Effect of chlortetracycline in a trace mineral salt mix on fertility traits in beef cattle females in Florida

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ABSTRACT: The study objective was to determine the effect of chlortetracycline in an ad libitum trace-mineralized salt mix given to heifers before and/or during bull exposure on the proportion pregnant and the time to conception in a fixed breeding period. Heifers (n = 768), 13 to 15 mo of age, were individually identified, immunized (Leptospira, Campylobacter fetus), examined (body condition score, vaginal lesion score, BW), and randomly allocated within 2 x 2 factorial blocks of treatments where trace mineral salt with and without chlortetracycline medication was provided in similar but nonadjacent pastures either before and/or during bull exposure. The chlortetracycline feeding period was about 30 d for each. In the 2 d immediately before bull exposure, heifers were examined (body condition score, vaginal lesion score, reproductive tract evaluation) and reallocated to treatment pastures. Pregnancy was determined by per rectal palpation at 45 d following bull removal. Mineral intake was below that expected for heifer groups and, as a result, chlortetracycline intake was estimated at less than one-third of that targeted. Many heifers were not cycling reproductively at the onset of bull exposure (n = 456, 60.3%, based on a reproductive tract score < 3). Despite these limitations, heifers receiving chlortetracycline treatment before breeding had a pregnancy percentage of 65% (chlortetracycline before and during breeding 67% and chlortetracycline before breeding 61.8%) compared to those receiving no treatment before breeding (53%, P < 0.03; no chlortetracycline before or during breeding 60.4% and chlortetracycline during breeding 47.4%). Heifers receiving chlortetracycline treatment before breeding were 57% more likely to become pregnant than those not treated before breeding. Change in vaginal lesion score was associated with the proportion pregnant, but neither body condition score nor average daily gain were.

Key Words: Beef Cattle, Chlortetracycline, Heifers, Puberty, Reproduction

Introduction

The raising of eligible replacement heifers is a challenge among beef producers in the southeast United States. Genotype (Zebu) and environment often interact to the detriment of heifer development for breeding at 1 yr of age. The use of chlortetracycline has shown promise for improving cyclicity and pregnancy percentage in yearling heifers and is, thus, very relevant to cow-calf economics.

In a series of trials in Florida, crossbred beef females were fed different chlortetracycline regimens relative to weaning and breeding to determine effects on fertility. In the first (Rae et al., 1993b), crossbred yearling heifers were either given chlortetracycline or no chlortetracycline in a grain supplement provided to all heifers for 30 d before natural breeding. More pregnancies occurred in the treated group compared with controls (71.5 and 56.8%, respectively; P < 0.01). Ureaplasma diversum was known to be endemic in these heifers, but no relationship was found between pregnancy percentage and Ureaplasma diversum culture. The possible effects of other tetracycline-susceptible pathogens were eliminated. In subsequent trials (Saltman et al., 1998) using crossbred yearling heifers and cows, treated females achieved a greater proportion of pregnancies than did the untreated animals and achieved them earlier in the breeding season. In each trial, body condition at breeding was a significant factor in outcome.

In our studies, chlortetracycline was delivered as a supplemented grain mix. It was postulated that chlortetracycline delivery could be simplified by administration in an ad libitum trace-mineralized salt. The objective of this trial was to determine the effect of chlortetracycline delivered in a trace-mineralized salt on percentage pregnant and time to conception within a
fixed breeding season in beef heifers treated before and/or during the breeding period and to evaluate body condition score (BCS), BW, and vaginal lesion score as they relate to pregnancy and chlortetracycline treatment.

**Materials and Methods**

Crossbred beef heifers to be exposed to Angus bulls during spring 1999 (n = 768) were selected on January 13 to participate in the trial while located at a contracted grazing facility. Each heifer was evaluated for body condition (1 = thin, 9 = obese; Herd and Sprott, 1986; Spitzer, 1986), vaginal lesion score (0 = no lesions, 4 = severe inflammation; Rae et al., 1993b), BW, and breed type (Simmental × Brahman, Hereford × Brahman, and Angus × Brahman). Each heifer, by breed type, was assigned by alternate chute sorting to one of four groups for assignment to a randomized block design having a 2 × 2 factorial arrangement of treatments, in which trace-mineralized salt with and without chlortetracycline medication (Aureomycin, Roche Vitamin, Inc., Parsippany, NJ; the use of chlortetracycline for this purpose is not currently approved by the U.S. Food and Drug Administration) was provided in similar but nonadjacent pastures either before and/or during bull exposure (Table 1). The four simple effect treatments were no chlortetracycline before or during breeding, chlortetracycline before breeding, chlortetracycline during breeding, and chlortetracycline before and during breeding. Number of heifers in each group are presented in Table 1. Animals received chlortetracycline for approximately 30 d before breeding and for 35 d during breeding.

Before the breeding season began (February 11), an examination was performed to determine reproductive tract score (1 = infantile reproductive tract, 5 = cyclic tract; Anderson et al., 1991), BCS, and vaginal lesion score. Vaginal lesion scores were determined before any reproductive evaluations by cleaning and parting the lips of the vulva, and examining the mucosal surface of the vagina for signs of inflammation. The degree of inflammation was scored as follows: 0 = no lesions, 1 = mild vesiculation, 2 = moderate vesicular inflammation, 3 = marked pustular inflammation, and 4 = severe inflammation and excoriation (Rae et al., 1993b).

Heifers were exposed to multisire natural mating (26 bulls; 25:1 service ratio), with half of the bulls allocated to each treatment pasture and managed in accordance with standard ranch protocol. Before the start of breeding, bulls were scored for body condition and tested for breeding soundness (Chenoweth et al., 1992) and *Tritrichomonas fetus*; both heifers and bulls were immunized against *Campylobacter fetus venerealis* and five serovars of *Leptospira interogens*. The medicated mineral feeding was discontinued on March 18. All heifers received or continued to receive the unmedicated trace-mineralized salt mixture.

The trace-mineralized salt was a custom mix that had been in use by the ranch for several years. Chlortetracycline was added to the mix at the feed mill in batch. In previous trials (Rae et al., 1993b), the target consumption of chlortetracycline in a grain supplement had been 1.1 mg·kg⁻¹·d⁻¹. Based on this and an expected trace mineral consumption of 50 to 100 g/d, the trace-mineralized salt mix was prepared with 4.928 mg of chlortetracycline to each kilogram of trace-mineralized salt. The medicated and unmedicated trace mineral was provided to the respective groups in weather-protected, ad libitum mineral feeders. Mineral consumption was monitored by recording dates and quantity of mineral distribution.

Heifers were transported from the site of contract grazing to the ranch proper about 70 d after initial bull exposure (April 22). This was done as a matter of grazing availability. At the home ranch, heifers continued their exposure to the bulls in two groups representing an even mix of groups. Bulls were removed from the heifers on May 15. Pregnancy examination was conducted about 45 d after breeding (June 29 and 30). This examination included determination of pregnancy status by per rectal palpation, gestational age of pregnant animals (range 45 to 135 d), BCS, vaginal lesion score, and BW.

Effects of chlortetracycline on pregnancy rate were examined using the CATMOD procedure of SAS (SAS Inst. Inc., Cary, NC). Analysis of variance (Proc MIXED) was used to examine treatment effects on time-to-conception and average daily gain. Logistic regression (Proc Logistic) tested the association of the before-breeding-period treatment and covariables of interest with respect to pregnancy status, such that

$$ Pg = CTC + ΔBCS + ΔVLS + ADG + \text{error} $$

where

- $Pg = \text{pregnancy state (0 = nonpregnant, 1 = pregnant)}$
- $CTC = \text{chlortetracycline or no chlortetracycline treatment in the 30-d period before breeding}$
- $ΔBCS = \text{change in body condition score (1 to 9) between initial and pregnancy examination, categorized to represent a loss, no change or a gain of BCS}$
- $ΔVLS = \text{change in vaginal lesion score (0 to 4) between initial and pregnancy examination, categorized to represent a reduction, no change or an increase in vaginal lesion severity}$
- $ADG = \text{average daily gain (kg) measured between the initial and pregnancy examination}$

Survival analysis procedures (Proc LifeTest and PHReg) evaluated survival and hazard functions by
treatment relative to conception events within the time interval of bull exposure.

Results

No significant differences were observed for the baseline variables BW, BCS, vaginal lesion score, or reproductive tract score by treatment group. Eight heifers were removed from the trial due to death or loss of identification: no chlortetracycline before or during breeding = four heifers, chlortetracycline before breeding = one heifer, chlortetracycline during breeding = two heifers, and chlortetracycline before and during breeding = one heifer.

The level of mineral consumption over the 64-d treatment period was estimated at 17 g heifer\(^{-1}\)d\(^{-1}\) for those receiving chlortetracycline and 24 g animal\(^{-1}\)d\(^{-1}\) for those not receiving chlortetracycline (total consumption 409 and 591 kg of mineral, respectively). The level of daily consumption in each group was similar for both the before-breeding and the during-breeding treatment periods. Given an estimated mean daily consumption of 17 g animal\(^{-1}\)d\(^{-1}\) for the chlortetracycline treatment group, the mean daily consumption of chlortetracyline was estimated at 84 mg d\(^{-1}\). Based on the mean starting heifer BW of 275 kg, the desired chlortetracycline daily intake for each treated heifer was 303 mg/d (or 61 g/d of trace-mineralized salt). This represented a dose of 0.3 mg-kg BW\(^{-1}\).d\(^{-1}\), about 28% of the targeted chlortetracycline dose.

Evaluation of heifer reproductive tract score suggested that many heifers were not cycling reproducitively at the onset of the breeding period (n = 456, 60.3%, reproductive tract score < 3). Effects of chlortetracycline treatment on pregnancy rate and heifer gain are presented as simple effects in Table 2. Heifers receiving chlortetracyline both before and after breeding gained more weight (P < 0.05) than did controls. Heifers receiving a full 64 d of chlortetracycline treatment gained 0.11 kg/d more than did those receiving no chlortetracycline (Table 2). The main effect of chlortetracyline before breeding was found to be associated with an increased percentage pregnant (65 and 53%, respectively, P < 0.03) and an increased daily gain (0.36 kg and 0.30 kg, respectively, P < 0.01) compared to those not receiving chlortetracycline, whereas the effect of chlortetracyline during the breeding period was not different for either pregnancy percentage and daily gain (57 and 60%, respectively, P = 0.57; 0.36 kg and 0.31 kg, respectively, P = 0.06).

The logistic regression model (Table 3), describing the association of treatment and factors related to pregnancy status in study heifers had a very good model fit (Hosmer-Lemeshow statistic, P = 0.908) and included treatment before the breeding period with or without chlortetracyline, change in BCS, change in vaginal lesion score, and average daily gain. Heifers receiving chlortetracyline before the start of the breeding season were 57% more likely to become pregnant than heifers not treated before breeding (P < 0.003). Those heifers with no change or an increased severity score of vaginal inflammation had from 35 to 114% increases in likelihood of pregnancy (P < 0.09 and P = 0.0001, respectively, Table 3) compared with those heifers having a reduction in vaginal lesion score. Heifers that maintained or gained BCS during the study period were from 6 to 53% more likely to become pregnant than heifers that lost condition; this, however, was not significant (P > 0.10). Likewise, there was a greater likelihood of pregnancy as average daily gain increased (P > 0.10) but did not show the same level of significance as the simple analysis of variance (Table 2).

The mean time to conception during the breeding period for all heifers was 63 d. Effects of chlortetracycline treatment on time to conception are presented as simple effects in Table 2. The cumulative proportion of heifers pregnant over the time period of bull exposure is depicted in Figure 1 (the inverse of the survival function). The pattern of pregnancy for at least one group (chlortetracycline during breeding) was different from others. Given the difference, the risk of pregnancy was evaluated by hazard analysis, that is, the hazard, or risk, of becoming pregnant during the bull exposure period. The instantaneous risk of a heifer becoming pregnant during the breeding period by treatment group is depicted in Figure 2. Heifers in all groups had rising risk levels but again varying as to height and duration of declining risk. Toward the end of the bull exposure period, all groups again had rising risk levels but again varying as to height and duration of rise.

Discussion

Heifers receiving chlortetracycline treatment before breeding had a pregnancy percentage of 65% (chlortet-
Table 2. Pregnancy rate, time to conception, and average daily gain of heifers receiving chlortetracycline (CTC) before and/or during the breeding season

<table>
<thead>
<tr>
<th>Item</th>
<th>No CTC</th>
<th>CTC before breeding</th>
<th>CTC during breeding</th>
<th>CTC before and during breeding</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals, n</td>
<td>189</td>
<td>191</td>
<td>190</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>Pregnant, %b</td>
<td>57.7e</td>
<td>61.8e</td>
<td>47.4f</td>
<td>67.4f</td>
<td>—</td>
</tr>
<tr>
<td>Time-to-conception, dc</td>
<td>60.4e</td>
<td>60.1e</td>
<td>69.4f</td>
<td>59.8e</td>
<td>2.3</td>
</tr>
<tr>
<td>Gain/d, kgd</td>
<td>0.29e</td>
<td>0.33f</td>
<td>0.32e</td>
<td>0.40f</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*aChlortetracycline was provided in a trace mineral mix for either 30 d before or the first 35 d during a breeding season. The CTC was consumed at a rate of approximately 0.3 mg·kg$^{-1}$·d$^{-1}$.

*bDetermined by rectal palpation 45 d after the end of the breeding season.

*cEstimated time to conception within the breeding season (February 11 to May 15) determined by rectal palpation.

*dDetermined between the initial and final examination days of the study (January 13 to June 30).

*e,fRow values with different superscripts differ from the no CTC controls ($P < 0.05$).

Heifers with a higher BCS or a greater ADG were more likely to become pregnant than heifers in lower BCS or lesser ADG; these were not, however, statistically significant in this study. The vaginal lesion score did follow a pattern similar to that observed in previous trials (Rae et al., 1993a); that is, on average, as the change in vaginal lesion score increased (indicating a greater lesion severity), a larger proportion of heifers were found to be pregnant. This was counter to our initial hypothesis; it was then modified to explain this now repeated finding (Rae et al., 1993a). It is supposed that with the approach of estrous cyclicity, either due to hormonal changes in the reproductive tract or increased sexual activity (sniffing, nuzzling), the vagina becomes more susceptible to infection or inflammation from organisms such as *Ureaplasma diversum*. So an increase in vaginal lesions may be associated with readiness of heifers to become pregnant. This is supported by the

Table 3. Logistic regression odds ratios, confidence intervals, and model estimates for variables associated with pregnancy in heifers treated with chlortetracycline (CTC) or without (no CTC) before the beginning of the fixed breeding period

<table>
<thead>
<tr>
<th>Predictor variables</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
<th>Estimate</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.57</td>
<td>1.16 - 2.11</td>
<td>-0.749</td>
<td>0.417</td>
<td>0.073</td>
</tr>
<tr>
<td>CTC before breeding</td>
<td>1.57</td>
<td>baseline comparison</td>
<td>0.450</td>
<td>0.152</td>
<td>0.003</td>
</tr>
<tr>
<td>no CTC before breeding</td>
<td>baseline comparison</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>ΔBCS = lost, 1 or more</td>
<td>1.53</td>
<td>baseline comparison</td>
<td>0.057</td>
<td>0.323</td>
<td>0.860</td>
</tr>
<tr>
<td>ΔBCS = 0 (no change)</td>
<td>1.56</td>
<td>0.56 - 2.00</td>
<td>0.424</td>
<td>0.235</td>
<td>0.237</td>
</tr>
<tr>
<td>ΔVLS = reduced, 1 or more</td>
<td>1.35</td>
<td>baseline comparison</td>
<td>0.302</td>
<td>0.177</td>
<td>0.088</td>
</tr>
<tr>
<td>ΔVLS = 0 (no change)</td>
<td>2.14</td>
<td>1.45 - 3.16</td>
<td>0.759</td>
<td>0.199</td>
<td>0.0001</td>
</tr>
<tr>
<td>ADG</td>
<td>1.61</td>
<td>0.86 - 3.04</td>
<td>0.479</td>
<td>0.323</td>
<td>0.139</td>
</tr>
</tbody>
</table>

*aΔBCS: Change in body condition score (BCS; 1 to 9) between initial and pregnancy examination, categorized to represent a loss, no change, or a gain of BCS.

*bΔVLS: Change in vaginal lesion score (0 to 4) between initial and pregnancy examination, categorized to represent a reduction, no change, or an increase in vaginal lesion severity.

ADG: Average daily gain measured between the initial and pregnancy examination.

Hosmer-lemeshow statistic (C) 3.391 with d.f. 8 ($P = 0.908$).
Figure 1. A cumulative proportion representation of heifers pregnant during the time of bull exposure by chlortetracycline treatment groups, that is, heifers treated with chlortetracycline (CTC) or without (no CTC) for \(\sim 30\) d before the start of the breeding period and (or) \(\sim 35\) d during the bull exposure period.

Figure 2. Risk of pregnancy at given points in time during a fixed breeding period for heifers treated with chlortetracycline (CTC) or without (no CTC) for \(\sim 30\) d before the start of the breeding period and (or) \(\sim 35\) d during the bull exposure period.

fact that, regardless of treatment, pregnant heifers had an increased mean vaginal lesion score compared with nonpregnant heifers. It did not appear that the chlortetracycline had an effect in reducing vaginal inflammation or lesions, as measured by our lesion scores.

The mechanism of chlortetracycline action on growth, body condition, and reproductive performance is not clear. It is hypothesized to have direct and (or) indirect effects on bacterial challenges in the animal, even at subtherapeutic levels. The proposed mechanisms of action include an inhibition of bacterial multiplication, or an altering of the bacterial cell wall, causing it to be more sensitive to immune clearance mechanisms (Lorian, 1980) and (or) a decreased ability of bacterial cells to colonize tissues and produce toxins (Chopra and Roberts, 2001). In the current study, the specific influence of chlortetracycline on reproductive pathogens was not measured.

The delivery of chlortetracycline by means of an ad libitum trace mineral supplement was not as successful as anticipated. Our target dosage of chlortetracycline was 1.1 mg-kg BW\(^{-1}\)-d\(^{-1}\) (or about 61 g of mineral (303 mg of chlortetracycline) per day for heifers averaging 275 kg at the study’s onset). Heifers received only about 28% of this target dose. The criteria for selection of this dose was based on that recommended for treatment of anaplasmosis. There was, however, an apparent positive benefit, and it appears that the positive benefits may be evident at lesser dosages. Nonetheless, controlled delivery of a specified dose by mineral administration is questionable, based on experience of this study.

The influence of chlortetracycline during breeding only on proportion pregnant during each time period was evident. This group had the lowest overall percentage pregnant (47%). The cause for the disparity in this group was unclear; it had similar baseline parameters, was managed similar to other groups, and maintained in pastures with either the group receiving no chlortetracycline or those receiving chlortetracycline both before and during breeding. We speculate that a difference may have existed in these heifers that was not explained by treatment effect or the contributing factors.

The measure of reproductive readiness to conceive, measured by reproductive tract score, suggested that a large number of heifers (60%) were not cycling at the beginning of the breeding period. The pattern of conception and graphic representation of risk of conception (Figures 1 and 2) suggest that heifers that were cycling at the beginning of bull exposure period became pregnant as expected. Then, either due to a reduced number of cycling heifers (heifers that still had not become pregnant) or due to nutritional challenge resulting in reduced expression of estrus, a nadir of risk potential for pregnancy resulted at the midpoint of bull exposure period. In the latter part of the bull exposure period, there is a pattern of increasing risk for becoming
Fertility of chlortetracycline-treated heifers

pregnant (Figure 2). The interpretation is made, with caution, that heifers receiving chlortetracycline both before and during breeding had the greatest rise and longest duration of increased risk or potential for pregnancy, followed by those receiving chlortetracycline before breeding, the controls receiving no chlortetracycline, and finally those receiving chlortetracycline only during breeding. The group receiving no treatment in either time period had a very strong instantaneous risk pattern during the early part of the breeding period but lacked all but a modest rise in risk for pregnancy in the latter portion of the breeding period. It is possible that the benefits of treatment were delayed in their manifestation or augmented with the addition of an improved plane of nutrition (i.e., the move to the home ranch).

Implications

There was evidence of an improved pregnancy percentage and an improved pattern of conception within the breeding season in heifers treated with the chlortetracycline trace mineral in the 30 d before the onset of the breeding period. Heifers treated both before and during the breeding season had the best reproductive performance. These results were observed despite consumption of chlortetracycline in trace-mineralized salt below the level anticipated. Treatment effects may have been delayed as depicted in the survival analysis instantaneous risk model. The mechanism of action by which the chlortetracycline has its effect is not clear. It is likely that it has a positive effect on daily gain of treated heifers but does not appear to appreciably influence the inflammation and lesions observed in the vagina.

Literature Cited


