Effects of Tween 80 on *In Vitro* Fermentation of Silages and Interactive Effects of Tween 80, Monensin and Exogenous Fibrolytic Enzymes on Growth Performance by Feedlot Cattle

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ABSTRACT : The effects of monensin, Tween 80 and exogenous fibrolytic enzymes on ruminal fermentation and animal performance were studied *in vitro* and *in vivo*. In Expt 1, the effects of the surfactant Tween 80 (0.2% wt/wt, DM basis) on ruminal fermentation of alfalfa, corn and orchardgrass silages were investigated using *in vitro* gas production techniques. Tween 80 did not affect ($p>0.05$) cumulative gas production at 24 h, but it reduced ($p<0.05$) the lag in fermentation of all three silages. With corn silage and orchardgrass silage, gas production rates and concentrations of total volatile fatty acids (VFA) were increased ($p<0.05$) by Tween 80; with alfalfa silage, they were reduced ($p<0.05$). Tween 80 increased ($p<0.05$) the proportion of propionate in total VFA, and reduced ($p<0.05$) acetate to propionate ratios (A:P) with all three silages. In Expt 2, exogenous fibrolytic enzymes (E; at 0, 37.5 or 75 g/tonne DM), monensin (M; at 0 or 25 ppm and Tween 80 (T; at 0 or 2 L/tonne DM) were added alone or in combination to backgrounding and finishing diets fed to 320 crossbred steers in a feeding trial with a 3×2×2 factorial arrangement of treatments. The backgrounding and finishing diets contained barley grain and barley silage in ratios of 57.8:42.2 and 93.5:6.5 (DM basis), respectively. Added alone, none of the additives affected DM intake ($p>0.1$) in the backgrounding or in the finishing period, but interactive M×T effects were observed in the finishing period ($p=0.02$) and overall ($p=0.04$). In the finishing period, T without M tended to reduce DM intake ($p=0.11$), but T with M increased ($p=0.05$) DM intake. Monensin increased average daily gain (ADG) during backgrounding ($p=0.07$) and finishing ($p=0.01$), and this ionophore also improved overall feed efficiency ($p=0.02$). Warm carcass weight was increased ($p<0.001$) by M, but dressing percentage was reduced ($p=0.07$). In the backgrounding period, T increased ADG by 7% ($p=0.06$). Enzymes increased ($p=0.07$) ADG by 5 and 6% (low and high application rates, respectively) during backgrounding, but did not affect ($p>0.10$) ADG during finishing, or overall feed efficiency. Whereas T enhanced the positive effects of M on ADG during backgrounding ($p=0.04$) and overall ($p=0.05$), it had no impact ($p>0.1$) on the effects of E. Interactions between M and T suggest that the surfactant may have potential for enhancing the positive effects of monensin on beef production, but this requires further research. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 7 : 968-978)***

Key Words : Enzymes, Feedlot Cattle, Growth Performance, Ionophore, Surfactant

INTRODUCTION

Attempts to maximize productivity of feedlot cattle often focus on improving feed digestion because feed accounts for approximately 70% of the total cost of production. Ruminal fermentation is intrinsically linked to feed utilization, and this microbial fermentative process can be manipulated through alterations to the diet, animal behaviour, or the microbial populations that carry out the fermentation (Nagaraja et al., 1997).

The most common anti-microbial additives used in beef cattle diets to manipulate rumen metabolism are ionophores, several of which consistently improve feed efficiency by 5 to 10% (Bergen and Bates, 1984). These compounds exert an array of favourable actions, including inhibiting ruminal deamination (Kobayashi et al., 1996) and decreasing the acetate:propionate ratio (Kook et al., 1999). Development of methods to enhance the potency of ionophores could further improve the efficiency of ruminant production.

Exogenous fibrolytic enzymes as feed additives improve productivity in the poultry industry (Geraert et al., 1996), and nutritionists have recently begun to investigate the potential benefits of including these agents in ruminant diets (Cheng et al., 1999; McAllister et al., 2001). Ruminant responses to exogenous enzyme supplements have varied with type of diet, form and composition of the enzyme preparation, and rate and method of enzyme application (Theurer et al., 1963; Beauchemin et al., 1995; Pritchard et al., 1996). To maximize the efficacy of this technology, the conditions under which feed enzymes will most likely improve animal performance must be more clearly defined. Another possibility is to investigate strategies to enhance the activity of the enzymes themselves.

Nonionic surfactants have been shown *in vitro* to exert a

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number of positive effects on degradative enzymes, such as preventing inactivation of cellulase (Park et al., 1992), increasing enzymatic hydrolysis of cellulose (Helle et al., 1993), and improving digestion of cellulose by mixed ruminal bacteria (Akin, 1980). Further, nonionic surfactants may increase production of enzymes by fungi and ruminal bacteria (Reese and Maguire, 1969; Kamande et al., 2000). These studies were conducted mainly with hay as substrate, and it is not known if similar effects could be realized with silage-based diets. The effects of surfactants on animal performance, has also rarely been examined. Adding Tween 80 (0.2% wt/wt) to diets for lactating dairy cows increased milk production by 2.5 to 3.5 kg/d (Shelford et al., 1996) but whether or not this positive response is diet specific, or a similar response can be realized in feedlot cattle, is unknown. This surfactant increased the anti-microbial activity of the ionophore salinomycin, possibly by promoting interaction of the ionophore with the cell membranes of target microorganisms (Hristov et al., 2000), but the significance of this finding for improving animal performance also has not been assessed.

This study was conducted to determine if Tween 80-mediated improvements of ruminal digestion observed *in vitro* with forage preserved as hay might also be realized with silage, and whether or not these direct effects and/or other observed positive effects of the surfactant on the activities of feed supplements commonly employed to improve feedlot cattle performance (i.e., ionophores, exogenous enzymes) would be carried through to improving growth performance by beef cattle fed diets containing barley silage.

MATERIALS AND METHODS

Experiment 1 - Effect of Tween 80 on ruminal fermentation of silage

Substrates, animals and inoculum : Alfalfa, orchardgrass, and corn silages, oven-dried (55°C) and ground to pass through a 1-mm screen, were used as substrates. Two ruminally cannulated non-lactating Holstein cows were used as donors of ruminal inoculum. The cows were fed (per day) 3 kg of rolled barley and 8 kg of orchardgrass hay (DM basis), plus a mineral vitamin supplement, with feed offered in two meals. Two hours after the morning feeding, approximately 2 L of ruminal fluid were collected from each cow and strained through four layers of cheesecloth and a layer of glass wool into a pre-warmed flask (39°C). Equal volumes of ruminal fluid from each of the cows were combined, maintained under anaerobic conditions, and used as inoculum within 30 min of collection.

Treatments and incubation procedure : Pre-warmed (39°C) phosphate-bicarbonate buffer/reducing solution (Goering and Van Soest, 1970) was measured in approximately 11 ml quantities into 50 ml Erlenmeyer flasks containing 100 mg of substrate. Appropriate volumes of diluent or Tween 80 stock solution were added to each flask to yield final concentrations of 0 or 0.2% (wt/wt) Tween 80 in each flask (total volume 12 ml). Triplicate flasks were prepared for each silage type (alfalfa, corn and orchardgrass) and level of surfactant (0 or 0.2%). To begin the incubation, 3 ml of inoculum were added to each flask. The flasks were flushed with CO_2 to promote anaerobiosis, then sealed. Incubation mixtures were allowed to equilibrate for 5 min prior to commencing data collection. The incubation was repeated three times.

Gas measurements and data processing : Gas production measurements were made with a computerized system adapted from Pell et al. (1998). Pressure within the 50 ml flasks was measured using transducers (0 to 103 kPa; 0 to 15 psi) that generated differential output voltages proportional to the pressures. Output voltages were determined using a Tempscan/1,000 voltmeter (Omega Engineering, Inc., Stamford, CT). Channels were configured to acquire data at 10 min intervals over a 24 h incubation period. Triplicate control flasks (containing ruminal fluid and buffer only) were included with each of the three runs and used to correct each channel for ruminal fluid activity. Incubation liquids from each treatment were sampled at the end of the incubation for gas chromatographic determination of VFA by the method of Dinn et al. (1998).

Experiment 2 - Effects of monensin, Tween 80 and exogenous fibrolytic enzymes on the performance of feedlot cattle

Experimental design : The experiment involved two periods, i.e., backgrounding (84 days) and finishing (93 days), and a 3×2×2 factorial arrangement of treatments. Treatments comprised three levels of enzymes (0, 37.5 and 75 g/t DM), two levels of monensin (0 and 25 ppm) and two levels of Tween 80 (0 and 2 L/tonne DM) added to diets fed in each period.

Animals and management : Three hundred and sixty crossbred steer calves purchased at weaning were transported to the Lethbridge Research Centre feedlot. The calves had no prior exposure to ensiled feeds. Upon arrival at the research feedlot, they were weighed, injected with vitamins A and D and selenium (Poten AD, Dystosel and Rogar/STB, London, ON), vaccinated against IBR, PI_3 and *Haemophilus somnus* (Resvac 2/Somubac, SmithKline-Beecham, Philadelphia, PA) and against *Clostridium* spp. (Tasvax 8, Mallinkrodt, St. Louis, MO), de-wormed (Ivermectin, Merial Canada Inc., Victoriaville, QC), branded and ear-tagged. Barley silage was offered free-choice for two weeks to familiarize the calves to feed bunks

Table 1. Composition of backgrounding and finishing diets and supplements

	Backgrounding diet	Finishing diet	Supplement without monensin	Supplement with monensin
Dietary ingredient (%, DM basis)				
Rolled barley	52.79	88.51		
Barley silage	42.21	6.49		
Supplement	5.00	5.00		
Chemical composition (g/kg DM)				
OM	918.8	923.4		
N	19.2	20.5		
NDF	356.7	253.3		
Composition of supplement (%, DM. basis)				
Barley grain			63.98	63.75
Urea			1.02	1.00
Limestone			23.71	23.70
Salt			6.23	6.20
Feedlot premix*			1.02	1.00
Dynamate			4.03	4.00
Monensin			-	0.35

* Mineral premix containing zinc sulfate, manganese sulfate, copper sulfate, cobalt sulfate, sodium selenite, and ethylene diamine diiodic acid (as an 80% preparation).

and ensiled feed. The calves were then weighed on two consecutive days. They received a hormonal implant (Component; Elanco, Calgary, AB) on the first weighing day and a *Haemophilus somnus* booster (Somnubac, SmithKline-Beecham, Philadelphia, PA) on the second day.

The steers were assigned to 12 dietary treatments in a randomized complete block design (15 animals per pen and two pens per treatment). They were weighed every four weeks prior to morning feed delivery. Feed was delivered once daily and the steers had *ad libitum* access to water throughout the experiment. Feed bunks were monitored closely to ensure that fresh feed was available at all times and to ensure that feed refusals did not exceed 10% of feed offered. Orts were collected weekly and weighed and analysed for DM content. Steers were kept in the same treatment groups in the backgrounding and finishing phases. The phases were separated by a 4-week period of adaptation to finishing diets. All steers were slaughtered at the completion of the finishing period (XL Beef, Calgary, AB) and carcasses were graded according to Canadian Beef Carcass Grading Regulations. Information collected included warm carcass weight, fat cover, ribeye area, marbling score, quality grade, and dressing percentage.

The steers and the ruminal fluid donor cows used in this study were all cared for according to the standards set by the Canadian Council on Animal Care (1993).

Experimental diets and feeding : The experimental diets were fed as total mixed rations (TMR) consisting of rolled barley grain, barley silage and a supplement (Table 1). The pelleted supplement contained ground barley and required vitamins and minerals, and two formulations (with and without monensin) were prepared. For diets containing monensin, a concentrated preparation (Rumensin premix, 144 g/kg, Elanco Animal Health, Calgary, AB) was incorporated into the supplement to provide a final concentration of 25 ppm (DM basis) in the diets. The exogenous fibrolytic enzyme used in this study was a 2:1 combination (wt:wt) of powdered xylanase and glucanase preparations from *Trichoderma longibrachiatum* (Biovance Technologies Inc., Omaha, NE). Sufficient enzyme to treat one-tonne batches of concentrate was dissolved in 10 L of water prior to application onto the concentrate. In a similar fashion, Tween 80 (T-Maz 80; Great West Chemical Company, Calgary, AB) was mixed with water for application onto the concentrate. When neither enzyme nor Tween 80 was included in the diet, concentrate was sprayed with water (10 L/tonne) to equalize the moisture contents among treatments.

Concentrates (supplement+rolled barley grain) were prepared weekly during the backgrounding phase and every third day during the finishing. Pelleted supplement and rolled barley were combined in a Tonne Marion Paddle Mixer (Rapid Machinery Company, Marion, IA), and treated during mixing with solutions of enzymes and/or Tween 80. The concentrates were stored in separate bins, and mixed with barley silage prior to feeding, using a feed wagon equipped with load cells for TMR formulations. Each TMR was sampled weekly and oven-dried for DM determination or frozen immediately (-40°C) for freeze drying prior to chemical analysis.

Laboratory analyses : Carboxymethylcellulase (CMCase; EC 3.2.1.4), xylanase (EC 3.2.1.8), amylase (EC 3.2.1.1) and β-glucanase (EC 3.2.1.6) activities in the crude enzyme preparations were determined using the method described by Hristov et al. (1998). Soluble protein in the enzyme preparations was determined using the protein-dye binding method described by Bradford (1976) with bovine serum albumin (Sigma, St. Louis, MO) as a standard.

Feed and orts samples were dried at 105°C for DM determination. Freeze-dried TMR samples were ground to pass through a 1.0 mm screen before chemical analysis for NDF (Van Soest et al., 1991), starch (Herrera-Saldana et al., 1990), total N (by flash combustion, chromatographic separation and measurement of thermal conductivity using a NA 1,500 mass spectrometer; Carlo Erba Instruments, Rodano, MI, Italy) and OM (by ashing at 550°C). Sodium sulfite and heat stable amylase were included in the solution for NDF analysis.

Calculations and statistical analyses

Experiment 1 : Cumulative gas production was calculated by the following nonlinear equation:

$$V=b(1-e^{-c(t-lag)}) \quad (1)$$

where V is cumulative gas produced (ml/100 mg DM) at time t (in h); b represents the asymptote (i.e., the maximum volume of gas that will be produced with time); and c is the specific gas production rate (/h). The parameters b, c and *lag* time were estimated by an iterative least-squares procedure (PROC NLIN) of SAS (1991). The parameters and VFA data generated for each silage were subsequently analyzed separately by the General Linear Models (GLM) procedure of the SAS (1991) using the following model:

$$Y_{ij}=\mu+\alpha_i+\beta_j+\varepsilon_{ij} \quad (2)$$

where Y_{ij} represents a measurement such as cumulative gas or VFA, and μ, α_i, β_j and ε_{ij} represent effects of the overall mean, treatment, replication, and the overall error term, respectively.

Experiment 2. Covariance analysis with steers' initial weights as covariant showed no effect of initial weight on animal performance and therefore initial weights were not used in the statistical analysis. Average daily DM intake (DMI), ADG and feed efficiencies (FE, measured as DMI/ADG) from the backgrounding and finishing periods were analyzed initially as separate periods. A second analysis of these parameters was also done using the average data from both periods. Results from the average data are referred to as results for the combined periods of the experiment. Average daily gain and carcass characteristics were computed on an individual basis with steer as the experimental unit, whereas DMI and FE were estimated using pen as the experimental unit. Average daily gain, DMI, FE and carcass characteristics were analysed statistically as a three-way ANOVA by the GLM procedure of SAS (1991) according to the following model:

$$Y_{ijkl}=\mu+M_i+T_j+En_k+P_l+(M\times T)_{ij}+(M\times En)_{ik}+(T\times En)_{jk}+(M\times T\times VEn)_{ijk}+\varepsilon_{ijkl} \quad (3)$$

where μ=overall mean; M_i=effect of monensin (0 or 25 ppm); T_j=effect of Tween 80 (0 or 2 l/t DM); En_k=effect of enzymes (0, 37.5 or 75 g/t DM); P_l = effect of pen (1 and 2); $(M\times T)_{ij}$=interaction between M_i and T_j; $(M\times En)_{ik}$= interaction between M_i and En_k; $(T\times En)_{jk}$=interaction between T_j and En_k; $(M\times T\times En)_{ijk}$=interaction between M_i, T_j and En_k; and ε_{ijkl}=random error. In Expts 1 and 2, treatments were compared by least square means (LS means) with PDIFF procedure at significance levels of P=0.05 and P=0.1, respectively.

Table 2. Effects of Tween 80 on the kinetics of gas production during *in vitro* fermentation of silages made from alfalfa, corn or orchardgrass (n=3)

Silage type	Parameter[2]	Treatment[1]		
		Control	Tween 80	SEM[3]
Alfalfa	Asymptote (ml/100 mg)	18.1[b]	19.5[a]	0.11
	Cumulative gas (ml/100 mg)	12.7[b]	13.2[a]	0.11
	Specific rate (/h)	0.067[a]	0.058[b]	0.0002
	Lag (h)	0.48[b]	0.16[b]	0.005
Corn	Asymptote (ml/100 mg)	26.6[a]	25.9[b]	0.10
	Cumulative gas (ml/100 mg)	13.6[b]	13.9[a]	0.08
	Specific rate (/h)	0.046[b]	0.049[a]	0.0001
	Lag (h)	0.77[b]	0.88[a]	0.003
Orchardgrass	Asymptote (ml/100 mg)	14.6[a]	13.8[b]	0.10
	Cumulative gas (ml/100 mg)	10.0[b]	10.5[a]	0.11
	Specific rate (/h)	0.070[b]	0.073[a]	0.0001
	Lag (h)	0.65[a]	0.41[b]	0.003

[a,b] Within a row, means without a common superscript differ ($p<0.05$).
[1] Incubations included 0 (Control) or 0.2% (wt:wt) Tween 80 (final concentrations).
[2] Asymptote: estimated maximum potential gas production. Cumulative gas: total gas produced at the end of 24 h incubation.
[3] Standard error of the mean.

RESULTS

Experiment 1

Gas production : Tween 80 did not affect ($p>0.05$) cumulative gas production during 24-h incubations of any of the silages (Table 2), although considerable variation among the three forage sources was observed. Tween 80 increased ($p<0.05$) the specific rate of gas production with corn silage (from 0.046/h to 0.049/h) and with orchardgrass silage (from 0.070/h to 0.073/h), but reduced ($p<0.05$) the rate associated with alfalfa silage (from 0.067/h to 0.058/h). Potential gas production (asymptote) during the fermentation of alfalfa silage increased ($p<0.05$), but the asymptotes of corn and orchardgrass silage were reduced ($p<0.05$) as a result of adding Tween 80 to the incubation mixtures. Incubation of the silages with Tween 80 also reduced ($p<0.05$) the lag time associated with the fermentations.

VFA production : Tween 80 increased ($p<0.05$) the total VFA present after 24 h fermentations of corn silage and of orchardgrass silage, by 16.8 and 21.9%, respectively (Table 3). In incubations of alfalfa silage, however, there was a 10% reduction ($p<0.05$) in total VFA when Tween 80 was included. Molar percentages of acetate were not affected ($p>0.05$) by Tween 80 during fermentation of corn or orchardgrass silage, but the proportion of acetate was reduced ($p<0.05$) by the surfactant during fermentation of

Table 3. Effects of Tween 80 on volatile fatty acid (VFA) profiles of *in vitro* fermentations of silages made from alfalfa, corn or orchardgrass (n=3)

Silage type	VFA (%, μmol/μmol)	Treatment[1]		
		Control	Tween 80	SEM[2]
Alfalfa	Acetate	67.3[a]	64.9b	1.45
	Propionate	25.8	26.1	0.31
	Butyrate	5.3	5.4	0.07
	Valerate	0.4	0.3	0.03
	Iso-butyrate	0.5[b]	1.1[a]	0.05
	Iso-valerate	0.8[b]	2.2[a]	0.04
	Acetate:propionate	2.6[a]	2.5[b]	0.01
	Total VFA (mmol/L)	54.7[a]	49.2[b]	2.47
Corn	Acetate	59.5	59.5	1.45
	Propionate	27.8[b]	28.9[a]	0.31
	Butyrate	7.8	6.9	0.19
	Valerate	2.0	2.0	0.05
	Iso-butyrate	1.0[b]	1.1[a]	0.03
	Iso-valerate	1.9[a]	1.7[b]	0.07
	Acetate:propionate	2.1	2.1	0.01
	Total VFA (mmol/L)	73.8[b]	86.3[a]	2.49
Orchardgrass	Acetate	54.7	55.0	1.42
	Propionate	30.2[b]	31.8[a]	0.21
	Butyrate	9.0	7.2	0.38
	Valerate	2.4	2.6	0.06
	Iso-butyrate	1.5	1.3	0.05
	Iso-valerate	2.3	2.1	0.06
	Acetate:propionate	1.8	1.7	0.01
	Total VFA (mmol/L)	74.3[b]	90.6[a]	2.52

[a,b] Within a row, means lacking a common superscript differ ($p<0.05$).
[1] Incubations included 0 (Control) or 0.2% (wt:wt) Tween 80 (final concentrations).
[2] Standard error of the mean.

alfalfa silage. Molar percentages of propionate were increased ($p<0.05$) by Tween 80 during fermentation of all three silages, resulting in lower ($p<0.05$) A:P ratios. Branched chain VFA (i.e., *iso*-butyrate and *iso*-valerate) were not affected ($p>0.05$) by Tween 80 during fermentation of corn or orchardgrass silages but were increased ($p<0.01$) during fermentation of alfalfa silage.

Experiment 2

Characterization of enzyme preparations : Although described by their manufacturer as a crude xylanase extract and a crude β-glucanase extract, the two enzyme preparations each exhibited a broad range of polysaccharidase activities (Table 4). Xylanase and β-glucanase activities accounted for 49% and 27%, respectively, of the total activity determined in the crude xylanase preparation. Activity of amylase was lowest among the polysaccharidase activities surveyed, accounting for only 7.5% of the total activity. In the crude β-glucanase preparation, β-glucanase activity accounted for only 35% of the total activity, with the remaining 65% of total activity evenly distributed among the other activities measured.

Table 4. Characterization of the xylanase and β-glucanase preparations from *Trichoderma longibrachiatum* that were used in the feedlot study

Characteristic	Xylanase preparation	β-glucanase preparation
Enzymic activity		
Carboxymethylcellulase (CMCase)	2.82	3.62
	(63.9)	(20.8)
Xylanase	7.91	4.78
	(179.4)	(27.4)
Amylase	1.23	3.24
	(27.9)	(18.6)
β-Glucanase	4.33	6.34
	(98.2)	(36.4)
Reducing sugars (RS, mg/g DM)	308.3	151.2
Soluble protein (mg/g DM)	44.1	174.2

[1] Expressed as μg RS released/(mg DM·min). Values in parentheses immediately beneath the primary values are specific activities (i.e., μg RS released / (mg soluble protein·min)).

Animal performance : Statistical analysis revealed no 3-way interactive effects ($p>0.1$) among monensin, Tween 80 and enzymes on any of the parameters of animal performance that were measured. Therefore, main effects of the relevant 2-way interactions only are presented for ADG, DMI, FE and carcass characteristics.

Average daily gain : Mean initial liveweight of all steers was 281±1.3 kg (mean±SE) and initial liveweights were similar ($p>0.1$) among treatments. Monensin increased the steers' ADG both during the backgrounding ($p=0.07$) and the finishing ($p=0.01$) periods of the experiment (Tables 5 and 6). Weight gains by steers were greater with Tween 80 in the diet than without during backgrounding ($p=0.1$) but not during finishing ($p>0.1$). Although no main effects of enzymes were observed, steers fed diets treated with 37.5 (E1) or 75 (E2) g enzyme per tonne DM had ADG 5% ($p=0.12$) and 6% ($p=0.07$) higher, respectively, than those fed the control diet during the backgrounding period.

Significant interactions between monensin and Tween 80 were detected (Table 5) when backgrounding data were analysed separately ($p=0.04$) and also when data from the backgrounding and finishing periods were combined ($p=0.05$). Compared with steers consuming diets containing monensin (M) or Tween 80 alone (T80), those fed diets containing both additives (M+T80) had higher ADG during backgrounding and overall. There was no interaction between monensin and enzymes with respect to ADG ($p>0.1$). During the backgrounding period, however, steers consuming diets containing Tween 80 and enzymes (T80+E2) had lower ($p=0.08$) ADG than those consuming diet with Tween 80 but no enzymes (T80).

Dry matter intake and feed efficiency : None of the

Table 5. Main effects of treatments on growth performance[1] by steers during backgrounding and finishing periods and over the entire feeding trial

Treatment[2]	Backgrounding (84d)			Finishing period (93d)			Overall experiment[3]		
	ADG	DMI	FE	ADG	DMI	FE	ADG	DMI	FE
Monensin (g/tonne)									
0	1,656	7.70	4.67	1,578	8.35	6.14	1,618	7.96	5.26
25	1,699	7.66	4.59	1,647	8.30	5.83	1,674	7.91	5.09
SEM[4]	15.5	0.058	0.053	17.5	0.079	0.076	13.6	0.059	0.047
Tween 80 (L/tonne)									
0	1,657	7.73	4.70	1,626	8.31	6.03	1,640	7.96	5.23
2	1,696	7.63	4.57	1,599	8.34	5.94	1,650	7.90	5.11
SEM	15.5	0.058	0.053	17.5	0.079	0.076	13.6	0.058	0.047
Enzymes (g/tonne)									
0	1,683	7.60	4.56	1,622	8.46	6.04	1,655	7.59	5.15
37.5	1,673	7.78	4.73	1,620	8.18	5.89	1,647	7.94	5.19
75	1,673	7.65	4.61	1,589	8.32	6.02	1,630	7.92	5.17
SEM	19.0	0.071	0.065	21.5	0.096	0.093	16.6	0.072	0.058
p Values (main and interactive effects)									
Monensin	0.072	0.599	0.309	0.012	0.670	0.013	0.004	0.591	0.024
Tween 80	0.098	0.276	0.106	0.188	0.793	0.405	0.659	0.607	0.111
Enzymes	0.913	0.230	0.205	0.487	0.162	0.470	0.478	0.961	0.883
M×T80	0.043	0.192	0.360	0.280	0.018	0.722	0.051	0.042	0.402
M×E	0.860	0.331	0.816	0.606	0.099	0.295	0.586	0.168	0.371
T80×E	0.068	0.130	0.379	0.465	0.603	0.610	0.591	0.233	0.675

[1] ADG: average daily gain (g); DMI: dry matter intake (kg/d); FE: feed efficiency, expressed as DMI/gain.

[2] Treatment levels are final concentrations in the diet (DM basis). Monensin was incorporated into the pelleted supplement. Tween 80 and enzyme preparation were dissolved in water and sprayed onto the rolled barley prior to mixing with pelleted supplement and barley silage.

[3] Overall data exclude the 4-wk transition period between backgrounding and finishing

[4] Standard error of the mean.

additives affected DMI ($p>0.1$) during backgrounding or during finishing (Tables 5 and 7). An interaction was observed, however, between monensin and Tween 80 during the finishing period ($p=0.02$) as well as overall, i.e., when data from the two feeding phases were combined ($p=0.04$). Tween 80 reduced the steers' DMI during the finishing period ($p=0.11$) and overall ($p=0.08$) only when diets did not include monensin. When monensin was present, the M×T80 interaction increased ($p=0.07$) DMI during finishing and overall.

A monensin-mediated improvement in feed efficiency (FE, Table 5) was evident during the finishing phase ($p=0.01$) and overall ($p=0.02$), but not during the backgrounding phase ($p=0.31$). Tween 80 tended to improve FE during backgrounding ($p=0.11$) and overall ($p=0.11$), but this was not evident during the finishing period ($p=0.40$). No interactive effects ($p<0.1$) of Tween 80 and monensin on FE were noted in either feeding period. Enzymes did not affect ($p>0.1$) DMI or FE in any period, nor were any interactive effects observed ($p>0.1$) between enzymes and monensin, or between enzymes and Tween 80.

Carcass characteristics : Carcass characteristics are presented in Table 8. Monensin increased warm carcass weight (323 *v.* 313 kg, $p=0.001$) and fat cover (10.99 *v.* 10.01 mm, $p=0.08$), but reduced dressing percentages of the carcasses (58.89 *v.* 59.49%, $p=0.07$). A numerical tendency of Tween 80 to reduce dressing percentage (58.89 *v.* 59.40%, $p=0.18$) was also observed, but enzymes did not affect ($p>0.1$) any of the carcass characteristics measured. Interactive effects of treatments on carcass characteristics were also not observed. Carcass marbling (data not shown), graded fat and ribeye area were not affected ($p>0.1$) by any of the treatments.

DISCUSSION

The improved feed efficiency reported with monensin supplementation of feedlot diets has arisen either by reducing feed intake or by increasing liveweight gain (Goodrich et al., 1984). In agreement with these earlier findings, while including 25 ppm of monensin in a TMR consisting of rolled barley and barley silage in the present study had no effect on feed intake (Table 6), ADG was significantly increased in both the backgrounding and the finishing periods (Table 5). The improved FE (Table 6) by monensin obtained in this study, can therefore be attributed to the enhanced growth rate of the steers, probably due physiologically to the alterations in ruminal microbial metabolism.

Monensin increases production of propionate, reduces methane and lactic acid production and, by reducing deamination, conserves amino acids in the rumen

Table 6. Average daily gain (n=30) by feedlot steers on backgrounding and finishing diets supplemented with monensin (M), Tween 80 (T80) and/or fibrolytic enzymes (E)

Item	Average daily gain (g)		
	Backgrounding period (84d)	Finishing period (93d)	Overall[1]
Diet[2]			
Control	1,610	1,619	1,614
E1	1,693	1,630	1,663
E2	1,708	1,578	1,619
T80	1,712	1,528	1,624
T80+E1	1,629	1,573	1,602
T80+E2	1,615	1,547	1,583
M	1,647	1,694	1,669
M+E1	1,645	1,604	1,625
M+E2	1,674	1,636	1,656
M+T80	1,765	1,646	1,726
M+T80+E1	1,725	1,673	1,700
M+T80+E2	1,723	1,596	1,663
SEM[3]	37.9	42.8	33.2

[1] Overall data exclude the 4-wk transition period between backgrounding and finishing.

[2] Control diet contained no additives; E1 and E2 indicate enzymes included at 37.5 or 75 g/tonne DM, respectively; T80 indicates Tween 80 included at 2 L/tonne DM; M indicates monensin included at 25 ppm (DM basis).

[3] Standard error of the mean.

(Kobayashi et al., 1996; Nagaraja et al., 1997). Any or all of these factors could have contributed to increased efficiency of energy metabolism and protein utilization by the steers in this study, and subsequently, to their increased ADG. These monensin-mediated effects were also evident in the higher carcass weight and thicker backfat cover of the carcasses of steers whose diets included monensin, compared to those that were not fed monensin (Table 7), even though their DMI were similar (Table 6).

One of the more notable observations in this study was the interaction between monensin and Tween 80 to increase ADG and DMI. Tween 80, an oleate ester of sorbitol and its anhydrides copolymerized with ethylene oxide, is a nonionic surfactant routinely used as an emulsifier. Nonionic surfactants increase enzymatic hydrolysis of cellulose (Helle et al., 1993), digestion of cellulose by mixed ruminal bacteria (Akin et al., 1980; Kamande et al., 2000), and production of enzymes by fungi (Reese and Maguire, 1969). Tween 80 also reduced A:P ratios during fermentation of silage by rumen microorganisms in this study (Table 3), although its effects on total VFA production and gas production were dependent upon silage type. These findings suggest that the effects of Tween 80 on ruminal fermentation are diet dependent, expressed either through altering the species composition of the rumen microbial population (as opposed to affecting the capacity of microbial populations to produce enzymes), or through altering the interaction between the enzymes and the target substrates.

The interactions between Tween 80 and monensin further support these conclusions regarding the mode of action of Tween 80. Tween 80 and monensin increased ADG in the backgrounding period and overall (Table 6), whereas Tween 80 and enzymes reduced ADG in the backgrounding period and had no effect overall. McAllister et al. (2000) also found that mixtures of Tween 80 and enzymes (differently sourced) did not improve feed digestibility or the growth performance of lambs.

The present study showed that Tween 80 in the TMR tended to increase ADG during backgrounding but not during finishing. Shelford et al. (1996) reported that adding

Table 7. Average dry matter intake and feed efficiency of feedlot steers fed diets supplemented with monensin (M), Tween 80 (T80) and enzymes (E), either alone or in combination (n=2)

	Dry matter intake (kg/head·d)			Feed efficiency (feed/gain)		
	Backgrounding	Finishing	Overall	Backgrounding	Finishing	Overall
Diet[2]						
Control	7.68	8.52	8.01	4.799	6.324	5.409
E1	7.94	8.53	8.18	4.731	6.144	5.296
E2	7.79	8.40	8.03	4.577	6.034	5.160
T80	7.56	8.18	7.81	4.435	6.256	5.164
T80+E1	7.82	8.24	7.99	4.839	5.997	5.302
T80+E2	7.41	8.20	7.73	4.654	6.089	5.228
M	7.46	8.33	7.81	4.595	5.833	5.091
M+E1	7.61	7.69	7.64	4.741	5.865	5.191
M+E2	7.87	8.37	8.07	4.746	5.979	5.239
M+T80	7.71	8.81	8.15	4.409	5.748	4.944
M+T80+E1	7.74	8.26	7.96	4.606	5.547	4.982
M+T80+E2	7.54	8.31	7.85	4.462	5.989	5.073
SEM[3]	0.142	0.192	0.144	0.1294	0.1852	0.1161

[1] Control diet contained no additives; E1 and E2 indicate enzymes included at 37.5 or 75 g/tonne DM, respectively; T80 indicates Tween 80 included at 2 L/tonne DM; M indicates monensin included at 25 ppm (DM basis). Backgrounding diet was fed for 84 d; finishing for 93 d. Overall data exclude the 4-wk transition period between diets.

[2] Standard error of the mean.

Table 8. Carcass characteristics of feedlot steers (n=30) fed barley grain/barley silage-based diets supplemented with monensin (M) and/or Tween 80 (T80) and/or enzymes (E)

	Warm carcass weight (kg)	Fat cover (mm)	Graded fat (mm)	Ribeye area (cm^2)	Dressing percentage
Diet[1]					
Control	312.75	9.81	8.60	85.28	59.85
E1	317.49	10.50	9.24	83.58	59.10
E2	314.97	9.25	8.28	83.76	60.23
T80	311.71	11.00	9.83	83.12	58.60
T80+E1	309.71	10.28	8.79	83.28	59.20
T80+E2	312.09	9.57	8.20	84.92	59.96
M	322.91	11.19	10.16	86.97	58.95
M+E1	317.82	10.15	8.99	84.95	59.40
M+E2	323.54	10.67	9.19	84.83	58.86
M+T80	322.94	10.53	9.21	82.92	58.59
M+T80+E1	327.79	10.36	8.88	85.57	58.83
M+T80+E2	323.75	11.74	10.17	85.19	58.19
SEM[2]	4.261	0.713	0.859	2.396	0.776
p Values (main interactive dffects)					
Monensin	0.004	0.094	0.221	0.432	0.069
Tween 80	0.920	0.436	0.837	0.596	0.176
Enzymes	0.944	0.777	0.663	0.979	0.787
M×T80	0.144	0.782	0.796	0.829	0.931
M×E	0.968	0.249	0.434	0.926	0.212
T80×E	0.914	0.770	0.823	0.483	0.817

[1] Control diet contained no additives; E1 and E2 indicate enzymes included at 37.5 or 75 g/tonne DM, respectively; T80 indicates Tween 80 included at 2 L/tonne DM; M indicates monensin included at 25 ppm (DM basis).
[2] Standard error of the mean.

Tween 80 (2 L/tonne diet DM) increased milk production in dairy cows by 2.5 to 3.5 kg/d. However, Tween 80 applied at 0.02% to both forage and concentrate diets for lambs affected neither digestibility nor animal performance (McAllister et al., 2000). This suggested that the response of animals to Tween 80 may be both dose-related and diet-dependent. Further research is needed to define the optimum application rates of Tween 80 under different feeding regimes.

The nature of the interaction between monensin and Tween 80 is not known but its mechanism may be related to alterations in transport of ions across cell membranes. Monensin has long been recognized for its ability to disrupt gradients across cell membranes to manipulate the metabolism of the cell (Pressman, 1976). Surfactants have also been shown to alter the structure of biological membranes by disrupting their lipid-protein structure (Schulman et al., 1955). Therefore, it is possible that the addition of Tween 80 modified the cell wall structure in a manner that enhanced the effects of monensin on ion gradients in target microorganisms. Recent work in our laboratory demonstrated synergism between salinomycin and Tween 80 (Hristov et al., 2000). Further research in this area is required to elucidate the mechanism of this interaction and to investigate if similar interactions exist between other ionophores and other nonionic surfactants. This synergism could have implications on beef production in its potential to further enhance the beneficial effects of ionophores on ruminal fermentation.

The potential benefit to animal performance by the enzyme mixture used in this study is demonstrated by the increased ADG during backgrounding achieved by steers receiving the higher rate of enzyme (75 g/tonne diet DM) relative to the controls. This was probably due to improved feed utilization when the enzyme was fed, given that there was no observable increase ($p>0.1$) in DMI with enzyme supplementation. Wang et al. (2001) found that the xylanase component of this enzyme mixture increased the number of cellulolytic bacteria, microbial protein synthesis, and feed particle-associated xylanase activity in the Rusitec. An increase in feed particle-associated microbial protein (i.e., in microbial attachment) in conjunction with exogenous fibrolytic enzymes was also observed in cows (Yang et al., 1998). Therefore, it is possible that the enzymes supplemented enhanced the cellulolytic activity and subsequently improved ruminal fermentation.

The increased ADG effect was not maintained by the same steers through the finishing period, which indicates that the effects of the enzyme mixture were diet-dependent. Similar diet-dependency of the action of other enzymes to enhance ruminal cellulolytic activity have been reported by others (Perry et al., 1966; Boyles et al., 1992; Beauchemin et al., 1995; Chen et al., 1995; Lewis et al., 1995; Stokes and Zhang 1995; Feng et al., 1996; McAllister et al., 1999,

2000). The method of application also influences the effectiveness of enzymes (Yang et al., 1998; Beauchemin et al., 1999; McAllister et al., 1999; Wang et al., 2000; Chiou et al., 2002). The absence of enzyme effects on animal performance when diets also contained monensin and/or Tween 80 suggests they were exerted primarily in the rumen and that they were not complementary to those of monensin and Tween 80.

Assay of the enzyme mixture used in this study confirmed multiple activities (Table 4) and other minor activities were likely present given that both preparations were crude extracts of cultures. The observed enzyme effects, therefore, can be attributed only to the combined effects of the mixtures and not to the activity of any single enzyme. Activity of the enzyme mixture was not assessed against complex substances such as feed substrates in the present study, but in a similar procedure with the same enzymes, they were shown to increase the level of free reducing sugars in diets (Wang et al., 2001). This suggests that enzymes applied onto dry feed prior to feeding solubilizes portions of the feed and releases readily digestible carbohydrates.

Feed efficiency values obtained during the backgrounding period in this study were better than those commonly reported by other researchers, although McCoy et al. (1995) did report feed:gain at 4.84 for steers fed a backgrounding diet comprising 45% alfalfa hay, 52% wet corn gluten feed and 3% protein-mineral-vitamin supplement. In that study, as in the present one, FE was lower during the finishing than during the receiving period. The reason for better than average FE during backgrounding in the present study is not known, but was likely due at least in part to the moderate weather conditions of the time. During the backgrounding period (November 1997 to February 1998) mean temperature was higher (2.35 *v.* 5.30°C), snow (7.2 *v.* 19.1 cm) and wind (11.378 *v.* 14.726 km) were less abundant, and wind speed was lower (15.7 *v.* 20.8 km/h) than long-term average values for the area (Source: Agriculture and Agri-Food Canada, Lethbridge Research Centre Weather Station, Lethbridge, AB). Christopherson et al. (1993) suggested that the effect of ambient temperature on digestion is approximately 0.2 units per degree Celsius in growing calves. Thus, the higher digestibility in the present study compared to more typical winters would be expected.

In conclusion, monensin supplementation increased ADG during both the backgrounding and finishing periods, and also improved FE during finishing and overall. Tween 80 included as the only additive in the diets of steers increased ADG and improved FE during the backgrounding period. A significant interaction between monensin and Tween 80 increased ADG during backgrounding and the overall feeding period, but this did not improve FE. Supplementing the barley grain-barley silage diets with enzymes increased ADG during backgrounding but not during the finishing period or overall. Further research is required to elucidate the mechanism of the action of exogenous fibrolytic enzymes in ruminants and the interactions between surfactants and ionophores.

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