Effects of lasalocid and intermittent feeding of chlortetracycline on the growth of prepubertal dairy heifers

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ABSTRACT

Forty Holstein heifers entered the 12-wk study at approximately 12 wk of age. At enrollment, heifers were blocked by birth date and assigned to 1 of 4 treatments: (1) carrier (30 g; control); (2) lasalocid + carrier (1 mg/kg of body weight; L); (3) chlortetracycline + carrier (22 mg/kg of body weight; CTC); (4) L + CTC + carrier (CTCL). Heifers on CTC and CTCL were provided treatment Monday through Friday and carrier only on Saturday and Sunday. These heifers were provided their respective treatment during wk 1 to 4, 6, and 10; wk 5, 7 to 9, and 11 to 12 heifers were provided the nonmedicated carrier. Heifers were individually fed a total mixed ration with treatments top-dressed at 1200 h daily. Dry matter intake was monitored for each heifer and feed provided was adjusted according to individual intakes. Skeletal measurements were taken weekly and blood samples were obtained every Monday, Wednesday, and Friday. Blood samples were analyzed for thyroxine concentration via radial immunoassay. Heifers supplemented with L had lower average daily gain, overall body weight gain, and trends for lower daily body length gain and overall girth gain compared with CTC heifers, but similar to control and CTCL heifers. Heifers fed L had lower hip height gain and overall hip height gain compared with CTCL heifers, but similar to control and CTC heifers. Heifers fed L had lower overall withers height gain compared with control heifers, but similar to CTC and CTCL heifers. No treatment effect on thyroxine concentrations was observed. These data indicate that L did not increase growth. Results from this experiment indicate that supplementing heifers with L was not beneficial and no benefits to supplementing heifers with CTC or the combination of CTC and L were evident compared with control heifers. Heifers in this study experienced minimal health problems and were regarded to be under low stress levels. Supplementing CTC and L may be

beneficial to growing heifers under conditions where disease exposure and stressors are greater.

Key words: heifer, lasalocid, chlortetracycline, thyroxine

INTRODUCTION

Raising healthy heifers is crucial to the subsequent productivity and longevity of the dairy operation. Cady and Smith (1996) indicate that, other than total feed cost, heifer rearing is the largest expense of a dairy herd. Growth is affected not solely by nutrition, but health and disease incidence as well. To decrease disease incidence and improve growth efficiency, 45.2% of dairy producers feed ionophores and 18.2% feed antibiotics other than ionophores to their heifers (USDA, 2008). Chlortetracycline (CTC) can be fed to control or treat respiratory infections in cattle caused by bacterial pathogens. Research indicates that CTC exposure to *Pasturella multocida*, *Mannheimia haemolytica*, and *Haemophilus somnus* can adversely affect growth kinetics (Reeks et al., 2005) and depress the expression of the gene responsible for production of the leukotoxin that can damage pulmonary tissue (Nanduri et al., 2005). Other research suggests that CTC affects the thyroid gland and the production of thyroxine, thereby altering metabolism (Rumsey et al., 1999), and may reduce mass of the small intestine (Baldwin et al., 2000). Lasalocid (L) is used extensively in the poultry and cattle industries to improve feed efficiency, increase rate of gain, and control coccidiosis. In 2006, the FDA approved the combined use of CTC and L to optimize weight gain and feed efficiency as well as to control coccidiosis and bacterial infections. Therefore, the objective of this experiment was to investigate the efficacy of CTC, L, and their combination on weight gain, skeletal measurements, and metabolic status in 12- to 24-wk-old dairy heifers.

MATERIALS AND METHODS

Experimental Design and Treatments

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(#100905). Forty Holstein heifers entered the study on the first Monday after achieving 12 wk of age and were assigned to 1 of 4 treatments: (1) carrier (control; **C**); (2) L + carrier (1 mg of L/kg of BW; **L**); (3) CTC + carrier (22 mg of CTC/kg of BW; **CTC**); (4) L + CTC + carrier (**CTCL**). All heifers were given the 30 g of carrier (distillers dried grains) per day and CTC, L, and CTCL treatments were adjusted weekly according to individual heifer weight. Heifers were blocked by date of birth and remained on study for 12 wk until they were approximately 24 wk of age. Heifers were individually fed a TMR with their designated treatments top-dressed at 1200 h daily. Heifers on CTC and CTCL were given treatment Monday through Friday and carrier only on Saturday and Sunday. These heifers were also only provided treatment for wk 1 to 4, 6, and 10; wk 5, 7 to 9, and 11 to 12, calves were given carrier. The medication regimen was recommended by the manufacturer. Dry matter intake was individually monitored for each heifer throughout the 12-wk study.

Management and Feeding

Heifers were housed in a naturally ventilated, freestall barn with mattress-covered stalls (129.5 × 72.5 cm), bedded lightly with kiln-dried sawdust. No competition for stalls occurred and water was provided ad libitum. Upon entering the study, heifers were fed the experimental TMR (Table 1) and were trained to use Calan feeding doors (American Calan Inc., Northwood, NH). Heifers were trained on doors by assisting with entry and exit until they were able to do so on their own.

Heifers were fed the experimental TMR (Table 1) individually at 1200 h daily. Feed was mixed and delivered using a mobile feed cart (Data Ranger; American Calan Inc.). The ration was fed to obtain 5 to 10% orts and the amount of feed provided was adjusted in respect to individual intakes. Treatments were top-dressed and hand mixed into each heifer's feed. Feed was stirred multiple times daily to encourage intake and prevent sorting. Because ionophores reduce ruminal degradation of peptides and AA, resulting in an increase in the flow of dietary protein to the small intestine (McGuffey et al., 2001), heifers in this experiment were fed diets that averaged 100.5 g in excess of NRC recommendations for RDP.

Feed Analysis

The amount of feed offered and refused was measured daily. Total mixed ration and ort samples were collected daily and frozen for later analysis. Frozen samples were thawed, dried in a forced hot-air convection oven at 55°C for 48 h (Binder, Bohemia, NY), ground through

a 1-mm screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) and composited into monthly TMR samples and by treatment for ort samples. Samples were analyzed for NDF and ADF using an Ankom 2000 Fiber Analyzer (Macedon, NY) and for CP using a Kjeltec 2200 (Foss, Höganäs, Sweden). A subsample was obtained from each composite sample and submitted to Agri-King Inc. (Fulton, IL) for analysis of fat (method 920.05), ash (method 942.05), Ca, P, Mg, K, Na, and S (method 985.01) according to AOAC International methods (AOAC International, 1999).

Skeletal Measurements

Skeletal measurements were taken weekly throughout the study. Withers and hip height (taken with a sliding scale height stick with a bubble level), body length, heart girth (taken with a measuring tape from point of withers to pin bone), and BW (scale model #708S serial; Cardinal Scale Manufacturing Co., Webb City, MO) were recorded at the beginning of the study period and weekly thereafter at 1100 h.

Blood Sampling and Analysis

Blood samples from heifers were obtained every Monday, Wednesday, and Friday at 1100 h for the duration of the 12-wk study period. Samples were taken via jugular venipuncture into 7-mL vacutainer tubes (Kendall, Mansfield, MA), allowed to clot at room temperature, and then centrifuged (CentraMP4R; International Equipment Co., Needham Heights, MA) at $1,310 \times g$ at 25°C for 20 min. The serum was aspirated and frozen at -20°C for thyroxine analysis. Thyroxine was analyzed at Kansas State University (Manhattan, KS) via RIA

Table 1. Ingredient composition of the experimental diet (range of use over the experiment)

Item	DM (%)
Corn silage	25.96–38.47
Hay crop silage	33.93–49.71
Canola meal	2.80–3.84
Soybean meal	9.00–12.15
Distillers grain	0.95–1.28
Urea	0.22–0.31
Soybean hulls	0.80–1.24
Corn grain	2.59–4.19
Molasses	0.30–0.45
Steam-flaked corn	1.00–4.19
Beet pulp	1.90–3.01
Vitamin and mineral mix ¹	2.04–2.20

¹Contained 19% Ca, 6% P, 3.5% Mg, 1.5% K, 2.5% S, 7.9% Na, 12.2% Cl, 25 mg/kg of Se, 0.26% Zn, 0.26% Fe, 0.26% Mn, 1,092 mg/kg of Cu, 15 mg/kg of I, 264,600 IU/kg of vitamin A, 110,250 IU/kg of vitamin D, and 2,205 IU/kg of vitamin E.

(Total T4 Coat-A-Count radioimmunoassay, Siemens, Tarrytown, NY).

Statistical Analysis

Weekly DMI, ADG for BW and skeletal measurements, FE, DMI as a percent of BW, and thyroxin concentration were analyzed as a randomized complete block design using the repeated measures determined in the MIXED procedure of SAS (Version 9.2; SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijk} = \mu + B_i + T_j + W_k + TW_{jk} + E_{ijk},$$

where Y = the dependent variable; μ = the overall mean; B_i = the random effect of block i ($i = 1, \dots, 10$); T_j = the effect of the j th treatment; W_k = the effect of the k th week ($k = 1, \dots, 12$); TW_{jk} = the effect of interaction between the j th treatment and the k th week; and E_{ijk} = the residual error $\sim N(0, \sigma_e^2)$.

Overall BW and skeletal measurement gains were analyzed as a randomized complete block design using the MIXED procedure of SAS according to the following model:

$$Y_{ij} = \mu + B_i + T_j + E_{ij},$$

where Y = the dependent variable; μ = the overall mean; B_i = the random effect of block i ($i = 1, \dots, 10$); T_j = the effect of the j th treatment; and E_{ij} = the residual error $\sim N(0, \sigma_e^2)$.

Initial weight, skeletal and thyroxin concentration were used as covariates in the experimental design. Degrees of freedom were calculated using the Kenward-Roger option of the MIXED procedure of SAS. The lowest Bayesian information criteria based on the following tests of covariance structure were used: autoregressive type 1, compound symmetry, unstructured, variance components, and Toeplitz. Least squares means were determined for each treatment and the Tukey's option of SAS was selected to differentiate least squares means between treatments. Significant treatment effects were noted at $P \leq 0.05$ and trends were noted at $0.05 \leq P \leq 0.10$.

RESULTS

The nutrient analysis of the TMR is presented in Table 2. Dry matter intake, FE and ADG for BW, and all skeletal measurements are presented in Table 3. A sample of CTC and L were sent for drug analysis. The expected level of CTC was 22.7 g/kg and tested 21.8 g/kg; the expected level of L was 10 g/kg and tested 8.26

g/kg. Acid detergent fiber analysis on ort samples were 28.20 ± 2.04 (mean $\pm$ standard deviation) for C-fed heifers; 26.84 ± 2.63 for L-fed heifers; 27.63 ± 2.78 for CTC-fed heifers; and 27.03 ± 2.92 for CTCL-fed heifers. Neutral detergent analysis on ort samples were 40.86 ± 1.15 for C-fed heifers; 40.38 ± 2.00 for L-fed heifers; 40.09 ± 2.18 for CTC-fed heifers; and 39.35 ± 2.05 for CTCL-fed heifers. Crude protein analysis on ort samples were 14.10 ± 2.12 for C-fed heifers; 12.76 ± 0.59 for L-fed heifers; 12.60 ± 1.01 for CTC-fed heifers; and 13.07 ± 0.34 for CTCL-fed heifers. These data suggest that sorting of the TMR was minimal.

Overall, heifers averaged 108 ± 12 kg of BW upon entering the study. Dry matter intake was typical for heifers of this age and DMI as a percent of BW were similar across treatments. Average daily gain was lower ($P = 0.02$) for heifers supplemented with L compared with heifers supplemented with CTC and similar to heifers supplemented with C and CTCL. Feed efficiency was similar across treatments. Hip height daily gain was lower ($P = 0.02$) for heifers fed L compared with heifers fed CTCL and similar to heifers fed C and CTC. Body length daily gain and heart girth daily gain tended to be lower ($P = 0.07$ and $P = 0.08$, respectively) for heifers fed L compared with heifers fed CTC and similar to heifers fed C and CTCL. Withers height gain was similar across treatments.

Overall gains for BW, hip height, withers height, body length, and girth are presented in Table 4. Overall BW gain was lower ($P = 0.01$) for heifers fed L compared with heifers fed CTC and similar to heifers fed C and CTCL (Figure 1). Overall hip height gains were lower for ($P = 0.01$) for heifers fed L compared with heifers fed CTCL and similar to heifers fed C or CTC (Figure 2). Overall withers height gain was lower ($P = 0.01$) for heifers supplemented with L compared with heifers supplemented with C and similar to heifers supplemented with CTC and CTCL (Figure 3). Overall heart girth tended to be lower ($P = 0.06$) for heifers supplemented with L compared with heifers supplemented with CTC

Table 2. Chemical analysis of experimental diet

Item	DM (%)
CP	18.61 ± 1.13
ADF	25.55 ± 1.57
NDF	38.96 ± 2.62
Fat	2.13 ± 0.31
Ash	7.53 ± 0.38
Ca	0.79 ± 0.07
P	0.43 ± 0.04
Mg	0.27 ± 0.02
K	1.69 ± 0.15
S	0.23 ± 0.01
Na	0.21 ± 0.03

Table 3. Growth measurements for heifers by treatment

Item	Treatment ¹				SEM	P-value
	C	L	CTC	CTCL		
Calves/treatment (n)	10	10	10	10		
Initial BW (kg)	108	107	104	113		
DMI (kg/d)	5.28	5.09	5.55	4.87	0.22	0.17
DMI (BW %)	3.48	3.58	3.46	3.29	0.15	0.57
ADG (kg/d)	1.04 ^{ab}	0.95 ^b	1.06 ^a	1.02 ^{ab}	0.03	0.02
Feed efficiency (kg of gain/kg of feed)	0.25	0.19	0.21	0.21	0.02	0.29
Hip (cm/d)	0.19 ^{ab}	0.17 ^b	0.18 ^{ab}	0.20 ^a	0.01	0.02
Withers (cm/d)	0.09	0.17	0.18	0.19	0.06	0.66
Body length (cm/d)	0.25 ^{ab}	0.24 ^b	0.30 ^a	0.26 ^{ab}	0.02	0.07
Girth (cm/d)	0.29 ^{ab}	0.24 ^b	0.29 ^a	0.29 ^{ab}	0.01	0.08

^{a,b}Means with different superscripts differ.

¹C = control; L = lasalocid; CTC = chlortetracycline; CTCL = combination of chlortetracycline and lasalocid.

and similar to heifers supplemented with C and CTCL. Thyroxine concentrations were not different among treatments (C = 56.6 ng/mL, L = 60.9 ng/mL, CTC = 59.6 ng/mL, and CTCL = 58.9; SE = 3.07).

DISCUSSION

Heifers were provided a diet high in forage (73.7%) as is typical for dairy heifer rations. Studies have indicated that high-forage rations also enhance the action of the ionophore when consumed by ruminants (Thonney et al., 1981; Yang et al., 2003). Goodrich et al. (1984) collected data from 16,000 beef cattle, which indicated that cattle supplemented with monensin consumed 6.4% less feed than cattle fed a control diet. Thonney et al. (1981) reported that cattle supplemented with monensin consumed less feed than cattle supplemented with L. When taste preference was ranked based on consumption, dairy heifers did not differentiate between control and L treatments (Erickson et al., 2004). Though DMI is negatively affected by monensin supplementation, this effect is not typically seen in L-supplemented animals, as observed in the present study. Erickson et al. (2004) reported that heifers previously fed L preferred L over monensin; however, if no previous exposure occurred, then no preference

was seen. Supplementation with CTC typically results in an increase in DMI compared with nonmedicated or ionophore-medicated diets. Jacobson et al. (1952) supplemented newly received feedlot cattle with CTC and saw an increase in DMI. The researchers hypothesized that the selective bacteriostatic action of CTC resulted in more favorable bacterial flora within the gastrointestinal tract, thus improving overall health and increasing appetite. When newly received feedlot cattle were supplemented with CTC at a level of 350 mg/d, an increase in feed intake and subsequent growth rate were observed (Perry et al., 1986), an increase in feed efficiency was also observed (Duff et al., 2000). Unlike the aforementioned studies, no effect of CTC on DMI was observed in our study. Young dairy heifers in commercial environments typically confront many stressors, including weaning, transport and adjustment to group housing, and changes to diet. Because of the weaning procedure at the university dairy facility where this study was completed, many of these stressors were limited.

Average daily gain and overall gain was lowest for heifers supplemented with L, and ADG of BW tended to be higher and was higher in overall BW gain in CTC-supplemented heifers. The results for the L-supplemented heifers is in contrast to the data of

Table 4. Overall gains by treatment

Item	Treatment ¹				SEM	P-value
	C	L	CTC	CTCL		
BW (kg)	87.2 ^{ab}	79.5 ^b	89.6 ^a	85.4 ^{ab}	1.8	0.01
Hip (cm)	15.7 ^{ab}	14.2 ^b	15.0 ^{ab}	16.4 ^a	0.4	0.01
Withers (cm)	16.9 ^a	13.7 ^b	15.5 ^{ab}	15.7 ^{ab}	0.6	0.01
Body length (cm)	22.6	20.9	25.1	22.8	1.2	0.18
Girth (cm)	24.2 ^{ab}	20.4 ^b	24.4 ^a	24.2 ^{ab}	1.2	0.06

^{a,b}Means with different superscripts differ.

¹C = control; L = lasalocid; CTC = chlortetracycline; CTCL = combination of chlortetracycline and lasalocid.

Figure 1. Body weight of heifers fed chlortetracycline (CTC), lasalocid (L), a combination of chlortetracycline and lasalocid (CTCL), or control (C) diets. Asterisks indicate weeks where heifers on CTC and CTCL diets were not receiving treatment. Color version available in the online PDF.

Anderson et al. (1988), who found that L-supplemented calves had greater feed intake and increased weight gain over control calves. Increases in BW gain in CTC-supplemented calves may have resulted from improvement in the microflora of the gastrointestinal tract or a reduction in respiratory pathogen load. Effect of L, CTC, and the combination affected individual skeletal measurement differently. Heifers on the C, CTC, and CTCL treatments tended to have greater daily hip height gain and gained the most hip height overall. The daily withers height gain was not different among treatments, but overall withers height gain was lowest for heifers supplemented with L and greatest for the other treatments. Overall body length gain was greatest in CTC-supplemented heifers. Overall girth tended to be lower for L-supplemented heifers. Supplementation with CTC has been associated with increased skeletal development (Bush et al., 1959) and growth. Jacobson et al. (1952) reported that though differences were small, trends were noted for CTC-supplemented calves to be taller at the withers and have greater chest depth and barrel circumference ($P = 0.01$) compared with calves that did not receive CTC. Little work has been done investigating the effect of L on skeletal growth measurements; however, Steen et al. (1992) report an increase

in heart girth with L supplementation compared with heifers not supplemented with L. Heifers on the CTC or CTCL treatment were not appreciably larger than C heifers. Current FDA regulations do not allow for the continuous feeding of CTC to dairy heifers intended for replacement (Feed Additive Compendium, 2011). In our study, CTC was provided at the therapeutic level on a schedule, which attempted to simulate commercial procedures when CTC is fed to dairy heifers as a management program to help alleviate respiratory disease for cattle fed in confinement. The incidence of respiratory disease cannot be predicted in a commercial operation, therefore, the schedule for administration of CTC used in this study tried to cover a time span when respiratory disease could be a health issue affecting the growth and productivity of replacement dairy heifers. Because the levels of stress and potential exposure to respiratory disease experienced by heifers in this study may not have been as severe as heifers raised in commercial facilities, its full effect may not have been realized. The treatment schedule may have affected growth, as Jacobson et al. (1952) indicated that removing CTC from the ration caused a diminution in rate of growth and adding CTC to the ration resulted in an early increase in growth rate. However, this is unlikely because

Figure 2. Hip height of heifers fed chlortetracycline (CTC), lasalocid (L), a combination of chlortetracycline and lasalocid (CTCL), or control (C) diet. Asterisks indicate weeks where heifers on the CTC and CTCL diets were not receiving treatment. Color version available in the online PDF.

the treatment by week interaction was not significant, indicating that growth parameters were not affected in CTC-supplemented heifers between on and off CTC treatment weeks.

Previous research suggests that CTC may affect endocrine function, specifically in relation to the pituitary and thyroid glands. Rumsey et al. (1999) observed a reduction in growth hormone and thyroid-stimulating hormone release after a challenge with their respective releasing hormones in CTC-supplemented steers caused by the reduction in pituitary gland sensitivity. Those authors postulated that CTC attenuates the release of growth hormone, thyroid stimulating hormone, and thyroxine, resulting in increased tissue deposition. In this study, thyroxine concentrations were not different among treatments, which may partially explain the lack of skeletal growth in reference to hip and withers height improvement associated with CTC. Similar to the results of this study, Reid et al. (2006) did not observe an increase in growth in CTC-supplemented dairy heifers or a difference in mean sera thyroxine concentrations. In our study, thyroxine may not have been affected either because of the CTC feeding regimen used or a potential breed difference in this response between beef and dairy heifers. Sizable differences were observed in serum

thyroxine concentrations from heifer to heifer possibly due to low animal numbers, which may obscure treatment effects. Feeding regimen may have been a factor, however, no treatment by week interactions were noted with respect to thyroxine. This indicates that thyroxine concentration was not affected when CTC was provided or removed from CTC-supplemented heifers.

CONCLUSIONS

Feeding L in this experiment was not beneficial, and no differences were observed among the other treatments indicating that nonmedicated heifers responded similarly to heifers medicated with CTC or the combination (CTCL). Provision of antibiotics presented no benefit in this experiment. Supplementing CTC or CTCL may have had a greater effect if experimental conditions more closely simulated commercial dairy heifer rearing facilities and management procedures where stress levels and environmental conditions may increase the potential for clinical respiratory and enteric disease to be present. Heifers on our study were apparently healthy and stressors were minimal. If stress and disease incidence had been greater, CTC and L may have affected growth more. Future research should

Figure 3. Withers height of heifers fed chlortetracycline (CTC), lasalocid (L), a combination of chlortetracycline and lasalocid (CTCL), or control (C) diets. Asterisks indicate weeks where heifers on the CTC and CTCL diets were not receiving treatment. Color version available in the online PDF.

focus on better identifying those conditions that predispose dairy heifers to both subclinical and clinical disease incidence so that feeding regimens for CTC and CTCL maybe more precisely designed and implemented to allow optimum growth performance of growing dairy replacement heifers.

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