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Comparative Response of Castrate and Intact Male Rats to Diethylstilbestrol (38602)[1,2]

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Diethylstilbestrol (DES) stimulates body weight gain when administered to growing cattle and sheep; the growth of rats, however, is decreased when several different doses of DES were studied (1–3). Since gain stimulation is primarily observed in castrate male cattle (steers) and sheep (wethers), with little or no stimulation in intact male cattle (bulls) at doses commonly used in steers (4), it seemed worthwhile to compare the growth effects of DES in intact and castrate male rats.

Procedures. One hundred ten male rats used for this experiment were a noninbred strain obtained from Sprague–Dawley, Madison, WI. Castration was performed on one half the rats 4 days after arrival. For 2 wk following castration, all rats were on limited feed intake to allow time for the incision to heal while maintaining weight gains at about the same level.

The design of the experiment was a completely randomized 2 × 5 factorial, using two sex conditions (castrate and intact male rats), each fed five levels of DES (0, 0.008, 0.04, 0.2 and 1.0 μg/g of diet). Three cages containing three rats from each sex condition were fed each of the above diets; their initial weight was 77.4 and 79.0 g for the castrate and intact rats, respectively.

DES was added to ground Purina Laboratory Chow by first preparing a stock solution (50 mg/100 ml of 95% ethanol). This solution was refrigerated throughout the experiment. To reach the desired concentration in the diet, 6 ml was removed and added to approximately 30 ml of ethanol; this was then thoroughly mixed with 3 kg of diet giving 1.0 μg DES/g. Subsequent diets were similarly prepared by diluting 2 ml of the previous dilution to 10 ml, mixing 6 ml in the feed as described above. The diets were allowed to stand at room temperature for 3 days or placed in a drying oven at 25° overnight to allow the ethanol to evaporate. The diets were mixed at approximately 10-day intervals throughout the trial. Fresh diet was weighed out daily and any uneaten feed was removed, weighed, and discarded. It was noted that certain cages of rats wasted more feed than others, however, no attempt was made to account for the feed spilled in the litter tray.

The experiment lasted 31 days at which time the rats were sacrificed by first anesthetizing with ether. Various organs and bones were removed for later measurement; testicle, seminal vesicle and adrenal glands were fixed in Bouins fluid.

The data were analyzed by least squares-analysis of variance (5). Each cage of three rats served as one experimental observation. Variance due to sex condition, DES treatment and the sex condition × DES treatment interaction was partitioned in the analyses.

Results. DES decreased feed intake and body weight gain in castrate and intact male rats, with the possible exception of intact males treated with 0.008 μg DES/g diet. Table I presents the data observed in this experiment. This response to DES is similar to other works where estrone (6) and stilbestrol (3, 7) were either injected or fed. Estrogens have generally reduced the growth of rats. The present study was undertaken, however, to see if the castrate male rat would respond to low levels of DES in the feed. The results presented here parallel other studies with estrogens. Analysis of the data revealed that sex condition did not materially affect body weight gain ($P < 0.09$). DES signifi-

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[2] The following paper was published while this manuscript was being reviewed: Bull, L. S., W. L. Hurley, W. S. Kennett, C. B. Tamplin and W. F. Williams. J. Nutr. **104:** 968 (1974).

TABLE I. INFLUENCE OF DES FEEDING ON GAIN IN BODY WEIGHT, FEED INTAKE AND CERTAIN BODY COMPONENTS[a].

Item	DES level (μg/g feed)					SE[b]
	0	0.008	0.04	0.2	1.0	
Gain in body-weight (g)						
Intact	219	224	188	133	96	5.9
Castrate	223	205	169	123	95	
Total feed intake (g)						
Intact	692	689	641	511	452	24.3
Castrate	652	614	611	489	455	
Adrenal gland weight (mg)						
Intact	37.8	37.2	43.9	47.3	50.2	2.7
Castrate	59.7	59.9	57.4	48.4	52.1	
Liver weight (g)						
Intact	15.3	15.9	13.6	10.5	9.3	0.50
Castrate	16.1	16.1	12.5	10.1	9.0	
Seminal vesicle gland weight (mg)						
Intact	652	689	571	59	44	24.0
Castrate	22	13	13	23	45	
Spleen weight (mg)						
Intact	670	660	660	550	470	47.0
Castrate	860	780	670	550	480	
Femur weight (mg)						
Intact	374	372	373	340	328	8.4
Castrate	342	352	345	316	317	
Femur length (cm)						
Intact	3.05	3.03	2.95	2.82	2.61	0.035
Castrate	2.92	2.90	2.80	2.72	2.69	
Testicle weight (g)	3.27	3.43	3.33	1.53	0.38	0.071

[a] Three rats per-pen—three pens per treatment.
[b] Standard error of the mean.

cantly ($P < 0.01$) reduced body weight gain. When body weight gain was adjusted statistically to equal feed consumption, differences in weight gain could be largely attributed to differences in feed intake, since feed consumption and weight gain were highly correlated ($r = 0.91$).

The adrenal glands were larger in the castrate rats compared to intact male rats ($P < .01$). The effect of DES was dependent upon sex condition as evidenced by the significant sex condition × DES interaction ($P < .05$). Adrenal weights increased when DES was fed to intact male rats but decreased somewhat when DES was fed to castrate rats. Liver weights were generally decreased by the feeding of DES, however, there was a high correlation ($r = 0.97$) between liver weight and final weight. When the data were adjusted to equal final live weight, DES treatments produced a linear and quartic effect on liver weight ($P < 0.05$). On the low levels of DES, the castrate rats had heavier livers than intacts. Both the castrate and intact rats had adjusted liver weight increases when 0.008 μg DES was fed in the diet. The 0.04 μg level resulted in adjusted liver weights similar to the control rats. The 0.2 and 1.0 μg levels of DES increased adjusted liver weights in both castrate and intact rats. Sex condition also appeared to have some effect ($P < .07$) on liver weight since castrate rats had heavier adjusted liver weights than intact rats, except on the 1.0 μg level. This is not in complete agreement with earlier work (6) but parallels later findings (7).

The seminal vesicle glands have been noted to increase in size with estrogen treatment (8). The weight of this gland was greatly affected by both sex condition and DES feeding ($P < 0.01$). In the castrate male it can be noted that high levels of DES increased

the weight of the seminal vesicle gland; when the data were adjusted to equal final live weight differences, the increase was more evident. Observations on intact males showed two points: First, intact males had heavier seminal glands; secondly the weight of the glands decreased as DES level increased, which is similar to other reports (2, 6, 8).

Castrate rats had heavier spleen weights than intact rats ($P < 0.01$); adjustment to equal final live weight emphasizes this response ($P < 0.05$). Treatment with DES did not significantly affect adjusted spleen weight ($P < 0.11$); therefore, differences in spleen weights were primarily a function of body weight.

The weight and length of the femurs were associated with body weight ($r = 0.76$) and ($r = 0.88$), respectively ($P < 0.01$). The intact males weighed more at the time of sacrifice and consequently had heavier, longer femur bones ($P < 0.05$). It may be noted from Table I that DES reduced the weight ($P < 0.01$) of the femur; however when adjusted to equal final live weight, femur weights of rats on DES were heavier than those of controls ($P < 0.01$). The effect of treatment on femur length was not significant even though length was decreased in treated rats. Final body weight did not appear to influence femur length.

The highest DES levels greatly reduced testicle weight ($P < 0.01$). Analysis of the data revealed a high correlation between testicular weight and final body weight ($r = 0.97$); however, adjustment for final live weight differences did not eliminate the depressing effect of the two higher DES levels.

Summary. Treatment of castrate and intact male rats with DES in the feed was used to note its comparative effect on feed intake and gain in body weight; adrenal, seminal vesicle, testicle, spleen and liver weights, and femur length and weight were also noted. DES similarly reduced gain and feed efficiently in both castrate and intact male rats. Thus, there appears to be a basic difference in the response of rats to DES, compared to cattle and sheep, that does not depend on whether they are intact or castrate males.

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