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Effects of dietary selenium on growth and selected reproductive parameters in young boars

Michael Chris Henson

To the Graduate Council:

I am submitting herewith a thesis written by Michael Chris Henson entitled "Effects of dietary selenium on growth and selected reproductive parameters in young boars." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

H. G. Kattesh, Major Professor

We have read this thesis and recommend its acceptance:

J. P. Hitchcock, S. A. Kincaid

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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We have read this thesis
and recommend its acceptance:

John P. Kletchock

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EFFECTS OF DIETARY SELENIUM ON GROWTH AND SELECTED REPRODUCTIVE PARAMETERS IN YOUNG BOARS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Michael Chris Henson

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ABSTRACT

Thirty-three crossbred boars (47-58 days of age) maintained similarly on concrete were randomly allotted to receive a corn-soybean meal ration containing selenium at the basal level of 50(t_1) ppb or supplemented with 100(t_2) or 250(t_3) ppb (from sodium selenite). All boars were fed their respective diets ad libitum until six months of age and were then hand-fed 2.2 kg/boar/day for the remainder of the study. Body weights and testicular widths were taken on each boar at biweekly intervals until six months of age and at monthly intervals thereafter. Blood samples were also taken at these times by vena cava puncture.

Libido was routinely scored upon exposure to ovariectomized-estrogenized gilts, beginning at approximately five months of age. When possible, ejaculates were collected and various sperm cell characteristics evaluated by both bright-field and phase-contrast microscopy. Seven animals from each group, from which ejaculates were collected, were slaughtered at nine months of age. Anatomical and histological appraisal of testicular and epididymal tissue was made soon after slaughter.

Regression of body weight and libido, measured in all 33 boars, and plasma testosterone levels for the 21 boars slaughtered were different ($P < 0.01$), over time, among the three treatment groups. Values for each parameter were found to be greater for boars in t_1 than for those in t_2, which were greater than those in t_3. A similar but less pronounced relationship to treatment ($P < 0.05$) was found for testicular

width as regressed over time. No treatment differences were observed in either weights or spermatozoan concentrations in the testes, capita-corporea, or caudae epididymides. Analysis of testicular lengths, weights, and circumferences also revealed no differences. Caudal epididymal spermatozoa, examined using both bright-field and phase-contrast microscopy, revealed no evidence of structural changes due to treatment. Measurements were made of epididymal epithelial heights, with those in capita tending ($P < 0.10$) to be greater in t_1 than in t_3. Testicular width as measured on the live animal was unrelated to total testicular spermatozoan concentration.

These results suggest that dietary selenium, fed at the levels of 150 and 300 ppb, may act to significantly retard the development of certain reproductive processes in young boars as analyzed over time.

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CHAPTER I

INTRODUCTION

Adequate nutrition is a vital factor in regard to optimum reproductive efficiency in swine. Research has defined certain levels of both macro- and micronutrients as inherent components of reproductive success, with their deficiencies leading to impaired production and subsequent financial loss to the producer. Reproductive efficiency is of prime importance to a growing pork industry, challenged by unpredictable market fluctuations and the ever-increasing costs of feed, labor, and materials. As all phases of the production process turn to the greater use of total confinement techniques, the importance of proper dietary nutrient levels for breeding animals becomes readily apparent.

Research has revealed a number of vital functions performed by selenium as a dietary constituent. A most important role is as a component in certain enzymatic reactions serving to prevent oxidative damage to cell membranes and other oxidant sensitive sites of cell structure. Studies further indicate that deficiency of the micronutrient in swine may result in severe physiological disturbances and perhaps death. Although research in females of many species has shown decreased dietary selenium to severely depress fertility and increase the incidence of embryonic mortality and retained placenta, only lately have deficiency-related reproductive anomalies in the male been subject to investigation. The models used in much of this work have included the small laboratory animals and, to a lesser extent, bulls. Although data involving these

species implicate selenium as an important factor in the genesis of normal spermatozoa and the maintenance of their structural integrity, effects on male swine have not been investigated.

Thus, selenium can be considered an essential micromineral, whose place in the scheme of animal nutrition and physiology has only been outlined and examined in the past 20 years. Although definitive selenium requirements have been established for growing-finishing swine and speculative levels advanced for both gilts and sows, the requirements for boars have remained largely unstudied. In light of the fact that selenium levels in feeds are dependent on its content in the soil, it is of some consequence that substantial grain producing areas of the Midwest, Northeast, far Southeast, and far Northwest are among those lowest in the mineral. As the conditions for possible deficiencies exist, it appeared that the effects of dietary selenium on male reproduction merited detailed investigation.

The objective of this study was to examine certain measurable parameters, including: 1) various semen evaluation criteria, 2) testicular growth and histology, 3) degree of exhibited libido, and 4) the assay of circulating testosterone levels. It was postulated that examination of these areas might be useful in assessing the effects of various amounts of dietary selenium on the reproductive processes of young boars.

CHAPTER II

REVIEW OF LITERATURE

A. THE INFLUENCE OF NUTRITION ON ANIMAL REPRODUCTION

General Introduction

Adequate nutrition has long been considered vital to the reproductive success of animals, as concluded by Maynard and Loosli (1962). In view of this, Lamming (1966) reported increased incidence of ovarian dysfunction in nutritionally deprived animal populations and amenorrhea in groups of women following periods of inanition. He also observed that proper diet was important for normal spermatogenesis. He concluded that while the gonads of both sexes served as target organs for a variety of both macro- and micronutrients, exclusion of still other similar nutrients reduced production or secretion of gonadotropins from the anterior pituitary, resulting in "pseudohypophysectomy." Supporting data from Hafez and Jainudeen (1975) indicated that secretion of hypothalmic-releasing factors might well be inhibited by the inadequate intake of certain nutrients. They also reported that decreased dietary energy and vitamin-mineral deficiencies led to reproductive inefficiency in females, with Faulkner and Carroll (1975) reaching the same conclusions for males of several species. Nalbandov (1976) reported that although a decreased plane of general nutrition impaired ovulation rates in swine and sheep and retarded sexual maturity in heifers, fertility in boars and bulls was unaffected. Dutt and Barnhart (1959) had previously noted this effect in boars. Nielson (1971), in his review of nutritional influences on

reproductive performance in swine, stated that the fertility of boars appeared relatively unchanged by decreased energy and protein levels. However, he was in agreement with Gunn and Gould (1970) and Underwood (1971, 1975) that normal spermatozoan development was totally dependent on adequate dietary amounts of certain essential trace minerals.

Microminerals in Various Species

Underwood (1975) reported 14 trace or microminerals as essential for life in the majority of higher species and agreed with Gunn and Gould (1970) that zinc, iron, copper, cobalt, molybdenum, manganese, iodine, and selenium were vital to spermatogenesis, spermiogenesis, or testicular endocrine function.

According to Underwood (1975), zinc has been associated with testicular atrophy and spermatogenic failure in rats since 1952. He also concluded that complete cessation of sperm cell formation and subsequent impairment of testicular growth occurred in ram lambs receiving zinc-deficient diets for 20-24 weeks. It was concluded that the dietary level necessary for simple somatic maintenance did not meet the needs for sexual development. Hidiroglou (1979) later reported that zinc deficiency inhibited testicular growth in calves, while deprived goats exhibited underdeveloped seminiferous tubules, immature germinal epithelium, testicular atrophy, and depressed libido. Impairment of sperm cell maturation was also noted in the final stages of spermiogenesis. Calvin (1979) had previously noted the incorporation of zinc in rat spermatozoa to occur in the dense fibers of the tail, with Gould (1970) having already revealed the binding of zinc late in spermatozoan development within the seminiferous tubules.

Gunn and Gould (1970) reported that manganese deficiency decreased libido and caused testicular atrophy and sterility in rats, mice, and rabbits. Sizes of accessory sex organs were also diminished. Hidiroglou (1979) noted depressed libido and decreased sperm number and motility among those deprived of sufficient dietary manganese. Increases in sperm cell abnormalities were also significant. Manganese had previously been shown by Gould (1970) to be incorporated in spermatozoa at several stages of their formation, as well as being a normal constituent of seminal plasma.

Although iron and copper are denied access to the spermatogenic pathway, they were reported by Gunn and Gould (1970) to be found in semen bound to proteins of spermatozoan tails and midpieces. It was further noted that failure of male reproductive processes due to deficiency of either element was probably traceable to systemic insufficiency rather than gonadal deficiency. In view of these findings, it is interesting to note that Hidiroglou (1979) reported decreased mortality and increased motility in spermatozoa from bulls fed supplemental copper. Iodine remains unincorporated and unbound to spermatozoa but was noted by Gunn and Gould (1970) to be vital to gonadal function through its influence on the thyroid hormones. Molybdenum and cobalt were reported as being found in very small quantities in the testes and epididymides of normal rats. It was not incorporated or bound by spermatozoa, although mild cobalt supplementation seemed to slightly stimulate spermiogenesis.

Deficiencies of many of the previously mentioned microminerals have also been shown to inhibit reproductive function in females of a number of species. Underwood (1975) implicated zinc as vital to the maintenance

of normal estrous cycles in rats. Deformities and high mortality rates among the young of deficient dams were also noted. Hurley, et al. (1968) and Williams (1977) reinforced these findings by reporting gross congenital abnormalities in 90% of the young of rats fed deficient diets from the beginning of gestation. Egan (1972) found that lowered conception rates in grazing ewes responded favorably to supplemental zinc. Hidiroglou (1979) later noted similar results in both sheep and cattle.

Maternal manganese deficiency was implicated by Underwood (1975) in precipitating neonatal ataxia in rats and guinea pigs. Williams (1977) added that high fetal mortality rates, skeletal abnormalities, and short postnatal survival times were indicative of maternal deprivation. Depressed reproductive efficiency was noted by Hidiroglou (1979) in both cattle and laboratory animals, with lowered conception rates observed among sheep, goats, and laboratory animals lacking in adequate dietary manganese. Egan (1972) supported these findings by reporting the favorable response of conception rates in ewes to supplemental manganese.

Underwood (1975) found that lambs born to copper-deficient dams exhibited a high incidence of neonatal ataxia, with similar findings advanced by Williams (1977) with respect to lambs, newborn guinea pigs, and rat pups. Hidiroglou (1979) indicated that lowered conception rates, implantation failure, and embryonic death were directly associated with hypocupriosis in cattle, sheep, and goats. He also reported that deficiencies of iron, iodine, and cobalt were highly correlated with poor conception rates in cattle, with total absence of estrous attributed to cobalt deprivation in cattle and sheep.

Microminerals in Swine

Although research concerning the relationship of trace elements to fertility in swine has not proceeded at the same pace as with other species, some definite trends have been revealed. Meduzov and Kudrjavcev (1964) observed the overall quality of spermatozoa to be significantly improved upon addition of supplemental zinc and manganese to the diet. Birth and weaning weights of pigs sired by boars fed additional zinc and manganese were also substantially greater than those of unsupplemented controls. Mankovskaja and Kovalenko (1965) reported an increase in the rate of spermatogenesis and total semen volume in boars supplemented with potassium iodide. Volumes of ejaculates increased an average of 26%, while sperm cell concentrations increased an average of 6% over controls. Partly on the basis of this work, Nielson (1971) was prompted to establish recommended dietary levels for iron, zinc, manganese, copper, cobalt, and iodine for breeding boars as 50, 75, 50, 10, 1, and 0.2 mg/kg of diet, respectively.

Micromineral requirements for all swine were reported by Cunha et al. (1973) for iron, zinc, manganese, copper, and iodine as 80, 50, 20, 6, and 0.2 mg/kg of diet, respectively. Although it was noted that the requirements for iron and copper met the needs of baby pigs, no mention was made of particular requirements for breeding boars. Slight changes were made in these standards by Teague et al. (1979), who reported modified values for breeding swine of either sex for manganese, copper, and iodine as 10, 5, and 0.14 mg/kg of diet, respectively. No attempt was made to differentiate the trace element needs of boars from those of gilts and sows.

Trace mineral requirements for optimum reproduction in sows were classifed as both important and confusing by Hansard (1970). He further reported that adequate dietary amounts were indeed very small in relation to the total diet. Pond and Jones (1964) had noted earlier that dietary zinc levels had to fall below 35 ppm/kg of feed to adversely affect the reproductive processes of gilts, while Newland and Davis (1961) found no reproductive problems in females maintained on a diet containing manganese at a level of 6 ppm/kg of feed. Tassel (1967) later suggested a manganese level of 5 ppm/kg of feed as adequate. Cromwell (1979) conceded that although confusion concerning the feeding of microminerals still existed, guidelines established by Cunha et al. (1973) were adequate.

B. THE ROLE OF SELENIUM AS AN ESSENTIAL TRACE ELEMENT

The Availability of Dietary Selenium

Although Underwood (1962) reported that interest in selenium as a micronutrient had existed for more than a century, intensive research into the physiological properties of the element has only been in progress for about 25 years. Van Fleet (1980) noted a shift of emphasis from consideration of selenium as a dangerous toxicant to our present appreciation of the micromineral as an essential nutrient for many species. Sedimentary rock was observed as providing most of the selenium incorporated into soils, with alkaline, well-aerated soils providing much higher amounts of the mineral to growing plants than acid, poorly-aerated ones. Selenium availability in soils was shown to be more highly correlated with the chemical form of the element than simply

with the concentration present. Soluble selenates predominated in alkaline soils, while small amounts of soluble selenite-iron salt complexes were found in acid environments. As selenium moved from soils into growing plants, it was noted as being incorporated into organic compounds, becoming mainly selenoproteins with abundant selenomethionine. Underwood (1966) had earlier characterized this interaction between soil type and selenium availability as responsible for the onset and severity of observed deficiency in production livestock. Geiger and Biehl (1979) further characterized selenium as a trace soil constituent with grains being the typical source of the element in animal diets. Amounts found in grains were reported to be determined by soil type and content. Heavily fertilized fields were observed to produce plants with lowered elemental uptake due to a decrease in pH along with added competition for selenium absorption into the plant. Characteristically, soils of the Midwest, Northeast, far Southeast, and far Northwest were among those lowest in the mineral.

Somatic Effects of Selenium Deficiency and Toxicity

Underwood (1966) reported the symptoms of established selenium deficiency as being easily recognized in a number of species. The first indications in cattle and horses were reported as loss of the long hair, lameness and soreness of the feet, and deformities of the hooves. Lameness was often seen in swine, along with depressed appetite, alopecia, and irregular hoof growth. Conditions attributable to dietary insufficiency were noted by Hartley and Grant (1961), Schubert et al. (1961), Nesheim and Scott (1961) and Arthur and Owen (1976) as including muscular dystrophy (white muscle disease) in lambs, calves, foals, goats,

and pigs; exhudative diathesis in poultry; and hepatosis dietetica in swine. Degenerative skeletal myopathy was later reported in adult cattle and horses as a result of insufficient dietary selenium. Van Fleet (1980) and Muth et al. (1971) had earlier described a wasting disease of selenium deficient squirrel monkeys, resulting in myopathy, hepatic degeneration, and death.

Teague's (1979) recommended selenium allowance of 0.15 mg/kg of feed for breeding boars, sows, and gilts, collectively, agreed with the figure advanced by Teague et al. (1979) for growing-finishing pigs. This level was found adequate for the prevention of the deficiency-related conditions of hepatosis dietetica, muscular degeneration, and "mulberry heart," as described by Lannek and Landsburg (1975) and Glienke and Ewan (1977).

Underwood (1971) noted that naturally occurring selenium toxicity was reported in Nebraska as early as 1856, where it was dubbed "blind staggers" or "alkali disease." He described the pathological mechanism involved as an inhibition of oxygen consumption by the tissues. Maag and Glenn (1967) observed controlled toxicity in both cattle and sheep and reported that similar results with swine were first published in 1937. Ullrey (1974), working with feeder pigs, established that toxicity occurred in some individuals at a dietary level of 7.5 ppm, while as much as 45 ppm was necessary to produce signs of toxicity in the remaining animals on trial.

Selenium Metabolism and Biochemistry

Although details concerning the metabolic functions and pathways of selenium are not yet resolved, certain facts are known. Mills (1957)

first established the existence of glutathione peroxidase (GSH-Px) as an erythrocytic enzyme responsible for catalyzing the oxidation of reduced glutathione by hydrogen peroxide. This was noted as a vital link in the protection of hemoglobin from oxidative breakdown brought about by low concentrations of ascorbic acid. Although the structure of GSH-Px was originally unknown, Roetruck et al. (1973) and Flohe et al. (1973) determined its nature as a selenoenzyme. In light of the findings of Underwood (1962), Hoekstra (1974), and Stadtman (1977), most of the pathological symptoms of selenium deficiency were related to peroxidation of unsaturated lipids in membranes. The primary function of GSH-Px was revealed to be the prevention of lipid peroxidation by either elimination of low molecular hydroperoxides or reduction of lipid hydroperoxides. These conclusions served to correlate the antioxidative effects of adequate dietary selenium with its function as an integral component of GSH-Px. In an extensive review of the subject, Ganther et al. (1976) revealed the selenium content of the purified enzyme to be 0.34%, or 4 gram atoms of selenium/mole of GSH-Px.

Further evidence for the role of dietary selenium as a catalyst in the genesis of GSH-Px was provided by Chow and Tappel (1974), who noted an increase in levels of the enzyme in the plasma and tissue of rats supplemented with 2 ppm dietary selenium for 11 days. Similar results in swine, noted by Chavez (1979), indicated that tissue and plasma GSH-Px levels were a reliable indicator of selenium status in that species also. Stadtman (1974, 1977) reported that formate dehydrogenase and glycine reductase were selenium-bearing compounds found to be manufactured in certain metabolic pathways by bacteria. It was further noted that only one

other selenoprotein was found in addition to glutathione peroxidase in mammalian systems. Although the structure of this compound, isolated from the heart and semitendinosus muscle of selenium-supplemented lambs, is unknown, analyses indicate the presence of aspartate, glutamate, lysine, glycine, leucine, methionine, and traces of cysteine or cystine. Little or none of this protein was found either in the muscles of selenium-deficient animals or in those already suffering from white muscle disease. Similar results seen in work by Godwin (1975) and Diplock (1976) further indicate a link between this unknown selenoprotein of low molecular weight and progressive muscular degeneration in lambs.

Selenium's Interaction with Vitamin E

Since the original observations of Evans and Bishop (1922) concerning an unknown element missing from the diets of sterile rats, much research has been devoted to vitamin E and its synergistic relationship with dietary selenium. Sondegaard (1967) examined this relationship in rats more closely and concluded that selenium could replace vitamin E in some of its non-antioxidative functions. This suggested a still unknown but common biochemical mechanism utilizing both nutrients. Green and Bunyan (1969) speculated that although selenium could not be classified as a potent antioxidant itself, it might act as a progenitor of other antioxidants in tissues. He further observed that the mineral may serve to enhance vitamin E absorption through cell walls.

The mystery surrounding their similar roles was clarified somewhat by the work of Diplock (1970) and Diplock and Lucy (1973), who postulated that specific complexes formed from the vitamin and some polyunsaturated lipids acted to block the otherwise high permeability of cell membranes

to endogenous phospholipases. Without the formation of this barrier, membranes were highly susceptible to degradation by normally occurring metabolic enzymes. This hypothesis was expanded by Hoekstra (1975) and Scott (1979), who reviewed the selenium-vitamin E relationship further. They noted that the selenoenzyme glutathione peroxidase was important in the destruction of hydrogen peroxide and organic hydroperoxides such as lipid hydroperoxides. This protected against oxidative damage to cell membranes and other oxidant-sensitive sites in the cell. While this selenium-dependent system destroyed lipid hydroperoxides and other peroxides, vitamin E prevented oxidative damage to membranes by preventing their formation through favoring alternate routes of oxygen metabolism.

Selenium's Role in Female Reproduction

Selenium's role in reproduction has been intensely contested according to Hidiroglou (1979). Hartley (1963) originally associated selenium levels with ewe fertility by noting increased embryonic mortality in deficient ewes during the first one to three weeks of gestation. He reported elimination of the problem by injecting 1-25 mg of selenium plus supplemental vitamin E, one month prior to breeding. Mudd and Mackie (1973) later supported these findings by injecting deficient ewes with 6 or 12 mg of selenium. Mean values of 1.22 and 1.28 lambs, born from ewes in groups 1 and 2, respectively, were significantly greater ($P < 0.05$) than that of 1.03 lambs born for group 3 controls. Animals were assigned to treatments, which contained 208, 214, and 2,399 ewes for groups 1, 2, and 3, respectively. In contrast to these findings, Mitchell et al. (1975) observed no difference in conception rates, lambing

percentage, or number of lambs born to ewes on diets containing 0.02 or 14.8 ppm selenium. Their data suggested that the interaction between selenium and other nutrients, possibly missing from the pastures of barren ewes, be investigated more closely. These reproductive effects of the interaction between selenium and vitamin E were later demonstrated by Buchanan-Smith et al. (1969), who demonstrated improved reproductive performance of ewes supplemented with both micronutrients. There was an increase in conception rates and the number of lambs born alive, with a significant decrease in embryonic mortality among selenium-vitamin E supplemented ewes. No progress was noted in groups receiving additional selenium or vitamin E supplementation alone. Similar conclusions were reached by Whanger et al. (1977), who also found a significant decrease in the incidence of white muscle disease among the lambs of dual-supplemented dams.

In a study involving the use of selenium alone, Halverson (1974) observed marked increases in the fertility of supplemented rats. Populations of females receiving diets containing 2.5 ppm selenium successfully conceived at a significantly higher rate than those receiving either 0.0 or 1.25 ppm selenium.

Conrad and Moxon (1980) postulated that decreased fertility among deprived females of ruminant species might suggest selenium as a component involved in the penetration of sperm through the zona pellucida at conception. It was further noted that <u>in vitro</u> motility of bovine spermatozoa was directly enhanced by supplementation, possibly linking selenium and post-coital spermatozoan movement.

Selenium has also been indicated to be an important nutrient in the diets of laying and breeding hens. Latshaw et al. (1977) demonstrated

that egg production and hatchability increased significantly when deficient diets were supplemented. The percentage of infertile eggs and early dead embryos produced by the same individuals decreased sharply. Combs and Scott (1979) later agreed that a dietary level of 0.05 ppm/kg of feed was adequate in maintaining optimum reproductive performance in poultry. They added, however, that a concentration of 0.10 ppm/kg of feed was necessary for use with vitamin E deficient diets.

Whitehair (1971) suggested that selenium-vitamin E supplementation of swine diets might be of significant importance for successful reproduction. Deficiency was said to result in general infertility, small litters, agalactica, and uterine atony, although the mode of action was unclear. Mahan et al. (1974, 1975) noted that sow ovulation rates were not affected by selenium availability but that fetal mortality was greatly increased. A larger quantitative transfer of selenium was observed from second litter sows to their progeny as opposed to first parity sows. This possibly indicated a reduction in the daily requirement of selenium for older females, thus allowing more of the nutrient to be passed by placental transfer to the young. It was further demonstrated that milk and colostrum selenium levels declined over several parities, illustrating a greater requirement necessary for lactation than for gestation. A dietary level of 0.1 ppm was judged adequate for all reproductive stages. Mahan et al. (1977) later examined their findings concerning the placental transfer of selenium more closely. Sow serum, milk, tissue, and hair selenium levels reiterated that the 0.1-ppm dietary selenium level previously studied was optimum for purposes of gestation and lactation. Young et al. (1977) studied groups of

selenium-supplemented gilts fed a basal diet containing 0.03 mg/g selenium on a dry-matter basis. Levels of supplementation were 0, 60, and 120 ppm. Although no gilts receiving the basal diet alone maintained pregnancy, there was no obvious influence of supplemented selenium on the reproductive performance of the gilts in this study. It must be noted, however, that the number of gilts per treatment was quite small. Piatkowski et al. (1979) noted that both the number of corporea lutea and viable fetuses at 105 days post-coitum were unaffected by diets containing as low as 0.02 ppm selenium. In retrospect, Wilkinson et al. (1977) had earlier reported that no significant reproductive benefits were realized by supplementing already adequate sow diets with an additional 1 ppm/kg of diet fed.

Although these later studies seem to indicate that a very low level selenium presence is necessary to preclude fetal mortality, they are in agreement with Mahan et al. (1979) that a dietary minimum of 0.1 ppm is important for lactating dams.

Selenium's Role in Male Reproduction

The use of radioisotopes has done much to clarify the role of minerals in the spermatogenic pathway. Rosenfield (1964) originally suggested that the effects of selenium on male reproduction should be explored more fully. Although impairment of spermatogenesis as a result of selenium deprivation was not observed, high concentrations of radioactive (tagged) selenium was noted in the testes of treated rats. Tracer doses of selenium-75 administered to rats and mice were observed by Gunn et al. (1967) and Gould (1970) to be definitely incorporated into developing sperm cells in much the same fashion as zinc, although

at an earlier stage of development (probably into late spermatocytes or early spermatids). In the later study, a measured amount of tagged selenium was subcutaneously injected and measured in various tissues at timed intervals. By day 7, selenium concentration in the testis was 3.5 times greater than that observed at one hour and ranked just below the liver and kidney in selenium content. As testicular levels fell, selenium-75 concentration in epididymides rose, reaching maximal concentrations at two to three weeks following injection, indicating definite incorporation into developing spermatozoa. Gould (1970) reached the same conclusions in reporting a rhythmic rise and fall within each section of the epididymis from caput to cauda following accumulation in the testis. Smith and Senger (1978), utilizing similar techniques, reported comparable findings in dairy bulls. High levels of selenium tracer found within the epididymis were noted as due to spermatogenic incorporation and not epididymal uptake per se. These findings had previously been noted by Gunn and Gould (1970), who added that an even greater quantity of the mineral was taken up by interstitial testicular tissue than by spermatogenic elements upon the same radioactive challenge. Autoradiography employed by Brown and Burk (1973) revealed that selenium incorporated into epididymal rat sperm was localized within the midpiece. This may have indicated a specific need for selenium by mitochondria, since they are found exclusively in that section of the gamete. Data concerning the epididymal transit time of injected selenium-75 agreed with those previously reported by Gould (1970) and Gunn et al. (1967).

The specific mode of selenium incorporation into developing spermatozoa was defined by Calvin (1978), who determined that selenium

was an important constituent of a selenopeptide he termed selenoflagellin. Radioactive tracer techniques using rats indicated that the protein-bound mineral was found selectively in the tail and midpiece of spermatozoa and was essential for proper tail assembly and integrity. Work with bull semen by Pallini and Bacci (1979) produced similar results. Sperm-bound selenium in that species was also found to be fixed to a mitochondrial protein complex closely resembling but differing from glutathione peroxidase. This selenopeptide was noted as playing an important part in the structural maintenance of the cell. Calvin (1978) alluded that its quantity within spermatozoa was not correlated with the level of glutathione peroxidase present. Smith et al. (1978) and Senger (1980) attempted to explain the dual function of testicular selenium in bulls as having one part bound to developing spermatozoa and the other found as a constituent of glutathione peroxidase, free in the seminal plasma. Both were described as vital roles necessary for the maintenance of cellular integrity and the prevention of oxidative damage to the gamete, respectively.

Specific effects of selenium deprivation on sperm cell morphology and action have been reported by several researchers. Wu et al. (1969) noted that the testicular weights of rats from selenium-deficient parents receiving 0.1 ppm dietary selenium was significantly greater than those of rats deprived of the nutrient. Seminal smears taken from caudae epididymides of deprived rats showed very few spermatozoa, with most of those present being morphologically abnormal. In contrast, high percentages of normal and actively motile cells were observed in animals receiving 0.01 to 0.08 ppm selenium. Spermatozoan motility

improved almost linearly in relation to increasing amounts of selenium added to the basal diet. Histological examination revealed that active spermatogenesis still occurred in the seminiferous tubules of animals exhibiting severely afflicted epididymal smears. Some tubules and corresponding epididymal ducts contained spermatozoa, while others were empty or filled with cellular debris.

These data suggest that selenium deficiency not only retarded the development and function of the testis, but also impaired the epididymal function relating to successful spermatozoan maturation. In a subsequent study incorporating two supplemental levels of vitamin E, Wu et al. (1971) found very similar results. Failure of spermatogenesis and cellular maturation, along with depressed motility, were again dramatic. Vitamin E given as a supplement had no effect. Wu et al. (1973, 1979) reiterated their previous findings and sharply defined the expected morphological changes due to selenium deficiency in rat spermatozoa. Breakage of the sperm cell near the midpiece or principal piece of the tail occurred in most of the spermatozoa of rats deprived of selenium for 12 weeks. These findings, along with the previously discussed data of Brown and Burk (1973), prompted the conclusion that selenium serves as both an important structural component of spermatozoa and as protection in seminal plasma from membrane damage by metabolic free radicals. Those findings concerning the negative effects of deficiency on sperm cell motility were recently more closely examined by Julien and Murray (1980). In an *in vitro* study utilizing bull semen, percent total motile sperm increased dramatically as selenium concentration increased from 0 to 1.0 ppm. Although supplemental concentrations greater than 1.0 ppm were cytotoxic,

it was concluded that selenium was definitely beneficial to sperm motility.

Although the consequences of dietary selenium deprivation on male reproduction poses a considerable obstacle to acceptable fertility, supplementary injections seem to be of little aid. Bartle et al. (1980) injected deficient dairy bulls with 5, 10, 20, or 40 mg selenium/90 kg body weight at 6, 16, 22, and 28 weeks, respectively. Bulls were considered moderately deficient if blood selenium levels were less than 0.05 ppm. Thirteen weeks following initiation of the experiment, semen from each bull was analyzed on a weekly basis. Selenium injections increased blood selenium ($P < 0.05$), blood glutathione peroxidase ($P < 0.005$), semen selenium ($P < 0.06$), and seminal plasma glutathione peroxidase ($P < 0.05$). No changes in semen volume, spermatozoan concentration, percent motile cells, or sperm morphology were noted over data collected prior to commencement of treatment. Segerson and Johnson (1981) later performed a similar study with yearling Angus bulls on a growing ration. No differences were observed between control and selenium-injected bulls with respect to spermatozoan concentration in the testis and caput, corpus and cauda epididymis. Treatment increased the selenium concentration of various tissues but had no apparent influence on sperm cell viability, number, or selenium content.

Segerson et al. (1980) achieved much the same results with boars fed a deficient diet of cornstarch and torula yeast and injected at 14-day intervals with 0.33 mg selenium/kg of body weight. At approximately four months after the first injection, some boars were sacrificed, along with controls. Tissues were retrieved and assayed for selenium.

Selenium concentrations were greater in testes, epididymides, serum, and accessory glands for the injected boars. At approximately five months after the first injection, four ejaculates were collected from each of the remaining boars at four to six-day intervals. No significant differences were noted in semen volume, percent normal spermatozoa, percent viability, or spermatozoan concentration/ml. However, selenium in whole semen, seminal plasma, and spermatozoa was greater in treated boars than for controls. These boars were later castrated, and no differences were found in testis length, diameter, weight, or spermatozoan concentration between treatment groups.

It appears that although the merits of injected supplementation are questionable, concensus of opinion calls for further study concerning the effects of dietary selenium on male fertility.

CHAPTER III

MATERIALS AND METHODS

Thirty-six crossbred boars of Duroc x Landrace or Duroc x Landrace x Yorkshire breeding were selected at the time of weaning from each of 12 litters and were distributed randomly to each of three treatment groups. Three of the boars were removed from treatment early in the trial due to illness. Ages ranged from 47 to 58 days ($\bar{x}$ = 54 days) when experimental diets were first administered on day 1. All animals were identically maintained in semi-enclosed concrete pens, originally in groups of five or six and later in groups of three. Boars had been farrowed and were maintained until conclusion of the experiment at the swine unit of the Blount Experiment Station at Knoxville, Tennessee. The study began on November 21, 1980 and was terminated with final slaughter by exsainguination on July 15, 1981 (day 247).

Experimental Diets

All animals received the corn-soybean meal ration, as outlined in Table 1, throughout the experimental period. Diets differed only in the amount of selenium present in the ration. Boars were fed diets containing selenium at the basal level of 50 ppb (treatment $_1$) or the basal diet supplemented with 100 ppb (treatment $_2$) or 250 ppb (treatment$_3$). Selenium added was in the form of sodium selenite. All boars were fed their respective diets ad libitum from two-hole self-feeders until approximately six months of age and then were hand-fed 2.2 kg/boar/day for the remainder of the study. Water was provided ad libitum

TABLE 1

BASAL RATION

Ingredient	Amount (lbs)
Corn	749
Soybean Meal	215
Dicalcium Phosphate	10
Limestone	10
Salt	5
UT-Experimental Premix H-1[a]	5
Selenium Premix[b]	5
Antibiotic	1

[a]Breakdown of UT-Experimental Premix:

Nutrient	Amount in 5 lbs Premix
Vitamin A	1.5 million IU
Vitamin D	0.6 million IU
Vitamin K compound	2.0 gm
Riboflavin	3.0 gm
Nicotinic acid	16.0 gm
D-pantothenic acid	12.0 gm
Choline	100.0 gm
Vitamin B_{12}	18.0 gm
Zinc	68.0 gm
Manganese	34.0 gm
Iodine	2.5 gm
Copper	9.0 gm
Iron	54.0 gm
Antioxidant[c]	45.0 gm

[b]Contributed 0, 100, or 280 ppm to diets 1, 2, and 3, respectively.

[c]Ground yellow corn, hominy feed, or other suitable carrier having low selenium-Vitamin E content.

throughout the experiment. Body weights of boars, to the nearest kilogram, were taken at biweekly intervals until approximately six months of age and at monthly intervals thereafter.

Measurement of Testicular Width

Testicular widths, to the nearest tenth of a centimeter, were made latitudinally along the midpoint (widest point) across both testicles, on all animals, using outside calipers. Measurements were taken and recorded at biweekly intervals beginning on day 70 of treatment and continuing until day 164. Thereafter, measurements were made at intervals of one month until slaughter on day 247.

Evaluation of Libido

Beginning on day 110 of treatment (approximately 5½ months of age), boars were subjected to routine subjective testing of sexual aggressiveness. Mating behavior was observed in standard ten-minute exposures at two-week intervals until day 146. Thereafter, tests were administered at intervals of one week until day 242. The reactions of single boars were observed upon exposure to one, two, or three gilts exhibiting standing estrus. Ovariectomized gilts were prepared for the test (day 0) by injecting them intramuscularly with 7.5 mg of progesterone on day -4, followed by 1.4 mg of estradiol benzoate on the next two days (days -3 and -2). On day 2, gilts received 7.5 mg of progesterone to suppress sexual receptivity.

The mating behavior test consisted of allowing each boar a three-minute familiarization period alone in a 9.2-m^2, concrete-floored pen

and then introducing the sexually receptive gilts. The sexual advances of the boar were scored subjectively using the method shown in Table 2. Sexual interest was evidenced by nosing the flanks of the gilt, naso-nasal contact, and ano-genital sniffs.

TABLE 2

LIBIDO SCORING

Score	Description of Activity
1	No interest
2	Interest
3	Interest w/mounting
4	Interest w/mounting and penile extension (w/wo intromission)
5	Interest w/mounting, penile extension, and ejaculation (w/wo intromission)

Evaluation of Collected Semen

Semen was collected from boars upon their mounting ovariectomized-estrogenized gilts by use of the gloved-hand technique according to Hancock and Hovell (1959). Semen collections were attempted on the occasion of each libido evaluation. Forty-one samples were obtained from 11 boars (three boars/treatment$_1$, four boars/treatment$_2$, and four boars/treatment$_3$) by the conclusion of the study.

Immediately upon collection, semen was strained through cheesecloth in order to remove the gel fraction. The sperm-rich fraction was then placed in a water bath at 40°C. A measurement of volume was made to the nearest millimeter. Both raw and extended samples were then subjected to bright-field microscopic evaluation in a manner prescribed by Sorenson (1979). Composition of extender and tissue preparations are outlined in Table 3.

Fresh semen was mixed (1:1 ratio) with extender on a warmed glass slide and observed as to motility of spermatozoa at 200 and 450X. A percentage of motile sperm cells observed in three fields was recorded to the nearest 10%. All microscopic procedures were performed by the same technician. A subjective measurement of rate of forward movement was also scored. A score of (1) was awarded when no spermatozoa were observed moving in a forward direction; (2) was awarded when movement was slow; (3) was awarded when moderate; and (4) was awarded when exceptionally fast. Concentration of spermatozoa/ml was estimated using a standard hemacytometer.

Drops of fresh semen, along with equal amounts of either live-dead stain or 2% glutaraldehyde fixative, were mingled and smeared on glass slides and allowed to dry on a warm surface. Within eight hours following preparation, they were examined for percentage of abnormal spermatozoa at 200 and 450X. Counts were made of the following spermatozoan anomalies: decapitate cells; tailless cells; abnormally short, coiled, or noticeably bent tails; acrosomal detachment; and the presence of cytoplasmic droplets. Percentages of abnormal cells/total cells in an ejaculate were determined by observing ten cells in each of ten fields

TABLE 3

EXTENDER AND TISSUE PREPARATIONS

Ingredient	Amount
Beltsville #1 semen extender (BL-1)[a]	
Glucose	29 gm
Sodium citrate	10 gm
$NaHCO_3$	2 gm
KCl	0.3 gm
Penicillin K	0.1 gm
Streptomycin sulfate	1 gm
Water (distilled)	1 liter
Live-dead stain[b]	
Eosin-B	1 gm
Nigrosin	5 gm
Sodium citrate	3 gm
Water (double-distilled)	100 ml
Glutaraldehyde fixative (2%)	
KH_2PO_4	4.36 gm
Na_2HPO_4	10.59 gm
NaCl	9.0 gm
$C_5H_8O_2$ (glutaraldehyde)	0.24 gm
Water (double-distilled)	1 liter

[a]If refrigerated, the solution will remain stable for two-three weeks.

[b]Mixture will remain stable for one year without refrigeration; however, refrigeration will prevent growth of bacteria.

on each slide prepared. Initial bright-field microscopic inspection of fresh cells utilized an American Optical standard laboratory microscope. Later examination of fixed and/or stained specimens employed a Weiss Dialux research microscope.

Acrosomal morphology was observed more closely by phase-contrast microscopy in a manner prescribed by Pursel and Johnson (1974). Cells fixed with a 2% glutaraldehyde preparation were observed closely for acrosomal retention and integrity as well as for vesicle formation within the spermatozoan head. Observations were made at 400, 630, and 1500X (oil immersion) using a Zeiss research microscope.

Histological Appraisal

Seven boars from each of the three treatment groups were slaughtered on day 247 and their testes and epididymides retrieved and placed on ice. All boars from which semen was collected were among those sacrificed. Within one hour following slaughter, the right testes and capita-corporea and caudae epididymides were separated and cleaned of connective tissue. Testicular circumference (cm), length (cm), and weight (gm) were recorded for each boar, as were the weights of epididymal segments.

Immediately following the recording of gross measurements, tissues were individually cut into slices of moderate size and homogenized in a Waring blender. Agitation was applied for approximately one minute in 200 ml of a saline-Triton-Merthiolate solution (Table 4) as dictated by Amann and Lambaise (1969) and Wettemann et al. (1976). Spermatozoan concentration within each anatomical division was then determined by use of a standard hemacytometer as prescribed by Kirton et al. (1967) and as modified by Wettemann et al. (1976).

TABLE 4

SALINE-TRITON-MERTHIOLATE SOLUTION

Ingredient	Amount
NaCl	9 gm
Triton X-100	0.5 gm
Merthiolate	100 ppm
Water (distilled)	1 liter

Spermatozoa in samples of homogenate were fixed in a 2% glutaraldehyde solution and examined for standard abnormalities as described previously for collected ejaculates. Bright-field and phase-contrast methods were employed as before. Decapitate and tailless cells were excluded from consideration due to the trauma of homogenization.

Using single-edged razor blades, 3-mm-thick blocks of tissue were cut from standardized locations on the left testis and capita, corporea, and caudae epididymides. The tissue samples were fixed in 10% buffered formalin, dehydrated through graded alcohols, and embedded in paraffin-low polymer plastics.[1] Seven micrometer sections were cut, mounted on albumized 0.0254 x 0.0762-m glass microscopic slides, and stained with hematoxylin and eosin. Preliminary examinations were conducted using bright-field microscopy. Testicular tissues were observed at 100X, and the percentages of apparently non-functional seminiferous tubules in

[1]Tissue Tek, Fisher Chemical Corporation, Atlanta, Georgia.

each of three representative fields were taken. Heights of epithelial layers lining the lumens of capita, corporea, and caudae epididymides were represented by the mean measurement taken at three locations within a representative section. Measurements were made using a standard ocular micrometer and recorded to the nearest micron (.001 mm). Care was taken to avoid the measurement of convoluted tissue and that obscured by misleading artifacts.

Spermatozoa within the lumens of caudae epididymides were microscopically examined for abnormalities in an identical manner to those previously observed in collected ejaculates. Initial observations were made at 400 and 787.5X (oil immersion) using bright-field microscopy. More precise inspections of acrosomal integrity utilized phase-contrast microscopy at 1500X (oil immersion).

Testosterone Quantification

Beginning on day 1, blood samples (approximately 20 ml) were taken from each boar by vena cava puncture, placed in heparinized tubes, centrifuged (2000 g), and stored as plasma at -20°C. Samples were taken at biweekly intervals until day 137 of treatment and then monthly until slaughter on day 247.

Each plasma sample taken from the 21 boars slaughtered was assayed for testosterone concentration by radioimmunoassay, in a manner prescribed by Kattesh et al. (1979). Rabbit antiserum[2] prepared against testosterone-3-oxime-human serum albumin was used. The radioimmunoassay procedure

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[2]Testosterone antibody (MSU #74) was kindly donated for this study by Dr. J. J. Ireland, Dairy Science Dept., Michigan St. University, East Lansing, Michigan.

was validated by extracting differing amounts of pooled boar plasma and by assaying 0.5-ml plasma samples to which various levels of testosterone were added. The results of these two validation procedures are shown in Table 5.

TABLE 5

RESULTS OF TESTOSTERONE VALIDATION

Amount Extracted/Added	n	$\bar{x}$ (ng/ml)	SE*
Plasma extracted (ml)			
0.5	4	1.81	0.07
1.0	4	1.87	0.14
1.5	4	1.61	0.10
2.0	4	1.68	0.19
Testosterone added (ng)			
0.0	5	1.74	0.11
0.5	5	2.35	0.11
1.0	5	2.76	0.15
2.5	5	4.62	0.12
5.0	5	6.81	0.21

*Standard error of the mean ($\bar{x}$).

Statistical Procedures

All data were analyzed by least-squares analysis of variance according to Goodnight (1979) for a randomized block design with incomplete subclass members. Variables measured on the live animals were analyzed using the following model:

$$Y_{ijk} = t_i + b_{ij} + \beta d_{ijk} + \beta(d - t)_{ijk} + e_{ijk}.$$

In the model, t_i represents treatment effect, b_{ij} is boar within treatment, and d_{ijk} is the date of sampling. The relationship of individual treatment over time is represented by $(d - t)_{ijk}$, with e_{ijk} being the residual. β is the regression coefficient for date. Treatment effect was tested using the boar within treatment mean square. Date of sampling and the treatment by date interaction was tested using the residual.

Data collected following slaughter was analyzed and tested with respect to treatment effect only.

Differences in least squares means were partitioned according to the method of Duncan (1955).

CHAPTER IV

RESULTS AND DISCUSSION

Parameters measured over the course of treatment, including body weight, libido, plasma testosterone level, and testicular width, were considered separately from data collected after slaughter. Post mortem criteria, selected for study, included histological and anatomical examination of testes and epididymides.

A. ANALYSIS OF PARAMETERS MEASURED PRIOR TO SLAUGHTER

Results of least-squares analysis of variance for pre-slaughter variables are shown in Table 6. Body weight, measured at intervals during the study, was found to be different ($P < 0.01$) between treatments as analyzed over time. Figure 1 illustrates that body weight tended to increase in a linear fashion among the three treatment groups with respect to age. However, weights for boars in treatments t_2 and t_3 were apparently less than those of boars in t_1, beginning at approximately 100 days of age. From these data, it appears that dietary selenium fed at the levels of 150 and 300 ppb may slightly inhibit weight gain in boars for a time shortly preceding sexual maturity.

Analysis of the subjective measurement of libido among boars indicated that the three treatment groups exhibited differences ($P < 0.01$) in sexual development. Figure 2 illustrates the linear plots of scored libido regressed over the continuous variable, age. An overall view of the relationship between treatments, as regressed over age, seems to

TABLE 6

MODEL FOR ANALYSIS OF SELECTED VARIABLES RECORDED DURING THE COURSE OF TREATMENT

Model	Body Weight			Libido			Testicular Width			Plasma Testosterone		
	df	MS	F	dp	MS	F	df	MS	F	dp	MS	F
Treatment	2	15.53	0.003	2	1.96	0.37	2	6.35	1.33	2	0.76	0.04
Boar (Treatment)	30	4996.16	17.44[a]	30	5.26	9.28[a]	30	4.79	2.54[a]	30	18.55	6.52[a]
Date of Sampling	1	5006729.41	17478.28[a]	1	28.99	51.22[a]	1	1485.71	788.06[a]	1	1286.52	36.79[a]
Date-Treatment	2	1459.07	5.09[a]	2	2.80	4.93[a]	2	5.87	3.11[b]	2	25.63	4.51[a]
Residual	426	286.45		441	0.57		248	1.89		270	2.84	

[a]Significant at ($P < 0.01$) level.

[b]Significant at ($P < 0.05$) level.

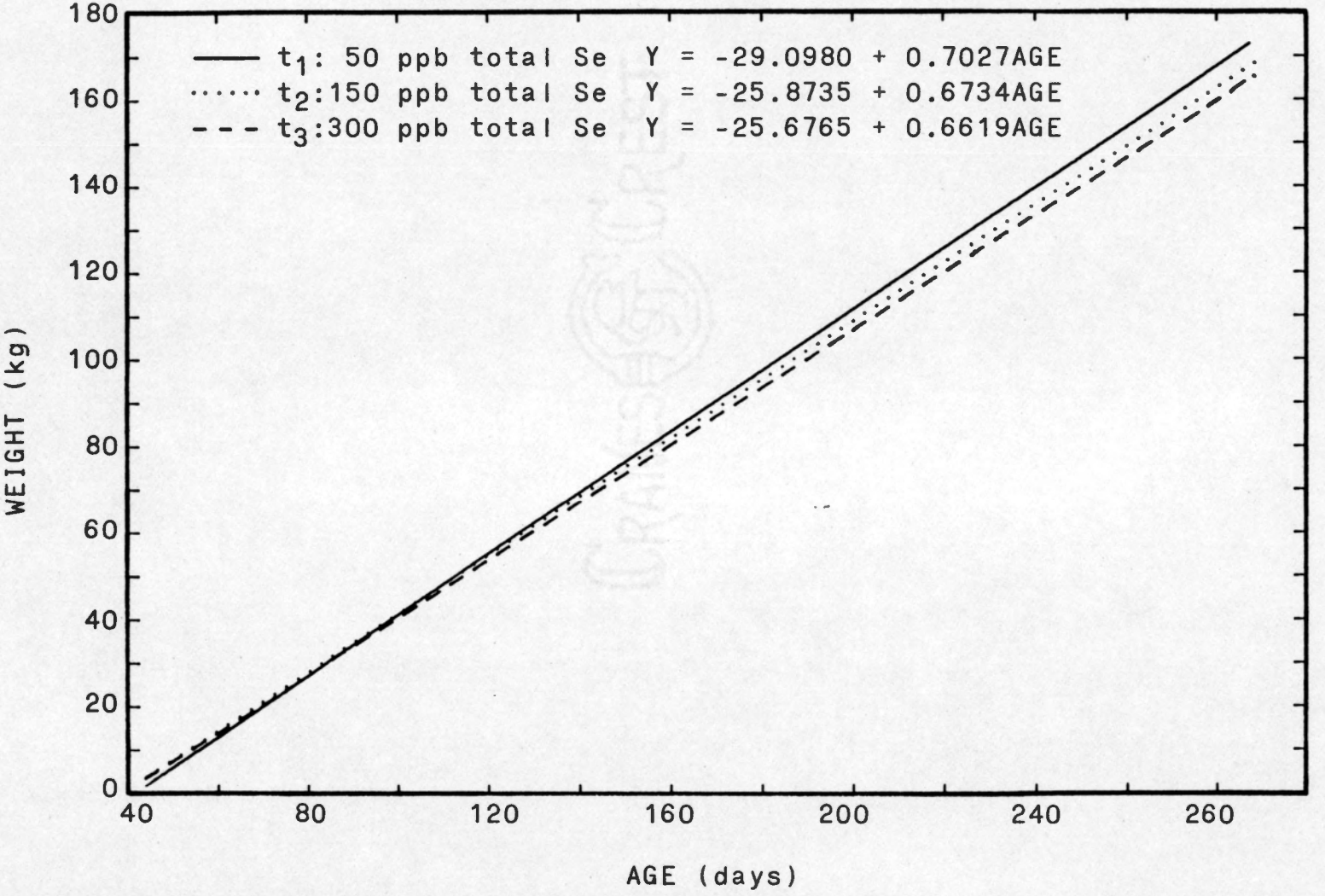

Figure 1. Relationship between the linear regressions of the weight (kg) of boars receiving three levels of dietary selenium (Se) over age (days). Treatments 1, 2 and 3 received 50, 150 and 300 ppb selenium (from sodium selenite), respectively.

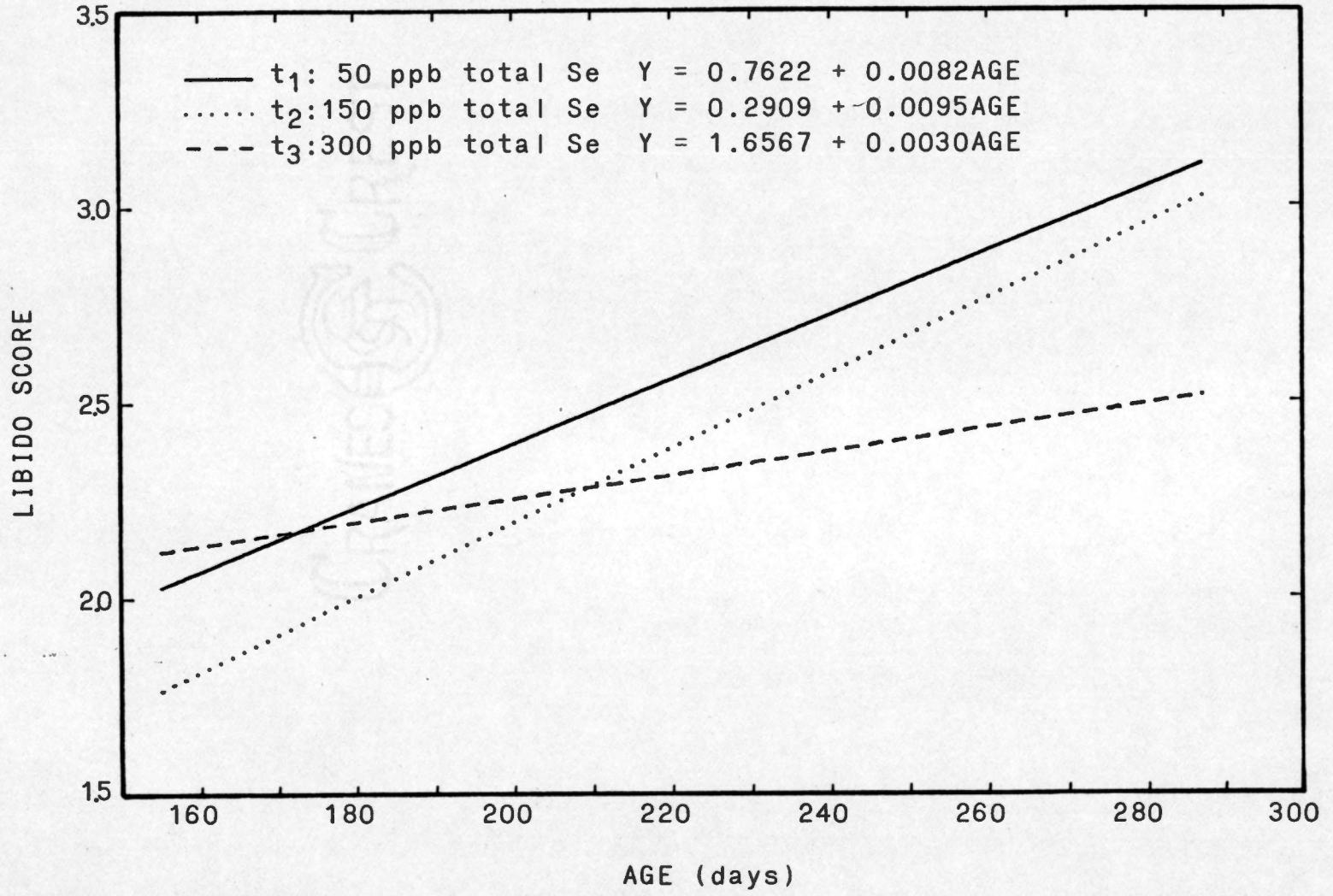

Figure 2. Relationship between the linear regressions of libido score (1-5) of boars receiving three levels of dietary selenium (Se) over age (days). Treatments 1, 2 and 3 received 50, 150 and 300 ppb selenium (from sodium selenite), respectively.

indicate a retarded sexual aggressiveness among the boars of t_3. Although boars of both t_1 and t_2 began the evaluation period with lower overall scores, both groups exhibited the expected tendency to improve their performances as their experience (gained in exposure to receptive females) and age increased. This is in agreement with Nelssen et al. (1980), who reported that subjective measurements of the libido of young boars increased linearly when measured at regular intervals over a period slightly preceding sexual maturity. These data may tend to suggest a significant "lag" time in the development of normal libido, inherent among boars fed a diet containing 300 ppb dietary selenium, as analyzed over time. Although boars scored in this study could be considered young by commercial breeding standards, Gray et al. (1971), Anderson (1974), and Pond and Houpt (1978) reported that boars could be expected to reach sexual maturity at between five and seven months of age. Libido, in this study, was first scored when boars were approximately 160 days old. Evaluations occurred at regular intervals until boars reached approximately 280 days of age. Although boars in t_1 and t_2 exhibited consistently higher scores through the first nine months of age, it is interesting to note the pronounced lack of improvement in boars of t_3.

In view of the relationships between trends in weight gain and libido score, some possible associations are noted. Libido among boars of t_1 and t_2 tended to increase in roughly the same fashion as did weight gain. This was in keeping with Gray et al. (1971), who reported that attainment of sexual maturity among boars was more closely mediated by somatic growth than by chronological age. Similar conclusions were reached by Pond and Houpt (1978). These findings are especially

noteworthy in light of the failure of boars in t_3 to increase in sexual aggressiveness. This similarity between weight gain and libido, expressed over time, seems to reinforce the theory of retarded sexual maturity among boars fed selenium at 300 ppb.

Blood samples were drawn at the same times that body weights were taken. Differences ($P < 0.01$) in plasma testosterone levels were found among treatment groups, as regressed over time (Table 6). The relationship of treatment with respect to age as a continuous variable (Figure 3) found testosterone concentration increasing in a quadratic fashion over time. Although all treatment effects were plotted in the same fashion, only data recorded for t_3 significantly ($P < 0.01$) adhered to the quadratic. Testosterone values were characterized as being higher for t_1 than t_2, which were both higher than t_3. Testosterone values found in peripheral plasma seemed to increase at a lower rate for boars in t_3 than that of both t_1 and t_2. Values attained by radioimmunoassay, for boars in t_1 and t_2, of approximately the same age, were in keeping with those found by Gray et al. (1971) and Kattesh et al. (1979). Levels assayed in boars of t_3 appeared somewhat lower with respect to these earlier findings. It is interesting to note that testosterone values for boars in t_3 appear to lag in a similar manner as the previously discussed libido scores. This trend may be associated with differences among these groups in weight gain, as analyzed over time. This situation tends to be an example of the unique relationship between somatic growth, testosterone production, and development of sexual maturity previously reported by Gray et al. (1971) and Pond and Houpt (1978).

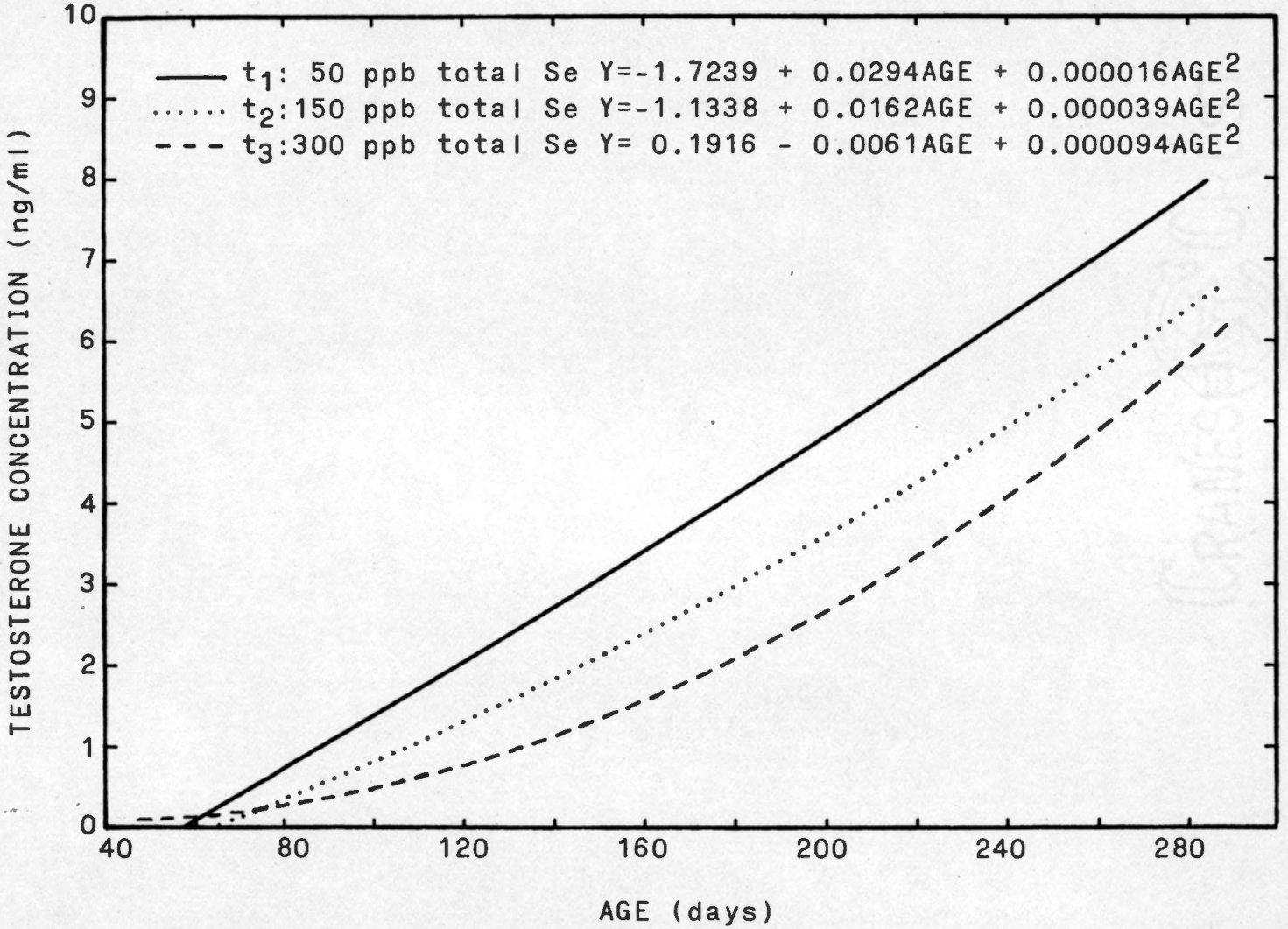

Figure 3. Relationship between the quadratic regressions of the plasma testosterone level (ng/ml) of boars receiving three levels of dietary selenium (Se) over age (days). Treatments 1, 2 and 3 received 50, 150 and 300 ppb selenium (from sodium selenite), respectively.

Analysis of testis width, as measured across both testes at the time body weights were taken, also exhibited differences ($P < 0.05$) between treatments over time (Table 6). As diagrammed in Figure 4, testis width is illustrated as having a linear relationship over age. This is in agreement with Mahone et al. (1979), who correlated increasing volume of testes with sexual maturity in boars. Although values recorded for boars in t_3 overtake those of t_1 and t_2 at approximately halfway through the experimental period, they seem to be noticeably lower prior to day 200. This may prove significant in view of the fact that testosterone levels assayed for t_3 also lagged behind those of the other treatments during roughly the same period. A possible explanation for this might be that the number or volume of steroid-producing Leydig cells (interstitial cells) within testes had previously been retarded by treatment for a period prior to sexual maturity. The possibility of damaging effects on Leydig cell function may have been suggested by Gunn and Gould (1970), when they reported that an even greater quantity of selenium was taken up by the interstitial tissue of rats than by developing spermatozoa. Although their selenium challenge was introduced by intramuscular injection, the resulting accumulations within testicular tissue may be worthy of note. This is especially true in light of the findings of Segerson et al. (1980), who found significantly ($P < 0.05$) greater volumes of selenium in the testicular tissues of boars than in other organs following injection.

An overall view of the collective data seems to indicate a significant association between weight gain, development of libido, and increases in testosterone level and testicular width in young boars.

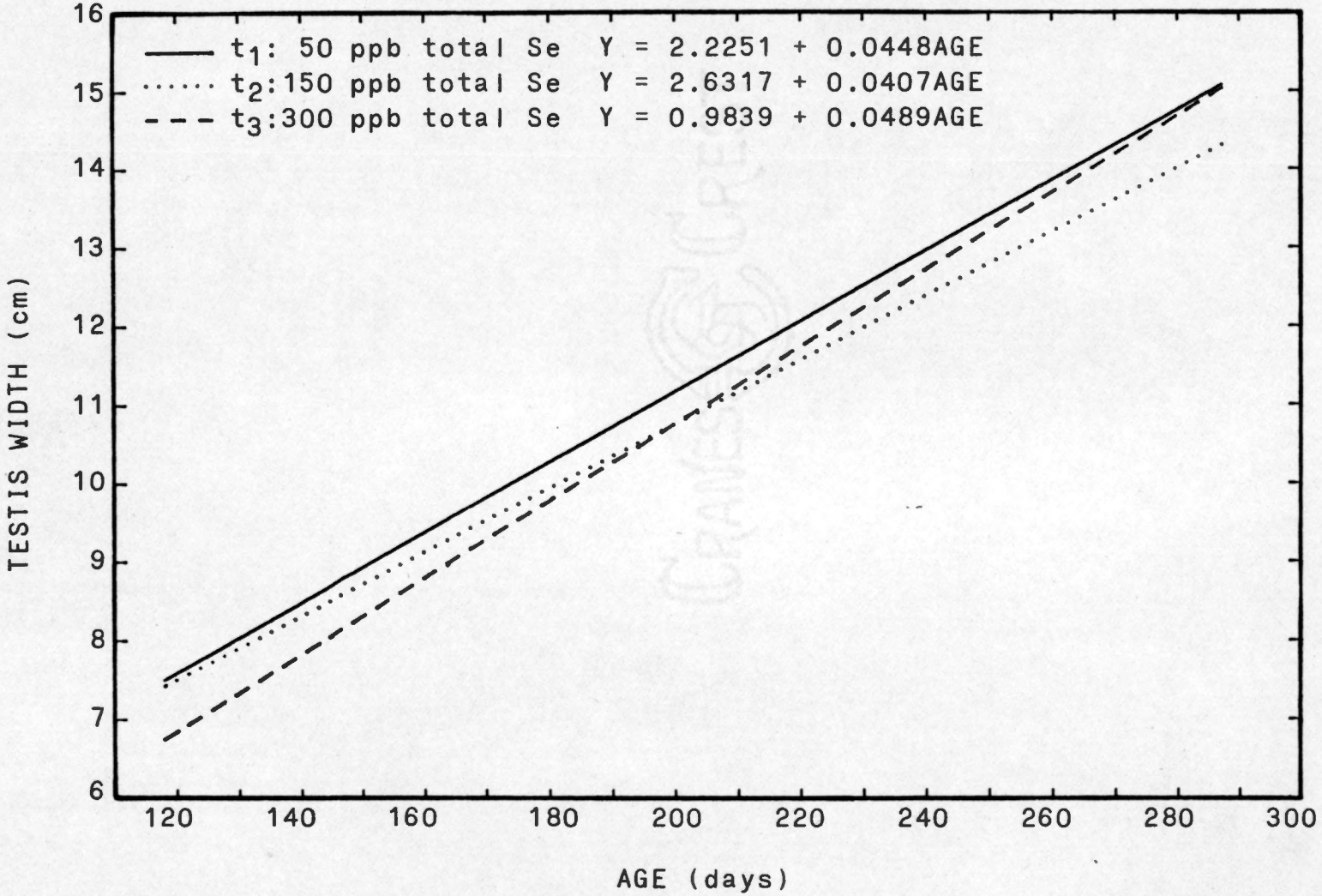

Figure 4. Relationship of the linear regressions of the testis width (cm) of boars receiving three levels of dietary selenium (Se) over age (days). Treatments 1, 2 and 3 received 50, 150 and 300 ppb selenium (from sodium selenite), respectively.

In view of the respective treatment over time effects, dietary selenium (from sodium selenite) at the level of 300 ppb may tend to slightly retard androgenesis and sexual aggressiveness for a short period prior to sexual maturity. In that the depression noted in testis width, libido score, and testosterone concentration appears most pronounced for the period from 100-200 days of age, slaughter and histological examination of some boars from each treatment group during that period might have yielded interesting data. It may have been that androgen-producing interstitial tissue was temporarily in a retarded state, resulting in a decreased influence over sexual maturation.

Although microscopic examination of collected semen was performed for types and numbers of spermatozoan abnormalities, no conclusions could be reached because an insufficient number of samples were collected.

B. CORRELATIONS AMONG PRE-SLAUGHTER VARIABLES

A listing of correlation coefficients between selected pre-slaughter variables is listed in Table 7. As expected, body weight was highly correlated ($P < 0.01$) with both age ($r = 0.97$) and testicular width ($r = 0.87$), as measured in the live animal. This may indicate that the technique used in taking width measurements was easily repeatable and a valid indicator of growth. Body weight was correlated ($P < 0.01$) with plasma testosterone levels ($r = 0.70$). This was in agreement with Gray et al. (1971) and Pond and Houpt (1978), who chose to define sexual maturity (as indicated by testosterone level) in terms of somatic growth. Also, plasma testosterone levels were positively correlated ($P < 0.01$) with age ($r = 0.71$) and testicular width ($r = 0.59$).

TABLE 7

CORRELATION COEFFICIENTS BETWEEN SELECTED VARIABLES TAKEN PREVIOUS TO SLAUGHTER

Variable[a]	A	B	C	D	E	F	G	H
A	1.00000	0.53702[b]	0.09787[c]	-0.05213	-0.03638	-0.00926	-0.07990	-0.01193
B		1.00000	0.18543[b]	-0.02608	-0.06361	-0.04461	-0.11570	-0.01718
C			1.00000	-0.00491	-0.09662[c]	0.17028[d]	-0.13363	-0.18554[b]
D				1.00000	0.97417[b]	0.82756[b]	0.03026	0.70807[b]
E					1.00000	0.87053[b]	0.00218	0.70198[b]
F						1.00000	0.10123	0.59007[b]
G							1.00000	0.08844
H								1.00000

[a]A = Boar; B = Breed; C = Treatment; D = Age; E = Weight; F = Testicular Width; G = Libido; and H = Plasma Testosterone.

[b]Significant at ($P < 0.01$) level.

[c]Significant at ($P < 0.10$) level.

[d]Significant at ($P < 0.05$) level.

It would appear from this that testicular width may be indicative of Leydig cell concentration and, thus, the rate of androgenesis.

C. ANALYSIS OF POST MORTEM PARAMETERS

Least-squares means of data collected immediately prior to and following slaughter are presented in Tables 8 and 9. There were no differences ($P > 0.10$) attributable to treatment for testicular weight, circumference, length, spermatozoan concentration, seminiferous tubule dysfunction or width, as measured on the live animal. Epididymal criteria also demonstrated no treatment effect ($P > 0.10$) in weight or spermatozoan concentration among caput-corpus or caudal segments. Differences in the heights of epithelial layers, lining the lumens of corporea and caudae epididymides, were also nonsignificant ($P > 0.10$). Relation of values for testis width, weight, and spermatozoan concentration, and for capita-corporea and caudae weights and spermatozoan concentration, were comparable to those reported by Davis et al. (1980) for boars of roughly the same age. Figures for spermatozoan concentrations in both testes and epididymal segments were also similar to those reported for control boars by Wettemann et al. (1976). Analysis of post mortem data seems to indicate that the possible retardation of testicular function, seen earlier in the treatment period, did not exist to the same degree at the time of slaughter. Histological examinations of Leydig cell morphology and number, made over the course of treatment and at slaughter, might have yielded interesting comparisons.

Treatment also did not appear to affect ($P < 0.10$) the morphology of caudal epididymal spermatozoa. Deficiency of dietary selenium,

TABLE 8

LEAST-SQUARES MEANS ($\bar{x}$ + SEM) OF TESTICULAR POST MORTEM PARAMETERS MEASURED ON BOARS ON THREE DIETARY TREATMENTS

Testis*	Treatment 1 (Se = 50 ppb)	Treatment 2 (Se = 150 ppb)	Treatment 3 (Se = 300 ppb)
Weight (gm)	327.50 ± 51.74	316.16 ± 53.61	333.67 ± 57.09
Length (cm)	32.46 ± 2.55	32.26 ± 2.69	31.48 ± 1.57
Circumference (cm)	20.94 ± 1.15	20.62 ± 1.62	21.33 ± 1.69
Sperm Concentration x 10^9 (per gm of tissue)	0.13 ± 0.03	0.14 ± 0.07	0.14 ± 0.07
Sperm Concentration x 10^9 (in total testis)	40.57 ± 8.33	44.78 ± 27.25	49.19 ± 35.45
Seminiferous Tubule Dysfunction (0-4)	0	0.86 ± 1.07	0.57 ± 1.13
External Testes Width (taken prior to slaughter)	14.3 ± 1.51	13.31 ± 1.26	13.90 ± 1.61

*No significant differences ($P > 0.10$) among treatment groups for testicular parameters.

TABLE 9

LEAST-SQUARES MEANS ($\bar{x}$ ± SEM) OF SELECTED EPIDIDYMAL PARAMETERS MEASURED ON BOARS ON THREE DIETARY TREATMENTS

Parameter	Treatment 1 (Se = 50 ppb)	Treatment 2 (Se = 150 ppb)	Treatment 3 (Se = 300 ppb)
Caput-corpus epididymis			
Weight (gm)	51.94 ± 19.56	44.64 ± 10.05	54.3 ± 7.08
Sperm concentration x 10^9 (per gm of tissue)	0.74 ± 0.25	1.26 ± 0.63	0.81 ± 0.31
Sperm concentration x 10^9 (in total caput-corpus)	36.49 ± 12.53	56.15 ± 27.23	44.16 ± 19.24
Cauda epididymis			
Weight (gm)	41.77 ± 10.81	36.97 ± 6.22	37.33 ± 5.33
Sperm concentration x 10^9 (per gm of tissue)	2.26 ± 0.97	2.67 ± 1.32	2.07 ± 0.90
Sperm concentration x 10^9 (in total cauda)	101.26 ± 68.53	103.09 ± 61.16	79.90 ± 42.61
Percent abnormal sperm in cauda	41.14 ± 4.63	40.29 ± 6.16	40.57 ± 6.90
Epididymal epithelial height (microns)			
Caput	124.86 ± 68.16*	89.71 ± 11.13	74.43 ± 20.02*
Corpus	102.71 ± 11.3	92.57 ± 15.33	103.43 ± 26.41
Cauda	59.86 ± 20.00	61.71 ± 18.05	69.50 ± 19.82

*A significant difference ($P < 0.10$) among treatment groups specified.

resulting in midpiece breakage among epididymal rat sperm, as reported by Wu et al. (1969, 1979), did not occur within boars in this study. This would indicate that dietary selenium levels as low as 50 ppb were adequate to prevent this occurrence in young boars. There were also no significant treatment differences ($P > 0.10$) in measurement of bent spermatozoan tails, acrosomal faults, or the presence of cytoplasmic droplets.

Caput epithelial heights for those boars in t_3 were less ($P < 0.10$) than those in t_1. Martan and Risley (1963) and Martan (1969) proposed two events which control the height of epididymal epithelial cell layers. In some instances, spermatozoan concentrations within lumina become so congested that epithelial cells are stretched to thinner proportions. Since boars in this study demonstrated no differences ($P > 0.10$) in spermatogenesis or epididymal storage of spermatozoa, this does not appear to be the case. The other possibility concerns work done with rats, which links the availability of testicular androgens to epithelial height. Although the mechanism is unknown, it appears that rats possessing lowered levels of testicular hormones also exhibit shortened epithelial height. Although boars in this study were producing essentially the same amount of testosterone at the time of slaughter, the lag appearing in androgenesis for t_3 early in the study might have temporarily retarded epithelial heights within capita for an extended period.

D. CORRELATIONS AMONG POST MORTEM VARIABLES

A listing of selected correlation coefficients between selected variables is found in Table 10. Testicular weight was highly

TABLE 10

CORRELATION COEFFICIENTS BETWEEN SELECTED VARIABLES TAKEN AT SLAUGHTER

Variable	Testicular Weight	Testicular Sperm Concentration	Testis Width	Caput-Corpus Weight	Caput-Corpus Sperm Concentration	Cauda Weight	Cauda Sperm Concentration	Cauda Sperm Abnormalities	Epithelial Height	
									Caput	Corpus
Testicular Weight	1.00000	0.49837[a]	0.78541[b]	0.63194[b]	0.34419	0.30682	0.31986	-0.27416	-0.00061	0.19979
Testicular Sperm Concentration		1.00000	0.31440	0.02656	0.55962[b]	0.22839	0.13401	0.30535	-0.15987	-0.07277
Testis Width (live measurement)			1.00000	0.49062[a]	-0.03832	0.46047[a]	0.26360	-0.36385[c]	0.24389	0.48400[a]
Caput-Corpus Weight				1.00000	0.20652	-0.07513	-0.09570	-0.22768	-0.16089	0.13166
Caput-Corpus Sperm Concentration					1.00000	-0.08854	0.22744	0.18204	-0.17333	-0.27418
Cauda Weight						1.00000	0.79635[b]	-0.29532	0.55577[b]	0.17865
Cauda Sperm Concentration							1.00000	-0.35834	0.51907[a]	-0.01968
Cauda Sperm Abnormalities								1.00000	-0.19729	-0.48663[a]
Caput Epithelial Height									1.00000	0.34004
Corpus Epithelial Height										1.00000

[a]Significant at ($P < 0.05$) level.

[b]Significant at ($P < 0.01$) level.

[c]Significant at ($P < 0.10$) level.

correlated ($P < 0.01$) with both caput-corpus weight ($r = 0.63$) and testicular width ($r = 0.78$) as measured on the live animal. Moderate correlation ($P < 0.05$) was also noted with testicular sperm concentration ($r = 0.50$), as previously reported by Pond and Houpt (1978). External measurement of testicular width, however, was unrelated ($P > 0.10$) to spermatozoan concentration in either testes or epididymal tissues. Although width was moderately correlated ($P < 0.05$) with the weight of capita-corporea ($r = 0.49$) and cauda ($r = 0.46$) epididymides, it cannot be linked with spermatozoan concentration. This seems to indicate that the testicular measurement of live boars, as a means of predicting rate of spermatogenesis or spermatozoan reserves, is not adequately represented by a simple measurement of width. Mahone et al. (1979) reported similar findings and demonstrated that a composite measurement, combining testicular width and height, was an excellent indicator of spermatozoan numbers.

In view of the correlations among post-mortem data, it would seem that the deficiencies in reproductive development seen over time could no longer be illustrated by the parameters examined. If any retardation of sexual development occurred during the course of the experiment, it appears to have been specific to a short period (100-200 days) immediately preceding sexual maturity.

CHAPTER V

SUMMARY

The objective of this study was to examine the relationship between three levels of dietary selenium and their effects on growth and selected reproductive parameters in young crossbred boars. Criteria selected for investigation during the course of treatment included: body weight, testicular width, libido, and circulating plasma testosterone levels. Semen was collected from boars at regular intervals during the study and inspected for certain spermatozoan anomalies, using both bright-field and phase-contrast microscopy. Seven animals of each group, from which ejaculates were collected, were slaughtered by exsainguination at approximately nine months of age. Measurements were taken of testes and epididymal segments, and histological sections were cut and microscopically examined. Spermatozoa within caudae epididymides were subjected to morphological appraisal.

The regression of body weight, libido, and testicular width for 33 boars over the course of this study were all found to display linear relationships among treatment groups. Testosterone levels for the 21 boars slaughtered were fitted to the quadratic. Analysis of the relationships among body weight, libido, and testosterone levels indicated that differences ($P < 0.01$) existed between treatments, as analyzed over time. Testicular width, as measured on the live animal, was also found to be different ($P < 0.05$) between treatments over time. Values for all four criteria were determined to be greater for boars in t_1 than for

those in t_2, which were each greater than those for t_3. Measurements of testicular width, taken on the live animal, were not correlated ($P > 0.10$) with spermatozoan concentrations in either testicular or epididymal tissue. It was postulated that a figure derived from both testicular height and width might prove more meaningful for determination of spermatozoan concentration in the live animal. No treatment differences were observed in either weights or sperm cell concentrations in testes or capita-corporea or caudae epididymides. Testicular lengths, weights, and circumferences were not different ($P > 0.10$) among treatments. Caudal epididymal spermatozoa, examined using both bright-field and phase-contrast microscopy, revealed no evidence ($P > 0.10$) of morphological changes due to treatment. Weights of caudae epididymides were highly correlated ($P < 0.01$) with concentration of total spermatozoa within caudae. Measurements of the width of epithelial layers, lining the lumens of capita epididymides, tended to be greater ($P < 0.10$) in t_1 than in t_3.

These results tend to suggest that young boars fed selenium at the levels of 150 or 300 ppb may exhibit some signs of retarded sexual development for a short period following weaning and preceding the onset of sexual maturity. Although it is unlikely that selenium toxicity occurred at the dietary levels in question, it might be speculated that such supplementation may act to create imbalances in the availability of other nutrients essential to reproductive development.

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