AN ABSTRACT OF THE THESIS OF

Daniel J. Drake for the degree of Doctor of Philosophy in Animal Science presented on May 20, 1988.

Title: Implications of Selenium Status for Various Metabolic and Performance Parameters in Beef Cattle.

Redacted for Privacy

Abstract approved: _____
James E. Oldfield

Metabolic and performance parameters of beef calves were compared between Se supplemented (Se) and control (C) dam treatment groups from known Se deficient cattle. Four trials were conducted at three locations involving 50, 36, 25 and 39 experimental dam/calf pairs. Se supplementation was either two 30 gram oral boluses (90% iron, 10% elemental Se) or intramuscular injection of sodium selenite (.055 mg Se/kg BW) and vit. E (.75 IU/kg BW) given to dams prepartum. Se boluses ($P<.01$) and injections ($P<.05$) increased calf whole blood Se at birth and about 90 days of age, but levels were similar at weaning. Calf whole blood Se at 90 d increased with dam Se levels, however calves had greater amounts of Se than their dams when dams were below 0.05 ppm Se. Serum levels for calves at birth and about 90 d were determined for GOT, GPT, LDH, alkaline phosphatase, bilirubin, urea

nitrogen, cholesterol, phosphorus, calcium, protein, albumin, immunoglobulins, and hemoglobin plus complete blood counts with differentials. Factors most consistently associated with Se treatment were GOT, GPT, LDH, total protein, albumin, hemoglobin, fibrinogen, and inorganic phosphorus. GOT, GPT, LDH, fibrinogen and hemoglobin were negatively related to Se levels. Total protein and albumin had a positive relationship with Se levels. Se treatment group calves had higher ($P<.03$) weight per day of age at weaning in one trial but not in two others. Gain at 90 d of age was similar for all treatments and trials. Regression models of WDA at 90 d using GOT, GPT, LDH, protein, albumin, hemoglobin, fibrinogen and phosphorus as variables had $R^2$ of .40-.88 and SE of .17-.40. Albumin ($P<.02$) and protein ($P<.03$) were more consistently significant in regressions than GOT, GPT or LDH. These trials demonstrate the difficulties with Se supplementation of beef cattle and the variable weight gain response. Se effects on protein and albumin serum levels and their significance on weight gain suggest possible important metabolic roles for Se in addition to its antioxidant relationship with GOT, GPT and LDH.

# Implications of Selenium Status for Various Metabolic and Performance Parameters in Beef Cattle

by

Daniel J. Drake

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed May 20, 1988

Commencement June 1989

APPROVED:

Redacted for Privacy

James E. Oldfield
Professor Emeritus Department of Animal Science

Redacted for Privacy

Steven L. Davis
Professor, Department of Animal Science

Redacted for Privacy

Lyle Calvin
Dean of Graduate School

Thesis presented May 20, 1988

Typed by Daniel J. Drake

ACKNOWLEDGMENT

Special thanks are extended to Ben B. Norman and Cooperative Extension, University of California for encouragement and sabbatical privileges to pursue this endeavor.

Dedication to my mother for the upbringing, teaching and confidence to follow through on my interests and ideas.

As a student of James E. Oldfield, I shall strive to reach and continue the standards of professional and personal conduct that he so quietly and unpretentiously, yet completely, conveys to those around him.

Recognition is also made to the faculty and students of Oregon State University for sharing this memorable time.

And finally, I want to express gratitude and appreciation for the understanding of my family during this project, and for their role in keeping life in perspective.

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## INTRODUCTION

Historically, biochemical attention to selenium was first focused on its toxicity, however since 1957 (Schwarz and Foltz, 1957) its nutrient essentiality has been recognized and practical deficiency situations involving it have been described in domestic livestock (Muth et al, 1958). Selenium deficiency symptoms have been identified in numerous animals, including mammal and avian species. These symptoms have been related to specific disease syndromes, such as cardiovascular problems like Keshan disease in humans, mulberry heart and white muscle disease in animals, pancreatic hypertrophy in chickens plus more general considerations taking the forms of reduced growth, impaired reproduction, lowered immune response and general unthriftiness. Depending on the severity and duration of the selenium deficiency, age of the individual, and surrounding environmental conditions, it may result merely in unsightly and unkempt appearance associated with slightly reduced performance of the individual, or in extreme instances may result in severe impairment, or mortality.

Selenium has been identified conclusively as a component of the enzyme glutathione peroxidase (GPX) which has antioxidant properties (Rotruck, 1973). This is the only well established metabolic role for selenium and is thought by some investigators to be responsible for most of the various symptoms that occur in its deficiency. Paradoxically however, most of an individual's total selenium content is not found in GPX (Hawkes et al, 1985a) which raises the question of alternate, metabolically active seleno compounds. Se has also been identified in numerous proteins, as components of cell membranes (Black et al, 1978; Hawkes et al, 1985b), associated with the mid-piece in spermatozoa (Wu et al, 1979) and possibly other structures (Whanger et al, 1973). Complete characterization of these proteins and elucidation of their functions have been difficult and knowledge of them is still incomplete. Moreover, a non selenium-dependent form of GPX exists (Lawrence and Burk, 1978) raising further questions as to the precise role of the seleno-glutathione peroxidase.

With specific reference to this study, selenium has been found to be an essential nutrient for beef cattle, as for many other domestic species and various methods of supplementation are available to prevent or reduce selenium deficiency in them. Under practical production

systems used in the United States, selenium supplementation of beef cattle poses some special challenges including: extensiveness of range cattle operations, which implies frequent inaccessibility of the animals, large variation in individual body size and variations in behavioral characteristics which cause problems in precise calculation of dosage, as well as the possibility of metabolic antagonism to selenium in natural feeds. There is some evidence that even when selenium-deficient cattle are adequately supplemented, according to presently-accepted standards, improved performance may not occur (Shirley et al, 1966; Allaway and Hodgson, 1964; Norman, 1983; Hidiroglou et al, 1987; pers. observ.) which suggests inadequate knowledge of dose-response relationships. In addition, the higher costs for conducting research with such large animals compound the problems associated with developing adequate scientific information regarding achievement of adequate selenium status for beef cattle.

The studies undertaken in this thesis were planned to add to existing knowledge of the metabolic relationships of selenium in beef cattle. Specifically, they examined responses of cows and their calves to approved levels of selenium supplementation in an area of known Se-deficient

soils in northern California. The selenium was administered by two routes: intramuscular injection, which provides a transitory increase in circulating selenium, and orally, via heavy intraruminal pellets which act over a longer period of time. Animal responses were measured in overall health and calf weight gains and also in terms of various biochemical parameters chosen to explore the involvement of selenium at several metabolic sites.

# REVIEW OF LITERATURE

## Occurrence of Selenium Deficiency

Virtually every continent has identified areas of selenium deficiency (Underwood, 1962; Allaway and Hodgson, 1964; Oksanen, 1967; Allaway, 1968; Mattsson, 1980). Selenium deficient areas are usually, but not solely, associated with soil of volcanic origin (Oldfield, 1972) and irrigation may aggravate the deficiency through leaching. Alternatively, soil selenium may exist in forms that are either unavailable to plants or poorly absorbed. For example, some Hawaiian soils contain higher total soil selenium than soils from South Dakota, yet while Dakotan soils produce plants with toxic levels of selenium, Hawaiian soils do not yield selenium toxic plants (Lakin, 1972). Fertilization with sulfur can lower plant selenium levels (Muth, 1955), however, the antagonism is apparently at the soil-plant level rather than at the animal level (Whanger et al, 1969). Phosphorus fertilization, even as superphosphate, can increase plant selenium concentration, perhaps through stimulating increased root tissue or through phosphorus replacing selenium in some reactions thereby making more selenium available for uptake by the plant (Carter et al, 1972) although stimulation of plant organic matter growth by phosphorus may result in actual reduced plant selenium concentration (Davies and

Watkinson, 1966). Animals, including humans, consuming food from selenium deficient soils, and especially animals like beef cattle which often consume food grown from a single location as their entire diet, are susceptible to selenium deficiency.

Selenium deficiency constitutes a worldwide problem and one of considerable magnitude in the United States. In 1970, the problem was estimated to cost $10,000,000 annually for sheep alone in the Pacific Northwest (Muth, 1970). Williams (1980) in Northern California and Koller et al (1983) in Idaho and Washington both identified a majority of beef cattle they randomly sampled and tested as selenium deficient.

## Nature of Selenium Deficiency Problems

A wide variety of selenium deficiency symptoms have been observed in numerous species. Symptoms vary by species and differences relate to both the species considered and the practical management or environmental conditions under which the animals are kept. Nonetheless, while specific symptoms may vary from species to species, many, if not most, selenium deficiency symptoms do occur across the various species involved with only the apparent magnitude and consequence of the symptom varying.

Selenium and Nutritional Muscular Dystrophy

The most widely recognized selenium deficiency symptom in cattle is a type of nutritional muscular dystrophy, frequently called white muscle disease. Nutritional muscular dystrophy is a degenerative dystrophy of skeletal and cardiac muscle, most commonly occurring in suckling beef calves up to the age of 3 months. Andrews et al (1968) described two forms: a congenital and a delayed nutritional muscular dystrophy. The congenital form often resulted in offspring born dead or dying within a few days of birth. Cardiac muscle with congenital selenium deficiency has extensive diffuse grayish-white discoloration while skeletal muscles are rarely affected. Conversely, with delayed onset selenium deficiency, skeletal muscles may be heavily involved. Body cavities have clear fluid with fibrin strands and the liver is congested. Calcification of the affected muscle fibers occurs. The two different forms of nutritional muscular dystrophy may occur at the same location and are likely variable responses to the same selenium deficiency with differences in the extent and physiological state at the time of selenium deficiency accounting for the symptom differences.

Jenkins and Hidiroglou (1972) summarized clinical signs of nutritional muscular dystrophy in lambs and calves as

variable depending on the extent of the tissues affected and extent of injury. Usual signs are stiffness or weakness, trembling of limbs, respiratory distress and elevated body temperature. Calves with severely degenerated skeletal muscles may not be able to suckle and may die of inanition. Secondary infections such as pneumonia are frequently found as is edema. The skeletal muscles commonly affected are those most active, such as diaphragm, intercostal, pelvic and hindleg muscles. Lesions are characteristically bilateral and symmetrical, with white striations.

Microscopically the lesions are seen as hyaline degeneration in beef cattle (Jenkins and Hidiroglou, 1972) and in pigs as myofibrillar lysis and disruption (Van Vleet et al, 1976). The thick muscle fibrils are somewhat more resistant, persisting longest. Additionally, disruption of mitochondria, sarcoplasmic reticulum and plasma membranes occurred in damaged myofibers. The basal lamina of the sarcolemma remained after destruction of the enclosed sarcoplasm and served as a start for regeneration.

Nutritional muscular dystrophy was associated with dietary selenium levels below 0.10 ppm (Oldfield, 1963;

Allaway and Hodgson, 1964) while whole blood selenium levels considered adequate to protect against selenium-responsive diseases are 0.01 ppm (Andrews et al, 1968; Anderson et al, 197), 0.02 (Andrews et al, 1976) and 0.05 (Hartley, 1967) in sheep, with 0.03 (Jenkins et al, 1974) and 0.04 (Williams, 1980) in beef cattle. In addition to low blood selenium, increased incidence of myopathy has been associated with spring turnout on range, and speculation has suggested a relationship with unaccustomed exercise, inclement weather (Allen et al, 1975) or high concentration of polyunsaturated fatty acids in new grass growth (McMurray and McEldowney, 1977; McMurray et al, 1980). Arthur (1981) established both a rise in serum linolenic acid and exercise as contributing factors associated with increased incidence of nutritional muscular dystrophy in calves at range turnout but could not eliminate the possibility of other unidentified factors.

Selenium and Weight Gain

Beef cattle, as well as other species, have shown reduced weight gains due to selenium deficiency and selenium responsiveness by increasing gain when supplemented (Allaway and Hodgson, 1964; Shirley et al, 1966; Andrews et al, 1968; Ammerman et al, 1980; Johnson et al, 1981; Koller et al, 1984; Morris et al, 1984). While numerous

studies have considered the relationship between selenium status and glutathione peroxidase (GPX), a Se containing enzyme functioning as an antioxidant, as responsible for the weight gain effects, no precise mechanism has been established for the decreased weight gain associated with selenium deficiency. The proposed antioxidant theory (Tappel, 1965; Hoekstra, 1975) has been assumed responsible but again no specific sites of action have been determined as critically significant toward reduced weight gain and the subsequent selenium responsiveness. Additionally, improved efficiency of feed conversion occurred in selenium supplemented rats, as compared to deficient rats (Ewan, 1976) and improved feed efficiency without increased weight gain has been observed in cattle (Norman, personal comm.).

Other growth and development symptoms associated with selenium deficiency include general unthriftiness (Ill thrift), weak calf syndrome and scours (diarrhea). Ill thrift has been best characterized in sheep and Andrews et al (1968) described it as varying from subclinical inability to maintain optimum growth rate to clinical unthriftiness leading to death. Affected lambs may thrive for several months only to show reduced weight gain then. Anorexia and diarrhea may be associated with it, and the fleece is harsh and dry. There is no

increase in SGOT (Serum Glutamate Oxalacetate Transaminase, renamed Aspartate Aminotransferase AST) levels. Necropsy findings are emaciation and osteoporosis with no characteristic microscopic lesions. Williams (1980) found cattle from Northern California ranches historically suffering from ill thrift to have blood selenium levels below 0.04 ppm, but no such association was found with weak calf syndrome. Kendall (1960) identified selenium deficient cattle in Northern California with scours as responsive to selenium supplementation by reduced scouring.

Selenium and Reproduction

Selenium deficiency effects have been observed in reproductive performance of both male and female bovines. Trinder et al (1969) observed a beneficial effect of selenium with vitamin E in reducing the incidence of retained placenta in dairy cattle. Blood selenium levels were lower in dairy herds with a higher incidence of retained placenta. Julien et al (1976) using a selenium and vitamin E injection significantly reduced incidence of retained placenta in four large Se-deficient commercial dairy herds. Andrews et al (1968) reviewed the conditions in New Zealand associated with reduced reproductive rate in ewes as early embryonic death; in cattle, reproductive performance was not affected when

they grazed with sheep suffering reduced reproductive performance. Williams (1980) did not find an association between selenium levels and either retained placenta or abortions in beef cattle. Differences in response between beef and dairy cattle may relate to components of the ration. Typical dairy rations contain corn silage that is often low in both vitamin E and selenium and perhaps ensiling factors that increase the rate of plasma selenium clearance may be involved (Reinhardt et al, 1978).

Spermatozoa from selenium deficient rats have shown reduced mobility and increased midpiece breakage (Wu et al, 1973, 1979) but it is unknown whether this is due to selenium-containing glutathione peroxidase (GPX). Selenium binding proteins in the range of 15-20,000 daltons have been identified in rat sperm (Calvin, 1978), bovine sperm mitochondria (Pallini and Bacci, 1979) and rat testis cytosol (McConnell et at, 1979). Calvin has suggested the function as "essential for proper assembly of the rat sperm tail". Radiolabeled selenium reaches a peak accumulation in whole semen in bulls (Smith et al, 1979) by 40 days after administration, and by 49 days in rams (Tripp et al, 1979). In bulls, the largest amount of selenium was retained in the epididymis and testis.

## Selenium and the Immune System

Selenium's involvement with the immune system has been measured in several different species experimentally and may be supported by its widespread use as a preventative and treatment for calf scours. Dietary supplements of either selenium, vitamin E or both did not reduce the incidence of mastitis in dairy cows but did shorten the duration of clinical symptoms (Smith et al, 1984). Norman and Johnson (1976) using a selenium/vitamin E injection observed increased antibody titers to Leptospira pomona vaccine in supplemented calves. Experimentally induced swine diarrhea was less prevalent in animals given dietary selenium and vitamin E supplementation (Teige et al, 1978). Increased responses to various antigens by selenium supplementation were seen in rats (Spallholz et al, 1973, 1974, 1975), swine (Peplowski et al, 1981) and rabbits (Berenstein 1972). Selenium supplementation increased weight gain of chickens exposed to coccidiosis (Eimeria tenella) whether they were immunized or not immunized (Colnago et al, 1984). Responses were variable depending on age (stage of development), sex and dose of antigen.

# Supplementation Methods

## Forms of Selenium

Selenium is commonly found in plants and plant products in organic forms such as selenocystine, selenocysteine,

Se-methylselenocysteine, selenohomocystine, selenomethionine, Se-methylmethionine, selenomethionine selenoxide, selenocystathione and dimethyl diselenide (NRC, 1983a). It appears that the primary selenium compounds consumed by livestock from natural plant foodstuffs are selenocystine, selenocysteine, selenomethionine, and Se-methylselenomethionine (Peterson and Butler, 1962; Shrift, 1969; and Olson et al, 1970).

Inorganic forms of selenium, usually as sodium selenite or selenate, are available, efficacious and approved for use in selenium supplements by various routes in several countries including the United States.

Gastrointestinal Actions and Absorption of Selenium

Absorption of selenium will vary with the animal species, selenium source and parameters used to measure absorption. Selenium absorption is greatly reduced in ruminants, as compared to simple stomached animals. Wright and Bell (1966) observed 77% retention of oral selenite in swine compared to 29% in sheep. Lowered absorption in the ruminant is apparently due to reducing actions by rumen microorganisms, which convert dietary selenium into insoluble forms. The conversion is greater for inorganic selenium than for organic selenium and is higher in high soluble-carbohydrate diets as compared to

high roughage diets (Cousins and Cairney, 1961; Peterson and Spedding, 1963; Whanger et al, 1968). While rumen microbes reduce the availability of selenium by forming insoluble forms, inorganic selenium can be metabolized to organic selenium compounds such as selenomethionine (Hidiroglou et al, 1968) by rumen microbes and organic selenium is incorporated into rumen microbe proteins (Paulson et al, 1968).

It is likely that dietary organic forms of selenium that escape the rumen are absorbed from the small intestine, while dietary inorganic forms of selenium may have at least three fates: 1.) formation of insoluble compounds that are not absorbed, 2.) incorporation into microbial organic compounds that are acted upon like dietary organic selenium forms or 3.) passage into the small intestine as inorganic forms such as selenite or selenate with subsequent gastrointestinal actions.

Gastric absorption of selenium has not been noted in sheep, swine or rats. Primary sites of absorption appear to be the small intestine, plus cecum and colon, in sheep, swine (Wright and Bell, 1966) and rats (Whanger et al, 1976).

Transport of selenium compounds across intestinal cell membranes has been studied *in vitro* using everted intestinal sacs of hamsters (McConnell and Cho, 1965). Selenomethionine was transported against a concentration gradient whereas selenite and selenocystine were not. The transfer of selenomethionine was inhibited by methionine but the sulfur analogs of selenite and selenocystine did not inhibit their transport.

## Efficacy of Different Forms of Selenium Supplementation

The efficiency of supplementation of animal diets with various forms of selenium is variable and depends on the parameter for determining efficiency, as well as the animals' physiological condition and previous selenium status. Selenium in alfalfa and milk proteins (Mathias et al, 1965) was comparable to sodium selenite for prevention of liver necrosis in rats but milk Se was superior for prevention of exudative diathesis (Mathias et al, 1967). Miller et al (1972) also found differences in effectiveness of various forms of selenium for prevention of exudative diathesis, decreasing from selenomethionine to selenite and fishery products. Similarly, Cantor et al (1975a) found selenomethionine more effective than sodium selenite for prevention of pancreatic fibrosis in the chick.

Consistently higher tissue selenium levels have been observed in cattle, sheep, swine and poultry when organic selenium has been fed as compared to sodium selenite. Selenium from grain was associated with higher muscle, liver, kidney and plasma contents than selenite when both were fed at 0.2 or 0.3 ppm in the diet of sheep and cattle (Ullrey et al, 1978). Mahan and Moxon (1978) found that when swine were fed selenium from different sources at 0.4 ppm, muscle selenium content was higher from fish meal or brewers' grains than from selenite but nonmuscular organ tissues, liver, kidney and testes, were similar. Organic forms of selenium such as selenomethionine, have also been more effective than selenite in raising tissue Se concentrations of muscle, liver and eggs of poultry (Latshaw and Osman, 1975; Osman and Latshaw, 1976; Latshaw and Biggert, 1981).

## Selenium and Glutathione Peroxidase

Selenium supplementation has also been shown to increase tissue glutathione peroxidase (GPX) levels in rats, chicks, sheep, cattle, mice, horses, swine, Japanese quail, deer and salmon (NRC, 1983b). Humans have also shown a correlation between blood selenium and GPX levels when consuming low selenium diets (McKenzie et al, 1978) but on adequate or high dietary levels of selenium the correlation disappears (Schrauzer and White, 1978). In

pregnant women, while blood selenium and GPX levels were not correlated (Butler et al, 1980, 1982) and Koller et al, (1984) reported sequestering of selenium by the bovine fetus, particularly at low selenium levels. The relationship between tissue GPX and dietary selenium has been reported by some as logarithmic (Omaye and Tappel, 1974; Smith et al, 1974), while others (Hafeman et al, 1974; Oh et al , 1976a,b) have reported tissue GPX plateaus (sigmoidal) at about 0.1 ppm and 0.5 ppm (Sunde et al, 1981) in the ration except for erythrocyte and pancreatic GPX.

While organic forms of selenium appear superior to inorganic forms in increasing tissue selenium levels, selenite is at least equal to or perhaps superior at low levels, in increasing GPX levels. Selenium given as a single large oral dose of selenite or selenomethionine to rats (Pierce and Tappel, 1977) and to chicks (Omaye and Tappel, 1974; Cantor, 1975b) resulted in similar increases in liver, kidney, and small intestine GPX activity. Noguchi et al (1973) found that at lower levels of selenium, 0.1 ppm dietary Se, selenite was twice as effective as selenomethionine in raising plasma, liver and heart GPX in the chick. In selenium deficient rats, Sunde et al (1981) found selenite and Se-methionine equal in biopotency to increase tissue GPX. Dietary

levels of methionine did not affect selenite biopotency but did alter Se-methionine biopotency. At dietary selenium levels of 0.1, 0.2 and 0.5 but not 1.0 ppm Se, the addition of methionine to the ration increased the biopotency of Se-methionine to equal that of selenite. This suggests that adequate dietary methionine is important for the utilization of organic selenium sources such as may be found in plants. McConnell and Cho (1967) have shown that Se-methionine and methionine compete for the same intestinal transport system in the hamster and factors that influence gut transport of methionine would presumably affect Se-methionine transport similarly. Methionine is transported by the sodium gradient dependent transporter called system A, which has been identified in liver, muscle, erythrocytes and salivary glands, showing evidence of adaptive regulation (i.e. substrate dependent repression and derepression acting at the level of gene transcription) and some trans-inhibition (intra-cellular concentrations of the carried molecules inhibit further transport).

## Supplementation Methods

Practical methods of providing additional selenium to that occurring naturally in the diets of beef cattle have included both parental and oral routes. Oral routes

include supplying selenium through water-solution drenches, as part of salt or feed mixes, or in the form of boluses and pellets that remain in the rumeno-reticulum area, slowly releasing selenium to the gastrointestinal tract.

Sodium selenite is the selenium form most frequently used in parenteral administrations. Experimentally, parenteral solutions may be prepared which contain solely a source of selenium, however commercially available products commonly contain mixtures of selenium salts and vitamin E. For example, Mu-Se[1] contains 5 mg/ml of selenium as sodium selenite and 68 international units/ml of alpha tocopherol.

In terms of efficacy of oral selenium supplementation, selenium added to the diets of deficient beef cows either as sodium selenite or organically in linseed meal (1186 ppb Se) increased selenium in the plasma, milk and liver of cows and plasma, muscle and liver of their nursing calves (Ammerman et al, 1980). In the same study, linseed meal diets produced higher selenium levels in calves (but not cows) than comparable amounts of selenium supplied in soybean meal plus sodium selenite. Milk

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[1] Burns Biotec, Inc.

selenium levels were increased shortly after calving but by 2 and 8 weeks after calving continued dietary selenium supplementation did not further increase milk selenium content. In goats, given intravenous jugular injection of radiotracer selenium Allen and Miller (1981) found radiation counts in milk peaking two hours after peak plasma $^{75}Se$ concentration. Selenium was primarily associated with the casein in milk of goats(Allen and Miller, 1981) and cattle (Yoshida et al, 1981). Seven days after dosing, both kidney and liver had higher selenium levels than mammary gland. The investigators suggested major changes in selenium metabolism affecting selenium concentration in milk occurs near parturition. In ewes parenterally supplemented with barium selenate, milk selenium levels were twice as high just prior to lambing as they were the first day after lambing and one week after lambing (Overnes et al, 1985), which also suggested changes in selenium metabolism near parturition.

Gleed et al (1983) used sodium selenate injections subcutaneously at 0.15 mg Se/kg bodyweight (BW) and observed increased serum selenium and GPX in Hereford/Friesian and Charolais steers. No breed effects on response were observed. Similarly, Thompson et al

(1980) using 0.1 mg Se/kg BW with nine month old calves found rapid increases in serum and liver selenium levels with an exponential decline with half-lives of 22.1 and 28.3 days, respectively. Whole blood selenium levels responded similarly but declined more slowly. Selenium concentration and GPX activity in erythrocytes increased more slowly and remained elevated for several months after liver and blood Se concentrations had declined. Injections of 0.1 mg Se/kg BW every two months were found necessary to maintain calves in good health on selenium deficient forage (0.018 mg Se/kg forage). Calves injected with 0.078 mg Se/kg BW and 5.4 International units of vitamin E/kg BW at birth had serum Se levels that were 87% higher at day 14, but 2 injections (double the dose) only produced serum Se levels that were 10% higher than non-treated controls by day 28 (Weiss et al, 1983); indicative of a non-linear response. A lag of 4-5 weeks occurred before erythrocyte GPX activity increased. Decline of serum selenium in calves not given supplemental selenium was linear from birth to 56 days of age.

Parenteral preparations of barium selenate have been found efficacious in elevating blood selenium concentrations in cattle and sheep for six months to two years at rates of approximately 1.2 mg. Se/kg. BW (Cawley

and McPhee, 1984; Overnes et al, 1985).

Scholz et al (1981) investigated the effects of oral selenium on Se concentration and GPX activity in various tissues and their responses to varying levels of supplementation. Calves from birth to twelve weeks of age were fed graded levels of selenium (0.03, 0.23 and 0.53 microgram Se/gm of solids) in whole milk diets. Se concentrations on a fresh tissue-weight basis were highest in kidney cortex, intermediate in kidney medulla, testes, liver and spleen and lowest in striated muscle, adipose and blood plasma. Se concentrations were increased with increased dietary selenium in liver, kidney cortex, spleen and heart but not in testes and adipose tissue. Glutathione peroxidase activity measured by hydrogen peroxide protection was highest in erythrocyte and testes and lowest in thymus, brain, striated muscle, adipose tissue and plasma. Marked differences were noted among tissues in GPX activity whether measured by hydrogen peroxide or cumene hydroperoxide, particularly in liver, lungs and adrenal glands. Arthur (1981), who supplemented calves on a Torula yeast-based diet with 0.1 mg Se/kg BW as sodium selenite found similar tissue selenium concentrations. Tissue selenium increases and GPX activity was highest in

kidney, followed by heart, liver, triceps muscle and semitendinosis muscle. Increased Se concentrations were also found in kidney, liver, seminal vesicle and testis, but not caput, corpus and cauda epididymus in yearling bulls injected with 50 mg. Se initially and 30 mg. monthly thereafter for 150 days (Segerson and Johnson, 1980). Additionally, extended whole semen (diluted with extenders; concentration was not adjusted for dilution) was higher in Se and apparently the Se was associated with the supernatant and not the sperm pellet fraction after centrifugation.

Oral supplementation of sheep with selenium results in the same general pattern of response as seen in cattle as reviewed by Andrews et al (1968) with highest concentrations in kidney cortex, pituitary and adrenals, intermediate levels in liver and relatively low levels in muscle, bone, adipose tissue and blood.

Research with sodium selenite in salt mixes indicates increased and adequate blood selenium concentration and GPX activity when offered for free choice consumption at 90 ppm but not at 20-30 ppm Se ( Williams, 1980; Hathaway et al, 1981; Koller et al, 1983). Supplementation of first calf Hereford heifers on alfalfa hay rations with

either soybean meal (0.313 mg Se/kg) or 90 ppm sodium selenite salt resulted in whole blood selenium concentrations of 0.250, 0.162 and 0.052 ppm in the dams at parturition, with corresponding calf levels of 0.242, 0.175 and 0.81 ppm, indicating some sequestering of selenium in utero when the dam is relatively low in selenium (Koller et al, 1984). Glutathione peroxidase activity showed a similar response to supplementation of the dam.

Sodium selenate was added to drinking water of first calf Santa Gertrudis heifers to receive 0.5, 1.0 or 2.0 mg. Se per day (Morris et al, 1984). Blood selenium concentration was significantly increased and rectilinearly related to the level of selenium supplementation. However after one year of continued supplementation, the levels in all supplemented animals were similar and about six times higher than control (deficient) animals. Supplementation at 1 mg selenium per animal/day resulted in blood Se levels generally considered adequate (5-8 microgram/dl).

Hidiroglou et al (1971; 1972) reported that use of subcutaneous silastic and magnesium stearate implants in sheep and calves increased their blood selenium concentrations.

Pellets of various selenium compounds with high-density carriers, frequently iron, given orally and remaining in the reticulum or rumen, have been shown effective in increasing tissue selenium levels (Kuchel and Buckley, 1969; Judson et al, 1980; Hudson et al, 1981; Hunter et al, 1981; Judson and McFarlane, 1984). Selenium sources included calcium selenate, barium selenate and elemental selenium (Kuchel and Buckley, 1969) and of these, elemental selenium (10%) pellets with iron granules (90%) are now available commercially (ICI, Australia). Pellets containing radiolabelled selenium have resulted in detectable amounts of selenium in plasma by 7 hours after administration (Handreck and Godwin, 1970). Over half of the total plasma selenium was bound to globulin and albumin with the predominant amount on globulin. Tissue distribution and urinary excretion of labelled selenium from the pellets followed the same patterns as seen with oral sodium selenite supplementation. Hunter et al (1981) observed maximum plasma Se concentrations 2 weeks after administration of either elemental Se pellets or oral sodium selenate, however the response to selenate declined rapidly thereafter while the pellets maintained plasma selenium concentration for 1 year in sheep. The authors found differences in actual selenium concentrations among 3 brands of pellets tested but all maintained adequate plasma selenium levels for 1 year. A

major factor in the long-term effectiveness of rumen pellets for sheep is granule size of the selenium (Hudson et al, 1981). In these studies, commercial sources of pellets varied in granule size from 4 to 40 microns and the larger granule selenium pellets maintaining adequate selenium levels for longer periods of time. After 28 days only a small percentage of elemental Se remained in pellets composed of small grain selenium, while approximately 50% remained in coarser grained pellets. It was postulated that the rapidly released selenium occurred as a result of iron oxidation and concomitant alteration of elemental selenium to iron selenide. Selenide is the most reduced state (-2) of selenium (NRC, 1983c). Subsequent work (Peter et al, 1981) with various non-Se and Se/Fe pellets has suggested electrochemical reactions as the likely events leading to release of selenium from the pellets and establishment of an anode/cathode relationship between pellets. Grinding or other physically abrasive actions were not major factors for the release of selenium. However, the processes appear complex and precise chemical forms of selenium released and subsequently absorbed by the animal are still unknown.

Supplementation of livestock through pasture fertilization has also been successful. Sprays of sodium

selenite (Grant, 1965) or selenious acid (Davies and Watkinson, 1966) or top dressing with superphosphate containing sodium selenate (Grant, 1965), elemental selenium (Grant, 1965; Watkinson and Davies, 1966a) and selenite with sodium, barium, iron or zinc (Grant, 1965; Davies and Watkinson, 1966b; Hartley, 1967) have proven efficacious.

## Metabolic Roles for Selenium

Selenium as a component of GPX has been clearly linked as an antioxidant (Rotruck, 1973; Hoekstra, 1975) but perhaps the significance of oxidants in physiology have been less clearly delineated. Background information on biological oxidants may prove useful in examining the precise function of GPX and its possible involvement in animal performance and selenium deficiency symptoms.

Oxidation may be defined as the removal of electrons and reduction as the gain of electrons. It follows then that oxidation is always accompanied by reduction of an electron acceptor. Peroxidation (or auto-oxidation) of lipids exposed to oxygen is responsible not only for rancidity of foods but also for damage to tissues in vivo (Mayes, 1985). The molecular precursor for the initiation process is generally hydroperoxide (ROOH)

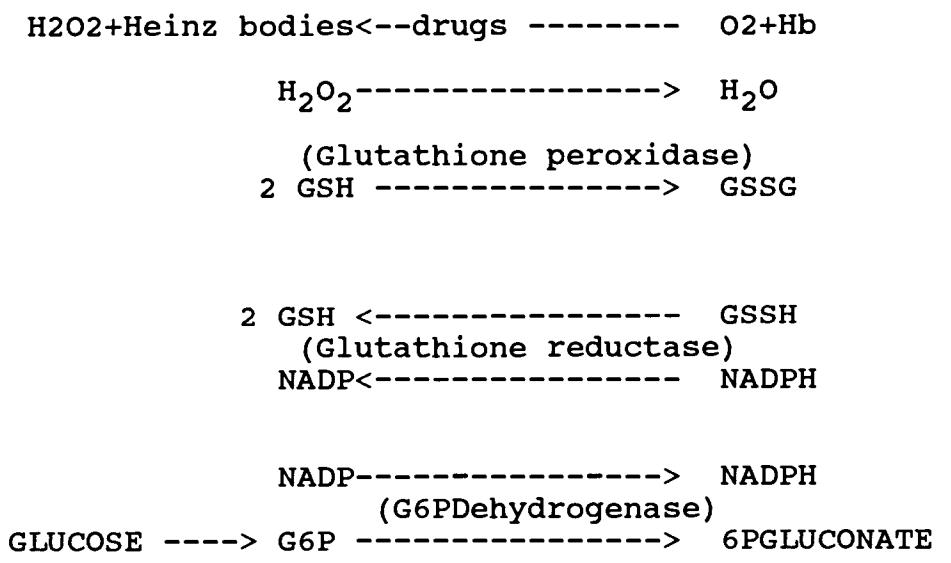
forming radicals (ROO, RO, OH), stimulating a chain reaction of lipid peroxidation with potentially damaging biological consequences. Peroxidation is also catalyzed in vivo by heme compounds and by lipoxygenases found in platelets and leukocytes. Hydrogen peroxide will be formed as the result of aerobic dehydrogenases (flavoproteins) which remove hydrogen from a substrate with hydrogen peroxide formed as the product.

A number of compounds, both natural and man-made, reduce lipid peroxidation such as butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) which are food additives and natural antioxidants, vitamin E, catalase, superoxide dismutase and GPX among others.

Enzymes utilizing hydrogen peroxide or an organic peroxide as a substrate are hydroperoxidases and two of these types are peroxidases found in milk, plants, leukocytes, platelets and erythrocytes; and catalase, found in blood, bone marrow, mucous membranes, kidney and liver of animals.

Glutathione peroxidase was shown in 1973 to be a selenoenzyme (Rotruck et al, 1973) and its role as an antioxidant can at least partially explain observed selenium deficiency symptoms (Hoekstra, 1975).

Glutathione peroxidase was discovered by Mills (1957), who found the enzyme in the presence of reduced glutathione would protect erythrocytes against hydrogen peroxide induced hemoglobin oxidation and hemolysis. Rotruck et al (1971; 1972) correctly identified glucose as a requirement to replenish glutathione for selenium to be protective against hemolysis, while vitamin E had no glucose requirement. Additionally glucose had no protective effect if the erythrocytes were from selenium deficient animals. The generally accepted reaction scheme for GPX as might occur in the erythrocyte is:




Where abbreviations are: GSH-reduced glutathione, GSSG-oxidized glutathione, G6P-glucose-6-phosphate and G6PD-glucose-6-phosphate dehydrogenase.

Oxidants from a variety of sources in the erythrocyte including the oxygen it carries can result in hydrogen peroxide and/or oxidant damage of hemoglobin seen as, for example, Heinz bodies. Oxidants are catalytically removed by GPX, if it is present in sufficient quantities, while glutathione is recycled with glucose 6 phosphate dehydrogenase (G6PD) providing adequate quantities of NADPH. As an indication of the importance of these linked pathways, in the mature erythrocyte which does not have mitochondria, the major role of the pentose phosphate pathway (PPP) is the generation of NADPH, required in the antioxidant protection provided by GPX (Kaneko, 1980). Several human and animal erythrocyte pathologies have been related to G6PD deficiency and Heinz bodies have been observed as a result of selenium deficiency in cattle (Morris et al, 1984). When considering the role of GPX in the erythrocyte it is further interesting to note two of the three major metabolic activities of the erythrocyte are directly linked to the function of GPX in the erythrocyte. These are maintenance of the cell membrane and its deformability, which is vital to its ability to transverse the miles of capillary beds, and maintenance of reducing potential in the form of NADPH to protect hemoglobin and enzyme protein from oxidative denaturation (Kaneko, 1980).

Glutathione peroxidase has been purified from several species and shown to consist of four identical subunits (Flohe et al, 1973; Ganther et al, 1976). The molecular weight of glutathione peroxidase varies between species and among tissues within a species but generally ranges from 76,000 to 95,000 (NRC, 1983d). Glutathione peroxidase contains no metals besides selenium of which it contains 4 gm atoms of Se per mole of GPX and in contrast to other peroxidases contains no heme or flavin groups (Flohe et al, 1973).

The selenium present in GPX is found as selenocysteine or a selenocysteine derivative (Forstrom et al, 1978; Wendel et al, 1978; Ladenstein et al, 1979) and is located with the polypeptide chain (Zakowski et al, 1978). The selenium atoms are arranged on the surface and due to their separation (21 Angstroms in a dimer) suggest only 1 selenium atom is present per active site. However, the number of active sites per tetramer has not been established nor whether all four selenium atoms are active catalytically (Ladenstein et al, 1979). Evidence in the rat suggests selenocysteine incorporation into glutathione peroxidase via a translational pathway (Hawkes and Tappel, 1983).

Glutathione peroxidase is quite specific for glutathione as its donor substrate, however, GPX will destroy a variety of organic hydroperoxides at rates similar to those for hydrogen peroxide, which is in contrast to catalases' specificity for hydrogen peroxide (Little and O'Brien, 1968). In light of this, assays for GPX have used either hydrogen peroxide, cumene hydroperoxide or t-butyl hydroperoxide. But due to possible presence of GSH S-transferases that have GPX activity with organic hydroperoxides (Lawrence and Burk, 1976; Prohaska and Ganther, 1976; Prohaska and Ganther, 1977), hydrogen peroxide is the recommended assay material. Lawrence et al (1978) termed the GSH S-transferase activity, non-Se dependent GPX since it has glutathione peroxidase activity towards organic peroxides, but not hydrogen peroxide, and does not contain selenium thus does not decreased with selenium deficiency.

Glutathione peroxidase activity, like selenium concentration, is not uniform between tissues nor species. In young calves, only Se-dependent glutathione peroxidase activity was found in spleen, cardiac muscle, erythrocytes, brain, thymus, adipose, striated muscles, uterine endometrium, intestinal epithelium, rumen epithelium and mesenteric, prescapular and prefemoral lymph nodes. Whereas both Se-dependent and non-Se-

dependent glutathione peroxidase activity was found in liver, lungs, adrenal glands, testes, kidney medulla and cortex (Scholz et al, 1981). Activity of glutathione peroxidase in erythrocytes, heart and spleen were 3, 10 and 12 times higher, respectively, than striated muscle glutathione peroxidase activity. The implications of this are a potential lack of oxidant protection in tissues, such as cardiac and striated muscle due to depletion of Se-dependent GPX as during Se deficiency and the concomitant lack of glutathione S-transferase to provide additional non-selenium glutathione peroxidase activity. In contrast liver has only approximately 25% of its glutathione peroxidase activity in the form of Se-dependent GPX.

Selenium supplementation has been shown to increase GPX activity above unsupplemented deficient control animals for several species including ruminants such as cattle (Anderson et al, 1979; Thompson et al, 1981) and sheep (Godwin et al, 1975, Oh et al 1976a,1976b; Whanger et al, 1977, 1978). Thompson et al (1981) found a correlation of 0.97 between blood selenium concentration and erythrocyte GPX activity from mixed age cattle on pasture of varying selenium content. Thompson et al (1976) in earlier work found correlations of 0.92, 0.59 and 0.27 for sheep, cattle and swine indicating lack of agreement

between trials and species. It should be noted that the earlier work used glutathione peroxidase activity in whole blood which may account for additional GPX activity from the plasma, however, the authors assayed for plasma glutathione peroxidase activity and did not feel it interfered. Presumably the reduced correlation was due to Se in compounds other than GPX. Furthermore careful examination of their later (1981) graphs show the relationship between Se concentration and glutathione peroxidase activity is much closer at selenium levels below 0.05 ppm whole blood Se. Response to sodium selenite was variable in two trials with dairy heifers receiving 5 mg. of selenium. In the first trial glutathione peroxidase activity per gm hemoglobin increased only 16% compared to a 167% increase in a second similar trial. Decline in activity reached deficient control values within 12-14 weeks (Hoffman et al, 1978). Yearling cattle showed a strong correlation (r=0.912) between selenium concentration and GPX activity per erythrocyte (Allen et al, 1975), however about four months elapsed from the start of supplementation and sampling so no evidence is available regarding the rapidity of response. Thompson et al (1981) found 6 weeks were required before erythrocyte GPX increased although liver and plasma activities increased by 4 weeks.

Studies on the biopotency of various selenium compounds for glutathione peroxidase have largely been conducted with rats and chicks. Generally selenite has shown greater biopotency for increasing GPX activity, especially at lower levels of supplementation. At higher selenium supplementation rates (about 0.2-0.3 or greater) selenomethionine and selenite have similar abilities to increase GPX. Considering the positive correlation between selenium concentration and glutathione peroxidase activity, during selenium deficiency the importance of non-Se dependent GPX (Glutathione-S-transferase) particularly in tissues such as liver with relatively large proportions of glutathione S-transferase, may be important. Glutathione S-transferase activity increases during selenium deficiency in the rat (Lawrence et al, 1978; Masukawa et al, 1984). This could afford oxidant protection to the liver during periods of selenium deficiency that resulted in reduced GPX activity and oxidant stress. It is of interest, however, that glutathione S-transferase will bind to non-substrate ligands that inhibits its activity (Ketley et al, 1975) and for the pathologic liver compounds such as bilirubin and hematin could act as inhibiting non-substrate ligands rendering the glutathione S-transferase less able to act with glutathione peroxidase activity. Burk and Correia (1978) have suggested selenium may have a role in

regulation of heme catabolism based on evidence of increased heme catabolism in selenium deficiency following phenobarbital injection. A possible cascade of events in the liver during selenium deficiency might include: 1.) decreased Se-dependent GPX, 2.) increased glutathione S-transferase affording oxidant protection, 3.) alterations in heme catabolism by low Se, and 4.) non substrate ligand inhibition of glutathione S-transferase (for further discussion see non-antioxidant actions).

Selenium, GPX and Other Antioxidants

Early work with selenium deficiency identified a relationship with vitamin E that has become more clear with further research. Increased knowledge of seleniums' antioxidant role has also lead to an integrated scheme for a variety of physiological antioxidants. Currently antioxidants such as vitamin E, glutathione peroxidase, catalase and glutathione S-transferases, have proposed roles that are distinct and complementary based upon cellular compartmentalization, enzyme kinetics and substrate affinities.

Vitamin E, being lipid soluble, reacts with free radicals and possibly singlet oxygen before they can attack cellular and intracellular membranes (McCay et al, 1978). Due to lipid solubility this action is thought to be

principally at the cell membrane. In the cytosol and mitochondrial matrix space glutathione peroxidase removes hydrogen peroxide and organic hydroperoxides, whereas catalase destroys hydrogen peroxide in peroxisomes. And superoxide dismutase degrades superoxide in the cytosol and mitochondria before superoxide can react with hydrogen peroxide to form hydroxy radical (NRC, 1983e).

GPX action appears limited to cytosolic and mitochondrial space due to its limited reaction with peroxidized phospholipids typically found in membranes. However, Grossmann and Wendel (1983) have suggested an interaction between GPX and phospholipase where phospholipase precedes GPX by an initial reaction with peroxidized phospholipids making them susceptible to GPX. This interaction between the two enzymes may confer some membrane protective function to GPX. This linking of actions has only been demonstrated in vitro and whether it occurs in cells and to what extent the reactions may provide oxidant protection, in vivo, have not been documented.

Glutathione peroxidase exhibits similar reaction rates to hydrogen peroxide and various organic peroxides including lipid, steroid, thymine, nucleic acid, and prostaglandin

hydroperoxides in contrast to catalases' specificity for hydrogen peroxide (Little and O'Brien, 1968). Glutathione S-transferases have glutathione peroxidase activity only toward organic peroxides and not hydrogen peroxide (Prohaska and Ganther, 1977) and Lawrence et al (1978) suggest hydrophobic peroxides are favored such as cumene hydroperoxide over t-butyl hydroperoxide. Additionally their work is consistent with an ordered enzymatic reaction for glutathione S-transferase compared to a ping-pong mechanism for glutathione peroxidase.

Vitamin E while complementary to those antioxidants previously discussed plays a somewhat different role in preventing formation of oxidants and particularly at membrane sites due to its lipid solubility. In the chain reactions of lipid peroxidation, vitamin E has the ability to transfer a phenolic hydrogen to a peroxyl (ROO·) radical with the phenoxy radical being stable and relatively unreactive (Martin, 1985b). In this manner vitamin E functions as a chain breaking antioxidant. In spite of this action some peroxides are formed and the organism has secondary lines of defense.

Acting against free radicals, such as superoxide, along with vitamin E is superoxide dismutase. Superoxide dismutase acts to join two superoxides with hydrogen to

form hydrogen peroxide and oxygen. Removal of the superoxide acts as a chain breaking step in the peroxidation process. Superoxide is formed when reduced flavins (ex. aerobic dehydrogenases like xanthine dehydrogenase) are reoxidized univalently by molecular oxygen (Martin, 1985b).

Non-Antioxidant Functions of Selenium

In addition to seleniums' role as an antioxidant, evidence suggests other possible functions for the element.

While glutathione peroxidase is the only proven selenoenzyme in animals, GPX accounts for only a minor amount of selenium in an animal. Recent work in Tappel's laboratory (Hawkes et al, 1985a, 1985b), and at least partially confirmed by Behne and Wolters (1983), indicate the distribution of selenoprotein in the rat and suggests some other still unknown role for selenium. Their work with radiolabelled selenium showed skeletal muscle, liver and blood accounted for 73 % of the total body Se and 95% of the Se-dependent glutathione peroxidase. Of the total body Se over 80 % was as selenocysteine but only one third of the total Se was accounted for in glutathione peroxidase. The presence of two thirds of the total body

Se in non-glutathione peroxidase, selenocysteine containing proteins suggests that there may be other important selenoproteins besides glutathione peroxidase.

Fifty one percent of the total selenium was in the soluble (essentially the cytosol) portion of liver, kidney and testes, the major selenium containing organs in the rat. And GPX was primarily found in the soluble fraction as expected of a cytosolic enzyme. However, of the remaining total selenium, 48% was associated with particulate portions and of that fraction only one third was GPX. Thus glutathione peroxidase was found in plasma membranes particularly of the liver, mitochondria in liver and kidney and microsomes of the testes. Katki and Myers (1980) have also identified a selenium containing glutathione peroxidase in liver and cardiac mitochondrial membranes. These results indicate that other selenium containing proteins besides glutathione peroxidase are present in membranes.

> "Since the non-glutathione peroxidase, selenium containing proteins accounted for the majority of the particulate selenium, it seems reasonable to expect that they have important roles in membrane biochemistry."
>
> Hawkes et al, 1985b

Behne and Wolters (1983) using different methodology (neutron activation) also found less than 40 % of the total body selenium of rats associated with glutathione

peroxidase, basically agreeing with the work from Tappel's lab of 33 %. Similarly, Beilstein et al (1981) found selenocysteine as the major form of Se in ovine heart and liver. Furthermore evidence from Tappel's lab suggests the selenocysteine was present in protein in a stable bond (amide or peptide bonding) and it was a relatively strong bond, suggesting more than an accidental or coincidental presence of the selenocysteine. Occurrence of a specific tRNA for selenocysteine in rat liver (Hawkes et al, 1982, 1983) also seems unlikely if only a single selenoenzyme, glutathione peroxidase, is produced.

Other work with rats indicates that the chemicals paraquat and diquat are toxic resulting in lipid peroxidation and liver necrosis. Selenium deficient rats were much more susceptible to lipid peroxidation as measured by ethane production and liver necrosis, measured histochemically, than control selenium adequate rats (Burk et al, 1980). Interestingly when selenium deficient rats were given supplemental selenium parentally, within 10 hours after receiving the selenium significant protection was provided toward diquat lipid peroxidation and mortality. This was in spite of no rise in glutathione peroxidase activity in liver, kidney, lung or plasma at 10 hours. While this evidence does not

preclude isolated, localized increases in GPX activity, it does suggest a selenium dependent factor in addition to glutathione peroxidase that protects against lipid peroxidation and liver necrosis.

Phagocytes, cells involved in the inflammatory and immune response, secrete a variety of arachidonic acid metabolites which are known to possess chemotactic and immunoregulatory properties. Selenium deficiency has been shown to differentially effect some of these pathways (Bryant and Bailey, 1980; Gairola and Tai, 1985). Bryant and Bailey (1980) found selenium deficiency decreased the activity of the 12-lipoxygenase pathway (pathway for leukotriene synthesis) and not the cyclooxygenase ($TXB_2$) pathway (prostaglandin synthesis), with Gairola and Tai (1985) observing the 5-lipoxygenase pathway (also leukotriene synthesis) decreased with selenium deficiency and no effect on the cyclooxygenase pathway. This work indicates no selenium effect at the level of phospholipase $A_2$ which generates arachidonate (the precursor for both leukotriene and prostaglandin synthesis) but suggests a selenium effect potentially at the enzymes 1.)5-lipoxygenase, 2.)12-lipoxygenase, 3.)$LTA_4$ synthase, 4.)$LTA_4$ hydrase and/or 5.)5-HPETE to 5-HETE. Whether any or all of these enzymes contain selenium, possibly as a cofactor, or are influenced by

glutathione peroxidase is unknown. Decreased synthesis of leukotrienes by selenium deficient phagocytes, due to either a glutathione peroxidase effect or some other selenoprotein effect, may play an important role in the impaired function of phagocytes observed with selenium deficiency.

## Selenium and Ketonuria

Selenium deficiency has also been associated with increased ketone bodies in the urine of rats (Olsson, 1985). Interestingly, the content of ketone bodies and glucose in blood and liver and liver glycogen were similar between selenium adequate and deficient rats. Therefore apparently the ketonuria in the Se deficient rat was not caused by increased liver ketone synthesis and ketonemia as is the usual case in starvation. The rate limiting enzyme (hydroxymethylglutaryl-CoA synthase: HMG-CoA synthase) in ketone formation is present in large quantities only in the liver, the ketone utilizing, rate limiting enzyme (3-oxoacid-CoA-transferase) is present in all tissues, except liver, and that reaction is reversible. It should be noted however, the synthesizing enzymes are present to some extent in non-hepatic tissues. Thus non-hepatic tissues might be synthesizing ketones. Fatty acids and ketones are the main fuels of

respiration in kidney cortex which is also an organ of high Se content. Olsson reported urinary ketone excretion decreased 50% after 2 days of Se supplementation suggesting the involvement of Se in ketone metabolism might be enzymatic. Whether this represents a function of glutathione peroxidase or some other selenoenzyme is unknown.

## Selenium and Heme Metabolism

Work in the laboratory of R.F. Burk has led them to suggest selenium is required for proper heme utilization in the rat and that this function is not related to glutathione peroxidase (Burk and Correia, 1981). Heme containing compounds would include hemoglobin, myoglobin, cytochromes and catalase. The rate limiting enzyme in heme synthesis is aminolevulinic acid synthase (ALA synthase) in the liver where the reaction occurs in mitochondria. This enzyme catalyzes the condensation of succinyl-CoA and glycine to form aminolevulinic acid. It is controlled essentially by decreasing amounts of heme increasing the amount of ALA synthase and diminished amounts in the presence of heme. This enzyme is inducible by a variety of drugs, most notably phenobarbital, which are metabolized by cytochrome P450, a specific hemoprotein. Any given xenobiotic affects only certain forms of cytochrome P450. During the metabolism

of these inducers, consumption of heme by cytochrome P450 increases which in turn diminishes the heme concentration. This latter effect derepresses ALA synthase and an increase in heme synthesis occurs (Martin, 1985b).

The catabolism of all of the heme proteins appears to be carried out in the microsome of reticuloendotheial cells by a complex of enzymes called heme oxygenase or MHO. This complex is substrate inducible and located near microsome electron transport systems (Martin, 1985b).

Selenium has been implicated in the proper function of the cytochrome system by Siami et al (1972) with the induction of the cytochrome P450 system by phenobarbital raising the Se requirement for adequate growth rate in rats. Similarly, administration of polychlorinated biphenyls to chicks (Combs and Scott, 1975) showed an increase in selenium requirements based on plasma glutathione peroxidase activity. The induction of cytochrome P450 and cytochrome $b_5$ was impaired during selenium deficiency in rats induced with phenobarbital but had no effect on basal levels of the hemoprotein (Burk and Masters, 1975; Ip, 1983). Ip's work showed an accentuated impairment of the phenobarbital induction in

rats with a high fat intake. Additionally, unlike the cytochrome P450, dietary fat had no influence on basal or stimulated glutathione peroxidase activity, regardless of the selenium status. With the work of Burk and Master (1975) the predominant effect of selenium deficiency was on the phenobarbital induced form of cytochrome P450 and to some extent ethylmorphine demethylase but not biphenyl 4-hydroxylase and 3-methylcholanthrene.

In male but not female selenium deficient rats (Shull et al, 1979) amino pyrine N-demethylation, monocrotaline metabolism and aniline hydroxylation was depressed. Phenobarbital induction of second generation females resulted in subnormal induction of monocrotaline metabolism, similar to the males, but normal inductions of amino pyrine N-demethylase and aniline hydroxylase. Maintenance of intestinal mucosal cytochrome P450 level in the rat (Pascoe et al, 1981) required selenium in the diet demonstrating a selenium and cytochrome P450 relationship in a site other than the liver. Therefore these studies suggest selenium deficiency impairs induction of cytochrome P450, and decreases some xenobiotic metabolism known to function through cytochrome P450.

Sissons et al (1982) have reported an apparent decrease

in the development of photosensitisation in sporidesmin afflicted, selenium-deficient sheep. No attempts were made to distinguish this as action of glutathione peroxidase or xenobiotic metabolism, it suggests the latter.

Comparative studies of selenium deficiency and adequacy on heme metabolism in rats (Correia and Burk, 1978) showed that selenium deficiency increased MHO, indicating heme catabolism was occurring and no defect in heme synthesis was observed. Similarly in lambs, Whanger et al (1977) found lower hepatic microsomal cytochrome P450 levels and total heme content in WMD (selenium deficient) lambs but no differences in hepatic ALA dehydrase activity (heme synthesis). After phenobarbital administration (Correia and Burk, 1978), heme synthesis increased in both selenium states but heme catabolism decreased in the selenium adequate controls and increased in selenium deficient rats. Their findings were interpreted as:

> Phenobarbital induces the synthesis of heme to be used primarily in the assembly of cytochrome P450. In control (selenium adequate) animals heme and apocytochrome P450 are produced and assembled; little heme is catabolized. In selenium-deficient animals the heme is produced but not efficiently assembled with the apoprotein. Consequently, less cytochrome P450

> is produced, and excess heme is present in the hepatocyte. This excess heme induces the catabolic enzyme microsomal heme oxygenase, which disposes of it.
>
> Burk, 1983

Several experiments have indicated the effect of selenium on heme metabolism is not through glutathione peroxidase. Comparing the MHO response of selenium deficient rats or vitamin E deficient rats to phenobarbital showed only the Se deficient rats had altered MHO activity (Correia and Burk, 1978). In Se repletion trials with rats, when Se was given 4 to 6 hours prior to phenobarbital administration, there was no significant stimulation of MHO by phenobarbital, yet there was also no increase in glutathione peroxidase (Correia and Burk, 1978). This suggests the effects of selenium were not mediated through GPX, but rather due to an undiscovered function of selenium.

## Selenium and Variable Response

Early work has recognized variability in responsiveness to selenium deficiency and supplementation. Allaway and Hodgson (1964) reported on six New York (USA) farms with three having a recurring history of white muscle disease and three without white muscle disease. Yet average selenium concentrations in forages from the farms was 0.05 ppm regardless of white muscle disease occurrences. Sulfur levels were also similar (0.27% and 0.29%).

Certainly factors other than selenium could account for these observations. The authors also reported on the selenium content of feed used in selenium injection field trials in Northern California. Five trials gave either an improved weight gain in cattle or reduced white muscle disease in sheep and five trials did not give a response, and yet the feed selenium contents were very similar (0.06, 0.02, 0.02, 0.04, 0.02 vs. 0.06, 0.04, 0.05, 0.04, 0.04, ppm respectively for responsive vs. non-responsive trials). The selenium values were also similar or below the New York data.

Cattle and sheep from Florida have also shown variable responses to selenium (Shirley et al, 1966). Calves on mixed clover and bahiagrass pasture with a selenium content of 0.02-0.03 ppm did not respond with increased weight gain over controls when supplemented by injection every 90 days of 5.5 mg selenium as sodium selenite to their dams. Lack of responsiveness may be related to inadequate supplementation of calves by treatment to their dams (an inditement of the efficacy of the supplementation method), however even control (unsupplemented group) calves showed no symptoms of white muscle disease suggesting a true lack of response. A second similar trial over two years of calves on St.

Augustine grass pastures somewhat less selenium deficient (0.04-0.06 ppm Se) showed, again, a lack of weight gain response or white muscle disease symptoms. Comparisons of lambs supplemented either from weaning to slaughter or birth to slaughter with selenium and unsupplemented controls either in drylot or bermuda grass pasture also showed no effect of selenium on weight gain.

When both calves and their dams were supplemented with selenium a slight increase ($P<.10$) in adjusted weaning weight and summer daily gains were seen (Spears et al, 1986), however during the winter phase no response was seen to selenium supplementation. These cattle were marginally deficient as indicated by blood selenium levels at birth, three and seven month of age of 0.042, 0.035, 0.032 and 0.058, 0.047, 0.57, respectively for two year averages of controls vs. supplemented.

In finishing cattle (Byers and Moxon 1980) supplemented with oral selenium as selenite at 0.048 and 0.132 ppm Se in the ration establishing blood selenium levels of 0.025 and 0.045 ppm, selenium supplementation did not increase average daily gain or improve feed conversion over deficient controls.

Weaned sheep supplemented with ruminal selenium pellets

also failed to show increased weight gain over deficient control (Hunter et al, 1982). Plasma selenium levels were 0.3 ppm and 0.7 ppm, respectively.

It is difficult to interpret these variable responses as to problems with inadequate treatments, type II statistical errors or true variable responses. Langlands et al (1980) have identified breed, tick treatment (formamidine compound) and nematode treatment (levamisole) in cattle as factors significantly effecting blood selenium levels and GPX activity. Sires within breeds were also significant in sheep but not cattle although this may have been due to larger numbers for the sheep trials. Thus some of the variable responses to selenium status may have been due to statistical design and analysis.

GPX activity was variable in sheep on the same diet and selenium intake and mating experiments suggested the variation was genetically determined (Atroshi et al, 1981). The low GPX activity animals showed improved performance as measured by reduced mortality, and larger weight gain and wool production. Further trials (Sankari and Atroshi, 1983) found sheep, on the same selenium content diet, of the high GPX activity type had higher blood selenium levels than low GPX activity type sheep.

This suggests adaptation to low selenium intakes and/or diversity in selenium absorption or metabolism.

# MATERIALS AND METHODS

Four separate planned experiments with beef cattle were conducted at three different but known selenium deficent locations in Siskiyou county, in northern California. The experiments compared Se supplemented cattle with their Se deficient herdmates for numerous metabolic and performance factors in calves. The variables measured were selected to evaluate Se effects on numerous body functions through metabolites with well documented pathways. Hypotheses for each variable were established and tested based on the recognized antioxidant properties of GPX and selenium's essentiality in GPX.

## General Experimental Design and Description

Treatments, either intramuscular (IM) injection of selenium (and vitamin E) or oral administration of selenium boluses, were randomly applied to beef females in various stages of gestation. Unsupplemented females were left as controls. Injection material was Mu-Se, a commercially available form of sodium selenite and alpha tocopherol[1], administered at the recommended rate of 0.055 mg Se/kg BW. Commercially available selenium boluses were 10% elemental selenium and 90% iron filings,

[1]Burns Biotec, Inc.

pressed into 30 gram pellets[2]. Administration was two boluses per animal per os. Pellets were from three different production lots, but from a single lot within trials.

All locations had historically experienced calf death losses from white muscle disease (WMD), even when various supplemental selenium injection programs were used. Location one (T.41 N., R.9 W., Section 23) and three (T.41 N., R.9 W., Section 24) were in Scott Valley and location two (T.46 N., R.5 W., Section 25) was in Shasta Valley, all in Northern California (Siskiyou County). Frequently, but not always, faster gaining male calves were found dead or nearly dead from WMD as identified by licensed veterinarians at autopsy. Deaths were most frequent in calves 0-30 days of age. Blood samples taken earlier from cows in these herds indicated selenium deficiencies (Williams, 1980).

Blood Sample Collection and Analysis

Whole blood samples were collected in EDTA vacutainer tubes[3] via jugular venipuncture, using 21 gauge needles, from cows and calves. Serum samples were prepared

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[2]ICI, Australia.

[3]Monoject, Sherwood Medical, St. Louis, MO.

similarly from blood obtained in plain tubes and allowed to clot. After removing clots, slight centrifugation (<5 minutes) was used to remove any remaining cellular debris. Samples for hematology were kept cool and processed within twenty four hours of collection, while those used for blood chemistry were frozen.

Selenium content of whole blood was determined by the method of Whetter and Ullrey (1978) modified using a Technicon BD-40 heating unit and secondary refluxing at 150 degrees Centigrade for 5 minutes. Assay detection limits were 0.010 ppm whole blood Se. Statistical analysis of repeated assays indicated standard deviation of 0.002 ppm. Therefore samples below assay detection levels were reported as 0.008 ppm Se.

Serum chemical analyses were performed via automated chemistry routines[4]. The items considered were: lactate dehydrogenase (LDH), glutamic oxaloacetate transaminase-GOT (AST), glutamic pyruvate transaminase-GPT (ALT), alkaline phosphatase (AP), calcium (Ca), inorganic phosphorus (P), total protein (PR), albumen (AL), blood urea nitrogen (BUN), bilirubin, and cholesterol. GOT is also referred to in the literature as aspartate

[4]Autotechnicon Analyser, Tarrytown, NY.

aminotransferase (AST) and GPT as alanine aminotrasferase (ALT). Serum globulins as a class of proteins were estimated by subtraction of albumen from total serum proteins. Immunoglobin G was determined by radial immunodiffusion[5] and fibrinogen levels by heat precipitation (Dodds, 1980). Blood cell counts were obtained on electronic cell counters[6] and blood cell differentials were established by staining[7].

Performance Data

Body weights of calves were unshrunk weights obtained on the cooperating ranches from certified scales. All weights were obtained on individual animals. Scale certification was to 0.2% of known weights by official weighmasters of the State of California.

Experiment 1

Treatments of selenium pellet (P, n=30) or IM injection (I, n=31) were administered on November 20, 1980 and at the same time, a non-treatment group (C, n=30) was

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[5]Miles Laboratory Inc., Elkhart, IN.

[6]Coulter Counter, Clinical Lab, College of Veterinary Medicine, Oregon State University, Corvallis, OR

[7]Methylene blue-eosin, Clinical Lab, College of Veterinary Medicine, Oregon State University, Corvallis, OR

assigned to a total experimental population of 91 spring calving commercial Hereford cows. Only females having previously calved were used and they ranged in age from 3 to 14 years, averaging 6.8 years.

Winter feed consisted of predominantly fescue (Festuca arundinacea) and ladino clover (Trifolium repens) hay, containing small amounts (<20%) of alfalfa (Medicago sativa). All other feed came from irrigated pasture consisting of fescue and ladino clover. Thus all feed was of local origin, produced in an area of known soil-selenium deficiency. Soil type is classified as Diyou series, a deep somewhat poorly drained flood plain. It formed in alluvium derived from mixed rock sources.

All cattle had been bred and raised on the ranch. Bulls were purebred Hereford, and were predominantly purchased from out of the area, though principally from California and Oregon locations with likely Se deficiencies. During the over 25 years of operation, some Shorthorn and Angus bulls had been used. Performance testing under Se deficient conditions and subsequent sire selection for growth traits may have indirectly placed some emphasis on ability to withstand a Se deficient environment. Replacement heifers were selected on size, age and

pregnancy status to calve as two year olds.

During the experiment, cows received only a single injection treatment or bolus administration. Whole blood samples were collected at the start of the trial(n=91) about 90 days prepartum, then again about 30 days prepartum i.e. 60 days after treatment, (January, 1980, n=88), when the calves were 90 days of age (May 22, 1981, n=88) and finally at weaning (October 27, 1981, n=76).

Calving occurred in February, March and April in pasture lots of approximately 40 acres. All calves received an injection of vitamins A, D and E and Se (0.055 mg Se/kg BW) within 24 hours of parturition and randomly selected calves (n=26) were blood sampled at this time just prior to treatment. Due to these constraints, calf blood samples at birth were most likely to have been obtained after suckling of dams by the calves.

Blood samples were taken from calves and analyzed for selenium (n=83), the various blood chemistry items (n=57) and immunoglobulin G (n=29) and were weighed (n=82) at approximately 90 days of age (May 22, 1981). At that time routine calfhood vaccinations were given, and castration performed on steer calves, which also received

an anabolic growth promotant[8].

Calf blood samples were analyzed for selenium (n=26) and the animals were weighed (n=76) at weaning (October 27, 1981).

Experiment 2A

Eighty mature cows at location two were randomly assigned to treatment groups C (n=10), I (n=30) and P (n=40) on January 15, 1982. Blood samples were drawn from cows and the P group cows received Se boluses at that time. They were resampled on June 3, 1982, October 7, 1982, and May 12, 1983. In October, approximately 30 days prepartum, the I group cows received the parenteral Se treatment.

The fall-calving cows were fed locally grown alfalfa and oat (Avena sativa) hay, ad libitum, in which some perennial grasses in dormant stages were present. The soils from which the hays were produced were predominantly stony clay and stony clay loam, formed in residual material derived from both extrusive and intrusive igneous rock and to some extent metamorphic rock, and again were known to be selenium-deficient.

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[8] Ralgro, IMC, Inc., Terre Haute, ID.

All cows in the trial were registered purebred Herefords, raised on the ranch. Bulls were also purebred Herefords and were predominantly purchased, although a few home-raised bulls were occasionally used. Purchased bulls were principally from California and Oregon areas with similar Se deficiencies. Replacement heifers were selected principally on size and weight with some emphasis on particular blood lines. Heifers were bred to calve at 3 years of age.

Calving occurred in October, November and December. Calves were individually ear tagged for identification and all calves received 0.055 mg Se/kg BW by IM injection. The calves were weighed on February 1983 at approximately 90 days of age at which time blood samples were taken and routine calfhood vaccinations were given.

Weaning weights of the calves were taken on May 12, 1983, and a complete set of cow and corresponding calf data values was assembled from C (n=9), I (n=9) and P (n=18) group treatment pairs.

Experiment 2B

Experiment 2B, also conducted at location two, was a second year of data involving 25 cows from Experiment 2A

that calved in October, November and December, 1985, assigned to a C group (n=14) that received no supplemental Se and a P group (n=11) that had been previously assigned to the P treatment.

Conditions of the experiment were similar to the preceding one, except that calves did not receive parenteral Se at birth. Blood samples were obtained at birth, weights were taken at approximately 90 days of age, and complete blood counts (CBC) were performed at 90 days of age.

Experiment 3

Heifers, from a third location, bred to calve in the spring of 1986 were randomly assigned to either C or P treatment groups on November 10, 1985. Initial blood samples were taken, and Se determinations and P treatments were made at that time. At birth every fourth calf was administered by IM injection the Se and vitamin E mixture, and designated the bSe group.

All the heifers used were born in the spring of 1984 and raised on the ranch. The only supplemental Se administered to them was given parenterally at birth and in April of 1985, about 280 days prepartum. Pasture

forage consisted of orchardgrass (Dactylis glomerata) and fescue with some mixture of ladino clover. Hay, fed in the winter, was an alfalfa and orchardgrass mix. All feeds were raised on the ranch. Soil types were very similar to those already described for Experiment 1 (the ranch was located just across a river from the Experiment 1 location).

All females used in this experiment were bred to a single Gelbvieh sire in April 1985. In contrast to the other experiments, the sire of these calves was from the lower Sacramento Valley of California, an area not generally recognized as Se-deficient.

During calving, blood samples were obtained generally within 24 hours after calving but after colostral suckling and before any supplemental treatment. Further blood samples and weights were taken at approximately 90 days of age (April 15, 1986).

## Statistical Analysis

Experiments were individually analyzed, statistically.

Selenium levels in cows were evaluated by analysis of covariance, with age of dam as the covariant. Mean separation was determined by LSD when treatment effects were significant ($P<.05$).

Blood chemistry and performance

Blood chemistry determinations and performance data were analyzed in a model considering age, sex, Se treatment, Se levels, and their two way interactions by the Multiple General Linear Hypothesis procedure of Systat (1985). The basic model considered:

$$y=b_0+(b_1)(age)+(b_2)(sex)+(b_3)(Se)+(b_4)(I_1)+(b_5)(I_2)+(b_6)(I_1*Se)+(b_7)(I_2*Se)+e$$

where $b_{1-7}$ represents the coefficients for each variable. with

y= dependent variable

$b_0$= Y intercept

$b_1$= age in days

$b_2$= sex with 0 or 1 (1=females)

$b_3$= Se as whole blood selenium levels, ppm

$b_4$= $I_1$ the indicator variable with value of 1= P treatment

$b_5$= $I_2$ the indicator variable with value of 1= I treatment

$b_6$= coefficient for the interaction between P treatment and Se level

$b_7$= coefficient for the interaction between I treatment and Se level

e= random error term

Indicator variables or codes were selected and assigned for each treatment to have a unique combination of the two indicators:

| | Indicators | |
|---|---|---|
| | $I_1$ | $I_2$ |
| Control treatment C | 0 | 0 |
| Pellet treatment P | 1 | 0 |
| Injection treatment I | 0 | 1 |

Thus $I_1$ represented P treatment due to zero as a factor (cancels during multiplication) for both C and I treatments, while $I_2$ worked similarly for the injection treatment.

Experiments with only a C or P treatment used a single indicator variable.

Use of indicators (as shown below) allowed betas (b's) 4 and 6 to represent only P treatment effects and betas 5 and 7 to represent only I treatment effects, which permits the general model to be reduced to the following equations for each treatment:

For C treatment:

$$y=b_0+(b_1)(age)+(b_2)(sex)+(b_3)(Se)+e$$

For P treatment:

$$y=b_0+(b_1)(age)+(b_2)(sex)+(b_3)(Se)+(b_4)(I_1)+(b_6)(I_1*Se)+e$$

For I treatment:

$$y=b_0+(b_1)(age)+(b_2)(sex)+(b_3)(Se)+(b_5)(I_2)+(b_7)(I_2*Se)+e$$

Betas (b's) for each variable are the coefficients in the regression model that represent the response due to that variable. Subsequently, P treatment effects could be tested by determining if coefficients $b_4$ and/or $b_6$ were significantly different from zero. Coefficient $b_4$ would indicate changes in the Y intercept, but not slope (the relationship between Se level and the dependent variable), while coefficient $b_6$ would indicate slope differences. The interaction term, i.e. $b_6$, when found to be an additional significant term along with $b_3$ would suggest additional impacts of an overall Se level and dependent variable relationship across all treatment levels ($b_3$). When only $b_6$ and not $b_3$ was found significant, a relationship between Se level and P calves but not among all treatments was suggested.

In an analogous manner, effects of I treatment were tested for $b_5$ and $b_7$. Additionally, differences between P and I treatments can be tested by substituting the

value of one treatment, b coefficient instead of zero (the C treatment) and conducting a hypothesis test.

In experiment 3, calves were subject to an additional treatment at birth, either supplemental parenteral Se or none. This treatment was evaluated by an additional indicator variable (bSe) as described for the dam treatments.

In Experiment 2B, statistical analysis with calf blood Se levels at 90d of age could not be conducted due to Se levels below detection (<0.01 ppm). Instead substitution was made by using calf Se levels at birth.

Residual analyses (Y vs. residuals) were conducted to test the appropriateness of the model. In cases of non-constant variance and/or suggested symmetrical residual variance, transformations (log, square root, square or reciprocal) were subsequently tested for improvements to the model.

These analyses tested the hypothesis that Se should effect levels of GOT, GPT and LDH as has been shown by other investigators. Furthermore, decreased antioxidant capacity resulting from Se deficiency would be expected to increase bilirubin, hemoglobin and cholesterol, while

decreasing albumin, red blood cell count and leaving unchanged Ca, P, globulins, immunoglobin G, WBC count, total protein, alkaline phosphatase, blood urea nitrogen, and WBC count.

Modeling Weight Gain

Regression analyses were used to determine the importance of parameters effected by experimental treatments for weight gain of calves. Weight gain as weight per day of age at 90s day was the dependent variable. Independent variables were selected for testing in the model from parameters affected in the experiments by treatments or extensively cited in the literature.

# RESULTS

## Experiment One

All dams were selenium deficient prior to treatment (mean whole blood selenium=.022 ppm). Unsupplemented, control (C) dams remained deficient throughout the experiment. Selenium injected (I) dams responded with slight increases in blood selenium throughout the duration of the experiment, but were still deficient, according to the generally-accepted standard of 0.04 ppm in whole blood. Selenium pellet treated dams showed greatly increased whole blood selenium levels, on the other hand, and were not deficient as measured by whole blood selenium (see Table 1 and Figure 1). Dams tended to respond the same to selenium treatment regardless of age except for the prepartum sampling where age of dam was a significant (P=.07) co-variant.

**Table 1.** Whole blood selenium (ppm) of cows by treatment and times during experiment 1.

| | Control | | Injection | | Pellet | |
|---|---|---|---|---|---|---|
| | n | Mean | n | Mean | n | Mean |
| Start (-90d) | 15 | 0.021[a] | 16 | 0.023[a] | 19 | 0.021[a] |
| Prepartum (-30d) | 15 | 0.020[a] | 16 | 0.027[a] | 19 | 0.072[b] |
| 90 days postpartum | 15 | 0.014[a] | 16 | 0.021[a] | 19 | 0.073[b] |
| Weaning | 11 | 0.020[a] | 13 | 0.030[a] | 16 | 0.075[b] |

Within rows treatments differ (P<.05) with different letters.

The full model for testing treatment effects on calf blood Se levels at about 90 days of age indicated I (P=.04)and P (P<.0001) treatment effects plus a tendency for sex (P=.13) and age of calf (P=.12) to influence calf Se levels. The residual plot indicated some non constance variance so the dependent variable calf Se was transformed by logarithm. This improved the residual plot and resulted in equation 1:

(1) Calf Se= antilog (-1.48-(.002)(age)+.09(I)+.33(P))
(P<.0001, $R^2$=.671, SE=.104)

where

Calf Se= calf blood selenium at 90d of age, ppm
Age= age in days (mean=89.4d) (P=.05)
P= code to indicate pellet treated calves only (P<.0001)
I=code to indicate injection treated calves only (P=.02)

With this transformation, calf age (P=.05), I (P=.02) and P (P<.0001) treatments of dams prepartum affected calf Se levels at 90 days of age. There was some tendency for female calves to have higher blood Se levels (P=.12). Blood Se levels in P treatment calves were higher (P=.02) than in I treatment calves, as shown in Table 2 and Figure 1.

Equation 1 reduces by treatment:

For calves from unsupplemented (C) dams

(2) Se= antilog (-1.48-.002(age))

For calves from I treated dams

(3) Se= antilog (-1.48-.002(age)+.09)
= antilog (-1.39-.002(age))

For calves from P treated dams

(4) Se= antilog (-1.48-.002(age)+.33)
= antilog (-1.15-.002(age))

Selenium levels at birth

At birth on a smaller subset (n=13) of the same calves, selenium injection to dams prepartum did not increase (P=.44) calf blood selenium levels while the selenium pellet treatment greatly increased (P<.001) calf whole blood selenium values. Raw data means were C=0.025, I=0.039, and P=.101. Means adjusted by the regression equation were C=.03, I=.03 and P=.10. Lack of significance for the differences in I treated calves may be due to insufficient numbers.

By weaning at 240 days of age, blood selenium values of calves were similar (C=0.022, I=0.017, P=0.023).

Prediction Equations

Equations to predict calf whole blood selenium levels at 90 days of age based on dams' blood selenium levels, and effects of supplementation, were developed for two time periods: 1.) dams' selenium value at 90 days postpartum and 2.) approximately 30 days prepartum. In addition to

the dams' selenium level, age of calf, age of dam and sex of calf were considered.

Using dam levels at 90 days postpartum to predict the calves' blood selenium levels indicated age of the calf ($P<.05$), sex ($P<.05$) and dams' selenium ($P<.06$) were important variables (Equations 6 and 7). Additionally, for pelleted cows an additional Y intercept factor ($P=.0002$, Eq. 12 and 13) and for I cows an additional linear factor (dams' Se level, $P<.04$, Eq. 8 and 9) were significant (Figure 2 for residual analysis).

The prediction equation was

(5) $Se=.032-.00015(age)+.14(dam\ Se)+.006(sex)$
$+.021(P)+.32(I*dam\ Se)$ ($P<.0001$, $R^2=.682$, $SE=.009$)

where

Se=calf Se at about 90 days of age
Age= age of calf ($P<.05$)
Sex= additional factor for females only ($P<.05$)
Dam Se= dams' Se at about 90 days postpartum ($P<.06$)
P=code to indicate P treated dams only ($P=.0002$)
I=code to indicate I treated dams only ($P<.04$)

This equation reduces by treatment:

For calves from C treated dams

(6) $Se\ males= .032-.00015(age)+.14(dam\ Se)$
(7) $Se\ females=.032-.00015(age)+.14(dam\ Se)+.006$
$=.038-.00015(age)+.14(dam\ Se)$

For calves from I treated dams

(8) Se males= .032-.00015(age)+.14(dam Se)+.33(dam Se)
(9) Se females=.032-.00015(age)+.14(dam Se)+.33(dam Se)+.006

which can be further condensed to

(10)Se males= .032-.00015(age)+.46(dam Se)
(11)Se females=.038-.00015(age)+.46(dam Se)

For calves from P treated dams

(12)Se males= .032-.00015(age)+.14(dam Se)+.021
(13)Se females=.032-.00015(age)+.14(dam Se)+.021+.006

which can be further condensed to

(14)Se males= .053-.00015(age)+.14(dam Se)
(15)Se females=.059-.00015(age)+.14(dam Se)

When actual data, adjusted to the mean age, are plotted (Figure 9) by this equation, they reveal that calves have higher blood Se levels than their dams when dams' levels are below about 0.05 ppm Se. Above that level in dams, calves' blood Se increases but calves no longer sequester greater amounts than their dams.

Similar analysis for predicting calf blood Se levels near 90 days of age based on Se levels of dams prepartum and selenium treatments were conducted. The full model considered age, sex, dam Se prepartum, I and P treatments and the two way interaction between treatment and Se level. Age ($P<.10$), sex ($P<.12$), P treatment ($P<.0001$) and prepartum dam Se ($P<.02$) for I treated calves were important variables. The residual plot indicated some non-constant variance so the dependent variable calf

blood Se was transformed by logarithm. This improved the residual plot (Figure 3) and resulted in

(16) Calf Se= antilog(-1.51-.0017(age) +.05(sex)
+.35(P)+4(I*damSe)) ($P<.0001$, $R^2=.703$, SE=.100)

where

Calf Se= calf Se at about 90 d of age
Age= age of calf ($P<.05$)
Sex= additional factor for females ($P<.10$)
P= code for P treatment ($P<.0001$)
I= code for I treatment
Dam Se= dam Se level at -30d prepartum ($P<.004$)

This equation reduces by treatment:

For calves from C treated dams

(17)Se males= antilog (-1.51-.0017(age))
(18)Se females= antilog (-1.51-.0017(age)+.05)
= antilog (-1.46-.0017(age))

For calves from I treated dams

(19)Se males= antilog (-1.51-.0017(age)+4(dam Se))
(20)Se females= antilog (-1.51-.0017(age)+4(dam Se)+.05)
= antilog (-1.46-.0017(age)+4(dam Se))

For calves from P treated dams

(21)Se males= antilog (-1.51-.0017(age)+.35)
= antilog (-1.16-.0017(age))

(22)Se females= antilog (-1.51-.0017(age)+.35+.05)
= antilog (-1.11-.0017(age))

Standard errors for both prediction equations, i.e. prepartum and postpartum, were about 0.009 ppm Se. Similarly $R^2$ was .68 using the postpartum equation and 0.70 with the prepartum equation.

**Table 2.** Calf whole blood selenium (ppm) means by treatment[4].

| | C | I | P | Effects |
|---|---|---|---|---|
| Raw data | .024 | .030 | .051 | |
| males | .024 | .026 | .05 | |
| females | .025 | .035 | .053 | |
| Adjusted[1] | .022 | .027 | .047 | I P<.02<br>Age P<.05<br>P P<.0001 |
| Predicted from post-partum values:[2] | | | | Age P<.05, Sex P<.05<br>dam Se P<.06 |
| male | .021 | .028 | .050 | P P<.0003 |
| female | .027 | .034 | .056 | IXdam Se P<.04 |
| Predicted from pre-partum values:[3] | | | | Age P<.05<br>Sex P<.10 |
| male | .022 | .028 | .049 | P P<.0001 |
| female | .024 | .031 | .055 | IXdam Se P<.004 |

[1] Se=antilog (-1.48-.002X89.4+.09(I)+.33(P))
(P<.0001, $R^2$=.671, SE=.104)

[2] Se=.032-.00015(age)+.14(dam Se)+.021(P)+.33(I*dam Se90d)
+.006(sex) (P<.0001, $R^2$=.682, SE=.009)

[3] Se=antilog (-1.51-.0017(age)+.34(P)+4(I*dam Se-30d)
+.05(sex)) (P<.00001, $R^2$=.703, SE=.100)

[4]Values:
Age= 89.4 d
Sex= additional factor for females only
Dam Se90 d post partum C=.014, I=.021, P=.073
Dam Se-30 d prepartum C=.020, I=.027, P=.072
P= code for P treatment
I= code for I treatment

## Blood chemistry and performance of calves

Mean blood chemistry values and performance as expressed by WDA at 90 days of age are shown in Table 3. Treatments and/or blood selenium levels affected GOT, LDH, albumin, protein, bilirubin and phosphorus levels (see Table 3 for specific effects and P levels).

Table 3. Blood chemistry and performance (WDA at 90 days of age) of calves in experiment 1.

| | C | I | P | Regression Models with P levels in Parenthesis[1] |
|---|---|---|---|---|
| Age days | | | | |
| Raw data | 86.1 | 90.0 | 91.4 | |
| LDH U/l | | | | |
| Raw data | 862 | 860 | 854 | |
| Adjusted | 864 | 861 | 853 | 873-395XSe (P<.05 $R^2$=.081, SE=20) |
| Alkaline Phosphatase U/l | | | | |
| Raw data | 344 | 380 | 365 | |
| Adj male | 299 | 299 | 299 | 657-4XAge+77Xsex (P<.0007 $R^2$=.266, SE=135) |
| Adj female | 376 | 376 | 376 | (.002) (.06) |
| GOT U/l | | | | |
| Raw data | 102 | 92 | 98 | |
| Adjusted | 97 | 87 | 88 | 1/(.012-.0003Xage+.04XSe+.004XI-.10XIXSe) (P<.009,$R^2$=.259, SE=.0016) (.03) (.05) (.004) (.02) (I&IXSe, P<.009) |
| Calcium mg/dl | | | | |
| Raw data | 10.0 | 10.2 | 10.4 | |
| Adj male | 10.4 | 10.4 | 10.4 | 10.4-.2Xsex (P<.04 $R^2$=.086, SE=.40) |
| Adj female | 10.2 | 10.2 | 10.2 | |
| Phosphorus mg/dl | | | | |
| Raw data | 9.21 | 9.22 | 8.8 | |
| Adjusted | 9.0 | 9.1 | 8.7 | antilog (1.01-.0009Xage+1.0XSe-.04XP) (P<.003 $R^2$=.266, SE=.039) (.009) (.09) (.03) |
| Protein gm/dl | | | | |
| Raw data | 6.03 | 6.17 | 6.30 | |
| Adjusted | 6.09 | 6.14 | 6.28 | 5.3+.007Xage+7XSe (P<.002 $R^2$=.253, SE=.27) (.04) (.009) |
| Albumin gm/dl | | | | |
| Raw data | 3.80 | 3.87 | 3.95 | |
| Adjusted | 3.83 | 3.85 | 3.93 | 3.73+4XSe (P<.03 $R^2$=.100, SE=.19) |
| Immunoglobins gm/dl | | | | |
| Raw data | 2.23 | 2.30 | 2.34 | |
| Adjusted | 2.29 | 2.29 | 2.29 | 1/(.58-.0016Xage) (P<.0004, $R^2$=.233, SE=.05) |
| BUN mg/dl | | | | |
| Raw data | 13.4 | 12.6 | 13.4 | |
| Adj male | 12.2 | 12.2 | 12.2 | 1/(.109-.0003Xage-.012Xsex) (P<.0005 $R^2$=.283, SE=.012) |
| Adj female | 14.2 | 14.2 | 14.2 | (.005) (.002) |
| Bilirubin mg/dl | | | | |
| Raw data | .37 | .23 | .22 | |
| Adjusted | .37 | .23 | .22 | .37-.14XI-.15XP (P<.00001 $R^2$=.597, SE=.06) (.00001) (.00001) |
| Cholesterol mg/dl | | | | |
| Raw data | 168 | 176 | 165 | |
| Adjusted | 172 | 172 | 172 | 1/(.0076-.00002Xage) (P<.04 $R^2$=.094, SE=.001) |
| WDA at 90 d kg | | | | |
| Raw data | 1.33 | 1.32 | 1.34 | |
| Adj male | 1.33 | 1.32 | 1.34 | 2.24-.009Xage-.18Xsex (P<.00001 $R^2$=.460, SE=.45) |
| Adj female | 1.23 | 1.23 | 1.23 | (.00001) (.003) |
| WDA at weaning kg | | | | |
| Raw data | 1.00 | 0.97 | 1.03 | |
| Adj male | 1.05 | 1.05 | 1.05 | 1.05-.11Xsex (P<.02, $R^2$=.140, SE=.14) |
| Adj female | 0.94 | 0.94 | 0.94 | (.02) |

Values[1]

AGE= age in days (mean=89.4)
Se= whole blood selenium of calves, ppm (mean=0.0364;C=0.024, I=0.030, P=0.050)
P= indicator for pellet treatment (coded as 1)
I= indicator for injection treatment (coded as 1)
Sex= males=0, females=1
WDA=weight per day of age from birth to about 90d of age as wt/age

Transformations of cholesterol, GOT, BUN and inorganic phosphorus were used to remove non-constant variances observed in plots of residual analysis.

The enzymes GOT (AST) and LDH both decreased linearly with higher blood selenium levels (P=.04). While the LDH relationship (Figure 5) was limited to this linear relationship, the GOT response was more complicated (Figure 4). GOT, in addition to the solely linear response for C and P calves, in the case of the I treatment calves an additional interacting factor (P=.02) plus a non-interacting factor (P=.003). When the raw data are used in the regression equation, adjusted to the mean age and the various effects combined, the GOT relationship to blood selenium level is similar for the C and P calves and is negative with Se levels (Figure 4). In contrast, for the I calves the overall relationship between GOT and Se level is positive. The graphic display suggests one calf may be an influential outlier for the I calves. However, even when values for this calf are removed the multiple regression coefficents remain significant (P<.05) and the specific value for each coefficient is within the confidence (P=.05) limits of the previously calculated coefficients. This strongly suggests the apparent outlier is in fact not influential. These results for GOT and LDH support the

hypothesis and confirm previous findings of others. However the observed differences between Se treatments given parenterally (I) and orally (P) suggest important influences due to the treatments. Several possible explanations are available, including different levels of Se supplementation, incorporation of Se into different metabolites due to treatment method, and differring time course of Se supplementation. The addition of vitamin E to the I treatment might have been influential, although this would not be expected on diets with adequate vitamin E.

GPT levels were below assay detection for all calves, so were not further evaluated.

Alkaline phosphatase, as stated in the hypothesis, was not affected by treatments or related to selenium levels.

Total serum protein (P=.008) and albumin (P=.02) decreased with lower selenium levels (Figures 6 and 7). Serum globulin levels were not affected by the treatments or related to selenium levels. Albumin synthesis occurs solely in the liver (Kaneko, 1980) implicating Se deficiency with impaired liver albumin synthesis.

Inorganic phosphorus tended to increase (P=.08) linearly with higher selenium levels except for calves from P treated dams which had lower inorganic phosphorus levels (Figure 8). Se deficiency effects on P levels have not been previously reported and were not anticipated as a result of direct antioxidant actions by Se containing GPX.

Serum bilirubin levels were not linearly related to selenium levels directly but both I and P treatments lowered bilirubin (P<.00001). The treatments I and P accounted for 60 % of the variation in bilirubin levels observed ($R^2$=.60). As a metabolite of hemoglobin, lower bilirubin levels were hypothesized as a consequence of improved antioxidant protection for red blood cells with higher levels of Se. Neither sex nor age had significant effects on bilirubin.

Neither WDA at 90 days of age nor age and sex-adjusted weaning weight were affected by treatments or related to blood Se levels. This lack of response was unexpected, considering the relatively low blood Se levels of the C calves and the adequate level of the P calves. The detection of other significant factors, albeit chemical items, suggests adequate power of the statistical tests.

The observations taken together suggest certain specific effects of Se on various metabolites, but a tolerance or compensation on the part of the calves to less than ideal metabolism resulting in weight gain differences that could not be measured.

## Experiment 2A

Eighty cows sampled at the beginning (January) of the experiment were selenium deficient with a mean whole blood selenium value of 0.026 ppm. A subset of these cows, whose calves were sampled extensively at 90 days of age, similarly had a mean blood selenium value of 0.026 ppm. Blood selenium levels by treatment group over the following 16 months with periodic sampling are shown (Table 4 and Figure 11). Unsupplemented (C) dams remaind deficient (Se<.02) throughout the experiment. Dams in treatment group I were deficient prior to supplementation (Se<.02) and when sampled 7 months after the single Se injection were similar to C dams, and still deficient. The long interval from treatment to sampling may have missed the rise in blood selenium expected from the selenium injection treatment. Dams from the P treatment showed a dramatic increase in blood selenium (P<.01), four times higher than levels of the C and I dams and maintained this level until 16 months following treatment when they were still higher (P<.01)

than C or I dams but only by a factor of two. Co-variant analysis did not indicate age of dam was a significant factor during any sampling.

**Table 4.** Whole blood selenium levels (ppm) of cows by treatment and times during experiment 2A.

| | Control | | Injection | | Pellet | |
|---|---|---|---|---|---|---|
| | n | mean | n | mean | n | mean |
| Start(-280d pre-partum) | 9 | .025 | 9 | .027 | 18 | .025 |
| Prepartum (-150d) | 9 | .017[a] | 9 | .018[a] | 17 | .080[b] |
| Prepartum (-30d) | 8 | .019[a] | 8 | .018[a] | 18 | .077[b] |
| Postpartum (185d) | 9 | .014[a] | 8 | .018[a] | 17 | .043[b] |

Within-rows treatments differ (P<.01) with different letters as determined by FPLSD.

Initial testing for treatment effects indicated calf blood Se levels at 90 days of age were affected by age of the calf (P=.08), sex (P=.02) and the P treatment (P=.10). However residual analysis suggested non-constant variance of the model. The dependent variable (calf blood Se level) was transformed (reciprocal) and the model was improved. Age of calf (P=.07), sex (P=.004), P treatment (P=.001) and I treatment (P=.06) all affected calf Se level (Table 5 and Fig. 11). The residual plot was improved and overall $R^2$ increased (.441 vs. .275). The coefficient for I treatment was not

different from the P treatment coefficient indicating similar effects of those treatments on calf Se levels at 90 days of age.

The regression equation was

(23) $Se=1/(19+.3(age)-12(sex)-15(P)-11(I))$
$(P=.001, R^2=.441, SE=11.9)$

where

Se= calf blood selenium at about 90 d, ppm
Age= age in days (mean=98.4) (P=.07)
Sex= additional factor for females only (P=.004)
P= code to indicate pellet treated calves only (P=.001)
I= code to indicate injection treated calves only (P=.06)

This equation reduces by treatment:

For calves from unsupplemented dams

(24) Se (males) = 1/(19+.3 (age))
(25) Se (females) = 1/(19+.3 (age) -12)
= 1/( 7+.3 (age))

For calves from I treated dams

(26) Se (males) = 1/(19+.3 (age) -11)
= 1/( 8+.3 (age))
(27) Se (females) = 1/(19+.3 (age) -11 -12)
= 1/(-4+.3 (age))

For calves from P treated dams

(28) Se (males) = 1/(19+.3 (age) -15)
= 1/( 4+.3 (age))
(29) Se (females) = 1/(19+.3 (age) -15 -12)
= 1/(-8+.3 (age))

**Table 5.** Experiment 2A: Calf whole blood selenium (ppm) means by treatment for 1.) raw data, 2.) adjusted values, and 3.) predicted from dam Se levels 30 days prepartum.

| | Control mean | Injection mean | Pellet mean | Effects |
|---|---|---|---|---|
| Raw data | .030 | .040 | .044 | |
| male | .019 | .037 | .039 | |
| female | .040 | .045 | .049 | |
| Adjusted[1] | | | | Age, P=.07 |
| male | .017[a] | .020[b] | .022[bc] | Sex, P=.004 |
| female | .021[a] | .027[b] | .030[bc] | P, P=.001 |
| | | | | I, P=.06 |
| Predicted from Prepartum (-30d)[2] | | | | Sex P=.009 |
| male | .025 | .038 | .036 | dam Se P=.006 |
| female | .038 | .051 | .049 | dam SeXI P=.02 |

Within-rows treatments differ: [ab] P=.06; [ac] P=.001.

[1] Se=1/(19+.3X98.4-12(sex)-15(P)-11(I))
(P=.001, $R^2$=.441,SE=11.9)

[2] Se=.035+.18(dam Se)+.7(dam Se*I)+.013(sex)
(P<.001, $R^2$=.425, SE=.013)

Dam selenium values at -30d prepartum were .019, .018, and .077, respectively for C, I and P treatments.

## Prediction Equations

Equations to predict calf blood selenium levels at about 90 days of age using dam blood Se levels prepartum were developed. Treatment and dam blood Se levels plus the two way interactions along with age of calf and sex were considered. Sex (P=.009), prepartum dam Se (P=.006), and the interaction between dam blood Se and I treatment (P=.02) were significant, and age of calf approached significance (P=.15).

The prediction equation was

(30) Se=.035+.18(dam Se)+.7(dam Se*I)+.013(sex)
(P=.001, $R^2$=.425, SE=.013)

where

Dam Se= dam blood selenium, ppm (P=.006)
Sex= additional factor for females only (P=.009)
I= code to indicate injection calves only (P=.02)

This equation reduces by treatment:

For unsupplemented dams (C) and P treated dams

(31)Se (males) = .035+.18(dam Se)
(32)Se (females) = .035+.18(dam Se)+.013

For I treated dams

(33)Se (males) = .035+.18(dam Se)+.7(dam Se)
(34)Se (females) = .035+.18(dam Se)+.7(dam Se)+.013

which can be further condensed to

(35)Se (males) = .035+.88(dam Se)
(36)Se (females) = .035+.88(dam Se)+.013

These prediction equations should be applicable to many practical production situations. Differences in slopes (.88 vs. .18; P<.001) suggest physiological differences in the animals, perhaps reflecting selenium placental or lactational transfer between I and C or P treated dams. It may, however, merely relate to treatment differences since the injectable Se was given 30d prepartum to I dams whereas there was a more steady-state Se status in the C and P treated dams at the time of sampling. Furthermore had dam blood Se samples been obtained 14-21 d post-injection, providing time for Se assimilation by the I dams prepartum, then similar slopes with C and P

dams might have been found.

To test whether the relationship between dam blood Se level prepartum and subsequent calf blood Se level was better represented quadratically, a quadratic expression for dam blood Se was substituted for the linear expressions in the equation. All of the terms remained significant but the fit of the equation ($R^2$) was reduced (.425 vs. .376). This suggests that calf blood selenium levels are better represented as a linear response to dam blood Se levels under the conditions of this experiment.

This linear response between dam blood Se levels prepartum and calf blood Se levels observed near 90 d postpartum is shown in Figure 12. It illustrates that calves from dams having blood Se levels below about 0.05 ppm have blood levels higher than their dams, while calves from dams above 0.05 ppm Se have blood Se levels below their dams.

Blood Chemistry at 90 days of age

Mean blood chemistry at about 90 days of age and performance of calves at weaning from C, I and P treatments are shown in Table 6.

Table 6 - Experiment 2A: Means by treatment for blood chemistry at about 90 days of age and performance at weaning of calves from control (C), injection (I) and pellet (P) treated dams[1].

| | C | I | P | Regression Models with P levels in Parenthesis |
|---|---|---|---|---|
| LDH U/l | | | | |
| Raw data | 868 | 832 | 903 | |
| Alkaline Phosphatase U/l | | | | |
| Raw data | 212 | 219 | 210 | |
| GOT U/l | | | | |
| Raw data | 116 | 93 | 124 | |
| Adjusted | 121 | 93 | 121 | GOT=121-28XI (P=.03, $R^2$=.130, SE=32) |
| GPT U/l | | | | |
| Raw data | 30 | 17 | 28 | |
| Adjusted | 26 | 17 | 26 | GPT=-13+.4Xage-9XI (P=.001, $R^2$=.328, SE=10.8) (.0005) (.04) |
| Calcium mg/dl | | | | |
| Raw data | 9.6 | 9.5 | 9.8 | |
| Phosphorus mg/dl | | | | |
| Raw data | 8.0 | 8.4 | 8.1 | |
| male only | 7.6 | 8.2 | 7.8 | P=7.8+.8Xsex-1021*P*$Se^2$+48*P*Se (P=.04, $R^2$=.229, SE=1.13) |
| female only | 8.3 | 8.6 | 8.4 | (.005) (.02) (.07) |
| Adjusted | | | | |
| male only | 7.8 | 7.8 | 7.66 | |
| female only | 8.6 | 8.6 | 8.46 | |
| Protein gm/dl | | | | |
| Raw data | 6.3 | 6.3 | 6.5 | |
| male only | 6.6 | 6.4 | 6.6 | Protein=6.55-.2Xsex-117XIX$Se^2$(P=.04, $R^2$=.181, SE=.36) |
| female only | 6.4 | 6.2 | 6.4 | (.10) (.04) |
| Adjusted | | | | |
| male only | 6.56 | 6.33 | 6.56 | |
| female only | 6.36 | 6.13 | 6.36 | |
| Albumin gm/dl | | | | |
| Raw data | 3.5 | 3.5 | 3.6 | |
| Immunoglobins gm/dl | | | | |
| Raw data | 2.7 | 2.8 | 2.9 | |
| BUN mg/dl | | | | |
| Raw data | 11.9 | 11.8 | 12.7 | |
| Adjusted | 9.8 | 10.4 | 10.7 | BUN=1/(.12-.6XSe) (P=.06, $R^2$=.100, SE=.03) (.06) |
| Bilirubin mg/dl | | | | |
| Raw data | .23 | .21 | .24 | |
| Cholesterol mg/dl | | | | |
| Raw data | 181 | 186 | 181 | |
| Adjusted | 185 | 185 | 185 | Cholesterol=87+1Xage (P=.04, $R^2$=.119, SE=36) (.04) |
| Adjusted weaning weight, kg | | | | |
| Raw data | 178 | 183 | 184 | |

1 Values:
Age= 93.4d
Se C=.030, I=.040, P=.044
$Se^2$ I=.0016, P=.0022
P= Pellet treatment
I= Injection treatment

Experimental Se treatments affected blood GOT, GPT, phosphorus, protein and BUN levels. On the other hand, blood levels of LDH, alkaline phosphatase, calcium, albumin, globulins, cholesterol and adjusted weaning weight were without significant differences due to treatment.

Serum GOT were similar for C and P treated calves but lower (P=.03) in I treated calves. Neither sex nor age were significant factors on GOT levels. Considering blood Se levels for I and P calves were similar at 90 days and yet P calves were not different from C calves in GOT, apparently the GOT levels in the I calves were not affected by the level of Se, per se. This suggests either the method of treatment, timing or addition of vitamin E as the influencing factor for differences in serum GOT levels between the I and P calves. Vitamin E is not likely deficient based on diet consumed, and supported by work of Albaugh et. al (1963) where similar cattle were not vitamin E responsive. In contrast to Experiment 1, it is extremely probable Se levels of I and P calves were similar during the last 30 days of gestation in this Experiment, due to P treatment much earlier. Therefore circumstantially, differences in the route of Se administration may be responsible for the observed effects on serum GOT levels. GPT and GOT levels

in the calves reacted similarly to treatment, however, unlike GOT, GPT was influenced by age of the calf (P=.005). Since both of these transaminase enzymes reacted similarly, the concept of an I treatment effect is further supported.

Serum proteins showed only minor effects from the treatments. Total protein was lower (P=.04) for I treated calves but albumin and total globulins were not affected by treatment. The I treatment effect on total serum proteins was somewhat similar to that observed for GOT and GPT. Higher calf blood Se levels were negatively associated with serum protein levels, which supports neither the hypothesis nor data from Experiment 1. This relationship was quadratically related to Se levels in the I treated calves. A linear response was initially selected from the full model (total protein=6.56-.2Xsex-6XI2XSe; P=.07, $R^2$=.15). Both sex (P=.10) and blood Se for I calves (P=.08) showed important relationships to total protein levels. However the residual plots suggested a quadratic response might better fit the data and this proved correct (P=.04, $R^2$=.18).

With all variables in the model testing treatment effects on BUN, blood Se level tended (P=.10) to be linearly

associated with BUN levels. However, when all of the clearly non-significant variables were removed, a linear relationship between blood Se and BUN became less strong (P=.15, $R^2$=.06). The quadratic blood Se term was substituted for the linear Se value for further testing using the reciprocal transformation of BUN as the dependent variable. The quadratic effect was not found to be related (P=.17) but the reciprocal of BUN transformation improved the equation. Using the reciprocal of the BUN level as the dependent variable, blood Se level as a linear variable (P=.06) decreased BUN levels ($R^2$=.100). This relationship is shown in figure 13. The quadratic term for blood Se again did not substitute well for the linear Se term when using the reciprocal transformation of BUN (P=.09, $R^2$=.08).

A relationship between Se and BUN has not been previously reported and is not, clearly, a direct action of Se-dependent GPX function. Indirectly, oxidant effects on muscle and/or protein metabolism might influence BUN levels. Thus this relationship between Se and BUN is unclear and considering the number of evaluations and the observed significance level (P=.06), further support of this relationship is needed.

Serum inorganic phosphorus levels were negatively

associated with calf blood Se levels only in P treatment calves. Both linear (P=.07) and quadratic (P=.02) coefficients for blood Se levels in calves from P treated dams acted to curvilinearly decrease serum inorganic phosphorus levels with higher blood Se levels (Fig. 14). This differs from Exeriment 1, where serum inorganic phosphorus levels of all calves were positively related to blood Se levels. The regression equation from Experiment 2A has a positive Se relationship as one component, confirming a positive relationship between higher blood Se levels and serum inorganic phosphorus. While both this positive component and the negative one, together are significant (P<.04), the predominant force is a negative relationship between serum inorganic phosphorus and blood Se (figure 15).

## Experiment 2B

The second year of data from location two utilized twenty five cows from Experiment 2A as the experimental units. They had a mean blood selenium level of 0.020 ppm in October, 1985 approximately 30 d prepartum. C cows (n=14) averaged 0.013 and P cows (n=11) were higher, at 0.030 ppm Se(P=.005) (Table 7 and Figure 16). Age of dam did not affect selenium levels (P=.95) however all cows were adults ranging from 5 to 11 years of age. Both treatment groups were lower in blood selenium by

approximately 90 days postpartum:, C dams averaged 0.008 and P dams higher, 0.013 ppm (P=.007). Age of dam was again not significant (P=.75).

**Table 7.** Whole blood selenium (ppm) of cows by treatment and times during experiment 2B.

| | Control | | | Pellet | | |
|---|---|---|---|---|---|---|
| | n | mean | SD | n | mean | SD |
| Start: Prepartum | 14 | 0.013 | .009 | 11 | 0.030[a] | .016 |
| Postpartum +90d | 14 | 0.008 | .0005 | 11 | 0.013[b] | .006 |

[a] P=.005
[b] P=.007

Calf Selenium levels

Blood selenium levels at birth of calves from C and P treated dams were 0.016 and 0.027 ppm, respectively. P treatment to dams affected (P<.003) calf blood selenium levels at birth (Table 8 and Figure 16). Neither sex (P=.74) nor age of dam (P=.70) influenced selenium levels of calves at birth. By 90 days of age calf blood selenium levels were similar (0.008 vs. 0.008 ppm). Increasing age (P=.002) was negatively related to selenium levels (extrapolating to a rate of -0.000019 ppm/day). In this trial sex was not influential on calf blood selenium levels at about 90 days of age (P=.38).

Prediction Equations

A multiple regression prediction equation using dam blood Se levels prepartum and whether or not the dams were supplemented with Se pellets, accounted for about 50% ($R^2$=.475) of the variation in calf blood selenium levels at birth. Dams' blood Se levels prepartum were linearly related (slope=.3, SE=.14) to calf selenium levels (P=.03) postpartum. P treatment to dams increased 90 day postpartum blood Se in their calves by an additional 0.006 ppm (P<.09, SE=.003). Substituting a squared term for prepartum dam blood Se indicated an exponential relationship (P=.04). However the overall exponential equation (P<.002) did not account for as much of the variation as the linear one, $R^2$=.475 vs. .467, respectively for the linear and exponential equations, although the difference was small.

This equation to predict calf blood Se at birth reduces to:

For calves from C dams (non Se supplemented)

(37) Se= .012 + .3 (dam Se)

For calves from P dams

(38) Se= .012 + .3 (dam Se) + .006
= .018 + .3 (dam Se)

where

Se= calf Se at birth, ppm
dam Se= Se of dams about 30d prepartum, ppm

These equations indicate calves from dams with prepartum blood Se levels below about .020-.030 (Figure 21) sequester larger amounts of Se than do their dams. As dam blood Se levels are higher than these levels prepartum, calves proportionately obtain less Se and at birth have blood Se levels lower than their dams.

Calf blood selenium levels by 90 days of age were all below 0.010 ppm except for one C calf (0.010) and one P calf (0.011). Since the majority of blood Se levels were below assay detection, further analysis was not possible.

**Table 8.** Experiment 2B: Calf whole blood selenium (ppm) means by treatment for 1.) raw data, 2.) adjusted values and 3.) predicted from dam Se levels.

| | Control mean | Pellet mean | Effects |
|---|---|---|---|
| Raw Data | | | |
| Birth | 0.016 | 0.027 | |
| Age 90d | 0.008 | 0.008 | |
| Adjusted | | | |
| Birth[1] | 0.016 | 0.027 | P, P=.003 |
| Age 90d[2] | 0.008 | 0.008 | Age, P=.002 |
| Predicted from prepartum dam Se | | | |
| Birth[3] | 0.016 | 0.027 | dam Se, P=.03<br>P, P=.09 |

[1] Se=.016+.011(P) (P=.003 $R^2$=.340, SE=.008)
[2] Se=.0101-.000019(AGE) (P=.002, $R^2$=.346, SE=.0006)
[3] Se=.012+.3(dam Se)+.006(P) (P=.001, $R^2$=.475, SE=.007)

where

P= indicator variable for P treatment
Age=age in days
Dam Se= Se level, ppm, of dams prepartum (-30d; C=.013, P=.030)

Calf Blood Chemistry and Performance at Birth

Blood chemistry and weights of calves at birth are shown in Table 9. Calf blood chemistry parameters at birth affected by dam selenium treatment included: serum GPT (P<.03), BUN (P<.0009), serum inorganic phosphorus (P<.04) and calcium (P<.07). Serum LDH (P<.06) was negatively related to blood selenium levels of the calves while serum GPT (P<.09) and bilirubin (P<.04) were positively related to Se levels of calves from P dams but not calves from C dams.

Serum GPT levels at birth are characterized differently for calves from P and C treated dams. P calves had both a different Y intercept and slope compared to those of C calves. Therefore, due to the Y intercept effect, C calves were lower in blood Se and their serum GPT levels were relatively higher and constant across Se levels (Figure 17), which supports previous findings (Walter and Jensen, 1964 and Whanger et al, 1970). In contrast and unexpectedly, serum GPT levels in calves from P treated dams showed a positive curvilinear (P<.09) relationship

Table 9 - Experiment 2B: Means by treatment for blood chemistry and birthweight of calves at birth from C and P treated dams.

| | Control | Pellet | Regression Models with P levels in Parenthesis[1] |
|---|---|---|---|
| LDH U/l | | | |
| Raw data | 819 | 686 | |
| Adjusted | 825 | 693 | LDH=1016-1965XSe (P=.06, $R^2$=.176, SE=246) |
| Alkaline Phosphatase U/l | | | |
| Raw data | 565 | 744 | |
| GOT U/l | | | |
| Raw data | 132 | 136 | |
| GPT U/l | | | |
| Raw data | 18.4 | 9.1 | |
| Adjusted | 22.7 | 7.4 | GPT=18-24XP+18339XPXSe$^2$ (P=.072, $R^2$=.254, SE=12) (.03) (.09) |
| Calcium mg/dl | | | |
| Raw data | 10.29 | 14.06 | |
| Adjusted | 10.00 | 14.00 | Calcium=10.00+4XP (P=.069, $R^2$=.163, SE=4.23) (.07) |
| Phosphorus mg/dl | | | |
| Raw data | 9.43 | 7.40 | |
| Adjusted | 9.40 | 7.40 | Phosphorus=9.4-2XP (P=.032, $R^2$=.219, SE=1.90) (.04) |
| Total Protein gm/dl | | | |
| Raw data | 5.67 | 6.27 | |
| Adjusted | | | |
| male only | 6.36 | 6.36 | Protein=6.36-0.9XSEX (P=.06 $R^2$=.174, SE=1.07) |
| female only | 5.46 | 5.46 | (.06) |
| Albumen gm/dl | | | |
| Raw data | 3.13 | 2.90 | |
| Immunoglobins gm/dl | | | |
| Raw data | 2.54 | 3.37 | |
| BUN mg/dl | | | |
| Raw data | 10.5 | 15.6 | |
| Adjusted | | | |
| male only | 9.02 | 13.98 | BUN=12-186XSe+7XP+2XSEX (P<.003, $R^2$=.555, SE=2.93) |
| female only | 11.02 | 15.98 | (.05)(.0007)(.10) |
| Bilirubin mg/dl | | | |
| Raw data | .34 | .87 | |
| Adjusted | .30 | .81 | Bilirubin=.3+19XPXSe (P=.036, $R^2$=.210, SE=.56) (.04) |
| Cholesterol mg/dl | | | |
| Raw data | 75 | 58 | |
| Birth weight kg | | | |
| Raw data | 77 | 78 | |
| Adjusted | | | |
| male only | 81 | 81 | Birth weight=81-7XSEX (P<.05, $R^2$=.165, SE=9.1) |
| female only | 74 | 74 | (.05) |
| Adjusted birth weight kg | | | |
| Raw data | 78 | 78 | |
| Adjusted | | | |
| male only | 82 | 82 | Adjusted birth weight=82-8XSEX (P<.04,$R^2$=.176, SE=9.0) |
| female only | 74 | 74 | (.04) |

[1]where
P= code for P treatment
Se= calf Se levels at birth, ppm
Age= age in days
Sex= additional factor for females only

to blood Se levels. At the higher Se levels in the P calves (near 0.04), GPT levels were very similar to those from C calves, with calves with lower Se levels having lower GPT levels. These data would indicate that blood Se status of the dam during gestation has a large effect on lowering GPT levels (the Y intercept change). But in addition and less clearly the relationship between blood Se levels of calves and GPT became positive when dams were treated with Se pellets. Both effects suggest changes in Se and/or GPT metabolism during gestational development due to treatment effects on the dam (i.e. different Se status of the dam). The importance of the dam treatment was further tested by substituting calf blood Se level squared for all calves as a non-interacting term. When this substitution was done, the curvilinear relationship was no longer significant (P=.28).

Bilirubin levels also responded differently for P calves than for C calves (Figure 19). P calves tended to have higher bilirubin levels due to a linear relationship with Se and the higher Se levels observed in calves from P treated dams, which differs from Experiment 1 where a negative association was observed and Experiment 2A where no relationship was found. Additionally, a positive relationship was not expected and remains unexplained.

In contrast to serum GPT and bilirubin, levels of serum LDH at birth had a linear relationship with blood Se for all calves, with an increase of 12 (SE=5.9) LDH units for each 1 ppm decrease in blood Se of the calves (Figure 18). This relationship showed some curvilinear response (P=.09) however the $R^2$ term was reduced. This supports the hypothesis.

Although treatment effects on GOT were anticipated, no treatment or Se relationship on serum GOT was actually found.

The regression relationship for BUN (Table 9) indicates similar negative slopes for C and P calves with a larger Y intercept for P calves. As blood Se increased BUN levels were lower, about .18 (SE=.09) units per ppm change in blood Se (Figure 20). This regression equation accounted for nearly 50% of the variation in BUN ($R^2$=.477). A curvilinear Se term (squared) could replace the linear function, however $R^2$ would be slightly reduced (.477 vs. .469).

Blood levels of both calcium and phosphorus in calves at birth were affected by the treatment to their dams. Calves of P-treated dams had higher (P<.07) calcium and

lower (P<.04) phosphorus blood levels than C calves at birth. Lower inorganic phosphorus levels in serum from P-treated calves were also observed in Experiments 1 and 2A. This response has not been previously reported and is unexplained. The repeatability of this finding lends further support to its metabolic significance.

Calf blood chemistry, hematology and performance at 90 and 205 days of age

Calf blood chemistry, hematology and performance data at 90 and performance at 205 days of age are shown in Table 10. When significant, data was adjusted to the mean age (98.8 days) and for sex of the calf. Additionally, treatment effects to calves' dams or relationships with blood Se levels of calves at birth, and the two-way interactions are also indicated. It should be remembered that these analyses differ from previous evaluations of 90 d data by using calf blood Se levels from birth rather than at 90 d. As previously discussed, this is due to nearly all calves having whole blood Se levels below detection (0.008 ppm) by 90 days of age. The blood selenium levels at birth thus serve as indicators of calf Se status from birth to 90 d of age.

With increasing age serum cholesterol (P<.07) and globulins (P<.07) tended to increase, while inorganic

serum phosphorus decreased (P<.0001). Female calves tended to have higher BUN levels (P<.006) and lower WDA at weaning (P<.02) than males.

Treatment effects were not observed for serum GOT, GPT, LDH, bilirubin, BUN, cholesterol, calcium, phosphorus, total protein, albumin or red blood cell count. This lack of response may be due to the small treatment effects resulting in corresponding small variations in Se status. Calf blood Se levels at birth for P group calves were only slightly higher than for C calves from previous trials and by 90 days of age, blood Se levels of all calves were very low and deficient for both treatments.

Globulin levels at 90 d were negatively related to calf blood Se levels at birth (P<.05). No relationship was found in Experiments 1 or 2A when blood Se levels of calves were higher. This relationship was not found for globulin levels at birth. This was an exponential relationship (squared function) with lower globulin levels at 90 d as Se levels at birth increased for both treatment groups (Figure 22).

In contrast to globulins, another immune system parameter, total white blood cell (WBC) count, exhibited

an interaction between treatment and birth Se levels ($P<.06$). Calves from P treated dams showed a curvilinear increase (Figure 24) in WBC count with increasing blood Se at birth. However, no linear or curvilinear relationship between blood Se level at birth and WBC count was observed with calves from C-treated dams.

The opposing associations of Se with globulins and WBC count indicate variable actions of Se with the immune system. Other researchers have investigated qualitative effects of Se on white blood cells such as increasing phagocytic actions of neutrophils (Arthur, 1981). These findings suggest quantitative effects, possible stimulatory actions, of Se on WBC proliferation. Lower globulin levels associated with increasing Se levels appears contrary to previous reports.

Hemoglobin levels at 90 d were lower ($P<.04$) in calves from P-treated dams than in calves from C-treated dams. Experimental evidence with laboratory animals indicates Se effects on heme metabolism (Burk and Correia, 1977, 1978, 1981). Although in these calves bilirubin, which is a metabolite of hemoglobin was similar, P-treatment lowered circulating hemoglobin levels. This is the first reported occurrence of alterations in hemoglobin levels due to Se treatments. Morris et. al. (1984) observed

Heinz bodies in cattle. Since Heinz bodies are a result of oxidative action on hemoglobin there is a close connection with GPX. Heinz bodies predispose red blood cells to splenic removal and hemolysis. Potentially elevated hemoglobin levels could arise due to increased red blood cell hemolysis.

Surprisingly, WDA at weaning and adjusted 205d weight were positively related to calf Se levels at birth for calves from P-treated dams ($P<.03$) but not from C dams (Figure 25). This is particularly surprising considering blood Se level differences between treatment groups were small and for only a short time period. Additionally, performance to 90 d as measured by WDA and adjusted (by birth weight) were not influenced by Se treatment or blood Se level.

Differences in weight gain might have been gradual over an extended period of time, such as from birth to weaning. Considering blood Se levels of calves were similar by 90 d of age, P treated calves were higher in blood Se than C-calves for only a very short period of neo-natal life. This would suggest that gestational factors associated with Se may have contributed some advantageous physiological mechanisms resulting in increased performance by weaning time.

Table 10 - Experiment 2B: Calf blood chemical and performance data at 90 and performance at 205 days of age by treatment.

| | Control | Pellet | Regression Models with P levels in Parenthesis |
|---|---|---|---|
| GOT U/l | | | |
| Raw data | 135 | 180 | |
| GPT U/l | | | |
| Raw data | 21.4 | 57.8 | |
| LDH U/l | | | |
| Raw data | 1176 | 1529 | |
| Alkaline Phosphatase U/l | | | |
| Raw data | 242 | 302 | |
| Bilirubin mg/dl | | | |
| Raw data | .079 | .109 | |
| BUN mg/dl | | | |
| Raw data | 13.8 | 13.4 | |
| Adjusted | | | |
| male only | 12.2 | 12.2 | 12.2+2.8XSEX (P<.006, $R^2$=.299, SE=2.3) |
| female only | 15.0 | 15.0 | |
| Cholesterol mg/dl | | | |
| Raw data | 236 | 227 | |
| Adjusted | 229 | 229 | 160+.7XAGE (P<.07, $R^2$=.145, SE=39) |
| Calcium mg/dl | | | |
| Raw data | 9.66 | 9.68 | |
| Phosphorus mg/dl | | | |
| Raw data | 8.38 | 8.74 | |
| Adjusted | 8.53 | 8.53 | 11.1-.026XAGE (P<.0001, $R^2$=.456, SE=.65) |
| Total Protein gm/dl | | | |
| Raw data | 6.13 | 6.14 | |
| Albumen gm/dl | | | |
| Raw data | 3.65 | 3.73 | |
| Immunoglobulins mg/dl | | | |
| Raw data | 2.48 | 2.41 | |
| Adjusted | 2.53 | 2.40 | 2.0-257Xcalf Se birth$^2$+.006XAGE (P<.05, $R^2$=.261, SE=.24) (.05) (.07) |
| WDA 90 d lbs. | | | |
| Raw data | .95 | 1.08 | |
| Adjusted | 1.00 | 1.00 | 1.4-.004XAGE (P=.03, $R^2$=.188, SE=.20) |
| Adjusted wt. 90 d lbs. | | | |
| Raw data | 88.9 | 95.2 | |
| WDA weaning lbs. | | | |
| Raw data | .98 | 1.10 | |
| Adjusted | | | |
| male only | 1.05 | 1.16 | 1.05-.14XSEX+4.3XSeXP (P<.02, $R^2$=.451, SE=.11) |
| female only | .91 | 1.02 | (.02) (.03) |
| Adjusted 205 d wt. lbs. | | | |
| Raw data | 201 | 223 | |
| Adjusted | | | |
| male only | 212 | 236 | 212-25XSEX+886XSeXP (P<.02, $R^2$=.434, SE=22) |
| female only | 187 | 211 | (.03) (.03) |

Table 10a - Experiment 2B: Calf hematology data at 90 days of age by treatment.

| | Control | Pellet | Regression Models with P levels in Parenthesis |
|---|---|---|---|
| PCV % | | | |
| Raw data | 38.0 | 36.3 | |
| Plasma Protein gm/dl | | | |
| Raw data | 6.66 | 6.49 | |
| Fibrinogen mg/dl | | | |
| Raw data | 371 | 309 | |
| Adjusted | 351 | 316 | $262+349145XSe^2+140XP-466511XSeXP$ ($P<.006$, $R^2=.464$, SE=100) (.003) (.09) (.002) |
| Red Blood Cells $X10^6$ | | | |
| Raw data | 11.5 | 10.9 | |
| White Blood Cells $X10^3$ | | | |
| Raw data | 11.3 | 11.8 | |
| Adjusted | 10.9 | 12.5 | $10.9+2238XSe^2XP$ ($P<.06$, $R^2=.162$, SE=2.5) |
| Hemoglobin gm/dl | | | |
| Raw data | 13.7 | 12.6 | |
| Adjusted | 13.7 | 12.6 | $13.7-1.1XP$ ($P<.04$, $R^2=.182$, SE=1.2) |
| Segmented Neutrophils % | | | |
| Raw data | 20.6 | 21.1 | |
| Monocytes % | | | |
| Raw data | 1.7 | 1.6 | |
| Lymphocytes % | | | |
| Raw data | 76.9 | 75.8 | |

## Experiment 3

Eighty-five days before the average calving date and prior to experimental treatment, heifers averaged 0.074 and 0.073 ppm blood Se for C and P treatment groups, respectively. By an average of 71 days postpartum, P-heifers were higher in blood Se with 0.105 ppm than C-heifers with 0.048 ppm (P<.0001).

**Table 11.** Whole blood selenium of cows by treatment and times during experiment 3.

| | Control | | | Pellet | | |
|---|---|---|---|---|---|---|
| | n | mean | SD | n | mean | SD |
| Start: Prepartum -85d | 20 | 0.074[a] | .013 | 19 | 0.073[a] | .016 |
| Postpartum 71d | 19 | 0.048[a] | .011 | 20 | 0.105[b] | .039 |

Within rows means with different superscripts differ (P<.0001).

### Calf Blood Selenium Levels

Selenium levels at birth of calves from C and P-treated dams were 0.049 and 0.089 ppm, respectively (Table 12). P treatment to dams increased (P=.002) calf blood Se at birth. Blood Se was similar for male and female calves (P=.76).

Whole blood selenium levels of calves at 71 days of age are listed in Table 12. By 71 days of age, calf blood

selenium levels were similar to birth levels. Calves from P-treated dams were higher, 0.071, than from C-dams, 0.047 ppm (P<.0001). From the raw data it appears that additional Se parenterally at birth increased Se levels at 71 days. However the actual data fail to reflect differences in actual age of the calves when the samples were taken.

**Table 12.** Experiment 3: Calf whole blood selenium means (ppm) by treatment and times during experiment.

| | Control | | | Pellet | | |
|---|---|---|---|---|---|---|
| | n | mean | SD | n | mean | SD |
| Birth | | | | | | |
| All calves | 10 | 0.049[a] | .010 | 9 | 0.089[b] | .031 |
| Male only | 5 | 0.045 | .008 | 3 | 0.089 | .045 |
| Female only | 5 | 0.052 | .012 | 6 | 0.088 | .028 |
| 71 Days of Age | | | | | | |
| Actual Data: | | | | | | |
| All calves | 20 | 0.047[a] | .018 | 18 | 0.071[b] | .023 |
| Males-No birth Se | 6 | 0.039 | .014 | 5 | 0.063 | .027 |
| Males-Birth Se | 3 | 0.043 | .029 | 3 | 0.084 | .004 |
| Females-No birth Se | 7 | 0.043 | .010 | 6 | 0.079 | .022 |
| Females-Birth Se | 4 | 0.067 | .018 | 4 | 0.061 | .011 |

Within rows, means with different superscripts differ (P<.002 and P<.0001).

When dam treatment, sex and age of the calf, supplemental Se at birth (bSe) and interactions between supplemental

birth Se and treatment or sex were considered, only dam treatment (P<.0001) and age of the calf (P<.03) were influential on blood Se levels at 71 d of age.

Table 13 indicates blood Se levels adjusted by treatment and age for the treatment groups by actual age and with age effects removed, i.e. adjusted to the mean age (71.6d).

**Table 13.** Experiment 3: Calf whole blood selenium at 71 days of age, adjusted by least squares for dam treatment and age of calf.

| | Control | | Pellet | |
|---|---|---|---|---|
| | Age | Se | Age | Se |
| Adjusted for dam treatment[1] | | | | |
| Males-No birth Se | 81.2 | 0.046 | 80.8 | 0.073 |
| Males-Birth Se | 50.0 | 0.055 | 82.0 | 0.072 |
| Females-No birth Se | 75.7 | 0.047 | 75.6 | 0.074 |
| Females-Birth Se | 39.9 | 0.058 | 63.8 | 0.079 |
| Adjusted for dam treatment and age[2] | | | | |
| Males-No birth Se | | 0.048 | | 0.076 |
| Males-Birth Se | | 0.048 | | 0.076 |
| Females-No birth Se | | 0.048 | | 0.076 |
| Females-Birth Se | | 0.048 | | 0.076 |

[1] Se=0.07-.0003(actual mean age)+.027(P)
(P<.0002, $R^2$=.395, SE=.018)

[2] Se=0.07-.0003(age 71.6)+.027(P)
(P<.0002, $R^2$=.395, SE=.018)

Lack of significant increase due to supplemental Se at birth, singly (P=.77) or as an interaction with treatment (P=.83) may be a consequence of the sampling interval. An actual increase may have occurred but was missed due to the sampling interval used. Additional limitations due to small numbers of calves may have restricted detection of significant differences.

## Predicting calf blood selenium levels from dam blood selenium levels

Equations were tested to predict calf blood selenium levels at birth based on dams' selenium levels approximately 90 days prepartum. Dependent variables considered were sex of calf, Se treatment, dam selenium level and the interaction of selenium level and treatment. The only significant variable was the interaction term between dams' selenium level and treatment (P<.0002). The equation was:

(39) Calf Se at birth=0.050+.66(dam Se*P)
($P<.0002$, $R^2=.604$, SE=.020)

where

Dam Se=dam's selenium level at 85 days prepartum
P= indicator for P treated dams only

This equation predicts that calves from C-dams, which had a mean prepartum blood Se of 0.074 ppm (range 0.047-0.094), will be 0.050 ppm (SE=0.020). Calves from P-

treated dams, which had a mean prepartum blood Se of 0.073 (range 0.038-.107), will have blood Se at birth of 0.050 ppm plus .66 times their dams' prepartum blood Se level (SE=.020). Thus the P-treatment increased (P<.0002) calf blood Se levels at birth by 0.048 ppm (.073 ppm Se X .66). It also re-confirms the previous observations that dams with blood Se higher than about 0.05 ppm will produce calves with Se levels lower than their dams'.

Similarly, an equation to predict calf blood Se levels at about 90 days of age from their dam's prepartum Se levels was developed. This equation also considered the effects of giving additional Se parenterally at birth and interaction of that with dam Se treatment. Age of calf was also used as an adjustment factor.

The only significant variable for predicting calf blood selenium levels at about 90 days of age from prepartum variables were P-treatment (P<.0001) and age of calf (P<.03). This equation was identical to that previously reported (footnote 1 of Table 13). When trying to predict calf blood Se levels at 90 days of age from prepartum dam Se levels, the addition of parental Se at birth did not (P=.77) improve the prediction equation.

While this equation did not account for as much of the variation in calf blood Se levels at 90 days as did the birth levels, the equations were similar in standard error.

Dam blood Se levels near 90 days postpartum can also be used to predict calf Se levels for calves at 90 days. Dam blood Se levels were positively related to calf Se levels ($P<.00001$). With this prediction equation, parental Se at birth also slightly (0.011 ppm) increased calf blood Se levels at 90 days of age ($P<.03$). As would be expected, data from this similar time period account for more of the variation in calf blood Se ($R^2=.716$) and the error was lower (SE=.013). This prediction equation was

(40) Calf Se 90d =0.036-.0003(age)+.05(dam Se)+.011(bSe)
($P<.00001$, $R^2=.716$, SE=.013)

where:

Age=calf age in days
Dam Se= dam Se, ppm, at about 90 days of age
bSe= additional parental Se administration at birth

Calf Blood Chemistry at Birth

Blood chemistry values of calves at birth are shown in Table 14. For most parameters, calf Se levels, Se treatment to calves' dams or sex of the calf had no significant influence on the parameter. However BUN was

lower (P<.03) for females and calcium was lower (P<.03) in calves from P treated dams. Calves in this trial were higher in whole blood Se than any of the previous trials.

**Table 14.** Experiment 3: Mean and standard deviations for calf blood chemistry levels at birth.

| | Control | | | Pellet | | |
|---|---|---|---|---|---|---|
| | N | Mean | SD | N | Mean | SD |
| GOT | 8 | 63.5 | 14.9 | 8 | 67.5 | 24.8 |
| GPT | 8 | 4.62 | 4.81 | 8 | 6.12 | 9.89 |
| LDH | 8 | 745 | 142 | 8 | 732 | 27 |
| Alkaline Phosphatase | 8 | 554 | 211 | 8 | 560 | 298 |
| Bilirubin | 8 | .25 | .27 | 8 | .28 | .47 |
| Blood Urea Nitrogen | 8 | 11.25 | 5.44 | 8 | 11.88 | 6.38 |
| Males only | 4 | 13 | 7.39 | 2 | 20.5 | 6.36 |
| Females only | 4 | 9.5 | 2.52 | 6 | 9 | 3.03 |
| Adjusted Males[1] | | 15.5 | | | 15.5 | |
| Adjusted Females[1] | | 9.2 | | | 9.2 | |
| Cholesterol | 8 | 81.1 | 25.7 | 8 | 84.5 | 24.8 |
| Phosphorus | 8 | 9.21 | 1.43 | 8 | 9.85 | .84 |
| Calcium | 8 | 11.55 | .47 | 8 | 10.59 | .97 |
| Adjusted[2] | | 11.55 | | | 10.59 | |
| Total Protein | 8 | 7.99 | .69 | 8 | 7.29 | 1.25 |
| Albumin | 8 | 3.03 | .39 | 8 | 3.18 | .48 |
| Globulins | 8 | 4.95 | .95 | 8 | 4.11 | 1.52 |

[1] BUN=15.5-6.3(sex) (P<.03, $R^2$=.301, SE=4.96)

[2] Calcium=11.55-.96(P) (P<.03, $R^2$=.314, SE=.76)

where

Sex=additional factor for females
P= effect due to Pellet treatment

Blood chemistry and performance of calves near 90 days of age

Means of all blood chemistry and performance factors

obtained are shown in Table A1 (appendix). Least square means are only shown for those factors with significant treatment, age or sex effects (Table 15). The significant effects from the statistical model are indicated. The mean whole blood Se level of 0.059 ppm across all calves was used in the regression equations and thus linear relationships between Se and a specific variable will not be readily apparent from the numerical values.

GPT levels were negatively associated with blood Se increases ($P<.04$) confirming earlier work. Surprisingly, GOT (Figure 26) was not related to blood Se levels of C-dams but was negatively related to blood Se levels of P-dams when their calves were given Se at birth ($P<.06$). The treatment of supplemental Se (IM injection) at birth tended to increase serum GOT levels regardless of dam treatment or dam blood Se levels ($P<.02$). Also LDH levels were similar for all calves.

The other enzyme considered, alkaline phosphatase, showed only weak positive association with blood Se levels ($P<.08$).

Table 15 - Experiment 3: Blood chemistry and performance values for calves at about 90 days of age, calculated from least squares regressions.

| | Control | | Pellet | | |
|---|---|---|---|---|---|
| | No Birth Se | Birth Se | No Birth Se | Birth Se | Regression Models with P levels in Parenthesis[1] |
| GOT U/l | 112 | 137 | 112 | 120 | 58+.76Xage+25XbSe-301XSeXPXbSe (P<.002, $R^2$=.359, SE=18) (.0002) (.02) (.06) |
| GPT U/l | 14.13 | 14.13 | 14.13 | 14.13 | 19-84XSe (P<.04, $R^2$=.119, SE=5.3) |
| LDH U/l | 974 | 974 | 974 | 974 | 797+2.5XAGE (P<02, $R^2$=.157, SE=121) |
| Alkaline Phosphatase U/l | 324 | 324 | 324 | 324 | 237+1507XSe (P<.08, $R^2$=.084, SE=115) |
| Bilirubin mg/dl | .02 | .02 | .02 | .055 | .02+.6X(SeXPXbSe) (P<.06, $R^2$=.095, SE=.057) |
| BUN mg/dl | 12.84 | 11.34 | 12.84 | 11.34 | 10+.04XAGE-1.5XbSe (P<.0008, $R^2$=.330, SE=2.06) (.02) (.06) |
| Cholesterol mg/dl | 103 | 103 | 125 | 125 | 123+22XP-343XSe (P<.03, $R^2$=.185, SE=21) (.009) (.07) |
| Phosphorus mg/dl | 8.79 | 8.79 | 8.79 | 8.33 | 8.3+17XSe-8X(SeXbSeXP)-.007Xage(P<.002, $R^2$=.45, SE=.53) (.0002) (.001) (.10) |
| Calcium male mg/dl female | 9.93 9.73 | 9.93 9.73 | 9.93 9.73 | 9.93 9.73 | 10.5-.008Xage-.2Xsex (P<.005, $R^2$=.258, SE=.32) (.003) (.05) |
| Protein gm/dl | 6.01 | 6.01 | 6.01 | 6.01 | 5.6+7XSe (P=.02, $R^2$=.141, SE=.40) |
| Albumin gm/dl | 3.53 | 3.53 | 3.73 | 3.73 | 3.53+3.4XSeXP (P<.0004, $R^2$.307, SE=.20) |
| Immunoglobulins gm/dl | | | | | |
| male | 2.25 | 2.25 | 2.25 | 2.25 | 2.25+.2Xsex (P<.06, $R^2$=.096, SE=.46) |
| female | 2.45 | 2.45 | 2.45 | 2.45 | |
| Hemoglobin gm/dl | 12.8 | 11.6 | 12.8 | 12.4 | 12.8-25X(SeXbSe)+20X(SeXbSeXP)(P<.02, $R^2$=.262, SE=1.13) (.004) (.04) |
| PCV % | 37.6 | 37.6 | 37.6 | 37.6 | 34+.05Xage (P<.04, $R^2$=.113, SE=3.0) |
| RBC count X$10^6$ | 11.4 | 9.7 | 11.4 | 11.67 | 11.4-1.7XbSe+34X(SeXbSeXP)(P<.002, $R^2$=.317, SE=1.24) (.003) (.0006) |
| Plasma protein gm/dl1 | 6.16 | 6.16 | 6.16 | 6.16 | 5.8+6.2XSe (P<.02, $R^2$=.156, SE=.33) |
| Fibrinogen mg/dl | 390 | 457 | 390 | 457 | 564+67XbSe-1.4Xage-1285XSe (P<.02, $R^2$=.254, SE=96.4) (.07) (.10) (.09) |
| Seg. Neut. % | 31 | 31 | 31 | 40.7 | 31+167X(SeXbseXP) (P<.004, $R^2$=.215, SE=9.2) |
| Bands % | .4 | .4 | .4 | .4 | .4+13XSe (P<.05, R=.106, SE=.9) |
| Lymphocytes % | 67 | 67 | 67 | 57.5 | 67-163X(SeXbSeXP) (P<.005, $R^2$=.203, SE=9.4) |
| Eosinophils % | | | | | |
| male | .3 | -.2 | .3 | -.2 | .3-.5XbSe+.5Xsex (P<.02, $R^2$=.220, SE=.7) |
| female | .8 | .3 | .8 | .3 | (.04) (.03) |
| IgG mg/dl | | | | | |
| male | 1631 | 2103 | 1631 | 2103 | 1631+8136X(SeXbSe)+506Xsex (P<.01, $R^2$=.232, SE=709) |
| female | 2137 | 2609 | 2137 | 2609 | (.03) (.04) |
| Wt. at 90 d kg | 77.8 | 72.3 | 77.8 | 72.3 | 48+.42Xage-96X(SeXbSe) (P<.00001, $R^2$=.567, SE=9.4) (.00001) (.06) |
| WDA at 90 d kg | 1.12 | 1.12 | 1.12 | 1.12 | 2.4-.018Xage (P<.00001, $R^2$=.683, SE=.26) |

[1]where

bSe=additional treatment of parental Se at birth
P=P treatment to dams' of calves
Sex=code for female calves
Se=whole blood Se level, ppm, of calves, mean=.059
Age=age in days, mean=71

Blood urea nitrogen levels were lower (P<.06) in calves treated with Se parenterally at birth. While P-treatment increased (P<.009) cholesterol levels (Figure 29), higher Se levels, without regard to treatment, were associated with lower (P<.07) cholesterol levels. This is paradoxical, but suggests differential response to treatment method.

Bilirubin levels were similar for all calves except those given both P treatment and additional Se at birth (P<.06) in which case bilirubin was elevated.

Total serum proteins increased (P<.02) with higher Se and albumin (figure 28) increased with higher Se levels only in calves from P-treated dams (P<.0004). Since the effects on albumin were restricted to the P-treatment calves, this suggests that the lower Se levels during gestation as in the C-dams compared to the P-supplemented dams, are associated with lower serum albumin. Since the liver is the sole site of albumin synthesis, this implies possible gestational liver compromise with lower Se levels during gestation.

Treatment effects on red blood cell count and serum hemoglobin were similar. Se given parenterally at birth,

regardless of dam treatment, tended to decrease RBC count ($P<.003$) and hemoglobin ($P<.004$). While together both P-treatment and parenteral Se at birth tended to increase RBC count (Figure 30) and hemoglobin levels (Figure 31). This latter effect tends to be confirmed with higher bilirubin levels associated with the combined treatments of P and parenteral birth-Se treatments. Taking the relationships from two independent observations on metabolically related compounds, it helps confirm the a strong relationship between Se and RBC/hemoglobin metabolism. The fact that this linear relationship occurs only when supplemental Se was given both prepartum and postpartum may indicate important temporal relationships during development.

Immunological parameters showed a variety of responses to treatments and Se levels. Total serum globulins were similar for all treatment groups. However IgG was postively related to Se levels ($P<.03$) in calves treated with additional Se at birth (Figure 33). Segmented neutrophils were also positively related to Se levels in calves from P-dams and receiving parenteral Se at birth (Figure 34). Band neutrophils were positively related to Se across all treatments. In contrast, lymphocytes were negatively related to Se level in calves from P-dams, given Se at birth (Figure 35). This identifies a

variable response for different components of the immune system to Se and should be considered when evaluating other trials.

Serum inorganic phosphorus showed a positive relationship with Se ($P<.0002$) (Figure 27). Additional effects were seen in calves from P treated dams given supplemental Se at birth by injection, in which there was a slight but significant ($P<.01$) negative association between serum inorganic phosphorus and Se.

Fibrinogen levels tended ($P<.10$) to be lower in calves given Se at birth regardless of Se treatment to dams (Figure 32).

Weight at about 90 days of age also tended ($P<.06$) to be lower in calves given Se at birth regardless of Se treatment to dams. However when weight per day of age was considered, performance of calves was similar for all treatments.

## Modeling Weight Gain

To help identify Se-related metabolic factors that were important for weight gain, a model of weight gain using

only those factors influenced in these experiments by Se treatments or cited extensively in the literature was developed.

The dependent variable was weight per day of age at 90d. The first model included as independent variables GOT, GPT, total serum protein, albumin, inorganic phosphorus and serum globulins, along with calf age, sex and blood Se level plus the two way interactions of the blood chemicals with Se level. The second model for experiments 2B and 3 included data for IgG levels, segmented neutrophils, RBC count, hemoglobin and fibrinogen.

For Experiment 1, the significant variables ($P<.05$) for weight per day of age were: age ($P<.0001$), sex ($P<.006$), GOT ($P<.004$), albumin ($P<.02$) and Se level X globulins interaction ($P<.02$).

(41) $WDA=2.95-.016(AGE)-.33(SEX)-.012(GOT)+.78(AL)-3.6(Se*G)$
($P<.00001$, $R^2=.623$, $SE=.39$)

where
WDA=weight per day of age at 90 days
AGE=age in days
SEX=code for females
AL=albumin
G=globulins
Se=calf Se level at 90 days, ppm

Both GOT and AL influence weight gain in the direction anticipated from known physiology. Surprisingly, as Se and globulins increase, weight gain is negatively impacted. Both GOT and AL can be replaced in this equation by a Se-interaction term with only slightly lower $R^2$ and higher standard error values.

In Experiment 2B for the first model, the significant variables ($P<.05$) for weight per day of age were: total serum protein ($P<.03$) and Se level X albumin ($P<.001$).

(42)WDA=1.68-.34(PR)+46(Se*AL) ($P<.004$, $R^2=.407$, SE=.18)

Albumin was again an influential variable and in the same manner as the previous experiment. Protein was an added factor while globulins were no longer significant. When the hematology factors were included in the model both PR ($P=.05$) and SeXAL ($P<.0003$) were again included plus RBC count X Se level ($P=.09$). $R^2$ and SE were only slightly improved.

In Experiment 3 for the first model, the significant variables were age ($P<.0001$), protein ($P<.0001$), phosphorus ($P<.006$), Se X GPT ($P=.07$) and SeXAL ($P<.009$).

(43)WDA=-1.05-.018(AGE)+.38(PR)+.15($P_i$)+.26(GPT*Se)
-1.49(AL*Se)
($P<.00001$, $R^2=.882$, SE=.17)

The $R^2$ was much higher and the SE lower than in the previous models for Experiments 1 and 2B. The signs for the coefficients of PR and AL are reversed from Experiment 2B, however the net effect of both terms is for higher albumin levels, as constituents of serum protein, to result in higher rates of gain. This model suffered in the residual analysis, however, from some linear pattern and non-constant variance. Transformations (log, square root and reciprocal of WDA) were substituted. The best model with these transformations was using the log of WDA. Both the GPT (P=.58) and ALXSe (P=.17) terms were not significant with the transformation. The improved model was:

$$(44)\ \log WDA=-.45-.005(AGE)+1.54(PR*Se)+.098(P_i)-1.07(P_i*Se)$$
$$(P<.00001),\ R^2=.883,\ SE=.05)$$

where $P_i$ was serum inorganic phosphorus levels.

Each individual term and both terms with $P_i$ together were significant (P<.0001).

For the second model using data from Experiment 3 the hematology items, segmented neutrophils, RBC count, hemoglobin, fibrinogen, IgG and their interactions with calf blood Se levels, were added to the previous factors. The initial model included age (P<.0001), protein

(P<.06), phosphorus (P<.06), IgG (P=.10) and RBC count (P<.05). However non-constant variance and a linear pattern was evident in the residual analysis. Using the log transformation of WDA the model was retested and the same model as previously discussed was developed, even though the additional items were included.

It is particularly interesting that albumin and serum protein appeared as important variables describing weight gain in two of the three trials. In contrast, well accepted indicators of Se deficiency and antioxidant damage were much less consistently included, for example GOT was included only in the model for Experiment 1 and GPT might be included in the model for Experiment 3. Additionally globulins and phosphorus levels each appeared as important variables in one experiment.

These models using only variables found with some consistency in the four experiments to be affected by Se treatments or related to Se levels or well established by others, strongly suggest metabolism in the liver is a crucial area relating both to Se deficiency and weight gain. Serum protein and/or albumin appear to be better indicators of growth rate than more commonly accepted values such as GOT or GPT in calves of questionable Se status.

# DISCUSSION

Despite historical evidence of Se deficiency in the test area and application of conventional treatments to supplement Se at the cooperating ranches, whole blood Se status of dams and their calves were variable and frequently less than judged adequate. This confirms the existence of difficulties in prescribing and administering adequate Se supplementation under typical range cattle conditions. Others have arrived at similar conclusions (Hathaway, 1987).

Finding lower serum levels of GOT, GPT and usually LDH in calves supplemented with Se than from control dams in these experiments confirms the findings of others and lends credence to the experimental procedures used.

While there were variable responses in the four experiments, the blood metabolites, in addition to GOT, GPT and LDH, with the most consistent response to Se treatments and blood Se levels were total serum protein, albumin, inorganic phosphorus, hemoglobin and fibrinogen.

Total serum protein levels were positively related to Se levels. There were some interesting specific relationships. Among these, albumin typically

constitutes 35-50% of the total serum proteins and it also was found to be positively related to Se levels. On the other hand, fibrinogen usually decreased with higher levels of Se, as did hemoglobin. It seems likely, therefore, that serum protein increases were accelerated largely due to the influence of albumin, which was specifically affected by Se. This, in turn, suggests reduced liver function as it relates to albumin synthesis and supports the concept that there are other metabolic mediators of Se activity in addition to GPX.

The weight gain models also suggest involvement of Se with metabolites other than GPX. In these models, GOT, GPT and LDH, all correlate negatively with GPX, as anticipated (Whanger et al, 1978). In contrast, if GPX was strongly involved in the selenium/albumin relationship, GOT as a negative GPX surrogate could substitute for albumin in the model. In fact, GOT does not substitute for albumin in the model and typically albumin explains more variation than GOT, and is included more consistently than GOT.

The GPX-induced antioxidant involvement in the selenium/albumin relationship seems highly unlikely considering that only about 25 % of the GPX activity in

liver is due to Se-dependent GPX, with GSH-S transferase (non Se-dependent GPX) occurring predominantly in the livers of selenium deficient animals (Scholz et al, 1981). Although GPX could still be involved directly, this item suggests no strong peroxidation challenge on the liver, and supports involvement of selenium in albumin plasma levels by some means not dependent on GPX.

Other protein-synthetic hepatocytes may be involved in Se metabolism, and efforts to more clearly identify them and to quantitate their relative effects through electrophoretic studies might prove fruitful.

Oxidative damage to erythrocytes was expected to result in higher bilirubin levels and this occurred in Experiment 1, however the opposite effect was seen in Experiment 3 while no significant treatment effects were observed in Experiments 2A and 2B. In both of the experiments where hemoglobin levels were measured, a negative relationship was found between them and Se levels. Erythrocyte numbers were also positively related to Se levels in the animals in Experiment 3.

These effects identifying increased damage to

erythrocytes, may logically involve autoxidation in the presence of reduced levels of GPX, with resultant increased hemoglobin levels and decreased RBC count in calves with lower Se levels. If this were the case there would need to be substantial RBC damage and data does not suggest this is the case. Nonetheless, adequate liver capacity appeared to maintain serum bilirubin levels in deficient calves comparable to those of calves with higher Se levels. Either reserve liver capacity or oxidative protection through GSH-S transferase, may explain the generally similar bilirubin levels between selenium deficient and selenium adequate cattle. This further supports a lack of GPX involvement in the selenium/albumin relationship.

The reasons for the relationships between Se treatment and serum inorganic phosphorus are difficult to explain, and may be fortuitous. In two of the three experiments, serum inorganic phosphorus was positively related to blood Se levels. Determinations of serum inorganic phosphorus can be misleading, since hemolysis of red blood cells accompanied by hydrolysis of phosphate esters can liberate phosphorus. This complication is considered unlikely, since particular care was taken during drawing and storage of blood to avoid hemolysis.

Immune system parameters were considered in Experiments 2B and 3. No direct evidence of a Se relationship with the immune system was observed in Experiment 2B and responses were variable in Experiment 3. Quantitatively, both band and segmented neutrophils increased with blood Se levels, while lymphocytes decreased. Antibody levels (IgG) increased with circulating Se. Several other studies have considered qualitative factors of Se on neutrophils, and have identified variable quantitative effects on both lymphocytes and neutrophils. How the quantitative and qualitative effects interrelate needs further investigation along with how these effects relate to seleniums' involvement with GPX or other mechanisms.

The lack of improved weight gain in young calves agrees with other reports of variable response. Considering the low blood Se levels in calves in these experiments and the considerable biochemical activities observed, this is particularly surprising. Apparently under the environmental and management conditions of these ranches, metabolic effects of Se treatments used were not substantial enough to affect weight gain.

Regression studies to model weight gain suggested that serum proteins levels, both total protein and albumin,

were important influences on weight gain. Thus the experiments have identified Se treatment effects on protein metabolism, with changes in albumin signaling specific involvement of the liver. These parameters appeared important to weight gain while commonly accepted indices of Se deficiency like GOT and GPT were less important. Here again, the effects of Se on albumin cannot be readily explained as a function of GPX, since GOT and GPT were not identified as significant determinants of weight gain.

These results suggest that Se influences metabolic parameters that cannot be easily explained through its involvement in GPX, and show that these parameters are important to growth of the young animal. More precise identification of the biochemical mechanisms involved will require further, carefully-controlled studies.

## Summary

1. Responses of beef cows and their calves to supplementary selenium given parenterally and intraruminally have been examined on three ranches in an area of known selenium-deficient soils, over a three year period.

2. Effectiveness of both routes of administration of selenium was established, in terms of elevated blood selenium levels in the calves. Changes in blood selenium of the dams were inconsistent, suggesting that the maternal organism preferentially protects the young in preserving adequate selenium status.

3. Whole blood maternal selenium levels of 0.05 ppm appear to be critical. Below this base level, supplementation resulted in higher selenium blood levels in the calves than in their dams.

4. Examination of control (unsupplemented) animals showed that calf blood selenium levels declined with age and tend to be higher in females than males.

5. Serum protein levels, and especially those of the albumin fraction, tended to rise in response to selenium administration. This interrelationship, which may have future use in establishing selenium status, is not readily explainable by the known antioxidant activity of the seleno-enzyme, glutathione peroxidase.

6. Varying relationships have been found between selenium and hemoglobin, serum fibrinogen and serum inorganic phosphorus. Possible significance of these to GPX-mediated functions for selenium is discussed.

7. Several immune system indices were generally not responsive to selenium treatment. Good animal health may have reduced responsiveness of these systems.

8. The use of mathematical models to estimate selenium status of animals from known blood values is described.

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# APPENDIX

Fig. 1 Exp. 1: Whole blood Se (ppm) levels of cows and their calves by treatment and times during Experiment 1. Cows represented by open symbols, calves by filled symbols.

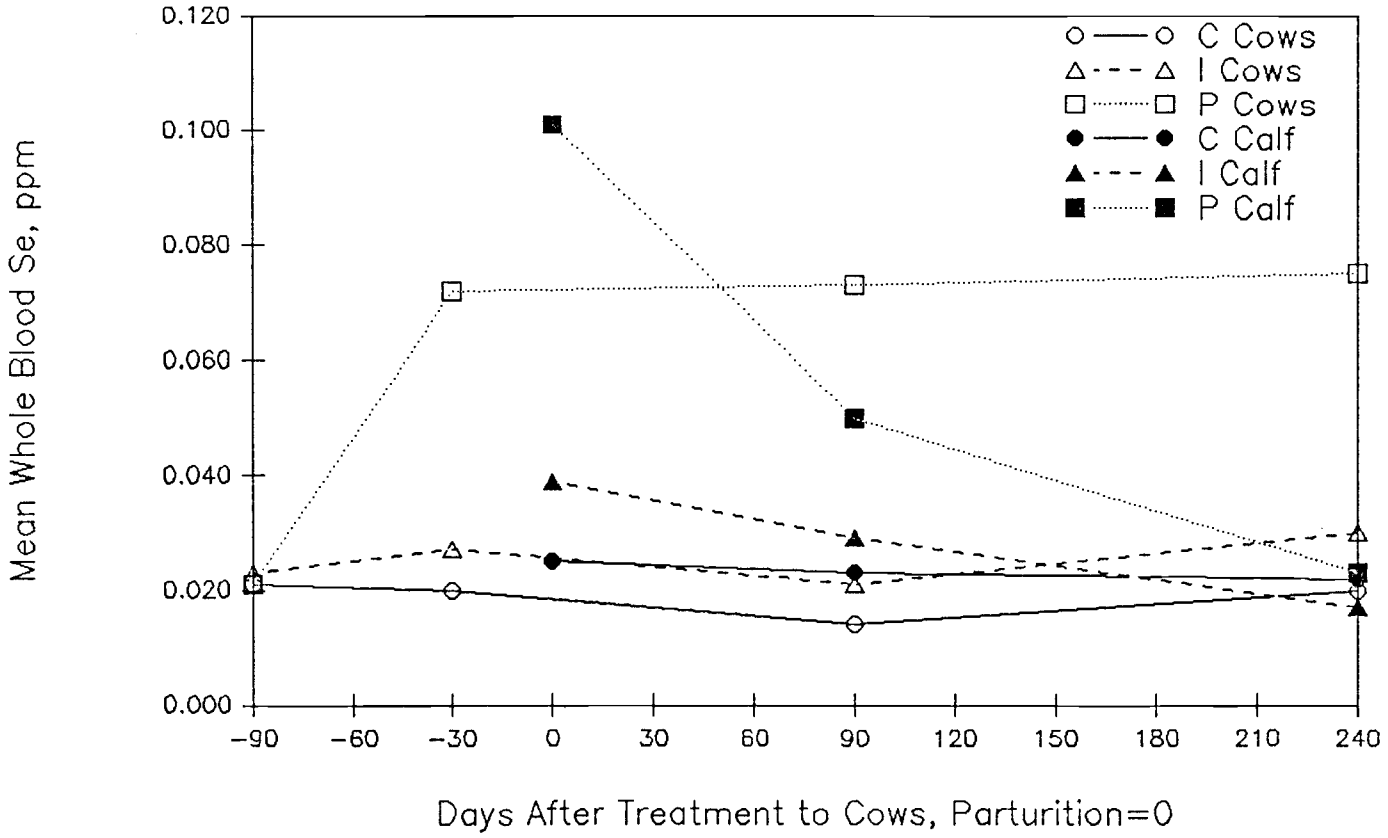

Fig. 2 Exp. 1: Residual analysis from equation to estimate calf whole blood Se levels at 90 days of age from dam Se levels at 90 days postpartum.

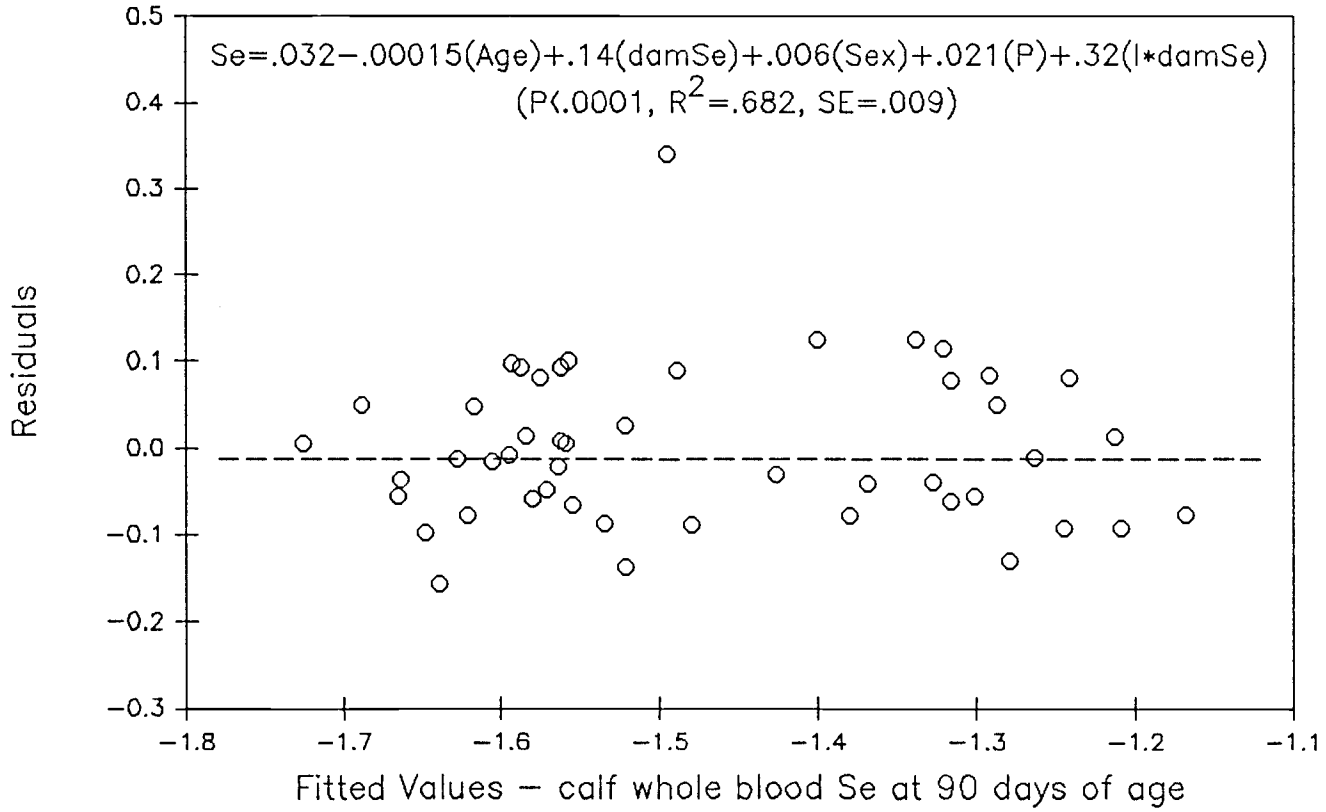

Fig. 3 Exp. 1: Residual analysis from equation to estimate calf whole blood Se levels at 90 days of age from dam Se levels 30 days prepartum.

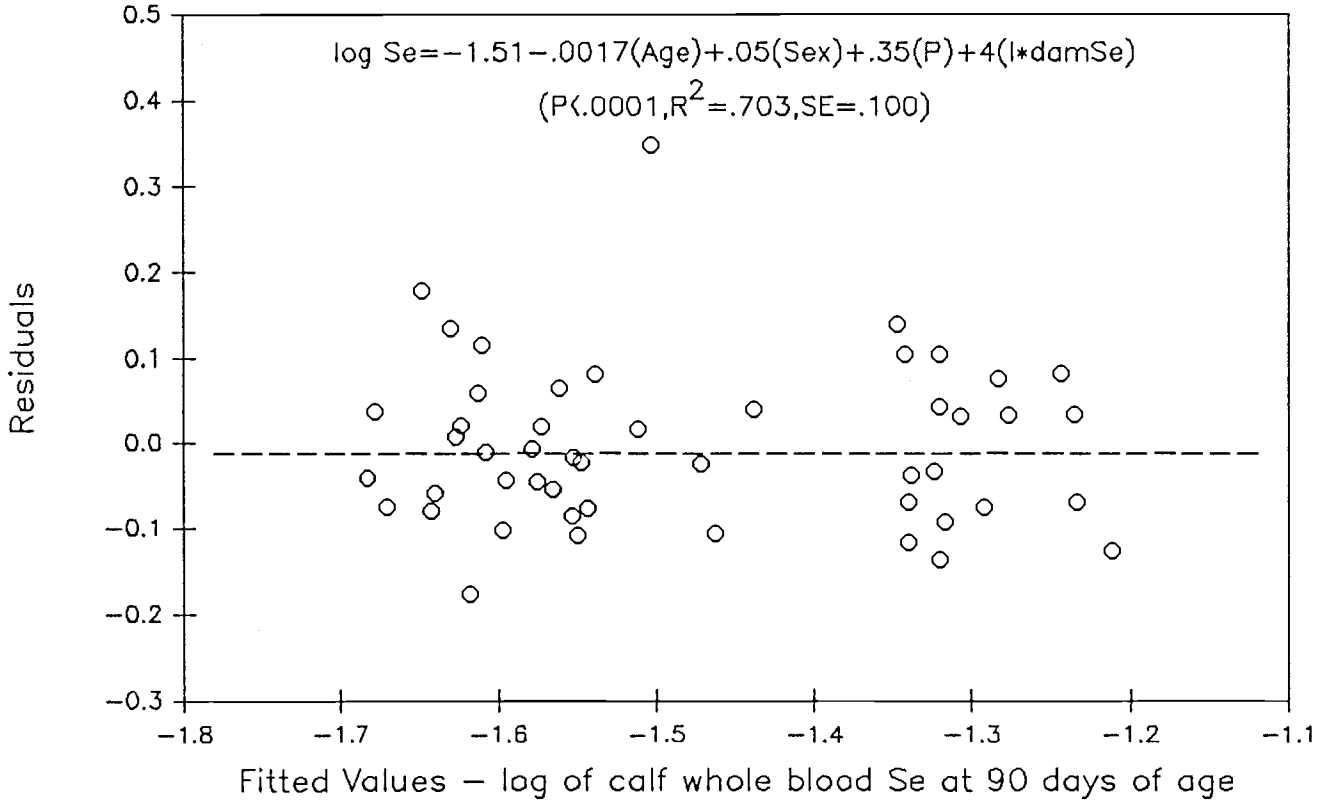

Fig. 4 Exp. 1: Serum GOT levels of calves, actual data (open symbols) and adjusted by age (to 89.4 d) filled symbols.

$GOT=1/(.012-.00003(Age)+.04(Se)+.004(I)-.1(Se*I))$

($P<.009$, $R^2=.259$, $SE=.0016$)

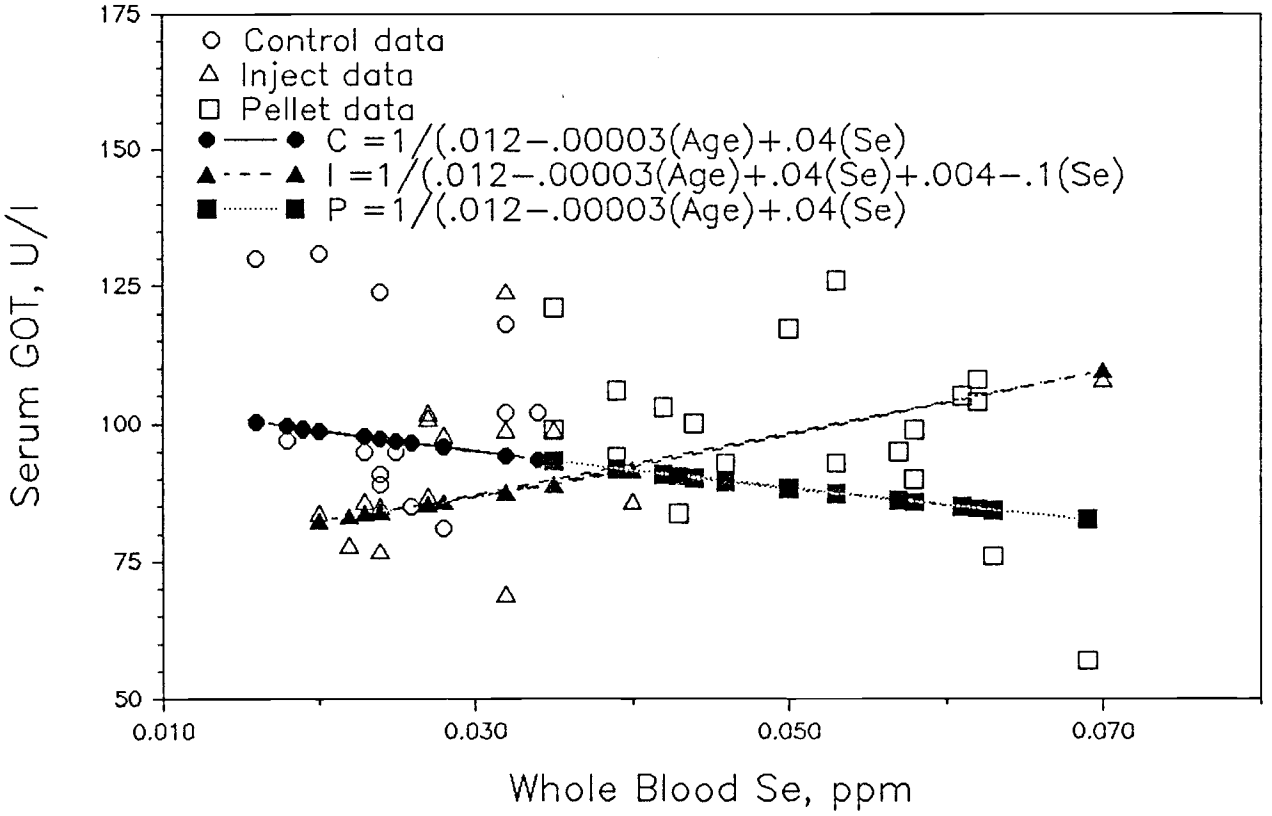

Fig. 5 Exp. 1: Serum LDH levels of calves, actual data (open symbols) and estimated by least squares regression (filled symbols).

LDH=873−395(Se)

($P<.05$, $R^2=.081$, SE=20)

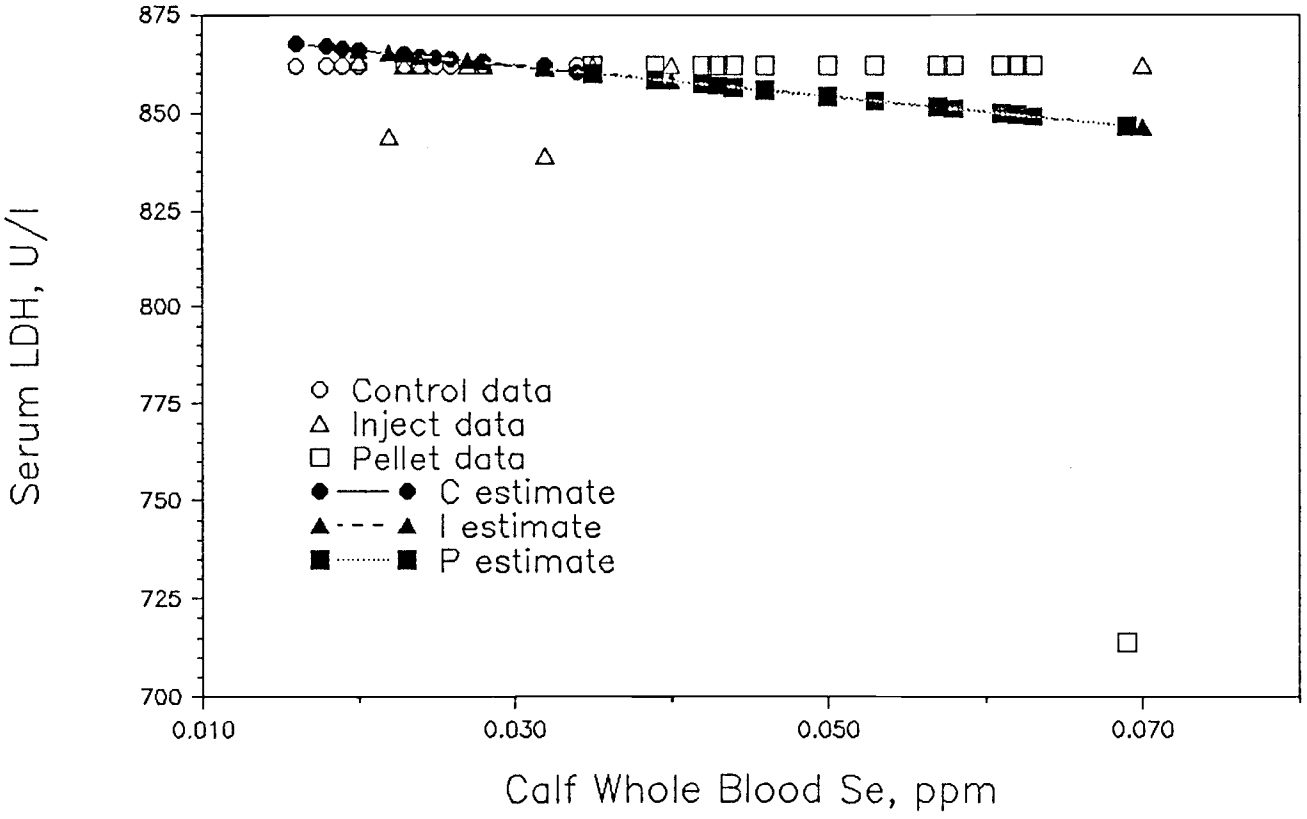

Fig. 6 Exp 1: Serum protein levels of calves, actual data (open symbols) and adjusted by age (to 89.4 d) filled symbols.

Protein=5.3+.007(Age)+7(Se)

(P<.002, $R^2$=.253, SE=.27)

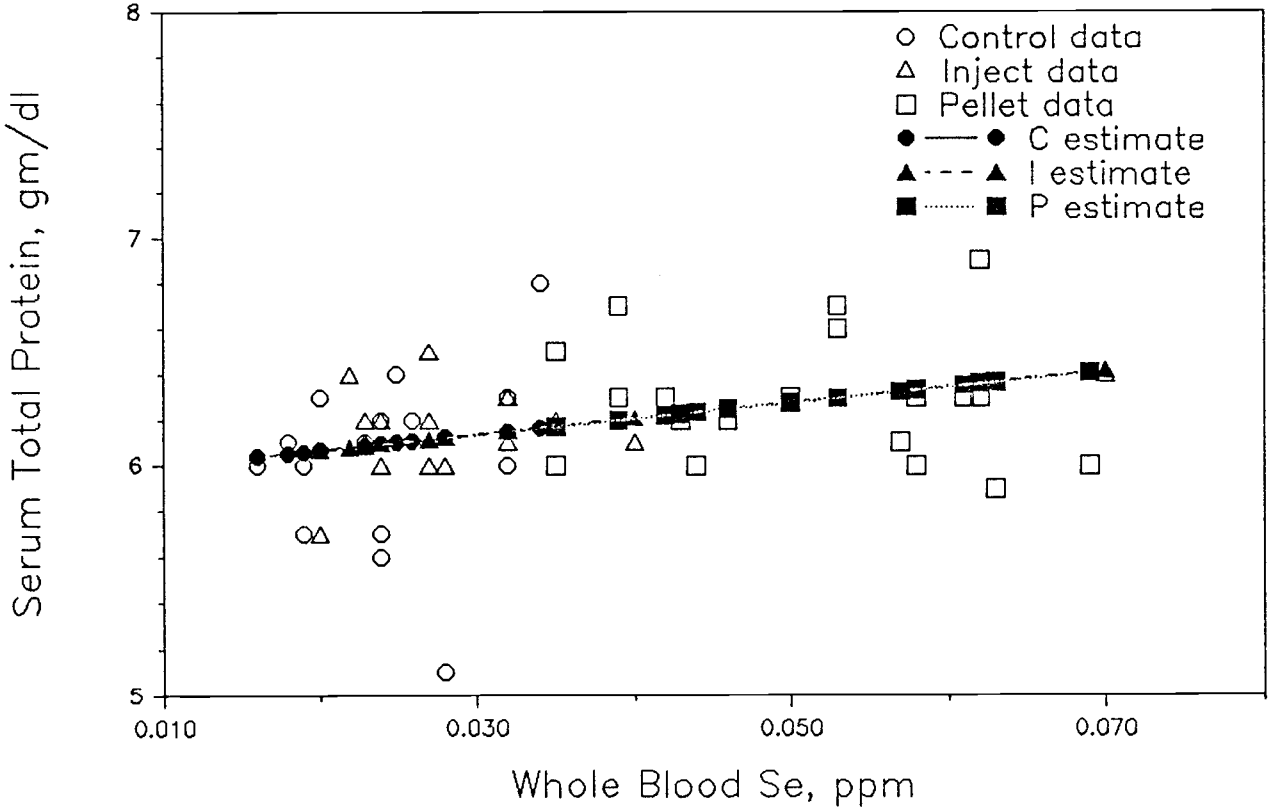

Fig. 7 Exp. 1: Serum albumin levels of calves, actual data (open symbols) and adjusted by least squares regression (filled symbols).

Albumin=3.73+4(Se)

($P<.03$, $R^2=.100$, SE=.19)

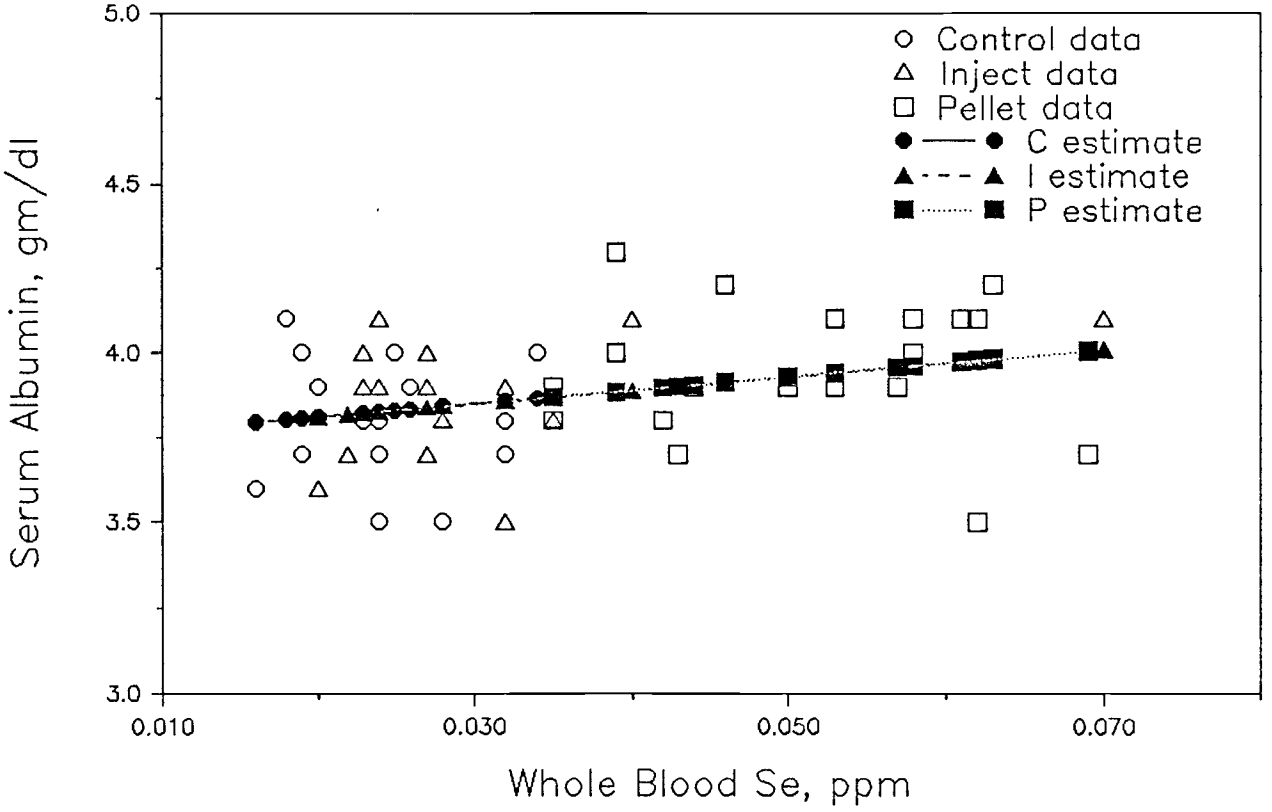

Fig. 8 Exp. 1: Serum inorganic Phosphorus levels of calves, actual data (open symbols) and adjusted by age (to 89.4 d) filled symbols.

$\log P_i = 1.01 - .0009(\text{Age}) + 1.0(\text{Se}) - .04(P)$

($P < .003$, $R^2 = .266$, SE=.039)

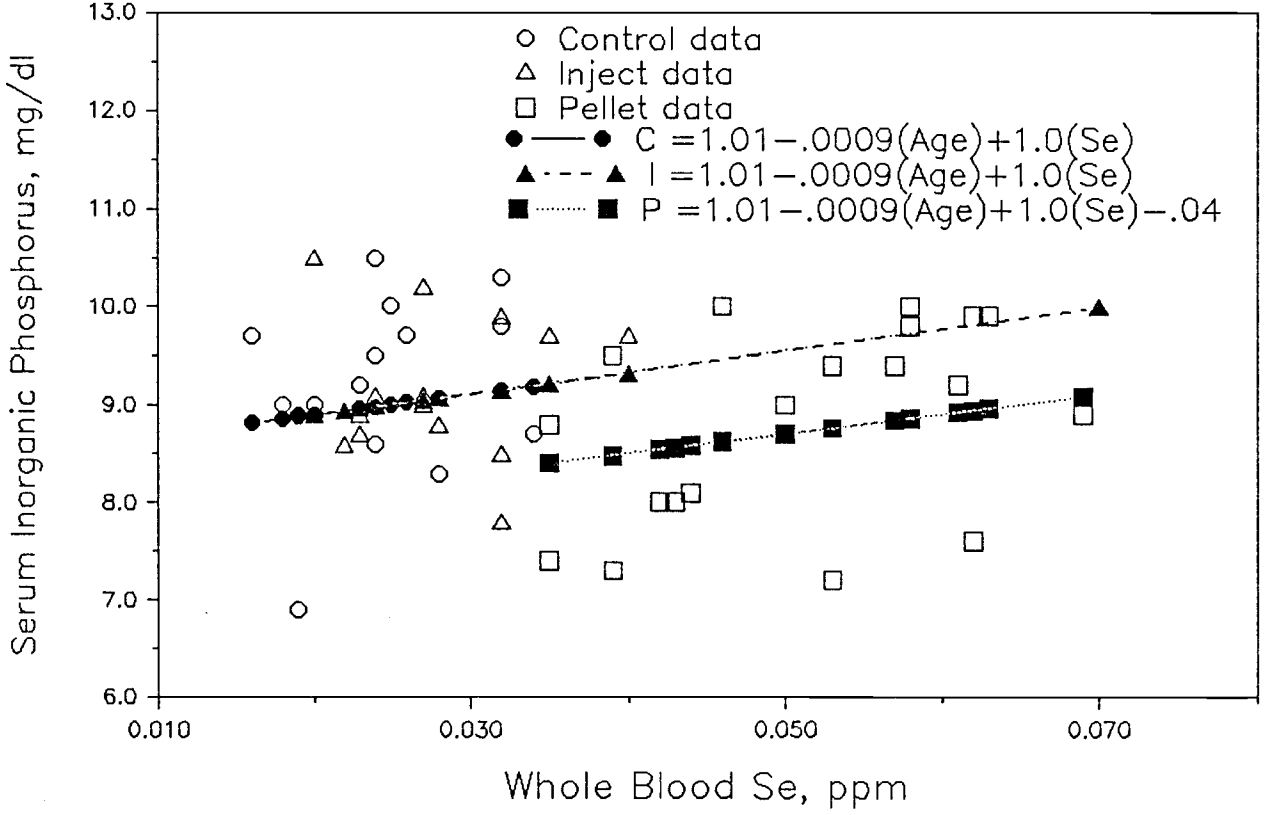

Fig. 9 Exp. 1: Calf whole blood Se levels vs. dam Se levels, actual data (open symbols), adjusted by age (to 89.4 d) and sex (filled symbols).

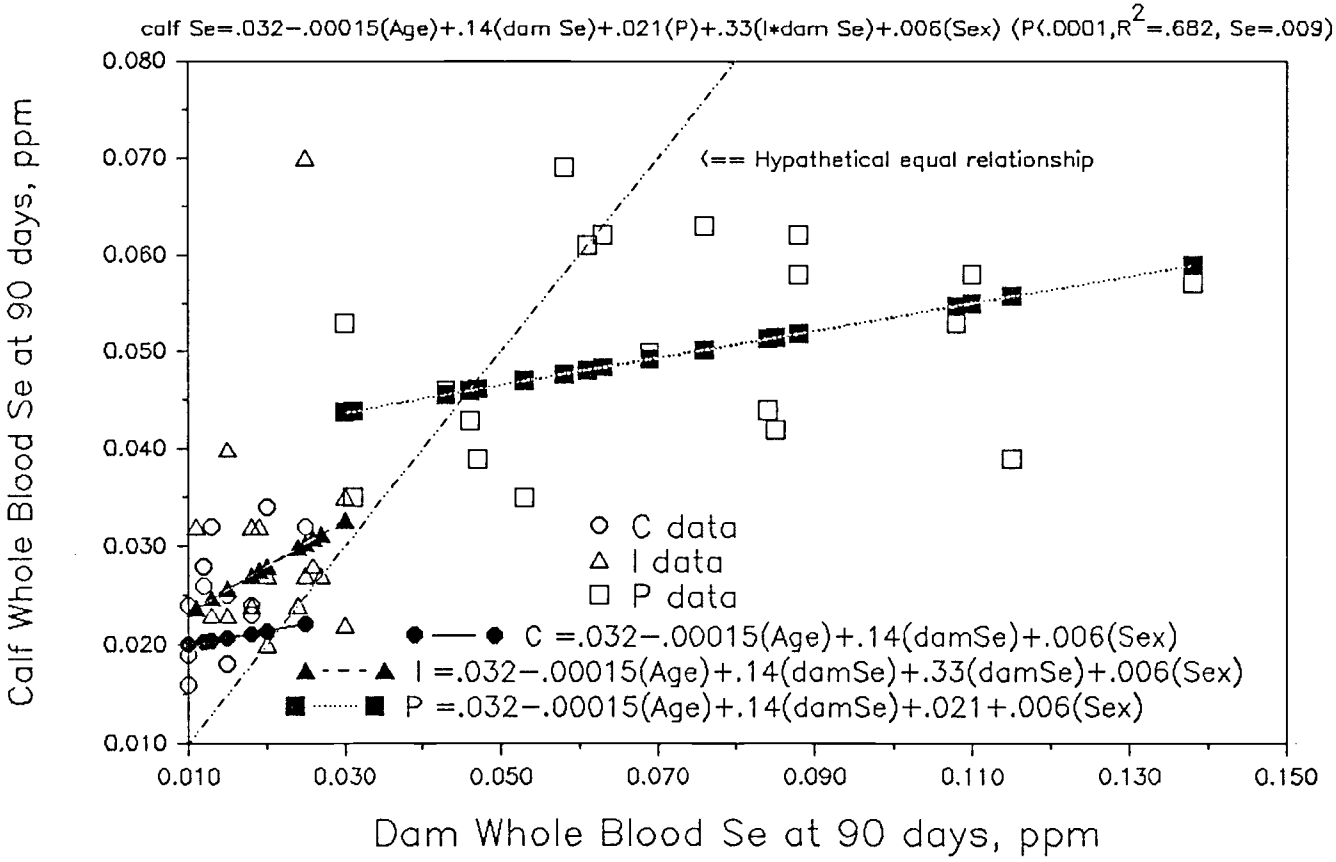

Fig. 10 Exp. 1: Calf whole blood Se levels vs. 30 day prepartum dam Se levels, actual data (open symbols), adjusted by age (to 89.4 d) and sex (filled symbols).

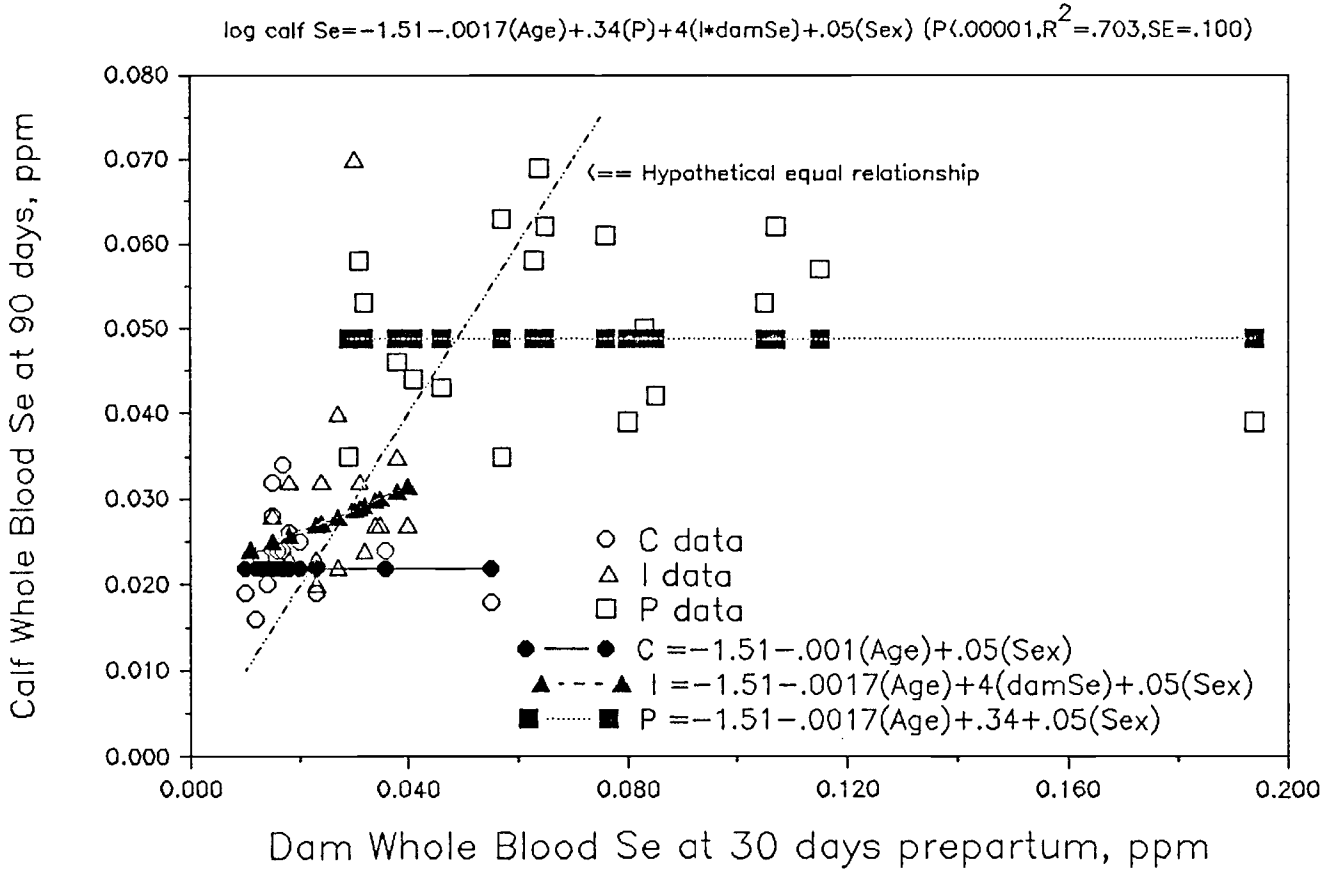

Fig. 11 Exp. 2A: Whole blood Se (ppm) levels of cows and their calves by treatment and times during Experiment 2A. Cows represented by open symbols, calves by filled symbols.

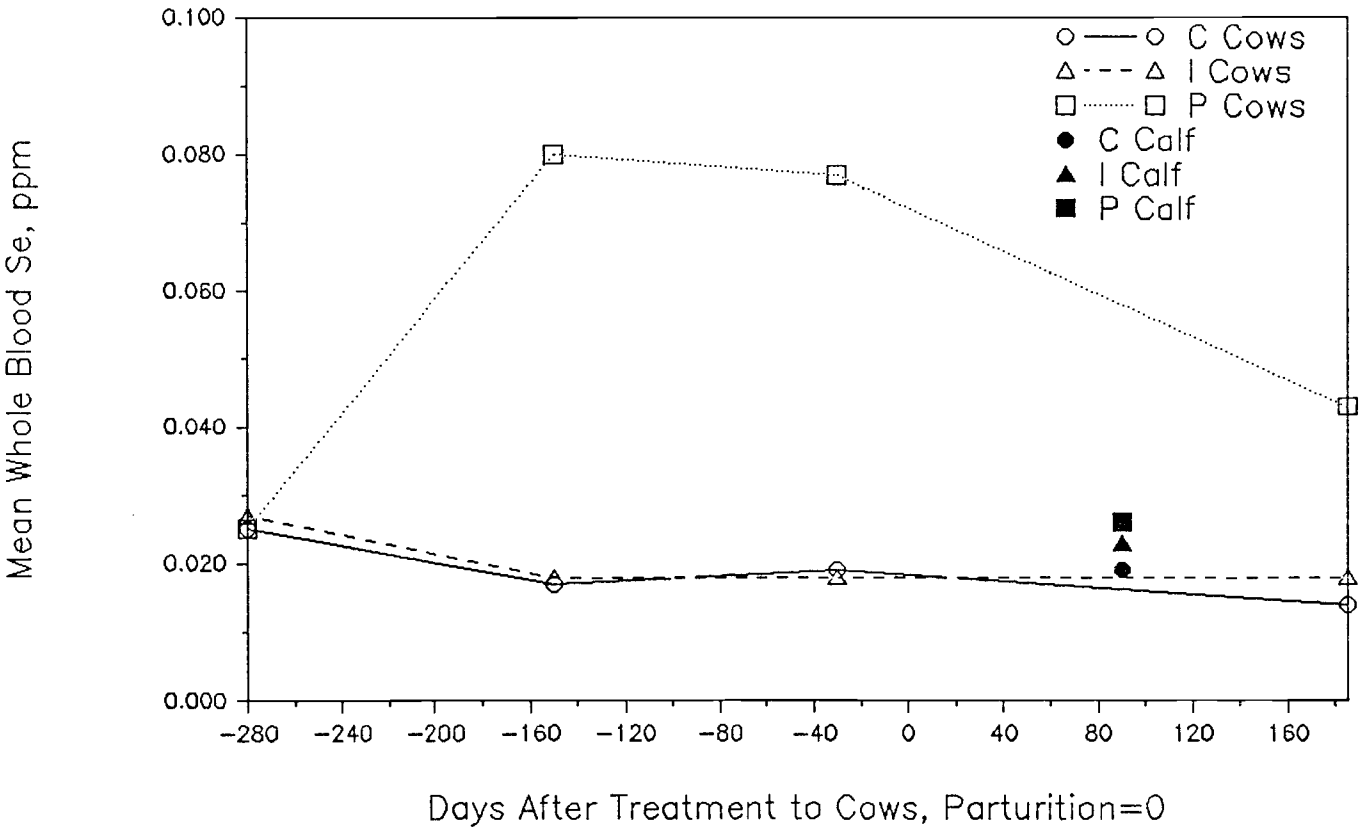

Fig. 12 Exp. 2A: Calf whole blood Se levels vs. 30 day prepartum dam Se levels, actual data (open symbols), and adjusted by sex (filled symbols).

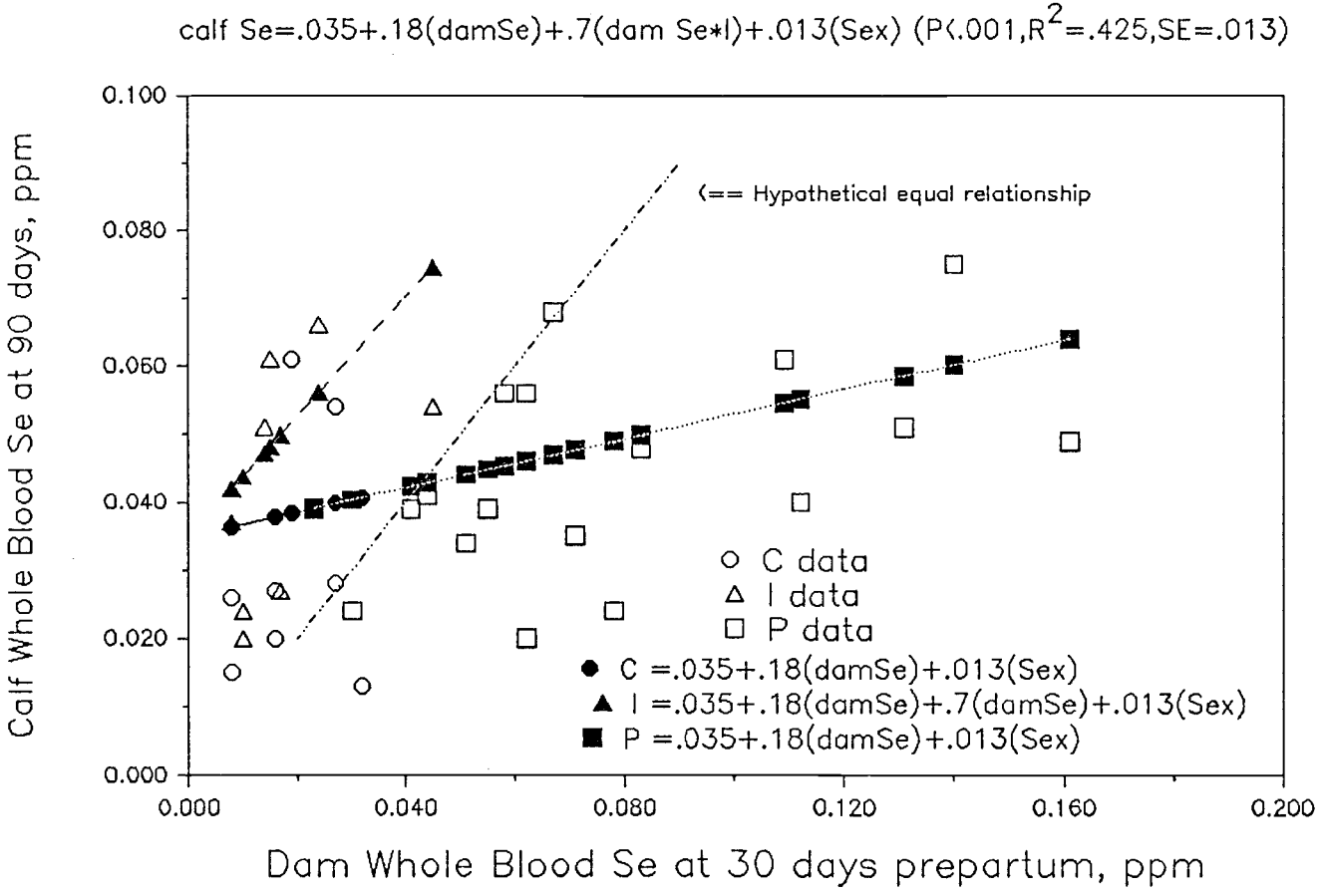

Fig. 13 Exp. 2A: Serum protein levels of calves, actual data (open symbols) and adjusted by sex (filled symbols).

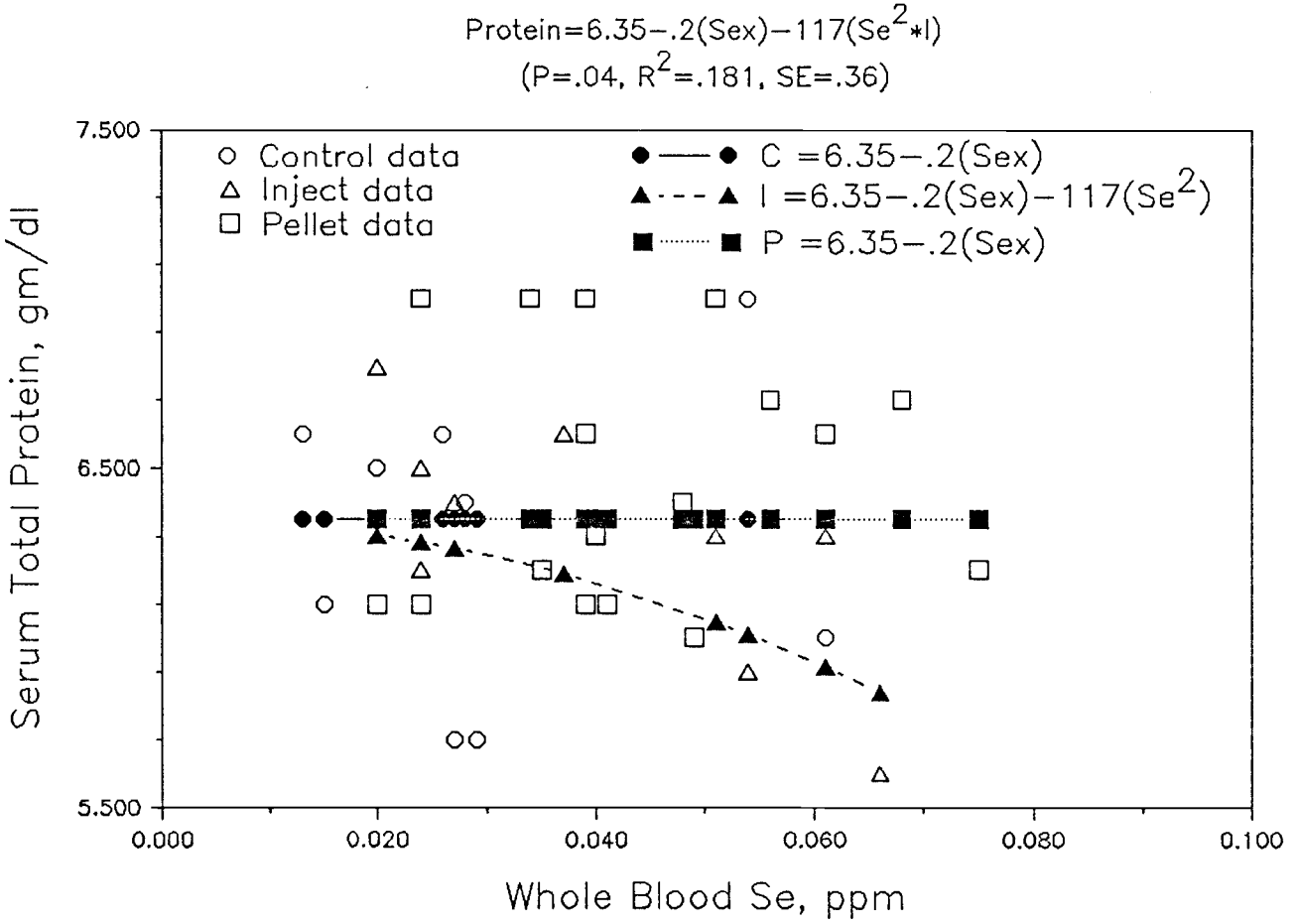

Fig. 14 Exp. 2A: Blood urea nitrogen levels of calves, actual data (open symbols) and adjusted by least squares regression (filled symbols).

$$BUN = 1/(.12 - .6(Se))$$

$$(P < .06,\ R^2 = .100, SE = .03)$$

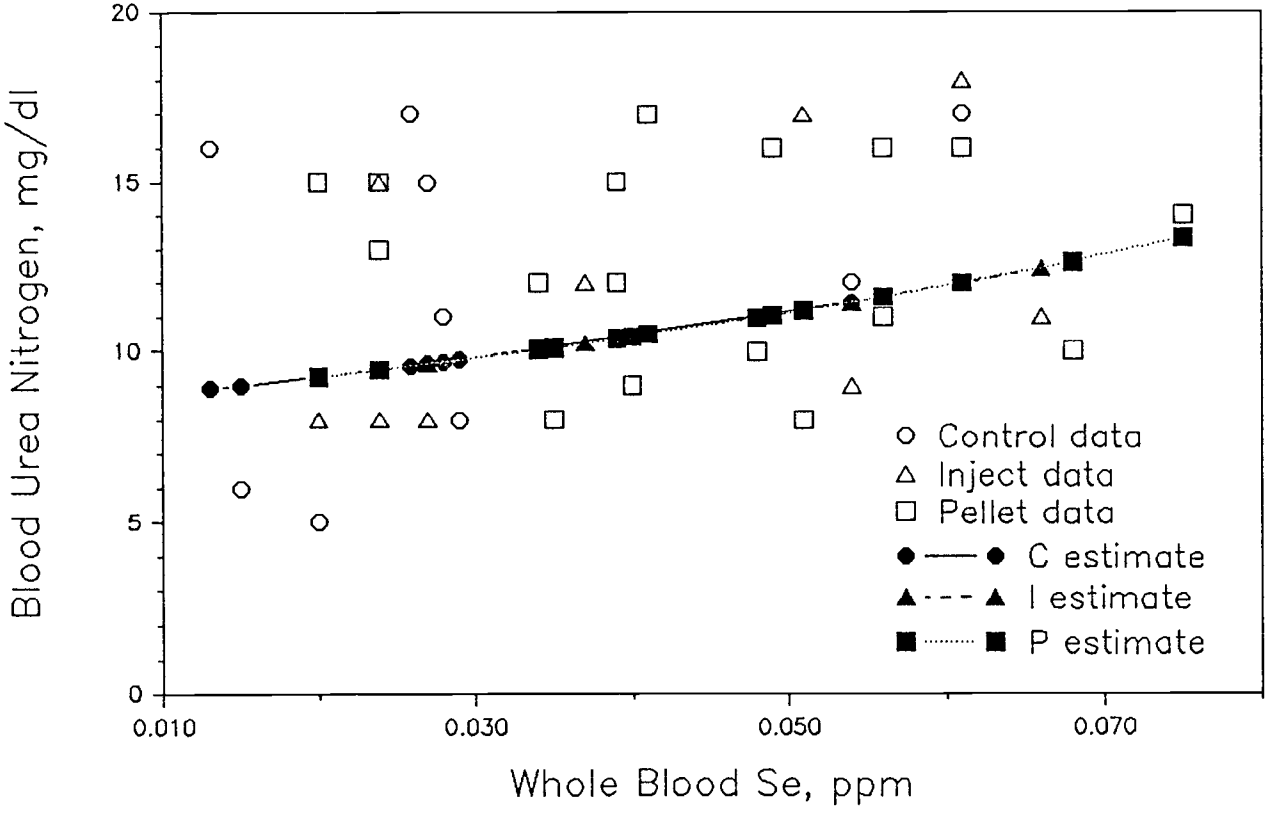

Fig. 15 Exp. 2A: Serum Inorganic Phosphorus levels of calves actual data (open symbols) and adjusted for sex (filled symbols).

$P_i = 7.8 + .8(Sex) - 1021(P*Se^2) + 48(P*Se)$

($P = .04$, $R^2 = .229$, $SE = 1.13$)

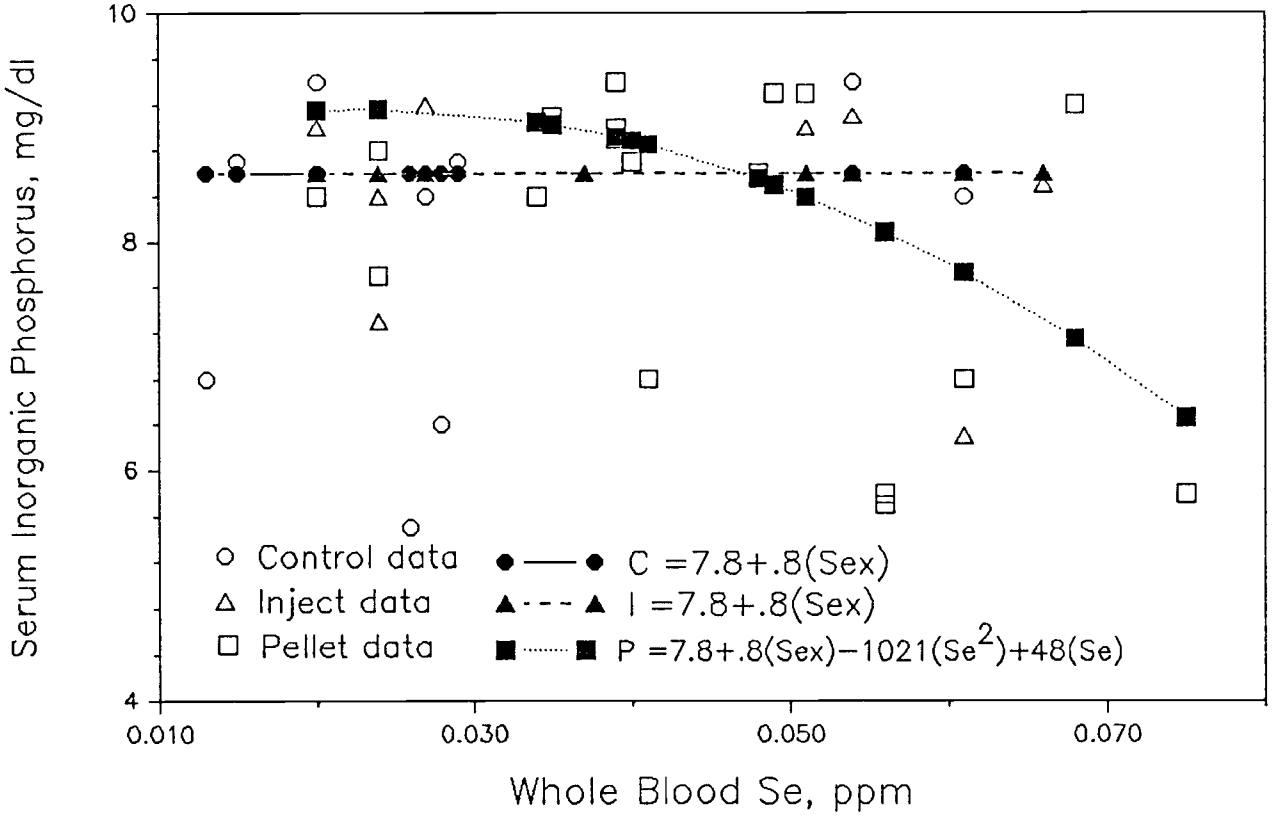

Fig. 16 Exp. 2B: Whole blood Se (ppm) levels of cows and their calves by treatment and times during Experiment 2B. Cows represented by open symbols, calves by filled symbols.

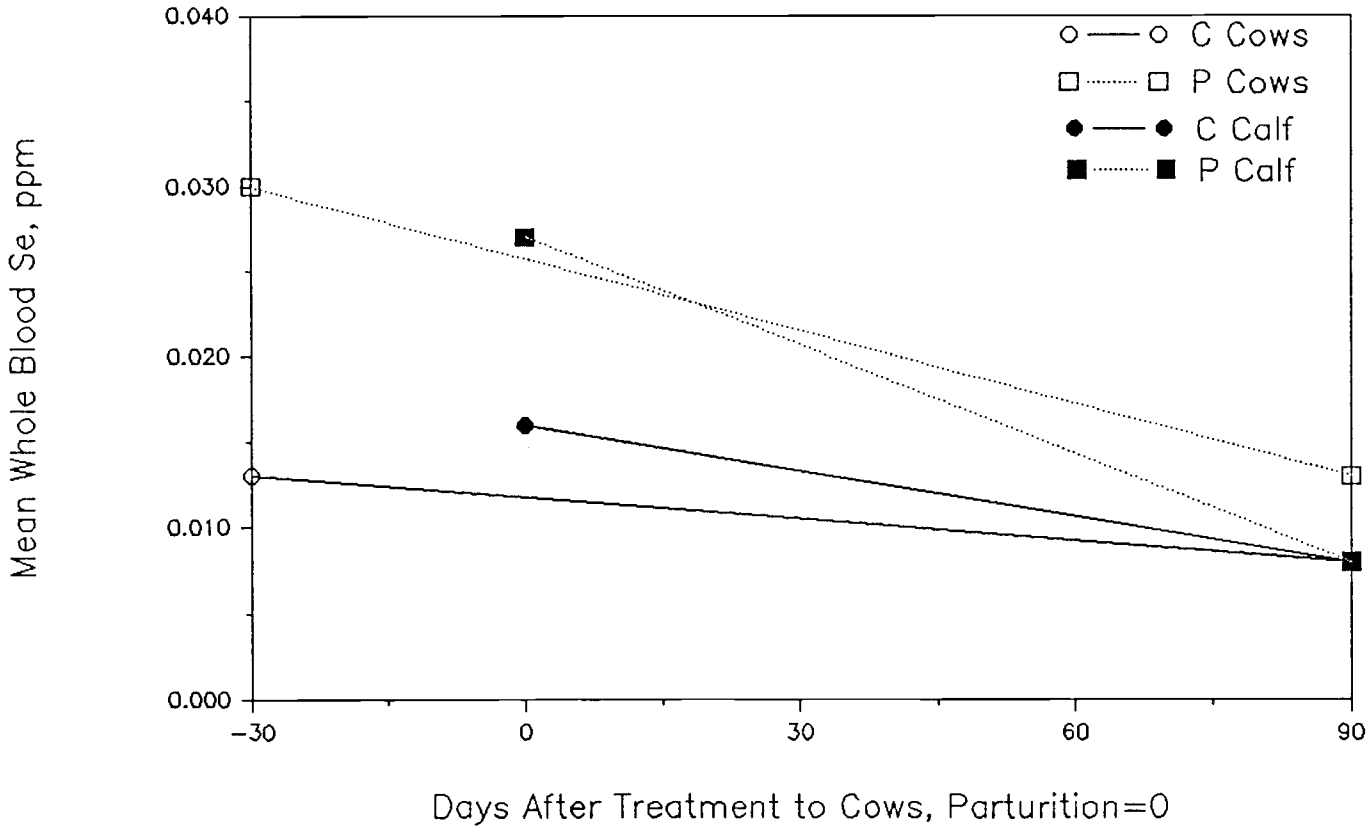

Fig. 17 Exp. 2B: Serum GPT levels of calves at birth, actual data (open symbols) and least squares regression estimates (filled symbols).

$GPT=18-24(P)+18339(Se^2*P)$

$(P<.072, R^2=.254, SE=12)$

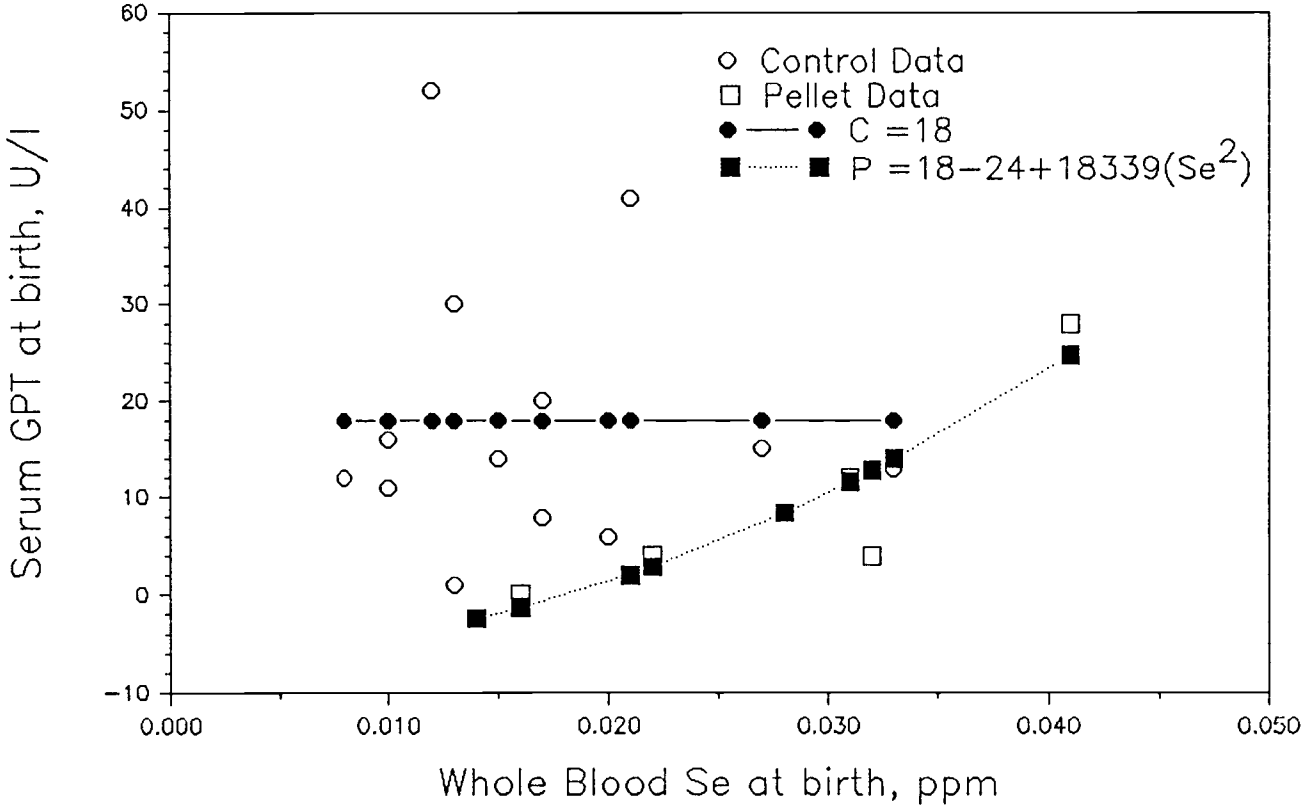

Fig. 18 Exp. 2B: Serum LDH levels of calves at birth, actual data (open symbols) and least squares regression estimates (filled symbols).

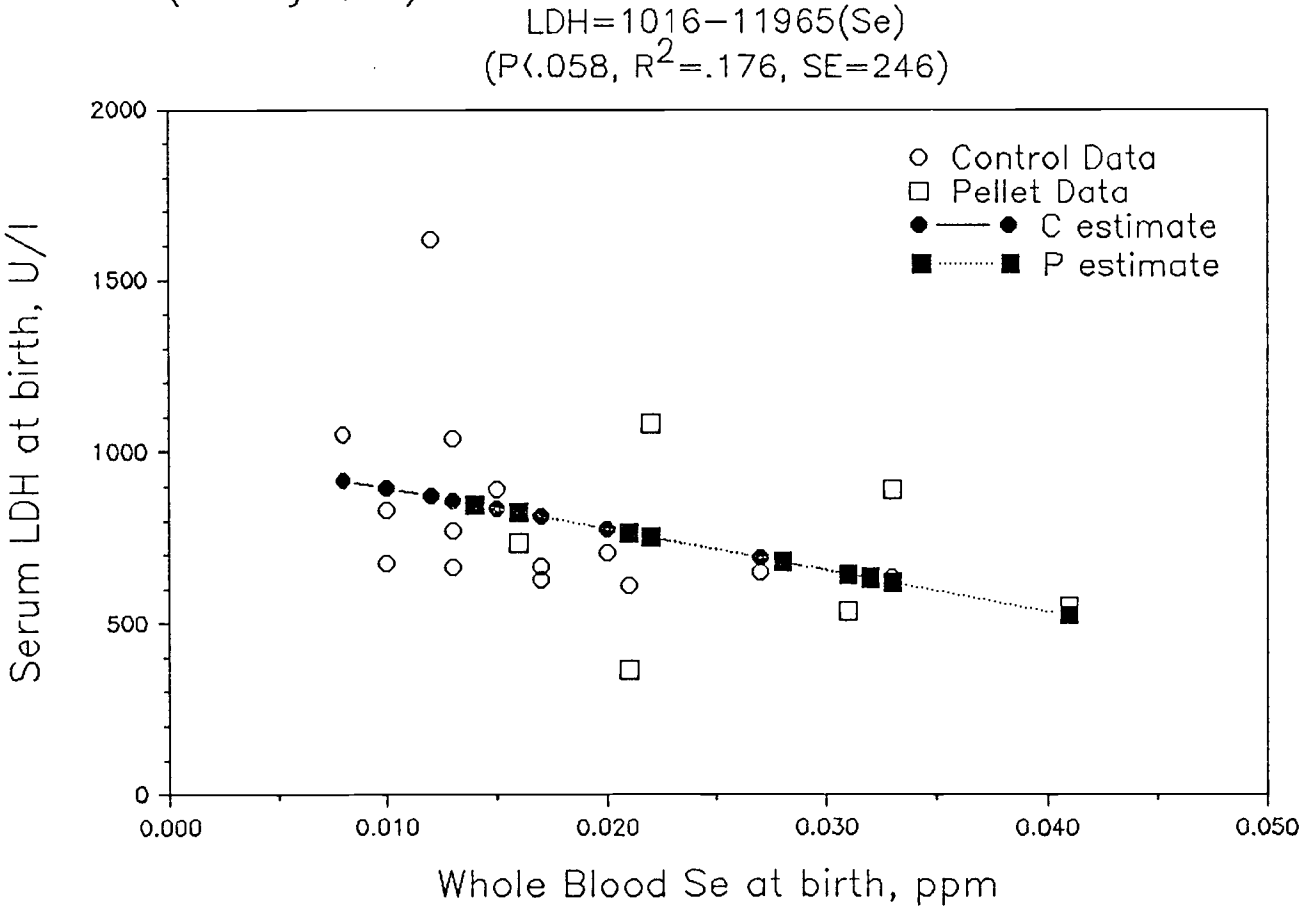

Fig. 19 Exp. 2B: Serum bilirubin levels of calves at birth, actual data (open symbols) and least squares regression estimates (filled symbols).

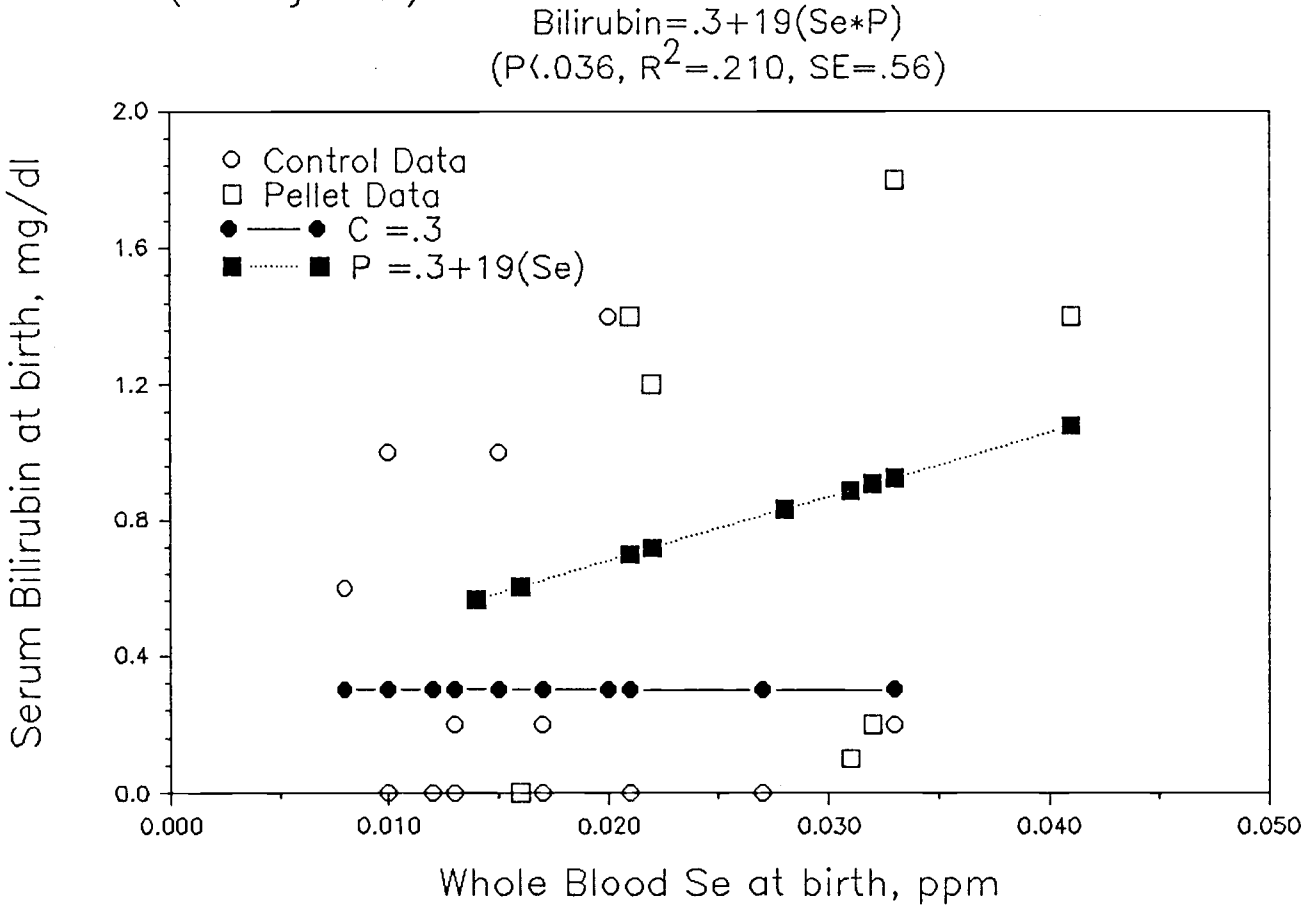

Fig. 20 Exp. 2B: Blood urea nitrogen levels of calves at birth, actual data (open symbols) and adjusted for sex (filled symbols).

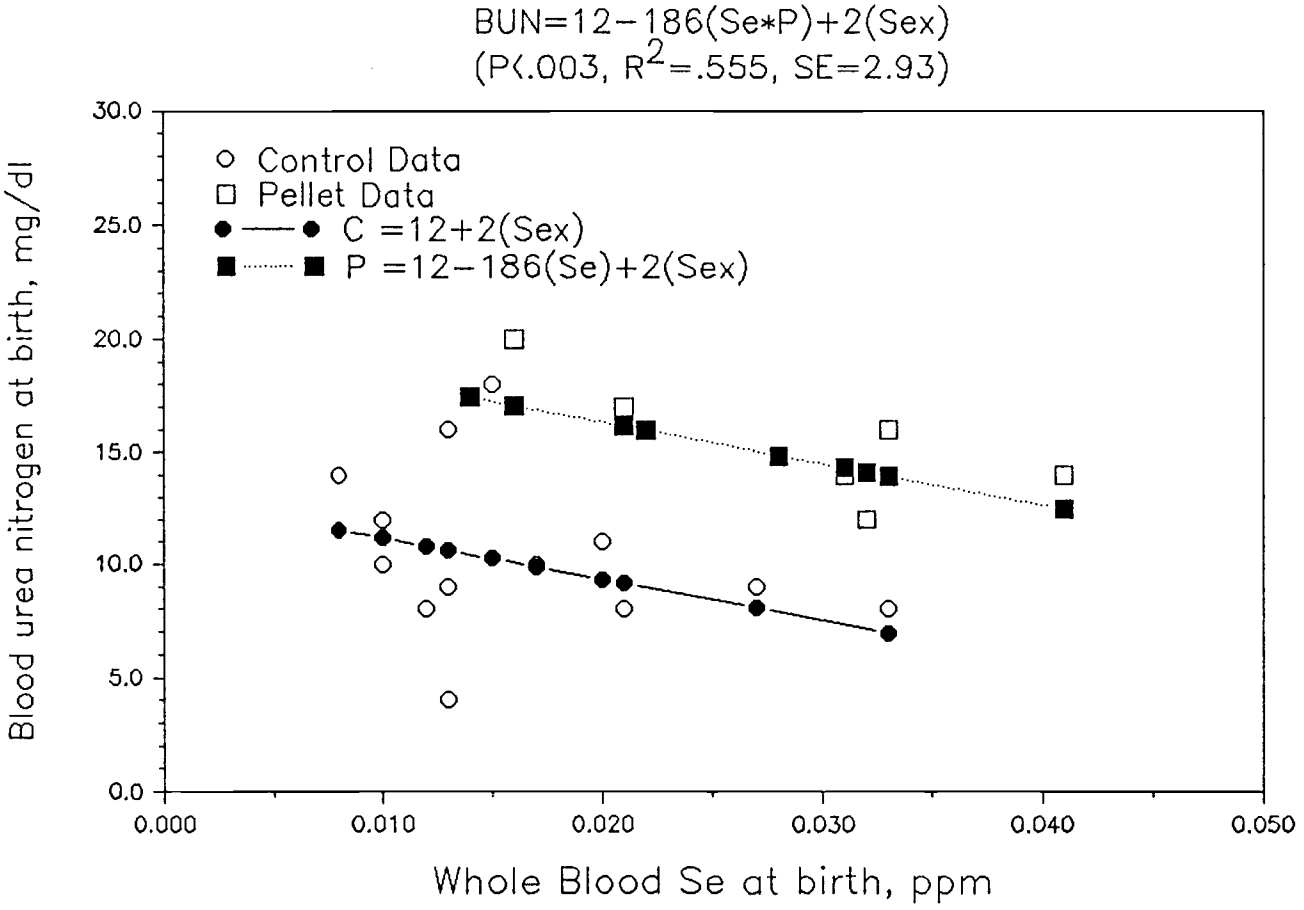

Fig. 21 Exp. 2B: Calf whole blood Se levels at birth vs. 30 day prepartum dam Se levels, actual data (open symbols) and predicted.

$$\text{Calf Se}_{birth} = .012 + .3(\text{dam Se}) + .006(P)$$

$$(P < .001,\ R^2 = .475,\ SE = .007)$$

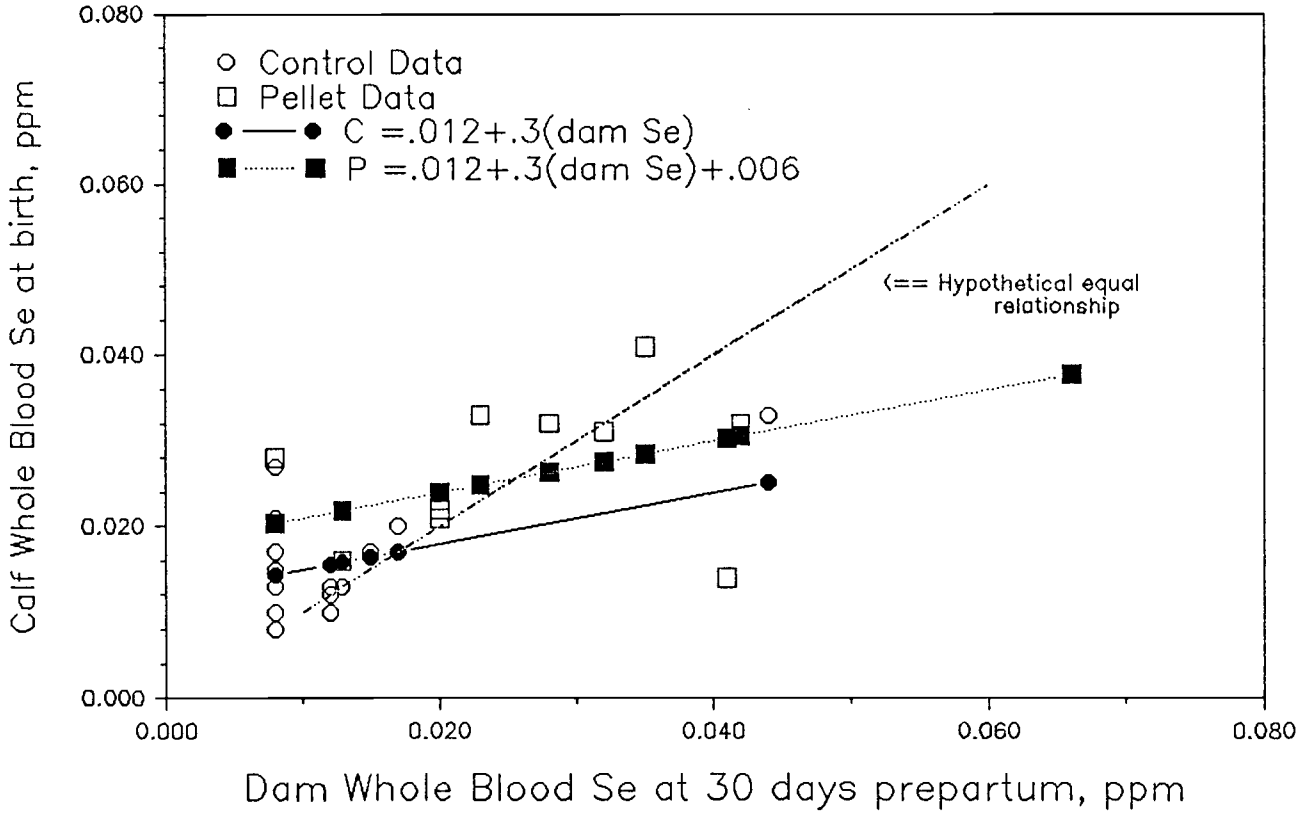

Fig. 22 Exp. 2B: Serum immunoglobulin levels of calves, actual data (open symbols) and adjusted for age (to 98.8 d) filled symbols.

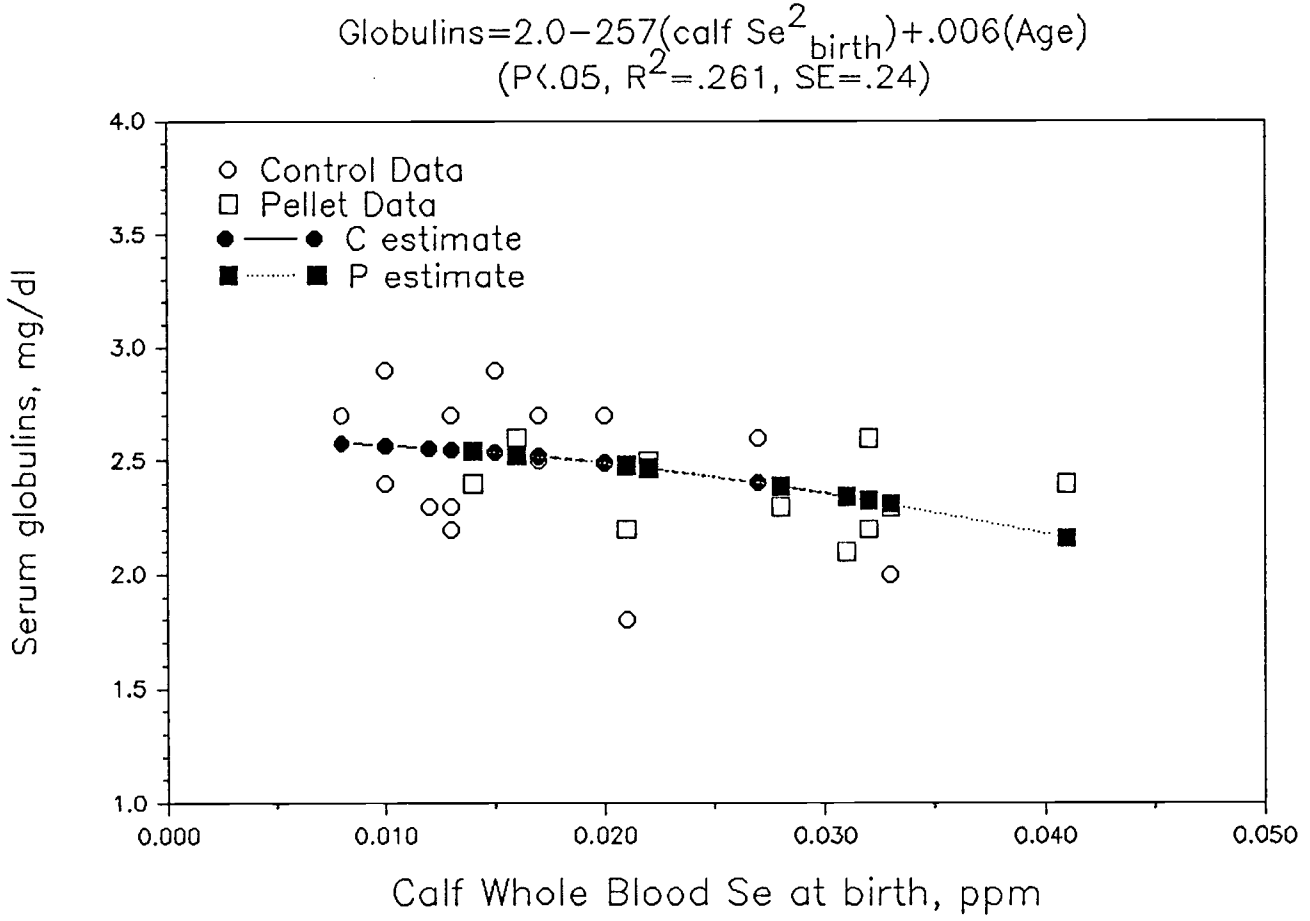

Fig. 23 Exp. 2B: Fibrinogen levels of calves at about 90 d, actual data (open symbols) and least squares regression estimates (filled symbols).

Fibrinogen=262+349145($Se^2$)+140(P)−466511(Se*P)

(P<.006, $R^2$=.464, SE=100)

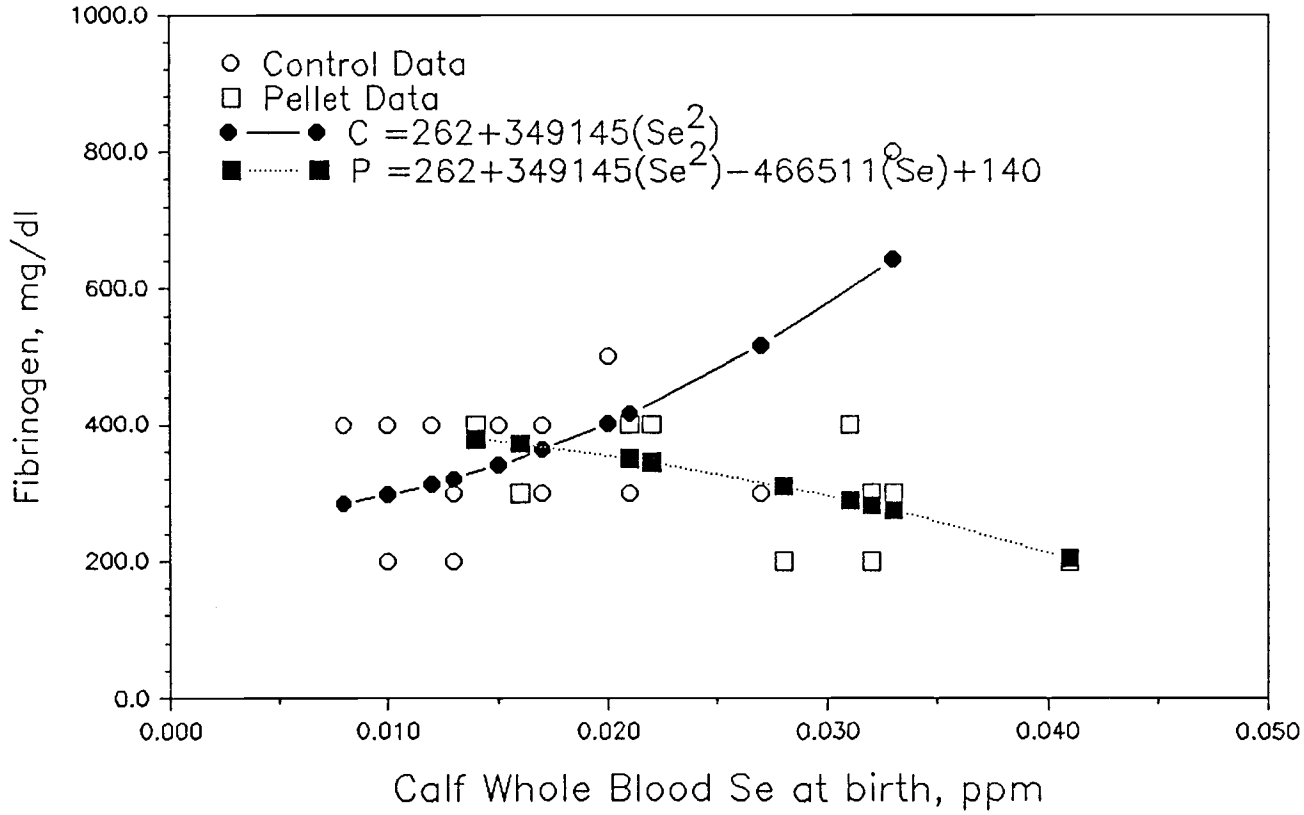

Fig. 24 Exp. 2B: White Blood Cells of calves at about 90 d, actual data (open symbols) and least squares regression estimates (filled symbols).

$WBC \times 10^3 = 10.9 + 2238(P*Se^2)$

$(P<.06,\ R^2=.162, SE=2.5)$

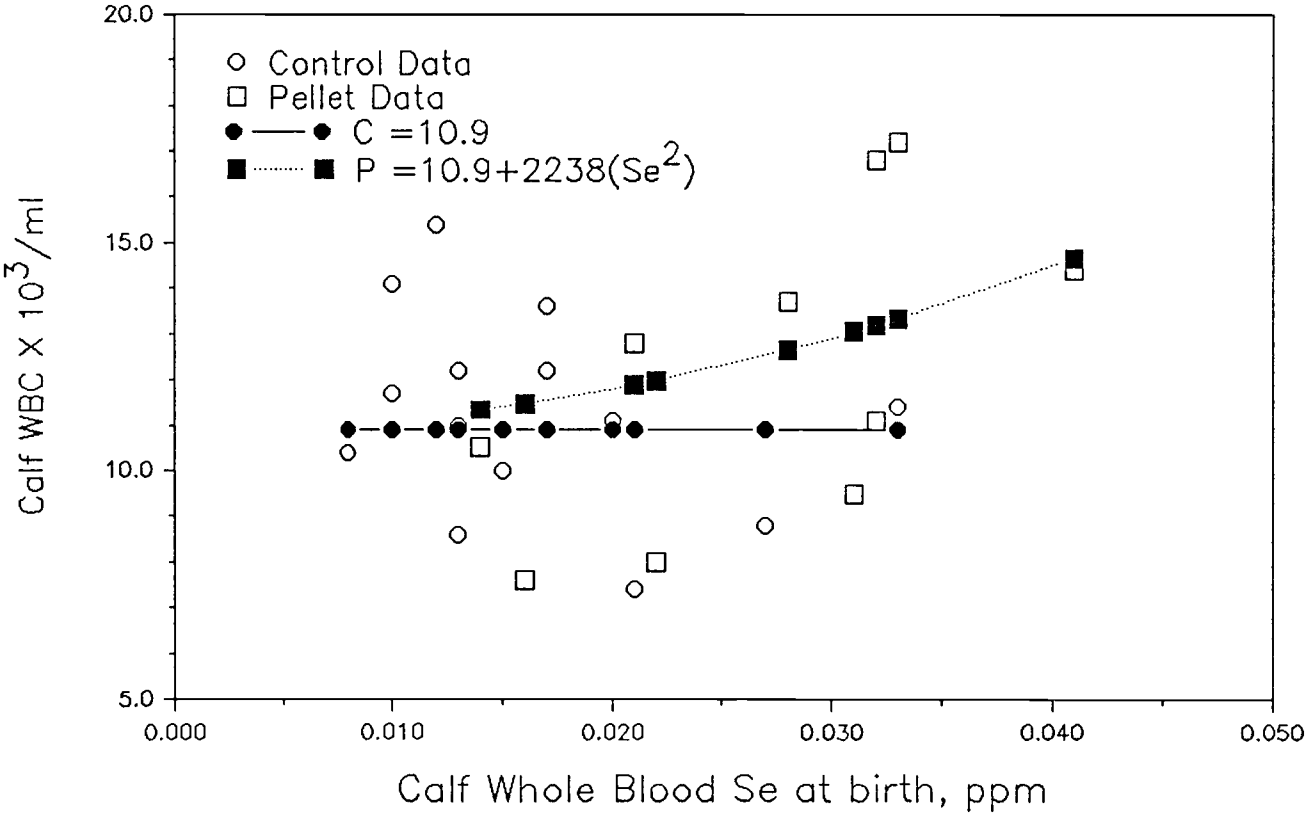

Fig. 25 Exp. 2B: Weight per day of age at weaning for calves, actual data (open symbols) and adjusted for sex (filled symbols).

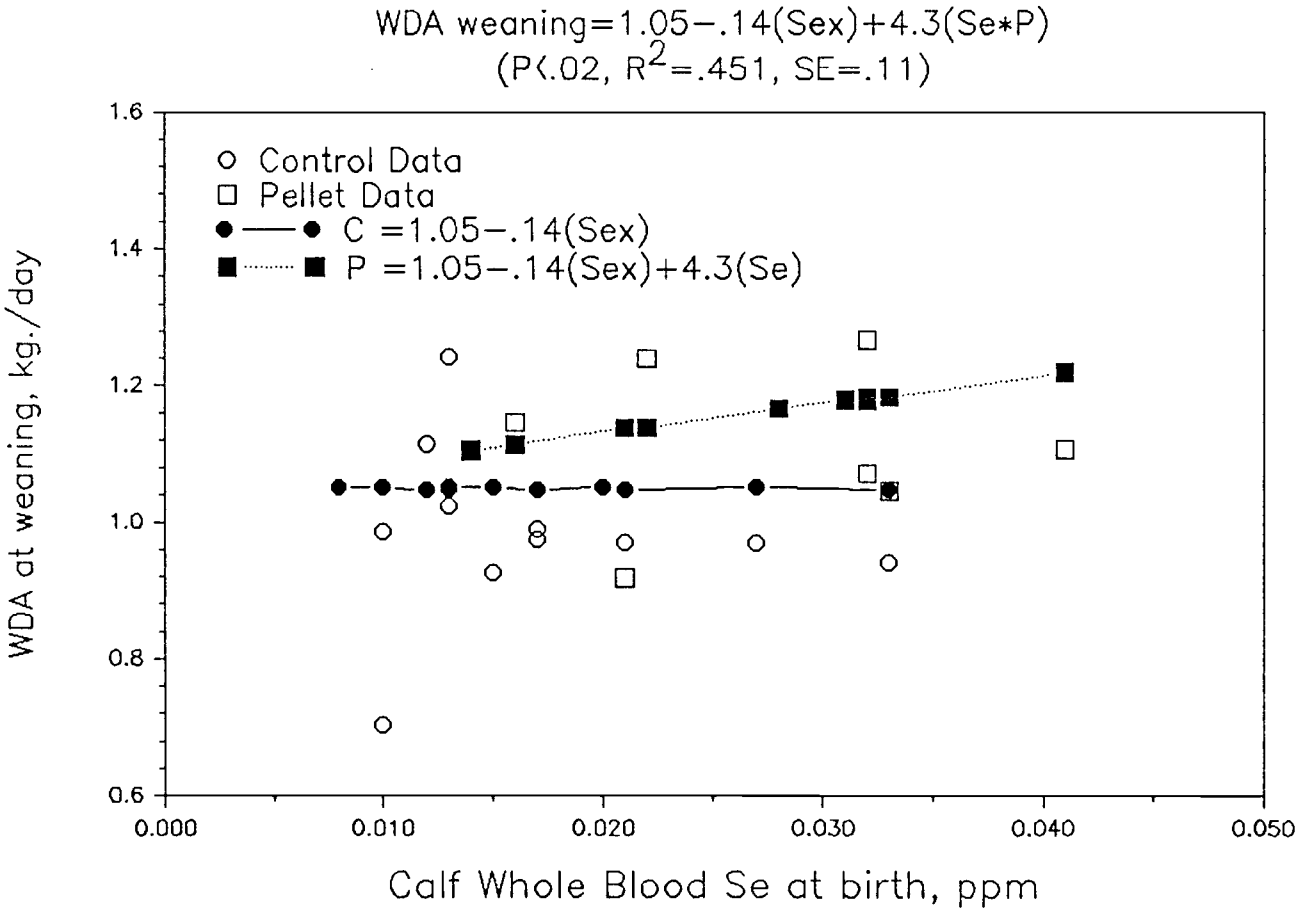

Fig. 26 Exp. 3: Serum GOT of calves, actual data (open symbols) and adjusted by age (to 71 days) filled symbols. Treatments: C no b Se=Controls without Se at birth, C yes b Se=Controls with Se at birth and similarly for P=Pellet treatments.

GOT=58+.76(Age)+25(bSe)−301(Se*P*bSe) (P<.002, $R^2$=.359,SE=18)

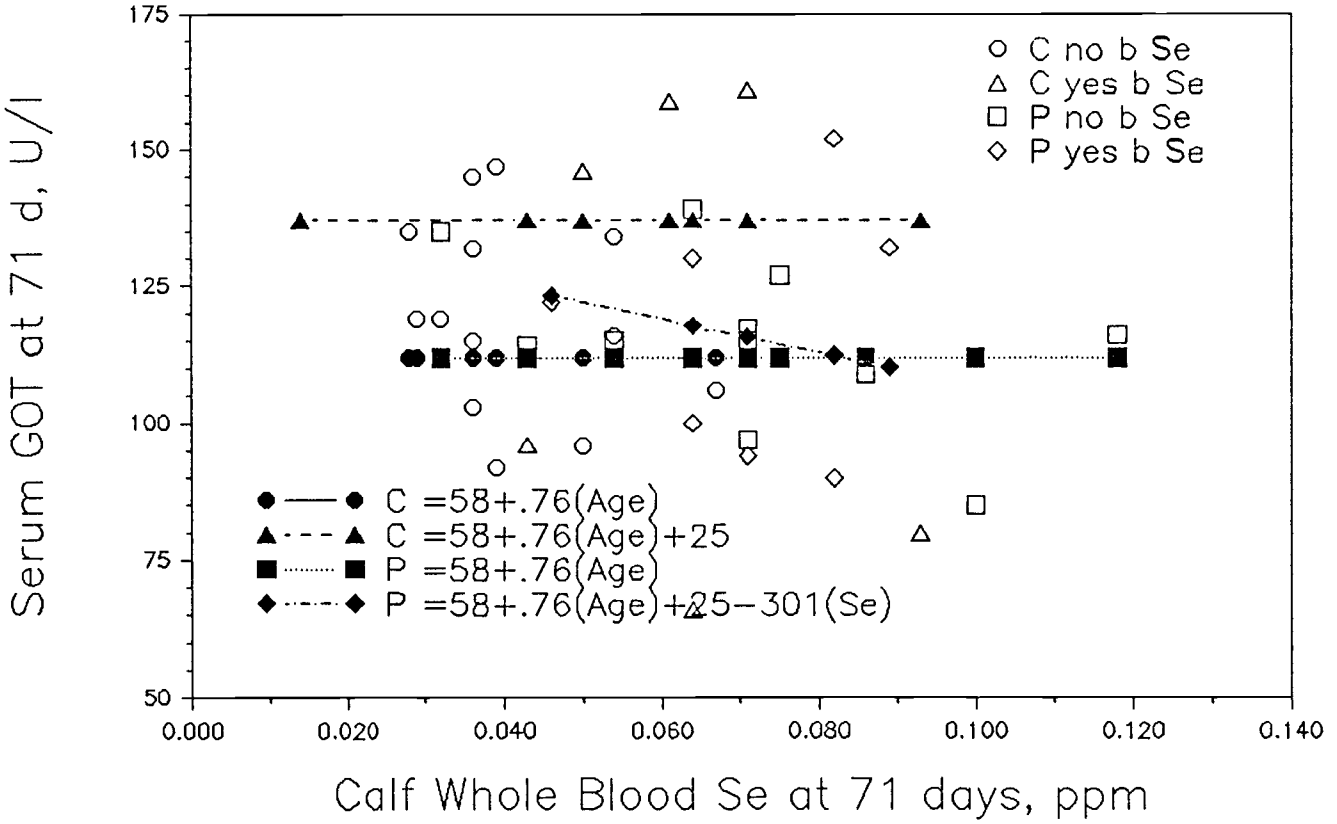

Fig. 27 Exp. 3: Serum inorganic phosphorus in calves at 71 days, actual data (open symbols) and adjusted by age (filled symbols).

$P_i = 8.3 + 17(Se) - 8(Se*P*bSe) - .007(Age)$

($P < .0002$, $R^2 = .449$, $SE = .53$)

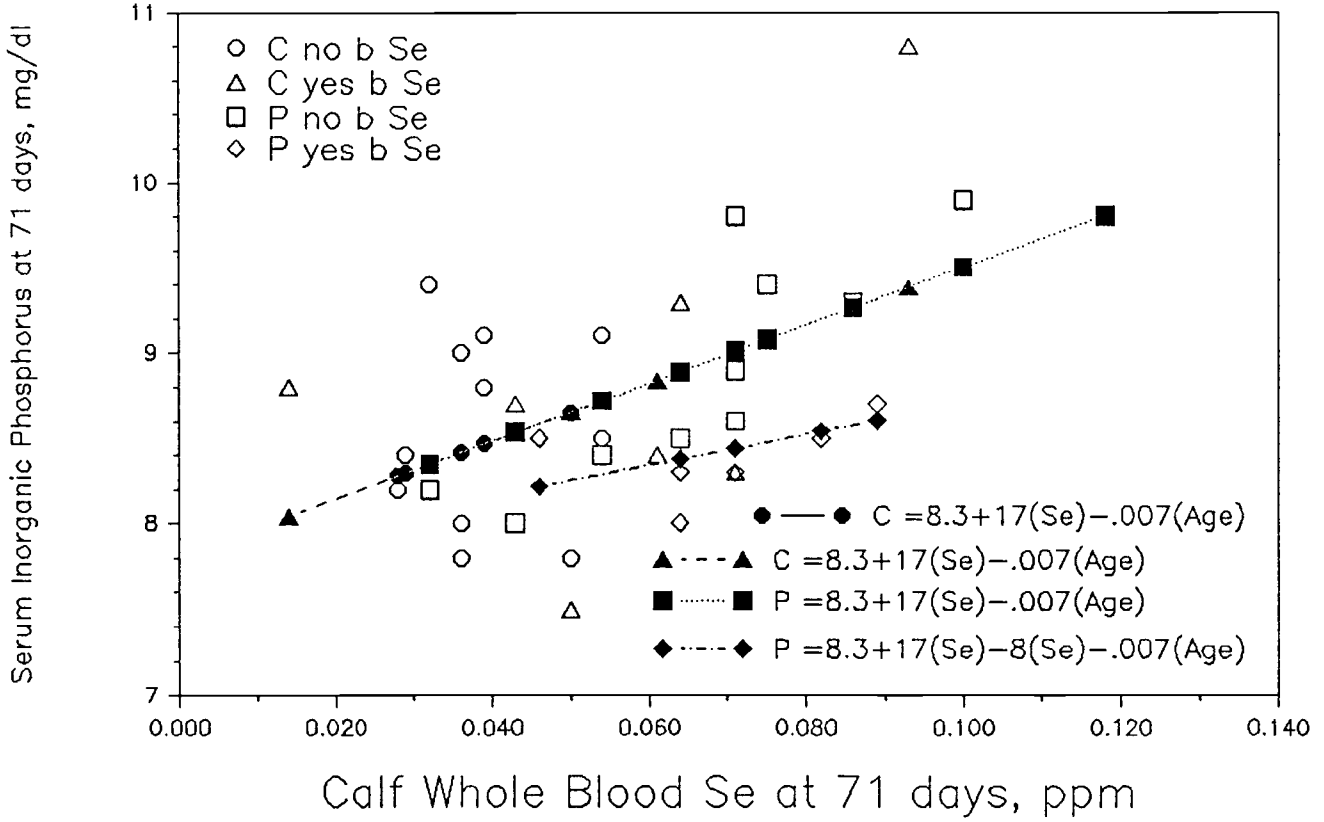

Fig. 28 Exp. 3: Serum albumin of calves at 71 days, actual data (open symbols) and adjusted by least squares regression (filled symbols). Treatments: C no b Se=Controls without Se at birth, C yes b Se=Controls with Se at birth and similarly for P=Pellet.

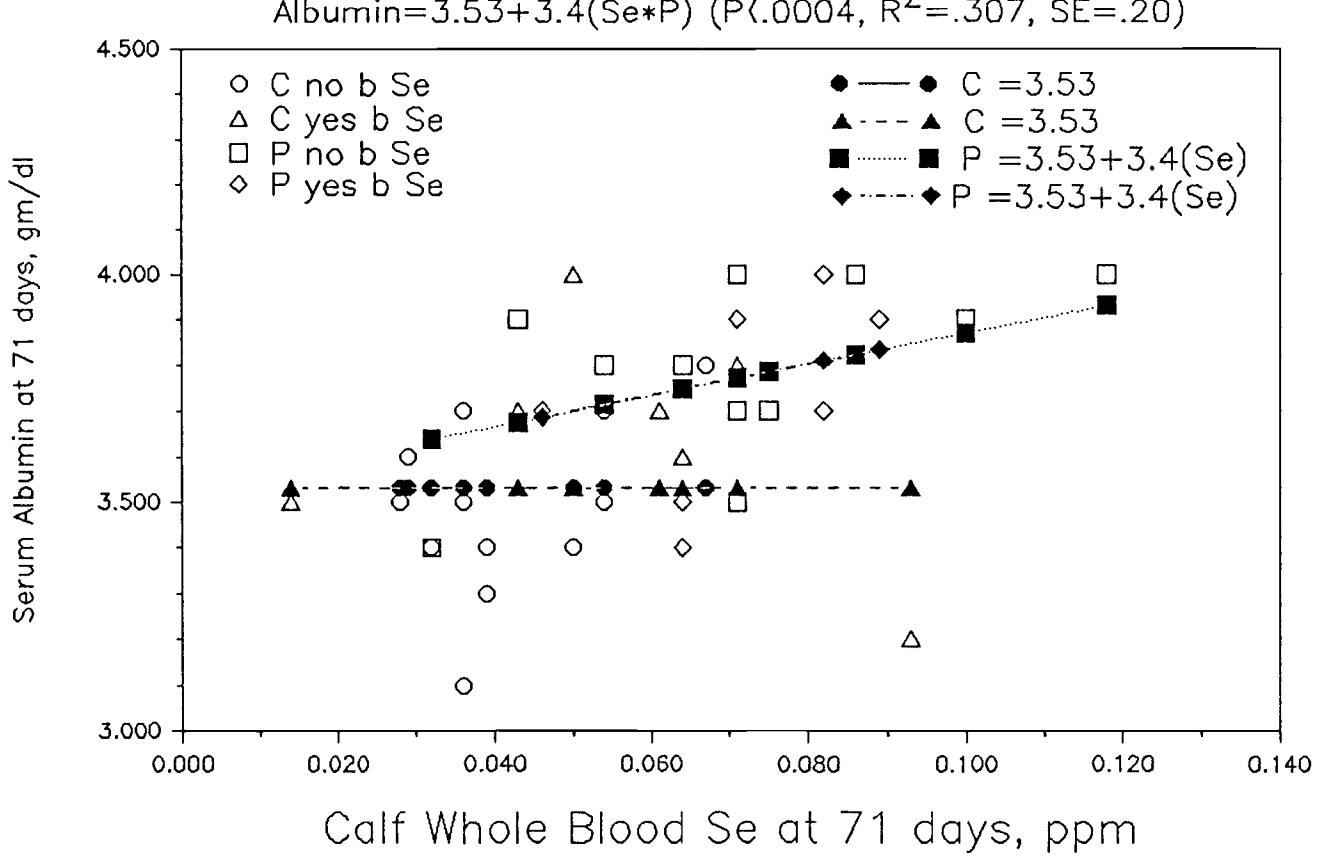

Fig. 29 Exp. 3: Serum cholesterol of calves at 71 days, actual data (open symbols) and least squares regression estimates (filled symbols). Treatments: C no b Se=Controls without Se at birth, C yes B Se= Controls with Se at birth and similarly for P=Pellet treatments.

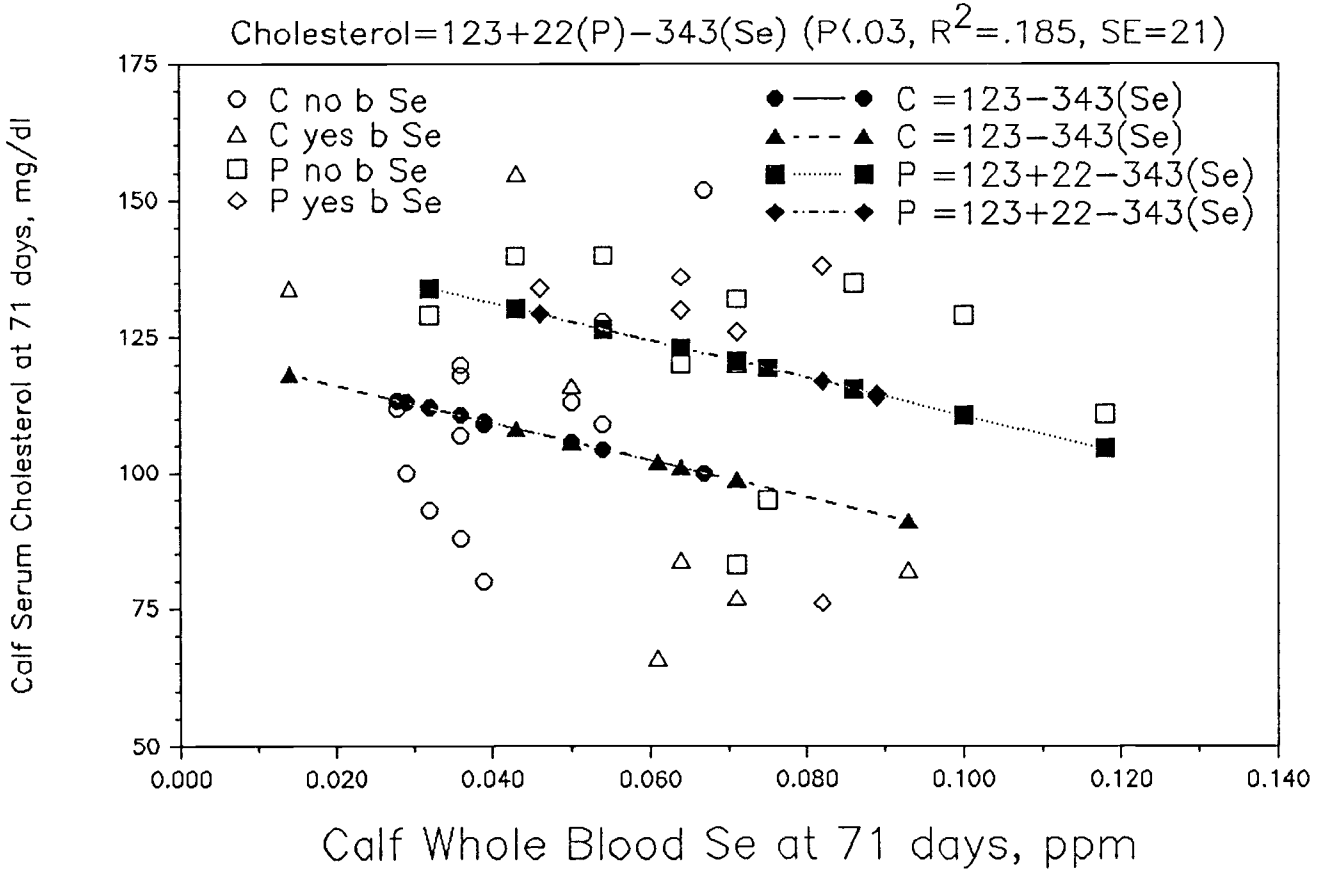

Fig. 30 Exp. 3: Red Blood Cells of calves at 71 days, actual data (open symbols) and adjusted by least squares regression (filled symbols). Treatments: C no b Se=Controls without Se at birth, C yes b Se=Controls with Se at birth and similarly for P=Pellet.

RBC=11.4−1.7(bSe)+34(Se*bSe*P) (P<.002, $R^2$=.317, SE=1.24)

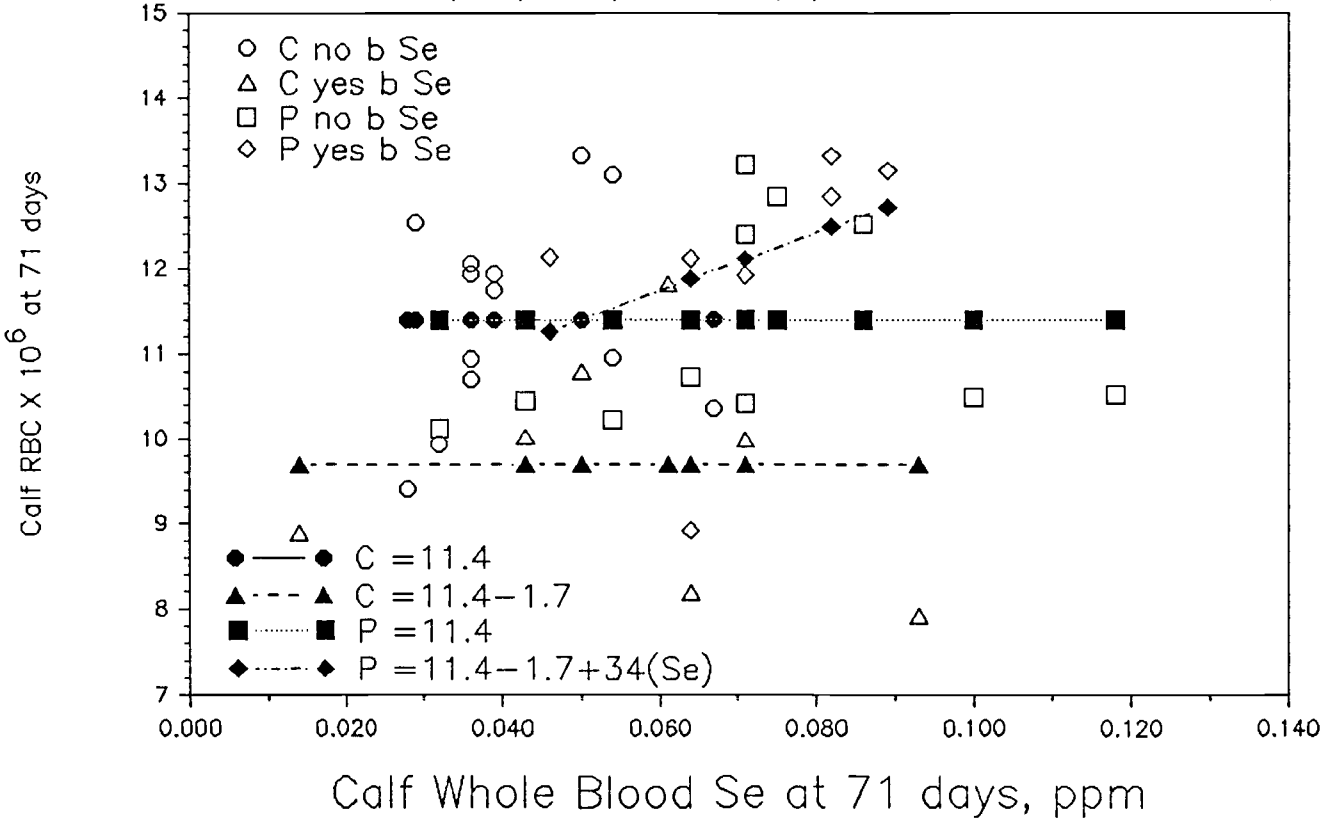

Fig. 31 Exp. 3: Hemoglobin of calves at 71 days, actual data (open symbols) and adjusted by least squares regression (filled symbols). Treatments: C no b Se=Controls without Se at birth, C yes b Se=Controls with Se at birth and similarly for P=Pellet.

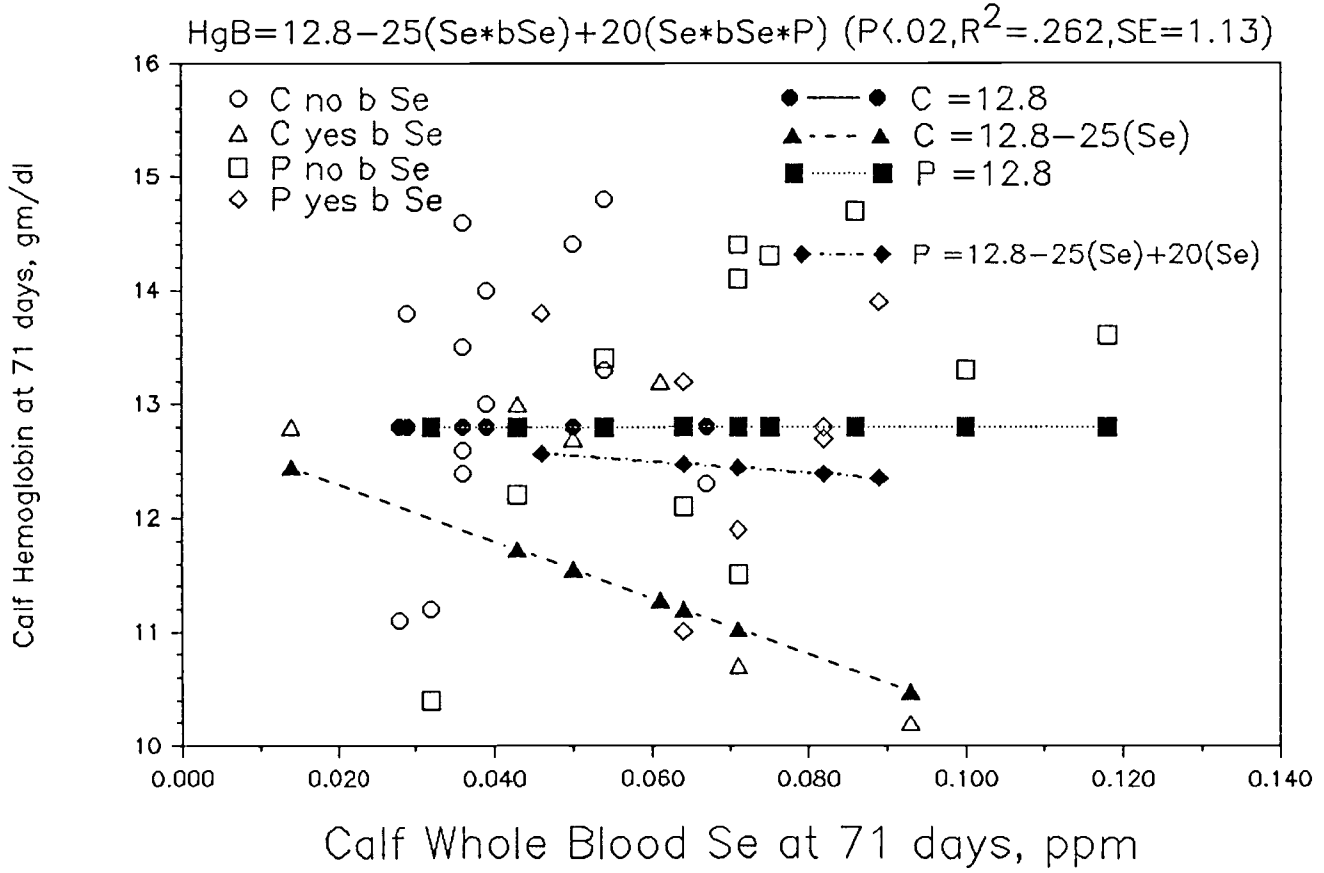

Fig. 32 Exp. 3: Fibrinogen levels of calves at 71 days, actual data (open symbols) and adjusted by age (filled symbols). Treatments: C no b Se=Control without Se at birth, C yes b Se=Control with Se at birth and similarly for P=Pellet treatments.

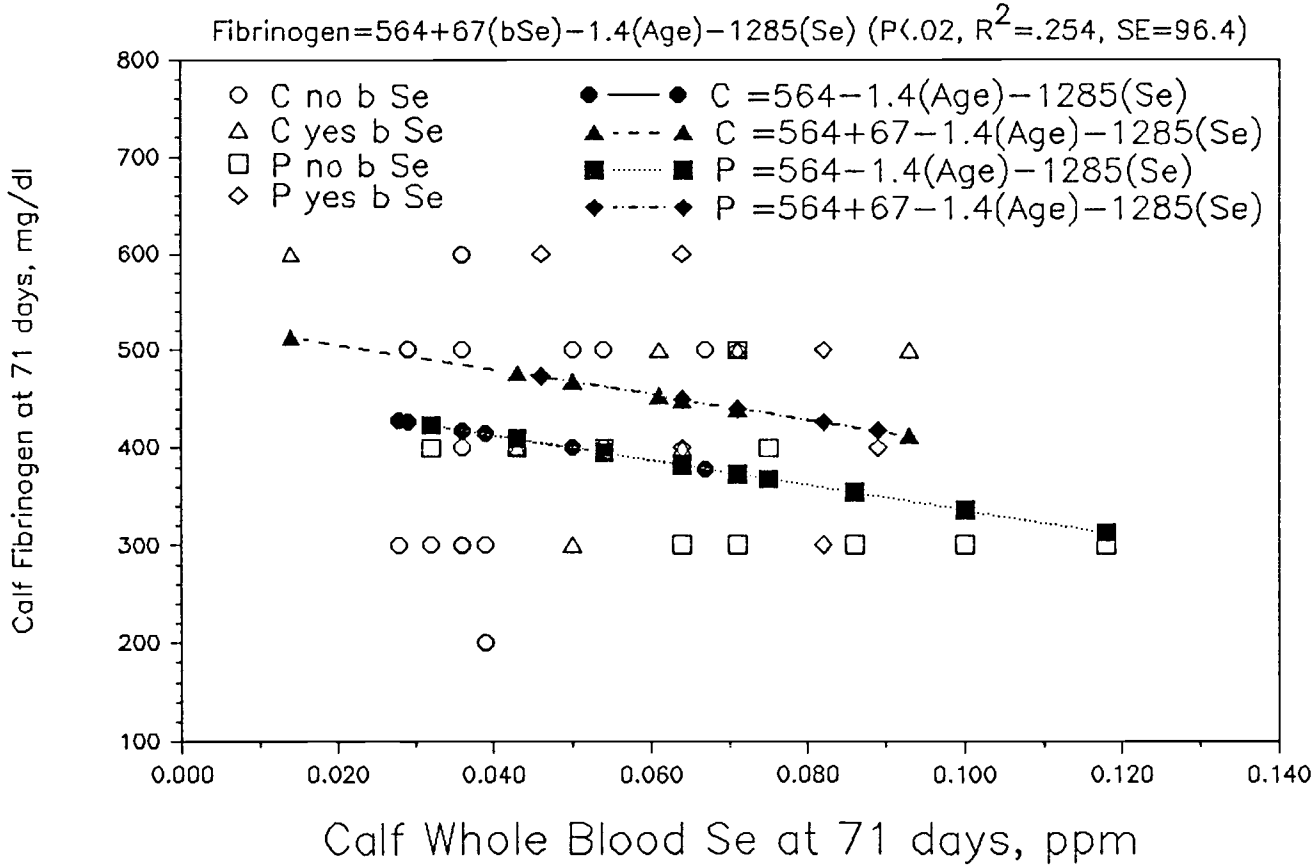

Fig. 33 Exp. 3: Calf immunoglobulin G at 71 days, actual data (open symbols) and adjusted for sex (filled symbols). Treatments: C no b Se=Controls without Se at birth, C yes b Se=Controls with Se at birth and similarly for P=Pellet treatments.

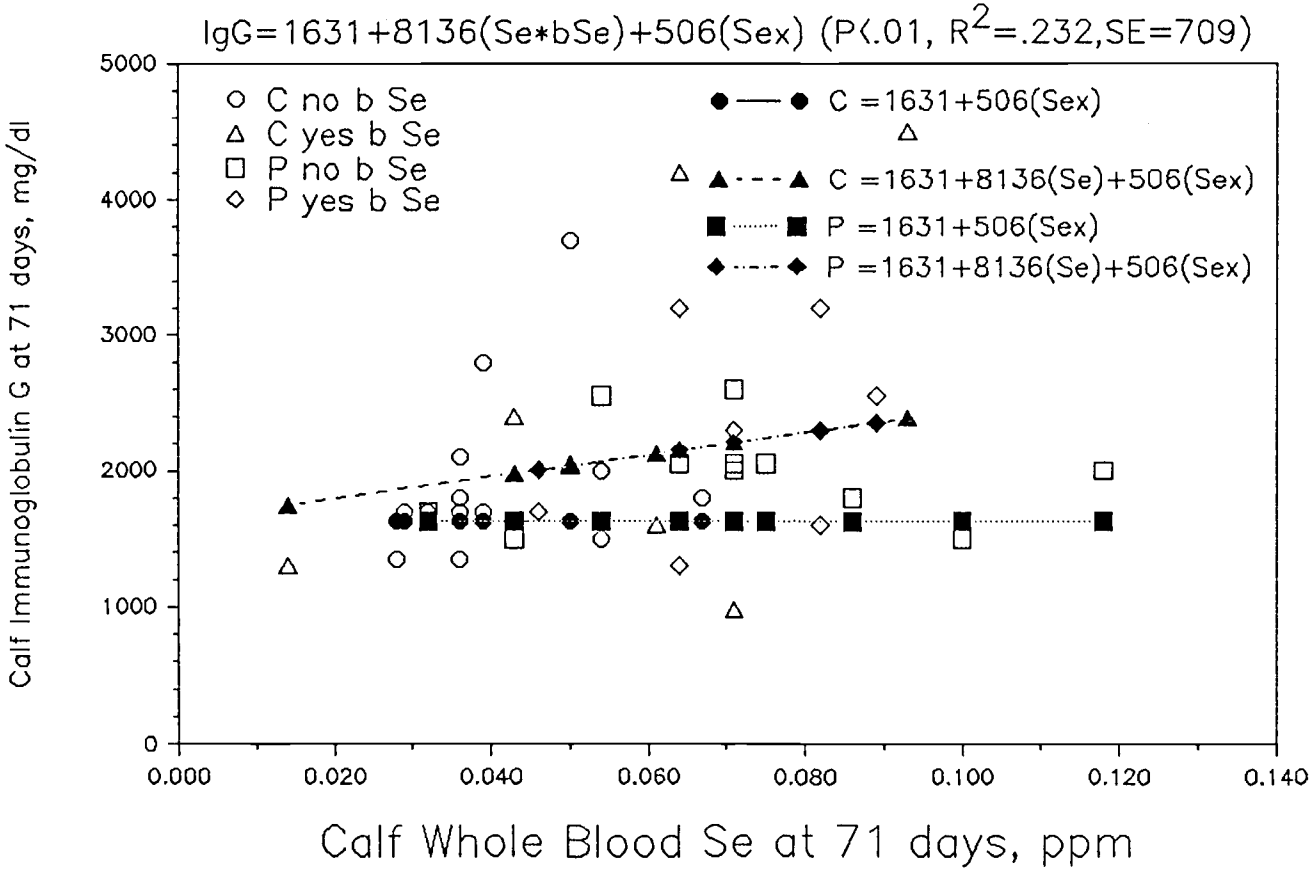

Fig. 34 Exp. 3: Segmented Neutrophils of calves at 71 days, actual data (open symbols) and least squares regression estimates (filled symbols. Treatments: C no b Se=Control without Se at birth, C yes b Se =Controls with Se at birth and similarly for P=Pellet treatments.

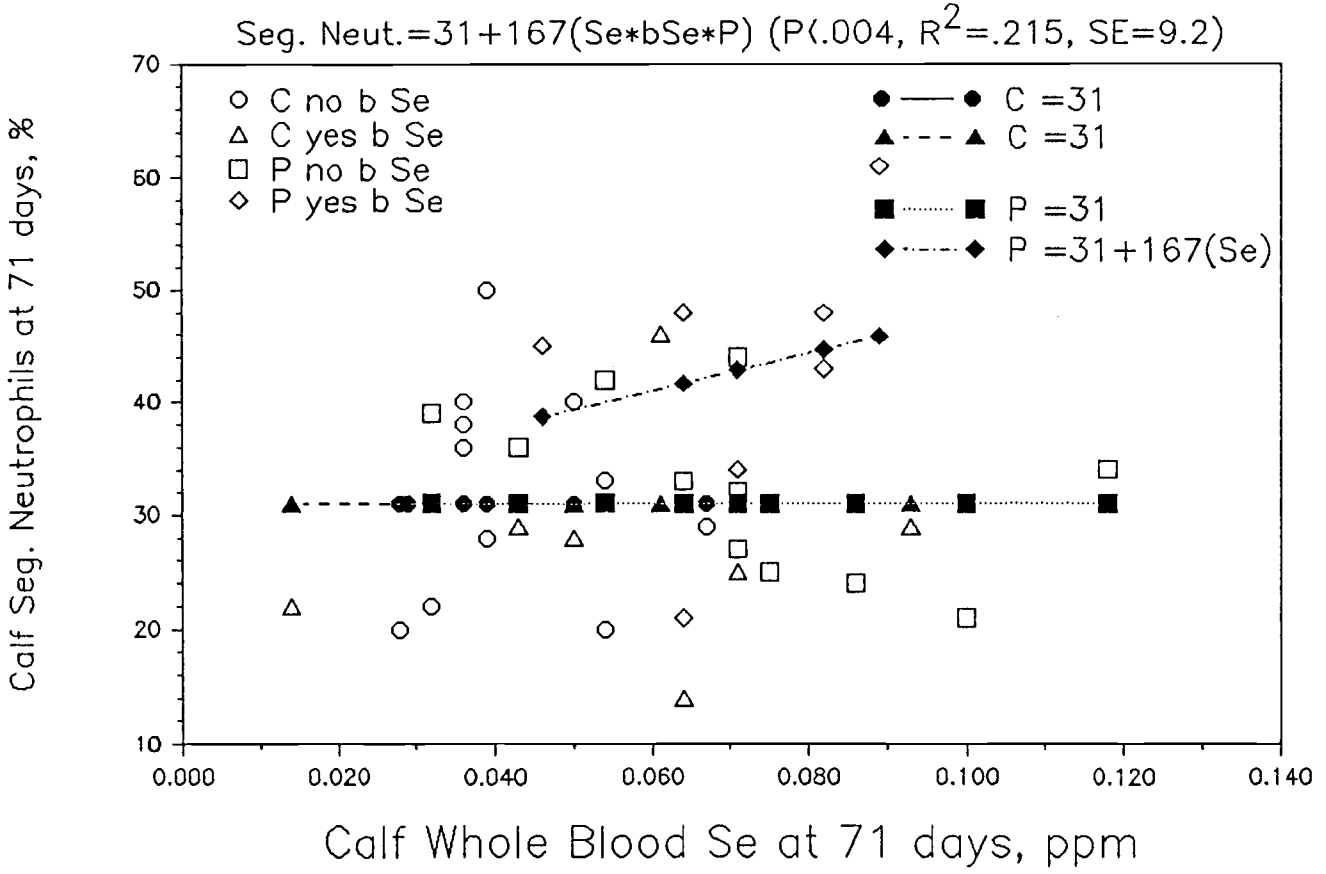

Fig. 35 Exp. 3: Lymphocytes of calves at 71 days, actual data (open symbols) and least squares regression estimates (filled symbols. Treatments: C no b Se=Control without Se at birth, C yes b Se =Controls with Se at birth and similarly for P=Pellet treatments.

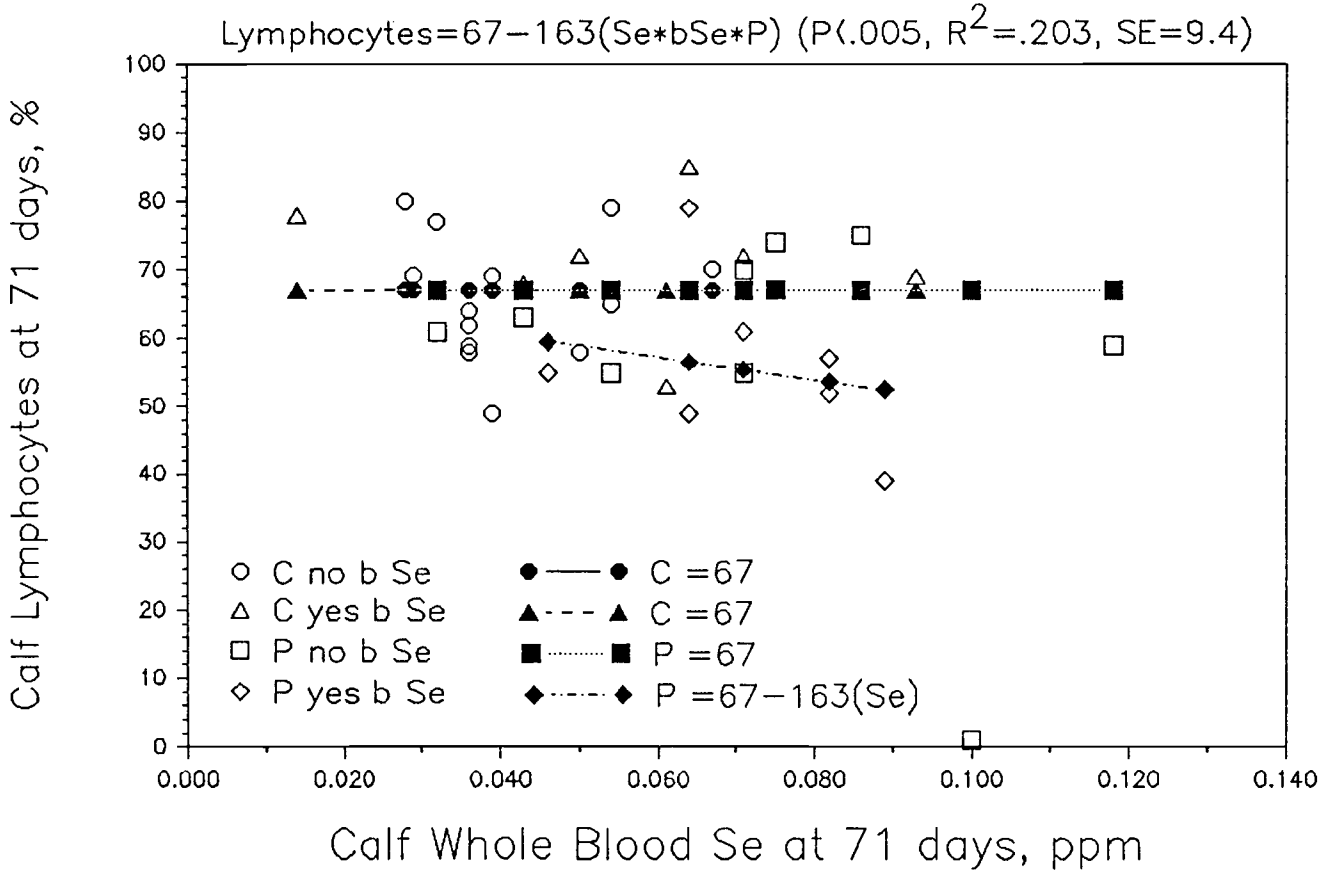

Table A1 - Experiment 3: Means and SD of blood chemistry and performance at 71 days of age.

CONTROLS

| | Males No birth Se | | | Males Birth Se | | | Females No birth Se | | | Females Birth Se | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | SD | n | Mean | SD | n | Mean | SD | n | Mean | SD |
| GOT | 6 | 118 | 13 | 3 | 131 | 33 | 7 | 121 | 22 | 4 | 113 | 46 |
| GPT | 6 | 13.7 | 1.6 | 3 | 19.7 | 15.9 | 7 | 14.1 | 2.7 | 4 | 14.5 | 9.0 |
| LDH | 6 | 984 | 70 | 3 | 1013 | 257 | 7 | 948 | 118 | 4 | 944 | 284 |
| AP | 6 | 311 | 60 | 3 | 324 | 111 | 7 | 325 | 125 | 4 | 366 | 116 |
| Bilirubin | 6 | .017 | .041 | 3 | 0 | 0 | 7 | .014 | .038 | 4 | 0 | 0 |
| BUN | 6 | 14.00 | 2.1 | 3 | 10.30 | 1.5 | 7 | 12.57 | 2.15 | 4 | 11.50 | 1.73 |
| Cholesterol | 6 | 117 | 20 | 3 | 122 | 40 | 7 | 104 | 16 | 4 | 87 | 21 |
| Phosphorus | 6 | 8.67 | .68 | 3 | 8.60 | .26 | 7 | 8.50 | .55 | 4 | 9.00 | 1.41 |
| Calcium | 6 | 10.02 | .33 | 3 | 10.33 | .35 | 7 | 9.57 | .28 | 4 | 9.98 | .56 |
| Protein | 6 | 5.87 | .24 | 3 | 5.67 | .31 | 7 | 6.00 | .49 | 4 | 6.48 | .61 |
| Albumin | 6 | 3.57 | .15 | 3 | 3.67 | .15 | 7 | 3.43 | .2 | 4 | 3.62 | .33 |
| Globulins | 6 | 2.30 | .23 | 3 | 2.00 | .26 | 7 | 2.57 | .5 | 4 | 2.85 | .84 |
| Hemoglobin | 6 | 12.52 | 1.35 | 3 | 12.17 | 1.27 | 7 | 13.70 | .78 | 4 | 11.82 | 1.38 |
| PCV | 6 | 37.50 | 3.8 | 3 | 36.30 | 2.1 | 7 | 39.29 | 2.5 | 4 | 34.8 | 3.5 |
| RBC count | 6 | 10.77 | 1.08 | 3 | 9.63 | .64 | 7 | 12.04 | 1 | 4 | 9.68 | 1.93 |
| WBC count | 6 | 8.23 | 1.49 | 3 | 7.10 | 2.52 | 7 | 7.94 | 1.96 | 4 | 8.15 | 2.13 |
| Plasma protein | 6 | 6.05 | .31 | 3 | 6.20 | .5 | 7 | 6.09 | .49 | 4 | 6.22 | .48 |
| Fibrinogen | 6 | 400 | 126 | 3 | 500 | 100 | 7 | 414 | 121 | 4 | 425 | 96 |
| Seg. Neutrophils | 6 | 31.2 | 8.9 | 3 | 25.3 | 3.5 | 7 | 34.3 | 9.6 | 4 | 29.2 | 13.1 |
| Bands | 6 | .5 | .55 | 3 | 1.0 | 1.0 | 7 | .1 | .4 | 4 | 0 | 0 |
| Lymphocytes | 6 | 68.0 | 9.23 | 3 | 72.7 | 5.0 | 7 | 64.4 | 9.5 | 4 | 69.8 | 13.2 |
| Monocytes | 6 | .2 | .4 | 3 | 1.0 | 1.0 | 7 | .7 | 1.1 | 4 | .8 | .5 |
| Eosinophils | 6 | .2 | .4 | 3 | 0 | 0 | 7 | .4 | .8 | 4 | .2 | .5 |
| Basophils | 6 | 0 | 0 | 3 | 0 | 0 | 7 | 0 | 0 | 4 | 0 | 0 |
| Reactive Lymph. | 6 | 1.25 | 1.37 | 3 | .33 | .58 | 7 | 1.36 | .90 | 4 | .88 | 1.18 |
| IgG | 6 | 1667 | 286 | 3 | 1560 | 745 | 7 | 2171 | 795 | 4 | 3087 | 1474 |
| Wt. at 90 d | 6 | 84.7 | 7.4 | 3 | 65.8 | 1.8 | 7 | 74.1 | 8.7 | 4 | 56.9 | 14.6 |
| Calf Se | 6 | .039 | .014 | 3 | .043 | .029 | 7 | .043 | .010 | 4 | .067 | .018 |
| Dam Se | 6 | .046 | .006 | 3 | .043 | .018 | 7 | .052 | .013 | 4 | .049 | .010 |
| Age | 6 | 81.2 | 5.2 | 3 | 50.0 | 3.6 | 7 | 75.7 | 11.6 | 4 | 46.5 | 39.9 |
| WOA at 90 d | 6 | 1.05 | .11 | 3 | 1.32 | .1 | 7 | 1.00 | .17 | 4 | 1.93 | 1.16 |

Table A1 - Experiment 3: Continued - Means and SD of blood chemistry and performance at 71 days of age.

PELLET

| | Males No birth Se | | | Males Birth Se | | | Females No birth Se | | | Females Birth Se | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean | SD | n | Mean | SD | n | Mean | SD | n | Mean | SD |
| GOT | 5 | 120 | 22 | 3 | 125 | 32 | 6 | 112 | 8 | 4 | 112 | 17 |
| GPT | 5 | 14.8 | 3.3 | 3 | 16.0 | 4.4 | 6 | 14.0 | 5.1 | 4 | 12.5 | 2.9 |
| LDH | 5 | 953 | 100 | 3 | 961 | 72 | 6 | 1020 | 90 | 4 | 970 | 118 |
| AP | 5 | 243 | 66 | 3 | 307 | 115 | 6 | 386 | 196 | 4 | 328 | 120 |
| Bilirubin | 5 | .040 | .056 | 3 | .100 | .173 | 6 | .017 | .041 | 4 | .025 | .050 |
| BUN | 5 | 16.00 | 1.73 | 3 | 12.33 | 1.53 | 6 | 13.5 | 2.81 | 4 | 12.00 | 2.45 |
| Cholesterol | 5 | 122 | 17 | 3 | 109 | 31 | 6 | 120 | 21 | 4 | 132 | 4 |
| Phosphorus | 5 | 8.80 | .82 | 3 | 8.57 | .12 | 6 | 9.13 | .60 | 4 | 8.28 | .21 |
| Calcium | 5 | 9.74 | .24 | 3 | 9.63 | .31 | 6 | 9.83 | .29 | 4 | 9.78 | .15 |
| Protein | 5 | 6.12 | .42 | 3 | 6.03 | .25 | 6 | 6.22 | .26 | 4 | 6.05 | .54 |
| Albumin | 5 | 3.74 | .21 | 3 | 3.87 | .15 | 6 | 3.83 | .21 | 4 | 3.62 | .22 |
| Globulins | 5 | 2.38 | .48 | 3 | 2.17 | .40 | 6 | 2.38 | .43 | 4 | 2.42 | .50 |
| Hemoglobin | 5 | 12.46 | 1.46 | 3 | 13.13 | .67 | 6 | 13.62 | 1.14 | 4 | 12.48 | 1.26 |
| PCV | 5 | 35.8 | 2.8 | 3 | 38.3 | 2.3 | 6 | 39.3 | 3.3 | 4 | 36.5 | 3.4 |
| RBC count | 5 | 10.93 | 1.09 | 3 | 13.10 | .24 | 6 | 11.55 | 1.31 | 4 | 11.28 | 1.57 |
| WBC count | 5 | 6.68 | .5 | 3 | 8.67 | 2.03 | 6 | 9.4 | 1.74 | 4 | 9.10 | 2.19 |
| Plasma protein | 5 | 6.18 | .39 | 3 | 6.13 | .12 | 6 | 6.27 | .29 | 4 | 6.25 | .33 |
| Fibrinogen | 5 | 360 | 55 | 3 | 400 | 100 | 6 | 350 | 84 | 4 | 525 | 96 |
| Seg. Neutrophils | 5 | 30.8 | 7.6 | 3 | 50.7 | 9.3 | 6 | 33.8 | 8.0 | 4 | 37 | 12.2 |
| Bands | 5 | 0 | 0 | 3 | 0 | 0 | 6 | .8 | 2.0 | 4 | .8 | 1 |
| Lymphocytes | 5 | 68.6 | 7.2 | 3 | 49.3 | 9.3 | 6 | 63.5 | 8.4 | 4 | 61.0 | 13.0 |
| Monocytes | 5 | .4 | .5 | 3 | 0 | 0 | 6 | 1.0 | .9 | 4 | 1.2 | 1.9 |
| Eosinophils | 5 | .2 | .4 | 3 | 0 | 0 | 6 | 1.2 | 1.0 | 4 | 0 | 0 |
| Basophils | 5 | 0 | 0 | 3 | 0 | 0 | 6 | 0 | 0 | 4 | 0 | 0 |
| Reactive Lymph. | 5 | 1.12 | 1.60 | 3 | 0 | 0 | 6 | 1.0 | 1.2 | 4 | .9 | 1.2 |
| IgG | 5 | 1760 | 277 | 3 | 2450 | 805 | 6 | 2167 | 328 | 4 | 2125 | 826 |
| Wt. at 90 d | 5 | 80.2 | 15.0 | 3 | 75.9 | 7.9 | 6 | 82.2 | 14.8 | 4 | 68.4 | 16.8 |
| Calf Se | 5 | .063 | .027 | 3 | .084 | .0004 | 6 | .079 | .022 | 4 | .061 | .011 |
| Dam Se | 5 | .110 | .066 | 3 | .089 | .190 | 6 | .120 | .026 | 4 | .080 | .021 |
| Age | 5 | 80.8 | 6.3 | 3 | 82.0 | 30.3 | 6 | 73.3 | 18.6 | 4 | 63.8 | 23.0 |
| WDA at 90 d | 5 | .99 | .16 | 3 | 1.02 | .38 | 6 | 1.17 | .28 | 4 | 1.10 | .11 |