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Bilaga 1*

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## Protein quality dynamics during wilting and preservation of grass-legume forage

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**Introduction** Solubility and degradability of forage protein change during wilting and preservation (Muck et al. 2003; Richardt & Steinhöfel 2008). Furthermore, only limited information is available on the effects of additives on silage protein quality (Slottner & Bertilsson 2006; Richardt & Steinhöfel 2008). It is imperative to evaluate changes in protein quality during storage of silage as differences in silage protein quality can affect intake and protein utilisation by ruminants (Broderick et al. 2007; Huhtanen et al. 2008). The objective of this study was to evaluate the effects of wilting, ensiling and silage additive on the protein quality of a grass-legume forage.

**Material and methods** A sward (77% grass, 18% clover, 5% lucerne) was mowed on 3 June, 2010 and wilted for ca 23 hours from 150 g/kg of dry matter (DM) to 350 g/kg of DM by wide spreading. Wilted forage was precision chopped and ensiled in 1.7-L silos at Lantmännen Dairy Research Farm Nötcenter Viken, Falköping, Sweden. The forage was either untreated or treated with KOFASIL® LIFE, containing *Lactobacillus plantarum* DSM 3676 and 3677 at an application rate of 400 000 cfu/g of forage or with KOFASIL® ULTRA K, containing sodium nitrite, hexamethylene tetramine, potassium sorbate, sodium benzoate and sodium propionate, at 2 L/ton forage (ADDCON EUROPE GmbH). The treated silages were compared to untreated silage. Forage was ensiled for 5, 10, 30 and 125 days (d) and was analysed for crude protein (CP) fractions according to Licitra et al. (1996) at LKS mbH, Lichtenwalde, Germany. Models by Kirchhof et al. (2006) were used to calculate rumen undegraded dietary protein (UDP). Data were analysed as a completely randomized design in PROC GLM of SAS 9.2, with treatment and storage length as fixed factors, using three replicates per treatment. For silages, data were analysed as treatment comparisons for each storage length separately and as main effect of storage length as no interactions between treatment and storage length were found. To correct for differences in DM content of the silages, silage DM content was used as a covariate in the model comparing untreated and treated silages. When the overall *P*-value was significant at 5% level, pair wise comparisons between MEANS and LSMEANS of forage treatments and storage lengths were done using Tukey's test.

**Results and discussion** The concentration of water soluble carbohydrates in unwilted and wilted forage was 215 g/kg DM. The mean CP content of forage and silage was 149 g/kg DM. Concentrations of neutral detergent fibre (NDF), acid detergent fibre (ADF) and ash of wilted forage were 375, 245 and 88 g/kg DM, respectively. *In vitro* organic matter digestibility of wilted forage was 917 g/kg (Lindgren, 1979). Concentrations of non-protein nitrogen (NPN), neutral detergent soluble protein (NDSP) and acid detergent soluble protein (ADSP) increased, while the buffer soluble protein (BSP) decreased during wilting, resulting in an increase in UDP at 8%/h ruminal passage rate (UDP8; Table 1). However, when the wilted forage was ensiled without additive for 125 d the NPN content increased, while the contents of BSP and NDSP decreased, resulting in a decreased UDP8 during ensiling (Table 1). Addition of KOFASIL LIFE and KOFASIL ULTRA K tended to increase the UDP8 of untreated silage after 125 d of storage (Table 2). The 11% increase in UDP8 by the additives was mostly achieved by their abilities to decrease the NPN but also by their tendency to increase the NDSP compared to untreated silage, with no differences between the additives (Table 2). No large differences in CP fractions were found between silage treatments at 5, 10 and 30 d of storage (data not shown).

As a mean over silage treatments, the NPN increased from 455 to 557 g/kg of CP (*P* < 0.0001) while the BSP decreased from 47 to 33 g/kg of CP (*P* < 0.05) from 5 to 30 d of storage. The NDSP decreased from 431 to 283 g/kg of CP from 5 to 125 d of storage (*P* < 0.0001). This decreased UDP8 from 264 to 218 g/kg of CP from 5 to 30 d of storage, when averaged over treatments (*P* < 0.0001). Furthermore, the ADSP and the acid detergent insoluble protein (ADIP) increased from 40 to 83 g/kg of CP and from 22 to 37 g/kg of CP, respectively, from 30 to 125 d of storage (*P* < 0.0001).

**Conclusions** Wilting increased protein quality of fresh forage by increasing UDP8, followed by a decrease during preservation. Use of additives tended to increase the UDP8 of the silage, with KOFASIL LIFE and KOFASIL ULTRA K being equally effective. None of the treatments affected the ADIP concentration.

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**Table 1.** Crude protein (CP), true protein (TP), CP fractions and rumen undegraded protein of forage as affected by wilting and ensiling (125 d of storage).

	Unwilted forage	Wilted forage	Untreated silage	SEM	P - value
CP, g/kg DM	150 <sup>a,b</sup>	143 <sup>b</sup>	152 <sup>a</sup>	2.1	< 0.05
TP, g/kg DM	132 <sup>a</sup>	118 <sup>b</sup>	62 <sup>c</sup>	1.8	< 0.0001
---g/kg CP <sup>1</sup> ---					
NPN	115 <sup>c</sup>	175 <sup>b</sup>	593 <sup>a</sup>	6.2	< 0.0001
BSP	352 <sup>a</sup>	180 <sup>b</sup>	33 <sup>c</sup>	6.9	< 0.0001
NDSP	475 <sup>b</sup>	550 <sup>a</sup>	259 <sup>c</sup>	8.9	< 0.0001
ADSP	17 <sup>b</sup>	61 <sup>a</sup>	79 <sup>a</sup>	5.9	< 0.001
ADIP	40	35	35	4.2	0.692
UDP8	292 <sup>b</sup>	350 <sup>a</sup>	210 <sup>c</sup>	7.4	< 0.0001

<sup>1</sup>NPN = non-protein nitrogen, BSP = buffer soluble protein, NDSP = neutral detergent soluble protein, ADSP = acid detergent soluble protein, ADIP = acid detergent insoluble protein, UDP8 = rumen undegraded protein at a ruminal passage rate of 8%/h. <sup>a,b,c</sup>MEANS with different superscripts within a row differ significantly at  $P < 0.05$ .

**Table 2.** Crude protein (CP), true protein (TP), CP fractions and rumen undegraded protein of untreated and treated silages after 125 d of storage.

	Untreated	KOFASIL LIFE	KOFASIL ULTRA K	SEM	P - value
CP, g/kg DM	150	153	148	2.1	0.224
TP, g/kg DM	61	71	68	2.2	0.097
---g/kg CP <sup>1</sup> ---					
NPN	597 <sup>a</sup>	540 <sup>b</sup>	535 <sup>b</sup>	10.4	< 0.05
BSP	31	49	39	5.2	0.192
NDSP	260	289	298	8.3	0.080
ADSP	77	84	87	12.1	0.869
ADIP	36	38	39	2.0	0.640
UDP8	210	233	232	4.6	0.060

<sup>1</sup>See footnotes to Table 1. <sup>a,b</sup>LSMEANS with different superscripts within a row differ significantly at  $P < 0.05$ .