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Whey Powder Supplementation Changes Chemical Composition, Improves Fermentation Quality, Increases the Utilization of Nutrients and Net Energy Lactation Value of Alfalfa Haylage in Kıvırcık Rams

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ABSTRACT

The aim of this study was to reveal the effect of whey powder (WP) supplementation on chemical composition, silage fermentation, *in vivo* digestibility and net energy lactation (NE_L) value of high dry matter (DM) alfalfa silage (Haylage). Alfalfa treated with different doses WP (0, 2, and 4%) were ensiled in plastic cans for 60 days. Nine Kıvırcık rams were used to estimated digestibilities of haylages in 3×3 Latin Square design with fifteen days experimental period. According to the results, the addition of WP significantly increased the DM of alfalfa haylage compared to the control whereas decreased ether extract, crude protein and crude ash. But, cell wall fraction rates of all groups were similar. Relative feed value and physical characteristics other than color improved in WP supplemented groups, and lactic acid rates increased in these groups. In terms of silo acids, the best values were found in the group with 4% WP. On the other hand, the addition of WP increased the organic matter digestibility of haylages, however the effect on other *in vivo* digestibility of crude protein, crude fiber, nitrogen free extract and neutral detergent fiber was insignificant. Moreover, total digestible nutrients (TDN) and NE_L values of haylages were found to be improved with the addition of WP. As a result, it was concluded that in order to obtain quality haylage in the ensiling of high DM and long chopped alfalfa, WP generally can be used as an alternative source of carbohydrates and a 4% whey powder dose can be recommended in the production of haylage especially in the sense of improving silage fermentation.

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Introduction

Alfalfa, which is one of the most significant roughage sources in many regions of the world with 35 million hectares planted in 80 countries, is a high yielding perennial legume forage legume (Martin et al., 2004; Radović et al., 2009). The reason why alfalfa is preferred particularly by dairy and beef cattle enterprises due to its high crude protein rate and digestibility, low cellulose content, and rich mineral and vitamin content (NRC, 2001). Beside these high advantages, it is less preferable as ensiling because of poor fermentation criteria with high buffering capacity and low water-soluble carbohydrate.

One of the most effective methods to minimize post-forming losses and maintain the nutritional quality of roughage is silage. Haylage is a silage with high dry matter content and makes some advantages according to silage that it reduces losses storage like dry matter, protects against leaf losses and avoids off odour. The most important risks are more being responsive to mold, more difficult to completely exclude of air during ensiling, and protein breakdown because of heating (Morse and Sedivec, 1990). Especially pre-ensiling material with unchopped (long stem) like bale silage causes more susceptible to yeast and mold growth, increasing the risk of spoilage. It is possible to a good fermentation by haylage with additives. The additive in haylage makes preservation against harmful microorganism growth and reduces extent of aerobic spoilage. However, studies investigating the effects of additives on fermentation and digestibility of long stem haylage are very limited (Coblentz and Akins, 2018). The aim of this study was to determine the effect of whey powder addition at different doses on the quality of silage and *in vivo* digestibility of unchopped alfalfa haylage.

Table 1. Chemical composition of alfalfa before ensiling, % dry matter (DM)

Alfalfa chemical composition			
DM*	55.00		
OM	49.8	NDF	52.18
CA	9.45	ADF	40.00
CP	16.90	ADL	8.90
EE	1.45	HEM	12.18
CF	27.81	CEL	31.10
NfE	44.36	WSC	2.47
NFC	20.02	BC	69.0*
Whey Powder Chemical Composition			
DM	95.0	EE	0.24
CA	6.50	CP	14.20
Lactose	75.60	pH	6.83

*Value of fresh matter silage; OM, organic matter; CA, crude ash; CP, crude protein; EE, ether extract; CF, crude fiber; NfE, nitrogen free extract; NFC, non-fibrous carbohydrate NDF, nötral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; HEM, hemicellulose; CEL, cellulose; WSC, water-soluble carbohydrate; BC, buffer capacity.

Table 2. Effect of WP on crude nutrients, NFC, RFQ and cell wall fractions values of alfalfa haylage, % dry matter (DM)

Item	Treatment of WP, %			P
	0	2	4	
DM	51.68±0.28[b]	55.93±0.31[a]	55.28±0.21[a]	<0.001
OM	84.32±0.07	85.22±0.41	85.24±0.15	0.070
CA	11.24±0.06[a]	9.31±0.01[c]	9.65±0.04[b]	<0.001
CP	19.12±0.05[a]	17.47±0.22[b]	16.25±0.04[c]	<0.001
EE	2.34±0.08[a]	1.95±0.06[b]	1.88±0.08[b]	0.001
CF	29.87±0.58[b]	31.38±0.18[a]	31.68±0.15[a]	0.004
NfE	38.87±0.32[b]	39.78±0.43[b]	41.56±0.52[a]	0.002
NFC	16.56±0.26[c]	22.38±0.44[b]	23.46±0.18[a]	<0.001
RFV	106.76±0.68[b]	109.57±0.50[b]	113.14±1.18[a]	0.005
NDF	49.62±1.04	49.42±0.95	48.60±0.77	0.770
ADF	38.56±0.61	38.30±0.59	37.34±0.27	0.210
ADL	8.05±0.11[b]	8.86±0.21[a]	8.76±0.11[a]	0.030
HEM	11.80±0.26	12.40±0.99	11.49±0.97	0.710
CEL	27.62±0.44[b]	30.16±0.19[a]	29.45±0.09[a]	0.020

a–c Means in the same row followed by different letters differ significantly (P<0.05). OM, organic matter; CA, crude ash; CP, crude protein; EE, ether extract; CF, crude fiber; NfE, nitrogen free extract, NFC, non-fibrous carbohydrate; RFV, relative feed value; NDF, nötral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; HEM, hemicellulose; CEL, cellulose.

Table 3. Effect of WP on fermentation characteristic of alfalfa haylage.

Items	Treatment of WP, %			P
	0	2	4	
Physical properties				
Color	1	1	1	
Smell	4	8	8	
Structure	1	2	2	
Total Quality class	6 Medium	11 Good	11 Good	
Chemical properties				
pH	6.00	5.52	5.21	
Lactic acid, DM%	7.68±0.03[c]	8.84±0.09[b]	9.40±0.15[a]	<0.001
Acetic acid, DM%	1.07±0.02[b]	0.96±0.04[b]	1.60±0.16[a]	<0.001
Butyric acid, DM%	0.00±0.00[b]	0.07±0.01[a]	0.00±0.00[b]	0.01

a–c Means in the same row followed by different letters differ significantly (P<0.05). Quality class: I: Very good (16-20 point), II: Good (10-15 point), III: Medium (5-9 point) ve IV: Low (0-4 point).

Material and Methods

Ethical Statement

The experiment was conducted at Ege University Experimental Farm (38° 27′ 26″ N, 27° 13′ 47″ E; İzmir, Turkey). This study protocol for care and use of animals used in the experiment was approved by the Ege University Animal Care and Use Committee (No:2017-015).

Alfalafa, Whey Powder and Rams

Alfalfa (*Medicago sativa* L.) was harvested in September and early flowering period at Ege University Faculty of Menemen Research, Application and Production Farm. Whey powder was purchased from Maybi (Malkara/Tekirdağ/Turkey). It was obtained from sweet fresh pasteurized whey and spray dried technology without using feed additives. The animal material of the experiment consisted of 1.5 years old Kıvırcık rams (mean CA, kg=55).

Ensiling Alfalfa Haylage

Harvested unchopped alfalfa (30-35 cm) was shade-dried until it reached 55% DM and treated with whey powder at different doses (0, 2, and 4% of fresh matter) for ensiling. Alfalfa was ensiled into 30-liter plastic cans with 7 replications accordance with silage making techniques (Kılıç, 2010). The fermentation period is 60 days.

Chemical Analysis

All samples were analyzed by following methods; crude nutrients (DM, Dry matter; CP, Crude protein; EE, Ether extract; CF, Crude fibre) by Weende analysis (Menke and Huss, 1975) and cell wall components (NDF, Neutral detergent fibre; ADF, Acid detergent fibre; ADL, Acid detergen lignin) by Fiber Bag System modified by Goering and Van Soest analysis (1970). Hemicellulose (HEM) content was calculated from NDF-ADF difference and cellulose (CEL) content from ADF-ADL difference. The water-soluble carbohydrates (WSC) of alfalfa before ensiling in spectrophotometer by an antron-thiourea method (Anonymus, 1986) and buffer capacity was detected using the method of Playne and McDonald (1966). The nitrogen-free extract (NfE) was calculated NfE as NfE% = 100 % – (% EE + % CP + % Ash + % CF), and the nonfibrous carbohydrate (NFC) was also calculated as NFC%= 100 - (NDF% + CP% + EE% + CA%) (all nutrients are in dry matter) (NRC, 2001).

Relative feed value (RFV) was calculated as Rohweder et al. (1978). RFV = (dry matter intake (DMI) * digestible dry matter (DDM) / 1.29, where: DMI, % of body weight (BW) = 120 / NDF, % of dry matter (DM); and DDM, % of DM = 88.9 – 0.779 × (ADF, % of DM). Physical characteristics of all silages were determined by three different observers for color, odor, and structure. Flieg score was calculated according to DM contents and pH values of the silages (DLG, 1987; Kılıç, 2010): Flieg score = 220 + (2 × DM% - 15) - 40 × pH. All samples' pH values were measured using a desktop pH meter (Hanna HI2211-02; Chennai, Tamil Nadu). Organic acids (acetic acid, butyric acid, lactic acid) by distillation method adapted from Lepper (Naumann and Bassler, 1993).

In Vivo Digestion Testing

The digestion testing lasted for a total of 12 days, as adjustment period (7 days) + measurement period (5 days) for each group. Feed intakes of animals were calculated as 1.2-1.5-fold of maintenance requirements. Feeding of rams was made according to 3x3 Latin square and daily at 8:30-16:30 and had ad libitum drinking water. The amount of manures by feed was recorded daily in the measurement period. Every day, 10% of the collected manures were stored in glass jars and at -20°C for chemical analysis (Menke and Huss, 1975; GfE, 1991). The apparent digestibility of coefficient (ADC) of the groups were calculated according to GfE, (1991): ADC, % = ((feed intake-throw out with manure)/feed intake) x 100. Total digestible nutrients (TDN) and NE_L values of them were also calculated according to NRC, (2001) and Küçük, (2019).

TDN (%) = ((CP (%) × ADC of CP) + (CF (%) × ADC of CF) + (NfE (%) × ADC of NfE) + (EE (%) × ADC of EE x 2.25),

Where;

CP = Crude protein
ADC = Apparent digestibility of coefficient
CF = Crude fiber
NfE = Nitrogen free extract
EE = Ether extract

NE_L (Mcal/kg) = 0.0245 × TDN (%) − 0.12.

Statistical Analysis

Data were performed to one-way analysis of variance (ANOVA) by employing the procedure SPSS 22.0 (IBM Corp., Armonk, NY, USA) package software (SPSS, 2013). The post-hoc Duncan's multiple range test was used to compare means when ANOVA was significant (P <0.05). The mathematical model of the experiment plan was: $y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where: yij – dependent variable, μ - overall mean, αi – fixed effect of treatment (i = 1 to 3), εij – random error. Correlation coefficients between all groups were computed using Pearson's correlation coefficients (SPSS, 2013).

Results and Discussion

Chemical Composition.

In the present study, it was found that DM, CF, NfE, NFC and RFQ rates were higher than control and CA, CP and EE rates were lower in whey powder added groups (P<0.05) and the effect of dose on CA, CP and NFC from these values was significant (Table 2). Nevertheless, there was no difference for NDF, ADF and HEM values between the groups. ADL and CEL rates were higher in whey powder added groups than in control, and the difference between groups was significant (P<0.05).

In our previous study, it was observed that addition of WP increased crude nutrients and decreased cell wall fractions in chopped alfalfa silage (Özüretmen et al., 2022). This indicates that DM ratio and chopping length of pre-ensiling are significant for effectiveness of additives and improvement of fermentation. Especially, decrease of CP rate in the haylages treated with WP was associated with the presence of air and the use of WP by aerobic microorganisms and so, proteolytic activity continued until O_2 was depleted. Anyway, the treatment of WP contributed to the enhance of the DM in haylage. Elizalde and Henríquez (2009) stated that the DM content of the alfalfa haylage decreased as the particle size increased. This research was inconsistent with the findings of our study may be due to the difference's microflora of alfalfa at harvest and fermentation changes during ensiling. Dash et al. (1974a) reported that the effect of 2% WP addition on the chemical composition was not significant. Lynch et al. (2014) reported that the addition of inoculant of alfalfa haylage (balyage) as a long stem has no effect on raw nutrients (excluding CP) and cell wall contents.

Fermentation Quality.

For physical properties, it was observed that the color (greener), smell (more aromatic) and structure (leaves intact) were better than control in the groups treated with WP (Table 3).

Table 4. Effect of WP on in vivo nutrient digestibility, TDN and NEL value in alfalfa haylage, % dry matter (DM)

Item	Treatment of WP, %			P
	0	2	4	
Crude nutrients				
DM	61.70±3.03	65.17±3.38	65.07±1.53	0.570
OM	55.24±1.83[b]	62.78±3.68[a]	62.74±1.67[a]	0.004
CP	71.56±1.72	71.58±2.62	72.95±1.34	0.820
CF	66.30±2.15	67.87±3.11	66.34±2.07	0.890
NfE	66.39±2.32	65.40±5.36	68.18±1.92	0.810
Cell wall fractions				
NDF	72.29±1.70	70.79±2.17	70.38±1.58	0.700
ADF	72.98±2.42	72.41±2.54	71.63±1.98	0.920
TDN, %	57.31±0.11[c]	63.65±0.02[b]	72.99±0.11[a]	<0.001
NE_L, Mcal/kg	1.40±0.01[c]	1.50±0.01[b]	1.79±0.01[a]	<0.001

a–c Means in the same row followed by different letters differ significantly (P<0.05). OM, organic matter; CP, crude protein; CF, crude fiber; NfE, nitrogen free extract; NDF, nötral detergent fiber; ADF, acid detergent fiber; TDN, total digestible nutrients; NEL, net energy lactation.

Table 5. Coefficients of correlations between TDN, RFV, cell wall fraction values of alfalfa haylage.

	TDN	RFV	NFC	NDF	ADF
TDN	1	0,914**	0,875**	-0,929**	-0,669*
RFV	0,914**	1	0,823**	-0,939**	-0,855**
NFC	0,875**	0,823**	1	-0,881**	-0,550
NDF	-0,929**	-0,939**	-0,881**	1	0,625
ADF	-0,669*	-0,855**	-0,550	0,625	1

**Correlation is significant at the P<0.01 level. *Correlation is significant at the P<0.05 level.

For chemical properties, WP decreased the pH value and increased the rate of LA in unchopped haylages (P<0.05). However butyric acid was found the group treated with 2% WP despite of no detecting in the control and group treated with 4% WP (P<0.05).

The WP treatment improved fermentation of alfalfa haylage but total acids rate were found insufficient for good fermentation in all groups. This poor rate was associated with limited fermentation due to high DM content of the haylages. Because anaerobic lactic acid bacteria grow less and produces less lactic acid in high DM (Kung and Shaver, 2001). Dash et al. (1974a,b) stated that WP addition enhanced fermentation in alfalfa haylage. Rizk et al. (2005) states the addition of inoculant to the chopped alfalfa haylage reduces pH values, increases LA, and WSC content. In present study, it was observed that long stem was also caused decreasing the fermentation. Borreani and Tobacco (2006) stated that chopping had no effect on the fermentation quality of alfalfa haylage (baylage); however, they stated that high DM causes less LA production since it is a limiting barrier on silage microflora activity. Lynch et al. (2014) reported that the addition of inoculant of alfalfa haylage (balyage) as a long stem reduced WSC content. They stated that the effect of inoculant addition on the chemical composition of alfalfa haylage may have restricted microorganism fermentation in silage due to the high DM in haylage. Accordingly, it concluded that the dry matter of ensiling fresh material firstly and then stem length secondly impressed the fermentation quality.

Apparent Digestibility.

The WP additive affected on some parameters of apparent digestibility in unchopped alfalfa haylages (Table 4). According to data, the groups treated with raised OM digestibility compared the control (P<0.05) but there was no dose-depending effect. However, TDN and NE_L values increased and the dose was significant (P<0.05).

The current study showed a strong positive correlation between TDN, RFQ and NFC in the unchopped alfalfa haylage (Table 5). Strong negative correlation was found between NDF and ADF values and TDN, RFQ and NFC.

In present study, it did not observe that WP affected on digestibility because there is no difference between chemical compositions of control and the groups treated with WP. Therefore, differences between digestibility of OM occurred due to effectiveness of WP on DM content of haylages. This also affected on the values of NEL and TDN, so that the values of them increased with dose. Dash et al (1974b) reported that WP increased digestibility of crude nutrients and cell wall fractions and the values of energy in alfalfa hay silage. The effect of whey on digestibility may have been observed due to lower DM content than our study.

Conclusion

Consequently, the additive did not affect digestibility except organic matter but helped to improve fermentation. It deduced that the addition of 4% whey powder can be used as an additive in the silage of long stem and high dry matter alfalfa and can enhance silage fermentation.

Authorship

Conceptualization: HHİ, SÖ and HÖ; Data curation: HHİ and SÖ; Formal analysis: HHİ; Methodology: HHİ and SÖ; Software: HHİ and SÖ; Validation: HHİ, SÖ and HÖ; Investigation: HHİ and SÖ; Writing - original draft: HHİ and SÖ; Writing - review & editing: HHİ and HÖ.

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Conflict of interest

The authors certify that they have no affiliations with or involvement in any organization or entity with financial or non-financial interests in the subject matter and materials discussed in this manuscript.

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