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Nutrient intake, digestibility, mastication and ruminal fermentation of Pelibuey lambs fed finishing diets with ionophore (monensin or lasalocid) and sodium malate

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ABSTRACT

The effects of fermentation and digestion characteristics of lambs fed high grain finishing diets with two ionophores (monensin and lasalocid) were compared, and the additive response of malate in rations containing ionophores were evaluated. Twenty 4-month-old Pelibuey lambs, weighing approximately 16 kg, were assigned to a completely randomize designed experiment with a 2×2 factorial arrangement of treatments (two ionophores with or without malate). The four treatment groups (diets) were: (1) diet with 30 parts per million (ppm) lasalocid, (2) diet with 30 ppm lasalocid and 0.3% malate, (3) diet with 30 ppm monensin, and (4) diet with 30 ppm monensin and 0.3% malate. Animals were confined to individual metabolic cages. Body weight during the sampling phase averaged 20.6 kg. Lambs fed diets with monensin had a 10.9% lower ($P < 0.05$) dry matter (DM) intake ($g/kg^{0.75}$) than those fed lasalocid. Malate had no effect ($P > 0.05$) on DM intake. Lambs fed monensin had lower ($P < 0.01$) NDF intake (37.8%) than those fed lasalocid. When malate was included in the diet, NDF intake was also reduced ($P < 0.05$) by about 25%. However, no difference ($P > 0.05$) in NDF or NFC digestibilities was observed between ionophores or by the addition of malate. Although time spent ruminating was 33% lower ($P < 0.05$) for lambs fed monensin diets, time dedicated to eating was not different ($P > 0.05$) between ionophores. Ruminal pH was similar ($P > 0.05$) for all treatments (5.8). Type of ionophore had no effect ($P > 0.05$) on concentration or molar percent of VFA, whereas the inclusion of malate increased ($P < 0.05$) acetic acid concentration in rumen fluid (60.5 mM vs. 48.2 mM). Nitrogen balance was greater for lambs fed lasalocid diets, which had a higher crude protein intake, than lambs fed diets with monensin. Lambs fed diets with monensin or malate consumed less NDF, and dedicated less time to ruminate than those fed lasalocid. Lambs on the lasalocid diets consumed more fiber, which might be attributed to a greater selection of fibrous components of the diet.

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1. Introduction

To improve productivity and meat quality, producers confine and feed lambs with finishing diets with more

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than 80% grain. Rumen acidosis is commonly caused by high grain consumption, which is highly fermented in the rumen. Feeding management (forage to concentrate ratio, forage and grain particle size, bunk management) and non-nutritive additives (ionophores, malate, direct-fed microbials, buffers) may help reduce ruminal acidosis and other health related problems (Owens et al., 1998).

Lasalocid, an ionophore, is a non-nutritive additive that is commonly included in finishing diets of lambs in feedlots. Ionophores modify ruminal fermentation, improve energy utilization and consequently increase feed efficiency (Bergen and Bates, 1984). Monensin is another ionophore that has been extensively used for more than 30 years in beef feedlot diets to improve feed efficiency and prevent acidosis. Monensin may exert an influence on electron flow by reorienting electrons to metabolic intermediates that may serve as electron sinks, resulting decreases in methane and acetate production, and an increase in propionate production. Ionophores inhibited growth of Gram-positive bacteria such as *Streptococus bovis* and altered ruminal fermentation (Bergen and Bates, 1984).

Malate, a four-carbon dicarboxylic acid, commercially available as the disodium salt (DL-sodium malate), instead of inhibiting microbial fermentation as do ionophores, appears to increase the utilization of ruminal lactate, especially by the predominant bacteria *Selenomonas ruminantium* (Nisbet and Martin, 1990, 1991, 1993, 1994). Since it is an intermediate of the citric acid cycle, malate accumulates in plant tissue. High quality forages have a considerable amount of malate and supplementation of malate in low forage diets may be beneficial in reducing acidosis (Martin et al., 2000).

Malate increased concentrations of propionate, total volatile fatty acids and pH in fermentation studies with mixed rumen microorganisms fed soluble starch and cracked corn (Martin and Streeter, 1995). Callaway and Martin (1996) reported that addition of malate to in vitro mixed rumen microorganism fermentations yielded beneficial results. Through increases in rumen pH and propionate production, these results were independent of monensin treatment. Reduced lactate accumulation was most likely accomplished by decreased lactate production by *S. bovis* and increased lactate utilization by *S. ruminantium*. Therefore, the objective of this experiment was to evaluate the effect of malate (0 and 0.3%) on intake, digestibility, rumen pH and VFA concentrations, and nitrogen balance of lambs fed high grain finishing diets containing two ionophores (lasalocid or monensin).

2. Experimental procedures

2.1. Animals and treatments

Twenty 4-month-old Pelibuey lambs, purchased at an average BW of 16 kg, were randomly assigned to four groups in a completely randomize designed experiment with a 2 × 2 factorial arrangement of treatments (two ionophores with and without DL-sodium malate): (1) diet with 30 ppm lasalocid, (2) diet with 30 ppm lasalocid and 0.3% sodium malate, (3) diet with 30 ppm monensin, and (4) diet with 30 ppm monensin and 0.3% sodium malate. The level of malate chosen was based on the study by Martin et al. (1999) and the recommendation of a Spanish company, NOREL while ionophore levels were based on the level recommended for beef cattle. The basal diet is presented in Table 1. A ton of feed was mixed and separated it into 4 different batches which were further mixed with the additives. Ionophores were included in the vitamin-trace mineral premix and 3 kg/ton of sodium malate was added. Lambs were weight twice, before and after the 7-d sampling phase, resulting in an average BW of 20.6 kg. This value was used to calculate DM intake on a metabolic BW basis ($kg^{0.75}$).

Table 1
Basal diet fed to lambs with one of two ionophores (monensin or lasalocid), and with or without sodium malate.

Ingredient	kg/ton
Sorghum, grain[a]	700
Soybean meal	115
Soybean hulls	100
Molasses	60
Calcium carbonate	14
Urea	5
Salt	4
Premix[b]	2
Chemical composition	
Crude protein, %	15.7
NEm, Mcal/kg[c,d]	1.91
NEg, Mcal/kg[c,d]	1.24
Neutral detergent fiber, %	19.3
Calcium, %	0.79
Phosphorous, %	0.35

[a] Grain: 50% whole and 50% ground.
[b] Trace minerals, vitamins A and E, monensin or lasalocid (30 g/ton). Sodium malate (3 kg) substituted sorghum grain in one of the diets.
[c] NEm, net energy for maintenance; NEg, net energy for gain.
[d] Calculated values (NRC, 2007).

2.2. Management and sample collection

Lambs were randomly assigned and confined to individual metabolism crates with metal slots (65 cm × 90 cm) and feed and water troughs. Lambs were dewormed, vaccinated against clostridial diseases, and injected with vitamins A, D and E prior to the onset of the experiment.

The study had two periods, 21 days to adapt animals to feed and management, and 9 days for sample and data collection. Lambs had free access to feed and water. Feed was offered twice daily (7:45 and 19:00 h). In order to determine voluntary feed intake, during the first seven day of adaptation period, an additional 10% of feed consumed in the previous day was offered the following day. During the collection phase, animals were fed at the voluntary intake determined during the adaptation phase.

During the first 7 days of the sample collection phase, total feces and urine were collected, weighted, identified and frozen. Offered and rejected feed samples were also obtained and frozen. Rejected feed samples were collected every 24 h, in individual plastic bags for each animal. Feed offered and feed refused were weighted and sampled (10%) for each lamb daily for 7 days. These sub-samples were further mixed into a composite sample by animal, before grinding. Representative samples were stored for analysis. On the ninth day of the sample collection phase, rumen fluid samples were obtained with a stomach tube, 2 h postprandial in the morning.

2.3. Sample analysis

Offered and rejected feeds, and total feces collected were dried individually in an air draft oven at 55 °C for more than 96 h until constant weight (AOAC, 1997). All feed and fecal samples were then ground through a Wiley mill with a 2 mm screen. To evaporate the residual water and calculate absolute DM content and nutrient composition on a dry matter basis, offered and rejected feeds, and fecal composite samples for each animal were dried in an air draft oven at 105 °C (AOAC, 1997). Ether extract (EE) was determined by the Soxhlet method (AOAC, 1997). Ash content was obtained after combustion in a muffle furnace at 550 °C for 3 h.

Nitrogen content of feed, feces and urine was determined using the micro-Kjeldahl method, and crude protein (CP) content was calculated as N × 6.25 (AOAC, 1997). Nitrogen balance (g/d) was calculated subtracting fecal and urinary N excretions from total N intake. Retained N (%) was then obtained by dividing N balance by total N intake (g/d).

Neutral detergent fiber (NDF) was analyzed in feed and fecal samples using the procedure outlined by Goering and Van Soest (1970), with modifications proposed by Harris (1970). Fiber residue was filtered under vacuum (0.75 hp vacuum pump) using Buchner funnels and Whatman #40 ash-less filter paper. Non-fiber carbohydrate (NFC) content, which is readily available for rumen fermentation, was calculated as follows: NFC = DM − (CP + EE + NDF + Ash).

2.4. Chewing activities

On the eighth day, after the 7-day sample collection period, during a 24-h period, eating and rumination activities were registered every 5 min, to determine eating, rumination and total chewing times (Lu et al., 2005).

2.5. Rumen fluid sampling and analysis

Rumen pH was measured immediately after sampling, using a Beckman model 390 pH meter. Rumen fluid samples were rapidly frozen to stop fermentation and centrifuged at 10,000 × *g* for 10 min. Five milliliters of supernatant were mixed with 1 ml of 25% metaphosphoric acid (w/v) containing an internal standard (2 g of ethylbutyric acid), and centrifuged at 10,000 × *g* during 10 min (Goetsch and Galyean, 1983). Aliquots of the supernatant fraction were subjected to a Varian Star 3400 gas chromatograph, to determine acetate, propionate, butyrate and total VFA concentrations.

2.6. Statistical analysis

Data were analyzed using a completely random design with a 2 × 2 factorial arrangement of treatments, using the SAS program (1990). The model used was: $Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + E_{ijk}$ where Y_{ijk} = response variable; μ = mean; A = ionophore type; B = malate; AB = interaction of ionophore type and malate; E_{ijk} = random error.

3. Results

3.1. Effect on DM intake and digestibility

During the 7-day period that was proceeding to the 9-day collection period, the mean DM intake (g/d) of lambs was not different ($P > 0.05$) between the lasalocid (665 g/d) and monensin (563 g/d) treatments, or between the 0 (521 g/d) and 0.3% malate (511 g/d) treatments. However, during the 9-day collection phase, DM intake (g/kg$^{0.75}$) was 10.9% lower ($P < 0.05$) for lambs fed diets with monensin compared with those fed lasalocid (Table 2). Malate had no effect ($P > 0.05$) on DM intake of lambs. Moreover, ionophore type or malate did not affect ($P > 0.05$) diet DM digestibility.

3.2. Effects on NDF and NFC intake and digestibility

Lambs fed monensin had lower ($P < 0.01$) NDF intake (37.8%) than those fed lasalocid (Table 2). When malate was included in the diet, NDF intake was also reduced ($P < 0.05$) by approximately 25%. However, no difference ($P > 0.05$) in NDF digestibility was observed between ionophores or with the addition of malate. Intake and digestibility of NFC were not affected ($P > 0.05$) by the inclusion of either ionophore type or sodium malate (Table 2).

3.3. Chewing activities

The time lambs spent eating was similar ($P > 0.05$) for both ionophores and was not affected ($P > 0.05$) by the addition of malate (Table 3). Rumination time was 33% lower ($P < 0.05$) for lambs fed diets with monensin in comparison with lasalocid, whereas it was 26% lower ($P < 0.05$) when malate was included in the diet. Monensin in the diet reduced ($P < 0.05$) total time lambs spent chewing (eating and rumination) by 22% in comparison with those fed lasalocid. Malate did not affect ($P > 0.06$) total chewing of lambs.

3.4. Effect on pH and VFA production

Rumen pH was not different ($P > 0.05$) among treatments. Average rumen pH of lambs varied from 5.76 to 5.83 (Table 4). Although ionophore type had no effect ($P > 0.05$) on rumen acetate concentration, ruminal acetate increased ($P < 0.05$) with the inclusion of malate from 48.2 to 60.5 mM. Neither ionophore type or malate affected ($P > 0.05$) rumen propionate, butyrate or total VFA concentrations. No differences were detected ($P > 0.05$) for molar percentages of acetate, propionate or butyrate. The acetate to propionate ratio was not different ($P > 0.05$) between ionophores or levels of malate.

Table 2
Effects of ionophores (I, lasalocid or monensin) and sodium malate (M) on dry matter, fiber, and non-fiber carbohydrate intake and digestibility of lambs fed finishing diets.

	Ionophore, 30 ppm		Malate, %		SE[a]	P[b]		
	Lasalocid	Monensin	0	0.3		Ionophore	Malate	I × M[c]
Initial weight, kg	20.8	20.3	21.3	19.8	1.52	0.77	0.33	0.84
DM intake								
g/d	710	624	704	630	42.7	0.06	0.11	0.18
g/kg$^{0.75}$	73.3	65.3	71.0	67.6	3.22	0.03	0.31	0.13
DM digestibility, %	71.8	69.6	73.4	68.0	3.05	0.49	0.10	0.87
Fiber carbohydrates								
NDF intake, g/d	196	122	182	136	16	0.01	0.01	0.03
NDF digestibility, %	56.5	57.3	60.3	53.4	3.9	0.88	0.23	0.07
Non-fiber carbohydrates								
NFC intake, g/d	305	318	320	303	23	0.59	0.45	0.58
NFC digestibility, %	83.9	81.1	83.9	81.2	1.92	0.17	0.18	0.14

[a] SE, standard error of the mean.
[b] P, level of significance.
[c] Ionofore and malate interaction.

Table 3
Effects of ionophores (I, lasalocid or monensin) and sodium malate (M) on chewing activities of pelibuey lambs fed finishing diets.

	Ionophore, 30 ppm		Malate, %		SE[a]	P[b]		
	Lasalocid	Monensin	0	0.3		Ionophore	Malate	I × M[c]
Chewing, min/d								
Rumination	175	117	168	124	20.1	0.012	0.046	0.693
Eating	142	130	142	130	18.3	0.544	0.527	0.501
Total chewing	317	247	310	254	27.8	0.025	0.064	0.874

[a] SE, standard error of the mean.
[b] P, level of significance.
[c] Ionophore and malate interaction.

Table 4
Effects of ionophores (I, lasalocid or monensin) and sodium malate (M) on rumen pH and volatile fatty acids (VFA) of pelibuey lambs fed finishing diets.

	Ionophore, 30 ppm		Malate, %		SE[a]	P[b]		
	Lasalocid	Monensin	0	0.3		Ionofore	Malate	I × M[c]
Rumen pH	5.83	5.76	5.76	5.83	0.15	0.611	0.639	0.435
VFA (mM)								
Acetic acid	55.6	53.1	48.2	60.5	5.41	0.647	0.038	0.637
Propionic acid	52.1	50.8	44.6	58.3	7.63	0.863	0.094	0.742
Butyric acid	13.0	13.0	13.0	12.9	3.56	0.997	0.985	0.361
Total VFA	120.7	116.8	105.8	131.7	12.98	0.769	0.065	0.522
VFA (molar percent)								
Acetic acid	46.4	46.1	45.9	46.8	2.87	0.858	0.735	0.847
Propionic acid	43.3	42.4	41.9	43.8	3.49	0.791	0.599	0.797
Butyric acid	10.1	11.5	12.2	9.4	2.31	0.536	0.234	0.532
Acetate:propionate	1.10	1.16	1.14	1.12	0.16	0.718	0.899	0.953

[a] SE, standard error of the mean.
[b] P, level of significance.
[c] Ionophore and malate interaction.

Table 5
Effects of ionophores (I, lasalocid or monensin) and sodium malate (M) on nitrogen (N) balance of pelibuey lambs fed finishing diets.

	Ionophore, 30 ppm		Malate, %		SE[a]	P[b]		
	Lasalocid	Monensin	0	0.3		Ionophore	Malate	I × M[c]
Urine, ml/d	319	326	341	304	60	0.916	0.537	0.741
N intake, g/d	17.9	15.6	17.8	15.8	1.1	0.050	0.091	0.218
N excreted in feces, g/d	7.3	7.0	6.7	7.6	0.9	0.685	0.336	0.455
N excreted in urine, g/d	3.8	4.9	4.5	4.2	0.2	0.261	0.708	0.948
N balance, g/d	6.8	3.8	6.6	4.1	1.4	0.053	0.106	0.601
Retained N, %	34.2	21.2	32.7	22.7	7.2	0.091	0.186	0.996
Apparent N digestibility, %	58.8	55.4	62.4	51.8	5.0	0.499	0.052	0.733

[a] SE, standard error of the mean.
[b] P, level of significance.
[c] Ionophore and malate interaction.

3.5. Effect on nitrogen retention

No differences for urinary volume between ionophores or with the inclusion of malate were obtained (Table 5). Nitrogen intake and excretion in feces and urine were not affected ($P > 0.05$) by ionophore type or malate in the diet, although N balance (g/d) was higher ($P = 0.05$) for lambs fed the lasalocid treatment. Although no differences in retained N or N digestibility were observed between ionophore treatments, malate in the diet decreased ($P = 0.052$) mean apparent N digestibility from 62.4 to 51.8%.

Nitrogen intake ($P < 0.05$) and N balance ($P = 0.053$) of lambs fed diets with lasalocid were greater than those fed diets with rumensin, which may be related to more DM and energy intakes with these diets. Malate did not affect N intake of lambs ($P > 0.05$). No changes ($P > 0.05$) in fecal or urinary N excretions were observed between ionophores or with the inclusion of malate.

4. Discussion

In this study, the inclusion of 30 ppm monensin (based on the level used in beef cattle feedlot diet recommendation), reduced DM intake ($g/kg^{0.75}$) of finishing lambs, in comparison with lasalocid (also 30 ppm), the only approved ionophore as a feed additive for sheep (Feed Additve Compendium, 2002). There was approximately a 10% difference in DM intake (expressed as g/d or $g/kg^{0.75}$) between monensin and lasalocid in lambs. A monensin level of 30 ppm used in this study may have depressed feed intake

of lambs. Monensin appears to restrict DM intake of sheep, as it does with beef cattle, reducing the consumption of NFC and the risk of acidosis. However, a lower level of monensin in lamb finishing rations may be more appropriate to allow a greater intake, and probably a better weight gain. Fox and Black (1984) reported that DM intake of beef cattle decreased 10% with 33 ppm monensin, whereas a reduction in intake was only 2% with lasalocid. It appeared that intakes reduction was less with lasalocid in both cattle and sheep, but the implication is not clear. There were evidences that monensin altered rumen motility, and influenced the dilution rate and the site of digestion of nutrients (Owens, 1980). Amount of DM digested (g/d) was similar ($P = 0.06$) between ionophore types, but greater ($P < 0.05$) for malate inclusion. Digestibility of DM components appears to be related primarily to DM intake of lambs. Carro et al. (2006) reported that malate did not influence diet intake or digestion of lambs.

Although feed offered was calculated to be 10% higher than the previous day feed intake, lambs fed lasalocid might have selected feed components with more fiber (27.6% vs. 19.6% NDF). Selection of more fibrous components may reduce the risk of acidosis in lambs fed high grain diets. Feed DM and NDF refusals were greater with monensin (122.6 and 54.7 g/d) than with lasalocid (70.6 and 45.2 g/d). Monensin restricts DM intake, a mechanism that reduces NFC intake and consequently reduced the risk of acidosis in beef cattle fed high grain rations (NRC, 1987). Carro et al. (2006) reported that malate had no effect on concentrate or straw intake and apparent digestibility of NDF or ADF of lambs.

Lambs fed diets with monensin ruminated less than those fed diets with lasalocid. The inclusion of malate also reduced the time lambs spent ruminating. Less rumination of lambs fed diets with monensin or malate may be related to their lower DM and NDF intake. Since time spent eating was not affected by ionophores or sodium malate inclusion, the lower total chewing time observed for lambs fed monensin and sodium malate was a reflection of change in rumination time.

Subclinical or subacute acidosis is characterized by a ruminal pH of 5.5–5.8 with little accumulation of lactate, whereas acute acidosis is characterized by a ruminal pH $\leq$5.0 with lactate accumulation (Britton and Stock, 1986; Nocek, 1997; Owens et al., 1998). In the present study, rumen pH values were similar among treatments, ranging from 5.76 to 5.83. Since 50% of sorghum in all diets was whole grain, greater rumination and total chewing with all diets may have increased saliva secretion, and therefore, reduced the effect of treatments on rumen pH. Kung et al. (1982) reported that malate had no effect on ruminal pH of early lactation dairy cows. Martin et al. (1999) observed that ruminal pH was greater for DL-malate than for control steers (6.07 vs. 5.77). In addition to the stimulation of lactate utilization by *S. ruminantium*, organic acid addition to in vitro mixed ruminal microorganism fermentations increased CO_2 concentrations (Callaway and Martin, 1996). Malate treatment may act to increase the pH of ruminal contents in two ways, increasing lactate utilization and CO_2 production by *S. ruminantium*. Carbon dioxide is an end product that is produced by *S. ruminantium*, which can account for up to 51% of the total viable bacteria in the rumen (Gottschalk, 1986).

In the present study, no differences in concentrations or molar percent of individual or total VFA production were observed between ionophores. Hadjipanayiotou et al. (1988) reported that addition of lasalocid increased molar proportion of propionate and decreased that of butyrate but had no effect on rumen pH or molar proportion of acetate of lambs. However, the addition of malate to the ration increased concentration of acetate in rumen fluid. In vitro studies have demonstrated that DL-malate can favorably alter rumen fermentation. Malate in the diet increased rumen pH and VFA production (Martin and Streeter, 1995; Callaway and Martin, 1996). Callaway and Martin (1996) concluded that supplementation of organic acids could be beneficial to feedlot cattle fed diets that are rapidly fermented by lowering the accumulation of lactic acid. They reported that DL-malate added to in vitro fermentation of cracked corn by mixed ruminal microorganisms increased pH and decreased the acetate to propionate ratio and reduced lactate accumulation. Organic acid addition to in vitro mixed ruminal microorganism fermentations yielded beneficial results independent of monensin treatment by decreasing the acetate to propionate ratio and increasing pH. In another in vitro study, in which different concentrations (0.03 and 10 mM) of L-malate were evaluated, Nisbet and Martin (1990) observed a dose–response effect of malate with respect to the utilization of lactate by *S. ruminantium*.

5. Conclusions

Malate did not improve the fermentation characteristics of lambs fed high grain diets that contained an ionophore. Malate may help alleviate acidosis in lamb fed finishing diets in which lasalocid, an antimicrobial additive, is not an option since its' use is not presently approved in many countries, including those of the European Community. In North America, although monensin has not been approved for sheep, a lower level than that used in this study may not affect DM intake, and be as effective as lasalocid in controlling acidosis. The use of whole sorghum grain in lamb finishing diets can stimulate rumination and saliva secretion, but may reduce the potential benefit of additives such as malate for increasing rumen pH. A study should be conducted with lambs fed finishing diets without an ionophore to determine if malate alone may help improve rumen fermentation conditions and performance.

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