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Effects of Monensin, Virginiamycin and Sodium Bicarbonate on Ruminal Fermentation and Acid-Base Status in Sheep

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Abstract: Four ruminally canulated sheep (55±10 kg initial BW) were used in a 4×4 Latin square design to evaluate the effects of monensin, virginiamycin and sodium bicarbonate on ruminal fermentation and acid-base balance in sheep fed a diet with 60% concentrate (DM basis). Treatments included control, monensin (25 mg d^{-1}), virginamycin (15 mg d^{-1}) and sodium bicarbonate (10 g d^{-1}) intraruminally. Each period included 14 d of adaptation and 4 d of sample collection. Ruminal fluid samples were collected at 0, 2, 4, 6, 8 and 10 h after the additive dose. Blood samples were collected at 0 and 6 h to determine pH, HCO_3, pCO_2, base excess, electrolytes (Na^+, K^+, Cl^-) and other metabolites (glucose, urea, L-lactate, NEFA). Intake was increased ($p<0.05$) with virginiamycin in comparison with sodium bicarbonate. Addition of sodium bicarbonate reduced significantly ($p<0.05$) DM intake. Sheep with monensin showed increased proportion of propionate at time of feeding, while virginiamycin lowered percentage of acetate in rumen liquor sampled 10 hours after feeding. Protozoa counts were not affected by the additives. Monensin, virginiamycin or sodium bicarbonate did not affected acid base status in sheep.

Key words: Monensin, virginiamycin, sodium bicarbonate, sheep, fermentation

INTRODUCTION

Sheep production in Mexico has been changed from grazing systems to intensive feeding with concentrates with the potential risks of subacute acidosis (Mendoza *et al*., 2007). Even when on a BW basis sheep can consume more feed than cattle (Slyter, 1976), generally are less susceptible of larges proportion of readily fermentable carbohydrates in the diet.

The use of additives in sheep production is not a common practice even when these modifiers may reduce metabolic disorders and improve gain or feed efficiency (Plata *et al*., 2004). In addition, dietary changes in ruminal fermentation could have an impact on acid balance status associated to the VFA from rumen to blood.

Ionophores have been used in cattle to manipulate ruminal fermentation, increasing propionate and reducing lactate producing bacteria (Russel and Strobel, 1989) and in sheep to improve weight gain or feed efficiency (Muwalla *et al*., 1994). Virginiamycin is an antibiotic which may alter rumen fermentation reducing the lactate ruminal and liver abscess (Godfrey *et al*., 1995; Nagaraja *et al*., 1995; Rogers *et al*., 1995) and improves sheep performance (Murray *et al.,* 1992). Sodium bicarbonate has been used as a buffer to maintenance of ruminal pH, but increases ruminal osmotic pressure and liquid dilution rate (Roger and Davis, 1982; Sanchez *et al*., 1997). Those additives may also have a beneficial effect on electrolytic balance in intensive systems of sheep production with increasing levels of concentrate. Physiological studies considering relationship between acid balance status and ruminal fermentation have been conducted in cattle with acute and subacute acidosis (Brown *et al*., 2000), however, data of additives in sheep are scarce. The objective of this experiment was to evaluate these additives on ruminal fermentation and in blood acid balance of sheep feed a diet with 60% concentrate.

MATERIALS AND METHODS

Four sheep (Dorset×Suffolk; 55±10 kg BW) were ruminally canulated and assigned to 1 of 4 treatments in a 4×4 Latin Square Design. Each period included 14 d of adaptation and 4 d of sample collection of ruminal fluid

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and blood. Treatments consisted of monensin (25 mg d^{-1}; Elanco™), virginiamycin (15 mg d^{-1}; Pfizer™), sodium bicarbonate (10 g d^{-1}) and a control without additive. Feed additives were placed daily through the ruminal cannula at the morning feeding time (08:00).

Sheep were fed with a complete diet with 30% alfalfa hay, 10% oat hay and 60% concentrate (15% CP) with corn grain (71%), molasses (12%) and soybean meal (17%) and a mineral premix. Intake was measured daily and feed and orts were sampled during each collection period, to determine Dry Matter (DM), Crude Protein (CP) (AOAC, 1990), Neuter Detergent Fiber (NDF) (Van Soest *et al*., 1991) and starch (Mendoza *et al*., 1999). Total diet composition (DM basis) was: 16.1% CP, 38.8% NDF and 32.8% starch.

Ruminal fluid (50 mL) was sampled at 0, 2, 4, 6, 8 and 10 h post feeding and pH was measured immediately. Ruminal contents were strained through four layers of cheesecloth, acidified with 1 mL of HCl 6 N and stored in a freezer for further analysis. Volatile Fatty Acids (VFA) were determined with gas chromatography in samples prepared with metaphosporic acid (Erwin *et al*., 1961). L (+) lactate was determined enzymatically with lactate dehydrogenase (Sigma Diagnostics, procedure No. 735, St. Louis, MO). A ruminal fluid sample with a 5-mL iodine solution was used to count protozoa (Dehority, 1984).

Osmolality of ruminal fluid was determined by freezing point depression using an osmometer Wiscor 5100C (Brown *et al*., 2000). Sheep were dosed on day 2 of collection period with 50 mL of Co-EDTA solution, prepared as described by Uden *et al.* (1980) to estimate rumen volume and fluid passage rate. Ruminal fluid samples were collected 0, 3, 6, 9, 12, 24 and 36 h after dosing. Rumen fluid was centrifuged (30,000×g for 15 min) and cobalt was measured by atomic absorption spectroscopy.

Venous whole blood was sampled by jugular puncture with vacutainer tubes and with heparinized syringe at 0 and 6 h. Blood was collected with heparin was used to determine blood gases, pH, base excess, HCO_3, pCO_2 with a pH/Blood Gas Analyzer (Corning Model 238; Ciba Corning Diagnostics, Medfield, MA). Blood collected with vacutainer tubes was used to determine plasma NEFA (Waco Chemicals, Code No. 994-75409F), L (+) lactate (Sigma Diagnostics, procedure No. 735, St. Louis, MO), urea, creatinine and glucose (Diagnostic Chemicals Limited, Cat No. 275-06, 221-30 and 220-32, Charlottetown, CA) using a chemical analyzer (Model Roche Cobas Mira, Roche Diagnostic, Basle, Switzerland). Serum was harvested from venous whole blood to determine Na^+, K^+ and Cl^- with an electrolytic analyzer (Ciba Corning 644, Ciba Corning Diagnostics, Medfield, MA). Data were analyzed as a 4×4 Latin Square Design and means were compared using the Tukey test. A repeated-measures analysis was used for variables sampled at different times, in order to detect time x treatment interaction. Blood metabolites and ruminal fermentation variables were subjected to correlation analysis (SAS, 1988).

RESULTS AND DISCUSSION

Addition of sodium bicarbonate reduced DM intake respect to the control (Table 1). Previous studies have reported minimal effect of virginiamycin on intake (Nagaraja *et al*., 1995; Rogers *et al*., 1995) or reductions in intake (Hedde *et al*., 1980; Murray *et al*., 1992). In this study monensin and bicarbonate did not have an effect ($p>0.05$) on dry matter intake, ruminal volume and fluid rate of passage (Table 1). In diets with 50% concentrate monensin had no effect on intake (Garcia *et al*., 2000). A reduced intake with monensin is usually observed in high grain diets (Lana *et al*., 1997), decreased of dilution rate and increased the percentage of feed digestion (Russell and Strobel, 1989). Yang and Russell (1993), feeding non-lactating Holstein cows with chopped timothy hay and soybean meal with monensin, did not detected changes in ruminal fluid kinetics with ionophore; in contrast, Lemenager *et al.* (1978) in steers fed with forage and monensin observed a substantial reduction on rumen volume, dilution rate and intake. In steers with 50% concentrate and 50% corn silage supplement with sodium bicarbonate, the researchers observed increased feed intake, water intake, rumen pH and fluid dilution rate (Rogers and Davis, 1982). Responses to sodium bicarbonate on intake have been inconsistent, which can be associated to differences of amount of grain in the diet, rate of starch digestion, effective fiber and saliva production (Mattiauda *et al*., 1995; Edwards and Poole, 1983).

Overall, additives did not affect ruminal pH, even when there was a tendency ($p = 0.06$) to increase with sodium bicarbonate at 2 h after feeding. Russel and Chow (1993) suggested that bicarbonates function not by increasing ruminal buffering capacity, but by increasing water intake, ruminal fluid dilution and flow of undegraded starch. Additions of bicarbonate not always increase rumen pH (Mattiauda *et al*., 1995; Khorasani and Kennelly, 2001) which can be related to other factors associated with saliva production or the intrinsic buffer capacity of feeds (Jasaitis *et al*., 1987). It has been proposed that sodium bicarbonate affects buffer capacity and ruminal osmotic pressure and liquid dilution rate (Rogers *et al*., 1979), however, our data related to fluid

Table 1: Effects of monensin (25 mg d^{-1}), virginiamycin (15 mg d^{-1}) and sodium bicarbonate (10 g d^{-1}) on intake and ruminal fluid characteristics in sheep feed a diet with 60% concentrate

Item	Treatment Control	Monensin	Virginiamycin	$NaHCO_3$	SE[1]
DM intake (kg d^{-1})	2.51[ab]	2.49[ab]	2.68[a]	2.44[b]	0.03
Rumen volume (l)	5.17	5.91	5.39	4.42	0.17
Rate of passage (%/h)	4.82	4.57	5.08	4.98	0.01
Osmolality (mOsm kg^{-1})					
0	261	244	264	270	11
2	276	252	271	297	11
4	257	265	254	272	12
6	286	292	287	268	9
8	304	302	301	300	11
10	309	303	317	305	10
Ruminal pH					
0	6.62	6.72	6.82	6.72	0.08
2	6.29	6.32	6.30	6.50	0.04
4	6.02	5.98	6.03	6.04	0.05
6	5.91	5.87	5.89	5.90	0.09
8	5.67	5.72	5.72	5.67	0.06
10	5.65	5.61	5.63	5.64	0.06
Protozoa, organisms×10^4					
0	178	144	157	149	10
2	104	77	109	74	14
4	137	80	105	90	24
6	146	119	120	105	12
8	147	108	123	112	16
10	167	122	51	113	21

[1]Standard error of the mean; [ab] Means with no common superscript in a row differ (p<0.05)

Table 2: Effects of monensin (25 mg d^{-1}), virginiamycin (15 mg d^{-1}) and sodium bicarbonate (10 g d^{-1}) on ruminal fermentation in sheep feed a diet with 60% concentrate

Item and hour	Treatment Control	Monensin	Virginiamycin	$NaHCO_3$	SE[1]
L (+)-lactate (mM)					
0	0.03	0.57	0.41	0.12	0.31
2	0.80	0.73	0.17	0.73	0.55
4	0.004	0.20	0.03	0.08	0.08
6	0.19	0.06	0.06	0.32	0.08
8	0.04	0.09	0.18	0.13	0.11
10	0.58	0.74	0.20	0.31	0.40
VFA (mM)					
0	83.88	79.25	73.82	74.40	9.07
2	95.89	101.48	82.34	84.73	5.42
4	91.76	105.12	100.05	93.02	10.20
6	105.31[ab]	88.33[b]	112.05[ab]	117.69[a]	5.54
8	111.21	111.68	108.27	115.93	6.78
10	120.98	107.93	111.35	115.14	8.93
Acetate (%)					
0	62.74	59.89	61.94	61.46	0.98
2	58.91	56.57	57.82	59.01	0.83
4	60.30	56.34	56.80	58.25	0.99
6	60.39	56.15	56.31	59.47	0.90
8	60.31	56.56	55.39	59.41	1.09
10	60.23[a]	57.11[ab]	55.36[b]	58.33[ab]	0.74
Propionate (%)					
0	23.03[b]	25.06[a]	21.42[b]	22.73[b]	0.38
2	26.82	28.91	27.01	26.14	0.63
4	26.05	29.00	27.65	26.39	0.76
6	25.65	29.49	28.68	26.03	1.12
8	25.86	29.35	29.27	25.53	1.18
10	26.69	29.71	28.96	25.65	1.09
Butyrate (%)					
0	15.19[ab]	13.74[b]	16.64[a]	15.64[ab]	0.41
2	15.22	13.93	15.57	15.04	0.43
4	14.44	13.52	15.32	15.42	0.80
6	14.82	13.44	14.89	15.06	0.80
8	14.52	12.98	14.82	15.88	0.86
10	14.48	13.17	14.83	15.32	0.72

[1] Standard error of the mean, [ab] Means with no common superscript in a row differ (p<0.05)

kinetics do not support that hypothesis. As observed in this study, monensin had not effect on ruminal pH as reported in diets with different levels of concentrates (Muwalla *et al*., 1994; Garcia *et al*., 2000). Addition of virginiamycin did not affect ruminal pH (Torres *et al*., 1999).

In this experiment, there was a negative relationship (p<0.01) between ruminal pH and osmolality (r = -0.71) and VFA concentration with ruminal pH (r = -0.88). The main solutes in ruminal fluid are minerals, VFA, lactate and glucose (Owens *et al*., 1998). Simple correlations indicated a poor relationship between ruminal pH and L-lactate concentration in rumen (r = 0.09, p = 0.60) and blood (r = -0.04, p = 0.82), which is confirmed by the absence of treatment effects on L-lactate concentration in rumen and blood.

Numbers of protozoa were not affected by additives (Table 1). Since ruminal pH and osmolality and dilution rate were not affected, conditions in rumen were similar to maintain protozoa population. The effects reported of feed additives on rumen protozoa have been variable. Several studies have reported that dietary monensin either reduces or tends to inhibit ruminal protozoan numbers (Hino, 1981; Mendoza *et al*., 1993). Also, virginiamycin has been reported to decrease numbers of protozoa (Murray *et al*., 1992; Nagaraja *et al*., 1995); however, other studies show no effect of this antibiotic on rumen ciliates (Coe *et al*., 1999).

Ruminal L(+) -lactate (Table 2) was not reduced by feed additives, even when monensin and virginiamycin affects growth of Gram positive lactate producing bacteria (Martin, 1998; Coe *et al*., 1999). A reduction in lactate with monensin, virginiamycin (Owens *et al*., 1998; Clayton *et al*., 1999) and sodium bicarbonate (Russell and Chow, 1993) was expected, however, in studies with induced subacute acidosis, were not able to detect differences in ruminal lactate concentrations (Brown *et al*., 2000). Monensin did not affect lactate concentration in sheep fed diets with 50% concentrate (Garcia *et al*., 2000) or in steers with subacute acidosis (Burrin and Britton, 1986). In several studies with cows fed 50% concentrate, sodium bicarbonate had no effect on ruminal lactate

Table 3: Effects of monensin (25 mg d^{-1}), virginiamycin (15 mg d^{-1}) and sodium bicarbonate (10 g d^{-1}) on blood gases, acid-base status, and blood metabolites in sheep feed a diet with 60% concentrate

Item and hour	Treatment Control	Monensin	Virginiamycin	$NaHCO_3$	SE[1]
Blood pH					
0	7.47	7.46	7.47	7.47	0.005
6	7.46	7.47	7.47	7.49	0.007
Bicarbonate (mEq L^{-1})					
0	24.1	24.2	24.7	24.9	0.4
6	24.2	24.6	25.2	26.3	0.5
pCO_2 (mm Hg)					
0	33.4	34.2	34.4	34.7	0.7
6	33.9	34.2	34.3	34.4	0.4
Base excess (mEq L^{-1})					
0	-0.18	-0.72	0.11	0.27	0.37
6	-0.21	0.20	0.76	2.09	0.59
Serum Na (mEq L^{-1})					
0	145.7	145.3	145.4	145.2	0.3
6	147.5	147.4	148.8	147.8	0.5
Serum K (mEq L^{-1})					
0	4.56	4.79	4.65	4.58	0.07
6	4.69	4.69	4.82	4.72	0.07
Serum Cl (mEq L^{-1})					
0	109.8	110.2	109.7	109.5	0.4
6	110.8	111.5	111.6	110.2	0.6
Glucose (mM)					
0	4.2	3.8	3.9	4.3	0.2
6	4.1	4.1	4.0	4.2	0.1
Urea (mM)					
0	8.4	8.0	8.1	8.1	0.2
6	8.7	8.6	9.3	8.8	0.3
Creatinine (µM)					
0	69.2	70.3	70.1	71.7	2.4
6	68.2	71.2	74.6	73.0	1.3
NEFA (mM)					
0	0.131	0.131	0.190	0.180	0.009
6	0.088	0.086	0.094	0.151	0.029
L(+)-lactate (µM)					
0	15.72	14.58	17.68	16.46	1.37
6	17.19	19.81	18.23	18.77	0.66

[1]Standard error of the mean

(Clayton *et al*., 1999; Kennelly *et al*., 1999). Khorasani and Kennelly (2001) did not find changes in ruminal lactate with diets with 50-75% concentrate, with our without buffer.

There were differences in VFA only at 6 post feeding. Propionate was highest with monensin only at 0 h. Virginiamycin reduced acetate proportion at 10 h (Table 2). In several studies monensin has increased propionate and reduced acetate (Garcia *et al*., 2000; Vagnoni *et al*., 1995; Lana *et al*., 1997), which is associated with some toxic effects on Gram positive bacteria (Van Nevel and Demeyer, 1977) and protozoa (Mendoza *et al*., 1993). The reduction in acetate with virginiamycin is generally associated to an increment in propionate *in vitro* and *in vivo* (Hedde *et al*., 1980; Nagaraja *et al*., 1987). Addition of sodium bicarbonate generally increases acetate proportion associated with reductions in propionate (Clayton *et al*., 1999; Kennelly *et al*., 1999; Khorasani and Kennelly, 2001). Rogers *et al*. (1979) conducted studies with Holstein steers infusing sodium bicarbonate with two diets; with high concentrate diet, dilution rate and molar proportion of acetate were increased and propionate was reduced, where as with a high roughage diet, dilution rate was augmented without effect on VFA pattern, as observed in this study.

Acid base status was not affected among treatments or by sampling time (Table 3). In general, the magnitudes of the changes in ruminal fermentation impact the acid base balance (Sanchez *et al*., 1997). It has been reported that subacute acidosis has minimal effects on blood gases and pH (Horn *et al*., 1979; Burrin and Britton, 1986; Brown *et al*., 2000), therefore, VFA and lactate in blood were buffered or concentration was too low to induce changes (Owens *et al*., 1998). The blood buffer systems are able to compensate certain amounts of organic acids. A basic aspect in body fluids is the electro neutrality and some modifications in electrolytes may affect the electrolytic balance of the organism and to have a direct effect on acid base equilibrium (Carlson, 1997). Since, concentrations of ions and cations were not affected by treatments (Table 3), electrolytic balance remained unchanged. Sánchez *et al*. (1997) observed that a mixture of $NaHCO_3$, NaCl and KCl in a mineral supplement for dairy cows showed minimal changes in the acid base equilibrium. Blood metabolites were not affected by additives, indicating that changes in VFA pattern were insufficient to affect energy metabolism. Monensin has improved glucose concentration in dairy cows and reduced blood NEFA (McGuffey *et al*., 2001), however, there was no effect in this experiment. It is important to indicate information relating ruminal modifiers and acid base status is scarce.

CONCLUSION

There were minimal effects of additives on ruminal fermentation, blood pH and acid-base balance in sheep fed 60% concentrate. The impacts of feed additives to modify systemic metabolic profile may depend on the level of grain, rate of starch digestion and fiber characteristics in the diet, which need more research in sheep kept in intensive systems.

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