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Plasma Estrogen Concentrations in Non-Implanted and Synovex-S Implanted Feedlot Steers

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Synovex-S[1], an ear implant formulation that contains 200 mg of progesterone and 20 mg of 17 β-estradiol benzoate per dose, is an approved hormonal growth promotant in feedlot steers. Growth responses with Synovex-S at Beltsville have been equal to or greater than those with diethylstilbestrol (DINIUS et al. 1978, RUMSEY 1978, KAHL et al. 1978). However, there is no information in the literature on the effect of Synovex-S on estrogen concentrations in steers under feedlot conditions.

The purpose of the present study was threefold: 1) to estimate the plasma concentrations and variability of estrone, 17 β-estradiol, and estriol found in non-implanted feedlot steers at any given time; 2) to determine the effect of Synovex-S implants on plasma estrogen concentrations in feedlot steers; and 3) to examine the correlation between plasma estrogen concentrations and growth rates of feedlot steers.

EXPERIMENTAL PROCEDURES

Feedlot Trials. The plasma samples used in this study were obtained from three feedlot trials conducted in the same feedlot facilities at Beltsville. All steers were purchased as feeder cattle at local auctions except for 16 dairy steers in trial 1 that were from the Beltsville dairy herd. All steers were housed on concrete slab lots, feed and water were available ad libitum, and half the steers in each trial were implanted with a single dose of Synovex-S (Syntex Laboratories, Inc., Palo Alto, CA 94304) at the beginning of each trial.

Trial 1, previously described as trial 2 in a report by RUMSEY (1978), consisted of 64 steers (359 kg average initial body weight) assigned to eight lots of eight steers per lot. The trial was designed to determine the response of sulfur addition (0 or 1.4 g of sulfur per kg of diet), dietary choline addition (0 or 750 parts per million), and Synovex-S implants in a factorial arrangement of treatments. The basic diet in this trial was 75.4% corn, 15.0% wheat, 6% molasses, 1.6% urea, and 2.0% minerals and salt. The steers were fed for a 112-day experimental period, and a blood sample from each steer was drawn into a heparinized centrifuge tube by venipuncture on the 113th day after implantation of Synovex-S, approximately 3 hr after the morning replenishment of feed.

Trial 2, previously described as experiment 2 in a report by DINIUS et al. (1978), consisted of 40 steers (331 kg average initial body weight) assigned to four lots of 10 steers per lot. The trial was designed to determine the response to monensin

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(Eli Lilly and Company, Greenfield, IN 46140) addition (0 or 30 parts per million) and Synovex-S implants in a factorial arrangement of treatments. The basic diet fed in this trial was 96% alfalfa hay, 3% animal fat, and 1% minerals. The steers were fed for a 105-day experimental period, and blood samples from each steer were collected on both the 60th and 106th day after implantation of Synovex-S as in trial 1.

Trial 3, previously described by KAHL et al. (1978), consisted of 80 steers (320 kg average initial body weight) assigned to four lots of 20 steers per lot. The four lots of steers were used to determine the response to the dietary addition of choline (0, 750, or 1500 parts per million as choline chloride or 750 parts per million as choline stearate). Half of the steers in each lot were implanted with Synovex-S at the beginning of the trial. The basic diet was the same as that used in trial 1. The steers were fed for a 119-day experimental period, and a blood sample was taken from each steer on both the 60th and 120th day after implantation of Synovex-S as in trial 1.

Estrogen Analysis. The blood samples were centrifuged, and the plasma was separated and frozen until analyzed. The plasma was analyzed for estrone (E_1), 17 β-estradiol (E_2), and estriol (E_3) by Hazleton Laboratories America, Inc., Vienna, VA, according to the radioimmunoassay method of CHUNG-HSIU WU and LUNDY (1971). The procedure in general was to extract plasma with diethyl ether, chromatograph in Sephadex LH-20 chromatography columns, and measure the estrogens by radioimmunoassay using antiserum specific to E_1, E_2, and E_3 produced at Hazleton Laboratories. For continuous checks of the accuracy and reproducibility of the radioimmunoassay, two plasma samples to which standard estrone was added and a blank or stripped plasma sample were routinely analyzed with each assay set.

Two sets of plasma samples to which standard estrogens had been added were analyzed to estimate analytical variation and recovery of the method for bovine plasma. The plasma used for each set of standard samples was taken from a common pool of plasma obtained from a non-implanted steer. The first set consisted of 20 standard samples, four samples for each of five concentrations of standard addition. The concentrations added were 0, 5, 25, 50, and 100 picograms per milliliter (pg/ml) of each estrogen added as a mixture; total estrogen concentration added was 0, 15, 75, 150, and 300 pg/ml. The second set of standard samples was a repeat of the first set, but there were 24 samples at standard concentrations for each estrogen of 0, 5, 10, 25, 50, and 100 pg/ml, and the standard samples were run in duplicate.

The limit of estimating estrogen concentration for this study was considered to be 1 pg/ml. Although recovery was not determined below 5 pg/ml and analytical quantitation was not

exact because 1 pg/ml was outside of the ideal range for the standard curve of bound labeled estrogen versus standard concentration, the lower limit of 1 pg/ml was sufficiently accurate to add valuable information relative to the low estrogen levels in steer plasma.

Statistical analysis. The data in this study were analyzed statistically by multiway classification analysis of variance (STEEL and TORRIE 1960) as outlined by RUMSEY (1978) for trial 1, DINIUS et al. (1978) for trial 2, and KAHL et al. (1978) for trial 3. For trials 2 and 3, sample time was considered in the statistical model by evaluating each trial as a split plot in time design.

RESULTS AND DISCUSSION

Known concentrations of estrogens that were added to bovine plasma were related by regression analysis to the concentrations measured in these standard samples. The regression curves for the individual estrogens and for total estrogens ($E_1 + E_2 + E_3$) were linear. On the basis of these curves, recoveries were 102, 101, 113, and 106% for E_1, E_2, E_3, and total estrogens, respectively. The respective R^2 values for these curves were .98, .99, .97, and .99, and the respective $S_{y.x}$ values were 4.7, 3.1, 6.8, and 10.0 pg/ml. The apparent high recovery for E_3 may be related to the difficulty of resolving the E_3 fraction by partition chromatography before radioimmunoassay analysis. For the individual estrogens, the fact that the lowest R^2 value and the highest $S_{y.x}$ value were obtained for the E_3 analysis suggests greater variability in the E_3 results. On the basis of the standard plasma samples, within assay and between assay coefficients of variation were 11.3 and 3.0, respectively, for E_1, 9.5 and 2.5 for E_2, and 13.6 and 3.4 for E_3. The relative variability tended to be greater at the lower concentrations; however, absolute variation was similar at all concentrations.

Growth response. The response to the use of Synovex-S in this study is shown in table 1, which shows that the implant treatment in each trial was physiologically effective. Synovex-S implanted steers gained 30% faster than non-implanted steers during the first half of trial 1 and 16% faster during the second half of the trial. These gain responses were 47% and 20%, respectively, for trial 2 and 19% and 30% for trial 3.

Plasma estrogen concentrations. In all trials, the treatments other than Synovex-S implants did not affect ($P>.05$) the concentrations of plasma estrogens; and in general, an effect of Synovex-S on plasma estrogen concentrations was noted at 60 days after implantation but not after 105 days. Table 2 shows the plasma concentrations of E_1 and E_2, and Table 3 shows the plasma concentrations of E_3 and total estrogens. In both trials 2 and 3, the fact that the average concentration of E_2 was lower ($P<.01$)

in non-implanted steers than in implanted steers suggests the absorption of estradiol from unabsorbed implants. At the same time, the range for E_2 concentrations was larger among the implanted steers and there was almost a complete overlapping of the ranges among the non-implanted and implanted steers. The larger range for E_2 concentrations for implanted steers at 60 days could reflect variation in implant absorption rate as affected by such factors as 1) breakage of implants at the time of implantation; 2) location of implant *in situ* relative to blood vessels; and 3) localized tissue reaction to the implants.

TABLE 1. EFFECT OF SYNOVEX-S IMPLANTS ON WEIGHT GAIN OF FEEDLOT STEERS, kg/steer/day

Item[a]	Treatment		$S\bar{x}$[b]
	Non-implanted	Implanted	
Trial 1			
0-60 days	1.29	1.68	
60-112 days	.94	1.09	.04
Trial 2			
0-60 days	.79	1.16	
60-105 days	.76	.91	.08
Trial 3			
0-60 days	1.65	1.96	
60-120 days	.89	1.16	.04

[a]Trial 1, RUMSEY, 1978; Trial 2, DINIUS *et al.*, 1978; Trial 3, KAHL *et al.*, 1978.

[b]Common standard error of mean from analysis of variance.

The average E_2 concentrations at the end of each trial were similar for both the non-implanted and implanted steers, and the concentration ranges were similar. Also, the average concentrations and ranges for both groups at the end of each trial were similar to those of the non-implanted steers at 60 days. The lack of differences at the end of each trial probably reflects nearly complete absorption of the Synovex-S implants. This complete absorption is verified by the absence of residual implants upon examination of the implant sites at slaughter. The concentrations found at 60 days in the non-implanted steers and at the end of each trial were similar to those reported by WETTEMANN *et al.* (1972), CORAH *et al.* (1974), and HENRICKS *et al.* (1977) in non-pregnant beef females but lower than values reported by BROWER and KIRACOFE (1978). The fact that values obtained by BROWER and KIRACOFE (1978) are higher than those in the current study or those reported for non-pregnant females is not clear. WETTEMANN *et al.* (1972) used a pooled sample of steer serum to standardize their radioimmunoassay procedures and reported an E_2 concentration in the pooled sample similar to the E_2 concentrations in the non-implanted steers in the current study.

TABLE 2

Estrone (E_1) and 17 β-Estradiol Concentrations (pg/ml) in Plasma of Non-Implanted and Synovex-S Implanted Feedlot Steers

Trial	Days on trial	Statistic[a]	Treatment: Non-implanted	Treatment: Implanted	$S\bar{x}$[b]
			E_1, pg/ml		
1	113	Range	<1.0-69.0	<1.0-91.0	
		Average	15.5	12.8	2.8
2	60	Range	<1.0-12.0	<1.0-14.0	
		Average	5.1	4.8	
	106	Range	<1.0-10.0	<1.0-24.0	
		Average	3.6	5.6	.9
3	60	Range	<1.0-26.0	<1.0-18.0	
		Average	2.4	4.3	
	120	Range	<1.0-20.0	<1.0-28.0	
		Average	5.4	5.7	1.1
			E_2, pg/ml		
1	113	Range	<1.0-23.0	<1.0-18.0	
		Average	3.8	5.7	1.1
2	60	Range	<1.0-20.0	6.9-181.0	
		Average[c]	7.1	31.1	
	106	Range	<1.0-12.0	<1.0-14.0	
		Average	3.0	3.0	4.3
3	60	Range	<1.0-14.0	<1.0-310.0	
		Average[c]	1.9	65.1	
	120	Range	<1.0-32.0	<1.0-81.0	
		Average	2.5	9.0	9.0

[a]Range: lower limit of estimating concentration was considered to be 1.0 pg/ml.

[b]Common standard error of mean from analysis of variance in which sampling time was included in the analysis for trials 2 and 3.

[c]Average concentration for the 60-day implanted steers was greater than the 60-day non-implanted steers or the 120-day averages at $P<.01$.

The data show that in all groups, the plasma concentrations of estrogens are not equally distributed about the group means but that a greater number of individual values fall within the lower part of the concentration range. This unequal distribution about the mean was particularly noticeable for the implanted groups in trials 2 and 3 at 60 days. Serial samples were not collected from steers in this trial to determine whether daily or diurnal variation contributed to the variation obtained with one-time sampling.

TABLE 3

Estriol (E_3) and Total Estrogen ($E_1 + E_2 + E_3$) Concentrations (pg/ml) in Plasma of Non-Implanted and Synovex-S Implanted Feedlot Steers

Trial	Days on trial	Statistic[a]	Treatment: Non-implanted	Treatment: Implanted	$S\bar{x}$[b]
			E_3, pg/ml		
1	113	Range	<1.0-22.0	<1.0-82.0	
		Average[c]	1.7	11.8	4.8
2	60	Range	<1.0-27.0	<1.0-17.0	
		Average	4.0	2.7	
	106	Range	<1.0-54.0	<1.0-33.0	
		Average	10.9	5.6	2.2
3	60	Range	<1.0-48.0	<1.0-138.0	
		Average	17.0	21.4	
	120	Range	<1.0-49.0	<1.0-44.0	
		Average	7.8	7.6	2.4
			$E_1 + E_2 + E_3$, pg/ml		
1	113	Range	5.9-7.15	4.3-208.7	
		Average	21.3	34.8	5.9
2	60	Range	4.9-37.0	12.3-194.0	
		Average[d]	16.1	38.6	
	106	Range	3.0-58.9	3.0-37.0	
		Average	17.5	11.8	3.8
3	60	Range	3.0-53.5	3.0-323.9	
		Average[d]	22.0	105.4	
	120	Range	3.0-88.3	3.0-110.0	
		Average	15.6	22.3	10.7

[a]Range: lower limit of estimating concentration was considered to be 1.0 pg/ml.

[b]Common standard error of mean from analysis of variance in which sampling time was included in the analysis for trials 2 and 3.

[c]Concentration affected by Synovex-S at $P<.05$.

[d]Average concentration for the 60-day implanted steers was greater than the average for the 60-day control steers or the 120-day averages at $P<.01$.

The concentrations of E_1 and E_3 were generally not affected by Synovex-S implant treatment. Although E_3 was lower ($P<.05$) in trial 1 for the non-implanted steers than for the implanted steers, this trend was not consistent with subsequent results. The differences noted between total estrogen concentrations appeared to reflect the differences in E_2 concentrations.

The overall coefficients of variation in this study were 108, 96, and 134% for trials 1, 2, and 3, respectively, for E_1; 122, 176, and 246% for E_2; and 285, 167, and 112% for E_3. These coefficients exceeded by approximately a factor of 10 the coefficients of less than 15% inherent in the analytical procedures and thus indicate the large biological variability in plasma estrogen concentrations of feedlot steers when determined from one-time sampling as in the current study. The variability also indicates the difficulty of determining whether a given plasma estrogen level in a feedlot steer is associated with the presence of an implant. Even though implanted steers had a faster rate of gain in these trials, rate of gain was not significantly ($P>.05$) correlated with plasma estrogen levels; thus the variability in plasma estrogen levels is not translated into variability in gain.

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