

**THE INFLUENCE OF DRIED WHEY SUPPLEMENTATION TO ALFALFA HAYLAGE ON RATION INTAKE AND DIGESTIBILITY IN WETHER SHEEP****Marina Vranić, K. Bošnjak, Jasna Pintar, J. Leto, Ivana Čačić, I. Stipić,  
Martina Protulipac, Marina Bukal****Summary**

The objective of this paper was to determine the effect of 5 % and 10% in dry matter (DM) of dehydrated whey (DW) supplemented to alfalfa haylage (AH) (*Medicago sativa L.*) on the ration *ad libitum* intake and in vivo digestibility in wether sheep.

The results show increase in *ad libitum* intake of DM ( $P < 0.005$ ) and organic matter (OM) ( $P < 0.05$ ) with DW supplementation but reduced in vivo digestibility of DM ( $P < 0.01$ ), OM ( $P < 0.05$ ) and crude protein (CP) ( $P < 0.001$ ). The level of DW supplementation (5% in comparison with 10%) did not influence AH *ad libitum* intake ( $P > 0.05$ ) neither in vivo digestibility ( $P > 0.05$ ).

It was concluded 5% and 10% of DW supplementation to AH in wether sheep encourages intake of AH but decreases in vivo digestibility probably due to increased rate of the ration passage through the digestive system.

**Key words:** *ad libitum* intake, alfalfa haylage, dried whey, in vivo digestibility.

**Introduction**

High protein forage like alfalfa haylage (AH) requires supplements rich in fermentable energy for more efficient nutrient utilization (Ørskov et al., 1970). Whey is a slightly acid, yellow-green liquid which is the residue obtained from the coagulation of milk by rennet or by the lowering of its pH. The composition of whey varies essentially with the type of cheese of which it is a by-product, with the method of preservation and with the origin of the milk (Matijević et al., 2011). It is composed essentially of lactose (70-73%) as an energy source which makes possible the utilization of non-protein nitrogen in the rumen. It also contains crude proteins (CP) (12-13%), mineral salts (7- 1%), lactic acid in variable quantity (0.5-10%), citric acid (about 1%) and some nonprotein nitrogen (0.5-0.8%). Increasing readily available carbohydrates in the diet of ruminants increases protozoal numbers (Kudo et al., 1991). Protozoa indirectly increase fiber breakdown of high-forage diets (Ushida et al., 1987) thus stimulate forage intake. The addition of a small quantity of whey (2% of the total DM content) to grass or maize silage in dried, concentrated or liquid form improves the digestibility of the main constituents of the mixture (Schingoethe, 1976). Ammonia nitrogen losses are reduced and the silage is made more palatable with beneficial effects reflected in the performance of the animals (Schingoethe and Beardsley, 1975).

Lactose as a source of energy could well replace a large part of the cereals in fattening rations for ruminants and enhance the use of non-protein nitrogen by the rumen microflora.

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For calves that are being weaned, the introduction of whey at the rate of 10% of the concentrate feeds increases feed intake (Morrill and Dayton, 1974), but if whey is introduced at a rate higher than 20%, the concentrate feed intake decreases. Previous papers reported no significant influence of whey supplementation to forage *in vivo* digestibility when fed to dairy cattle (Salem and Fraj, 2007) or increased DM digestibility in heifers (Schingoethe et al., 1980).

The aims of the present study were to examine the effect of 5% and 10% of DW supplementation to AH (*Medicago sativa* L.) on feed and water intake and *in vivo* digestibility in wether sheep. The hypothesis of the present study was that DW supplementation to AH would increase feed and water intake and *in vivo* digestibility.

## *Materials and Methods*

### *Alfalfa haylage*

Alfalfa haylage (AH) was made in 2012 (May 22) at the early alfalfa flowering stage (20 % of flowering crop). The crop was mown and allowed to wilt for 24 h before being harvested at 400-500 g dry matter (DM) kg<sup>-1</sup> fresh sample with a round baler. Bales were wrapped in four to six layers of 500-mm wide white plastic film.

Prior to each out of 3 feeding periods, AH was chopped to approximately 3-5 cm using a commercial chopper. The chopped material was compressed into plastic bags (approximately 10 kg AH bag<sup>-1</sup>) and stored in the cold chamber at a temperature of 4°C by the time of feeding.

### *Dried whey*

Dried whey (pasteurized sweet whey defence) in 2 packages, each of 25 kg, for the research needs was provided from Dukat d.d., M. Čavić 9, 10000 Zagreb, Croatia.

### *Dietary treatments*

The experiment consisted of 3 feeding treatments incorporating AH alone and AH supplemented with 5 or 10 % of dried whey (AH5 and AH10, respectively) kg<sup>-1</sup> consumed DM.

Whey (5 or 10 % kg<sup>-1</sup> consumed DM) was stirred with 15 ml of water, dispersed by haylage, than mixed together with AH and fed to animals. With the feeding treatment AH only, 15 ml of water was dispersed by AH before feeding. No supplementary feeds were provided.

### *Animals, management and experimental design*

Three crossbreed wethers were selected on the basis of live weight (mean body weight 35 kg, s.d. 3.8 kg) and condition score. All animals were treated for internal parasites prior to the experiment. The sheep were subjected to artificial lightening from 08:00 a.m. to 08:00 p.m. daily.

A feeding trial with the three wethers and three treatments was carried out in three periods using a balanced 3x3 Latin square design. Sheep were allocated at random to the three dietary treatments. A 10-day acclimatization period was followed by an 11-day measurement period (4-day *ad libitum* intake was followed by 7-day *in vivo* digestibility measurements), in which feed offers and refusals were measured and total faeces collected. The animals were housed in individual pens

(1.5 x 2.2 m) over the acclimatization period and in individual crates (0.136 m x 0.53 m x 0.149 m) during the measurement period. Animals were fed twice a day (09:00 a.m. and 04:00 p.m.) in equal amounts. Wethers were fed *ad libitum* during the 4-day period while over the following 7-day period the rations were designed so as to ensure a refusal margin of 10–15 % AH each day.

#### *Voluntary feed intake*

During the measurement period, fresh weights and DM contents of AH, AH5 and AH10 treatments offered and refused were recorded daily. Subsamples of offered feed were taken daily and stored at a temperature of  $-20\text{ }^{\circ}\text{C}$  until the end of the experiment, when they were bulked prior to chemical analyses. Daily subsamples of refusals were bulked on an individual animal basis and stored at a temperature of  $-20\text{ }^{\circ}\text{C}$  prior to chemical analyses.

Daily production of faeces was collected. Total daily faecal production of each animal was stored frozen until completion of the collection period. Bulked faecal output from each animal was then weighed and subsampled prior to subsequent analyses. The sheep were weighted on the 10th, 14th and 21st day of each period and the mean weight was used to calculate the daily voluntary intake of fresh matter (FM) and DM expressed per unit of metabolic weight, i.e.,  $\text{g kg}^{-1} \text{M}^{0.75}$ .

The experiment conducted followed the Council Directive issued by the European Economic Community (EEC) (1986) on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes.

#### *Chemical analysis*

DM contents of feed offered, feed refused and faeces were determined by oven drying to a constant weight at  $60\text{ }^{\circ}\text{C}$  in a fan-assisted oven (ELE International). Ash of feed offered, feed refused and faeces was measured by igniting samples in a microwave oven (Milestone PIYRO, Italy) at  $550\text{ }^{\circ}\text{C}$  for 3h. Total N concentrations of feed offered, feed refused, faeces and urine were determined by the Kjeldahl method (AOAC 1990, ID 954.01) using a Gerhardt nitrogen analyzer. In addition, N concentration was expressed as CP (total N x 6.25)  $\text{g kg}^{-1}$  DM for feed offered, feed refused and faeces.

Neutral Detergent Fibre (NDF) and ADF were analyzed using the procedure of Van Soest et al. (1991) by the Ancom Filter bag technology (USA) with an Ancom fiber analyzer. Silage pH was determined in a water extract from 10 g of fresh silage and 100 mL distilled water using a pH meter 315i (WTW). HMC volatile fatty acids (VFA) were measured by liquid gas chromatography and lactic acid was determined enzymatically on an Express Auto biochemical analyzer using the juice expressed from silage.

The average amount of lactose in two samples of dried whey was determined by an enzymatic method (HRN ISO 5765-1:2003) at the Department of Dairy Science, University of Zagreb Faculty of Agriculture.

### Statistical analysis

Data were analyzed using mixed model procedures (SAS, 1999). Model applied:  $Y_{ij} = \mu + T_i + P_j + e_{ij}$ , where  $Y$  is the overall model,  $\mu$  = grand mean,  $T$  = treatment,  $P$  = period,  $e$  = experimental error,  $I$  = number of treatments, and  $j$  = number of periods.

Mean separation was calculated if the F-test was significant at  $p=0.05$ .

### Results and Discussion

Chemical composition of AH used in the experiment is given in table 1.

Table 1. – CHEMICAL COMPOSITION OF ALFALFA HAYLAGE

Parameter	Mean value	Min	Max	CV (%)
DM (g kg <sup>-1</sup> fresh sample)	534.7	512.0	578.5	5.04
OM (g kg <sup>-1</sup> DM)	916.5	910.9	927.1	0.62
CP (g kg <sup>-1</sup> DM)	171.4	148.2	191.2	8.38
NDF (g kg <sup>-1</sup> DM)	459.3	368.9	505.3	10.47
ADF (g kg <sup>-1</sup> DM)	378.5	300.6	442.9	12.37
Fermentation characteristics				
Lactic acid (g kg <sup>-1</sup> DM)	16.9	11.4	27.1	35.7
Acetic acid (g kg <sup>-1</sup> DM)	14.5	11.7	30.7	86.5
Butiric acid (g kg <sup>-1</sup> DM)	0.0	0.0	0.0	0.0
pH	5.0	4.9	5.1	1.46
NH <sub>3</sub> -N (g N kg <sup>-1</sup> total N)	79.8	76.0	83.5	3.8

AH, alfalfa haylage; DM, dry matter; OM, organic matter; CP, crude proteins; NDF, neutral detergent fibre; ADF, acid detergent fibre; NH<sub>3</sub>-N, ammonium N; mean, mean value; min, minimum value; max, maximum value; CV, coefficient of variation.

Relatively high average DM content of alfalfa haylage (534.7 g kg<sup>-1</sup> fresh sample) was a result of 24-hour wilting prior to harvest. This meets the DM requirements of fresh plant material (400-600 g kg<sup>-1</sup>DM) prior to ensiling into big bales wrapped with plastic (Chamberlain and Wilkinson, 1996). The average CP content of AH used in this experiment of 171.4 g kg<sup>-1</sup> DM is slightly lower than 180 g CP kg<sup>-1</sup> DM if harvested at the beginning of flowering (Ball et al., 2002) or lower than the average CP content in alfalfa haylage (193 g kg<sup>-1</sup> DM) reported by Frame et al. (1988), but it is within the range of 150-175 g CP kg<sup>-1</sup> DM reported for ideal haylage (Chamberlain and Wilkinson, 1996).

Higher DM in AH resulted in restricted fermentation (Table 1), lower lactic acid and higher pH value than usually reported for fermented feeds (Chamberlain and Wilkinson, 1996). AH was free from butyric acid. Concentration of NH<sub>3</sub>-N in AH was higher than recommended (lower than 50 g NH<sub>3</sub>-N kg<sup>-1</sup> total N; Chamberlain and Wilkinson, 1996), but higher DM concentration of fresh plant material prior to ensiling means also prolonged proteolysis, which might result in a higher NH<sub>3</sub>-N concentration.

The chemical composition of dried whey (according to producers' declaration) is given in table 2.

Table 2. – CHEMICAL COMPOSITION OF DRIED WHEY

Parameter	Mean	Unit
DM	98.14	%
ME	3.317	kcal/kg
CP	11.2	%
Total fat	0.25	%
Ash	7.19	%
Lactose	700	g kg <sup>-1</sup> DM

DM, dry matter; CP, crude protein, ME, metabolizable energy

The composition of whey varies essentially with the type of cheese of which it is a by-product, with the method of preservation and with the origin of the milk. The dried whey used in this research composed in DM mainly of lactose which fitted the usual range of 70 - 73% (Blaschek et al., 2007). The highest lactose concentration in whey DM agrees with previous research describing the main component of whey is the carbohydrate lactose that supplies energy (Norgaarda et al., 2005). Whey protein is a complete, high quality protein with a rich amino acid profile which is important in tissue growth and repair (Anandharamakrishnan et al., 2007). Declared CP concentration in the dried whey was lower than described previously (12-13% in DM) (Blaschek et al., 2007).

### Diet intake

Table 3 shows the effect of dried whey supplementation to AH on the ration *ad libitum* intake by wether sheep.

Table 3. – THE EFFECT OF DRIED WHEY SUPPLEMENTATION TO AH ON THE RATION *AD LIBITUM* INTAKE BY WETHER SHEEP

Feeding treatment	Water intake d <sup>-1</sup>		Ration intake g d <sup>-1</sup>			Ration intake g kg <sup>-1</sup> M <sup>0.75</sup>		
	l d <sup>-1</sup>	ml kg M <sup>0.75</sup>	DM	OM	CP	DM	OM	CP
AH	2,01 <sup>a</sup>	139 <sup>a</sup>	775 <sup>a</sup>	715 <sup>a</sup>	101	54 <sup>a</sup>	49 <sup>a</sup>	6.8
AH5	2,37 <sup>b</sup>	164 <sup>b</sup>	868 <sup>b</sup>	797 <sup>b</sup>	96	58 <sup>b</sup>	55 <sup>b</sup>	6.2
AH10	3,39 <sup>b</sup>	161 <sup>b</sup>	884 <sup>b</sup>	804 <sup>b</sup>	98	59 <sup>b</sup>	54 <sup>b</sup>	6.1
SEM	0,11	7.8	22.1	30.1	4.3	1.4	2.0	0.28
Sig.	*	*	*	*	N.S.	**	*	N.S.

AH, alfalfa haylage; AH5, alfalfa haylage supplemented with 5 % of dried whey kg<sup>-1</sup> dry matter intake; AH10, alfalfa haylage supplemented with 10 g of dried whey kg<sup>-1</sup> dry matter intake; DM, dry matter; OM, organic matter, CP, crude proteins; M<sup>0.75</sup>, metabolic body weight; SEM, standard error of the mean; Sig., significance; N.S., non-significant, P>0.05; \*, P<0.05, \*\*P<0.01. <sup>a,b,c</sup>, means labeled with same letter are not significantly different (P>0.05).

The obtained results of *ad libitum* intake are roughly equal to the results of DM intake in wether sheep that ranged from 770 – 780 g d<sup>-1</sup> (ZoBell et al., 2004).

Supplementation of DW significantly increase DM and OM intake (P<0.05) in comparison with AH treatment which is in agreement with the results reported by ZoBell et al. (2004). No statistically significant differences in *ad libitum* intake between the supplementation levels (AH5 and AH10) were noticed (P>0.05). The results corresponds with the findings of King and

Schingoethe (1983) who reported higher DM intake of steers fed forage supplemented with DW which replaced 86% of concentrate in the diet. Casper and Schingoethe (1986) also reported higher DM intake in dairy cows ration based on forage supplemented with DW in comparison with just the addition of urea. In contrast to the mentioned, in some other studies no influence of whey supplementation on cattle DM intake was recorded when whey replaced only a part of the concentrate (Remond et al., 1978; Coulon et al., 1979) or other component of the meal (Remond et al., 1978; Schingoethe et al., 1980; Schingoethe and Skyberg, 1981).

For calves that are being weaned, the introduction of whey at the rate of 10% of the concentrate feeds increases feed intake (Morrill and Dayton, 1974), but if whey is introduced at a rate higher than 20%, the concentrate feed intake decreases.

The higher water intake of AH5 and AH10 in comparison with AH ( $P < 0.05$ ) are in agreement with increased water intake in cows fed whey products (Charbonneau et al. 2006) as a result of altered rumen fermentation due to greater mineral intake.

### *Diet in vivo digestibility*

Total tract in vivo digestibility of AH, AH5 and AH10 is shown in table 4.

Table 4. – THE EFFECT OF DRIED WHEY SUPPLEMENTATION TO ALFALFA HAYLAGE ON TOTAL TRACT *IN VIVO* DIGESTIBILITY IN WETHER SHEEP

Feeding treatment	<i>In vivo</i> digestibility		
	OM	DM	CP
AH	498 <sup>a</sup>	501 <sup>a</sup>	532 <sup>a</sup>
AH5	470 <sup>b</sup>	483 <sup>b</sup>	473 <sup>b</sup>
AH10	476 <sup>b</sup>	489 <sup>b</sup>	488 <sup>b</sup>
SEM	13.2	12.2	15.2
Significance	*	**	***

AH, alfalfa haylage; AH5, alfalfa haylage supplemented with 5 g of dried whey  $\text{kg}^{-1}$  dry matter intake; AH10, alfalfa haylage supplemented with 10 g of dried whey  $\text{kg}^{-1}$  dry matter intake; DM, dry matter; OM, organic matter, CP, crude proteins; D-value, the digestibility of the organic matter in the dry matter; SEM, standard error of the mean;  $P > 0.05$ ; \*,  $P < 0.05$ ; \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . <sup>a,b,c</sup> - means labeled with same letter are not significantly different ( $P > 0.05$ ).

Supplementation of DW to AH reduced DM digestibility ( $P < 0.01$ ) and OM digestibility ( $P < 0.5$ ) probably due to AH higher nutritional value as feeding energy supplements to higher quality forages cause negative associative effects on digestibility (Pordomingo et al., 1991). A better way of increasing intake and digestibility is to supplement low quality forage with higher quality forage (Matejovsky and Sanson, 1995).

The both levels of DW supplements to AH decreased CP digestibility ( $P < 0.001$ ). The use of whey in ruminant feeding could result in an inefficient utilization of the protein that it contains which is confirmed with previous research where energy supplements to forage based ration also reduced CP digestibility (Ferrell et al., 1999; Migwi et al., 2011).

Increased feed intake and decreased digestibility with whey substitution seemed to be a function of an increased rate of ruminal DM disappearance via outflow and digestion because the digestibility of rations is depressed as the level of intake increases (Andersen et al., 1959). If greater dietary sugar supply increases the rate of passage (Sutoh et al. 1996) or production of

microbial mass (Ribeiro et al. 2005), less organic matter (OM) would be available for fermentation and acid production (Allen 1997). Since forages may influence passage rates differently, the effect of increased forage intake may differ with physical and chemical characteristics of the forage.

### Conclusions

It was concluded that dried whey supplementation to alfalfa haylage increases ration dry matter and organic matter intake in wether sheep. On the other side, the dried whey supplementation reduces *in vivo* digestibility of the diet probably due to higher rate of particle passage through the digestive tract.

No differences of dried whey supplementation levels to alfalfa haylage in intake and digestibility parameters were recorded.

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## UTJECAJ DODATKA SIRUTKE SJENAŽI LUCERNE NA KONZUMACIJU I PROBAVLJIVOST OBROKA U HRANIDBI KASTRIRANIH OVNOVA

### Sažetak

Cilj istraživanja bio je utvrditi utjecaj dodatka 5% i 10% sirutke u prahu sjenaži lucerne (SL) (*Medicago sativa* L.) na *ad libitum* konzumaciju i *in vivo* probavljivost obroka u hranidbi kastriranih ovnova.

Dodatak sirutke u prahu SL je povećao *ad libitum* konzumaciju ST ( $P < 0.005$ ) i organske tvari (OT) ( $P < 0.05$ ), ali smanjio *in vivo* probavljivost ST ( $P < 0.01$ ), OT ( $P < 0.05$ ) i sirovih proteina (SP) ( $P < 0.001$ ). Razina dodatka osušene sirutke (5% u usporedbi s 10%) nije statistički značajno utjecala na *ad libitum* konzumaciju SL ( $P > 0.05$ ) niti na *in vivo* probavljivost ( $P > 0.05$ ).

Zaključeno je da dodatak osušene sirutke SL u hranidbi kastriranih ovnova u količini od 5% i 10% potiče konzumaciju SL ali smanjuje *in vivo* probavljivost kao posljedicu brže pasaže hrane kroz probavni sustav.

Ključne riječi: *ad libitum* konzumacija, sjenaža lucerne, osušena sirutka, *in vivo* probavljivost.

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