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Western Industrial Clay Products

Mode of Action of Red Lake Earth in Ruminant Rations

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Executive Summary

Red Lake Earth (RLE), a product distributed by Western Industrial Clay products, is comprised of diatomaceous earth (DE) and montmorillonite. Traditionally, RLE has been included in livestock rations as an anti-caking agent. In the recent past, however, there have been suggestions that products such as RLE may have additional value, over and above their anti-caking benefits, when incorporated into livestock rations. As such, a research trial was conducted to examine the effect of RLE fed at 0%, 0.5%, 1.0% and 2.0% as fed basis of the total diet on dry matter intake (DMI), feed digestibility and rumen metabolism.

The main results generated from this trial are summarized in the following points:

- Addition of RLE did not impact intake or digestibility of the diets
- Although there were no significant differences between the four diets, rumen pH did reach subacidotic levels in the rumen of animals which received the diet containing 0% RLE. This suggests that RLE may have either alkalinizing or buffering properties.
- RLE did not interfere with rumen fibre or protein degradation but did decrease the rumen solubility of barley grain. The observed decrease in solubility may serve to prevent a rapid drop in rumen pH, as well as the acidotic conditions which are associated with such a drop.
- Addition of RLE did not alter the composition of the feces in terms of moisture content, nor did it alter the percent nitrogen, phosphorus, volatile fatty acid composition, ADF, or NDF of the feces. The highest rate of inclusion of 2.0% did result in significantly higher ash content in the feces.
- Although there were no significant differences between the four dietary concentrations of

RLE, it appears that diets containing RLE reduced hydrogen sulfide levels in manure.

Rationale for the Research:

Red Lake Earth (RLE), a product distributed by Western Industrial Clay products, is comprised of diatomaceous earth (DE) and montmorillonite. This product (see Table 1 for a chemical analysis) is used by feed manufacturers as an anti-caking agent. The Canada Feeds Act permits the use of DE as an anti-caking agent or carrier in feed stuffs at concentrations not exceeding 2% of the total diet. More recently, there has been interest in examining the impact of DE in livestock rations. As such, a research trial was initiated to determine if Red Lake Earth has the potential to a) influence dry matter and nutrient intake; b) alter the digestion and metabolism of feedstuffs in the rumen; and c) reduce odour and the excretion of excess nutrients in beef cattle manure.

Objective:

The objective of this trial was to examine the effect of RLE fed at 0%, 0.5%, 1.0% and 2.0% as fed basis of the total diet on:

- dry matter intake (DMI)
- digestibility of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF) in diets
- rumen metabolism as measured by rumen pH, rumen volatile fatty acids (VFA's), rumen ammonia and blood urea nitrogen (BUN)
- manure nutrient profile as measured by nitrogen (N), phosphorus (P) and VFA concentrations
- manure odour as estimated by ammonia and hydrogen sulfide

Materials and Methods:

Eight animals (4 cannulated Jersey steers and 4 non-cannulated Holstein steers), weighing an average of 321 kg, were fed a 60% barley-based concentrate, 40% alfalfa-grass hay and grass hay ration, containing four dietary concentrations of DE (0%, 0.5 %, 1.0 % and 2.0 %) for four 21-day periods in a replicated 4 X 4 Latin square design. This relatively high concentrate:forage ratio was selected to model the rumen conditions present in high producing dairy cows and beef steers being finished or backgrounded at relatively high rates of gain, allowing an assessment of the addition of RLE under such conditions. High concentrate diets are used in all feedlots and dairy operations, as well as in some backgrounding operations. It is in these intensive cattle production systems that one might expect a response to the addition of RLE.

At the onset of each of the four periods, animals were fed the assigned diet (see Table 2) for 14 days. Following this 14-day adaptation period, rumen and blood samples were collected from the cannulated steers at two-hour intervals starting two hours prior to feeding and ending six hours post-feeding for two consecutive days (days 15-16) and analyzed for rumen pH, VFA's, ammonia, and blood urea nitrogen respectively. The pH was determined by Accumet pH meter, model 810 (Fisher Scientific) with a Gel-filled combination electrode (Orion model 91-05). Five mL of the rumen fluid and 1 mL 25% metaphosphoric acid were transferred to a 10 mL centrifuge tube, mixed and frozen overnight. The mixture was thawed and centrifuged at 3000 rpm for 15 min. The supernatant was injected directly into gas chromatograph for VFA determination as outlined by Erwin et al. (1961). The NH_3N concentration was determined by

the colorimetric procedure of Novozamsky et al. (1974). The blood urea nitrogen (BUN) concentration was determined with Sigma Kit No. 535-B (Sigma Chemical Company, St. Louis, MO) using an Ultrospec 2000 UV/visible spectrophotometer (Pharmacia Biotech, Cambridge, UK).

Feed intake was measured in all animals on a daily basis for 5 days (d 17-21) following the two days of blood and rumen fluid collection. Feed samples were collected concurrently, composited for each period, dried in a convective oven at 60°C for 48 hours, ground through a 1 mm screen and analyzed for CP (Kjeldahl method, Method No. 984.13, AOAC, 1990) using a Tecator 1030 analyser, NDF (Komarek et al. 1994) and ADF (Komarek et al, 1993) using ANKOM's Fibre Analyser #F200 (Fairport, NY) and ash (Method No. 942.05, AOAC, 1990).

Fecal collections were conducted (d 17 - d 21) twice daily (from 8:00 am to 4:00 pm and 4:00 pm to 8:00 am) in all eight steers to determine apparent digestibility of DM, CP, ash, NDF, and ADF, as described above. Five per cent of the total fecal output produced in each of the two daily collections by each animal were sampled, dried in a convective air oven at 60°C for 48 hours, ground and composited by period.

Rumen degradability of individual feedstuffs was determined on day 17 - 21 in the four cannulated steers using the nylon bag technique described in Moshtaghi Nia (1994). One gram samples of canola meal, barley, and hay were placed in 4 X 6 cm heat-sealed nylon bags with a porosity of 50 µm (Felco Industries Ltd., Concord, ON). Three bags of each feedstuff and two empty bags, which served as blanks to correct for feed particles and microorganisms that adhered to the bags, were placed in large mesh nylon bags and incubated in each of the four cannulated steers for 0, 2, 4, 8, 12, 16, 24, 32, 48, 72, and 96 hours. Upon removal, all bags were immersed

in cold water, rinsed in a wringer-type washer for 10 minutes, drained, rinsed again for 5 minutes, and dried in a forced air oven at 60 °C for 48 hours. All nylon bags were analyzed for DM (Method 7.013, AOAC, 1984) and CP (Kjeldahl method, Method No. 984.13, AOAC, 1990).

In addition to the fecal samples taken for apparent digestibility as described above, an additional 250 gram sample of feces was collected and analyzed for VFA (Erwin et al. 1961), N (Kjeldahl method, Method No. 984.13, AOAC, 1990) and P (Method 965.17, AOAC 1990). The feces which remained from the total fecal collections was mixed with the urine collected. Four litres of this mixture (2 litres from each steer on a given treatment) was placed in a 77-litre container and stored at room temperature (20.1 °C), twice daily during each day of the 5-day digestibility trial. Hydrogen sulfide and ammonia levels were determined using a Jerome meter and colorimetric tubes, respectively, from the head space of the 77-litre container on a daily basis for the first week. Air samples were drawn concurrently using a 15 mL syringe and analyzed for methane, CO_2 and nitrous oxide. Thereafter, the containers holding the feces and urine were placed in a temperature-controlled room (20.0°C). Manure gases were measured in the headspace of the manure storage containers before and 2 hours after stirring on a weekly basis for four weeks. The values before stirring represent the emission potential of the feces and urine while those obtained after stirring represent the in-manure gas production. The effect of the crust can then be determined by comparing the values before and after stirring.

Intake and digestibility data, as well as rumen parameters, fecal nutrient data and manure gas data were analyzed using the Mixed Procedures of the Statistical Analysis System Institute, SAS (1988). Least square means of DM intake, digestibility of DM, CP, ADF and NDF and

fecal DM, ADF, NDF, ash, N, P and VFA were analyzed in a model where the effect of dietary treatment was tested against the overall error which included period and animal. Emission potential and crust effect of the manure gases were analyzed in a model where effects of diet, treatment and week were tested against the overall error term which included period, animal and week. Rumen parameters including pH, VFA's, BUN and ammonia were analyzed in a model that included dietary treatment, time before and after eating and dietary treatment by time. Statistical differences among the treatment least square means were tested using the Bonferroni test (SAS, 1988).

Percentage disappearance of DM in the rumen at each incubation time was calculated as described in Nia (1994) where data were fitted to the nonlinear regression equation of McDonald (1981):

$$p = a + b\,(1-e^{-ct})$$

where p is the disappearance of DM in the rumen at time t; *a* is the soluble fraction, *b* is the potentially degradable fraction; *c* is the rate of degradation. Effective degradabilities were estimated using the equation of Orskov and McDonald (1979):

$$ED = a + bc/(c+k^{-1})^{-1}$$

where *ED* is the effective degradability of DM; *a*, *b*, and *c* are as described above and *k* is the rumen particulate outflow rate. Effective degradabilities were estimated at rumen outflow rates of 0.04 and 0.06 h^{-1} (Agricultural Research Council, 1984).

The ammonia and hydrogen sulfide data collected before stirring were analyzed using quadratic regression analyses (SAS, 1988). A randomized block design with repeated measures was used for the four weekly gas samples analyzed after stirring. Linear contrasts were also

used on the gas data to examine the impact of the presence or absence of RLE.

For all data, differences between diets were consider significant when $P < 0.05$ and trends when $P > 0.5$ but less than 0.1.

Results and Discussion

Mixing and Feeding RLE

Addition of RLE to the concentrate component of the diet presented several challenges. The light, fine particles were readily dispersed into the air in the initial mix. Subsequently, wheat middlings were added as a carrier for RLE. This addition still resulted in uneven mixing and loss of RLE in the air. Finally, one kilogram of water and 1 kilogram of molasses were added to the RLE premix to minimize particle dispersion and to reduce particle separation when the premix was added to barley.

Feeding RLE in a hay and concentrate ration also presented challenges as the animals appeared to sort through the feed. This was apparent upon examination of the feed which was refused, as it was comprised primarily of fines.

Effect of RLE on Intake, Digestibility and Metabolism

Examination of the nutrient analysis of RLE indicates that it is a mineral-based product which does not contribute to the energy or protein concentration of the diet. As such, high inclusion rates of RLE would be expected to decrease the nutrient density of the diet, resulting in a decrease in total dietary energy or protein. This decrease in density may be an important consideration when nutrient dense diets, such as that delivered to finishing beef cattle or high

performance dairy cattle, are essential for optimum performance.

The addition of Red Lake Earth (RLE) to the diet, did not negatively impact intake or digestability of the diets as DM intake (P=0.8464), fecal output (P=0.9415) and digestibility of DM (P=0.4723), ADF (P=0.1205), NDF (P=0.7618) and CP (P=0.5571) were not significantly different between the four diets (Table 3). Although addition of RLE is expected to reduce nutrient density, examination of crude protein intake across the four diets indicated that the addition of RLE did not significantly (P=0.6427) impact crude protein intake. Variation in dry matter intake and therefore crude protein intake due to variation in individual animal feeding behavior is expected. This variation, however, minimizes the ability to detect significant differences in protein intake.

Ash intake, which represents the concentration of inorganic dietary components and total DM intake, was also not significantly different among diets (P=0.6450). This similarity in intake may be attributed to the high ash content of the grass and legume hays (Table 2). Inclusion of RLE into the concentrate increased concentrate ash levels from 3.99 to a high of 6.14% DM basis. This ash component has no energy or protein value, however, it does contain minerals essential for the growth and well-being of the animal.

To ensure efficient rumen function, it is important to determine the effect of RLE on the rumen metabolism as measured by pH, VFA's, rumen ammonia nitrogen and BUN. These results are summarized in Table 4. Overall rumen pH was not significantly different between the four diets (P=0.4898). Although, rumen pH immediately prior to, during and after feeding followed a similar pattern for each of the four dietary treatments, as is depicted in Figure 1, it should be noted that only the diet containing 0.0% RLE reached a lower critical rumen pH value

of 5.8 at 6 hours post feeding. Subacute acidosis has been defined by rumen pH values of less than 6. These results suggest that RLE may have either alkalinizing or buffering properties which would serve to protect the animal from a drop in rumen pH. This buffering capacity is important for ruminants consuming high starch diets.

Volatile fatty acids are the end product of microbial breakdown of carbohydrates and protein in the rumen and are the major source of energy for ruminants. Total and relative proportions of VFA'a were examined to determine the potential influence of RLE on microbial populations responsible for breakdown of structural and nonstructural carbohydrates (fibre and starch). Although the addition of RLE did not affect total VFA production (P=0.5987), it did significantly increase the percent of acetic acid (P=0.001), isobutryic acid (P=0.0037) and isovaleric acid (P=0.001) and decrease the percent of butryic acid (P=0.0001). Situations in which total VFA production remain high but proportions of individual VFA's change can result from either shifts in microbial populations or from selective use of nutrients by the microorganisms. This data suggests that RLE does affect microbial activities as is evidenced by the change in VFA profile. This change, however, does not affect energy utilization of the feed. The observed increase in acetic acid and commensurate decline in butyric acid is unusual in that acetic and butyric acid usually follow the same pattern.

Rumen ammonia is generated by the microbes in the rumen. In a well-functioning rumen, ammonia is the major source of nitrogen for the growth of bacteria which are later digested, along with feed protein that has not been degraded. Concentrations may range from 3 mg/dl to 60 mg/dl. Excess ammonia which is not utilized by bacteria enters the bloodstream and is transported to the liver where it is converted to urea. The urea, which can be excreted by

the kidney into the urine or recycled into the rumen through the saliva, may be measured in the blood (blood urea nitrogen). Blood urea nitrogen levels may vary from 6 to 24 mg/dl. In this study, both rumen ammonia and blood urea nitrogen were measured to determine if RLE adversely affects assimilation of rumen ammonia and results in excess excretion of nitrogen in the urine. Addition of RLE did not significantly alter rumen ammonia concentrations (P=0.2441), as indicated in Table 4. Although rumen ammonia levels follow the same pattern for all diets at -2, 0, 2 and 4 hours after feeding, there is somewhat greater variability in the concentration at 6 hours post feeding, as indicated in Figure 2. Blood urea nitrogen concentrations were significantly impacted by diet (P=0.0036). Levels were lowest with the addition of 1.0% RLE and highest with the addition of 0.5% RLE. This data is shown graphically in Figure 3. The apparent differences in BUN levels between the diets can not be attributed to differences in protein intake as intake was not significantly different between diets and as such, might be a function of RLE in the animal's diet. The lack of a consistent response to sequential increases of RLE in the diets make interpretation of this data difficult. It is important to note that although significant differences in BUN were observed, all BUN values were within the range allowing for normal N metabolism

The effect of RLE on rumen DM degradability of hay, barley and canola is presented in Table 5. These feedstuffs were selected as they are high in fibre, starch and protein respectively, and serve as ideal models for examining the rumen degradability of each of these components. Furthermore, each of these feedstuffs are frequently used in ruminant rations. As described in the Materials and Methods, **a** is the fraction which is soluble and readily available to bacteria in rumen fluid immediately after eating, **b** is the fraction which is potentially degradable and **c**

represents the rate of degradation. None of the diets significantly changed the soluble fraction of the hay (P=0.7916) or the canola (P=0.2846), but did appear to alter the soluble fraction of barley as the control diet did have a significantly higher soluble fraction than that of the diet which contained 1.0% RLE . Neither the potentially degradable fractions, the rate of degradation nor degradability at outflow rates of 0.04 or 0.06 of hay, barley or canola were significantly different between the four dietary treatments. Dry matter disappearance of hay, barley and canola meal are represented graphically in Figures 4, 5 and 6, respectively. Addition of RLE to the diet did not impact protein degradability as none of the diets significantly changed the soluble fraction, the potentially degradable fraction, the rate of degradation or degradability in hay, canola or barley, as indicated in Table 6. Crude protein disappearance in hay, barley and canola meal is represented graphically in Figures 7, 8 and 9. Thus, it appears that although RLE did not interfere with rumen fibre degradation or protein degradation, it did appear to decrease the solubility of the starch component. The latter effect is not adverse as rapid starch degradation can lead to a rapid drop in rumen pH resulting in less than optimal feed utilization.

Effect of RLE on Manure Composition and Odour

In order to assess the impact of RLE on the composition of the feces and its subsequent impact on the environment, fecal DM, N, P, ADF, NDF , and ash concentrations were measured. The effect of the addition of RLE on the composition of the feces is given in Table 7. The addition of RLE did not significantly alter the percent DM, ADF, NDF, N, or P content of the feces. The ash content of the feces was, however, significantly higher for the diet to which 2.0% RLE had been added. This suggests that RLE may have some effect on manure properties

during storage.

The effect of RLE on manure odour was assessed by measuring VFA's, as well as hydrogen sulfide and ammonia. Greenhouse gas concentrations were assessed by measuring carbon dioxide, methane and nitrous. As indicated in Table 8, there were no significant differences between the diets in any of the gases measured. It is important to note, however, that there is a trend toward reduced hydrogen sulfide production in the manure when RLE is included in the diet. Further analysis of the data using linear contrasts verified that the addition of RLE reduced hydrogen sulfide levels in manure. Using the regression equations described in Table 9, the hydrogen sulfide and ammonia concentrations are represented graphically in Figures 10 and 11 respectively. Hydrogen sulfide concentrations appear to decline from the manure of all diets until approximately day 20. At this point, levels begin to increase from the manure of the diets containing 0.0% and 1.0% RLE. A similar increase in hydrogen sulfide from the manure of the diets containing 0.5% and 2.0% is also apparent but does not occur until day 30. This suggests that differences exist between diets with regards to the emission potential of the feces/urine mixture.

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Tables and Figures

Table 1. Summary of the chemical analysis of Red Lake Earth (as provided by Western Industrial Clay Products from Chemex Labs Ltd, March 19, 1999).

Chemical element	Total % of sample	% Element in Sample
AL_20_3(ALUMINUM OXIDE)	14.13	7.48
CA0 (CALCIUM OXIDE)	1.01	.72
FE_20_3(IRON OXIDE)	5.73	4.01
K_20 (POTASSIUM OXIDE)	.68	.56
MG0(MAGNESIUM OXIDE)	.84	.51
NA_20(SODIUM OXIDE)	.62	.46
$SI0_2$ (SILICON DIOXIDE)	62.14	29.05
$TI0_2$(TITANIUM OXIDE)	.75	.45
CRO_3(CROMIUM OXIDE)	<.01	.01
MNO(MAGANESE OXIDE)	.01	.01
P_20_5(PHOSPHORUS OXIDE)	.07	.03

Table 2. Ingredients and ingredient analysis of dietary treatments containing varying levels of Red Lake Earth (RLE)

Ingredient	**% of diet (DM basis)**			
	0.0% RLE	**0.5% RLE**	**1.0% RLE**	**2.0% RLE**
Chopped alfalfa hay	32.1	32.1	32.1	32.1
Chopped grass hay	8.2	8.2	8.2	8.2
Concentrate				
barley	56.0	55.5	55.0	54.0
wheat middlings	2.8	2.8	2.8	2.8
RLE[1]	0.0	0.5	1.0	2.0
molasses	0.5	0.5	0.5	0.5
mineral	0.2	0.2	0.2	0.2
salt	0.2	0.2	0.2	0.2

Ingredient composition, % DM basis

DM	**CP**	**ADF**	**NDF**	**Ash**

Alfalfa hay	89.8	17.2	34.1	44.7	10.1
Grass hay	91.0	11.3	39.6	64.9	10.8
0.0 % concentrate	90.6	14.2	5.9	15.2	4.0
0.5 % concentrate	90.2	13.9	5.9	15.6	4.3
1.0 % concentrate	90.4	13.9	6.1	15.7	5.0
2.0% concentrate	90.0	13.7	6.3	15.8	6.1

[1]See Table 1 for composition of RLE

Table 3. Effect of the addition of RLE on dry matter, crude protein, and ash intake and fecal output and nutrient digestibility

Parameter	Diet				SE	P
	0.0%	0.5%	1.0%	2.0%		
Intake						
DM (kg/d)	8.02	7.39	7.52	7.28	0.6297	0.85
CP (g/day)	1221.7	1112.8	1111.3	1087	83.16	0.64
Ash (g/day)	515.8	496.1	524.7	560.7	36.29	0.65
Fecal DM output (kg/d)	2.2	2.13	2.09	2.11	0.236	0.94
Digestibility (%)						
DM	72.7	70.5	72.2	71.4	1.6415	0.47
ADF	51.2	51.3	48.4	43.5	3.9291	0.12
NDF	53.0	55.1	52.9	52.1	3.5938	0.76

CP	69.8	67.5	68.9	69.7	1.9957	0.56

Table 4. Effect of the addition of RLE on rumen parameters

Parameter	Diet				SE	P (diet)	P (diet*time)
	0.0%	0.5%	1.0%	2.0%			
pH	6.11	6.08	6.13	6.16	0.098	0.49	0.97
VFA's (% of total)							
acetic acid	65.52[b]	65.02[b]	67.36[a]	67.85[a]	0.879	0.01	0.76
propionic acid	16.49	16.12	15.16	15.07	0.824	0.06	0.9
isobutyric acid	0.75[b]	0.88[ab]	0.98[a]	1.02[a]	0.075	0.01	0.99
butyric acid	14.20[a]	14.10[a]	13.02[b]	12.48[b]	0.657	0.01	0.92
isovaleric acid	1.250[c]	1.908[a]	1.652[b]	1.775[ab]	0.174	0.01	0.99
valeric acid	1.816	1.853	1.842	1.761	0.074	0.42	0.36
Total VFA's (mmoles/dL)	124.85	119.05	118.07	119.17	8.674	0.6	0.93
Blood urea N (mmoles/dL)	8.912[ab]	9.499[a]	8.281[b]	9.115[ab]	0.686	0.01	0.99
Rumen ammonia N (mmoles/dL)	26.21	24.72	23.17	22.85	3.148	0.24	0.97

Table 5. Effect of RLE on DM disappearance and degradability of hay, barley and canola meal in the rumen

Diets	Disappearance parameters			Degradability	
	a	**b**	**c**	**k=0.04**	**k=0.06**
Hay					
R_1	20.01	34.73	0.08	41.26	38.02
R_2	20.14	30.83	0.07	38.60	35.70
R_3	19.70	29.45	0.06	38.17	35.11
R_4	19.63	31.88	0.06	38.37	35.10
SE	0.701	2.517	0.011	2.000	1.871
P	0.79	0.43	0.60	0.13	0.14
Barley					
R_1	23.84a	49.97	0.303	66.12	63.21
R_2	22.28ab	47.80	0.263	64.02	61.74
R_3	21.46b	48.13	0.266	63.44	61.02
R_4	22.14ab	48.52	0.230	64.47	62.05
SE	1.553	0.694	0.074	2.776	3.193
P	0.03	0.16	0.90	0.19	0.45
Canola					
R_1	25.00	41.21	0.069	50.31	46.35
R_2	24.69	41.69	0.069	49.89	45.92
R_3	24.14	39.24	0.068	49.12	45.15
R_4	23.99	39.54	0.072	49.57	45.62
SE	0.650	1.208	0.011	2.156	2.191
P	0.29	0.47	0.99	0.83	0.85

Table 6. Effect of RLE on protein disappearance and degradability of hay, barley and canola meal in the rumen

Diet	Disappearance parameters			Degradability	
	a	**b**	**c**	**k=0.04**	**k=0.06**
Hay					
R_1	32.46	56.16	0.104	68.20	63.34
R_2	29.50	54.44	0.107	67.74	63.19
R_3	30.69	53.51	0.082	68.21	63.20
R_4	33.91	53.72	0.066	69.25	63.93
SE	2.842	1.814	0.0258	5.099	5.406
P	0.42	0.73	0.60	0.99	0.99
Barley					
R_1	25.80	70.44	0.189	81.02	75.75
R_2	26.04	68.65	0.132	80.71	76.02
R_3	26.81	68.31	0.143	83.69	79.50
R_4	26.66	67.95	0.145	83.22	79.06
SE	2.657	0.694	0.0418	4.689	5.431
P	0.99	0.09	0.71	0.75	0.59
Canola					
R_1	32.11	62.02	0.065	68.70	62.68
R_2	31.94	62.26	0.069	69.79	64.02
R_3	31.40	58.23	0.071	69.44	63.96
R_4	29.90	59.12	0.064	67.29	61.57
SE	0.864	1.537	0.0119	3.457	3.507
P	0.20	0.28	0.85	0.63	0.55

Table 7. Effect of RLE on composition (% DM basis) of fecal material

	Diets				SE	P
	0.0%	**0.5%**	**1.0%**	**2.0%**		
DM	19.3	18.9	20.9	21.1	0.88	0.06
pH	7.38	7.36	7.06	7.37	0.11	0.07
ADF	31.75	30.43	29.79	33.88	1.03	0.05*
NDF	48.35	44.75	45.59	47.74	1.29	0.07
N	2.61	2.66	2.7	2.51	0.04	0.05**
ash	12.30b	13.00b	14.11ab	16.01a	0.6	0.01
P	1.09	1.07	1.14	1.14	0.06	0.35
VFA's (% of total)						
acetic	65.89	65.06	62.74	61.94	2.11	0.46
propionic	17.20	17.37	17.84	18.35	0.99	0.46
butyric	11.00	11.77	13.78	12.39	1.05	0.37
isovaleric	2.85	2.37	2.34	3.05	0.47	0.42
valeric	2.92	2.70	2.66	3.63	0.69	0.42

* Using Bonferroni mean comparison the P value of the diet containing 1.0% RLE vs. the diet containing 2.0% RLE is 0.0646.
**Using Bonferroni mean comparison the P value of the diet containing 1.0% RLE vs. the diet containing 2.0% RLE is 0.0568.
*** No isobutyric found

Table 8. Effect of RLE on ammonia and hydrogen sulfide from feces/urine stored over a four week period

	Diets						
	0.0%	**0.5%**	**1.0%**	**2.0%**	**SE**	**P**	**P***
Ammonia							
after stirring (ppm)	71.95	77.95	61.31	73.35	7.822	0.12	0.85
after-before (ppm)	9.3	7.61	-6.07	1.31	7.705	0.2	0.20
Hydrogen sulfide							
after stirring (ppm)	5.36	3.62	4.14	3.36	0.86	0.09	0.02
after-before (ppm)	1.92	1.09	0.85	0.7	1.04	0.45	0.12
Methane							
after stirring (ppm)	202.34	49.54	177.02	185.63	114.50	0.35	0.58
after-before (ppm)	17.63	-130.84	-56.93	-114.09	82.77	0.59	0.24
Nitrous oxide							
after stirring (ppm)	0.33	0.33	0.34	0.35	0.01	0.59	0.41
after-before (ppm)	0.01	-0.01	0.01	0.02	0.02	0.81	0.69
Carbon dioxide							
after stirring (ppm)	0.75	0.59	0.69	0.49	0.14	0.60	0.28
after-before (ppm)	-0.10	-0.22	-0.39	-0.57	0.42	0.76	0.45

*Analysed using linear constrasts

Table 9. Regression equations for ammonia and hydrogen sulfide in the container head space for the feces/urine mixture before stirring[1]

Ammonia

Diet	Equation	R^2
0.0%	NH_3=255.2-24.87 day+0.999 day^2-0.012 day^3	0.582
0.5%	NH_3=218.7-19.65 day+0.787 day^2-0.010 day^3	0.617
1.0%	NH_3=178.2-19.83 day+0.960 day^2-0.014 day^3	0.326
2.0%	NH_3=233.2-23.10 day +1.022 day^2-0.014 day^3	0.7269

Hydrogen Sulfide

Diet	Equation	R^2
0.0%	H_2S=7.367-0.318 day+0.0068 day^2	0.371
0.5%	H_2S=7.535-0.327 day+0.0056 day^2	0.262
1.0%	H_2S=6.197-0.294 day+0.0072 day^2	0.2002
2.0%	H_2S=7.170-0.273 day+0.0044 day^2	0.4265

[1]Values for period one are not included