TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2014) 38: 285-294
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doi:10.3906/vet-1311-91

Conservation characteristics and protein fractions of cereal silages ensiled with additives at the booting and dough stages of maturity

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Received: 25.11.2013 • **Accepted:** 29.01.2014 • **Published Online:** 21.04.2014 • **Printed:** 20.05.2014

Abstract: The experiment was conducted to investigate the effects of organic acid and bacterial inoculant on conservation characteristics and protein fractions as defined by the Cornell Net Carbohydrate and Protein System of barley, wheat, rye, triticale, and oat silages ensiled at their booting or dough stage of maturity. The cereal crops were not treated or were treated with bacteria (*L. buchneri*, *L. plantarum*, *E. faecium*) or organic acid (formic acid, propionic acid, sodium formate) and ensiled in 1.0-L anaerobic jars for 45 days. Bacterial inoculant improved the fermentation profile for all cereal silages and increased the dry matter (DM) recovery at both maturity stages. The benefit of bacteria inclusion in the silage was more pronounced when applied at dough stages. Organic acid also improved the fermentation profile for all cereal silages, but it was less effective than the bacteria at both booting and dough stages of maturity. The reduction ($P < 0.001$) in in vitro DM digestibility was not as sharp as the decrease in crude protein (CP). Protein A fraction and digestible CP were higher ($P < 0.001$) at the booting stage, while all B fractions and C fraction were higher ($P < 0.001$) in cereal silages ensiled at the dough stage. It was concluded that additives, in particular bacterial inoculant, can improve fermentation and protein quality at both stages examined.

Key words: Additives, cereal silages, fermentation, protein fractions, stage of maturity

1. Introduction

Harvesting cereal crops for hay or silage production at the booting or dough stage of maturity is most common. However, the benefits of higher nutritive value and earlier harvest date at the booting stage are offset by lower dry matter (DM) production compared to harvesting at the dough stage (1–3). Maturity differences among species also have a great impact on their nutritive value (1). Unlike leguminous species, there is an obvious reduction in nutritive value, especially crude protein (CP), with advancing maturity in cereal species (4). Given the huge difference in CP content of cereal crops between the booting and dough stage of growth, it would be wise to characterize the protein fractions for more effective use in ruminant nutrition. If protein degradation is rapid or nonprotein nitrogen (NPN) value is higher than the capacity of ruminal microbes to utilize released amino acids or ammonia, this could lead to inefficiencies in ruminant nutrition. The nutritional quality of CP in forages is determined by its rate and extent of degradation in the rumen, and this can be enhanced by increasing true protein that is resistant to microbial degradation in the rumen. Choosing the most efficient combination of forage species, timing the harvest, and silage additives could increase CP quality for ruminant production (5,6).

The DM content of cereal species harvested at the booting stage is approximately 40% lower than in those harvested at dough stage (4). The low DM content of cereal crops at booting requires longer wilting times, which could be a major challenge, particularly in wet spring conditions. This could be associated with undesirable silage fermentation resulting in high ammonia-N concentrations. However, cereal species with an average DM of over 350 g kg^{-1} at their dough stage do not require prewilting prior to ensiling to obtain satisfactory silage fermentation (7).

Under conditions where the DM content of cereal species is low, organic acids generally restrict the fermentation processes to obtain good quality silage (8) and reduce the minimum DM necessary to produce well-fermented silages (9). Most common silage additives like organic-acid–based and bacterial inoculant can reduce proteolysis and increase the true protein content of cereal silages. Guo et al. (6) reported that organic acid reduced

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the NPN content of alfalfa silages. Similarly, Davies et al. (10) reported reduced proteolysis in grass and clover silages after inoculation with a bacterial inoculant. However, there are limited data on protein fractions of cereal silages harvested at different stages of maturity. Furthermore, most of the work done with silage additives only investigated the end point of protein degradation, ammonia-N.

The objective of this experiment was to assess the effects of cereal species, stage of maturity at harvest, and type of silage additives and their interactions on nutritive value, fermentation characteristics, and protein fractions in barley, wheat, rye, triticale, and oat silages for their more efficient use in high-yielding ruminants.

2. Materials and methods

2.1. Ensilage

Cereal grains of barley (*Hordeum vulgare* L. 'Beysehir'), wheat (*Triticum aestivum* L. 'Goksu'), rye (*Secale cereale* L. 'Aslim'), triticale (X *Triticosecale wittmack* 'Tatlicak'), and oat (*Avena sativa* L. 'Faikbey') grown in Bahri Dağdaş International Agricultural Research Institute research field (37°51′N, 32°33′E, 1008 m, a.s.l.), Konya, Turkey, used for silage production. Crops were seeded at rates typical for the region: 210 kg ha^{-1} for wheat, 200 kg ha^{-1} for triticale, 172 kg ha^{-1} for rye, 166 kg ha^{-1} for barley, and 146 kg ha^{-1} for oats, grown in 16 m × 78 m plots. Based on soil test results, a total of 100 kg ha^{-1} of fertilizer (18% N and 46% P_2O_5) was applied at sowing. Crops were harvested at booting and at the dough stage of maturity. At the dough stage, oat was at soft dough, while other crops were at the hard dough stage. Crops were cut leaving a stubble height of approximately 5 cm and wilted to a target DM of about 350 g kg^{-1} at the booting stage. At the dough stage they were ensiled without prewilting as their DM content was over 350 g kg^{-1}. Crops were chopped at a theoretical cut length of 2.0 cm at both stages, and then the following additive treatments were applied: (1) control: no additive, (2) bacterial inoculant (1.5×10^5 cfu g^{-1}; Pioneer 11GFT, *L. buchneri, L. plantarum, E. faecium*; Pioneer Hi-Bred, Int., Inc., USA), and (3) organic acid (4 L t^{-1}; organic acid + propionic acid + sodium formate; Silofarm Combi Liquid, Farmavet, Turkey). Bacterial inoculant in powder form was dissolved in 20 mL of deionized water, while 32 mL of organic additives were used directly. The additives were spread over 8 kg of chopped herbage each with a hand sprayer. Twenty milliliters of water was also spread on the 8 kg of control herbage. The additives were aseptically applied to the herbage in a uniform manner with constant mixing. Herbages were ensiled into 1.0-L anaerobic jars (Weck, Wher-Oftlingen, Germany) equipped with lids and rubber seals that enabled the release of fermentation gases only. A total of 90 jars (5 cereal crops × 3 additives × 2 stages of maturity × 3 replicates) were ensiled for 45 days at ambient temperature. At the end of the ensiling, the jars were emptied into a container, mixed thoroughly, and sampled.

2.2. Analytical procedures

Silage samples were assayed for DM by oven drying at 60 °C for 48 h. Ash and crude fat (CF) were determined by AOAC (11). Neutral detergent fiber (aNDF), acid detergent fiber (ADF), and lignin (sa) were assayed according to Van Soest et al. (12). The NDF was analyzed with the inclusion of a heat-stable amylase and sodium sulfite, but both NDF and ADF were expressed inclusive of residual ash. Neutral (NDICP) and acid (ADICP) detergent-insoluble CP was determined on the samples obtained from NDF and ADF residues. Nonfiber carbohydrates were 1000 – (aNDF + ash + CP + CF). In vitro true DM digestibility (DMD) was determined with the Ankom DAISY^{II} incubator. Ruminal fluid used for DMD was collected from a nonpregnant, dry cow fed an alfalfa pellet and concentrate (60:40). Samples analyzed for in vitro true DM digestibility were further analyzed for CP content to obtain the digestible CP (DCP). The Kjeldahl method according to AOAC (11) was used to determine CP content of all samples (Gerhart, with automated distillation and titration, Germany). Borate-phosphate-buffer–soluble CP and CP not precipitated with trichloroacetic acid were determined according to the method of Licitra et al. (13).

Twenty grams of sampled silage was blended (8010ES blender, Waring Laboratory, Torrington, CT, USA) with 180 mL of distilled water for 1 min at high speed. The resulting homogenate was filtered through Whatman 1 filter paper. The pH of the filtrate was measured with a pH meter (Inolab 720, WTW, Germany). A proportion of the filtrate (50 mL) was acidified with 100 µL of 50% H_2SO_4 and then frozen before being used for determination of concentration of lactic acid (14), water soluble carbohydrates (WSC) (15), and ammonia-N (16).

2.3. Crude protein fractionation

Protein fractionation as percentage of total CP was made by the Cornel Net Carbohydrate and Protein System (CNCPS) (17). According to CNCPS, CP is partitioned into 3 fractions. Briefly, the A fraction is nonprotein N (NPN), the B fraction is a degradable protein, and the C fraction is an undegradable and unavailable protein. The B fraction is further divided into 3 fractions according to solubility and rate of ruminal degradation. The degradation rates in the rumen of borate-phosphate-buffer–soluble B1, neutral-detergent–soluble B2, and acid-detergent–soluble B3 fractions are rapid, intermediate, and slow, respectively. Rumen-undegradable CP (RUP) of silages was calculated according to CNCPS using a ruminal passage rate of 0.045 h^{-1} and digestion rate constants of 2.5, 0.13, and 0.011 h^{-1} for B1, B2, and B3, respectively.

2.4. Statistical analysis

The experimental data were analyzed by a 3-way analysis of variance in a completely randomized design using a model that accounted for the main effects of crops, stage of maturity, and additives; for all 2-way and 3-way interactions; and for error, using the general linear model procedure of SPSS 10 (18). Differences were deemed significant at $P < 0.05$.

3. Results

3.1. Nutritive value of silages

The chemical compositions of the cereal silages are presented in Table 1. The main effect of maturity was significant for several variables. The CP, ash, CF, DMD, and metabolizable energy (ME) were higher ($P < 0.001$), while lignin and NFC were lower ($P < 0.001$) at the booting stage. Barley silage had the highest ($P < 0.001$) ash and CP contents, while oat silage had the highest ($P < 0.05$) CF and cell wall content. Wheat and rye silages had higher ($P < 0.001$) NFC content than triticale and oat silages. The ME content of wheat silages was lower ($P < 0.05$) than that of rye and oat silages. The addition of bacteria slightly decreased ($P < 0.05$) the ash and NDF contents of silages but increased ($P < 0.05$) the NFC. There were significant 2-way interactions between the maturity and cereal silages for all chemical compositions. CP, ash, lignin, DMD, and NFC content of all cereal silages gave similar responses to advancing maturity. However, NDF values were lower in rye and wheat silages ($P < 0.05$) while they were higher in barley silages ($P < 0.01$) when they ensiled at their dough stage. By contrast, CF content and ME value of oat silages did not decrease ($P > 0.05$) with advancing maturity. Rye silages had lower ($P < 0.05$) ADF content at the dough stage compared to ensiling at the boot stage. Bacteria slightly decreased ($P < 0.05$) ash value at the dough stage. There were significant 2-way interactions between cereal silages and additives only for NDF and ME values of cereal silages. The addition of both additives decreased the NDF ($P < 0.05$) content of oat silages, but wheat silages had the lowest ($P < 0.05$) ME values and increased ($P < 0.05$) in NDF content with the addition of organic acid. There were no ($P > 0.05$) 3-way interactions for the chemical composition of the cereal silages.

3.2. Fermentation characteristics and DMR of silages

Fermentation characteristics of the cereal silages are presented in Table 2. The main effect of maturity was significant for fresh and silage DM, pH, gas losses, DMR, LA, and ammonia-N. Dry matter contents of cereal silages were 6% and 4% lower than fresh forage at boot and dough stages, respectively. Only DM and DMR were higher with advancing maturity, while other variables were higher ($P < 0.05$) at the booting stage.

Except for DMR, all fermentation characteristics were affected by cereal species. Wheat silages had the highest ($P < 0.001$) DM content and the lowest ($P < 0.001$) ammonia-N content. The pH was lowest ($P < 0.001$) in the triticale silages, which also had the highest ($P < 0.001$) LA content together with barley silages. Wheat and oat silages had the highest ($P < 0.001$) gas losses, while they had the lowest ($P < 0.001$) WCS content. The main effect of the additives was significant for all variables but WSC content. The addition of both additives decreased ($P < 0.001$) pH, gas losses, and ammonia-N content of silages but resulted in more DMR, especially with bacteria. The bacteria also increased ($P < 0.001$) the DM and LA content of silages. There were significant 2-way interactions between maturity and cereal silages for all fermentation characteristics but not DMR. Only wheat and oat silages ensiled at the dough stage had higher ($P < 0.001$) pH compared to their pH values at the booting stage. Only the gas losses of triticale silages were not affected ($P > 0.05$) by the maturity stage. Rye and oat silages had lower ($P < 0.05$) ammonia-N content when they were ensiled at the dough stage. Barley and wheat had lower ($P < 0.001$) and rye and triticale had higher ($P < 0.001$) WSC content when ensiled at the dough stage. The effect of additives on silage pH and ammonia-N was more pronounced ($P < 0.05$) at the dough stage, especially with bacteria. Both additives increased ($P < 0.05$) WSC content of silages only at the booting stage. The effect of bacteria on LA was more pronounced ($P < 0.05$) at the dough stage. There were also significant 2-way interactions between the cereal silages and additives for silage DM, pH, gas losses, DMR, LA, and WSC values. The additives increased ($P < 0.001$) DM of wheat, triticale, and oat silages, while organic acid increased ($P < 0.001$) only the DM of triticale silages. The bacteria increased the pH of all cereal silages, while organic acid increased ($P < 0.001$) the pH of barley, rye, and oat silages. Bacteria did not reduce ($P > 0.001$) gas losses in rye silages only, while organic acid reduced ($P < 0.001$) only the gas losses of wheat and triticale silages. Both additives increased ($P < 0.01$) DMR in triticale silages, while bacteria increased the DMR of wheat and oat silages. Bacteria increased ($P < 0.05$) the LA content of all silages, while organic acid had no effect ($P > 0.05$) on the LA content of silages. Both additives increased ($P < 0.05$) the WSC content in wheat silages only. Organic acid also increased ($P < 0.05$) the WSC content of triticale silages. A 3-way interaction was significant ($P < 0.05$) for several variables where the effect of bacteria for reducing pH, gas losses, and ammonia-N of cereal silages was generally more pronounced than the effect of organic acid at both maturity stages.

3.3. Protein fractions

Protein fractions, RUP, and digestible CP content of cereal silages are presented in Table 3. The A fraction and DCP decreased ($P < 0.001$) with the later harvest, but this

Table 1. Effect of species, maturity, and additives on chemical composition of silages (g kg^{-1} DM).

Factors			Chemical composition[1]								
Stage	Silage	Add.	CP	Ash	CF	NDF	ADF	ADL	NFC	DMD	ME[2]
Boot	Barley	Organic	164	103	47	507	311	42	178	685	9.0
Boot	Barley	Bacteria	168	103	41	524	315	42	165	680	8.9
Boot	Barley	Control	165	105	43	516	317	51	170	685	8.7
Boot	Wheat	Organic	142	99	45	555	340	52	160	684	8.5
Boot	Wheat	Bacteria	147	97	44	544	340	59	168	680	8.5
Boot	Wheat	Control	140	99	46	543	351	54	172	658	8.6
Boot	Rye	Organic	166	93	50	560	341	46	130	699	8.8
Boot	Rye	Bacteria	166	95	52	559	340	47	128	684	8.9
Boot	Rye	Control	166	96	53	556	331	38	130	698	8.9
Boot	Triticale	Organic	163	109	47	541	327	52	140	660	8.6
Boot	Triticale	Bacteria	164	109	47	540	328	52	141	658	8.7
Boot	Triticale	Control	162	109	49	545	338	55	135	662	8.6
Boot	Oat	Organic	133	104	54	562	367	53	147	661	8.6
Boot	Oat	Bacteria	131	101	54	540	348	51	173	653	8.7
Boot	Oat	Control	134	103	51	591	367	55	120	694	8.3
Dough	Barley	Organic	102	80	37	540	327	67	241	595	8.2
Dough	Barley	Bacteria	101	77	35	541	326	69	245	586	8.1
Dough	Barley	Control	100	79	34	553	321	64	233	604	8.1
Dough	Wheat	Organic	93	75	33	576	345	69	223	613	7.8
Dough	Wheat	Bacteria	93	62	32	481	320	64	332	656	8.5
Dough	Wheat	Control	94	69	34	498	330	60	305	628	8.5
Dough	Rye	Organic	77	51	31	565	319	68	277	573	8.1
Dough	Rye	Bacteria	78	44	30	499	319	62	350	595	8.4
Dough	Rye	Control	77	53	31	531	316	66	308	600	8.4
Dough	Triticale	Organic	85	67	39	563	344	66	247	596	8.2
Dough	Triticale	Bacteria	82	58	37	566	338	65	258	592	8.4
Dough	Triticale	Control	83	68	39	567	333	76	244	628	8.0
Dough	Oat	Organic	94	71	54	548	349	68	233	604	8.6
Dough	Oat	Bacteria	92	69	56	538	350	63	245	579	8.7
Dough	Oat	Control	92	72	55	582	360	65	199	572	8.5
SE[3]			1.8	2.8	4.2	15.2	8.5	3.9	17.6	15.4	0.14
P_M			***	***	***	NS	NS	***	***	***	***
P_{CS}			***	***	***	*	***	*	**	NS	*
P_A			NS	*	NS	*	NS	NS	*	NS	NS
$P_{M \times CS}$			***	***	**	*	*	*	***	*	**
$P_{M \times A}$			NS	*	NS	NS	NS	NS	NS	NS	NS
$P_{CS \times A}$			NS	NS	NS	*	NS	NS	NS	NS	*
$P_{M \times CS \times A}$			NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]: CP: crude protein; CF: crude fat; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFC: nonfiber carbohydrates; DMD: in vitro dry matter digestibility.
[2]: ME: metabolizable energy (MJ/kg DM). Calculated according to tabular value of NRC (19).
[3]: for the 3-way interactions. * = P < 0.05; ** = P < 0.01; *** = P < 0.001.
M = maturity stage, CS = cereal silage, A = additive; NS: not significant.

Table 2. Fermentation characteristics and DMR of cereal silages.

Factors			Fermentation characteristics[1]							
Stage	Silage	Add.	FDM	SDM	pH	LA	NH_3-N	WSC	GL	DMR
Boot	Barley	Organic	344	331	4.5	32	101	39	11.1	946
Boot	Barley	Bacteria	336	318	3.9	56	102	30	10.5	933
Boot	Barley	Control	345	327	4.6	33	115	22	12.6	931
Boot	Wheat	Organic	349	320	4.2	30	104	26	17.9	895
Boot	Wheat	Bacteria	349	343	3.9	49	96	35	9.6	970
Boot	Wheat	Control	346	315	4.3	32	117	16	20.6	883
Boot	Rye	Organic	328	304	4.6	34	122	11	12.7	913
Boot	Rye	Bacteria	328	310	4.6	36	132	10	13.1	927
Boot	Rye	Control	336	317	4.9	37	129	14	14.4	925
Boot	Triticale	Organic	333	318	4.3	38	104	29	10.9	941
Boot	Triticale	Bacteria	331	315	4.1	54	120	11	11.3	936
Boot	Triticale	Control	336	301	4.4	45	131	19	13.1	880
Boot	Oat	Organic	336	311	4.4	27	110	12	15.7	903
Boot	Oat	Bacteria	338	329	4.0	55	100	24	9.9	959
Boot	Oat	Control	342	313	4.5	29	126	11	16.3	895
Dough	Barley	Organic	372	351	4.1	29	112	11	9.8	931
Dough	Barley	Bacteria	372	360	3.9	43	97	14	8.6	955
Dough	Barley	Control	374	360	4.4	30	133	35	9.4	950
Dough	Wheat	Organic	528	505	4.4	20	90	17	10.5	938
Dough	Wheat	Bacteria	519	512	4.1	34	94	14	6.9	973
Dough	Wheat	Control	522	498	4.4	22	115	17	11.3	934
Dough	Rye	Organic	445	432	4.0	34	117	33	9.1	954
Dough	Rye	Bacteria	449	438	3.9	36	96	45	8.9	959
Dough	Rye	Control	456	440	4.4	21	136	48	9.8	947
Dough	Triticale	Organic	455	436	4.2	23	110	34	12.4	940
Dough	Triticale	Bacteria	457	438	3.9	38	107	29	9.7	943
Dough	Triticale	Control	455	433	4.2	26	141	20	13.4	929
Dough	Oat	Organic	446	424	4.4	28	99	13	13.1	930
Dough	Oat	Bacteria	441	425	4.1	37	95	11	7.8	951
Dough	Oat	Control	446	417	4.6	21	101	10	13.6	914
SE[2]			3.9	2.8	0.02	3.2	5.3	4.0	0.7	11.1
P_M			***	***	***	***	*	NS	***	***
P_{CS}			***	***	***	*	***	***	***	NS
P_A			***	***	***	***	***	NS	***	***
$P_{M \times CS}$			***	***	***	*	*	***	***	NS
$P_{M \times A}$			NS	NS	***	*	*	*	NS	NS
$P_{CS \times A}$			***	***	***	*	NS	*	***	**
$P_{M \times CS \times A}$			***	***	***	NS	*	***	*	NS

[1]: FDM: fresh dry matter, g kg^{-1}; SDM: silage dry matter, g kg^{-1}; LA: lactic acid, g kg^{-1} DM; NH_3-N: ammonia-N, g kg^{-1} N; WSC: water-soluble carbohydrates, g kg^{-1} DM; gas losses, g kg^{-1}; DMR: dry matter recovery, g kg^{-1}.
[2]: for the 3-way interactions. NS: not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.
M = maturity stage, CS = cereal silage, A = additive.

Table 3. Protein fractions, RUP, and digestible CP of silages.

Factors			Protein fractions (g kg^{-1}crude protein)							
Stage	Silages	Add.	A	B1	B2	B3	B	C	RUP	DCP
Boot	Barley	Organic	565	218	131	17	366	69	147	866
Boot	Barley	Bacteria	518	216	169	28	413	69	172	880
Boot	Barley	Control	594	160	163	23	345	61	155	886
Boot	Wheat	Organic	474	231	168	32	431	95	203	859
Boot	Wheat	Bacteria	476	225	193	31	449	75	192	854
Boot	Wheat	Control	583	137	184	12	333	85	177	864
Boot	Rye	Organic	495	202	217	12	430	74	182	890
Boot	Rye	Bacteria	485	199	232	8	440	75	187	879
Boot	Rye	Control	610	100	200	23	324	66	174	876
Boot	Triticale	Organic	540	259	118	14	391	69	140	904
Boot	Triticale	Bacteria	532	267	125	10	403	65	136	905
Boot	Triticale	Control	696	124	108	5	237	66	120	888
Boot	Oat	Organic	418	265	219	10	495	88	197	811
Boot	Oat	Bacteria	373	300	218	8	527	100	209	875
Boot	Oat	Control	350	356	182	27	564	86	199	816
Dough	Barley	Organic	382	279	193	61	534	85	231	796
Dough	Barley	Bacteria	313	227	321	11	560	127	279	795
Dough	Barley	Control	394	173	267	55	495	111	279	833
Dough	Wheat	Organic	226	207	382	51	640	134	348	795
Dough	Wheat	Bacteria	316	265	280	37	582	102	261	774
Dough	Wheat	Control	340	229	297	25	550	109	262	769
Dough	Rye	Organic	268	343	214	48	605	127	273	779
Dough	Rye	Bacteria	283	350	205	28	583	133	256	774
Dough	Rye	Control	433	262	141	32	435	132	230	775
Dough	Triticale	Organic	221	445	182	20	647	132	242	776
Dough	Triticale	Bacteria	297	331	194	44	570	133	267	774
Dough	Triticale	Control	391	294	157	41	493	117	230	775
Dough	Oat	Organic	362	222	252	34	509	129	274	794
Dough	Oat	Bacteria	348	256	233	32	521	131	266	802
Dough	Oat	Control	347	146	357	19	522	131	304	799
		sem[1]	26.1	20.5	22.1	7.5	28.0	5.3	9.8	24.3
P			***	***	***	***	***	***	***	***
P_{CS}			***	***	***	*	***	***	***	NS
P_{A}			***	***	NS	NS	***	NS	*	NS
$P_{M \times CS}$			***	***	***	NS	***	***	*	*
$P_{M \times A}$			NS	NS	NS	NS	NS	NS	NS	NS
$P_{CS \times A}$			***	**	*	*	***	***	***	NS
$P_{M \times CS \times A}$			NS	***	*	*	NS	*	**	NS

[1]: for the 3-way interactions. NS: not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.
RUP: rumen undegradable protein; DCP: digestible crude protein.

increased (P < 0.001) all other protein fractions and RUP value. True protein and RUP values of wheat and oats silages were the highest (P < 0.001), and A fraction was the lowest (P < 0.001). Oat had the highest (P < 0.001) while barley had the lowest (P < 0.001) C fraction. Both additives decreased (P < 0.001) the A fractions but increased (P < 0.05) the B1 and B fractions and RUP value of silages.

Two-way interactions occurred between stage of maturity and crops for A, B_1, B_2, B, and C fractions and RUP and DCP values. Only oat silages had similar (P > 0.05) A and B fractions and DCP values at both ensiling times. A 2-way interaction between stage of maturity and additives was not significant (P > 0.05) for all measurements. Two-way interactions between cereal silages and additives occurred for all protein fractions and RUP values. Except for oat silages, both additives decreased (P < 0.001) A fraction but increased (P < 0.001) B1 fraction content of the cereal silages compared to the untreated control. Both additives increased (P < 0.01) the B2 fraction in rye silages, but only organic acid increased (P < 0.01) the B2 fraction of barley and wheat silages. Additives increased (P < 0.05) the B3 fraction of wheat silages, but the bacterial inoculant decreased (P < 0.05) B3 fraction in barley silages. Additives increased (P < 0.001) total true protein content of all crops, except for oat silages. The addition of organic acid decreased (P < 0.001) RUP values of barley; however, it increased (P < 0.001) RUP values of wheat and rye. The bacterial inoculant only increased (P < 0.001) the RUP values in triticale.

Three-way interactions occurred for B1 (P < 0.001), B2 (P < 0.001), B3 (P < 0.01), and C (P < 0.05) fractions and RUP values (P < 0.001). Additives increased (P < 0.001) the B1 fraction of all silages, except for the oat silage at the booting stage, but only rye and oat silages had a higher (P < 0.001) B1 content at the dough stage with both additives. Organic acid also increased (P < 0.001) the B1 content of barley and triticale silages at the dough stage. Both additives had no effect (P > 0.05) on the B2 content of silages ensiled at the booting stage. At the dough stage, additives increased (P < 0.001) the B2 fraction of rye silage; however, oat silages treated with additives had lower (P < 0.001) B2 fractions compared to the untreated control. Only organic acid increased (P < 0.01) the B3 fraction of wheat silages at the dough stage. Wheat silages treated with organic acid had higher (P < 0.05) C fractions at the dough stage. Organic acid decreased (P < 0.05) the C fraction and RUP value of the barley silages, but wheat and rye silages treated with organic acid had higher (P < 0.001) RUP values at the dough stage. The bacterial inoculant increased (P < 0.001) the RUP value of triticale at the dough stage, but both additives decreased (P < 0.001) the RUP value of oat silages at the dough stage.

4. Discussion

4.1. Nutritive value of silages

Nutritive values of cereal silages were higher when they were ensiled at the booting stage as evidenced by having higher CP, ash, CF, DMD, and ME values. The CP content decreased for all cereal silages with maturity. This is in line with the results reported by Helsel and Thomas (1) for barley, wheat, rye, and oat and by Bect et al. (3) for wheat. However, the reduction in DMD was not as sharp as in CP, which was also reported by Crovetto et al. (2). This was due to accumulation of NFC with maturity.

If the cell wall component is an important factor affecting the feeding value of cereal silages due to its effect on reduced DM intake, as suggested by Khorasani et al. (4), then there were no differences between cereal crops harvested at the booting or dough stages in the present study. In a study of growing calves fed with 20% or 40% wheat hay or silages, the feeding value of hay or silages was similar when harvested at the booting or dough stages (3). However, because of their rough structure compared to legume or grass silages, the nutritive value of cereal silages rarely reflects their feeding value. Bolsen et al. (20) reported that in a 2-lamb feeding trial with awnless wheat, awned wheat, and barley silages produced at the booting, milk, dough, or ripe stages, lambs fed with awnless wheat or dough-maturity silages performed best. Similarly, Emile et al. (21) reported decreased DM intake by sheep fed barley and triticale silages cut at the late milk–early dough stage compared to silage from 2 awnless wheat cultivars because of the rough barbs of the barley and triticale cultivars. The reason for choosing the cereal species bred for their grain production rather than their forage production in this study is that there is a trend towards using these cereals for dual purposes (22). For this reason there need to be more comprehensive studies comparing the feed value of cereal species and covering their palatability to livestock in addition to nutritive value, especially after the booting stage.

4.2. Fermentation characteristics and DMR of silages

The pH values of the silages generally ranged from 4.0 to 4.6 along with DM contents between 350 and 500 g kg^{-1}. According to Weissbach (23), these values were satisfactory to ensure effective conservation. In addition to pH, a moderately low concentration of ammonia-N indicated that all cereal silages were well preserved when ensiled at either the booting or dough stages.

A previous study suggested cereal crops at their dough stage contain about 350 g kg^{-1} DM (24). However, in the present experiment cereal crops reached the dough stage at over 400 g kg^{-1}, except for barley, which reached dough stage at 370 g kg^{-1} DM. A higher DM content of wheat silage at the dough stage (467 g kg^{-1}) was previously reported (3). The cereal species used in this experiment were originally

bred for grain production not for forage, and this may have led to the higher DM, in part. However, higher DM in cereal crops also poses a challenge when making high-DM baled silage where there is no precision chopping (25). Among the cereal species, rye had the lowest DM content at the booting stage, which was also reported by Helsel and Thomas (1). This could be a challenge when ensiling rye as it required more drying time at the booting stage especially when drying conditions were not favorable in early spring.

The differences in DM content of cereal crops ranging from 331 to 348 g kg^{-1} prior to ensiling were not large enough to affect silage fermentation at the booting stage. However, there were significant differences in DM content of cereal crops at the dough stage. For instance, barley had 373 g kg^{-1} DM, and wheat had 523 g kg^{-1} DM before ensiling at the dough stage. These differences in forage DM prior to ensiling suggested that different fermentation patterns would occur. Thus, for silages that are ensiled at the dough stage of maturity, a restricted fermentation (with a higher pH, more residual WSC, and lower LA) would be expected. However, cereal crops that were ensiled at the dough stage resulted in silages with lower LA content for all cereal silages, but only wheat and oat silages had a slightly higher pH value compared to pH at the booting stage. This result suggested that the restricted fermentation with a lower LA content in this experiment could produce well-fermented silages with a lower pH and ammonia-N content at the dough stage compared to ensilaging at the booting stage. This finding also showed that a higher DM content in cereal crops before ensiling requires a lower acidic environment to attain a low pH. The gas losses were lower in silages ensiled at the dough stage compared to silages ensiled at the booting stage for all cereals. The DMR was 2.3% higher in silages ensiled at the dough stage. However, these values were for cereal crops that were precision chopped before ensiling at the dough stage. Seale et al. (26) reported that the mechanical treatment of herbage prior to ensilage (chopping and mincing) produces a rapid release of fermentable nutrients, and this makes the number of lactobacilli present initially less important. Precision chopping also increases the DM density. Furthermore, Keles and Demirci (25) reported that the high-herbage DM and harsh stalks of triticale reduced the efficiency of the chopping units of the round baler, which has stationary cutting blades, and this resulted in long particle lengths. For these reasons, when making cereal silages with high DM at the dough stage, special attention must be given to fine chopping.

Under conditions where the DM of cereal crops is insufficient for the desirable silage fermentation, organic acid generally restricts the fermentation (8), and in such cases the addition of organic acid reduced the requirement for minimum DM necessary to produce well-fermented silages from 260 to 240 g kg^{-1} DM (9). In this experiment the DM of cereal crops prior to ensiling was sufficient to ensure well-preserved silages at both maturity stages, and the effect of organic acid on LA content of cereal silages was absent and did not result in silage with restricted fermentation. For these reasons the effect of organic acid on fermentation characteristics of cereal silages is low. The addition of bacteria, on the other hand, reduced pH and increased the LA content of all cereal silages, and their effect on the fermentation characteristics of cereal silages was more obvious than the effect of organic acid. The effects of organic acid or bacteria on cereal silages were similar to the results reported by Davies et al. (10), who also noted that the effects of bacteria on pH, LA, and ammonia-N content of grass silages were more pronounced than the effects of organic acid. After successful inoculation with bacteria, mean DMR was 3.5% higher than in the control. This suggested that even though the DM of cereal forage was sufficient to ensure desirable silage fermentation, the addition of bacteria would still be beneficial for cereal silages.

4.3. Protein fractions

In all the cereal silages evaluated in this study CP decreased while carbohydrate content increased when cereal silages were ensiled at the dough stage compared to the earlier booting stage. The large decline in CP content with increasing maturity found in this study is in agreement with the findings of Khorasani et al. (4) in barley, triticale, oats, and a barley–triticale mixture. The increase in crude protein associated with cell wall contents as maturity increased was also reported by Acosta et al. (27) in barley, Johnson et al. (28) in maize, and Mustafa and Seguin (29) in oat silages. Crovetto et al. (2) reported that nitrogen digestibility of wheat silages decreased between the booting and dough stages of maturity. This was also in line with our finding that in vitro DCP decreased with increasing maturity, probably because of increasing CP associated with cell wall content. Nonprotein nitrogen content of all silages decreased, while total true protein increased at the dough stage compared to the booting stage, which is in agreement with the findings of Mustafa and Seguin (29). Differences in protein (B fractions) reflected differences in the NPN values of silages and CP associated with cell wall content between the maturity stages for all cereal silages. The 10.5% decrease in DCP corresponded with a 35% increase in RUP value on average for all silages, and the greater CP associated with cell walls as maturity increased seemed related to an increase in RUP value in all cereal silages.

A higher C fraction in wheat and oat silages when they were ensiled at the booting stage mostly related to higher RUP values. Because the C fraction is defined as an unavailable and bound protein, it is not degradable in the

rumen and is not digestible in the intestine (17). However, the high RUP values of wheat and oat silages at the booting stage have also been found at the later dough stage without any reductions in DCP content compared to other cereal silages. Together with high RUP value, wheat and oat also had lower A fraction content, and hence, may possess nutritional advantages for high-yielding ruminants over other cereal silages.

Both bacterial and organic acid additives reduced the breakdown of protein at the booting and dough stages with reduced NPN values in all crops except oats. Similar effects of organic acid were also reported by Guo et al. (6) in alfalfa silage. Both additives seemed to cause a rapid drop in pH in the silage because plant proteases are more active between pH 6 and 7 than at pH 4 (30). In silages, proteolysis mostly occurs within the first 2 days of ensiling (29,30), and the more rapid the drop in pH, the less extensive the breakdown of protein. The reduced proteolysis with the additives resulted in an increase in B1 fraction for all cereal silages. The effect of organic acid on increasing B1 fraction was more prominent with barley and triticale ensiled at the dough stage than other treatments. Similarly, Guo et al. (6) reported the addition of organic acid increased the B1 and B3 fraction content of alfalfa silage. The increase in B1 fraction and decrease in A fraction with the addition of silage additives suggests that additives can increase the true protein; considering silages have more NPN than dried forages (31), this could pose a nutritional advantage for ruminant nutrition.

In conclusion, the fermentation profile of silages suggests that all cereal crops can be harvested at either the booting or dough stages for high-quality silages, but delaying harvest until the dough stage is more promising for the production of well-fermented silages with high DMR. High DM content at dough stage does not prevent the attainment of well-fermented cereal silages when they are chopped effectively. Bacterial inoculants can improve fermentation of all cereal silages and increase DMR at both maturity stages but especially at the dough stage. Organic acid can also be applied to produce well-fermented cereal silages, but it is less effective than bacteria at both examined stages. Delaying the harvest time from boot to dough stage could increase the true protein content and RUP value of barley, wheat, rye, triticale, and oats. Both bacterial and chemical additives evaluated can increase the true protein content of all the cereal crop silages.

Acknowledgment
We would like to acknowledge the financial support of Selçuk University (project: BAP 11401094).

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