

Effects of Oral Chlortetracycline and Dietary Protein Level on Plasma Concentrations of Growth Hormone and Thyroid Hormones in Beef Steers Before and After Challenge with a Combination of Thyrotropin-Releasing Hormone and Growth Hormone-Releasing Hormone^{1,2,3}

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ABSTRACT: The objective of this study was to determine the effect of a subtherapeutic level of chlortetracycline (CTC) fed to growing beef steers under conditions of limited and adequate dietary protein on plasma concentrations of GH, thyroid-stimulating hormone (TSH), and thyroid hormones before and after an injection of thyrotropin-releasing hormone (TRH) + GHRH. Young beef steers ($n = 32$; average BW = 285 kg) were assigned to a 2×2 factorial arrangement of treatments of either a 10 or 13% crude protein diet (70% concentrate, 15% wheat straw, and 15% cottonseed hulls) and either a corn meal carrier or carrier + 350 mg of CTC daily top dressed on the diet. Steers were fed ad libitum amounts of diet for 56 d, and a jugular catheter was then placed in each steer in four groups (two steers from each treatment combination per group) during four consecutive days (one group per day). Each steer was injected via the jugular catheter with 1.0 $\mu\text{g}/\text{kg}$ BW TRH + .1 $\mu\text{g}/\text{kg}$ BW GHRH in 10 mL of saline at 0800. Blood samples were collected at -30, -15, 0, 5, 10, 15, 20, 30, 45, 60, 120, 240, and 360 min after

releasing hormone injection. Plasma samples were analyzed for GH, TSH, thyroxine (T_4), and triiodothyronine (T_3). After 84 d on trial, the steers were slaughtered and the pituitary and samples of liver were collected and analyzed for 5'-deiodinase activity. Feeding CTC attenuated the GH response to releasing hormone challenge by 26% for both area under the response curve ($P < .03$) and peak response ($P < .10$). Likewise, CTC attenuated the TSH response to releasing hormone challenge for area under the response curve by 16% ($P < .10$) and peak response by 33% ($P < .02$), and attenuated the T_4 response for area under the curve by 12% ($P < .08$) and peak response by 14% ($P < .04$). Type II deiodinase activity in the pituitary was 36% less ($P < .02$) in CTC-fed steers than in steers not fed CTC. The results of this study are interpreted to suggest that feeding subtherapeutic levels of CTC to young growing beef cattle attenuates the release of GH and TSH in response to pituitary releasing hormones, suggesting a mechanism by which CTC may influence tissue deposition in cattle.

Key Words: Antibiotics, Chlortetracycline, Cattle, Somatotropin, Thyroxine, Triiodothyronine

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Introduction

Antibiotics have been used at subtherapeutic levels in diets of cattle for approximately 40 yr. A growth-promoting effect has been attributed to this subtherapeutic use, but the mechanism for growth promotion is not known. Most hypotheses for growth promotion in ruminants relate to the effects of these antimicrobials on digestive tract microorganisms or digestive tract thinning (Vissek, 1978). Based on carcass composition of calves that were studied from birth to 12 or 16 wk of age, Landagora et al. (1957) suggested that chlortetracycline (CTC) may affect growth via an endocrine axis. However, there is a void of

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information on the effects of antibiotics on endocrine regulation of growth in ruminants.

Hathaway et al. (1996) recently reported that a combination of chlortetracycline, sulfamethazine, and penicillin (ASP-250, American Cyanamid, Princeton, NJ) fed to pigs increased plasma concentrations of IGF-1. Growth hormone released from the pituitary is a regulator of IGF-1 concentrations (Copeland et al., 1980), and IGF-1 concentrations are positively related to growth (Clemmons et al., 1987; Rumsey and Elsasser, 1989). In poultry, feeding CTC caused a hypothyroid condition (Hsu et al., 1970) and increased body fat in chicks studied from 3 to 28 d of age (Yok, 1975). Rumsey et al. (1996, 1997) found that pituitary release of GH and thyroid stimulating hormone (**TSH**) in steers was positively related to type-II 5'-monodeiodinase (**5'-D**) activity in the pituitary.

Because both GH and thyroid status is regulated via the hypothalamic-pituitary axis, the objective of this study was to determine whether CTC fed to beef steers influences their sensitivity to pituitary releasing hormones. The study included a mixture of thyrotropin-releasing hormone (**TRH**) plus **GHRH** as recently reported by Rumsey et al. (1996, 1997).

Materials and Methods

Animal Care. The animal protocol for the research in this report was approved by the Beltsville Agricultural Research Center Institutional Animal Care and Use Committee. This study was part of a larger study designed to relate the feeding of CTC to performance, carcass, and plasma measurements and gastrointestinal tract tissue growth.

Experimental Protocol. Young beef steers ($n = 32$) of predominantly Angus breeding were purchased at an annual fall feeder calf sale in central Virginia. The steers were initially housed on pasture for 30 d upon arrival at the Beltsville Agricultural Research Center during which time they were dewormed and treated for ring worm (a problem that was particularly severe in the Eastern United States during the fall, winter, and spring of 1996–1997). After 30 d, the steers were placed in individual pens and adapted over a 6-wk period to a near ad libitum intake of a standardization pelleted diet of 41% cracked corn, 40% orchardgrass hay, 9% dehydrated cane molasses, 8% soybean meal (48% minimum CP), 1% trace-mineralized salt, and 1% calcium phosphate plus vitamin A, D, and E. Standard protocol during this period was that steers were released from their pens daily at 0600 for exercise and pen cleaning, fed once daily at 0800 with orts recorded daily before the 0800 feeding, provided water continuously, weighed once weekly, and adapted to handling and a halter. Adaptation to handling and a halter was accomplished by returning

steers from exercise to their pens via the animal working chute system and by haltering in pens daily. At the end of the 6-wk adaptation period, steers were assigned to four groups in a 2×2 factorial arrangement of treatments to equalize average body weight across groups. The treatments were either 10% crude protein or 13% crude protein pelleted diets and either 500 g of corn meal carrier or 500 g of carrier plus CTC as a daily top dressing to provide 350 mg of CTC/d. The 10% crude protein diet consisted of 43.5% cracked corn, 20% cottonseed hull, 20% wheat straw, 9% dehydrated cane molasses, 5.5% soybean meal, 1% trace-mineralized salt, and 1% calcium phosphate, plus vitamin A, D, and E. The 13% crude protein diet was the same as the 10% diet except cracked corn was included at 36.5% and soybean meal was added at 12.5%.

During the last week of the 6-wk adaptation period, the steers were switched to ad libitum levels of their respective experimental diets, and, at the end of the 6-wk adaptation period, carrier and carrier plus CTC feeding was started. This was done by placing 1 kg of experimental diet in each feeder at 0800, top dressing with carrier or carrier plus CTC, and briefly hand mixing. After this initial portion of the diet was consumed, the remainder of the daily allotment of feed was placed in the feeders, typically by 1000. The animal handling protocol during the experiment was the same as during the adaptation period, except daily haltering was not continued.

Fifty-six days after the steers received their experimental diets, a jugular catheter was placed in each of eight steers (initial injection group, two from each treatment combination). The following day, these eight steers were injected via the catheters at 0800 with 10 mL of saline containing a dose of 1.0 μg of TRH plus .1 μg of GHRH per kg BW. Blood samples were taken via the jugular catheters at -30, -15, 0, 5, 10, 15, 20, 30, 45, 60, 120, 240, and 360 min after injection of releasing hormones for measurement of plasma concentrations of GH, TSH, thyroxine (**T₄**), and triiodothyronine (**T₃**). Plasma samples were stored frozen at -40°C until analyzed. Similarly, injection groups of eight steers (two/treatment) were catheterized, challenged, and sampled beginning on the three consecutive days following the catheterization of the initial group of steers.

After 84 d on the experimental diets, steers were slaughtered in groups of four per day over a period of 2 wk for digestive tract tissue studies. At the same time, the pituitary gland and samples of liver were weighed, frozen in liquid nitrogen, transported to the laboratory, stored at -81°C , and later analyzed for Type II and Type I 5'-D activity in pituitary and liver tissue, respectively.

Sample Analysis. Growth hormone concentrations in plasma were determined with a RIA (Elsasser et al.,

1989) with inter- and intraassay CV of 9.6 and 12.0%, respectively. The TSH concentrations in plasma were determined with a RIA (Elsasser et al., 1992) with an intraassay CV of 14% (one assay set). Thyroxine and T_3 concentrations were determined with a RIA as reported by Kahl et al. (1992), with inter- and intraassay CV of 4.8% and 6.3% for T_4 and 5.2% and 7.8% for T_3 , respectively. The GH, TSH, T_4 , and T_3 response curves were evaluated for area under the curve, peak response (visual evaluation of plasma concentrations), and time from challenge to peak. Outer-ring 5'-D activity was determined by quantifying the ^{125}I -released from [^{125}I]-reverse T_3 as previously described (Kahl et al., 1995) with minor modifications for Type II 5'-D assay in pituitary tissue. Tissue samples of liver (Type I 5'-D) and pituitary gland (Type II 5'-D) were homogenized in .01 M HEPES buffer (pH 7.0, .25 M sucrose, 5 mM EDTA). After centrifugation (30 min at $2,000 \times g$), the supernatant was incubated for 5 min (liver) or 60 min (pituitary) in .1 M phosphate buffer (pH 7.0, 5 mM EDTA) in the presence of 5 mM (liver) or 20 mM (pituitary) dithiothreitol at 37°C with 500 nM (liver) or 3.2 nM (pituitary) reverse T_3 and 80,000 cpm [^{125}I]-reverse T_3 (DuPont-New England Nuclear, Boston, MA). Type II 5'-D in the pituitary gland was determined in the presence of 1 mM propylthiouracil in the incubation mixture. Activity of 5'-D was expressed as nmol (liver) or pmol (pituitary) of I^- produced per mg of protein/h. Protein concentration in homogenates was determined with bicinchoninic acid reagent (Pierce Chemical Co., Rockford, IL) and BSA as a standard.

Statistical Analysis. Statistical analysis procedures were those outlined by SAS (1989). The plasma concentrations of GH, TSH, T_4 , and T_3 were analyzed with GLM procedures as a 2×2 factorial split-plot-in-time. The initial model included injection group as a factor, which was removed from the final model because of lack of significance ($P > .05$). Heterogeneity of variance within each time was not significant, thus data were not transformed before analysis. Each response curve measurement for the GH, TSH, T_4 , and T_3 curves was analyzed with GLM procedures as a 2×2 factorial with injection group in the initial model. Injection group was dropped for the final model because of lack of significance ($P > .05$). Liver and pituitary deiodinase activity was analyzed as a 2×2 factorial as described above.

Results

Growth Hormone. The effect of CTC on changes in plasma GH concentrations over time in relation to the injection of TRH+GHRH is shown in Figure 1. The effect of time was significant ($P < .001$). Concentration of GH increased following injection and returned

to baseline by 120 min. Concentrations of GH were lower ($P < .001$) following injection of TRH+GHRH for CTC-fed steers than for steers not fed CTC.

A summary of the effects of CTC and dietary protein level on characteristics of the GH response curve is shown in Table 1. There were no CTC by protein level interactions. Baseline concentrations of GH (the average of samples obtained at -30, -15, and 0 min relative to injection time) were not different ($P > .10$) between treatments. On average, time to peak tended to be longer ($P < .10$) for CTC-fed steers than for steers not fed CTC and tended to be shorter ($P < .09$) for steers fed the 13% protein diet than for steers fed the 10% protein diet. Peak concentration of GH following injection of TRH+GHRH tended to be less ($P < .10$) for steers fed CTC than for steers not fed CTC, and area under the response curve was less ($P < .03$) for CTC-fed steers than for steers not fed CTC. Dietary protein level did not affect ($P > .10$) either peak concentration of GH or area under the response curve.

Thyroid Stimulating Hormone. The effect of CTC on changes in plasma TSH concentrations over time in

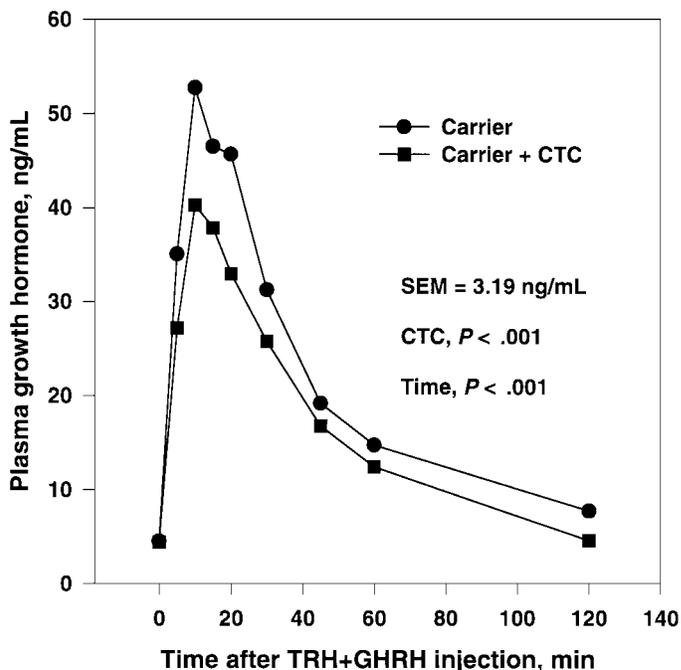


Figure 1. Plasma growth hormone concentration in beef steers fed the same diets top dressed with either corn meal carrier or carrier plus 350 mg of chlortetracycline (CTC) per day and injected at time zero with a combination of thyrotropin-releasing hormone (TRH) plus GHRH. The amount injected was 1.0 μg TRH plus .1 μg GHRH per kg BW. Plasma growth hormone concentration changed with time in response to the releasing hormone challenge ($P = .0003$) and was lower ($P = .0003$) for CTC-fed steers than for steers not fed CTC. Each point on the graph represents a mean of 16 steers.

Table 1. Influence of oral chlortetracycline (CTC) and dietary protein level on growth hormone (GH) and thyrotropin-releasing hormone (TSH) responses to a releasing hormone challenge in growing beef steers, $n = 16^a$

Item	CTC, mg/d		Protein level, %		SEM ^b	P-value	
	0	350	10	13		CTC	Protein
GH							
Baseline, ng/mL ^c	4.52	4.39	4.78	4.13	.92	.94	.63
Time to peak, min	11.3	14.4	14.4	11.3	1.3	.10	.09
Peak-baseline, ng/mL	54.33	40.22	45.72	48.83	6.28	.10	.80
Area, (ng/mL)*min	1,951	1,439	1,615	1,775	167	.03	.42
TSH							
Baseline, ng/mL	.77	.68	.73	.71	.05	.25	.86
Time to Peak 1, min	25.9	28.4	29.1	25.3	2.3	.45	.26
Time to Peak 2, min	120.0	116.3	116.3	120.0	2.7	.32	.32
Peak 1-baseline, ng/mL	1.62	1.38	1.32	1.68	.19	.37	.18
Peak 2-baseline, ng/mL	2.18	1.47	1.71	1.94	.21	.02	.43
Area, (ng/mL)*min	728	609	661	677	50	.10	.82

^aThere were no CTC \times protein interactions.

^bCommon standard error of the mean from analysis of variance.

^cAverage across samples taken at 30, 10, and 0 min before the injection of thyrotropin-releasing hormone + growth hormone-releasing hormone (1.0 + .1 μ g/kg BW).

relation to the injection of TRH+GHRH is shown in Figure 2. The effect of time was significant ($P < .001$). Concentration of TSH increased following injection and returned to near baseline at 6 h after challenge. The CTC \times time interaction after challenge was significant ($P < .01$), with TSH concentration a 2 h being lower ($P < .01$) for the CTC-fed steers than for the steers not fed CTC.

The effects of CTC and dietary protein level on the characteristics of the TSH response curve are shown in Table 1. The response curve consisted of two peaks; presumably, the first peak reflects the release of stored TSH, whereas the second peak reflects the up-regulation of TSH synthesis. Peak 2, an average of the individual peak concentrations, was lower ($P < .02$) and area under the response curve tended to be lower ($P < .10$) for the CTC-fed steers than for the steers not fed CTC.

The TSH response to TRH+GHRH challenge was not influenced ($P > .10$) by dietary protein level or the CTC \times protein level interaction.

Thyroid Hormones. The effect of CTC on changes in plasma T_4 concentrations over time in relation to the injection of TRH+GHRH is shown in Figure 3. In this study, the response to TRH+GHRH challenge injection of plasma T_4 concentrations seemed slower than in a previous study that served as the basis of our sampling protocol (Rumsey et al., 1997), thus a complete response curve was not obtained. However, based on individual steer data that showed that the highest concentrations for some of the steers occurred before 360 min after challenge injection, it appeared that the peak concentration, on average, was occurring at approximately 360 min after injection. Thyroxine concentration was increased ($P < .001$) after challenge injection, and, in all steers, the increase in

T_4 concentration over time after injection was less ($P < .02$) for steers fed CTC than for steers not fed CTC. The effect of dietary protein on response of T_4 to challenge injection was not significant ($P > .10$; summary not shown).

A summary of the effects of CTC and dietary protein level on characteristics of the T_4 response curve is shown in Table 2. There were no CTC \times protein level interactions. Baseline T_4 concentration in the plasma was not different between treatments ($P > .10$). Time to maximum response detected was not analyzed statistically because the highest values for some of the steers was at 360 min after challenge injection. The maximum response observed for plasma concentration of T_4 following challenge injection was lower ($P < .04$) for CTC-fed steers than for steers not fed CTC, and response area tended to be lower ($P < .08$) for CTC-fed steers than for steers not fed CTC. Neither maximum response nor area were different between protein levels ($P > .10$).

The effect of dietary protein level on changes in plasma T_3 concentrations over time in relation to the injection of TRH+GHRH is shown in Figure 4. Triiodothyronine concentration was increased ($P < .001$) after challenge injection, and the increase in T_3 concentration was less ($P < 0.03$) for steers fed the 13% protein diet than for steers fed the 10% protein diet. The effect of CTC on response of T_3 to challenge injection was not significant ($P > .10$; Table 2). A summary of the effects of CTC and dietary protein level on characteristics of the T_3 response curve is shown in Table 2. There were no CTC \times protein level interactions. Neither baseline T_3 concentration in the plasma, time to peak concentration, peak concentration, nor area under the response curve were different between treatments ($P > .10$).

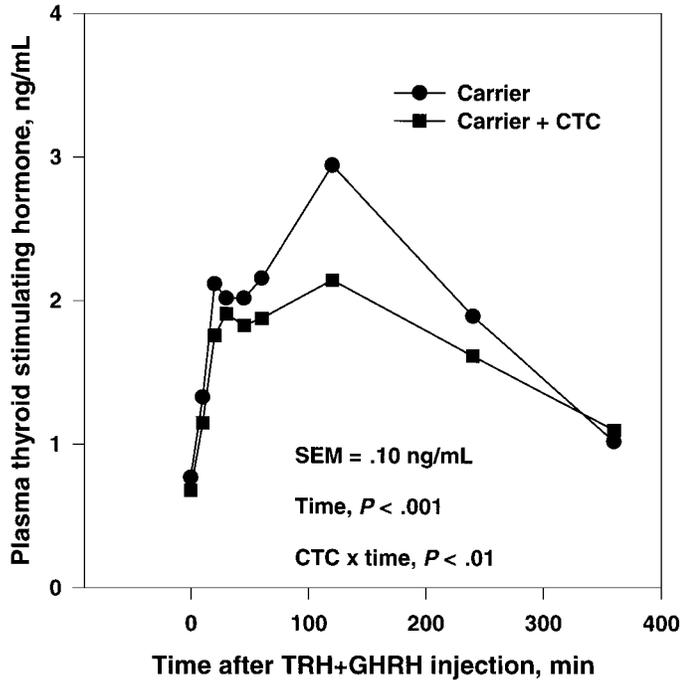


Figure 2. Plasma thyroid-stimulating hormone concentration in beef steers fed the same diets top dressed with either corn meal carrier or carrier plus 350 mg of chlortetracycline (CTC) per day and injected at time zero with a combination of thyrotropin-releasing hormone (TRH) plus GHRH. The amount injected was 1.0 μg TRH plus .1 μg GHRH per kg BW. Plasma thyroid-stimulating hormone concentration changed with time in response to the releasing hormone challenge ($P = .0001$), and the response over time after injection was less ($P = .003$) for CTC-fed steers than for steers not fed CTC. Each point on the graph represents a mean of 16 steers.

Deiodinase Activity. A summary of the effects of CTC and dietary protein level on 5'-D activity in the liver and pituitary is shown in Figure 5. There were no treatment interactions ($P > .10$). Deiodinase activity in the liver, which is Type I 5'-D, was not different between treatments ($P > .10$). Deiodinase activity in the pituitary, which is Type II 5'-D, was lower ($P < .02$) in the CTC-fed steers than in steers not fed CTC and was not different ($P > .10$) between dietary protein levels.

Discussion

Most of the research on the effects of subtherapeutic feeding of antibiotics to cattle was published in the 1950s. A review of the mode of action of antibiotics with regard to their growth-promoting effects in animals has been published (Visek, 1978). Hypotheses for growth promotion, particularly in cattle, have focused on the effects of antibiotics on microorganisms of the digestive tract, either stimulat-

ing the proliferation of microorganisms that positively affect nutrient utilization or inhibiting the proliferation of microorganisms that may produce antigrowth factors. Digestive tract thinning and associated effects on nutrient absorption has also been proposed. Some work with cattle and lambs would suggest a more direct effect on mechanisms that regulate tissue deposition. In a study with dairy calves from birth to 16 wk of age, Rusoff et al. (1954) compared untreated calves with calves either fed or injected with CTC to remove the influence of ruminal changes. Both fed and injected calves had increased gain and skeletal frame size and a greater amount of edible meat. In a later study (Landagora et al., 1957), calves either fed or injected with CTC had increased gain, frame size, and dressing percentage and a greater amount of higher priced meat cuts. Perry et al. (1958) reported that CTC fed for 233 d to feedlot steers starting at 233 kg of BW increased gains and carcass grade. In lambs, starting at approximately 29 kg of BW and fed for 84 d, Jordan et al. (1956) did not observe an effect on performance with oral CTC, but they observed a

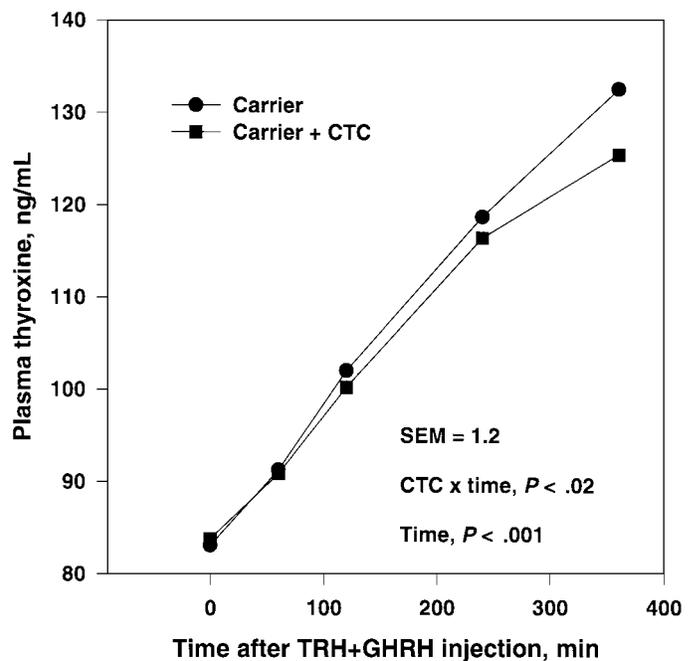


Figure 3. Plasma thyroxine concentration in beef steers fed the same diets top dressed with either corn meal carrier or carrier plus 350 mg of chlortetracycline (CTC) per day and injected at time zero with a combination of thyrotropin-releasing hormone (TRH) plus GHRH. The amount injected was 1.0 μg TRH plus .1 μg GHRH per kg body weight. Plasma thyroxine concentration changed with time in response to the releasing hormone challenge ($P = .0001$), and the response over time was less ($P = .02$) for CTC-fed steers than for steers not fed CTC. Each point on the graph represents a mean of 16 steers.

Table 2. Influence of oral chlortetracycline (CTC) and dietary protein level on the thyroxine (T₄) and triiodothyronine (T₃) hormone responses to a releasing hormone challenge in growing beef steers, n = 16^a

Item	CTC, mg/d		Protein level, %		SEM ^b	P-value	
	0	350	10	13		CTC	Protein
T₄							
Baseline, ng/mL ^c	83.1	83.8	86.1	80.7	3.1	.87	.22
Time to peak, min	360	353	353	360	—	—	—
Peak-baseline, ng/mL	49.6	42.6	46.1	46.1	2.3	.04	.98
Area, (ng/mL)*min	9,467	8,361	8,783	9,045	427	.08	.66
T₃							
Baseline, ng/mL ^c	2.72	2.86	2.87	2.72	.07	.17	.14
Time to peak, min	270	236	251	255	16	.15	.87
Peak-baseline, ng/mL	1.29	1.26	1.38	1.17	.13	.90	.27
Area, (ng/mL)*min	341	305	349	297	26	.35	.17

^aThere were no CTC × protein interactions.

^bCommon standard error of the mean from analysis of variance.

^cAverage across samples taken at 30, 10, and 0 min before the injection of thyrotropin-releasing hormone + growth hormone-releasing hormone (1.0 + .1 μg/kg BW).

slightly increased carcass grade. The above mentioned studies suggest that CTC may affect tissue deposition in ruminants via an endocrine involvement as suggested earlier by Landagora et al. (1957), particularly as related to an increase in the proportion of fat. It should be pointed out, however, that the above studies represent a small number of the many studies conducted during the 1950s that showed quite varied performance responses to feeding CTC. Few studies on subtherapeutic feeding of antibiotics to ruminants have been reported since the 1950s, and there is a void of information on the effects of subtherapeutic feeding of antibiotics on endocrine factors as related to growth in ruminants.

This seems to be the first study reported in the literature on the effects of subtherapeutic feeding of CTC on pituitary function of cattle. Feeding CTC reduced both the area under the response curve and peak response to releasing hormones by 26% for GH, reduced area under the response curve by 16% and peak response by 33% for TSH, reduced area under the response curve by 12% and peak response by 14% for T₄, and reduced Type II 5'-D activity in the pituitary by 36%. Type I 5'-D activity in the liver was not affected. The reduced T₄ response in CTC-fed steers is interpreted as reflecting the reduction in the release of TSH from the pituitary in response to the acute challenge with releasing hormone.

After adaptation to treatments, daily gain and feed efficiency for the steers on this study averaged 1.55 kg of gain/d and 152.5 g of gain/kg of DM intake, respectively. Gain was numerically 7.5% lower for CTC-fed steers than for steers not fed CTC, and efficiency of gain was similar between – and + CTC treatments. At slaughter, fat over the longissimus muscle was greater and marbling tended to be greater for CTC-fed steers than for steers not fed CTC (data not shown).

The GH results are not consistent with expected results, considering the studies with swine that showed circulating IGF-1 concentrations were increased by feeding antimicrobials (Hathaway et al., 1996). If GH was influencing IGF-1 concentrations, one would expect greater output of GH from the pituitary in this study based on the work with swine (Hathaway et al., 1996). There are two primary differences between the swine studies of Hathaway et al. (1996) and the current study other than species; CTC was fed in combination with sulfamethazine and penicillin, and total antibiotic dose rate was greater in the swine studies. In addition, the duration of antibiotic feeding may be a factor. In our study, we did not observe a difference in plasma IGF-1 concentrations, except a trend for greater concentrations during the initial 4 wk of CTC feeding in steers on the lower protein diet (data not shown), nor were IGF-1 concentrations lower in CTC fed steers. This may indicate that, after extended feeding, the GH response in this study was not great enough to influence IGF-1 concentrations or other factors that regulate IGF-1. Thus, an effect on growth via GH involvement may not be expected in ruminants.

The TSH and T₄ data are consistent with reports in the literature on the biological effects of CTC in other species. Calesnick et al. (1954) reported that CTC fed to rats increased thyroid weight and reduced I¹³¹ uptake by the thyroid. Even though these results in rats could not be repeated by Grant (1954) and Libby and Mietes (1954), Holtzman and Visek (1965) reported reduced maintenance requirements in rats fed CTC. Later, Hsu et al. (1970) and Yok (1975) showed in work with growing chicks that CTC stimulated growth, increased thyroid gland weight, and caused a hypothyroid condition. Their responses were found in both untreated and alkali-treated CTC. Alkali treatment of CTC was shown to remove any

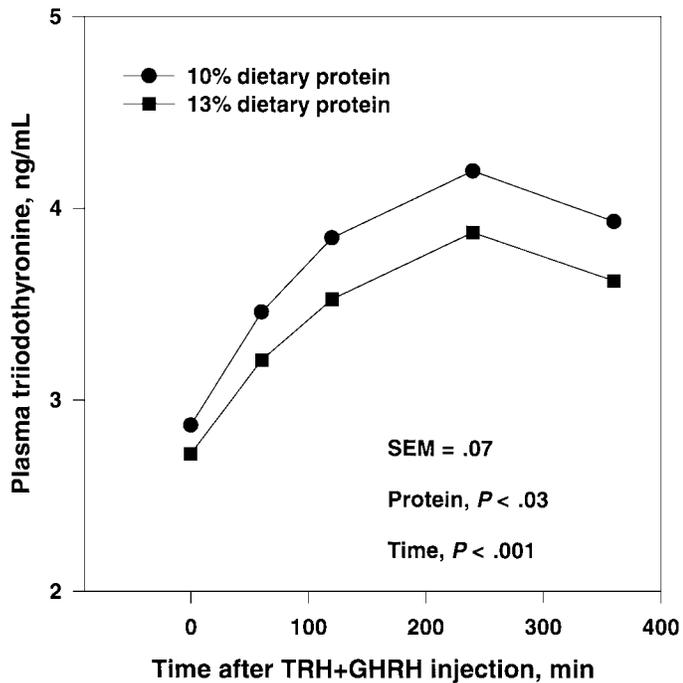


Figure 4. Plasma triiodothyronine concentration in beef steers fed the same diets top dressed with either corn meal carrier or carrier plus 350 mg of chlortetracycline (CTC) per day and injected at time zero with a combination of thyrotropin releasing hormone (TRH) plus GHRH. The amount injected was 1.0 μg TRH plus .1 μg GHRH per kg body weight. Plasma triiodothyronine concentration changed with time in response to the releasing hormone challenge ($P = .0001$) and was lower ($P = .03$) for steers fed 13% dietary protein than for steers fed 10% dietary protein. Each point on the graph represents a mean of 16 steers.

antimicrobial activity, thus removing this property as a factor in the observed responses. Hsu et al. (1970) suggested that the neutral pH of the digestive tract could partially remove antimicrobial activity while allowing retention of other biological properties. Additionally, Begin (1971) reported that CTC fed to chicks starting at 1 d of age and fed for 28 d increased gain and energy efficiency. These results are consistent with a negative effect of CTC on the release of TSH from the pituitary and the observed reduction of T_4 release from the thyroid following challenge with releasing hormones. This is also consistent with the reports for ruminants discussed earlier in which, under certain situations, there was a trend toward increased proportions of fat deposition. However, in poultry, the effect of CTC seems to be more consistent than in ruminants, possibly because of an involvement of the direct effects of CTC by ruminal or digestive tract factors.

Chlortetracycline also is used as a probe for Ca^{+2} and Mg^{+2} by binding these elements in the cytoplasm and has been used to monitor membrane bound Ca

that is extracted by TRH (Gershengorn and Thaw, 1982) as part of the cascade of events involved in the release of hormones by TRH. Other compounds have been used to bind Ca and block the release of prolactin by TRH (Thaw et al., 1982). Kolesnick and Gershengorn (1985) demonstrated that arachidonic acid regulates cellular Ca^{+2} and inhibits TRH-induced elevation of cytoplasmic free Ca^{+2} . Thus, the ability of CTC to bind Ca could be one factor that causes an attenuation of the response to releasing hormones.

The reduction in Type II 5'-D activity in the pituitary and no effect on Type I 5'-D activity in the

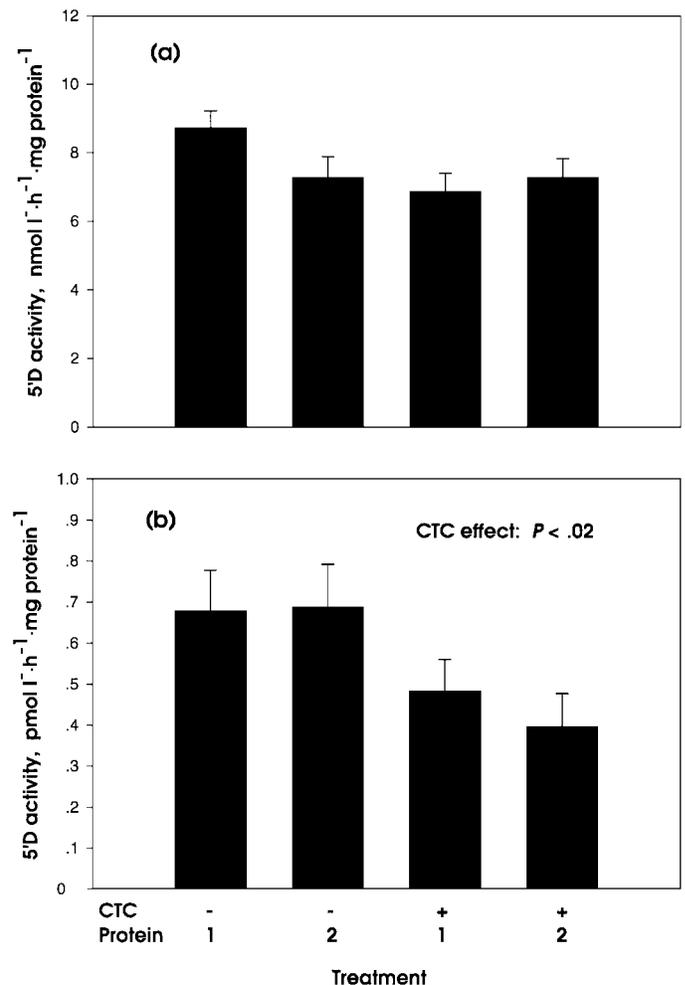


Figure 5. The effect in beef steers of dietary protein level and oral chlortetracycline (CTC) on 5'-deiodinase (5'-D) activity in liver (A; Type I 5'-D) and pituitary tissue (B; Type II 5'-D). Steers were fed 10% crude protein (Protein 1) or 13% crude protein (Protein 2) diets and either corn meal carrier (CTC -) or carrier plus 350 mg CTC per day (CTC +) in a factorial arrangement for 84 d before sample collection. Type I 5'-D activity in the liver was not affected by treatment ($P = .11$). Type II 5'-D activity in the pituitary was lower ($P = .02$) in steers fed CTC. Each bar on the graph represents a mean of eight steers.

liver is difficult to explain, although activities of Type II and Type I 5'-D are regulated differently. Reduced 5'-D activity in the pituitary would suggest a localized reduction in energy status and reduced cAMP, a factor also required for membrane transport of hormones from the pituitary (Dickson, 1984). Systemic levels of T₃ are primarily determined by the conversion of T₄ to T₃ in the liver by Type I 5'-D activity. The lack of an effect on Type I 5'-D activity is consistent with the lack of a treatment effect on circulating T₃ concentrations.

Even though in the present study 5'-D activity was estimated in whole pituitaries, previous studies indicate that somatotrophs show the highest 5'-D activity among the secretory cells of stimulated pituitary gland (Koenig et al., 1984). Lower GH release following TRH+GHRH challenge in roasted soybean-supplemented steers compared with steers fed soybean meal, and neutralization of this effect with an estrogen implant could be related to changes in local production of T₃ by type II 5'-D activity within the pituitary (Rumsey et al., 1996, 1997). Thyroid hormones have been suggested to regulate GH gene expression and GH synthesis and secretion (Valcavi et al., 1992).

In summary, this study demonstrated that the subtherapeutic feeding of CTC to young growing beef steers attenuates the response of the pituitary to a challenge of TRH+GHRH. This effect is consistent with known biological changes reported in the literature that have been seen when CTC has been the single antibiotic used at subtherapeutic levels and may, under certain circumstances, be the route by which CTC changes tissue deposition in ruminants.

Implications

Chlortetracycline has been used in cattle diets at subtherapeutic levels since the 1950s. Growth promotion, or fat deposition in particular, to varying degrees has been attributed to this practice, but the literature is devoid of studies that investigate the direct effects of chlortetracycline feeding on endocrine mechanisms in ruminants. This study showed that subtherapeutic feeding of chlortetracycline to young growing beef steers reduced the sensitivity of the pituitary to a releasing hormone challenge. This may be a factor that helps explain the results of earlier studies that showed an effect of chlortetracycline on performance, carcass composition, and(or) quality, and may promote improved energy efficiency of tissue deposition but not necessarily promote muscle deposition.

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