COMPARISON OF ANABOLIC IMPLANTS ON REPRODUCTIVE FUNCTION, PERFORMANCE AND CARCASS CHARACTERISTICS IN WEANED BEEF BULLS[1]

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Sixty-six Angus bulls, averaging 7 mo of age and 282 kg were utilized to study the effects of implants on performance, carcass characteristics and reproductive function of intact males. Bulls were randomly assigned to one of four treatments, nonimplanted (N), 36 mg of Ralgro® (R), 220 mg of Synovex-S® (S) or 24 mg of Compudose® (C). Bulls remained on test for 217 d. Blood samples were analyzed for testosterone, growth hormone (GH) and luteinizing hormone (LH). Nonimplanted bulls had larger ($P<0.05$) final scrotal circumference (39.0 vs. 37.7 cm) than S-implanted bulls, but were not different from R (38.8 cm) or C (38.6 cm) bulls. No differences were detected in sperm chromatin structure among treatment groups as determined by flow cytometry. There were no treatment differences in average daily gain, feed per gain, testicular weight, and testosterone or LH levels. Synovex implanted bulls had higher GH levels ($P<0.05$) compared to N bulls, but were not different from C or R groups. Carcasses from C (331 kg) and S (332 kg) were heavier ($P<0.05$) than those from R (307 kg) but not different from N (318 kg) animals. Fat thickness at the 12th rib was greater ($P<0.01$) for S (11.6 mm) than N (9.0 mm), C (8.8 mm) and R (8.3 mm) treatments. The greater fat thickness of S bulls increased the yield grade compared to N and C ($P<0.05$) and R-treated bulls ($P<0.01$). Dressing percent was higher ($P<0.05$) for C (63.2%) and S (62.6%) than R (61.7%) and N (61.0%) groups. No differences were detected for longissimus muscle area or USDA quality grades.

Key words: Implants, beef bulls, sperm chromatin structure, carcass, hormones

[Comparaison des effets d'implants anaboliques sur les fonctions reproductives, le rendement et les caractéristiques de la carcasse des taurillons de boucherie sevrés.]
Titre abrégé: Effets des implants chez les taurillons.
Nous avons utilisé 66 taurillons Angus âgés en moyenne de 7 mois et pesant en moyenne 282 kg pour étudier les effets des implants sur le rendement, les caractéristiques de la carcasse et les fonctions reproductives de mâles intacts. Les sujets ont été répartis au hasard en quatre groupes: sans implant (N); 36 mg de Ralgro® (R), 220 mg de Synovex-S® (S) ou 24 mg de Compudose® (C). La période d'essais a duré 217 jours. Nous avons mesuré la teneur du sang en testostérone, en hormones de croissance (GH) et en hormone lutéinisante (LH). Les taurillons sans implant présentaient une circonférence finale de scrotum plus grande ($P<0,5$) que les taurillons S (39,0 comparativement à 37,7 cm) mais ils n'étaient pas différents de ce point de vue des taurillons R

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(38,8 cm) ou C (38,6 cm). Aucune différence n'a été observée dans la structure de la chromatine des spermatozoïdes, telle que déterminée par cytométrie de flux, entre les groupes expérimentaux. Nous n'avons pas non plus observé de différence, entre les groupes, quant au gain moyen quotidien, au rapport aliments-gains, au poids des testicules et aux teneurs en testostérone et en LH. Les taurillons portant l'implant de Synovex présentaient des teneurs plus élevés en GH ($P<0,5$) comparativement aux taurillons N, mais ils n'étaient pas différents de ce point de vue des taurillons C ou R. Les carcasses des taurillons C (331 kg) et S (332 kg) étaient plus lourdes ($P<0,5$) que celles des taurillons R (307 kg) mais elles n'étaient pas différentes de celles des taurillons N (318 kg). L'épaisseur du gras dorsal au niveau de la douzième côte était plus grande ($P<0,1$) chez les taurillons S (11,6 mm) que chez les taurillons N (9,0 mm), C (8,8 mm) et R (8,3 mm). Cette épaisseur plus grande du gras dorsal chez les taurillons S les a fait passer dans une classe de rendement supérieure à celle des taurillons N et C ($P<0,5$) et des taurillons R ($P<0,1$). Le rendement en viande était plus élevé ($P<0,5$) chez les taurillons C (63,2%) et S (62,6%) que chez les taurillons R (61,7%) et N (61,0%). Aucune différence n'a été observée quant à la superficie du muscle longissimus ou à la catégorie de qualité de l'USDA.

Mots clés: Implants, taurillons de boucherie, structure de la chromatine des spermatozoïdes, carcasses, hormones

Little information is available comparing the response of different anabolic implants in a single experiment with beef bulls. Most of the information on implanting bulls is from work using only Ralgro® implants. Increased growth performance due to implants without impairment of reproductive performance in weaned beef bulls would be of economic importance to seed stock producers.

Implanting bulls before weaning until slaughter with Ralgro® improves performance, behavior and carcass characteristics (Greathouse et al. 1983). Also, it may improve marbling score, lean texture and palatability traits (Gray et al. 1984). In contrast, postweaning implanting of young bulls has produced varying growth response (Gregory and Ford 1983). The use of Ralgro® in intact males implanted at less than 90 d of age or throughout the suckling and finishing periods is hypothesized to impair testicular function by inhibiting the production of gonadotropin releasing hormone (Baker and Gonyou 1986). Zeranol inhibits testicular development in bulls (Ralston 1978) and decreases testosterone levels (Juniewicz et al. 1985; Straigmiller et al. 1985). Juniewicz et al. (1985) reported the effects of postweaning Ralgro® implants on testicular function and spermatogenesis were not permanent. However, preweaning Zeranol implants resulted in impairment of testicular development and spermatogenesis 1 yr later (Ballachey et al. 1986). Therefore, the physiological effects of implanting appear to depend upon age at implantation.

The primary mode of action of anabolic agents appears to be increased growth hormone (GH) levels. Steers implanted with DES®, Synovex-S® and Ralgro® had increased GH levels 20 d after implantation (Gopinath and Kitts 1984). Borger et al. (1973) noted in steers implanted with Ralgro® that GH levels tended to rise and then fall. Little information is available in the literature on GH levels in bulls implanted after weaning.

The purpose of this research was to compare the response of growth-promoting implants in weaned beef bulls on performance, sperm chromatin structure, GH, testosterone, luteinizing hormone (LH) levels, and carcass characteristics.

MATERIALS AND METHODS

After weaning, 66 Angus bulls averaging 211 ± 2.1 d of age and 282 ± 2.8 kg were randomly assigned to one of four treatments: nonimplanted (N), 36 mg of Ralgro® (R), 220 mg Synovex-S® (S) or 24 mg Compudose® (C). Bulls in treatments R and

S were reimplanted at 275 and 340 d of age and bulls in the C treatment at 390 d of age. Implants were placed according to the manufacturers standard recommendations. Each treatment was divided in two pens of eight or nine bulls to determine feed per gain and feed intake with similar treatments penned side by side. All animals were in outdoor drylots, and remained on test for 217 d.

All animals were initially fed an ad libitum diet of 45% corn and 55% alfalfa hay (13.6% crude protein) for 95 d. The ration was progressively changed over a 21-d period to a final finishing ration of 73% corn, 22% corn silage and 5% mineral-protein supplement (10.6% crude protein) for 101 d.

Individual body weights were recorded at 28-d intervals following the initial weighing. Scrotal circumferences, measured with a steel tape were taken at initiation and termination of the trial.

At trial termination one pen per treatment was transported to a commercial packing plant and the remaining bulls were slaughtered 1 wk later. Hot carcass weights were recorded and testes recovered from each bull. Testes were trimmed of connective tissue to the tunica vaginalis and the epididymis removed.

Carcass data were obtained 24 h postmortem with the assistance of a USDA grader. The data collected consisted of marbling, maturity score, final quality grades, adjusted fat thickness and kidney, heart and pelvic fat. The longissimus muscle was traced at the 12th rib for each carcass and the area later determined by a compensating planimeter (Model 620015, Keuffel and Esser).

Blood samples were collected weekly for 9 wk, and then monthly thereafter. The initial sample was taken at the time of first implanting and final collection prior to slaughter. Blood samples were collected between 1000 and 1200 h by jugular venipuncture in vacutainer tubes and immediately placed on ice. After centrifugation serum was decanted into duplicate tubes, stored at −24°C and later assayed for testosterone, GH and LH.

The testosterone assays (Diagnostic Products Company, DPC, Los Angeles, Calif.) were a solid-phase radioimmunoassay based on a specific testosterone antibody immobilized to the wall of polypropylene tubes. An aliquot of 25 μL was used for each sample and samples were analyzed in duplicate. Testosterone labeled with (^{125}I) Na-iodide had a high specific activity, with total counts of approximately 75 000 dpm. Maximum binding was approximately 45%. The antiserum was specific for testosterone with a cross reactivity of 100% to testosterone standards and sensitivity of 0.11 ng mL^{-1}. The standard curve was linear between 0.3 and 30.0 ng mL^{-1}. Increasing volumes of steer serum and spiked (7.75 ng mL^{-1}) steer serum paralleled the standard curve. Recovery was determined by adding 1 ng mL^{-1} of testosterone (Sigma, St. Louis, Mo.) to steer serum and was 103%. Intra- and interassay coefficients of variation were 4.2 and 9.8%, respectively.

Concentrations of LH were determined by the double antibody radioimmunoassay procedure (Niswender et al. 1969) with modification. Cross reactivity existed between ovine and bovine LH. The maximum binding ranged from 40 to 55%. Increasing volumes of steer serum and spiked (10 ng mL^{-1}) steer serum paralleled the standard curve which ranged from 0.063 to 25 ng mL^{-1}. Recovery was determined by adding 1 ng mL^{-1} of LH (DPC) to steer serum and was 104%. The intra- and interassay coefficients of variation were 12.0 and 21.0%, respectively. Samples were run in duplicate. For each sample 100 μL of serum was added to tubes containing LH antibody and labeled antigen. After 3 d of incubation at 4°C, the second antibody was added and incubated an additional 2 d. The assay was then stopped, samples centrifuged and counted.

Growth hormone levels were determined by a double antibody, radioimmunoassay similar to LH. Maximum binding was 41.5%. Increasing volumes of steer serum and spiked (10 ng mL^{-1}) steer serum paralleled the standard curve which ranged from 0.05 to 50 ng mL^{-1}. Recovery was determined by adding 1 ng mL^{-1} of GH (NIADDK-oGH-1-3-APF5285C) to steer serum and was 106%. Intra- and interassay coefficients of variation were 4.4% and 11.0%, respectively.

The ductus deferens were removed from testes at the slaughter plant and sperm stripped into tubes, and mixed with TNE buffer (0.01 M Tris-buffer, 0.15 M NaCl and 0.001 M disodium ethylenediamine tetracetate EDTA, pH 7.4) and 10% glycerol and placed on ice. The testes were also placed in ice and weights recorded later. Ductus sperm were frozen at −20°C for 8 h and then at −100°C and evaluated later by flow cytometry. Sperm samples were analyzed by the sperm chromatin structure assay (Darzynkiewicz 1979; Evenson 1986; Ballachey et al. 1987, 1988). In the sperm chromatin structure assay, sperm are first treated for 30 s at an acid pH (1.2) which induces DNA denaturation in sperm with abnormal chromatin structure (Evenson et al. 1985). Cells were then stained with acridine orange which, when intercalated into

double stranded DNA, will fluoresce green and when associated with single stranded DNA will fluoresce red. The extent of DNA denaturation per cell was quantitated by red (red + green)$^{-1}$ fluorescence and is termed alpha $_t$ (α_t). Fluorescence measurements were made in a Cytofluorograf II interfaced to an Ortho Diagnostics 2150 data handler (Ortho Diagnostics, Inc. Westwood, Mass). Recorded measurements began 3 min after staining at a flow rate of about 200 cells s^{-1}. Red fluorescence ($\geq$600 nm) and green fluorescence (515–530 nm) were recorded for 5000 cells per sample. Alpha$_t$ was calculated by a computer protocol and the distribution recorded for each sample.

Treatments were compared using a one way-analysis of variance procedure – Statistical Analysis System Institute (1985). Feed intake and conversion were analyzed using pen means, and average daily gain by individual animal performance. Hormone levels were analyzed by a repeated measure analysis of variances with treatment and time. Significant differences between treatments were separated using Fisher's protected LSD procedure. Final weight was used as a covariate in the model for scrotal circumference, testicular weight and carcass traits.

RESULTS AND DISCUSSION

Two animals died in the trial unrelated to treatment effects during the course of the study (one each in groups N and C). Performance data (final weight, average daily gain, feed per gain and intake) did not differ due to treatments (Table 1). This lack of difference in performance was consistent with the results obtained by Price et al. (1983) with Ralgro®. Feed intake and feed per gain are higher than would be anticipated by bulls of this weight. This may be due to the low temperature and wet conditions during the first 100 d of the trial.

Least-square means for testicular parameters are also shown in Table 1. There was no difference ($P>0.05$) in initial scrotal circumference. Staig Miller et al. (1985) reported that implanting bulls with Ralgro® at 48 d of age resulted in decreased scrotal circumference, but implanting bulls at 215 d of age had no effect on scrotal circumference. Similar results were obtained in this trial. Final scrotal circumference was similar between N and R groups. However, bulls in the S group had smaller ($P<0.05$) scrotal circumference than bulls in the N group. Group C bulls responded similarly to R bulls in regard to scrotal circumference. Implanting decreased testicular weights in all groups but not significantly ($P>0.05$). Response in testicular weight to implanting (Table 1) was similar to scrotal circumference being highest in the N group, intermediate in R and C and lowest in the S group.

Least-square means for carcass data are presented in Table 1. Carcass weights from R-implanted bulls were lower ($P<0.05$) than carcass weights from S and C bulls, but not different from N bulls. Johnson et al. (1984) reported no difference in carcass weights in post-weaned bulls implanted with Ralgro®, Synovex®, or Compudose®. However, since their ration was higher in energy it may have masked the response of implants on carcass weight.

Longissimus muscle area, kidney, heart and pelvic fat, marbling and USDA quality grades were similar among treatments (Table 1). Bulls in the R group, however, exhibited the lowest quality grade "good" and marbling score "slight" (3.9) which coincide with less fat thickness. The remaining treatments had "low choice" quality grades and "small" marbling scores.

Fat thickness measured at the 12th rib was greater ($P<0.01$) for S than N, C and R bulls. The greater degree of backfat in S bulls increased ($P<0.05$) the USDA yield grade compared to the other three treatments. Implanting increased ($P<0.05$) dressing percent in the C and S groups.

The effects of implanting on α_t variables were minimal and are also presented in Table 1. The S bulls had a lower, although nonsignificant, α_t peak channel value than R, C and N bulls. Ralgro®-implanted bulls had nonsignificantly higher ($P>0.05$) SD α_t and COMP α_t values (cells outside the main population). Higher α_t values correspond to poorer sperm quality and reduced fertility (Ballachey et al. 1986, 1987, 1988). Implanting postweaned bulls thus resulted in

Table 1. Least-squares means (±SE) for performance, testicular parameters carcass traits and sperm variables

Item	Nonimplanted	Ralgro†	Synovex†	Compudose‡
No. of observations	15	17	17	15
Initial age (d)	211.4 ± 4.27	207.2 ± 4.14	210.9 ± 4.14	214.9 ± 4.27
Initial wt (kg)	280.0 ± 5.4	274.0 ± 5.2	282.0 ± 5.2	289.0 ± 5.4
Final wt (kg)	521.0 ± 9.9	497.0 ± 9.3	531.0 ± 9.3	524.0 ± 9.9
Avg daily gain (kg d^{-1})	1.1 ± 0.04	1.03 ± 0.04	1.14 ± 0.04	1.08 ± 0.04
Feed intake (kg d^{-1})	12.65 ± 0.32	12.26 ± 0.32	12.30 ± 0.32	12.84 ± 0.32
Feed $gain^{-1}$	8.57 ± 0.31	9.01 ± 0.31	7.89 ± 0.31	8.61 ± 0.31
Scrotal circumference (cm)				
Initial	25.8 ± 0.60	25.8 ± 0.59	26.1 ± 0.59	27.6 ± 0.60
Final	39.6*a* ± 0.44	38.8*ab* ± 0.41	37.8*b* ± 0.41	36.6*ab* ± 0.44
Testicular wt (g)	602.1 ± 27.20	581.7 ± 25.20	522.3 ± 25.50	584.3 ± 27.20
Hot carcass wt (kg)	318.0*ab* ± 6.5	307.0*b* ± 6.1	332.0*a* ± 6.1	331.0*a* ± 6.5
Fat thickness (mm)	9.05*a* ± 0.71	8.32*a* ± 0.66	11.55*b* ± 0.66	8.72*a* ± 0.71
Est KPH fat (%)§	1.4 ± 0.11	1.4 ± 0.10	1.5 ± 0.10	1.6 ± 0.11
Longissimus muscle area (cm^2)	78.8 ± 1.9	76.2 ± 1.7	78.7 ± 1.7	81.0 ± 1.9
USDA yield grade	2.43*a* ± 0.11	2.38*a* ± 0.11	2.82*b* ± 0.11	2.44*a* ± 0.11
Marbling score¶	4.36 ± 0.19	3.90 ± 0.18	4.19 ± 0.18	4.26 ± 0.19
USDA quality grade‖	6.00 ± 0.25	5.00 ± 0.24	6.00 ± 0.24	6.00 ± 0.25
Dressing percentage	61.0*a* ± 0.46	61.7 *a* ± 0.44	62.6*b* ± 0.44	63.2*b* ± 0.46
Cytometric evaluations of semen quality				
Peak††	203.48 ± 3.05	201.15 ± 2.86	194.48 ± 2.86	202.42 ± 3.05
Means ‡‡	203.57 ± 5.39	212.28 ± 5.05	205.17 ± 5.05	212.15 ± 5.39
Standard deviation§§	37.64 ± 4.46	43.72 ± 4.19	38.31 ± 4.19	37.17 ± 4.46
COMP¶¶	6.12 ± 1.91	8.67 ± 1.79	5.72 ± 1.79	7.74 ± 1.91

† Implanted at day 0 and every 60 - 70 d.
‡ Implanted at days 0 and 180.
§ Estimated kidney, pelvic and heart fat.
¶ Marbling 1-8, 1 = practically devoid, 4 = small, 8 = moderately abundant.
‖ Quality grade 1-8, 1 = low standard, 5 = high good, 8 = high choice.
†† Peak at the α_t distribution.
‡‡ Mean of the α_t distribution.
§§ Standard deviation of the α_t distribution.
¶¶ COMP α_t cells that fall outside the main population.
ab Means in the same row not bearing the same letter differ ($P<0.05$).

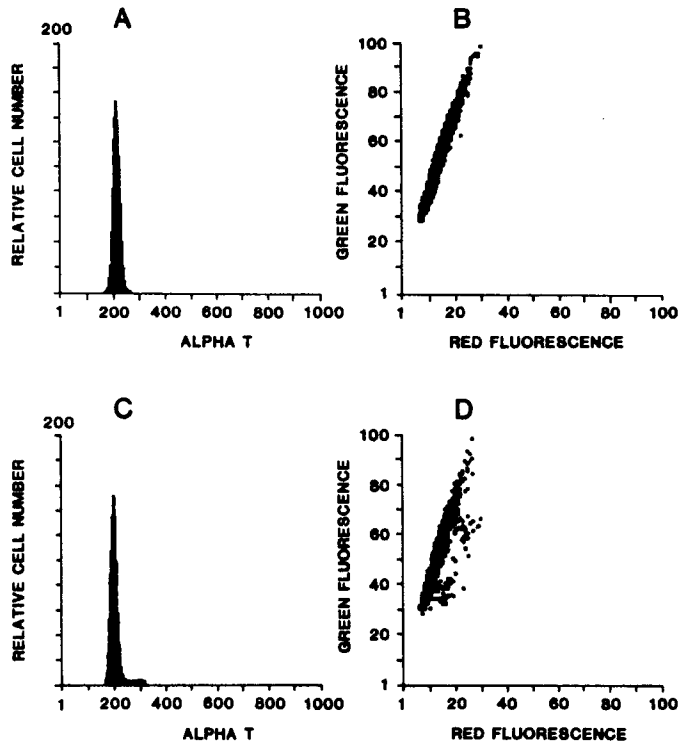

Fig. 1. Red and green fluorescence cytograms (B, D) corresponding to α_t frequency histograms (A, C) of sperm cells stained with acridine orange and measured by flow cytometry. Red and green fluorescence correspond to single-stranded and double-stranded DNA, respectively. A and B represent cells from one of the best, and C, D from one of the poorest, sperm samples.

little variation in α_t parameters. Juniewicz et al. (1985) using conventional methods of semen evaluation reported similar result with Ralgro®. Ballachey et al. (1986), utilizing the sperm chromatin structure assay, reported an apparent permanent effect of Ralgro on testicular development in beef bulls. However, in their study bulls were implanted preweaning compared to postweaning in the present study. These results indicate weaned bulls can be implanted with anabolic agents without affecting sperm chromatin structure.

An example of flow cytometry data on ductus sperm from two bulls is presented in Fig. 1. Generally, little variation was noted due to implants in flow cytometry measurements. The graphs are examples of one of the best (1 A,B) and poorest (1 C,D) sperm samples. The cytograms of green vs. red fluorescence in Fig. IB and ID correspond to the α_t frequency histogram 1A and 1C, respectively. Cytogram IB is typical of a high-quality semen sample. The distribution produces an α_t frequency histogram that has a narrow distribution and therefore low values for SD α_t as seen in Fig. 1A. Likewise, few cells are outside of the main population, i.e., a low COMP α_t value. Cytogram ID is typical of semen samples from lower fertility bulls but would still fall within an acceptable range of fertility (Ballachey et al. 1986, 1987, 1988). This distribution equates with a higher SD and COMP α_t as seen in Fig. 1C.

Testosterone levels fluctuated throughout the trial period as seen in Fig. 2. There were no differences in testosterone levels except

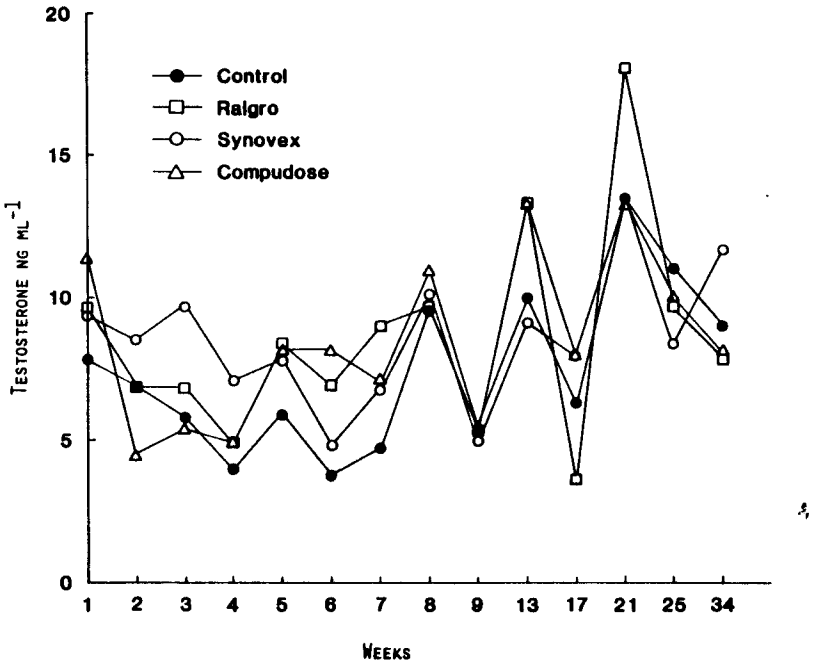

Fig. 2. Effects of implants on serum testosterone concentrations in weaned bulls. Mean ± SE testosterone concentrations for the trial were 7.39 ± 0.76 (223), 8.93 ± 0.74 (236), 8.27 ± 0.74 (236), and 8.56 ± 0.76 (221) ng mL^{-1} for the Control, Ralgro, Synovex and Compudose treatments, respectively (values in parentheses are the number of observations).

during weeks 6 and 17 ($P<.05$). At week 6 testosterone levels were 8.20, 3.65, 4.84 and 6.94 ng mL^{-1} for C, N, S and R, respectively. Compudose®-implanted bulls also had higher ($P<0.05$) testosterone levels on wk 17. Mean testosterone levels for the trial were 8.93, 8.56, 8.27 and 7.39 ng mL^{-1} for R, C, S and N, respectively. Juniewicz et al. (1985) and Staigmiller et al. (1985) found serum testosterone concentrations decreased in Ralgro®-implanted bulls compared to nonimplanted bulls when implants were done at an early age, but by the time of slaughter testosterone concentrations were equal in control and implanted bulls.

There was no effect of implants on mean LH levels which were 0.14, 0.13, 0.11 and 0.11 ng mL^{-1} for S, N, R and C, respectively. Similar LH patterns were present in all four groups except during weeks 17 and 21. During week 17 N bulls and during week 21 S bulls had higher ($P<0.05$) LH levels. Both LH and testosterone concentrations followed the same general pattern for the duration of the experiment. These data indicate there is little response of implants in weaned bulls on circulating levels of testosterone and LH.

During the trial GH patterns were similar for the four groups (Fig. 4). All three implants resulted in numerically higher mean GH concentrations than controls, with mean values of 54.8, 46.8, 44.6 and 32.0 ng mL^{-1} for S, R, C, and N, respectively. However, the only significant increase ($P<0.05$) in mean GH levels occurred in the S group. In week 3, Ralgro®-implanted bulls had higher ($P<0.05$) GH levels than the other three groups. Synovex®-implanted bulls had higher ($P<0.05$) GH levels in weeks 7 and 8 than the other groups.

The present investigation suggests limited response of weaned bulls to anabolic implants. No differences were present between non-

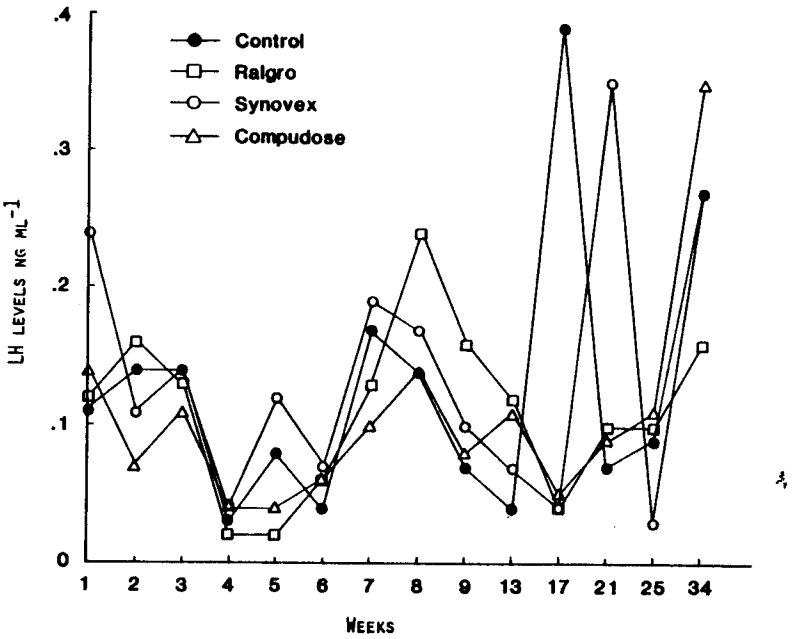

Fig. 3. Effects of implants on serum luteinizing hormone (LH) concentrations in weaned bulls. Mean ± SE LH concentrations for the trial were 0.13 ± 0.02 (223), 0.11 ± 0.02 (236), 0.14 ± 0.02 (236), and 0.11 ± 0.02 (221) ng mL^{-1} for the Control, Ralgro, Synovex and Compudose treatments, respectively (values in parentheses are the number of observations).

implanted bulls and bulls implanted with Ralgro®, Synovex® or Compudose® in sperm chromatin structure, testicular weight, performance, KPH fat, marbling score, USDA quality grade or LH and testosterone levels. There was, however, some difference due to implanting on other traits. Bulls implanted with Synovex® had smaller scrotal circumference and higher GH concentrations than nonimplanted bulls, and higher backfat thickness and yield grade than nonimplanted bulls and bulls implanted with Ralgro® or Compudose®. Ralgro® implanting decreased carcass weight compared to Synovex® and Compudose® implanted bulls. Both Synovex® and Compudose® resulted in increased dressing percent compared to nonimplanted and Ralgro®-implanted bulls. Results of this study indicate no advantage of implanting weaned bulls with any of the growth promotants due to the lack of response in performance traits, even though there were no apparent adverse effects on the reproductive parameters measured.

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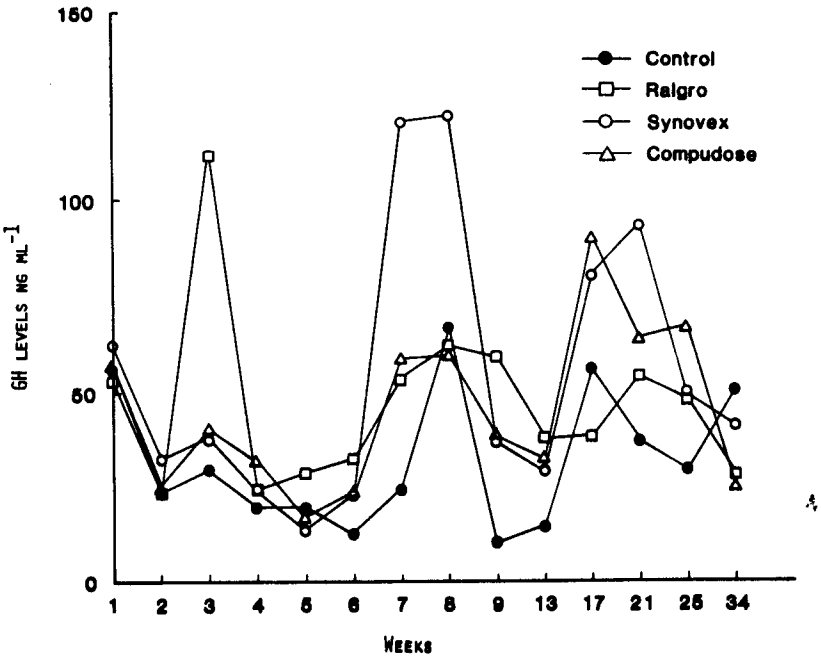

Fig. 4. Effects of implants on serum growth hormone (GH) concentrations in weaned bulls. Mean ± SE GH concentrations for the trial were 32.0 ± 5.5 (223), 46.5 ± 5.4 (236), 54.8 ± 5.4 (236), and 44.6 ± 5.5 (221) ng mL^{-1} for the Control, Ralgro, Synovex and Compudose treatments, respectively (values in parentheses are the number of observations).

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