CONSUMPTION OF BEEF FROM CATTLE ADMINISTERED ESTROGENIC GROWTH PROMOTANTS DOES NOT RESULT IN PREMATURE PUBERTY AND OBESITY USING THE SWINE MODEL

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Abstract – The objective was to investigate the effects of ground beef from cattle administered commercial growth promotants on puberty attainment and body composition in female swine. Twenty-four gilts were selected based on strict selection criteria to reduce piglet variation. Treatments were randomly assigned based on BW (24.5 ± 3.2 kg) and litter number, and included daily feedings of a 113-g beef patty from non-implanted steers (NAT), a 113-g beef patty from steers receiving estrogenic implants (IMP), a 198-g piece of tofu (TOFU), or a negative control receiving no supplemental treatment (CON). All gilts received the very low estrogen base diet fed at approximately 3.5% of BW and adjusted weekly to account for intake. Gilts were slaughtered at the NDSU Meats Lab a minimum of 3 d following first visual heat. Data were analyzed using the PROC MIXED function of SAS, with litter as a random variable. No treatment effect was observed with any growth parameter ($P \geq 0.32$), measurement of puberty including age at first heat ($P \geq 0.46$), or carcass composition measurement ($P \geq 0.35$). These data indicate that consumption of beef from cattle receiving estrogenic growth promotants does not influence growth rate, body composition, or attainment of puberty.

Key Words – estrogen, gilt, puberty

I. INTRODUCTION

Nebesio [1] reported that young American girls are reaching puberty at an earlier age than previously reported acknowledging that the trigger for precocious puberty is a complex interaction between genetics, hormones, and environmental factors. The authors list industrial chemicals, pesticides, estrogen-containing cosmetics, and phytoestrogens as possible causes. Anderson [2] stated that hormone residues in meat obtained from animals administered sex-steroid hormones that promote growth were largely ignored as a possible cause of precocious puberty and was a "very likely cause". Further, Cuffman [3] suggested that a mother's consumption of beef obtained from estrogenically implanted cattle may have an influence on the development of the unborn fetus which could lead to early onset of puberty in her female offspring.

The pig's gastrointestinal system, body composition, and nutrient requirements favor the use of the pig as an ideal model for evaluation of how diet influences physiological responses in growth and development [4]. Hughes [5] reported that factors such as genotype, social environment, season of the year, boar exposure, growth rate, body composition, and age influence the development and onset of first estrus in gilts. Just as described above for gilts, there are complicated combinations of factors that can contribute to the onset of puberty in young women. The objective was to investigate the effects of dietary estrogen intake on the onset of puberty and body composition using the pig as a biomedical model. The advantage of using swine as a model is that these extrinsic factors previously discussed can be controlled to allow for an accurate assessment of the influence of the dietary treatments.

II. MATERIALS AND METHODS

Animals

Commercial crossbred gilts (n = 56; Danbred North America, Columbus, NE) were identified at birth from a common sire (Danbred 610) and dam line (Danbred 241) based on strict parameters. At weaning (18 d), gilts were reassessed for uniformity and 33 were transported 725 km to the Animal Nutrition and Physiology Center at North

Dakota State University. Gilts were allowed *ad libitum* access to a pelleted commercial nursery diet and were vaccinated with Ingelvac Circo Flex and Ingelvac MycoFlex (Boehringer Ingelheim, Ridgefield, CT). At 54 d of age, 24 gilts were selected for the project based on uniformity of littermates and weight (24.5 ± 3.2 kg) and assigned to individual pens. A minimum of 2 and a maximum of 4 gilts per sow were included in the project and were stratified over the 4 dietary treatments based on BW rank within litter. Following the attainment of 70 kg BW, longissimus muscle area and subcutaneous fat depth was obtained via ultrasonography biweekly to monitor muscle and adipose development over time on test.

Diet and Treatment

A corn and canola-base diet was formulated to meet or exceed *National Research Council* nutrient requirements for maximizing lean growth for all phases of production. Canola was selected as a replacement for soybean meal in an effort to reduce the overall estrogenicity of the base diet. The base diet was fed as a percentage of BW and adjusted weekly to equal that consumed by the gilt with the lowest feed intake. In this way energy intake was restricted, ensuring that all treatment supplements were consumed, and allowed for consistency across animals and treatments as intake was equal on a percent of BW basis (approximately 3.5% BW). Four treatments were administered beginning at 61 d of age: 1) negative control- (CON; low estrogenicity base diet only), 2) positive control- (TOFU; base diet + 198 g [pre-cooked wt] tofu patty), 3) Natural (NAT: base diet + 113 g [pre-cooked wt] beef patty obtained from feedlot steers raised without the use of growth promotant technology (GPT), and 4) Implanted- (IMP; base diet + 113 g [pre-cooked wt] beef patty obtained from feedlot steers provided GPT (Synovex Choice implants, 100mg of trenbolone acetate and 114 mg of 17β-estradiol) administered to the steers at weaning (approximately 230 kg BW) and again at approximately 410 kg BW). Tofu and beef patties were cooked to internal temperature of 76˚C, cooled, and fed daily at 1600 h prior to distribution and consumption of the base diet. The required weight of the tofu patty was calculated to provide equivalent caloric and nitrogenous intake to the burgers. Table 1 contains the compositional analysis of the supplemental treatments.

Table 1. Compositional analysis (%) average (SD) of supplemental dietary treatments.

	Treatments[1]		
	NAT	**IMP**	**TOFU**
Raw patty weight[2], g	113.0	113.0	198.0
DM (as-fed), %	44.61	39.19	17.85
E2 equivalents[3], ng/kg	10.0 (3.4)	14.0 (3.7)	5133.0 (643.0)
Nutritional composition, %			
Crude protein	75.00	85.68	57.99
Crude fat	19.81	9.74	24.24
Calcium	0.77	0.07	0.82
Phosphorus	0.58	0.63	0.90
Gross energy, Mcal/kg	6.20	5.68	6.03

[1]**NAT** = cooked beef patty obtained from a feedlot steers raised without the use of growth promoting technology; **IMP** = cooked beef patty obtained from feedlot steers provided Synovex Choice (100 mg of trenbolone acetate and 14 mg of estradiol 17β) at approximately 230 (weaning) and 410 kg BW; **TOFU** = cooked tofu.
[2]All supplemental treatments were cooked to 76C and cooled prior to feeding.
[3]E-Screen analysis of estradiol equivalents, mean ± S.D. of three extractions.

Estrus Detection

Gilts were physically isolated from boars to prevent precocious puberty. Upon reaching 90 kg BW, gilts were allowed access to a community pen for 30 minutes a day, where visual signs of estrus were monitored. Visual estrus was confirmed chemically based on presence of circulating progesterone greater than 1ng/mL. Two gilts (one CON, and one TOFU) failed to exhibit heat before termination of the experiment. Gilts were slaughtered on Tuesdays at the NDSU Meat Science abattoir a minimum of three days following the first visible sign of estrus

Analysis of Serum Progesterone

Blood samples were collected once a week beginning at an average BW of 24 kg and then twice weekly beginning at an average BW of 68 kg. Serum samples were refrigerated for 2 hours, centrifuged, separated, transferred to 2 ml vials, and frozen until analysis. Harvested plasma was stored at -20^{o}C until analysis. Progesterone concentration was analyzed using the Immulite

1000 (Siemens Medical Solutions Diagnostics, Los Angeles, CA, catalog number: LKPG1).

Analysis of Estrogenicity

Extraction of Rations
The base diet for each phase (Grower 1 and 2, and Finisher 1 and 2) were extracted as previously described for sugarbeet by-product feed samples [6] with exception of an additional hexane extraction step. Dry eluates were stored at -20°C for later analysis. While rations were not expected to contain known phytoestrogens, extraction efficiency was assessed using genistein as a surrogate (400µg genistein/g of ration, using a stock solution of 2.5 mg/mL in EtOH).

Extraction of Tofu Burger
Cooked tofu burgers were extracted by the method described by Murphy [7] for isoflavones analysis of foods modified with an additional hexane extraction step. Extraction efficiency was assessed with genistein, adding 800 µg of genistein/ 3 g tofu burger, to ensure estrogenic activity greater than what was due to the endogenous genistein contained in the tofu samples.

Extraction of Ground Beef
Cooked ground beef burgers were extracted in a modification of a method for extraction estrogens from fish [8]. Crumbled burger (5g as fed) was mixed with approximately 2 g of diatomaceous earth (ASE Prep DE, Dionex Corp., Salt Lake City, UT) that had been previously processed through the solvent extraction method to be used on the Accelerated Solvent Extractor (ASE 200, Dionex). Extraction efficiency was assessed by fortifying ground beef with 17ß-estradiol (17β-E_2, 600 pg/5 g of beef).

E-Screen
Estrogenic activity was determined by E-Screen and *in vitro* assay that assesses estrogen-dependent proliferation of human mammary epithelial cell line (MCF7-BOS, from the laboratory of Drs. Anna Soto and Carlos Sonnenschein, Tufts University School of Medicine, Boston, MA). The assay, as described Shappell [9], was performed with the only change being an increase in cell plating density to 4 X 10^3 cells/ well in the 96 well plate.

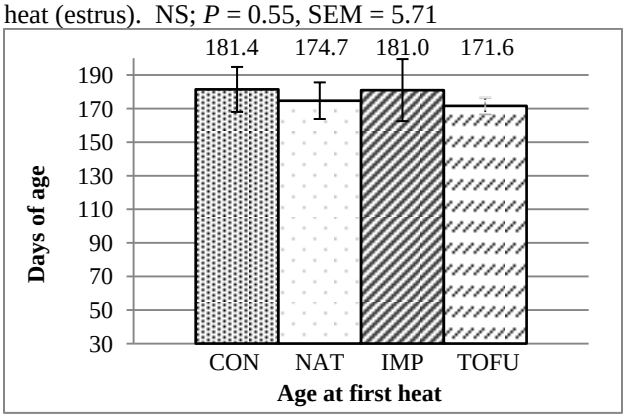
Statistical analysis
Differences in growth performance, carcass composition, and measurement of puberty attainment were analyzed using the PROC MIXED procedure of SAS (SAS Institute, Cary, NC; version 9.2). Treatment served as a fixed effect, litter as a random variable, and gilt was the experimental unit. Statistical significance was set at $P < 0.05$.

III. RESULTS AND DISCUSSION

Data presented in Table 1 show no significant difference in the amount of estradiol (E2) between NAT and IMP beef and the E2 level of beef is extremely low compared to the estrogen content of TOFU. TOFU contributed considerably more estrogenicity to the overall diet consumed than CON, NAT, or IMP treatments.

Hartmann [10] stated that 90% of the hormones that are ingested are inactivated by the first-pass-effect of the liver and believed the exposure to phytoestrogens from plants or environmental chemicals has a greater influence on human beings than the exposure to natural occurring hormones from food. These findings are supported by the present study relative to the influence of diet on the early onset of puberty in gilts. We observed no difference ($P = 0.55$) in the number of days to first estrus across supplemental treatments (Figure 1).

Figure 1. Day of age and SE of gilts at first observed heat (estrus). NS; $P = 0.55$, SEM = 5.71



Our data also suggest that the amount of estrogen consumed did not have an effect on any growth trait or carcass parameter. ADG, final BW, 10th rib fat depth, loin muscle area, and percent fat free lean were not different across treatments ($P > 0.25$). Furthermore, there were no observed treatment differences ($P > 0.26$) in uterine weight, uterine length, or ovarian weight.

In the present study, genetic and environmental factors were completely controlled, which has been the limiting factor of human studies evaluating the same objectives. Furthermore, total dietary intake of "hormones" must be evaluated by considering that cooking meat reduces the amounts of E1, E2, and catechol estrogens in ground beef which appears to be related to the fat content of the beef, with greater losses occurring in fattier samples [11]. Tittlemier [11] explains that uncooked regular ground beef contains approximately 25% fat, and the juices collected from cooked regular ground beef are predominantly (~75%) fat. The increased level of chemical losses observed in the regular ground beef patties in the present study may have been due to their association with the fats, which liquefied during cooking and were easily lost from the patties. The estrogenic content of beef reported in the literature is frequently that of uncooked tissues. For example Anderson [2] provided no indication that the meat samples evaluated in their study were cooked, thus the values reported may be uncharacteristically elevated relative to levels that would be consumed as part of a normal human diet.

IV. CONCLUSION

These data show that the consumption of beef from cattle receiving growth promoting implants does not impact growth rate, body composition, or onset of puberty using swine as a biomedical model. Furthermore, these data confirm that cooked beef has a relatively low level of estrogenicity. In contrary, the analyses confirm a high level of estrogen in tofu, which also contains a high level of phytoestrogen. While much attention has been focused on beef being a leading cause of precocious puberty, these results verify that beef is not a contributing factor to the early onset of puberty.

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