDF_f decreases at increasing intake of NDF_f /BW (Nørgaard et al., 2010; Schulze et al. 2015), whereas the ratio between eating time and ruminating time increases at increasing intake of NDF_f and at decreasing iNDF/NDF ratio of the forage (Schulze et al., 2014a). Time spent eating per kg DM intake and per kg NDF_f intake appears to increase at increasing feeding level up to *ad libitum* intake (Schulze et al 2014a). In addition, increasing lignification of NDF and increasing iNDF/NDF ratio in forages is considered to increase rumination time per kg NDF_f according to the Nordic Chewing index system (Nørgaard et al., 2010; Nørgaard et al., 2011), and this effect has been supported by the observation by Schulze et al. (2015). When using a ruminating monitoring system (RuminAct-Milkline, Gariga di Podenzano, Italy) on lactating dairy cows, variation in daily rumination time was to a lesser extent explained by variation in intakes of dietary fractions, such as fibre, starch and sugar, than to the individual variations between cows. Furthermore, rumination time in minutes per kg of DM intake was negatively related to milk yield and milk protein content but positively related to milk fat content (Byskov et al., 2015).

Faeces characteristics

Faeces characteristics in cattle, sheep and goats are strongly affected by the forage intake. Plant species, stage of maturity at harvest, NDF content, lignification of NDF, digestibility and physical form of forages affect faeces characteristics. Faeces have been characterized by the content of DM, the content of particle dry matter in DM (PDM), particle size and distribution of particle size dimensions in the PDM fraction (Table 2). The PDM values of faeces from cattle and sheep generally increase at increasing stage of maturity at harvest, increasing ADL/NDF ratio of forage (Jalali et al., 2015), increasing NDF content and at decreasing apparent digestibility of NDF (Schulze et al., 2014a,b; Schulze et al., 2015; Figure 1).

The dimension size of the faeces particles in the PDM fraction has been characterized by sorting of PDM matter in different sieving fractions, and by density and accumulated distribution functions of particle length and width values (Jalali et al., 2012a,b). Figure 2 shows the left skewed density distribution of particle length in faeces PDM from small ruminants fed either artificially dried grass hay or grass seed straw (Jalali et al., 2012b). The length and width distributions of particles in faeces from ruminants are characterized by many short and thin particles and a few long and wide particles. The most frequent (mode) width values of faeces particles from ruminating animals fed grasses and legumes are found to range between 0.07 and 0.3 mm, and with increasing mode value due to delayed harvest and increased lignification of NDF (Table 2). Likewise, Schulze et al. (2015) observed a decreasing proportion of small particles < 0.1 mm due to delayed harvest of grass-clover forage conserved as silage or hay. The density distribution for width values of faeces particles from cattle fed forage legumes, such as lucerne and clovers show two peaks (Kornfelt et al., 2013a,b). The second peak value indicates much wider faeces particles compared to faeces particles from cattle or sheep fed grasses, which might be associated with much higher lignification of legumes compared to grasses.

Table 2 Effect of harvest time (early, medium, late) of different forage types on chewing activity and faeces characteristics in ruminants.

	Pregnant ewes Ad libitum			Dry cow 80% of ad libitum					
	Grass silage^1			Lucerne silage^2		White clover silage^3		Red clover silage^3	
	Early	Medium	Late	Early	Late	Early	Late	Early	Late
Intake, g NDF/kg BW	11	13	13	11	11	3.1^x	4.8^y	5.4^y	7.7^z
Chewing time									
Eating,min/kg NDF	325	359	357	108^x	117^y	165	139	116	107
Rumination, min/kg NDF	343	323	360	96	106	135	127	125	135
Total, min/kg NDF	668	682	717	201^x	224^y	300	266	241	243
Faeces characteristics									
Dry matter (DM), %	27^x	31^y	35^z						
Particle DM , % of DM	41^x	60^y	68^z	62	71				
Sieving particle DM									
Mean particle size, mm	0.18^x	0.20^x	0.23^y			0.23^x	0.21^{xy}	0.19^y	0.26^z
LP > 1 mm, %	2.9^x	2.4^{xy}	1.8^y			8	4	6	4
Image analysis of particles									
Mode particle length^4, mm	0.37	0.31	0.34	0.35	0.38	0.19^x	0.25^x	0.27^x	0.44^y
Mode particle width, mm	0.071	0.064	0.074	0.10	0.10	0.06	0.07	0.06	0.08
Mean particle length, mm	0.82	0.78	0.88	1.16	1.18	0.91^x	0.94^x	0.81^y	1.11^z
Mean particle width, mm	0.11^x	0.13^x	0.16^y	0.25^x	0.27^y	0.21^x	0.20^x	0.18^y	0.25^z
95 percentile length^5, mm	3.8	3.2	3.5	4.3	4.4	4.6	4.3	4.2	4.6

[1] Jalali et al. 2012a
[2] Kornfelt et al. 2013b
[3] Kornfelt et al. 2013a
[4] Most frequent particle length, see Figure 5
[5] Fractile value, which defines the minium length of the 5% longest particles
$^{x, y, z}$Values within the same row and experiment without common superscript differ ($P < 0.05$)

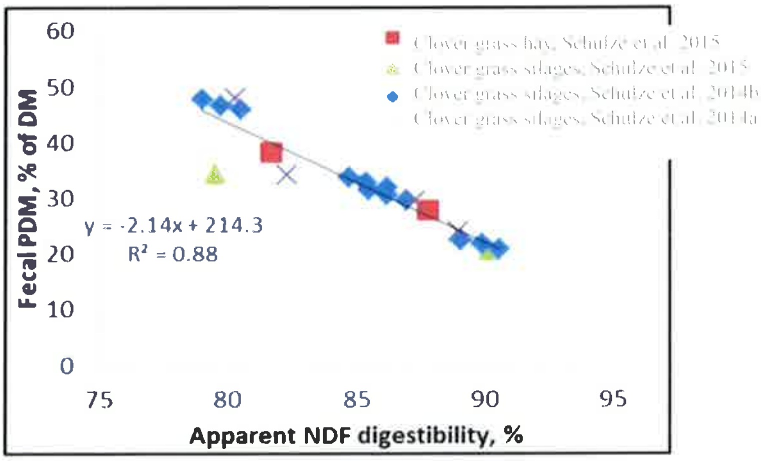
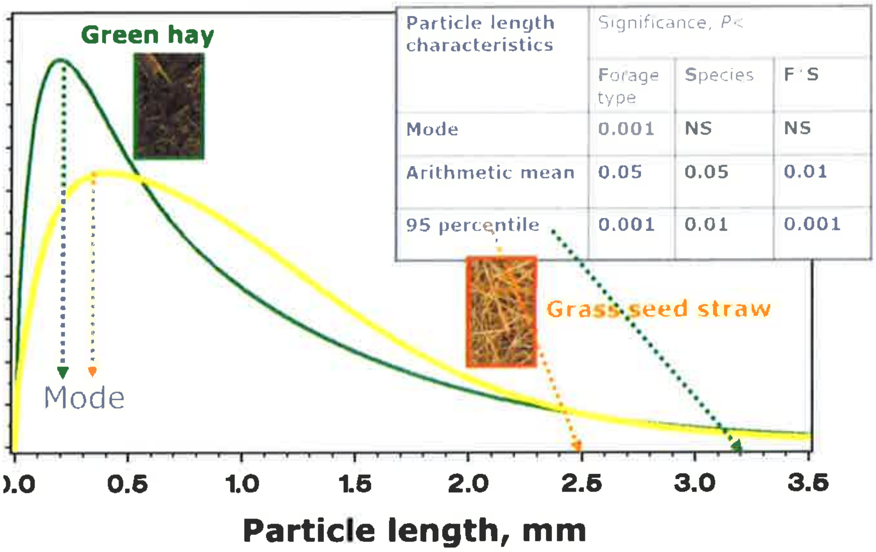


Figure 1 Relationship between the content of faecal particle dry matter (PDM) and the apparent NDF digestibility in heifers fed highly digestible grass-clover silages or hay (Schulze, 2014).

The large particles (LP) in faeces have been defined as the particles larger than the critical particle size (CPS) of 1.18 mm or as particles longer than the critical particle length (CPL) value of 5 mm in cattle and about 3-4 mm in small ruminants. The CPS and the CPL have been defined as the 95% percentile value for the accumulated distribution of particle matter by sieving and particle length value from image analysis (Table 2). The mean particle size in faeces from ruminant animals increases due to increasing BW, increasing lignification of NDF and increasing intake of forage NDF relative to BW (Jalali et al., 2015).

Rumination is considered as the major process for the physical break down of feed particles, and rumen particles larger than CPS or longer than CPL are selectively retained in the reticulo-rumen system. Schulze et al. (2014a) observed a negative relationship between the mean particle size in faeces and rumination time per g iNDF and between the mean particle size in faeces and rumen degradation of NDF and DNDF. This indicates that a high degradation of DNDF is promoted by an effective particle size reduction during rumination.



Particle length characteristics	Significance, $P<$		
	Forage type	Species	F*S
Mode	0.001	NS	NS
Arithmetic mean	0.05	0.05	0.01
95 percentile	0.001	0.01	0.001

Figure 2 Density distribution of particle length of washed faeces particles from different animal species (S) fed different forage types (F) and the interaction between forage type and animal species (F*S). F: Green hay and Grass seed straw S: Goats, sheep and llamas (Jalali et al. 2012b).

Feed intake

The intake is generally dependent on both animal and feed characteristics, and management (Mertens, 2007). Forage intake generally increases due to increasing digestibility, and decreasing NDF content, whereas the intake of forage decreases due to increased supplementation of concentrates (Mertens, 2007). Randby et al. (2012) observed an intake of 1.5% NDF_f of BW in lactating dairy cows, whereas Nadeau et al. (20015a) observed and intake of 2% NDF_f of BW in nursing ewes fed medium cut grass silage. Several models have been established for prediction of intake by dairy cows from concentrate supplementation and forage characteristics, such as digestibility, net energy (NE) content (NE/kg DM), NDF or crude fibre content, DM content and content of fermentation products (Jensen et al., 2015). Jensen et al. (2015) evaluated five recent models on a dataset of 140 treatment means of DM intake values by lactating dairy cows, and observed that models generally over predicted high intake and that the performance of the predictions ranged in RMSPE from 1½ to 3 kg DM per day. Nørgaard and Mølbak (2001) observed a decreasing intake of NE at increasing dietary chewing index value (min/NEI) of lactating dairy cows, dry cows and growing cattle. The intercept value, predicted as the chewing index approaching zero, has been interpreted as the metabolic capacity for intake of net energy (NE_0). Furthermore, the decrease in NE intake at increasing chewing index values has been found to be proportional with NE_0^2. The NE_0 has been parameterized in lactating dairy cows to be depending on the metabolic body size ($BW^{0.75}$), lactation performance and days in milk (Jensen, 2015; Jensen et al., 2016). In addition, Nielsen (2016) found that the $NE_0/BW^{0.75}$ values did not differ between pregnant Hereford and pregnant large Charolais beef cows, but the ranking order of $NE_0/BW^{0.75}$ values was lactating dairy cows > nursing ewes > pregnant ewes and pregnant beef cows. As an implication of the new model, Jensen (2015) showed an increasing substitution rate of forage for concentrate due to increased supplementation of concentrate, decreasing NE_0 value and decreasing dietary chewing index values. Nielsen et al. (2015) observed decreasing intakes of metabolizable energy (ME) by pregnant ewes at increasing dietary chewing index values, which were corrected for the lower BW of ewes compared to dairy cows. These new findings of a linear relationship between the dietary chewing index value and the intake of energy appears to be a potential for modelling energy intake of different levels of supplementation and different forage qualities by use of the same intake model across different ruminating species of different body sizes.

Effects of forage feed value on intake and performance by ruminants

Delayed harvest of forages results in decreased intake and a need for increased supplementation of concentrate in order to maintain intake. The intake of NE is the principal driver for milk yield and daily gain in cattle, sheep, goats, and wild ruminants. Randby et al. (2012) observed decreasing forage intake, decreasing milk yield, increasing body weight loss and increasing milk acetone content in dairy cows due to decreased feed value of grass silage, which was supplemented with different levels of concentrates. In addition, the intake of NDF_f/BW was negatively related to the energy balance and positively related with the acetone concentration in milk. Likewise, Helander et al. (2014) and Nadeau et al. (2015a) observed decreasing ME intake in nursing ewes and decreasing performance of their lambs due to decreased feed value of grass silages, which were supplemented with concentrates. Randby et al. (2010) observed decreasing NE intake and daily gain in growing bulls due to delayed harvest of timothy grass silage with or without concentrate supplementation. Dairy cows and growing cattle require a minimum intake of forage and NDF_f in order to prevent rumen digestive disorders. The intake of NDF_f is the major source of physically effective fibre (peNDF). Mertens (1997) recommended a minimum content of peNDF in diets for dairy cows of about 20% in order to avoid low rumen pH and low milk fat content. Nørgaard et al. (2011) recommended a minimum dietary chewing index value of 30 minutes per kg DM in order to prevent digestive disorders.

In a Swedish study conducted in 26 dairy herds, which differed in forage types fed to the cows, results showed that combining grass-clover silage with maize silage increased the concentration of undigested NDF in the faeces, contributing to a firmer consistency of the faeces compared to feeding grass-clover silage as the sole forage in the diet ($P < 0.05$; Mgbeahuruike et al., 2016). Furthermore, increasing forage DM intake decreased faecal DM concentration ($r = -0.54$, $P < 0.01$) but tended to result in cleaner cows, whereas increasing DM intake of concentrate increased faecal DM concentration ($r = 0.63$, $P < 0.01$), but might result in dirtier cows (Mgbeahuruike et al., 2016).

Based on a large amount of data from maize cultivars grown in different locations in Sweden, Mussadiq et al. (2013) concluded that the starch concentration and fibre digestibility of the whole-crop maize were the most important parameters for predicted milk yield per Mg DM according to MILK 2006 (Shaver et al., 2006). The importance of NDF digestibility of maize silage on milk yield of dairy cows were confirmed by Krämer-Schmid et al. (2016) in a meta-analysis using 96 dietary treatment means from 29 published experiments. A 0.01 unit increase in NDF digestibility of maize silage improved daily milk yield by 82 g ($P = 0.04$) and daily weight gain by 12 g ($P = 0.03$). The effect of improved fibre digestibility of maize silage on milk yield has previously been shown by Oba and Allen (1999), who reported 2.6 kg higher 3.5% fat-corrected milk yield (41.0 *vs.* 38.4, $P < 0.0001$), when cows were fed the brown midrib 3 mutant (*bm3*) maize silage with improved NDF digestibility compared to its normal counterpart. Intakes of DM, starch and NDF were 2.1 kg, 0.7 kg and 0.5 kg higher, respectively, for cows fed the *bm3* maize silage, thus a higher energy intake. The *in vitro* NDF digestibility at 30 hours of incubation was 49.1% and 39.4% and the lignin concentration was 17 and 25 g/kg DM for the *bm3* maize silage and its normal counterpart, respectively (Oba and Allen, 1999). Increased energy intake also was the driver for increased live-weight gain of bulls of Swedish Holstein and Swedish Red breeds (initial live weight: 390 kg, slaughtered live weight: 638 kg) when maize silage was fed as the sole forage compared with equal proportions of maize silage and grass silage on a DM basis in a total mixed ration (TMR) containing 60-65% forage of diet DM (Zaralis et al., 2014). The daily ME intakes were 140 and 133 MJ ($P < 0.01$) and the daily live weight gains were 1.78 and 1.67 kg ($P < 0.01$) for bulls fed 100% maize silage and bulls fed 50% maize silage and 50% grass silage of diet DM (Zaralis et el., 2014). When feeding the same forage treatments in TMR to growing lambs, live weight gain was unaffected (mean live weight gain: 445 g/day; Helander et al., 2015). The authors concluded that daily intake of crude protein and dietary metabolizable protein to ME ratio were closely related to live weight gain of the lambs. Furthermore, including maize silage in the forage component of the diet has a daily concentrate sparing effect in diets to ruminants (Keady, 2014).

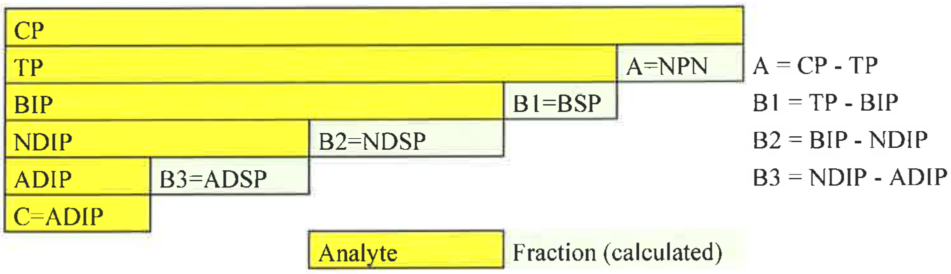
PROTEIN QUALITY OF FORAGES

Forage is an important protein source for ruminants but a large part of the protein in ensiled forage is already in the form of free amino acids and ammonia and as rumen degradable protein (RDP), which makes the utilization of forage protein in ruminants a challenging topic (Givens and Rulquin, 2004). To capture the free amino acids and ammonia for microbial protein synthesis, instant energy sources, such as sugars are needed, and for the RDP, also digestible fibre is needed as energy source, which tells us that the energy concentration of the forage is at least as important as its CP concentration as a majority of the metabolizable protein (MP) from forage originates from microbial protein (Merchen and Bourquin, 1994). Secondary fermentation of the silage can result in production of biogenic amines, which decrease intake and impair animal health (Pahlow et al., 2003; Krizsan et al., 2007; Saleem et al., 2012)

Protein quality can be described as plant CP fractions according to the Cornell Net Carbohydrate and Protein System (CNCPS; Sniffen et al., 1992) as described below. The fractions differ in solubility and rumen degradation and one fraction is considered indigestible. The fractions give us useful information on the types of energy sources needed for optimal protein utilization by the ruminant animal. It needs to be considered, though, that a portion of the soluble non-ammonia N may escape the rumen degradation and be available for absorption in the small intestine (Choi et al., 2002). There are several plant and management factors that affect the proportions of these CP fractions of which we will give an overview of the most important ones.

Crude protein fractionation

Licitra et al. (1996) divide the crude protein according to the CNCPS (Sniffen et al., 1992) into five different fractions A, B1, B2, B3, C (Pichard and Van Soest, 1977). Fraction A is the non-protein nitrogen (NPN), which is the nitrogen passing into the filtrate after precipitation with tungstic acid (Figure 3). B1 is the true protein soluble in borate-phosphate buffer at rumen pH and is degraded rapidly in the rumen. B2 is the true protein insoluble in borate-phosphate buffer, but soluble in the ND solution. Fraction B2 means the protein within the plant cell with high molecular weight and has variable degradation. B3 is the protein insoluble in the ND solution but soluble in the AD solution. This protein is normally cell wall-bound, digestible, but slowly degradable of which most occur post-ruminal. The ND solution is used without sodium sulfite, because sulfite cleaves disulfide bridges in cysteine and reduces the protein content in NDF (Licitra et al., 1996). Fraction C is the protein insoluble in the AD solution and is regarded as indigestible (Figure 3). This fraction is also called ADIN (acid-detergent insoluble nitrogen) and means nitrogen associated with lignin, Maillard products or none-enzymatic browning reaction caused by heating and drying (Licitra et al., 1996).



CP (crude protein), TP (true protein), NPN = non-protein nitrogen, BIP (buffer-insoluble protein), BSP (buffer soluble protein), NDIP (ND-insoluble protein), NDSP (ND-soluble protein), ADIP (AD-insoluble protein), ADSP (AD-soluble protein)

Figure 3 Analysis and calculation of crude protein fractions.

Shannak et al. (2000), Kirchhof (2007) and Edmunds et al. (2012) found strong correlations between *in situ* rumen undegraded protein (RUP) content for different feed stuffs and the crude protein fractions. The RUP content of different feedstuffs can be calculated for three rumen passage rates (2% / h, 5% / h, 8% / h) by use of different formulas. The regression equations by Kirchhof (2007) and Kirchhof et al. (2010) can used to calculate RUP for forage (Table 3).

The lower molecular weight proteins are grouped together in fraction B1, which together with fraction A form the parameter 'protein solubility' (A+B1). Normally, fraction C (ADIP) is between 2-8% of CP (Richardt et al. 2011, Nadeau et al., 2012b). Higher values are indicators for heat damaged protein (Weiss et al., 1986).

Table 3 Regression equations for estimating the ruminally undegraded feed protein (RUP) proportion (g/kg crude protein) assuming passage rates of 2, 5 and 8% / h (RUP2, RUP5, RUP8; mod. Kirchhof et al., 2010)

	RUP2	RUP5	RUP8
Intercept	204.3207	321.923	285.5459
C	1.0753		1.2143
ADF		0.1676	
CP x (A+B1)	-0.0014	-0.0022	
CP x C x C		0.0001	
NDF x B2			0.0005
(A+B1) / NDF			-110.1740
R^2	0.51	0.52	0.56

C = ADIP = acid-detergent insoluble protein, g/kg CP
A = NPN = non-protein nitrogen, g/kg CP
B1 = BSP = buffer-soluble protein, g/kg CP
B2 = NDSP = ND-soluble protein, g/kg CP
CP, ADF and NDF in g/kg DM

Effects of forage species and cultivars

Kirchhof et al. (2010) compared fresh forage legumes in the spring growth cycle and found greater proportions of NPN (fraction A) in white clover and kura clover (*Trifolium ambiguum* M. Bieb.) than in lucerne and birdsfoot trefoil (*Lotus corniculatus* L.) with red clover being intermediate. Lucerne had the greatest proportion of buffer-soluble true protein (BSP, fraction B1) whereas birdsfoot trefoil had the smallest, and white clover, red clover and kura clover were intermediate. Birdsfoot trefoil had a much greater proportion of ND-soluble protein (NDSP, fraction B2) than the other legumes, which did not differ as much. Red clover contained more of the AD-soluble protein (ADSP, fraction B3) than the other legumes. The AD-insoluble protein (ADIP, fraction C) was somewhat higher in red clover than in the other legumes. These differences in CP fractions resulted in greater RUP for red clover and birdsfoot trefoil than for the other legumes (Kirchhof et al., 2010). In comparison, Nadeau et al. (2016) showed lower NPN and NDSP concentrations but higher ADSP concentration in red clover than in lucerne. Furthermore, Fijalkowska et al. (2015a) showed greater proportion of true protein (TP) but smaller proportion of BSP in red clover than in lucerne. Krawutschke et al. (2011) reported lower concentrations of NPN and BSP but higher concentrations of ADSP and ADIN in red clover than in white clover. The soluble protein fraction can differ in rumen degradability between forage species. Hedqvist and Udén (2006) showed lower *in vitro* degradation rate and lower effective protein degradation of the soluble protein fraction in fresh red clover than in fresh white clover, birdsfoot trefoil and perennial ryegrass (*Lolium perenne*), which did not differ. Furthermore, lucerne silage had greater effective protein degradability than silages of red clover and red fescue (*Festuca rubra* L.), which did not differ (Purwin et al., 2014).

When comparing 27 cultivars of lucerne over two years, Tremblay et al. (2000) concluded that there is variability for protein degradability for cultivars with similar yield potentials. Thus, genetic selection for low rumen degradability and high DM yield is feasible. However, in a more limited experiment, no differences in the CP fractions (A, B1, B2, B3 and C) were found between four lucerne varieties (Nadeau et al., 2016). Similar results were obtained by Krawutschke et al. (2011) when comparing three red clover cultivars. However, when 133 entries of red clover, originating from different countries around the world, were compared in protein characteristics, degradation rate and estimated rumen escape ranged from 0.088 to 0.146/h and from 287 to 409 g CP/kg CP with a normal distribution. Hence, these results are promising in developing lines of red clover with improved protein utilization by ruminants (Broderick et al., 2004).

Effects of forage maturity

The *in vitro* CP degradation decreased with advancing maturity of fresh lucerne (bud, 1/10 bloom, full bloom) and smooth bromegrass (*Bromus inermis*; boot, early reproductive, seeded) in spring growth cycle as both NPN and soluble protein decreased (Kohn and Allen, 1995). Likewise, *in situ* degradation of CP decreased from 0.693 to 0.597 at advanced maturity of cooksfoot (*Dactylis glomerata* L.) from heading to flowering (Aufrère et al., 2003). Similar trends were seen in the CP fractions of legumes, where the ADSP (fraction B3) and the ADIN (fraction C) increased by 60% and 80%, respectively, from late vegetative to mid flowering stage of maturity. This resulted in increased RUP from 192 to 257 g/kg CP (Kirchhof et al., 2010). Also, Grabber (2009) reported increased ADSP with advancing maturity of ensiled lucerne and red clover.

Effects of nitrogen fertilization

Tremblay et al. (2005) did an extensive study on the effects of nitrogen fertilization rate on silage quality of timothy (*Phleum pratense* L.). The experiment involved four rates of N fertilization (0, 60, 120 and 180 kg N/ha) prior to the start of the growth in spring at two locations in Canada over two years. The main results were decreased concentration of water soluble carbohydrates (WSC), increased buffering capacity (BC) and nitrate concentrations, primarily in the early stages of development. Hence, the ensiling ability of timothy was diminished when high rates of N were applied. Silage pH, NPN, soluble-N and NH_3-N concentrations increased with increasing N-fertilization rates, especially at the early development stages. Thus, silage quality was reduced at increasing N-fertilizer application rates and the effect was more evident at the early stages of maturity of timothy (Tremblay et al., 2005). Similarly, Keady and O'Kiely (1996) showed increased BC, pH and NH_3-N concentrations of grass silage with increasing N application rates. Nitrogen fertilization increases the CP of plants with a greater increase in NPN than in protein-N (Fijalkowska et al., 2015b).

Effects of wilting and ensiling

Proteolysis during wilting seems to be affected by species that differ in NPN. Among the legumes, lucerne and white clover usually have higher levels of proteolysis during wilting than red clover (Owens et al., 1999; Krawutschke et al., 2011), which could be related to the presence of polyphenol oxidase, which produces phenolic compounds that inhibit proteolysis in red clover (Jones et al., 1995). Birdsfoot trefoil, which has low levels of tannins, had intermediate levels of proteolysis relative to lucerne and red clover (Papadopoulos and McKersie, 1983). In agreement with previous mentioned studies, Fijalkowska et al. (2015a) reported extensive proteolysis of lucerne during wilting and ensiling but very limited proteolysis in red clover silage. However, the ADIN was substantially higher in the silage than in the fresh forage of red clover, which will decrease the utilization of the protein by ruminants. Recently, Nadeau et al. (2016) reported that BSP (B1 fraction) decreased from 169 to 74 g/kg CP while the ADSP (B3 fraction) increased from 26 to 72 g/kg CP during wilting of lucerne (90%) /white clover (10%) forage to 40% DM in sunny weather for 6 hours. There was no effect on the NPN concentration during wilting (Nadeau et al., 2016). During wilting of white clover and red clover to 40% DM in another study, the TP decreased while the NPN increased and the proteolysis continued during ensiling (Krawutschke et al., 2011).

Wilting of early harvested grass-dominated forage (77% grass, 18% clover, 5% lucerne) from 15% to 35% DM for 23 hours in good weather conditions decreased BSP while the NPN, NDSP and ADSP increased resulting in an increased RUP at 8% passage rate per hour (Table 4; Nadeau et al., 2012b). When the wilted grass-dominated forage was ensiled for 125 days, there was a further decrease in BSP from 180 to 33 g/kg CP (Table 4). In addition, the NDSP decreased while the NPN further increased from 175 to 593 g/kg CP, which resulted in a decreased RUP (Table 4; Nadeau et al., 2012b). Changes in CP fractions over the course of ensiling until 125 days are shown in figure 4. Most of the proteolysis occurred during the first 10 days of ensiling and thereafter the rate of proteolysis decreased and stabilized after 30 days. Instead, there was a conversion from NDSP (B2) to ADSP (B3) after 30 days of ensiling (Nadeau et al., 2012b).

In an experiment where both extent and rate of wilting on CP fractions of grass silage were evaluated, it was reported that NPN decreased quadratically with increasing DM from 20 to 65% (Edmunds et al., 2014). Rapid wilting also decreased NPN, implying decreased proteolysis during wilting due to shorter wilting time. Furthermore, fast wilting resulted in more NDSP than slow wilting at all DM concentrations. Fast wilting and increasing DM concentration resulted in increased ADSP compared to slow wilting and decreasing DM concentrations of the grass silage (Edmunds et al., 2014). Likewise, McEniry et al. (2007) showed decreased NH_3-N in grass silage with more rapid wilting. Ensiling alters the amino acid profile of the protein (Edmunds et al., 2014; Purwin et al., 2015), but rumen exposure basically reverts the amino acid profile to the profile found in the forage before ensiling (Edmunds et al., 2014).

Table 4 Crude protein (CP), true protein (TP), CP fractions and rumen undegraded protein of forage as affected by wilting and ensiling (125 d of storage; adapted from Nadeau et al., 2012b).

	Unwilted forage	Wilted forage	Untreated silage	SEM	*P* - value
CP, g/kg DM	150^{a,b}	143^{b}	152^{a}	2.1	< 0.05
TP, g/kg DM	132^{a}	118^{b}	62^{c}	1.8	< 0.001
----g/kg CP[1]----					
NPN (A)	115^{c}	175^{b}	593^{a}	6.2	< 0.001
BSP (B1)	352^{a}	180^{b}	33^{c}	6.9	< 0.001
NDSP (B2)	475^{b}	550^{a}	259^{c}	8.9	< 0.001
ADSP (B3)	17^{b}	61^{a}	79^{a}	5.9	< 0.001
ADIP (C)	40	35	35	4.2	NS
RUP8	292^{b}	350^{a}	210^{c}	7.4	< 0.001

[1] NPN = non-protein nitrogen, BSP = buffer soluble protein, NDSP = neutral detergent soluble protein, ADSP = acid detergent soluble protein, ADIP = acid detergent insoluble protein, RUP8 = rumen undegraded protein at a ruminal passage rate of 8%/h. ^{a,b,c}Means with different superscripts within a row differ significantly at $P < 0.05$. NS = none significance.

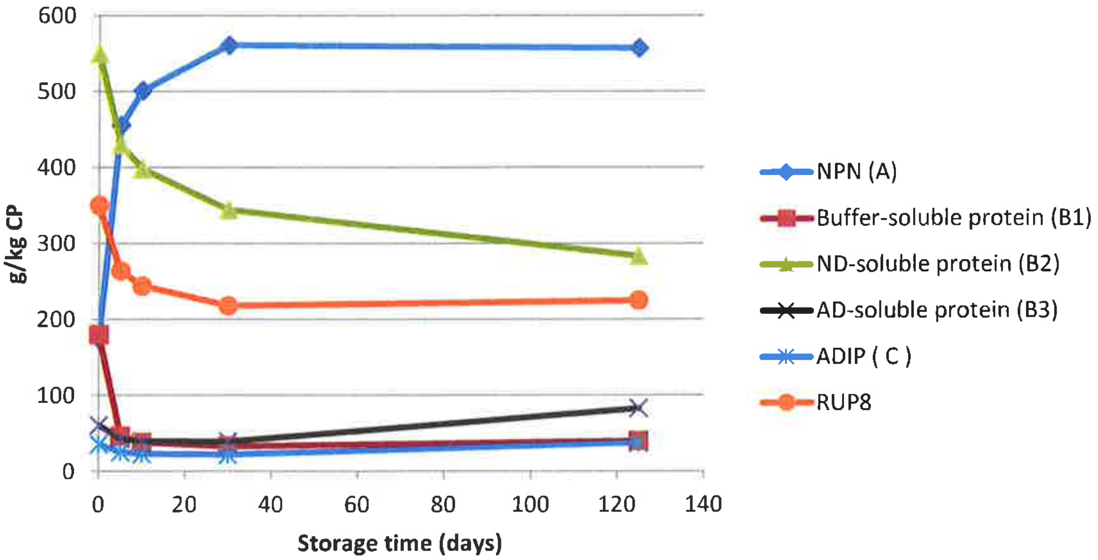


Figure 4 Changes in CP fractions and rumen undegraded protein at 8% passage rate per hour (RUP8) during ensiling of grass silage until 125 days of ensiling. Values are means over untreated and additive-treated silages, n=9 (Nadeau et al., 2012b).

Effects of silage additives

Both chemical and biological additives can reduce proteolysis during ensiling (Nadeau et al., 2000; Slottner and Bertilsson, 2006; Nadeau et al., 2012a,b; Nadeau and Auerbach, 2014). The effectiveness of inoculants on proteolysis is dependent on the WSC concentration of the forage. Inoculants will restrict proteolysis of forages with moderate to high WSC concentrations but will be limited in their actions at low forage WSC concentrations and under those conditions, formic acid is more effective (Davies et al., 1998). This was recently confirmed by Nadeau et al. (2016), who reported no effect of an inoculant containing homofermentative lactic acid bacteria (Kofasil Lac, Addcon Europe GmbH) on the proteolysis of lucerne silage, whereas the use of a formic acid based additive (GrasAAT SP, Addcon Nordic AS) decreased the NPN of the silage from 612 to 554 g/kg CP. Furthermore, Purwin et al. (2014) reported increased effective protein degradability in formic acid treated lucerne silage but similar effect was not found in silages of red clover and red fescue. When highly digestible grass with a WSC content of 215 g/kg DM was ensiled, both a bacterial inoculant and a salt-based additive decreased NPN from 597 to 537 g/kg CP and increased RUP at 8% passage rate per hour from 210 to 233 g/kg CP (Nadeau et al., 2012b). The NH_3-N concentration was 73, 54 and 69 g/kg total N of the untreated, inoculant treated (Kofasil Life, Addcon Europe, GmbH) and chemically treated (Kofasil Ultra K, Addcon Europe GmbH) silages at 125 days of ensiling (Nadeau et al., 2012a). Others also have found decreased NPN by use of a bacterial inoculant to grass silage (Kramer et al., 2012).

Effects on ruminant performance

When red clover silage has been compared to lucerne silage in production trials with dairy cows, red clover has resulted in higher feed efficiency in kg milk/kg DM intake, higher nitrogen efficiency in milk N/N intake and lower milk urea content (Broderick et al., 2001). The authors related the results to the lower NPN concentration of the red clover silage and the higher apparent digestibility of dietary DM, OM and fibre resulting in higher net energy for lactation of the red clover silage diets compared to the lucerne silage diets. The improved apparent nitrogen efficiency of red clover diets compared to lucerne diets also was shown in later trials, although the response in milk yield was lacking, which partly was explained by energy partitioning into fat storage rather than milk fat secretion (Broderick et al., 2007). There also was an elevated ADIN concentration in the red clover silage that could have impaired the N utilization by the cows (Broderick et al., 2007). When red clover silage partially replaced grass silage in diets, the microbial protein flow from the duodenum per unit of DM intake was decreased. However, lower rumen degradable protein could have increased the flow of undegraded feed protein to the duodenum, resulting in similar total protein flow (Merry et al., 2006; Moorby et al., 2009). Increasing proportions of red clover in the diets increased DM intake and milk yield and the proportion of C18 poly-unsaturated fatty acids but decreased concentrations of fat and protein in the milk (Moorby et al., 2009). Birdsfoot trefoil, with higher levels of condensed tannins, has been shown to increase milk yield, milk protein yield, decrease milk urea content and improve milk nitrogen use efficiency compared to lucerne silage in diets to dairy cows (Hymes-Fecht et al., 2013).

As mentioned previously, wilting improves RUP of grass silage (Nadeau et al., 2012b) and Kebreab et al. (2000) reported increased content and yield of milk protein and decreased proportion of N excretion in urine of total manure excretion of dairy cows when fed grass silage, which was wilted for 24 hours to a DM of ca 30% compared to direct cut grass silage at ca 20% DM. The same response on milk protein and N excretion in urine was achieved when a medium application rate of 75 kg of N/ha in the spring before the first harvest was used instead of a high application rate of 150 kg of N/ha. Also, early harvested grass fertilized at a medium N application rate resulted in higher milk protein yield and a lower proportion of total manure N excretion being excreted in the urine than early and late harvested grass fertilized with high N application rate. In summary, early harvest of grass, which is fertilized with a medium rate of N and wilted to ca 30% DM is preferred to dairy cows (Kebreab et al., 2000). Increased RUP will not necessarily result in improved dairy cow performance if its amino acid profile will not meet the requirements of the first limiting amino acids. Edmunds et al. (2013) concluded that rumen degradation changes the amino acid composition of forage and that the amino acid composition of RUP is more similar between forages than to their original composition. This information can help decreasing the number of samples that need to be analysed to gain more knowledge on the effect of rumen exposure on the amino acid composition of forages (Edmunds et al., 2013).

In agreement with Nadeau et al. (2016), Broderick et al. (2007) reported decreased proteolysis and, thereby, lower contents of NPN, ammonia N and free AA N in lucerne silage treated with ammonium tetraformate (GrasAAT, Norsk Hydro ASA) compared to untreated lucerne silage. When fed to dairy cows, the daily DM intake increased by 1.0 kg and the 3.5% fat-corrected milk increased by 2.1 kg. Content and yield of milk true protein increased and nitrogen efficiency in milk N per unit of N intake increased by 1.3 units (Broderick et al., 2007). This production response was, though, not repeated in a second trial.

In an experiment by Nadeau et al. (2014) dairy cows were fed a diet containing 52% grass silage of total DM intake and 170 g CP/kg DM. The silages were treated with the bacterial inoculant Kofasil Life (*Lactobacillus plantarum* DSM 3676, 3677; Addcon Europe GmbH), with the chemical additive Kofasil Ultra K (sodium nitrite, hexamine, sodium benzoate, potassium sorbate; Addcon Europe GmbH) or left untreated. The additives decreased NH_3-N (5.8 *vs.* 7.3% of total N, $P < 0.001$) increased BSP (6.1 *vs.* 2.4% of CP, $P < 0.01$) and tended to increase the more slowly degradable ADSP (7.4 *vs.* 6.1% of CP, $P < 0.10$) compared to untreated silage. The chemical additive decreased contents of urea in milk without affecting the daily nitrogen intake and milk yield, indicating improved protein utilization (Table 5). The excretions of purine derivatives in urine were higher from cows fed silage treated with the chemical additive, indicating a tendency for increased microbial protein flow to the duodenum. Furthermore, the chemical additive decreased the somatic cell count in milk (Table 5). The cows increased in live weight by 5 kg during the 20-day period, which corresponds to a daily milk yield of ca 1.8 kg calculated on an energy basis (GfE, 2001). Use of silage additives can decrease proteolysis during ensiling resulting in potentially improved protein utilization and udder health of dairy cows (Nadeau et al., 2014).

Table 5 Intake, live weight, milk yield, milk and urine components of dairy cows fed diet containing grass silage treated with or without additives[1] (Nadeau et al., 2014).

	Control	Bacterial inoculant	Chemical additive	SEM	*P* - value
Dry-matter intake (% of liveweight)	3.62	3.46	3.54	0.109	NS
Crude protein intake (kg/day)	3.92	3.84	3.84	0.112	NS
Live weight (kg)[2]	645^{b}	650^{a}	650^{a}	8.6	< 0.05
Energy-corrected milk (kg/day)	40.0	39.9	39.4	0.74	NS
Milk urea (mg/l)	240^{b}	248^{a}	230^{c}	4.2	< 0.0001
Milk somatic cell count (no./ml)	$92\ 046^{a}$	$58\ 787^{a,b}$	$51\ 766^{b}$	16 351	< 0.05
Urea in urine (g/day)	383	409	395	13.7	NS
Allantoin in urine (g/day)	91	97	109	6.1	< 0.10
Purine derivatives in urine (g/day)	95	108	115	10.6	< 0.10

[1] control; without additive, bacterial inoculant Kofasil Life, chemical additive Kofasil Ultra K (Addcon Europe GmbH)

[2] SEM is calculated from the variance of the random factor cow within treatment and the variance of the error term in the model of which the factor cow (=variation between cows within treatment) is much greater than the variance of the error term in the model for live weight. When the treatments are compared, the variance of the error term is used. NS = none significance

In a later experiment, grass silage was treated with the inoculant Kofasil Duo (*Lactobacillus plantarum/Lactobacillus buchneri*, 200,000 cfu/g) or with the chemical additive Kofasil Ultra K (Addcon Europe GmbH), which were compared with untreated silage (Nadeau et al., 2015b). The silage contained 15% CP, 47% NDF, 3.3% WSC, 8.2% lactic acid, 2.1% acetic acid and 0.25% NH_3-N of DM with minor differences between treatments. The RUP of the silage at 5% passage rate per hour was 20% of CP for the control and 22% of CP for the inoculant and the salt-based additive. Diets were isonitrogenous (15% of DM) and isoenergetic (11.1 MJ ME/kg DM) varying in RUP (4.9% (high) and 2.9% (low) of DM). Dietary forage proportion of the TMR was 58% of DM. High RUP diet had higher milk yield than low RUP diet (29.4 *vs.* 27.9 kg; $P < 0.05$). The DM intake was not affected by RUP and silage treatment. Yields of milk and ECM were higher for the diets containing additive treated silages than for the control diet at low RUP (28.9 *vs.* 26.0 kg milk, $P < 0.01$; 30.6 *vs.* 27.1 kg ECM, $P < 0.001$) whereas there was no effect of additive treatment in the high RUP diet. Milk fat and protein percentages did not differ between silage treatments. Feed efficiency was higher for the diets containing the adidtive-treated silages than for the control diet at the low RUP (1.6 *vs.* 1.3 kg of ECM/kg DM intake, $P < 0.001$) but not at the high RUP. The increased milk yield and feed efficiency when fed a diet with low RUP can partly be explained by increased RUP of the additive-treated silages (Nadeau et al., 2015b).

CONCLUSIONS

Forage characteristics, as affected by intrinsic plant factors in combination with management factors during harvest, storage and feed out, have major impact on the concentrations and fractionations of fibre and protein in forages, resulting in differences in solubility and digestibility of protein and digestibility of fibre and DM of forages. Variations in nutrient contents of forages determine the amounts of concentrates needed for maintaining growth rate and milk yield of ruminant animals. Forage fibre content affects DM intake and time spent chewing for mastication of forage fibre and rumen retention time for microbial digestion of forage fibre to a size that is small enough to leave the rumen and be present in the faeces. Improved fibre digestibility of forages can increase milk production and live weight gain of ruminants. The energy released during fermentation of carbohydrates is used for the microbial protein synthesis from ammonia, free amino acids and peptides from NPN and from degraded true protein in the rumen. The microbial protein forms together with the rumen undegraded feed protein metabolizable protein that is used for growth and milk yield by ruminant animals.

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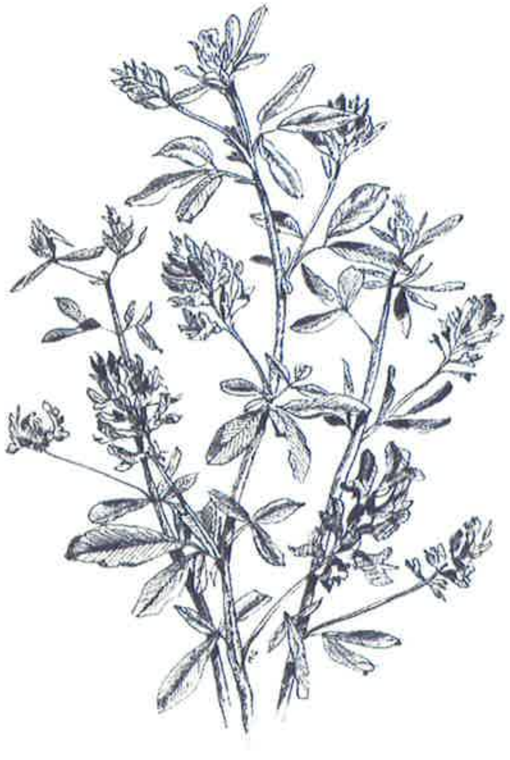

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