Journal of Agricultural Science and Technology A 1 (2011) 1095-1102
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Fermentation Characteristics, *In Situ* Rumen Degradation and Nutritional Value of Whole Crop Barley Ensiled with Microbial Inoculant or Ammonium Propionate for Lactating Dairy Holstein Cows

M. Vatandoost, M. Danesh Mesgaran and A. R. Vakili

Department of Animal Science, Excellence Centre for Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, P.O. Box 91775-1163, Iran

Received: May 11, 2011 / Published: November 20, 2011.

Abstract: Various microorganisms and chemical compounds might be added to forage, maintain or improve the nutritive value of a crop ensiled. The aim of the present experiment was to evaluate the fermentation characteristics and *in situ* rumen degradation of whole crop barley ensiled with *Lactobacillus plantarum* or ammonium propionate and its effect on feed intake and milk production of lactating dairy Holstein cows. Whole crop barley was harvested (32.5% DM), chopped and then ensiled alone (UT) or with *Lactobacillus plantarum* (8×10^{10} CFU g^{-1} fresh forage; LP) or ammonium propionate (1 g kg^{-1} DM; AP). Chemical composition, silage extract pH, NH_3-N concentration and *in situ* ruminal degradation parameters of dry matter (DM), crud protein (CP) and neutral detergent fiber (NDF) were determined. Microbial inoculant had a significant ($P < 0.05$) effect on NDF content (LP = 545 vs. UT = 525 and PA = 522 g kg^{-1} DM) of whole crop barley silage (WCBS). *In situ* dry matter degradable coefficient of fraction (b) was affected by the treatments (LP = 0.48 ± 0.01 and PA = 0.47 ± 0.02 vs. UT = 0.45 ± 0.02). Use of LP caused to a decrease in CP degradability in fraction (b); (LP = 0.39 ± 0.02 and PA = 0.43 ± 0.05 vs. UT = 0.43 ± 0.04 % DM), and enhanced effective degradability of CP about 0.04 in contrast with the untreated silage. Treatment had no significant effect on dry matter intake, milk yield, milk fat and lactose concentrations, but milk protein yield for cow fed LP increased significantly compared with those of the other animals.

Key words: Whole crop barley silage, *Lactobacillus plantarum*, ammonium propionate, *in situ*, milk production.

1. Introduction

Whole crop cereal silages are the most important fodder crops for feeding dairy cows in Iran. Various microorganisms, enzymes and chemical compounds have been added to forage, maintain or improve the nutritive value of a crop ensiled [1-3]. As is the nature of most biological systems, there is a considerable variation in the outcome of using these additives. Some additives, which have proven to be effective in this respect, include chemical additives, based on volatile fatty acids, such as propionic, formic and acetic acid and biological additives based on bacteriocin producing microorganisms such as lactobacilli and bacilli [3-5]. Microbial inoculants are applied to forage at the time of ensiling to establish a desirable microbial flora in silage, to accelerate the decline of pH during the initial stage of silage fermentation [6-9]. This might preserve plant carbohydrates through homofermentation and protein by decreasing proteolysis and deamination [10] to result in higher retention of soluble components. In case of lactic acid bacteria (LAB) previous study reported that it caused to ferment water-soluble carbohydrates to organic

Corresponding author: M. Danesh Mesgaran, Ph.D., professor, research fields: protein digestion and metabolism in sheep and dairy cows. E-mail: danesh@um.ac.ir.

acids, mainly lactic acid, under anaerobic conditions. The production of organic acids lead to decrease in pH and the silage is preserved [5]. The low pH in combination with anaerobic condition and undissociated acids prevents growth of unwanted bacteria, moulds and yeasts [11]. Previous results suggested that an improvement in milk yield was attributed mainly to the increase in metabolic energy intake, either because of increased DM intake or improved DM and fiber digestibility [5, 12-14], which resulted in improved animal performance [14, 15]. Whole crop barley (WCB) has low buffering capacity and abundant fermentable carbohydrates and is considered relatively easy to ensile [16]. Despite its ease of ensiling, results of previous experiments indicated that lactic acid bacteria-based inoculants have the potential to improve barley silage fermentation [17, 18], digestibility of whole crop barley silage (WCBS), nutrient intake and average daily gain by cattle [18, 19]. Propionic acid-based preventatives have also been used to improve whole crop cereal silages fermentation successfully [20, 21]. In recent years, marked changes have been made to the formulations and recommended application rates of additives containing propionic acid [21]. An advantage of these salts is that they are easier and safer to handle than their corresponding acids. Current recommendation for use of buffered propionic acid additives are considerably lower (0.1% to 0.2% of fresh forage weight) than classical recommendation for use of the unbuffered acid as 0.75% to 1.5% [3, 21]. The aim of the present experiment was to evaluate the fermentation characteristics and *in situ* rumen degradation of whole crop barley ensiled with *Lactobacillus plantarum* or ammonium propionate and its effect on feed intake and milk production of lactating dairy Holstein cows.

2. Materials and Methods

2.1 Ensiling Procedures

Whole crop barley was harvested (32.5% DM), chopped, and then ensiled for 40 days (n = 4). The forage was ensiled as untreated (UT) or treated with the following additives; *Lactobacillus plantarum* (8×10^{10} CFU g^{-1} fresh forage; LP) or ammonium propionate (1 g kg^{-1} DM, AP).

2.2 Chemical Analysis

Representative samples of fresh chopped WCB and the silages were collected, oven dried to a constant weight at 60 °C, and ground to pass through a 2 mm-screen for later analysis. Standard procedures were used to determine the chemical composition of the samples. Crude protein (CP) was determined according to the Kjeldahl procedure (AOAC, 2004) on the Tecator Auto-analyzer (1030). Determination of neutral detergent fiber (NDF) was made using the method of Van Soest et al. [22]. Samples of fresh silage (approximately 50 g) were mixed with 450 mL distilled water, and the silage extraction was made. Then, silage pH was determined using a portable pH meter (Metrohm 691, Swiss). 5 mL of the silage extract was mixed with 5 mL of 0.2 N HCl. Ammonia-N degradation of the acidified silage extract was determined using distillation method (Kjeltec 2300 Autoanalyzer, FossTecator AB, Hoganas, Sweden).

2.3 In Situ Technique

The ruminal degradable parameters of dry matter (DM), NDF and CP of the silages were determined using *in situ* procedure [23]. Four sheep (44 ± 3 kg, body weight) fitted with rumen fistulae were used in the present study. The bags (10 cm × 12 cm) were made of polyester nylon cloth with a pore size of 48 μm. Approximately, 5 g DM of each sample was placed in each bag, and four bags for each treatment were incubated for each time (2, 4, 8, 16, 24, 48, 72, 96 h). After removal the bags from the rumen, they were washed in cold running water and dried in an air-forced oven (60 °C, 48 h). Zero time disappearance was obtained by washing rumen-unincubated bags in a similar way. After that, the bags were weighted and analyzed to determine the

CP and NDF concentrations.

2.4 Cows, Management and Experimental Design

Eighteen Holstein lactating multiparous dairy Holstein cows (33 ± 5 kg milk d^{-1}) were used in a complete randomized design for 7 weeks (6 animals per each treatment). Cows were kept in individual stalls and had free access to water. Diets and chemical composition of total mixed rations are presented in Table 1. Cows were fed total mixed rations in two separate feedings at 08:00 and 18:00 to allow 5% orts. The a.m. and p.m. daily dry matter intake was recorded. Cows were milked three times daily at 06:00, 13:00 and 21:00 and samples of milk were collected at the end of each week. Milk samples were analyzed for fat, protein and lactose concentrations using milko-tester (Foss Electric, Conveyor 4000). Blood samples were collected into heparinized tubes from the jugular vein of each cow at 0.0 and 4 h after the morning feeding in weeks 3 and 6. Samples were centrifuged (3,500 rpm, 10 min) and plasma were analyzed for glucose and blood urea nitrogen (BUN), [24, 25] using spectrophotometer procedure.

2.5 Calculating and Statistical Analysis

The equation of $P = a + b (l - e^{-ct})$ was applied to determine the coefficients of a = quickly degradable, b = slowly degradable and c = constant rate of degradation of the incubated samples at t = time [26]. Effective Degradability (ED) of DM, CP and NDF was then calculated according to the equation of Ørskov & McDonald. [26], where $ED = a + [(b \times c)/(k + c)]$ where k is the rumen outflow rate assumed to be 2, 4 or 6% h^{-1} and a, b and c are as described before. Data of silage chemical components (PH, NDF, NH_3-N and CP) were statistically analyzed using complete randomized design. The statistical model was $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y_{ij} = dependent variable, μ = dependent valuable mean, T_i = effect of treatment, ε_{ij} = residual error term. The Duncan procedure was used to test the mean significant difference at $P < 0.05$.

Table 1 Ingredient and chemical composition of ration (% DM).

	Diets[1]		
	UT	LP	PA
Ingredients			
Untreated whole crop barley silage	19.3	-	-
Microbial inoculant treated whole crop barley silage	-	19.3	-
Ammonium propionate treated whole crop barley silage	-	-	19.3
Alfalfa hay, chopped	21.9	21.9	21.9
Barley grain, ground	16.8	16.8	16.8
Com grain, ground	12.6	12.6	12.6
Soybean meal	11.3	11.3	11.3
Cotton seed meal	9.3	9.3	9.3
Wheat bran	7.1	7.1	7.1
Salt	0.3	0.3	0.3
Vitamin-Mineral premix2	0.7	0.7	0.7
Calcium carbonate	0.5	0.5	0.5
Sodium bicarbonate	0.3	0.3	0.3
Chemical composition (%)			
Crud protein (g kg^{-1} DM matter)	174	175	175
Neutral detergent fibre (g kg^{-1} dry matter)	315	319	314
Acid detergent fibre (g kg^{-1} dry matter)	195	196	195
Ether extract (g kg^{-1} dry matter)	28	28	28

[1]UT = untreated; LP = *Lactobacillus plantarum* 8×10^{10} CFU g^{-1} of fresh forage; PA = Propionate ammonium 1 g kg^{-1} of DM.
[2]Premix contained (DM basis): 190,000 mg kg^{-1} Ca, 90,000 mg kg^{-1} P, 50,000 mg kg^{-1} Na, 9,000 mg kg^{-1} Mg, 3,000 mg kg^{-1} Fe, 3,000 mg kg^{-1} Zn, 2,000 mg kg^{-1} Mn, 100 mg kg^{-1} Co, 300 mg kg^{-1} Cu, 100 mg kg^{-1} I, 1 mg kg^{-1} Se, 500,000 IU kg^{-1} vitamin A, 100,000 IU kg^{-1} vitamin D3, 100 mg kg^{-1} vitamin E, 3,000 mg kg^{-1} antioxidant (B.H.T).

Effects of treatments on feed intake, cow performance and blood metabolites were analyzed as repeated measures in time using the mixed procedure of SAS (Version 9). The statistical model was Y = treatment + cow (treatment) + lactation week + treatment by lactation week, where cow (treatment) was used to test the treatment effect. Data were analyzed using the GLM procedure of SAS. Statistical significance effects were determined at $P < 0.05$.

3. Results and Discussion

3.1 Chemical Composition

Chemical composition of the untreated and treated whole crop barley silage (WCBS) is shown in Table 2.

Table 2 Chemical composition of whole crop barley silage treated with *Lactobacillus plantarum* or ammonium propionate.

Item	Fresh forage	Treatments[1]			S.E.M	*P*-Value[2]
		UT	LP	PA		
pH	6.78	4.27[a]	4.14[b]	4.19[b]	0.04	*
NDF (g kg^{-1} DM)	640	525[b]	545[a]	522[b]	5.46	**
CP (% DM)	7.54	8.18[b]	8.42[ab]	8.63[a]	0.14	*
NH_3-N (mg dL^{-1})	-	17.3	16.0	17.1	1.44	NS[3]

[a-c] Means in each row with unlike superscript letters differ significance at $P < 0.05$.

[1] UT = untreated; LP = *Lactobacillus plantarum* 8×10^{10} CFU g^{-1} of fresh forage; PA = Propionate ammonium 1 g kg^{-1} of DM.

[2] *: ($P < 0.05$); **: ($P < 0.01$).

[3] NS: non-significant.

In the present study, the pH was declined (4.14 ± 0.04) when LP was applied. There are different reports about the effect of microbial inoculation on silage fermentation characteristics. Results of various experiments showed that microbial inoculation to silage has a positive effect on the silage fermentation by decreasing pH [27-31]. Ammonium propionate treated silage also had lower pH than untreated silage. This finding supports previous results of Arbabi et al. [3], and Kung et al. [21]. Addition of the buffered propionic acid-based additive decrease pH which Kung et al. [32] suggests partially reduced the metabolism of some aerobic microorganisms.

Lactobacillus plantarum had a significant effect on NDF content and caused an increase (20 g kg^{-1} DM) in WCBS compared with that of the untreated silage. These data support previous finding [29, 33, 34] which indicated LAB might increase NDF content of grass silage. The modifying effect of ensiling on carbohydrate concentration of grass herbage however is complicated, because in addition to hydrolysis of NDF, the concentration is affected by nutrient losses in respiration, effluent and fermentation [35]. Only limited data are available to substantiate these reports and on which to propose a causal mechanism of that. In the present study, the CP was increased (8.63 ± 0.14) when LP was applied. In this regard it seems that *Lactobacillus plantarum* additive was more effective in limiting the degradation of protein rather than PA or untreated silage. Kung & Ranjit [28] reported that lower degradation of protein in the silage may be resulted from higher rate of lactic and acetic fermentation via inoculants and greater amount of propionic acid which inhibits the growth of proteolytic bacteria. Non-significant difference was observed for ammonia-N concentration between the treated and untreated silages.

3.2 In Situ

Data of ruminal *in situ* degradation parameters of DM, CP and NDF are shown in Table 3. Present data of dry matter degradable coefficients showed that both LP and PA caused a significant decrease in fraction

Table 3 *In situ* dry matter, NDF and crude protein degradable coefficients of whole crop barley silage treated with *Lactobacillus plantarum* or ammonium propionate.

Items	Treatments[1]				
	Coefficients[2]	UT	LP	PA	S.E.M
Dry matter	a	0.37[a]	0.34[b]	0.34[b]	0.011
	b	0.45[b]	0.48[a]	0.47[ab]	0.015
	c	0.04	0.05	0.05	0.005
	ED (0.02)	0.68	0.69	0.69	
	ED (0.04)	0.60	0.62	0.61	
	ED (0.06)	0.56	0.57	0.56	
Neutral detergent fibre	a	0.12	0.15	0.14	0.022
	b	0.69	0.66	0.66	0.042
	c	0.03	0.03	0.03	0.006
	ED (0.02)	0.54	0.54	0.55	
	ED (0.04)	0.42	0.43	0.44	
	ED (0.06)	0.35	0.37	0.37	
Crude protein	a	0.40	0.42	0.41	0.017
	b	0.43	0.39	0.43	0.036
	c	0.03[ab]	0.04[a]	0.02[b]	0.007
	ED (0.02)	0.65	0.68	0.64	
	ED (0.04)	0.58	0.62	0.57	
	ED (0.06)	0.54	0.58	0.53	

[a,b] Means in each row with unlike superscript letters differ significance at $P < 0.05$.

[1] UT = untreated; LP = *Lactobacillus plantarum* 8×10^{10} CFU g^{-1} of fresh forage; PA = Propionate ammonium 1 g kg^{-1} of DM.

[2] a = rapidly degradable, b = slowly degradable, c = fractional degradation rate constant. ED = a + [(b × c)/(k + c)] where k is the rumen outflow rate assumed to be 0.02, 0.04 or 0.06 h^{-1}.

(a), while LP increase fraction (b) compared with those of the UT silage. This finding support previous data reported for improvement ruminal DM degradability of WCBS [18, 29, 36] or grass silage [37] as a result of inoculation. In the present study, degradation parameters of NDF were not affected by the treatment applied. In the case of the data of CP degradability, significant difference between LP and PA was observed, while both of them did not have significant difference relative to the untreated silage. *Lactobacillus plantarum* has a most effective degradability of CP and caused to increase about 0.04 in contrast with the untreated silage (Table 3). As a whole, the data of degradation coefficients and effective degradability of treatments showed that LP had a greater effect than that of the AP.

3.3 Feeding Trail

Dry matter intake (DMI), milk yield and the composition are presented in Table 4. There was a non-significant effect of the treatments on DMI and milk yield. The data of milk composition showed that milk yield or milk protein production for cows fed LP treated silage was increased significantly ($P < 0.05$). Several studies have reported positive [7, 12] or no effects [38] of silage inoculation on milk yield. However, reasons for improved animal performance as a result of silage inoculation are not fully understood. One hypothesis is a probiotic effect, in which specific LAB strains interact with rumen microorganisms to enhance rumen functionality and animal performance [39]. Another hypothesis is the involvement of a variety of antimicrobial substances such as bacteriocins, which produced by LAB [5, 40] that caused intra-species antagonistic effects and inhibit detrimental microorganisms in the silage [41]. In this regard, it is well known that LAB produces a variety of antimicrobial substances such as bacteriocins [41, 42]. In previous studies [5, 14] treating silages with bacterial inoculants improved total tract DM and fiber digestibility [5, 13, 14], resulted in improved animal growth rate [14]. In this regards, researchers attributed the positive impact of inoculation on animal performance to improved fiber digestibility [43, 44]. Fellner et al. [14] found that increase in growth rate with bacterial inoculation was achieved without changes in feed intake or nutrient digestion, suggesting that the response may be related to improved efficiency of metabolisable energy utilization. However, other offered no explanations [7, 45]. Data of blood glucose and urea-nitrogen concentrations are presented in Table 5. No significant difference was observed between animals fed the experimental diets for these data.

Table 4 Dry matter intake, milk yield and milk compositions of lactating cows fed diets containing whole crop barley silage as untreated or treated with *Lactobacillus plantarum* or ammonium propionate.

Item	Treatment effect[1]			Diet effect		Time effect	
	UT	LP	PA	S.E.M	P^2	S.E.M	P^2
Dry matter intake (g kg^{-1})	22.7	23.4	23.5	0.92	NS[3]	0.55	**
Milk yield (kg day^{-1})	32.8	33.9	33.0	1.37	NS	0.81	**
Milk fat (%)	3.1	3.0	3.2	0.13	NS	0.09	***
Milk protein (%)	2.6^b	2.9^a	2.7^b	0.06	**	0.04	***
Milk lactose (%)	4.2	4.2	4.3	0.09	NS	0.06	***
Milk fat (g day^{-1})	997	1,023	1,041	30.7	NS	23.0	***
Milk protein (g day^{-1})	860^b	993^a	907^b	34.3	*	21.7	***
Milk lactose (g day^{-1})	1,360	1,430	1,400	53.4	NS	32.9	***

$^{a-b}$ Means in each row with unlike superscript letters differ significance at $P < 0.05$.

[1] UT = untreated; LP = *Lactobacillus plantarum* 8×10^{10} CFU g^{-1} of fresh forage; PA = Propionate ammonium 1 g kg^{-1} of DM.

[2] *: ($P < 0.05$); **: ($P < 0.01$); ***: ($P < 0.001$).

[3] NS: non-significant.

Table 5 Blood glucose and urea-nitrogen concentrations of lactating Holstein dairy cows (mg dL^{-1}) fed diets containing whole crop barley silage as untreated or treated with *Lactobacillus plantarum* or ammonium propionate.

Item	Sampling (weeks)	Time (h) [1]	Treatments [2]			S.E.M	*P*-Value [2]
			UT	LP	PA		
Blood glucose							
	3	0.0	58.5	56.4	59.0	2.11	NS [3]
		4	59.1	61.1	56.9	1.96	NS
	6	0.0	60.7	58.5	62.4	2.16	NS
		4	61.2	60.9	62.8	1.77	NS
Blood urea nitrogen							
	3	0.0	20.8	22.9	20.6	0.59	NS
		4	19.1	22.6	21.0	0.99	NS
	6	0.0	23.0	24.5	23.5	0.50	NS
		4	22.3	24.1	20.9	0.84	NS

[1] Samples were taken 0.0 and 4 h past the morning feeding.
[2] UT = untreated; LP = *Lactobacillus plantarum* 8×10^{10} CFU g^{-1} of fresh forage; PA = Propionate ammonium 1 g kg^{-1} of DM.
[3] NS = non-significant ($P < 0.05$).

4. Conclusion

As a result of the present study, it was concluded that LP was more effective in limiting the degradation of protein rather than the untreated silage which caused to improve the silage quality. Data of *in situ* degradability suggested that *Lactobacillus plantarum* has a most considerable effect on NDF content and *in situ* degradation coefficients of WCBS. This data showed that the content of NDF in silage treated with LP increased about 2% relative to the untreated. This treatment improved *in situ* degradability of DM by decrease (3%) in fraction (a) and increase (3%) in fraction (b). As a whole, *Lactobacillus plantarum* had a positive effect on effective degradability for DM, NDF and CP. Results of the present experiment do not present a significant effect of the WCBS treated whit LP or AP on feed intake and milk yield of the dairy cows fed the experimental diets. It may be related to the silage dry matter included in the diets. The silages were accounted for 19.3% of diet DM, which is less than of 50% of the whole crop forage provided. Therefore, there is a need to evaluate the effect of the WCBS treated with LP or AP while introduce to the lactating dairy cow diets with a higher proportions than that used in the present study.

Acknowledgment

The authors wish to acknowledge the financial and technical support received from Ferdowsi University of Mashhad (Project number 14789/1).

References

[1] L.Jr. Kung, C.C. Taylor, M.P. Lynch, J.M. Neylon, The effect of treating alfalfa with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for lactating dairy cows, J. Dairy. Sci. 86 (2003) 336-343.

[2] M. Vatandoost, M. Danesh Mesgaran, R. Valizadeh, H. Nasiri Moghaddam, Effect of whole crop silages (triticale or barley) versus corn silage on performance of Holstein lactating dairy cows, J. Anim. Vet. Adv. 6 (2007) 344-348.

[3] S. Arbabi, T. Ghoorchi, S. Hasani, The effect of delayed ensiling and application of an propionic acid-based additives on the nutrition value of corn silage, Asian. J. Anim. Sci. 2 (2008) 26-34.

[4] L.E. Phillip, V. Fellner, Effects of bacterial inoculation of high-moisture ear corn on its aerobic stability, digestion, and utilization for growth by beef steers, J. Anim. Sci. 70 (1992) 3178.

[5] Z.G. Weinberg, R.E. Muck, New trends and opportunities in the development and use of inoculants for silage, FEMS, Microbiol. Rev. 19 (1996) 53-68.

[6] C.S. Mayne, An evaluation of an inoculant of *Lactobacillus plantarum* as an additive for grass silage for dairy cattle, Anim. Prod. 51 (1990) 1-13.

[7] F.J. Gordon, An evaluation through lactating cattle of a bacterial inoculant as an additive for grass silage, Grass Forage Sci. 44 (1989) 169-179.

[8] T.W.J. Keady, R.W.J. Steen, Effects of treating low dry matter grass with a bacterial inoculant on the intake and performance of beef cattle and studies on its mode of action, Grass Forage Sci. 49 (1994) 438-446.

[9] T.W.J. Keady, R.W.J. Steen, The effects of treating low dry matter, low digestibility grass with a bacterial inoculant on the intake and performance of beef cattle, and studies on its mode of action, Grass Forage Sci. 50 (1995) 217-226.

[10] D. Seale, Bacterial inoculants as silage additives, J. Applied Bacteriol. 61 (1986) 9-26.

[11] K.A. Scudamore, C.T. Livesey, Occurrence and

significance of mycotoxins in forage crops and silage: a review, J. Sci. Food Agric. 77 (1998) 1-17.

[12] B.A. Kent, M.J. Arambel, J.L. Walters, Effect of bacterial inoculant on alfalfa haylage: ensiling characteristics and milk production response when fed to dairy cows in early lactation, J. Dairy Sci. 71 (1988) 2457-2561.

[13] K. Martinsson, A study of the efficacy of a bacterial inoculant and formic acid as additives for grass silage in terms of milk production, Grass Forage Sci. 47 (1992) 189-198.

[14] V. Fellner, L.E. Phillip, S. Sebastian, E.S. Idziak, Effects of a bacterial inoculant and propionic acid on preservation of high-moisture ear com, and on rumen fermentation, digestion and growth performance of beef cattle, Can. J. Anim. Sci. (2001) 273-280.

[15] A.N. Hristov, T.A. McAllister, Effect of inoculants on whole-crop barley silage fermentation and dry matter disappearance *in situ*, J. Anim. Sci. 80 (2002) 510-516.

[16] Y.M. Acosta, C.C. Stalings, C.E. Polan, C.N. Miller, Evaluation of barley silage harvested at boot and soft dough stages, J. Dairy Sci. 74 (1991) 167-176.

[17] S.A. Moshtaghi Nia, K.M. Wittenberg, Use of forage inoculants with or without enzymes to improve preservation and quality of whole crop barley forage ensiled as large bales, Can. J. Anim. Sci. 79 (1999) 525-532.

[18] T.A. McAllister, L.B. Sehnger, L.R. McMahon, H.D. Bae, T.J. Lysyk, S.J. Oosting, et al., Intake, digestibility and aerobic stability of barley silage inoculated with mixtures of *Lactobacitus plantarum* and *Enterococcus faecium*, Can. J. Anim. Sci. 75 (1995) 425-432.

[19] D.G. Chamberlain, Effect of added glucose and xylase on the fermentation of perennial ryegrass silage inoculated with *Lactobacillus plantarum*, J. Sci. Food Agric. 46 (1982) 129-138.

[20] D.G. Britt, J.T. Huber, A.L. Rogers, Fungal growth and acid production during fermentation and refermentation of organic acid treated corn silages, J. Dairy Sci. 58 (1975) 532-539.

[21] L.Jr. Kung, J.R. Robinson, N.K. Ranjit, J.H. Chen, CM. Golt, J.D. Pesek, Microbial populations, fermentation end-products and aerobic stability of com silage treated with ammonia or a propionic acid-based preservative, J. Dairy Sci. 83 (2000) 1479-1486.

[22] P.J. Van Soest, J.B. Robertson, B.A. Lewis, Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition, J. Dairy Sci. 74 (1991) 3583-3597.

[23] M.H. Fathi Nasri, M. Danesh Mesgaran, J. France, J.P. Cant, E. Kebreab, Evaluation of models to describe ruminal degradation kinetics from *in situ* ruminal incubation of whole soybeans, J. Dairy Sci. 89 (2006) 3087-3095.

[24] L. Kerscher, J. Ziegenhom, Urea, in: H.U. Bergmeyer (Ed.), Methods of Enzymatic Analysis, Verlag. Chemie, Weinheim, 1985, pp. 444-453.

[25] R. Jafari Jafarpoor, M. Danesh Mesgaran, A.R. Heravi Moussavi, S. Danesh Mesgaran, F. Tabatabai, Effect of protein sources of isonitrogenous whole crop barley silage based diets on perfonnance and blood metabolites of early lactating holstein cows, J. Anim. Vet. Adv. 8 (2009) 2100-2105.

[26] E.R. Ørskov, I. McDonald, The estimation of protein degradability in the rumen from incubation measurements weighted according to rates of passage, Journal of Agricultural Science 92 (1979) 499-503.

[27] J.A. Rooke, F.M. Maya, J.A. Arnold, D.G. Armstrong, The chemical composition and nutritive value of grass silage prepared with no additive or with the application of additives containing either *Lactobacillus plantarum* or formic acid, Grass Forage Sci. 43 (1998) 87-95.

[28] L.Jr. Kung, N.K. Ranjit, The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage, J. Dairy Sci. 84 (2001) 1149-1155.

[29] M. Vatandoost, M. Danesh Mesgaran, A. Heravi Mousavi, A.R. Vakili, Effect of biological and chemical additives on fermentation responses and degradation characteristics of whole crop barely silage, J. Anim. Vet. Adv. 9 (2010) 1452-1457.

[30] S.J. Kennedy, An evaluation of three bacterial inoculants and formic acid as additive for harvest grass, Grass Forage Sci. 45 (1990) 281-288.

[31] T. Aksu, E. Baytok, M.A. Karsl, H. Muruz, Effects of formic acid, molasses and inoculant additives on com silage composition, organic matter digestibility and microbial protein synthesis in sheep, Small Rum. Res. 61 (2006) 29-33.

[32] L.Jr. Kung, A.C. Sheperd, A.M. Smagala, K.M. Enders, C.A. Besset, N.K. Raryit, et al., The effect of preservatives based on propionic acid on the fermentation and aerobic stability of com silage and a total mixed ration, J. Dairy Sci. 81 (1998) 1322-1330.

[33] V.L. Nsereko, B.K. Smiley, W.M. Rutherford, A. Spielbauer, K.J. Forrester, G.H. Hettinger, et al., Influence of inoculating forage with lactic acid bacterial strains that produce ferulate esterase on ensilage and ruminal degradation of fiber, Anim. Feed Sci. Technol. 145 (2008) 122-135.

[34] Z.G. Weinberg, O. Shatz, Y. Chen, E. Yosef, M. Nikbahat, D. Ben-Ghedalia, et al., Effect of lactic acid bacteria inoculants on *in vitro* digestibility of wheat and corn silage, J. Dairy Sci. 90 (2007) 4754-4762.

[35] S. Jaakkola, V. Kaunisto, P. Huhtanen, Volatile fatty acid

proportions and microbial protein synthesis in the rumen of cattle receiving grass silage ensiled with different rates of formic acid, Grass Forage Sci. 61 (2006) 282-292.

[36] H. Zahiroddini, J. Baaha, W. Absalomb, T.A. McAllister, Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barley silage, Anim. Feed Sci. Tech. 117 (2004) 317-330.

[37] P. Mandebvu, J.W. West, M.A. Froetschel, R.D. Hatfield, R.N. Gates, G.M. Hill, Effect of enzyme or microbial treatments of Bermuda grass forages before ensiling on cell wall composition, end products of silage fermentation and *in situ* digestion kinetics, Anim. Feed Sci. Technol. 77 (1999) 317-329.

[38] T.W.J. Keady, J.J. Murphy, The effects of treating low dry matter herbage with a bacterial inoculant or formic acid on the intake and performance of lactating dairy cattle, Anim. Sci. 64 (1997) 17-24.

[39] H.V. Petit, P.M. Flipot, Intake, duodenal flow, and ruminal characteristics of long or short chopped alfalfa-timothy silage with or without inoculant, J. Dairy Sci. 73 (1990) 3165-3171.

[40] T. Aksu, E. Baytok, D. Bolat, Effects of a bacterial inoculant on corn silage fermentation and nutrient digestibility, Small Rum. Res. 55 (2004) 249-252.

[41] P.A. Vandenbergh, Lactic acid bacteria, their metabolic products and interference with microbial growth, FEMS Microbiology Reviews 12 (1993) 221-238.

[42] T. Muller, U. Behrendt, M. Muller, Antagonistic activity in plant-associated lactic acid bacteria, Microbiological Research 151 (1996) 63-70.

[43] L.Jr. Kung, J.H. Chen, E.M. Creck., K. Knusten, Effect of microbial inoculants on the nutritive value of corn silage for lactating dairy cows, J. Dairy Sci. 76 (1993) 3763-3770.

[44] R. Meeske, H.M. Basson, C.W. Cruywagen, The effect of a lactic acid bacterial inoculant with enzymes on the fermentation dynamics, intake and digestibility of *Digitaria eriantha* silage, Anim. Feed Sci. Technol. 81 (1999) 237-248.

[45] J.A. Rooke, F. Kafilzadeh, The effect upon fermentation and nutritive value of silages produced after treatment by three different inoculants of lactic acid bacteria applied alone or in combination, Grass Forage Sci. 49 (1994) 42-53.