Journal of Animal and Veterinary Advances 2 (9): 531-535, 2003

Influence of Subtherapeutic Chlortetracycline and Dietary Protein on Circulating Concentration of Insulin-Like Growth Factor-1 in Growing Beef Steers

[1]K. R. McLeod, [2]R. L. Baldwin, [3]T. S. Rumsey, [3]T. H. Elsasser, [3]S. Kahl and [4]M. N. Streeter
[1]U. S. Department of Agriculture, Agricultural Research Service, [2]Animal and Natural Resources Institute, Bovine Functional Genomics Laboratory, [3]Growth Biology Laboratory, [4]Beltsville, MD 20705 and Alpharma Inc., Animal Health Division, Fort Lee, NJ 07024

Abstract: The objective of this study was to determine the effects of oral administration of a subtherapeutic amount of chlortetracycline (CTC) on plasma concentrations of insulin-like growth factor-1 (IGF-1) in bovine receiving two levels of dietary protein. Thirty-two beef steers, weighing 286 ± 3 kg, were allotted randomly by weight to a factorial arrangement of treatments consisting of diets containing either marginal (10%) or adequate (13%) amounts of crude protein supplemented with a corn meal carrier (500 g d^{-1}) containing either 0 or 350 mg CTC. Blood samples were collected from each steer via jugular puncture on d 0, 7, 14, 28, 56, and 84 and plasma concentrations of IGF-1 were determined by radioimmunoassay. Across the 84-d sampling period, mean plasma IGF-1 concentration was greater ($P < 0.03$) in steers fed 13% protein compared with those fed 10% protein. However, there was a tendency ($P < 0.11$) for a CTC × dietary protein interaction. Analysis within d revealed that on d 7 and 14 plasma IGF-1 concentration in steers fed 10% protein was greater for those receiving CTC compared with those fed carrier alone (CTC × dietary protein, $P < 0.10$). However, by d 28 only a numerical difference was observed and by d 56 this temporal effect of CTC was no longer apparent. Results from this study suggest that oral administration of a subtherapeutic amount of CTC increases circulating IGF-1 concentrations in a temporal fashion in steers when dietary protein supply is marginal.

Key words: Insulin-Like growth factor-1, chlortetracycline, protein, bovine

Introduction

Oral administration of subtherapeutic levels of antibiotics has been shown to promote growth in a number of species studied, including bovine (Visek, 1978). The mechanism(s) by which antibiotics stimulate growth has not been elucidated. However, it is generally hypothesized that the growth promoting actions of antibiotics is directed through alterations in gut microbial flora and(or) thinning of the digestive tract and subsequent increase in nutrient absorption (Visek, 1978 and Hays, 1991). This hypothesis is supported by experiments which have demonstrated that subtherapeutic levels of antibiotics fail to promote growth under germ-free conditions (Whitehair and Thompson, 1956; Coats *et al.*, 1963 and Freeman *et al.*, 1975). Moreover, the growth promoting effects of antibiotics have been shown to be enhanced when animals are fed diets marginal in essential nutrients, such as protein (Hays, 1991).

Recent studies indicate that subtherapeutic levels of antibiotics alter the endocrine growth axis. Oral administration of antibiotics has been shown to increase plasma concentrations of insulin-like growth factor-1 (IGF-1) in growing swine (Hathaway *et al.*, 1996). Additionally, we have demonstrated that oral administration of chlortetracycline (CTC) decreases pituitary release of growth hormone (GH) and thyroid-stimulating hormone in bovine challenged with GH-releasing hormone (GHRH) and thyroid-releasing hormone (Rumsey *et al.*, 1999). Currently, there are no published reports on the effects of subtherapeutic levels of antibiotics on circulating IGF-1 concentrations in bovine. Therefore, the objective of this experiment was to determine the effects of a subtherapeutic level of CTC on circulating IGF-1 concentrations in growing bovine receiving diets containing adequate or marginal amounts of protein.

Materials and Methods

Angus crossbred beef steers were purchased from a regional calf sale in central Virginia and delivered to the ARS research facilities at Beltsville, MD. Prior to implementation of the experimental protocol, steers were given a parasiticide (ivermectin; Ivomec, Merck and CO., Inc, Whitehouse Station, NJ), treated for ring worm (griseofulvin, 3 d; Fulvicin-U/F, Schering-Plough Animal Health Corp., Kenilworth, NJ), and housed on a fescue and orchard grass pasture for a 30-d quarantine period. All procedures involving the steers were approved by the Beltsville Agricultural Research Center Institutional Animal Care and Use Committee (protocol #96-022).

Thirty-two steers were housed individually in covered pens (1.7 m × 6.1 m) with concrete flooring and continuous access to water. Steers were subjected to a 42-d adaptation period. During the initial 35 d of

adaptation, steers were fed a pelleted 60% concentrate diet containing 13% crude protein (CP). The amount of feed offered was limited to provide an adequate amount of metabolizable energy to achieve 1.25 kg d^{-1} of body weight gain (NRC, 1984). After the initial 35 d, steers (285.8 ± 5.9 kg) were stratified by weight and steers within each stratification were assigned randomly to a 2 × 2 factorial arrangement of treatments. Treatments included pelleted diets (Table 1) containing either 10 or 13% CP supplemented with 500 g d^{-1} of a ground corn carrier containing either 0 or 350 mg CTC (Aureomycin, Roche Vitamins Inc., Parsippany, NJ). The 10 and 13% CP diets were formulated to provide marginal and adequate amounts of CP necessary to achieve maximal growth rates, respectively (NRC, 1984). Steers were fed their respective experimental diets (minus supplement) for a 7-d transition period in order to adjust to *ad libitum* intakes. At the onset of the 84-d experimental period (i.e., end of the adaption period), carrier or carrier plus CTC was introduced into the diet by placing 1 kg of experimental diet into each feeder and top dressing it with the appropriate supplement (0800). After this portion of the diet was consumed, the remainder of the daily allotment of feed was placed into the feeders (1000). Orts were recorded daily (0730) and the amount of feed offered was adjusted to maintain approximately 10% orts. Steers were exercised daily and body weights were recorded weekly prior to feeding (0700).

Table 1: Composition of Adaptation and Experimental Diets (Dry Matter Basis)

		Experimental diet	
Item	Adaptation diet[a]	10% Protein	13% Protein
Ingredient, %			
Cracked corn	41.0	43.5	36.5
Wheat straw	---	20.0	20.0
Cottonseed hulls	---	20.0	20.0
Orchard grass hay	40.0	---	---
Molasses, cane, black-strap	9.0	---	---
Molasses, cane, dehydrated	---	9.0	9.0
Soybean meal, 48%	8.0	5.5	12.5
Trace mineral salt[b]	1.0	1.0	1.0
Dicalcium phosphate	1.0	1.0	1.0
Vitamins A,D,E[c]	[+]	[+]	[+]
Metabolizable energy, Mcal/kg[d]	2.75	2.41	2.40

[a]Formulated to contain 13% crude protein.
[b]Trace mineral composition (g/kg): Mn, 2.00; Fe, 1.60; Cu, .33; S, .11; Co, .10; Zn, .10; I, .07.
[c]Provided 2,497,000 IU vitamin A, 550,000 IU vitamin D3, and 275 IU vitamin E per 1,000 kg of diet (as fed).
[d]Calculated based on NRC (1996) published values

Blood samples were collected from each steer via jugular puncture on d 0, 7, 14, 28, 56, and 84 immediately prior to a.m. feeding. All blood samples (10 mL) were collected into plastic syringes and transferred to glass tubes, containing 15 mg EDTA, and maintained on ice. Plasma was harvested following centrifugation (1,800 × *g* at 4°C) and stored at -20°C until assayed for IGF-1 by double antibody radioimmunoassay (Elsasser *et al.*, 1989). Assay sensitivity was 12.3 ng mL^{-1} and the intra-assay coefficient of variation was 7.6%.

All statistical analyses were conducted using GLM procedures of SAS (1989). For these analyses, data were adjusted so that IGF-1 patterns across d reflected a common zero-time mean. Data were analyzed as a split-plot over time (Gill, 1978). The initial model included CTC, dietary protein concentration (PRO), d and interactions of CTC × PRO, animal within CTC × PRO, CTC × d, PRO × d, and CTC × PRO × d as possible sources of variation. The main effect of treatment and treatment interactions were tested using animal within CTC × PRO interaction as the error term, while effects of d and treatment × d interactions were tested using residual error. The three-way interaction was non-significant ($P > 0.01$) and subsequently eliminated from the model. Analysis of treatment within d was conducted using CTC, PRO, and CTC × PRO in the model. The effects of treatment and treatment interactions were tested using the residual error term and significant treatment interactions were separated using Least Significant Differences (SAS, 1989). Additionally, because subtherapeutic amounts of CTC are applied both acutely and chronically to animal production diets, data were analyzed and presented as two discrete periods. Period 1 (d 7, 14, and 28) was considered to be the transition period, or the period which reflects the acute effects of CTC, while Period 2 (d 56 and 84) was considered to reflect the long-term or sustained affects of CTC. For this analysis, average IGF-1 concentrations for each steer were calculated for each period and analyzed according to procedures described for treatment within d analysis.

Results

Rates of body weight gain and dry matter intake for Periods 1 (2.3 ± 0.1 kg d^{-1} and 8.7 ± 0.3, respectively) and 2 (1.5 ± 0.1 kg d^{-1} and 10.4 ± 0.6 kg d^{-1}, respectively) were typical of growing steers under similar conditions and were largely unaffected by treatment (Rumsey *et al.*, 2000). However, efficiency of gain (i.e. body weight gain per unit of dry matter intake) during Period 2 was greater ($P < 0.05$) for steers fed adequate dietary protein (13%) compared with steers fed marginal dietary protein (10%). This increase in gain efficiency was reflective of a numerical increase in rate of body

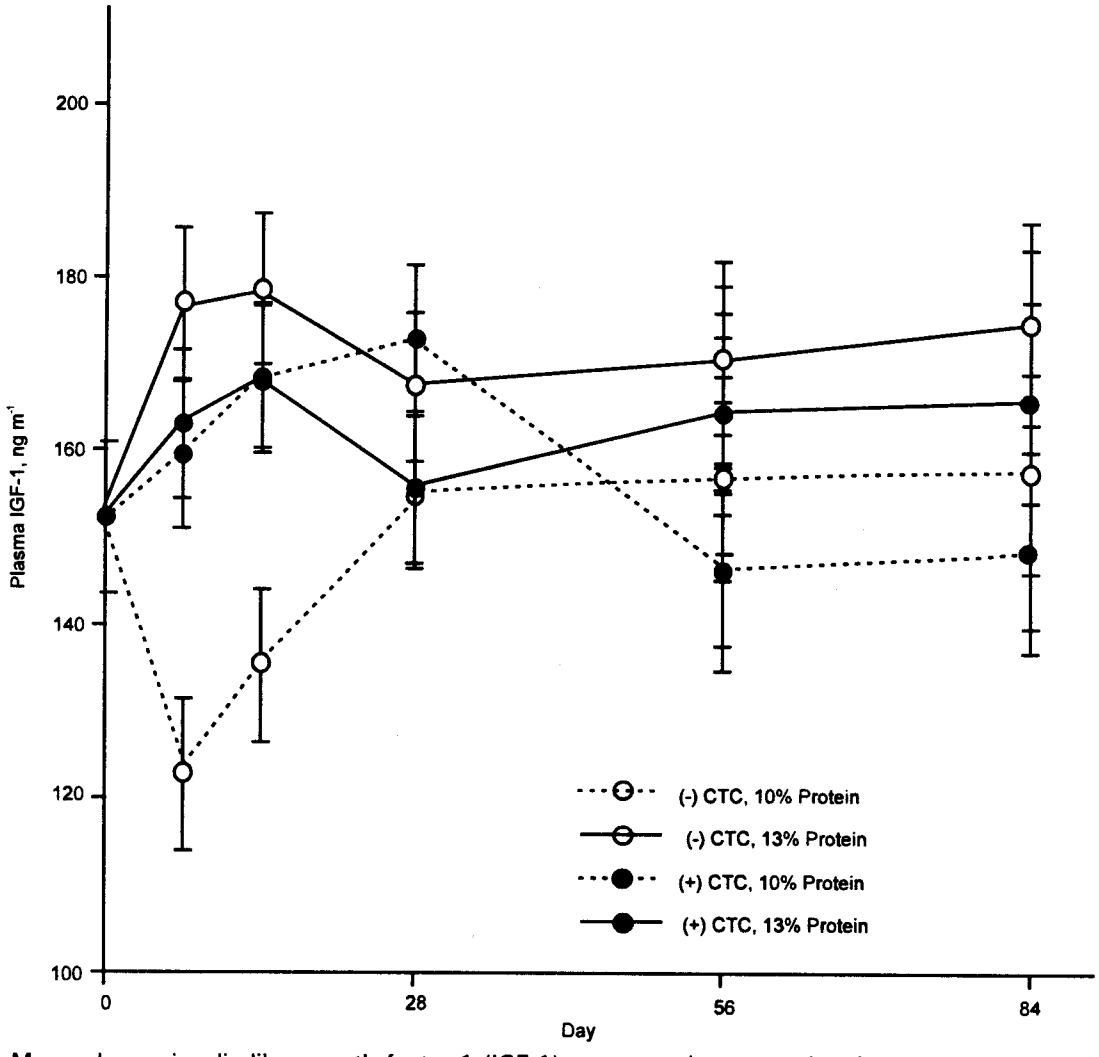

Fig. 1: Mean plasma insulin-like growth factor-1 (IGF-1) concentrations over time in steers fed diets containing either 10% or 13% protein (PRO) supplemented with either carrier (-CTC) or carrier plus chlortetracycline (+CTC). Each point represents means with attached SEM (n=8). Means were adjusted, within each treatment group, to a common d 0 concentration. Days 7 and 14 exhibited a CTC×PRO interaction ($P < 0.10$).

weight gain (12.4%) and a slight numerical decrease (3.9%) in dry matter intake for steers fed 13% protein compared with those fed 10% protein.

Across the 84-d sampling period (Fig. 1), circulating IGF-1 concentrations were unaffected ($P > 0.10$) by CTC and d and potential treatment by d interactions were not significant ($P > 0.10$). Residual error was 11.9 ng mL^{-1} and steer (CTC × PRO) was significant ($P < 0.02$), factors which may have prevented d by treatment interactions from being detected. Mean IGF-1 concentration was higher ($P < 0.03$) in steers fed 13% protein (169.7 ng mL^{-1}) compared with steers fed 10% protein (152.6 ng mL^{-1}). However, there was a tendency ($P < 0.11$) for a CTC × PRO interaction. Within d analysis shows that this overall interaction was attributable to responses observed on d 7 and 14 (CTC × PRO interaction, $P < 0.10$). In each case, CTC increased ($P < 0.10$) plasma IGF-1 concentration in steers fed 10% protein, but did not affect IGF-1 concentration in steers fed 13% protein. By d 28, the positive effect of CTC on plasma IGF-1 concentration in steers fed 10% protein was negated ($P > 0.10$) as a result of a rebound from the initial depression in IGF-1 concentration observed for steers administered carrier alone.

Evaluation of plasma IGF-1 concentration by period

(Fig. 2) showed that mean concentration for Period 1 exhibited a CTC × PRO interaction ($P < 0.05$). Oral administration of CTC increased ($P < 0.05$) circulating concentration of IGF-1 in steers fed 10% protein, but did not affect IGF-1 concentration in steers receiving 13% protein. For Period 2 (d 56 and 84), mean plasma IGF-1 concentrations were not affected ($P > 0.10$) by treatment. However, on a numerical basis, IGF-1 concentrations were 11% greater when adequate dietary protein was supplied (170.0 vs.153.5 ng mL^{-1}) and were 6% lower when CTC was administered (157.4 vs. 166.1 ng mL^{-1}).

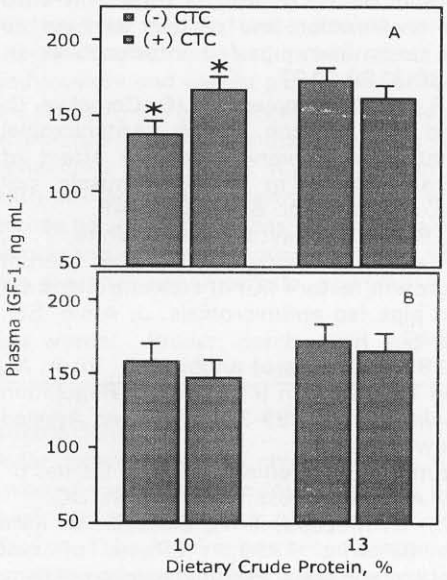

Fig. 2: Average plasma insulin-like growth factor-1 (IGF-1) concentrations for Period 1 (d 7,14, and 28; panel A) and Period 2 (d 56 and 84; panel B) in steers fed diets containing either 10% or 13% protein (PRO) supplemented with either carrier (-CTC) or carrier plus chlortetracycline (+CTC). Each bar represents means with attached SEM (n = 8). Plasma IGF-1 concentrations for period 1 exhibited a CTC × PRO interaction ($P < 0.05$). Asterisks denote treatments which differ ($P < 0.05$)

Discussion

A number of studies have demonstrated the growth promoting actions of antibiotics when fed at subtherapeutic levels (Visek, 1978 and Hays, 1991). However, there is a paucity of information available on the effects of subtherapeutic levels of antibiotics on the endocrine growth axis. Recent studies have demonstrated that feeding subtherapeutic levels of antibiotics to swine increases circulating IGF-1 concentrations (Hathaway *et al.*, 1990a; Hathaway *et al.*, 1990b and Hathaway *et al.*, 1996). The current experiment adds to these findings by demonstrating that oral administration of CTC at a subtherapeutic level increases, at least initially, circulating IGF-1 in bovine when dietary protein is marginal, but does not appear to affect IGF-1 concentrations when dietary protein is supplied in adequate amounts.

Although direct effects of CTC on IGF-1 can not be eliminated, the positive effects of CTC on circulating IGF-1 concentrations in steers fed 10% protein during Period 1 and the lack of an effect of CTC on IGF-1 concentrations in steers fed 13% protein, suggests that the action of CTC on circulating IGF-1 is mediated through an indirect mechanism(s). The addition of subtherapeutic levels of antibiotics to the diet have been shown to reduce the amount of dietary protein necessary to achieve maximal growth rates in animals (Hays, 1991). This protein sparing effect is presumably mediated via an increase in splanchnic output of amino acids due to a reduction in gut microbial load and epithelial mass (Visek, 1978; Hays, 1991 and Gill *et al.*, 1989). Because dietary protein has been shown to be the primary nutritional determinate of circulating basal concentrations of IGF-1 in bovine (Elsasser *et al.*, 1995), CTC may have augmented circulating IGF-1 concentrations in the current study via a protein sparing effect. Although microbial load was not determined in this experiment, CTC significantly reduced (10%) small intestinal mass (Baldwin *et al.*, 2000). Alternatively, because the immune status of the steers fed 10% protein may have been compromised, CTC could have blunted the negative influence of chronic bacterial loads. Reduced circulating IGF-1 concentrations have been demonstrated in bovine challenged with parasitic loads and bacterial endotoxin (Elsasser *et al.*, 1988 and Elsasser *et al.*, 1995).

Our finding that CTC did not influence circulating IGF-1 concentrations in steers fed 13% protein is in contrast to previous work in swine. Hathaway *et al.* (1996) reported feeding subtherapeutic levels of antibiotic over a 5-wk period increased circulating concentrations of IGF-1 in young growing swine fed a diet with adequate protein. In addition to species, several fundamental differences exist between the current study and that of Hathaway *et al.* (1996). Hathaway *et al.* fed CTC in combination with sulfamethazine and penicillin (ASP-250), thus the additional antibiotics or the combination of antibiotics acting in concert may have affected circulating IGF-1 concentrations differently than CTC alone. Additionally, because Hathaway *et al.* used weaned piglets 34 d of age, subtherapeutic antibiotics may have had a greater impact due to immune status and(or) greater amino acid demands for growth. Dry matter intake was increased in piglets fed ASP-250, but CTC did not effect intake in steers in the current study. Because circulating IGF-1 concentrations are

influenced by dietary nutrient supply, the differential effects on IGF-1 concentrations between the current study and that of Hathaway *et al.* may be explained by nutritional status.

The temporal effect of CTC on circulating IGF-1 concentrations in steers fed 10% protein is difficult to interpret. It is possible that in period 1, specifically for d 7 and 14, CTC reduced stress associated with transition onto *ad libitum* amounts of the marginal protein diet by sparing protein, as described earlier. In contrast, by period 2 (d 56 and 84) the 10% protein diet may no longer have been marginal due to a decreased amino acid requirement associated with physiological maturation. This contention is consistent with the observed differences in initial body weight (285 vs 346 kg) and rate of body weight gain (2.3 vs 1.6 kg d^{-1}) between periods 1 and 2 for steers administered CTC and fed the 10% protein diet (Rumsey *et al.*, 2000). However, it is worth noting that CTC attenuated pituitary release of GH and TSH in response to a challenge with GHRH and TRH in these same steers at d 56 (Rumsey *et al.*, 1999). It is unclear whether this inhibitory affect of CTC on GH release is limited to sustained or long-term administration. However, if this response is limited to sustained administration, this could explain the temporal affects of CTC on plasma IGF-1 concentrations because GH is the primary regulator of circulating IGF-1concentrations (Ehterton, 1991).

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