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Effects of lasalocid on coccidial infection and growth in young dairy calves

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Summary: *Effects of lasalocid on coccidial infection and on calf growth were examined in 16 Holstein bull calves. Calves were assigned randomly to a 2 × 2 factorial arrangement of starter ration containing 0 or 40 mg of lasalocid/kg of starter, beginning when calves were 3 days old (SE = 0.046), and single oral inoculation with 0 or 30,000 sporulated oocysts* (Eimeria bovis) *at 28 days. Pelleted calf starter was fed ad libitum from day 1; milk replacer was fed at a rate of 3.6 kg/d until day 28. Mean daily gain, dry-matter intake, and body weight were increased in calves fed lasalocid and decreased in those inoculated with coccidia. Addition of lasalocid to the feed improved gains by 8% in uninoculated calves and by 50% in inoculated calves. Fecal oocyst numbers were reduced when lasalocid was fed to inoculated calves. Feces were more abnormal in calves inoculated with coccidia. Respiration rates, rectal temperatures, PCV, and serum sodium and potassium concentrations were unaffected by treatment. On the basis of findings in this study, lasalocid minimized effects of coccidial challenge inoculation and increased growth of calves.*

Coccidiosis in young calves is an important source of economic loss in many parts of the United States. Incidence of coccidiosis is the highest of all calf diseases, and morbidity approaches 53%.[1] Calves <1 year old are most severely afflicted,[2] and mortality may reach 24% in severe outbreaks.[1]

Intestinal coccidiosis in cattle is caused by protozoan parasites of genus *Eimeria*,[3] which is characterized by a sporulated oocyst with 4 sporocysts, each with 2 sporozoites.[2] Coccidia are transmitted by ingestion of sporulated oocysts in contaminated water and feed, by soiled pastures, and by hair-licking. After infection, calves may develop diarrhea with or without blood, dehydration, anorexia, anemia, weakness, and weight loss.[4]

Researchers have reported various degrees of prevention of coccidiosis in young calves fed lasalocid.[5,6] Inclusion of ionophores such as lasalocid in early weaning programs improved growth and prevented or controlled coccidiosis in young calves.[5] However, it is not clear whether improvements in growth of animals resulted from control of coccidia or altered ruminal fermentation, as reported by Anderson et al.[7] Additive or synergistic effects of lasalocid in growth responses of young calves infected with coccidia would be of economic benefit to dairy producers raising replacement animals. Therefore, the objective of the study reported here was to evaluate the efficacy of lasalocid on growth and coccidial challenge inoculation in young dairy calves.

Materials and Methods

Animal assignments and management—Sixteen Holstein bull calves were assigned randomly at birth to a 2 × 2 factorial arrangement of calf starter containing 0 (L^-) or 40 (L^+) mg of lasalocid[a]/kg of starter, and oral inoculation with 0 (C^-) or 30,000 (C^+) sporulated oocysts (*Eimeria bovis*). Calves were left with dam until they were 3 days old, then they were moved to individual pens in an open-sided barn bedded with sawdust. They were fed 1.8 kg of milk replacer twice daily (8:00 AM and 4:00 PM) from nipple-bottles until weaning at 28 days. Beginning when the calves were 3 days old (SE = 0.046) and continuing for 12 weeks, experimental calf starters were offered once daily at 8:30 AM at 110% of expected daily intake. Refused calf starter and milk replacer were weighed back daily and reported weekly. Fresh water was available at all times.

Single oral inocula of 0 or 30,000 sporulated oocysts of *Eimeria bovis* were administered 28 days after initiation of the study. Sporulated oocysts[b] were administered orally in a single gelatin capsule.

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[a]Bovatec, Hoffman-LaRoche, Inc, Nutley, NJ.

[b]Provided by Dr. C. A. Speer, Veterinary Research Laboratory, Montana State University, Bozeman.

Experimental calf starters were prepared commercially and contained 17% crude protein (CP) and 75% total digestible nutrients on a dry-matter (DM) basis. Although concentrations were slightly lower than those recommended for calf starter by the National Research Council (CP = 18% of DM; total digestible nutrients = 80% of DM),[6] starters contained high-fiber by-products (acid detergent fiber = 7.5% of DM) to stimulate ruminal development. Lasalocid was mixed into meal prior to pelleting at 40 mg/kg and replaced an equal amount of corn. Remaining ingredients were similar to control calf starter.

Sampling and analysis—Starters were sampled once weekly and stored (−20 C) prior to monthly compositing and analysis for DM, CP, ether extract,[9] acid detergent fiber, neutral detergent fiber,[10] calcium, potassium, magnesium, sodium, and phosphorus.[11]

Body weights (BW) were measured on day 0 (first day of the study) and every 7 days thereafter.

Fecal scores were determined daily at AM feeding to indicate fecal consistency. Feces were scored by the same individual according to the following scale: 1 = normal feces; firm, hard appearance; 2 = slightly loose feces; soft, somewhat liquid; 3 = moderate scours; feces moderately thin and watery; and 4 = severe scours; thin, watery; may contain blood.

A sample of feces was collected per rectum from each calf once weekly, and fecal oocysts were counted by use of the modified McMaster technique.[6] Respiration rate (breaths/min) and rectal temperature were determined daily at AM feeding from day 28 to the end of the study. Approximately 10 ml of blood was collected once weekly by jugular venipuncture into evacuated tubes containing no anticoagulant. Blood was collected approximately 5.5 hours after AM feeding. Serum was removed after coagulation and stored (−20 C) prior to analysis of sodium and potassium concentrations by atomic absorption spectrophotometry. A second sample of jugular blood (approx 3 ml) was collected into evacuated tubes[c] (45 *United States Pharmacopeia* units lithium heparin as anticoagulant) for measurement of PCV by centrifugation.

Statistical analysis—Data were analyzed as a split-plot design by use of a generalized linear mixed models algorithm.[12] The model used follows:

$$Y_{ijk} = \mu + T_i + A_{(i)j} + W_k + (TP)_{ik} + \epsilon_{(ijk)}$$

where Y_{ijk} = dependent variable, μ = overall mean, T_i = effect of ith treatment, $A_{(i)j}$ = effect of jth animal nested within ith treatment, W_k = effect of kth week of study, $(TP)_{ik}$ = effect of treatment × period interaction, and $\epsilon_{(ijk)}$ = residual.

Term $A_{(i)j}$ was used as error term to test differences attributable to treatment. Treatments and periods were defined as fixed effects, and animal was defined as a random effect. Fecal oocyst counts were transformed to logarithms prior to analysis.

Initial and final BW were analyzed as a completely randomized design by ANOVA.

Included in analyses were single degrees of freedom contrasts evaluating lasalocid (L^- vs L^+), coccidial infection (C^- vs C^+), and interaction. Probability of 0.05 was used unless otherwise noted.

[c]Vacutainer, Becton Dickinson, Rutherford, NJ.

*Table 1—Chemical composition of feeds**

Item	Control	Lasalocid
Dry Matter (DM), %	89.4	90.3
	——(% of DM)——	
Crude protein	19.2	19.5
Neutral detergent fiber	35.7	35.4
Ether extract	0.38	0.48
Calcium	0.89	0.92
Phosphorus	0.64	0.64
Magnesium	0.32	0.32
Potassium	1.21	1.21
Sodium	0.41	0.34

*Mean of 5 observations.

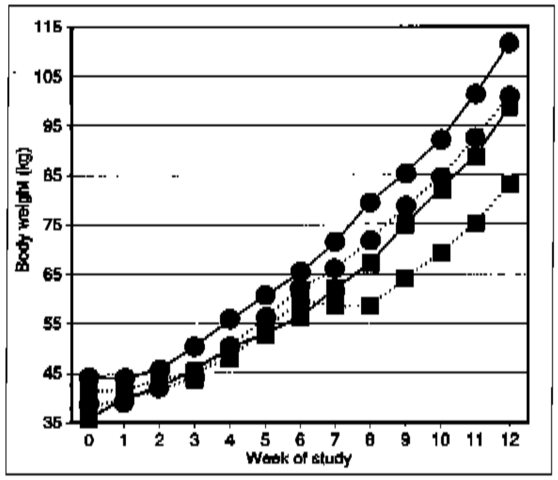

Figure 1—Least-squares means of body weight (kg) in calves fed starter containing 0 (L^-) or 40 (L^+) mg of lasalocid/kg of starter and inoculated with 0 (C^-) or 30,000 (C^+) sporulated oocysts (Eimeria bovis) *at week 4. Standard error of the mean = 2.0. —■— C^-L^-, —●— C^-L^+, ··■·· C^+L^-, ··●·· C^+L^+*

Results

Calf starter averaged 19.4% CP, which was higher than formulated, and 35.6% neutral detergent fiber, because of inclusion of cottonseed and soybean hulls at 7.5 and 20% of the diet, respectively (Table 1).

Calves on treatment C^-L^- and C^+L^+ weighed less initially than other calves (Table 2; $P < 0.01$), although they were randomized to treatments. To test whether initial BW affected measures of performance significantly, data were reanalyzed by analysis of covariance, using the described model, but with initial BW included as a covariable. No measures of performance except serum potassium concentration ($P < 0.03$) were affected by initial BW; therefore, unadjusted means are presented.

Table 2—Least-squares means of body weight (BW), gain, and intake in calves on experimental treatment

Item	Treatments*				SE	Contrasts†		
	C^+L^+	C^+L^-	C^-L^+	C^-L^-		1	2	3
Body weight (kg)								
Initial	38.2	41.3	44.2	35.9	1.7	NS	NS	a
Final‡	101.0	83.2	111.8	99.0	5.5	b	b	NS
Daily gain§	0.75	0.50	0.81	0.75	0.11	b	b	NS
Gain-to-feed§	0.38	0.27	0.34	0.44	0.05	NS	b	a
Intake (kg/d)								
Dry Matter§	1.8	1.4	2.1	1.7	0.3	a	c	NS
Milk	0.13	0.13	0.13	0.13	0.06	NS	NS	NS
Grain§	1.7	1.3	1.9	1.5	0.3	a	NS	NS
Crude protein§	0.47	0.38	0.51	0.43	0.04	a	c	NS
NDF§	0.78	0.64	0.85	0.72	0.08	a	c	NS
TDN§‖	1.5	1.2	1.7	1.4	0.2	a	c	NS

*Treatment = oral infection with 0 (C^-) or 30,000 (C^+) coccidial oocysts; and feeding 0 (L^-) or 40 (L^+) mg of lasalocid/kg of starter. †Contrasts: 1 = lasalocid vs no lasalocid; 2 = coccidia vs no coccidia; 3 = interaction; NS = $P > 0.10$; a = $P < 0.01$; b = $P < 0.05$; c = $P < 0.10$. ‡Final BW at 84 days after initiation of the study. §Significant week × treatment interaction ($P < 0.05$). ‖Calculated value of grain (TDN = 80% of DM) + Milk (TDN = 129% of DM).
NDF = neutral detergent fiber; TDN = total digestible nutrients.

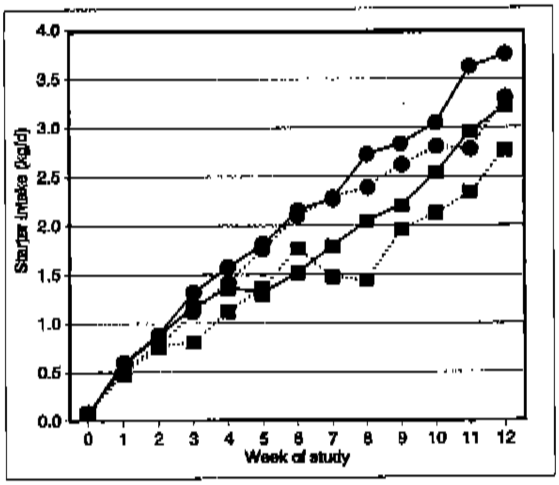

Figure 2—Least-squares means of starter intake (kg/d) in calves fed starter containing 0 (L^-) or 40 (L^+) mg of lasalocid/kg of starter and inoculated with 0 (C^-) or 30,000 (C^+) sporulated oocysts (Eimeria bovis) at week 4. Standard error of the mean = 0.12. See Figure 1 for legend.

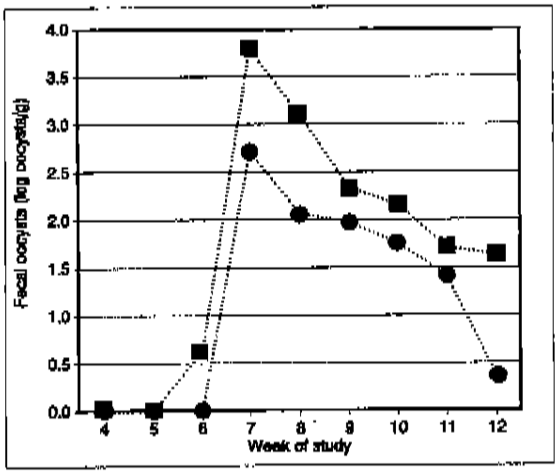

Figure 3—Least-squares means of logarithms of coccidial oocysts (oocyst/g) in feces from calves fed starter containing 0 (L^-) or 40 (L^+) mg of lasalocid/kg of starter and inoculated with 0 (C^-) or 30,000 (C^+) sporulated oocysts (Eimeria bovis) at week 4. Standard error of the mean = 0.18. See Figure 1 for legend.

Least-squares means of final BW (Table 2) were significantly affected by treatment and indicated that calves fed lasalocid ($P < 0.02$) and those uninfected with coccidia ($P < 0.03$) were heavier than other calves. However, when covariately adjusted for initial BW, lasalocid × coccidia interaction approached significance ($P < 0.11$). Covariately adjusted least-squares means were 103.8, 80.8, 104.7, and 105.7 kg for C^+L^+, C^+L^-, C^-L^+, and C^-L^- treatments, respectively. These data indicated that calves on C^+L^- treatment were lightest of all calves. This trend was further observed in least-squares means of weekly BW (Fig 1), which showed a reduction in BW of calves on C^+L^- treatment during weeks 7 and 8. Thereafter, rate of weekly BW change was similar to that for other treatments.

Mean daily gains (Table 2) increased when calves were fed L^+ and decreased when calves were infected with C^+. Addition of L^+ to the feed improved gains by 8% in uninfected calves and by 50% in infected calves. A significant week × treatment interaction indicated that mean daily gain was increased by L^+ during weeks 5 to 8, and C^+ effect was mainly apparent during weeks 7 to 8.

Gain-to-feed ratio (Table 2) was reduced when calves were infected with C^+, but a highly significant ($P < 0.01$) interaction indicated that calves on C^+L^- treatment had markedly reduced feed efficiency. Gain-to-feed ratio was most affected during weeks 7 and 8 in calves inoculated with coccidia. Mean gain-to-feed ratios were 0.29, −0.08, 0.41, and 0.41 for C^+L^+, C^+L^-, C^-L^+, and C^-L^- treatments, respectively, during weeks 7 and 8.

Intake of DM was increased ($P < 0.01$) by feeding L^+, and tended to be reduced by coccidial infection (Table 2). Calves on treatment C^+L^- tended to consume less grain, although interactions were not significant. Intake of milk was unaffected by lasalocid treatment. A significant week × treatment interaction indicated that intake response was affected by week of study. During weeks 6 to 8, intake of starter decreased (Fig 2)

Table 3—Least-squares means of fecal score, rectal temperature, respiration rate, logarithms of coccidia, PCV, and serum sodium and potassium concentrations in calves on experimental treatment

Item	Treatments*				SE	Contrasts†		
	C^+L^+	C^+L^-	C^-L^+	C^-L^-		1	2	3
Fecal score‡,§	1.9	2.0	1.6	1.4	0.1	NS	a	b
Body temp (C)	38.5	38.5	38.4	38.4	0.1	NS	NS	NS
Respiration (breaths/min)	35.3	31.4	34.7	33.6	0.6	NS	NS	NS
Coccidia‡,‖	1.1	1.7	0	0	0.3	a	a	a
Hematocrit (% PCV)	31.4	31.7	32.4	32.8	0.7	NS	NS	NS
Serum Sodium, mg/dl	373.6	372.7	370.8	378.8	2.6	NS	NS	NS
Serum Potassium, mg/dl	18.3	18.3	18.1	18.9	0.4	NS	NS	NS

*Treatment = oral infection with 0 (C^-) or 30,000 (C^+) coccidial oocysts; and feeding 0 (L^-) or 40 (L^+) mg of lasalocid/kg of starter. †Contrasts: 1 = effects of lasalocid vs no lasalocid; 2 = effects of coccidia vs no coccidia; 3 = interaction; NS = $P > 0.05$; a = $P < 0.01$; b = $P < 0.05$. ‡Significant week × treatment interaction ($P < 0.05$). §Fecal score: 1 = normal feces, firm hard appearance; 2 = slightly loose feces; soft somewhat liquid; 3 = moderate diarrhea; feces do not hold firm, are thin and watery; 4 = severe diarrhea; thin, watery; may contain blood. ‖Log of oocysts/g of feces. Data are for week 4 to 12.

in calves on treatment C^+L^-, presumably because of loss of appetite caused by coccidiosis.

Least-squares means of logarithms of coccidial numbers (Table 3) showed a marked increase in calves infected with C^+. However, in calves fed L^+, logarithm of fecal oocysts after inoculation was reduced ($P < 0.01$) from 1.71 to 1.14. Oocysts (all *Eimeria bovis*) began appearing in feces of C^+L^- and C^+L^+ calves approximately 21 days after inoculation (Fig 3). Calves receiving L^+ shed fewer oocysts than did untreated calves ($P < 0.01$). Approximately 4 weeks after inoculation, numbers of oocysts shed by calves infected with C^+ decreased (Fig 3) through the end of the study, suggesting that calves developed resistance to the coccidia, or that no reinfection by coccidial oocysts occurred. Lasalocid effectively reduced the number of oocysts shed by calves on treatment C^+L^+, although coccidiostatic effect was not complete.

Diarrhea was observed in calves 3 weeks after coccidial inoculation. Calves on C^+L^- treatment developed moderate to severe diarrhea (fecal score >3) during the shedding of oocysts, and 1 of these developed bloody diarrhea. Calves on C^+L^+ treatment developed moderate diarrhea (fecal score ≥ 2) during the shedding of oocysts (Table 3). One calf on C^-L^+ treatment had diarrhea during the study. Feces from this calf did not contain oocysts or blood.

As a result of diarrhea in calves inoculated with C^+, fecal scores were increased significantly (Table 3). Mean fecal scores were 1.9 and 1.5 in calves inoculated with C^+ and C^-, respectively. Calves on treatment C^-L^+ had a slight increase in fecal score, compared with C^-L^- calves, whereas those on treatment C^+L^+ had a decrease compared with C^+L^- calves. As expected, fecal scores increased at 21 days after inoculation, similar to shedding of coccidial oocysts.

Respiration rates and rectal temperature were unaffected by treatment. Respiration rates ranged from 31.4 to 35.3 breaths/min (Table 3). Packed cell volume and serum sodium and potassium concentrations were unaffected by treatment (Table 3), and the means were 32% and 374 and 18 mg/dl, respectively.

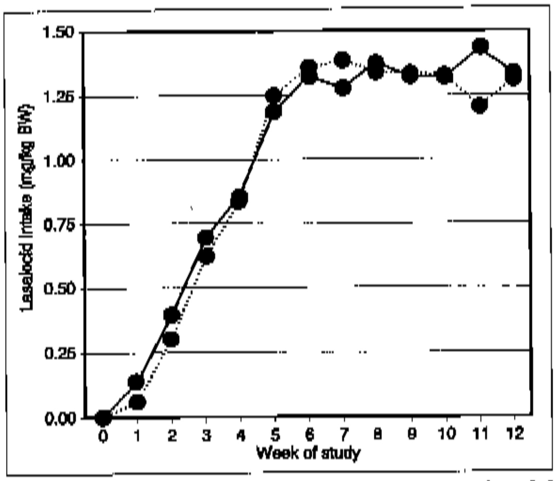

*Figure 4—Mean lasalocid intake (mg/kg BW) in calves fed starter containing 40 (L^+) mg of lasalocid/kg of starter and inoculated with 0 (C^-) or 30,000 (C^+) sporulated oocysts (*Eimeria bovis*) at week 4. Standard error of the mean = 0.04. See Figure 1 for legend.*

Discussion

Results of this study indicated effects of coccidial challenge inoculation with a moderate dose of *Eimeria bovis* on growth, intake, and some metabolic factors of calves older than 4 weeks. Approximately 3 weeks after dosing, effects of challenge inoculation were apparent in calves on C^+L^- treatment. Body weights decreased during weeks 6 to 8; thereafter, calves may have developed natural resistance to coccidia.[1]

Body weight gain was reduced only during weeks 7 and 8 when fecal oocysts exceeded approximately 1000/g of feces. At no time did fecal oocysts reach this number in calves on C^+L^+ treatment, and BW gain was unaffected. Calves not infected with coccidia never discharged oocysts, indicating no cross-contamination between treatment groups. Calves were housed in a barn previously uninhabited by other cattle; therefore, the absence of oocysts in feces of noninoculated calves was expected.

Feed efficiency also was influenced by coccidial challenge inoculation. The reduction in feed efficiency of nearly 30% was probably attributable to a loss of nutrient absorption in the small intestine.

Coccidia destroy the lining of the small intestine, causing nutrients to be incompletely absorbed.[2] Anorectic effects of coccidiosis,[13] coupled with depressed intestinal absorption, would markedly reduce feed efficiency in calves on $C^{+}L^{-}$ treatment.

Although coccidial challenge inoculation resulted in moderate scours, loss of BW, and lower feed intake, other indices of coccidiosis were unaffected by treatment. Respiration rates and body temperature compared favorably with normal values,[14] and serum sodium and potassium concentrations were within normal ranges.[15] Changes in serum sodium and potassium concentrations are generally minor, unless signs of coccidiosis are severe.[15]

Although intake of lasalocid (Fig 4) was generally lower than 200 mg/head/d fed for maximal growth responses, it appeared that lasalocid did affect the environment of the developing rumen, and subsequent growth. Rates of gain were higher than those in other reports from our laboratory[16] because of the influence of lasalocid. Additionally, intake of feed was greater in calves fed lasalocid during the last 6 weeks of the study, suggesting that as ruminal function was established, lasalocid modified the ruminal environment. Also, Anderson et al[7] reported increased feed intake in calves fed calf starters containing 44 mg of lasalocid/kg of starter after the calves were 6 weeks old, as well as increased rates of ruminal fermentation and microbial numbers. Apparently, lasalocid altered intake and growth by increasing ruminal fermentation as well as control of coccidia.

The finding that calves fed L^{+} did not have improved feed efficiency compared with calves fed L^{-} suggested an increased rate of digesta passage. Calves fed L^{+} had higher intakes (Table 2), therefore digestibility of feed probably was lower. Foreyt et al[17] reported that increased feed efficiency in cattle given ionophores may be attributable to changes in volatile fatty acid composition in the rumen (ie, acetic acid concentration decreases and propionic acid concentration increases, whereas total concentration of volatile fatty acids remains unchanged). These changes result in less energy lost as methane.[18-20]

Investigators[6,21] have reported effects of coccidiostats in 6- to 10-week-old dairy calves. However, greater attention should be given to the effects of coccidiosis in younger calves (4 weeks old). Assuming a 3-week incubation period and manifestation of clinical signs in 3- to 6-week-old calves,[22] it is clear that infection by coccidia occurs early in life of the young calf. Also, because early-weaned calves may not consume large amounts of starter prior to weaning (as in this study), combination of weaning stress and coccidiosis may significantly increase morbidity and mortality.

Our findings suggest that lasalocid fed at a rate of 40 mg/kg of starter is effective in reducing effects of coccidial challenge inoculation and increasing growth in early-weaned dairy calves when coccidial oocysts are introduced when the calves are 4 weeks old. Lasalocid fed at a rate of 40 mg/kg of calf starter may benefit calves exposed to coccidia immediately after weaning at 4 weeks from increased rate of gain, improved efficiency of feed utilization, and reduced clinical signs of coccidiosis. Further, reduced shedding of coccidial oocysts may also reduce the degree of infection in the herd.

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