Effect of *Lactobacillus buchneri* 40788 and Buffered Propionic Acid on Preservation and Nutritive Value of Alfalfa and Timothy High-moisture Hay

J. Baah[1], T. A. McAllister[1,*], L. Bos[2], F. Van Herk[1] and R. C. Charley[3]

[1]Lethbridge Research Centre Agriculture and Agri-Food Canada. Lethbridge. AB. T1H 4B1. Canada

ABSTRACT : The effects of *Lactobacillus buchneri* 40788 and buffered propionic acid on preservation, intake and digestibility of alfalfa (*Medicago sativa*) and timothy (*Phleum pratense*) hay were investigated. During baling, forages were treated with *L. buchneri* 40788 (1.2×10^9 CFU/g) as a liquid (LLB) or as a granular preparation (GLB), with buffered propionic acid (10 mL/kg, BPA), or left untreated (control). Triplicate 500 kg round bales of each treatment were put up at two moisture levels for each forage: 17%±0.33% and 20%±0.30% for timothy and 17%±0.20% and 19%±0.27% for alfalfa (mean±SD). Bales were sampled for chemical and microbiological analyses after 0, 30 and 60 d of storage. Compared to controls, all preservatives reduced ($p<0.05$) heating of both forages at all moisture levels with the exception of alfalfa baled at 19% moisture. After 60 d of storage, GLB reduced ($p<0.05$) moulds in 17% timothy hay as compared to other treatments, but at 20% moisture, moulds were reduced in LLB- and BPA-treated timothy as compared to controls. In alfalfa at 17% moisture, total bacteria were lower ($p<0.05$) in GLB-treated bales than LLB or control bales, but yeast and total bacteria were only reduced in BPA-treated alfalfa at 19% moisture. *In situ* DM disappearance of timothy (both moisture levels) and alfalfa (19% moisture level) increased ($p<0.05$) with LLB treatment compared to control. Digestibility of both forages did not differ ($p>0.05$) among treatments, however, voluntary DM intake of LLB-treated timothy (1.32 kg/d) was 22.3% higher ($p<0.05$) than control, and 14.1% higher than BPA-treated timothy. Treating timothy and alfalfa hay with *L. buchneri* 40788 or buffered propionic acid may improve the nutritive value of the hay when baled at 17 to 20% moisture. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 5 : 649-660)***

Key Words : Alfalfa, Hay, *Lactobacillus buchneri*, Nutritive Value, Timothy

INTRODUCTION

Rapid wilting of forage to an optimum moisture level in the field before baling is essential in order to reduce field and storage dry matter (DM) losses. However, due to unpredictable field conditions, optimum moisture levels are seldom attained, and as a result significant losses in DM and quality of hay occur both in the field and during storage (Ball et al., 1996). Leaf separation from stems is a major cause of DM loss in hay during baling and any significant leaf separation from stems invariably leads to significant losses in protein and mineral content of the final product. Baling at higher moisture levels tends to reduce the extent of leaf separation (Nelson et al., 1989). However, at such high moisture levels, biochemical changes brought about by plant cell respiration and microbial growth can also contribute to a reduction in forage quality. Furthermore, the preservation of hay in large bales, although convenient, reduces aeration and the rate of internal moisture escape, forming an environment conducive to microbial growth. Dry matter losses, reduced nutrient quality, and increased dust during storage are the direct result of the microbial spoilage of hay (Wittenberg, 1991).

Organic acids have proven to be effective in reducing the growth of yeasts, moulds and bacteria in high-moisture hay, but producers are reluctant to use these products because of corrosive damage to equipment and increased preservation cost (Wittenberg, 1999). As a result, producers are increasingly using additives such as buffered organic acids, that are safer to handle.

Lactobacillus buchneri 40788® is a heterolactic bacterium capable of producing lactic and acetic acid and has been shown to improve the aerobic stability of barley and corn silage (Ranjit and Kung, 2000; Kung and Ranjit, 2001; Ranjit et al., 2002). Most studies on the effect of microbial inoculants on forage preservation have involved silage or haylage with little data on the effects of these types of additives on the quality or chemical composition of moist, uncovered hay (Rotz et al., 1988; Tomes et al., 1990; Wittenberg and Moshtaghi-Nia, 1990; Rotz et al., 1992).

The objectives of the current study were to determine the effect of *L. buchneri* 40788® and a buffered propionic acid preparation on changes in (1) nutrient composition, (2) microbiological profiles, (3) aerobic stability, (4) *in situ* disappearance of DM, and (5) voluntary intake and digestibility of timothy and alfalfa hay baled above optimum moisture levels.

* Corresponding Author: T. A. McAllister. Agriculture and Agri-Food Canada, Research Centre, P.O. Box 3000, Lethbridge, Alberta, T1J 4B1, Canada. Tel: +1-403-317-2240, Fax: +1-403-382-3156, E-mail: mcallister@agr.gc.ca

[2] Wageningen University, Department of Animal Sciences, Marykeweg 40, Wageningen, The Netherlands.

[3] Lallemand Animal Nutrition Canada, Niagara-on-the-Lake, Ontario, Canada.

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MATERIALS AND METHODS

Forages and baling conditions

Separate fields of first-cut alfalfa (*Medicago sativa* var. AC Beaver) and timothy (*Phleum pratense* var. Climax) in mid-bloom were cut on separate days with a discbine mower-conditioner and wilted on the field for 42 to 48 h prior to baling. The alfalfa and timothy fields were 32 ha and 64 ha, respectively. The entire field was cut and allowed to wilt to the desired moisture ranges. Alfalfa and timothy were each baled at two moisture levels. Mean moisture levels for alfalfa were 17%±0.2% SD and 19%± 0.3% SD, and those for timothy bales were 17%±0.3% SD and 20%±0.3% SD. Moisture content of the forages was estimated in the field with a Dani Portable Hay Moisture Tester (Dani Farm Supply, Red Deer, AB, Canada) and later confirmed by oven DM in the laboratory. Once the forages had attained the desired moisture levels, three windrows were selected randomly from the field and within each windrow, 500 kg large round bales were made consecutively for each preservative and moisture level to prevent the confounding effect of treatment and field variation. The selected windrows served as replicates, and triplicate bales of each forage were put up for each preservative and moisture level.

The forages were treated with one of the following preservatives just prior to pickup by the baler: 1) 400 g of Biotal Buchneri® (Biotal Canada, Ltd., Niagara-on-the-Lake, ON, Canada) dissolved in 50 L of water and applied on the windrow at a rate that yielded 1.2 million cfu of *L. buchneri* 40788® per g of fresh forage (LLB), 2) a granular preparation of *L. buchneri* 40788® applied directly to the window at a rate that yielded 1.2 million cfu/g of fresh forage (GLB), 3) a buffered propionic acid hay preservative (BPA) manufactured by American Farm Products Inc. (Ypsilanti, MI, USA), applied at a rate of 10 mL/kg of fresh forage, and 4) no preservative (control). The two liquid additives were applied with a three-nozzle sprayer mounted on the pickup of the baler, and the granular additive was applied using a Jumbo Gandy applicator (Owatonna, MN, USA) also mounted on the pickup. The nozzles and applicator openings were selected to ensure delivery of the recommended amounts of preservatives to the forage. Weather conditions during baling and application of treatments were dry, hot (i.e., 39°C day time high), and windy. There was no precipitation during this period. All treatments of the same forage type were put up the same day, however, timothy bales were put up 2 d after the alfalfa bales.

Both forms of *L. buchneri* 40788® preparations were confirmed by standard plating technique to contain 4.95×10^{10} cfu/g of *L. buchneri* 40788®, as well as 5,250 IU/g of β-glucanase, 2,850 IU/g of xylanase, 2,625 IU/g of amylase, and 480 IU/g of galactomannase (McAllister et al., 1998). The BPA contained active ingredients (wt/wt) propionic acid (0.56), ammonium hydroxide (0.30) and acetic acid (0.14). Each bale was tagged immediately after production and gathered from the field onto a flat-bed truck. All bales were removed from the field within 24 h of production and unloaded randomly into storage. Timothy bales were stored in a single layer in a well ventilated rectangular barn, and alfalfa bales were stored under a pole shed, also in a single layer. All bales were stored for a minimum of 60 d.

Chemical composition, microbiology and aerobic stability studies

Core samples for chemical and microbiological analyses were obtained from six different locations on each bale immediately (i.e., within 30 min) after baling (d 0) and after 30 and 60 d of storage. Procedures outlined by McAllister et al. (1998) were used for enumerating total bacteria, lactic acid producing bacteria (LAB), yeasts and moulds, and for determining concentrations of water soluble carbohydrates (WSC) and ammonia. Dry matter (DM), organic matter (OM), and crude protein (CP) were determined according to AOAC (1990) procedures, and neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent insoluble nitrogen (ADIN) as described by Van Soest et al., (1991). Interior temperature of bales was monitored continuously using Type T temperature probes inserted in each bale and connected to an AM25T multiplexer linked to a CR7X data logger (Campbell Scientific, Logan, UT, USA) for 35 d in alfalfa and 24 d in timothy.

In situ dry matter disappearance

Subsamples of the d 60 core samples from the three bales of each moisture level × preservative combination were composited for each forage type. The rate and extent of *in situ* DM disappearance of the samples was determined using two ruminally cannulated Jersey cows fed a diet of (DM basis) of timothy hay (0.50) and barley silage (0.50). The cows were housed in individual pens and given *ad libitum* access to feed and water. Duplicate nylon bags containing approximately 5 g of sample DM (alfalfa or timothy) were incubated in the rumen of each cow for 2, 4, 8, 12, 24, 48 and 72 h. The bags were 5 cm × 20 cm and constructed of monofilament polyester mesh with a 53-μm pore size (Ankom, Fairport, NY, USA). The cows were adapted to their diet for 2 weeks before the incubations were begun. Upon withdrawal from the rumen, the bags were rinsed immediately under cold tap water until all ruminal content on the outside of the bags was been removed, then transferred to a household automatic washing machine and washed in cold water for three 10 min washing cycles (delicate agitation; no spin). All bags were dried in a forced-air oven at 55°C for 48 h. Duplicate bags of each

Table 1. Mean chemical composition of core samples taken from bales of timothy and alfalfa hay immediately after baling

Composition	Forage type and moisture concentration immediately after baling							
	Timothy				Alfalfa			
	17% moisture	SD	20% moisture	SD	17% moisture	SD	19% moisture	SD
Dry matter (g/kg)	834[a]	3.0	803[b]	3.0	830[a]	2.0	809[b]	3.0
Organic matter (g/kg DM)	947	1.0	945	1.0	892	1.0	894	1.0
NDF (g/kg DM)	699	7.0	696	7.0	404	2.0	425	2.0
ADF (g/kg DM)	418	7.0	412	6.0	311	7.0	313	5.0
Crude protein (g/kg DM)	81	3.0	82	3.0	233	4.0	234	3.0
ADIN (% of total N)	5.9	0.37	6.8	0.36	4.1	0.19	4.0	0.13
Ammonia (mmol/L)	0.67	0.082	0.78	0.074	0.58	0.051	0.58	0.034
WSC (mg/g of forage)[1]	37.7	3.13	37.7	2.82	13.1	1.36	17.4	1.85

[1] WSC: water soluble carbohydrates.

[a, b] Within a row and forage type, values followed by different letters differ ($p<0.05$).

treatment that had not been ruminally incubated were washed alongside the bags above and used to estimate 0 h DM disappearance. Disappearance of DM was calculated from the proportion of DM loaded initially that was remaining in the bag after each incubation interval. The DM disappearance data were fitted to a modified version of the exponential model of Ørskov and McDonald (1979) with a lag phase:

$$p = a+b\ (1\text{-}e^{-c(t-lag)}) \text{ for } t>\text{lag}$$

where p is the proportional DM disappearance as at time t (in hours), a is the rapidly disappearing fraction, b is the slowly disappearing fraction, and c is the fractional rate of disappearance (/h) of fraction b. Variables a, b, c, and *lag* were estimated by an iterative nonlinear procedure (Marquardt method) with the SAS (1990) software package. Effective disappearance of DM was estimated on the basis of an assumed fractional outflow rate of 0.06/h.

Voluntary intake and digestibility

The effect of the preservatives on nutrient intake and digestibility were evaluated in a feeding trial involving twelve Canadian Arcott wethers (initial body weight 70.7kg±6.3 kg; mean±SD). Single bales were selected from each of the control, LLB- and GPA-treated timothy baled at 20% moisture and alfalfa baled at 19% moisture for use in the feeding trial. The selected bales were chopped on the same day and stored in separate piles in the same shed. Forage quantity was sufficient for the entire experiment. Timothy and alfalfa were evaluated concurrently using six wethers in a replicated randomized complete block design with two wethers per treatment and three 28-d periods.

The wethers were housed individually on floor pens during the initial 21 d of each period and given *ad libitum* access to water and their respective hay diets, as well as a daily allotment of 182 g (DM basis) of a pelleted concentrate, as described by McAllister et al. (1995). The first 14 d of each period were dedicated to adaptation to diet, after which voluntary feed intake was determined over 6 d. The wethers were transferred to individual digestibility crates, allowed 2 d to adjust to the novel environment, and then offered the experimental forages at 0.90 of their recorded *ad libitum* intake. Feed consumption and total faecal output were recorded during the next 6 d. Feed and orts from d 23 to d 27 were composited for each period and animal, and frozen for later analysis. Each day, total faecal collection for each wether was mixed thoroughly, and a subsample (0.10) was transferred to frozen storage. Orts, feeds, and faecal samples for were pooled for each wether and period, and subsampled for analysis of DM, OM, NDF, ADF and nitrogen as described above.

All of the wethers used in this study were cared for according to the standards set by the CCAC (1993).

Statistical analyses

All data were analysed using the General Linear Models procedure (PROC GLM) of SAS (1990) to test for significant differences among least square means. Chemical and microbiological data for each forage type, moisture level, and day of sampling were analysed separately using the following model:

$$Y_{ij} = \mu+\alpha_i+\beta_j+e_{ij}$$

where Y_{ij} is an observation, μ is the overall mean; α_i is the effect of preservative (control, LLB, GLB, or BPA); β_j is replication or bale, and e_{ij} is the residual error. Microbiological data were first transformed to $\log_{10}$ before performing statistical analysis on the log transformed data. *In situ* disappearance data of each forage and moisture level were analysed separately using the following model:

$$Y_{ijk} = \mu+\alpha_i+\beta_j+\gamma_k+e_{ijk}$$

where Y_{ijk} is an observation, μ is the overall mean; α_i is the effect of preservative (control, LLB, GLB, or BPA); β_j is cow (1 or 2), γ_k is the k^{th} nylon bag in the j^{th} cow, and e_{ijk} is the residual error.

Table 2. Effect of moisture content at baling and preservative on chemical composition of timothy hay stored for 60 days

Composition	Moisture content and preservative added at baling[1]									
	17% moisture					20% moisture				
	Control	LLB	GLB	BPA	SEM	Control	LLB	GLB	BPA	SEM
Dry matter (g/kg)	899	908	901	900	4.0	901	903	908	885	1.0
Organic matter (g/kg DM)	939	938	941	941	2.0	940	938	942	939	1.0
NDF (g/kg DM)	729	689	735	697	1.0	758^{a}	744^{a}	728^{a}	695^{b}	1.0
ADF (g/kg DM)	436	442	445	431	1.0	454^{a}	447^{ab}	426^{bc}	420^{c}	1.0
Crude protein (g/kg DM)	95	100	10	10	1.0	98	108	98	97	5.0
ADIN (% of total N)	11.3	10.9	11.2	10.3	1.85	14.7	10.3	11.1	9.9	1.41
Ammonia (mmol/L)	0.3^{b}	0.3^{b}	0.3^{b}	1.9^{a}	0.16	0.2^{b}	0.2^{b}	0.4^{b}	2.5^{a}	0.19
WSC (mg/g of forage)[2]	56.3	58.5	56.6	67.7	6.74	38.7^{c}	58.9^{ab}	45.5^{bc}	64.2^{a}	6.23

[1] Control, no additive applied; LLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a liquid formulation; GLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a granular formulation; BPA, buffered propionic acid applied at a rate of 10 mL/kg of hay. All preservatives were applied immediately prior to baling.

[2] WSC: water soluble carbohydrates.

[a-c] Within a row and moisture content, values followed by different letters differ ($p<0.05$).

Table 3. Effect of moisture content at baling and preservative on chemical composition of alfalfa hay stored for 60 days

Composition	Moisture content and preservative[1]									
	17% moisture					19% moisture				
	Control	LLB	GLB	BPA	SEM	Control	LLB	GLB	BPA	SEM
Dry matter (g/kg)	921	912	917	896	17.0	893	888	870	879	11.0
Organic matter (g/kg DM)	886	891	883	885	7.0	888	892	891	889	3.0
NDF (g/kg DM)	446	424	442	401	23.0	438	419	412	414	16.0
ADF (g/kg DM)	353^{a}	318^{b}	362^{a}	315^{b}	8.0	308	319	310	320	15.0
Crude protein (g/kg DM)	199	219	211	232	14.0	225	226	238	225	7.0
ADIN (% of total N)	8.3	8.0	9.7	7.1	1.15	7.7	7.6	5.8	6.9	0.61
Ammonia (mmol/L)	0.6^{b}	0.5^{b}	0.5^{b}	1.6^{a}	0.20	1.4^{ab}	0.9^{b}	0.9^{b}	1.8^{a}	0.29
WSC (mg/g of forage)[2]	31.5^{a}	20.7^{b}	28.2^{a}	16.9^{b}	1.32	17.8	20.0	19.1	22.0	1.98

[1] Control, no additive applied; LLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a liquid formulation; GLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a granular formulation; BPA, buffered propionic acid applied at a rate of 10 mL/kg of hay. All preservatives were applied immediately prior to baling.

[2] WSC: water soluble carbohydrates.

[a, b] Within a row and moisture content, values followed by different letters differ ($p<0.05$).

Voluntary intake and digestibility data for each forage type were analysed separately. The model used in each analysis was:

$$Y_{ijk} = \mu+\alpha_i+\beta_j+\gamma_k+e_{ijk};$$

where Y_{ijk} is the observation, μ is the overall mean; α_i is the effect of preservative, β_j is the effect of period, γ_k is the effect of sheep, and e_{ijk} is the overall error. Treatment least square means were deemed significantly different when the probability of the difference (PDIFF) between the least square means was less than 0.05.

RESULTS

Chemical composition

Within forage group, there were no differences ($p>0.05$) in chemical composition (except DM) of bales sampled immediately after baling (Table 1). Similarly, other than the higher ammonia concentration in BPA-treated bales, there were no differences ($p>0.05$) in chemical composition between forage samples collected on d 0 (immediately after baling) and d 30. Therefore, only the chemical composition of the forage samples collected immediately after baling and those collected on day 60 are reported, as they reflect the composition of the starting material and final product (Table 2).

Timothy hay : After 60 d in storage, concentrations of WSC and ammonia were higher in BPA-treated bales at 20% moisture than in control and GLB-treated bales (Table 2). The ammonia concentration in 20% moisture BPA-treated bales was also higher ($p<0.05$) than in LLB-treated bales. However, within moisture group, NDF and ADF concentrations were lower in BPA-treated bales as compared with control bales. The elevated level of ammonia in BPA-treated bales was most likely the result of the ammonium hydroxide in the BPA preparation.

Alfalfa hay : Similar to the observation made in timothy hay, BPA-treated alfalfa bales had higher ($p<0.05$) ammonia concentrations on d 60 than did compared to LLB- and GLB-treated alfalfa bales (Table 3). However, the ammonia concentrations in control (1.4 mmol/L) and BPA-treated

Table 4. Effect of moisture content at baling and preservative on microbial numbers recovered from timothy hay during storage

Sample	Population[2]	Moisture content and preservative[1]									
		17% moisture					20% moisture				
		Control	LLB	GLB	BPA	SEM	Control	LLB	GLB	BPA	SEM
Day 0	Lactobacilli	0.92	2.44	1.13	1.24	0.494	0.40^{bc}	0.95^{b}	3.10^{a}	0.00c	0.270
	Yeasts	5.27^{b}	5.79^{a}	4.95^{c}	5.17^{bc}	0.124	5.14	5.14	5.00	4.74	0.176
	Moulds	3.67^{a}	4.12^{a}	3.62^{a}	2.10^{b}	0.449	3.49	3.50	3.66	3.18	0.181
	Total bacteria	6.23	6.72	6.36	6.44	0.171	6.34^{ab}	6.63^{a}	6.52^{a}	6.09^{b}	0.134
Day 30	Lactobacilli	0.32^{a}	0.00	0.00	0.00	0.103	0.27^{b}	0.37^{b}	1.65^{a}	0.08^{b}	0.156
	Yeasts	4.99^{a}	4.24^{b}	5.25^{a}	4.81^{ab}	0.260	4.90^{b}	4.95^{b}	5.67^{a}	4.79^{b}	0.243
	Moulds	3.38^{a}	3.32^{a}	2.83^{b}	2.78^{b}	0.165	2.30	2.12	3.11	1.45	0.497
	Total bacteria	5.25^{c}	6.75^{a}	5.79^{b}	6.10^{b}	0.221	5.75	5.96	6.31	5.59	0.236
Day 60	Lactobacilli	0.20	0.00	0.00	0.39	0.144	0.45^{a}	0.00^{b}	0.54^{a}	0.00^{b}	0.156
	Yeasts	3.53	4.56	5.37	5.20	0.602	3.49^{b}	5.36^{a}	4.97^{a}	3.86^{b}	0.489
	Moulds	3.08^{a}	3.30^{a}	0.94^{b}	2.01^{ab}	0.680	3.51^{a}	0.00^{b}	2.91^{a}	0.78^{b}	0.324
	Total bacteria	6.38	6.28	6.12	5.99	0.173	6.25^{a}	6.09^{a}	6.66^{a}	5.24^{b}	0.266

[1] Control, no additive applied; LLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a liquid formulation; GLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a granular formulation; BPA, buffered propionic acid applied at a rate of 10 mL/kg of hay. All preservatives were applied immediately prior to baling.

[2] Populations (expressed as log cfu/g) were enumerated after 24 h at 25°C on MRS agar with cotton blue, tartaric acid and cycloheximide (lactobacilli), on Sabouraud's dextrose agar with tetracycline and chloramphenicol (yeasts and moulds), or on nutrient agar with cycloheximide (total bacteria).

[a-c] Within a row and moisture group, values followed by different letters differ ($p<0.05$).

Table 5. Effect of moisture content at baling and preservative on microbial numbers recovered from alfalfa hay during storage

Sample	Population[2]	Moisture content and preservative[1]									
		17% moisture					19% moisture				
		Control	LLB	GLB	BPA	SEM	Control	LLB	GLB	BPA	SEM
Day 0	Lactobacilli	3.42^{a}	3.76^{a}	3.25^{ab}	2.70^{b}	0.260	3.69^{a}	3.85^{a}	3.19^{b}	2.82^{b}	0.144
	Yeasts	5.13^{a}	4.86^{b}	5.51^{a}	5.13^{ab}	0.169	5.01	5.37	5.4^{8}	5.30	0.128
	Moulds	1.36	2.03	1.96	0.92	0.576	1.04^{b}	1.78^{ab}	2.27^{a}	0.97^{b}	0.346
	Total bacteria	6.32	6.05	5.91	5.74	0.175	6.01^{a}	6.26^{ab}	5.86^{b}	5.79^{b}	0.115
Day 30	Lactobacilli	1.46	0.38	0.37	2.00	0.311	3.59^{a}	3.85^{a}	3.19^{b}	2.82^{b}	0.144
	Yeasts	4.82	4.55	3.90	4.63	0.538	5.01	5.37	5.48	5.30	0.128
	Moulds	0.00^{c}	2.21^{a}	1.11^{b}	1.22^{ab}	0.488	1.04^{b}	1.78^{ab}	2.27^{a}	0.99^{b}	0.346
	Total bacteria	5.21^{a}	5.09^{a}	3.37^{b}	5.13^{a}	0.732	6.01^{ab}	6.26^{a}	5.86^{b}	5.79^{b}	0.115
Day 60	Lactobacilli	1.70	1.58	1.24	1.40	0.198	1.59	1.54	1.10	1.27	0.316
	Yeasts	4.93	5.10	4.85	4.72	0.102	5.09^{a}	5.30^{a}	5.04^{ab}	4.70^{b}	0.143
	Moulds	1.67	2.16	2.28	1.92	0.217	1.57	1.71	2.54	2.07	0.358
	Total bacteria	5.70^{ab}	5.77^{a}	5.34^{c}	5.44^{bc}	0.119	5.67^{a}	5.90^{a}	5.73^{a}	5.39^{b}	0.131

[1] Control, no additive applied; LLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a liquid formulation; GLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a granular formulation; BPA, buffered propionic acid applied at a rate of 10 mL/kg of hay. All preservatives were applied immediately prior to baling.

[2] Populations (expressed as log cfu/g) were enumerated after 24 h at 25°C on MRS agar with cotton blue, tartaric acid and cycloheximide (lactobacilli), on Sabouraud's dextrose agar with tetracycline and chloramphenicol (yeasts and moulds), or on nutrient agar with cycloheximide (total bacteria).

[a-c] Within a row and moisture group, values followed by different letters differ ($p<0.05$).

(1.8 mmol/L) bales were similar. At 17% moisture, WSC concentrations were lower in LLB- and GLB-treated bales than in the control, but this relationship was not observed in alfalfa at 19% moisture. After 60 d of storage, concentrations of ADF in control and GLB-treated bales at 17% moisture were higher ($p<0.05$) than in LLB- and BPA-treated bales (Table 3).

Microbial populations

Timothy hay : Populations of LAB and total bacteria at d 0 in timothy hay baled at 17% moisture did not differ ($p>0.05$) among treatments (Table 4). When baled at 20% moisture however, the d 0 numbers of LAB and total bacteria were higher ($p<0.05$) in GLB-treated timothy than in BPA-treated timothy. The d 0 yeast populations were greater ($p<0.05$) in LLB-treated timothy at 17% moisture as compared with other treatments. In this same forage, the moulds were less numerous ($p<0.05$) with application of BPA than with the other treatments. The GLB-treated timothy had the greatest d 0 LAB population ($\log_{10}$ 3.10 cfu/g) among the bales put up at 20% moisture. Populations of LAB had decreased to <10 cfu/g in all samples by d 30, with the exception of GLB-treated bales. The control and LLB-treated 17% moisture bales yielded the highest or nearly highest counts of moulds on all sampling days. Among the 17% moisture bales, the greatest number of

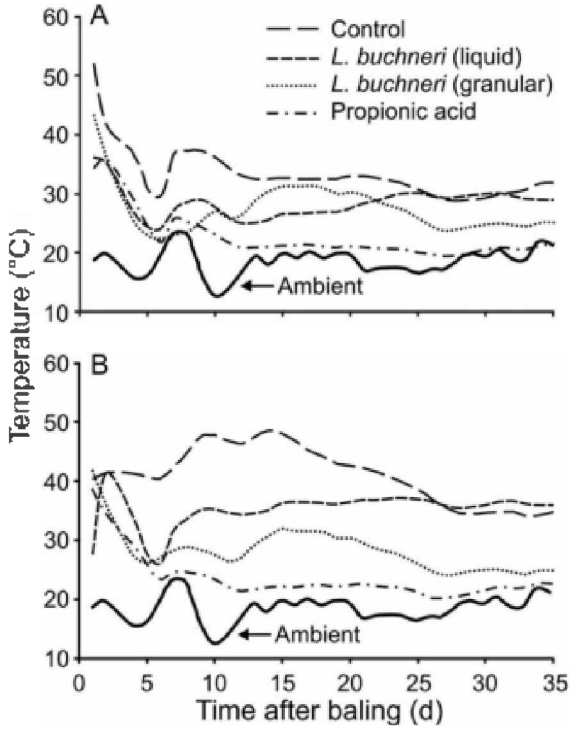

Figure 1. Effect of preservative added immediately prior to baling on internal temperature of 500 kg round bales of timothy hay baled at 17% moisture (A) or at 20% moisture (B) during 35 d of storage in single layer in a well-ventilated rectangular barn. Control bales had no preservative applied. *Lactobacillus buchneri* 40788 preservative (liquid or granular) was applied at a rate of 1.2×10^5 cfu/g of fresh forage. Buffered propionic acid was applied at a rate of 10 mL/kg of fresh forage.

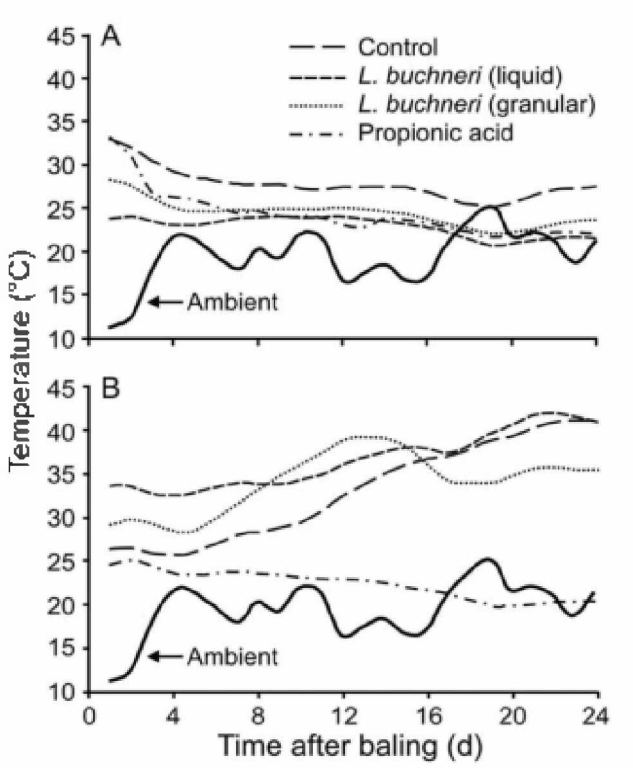

Figure 2. Effect of preservative added immediately prior to baling on internal temperature of 500 kg round bales of alfalfa hay baled at 17% (A) or at 19% moisture (B) during 35 d of storage in single layer under a pole shed. Control bales had no preservative applied. *Lactobacillus buchneri* 40788 preservative (liquid or granular) was applied at a rate of 1.2×10^5 cfu/g of fresh forage. Buffered propionic acid was applied at a rate of 10 mL/kg of fresh forage.

yeast colonies recovered from samples taken on d 0 ($\log_{10}$ 5.79 cfu) was from LLB-treated bales. The yeast population at d 30 was lower ($p<0.05$) in LLB-treated bales put up at 17% moisture than in control or GLB-treated bales. In d-30 samples from bales put up at 20% moisture, however, yeast numbers were higher ($p<0.05$) in those treated with GLB ($\log_{10}$ 5.67 cfu/g) than in all other treatments in that moisture group. Neither *L. buchneri* 40788® preparation reduced yeast populations in d 60 samples, relative to controls. However, LLB was effective in eliminating moulds in bales at 20% moisture (as seen from d 60 samples), but not in bales at 17% moisture. The BPA preparation was also effective in reducing the population of moulds in bales at 20% moisture. Within the 17% moisture group, only GLB was effective in reducing mould counts. After 60 d of storage, total bacterial populations did not differ among bales at 17% moisture, but among bales at 20% moisture, they were lower ($p<0.05$) in BPA-treated bales than in all other treatments.

Alfalfa hay : Other than on d 0, LAB numbers in any sample did not exceed 100 cfu/g (Table 5). The LAB populations in all d 60 samples from both moisture groups were <100 cfu/g. In comparison to the control, none of the preservatives was effective in eliminating yeasts and moulds after 60 d of storage of hay baled at 17% moisture. At 19% moisture, however, the BPA- and GLB treatments reduced ($p<0.05$) the population of yeasts compared to control and LLB treatments.

Temperature profiles

Timothy hay : Ambient temperature in the bale storage area fluctuated between 15 and 23°C during the storage period. The highest temperature (52°C) was recorded on d 1 in control bales at 17% moisture (Figure 1a). There was a steep but transient rise in mean temperature from d 6 to d 9 in all of the bales prepared at 17% moisture. During this period, the highest temperatures were again registered in control bales, whereas the lowest temperatures were recorded in BPA-treated bales. After the transient rise, the temperature in BPA-treated bales stabilized on d 6, and did not exceed 22°C thereafter. In contrast, mean temperature in GLB-treated bales rose sharply again, from 26°C on d 12 to approximately 32°C on d 15. It remained at approximately 30°C for the next 7 d, then stabilized at approximately 25°C.

The temperature in LLB-treated bales also rose steadily from d 12 until d 28, reaching 28°C at the end of the monitoring period.

In LLB-treated bales prepared at 20% moisture, mean daily temperatures increased sharply from approximately 27°C on d 1 to 42°C on d 2 (Figure 1b). Temperatures in these bales then fell to their lowest level of 26°C on d 6, after which they increased to finish at approximately 36°C on d 35. As was observed in the 17% moisture bales, the BPA-treated bales prepared at 20% moisture exhibited lower mean daily temperatures over the storage period than did the control bales. Mean daily temperatures of GLB-treated bales were also notably lower than LLB-treated bales during the storage period. None of the temperatures achieved was indicative of severe heating as a result of microbial decomposition.

Alfalfa hay : Average ambient temperature was at its lowest (12°C) on d 1 and fluctuated between 22°C and 17°C from d 5 to d 16. Among the alfalfa bales put up at 17% moisture, mean daily temperature was higher in the control bales than in all other treatments over the entire storage period (Figure 2a). The temperatures in LLB-, GLB- and BPA-treated bales were consistently lower than in the controls, with LLB-treated bales exhibiting the most consistent temperature during the storage period. Average temperature in LLB bales was about 23°C during the first 10 d of storage and did not vary by more than 2.5°C during the entire monitoring period. Among the bales at 19% moisture, temperature declined steadily during the storage period in the BPA-treated bales only, from a high of 25°C on d 2 to 22°C on d 24. The temperature in all other bales increased from d 1 onward and had not stabilized by d 24 (Figure 2b). The temperature in control bales at 19% moisture had climbed from 27°C on d 1 to 42°C by d 24.

In sacco dry matter disappearance

Timothy hay : Within the 17% moisture group, the most rapid ($p<0.05$) DM disappearance (0.081/h) was observed in the bales treated with LLB, whereas the lowest ($p<0.05$) rate (0.061/h) was observed in GLB-treated bales. The rates for control and BPA-treated bales were similar ($p>0.05$) and intermediate (Table 6). Among bales at 20% moisture, however, the rates of DM disappearance from GLB-treated and LLB-treated bales were similar ($p>0.05$), and higher ($p<0.05$) than control or treatment BPA ($p<0.05$).

The longest ($p<0.05$) lag periods in the 17% moisture group were recorded with LLB-treated and control bales (3.1 h), and the shortest (1.6 h; $p<0.05$) was with BPA-treated bales. In addition, treatments GLB and BPA increased ($p<0.05$) the potentially digestible DM fraction relative to the control and LLB-treated bales. With the 20% moisture bales, however, the lag period for control hay (3.0 h) was shortest ($p<0.05$) of the four treatments (LLB>GLB and BPA; $p<0.05$), and all three preservatives increased ($p<0.05$) the potentially digestible fraction compared with control. In the 17% moisture samples, the proportion of rapidly soluble DM was higher ($p<0.05$) with treatment LLB than with control, but was decreased ($p<0.05$) by treatment GLB whereas in the 20% moisture group, the rapidly soluble fraction was lowest ($p<0.05$) in the control.

Table 6. Effect of preservative on *in situ* DM digestion kinetics of timothy hay baled at two moisture levels and incubated ruminally in Jersey cows

Moisture content and parameter[2]	Preservative[1]				
	Control	LLB	GLB	BPA	SEM
17% moisture					
Rapidly soluble fraction (%)	22.8^{b}	23.3^{a}	22.4^{c}	22.6^{bc}	0.11
Slowly disappearing fraction (%)	39.2^{b}	38.8^{b}	41.0^{a}	41.4^{a}	0.21
Fractional rate of disappearance (/h)	0.070^{b}	0.081^{a}	0.061^{c}	0.069^{b}	0.002
Potentially digestible fraction (%)	62.0^{b}	62.1^{b}	63.4^{a}	64.0^{a}	0.32
Lag (h)	3.1^{a}	3.1^{a}	2.3^{b}	1.6^{c}	0.11
Effective disappearance (%)	40.3	41.8	40.5	41.5	0.92
20% moisture					
Rapidly soluble fraction (%)	22.1^{c}	23.7^{ab}	23.8^{a}	23.4^{b}	0.11
Slowly disappearing fraction (%)	40.0^{a}	39.3^{b}	40.3^{a}	40.1^{a}	0.21
Fractional rate of disappearance (/h)	0.075^{b}	0.083^{a}	0.081^{a}	0.069^{c}	0.002
Potentially digestible fraction (%)	62.1^{b}	64.0^{a}	64.1^{a}	63.5^{a}	0.32
Lag (h)	3.0^{c}	4.0^{a}	3.3^{b}	3.3^{b}	0.11
Effective disappearance (%)	40.7	41.6	42.7	41.0	0.92

[1] Control, no additive applied; LLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a liquid formulation; GLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a granular formulation; BPA, buffered propionic acid applied at a rate of 10 mL/kg of hay. All preservatives were applied immediately prior to baling.

[2] Parameters were calculated from the fitted equation: $p=a+b[1-e^{-c(t-lag)}]$ for $t>lag$; where p is the proportion (%) of DM disappearing from nylon bags after t hours of incubation; a is the rapidly soluble fraction (%); b is the slowly disappearing fraction (%); c is the fractional rate of disappearance (/h) of fraction b; and lag (h) is the interval before commencement of digestion.

[a-c] Within a row, means followed by different letters differ ($p<0.05$).

Table 7. Effect of preservative on *in situ* DM digestion kinetics of alfalfa hay baled at two moisture levels and incubated ruminally in Jersey cows

Moisture content and parameter[2]	Preservative[1]				
	Control	LLB	GLB	BPA	SEM
17% moisture					
Rapidly soluble fraction (%)	33.9^{c}	35.9^{a}	33.7^{c}	34.5^{b}	0.24
Slowly disappearing fraction (%)	41.2^{b}	39.7^{c}	42.4^{a}	41.3^{b}	0.31
Fractional rate of disappearance (/h)	0.106	0.107	0.108	0.103	0.006
Potentially digestible fraction (%)	75.1	75.6	76.1	75.8	0.55
Lag (h)	1.2^{b}	1.4^{a}	0.9^{c}	0.7^{d}	0.07
Effective disappearance (%)	58.0	58.8	58.6	58.8	1.78
19% moisture					
Rapidly soluble fraction (%)	34.6^{c}	35.6^{a}	32.0^{d}	35.1^{b}	0.24
Slowly disappearing fraction (%)	43.3^{b}	40.7^{c}	45.4^{a}	41.5^{c}	0.31
Fractional rate of disappearance (/h)	0.088^{b}	0.118^{a}	0.094^{b}	0.113^{a}	0.006
Potentially digestible fraction (%)	77.9	76.3	77.4	76.6	0.55
Lag (h)	0.9^{d}	1.6^{a}	1.2^{c}	1.4^{b}	0.07
Effective disappearance (%)	58.0	59.7	57.0	57.8	1.78

[1] Control, no additive applied; LLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a liquid formulation; GLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a granular formulation; BPA, buffered propionic acid applied at a rate of 10 mL/kg of hay. All preservatives were applied immediately prior to baling.

[2] Parameters were calculated from the fitted equation: $p = a-b[1\text{-}e^{-c(t-lag)}]$ for $t>lag$; where p is the proportion (%) of DM disappearing from nylon bags after t hours of incubation; a is the rapidly soluble fraction (%); b is the slowly disappearing fraction (%); c is the fractional rate of disappearance (/h) of fraction b; and lag (h) is the interval before commencement of digestion.

[a-d] Within a row, means followed by different letters differ ($p<0.05$).

Alfalfa hay : With alfalfa hay baled at 17% moisture, there were no differences ($p>0.05$) among treatments in the rate of DM disappearance, the potentially digestible fraction, or effective disappearance of DM (Table 7). The rapidly soluble fraction in samples from these bales was largest ($p<0.05$) with treatment LLB (35.9%) and smallest ($p<0.05$) in control (33.9%) and GLB-preserved bales (33.7%). Lag times for DM disappearance varied with treatment, from less than 1 h for GLB- and BPA-preserved bales to 1.2 h ($p<0.05$) in control, and 1.4 h ($p<0.05$) in LLB-preserved alfalfa hay. Samples from alfalfa bales at 19% moisture and preserved with LLB had the largest ($p<0.05$) proportion of rapidly soluble DM fraction (35.6%) and the most rapid ($p<0.05$) rate of DM disappearance (0.118/h). However, the longest ($p<0.05$) lag period was also found in LLB-preserved bales (1.6 h), whereas the shortest ($p<0.05$) lag was in control bales (0.9 h). There were no differences ($p>0.05$) in potentially digestible fractions or effective disappearances of DM among untreated or preserved alfalfa hays baled at 19% moisture.

Voluntary intake and digestibility

Timothy hay : Total voluntary DM intake by wethers (expressed as g/d or as g/kg $BW^{0.75}$; Table 8) was higher ($p<0.05$) with treatment LLB (1,506.1 g/d or 63.8 g/$kg^{0.75}$, respectively) than with control (1,264.6 g/d or 53.0 g/$kg^{0.75}$) or BPA-treated (1,342.3 g/d or 56.1 g/$kg^{0.75}$) forages. Expressed as a percentage of body weight, DM intake tended ($p=0.07$) to be higher with treatment LLB than with control or BPA (2.2% versus 1.8 and 2.0%, respectively). As expected, forage intake patterns followed those of DM intake by the wethers, with greater intake ($p<0.05$) of LLB-preserved hay (1,322.9 g/d) than of BPA-preserved (1,159.1 g/d) or control (1,081.5 g/d) hay. However, nitrogen intake and coefficients of apparent digestibility of nutrients did not differ ($p>0.1$) among treatments.

Alfalfa hay : Applying preservative (LLB or BPA) to alfalfa hay immediately prior to baling did not affect ($p>0.05$) voluntary intake or apparent digestibility of the components measured, relative to the control (untreated) hay, other than to increase ($p<0.05$) the apparent digestibility of nitrogen (Table 9). Nitrogen digestibility was greatest ($p<0.05$) in wethers consuming LLB-treated hay (78.5%), followed by the BPA treatment (77.4%), and then ($p<0.05$) by those consuming the control diet (76.3%).

DISCUSSION

The addition of preservatives to high moisture forage can prevent prolonged temperature build-up and reduce the extent of heat damage. Heating in moist hay is usually caused by biochemical changes brought about by plant cell respiration and the activities of microbes (Wittenberg, 1999). These activities are influenced by a number of factors, including the numbers and types of epiphytic microbial organisms, water activity, and chemical composition of the forage (Undi et al., 1997).

Propionic acid has been used successfully as a chemical preservative to reduce microbial activity and temperature build-up in moist agricultural products (Goering and

Table 8. Effect of preservative on chemical composition, voluntary intake and coefficients of apparent digestibility of timothy hay by sheep

Parameter	Preservative[1]			
	Control	LLB	BPA	SEM
Chemical composition				
Dry matter (g/kg)	909	914	918	-
Organic matter (g/kg DM)	943	938	942	-
Neutral detergent fibre (g/kg DM)	747	733	683	-
Acid detergent fibre (g/kg DM)	455	451	414	-
Crude protein (g/kg DM)	93	94	98	-
Water soluble carbohydrates (mg/g)	50.43	46.8	52.4	-
Voluntary intake				
Dry matter (g/d)	1,264.6^{b}	1,506.1^{a}	1,342.3^{b}	58.43
Dry matter (g/kg$^{0.75}$)	53.0^{b}	63.8^{a}	56.1^{b}	3.01
Dry matter (% of body weight)	1.8^{B}	2.2^{A}	2.0^{B}	0.11
Forage (g/d)	1,081.5^{b}	1,322.9^{a}	1,159.1^{b}	58.25
Nitrogen (g/d)	21.1	20.0	19.3	0.97
Apparent digestibility (%)				
Dry matter	55.2	56.4	58.1	1.27
Nitrogen	55.7	55.0	58.0	1.41
Neutral detergent fibre	54.6	53.9	54.9	1.35
Acid detergent fibre	45.5	45.7	48.9	1.54

[1] Control, no additive applied; LLB, *Lactobacillus buchneri* 40788 (1.2×10^{6} cfu/g of hay) applied in a liquid formulation; BPA, buffered propionic acid applied at a rate of 10 mL/kg of hay. The preservatives were applied immediately prior to baling.
[a,b] Within a row, values followed by different letters differ ($p<0.05$); [A,B] ($p=0.07$).

Gordon, 1973). As with other organic acids, a major problem with propionic acid is that losses of up to 70% through volatilization can occur during application (Lacey et al., 1980). Under field conditions, therefore, applications rates as high as 1% (wt/wt) are often required for complete control of mould growth in high moisture hay (Jafri et al., 1979). Cost and the risks to human health associated with using such high levels of acid, as well as the corrosive effects of the acid on farm machinery are among the factors that have limited the popularity of propionic acid. Buffered acid products have been introduced in an attempt to reduce these risks.

Increasingly, microbial inoculants are also being developed as alternatives to organic acids (Wittenberg, 1999). *Lactobacillus buchneri* 40788® is a heterolactic bacterium that produces lactic acid and acetic acid as by-products of fermentation (Ranjit and Kung, 2000). Both by-products have been suggested to prevent heating in silage (Dreihuis et al., 1996; Muck, 1996). The principle behind inoculating stored agricultural products with microbes is to introduce live organisms into an existing community under conditions that will enable them to out-compete and thereby dominate the epiphytic microorganisms that cause the deterioration of stored forages. Successful competition by inoculant organisms depends on the amount and type of inoculant applied and on the type and numbers of indigenous organisms.

The higher ammonia concentration detected in samples taken from BPA-treated bales is probably a reflection of the ammonium hydroxide in the preparation and not a result of increased proteolysis in the forage. Differences in total numbers of yeasts, moulds and bacteria recovered from forage samples taken on d 0 may simply be a reflection of their natural occurrence and variation on forages and not necessarily due to the treatments applied.

Although the recorded temperatures were lower in most of the treated bales than in the controls, the microbial populations were not necessarily the lowest in those bales. This indicates that neither the lower temperature recorded in BPA-treated bales, nor the relatively high temperature recorded in control bales was due solely to microbial populations. For instance, whereas the d 60 populations of yeasts, moulds and total bacteria in BPA-treated 17% moisture alfalfa and timothy bales did not differ from those in the other treatments, very little heating occurred in the BPA-treated bales compared to the other treatments. A similar observation was found in bales put up at 19% and 20% moisture, respectively. Lower temperatures in BPA-treated bales may have resulted from inhibition of both plant and microbial metabolic activities unaccompanied by changes in the number of microorganisms present.

Atwal and Heslop (1987) reported that temperatures in large, medium-moisture (23.6%) round bales of alfalfa hay preserved with 3% propionic acid peaked at 57°C whereas bales preserved with 0.3% of the acid exhibited a peak temperature of 85°C. Obviously, the concentration of acid is an important determinant of its effectiveness. None of the bales preserved at the lower moisture levels in the present study achieved temperatures that would indicate severe spoilage. Lacey and Lord (1977) found that with uniform

Table 9. Effect of preservative on chemical composition, voluntary intake and apparent digestibility coefficients of alfalfa hay by sheep

Parameter	Preservative[1]			
	Control	LLB	BPA	SEM
Chemical Composition				
Dry matter (g/kg)	894	901	892	
Organic matter (g/kg DM)	891	892	891	
Neutral detergent fibre (g/kg DM)	438	464	389	
Acid detergent fibre (g/kg DM)	321	346	296	
Crude protein (g/kg DM)	231	232	239	
Water soluble carbohydrates (mg/g)	13.8	22.0	22.7	
Voluntary Intake				
Dry matter (g/d)	2,045.4	2,000.0	1,942.8	86.10
Dry matter ($g/kg^{0.75}$)	80.5	77.5	74.6	3.24
Dry matter (% of body weight)	2.7	2.6	2.5	0.11
Organic matter (g/d)	1,731.2	1,709.5	1,736.8	70.24
Forage (g/d)	1,862.2	1,816.8	1,759.7	86.10
Nitrogen (g/d)	63.6	62.5	63.4	3.63
Apparent Digestibility (%)				
Dry matter	68.1	69.3	66.4	1.15
Nitrogen	76.3[c]	78.5[a]	77.4[b]	0.35
Neutral detergent fibre	61.0	55.0	50.7	3.11
Acid detergent fibre	57.5	52.2	47.3	3.75

[1] Control, no additive applied; LLB, *Lactobacillus buchneri* 40788 (1.2×10^6 cfu/g of hay) applied in a liquid formulation; BPA, buffered propionic acid applied at a rate of 10 mL/kg of hay. The preservatives were applied immediately prior to baling.

[a-c] Within a row, values followed by different letters differ ($p<0.05$).

distribution, propionic acid concentrations of approximately 0.3 to 0.4% are required to inhibit mould growth in hay at 25 to 30% moisture. Under rapidly changing field conditions, however, attaining a uniform distribution of preservative on forage is usually a daunting task. In the present study, propionic acid was applied at 1% (wt/wt) in anticipation of field losses. Temperatures in BPA-preserved bales declined steadily from the day of baling, and did not exceed 45°C, indicating that the application rate was probably adequate for the conditions of this study. Wittenberg (1991) reported that application of a buffered propionic acid formulation (2.1% of water content) to hay baled at 20-25% moisture did not reduce fungal activity of hay during storage relative to untreated hay of the same moisture content. That same study also reported that hay treated with the buffered propionic acid (1.9% of water content) at 25-30% moisture recorded lower temperatures during the first 20 d of storage than did untreated control bales at the same moisture content. Beyond 20 d of storage, however, temperatures in the acid-treated bales were 5 to 6 Celsius degrees higher than in untreated bales, suggesting that microbial activity was not sufficiently suppressed during the entire storage period to prevent forage degradation.

According to Magan and Lacey (1984), the most important factors dictating the types and numbers of microbial species in stored agricultural products are moisture, temperature, substrates, and pH. Those researchers noted that individual microbes (bacteria, yeasts, or moulds) seldom occur as monocultures in stored agricultural products, existing instead in association with other microbes. Invariably, the conditions under which each of them grow, and are therefore detected in stored products, are those under which the microbes best survive or compete, and not necessarily those at which they grow best. Therefore, the continual detection of yeast and moulds and the variations in their numbers at various times during the storage period could also be a reflection of changing conditions within the bales. In a study of the effect of moisture content at baling on fungal growth during storage of alfalfa forage, Undi et al. (1997) reported that the highest temperatures recorded in high (35.9%), medium (28.1%), and low (24.6%) moisture forage were 54.5, 50.0 and 44.0°C, respectively, and that by the time the peak temperatures were attained in all forages, microbial species with optimum temperatures for growth of about 25°C and water activity of 0.99-1.00 had all been eliminated. In addition, it was observed that a combination of high temperature and high moisture favoured proliferation of fungal species, and that yeasts were predominant only in the early stages of storage and were eliminated by d 9. Although individual microbial species were not identified in the present study, it is possible that the enumerations recorded during storage may be attributable to populations and successions of different species thriving under the changing conditions within the bales and not necessarily the persistence of any given species.

Compared to untreated bales, both LLB- and GLB treatments were as effective as BPA in reducing the extent of heating in alfalfa bales at 17% moisture. The fact that

LLB inhibited mould growth to the same extent as the BPA preparation in timothy bales at 20% moisture, together with the observation that GLB also inhibited mould growth in 17% moisture bales, suggests that *L. buchneri* 40788® is effective for inhibiting mould growth in timothy hay baled at moisture levels above those normally targeted (i.e., <12%). The finding that both forms of the *L. buchneri* preparation were not consistent in inhibiting mould growth at the two moisture levels, however, is difficult to explain.

Although the bacteria used in this study were in different carriers (liquid or granular), one would expect that once applied to the forage, their products of fermentation would be similar and so, therefore, would be their effects on the forages. However, only the granular form of *L. buchneri* 40788® was effective for preventing heating of timothy hay to any appreciable extent (compared to control) when baled at either 17% or 20% moisture. The granular form also inhibited mould growth in bales at 17% moisture, but not at 20% moisture. In contrast, the liquid form of *L. buchneri* 40788® inhibited mould growth in bales at 20% moisture but not at 17% moisture. This variability may reflect differences in the dispersion of liquid- versus granular-applied *L. buchneri* 40788® products.

Intake and digestibility studies can provide true estimates of the nutritive value of forages. The average moisture content of bales selected for the feeding trial was 18.9% on the day of baling. The average moisture in the hay bales at the time they were fed was 8.6%. The sheep given timothy hay preserved with *L. buchneri* 40788® registered forage intake 22.3% and 14.1% higher than those fed the control and BPA-preserved hay, respectively, which may indicate enhanced palatability of the 19% moisture, LLB-treated hay as compared with timothy hay untreated or preserved with BPA and baled at the same moisture content. This positive response was not observed with alfalfa forage, however. Davies and Warboys (1982) also reported no differences in DM intake between ewes fed propionic acid-treated alfalfa hay and those fed untreated alfalfa hay.

The type of preservative used in the present study also did not affect digestibility coefficients of nutrients of either timothy or alfalfa hay. Jafri et al. (1979) also found that apparent digestibilities of DM, OM, NDF and crude protein recorded when dairy cows consumed diets containing alfalfa hay treated with a combination of propionic acid and formalin were similar to the controls. Deetz et al. (1989), however, reported that digestibilities of NDF and hemicellulose were enhanced when alfalfa forage was treated with a commercial low-acid hay stabilizer (Fresh Cut®, Kemin Industries, Inc., Des Moines, IA, USA).

The dramatic increase in intake of the LLB-preserved timothy forage cannot be attributed to a single factor. Any number of factors or combinations of factors including individual chemicals and their interactions, nature of the fibre, forage odour, or rate of particle size reduction could have given rise to the differences in intake observed in this study. Thus, measurements routinely undertaken to evaluate the efficacy of inoculants (e.g., changes in chemical composition, microbiology, colour, dust, etc.) may not give an accurate reflection of the efficacy of an inoculant for improving overall feeding value.

The higher fractional rate of DM disappearance of the 19% moisture timothy preserved with LLB (as observed during the *in sacco* trial in this study) may be partly responsible for the higher DM intake observed in this study. A higher rate of DM disappearance is usually associated with a higher rate of particle size reduction and a shorter retention time in the rumen. Shorter retention time usually results in an increase in DM intake, and a depression in DM digestibility, although in the present study the increase in intake of timothy DM was accompanied by a slight increase (though not statistically significant) in digestibility.

It is important to reiterate the fact that the effects of the different forms of *L. buchneri* 40788® on the two forages were not consistent across treatments. For example, the reason that the granular preparation of *L. buchneri* 40788® was effective for preventing heating in medium-moisture timothy bales but not in medium-moisture alfalfa hay remained unexplained. In the same light, the reason why the liquid preparation was effective in preventing heating in the low-moisture timothy bales but not in the low-moisture alfalfa bales is also not obvious. These inconsistencies may be related to variations in temperature, water activity, chemical composition, buffering capacity, or ecological succession and interactions of microbes in the forage. Despite these differences in outcome, this study has shown that the heterolactic bacterium *L. buchneri* 40788® may have a role to play in preventing excessive heating in timothy and alfalfa hay baled at moisture concentrations of 14 to 22%. However, further research is required to understand the interactions between the different forms of the *L. buchneri* 40788® preparation and the moisture content of forages.

IMPLICATIONS

From a producer's perspective, forage would normally not be baled at moisture levels above 14%; rather, levels below 12% are typical. However, unpredictable weather conditions sometimes makes it necessary to bale at moisture levels above 12%, which invariable leads to heating and mould growth in the bales during storage, with resultant reduction in nutritive and market value. The price of hay depends on its quality, which on the spot market, is determined by the nutrient composition and visual characteristics such as colour and dustiness. This study has shown the potential of *L. buchneri* 40788, and the ability of buffered propionic acid to minimize mould growth and to

prevent excessive heating in timothy and alfalfa forage baled at above-optimum moisture content. With *L. buchneri* 40788®, producers may have a microbial alternative to the use of direct acidification as a means of preserving high moisture hay.

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