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# EFFECTS OF LASALOCID AND NITROGEN SOURCE ON DIGESTION AND NITROGEN BALANCE IN RAMS

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## ABSTRACT

Four Creole rams (57 ± 3.5 kg BW), fitted with ruminal cannulae, were used to evaluate the effects of lasalocid on digestion, ruminal kinetics and nitrogen balance using two diets with different nitrogen source e.g. blood meal and poultry litter. Dry matter intake was not affected by blood meal or poultry litter. Total tract digestion of organic matter, crude protein, neutral detergent fiber and acid detergent fiber was greater ($P < 0.05$) for poultry litter. Rams fed poultry litter excreted more ($P < 0.001$) urinary N than those receiving blood meal; therefore, the diet containing blood meal elicited a better N retention ($P < 0.001$). The poultry litter diet caused a larger ($P < 0.05$) ruminal degradation of dry matter, nitrogen and neutral detergent fiber, as well as ruminal ammonia-N concentration compared to the blood meal diet. Lasalocid decreased ruminal bacteria population, and as a consequence total tract digestion only in rams fed the blood meal diet ($P < 0.05$).

**Key words:** ionophores, degradation, fermentation, nitrogen excretion.

## INTRODUCTION

Feeding poultry litter to ruminants is controversial and despite evidence that this feedstuff is generally safe (Obeidat *et al*., 2011, 2012; Azizi-Shotorkhoft *et al*., 2013), some countries have chosen to ban its use as animal feed. All applicable local, state, and federal regulations should be considered before using poultry litter as a feed ingredient (Boland *et al*., 2010). In several countries poultry is used as a low cost nitrogen source for ruminants because its N content is about 45 g/kg of dry matter (DM; Suppadit, 2010). Ruminal degradability of N in poultry litter is high (78%), whereas its rumen-bypass N has a low degradability (27%) and contains less than 10% as true protein N (Álvarez Zapata and Combellas Láres, 2005). The degradation rate of uric acid in the rumen is apparently higher than urea, according to ruminal ammonia accumulation (McDonald *et al*., 2002). In contrast, blood meal with 821 to 935 g of crude protein (CP)/kg DM (Grummer and Klopfenstein, 1996) shows 89.6 to 97.3% of rumen-undegradable protein (RUP). Lasalocid is a carboxylic polyether ionophore that mainly reduces gram-positive bacteria growth by the ion flux across cell membranes (Russell and Strobel, 1989). Lasalocid, therefore, shifts the microbial populations in the rumen improving nitrogen metabolism by a reduction of the rate of ammonia production by some bacteria (Chen and Russell, 1989); however, apparent digestibility of nitrogen sources is increased likely because of an increase in the ratio of dietary escape protein to microbial protein flow from the rumen (Ruiz *et al.*, 2001). Duffield *et al.* 2008 reviewed ionophores mechanisms of action in dairy cattle and point out that the large variation of the results may be due to the fact that the ruminal ecosystem is highly dependent on diet, including nitrogen source.

Because poultry litter is a highly rumen degradable protein source and blood meal is a highly RUP source, the capability of lasalocid to reduce rumen protein deamination by inhibiting rumen urease activity (Starnes *et al* 1984) could be different. Thus, lasalocid would modify ammonia-N concentrations from diets containing poultry litter and then enhance nitrogen balance. Therefore, the objective of this study was to evaluate the effects of lasalocid on ruminal digestibility, total tract digestion, and nitrogen balance in rams fed diets with blood meal or poultry litter.

## MATERIALS AND METHODS

All the experiments were approved by the Academic Committee of the Animal Science Department, Colegio de Postgraduados, campus Montecillo, according to regulations established by the Animal Protection Law enacted by the Estado de México. Four Creole rams (57 ± 3.5 kg body weight) fitted with rumen cannulae (Bar Diamond Inc., Parma, ID) were used. Rams, housed in 1.4 m × 2.6 m individual metabolic pens, received diets (Table 1) *ad libitum* and water at 07:00 and 16:00 h, allowing 5% orts. Diets were formulated to maintain 13% CP, according to Ludden *et al*. (2002). Amounts of feed

offered and orts were recorded daily and averaged weekly.

The experimental design was a replicated 4 x 4 Latin square balanced for residual effects. There were eight 21 day experimental periods: 13 days for adaptation, 5 days for sampling urine and feces, and 3 days for *in sacco* incubations and sampling ruminal fluids. Treatments were as follows: 0 or 1 mg lasalocid per kg of body weight, and blood meal or poultry litter diets. Lasalocid (Bovatec, Hoffman-La Roche, Inc., Nutley, NJ, USA) dose was divided into two equal portions and administered orally to each ram within a paper pellet 15 min prior to each feeding. A similar pellet, without ionophore, was administered to the control rams. Poultry litter (NOM-061-ZOO-1999) and blood meal (NOM-Y-012-SCFI-2006) were purchased at an animal feed store (Texcoco, Estado de México) and they fulfilled the animal feeds specifications enacted by Mexican laws. Poultry litter was a mixture of bedding (wheat straw) and poultry manure from a large broiler house.

Samples were ground (Arthur H. Thomas, Philadelphia, PA, USA) through a 1 mm screen for chemical analysis or a 2 mm screen for ruminal *in situ* degradation determination. On days 13, 14, 15, 16 and 17 of each experimental period, orts, feces and urine were collected, composited by animal and period, and a 5% aliquot was frozen (-4 ºC). Determinations of DM, ash, N (AOAC, 2006), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest *et al.,* 1991) were carried out. Rumen-degradable protein and RUP in diets were calculated using the NRC (1996) software for beef cattle. To estimate total tract nutrient digestion, orts and feces were collected daily during 5 days. After weighing, samples (200 g/kg total feces) were retained for chemical analysis. Urine was collected using buckets placed under the metabolic pens, the volume was recorded and urine samples were acidified with hydrochloric acid (0.18 M) to attain a final pH 4.0. Nitrogen balance was calculated using N values of feed, feces and urine (Harris, 1970).

To determine *in sacco* DM, N and NDF degradation, 18 bags (5 x 7.5 cm; pore size 52 ± 10 µm) each with 3 g of the diet (DM basis) were placed in the rumen of each ram at 08:00 h and removed at 3, 6, 9, 12, 24, 48, and 72 h(three bags for each hour). Before insertion into the rumen, three additional bags per sample were manually rinsed with water (39 ºC) for 20 min until clear water was obtained; afterwards, the soluble fraction was calculated. After incubation, bags were processed similarly. *In sacco* DM and NDF degradation of diets were calculated using the values determined before and after rumen incubation of bags.

Rumen fluid samples were collected from each ram at 0, 3, 6, 9, 12, and 24 h after the morning feeding; samples at 0 and 24 h were collected 5 min before the morning feeding. Filtrate pH was recorded immediately and the samples were acidified with 3 M metaphosphoric acid by a 1:10 dilution, cooled (4 ºC) for 30 min, and centrifuged at 25,000 x *g* at 4 ºC for 20 min. Supernatants were removed and frozen (-4 ºC). Ammonia-N concentration ($NH_3N$) was determined according to McCullough (1967).

Rumen bacteria population was evaluated using the most probable number technique (Harrigan and McCance, 1979). Fresh ruminal fluid was collected 3 h after the morning feeding and a 0.5 mL sample was mixed with 4.5 mL of medium, as described by Cobos *et al.* (2002). Bacterial growth was confirmed by turbidity after 24 h of culture incubations at 38 ºC according to Harrigan and McCance (1979).

Ruminal kinetics of DM, N and NDF were calculated using the Gompertz model (Susmel *et al* 1999) as: $dis_{(t)} = (a + b)\ \exp[(-C)\ \exp\ (-Dt)])$, where: $dis_{(t)}$ is the degradation (g/kg) from the bag at time *t*; *a* is the ruminally soluble DM, N, or NDF fraction (g/kg) at *t* = time (h); *b* is the insoluble, but potentially degradable fraction (g/kg); *C* is the fractional degradation rate of (*a* + *b*); and *D* is a parameter to measure rate of degradation. According to the Gompertz model, the fractional rate of degradation varies as a function of time, and the average value (i.e., a constant comparable to the exponential rate of degradation) is derived as: $c = D/C$.

Data of DM, N, or NDF remaining at each incubation time were used to fit nonlinear regression models using the NLIN procedure of SAS V8 (The SAS Institute, Cary, N.C., USA). Ruminal pH and ammonia N concentrations were analyzed using the MIXED procedure in which the covariate structure that resulted in the lowest Akaike's information criterion was first-order autoregressive (The SAS Institute, Cary, N.C., USA). Bacterial numbers were analyzed by confidence intervals as described by Harrigan and McCance (1979). Differences were considered significant at $P < 0.05$.

## RESULTS AND DISCUSSION

According to the chemical composition, DM, CP, NDF, ADF contents and gross energy values were similar for both experimental diets (table 1) but, as expected, the calculated RUP was greater for the blood meal diet, as compared to the poultry litter diet. Rams fed the experimental diets showed a similar DM intake (Table 2). Diets with poultry litter elicited greater total tract digestion of OM, CP, NDF and ADF, as compared to blood meal diets, whereas there was no effect of lasalocid on total digestion of fiber fractions. Fecal N was similar among treatments; however, rams fed poultry litter showed less nitrogen retention since they excreted more urine N as compared to rams fed blood meal. Besides, addition of lasalocid reduced the N retained only in rams receiving blood meal (Table 2). The nitrogen source affected total N degradation which was higher for

poultry litter than for blood meal diets and is also related to a significant interaction with lasalocid (Table 3) since this variable was decreased in rams fed the blood meal plus lasalocid diet, a negative effect that may probably be due to a reduced ruminal microbial activity in those rams. This is supported by the fact that bacterial number in the rumen was 2.5 x $10^{10}$/mL in rams fed blood meal plus lasalocid, compared to 140 x $10^{10}$/mL in rams receiving either blood meal, poultry litter or poultry litter plus lasalocid diets. Ionophores have decreased rumen CP degradation as a result of a reduction in rumen bacteria that utilize amino acids and peptides (Russell and Strobel, 1989). It is therefore possible that addition of lasalocid to the blood meal diet might increase total amount of dietary true protein escaping the rumen (Faulkner *et al.,* 1985). According to Steen *et al.* (1992), lasalocid plus a higher amount of RUP may impair microbial protein synthesis which can be avoided if an adequate amount of rumen-degradable N compound is present in the feed, as would be the case with poultry litter as a nitrogen source (Álvarez Zapata and Combellas Láres, 2005).

**Table 1. Ingredients and chemical composition of experimental diets (DM basis) fed to rams for determination of nitrogen balance and rumen kinetics.**

| | Diet | |
|---|---|---|
| | **Blood meal** | **Poultry litter** |
| Ingredient (% DM) | | |
| Corn grain, ground | 43.6 | 33.0 |
| Corn stover, ground | 33.3 | 21.2 |
| Cane molasses, liquid | 15.0 | 15.0 |
| Blood meal[1] | 8.1 | |
| Poultry litter[2] | | 30.8 |
| Chemical composition | | |
| Dry matter (%) | 85.5 | 85.1 |
| Crude protein (% DM) | 12.5 | 12.9 |
| Rumen-degradable protein (% DM)[3] | 6.0 | 9.6 |
| Rumen-undegradable protein (% DM)[3] | 6.5 | 3.3 |
| Acid detergent fibre (% DM) | 23.0 | 31.2 |
| Neutral detergent fibre (% DM) | 38.9 | 41.2 |
| Gross energy (MJ/kg) | 17.0 | 15.0 |

[1] DM, 90 %; CP, 82 % DM basis.
[2] DM, 84%; CP, 27% DM basis.
[3] Calculated according to NRC (1996).

**Table 2. Effect of lasalocid on *in vivo* digestion and nitrogen balance in rams fed diets containing blood meal or poultry litter.**

| | Treatment[1] | | | | | | Significance[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | **BM-L** | **BM+L** | **PL-L** | **PL+L** | **SEM[3]** | **SD[3]** | **N** | **L** | **N x L** |
| Dry matter intake (g/d) | 1791 | 1658 | 1801 | 1852 | 85.2 | 477.1 | NS | NS | NS |
| Total tract digestion (%) | | | | | | | | | |
| Dry matter | 69.0 | 67.0 | 68.2 | 67.7 | 0.64 | 3.6 | NS | NS | * |
| Organic matter | 69.9 | 67.7 | 70.5 | 69.9 | 0.65 | 3.6 | ** | NS | * |
| Crude protein | 60.7 | 58.0 | 62.9 | 62.8 | 0.79 | 4.4 | * | NS | * |
| Neutral detergent fiber | 57.4 | 55.3 | 65.0 | 63.6 | 1.53 | 8.6 | *** | NS | NS |
| Acid detergent fiber | 48.9 | 47.0 | 55.4 | 56.7 | 1.79 | 10.0 | *** | NS | NS |
| N balance (g/d) | | | | | | | | | |
| Intake | 36 | 33 | 36 | 37 | 1.1 | 6.2 | NS | NS | NS |
| Feces | 15 | 14 | 13 | 15 | 0.6 | 3.3 | NS | NS | NS |
| Urine | 7 | 7 | 16 | 16 | 1.6 | 8.9 | *** | NS | NS |
| Retained | 14 | 12 | 7 | 6 | 1.2 | 6.7 | *** | NS | * |

[1] BM-L, blood meal; BM+L, blood meal and lasalocid; PL-L, poultry litter; PL+L, poultry litter and lasalocid; N, nitrogen source; L, lasalocid level; N x L, interaction nitrogen source x lasalocid level.
[2] NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001
[3] SEM = standard error of mean, SD = standard deviation

At 3, 6 and 9 h after feeding, as well as between 0 to 12 h, rumen pH of rams fed poultry litter diets was higher than in rams receiving blood meal diets (Fig. 1), whereas pH was not affect by lasalocid. Differences found on digestion and ruminal degradation and fermentation characteristics between poultry litter and blood meal could be due to variations of RUP contents. Thus, poultry litter has a higher N soluble fraction which is responsible for the higher rumen ammonia concentrations observed in our study, and more N was available to increase nutrient digestion (Paterson *et al.,* 1983), mainly on fiber fractions (Nurfeta, 2010). Because blood meal is a feedstuff with high RUP value (Legleiter *et al*., 2005), urinary N concentration was lower and N retention greater as compared to poultry litter, which elicited higher rumen ammonia-N concentrations and increased pH values (Capucille *et al* 2004). Only in rams fed poultry litter diets, lasalocid increased ruminal ammonia-N concentration at 6 h after feeding (Fig. 2), which might be due to an interaction between poultry litter as a source of N and lasalocid effect on rumen microbes. Indeed, ionophores (i.e., monensin) have increased ammonia-N in rumen via blood or saliva (Ruiz *et al.,* 2001).

**Table 3. Effect of lasalocid on ruminal degradation fractions of diets with blood meal or poultry litter in rams.**

| | Treatment[1] | | | | | | Significance[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | **BM-L** | **BM+L** | **PL-L** | **PL+L** | **SEM[3]** | **SD[3]** | **N** | **L** | **N x L** |
| Dry matter | | | | | | | | | |
| Soluble fraction (%DM) | 26.6 | 28.1 | 37.3 | 37.1 | 1.51 | 8.4 | *** | NS | NS |
| Potentially degradable fraction (% DM) | 44.6 | 43.6 | 43.0 | 43.8 | 1.91 | 10.7 | NS | NS | NS |
| Total degradation | 71.2 | 71.7 | 80.3 | 80.9 | 1.61 | 9.0 | *** | NS | NS |
| Degradation rate (%/h) | 5.8 | 5.8 | 5.8 | 5.6 | 0.45 | 2.5 | NS | NS | NS |
| Nitrogen | | | | | | | | | |
| Soluble fraction (%DM) | 38.4 | 38.2 | 57.8 | 55.5 | 2.48 | 13.9 | *** | NS | NS |
| Potentially degradable fraction (% DM) | 36.6 | 33.6 | 30.7 | 34.6 | 2.38 | 13.3 | *** | NS | NS |
| Total degradation | 75.0 | 71.8 | 88.5 | 90.1 | 2.58 | 14.4 | *** | NS | * |
| Degradation rate (%/h) | 5.1 | 5.4 | 5.4 | 5.1 | 0.26 | 1.4 | NS | NS | NS |
| Neutral detergent fibre | | | | | | | | | |
| Potentially degradable fraction (% DM) | 63.4 | 60.4 | 70.0 | 69.3 | 1.14 | 6.3 | *** | NS | NS |
| Degradation rate (%/h) | 3.6 | 3.7 | 4.7 | 4.5 | 0.20 | 1.2 | ** | NS | NS |

[1] BM-L, blood meal; BM+L, blood meal and lasalocid; PL-L, poultry litter; PL+L, poultry litter and lasalocid; N, nitrogen source; L, lasalocid level; N x L, interaction nitrogen source x lasalocid level.
[2] NS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001
[3] SEM = standard error of mean, SD = standard deviation

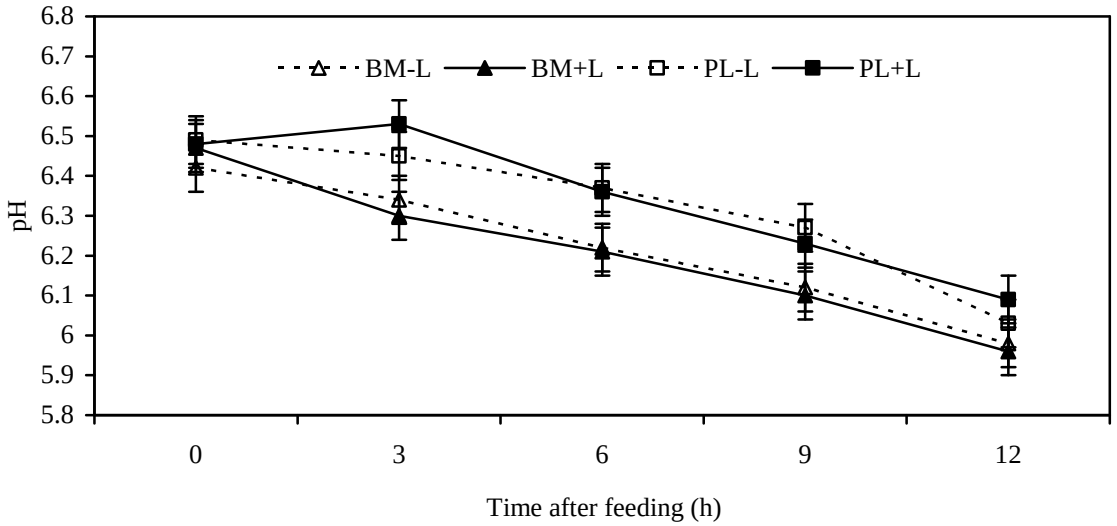


**Fig. 1. Rumen pH values of rams fed diets with blood meal (BM–L), blood meal and lasalocid (BM+L), poultry litter (PL–L) or poultry litter and lasalocid (PL+L). Error bars are expressed as standard error of means.**

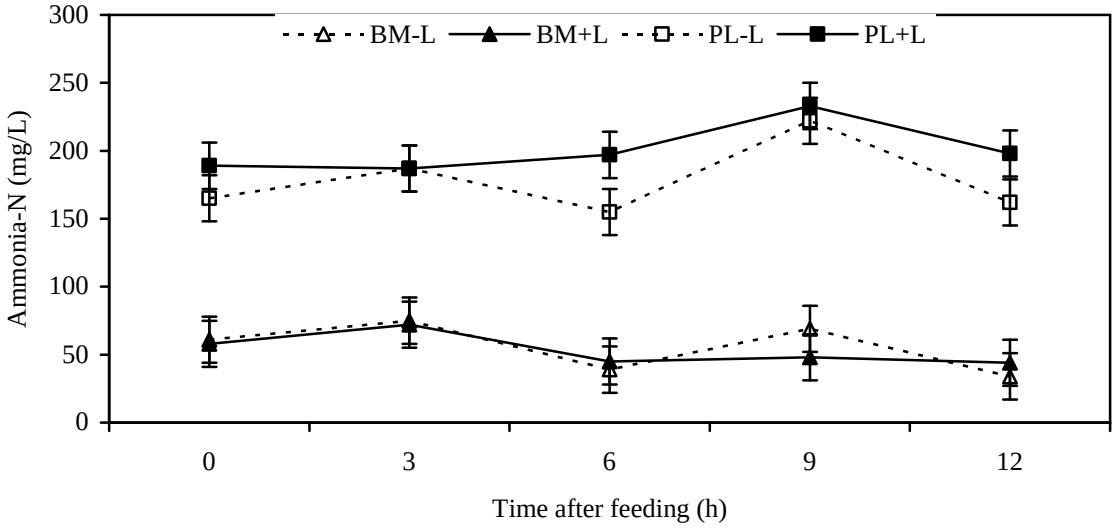


**Fig. 2. Rumen ammonia-N concentrations of rams fed diets with blood meal (BM–L), blood meal and lasalocid (BM+L), poultry litter (PL–L) or poultry litter and lasalocid (PL+L). Error bars indicate standard error of means**

**Conclusion:** It can be concluded that the dietary nitrogen source affected digestion of nutrients, rumen ammonia concentrations and N balance. Lasalocid reduced total tract digestion of crude protein in rams fed blood meal presumably as a result of the decreased rumen bacteria numbers, which finally reduced urine excretion and enhanced nitrogen retention. The results obtained when the ionophore was included in the poultry litter diet were only partially explained, requiring further studies about this subject.

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