Effects of dietary addition of zinc and(or) monensin on performance, rumen fermentation and digesta kinetics in beef cattle

H. M. Arelovich[1]*, H. E. Laborde[2], M. I. Amela[3], M. B. Torrea[3] and M. F. Martínez[2]

[1] Departamento de Agronomía. CERZOS. Universidad Nacional del Sur. Comisión de Investigaciones
Científicas de la Provincia de Buenos Aires (CIC). San Andrés, 800. 8000 Bahía Blanca. Argentina
[2] Departamento de Agronomía. CERZOS-CONICET. Universidad Nacional del Sur.
San Andrés 800. 8000 Bahía Blanca. Argentina
[3] Departamento de Agronomía. Universidad Nacional del Sur.
San Andrés 800. 8000 Bahía Blanca. Argentina

Abstract

Two experiments (Exp1 and Exp2) were conducted to investigate the effect of dietary inclusion of Zn and(or) monensin on performance and rumen fermentation in beef cattle fed a barley grain, alfalfa hay and sunflower meal. In Exp1, 20 calves were assigned to one of the following treatments: CON = control; Z = 430 mg Zn kg^{-1} of DM; M = 35 mg monensin kg^{-1} of DM; and ZM = both Zn and monensin. Dry matter intake (DMI), DM digestibility (DMD), average daily gain (ADG), and feed to gain (F/G) ratio were determined. Blood analyses included hematocrit, glucose, urea, total protein, alkaline phosphatase and creatinine. In Exp2, the rumen fluid of four cannulated steers, in identical treatments, was studied for Zn concentration, pH, NH$_3$-N, VFA. Rumen dilution rate, turnover time, and volume; *in situ* DM disappearance of barley and alfalfa were also determined. In Exp1, no treatment response was observed for DMI, ADG or DMD (P > 0.10). Differences in F/G were not significant, despite a numeric F/G decrease in M (6.6 kg kg^{-1}). No differences were detected in blood variables. In Exp2, an interaction of treatment × time (P = 0.0174) for Zn concentration was detected, where ZM, followed by Z, had highest mean values at all time intervals. Ruminal parameters, kinetics or DM degradability were not modified by treatments; pH reached the lowest value (6.1; P < 0.05) 12 h after supplement feeding. Overall, supplying more than 20 times the Zn requirement has not substantially affected performance or digestion.

Additional key words: additives, blood parameters, intake, rumen kinetics, ruminants, weight gain.

Resumen

Efecto de la adición a la dieta de Zn y/o monensina sobre la respuesta productiva, fermentación ruminal y cinética digestiva en bovinos de carne

En dos experimentos (Exp1 y Exp2) se investigó el efecto de la inclusión en la dieta de Zn y/o monensina sobre la productividad y fermentación ruminal de bovinos alimentados con grano de cebada, alfalfa y harina de girasol. En Exp1, 20 terneros fueron asignados a tratamientos: CON = control; Z = 430 mg Zn kg^{-1} de MS; M = 35 mg monensina kg^{-1} de MS y ZM = Zn + monensina. Se determinó consumo de materia seca (DMI), digestibilidad (DMD), ganancia de peso (ADG) y eficiencia de conversión alimenticia (F/G). Análisis de sangre incluyeron: hematocrito, glucosa, urea, proteína total, fosfatasa alcalina y creatinina. En Exp2, el líquido ruminal de cuatro novillos fistulados sujetos a tratamientos idénticos fue analizado para: concentración de Zn, pH, NH$_3$-N, VFA. Se determinó dilución de fase líquida, tiempo de recambio y volumen ruminal; y degradación *in situ* de cebada y alfalfa. En Exp1 no se observó respuesta a los tratamientos para DMI, ADG y DMD. Las diferencias no fueron significativas para F/G, a pesar de una disminución en M (6,6 kg kg^{-1}). Tampoco fueron observadas diferencias para variables sanguíneas. En Exp2, se detectó una interacción tratamiento × tiempo (P = 0,0174) para concentración de Zn; ZM resultó mayor para todos los intevalos de tiempo, seguido de Z. Los tratamientos no alteraron parámetros y cinética ruminal o desaparición de DM; pH decreció (6,1; P < 0,05) 12 h posterior a la suplementación. Suministrando más de 20 veces el requerimiento de Zn no afectó substancialmente productividad o digestión.

Palabras clave adicionales: aditivos, cinética ruminal, consumo, ganancia de peso, parámetros sanguíneos, rumiantes.

Introduction

Generally, increased rumen fermentation efficiency leads to improved animal performance. To promote this biological response, scientists have made numerous attempts to manipulate rumen fermentation by the dietary addition of a large variety of feed additives. Monensin, for example, increases rumen propionate production while decreasing methane production and protein degradation, which results in improved energy metabolism and protein utilization (Callaway *et al.*, 2003). Additionally, monensin decreases methane and spares rumen amino acids from ruminal degradation (Goodrich *et al.*, 1984).

Although monensin is legally employed in many countries as a growth promoter, the European Union has banned the use of monensin sodium as well as other antimicrobials in animal feeds (Papademetriou, 2006). Hence, several other additives that could be used to manipulate fermentation of dietary substrates by rumen microbes are currently under study. Various metal ions could affect rumen fermentation (Martínez *et al.*, 1970; Spears and Hatfield, 1978; Rodríguez *et al.*, 1995; Arelovich *et al.*, 2000; Faixova and Faix, 2002). Previous research indicated that dietary addition of 250 to 400 mg Zn kg^{-1} DM to low-quality forage altered rumen fermentation by retarding ammonia accumulation and increasing molar proportions of propionate (Arelovich *et al.*, 2000). Therefore, feed efficiency and beef production could be improved by Zn inclusion in cattle diets. It was also shown that when administered at over 20 times the daily NRC (2000) requirement, Zn could increase the concentration of rumen propionate (Arelovich *et al.*, 2000) and decrease the acetate:propionate ratio (Bateman II *et al.*, 2004).

Thus, for ruminants, Zn could hypothetically become an alternative growth promoter to ionophores when included in the diet at a higher concentration than the animal's requirement. Considering the present restrictions imposed on ionophores as growth promoters, the impact of dietary Zn addition should be studied for high-quality diets as well as for forage-based diets. However, available scientific data concerning the use of Zn as an additive is scarce. The Zn source, as well as the achieved rumen concentration of Zn, the feeding procedures, the type of diet, and the interaction with other dietary components or additives may be some of the factors influencing the effect of Zn in ruminal fermentation. Alfalfa (*Medicago sativa* L.) hay and barley (*Hordeum vulgare* L.) grain are common ingredients used for growing-finishing diets in beef cattle. Animal performance on this type of diet could be increased by the addition of Zn in the diet (Arelovich *et al.,* 2000; Faixova and Faix, 2002; Bateman II *et al.,* 2004). One of the advantages of using Zn as an additive is that various inorganic Zn salts can be easily obtained in the market, and usually they have a lower cost when compared to monensin or similar feed additives. The objectives of this study were to investigate the effects of dietary inclusion of Zn —as an alternative to monensin— on animal performance, blood metabolite changes, digestion and rumen fermentation measurements in beef cattle receiving a diet of barley grain, alfalfa hay and sunflower (*Helianthus annus* L.) meal (SFM). Since the impact of both Zn and monensin could potentially exhibit additive effects on these parameters, a treatment including both the mineral and the ionophore was included.

Material and Methods

The general principles for animal care and welfare suggested by CIOMS (1985) and OIE (2002) were followed. Two feeding experiments were conducted at the National University of the South (Universidad Nacional del Sur), Argentina (Argerich Experimental Field; 38° 46'S, 62° 38'W). The trials included growing steers (Exp 1) to measure performance, and ruminally-cannulated steers (Exp 2) to evaluate rumen fermentation characteristics. Immediately before commencing the experiments, all animals were treated with Ivermectin to prevent parasitic infections.

Abbreviations used: ADF (acid detergent fiber), ADG (average daily gain), ADL (acid detergent lignin), AIA (acid-insoluble ash), ALKP (alkaline phosphatase), CIOMS (Council for International Organizations of Medical Sciences), CON (control treatment), CP (crude protein), CRT (creatinine), DM (dry matter), DMD (DM digestibility), DMI (DM intake), ED (effective degradability), FDR (rumen fluid dilution rate), F/G (feed to gain ratio), FTT (rumen fluid turnover time), GLU (glucose), HTC (hematocrit), M (monensin treatment), NDF (neutral detergent fiber), NH3-N (rumen ammonia nitrogen), OIE (World Organisation for Animal Health), RFV (rumen fluid volume), SEM (standard error of the mean), SFM (sunflower meal), TP (total protein), U (urea), VFA (volatile fatty acids), Z (Zn treatment), ZM (Zn + monensin treatment).

Experiment 1

Animals and treatments

Twenty Aberdeen Angus steers, 11 months of age and averaging 213 ± 4.8 kg of initial body weight, were randomly allocated to individual indoor pens. Steers were fed a mixed ration of cracked barley grain, chopped alfalfa hay and sunflower meal plus a supplement with or without Zn and(or) monensin (Table 1). All supplements contained NaCl and wheat middlings, the latter as a carrier for monensin (Table 2). Animals were grouped by weight and randomly assigned to one of the following treatments: (1) CON (Control), no Zn or monensin added; (2) Z, anhydrous $ZnCl_2$ was included to provide 430 mg Zn kg^{-1} of feed DM; (3) M, monensin was added as a commercial premix to supply 35 mg monensin kg^{-1} of feed DM; and (4) ZM, included both 430 mg of Zn and 35 mg of monensin kg^{-1} feed DM. The experiment was a completely randomized design with five replications per treatment. Feed was offered daily, *ad libitum*, in two equal meals at 08:00 and 17:00 h. Daily feeding quantities were computed so that 15-20% surplus was left in the feeder. The supplements were mixed with the morning meal. The Zn in the diet was 22-fold the required Zn level, but less than 500 mg kg^{-1} of feed DM, the tolerable concentration suggested by NRC (2000). The total evaluation period was 77 days.

Sampling procedures and chemical analyses

Daily, feed offered and rejected, and fresh fecal grab samples were collected. These samples were composite

Table 1. Ingredients and chemical composition of the basal diet

Ingredients, dry matter basis (g kg^{-1})	
— Cracked barley grain	482
— Chopped alfalfa hay	393
— Sunflower meal	99
— Supplement[1]	26
Chemical composition (g kg^{-1})	
— Dry matter	897
— Crude protein	148
— Neutral detergent fiber	493
— Acid detergent fiber	232
— Acid detergent lignin	52

[1] Supplement content by treatment was: CON, control, neither Zn nor monensin added; Z, Zn added; M, monensin added; ZM, both Zn and monensin added.

Table 2. Ingredient composition of the supplements by treatment

Item (g kg^{-1})	CON[1]	Z	M	ZM
Zinc chloride[2]	—	35	—	35
Monensin[3]	—	—	15	15
Sodium chloride	150	150	150	150
Wheat middlings	840	815	835	800

[1] CON: control. Z: Zn added. M: monensin added. ZM: both Zn and monensin added. [2] Anhydrous $ZnCl_2$, included to provide 430 mg kg^{-1} DM. [3] Monensin commercial premix, included to provide 35 mg of monensin kg^{-1} DM.

by animal within treatment, and were dried in a forced air oven at 60°C for 72 h. After drying, each sample was ground through a 2-mm screen in a Wiley mill (Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) and stored for later analyses. Samples of dry feed, orts and ground feces were analyzed for DM; crude protein (CP = g N per 100 g DM × 6.25) using macro-Kjeldahl N analyses (AOAC, 1990); neutral detergent fiber (NDF); acid detergent fiber (ADF); and acid detergent lignin (ADL) (Goering and Van Soest, 1970). Unshrunk animal weights were recorded at 09:00 h and simultaneously, blood samples were taken by jugular venipuncture at 0, 25, 51 and 77 d after the initiation of the experiment. After a 15-d adaptation period, and for the length of the trial (77 d), samples of alfalfa, barley and sunflower meal were obtained once daily before feeding the first meal. Feed samples were saved at room temperature for chemical analyses. Orts and fresh fecal grab samples from individual pens were collected once daily, before feeding the morning meal, during the 77 d-period. The samples were frozen and preserved in plastic bags. At the end of the trial, orts and feces samples were unfrozen, composited by animal, dried, ground as indicated above, and saved for chemical analyses. For each animal, daily DM intake (DMI) was determined by recording the weight of feed offered each day minus the rejected feed collected the morning after. Total feed DM digestibility (DMD) was estimated using lignin as an internal marker. Average daily gains (ADG) and feed to gain (F/G) ratio were computed from liveweight differences and intake data. Blood serum analyses were performed for hematocrit (HTC). Glucose (GLU), urea (U), total protein (TP), alkaline phosphatase (ALKP) and creatinine (CRT) were also determined by automated methods.

Experiment 2

Animals, diets and experimental procedure

Four Aberdeen Angus steers (averaging 404 ± 9.7 kg initial liveweight) were randomly allocated to individual pens. Animals were allocated to a 4×4 Latin square design and received the same diets described for Exp 1. DMI was fixed for all treatments at 10.3 kg DM d^{-1} (divided in two equal meals at 08:00 and 17:00 h). Daily consumption was restricted to 10-20% below maximum intake to facilitate rumen sampling. Each period of the Latin square consisted of: i) dietary adaptation on days 1 through 7; ii) sampling of rumen contents on day 8; and iii) marker labeling of the fluid phase and *in situ* incubation of Dacron bags on days 9 through 11. Fecal grab samples were also collected twice daily (at 09:00 and 17:00 h) on days 9 through 11, composite within animal and period, dried at 60°C and ground through a 2-mm screen to estimate DMD by using acid-insoluble ash (AIA) from the ADF fraction as an inherent marker (Van Soest *et al*., 1991).

Chemical determinations in ruminal fluid

On day 8 of each period, ruminal fluid was obtained at 2, 4, 8, 12, and 24 h after the morning meal and filtered through four layers of cheesecloth. The pH of the filtrate was measured immediately. To stop microbial activity, 200 mL of filtered fluid were acidified with 2 mL of a 7.2 N HCl solution (Merchen *et al*., 1986) and frozen for future analyses. Rumen liquid solutions were prepared from rumen samples according to the acid-soluble Zn protocol (AOAC, 1990). Next, Zn concentrations in rumen fluid were measured by atomic emission spectrophotometry (ICPS Shimadzu model 1000III). A colorimetric procedure was used to evaluate NH_3-N concentrations using 50-µL aliquots from ruminal samples and a Beckman spectrophotometer (Beckman, DU 64, Beckman Instruments, Inc., Fullerton, CA) as previously described (Broderick and Kang, 1980). Samples were deproteinized with ortophosphoric acid (2%, v/v), centrifuged at $10,000 \times$ g during 20 min at 4°C, and the supernatant was kept frozen until VFA (volatile fatty acids) analyses were carried out. Ruminal fluid VFA concentrations were determined by using a SHIMADZU GC-14A chromatograph equipped with a FID detector and a NUKOL column (30 m $\times$ 0.25 mm $\times$ 0.25 µm). Helium was used as a carrier and 4-methylvaleric acid as the internal standard.

Ruminal fluid phase labeling and kinetics calculations

A Co-EDTA marker was used to estimate rumen fluid passage (Uden *et al*., 1980). Subsequently, fluid dilution rate (FDR), fluid turnover time (FTT), and rumen fluid volume (RFV) were evaluated by sampling the rumen contents at different time intervals. On day 11, each steer was dosed with 965 mg of Co in 200 mL of Co-EDTA and fluid rumen samples were obtained at 0, 2, 4, 8, 12 and 24 h after dosing. Rumen fluid solutions were prepared from rumen samples according to the acid-soluble Co protocol (AOAC, 1990) and the Co concentration was measured by atomic absorption spectrophotometry (ICPS Shimadzu model 1000III). The FDR and the RFV were calculated from the regression on time of the natural logarithm of marker concentrations, while FTT was estimated from the inverse of passage rate.

In situ *incubation of dietary components*

For the *in situ* study 10×20 cm dacron bags (Ankom Technology Corporation, Fairport, USA) were dried, weighed, filled with 5 g of 2-mm ground alfalfa hay or barley grain, and heat-sealed. The bags were sewed to a polyester cord with a weight attached to its extreme in order to achieve proper immersion in the ventral sac and thus be adequately exposed to the rumen environment for 0, 2, 4, 8, 12, 24, 36 and 72 h after supplement feeding. Prior to insertion through the rumen cannula, all bags were soaked in water at 20°C. After extraction at the corresponding incubation times, bags were immediately rinsed under tap water and subsequently frozen to stop fermentation. They were later rinsed in a washing machine, dried at 60°C, and re-weighed.

The weight data were analyzed by a computer program for estimating degradability constants, by fitting them into the nonlinear equation $PD = a + b\,[1 - e^{(-ct)}]$, where PD was the potential DM degradability after time, t; a, the soluble fraction; b, the potentially degradable insoluble fraction; and c, the rate of degradation of the rumen degradable fraction b (Ørskov and McDonald, 1979). The equation $ED = a + [bc/(c + k)]$ allowed to compute effective rumen degradation of DM for alfalfa hay and barley grain (Ørskov and McDonald, 1979), where ED was effective degradability; a, b and c were the same variables as described above for the non-linear equation; and k, the rumen fractional dilution rate. The

Table 3. Performance parameters of Aberdeen Angus steers receiving a barley grain + alfalfa hay + sunflower meal diet, with Zn and(or) monensin added

Item	CON[1]	Z	M	ZM	SEM[2]	P value
Average daily gain (g d^{-1})	1,013	1,008	1,026	982	53.4	0.949
DM intake (kg d^{-1})	7.74	7.99	7.09	7.36	0.27	0.122
Feed/gain ratio (kg kg^{-1})	7.74	8.09	6.91	7.52	0.45	0.338
DM digestibility[3] (g kg^{-1})	623	632	665	655	2.45	0.628

[1] CON: control. Z: Zn added. M: monensin added. ZM: both Zn and monensin added. [2] SEM: standard error of the mean. [3] DM digestibility calculated using lignin as internal marker.

fractional dilution rate (which refers to the particulate outflow rate) used was $k = 3.61\%$ for barley and $k = 3.29\%$ for alfalfa, as proposed by Rodríguez Cortés (1996).

Statistical analysis

Analyses of variance for both experiments were performed using the GLM procedure of SAS (1996) for a completely randomized design (Exp 1) and a 4×4 Latin square design (Exp 2). In Exp 1, treatment was included in the model and the residual error was used to test its effect. In Exp 2, digesta flow, digestibility, and rumen feed degradation were analyzed with treatment, period, and animal within square as effects in the model tested against the residual error term. For time-sequence data (Zn concentration, pH, NH$_3$-N, and VFA), the effects of treatment were tested with the treatment $\times$ animal interaction, and sampling hour was tested with the treatment $\times$ sampling hour interaction, as the error term. When a significant *F*-test was detected ($P < 0.05$) for treatment or hour main effects, Tukey's test was used as the mean separation procedure. In case of missing values, least square means, rather than means, are reported.

Results

Animal performance and blood profile

No treatment response was observed at earlier time periods for ADG and DMI (days 1 to 25, 26 to 51, and 52 to 77; $P > 0.10$). Therefore, only the treatment means for these two variables, as well as the DMD and the F/G ratio, are reported in Table 3. Overall ADG, DMI and DMD values were 1007 g d^{-1}, 7.6 kg DM d^{-1}, and 644 g kg^{-1} DM, respectively. None of these performance parameters were significantly affected by the addition of Zn, monensin or both ($P > 0.10$). Likewise, the averages of the blood serum measurements for each animal across sampling dates are shown, since there were no effects detected for treatment or time ($P > 0.10$; Table 4).

Ruminal and digestion measurements

The concentration of Zn was affected by an interaction between the treatment and the sampling time ($P = 0.0174$). Table 5 shows the ruminal levels of Zn by sampling hour for every treatment. Overall, Zn concentration exhibited a large variability, with averages of 3.01 ± 0.63,

Table 4. Blood serum chemical composition of Aberdeen Angus steers receiving a barley grain + + alfalfa hay + sunflower meal diet, with Zn and(or) monensin added

Item	CON[1]	Z	M	ZM	SEM[2]	P value
Hematocrit (%)	37.5	39.2	36.7	37.0	0.89	0.219
Glucose (g L^{-1})	0.91	0.96	0.87	0.89	0.04	0.437
Urea (g L^{-1})	0.38	0.40	0.39	0.39	0.02	0.949
Total protein (g L^{-1})	73.9	73.4	72.4	72.7	1.34	0.834
Alkaline phosphatase (UI L^{-1})	320	292	358	346	38.3	0.614
Creatinine (mg L^{-1})	13.0	12.7	13.5	13.6	0.54	0.449

[1] CON: control. Z: Zn added. M: monensin added. ZM: both Zn and monensin added. [2] SEM: standard error of the mean.

Table 5. Zn levels (mg L^{-1} of ruminal fluid) in cannulated Aberdeen Angus steers receiving a barley grain + alfalfa hay + sunflower meal diet, with Zn and(or) monensin added

Sampling time[1] (h)	CON[2]	Z	M	ZM	SEM[3]
2	3.71	3.53	3.17	8.20	1.33
4	3.37	4.39	2.76	5.65	0.76
8	3.05	3.96	2.32	5.07	0.71
12	2.88[a]	3.40[ab]	2.77[c]	4.12[b]	0.24
24	2.02	2.15	1.92	2.99	0.24

[1] Indicates sampling time after the supplement was fed. [2] CON: control. Z: Zn added. M: monensin added. ZM: both Zn and monensin added. Interaction of treatment × sampling time (P = 0.0174). [3] SEM: standard error of the mean. [a,b,c] Means in the same row with different superscript differ (P < 0.05).

3.49 ± 0.84, 2.59 ± 0.48 and 5.21 ± 1.95 mg L^{-1} of ruminal fluid for the CON, Z, M and ZM treatments, respectively. Ruminal Zn showed the highest numeric values for ZM, followed by Z, between sampling hours 4 and 24. However, significant differences were only detected at 12 h.

Ruminal pH, NH_3-N or VFA profile were not significantly affected by treatment, but differences between sampling hours were found for all parameters (Table 6). The average pH level was 6.48 ± 0.26, and it was lowest (P < 0.05) 12 h after the supplement was given. The NH_3-N concentration ranged during the day from 56 mg L^{-1} to 152 mg L^{-1}, with its lowest level observed before each meal was offered.

Concentrations of acetate exhibited a non-significant decrease of 2.5% for M and of 7.0% for ZM with respect to the average of the CON and Z treatments (P = 0.2283). Although propionate increased as acetate decreased with an acetate/propionate ratio 9% (for M) and 22% (for ZM) lower than the average of CON and Z, none of these treatment effects were significant on VFA molar proportions (P = 0.2587). Across time periods, acetate remained stable, propionate increased, and butyrate decreased after a meal (P < 0.05). At hour 24, when valerate was lowest, isobutyrate and isovalerate reached their highest values (P < 0.05). Isobutyrate and isovalerate increased immediately before the morning meal (P < 0.05).

The ruminal FDR, FTT and RFV were not significantly affected (P > 0.10) by the addition of Zn or monensin (Table 7). There was a trend for the average RFV (P = 0.1284) to increase with Zn addition in Z and ZM, when compared with CON or M.

Total tract DM digestibility as well as rumen DM degradability of different fractions are shown in Table 8. Differences were found only for the potentially

Table 6. Least square means of ruminal parameters in cannulated Aberdeen Angus steers receiving a barley grain + alfalfa hay + sunflower meal diet, with Zn and(or) monensin added

Item	Treatment[1]					Sampling time (h)[2]					
	CON	Z	M	ZM	SE[3]	2	4	8	12	24	SEM[3]
pH	6.6	6.6	6.6	6.5	0.03	6.6[a]	6.4[b]	6.5[ab]	6.1[c]	6.8[d]	0.04
NH_3-N (mg L^{-1})	115	111	112	96	0.80	134[ac]	109[a]	56[b]	152[c]	109[a]	0.98
Total VFA (mmol L^{-1})	40.5	47.7	42.5	45.9	2.83	41.5[a]	47.4[a]	41.2[a]	52.8[b]	38.0[c]	3.17
Acetate (mmol mol^{-1})	505	504	492	469	2.7	499	493	490	489	491	3.0
Propionate (mmol mol^{-1})	244	219	252	291	4.0	234[a]	255[b]	263[ab]	278[b]	228[c]	4.4
Butyrate (mmol mol^{-1})	187	209	192	180	3.6	201[a]	192[ab]	187[b]	174[c]	206[a]	4.0
Isobutyrate (mmol mol^{-1})	17	17	18	19	1.0	17[a]	16[a]	16[a]	16[a]	23[b]	1.1
Valerate (mmol mol^{-1})	20	20	20	17	0.6	21[a]	20[ab]	18[b]	20[ab]	17[c]	0.7
Isovalerate (mmol mol^{-1})	27	32	26	25	0.9	27[a]	24[a]	26[a]	23[a]	36[b]	1.0
Acetate/propionate ratio	2.1	2.3	2.0	1.7	0.04	2.2[a]	2.0[b]	1.9[c]	1.8[c]	2.3[a]	0.04

[1] CON: control. Z: Zn added. M: monensin added. ZM: both Zn and monensin added. No treatment effect was found for any of the measurements (P > 0.10). [2] Indicates sampling time after the supplement was fed. [3] SEM: standard error of the mean. [a,b,c] Sampling time effect: means in the same row with different superscript differ (P < 0.05).

Table 7. Ruminal fluid kinetics for cannulated Aberdeen Angus steers receiving a barley grain +
+ alfalfa hay + sunflower meal diet, with Zn and(or) monensin added

Item[1]	CON[2]	Z	M	ZM	SEM[3]	P value
FDR (% h^{-1})	12.94	12.42	12.97	12.24	0.25	0.197
FTT (h)	7.73	8.08	7.70	8.20	0.17	0.192
RFV (L)	44.50	54.38	46.53	56.30	3.45	0.128

[1] FDR: rumen fluid dilution rate. FTT: rumen fluid turnover time. RFV: rumen fluid volume. [2] CON: control. Z: Zn added. M: monensin added. ZM: both Zn and monensin added. [3] SEM: standard error of the mean.

degradable insoluble fraction of the DM (fraction *b*) in alfalfa (P = 0.0038), with a trend for barley grain (P = 0.0653).

Discussion

Animal performance and blood profile

Despite the numeric decrease in the F/G ratio for M (6.6 kg kg^{-1}) with respect to the averages of the other treatments, the differences observed were not significant. Generally, monensin increases ruminal propionate and decreases ammonia and lactate thus improving overall feed efficiency (Russell and Houlihan, 2003). Certain factors, such as feeding procedures and type and proportion of feed components, could make animal response more variable to the dietary addition of monensin. With regards to monensin, no effects we reported on feed efficiency or other performance parameters when cattle was fed *ad libitum* compared with clean bunk programs (Erickson *et al.*, 2003; Berthiaume *et al.*, 2006). Similar results —regarding monensin addition— were found when high roughage levels were included in the diet (Stock *et al.*, 1990; Meinert *et al.*, 1992; Morais *et al.*, 1993; Berthiaume *et al.*, 2006). The latter relates to an increase in the NDF content. In the present trial, the diet was fed *ad libitum* with a NDF content

Table 8. Total tract diet dry matter digestibility and rumen *in situ* degradability of the main dietary components alfalfa and barley

Item	CON[1]	Z	M	ZM	SEM[2]
DM digestibility[3] (g kg^{-1})	667	672	704	676	18.1
Alfalfa hay[4]:					
a (g kg^{-1})	168.0	145.8	166.5	171.8	12.5
b (g kg^{-1})	446.5^a	464.5^a	454.0^a	420.7^b	7.8
c (% h^{-1})	0.076	0.084	0.064	0.070	0.009
DMED[5] (g kg^{-1})	477.9	464.5	478.7	458.1	1.25
DMPD[6] (g kg^{-1})	614.5	620.5	610.3	592.5	1.01
Barley grain[4]:					
a (g kg^{-1})	259.0	253.8	269.0	257.8	7.1
b (g kg^{-1})	539.5	551.0	520.8	532.3	9.9
c (% h^{-1})	0.65	0.58	0.63	0.68	0.04
DMED (g kg^{-1})	772.3	763.9	775.2	765.2	5.2
DMPD (g kg^{-1})	798.5	789.8	804.8	790.0	6.5

[1] CON: control. Z: Zn added. M: monensin added. ZM: both Zn and monensin added. [2] SEM: standard error of the mean. [3] Total tract DM digestibility estimated by ADF-AIA as an internal marker. [4] *a:* soluble fraction. *b:* potentially degradable fraction. *c:* rate of degradation of fraction *b*. Fractional dilution rate (k) = 3.29 for alfalfa and 3.61 for barley. [5] DMED: dry matter effective degradability. [6] DMPD: dry matter potential degradability. ^{a,b} Means in the same row with different superscript differ (P < 0.05).

of 493 g kg^{-1} DM. These factors could have increased variability of response to Zn addition in performance parameters as well.

Blood data contrasted with reference values (Boyd, 1985) showed that HTC, TP, and CRT were within the normal ranges. However, regardless of supplementation treatments, GLU, U, and ALKP exceeded the reference values of 0.42–0.75 g L^{-1}, 0.08-0.25 g L^{-1} and 18-153 UI L^{-1}, respectively (Boyd, 1985). Nevertheless, since animals did not show clinical signs or pathological symptoms they were considered «healthy».

Propionate, the main precursor of blood GLU, usually increases with monensin supply (Goodrich *et al*., 1984). Propionate could potentially be increased with high Zn levels as well (Arelovich *et al*., 2000). However, serum GLU levels do not always seem to respond in the same way to changes in propionate concentration. Thus, blood GLU either increased (Broderick, 2004) or was not affected by monensin supply (Plaizier *et al*., 2005), since it depends on the monensin level in the diet, the type of animal, and the dietary components. However, if Zn can alter VFA production, we should at least expect similar factors to influence blood GLU in response to dietary Zn feeding.

The U values across treatments averaged 0.39 mg L^{-1}, which was higher than the proposed reference interval. Feeding U with 400 mg Zn kg^{-1} of DM, blood U was higher and remained high for a longer period of time because Zn delayed the ureolysis rate in the rumen (Arelovich, 1998). This would benefit N recycling, particularly in low-quality diets. In this study, however, only true protein was fed, and the 430 mg Zn or 35 mg monensin kg^{-1} DM added did not alter blood U level compared with the CON treatment. Other authors found no significant effects on blood U-N when Zn (Engle *et al*., 1997) or monensin (Plaizier *et al*., 2005) were included in the diet.

Average ALKP values in this study were much higher than those found by Arelovich (1998) and Shaeffer (2006). Even animals in the CON treatment including only 30 mg Zn kg^{-1} DM had high values. While a Zn deficiency could cause a decrease in ALKP activity or concentration (Underwood and Suttle, 1999), adequate or high dietary levels of Zn would enhance ALKP concentration (Engle *et al.,* 1997; Arelovich, 1998; Shaeffer, 2006). However, high ALKP could be related to the age of cattle rather than to metabolic aspects or liver malfunction. Young cattle, similar to those used in this trial, had serum values several times higher than mature cattle (Otto *et al.,* 2000).

Ruminal fluid chemistry

Exp 2 was carried out in an attempt to find more information to support the results on animal performance (Exp 1). One of the aspects considered was the Zn concentration achieved in the rumen with regard to time. The variability observed in the ruminal concentration of Zn could be attributed to the fact that the Zn supplement was fed with the morning meal and completely consumed within a 1-2 h period. Therefore, Zn must have entered the rumen progressively, and most likely at different rates for each animal. In a previous study with cannulated steers, the whole amount of Zn was given at once through a rumen cannula rather than fed (Arelovich, 1998). For that experiment, the ruminal concentration of Zn as well as its rate of disappearance were more stable than those observed in the present trial. Nonetheless, this aspect should not pose an issue since in everyday farm practices, supplements are fed rather than supplied through a cannula. In consequence, the impact of Zn supplementation on rumen parameters, if any, should be expected under practical as well as experimental conditions. Sheep receiving a low-quality diet plus supplementary Zn and following similar feeding procedures exhibited different effects on their fermentation patterns (Arelovich *et al*., 2003). Therefore, it seems that besides the Zn concentration in the diet, the supplying method, the type of diet, and probably the Zn source interact in their effect on ruminal fermentation and digestion.

Acetate concentration decreased only numerically while propionate increased with an acetate/propionate ratio of 9 and 22% —for M and ZM, respectively— lower than the average for CON + Z (P = 0.2587). If these results had been significant, we could speculate on the apparent additive effect of Zn and monensin, at least when there was a low response to monensin alone. There was a non-significant increase in total VFA with the addition of Zn and(or) monensin compared with the CON (P = 0.8530).

Across time periods, acetate remained stable, propionate increased, and butyrate decreased after a meal. The average pH level (6.5) at 12 h was coincident with the highest total VFA concentration, lowest acetic/propionic ratio and highest NH$_3$-N levels. Low ruminal pH has been associated to VFA accumulation in the rumen (Burrin and Britton, 1986). In this study, the fluctuations observed in ruminal parameters along the day seem more associated to the feeding pattern rather than to the imposed treatments. Nevertheless, the mag-

nitude of the pH fluctuations was not large enough to depress fiber digestion, as it may occur with chronic pH decrease with a much larger grain proportion than was fed in the diets used in this experiment.

The observed range of NH_3-N, 56 to 152 mg L^{-1}, seemed adequate for maximum digestion rate at all sampling hours, and should not have limited bacterial growth (Satter and Slyter, 1974). Previous data indicated that Zn could modulate NH_3-N concentrations when urea was fed (Arelovich *et al*., 2000), and an improved rumen utilization of N was also expected by Zn addition with degradable true protein as done in this trial. Also, beef steers fed high-energy diets responded to monensin when supplemented with true protein. This response improved overall utilization efficiency of feed and N, sparing amino acids from wasteful ruminal degradation (Lana *et al*., 1997). Thus, when contrasting the results of this experiment with the previously mentioned data, Zn seems to improve rumen N economy when low quality forages plus non-protein N are fed; while monensin performs better with high-concentrate diets and true protein with regard to N utilization.

Several strains of rumen bacteria require branched-chain volatile fatty acids for normal growth (Allison *et al*., 1962). However, additives or dietary components can influence branched-chain VFA availability. Thus, these necessary fermentation products can decrease with the addition of monensin or if a source of low degradability-protein, such as fish meal, is supplied (Broderick, 2004). In this study, isobutyrate and isovalerate were particularly high 24 h after receiving the supplement. This could be related to the feeding pattern, indicating a higher catabolism of ruminal amino acids, most likely available from the microbial rumen population, later in the day and immediately before receiving the morning meal.

Ruminal fluid kinetics and degradation of dietary components

The outflow rate for each rumen pool is different, but both the fluid and particulate phases will wash out microbial protein (Illius *et al*., 2000). It is generally accepted that a faster passage rate and an increased turnover time would improve microbial efficiency due to a faster change of rumen population and better supply of microbial protein to the lower tract. Bateman II *et al*. (2004) found that both Zn (500 mg kg^{-1}) and monensin (40 mg kg^{-1}) increased the fluid passage rate when a

50:50 alfalfa:concentrate diet was fed to Holstein cannulated steers. However, «the alfalfa hay was top-dressed with the concentrate» (ground corn + soybean meal + minerals) and fed once daily, while in our experiment, for a similar diet, the supplement was mixed and fed with the morning meal. These differences in the feed processing and the feeding procedures could partially explain the differential effects in fluid kinetics found between both trials. Therefore, an improvement in microbial efficiency that can impact animal performance should not be expected by the addition of Zn and(or) monensin under the same conditions of this experiment.

As expected, in the *in situ* study, the average DMED was lower for alfalfa and higher for barley. This is related to the higher concetration of NDF in alfalfa and the highly degradable starch content in barley grain. Total tract DM digestibility was not affected by the treatment. Even so, a slight, non-significant digestibility increase was observed for M, but not ZM, on each experiment. Lignin is quite satisfactory in rations where lignin content is over 50 g kg^{-1} DM (this diet contained 52 g kg^{-1} DM); and the ADF-AIA procedure is adequate since it recovers all silica (Van Soest, 1994). As fallible as digestibility markers can be, relative estimates should be quite accurate, therefore, neither lignin nor ADF-AIA disappearance was expected to be altered by Zn or monensin addition.

In conclusion, previous findings suggested a potential for high Zn concentrations to alter rumen fermentation patterns thus increasing feed efficiency and consequently animal productivity, in a way that resembled monensin's effects. However, the addition of a 430 mg of Zn and(or) 35 mg of monensin kg^{-1} diet DM did not substantially affect animal performance, digestion or rumen kinetics measurements in beef cattle receiving an alfalfa hay, barley grain and sunflower meal diet. The type of diet, feed processing, source of Zn, and feeding frequency of the supplement could differentially affect animal response to Zn supply. In the view of these results more studies emphasizing these topics would be necessary, since Zn sources could be more widely accepted and economically affordable as a feed additive than monensin.

Acknowledgments

Nacional del Sur (UNS), the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC) and to CERZOS-UNS for assistance in this project.

References

ALLISON M.J., BRYANT M.P., KATZ I., KEENEY M., 1962. Metabolic function of branched-chain volatile fatty acids, growth factors for ruminococci II. Biosynthesis of higher branched-chain fatty acids and aldehydes. J Bacteriol 83, 1084-1093.

AOAC, 1990. Official methods of analyses, 15th ed. Association of Official Analytical Chemists. Washington, DC.

ARELOVICH H.M., 1998. Effects of zinc and manganese on digestion, ruminal and blood parameters of cattle fed prairie hay. Doctoral thesis. Oklahoma State University Stillwater, OK, USA. 141 pp.

ARELOVICH H.M., OWENS F.N., HORN G.W., VIZCARRA J.A., 2000. Effects of supplemental zinc and manganese on ruminal fermentation, forage intake and digestion by cattle fed prairie hay and urea. J Anim Sci 78, 2972-2979.

ARELOVICH H.M., AMELA M.I., MARTÍNEZ M.F., TORREA M.B., LABORDE H.E., 2003. Diferentes fuentes de zinc en el suplemento proteico de ovinos alimentados con un forraje de baja calidad. 2. Parámetros ruminales. Rev Argentina Prod Anim 23(Supp 1), 88. [In Spanish].

BATEMAN H.G. II, WILLIAMS C.C., GANTT D.T., CHUNG Y.H., BEEM A.E., STANLEY C.C., GOODIER G.E., HOYT P.G., WARD J.D., BUNTING L.D., 2004. Effects of zinc and sodium monensin on ruminal degradation of Lysine-HCl and liquid 2-hydroxy-4-methylthio-butaoic acid. J Dairy Sci 87, 2571-2577.

BERTHIAUME R., MANDELL I., FAUCITANO L., LAFRENIERE C., 2006. Comparison of alternative beef production systems based on forage finishing or grain forage diets with or without growth promotants, 1. Feedlot performance, carcass quality, and production costs. J Anim Sci 84, 2168-2177. doi:10.2527/jas.2005-328.

BOYD J.W., 1985. The interpretation of serum biochemistry test results in domestic animals. Vet Clin Pathol 13, 7-14.

BRODERICK G.A., 2004. Effect of low level monensin supplementation on the production of dairy cows fed alfalfa silage. J Dairy Sci 87, 359-368.

BRODERICK G.A., KANG J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. J Dairy Sci 63, 64-75.

BURRIN D.G., BRITTON R.A., 1986. Response to monens in in cattle during subacute acidosis. J Anim Sci 63, 888-893.

CALLAWAY T.R., EDRINGTON T.S., RYCHLIK J.L., GENOVESE K.J., POOLE T.L., JUNG Y.S., BISCHOFF K.M., ANDERSON R.C., NISBET D.J., 2003. Ionophores, their use as ruminant growth promotants and impact on food safety. Curr Issues Intest Microbiol 4, 43-51.

CIOMS, 1985. International guiding principles for biomedical research involving animals. Council for International Organizations of Medical Sciences. Available in: http//www.cioms.ch/frame_1985_texts_of_guidelines.htm [10 March, 2007].

ENGLE T.E., NOCKELS C.F., HOSSNER K.L., KIMBERLING C.V., TOOMBS R.E., YEMM R.S., WEABER D.L., JOHNSON A.B., 1997. Marginal zinc deficiency affects biochemical and physiological parameters in beef heifer calves. Asian Australasian J Anim Sci 10, 471-477.

ERICKSON G.E., MILTON C.T., FANNING K.C., COOPER R.J., SWINGLE R.S., PARROTT J.C., VOGEL G., KLOPFENSTEIN T.J., 2003. Interaction between bunk management and monensin concentration on finishing performance, feeding behavior, and ruminal metabolism during an acidosis challenge with feedlot cattle. J Anim Sci 81, 2869-2879.

FAIXOVA Z., FAIX S., 2002. Influence of metal ions on ruminal enzymes activities. Acta Vet BRNO 71, 451-455.

GOERING H.K., VAN SOEST P.J., 1970. Forage fiber analyses (apparatus, reagents, procedures and some applications). USDA-ARS, Agricultural Handbook, US Department of Agriculture, No. 379, Washington, DC.

GOODRICH R.D., GARRETT J.E., GAST D.R., KIRICK M.A., LARSON D.A., MEISKE J.C., 1984. Influence of monensin on the performance of cattle. J Anim Sci 58, 1484-1498.

ILLIUS A.W., JESSO N.S., GILL M., 2000. Mathematical models of food intake and metabolism in ruminants. In: Ruminant physiology, digestion, metabolism growth and reproduction (Cronjé P.B., ed). CABI Publishing, Wallingford, UK, pp. 21-40.

LANA R.P., FOX D.G., RUSSELL J.B., PERRY T.C., 1997. Influence of monensin on holstein steers fed high-concentrate diets containing soybean meal or urea. J Anim Sci 75, 2571-2579.

MARTÍNEZ A., CHURCH D.C., 1970. Effect of various mineral elements on in vitro rumen cellulose digestion. J Anim Sci 31, 982-990.

MEINERT R.A., YANG C.M.J., HEINRICHS A.J., VARGA G.A., 1992. Effect of monensin on growth, reproductive performance, and estimated body composition in Holstein heifers. J Dairy Sci 75, 257-261.

MERCHEN N.R., FIRKINS J.L., BERGER L.L., 1986. Effect of intake and forage level on ruminal turnover rates, bacterial protein synthesis and duodenal amino acid flows in sheep. J Anim Sci 62, 216-225.

MORAIS C.A.C., FONTES C.A.A., LANA R.P., SOARES J.E., QUEIROZ A.C., CAMPOS J.M.S., 1993. Influencia da monensina sobre o ganho de peso, consumo e conversao alimentar em bovinos castrados e nao castrados. Rev Soc Brasileira Zoot 22, 64-71. [In Portuguese].

NRC, 2000. Nutrient requirements of beef cattle. 7th ed. update. National Research Council, National Academy Press, Washington DC. 242 pp.

OIE, 2002. On line report of the Meeting of the OIE Working Group on Animal Welfare. World Organization of Animal

Health. Available in http://www.oie.int/eng/bien_ etre/ AW_WG_october2002_eng.pdf, 16 pp. [12 February, 2007].

ØRSKOV E.R., MCDONALD I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J Agr Sci 92, 499-503.

OTTO F., VILELA F., HARUN M., TAYLOR G., BAGGASSE P., BOGIN E., 2000. Biochemical blood profile of angoni cattle in Mozambique. Israel J Vet Med [Serial On line], 55(3). Available in http://www.isrvma.org/ article/55_3.htm [17 April 2007].

PAPADEMETRIOU T., 2006. Recent developments in the European Union. World Law Bulletin. The Law Library of Congress. Available in http://www.fas.org/sgp/ othergov/wlb/200602.pdf [18 December, 2007].

PLAIZIER J.C., FAIRFIELD A.M., AZEVEDO P.A., NIKKHAH A., DUFFIELD T.F., CROW G.H., BAGG R., DICK P., MCBRIDE B.W., 2005. Effects of monensin and stage of lactation on variation of blood metabolites within twenty-four hours in dairy cows. J Dairy Sci 88, 3595-3602.

RODRÍGUEZ B.T., ARELOVICH H.M., VILLALBA J.J., LABORDE H.E., 1995. Dietary supplementation with zinc and manganese improves the efficiency of nitrogen utilization by lambs. J Anim Sci 37,1233 (Abstract).

RODRÍGUEZ CORTÉS C.A., 1996. Estudio de la colonización microbiana de los alimentos en el rumen. Implicaciones sobre la estimación de la degradabilidad ruminal de las materias nitrogenadas mediante técnicas *in situ*. Doctoral thesis. Universidad Politécnica, Madrid. 288 pp. [In Spanish].

RUSSELL J.B., HOULIHAN A.J., 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microb Rev 27, 65-74. doi:10.1016/ S0168-6445(03)00019-6.

SAS, 1996. The SAS System for Windows (release 6.08). SAS Inst Inc Cary, NC.

SATTER L.D., SLYTER L.L., 1974. Effect of ammonia concentration on rumen microbial protein *in vitro*. Br J Nutr 32, 199-208.

SHAEFFER G.L., 2006. Evaluaton of basic zinc chloride as a zinc source for cattle. Doctoral Thesis. North Carolina State University Raleigh, NC. 68 pp.

SPEARS J.W., HATFIELD E.E., 1978. Nickel for ruminants. Influence of dietary nickel on rumen urease activity. J Anim Sci 47, 1345-1350.

STOCK R.A., SINDT M.H., PARROTT J.C., GOEDEKEN F.K., 1990. Effects of grain type, roughage level and monensin level on finishing cattle performance. J Anim Sci 68, 3441-3455.

UDEN P., COLUCCI D.E., VAN SOEST P.J., 1980. Investigation of chromium, cerium and cobalt as markers in digesta rate of passage studies. J Sci Food Agric 31, 625-632.

UNDERWOOD E.J., SUTTLE N.F., 1999. Zinc. In: The mineral nutrition of livestock (Underwood E.J., Suttle N.F., eds). CABI Publishing, NY, USA. pp. 477-512.

VAN SOEST P.J., 1994. Nutritional ecology of the ruminant. Cornell Univ Press, Ithaca, USA. 476 pp.

VAN SOEST P.J., ROBERTSON J.B., LEWIS B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74, 3583-3597.