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Effects of Immunization of Heifers against Estradiol on Growth, Reproductive Traits, and Carcass Characteristics (41866)

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Abstract. An experiment was performed to evaluate the effects of active immunization of heifers against estradiol on feedlot performance (growth and efficiency), carcass characteristics, and reproductive functions. Seventy-two crossbred heifers were divided into four equal treatment groups consisting of controls, ovariectomized heifers, and heifers actively immunized against keyhole limpet–estradiol antigen and bovine serum albumin–estradiol antigen. Heifers were fed *ad libitum* for 170 days. Both groups of heifers immunized against estradiol had higher ($P < 0.05$) average daily gains than controls. Heifers immunized against bovine serum albumin–estradiol had increased feed efficiency ($P < 0.05$) over controls. Ovariectomized heifers did not perform at levels sufficient to compensate for the initial setback from surgery. No differences were noted in carcass grade, quality, or concentration of water, fat, or protein. Uterine weights were increased in estrogen-immunized animals but were not significantly greater than controls. Ovarian weights and numbers of large follicles (>9 mm diam) in immunized animals were significantly greater than in controls. Twenty-eight percent of the animals ($n = 5$) in the bovine serum albumin–estradiol-immunized group had cystic follicles (>20 mm diam) and 50% ($n = 9$) of this group had no detectable corpus luteum. Low titer (1:100) systemic binding of estrogens may act as a steroid reservoir in which systemic estrogen clearance is decreased and availability to target tissues is increased.

Estrogens and related estrogenic compounds (diethylstilbestrol) have been proven to be excellent anabolic growth promotants for ruminants. These compounds improve both growth rate and feed conversion (1–3). The administration of diethylstilbestrol to steers in the feedlot increases the carcass protein and moisture content, and retail yield (2, 3). Growth rate and feed conversion are generally lower for heifers than steers.

Estrogen levels in animals immunized against estrogens are considerably elevated (4–6) and may produce an *in vivo* estrogenic growth effect depending on how much of the bound estrogen is available to target tissue. The goals of this experiment were to evaluate the effects of active immunization against estradiol on (1) feedlot performance, (2) reproductive effects, and (3) carcass characteristics in heifers.

Materials and Methods. *Animals and treatments.* Seventy-two crossbred heifers were randomized by breed and weight to four groups: Group I ($n = 18$) contained controls injected with 1 ml saline (9 g NaCl/liter); Group II ($n = 18$) contained ovariectomized animals injected with 1 ml saline; Group III ($n = 18$) animals were immunized against 1,3,5,(10)-estratrien-3,17β-diol-6-one carboxymethoxime (Steraloids, Inc., Wilton, N.H.) conjugated to keyhole limpet hemocyanin (KLH; Calbiochem, La Jolla, Calif.); and Group IV ($n = 18$) animals were immunized against 1,3,5,(10)-estratrien-3,17β-diol-6-one carboxymethoxime conjugated to bovine serum albumin (BSA). Steroids were coupled to the carrier protein (KLH or BSA) by the carbodiimide method of Goodfriend *et al.* (7).

Animals were injected along the tailhead with 5 mg antigen for Group III and 1 mg of antigen for Group IV in 1 ml of Freund's complete adjuvant:saline (1:1 v/v) and 0.5 ml each of diphtheria, tetanus, and pertussis toxoids (Wyeth Lab, Marietta, Pa.). Booster injections were given in Freund's incomplete adjuvant 60 and 90 days after the initial injection. Animals were weighed at the time of each injection.

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Serum obtained at slaughter was submitted to dextran–charcoal radioassay to measure the estradiol binding titer (8). Aliquots of diluted serum (1:100, 1:1000 in phosphate-buffered saline) were incubated with 20,000 dpm of [1,2,6,7,16,17-^{3}H]estradiol-17β (sp act 137 Ci/mmole; New England Nuclear Corp., Boston, Mass.). At the time of slaughter, the reproductive tract was removed; then ovarian, corpora luteal, and uterine weights were recorded. Ovarian surface follicles were counted and divided into three size groups: small ($\leq$4 mm diam), medium (5–8 mm diam), and large (>9 mm diam).

Feeding regime and carcass analysis. Six heifers within each treatment were randomly assigned to each of three pens (total of 12 pens) and fed the diet indicated in Table I *ad libitum* for 170 days. Mean (±SE) heifer age at the initiation of the study was 307 ± 1.2 days (range 288–324). Heifers were weighed on two consecutive days at the beginning and end of the study and at 28-day intervals during the study. Feed samples were taken and frozen daily throughout the diet study, composited for each 28-day period, and subsequently analyzed for dry matter and crude protein. Weights of orts were recorded weekly. Heifers were slaughtered at the research center abattoir at the end of the study. Hot carcass weight was obtained. Fat thickness at the 12th rib and longissimus muscle area were determined; marbling score and percentage kidney, heart, and pelvic (KHP) fat were subjectively evaluated approximately 24 hr after slaughter.

TABLE I. DIET DRY MATTER COMPOSITION

Ingredient	Percentage of diet dry matter[a]
Corn silage	25.00
High moisture corn	71.67
Supplement	3.33
Urea	30.63
Dicalcium phosphate	15.63
Limestone	36.34
Salt	15.00
Chelated trace mineral	0.60
Vitamins A, D, and E[b]	1.80

[a] Diet contains 2.99 Mcal ME/kg, 12.1% crude protein, 0.61% calcium, and 0.40% phosphorus.

[b] Vitamin A, D, and E supplements contained 2.65 g vitamin A, 22 mg vitamin D, and 88 g vitamin E per kilogram.

Carcass water, fat, and protein concentrations were estimated from fat thickness, longissimus area, marbling score, and KHP fat (9).

Statistical analysis. Data were analyzed by analysis of variance and χ^2 to determine effects of treatment. When analysis of variance indicated differences, means were compared by Student's *t* test. Feed intake and efficiency of feed conversion were calculated on a pen basis (n = 6/pen; 3 pens/treatment). Linear and quadratic relationships between weight and time, cumulative feed intake and time, and weight and cumulative feed intake were evaluated by least-squares procedures.

Results. *Antibody response.* The pattern of anti-estradiol titer increased in immunized animals to 1:10 at the 60-day booster and approached a 1:100 dilution by the 90-day booster. At slaughter time, the titer of anti-estradiol was 1:100 (2–90% binding) in all immunized animals. Animals immunized against the BSA–estradiol antigen had a final average serum binding of 44% at 1:100 and those immunized against KLH–estradiol bound 31% (1:100). Control and ovariectomized animals bound less than 1% at 1:100 dilution. The cross-reactivity for estrone was 5% for the KLH–estradiol antigen and less than 1% for the BSA–estradiol antigen (determined by mass of steroid required for 50% displacement of [^{3}H]estradiol).

Feedlot performance. Of the animals immunized against estradiol (KLH–estradiol and BSA–estradiol), 50% had average daily gains greater than 1 kg/day whereas only 14% of the controls reached this level. Animals immunized against the BSA–estradiol antigen had a higher average daily gain and feed efficiency over the 170-day feeding period than the other treatment groups and controls (Table II). Heifers immunized against the KLH–estradiol antigen had a higher average daily gain than controls (Group I) but efficiency (gain/feed intake) did not differ significantly. Animal variation is high in response both to the immunization procedure (titers developed) and to the possible growth effects from the sequestered estrogen. Many animals with low binding (1–2% at 1:100) had good average daily weight gains (1.2–1.3 kg/day) whereas others with 60–80% estradiol binding (1:100) gained less than 0.7 kg/day. Ovariectomized heifers gained weight at a similar rate as con-

TABLE II. PERFORMANCE CHARACTERISTICS OF CONTROL, OVARIECTOMIZED, AND IMMUNIZED CROSSBRED HEIFERS DURING A 170-DAY POSTWEANING FEEDING PERIOD

Trait	Group I (control)	Group II (ovariectomized)	Group III (KLH–estradiol)	Group IV (BSA–estradiol)	SE
Initial weight (kg)	304[b]	278[a]	296[b]	296[b]	2.2
Final weight (kg)	446[a,b]	429[a]	457[b]	461[b]	4.2
Daily gain (kg)	0.83[a]	0.88[a,b]	0.95[b,c]	0.97[c]	0.017
Dry matter intake (kg/day)	7.12	6.45	7.49	6.79	0.27
Gain/feed consumption (g/kg)	118[a]	140[b]	126[a,b]	142[b]	1.9

[a–c] Means within a row with different superscripts differ ($P < 0.05$).

trol heifers but were more efficient; final weights were lower due to the initial setback from the surgery. When initial weight was included as a covariate in the statistical model, final weight and average daily gain were significantly higher in Groups III and IV than in controls, and these values in ovariectomized heifers were intermediate between those of immunized and control heifers. When animals were adjusted for initial body weight, feed efficiency did not differ significantly among treatments but tended to be greater for heifers immunized against the BSA–estradiol antigen (144 g/kg) than for other heifers (128 g/kg; residual standard deviation = 11, error $df = 6$).

Figure 1 shows the regression of live weight on cumulative feed intake. These relationships were curvilinear ($P < 0.05$) for control and ovariectomized animals but linear for immunized animals, suggesting that efficiency decreased for the control and ovariectomized heifers but was constant for immunized heifers during the study. Not only were ovariectomized animals penalized at the beginning of the experiment from the surgery, but also apparently matured more rapidly than other heifers.

Carcass characteristics. Ovariectomized heifers had lower carcass weights and longissimus muscle areas than other heifers due to lower final weights in the feedlot (Table III). Ovariectomized heifers had greater ($P < 0.01$) marbling scores and tended ($P = 0.10$) to have more KHP fat than controls. No differences were noted in relation to estimated carcass water, fat, or protein concentrations among the four treatment groups. Ovariectomized heifers had greater ($P < 0.05$) marbling scores, higher concentrations of fat, and lower concentrations of water and protein than other heifers when carcass data were evaluated at

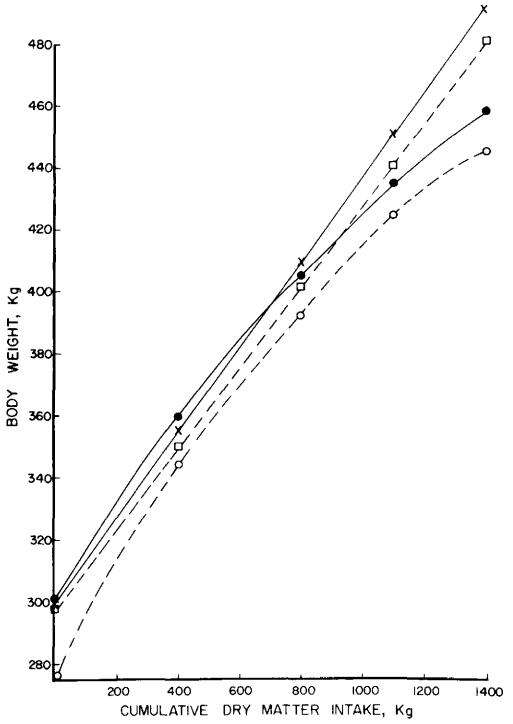

FIG. 1. Regression of live weight (kg) on cumulative dry feed intake (kg) for control (●), ovariectomized (○), BSA–estradiol-immunized (×), and KLH–estradiol-immunized (□) groups of heifers. Control ($Y = 301 + 0.16X - 0.000034X^2$, $r = 0.97$) and ovariectomized ($Y = 277 + 0.186X - 0.000047X^2$, $r = 0.98$) are quadratic ($P < 0.05$) whereas BSA–estradiol ($Y = 299 + 0.137X$, $r = 0.99$) and KLH–estradiol ($Y = 298 + 0.13X$, $r = 0.97$) are linear ($P < 0.05$).

TABLE III. CARCASS CHARACTERISTICS OF CONTROL, OVARIECTOMIZED, AND IMMUNIZED CROSSBRED HEIFERS

	Group I (control)	Group II (ovariectomized)	Group III (KLH–estradiol)	Group IV (BSA–estradiol)	SE
Carcass weight (kg)	282[b,c]	266[b]	289[c]	290[c]	3.5
Fat thickness (cm)	0.84	0.84	0.79	0.83	0.033
Adjusted fat thickness (cm)	0.78	0.76	0.73	0.76	0.030
Longissimus area (cm^2)	77.7[c]	72.2[b]	74.5[b,c]	76.5[c]	0.87
Kidney, heart, and pelvic fat	3.65[b]	3.88[b,c]	3.75[b,c]	3.92[c]	0.05
Marbling score[a]	4.10[b]	4.88[c]	4.36[b,c]	4.42[b,c]	0.11
Water (g/kg)	520	511	519	515	2.6
Fat (g/kg)	321	334	323	328	3.4
Protein (g/kg)	158	154	157	156	0.86

[a] Marbling score: 4.0 = small, 5.0 = modest.
[b,c] Means within a row with different superscripts differ ($P < 0.05$).

constant carcass weight by covariate analysis.

Reproductive effects. Differences in reproductive tissues at slaughter were monitored as a biological index of increased estrogens due to the binding antibodies. Uterine weights tended to be greater in immunized groups (Table IV) but were not significantly different from controls. Ovarian weight and numbers of large follicles on the ovary were significantly greater in controls than in the estradiol-immunized groups. Two animals in each estradiol-immunized group had double ovulations. Fifty percent ($n = 9$) of the BSA–estradiol group were acyclic as no corpora lutea were noted and 22% ($n = 5$) had cystic follicles (>20 mm diam). No differences were noted between the control and KLH–estradiol groups in relation to number of corpora lutea or cystic follicles.

Discussion. The increased growth rate and efficiency noted in animals immunized against estradiol may be related not only to the mitogenic effects estrogen has at the cellular level but also to augmentation by growth hormone. Although growth hormone relationships with improved gain and efficiency have not been consistent (10–12), increased growth hormone secretion has been accepted as the general mode of action by which estrogenic anabolic agents improve the rate of gain and feed con-

TABLE IV. REPRODUCTIVE TRAITS OF CONTROL, OVARIECTOMIZED, AND IMMUNIZED CROSSBRED HEIFERS

	Group I (control)	Group II (ovariectomized)	Group III (KLH–estradiol)	Group IV (BSA–estradiol)	SE
Uterus weight (g)	192[b]	55[a]	208[b]	211[b]	5.2
Ovary weight (g)	13.2[a]	—	20.6[b]	19.8[b]	1.3
No. corpus luteum	0.83[b]	—	0.96[b]	0.50[a]	0.08
No. large follicles (>9 mm diam)	1.7[a]	—	2.3[a,b]	2.7[b]	0.2
No. medium follicles (5–8 mm diam)	5.6	—	7.0	5.7	0.7
No. small follicles (≤4 mm diam)	39.0	—	50.0	52.0	4.2
No. cows with follicles >20 mm (cystic)	0.0[a]	—	1.0[a]	5.0[b]	0.03
Percentage estradiol serum binding at 1:100 dilution	0.39[a]	0.42[a]	31.0[b]	43.5[c]	2.4

[a–c] Means within a row with different superscripts differ ($P < 0.05$).

version of ruminants (3). Increased feed efficiency from anabolic steroids is thought to be due to increased cellular protein accretion and reduced turnover rates (13). A major effect of the anti-estradiol titers upon feedlot performance may be interpreted as an extended growth phase, whereas control and ovariectomized animals matured at a lower body weight (Fig. 1).

Since animals were in unknown stages of the estrous cycle throughout the study, systemic estrogens were not quantitated. Interpretation of blood estrogen concentrations and growth rate is difficult when the data are confounded with the stage of the estrous cycle. Uterine and ovarian weights (Group I, III, IV) provide a method of estimating estrogen activity on known target tissues (14, 15) and indicate that immunized animals have a greater availability of estrogens at target tissues. Animals given diethylstilbestrol have increased uterine weights (16–18) but not necessarily ovarian weights (16, 18). The increased ovarian weights in immunized animals may indicate that the mechanism of action of the animal's own estrogen bound to antibodies may not be exactly the same as that of exogenously administered estrogenic growth stimulants. Increased ovarian function from estrogen immunization has been observed in ewes by Scaramuzzi *et al.* (6). The correlation of ovarian and uterine weights with average daily gain ($r = 0.39$ and 0.21; $P < 0.05$, respectively) may indicate that estrogen affected other body tissues as well as reproductive tissues.

Changes in sex-binding globulins can regulate steroid clearance because hepatic clearance of gonadal steroids is proportional to the extent bound (19). The rate of steroid clearance and metabolism to more polar metabolites is inhibited in immunized animals (20). The mode of action by which immunization against estradiol increases animal feedlot performance is unknown, but may involve behavior aspects (e.g., decreased estrous activity) due to antibody-bound estrogens being less available to the higher brain centers (19). As noted in this study (the BSA–estradiol-immunized animals), animals with high titers become anovulatory (4, 5, 21) and thus may be more efficient under feedlot conditions because of diminished estrous activity.

At the anti-estradiol titers generated in this study, the antibody may act as a mass-action reservoir for estrogens that functions to increase estrogen availability to target tissues (8). Alternatively, as the antibody binding of estrogen increases, albumin-bound estrogen decreases and total hormone concentration may increase to offset the decrease in the albumin-bound fraction (19). This may not only decrease the clearance rate of bound estrogen, but also elevate the quantity of hormone available to target tissues.

Earlier studies have shown that little advantage accrues from ovariectomizing heifers that are going into the feedlot (22–24) but the decreases in rate of gain or feed efficiency in ovariectomized animals noted in the above studies were not observed in this experiment (Table II). The decreased performance of ovariectomized animals observed in this study was most likely due to the trauma of ovariectomy and the fact that ovariectomized animals seemed to reach maturity at a lower weight (Fig. 1). The initial weight loss due to surgery and lack of full compensation in the feedlot as well as carcass differences suggest that disadvantages probably offset the advantages of the procedure.

The influence of estrogenic growth stimulants on carcass composition of castrated male ruminants is to increase carcass protein and water content and decrease carcass fat (2, 11, 25, 26). However, in female ruminants, estrogenic growth stimulants appear to have less effect on carcass composition (2, 24) though estrogenic effects upon ovariectomized animals are unknown. The increased utilization of the animals' own estrogen to increase growth and efficiency from the active immunization against estrogens may provide the beef industry with a useful management tool.

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