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## **Serum concentrations of trenbolone-17 beta and estradiol-17 beta and performance of heifers treated with trenbolone acetate, melengestrol acetate, or estradiol-17 beta**

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# Serum Concentrations of Trenbolone-17 $\beta$ and Estradiol-17 $\beta$ and Performance of Heifers Treated With Trenbolone Acetate, Melengestrol Acetate, or Estradiol-17 $\beta$ <sup>1,2</sup>

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**ABSTRACT:** The effects of the growth-promoting steroids estradiol-17 $\beta$  (E<sub>2</sub>), trenbolone acetate (TBA), and melengestrol acetate (MGA) in heifers on serum concentrations of E<sub>2</sub> and trenbolone-17 $\beta$  (TBOH) were examined. Feed intake and growth performance were also measured. Serum concentrations of E<sub>2</sub> and TBOH were measured on d 0, 1, 3, 5, 7, 13, 21, 28, 42, 56, 84, 112, and 140 in finishing heifers administered the following treatments: 1) control; 2) MGA, .5 mg per heifer daily; 3) Revalor-H (140 mg TBA + 14 mg E<sub>2</sub>); 4) Revalor-H + MGA; 5) Finaplix-H (200 mg TBA); and 6) Finaplix-H + MGA. Revalor-H implantation (Treatments 3 and 4) increased ( $P < .05$ ) serum E<sub>2</sub> concentrations; peak concentrations (67.5 pg/mL) occurred between d 21 and 56. Feeding MGA (Treatment 4) had no effect ( $P > .05$ ) on this increase in serum E<sub>2</sub> concentrations (63.3 pg/mL). From d 84 until d 140, serum E<sub>2</sub> was greater ( $P < .05$ )

for the Revalor-H treatment (average of 19 pg/mL) than for the control (7 pg/mL) or Finaplix-H treatments (6.5 pg/mL). Serum E<sub>2</sub> concentrations increased numerically two- to threefold from d 56 to 140 in controls fed MGA, compared with controls not fed MGA. There was the expected increase in serum TBOH concentrations after TBA implantation in the Revalor-H and Finaplix-H treatments; concentrations were similar ( $P > .05$ ) for Revalor-H (221 pg/mL) and Finaplix-H (280 pg/mL). After d 56, serum TBOH concentrations decreased in both treatments to 10 and 20% of these concentrations, respectively. Feeding MGA increased serum TBOH ( $P < .05$ ). Dry matter intake by heifers did not differ among treatments. Feeding of MGA improved gains ( $P = .12$ ) and efficiencies ( $P < .01$ ) in nonimplanted heifers and had no effect ( $P > .4$ ) on gains or efficiencies in Finaplix-implanted heifers.

Key Words: Cattle, Trenbolone, Estradiol, Growth Promoters, Growth, Anabolic Steroids

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## Introduction

Exogenous sex steroids are efficacious growth promoters in cattle. Average daily gain is increased 8 to 20% depending on the sex, implant strategy, and duration of feeding (Anderson, 1990). Trenbolone

acetate (TBA) is 8 to 10 times more active anabolically than testosterone (Bouffault and Willemart, 1983). Heitzman (1976) hypothesized that androgens and estrogens were necessary to realize maximum growth in cattle. When trenbolone was combined with estradiol-17 $\beta$  (E<sub>2</sub>) in steers, growth performance was greater than with either alone (Anderson, 1990). Serum trenbolone-17 $\beta$  (TBOH) and E<sub>2</sub> concentrations were not measured during most of the growth trials. Hormonal profiles over the growth period could be used to evaluate hormone implant composition and efficacy. Hormone profiles in individual animals may provide insights into the hormone concentrations required for optimal growth.

The U.S. Food and Drug Administration requires a rigorous assay of blood and tissues from animals implanted with anabolic steroids to determine the hormonal residues and efficacy of the treatment, and RIA is the technology of choice.

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This study was conducted primarily to report a valid, practical RIA for the measurement of TBOH in blood serum and to characterize serum TBOH and E<sub>2</sub> profiles in heifers following administration of different combinations of E<sub>2</sub>, TBA, and melengestrol acetate (MGA). Secondly, growth of heifers was measured for each treatment, and these data were related to the hormonal profiles of TBOH and E<sub>2</sub>.

### Materials and Methods

**Animals.** Thirty crossbred beef heifers (303 kg) of approximately 9 to 11 mo of age and with no previous anabolic treatments were used. Heifers were assigned to one of five weight blocks and were assigned randomly to one of six treatments within each block: 1) negative control, 2) MGA fed .5 mg per heifer daily; Pharmacia Upjohn, Kalamazoo, MI), 3) Revalor-H implant (140 mg TBA + 14 mg E<sub>2</sub>; Hoechst-Roussel, Warren, NJ), 4) Revalor-H implant + MGA, 5) Finaplix-H implant (200 mg TBA; Hoechst-Roussel), and 6) Finaplix-H implant + MGA. Heifers were individually housed and fed a corn-based finishing diet in open-front barns in a 140-d study. Heifers were brought up to full feed on the finishing diet before the experiment began. Final diets provided 275 mg of monensin (Elanco, Indianapolis, IN) and 90 mg of tylosin (Elanco) per heifer daily. All implants were placed in the middle one-third of the right ear of heifers, and ears were checked on d 13 for implant presence and lack of infection at the implant site. Animals were weighed every 28 d. Individual intakes were measured; feed refusals were collected and weighed daily for the first 14 d and once weekly thereafter. Carcass data were collected from all heifers at the conclusion of the experiment from a commercial slaughter facility. Hot carcass weights were recorded at slaughter, whereas other carcass traits were measured following a 24-h chill.

**Serum Collection and Hormone Assays.** Blood samples were obtained by jugular vein puncture with syringes, and blood was immediately transferred to vacutainer tubes. Blood was collected two times from the vein ipsilateral to the ear containing the implant at 45-min intervals on d 0, 1, 3, 5, 7, 13, 28, 42, 56, 84, 112, and 140. Serum was harvested following refrigeration for 24 h, and the two samples were composited within day for each animal and stored at -4°C pending hormone analysis.

**Estradiol-17β Assay.** Serum E<sub>2</sub> was measured with a validated RIA procedure described by Skaggs et al. (1986), with modifications described by Perry et al. (1991).

**Trenbolone-17β Assay.** Trenbolone-17β, the conversion product of TBA, was measured in the serum with a RIA. The antisera was a gift from Roussel-Uclaf (Paris, France). It was used in a dilution of 1:5,000 (initial dilution), and it had less than 1% cross-

Table 1. Cross-reactivity of various steroids with trenbolone-17β antisera

Steroid	% Cross-reactivity <sup>a</sup>
Trenbolone-17β	100.00
Trenbolone-17α	< .01
Trenbolone acetate	< .01
Diethylstilbestrol	< .01
Estradiol-17β	< .01
Estrone	< .01
Estradiol-17α	< .01
Estriol	< .01
Androsten-3, 17-dione	< .01
Cholesterol	< .01
Corticosterone	< .01
Dehydroisoandrosterone	< .01
Dihydrotestosterone	< .01
Pregnenolone	< .01
Testosterone	< .01
Deoxycorticosterone	< .01
Progesterone	< .01

<sup>a</sup>Percentage cross-reactivity of potential cross-reactant (×) equals quantity of trenbolone-17β at ED at Bo/T = 50% divided by quantity of × at ED at Bo/T = 50%. ED = estimated dose, Bo = cpm bound at 0 quantity of standard, and T = total cpm.

reactivity with 17 steroids, including TBOH-17α and TBA (Table 1). The limit of quantification (least value that did not overlap with the assay blank) was 10 pg of TBOH.

Reverse-phase chromatography of serum aliquots proceeded as follows. Octadecyl columns (SPE-21 System, J. T. Baker, Phillipsburg, NJ) were prewashed with two volumes of distilled acetone, two volumes of HPLC-grade methanol, and two volumes of distilled water. Steroid-free serum (.5 mL) was aspirated through the column, followed by 2 mL of 1:4 acetone:water solution. The column was dried under vacuum for 5 min then rinsed with two volumes of HPLC-grade methanol and two volumes of distilled water. Then, .5 mL of the prepared serum sample was pipetted onto the column, aspirated, and rinsed with 2 mL of 1:4 acetone:water solution. After drying under vacuum, the TBOH-17β fraction was collected by adding and aspirating two .5-mL aliquots of methanol. The warmed methanol was gently evaporated under a stream of filtered, dry air. Reagents for the standard curve tubes (total bound, total count, and nonspecific bound tubes) were likewise subjected to the column extraction procedure. Hence, all tubes were subjected to the same procedures, and no blank was subtracted from each sample's value. Seven quantities of TBOH ranging from 3.1 to 200 pg/100 mL in serial dilution were pipetted to prepare a standard curve. Following chromatography of a set of serum samples, the dried extracts were assayed for TBOH. Recovery during chromatography of [<sup>3</sup>H]TBOH-17β from spiked aliquots of a serum pool averaged 92.6%.

The extraction and assay procedures were validated by assay of aliquots of a serum pool from a nonimplanted heifer spiked with 0, 15.6, 62.5, and 250 pg

TBOH-17 $\beta$ . This experiment was repeated five times. As shown in Table 2, the quantities measured demonstrated acceptable accuracy for the assay. Intraassay and interassay CV were 8.8 and 10.7%, respectively, indicating acceptable precision for the assay.

**Statistical Analyses.** Performance data were analyzed as a 2  $\times$  3 factorially arranged, randomized complete block design (**RCBD**), with five blocks and six treatments using GLM procedures of SAS (1991). The model included block, implant, MGA supplementation, and the implant  $\times$  MGA interaction. Serum data and intake data for the first 14 d were analyzed as split-plots in time, the main plot being a RCBD. The model included block, implant, MGA supplementation, implant  $\times$  MGA, block  $\times$  implant  $\times$  MGA, day, day  $\times$  implant, day  $\times$  MGA, and day  $\times$  implant  $\times$  MGA. The block  $\times$  implant  $\times$  MGA term was used as the error term for testing main plot effects of implant, MGA, and implant  $\times$  MGA.

## Results and Discussion

**Serum Profiles.** Serum E<sub>2</sub> concentrations were not affected by feeding MGA ( $P > .7$ ), nor did MGA interact with either implant or time to alter serum E<sub>2</sub> ( $P > .7$ ; Table 3). Although not statistically different, feeding MGA (Treatment 2) increased serum E<sub>2</sub> approximately two- to threefold that for control heifers from d 56 to 140. Revalor-H, alone or with MGA, increased ( $P < .01$ ) serum E<sub>2</sub> concentrations compared with other treatments (Table 3; Figure 1). Heifers implanted with Revalor-H, alone or with MGA, had serum E<sub>2</sub> that increased from a baseline of 4 pg/mL to a mean of 26 pg/mL between d 1 and 13. Serum E<sub>2</sub> then increased to a mean of 67 pg/mL between d 21 and 56, before decreasing to a mean of 19 pg/mL between d 84 and 140. In summary, Revalor-H increased serum concentrations of E<sub>2</sub> for up to 140 d after implantation, with a decrease of approximately 72% observed after d 56. Heifers implanted with Finaplix-H (200 mg TBA), alone or with MGA, had serum E<sub>2</sub> values similar to those for control heifers ( $P > .95$ ).

There are no reports on serum anabolic hormone concentrations available for heifers implanted with 140 mg TBA + 14 mg E<sub>2</sub>. These serum concentrations of E<sub>2</sub> and TBOH can be compared to several studies that used steers. Johnson et al. (1996) reported that steers implanted with Revalor-S (120 mg TBA + 24 mg E<sub>2</sub>) had serum E<sub>2</sub> that reached 16 pg/mL and remained above 12 pg/mL for the entire 150-d implant period. Hunt et al. (1991) reported serum E<sub>2</sub> concentrations of 15 to 20 pg/mL on d 22 after implanting steers with Revalor-S; serum E<sub>2</sub> subsequently fell to approximately half that level on d 82 and 134. The higher concentrations of serum E<sub>2</sub> in our study with heifers, compared with studies with steers, may be due to additional contributions from the ovaries, a difference in liver catabolism of E<sub>2</sub> between the sexes, or the difference between assays. In any case, Revalor-H and Revalor-S caused serum levels of E<sub>2</sub> to be greatly elevated compared with those of control heifers and steers.

Serum TBOH concentrations remained essentially zero over time for nonimplanted heifers but increased markedly following implantation with either Revalor-H or Finaplix-H (Table 4; Figure 2). Serum TBOH concentrations were affected by MGA feeding when an implant was present, as shown by MGA  $\times$  day and MGA  $\times$  implant  $\times$  day interactions (Table 4). Feeding MGA when an implant was present increased serum TBOH concentrations ( $P < .05$ ). A large portion of the interaction between implant type and MGA resulted because TBOH was not present in the serum of nonimplanted heifers, regardless of whether MGA was fed, whereas fluctuations in serum TBOH due to MGA did occur for implanted heifers. The large TBOH peak on d 1 for heifers implanted with Finaplix-H and fed MGA contributed a large portion of the MGA  $\times$  implant  $\times$  day interaction. Because much of the effect of MGA was observed in samples collected the first week following implantation, and for ease of presentation, the main effect of implant type on serum TBOH concentration is shown in Figure 2. Over the entire experiment, serum TBOH concentrations tended to be higher ( $P = .14$ ) for heifers implanted with Finaplix-H than for those implanted with Revalor-H. Apparently, the higher dosage of TBA in Finaplix-H (200 mg) than in Revalor-H (140 mg) was not great enough to affect serum levels of TBOH. Serum TBOH decreased

Table 2. Quantity of trenbolone-17 $\beta$  measured vs quantity of trenbolone-17 $\beta$  added to control heifer serum

Quantity added, pg/mL	n	Quantity measured $\pm$ SD, pg/mL	Recovery, <sup>a</sup> %	CV, %
Serum	25	.05 $\pm$ .19	—	—
Serum + 15.6	25	13.60 $\pm$ 3.50	87.2	25.7
Serum + 62.5	25	63.17 $\pm$ 7.38	101.0	11.7
Serum + 250.0	25	242.3 $\pm$ 20.64	96.9	8.5

<sup>a</sup>[(Quantity measured minus serum blank)  $\div$  (quantity added)]  $\times$  100. Serum blank = .05.

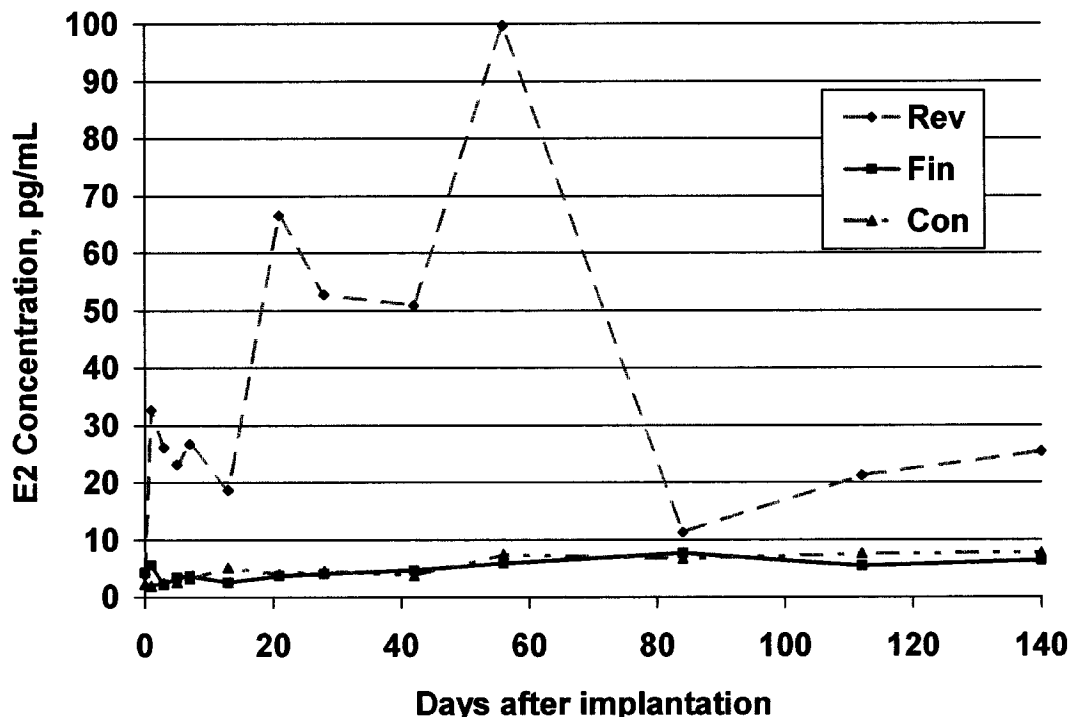


Figure 1. Mean serum estradiol-17 $\beta$  (E2) concentrations in heifers implanted with Revalor-H (Rev) or Finaplix-H (Fin) for 140 d. Con = control.

from d 84 until the end of the experiment in all treatments receiving the TBA implant, more so in the Revalor-H treatments.

The serum TBOH concentrations in heifers can be compared to an earlier experiment with heifers (Henricks et al., 1982) implanted with 300 mg of TBA for 120 d. In that study, serum TBOH increased to a mean of 900 pg/mL, then remained greater than 500

pg/mL until d 60, after which it decreased to less than 100 pg/mL by d 120. These significantly greater concentrations can be attributed to the greater quantity of TBA implanted in the earlier compared with the present experiment (300 mg vs 140 mg). In the experiment by Johnson et al. (1996) using steers, serum TBOH concentrations (blood samples were assayed in our laboratory) were 300 pg/mL on d 2, 120

Table 3. Serum estradiol-17 $\beta$  concentrations (pg/mL) in heifers treated with anabolic implants and melengestrol acetate (MGA)

Day	Control		Revalor-H <sup>a</sup>		Finaplix-H <sup>b</sup>		Statistics	
	0	MGA <sup>c</sup>	0	MGA <sup>c</sup>	0	MGA <sup>c</sup>	Effect <sup>d</sup>	P =
0	2.4	2.2	6.4	1.6	5.8	2.9		
1	2.6	1.5	31.9	33.5	5.3	5.8	Implant (I)	< .01
3	2.9	2.2	26.7	25.4	2.3	2.1	MGA	.75
5	2.6	2.6	18.6	27.8	4.8	2.1	I $\times$ MGA	.71
7	2.9	3.5	28.1	25.3	5.0	2.5	Day	< .01
13	5.9	4.2	16.3	21.1	2.2	3.0	I $\times$ day	< .01
21	3.5	4.5	62.7	70.4	4.6	2.8	MGA $\times$ day	.88
28	3.3	5.7	60.9	44.6	4.8	3.4	I $\times$ MGA $\times$ day	.78
42	2.7	4.8	46.2	55.6	5.1	4.3		
56	3.1	11.7	117.0	82.5	8.7	3.2		
84	3.6	9.8	13.0	9.6	12.2	3.1		
112	5.2	9.9	15.3	27.0	6.2	4.5		
140	3.8	11.5	32.3	18.4	7.9	4.9		

<sup>a</sup>140 mg trenbolone acetate plus 14 mg estradiol-17 $\beta$ .

<sup>b</sup>200 mg trenbolone acetate.

<sup>c</sup>Fed at a level of .5 mg per heifer daily.

<sup>d</sup>Pooled SEM = 6.7

Table 4. Serum trenbolone-17 $\beta$  concentrations (pg/mL) in heifers treated with anabolic implants and melengestrol acetate (MGA)

Day	Control		Revalor-H <sup>a</sup>		Finaplix-H <sup>b</sup>		Statistics	
	0	MGA <sup>c</sup>	0	MGA <sup>c</sup>	0	MGA <sup>c</sup>	Effect <sup>d</sup>	P =
0	2	4	2	5	7	2		
1	0	0	578	624	390	1203	Implant (I)	<.01
3	0	0	369	623	407	667	MGA	.04
5	0	1	178	236	256	319	I $\times$ MGA	.12
7	0	3	144	206	156	264	Day	<.01
13	0	0	71	77	140	138	I $\times$ day	<.01
21	0	0	252	367	181	282	MGA $\times$ day	.03
28	0	0	241	182	331	249	I $\times$ MGA $\times$ day	.09
42	0	0	126	140	75	366		
56	0	0	332	404	271	640		
84	0	0	6	7	90	72		
112	2	0	16	61	64	127		
140	0	0	56	11	32	7		

<sup>a</sup>140 mg trenbolone acetate plus 14 mg estradiol-17 $\beta$ .

<sup>b</sup>200 mg trenbolone acetate.

<sup>c</sup>Fed at a level of .5 mg per heifer daily.

<sup>d</sup>Pooled SEM = 82.

pg/mL on d 40, and 70 pg/mL on d 115. Thus, heifers in our study and steers in the study of Johnson et al. (1996), both implanted with similar amounts of TBA, had similar initial serum TBOH levels. However, from d 21 until 56, the levels in the heifers were twofold higher than in steers. From d 56 to 140, serum TBOH was less than 100 pg/mL in both sexes. Heitzman et al. (1981) reported results using steers implanted with 140 mg TBA or with 140 mg TBA + 20 mg E<sub>2</sub>, in

the same ear or in separate ears that were very similar to our results. In the separate ear implant group, they found a consistently high level of TBOH (200 to 300 pg/mL) for the entire 100-d period. Finally, in the present study, feeding MGA increased ( $P < .05$ ) serum TBOH during the overall implant period.

**Performance.** The effect of anabolic treatment on heifer performance for the 140-d study is shown in

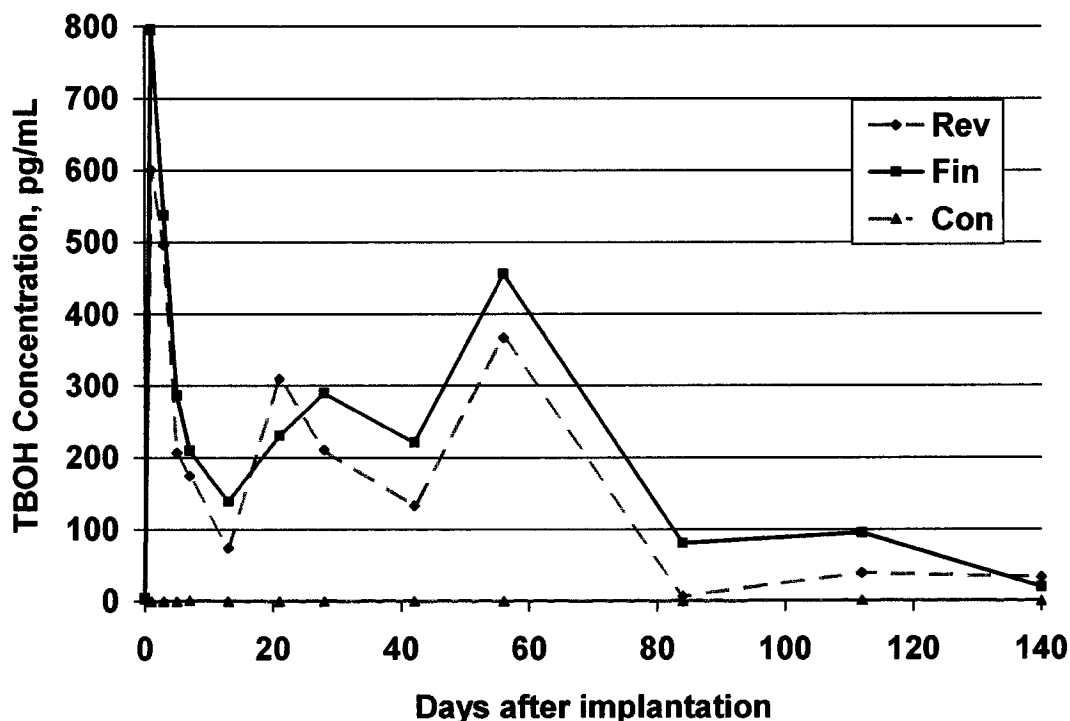


Figure 2. Mean serum trenbolone-17 $\beta$  (TBOH) concentrations in heifers implanted with Revalor-H (Rev) or Finaplix-H (Fin) for 140 d. Con = control.

Table 5. Effect of anabolic treatment on heifer performance for a 140-day period

Item	Control		Revalor-H <sup>a</sup>		Finaplix-H <sup>b</sup>		SEM	P-value		
	0	MGA <sup>c</sup>	0	MGA <sup>c</sup>	0	MGA <sup>c</sup>		I <sup>d</sup>	MGA	I × MGA
No. of heifers	5	5	5	5	5	5				
Feed, kg DM/d	8.6	8.4	9.1	8.7	8.7	8.3	.38	.54	.28	.95
Gain, kg/d	1.33	1.52	1.72	1.46	1.56	1.46	.08	.18	.42	.04
Gain/feed	.154	.182	.190	.169	.181	.175	.006	.15	.97	<.01
Hot carcass weight, kg	308	318	344	307	322	311	8	.30	.06	.03
Dressing percentage	61.8	61.7	61.9	60.7	60.8	61.2	.55	.40	.57	.32
12th rib fat, cm	2.0	2.2	2.0	1.9	1.7	2.1	.30	.79	.46	.70
KPH <sup>e</sup> fat, %	2.6	2.8	3.0	2.7	2.9	2.9	.25	.72	.87	.62
Marbling <sup>f</sup>	5.8	6.8	5.7	6.6	5.0	5.9	.44	.12	.02	.99
Longissimus muscle area, cm <sup>2</sup>	67.7	74.8	78.7	71.6	75.5	73.5	3.0	.41	.78	.09

<sup>a</sup>140 mg trenbolone acetate plus 14 mg estradiol-17 $\beta$ .

<sup>b</sup>200 mg trenbolone acetate.

<sup>c</sup>Fed at a level of .5 mg per heifer daily.

<sup>d</sup>I = Implant.

<sup>e</sup>KPH = kidney, pelvic, and heart.

<sup>f</sup>5 = small, 6 = modest, 7 = moderate.

Table 5. Feed intakes for the first 2 wk tended ( $P = .10$ ) to be depressed in the groups fed MGA, and this effect became less evident over the course of the experiment (data not shown). Implanting with either Revalor-H or Finaplix-H had no effect ( $P = .87$ ) on dry matter consumption during the first 2 wk (data not shown) or for the entire study. Hutcherson et al. (1993) reported that mean intake was not affected by MGA fed to heifers in 19 comparisons. Brandt et al. (1995) reported that MGA significantly increased dry matter intake by heifers by 7% compared with controls. Feeding of MGA interacted with implant on weight gain ( $P = .04$ ) and gain efficiency ( $P < .01$ ). Whereas feeding of MGA improved gains ( $P = .12$ ) and efficiencies ( $P < .01$ ) of nonimplanted heifers, it reduced ( $P < .05$ ) gains and efficiencies of Revalor-H-implanted heifers but did not affect ( $P < .04$ ) gains and efficiencies of Finaplix-H heifers.

With the exception of the periods from d 0 to 28 and d 112 to 140, rate of gain was numerically greatest in each 28-d period for heifers implanted with Revalor-H and not fed MGA (performance data for individual 28-d periods not shown). Except for the first 28-d period, heifers receiving Finaplix alone did not do as well as those treated with Revalor-H. Overall, it ranked with MGA alone. Rates of gain were low in the final period (d 112 to 140) because heifers were overfinished by the end of the study (average of 2.0 cm of backfat). Good performance through d 112 necessitated overfeeding the heifers to obtain the 140-d serum sample. Carcass weights reflected the differences in live weight gain among the six treatments, but longissimus muscle area, dressing percentage, backfat thickness, and kidney, pelvic, and heart fat were not different among treatments. However, marbling scores were greater ( $P = .02$ ) when heifers were fed MGA (Table 5).

The serum hormone profiles should be interpreted carefully. Three factors should be taken into account: 1) not all metabolites of E<sub>2</sub> and trenbolone acetate were measured; 2) interconversions of steroid hormones were not measured; and 3) synergism between TBA and E<sub>2</sub> may be more important than serum concentrations of the hormones individually. It was disappointing, but not surprising, that heifer performance in this study was not closely correlated with serum E<sub>2</sub> or TBOH concentrations when individual animal gain was compared with serum concentrations of one of the hormones. Even though serum patterns of TBOH from Finaplix-H and Revalor-H were similar to each other, growth performance was numerically better with Revalor-H. The MGA had a slight numerical effect on serum E<sub>2</sub> concentrations. Many studies such as this will be needed to arrive at a better understanding of the relation between serum hormone concentrations and rate and efficiency of gain in beef cattle treated with growth-promoting steroids. Having this understanding should help to develop better use of these anabolic steroids.

## Implications

A practical assay for the accurate, precise measurement of trenbolone-17 $\beta$  (TBOH) concentrations in bovine serum was established. Implantation of heifers with Revalor-H or Finaplix-H increased serum TBOH concentrations throughout the 140-d experiment; these increases were particularly great during the first 56 d after implantation. Serum estradiol-17 $\beta$  also was increased by implantation with Revalor-H throughout the 140-d experiment; the increases were most notable during the first 56 d. Although animal numbers were small, feeding of melengestrol acetate

improved gain and efficiency of nonimplanted heifers but reduced gain and efficiency of implanted heifers, demonstrating that important interactions exist among anabolic agents.

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