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EFFECT OF EXCESS DIETARY SELENIUM SUPPLEMENTATION ON HOLSTEIN COWS

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Roger George Ellis

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EFFECT OF EXCESS DIETARY SELENIUM SUPPLEMENTATION ON HOLSTEIN COWS

By

Roger George Ellis

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ABSTRACT

EFFECT OF EXCESS DIETARY SELENIUM SUPPLEMENTATION ON HOLSTEIN COWS

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Twenty-four non-lactating cows were fed 0, 3, 20, 50 or 100 mg supplemental Se/head/day. Over the treatment period, mean Se concentrations (serum, whole-blood, liver, urine, feces) did not differ between the unsupplemented control group and the 3-mg Se/head/day group. However, within two days of initial supplementation, serum Se in both the 20-and 50-mg groups exceeded controls ($P < 0.01$). Whole-blood Se exceeded controls ($P < 0.01$) at one week post supplementation in the 50-mg group and at seven weeks in the 20-mg group. Liver Se concentrations of the 20- and 50-mg groups were higher than controls at 90 days ($P < 0.01$). No significant differences between groups were detected at any time for complete blood counts; serum activities of AST, CPK, SDH and GGT; immunological variables and general health. Sodium selenite supplementation at as much as 50-mg Se/head/day for 100 days and 100-mg Se/head/day for 28 days had no detectable harmful effects in non-lactating cattle.

DEDICATION

To my family, Claudia, Lisa and Timothy for their never failing support. **THANK YOU!**

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LITERATURE REVIEW

Selenium (Se) is an essential trace mineral in animal nutrition. The discovery of Se is attributed to the Swedish scientist Berzelius in 1818.[1] The biological importance of high concentrations of Se became evident when it was associated with "alkali disease" and "blind staggers" in the 1930's. These syndromes were related to feeds grown on seleniferous soils (soils having high Se concentrations) of the northern Great Plains of the United States.[2] Descriptions of toxic feed producing similar syndromes have been reported dating back to the writings of Marco Polo. The perceived negative effects of Se were supported by reports in the 1940's that Se had carcinogenic properties. Consequently, for the next two decades Se was viewed as a toxic element with no beneficial nutritional effect.

Toxicity:

Toxicity in dairy cattle is usually of the chronic form (alkali disease or blind staggers); however, there have been reports of acute toxicity or "high-concentration" toxicity. Signs of acute toxicity include anorexia and decreased milk production with single doses of 7-10 mg Se/kg of body weight.[3] Single doses above 11 mg Se/kg produce signs of excessive salivation, respiratory distress, garlic-smelling breath, and death within 48 hours. These cases usually only occur when cattle have no other feed

except seleniferous plants (plants which accumulate high concentrations of Se) or when large amounts of Se are accidentally or experimentally administered. Chronic selenosis, in the form of alkali disease, is characterized by signs of alopecia, hoof malformations and loss, emaciation and reproductive failure accompanied by increases in serum transaminases and alkaline phosphatase.[4] Chronic ingestion of seleniferous plants also produces the syndrome of blind staggers. Animals affected wander aimlessly, stumble, appear to have impaired vision, and have signs of respiratory distress. This characterizes the blind staggers type of toxicity and can be reproduced with Se-free water extracts from the plants. Based on these findings it has been proposed that the cause of these signs is the water soluble alkaloids found in seleniferous plants and that Se is not the cause of "blind staggers".[4]

Essentiality and Biochemical Functions:

The dietary essentiality of Se became evident in the late 1950's. Researchers working independently discovered that Se played an important role in preventing liver necrosis in rats[5] and exudative diathesis in chicks.[6] During the next three decades, Se deficiency has been associated with many diseases. Selenium supplementation of animal feeds today, is a relatively common practice. A common concern is the perceived ease of producing toxicity with small deviations from recommended concentrations.

The biochemical importance of Se is related to Se being a component of the enzyme glutathione peroxidase (GSH-Px).[7] This enzyme is located in the cytosol where it is important in converting toxic free radicals to water. Research continues to identify other biochemical functions of Se which may help to explain further the causes of Se

deficiency diseases. The functions of Se are closely related to vitamin E. Se alone may alleviate or decrease the severity of some vitamin E-responsive diseases.[8]

Sources and Factors Influencing Requirements:

Selenium is normally obtained by the body through the diet. The Se content of a feed is related to the content and availability of Se in the soil on which the feed is grown, the plant species and any supplemental source of Se added to the feed. The Se availability to plants depends upon soil type, pH and climatic conditions. Soils are divided into three groups based on Se availability: toxic seleniferous, nontoxic seleniferous and low Se soils.[9] Plant species utilize and accumulate Se to various degrees producing different concentrations of Se. Plants have been divided into three groups: primary, secondary, and non-Se accumulators.[10] Primary accumulators such as the genera Astragalus and Stanleya have the ability to accumulate Se in concentrations of from 1000 to greater than 7000 ppm.[11] Secondary accumulators such as the genera Atriplex and Mentzelia accumulate Se up to 100 to 200 ppm. Non-Se accumulators include plants routinely used as animal feeds such as cereal grains, grasses, and legumes. These plants may accumulate Se up to 20-50 ppm depending on soil condition and Se content. Grasses tend to have higher Se concentrations than legumes.

The many factors which influence the Se concentration in plants make it possible for animal feed Se concentrations to vary from less than 0.01 ppm to greater than 10,000 ppm. Forages produced on neighboring farms may be very different in Se concentration.[12] Maps defining areas with forages containing low, variable, adequate, and toxic Se concentrations are constantly being expanded and updated as new

information becomes available (Figure 1). In North America, areas containing forages with toxic concentrations of Se are limited to the Great Plains of the United States. Areas in North America having forages low in Se content include the Eastern and Western coastal regions and the areas surrounding the Great Lakes in Canada and the Northeast, Atlantic Seaboard, areas around the Great Lakes, and the Pacific Northwest in the United States.

Absorption:

The absorption of Se is significantly lower in ruminants than monogastrics. The retention of oral Se as sodium selenite was 77% in swine while only 29% in sheep.[13] Absorption from the stomach area is essentially absent in both monogastrics and ruminants. Most absorption in the ruminant occurs in the small intestine and the cecum. In monogastric species, absorption occurs in the last part of the small intestine, cecum, and colon. In the rat, the availability of Se has been shown to be related to the form of Se. Organic Se compounds were found to be 138%-180% more available when compared to inorganic forms.[14] A significant amount of the availability at low concentrations of Se supplementation is related to the amount of Se absorbed. Everted intestinal sacs of hamsters have demonstrated that selenomethionine is transported against a concentration gradient where selenite and selenocystine are not. Transport of selenomethionine is inhibited by methionine where selenite and selenocystine are not inhibited.[15] This indicates selenite is passively absorbed along concentration gradients while selenomethionine is actively transported during absorption.

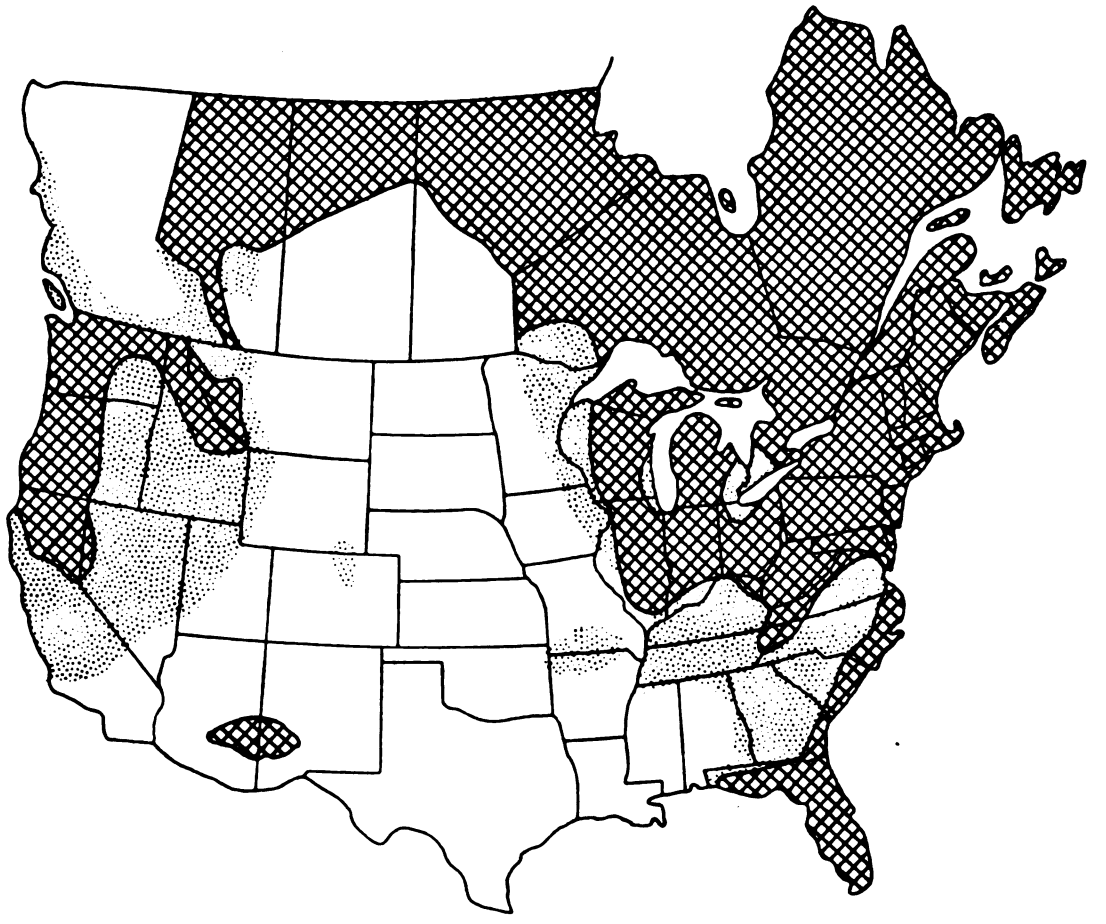

Figure 1. Selenium concentrations in forages and grains from different regions of the United States and Canada.[a]

[a] National Research Council. 1983. Selenium in Nutrition, revised ed. National Academy Press, Washington, D.C. pp 24.

Chemically, the differences in availability of Se from various seleno-compounds has been related to a number of criteria in the rat. The two carbon chains attached to the Se atom affect Se availability. Even numbered chains have low availability while uneven numbered chains have relatively high availability. The location of the Se atom near the center of the molecule, combined with odd numbered carbon chains, gives very high availability. Short alkyl chains with 3 or 4 carbons significantly depress Se availability by allowing the formation of 5-6 member rings. Finally acid amides of seleno-carboxylic acids are more potent than the free acids unless the compound contains more than one Se atom.[16] Further, availability was greatly influenced by the structure of the alkanoic moiety of the molecule, and the presence of a quaternary carbon atom in the chain almost totally eliminated availability. Biopotency was further decreased by the introduction of methyl or nitro groups in the fourth position of the ring in the benzylseleno-carboxylic compounds.[17] Unfortunately, no data could be found comparable to these data for ruminants. Rumen microbes do play a major role in changing the form of ingested Se.

The absorption of Se is also related to whether Se is bound or unbound in protein with inorganic Se being absorbed more readily.[18] Biosynthesis of seleno-compounds from inorganic Se or unbound Se occurs in the rumen of sheep.[19] Specifically Se^{75} selenomethionine has been found to be formed after incubation of Se^{75} selenite with rumen microbes.[20] Rumen microbes have been shown to not only convert Se to insoluble forms by reduction but also to incorporate Se, in the form of selenomethionine, into bacterial protein. Studies have also shown that inorganic Se may be substituted for inorganic sulfur during rumen microbial amino acid synthesis and that the resulting seleno-amino acids are incorporated into microbial protein.[21] The preceding would

indicate that rumen microorganisms are responsible for the lower absorption of Se in the ruminant compared to the non-ruminant. The ruminant microbes impair absorption by reducing ingested Se to insoluble forms (bound or unbound Se) which occurs to a greater extend with inorganic Se compared to organic forms. Further data indicate that a ruminant consuming a high carbohydrate diet provides a better environment for the conversion of Se to insoluble forms than when consuming a high roughage diet.[22] The solubility of inorganic Se is related to the oxidation states of Se which include -2 (selenide), 0 (elemental Se), +4 (selenite), and +6 (selenate). The selenate form of Se is the most soluble with elemental Se being insoluble and selenite in between.

Biological Availability:

All Se absorbed is not utilized physiologically. Biological availability is a measure of how much Se is available to the tissues from a specific Se compound after the compound has been exposed to several physiological and metabolic processes. These include digestion, absorption, and metabolism which may be affected by the Se status of the animal. Selenium bioavailability is only an estimate of Se utilization derived from experimental values, and must be considered in the light of biological response(s).[20] Selenium compounds are classified into three different groups based on biological availability. The first group consists of the more reduced and insoluble forms which have very low bioavailability. Secondly, Se in most animal products other than protein has low to moderate bioavailability. Thirdly, the common selenoamino acids like selenomethionine or selenocystine which have relatively good bioavailability.

Excretion:

Selenium is excreted from the body by three major routes. These include fecal, urinary, and respiratory excretion. Respiratory excretion is minimal at dietary Se concentrations less than 1 ppm. At higher concentrations, respiratory excretion becomes more significant.[23] Excretion of Se depends on the form of Se ingested and the amount of Se fed. In sheep fed sodium selenite and selenomethionine at the same low concentrations, the amount of Se excreted in the feces was about equal for each source. The major route of excretion is through the feces. However, the Se in the feces of the sheep fed sodium selenite was much more insoluble than the feces of sheep fed selenomethionine. This supports the theory that rumen microbes convert ingested Se into insoluble forms, especially if the Se is in the inorganic form.

Urinary excretion of Se was higher in the sheep fed sodium selenite compared to sheep fed selenomethionine. This suggests that selenomethionine is better utilized by tissues (higher biological availability) than sodium selenite leaving more sodium selenite available for excretion in the urine.[24] Selenomethionine may also be incorporated into non Se requiring proteins making very little Se available for body needs. It is however, important to note that selenite is more easily excreted by the kidney when compared to seleno amino acids. Toxic concentrations of Se cause Se excretion in the urine to approach and even exceed Se excretion in the feces.[25] The major excretory product in rat urine fed excessive amounts of Se is trimethylselenonium.[26] In animals receiving adequate concentrations of Se, little or no trimethylselenonium is found in the urine; however, at excess concentrations of Se, the concentration of trimethylselenonium increases.[27] This has been suggested as a possible method for evaluating the adequacy

of the concentration of Se supplementation. However, the necessity of a twenty four hour urine collection for accuracy limits it's practical application. The observations of this excretory product have been limited to the urine of monogastrics. Presence of this compound in ruminant urine must, therefore, be confirmed. It has been observed in ruminants that Se excretion in the urine does increase with excess concentrations of supplementation.[25]

Deficiency:

Numerous livestock problems have been related both independently and in conjunction with other nutrients, to Se deficiency. Selenium most frequently is associated with vitamin E but also has been associated with the elements calcium, copper, sulfur, arsenic and cadmium. In ruminants, Se deficiency has been associated with a multiplicity of disease syndromes including nutritional muscular dystrophy (White Muscle Disease) in sheep, cattle, pigs, goats and horses,[28] infertility in sheep, pigs, and cattle,[29] retained placenta in cattle,[30,31] cystic ovarian disease in cattle,[32] abortion in sheep, cattle, pigs, and horses,[33] and untriftiness in both sheep and cattle.[34] Recently, Se deficiency has also been related to impaired function of both the humoral and cell-mediated immune systems in ruminants.[35,36,37] In cattle the influence of Se on the immune system has been specifically manifest in the duration of clinical signs of mastitis.[38,39]

Reproductive problems in cattle, including retained placenta, cystic ovarian disease, fertilization, sperm viability and abortion, have been related to Se deficiency. The literature in these areas is both supportive and non-supportive of these relationships.

It is impossible to disassociate Se from vitamin E in most cases. The following review will focus on the effects of Se.

Retained Placenta:

Retention of the placenta during the early postpartum period is a common problem in dairy cattle and has received much attention over the years. The overall incidence of retained placenta is reported by numerous investigators to be ten percent in North America.[40,41,42] Sporadic incidences exceeding fifty percent have been reported.[43] Retained placenta in cattle is characterized as failure of the fetal placenta to separate from the maternal crypts, which normally occurs within 2-8 hours postpartum. Retention is defined as the placenta remaining intact for greater than 12 hours.[44] Retained placenta is associated with decreased fertility in the postpartum dairy cow as measured by increases in calving interval and days to conception due to various factors. A major factor is an increased rate of uterine infection, up to 54% from a normal of 10%.[45] Historically, nutrition has been associated with the various causes of increased incidence of retained placenta.[46]

During the late 1960's and early 1970's, an association between white muscle disease and retained placenta was first reported by Trinder in the British Isles.[47] This was the first reported association of Se and vitamin E deficiency with retained placenta. Since that time many investigations of this relationship have been conducted in different locations in the world with some investigators, interestingly, concluding Se and/or vitamin E to be the cure for retained placenta and some investigators finding no evidence to support a relationship.

Effective experimental prevention of retained placentas with Se supplementation has been reported in Ohio herds consuming a ration formulated with feeds containing 20-40 ng Se/g DW.[44] This was accomplished either by injecting 50 mg of Se 1-3 weeks prepartum or supplementing the ration with 1 mg sodium selenite daily (a very low concentration of supplementation). The same group of investigators reported at about the same time that a single dose of 50 mg of sodium selenite and 680 IU of vitamin E as alpha-tocopherol, administered 21 days prepartum, reduced the incidence of retained placenta from 51% to 9% in 113 cows.[43] At the same time, no improvement in retained placenta incidence was observed with sodium selenite supplementation in South Dakota where Se is adequate in feeds.[48] Collectively, of these observations seemed to support Se deficiency being related to the incidence of retained placenta and making Se supplementation a logical preventative.

Subsequent observations indicated that the concentration of Se supplementation to prevent retained placenta varied relative to the concentration of protein and type of diet during the prepartum period. The same concentration of Se supplementation was shown to be more effective in preventing retained placentas when cows were fed pasture compared with higher protein alfalfa silage as the main component of their prepartum diet.[49]

Calcium concentrations in the ration are related to the amount of Se absorbed; however, this was not directly related to the incidence of retained placenta. Maximal Se absorption was reported when Ca represented 0.8% of dry matter intake. Higher or lower concentrations of calcium in the diet resulted in decreased Se absorption.[50] The combination of these two observations may be related due to the higher predicted calcium

content of alfalfa silage when compared to pasture. The high protein concentration of the silage may not be related to the lower Se tissue concentrations and higher incidence of retained placenta.

Selenium has been closely linked with vitamin E in a controlled experiment. It showed no effect of vitamin E or Se supplementation individually when administered orally, or by injection, however, a reduction of the incidence of retained placenta from 17.5% to 0% was observed in animals supplemented with both vitamin E and Se.[51] Adding even more confusion, a study in Israel of cattle consuming Se-low diets showed a decreased incidence of retained placenta (approx. 10%) in relatively low doses of supplemental Se (2.3 mg/day, 21 days prepartum) and that Se alone was just as effective as vitamin E and Se together.[52]

A specific mechanism has not been demonstrated relating Se deficiency to retained placenta. The theory of decreased uterine muscle function in the early postpartum period has been considered. In a limited number of cows with retained placenta, the Se concentrations in the cotyledons and caruncles were 27.5% and 33% lower, respectively, than for cows with normal placenta expulsion.[53] A similar observation has been made in both the placenta and caruncle of the ewe suggesting that these tissues might be particularly prone to Se deficiency.[54]

Numerous investigators, however, have found no reduction of retained placenta incidence when Se and/or vitamin E were supplemented.[55] Some of these observations were made in geographical locations (Nebraska) with adequate concentrations of Se in feedstuffs and with confirmed adequate serum Se concentrations of .082 ug Se/ml.[56] Another large study recently examined 627 parturitions at a large university research

farm in the province of Ontario in Canada.[57] These investigators concluded that retained placenta was not a Se-responsive disease. This conclusion was based on the lack of significant differences in the retained placenta incidence between controls, Se and/or vitamin E supplemented cows. It is important to note, however, that the parturition plasma Se concentrations of control animals were in the low adequate range (.07 ug/ml) and the concentrations of the treated animals were in the middle of the expected adequate range (.083-.089 μg/ml). This fact would support a different conclusion; e.g. that retained placenta is not Se-responsive in cattle which have an adequate level of Se. The study did not demonstrate what the incidence of retained placenta was in deficient animals.

The most logical approach to the relationship of Se and vitamin E to the incidence of retained placenta in cattle is demonstrated in a study conducted in four herds in North Carolina during the early 1980's.[58] A single injection of 50 mg Se and 680 IU alpha-tocopherol was used at 21 days prior to expected parturition. The Se status of the cattle, based on serum Se concentration prior to treatment, was correlated with the incidence of retained placenta. There was no effect of Se and vitamin E supplementation in cattle with a pretreatment Se status of adequate ($\geq$ 0.08 ppm) or extremely deficient ($\leq$ 0.05 ppm). A significant difference was demonstrated in cattle with borderline Se status (0.05-0.08 ppm). This supports the hypothesis that one of the factors affecting the incidence of retained placenta is the deficiency of Se and/or vitamin E. Since the concentration of vitamin E provided by the injection is low, it is most likely Se is the important component. Further, it demonstrates that the incidence of retained placenta will not be changed if Se status is adequate and additional Se is provided. Likewise,

insufficient Se provided to severely deficient cattle to correct their Se status will not decrease the incidence of retained placenta. Indiscriminant use of standard dosages of Se and vitamin E injections in prepartum dairy cattle are not indicated. Pretreatment evaluation of Se status of the population should be accomplished prior to making the clinical decision to supplement the population.

Cystic Ovarian Disease:

A negative correlation has been demonstrated between cystic ovarian disease and plasma Se concentrations ($r = 0.83$) and GSH-Px activity ($r = 0.69$). Correlation, however does not indicate a causal relationship. Subsequently, it was demonstrated that injected Se reduced the incidence of cystic ovaries by 28% compared to controls.33 It is important to note that the incidence of cystic ovaries has been shown to be related to other post-partum problems such as retained placenta and milk fever. The investigations do not allow conclusions regarding whether Se has a direct causal effect on cystic ovarian disease incidence or whether the reduced incidence is simply related to a decrease in the incidence of retained placenta which has been related to Se.

Fertilization:

Decreased fertility in a group of cattle used for embryo transfer in Ohio has been related to low Se concentrations.[59] This group of cattle was also consuming a diet low in protein, energy and vitamin A. A subsequent trial showed 100% fertilization of ova when cattle were provided a adequate diet supplemented with Se and vitamin E and only a 40% rate of fertilization in unsupplemented group. Other investigators have reported

no change in number of fertilized ova in Se- and vitamin E-treated Charolais cattle but did report an increased number of sperm associated with ova in supplemented cattle. On this basis these investigators suggested that ova fertilization has no association with Se status but that Se supplementation may increase sperm transport.[60] Supporting data relating Se to fertility are reported for sheep where in Se supplementation was related to increased ova fertilization, embryonic survival and stronger contractions of uterine muscle, possibly improving sperm transport.[61,62,63] However, one paper reports normal calving percentages in cattle grazing the same pasture with sheep exhibiting very low lambing percentages. Lambing percentages were improved from as low as 25% to 80-120% with Se supplementation immediately prior to breeding.

The evidence for a relationship between Se and fertilization in cattle is unclear. The only evidence suggestive of a relationship is an increased number of sperm associated with fertilized ova. This was in a relatively small group of cattle with no increase in numbers of fertilized ova in Se supplemented animals.

Sperm Viability:

The addition of Se at the rate of 1 ppm to diluted semen was reported to increase sperm motility and sperm oxygen consumption in 13 of 15 ejaculates.[64] However, other investigators, working with twenty-four yearling Angus bulls, were able to show increased Se concentration in serum and various semen components as a result of Se supplementation but no difference in percent viability thawed semen.[65] They concluded that Se was not associated with in vitro sperm viability; however, no in vivo fertility

observations were made. Conflicting evidence about the association of Se to semen viability is the result.

Abortion:

In the past decade, reports have surfaced associating the frustrating problem of undiagnosed abortion in the bovine with Se deficiency. In western Canada liver Se concentrations in 69 of 243 aborted fetus were in the severely Se-deficient category. Thirty-five of the Se- deficient fetuses had no other detectable cause of abortion while most of the other thirty-four had bacterial and viral isolates usually not associated with bovine abortion.[66] In Michigan, of seventy-four bovine fetuses with an undetermined cause of abortion after complete necropsy, histology, bacteriology and virology, nutritional analysis demonstrated that 32% of the liver Se values were in the deficient category and that 28% had deficient concentrations of Vitamin E.16 More recently another study in Michigan demonstrated a 31% incidence of low liver Se in 301 fetuses reviewed. One hundred and forty one fetuses in this group had no other demonstrable cause of abortion; of these, twenty-eight had low liver Se and thirty-eight had both low liver Se and vitamin E concentrations.[67] A review of aborted fetuses from 1976-86 submitted to the Veterinary Diagnostic Laboratory at Oregon State University showed an increase in Se-deficient fetuses compared to liver Se between 1982-86.[68]

A hypothesis is proposed by Taylor to explain the relationship of Se to abortion. A common thread through the many abortion syndromes suggests vascular damage which leads to degeneration and necrosis of cells in the particular target organ. Taylor proposed that a common denominator is the protection of biological membranes by Se

and vitamin E. In the deficient state, more severe damage may be prostaglandin-mediated. Arachidonic acid is released from the damaged membranes via phospholipase A2 which is activated by many stimuli, including membrane damage comparable to that which may occur with Se deficiency. Two of the resulting prostaglandins (F2a and TXA2) have physiological effects which include producing thrombosis and vascular damage. This is the common histological lesion in nutritional muscular dystrophy (WMD) as well as in aborted fetuses and placenta. Likewise PGF2a is produced which has a luteolytic effect and initiates strong uterine contractions.[66] A strong case is developing for a relationship between Se deficiency and abortion in the bovine.

Selenium Status:

Selenium status is assessed directly by measuring the concentration of Se in serum, plasma, whole blood and/or tissues flurometrically.[69] An indirect measure of Se status is plasma, erythrocyte or tissue GSH-Px activity using a coupled colormetric procedure.[70] Plasma and serum Se concentrations indicate the present Se status.[71] Whole blood Se concentration and erythrocyte GSH-Px activity, tend to reflect prior Se status.[72] This is thought to be due to the incorporation of GSH-Px and Se into erythrocytes during erythropoiesis. The rate of erythrocyte turnover is reflected in the whole blood Se concentration and erythrocyte GSH-Px activity.[73] Table 1 provides a summary of values used in the assessment of Se status in cattle.[74]

Table 1. Assessment of Selenium status in cattle.[1]

			Selenium Concentration		
Reference	Tissue	Units	Adequate	Marginal	Deficient
Julien et al. 1976b; Sergerson et al. 1981	Serum	μg/ml	>.08	.05-.08	<.05
Puls 1981	Serum	μg/ml	.07-.3	.02-.04	.002-.008
	Liver	μg/g WW[2]	.25-.50	.12-.25	.02-.07
Koller et al. 1983	Whole Blood	μg/ml	>.10	.051-.10	<.05
	GSH-PX[3]	mU/mg Hb	<15	15-30	>30a
Maas 1983; Maas and Koller 1985	Whole Blood	μg/ml	.07->.10	.05-.06	.01-.04
	GSH-Px	mU/mg Hb	0-15	15-25	25-500
	Liver	μg/g DW[2]			<.2-.3
Miller and Thompson 1983	Whole Blood	μmole/L	.8-2.5	.4-.8	<.4
	Liver	μmole/kg DW			<3.0
Van Vleet 1980	Liver	μg/g WW			<10
	Whole Blood	μg/ml			<.05
Carlstrom et al. 1979	GSH-Px	μkat/L	>500	200-500	<200
Thompson et al. 1980	Whole Blood	μmole/kg	>.191	.127-.191	<127
	Liver	μmole/kg WW	>.635	.254-.635	<254

[1]from Van Saun 1988
[2]Liver wet weight (WW) or dry weight (DW)
[3]Erythrocyte glutathione peroxidase activity

Supplementation:

Supplemental Se has been provided to livestock per os and by parenteral injection.[75] The parenteral (injection) Se supplementation method has several limitations. These include labor requirements to administer repeated individual injections, relative short period of effect (1-2 weeks), drug and tissue reactions to the parenteral product, and the additional cost required to provide a sterile product suitable for injection. In fact, Se supplementation on a unit per unit basis of Se costs greater than twenty-five times more in the parenteral form compared to oral Se supplementation. Selenium supplementation has only been allowed in some livestock feeds since 1974.[76,77,78] This was due to the concern of possible toxicosis resulting from over supplementation. The allowable concentrations have also been very conservative reflecting data collected mainly in monogastric animals.[79] The current allowable concentration of supplementation is 0.3 ppm of the total ration for ruminants.[78]

Researchers have speculated that ruminants probably have a higher tolerance for supplemental Se.[12] Concentrations approaching 10 ppm have been fed to lactating dairy cattle for a short period of time (8 days) with no apparent problems.[80] Livestock producers and veterinarians report an apparent biological effect in the absence of toxicity at concentrations of Se supplementation above allowable concentrations.[81] The exposure of supplemental Se to the microbial flora probably is a significant factor affecting the amount of absorption of Se.[82]

BIBLIOGRAPHY

1. Schamberger RJ, Biochemistry of Selenium. Plenum Press, New York (1983).

2. Franke KW, A new toxicant occurring naturally in certain samples of plant foodstuffs. J Nutr 1934;8:597.

3. Miller WT, Williams KT. Minimum lethal doses of selenium, as sodium selenite, for horses, mules, cattle and swine. J Agric Res 1940;60:163.

4. Maag DD, Glenn MW. Toxicity of Selenium: Farm animals. 127-140. Symposium: Selenium in Biomedicine. 1976:AVI Publishing Co. West Port Conn.

5. Schwarz K, Foltz CM, Selenium as an integral part of factor-3 against dietary necrotic liver degeneration. J Am Chem Soc 1957;79:3292-3293.

6. Patterson EL, Mostrey R, Stockstad ELR, Effect of selenium in preventing exudative diathesis in chicks. Proc Soc Exp Biol Med 1957;95:617.

7. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG, Selenium:Biochemical role as a component of glutathione peroxidase. Science 1973;179:588-590.

8. Putnam ME, and Comben N, Vitamin E. Vet Rec 1987;121:541-545.

9. NRC-Subcommittee on Selenium (1983) Selenium in nutrition. National Academy Press. Washington, DC.

10. Mayland HF, Selenium in soils and plants. In: Selenium Responsive Diseases in Food Animals. Proc Symposium Western States Vet Conf, Las Vegas, Nevada, Veterinary Learning Systems 1985;5-10.

11. Scott ML, Selenium. In: Comar CL, Bronner F, eds. Mineral Metabolism. Vol IIB, New York: Academic Press, 1962.

12. Stevens JB, Olson WG, Kraemer R, Archambeau J, Serum selenium concentrations and glutathione peroxidase activities in cattle grazing forages of various selenium concentrations. Am J Vet Res 1985;46:1556-1560.

13. Wright PL, Bell MC. Comparative metabolism of selenium and tellurium in sheep and swine. Am J Physi 1966;211:6-10.

14. Schwarz K, Fredga A. Biological potency of organic selenium compounds. Aliphatic monoseleno- and diseleno-dicarboxylic acids. J Bio Chem 1969;244:2103.

15. McConnell KP, Cho GJ. Transmucosal movements of selenium. Am J Physi 1965;208:191.

16. Schwarz KA, Fredga A. Biological potency of organic selenium compounds. II. Aliphatic seleno-carboxylic acids and acid amides. Bioinorg Chem 1972;2:47.

17. Schwarz K, Fredga A. Biological potency of organic selenium compounds, III. Phenyl-, Benzyl-, and phenylethylseleno-carboxylic acids, and related compounds. Bioinorg Chem 1972;2:171.

18. Thomson CD, Robinson BA, Stewart RDH, Robinson MF. Metabolic studies of Se-75 selenocystine and Se-75 selenomethionine in the rat. Brit J Nutr 1975;43:501.

19. Rosenfeld I. Biosynthesis of seleno-compound from inorganic selenium; Proc Soc Exper Bio Med 1962;111:670.

20. Hidiroglou M, Heaney DP, Jenkins KJ. Metabolism of inorganic selenium in rumen bacteria. Canadian J Physi Pharm 1968;46:229.

21. Paulson GD, Baumann CA, Pope AL. Metabolism of 75-Se-selenite, 75-Se-selenate, 75-Se-selenomethionine, and 35-S-sulfate by rumen microorganisms in vitro. J Ani Sci 1968b;27:497.

22. Whanger PD, Wewig PH, Muth OH. Metabolism of 75-Se-selenite and 75-Se-selenomethionine by rumen microorganisms. Fed Proc 1968;27:418.

23. Burk RF, Brown DG, Seeley RJ, Scaief CC. Influence of dietary and injected selenium on whole body retention, route of excretion, and tissue retention of 75-Se(-2) in the rat. J Nutr 1972;102:1049.

24. Ehlig CF, Hogue DE, Allaway WH, Hamm DJ. Fate of selenium from selenite or selenomethionine with or without vitamin E, in Lambs. J Nutr 1967;92:121.

25. Rosenfeld I. Metabolic effects and metabolism of selenium in animals. University of Wyoming Agricultural Exp Sta Bull 1964:414.

26. Palmer IS, Fischer DD, Halverson AW, Olson OE. Identification of a major selenium excretory product in rat urine. Biochimica et Biophysica Acta 1969;177:336.

27. Nahapetian AT, Janghorbani M, Young, VR. Urinary Trimethylselenonium excretion by the rat: Effect of level and source of selenium-75. J Nutr 1983;113:401.

28. Muth OH, Oldfield JE, Schubert JR, Remmert LF, White muscle disease (myopathy) in lambs and calves. VI. Effects of Selenium and Vitamin E on lambs. Am J Vet Res 1959;20:231.

29. Hartley WJ, Selenium and ewe fertility. Proceedings of the N Z Soc Ani Prod 1963;23:20.

30. Trinder N, Woodhouse CD, Rentan CP. The effect of vitamin E and selenium on the incidence of retained placentae in dairy cows. Vet Rec 1969;85:550.

31. Julien WE, Conrad HR, Jones JE, Moxon AL, Selenium and Vitamin E and the incidence of retained placenta in parturient dairy cows. J Dairy Sci 1976;59:1954.

32. Harrison JH, Hancock DD, Conrad HR. Selenium deficiency and ovarian function in dairy cattle. Fed Pro 1982;41:786.

33. Yamini B, Mullaney TP, Vitamin E and selenium deficiency as a possible cause of abortion in food animals. Proc 28th Annual Meeting Am Assoc Vet Lab Diagnosticians 1985;131-144.

34. Andrews ED, Hartley, WJ, Grant AB. Selenium-responsive diseases of animals in New Zealand. N Z Vet J 1968;16:3.

35. Norman BB, Johnson W. Selenium responsive disease. Ani Nutr Health 1976;31:6.

36. Gyang EO, Stevens JB, Olson WG, Tsitsamis SD, Usen KEA. Effects of selenium-vitamin E injections on bovine polymorpho-nucleated leukocytes phagocytosis to killing of Staphylococcus aureus. Am J Vet Re 1984;45:175.

37. Swecker WS, Eversole DE, Thatcher CD, Blodgett DJ, Schurig GG, Meldrum JB. Influence of supplemental selenium on humoral immune responses in weaned beef calves. Am J Vet Res 1989;50:1760.

38. Smith KL, Harrison JH, Hancock DD, Todhunter DA, Conrad H,R. Effect of vitamin E and selenium supplementation on the incidence of clinical mastitis and duration of clinical symptoms. J Dairy Sci 1984;67:1293-1300.

39. Erskine RJ, Eberhart RJ, Grasso MS, Scholz RW. Induction of Escherichia coli mastitis in cows fed selenium-deficient or selenium-supplemented diets. Am J Vet Res 1989;50:2093.

40. Erb RE, Hinze PM, Gildow EM, Morrison RH. Retained Fetal Membranes - The effect of prolificacy of Dairy Cattle. J Am Vet Med Assoc 1958;133:489.

41. Muller LD, Owens MJ. Factors associated with the incidence of retained placentas. J Dairy Sci 1974;57:725.

42. Wetherhill GD, Retained placenta in the bovine. A brief review. Can Vet J 1965;6:290.

43. Julien WE, Conrad, HR, Moxon AL, Selenium and Vitamin E and incidence of retained placenta in parturient dairy cows. II. Prevention in commercial herds with prepartum treatment. J Dairy Sci 1976b;59:1960.

44. Black WG, Ulberg LC, Kidder HE, Sinvi J, McNutt SH, Casida LE. Inflammatory response of the bovine endometrium. Am J Vet Res 1953;14:179.

45. Callahan CJ, Post parturient infection of dairy cattle. J Am Vet Med Assoc 1969;155:1963.

46. Guieero RTC. Retained fetal membranes in cattle. J Am Vet Med Assoc 1959;135:475.

47. Trinder N, Rentan CP, The relationship between the intake of selenium and vitamin E on the incidence of retained placenta in dairy cows. Vet Rec 1973;93:641.

48. Williams WF, Yuer DR, Diefeuderfer DL, Douglas L.W., and Vandersall, Influence of prepartum selenium-vitamin E on retained placenta in dairy cattle, Proc Forage Research Farm Field Days, Maryland Agri Exp Sta, Univ Maryland 1977:24.

49. Reinhardt TA, Conrad HR, Julien WE, Moxon AL. Alfalfa silage, selenium injections and retained placentas. J Dairy Sci 1978;61(supp. 1):185.

50. Harrison JH, Conrad HR. Effect of dietary calcium on selenium absorption by the non-lactating dairy cow. J Dairy Sci 1984;67:1860.

51. Harrison JH, Hancock DD, Conrad HR. Vitamin E and selenium for reproduction of the dairy cow. J Dairy Sci 1984;67:123.

52. Eger S, Drori D, Kadoori I, Miller N, Schindler H. Effects of selenium and vitamin E on incidence of retained placenta. J Dairy Sci 1985;68:2119.

53. Bostedt H, Schramel P. Investigation to the Influence of Selenium in Veterinary Medicine: Nutritional Muscle Dystrophy and Retained Placenta. Trace Element Analytical Chemistry in Medicine and Biology, Walter de Gruyter and Co., New York, N.Y., pg 83 (1980).

54. Hidiroglou M, Hoffman I, Jenkins KJ. Selenium distribution and radiotocopherol metabolism in the pregnant ewe and fetal lamb. Can J Physi Pharm 1969;47:953.

55. Gwazdauskas FC, Bibb ML, McGillard ML, Lineweaver JA. Effect of prepartum selenium-vitamin E injection on time for the placenta to pass and on reproductive functions. J Dairy Sci 1979;62:978.

56. Ishak MA, Larson LL, Owen FG, Lowry SR, Erickson ED. Effects of selenium, vitamins and ration fiber on placental retention and performance in dairy cattle, J Dairy Sci 1983;66:99.

57. Hidiroglou M, McAllister AJ, Williams CJ. Prepartum supplementation of selenium and vitamin E to dairy cows: Assessment of selenium status and reproductive performance, J Dairy Sci 1987;70:1281.

58. Segerson EC, Riviere GJ, Dalton HL, Whitacre MD. Retained placenta of Holstein cows treated with selenium and vitamin E. J Dairy Sci 1981;64:1833.

59. Segerson EC, Murray FA, Moxon AL, Redman DR, Conrad HR. Selenium and Vitamin E: Role in fertilization of the bovine ova. J Dairy Sci 1977;60:1001.

60. Segerson EC, Libby DW. Ova fertilization and sperm number per fertilized ovum for selenium and vitamin E-treated Charolais cattle. Theriogenology 1982;17:333.

61. Hartley WJ, Grant AB. A review of selenium responsive diseases of New Zealand livestock. Fed Proc 1961;20:679.

62. Segerson EC, Ganapathy SN. Fertility of ova in ewes receiving selenium and vitamin E supplementation. J Ani Sci 1979;49 (supp. 1):335.

63. Segerson EC, Ganapathy SN. Fertilization of ova in selenium/vitamin E treated ewes maintained on two planes of nutrition. J Ani Sci 1983;51:386.

64. Julien WE. Murray FA. Effect of selenium and selenium and vitamin E on in vitro motility of bovine spermatozoa. Proceedings of the American Society of Animal Science, 69th annual meeting, Madison, U. of Wisconsin, pg. 174 .

65. Segerson EC, Johnson BH. Selenium/Vitamin E and reproductive function in yearling Angus Bulls. J Ani Sci 1980;51:395.

66. Taylor RF, Puls R, MacDonald KR. Bovine abortions associated with selenium deficiency in Western Canada. Proceedings of the 22nd Annual Meeting of the Am Assoc Vet Lab Diagnos 1979:77.

67. Yamini B, Trapp AL, Stowe HD. Congenital myopathy, cardiomyopathy, Purkinjie cardiocyte degeneration and abortion associated with Vitamin E and/or selenium deficiency in the bovine Am J Vet Res 1989? (accepted for publication).

68. Hedstrom OR, Maas JP, Hultgren DD, Lassen ED, Wallner-Pendelton EA, Synder SP. Selenium deficiency in bovine, equine, and ovine with emphasis on its association with chronic disease. Proceedings of the 29th Annual Meeting of the Am Assoc Vet Lab Diagno 1986:101.

69. Olson OE. Flourometric analysis of selenium in plants. J Assoc Off Anal Chemists 1969;52:627-634.

70. Agergarrd N, Jensen PT. Procedure for blood glutathione peroxidase determination in cattle and swine. Acta Vet Scand 1982;23:515-527.

71. Thompson KG, Fraser AJ, Harrop BM, Kirk JA. Glutathione peroxidase activity in bovine serum and erythrocytes in relation to selenium concentrations of blood serum and liver. Res Vet Sci 1980;28:321-324.

72. Thompson KG, Fraser AJ, Harrop BM, Kirk JA, Bullians J, Cordes DO. Glutathione peroxidase activity and selenium concentration in bovine blood and liver as indicators of dietary selenium intake. N Z Vet J 1981;29:3-6.

73. Oh SH, Sunde RA, Pope AL, Hoekstra WG, Glutathione peroxidase response to selenium intake in lambs fed a torula yeast-based, artificial milk. J An Sci 1976a;42:977-983.

74. VanSaun RJ. Selenium and Vitamin E: Relationships between the pregnant dairy cow and fetus. MS Thesis 1988:36.

75. Muth OH, Schubert JR, Oldfield JE, White muscle disease (myopathy) in lambs and calves. VII. Etiology and prophylaxis Am J Vet Res 1961;22:466.

76. Food and Drug Administration. Food Additives: selenium in animal feed. Fed Reg 1974;39:1355.

77. Food and Drug Administration. Food additives permitted in feed and drinking water of animals: selenium. Fed Reg 1979;44:5392.

78. Food and Drug Administration. Food Additives: selenium in animal feed. Fed Reg 1987;52:10,668.

79. Combs, GF, Combs SB. The Role of Selenium in Nutrition. Academic Press, New York 1986:465.

80. Fisher LJ, Hoogendorn C, Montemurro J. The effect of added dietary selenium on the selenium content of milk, urine and feces. Can J An Sci 1980;60:79

81. Ellis RG. (personal communications).

82. National Research Council Subcommittee on Selenium; Selenium in Nutrition; 1983:72.

INTRODUCTION

Selenium (Se) is an essential trace mineral in the nutrition of cattle. However, cattle feed that contains high Se concentrations is reported to produce acute and chronic toxicity.[1,2,3] Signs of acute toxicity include anorexia, decreased milk production, excess salivation, respiratory distress, breath with a garlic-like odor, and occasionally death.[4] Clinical signs of chronic toxicity in cattle are frequently that of a chronic wasting disease. These signs include anorexia, emaciation, dullness, listlessness, rough hair coat, alopecia, hoof sloughing, joint erosions, liver cirrhosis and death.[4]

Research during the last three decades has related numerous livestock problems to Se deficiency; both independently and in conjunction with other nutrients. Selenium deficiency is frequently associated with vitamin E deficiency but also has been demonstrated to interact with and be affected by, the elements calcium, copper, sulfur, arsenic and cadmium. In cattle, Se deficiency has been associated with numerous disease syndromes. These include nutritional muscular dystrophy (White Muscle Disease),[5] infertility,[6] retained placenta,[7,8] cystic ovarian disease,[9] abortion,[10] untriftiness,[11] impaired function of both the humoral and cell-mediated immune systems,[12,13] and mastitis.[14] Selenium's major biological function is related to it's structural role in the enzyme glutathione peroxidase (GSH-Px).[15] Selenium is also associated with the

function of hepatic enzymes involved with metabolism and detoxification of drugs and other foreign substances.[16]

The maximum legal concentration of Se in feed for dairy cows has recently been raised from 0.1 to 0.3 ppm of total dry matter.[17] Consequently the Se concentration in supplements and premixes has been raised. These changes, while most likely being beneficial to animal health and production, also, increase the possibility of dietary Se intake inadvertently exceeding legal limits. This could result from multiple Se sources or errors in feed formulation. The authors are aware of some herds consuming 19 mg of supplemental Se/head/day and have seen bags of Se-supplement premix containing 50 mg Se/ounce (approximately 10X higher than intended) mistakenly delivered to dairy farms on Se supplementation programs.

The need to document any signs of excess dietary Se intake in Holstein cows is evident. Likewise it is necessary to identify tests to determine the Se status of cattle suspected to be receiving excess dietary Se whether from natural or supplemental sources.

The objectives of this experiment were twofold. First, to determine the response(s) of Holstein cows to high concentrations of dietary Se as sodium selenite. Secondly, to compare the values of various measures of Se status in Holstein cows to three different dietary Se concentrations. The experiment consisted of two concurrent trials to be referred to as trial one and two.

MATERIALS AND METHODS

General:

The research cows were housed in individual tie stalls in a common stable . Feeders were partitioned to assure that consumption of the Se supplement was limited to the designated cow. All cows were fed mixed hay (ad libitum). A corn meal concentrate (Table 2) containing the supplemental Se was fed once daily. Blood samples were collected from the coccygeal vessels into tubes containing heparin, EDTA or no anticoagulant. Samples of whole blood and serum for Se analysis and rabies titers were frozen at -20°C for future analysis. Blood samples for hematology studies, enzyme activity determinations, and lymphocyte response testing were analyzed immediately.

Antemortem liver samples were obtained using a percutaneous liver biopsy technique.[18] This procedure uses a illuminating endoscopic device[a] to visualize the liver. The endoscope has a plastic stylet which, after making a small skin incision, is bluntly introduced into the abdomen via the eleventh or twelfth right intercostal space. After removal of the stylet, a custom-made biopsy instrument was introduced through the endoscope to collect a 3-6 gram sample of liver tissue. Postmortem liver samples were obtained when the cows were slaughtered at the end of the trials. Liver samples were frozen at -20°C until analyzed.

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[a] Welch Allyn, Skaneateles Falls, New York

Table 2. Composition of concentrate fed to cows during the trials.[1,2]

		mg supplemental Se/hd/d				
Ingredients	units	0	3	20	50	100
Ground corn	%	87	87	87	87	87
Trace mineral salt	%	6.7	6.7	6.7	6.7	6.7
Molasses	%	3.7	3.7	3.7	3.7	3.7
Limestone $\times 10^{-2}$	%	2.5	2.4	2.0	1.2	0.0
Sodium Selenite $\times 10^{-2}$	%	0.0	.074	.5	1.3	2.5
Vitamin A	IU[3]/kg	55	55	55	55	55
Vitamin D	IU[3]/kg	5	5	5	5	5
Vitamin E	u/kg	166	166	166	166	166

[1].9 kg of concentrate fed daily
[2]in addition to ad libitum mixed legume hay - estimated Se content .05 ppm

Variables tested included Se concentrations in serum, whole blood and liver. Urinary and fecal Se concentrations were also measured. Hematologic measurements included packed cell volume and the concentrations of white blood cells, red blood cells and hemoglobin. Serum enzyme activities of aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), sorbitol dehydrogenase (SDH) and creatine phosphokinase (CPK) were also determined. General health variables included daily measurements of body temperature, heart rate and respiratory rate. At the beginning of the trial, a groove was cut 5mm below the coronary band on the dorsal and lateral surfaces of diagonal feet. Monthly, the distance between the groove and the coronary band was measured to quantify hoof growth, body weight was estimated by heart girth measurement, body-condition scores were recorded and the animals were videotaped. Lymphocyte response tests to the non-specific mitogens concanavalin A, phytohemagglutinin and pokeweed mitogen were measured approximately every 45 days during the trial. All samples were collected in the morning prior to the feeding of the concentrate containing the Se supplement.

Analysis for serum, whole blood, liver, urine and fecal Se concentrations used the improved flurometric method.[19] Hematological analysis of fresh, EDTA-treated blood used the Technicon H-1 automated hematology analyzer.[b] Analysis of serum enzymes was done on fresh serum using a Flexi-Gem centrifugal analyzer.[c]

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[b]Technicon Instruments Corp., Tarrytown, N.Y.

[c]Pharmacia E.N.I. Diagnostics Inc., Fairfield, N.J.

Trial One:

Twenty-four adult, non-lactating Holstein cows were used as research animals. After a two-week period of adjustment on the base diet, they were divided into four groups balanced for serum Se concentrations. The groups were supplemented with 0, 3, 20 and 50-mg Se/head/day (Control, 3, 20, and 50-mg groups, respectively) for 90 days. A summary of the sampling dates for each variable measured is presented in (Table 3).

The cows were vaccinated on days 45 (primary) and 73 (secondary) with a commercial rabies vaccine[d] licensed for use in cattle. Rabies vaccine was used because it is safe and effective and cows were not likely to have baseline titers. Rabies titers were measured on days 0, 7 and 14 after primary and secondary vaccinations to measure primary and secondary immune response.

On the last day of the trial, voided urine samples and random samples of fresh feces were collected from each cow.

Variables measured were tested by a split-plot, repeated measures analysis of variance with main effects of dietary Se and time over treatment.[20] The linear model is as follows:

$$Y_{ijk} = +S_j + A_{(i)j} + T_k + ST_{jk} + E_{ijk}$$

Where

- Y = the individual dependent variables measured
- S = dietary Se; 0, 3, 20, 50 mg/head/day
- A = animal, $A_{(i)j}$ = animal within treatment group = error term for testing treatment effects.
- T = time
- ST = time by Se interaction
- E = random error

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[d]Rabguard TC, Norden Laboratories Inc., Lincoln, Neb.

An example of the ANOVA table used follows:

TYPE III

Source of Variation	df	SS	MS	F value	Sig
Among Subjects					
Treatmemts	3	74.469	24.823	17.68	.0001
Subjects ($error_a$)	20	28.077	1.4038		
With Subjects					
Time	1	57.028	57.028	56.74	.0001
Time * Treatments	3	63.644	21.214	21.11	.0001
$Error_b$	20	20.101	1.005		

Minimum Significant Difference (MSD) equations used are:
1) $MSD = V\,[q_k]\,(t_k)$, where
2) $V_E = (0)^2 / \{[(MS_{D/A} + (B\text{-}1)\,(MS_E)^2]/ab^2_{(r\text{-}1)}\}$, and
3) $t_{D,.05,3,20}$, and
4) $V\,(q_k) = (C_i^2)\,(0^2/r)$, and
5) $0^2 = [MS_{D/A} + (b\text{-}1)\,MS_E] / b$

Specific contrasts between controls (0mg Se) and treatments were assessed with Dunnet's t distribution.

In this analytical design, time trends, as well as overall mean differences in dependent variables were assessed.[21]

Table 3. **Sampling schedule for trial 1.**

Variables	Days of the Trial											
	0	2	6	13	18	23	28	42	56	70	84	90
Serum selenium	*	*	*	*	*	*	*	*	*	*	*	*
Whole blood selenium	*	*	*	*	*	*	*	*	*	*	*	*
Liver biopsies	*											*
Urinary selenium												*
Fecal selenium												*
White blood cell	*				*				*			*
Erythrocyte	*				*				*			*
Hemoglobin	*				*				*			*
Packed cell volume	*				*				*			*
Serum asparate amino transferase	*				*				*			*
Serum gamma-glutamyl transferase	*				*				*			*
Serum sorbitol dehydrogenase	*				*				*			*
Serum creatine phosphokinase	*				*				*			*
Body weights	*				*				*			*
Body condition scores	*				*				*			*
Hoof growth	*				*				*			*
Rabies titers							*	*	*	*	*	*
Unstimulated lymphocytes	*							*				*
PHA-stimulated lymphocytes	*							*				*
Con A-stimulated lymphocytes	*							*				*
Pokeweed-stimulated lymphocytes	*							*				*

Trial two:

Trial two was conducted because no detectable signs of toxicity had developed in any of the groups after 90 days of daily feeding in trial one.

At the end of trial one, the 6 cows in the 50-mg group continued to receive the concentrate feed containing 50 mg of supplemental Se for 10 additional days. Starting on day 100 they were fed the same concentrate re-formulated to provide 100-mg Se/head/day (100-mg group) for an additional 28 days. After day 128 no concentrate was fed so that changes occurring in response to the withdrawal of Se could be monitored. A summary of the sampling dates to assess each variable can be found in (Table 4). Analysis of variables measured at the beginning and end of the 100 mg supplementation was done using paired t tests.

Table 4. Sampling schedule for trial 2.

Variables	Days of the Trial											
	100[1]	102	108	120	128	132	136	149	156	163	176	184
Serum selenium	*	*	*	*	*	*	*	*	*	*	*	*
Whole blood selenium	*	*	*		*	*	*	*	*	*	*	*
Liver biopsies	*				*							*
Urinary selenium	*				*							*
Fecal selenium	*				*							*
White blood cell	*				*					*		*
Erythrocyte	*				*					*		*
Hemoglobin	*				*					*		*
Packed cell volume	*				*					*		*
Serum asparate amino transferase	*				*					*		*
Serum gamma-glutamyl transferase	*				*					*		*
Serum sorbitol dehydrogenase	*				*					*		*
Serum creatine phosphokinase	*				*					*		*
Body weights	*				*				*			*
Body condition scores	*				*				*			*
Unstimulated lymphocytes	*				*							
PHA-stimulated lymphocytes	*				*							
Con A-stimulated lymphocytes	*				*							
Pokeweed-stimulated lymphocytes	*				*							

[1]After the start of trial 1

RESULTS

Trial One:

Mean serum Se concentrations increased with treatment in all groups (Figure 2 and Table 1 of Appendix A). Differences between the mean serum Se concentrations of the control and 3-mg groups were never significant ($P > .05$) and remained below reference concentrations for the entire trial.[22,23] Serum Se concentrations increased rapidly in the 20 and 50-mg groups becoming and remaining significantly different ($P < .05$) from the control group within 2 days after the beginning of Se supplementation. The mean serum Se concentration of the 20-mg group remained within, while the 50-mg group exceeded the reported reference range at the end of the trial.

Whole-blood Se concentrations increased gradually with treatment in all groups with values becoming significantly different from the control group ($P < .05$) at days 5 and 70 of the trial in the 50- and 20-mg groups, respectively (Figure 3 and Table 2 of Appendix A). The mean values of whole blood Se concentrations in the 3-mg group were never significantly different ($P \geq .05$) from the control group.

Mean liver Se concentrations in the control and 3-mg groups did not change significantly between day 0 and day 90 of the trial (Figure 4 and Table 3 of Appendix A). In the 20- and 50-mg groups Se concentrations were significantly different ($P < .05$) from the controls at day 90.

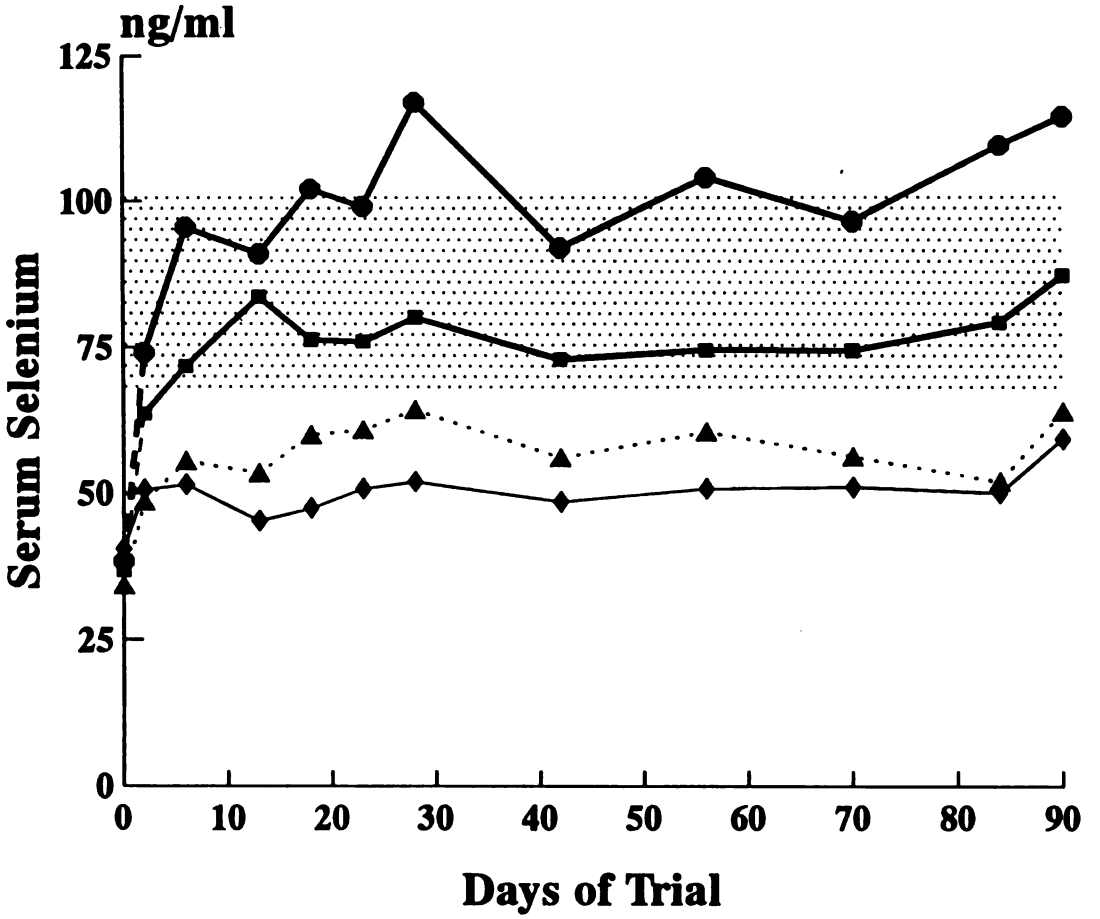

Figure 2. Mean serum selenium concentrations for cows in trial 1 supplemented with 0 (control) ◆—◆, 3 ▲····▲, 20 ■---■ and 50 ●- -● mg Se/head/day. |⠿⠿⠿⠿⠿⠿⠿⠿⠿| reference range (70 - 100 ng/ml), ▬ significantly different from control group ($P \leq .01$).

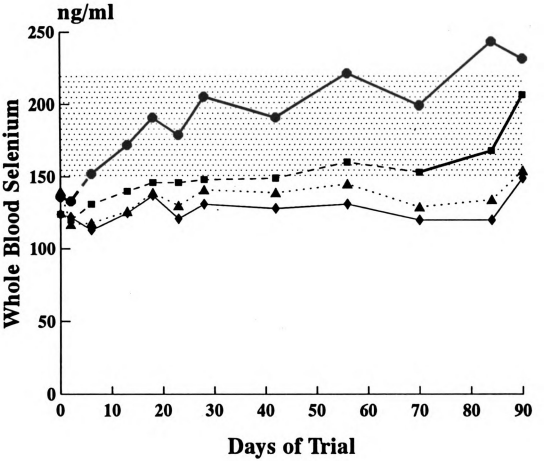

Figure 3. Mean whole blood selenium concentrations for cows in trial 1 supplemented with 0 (control) ♦—♦, 3 ▲····▲, 20 ■---■ and 50 ●- -● mg Se/head/day. |⁝⁝⁝⁝⁝⁝⁝⁝| reference range (150 - 220 ng/ml), ▬ significantly different from control group ($P \leq .01$).

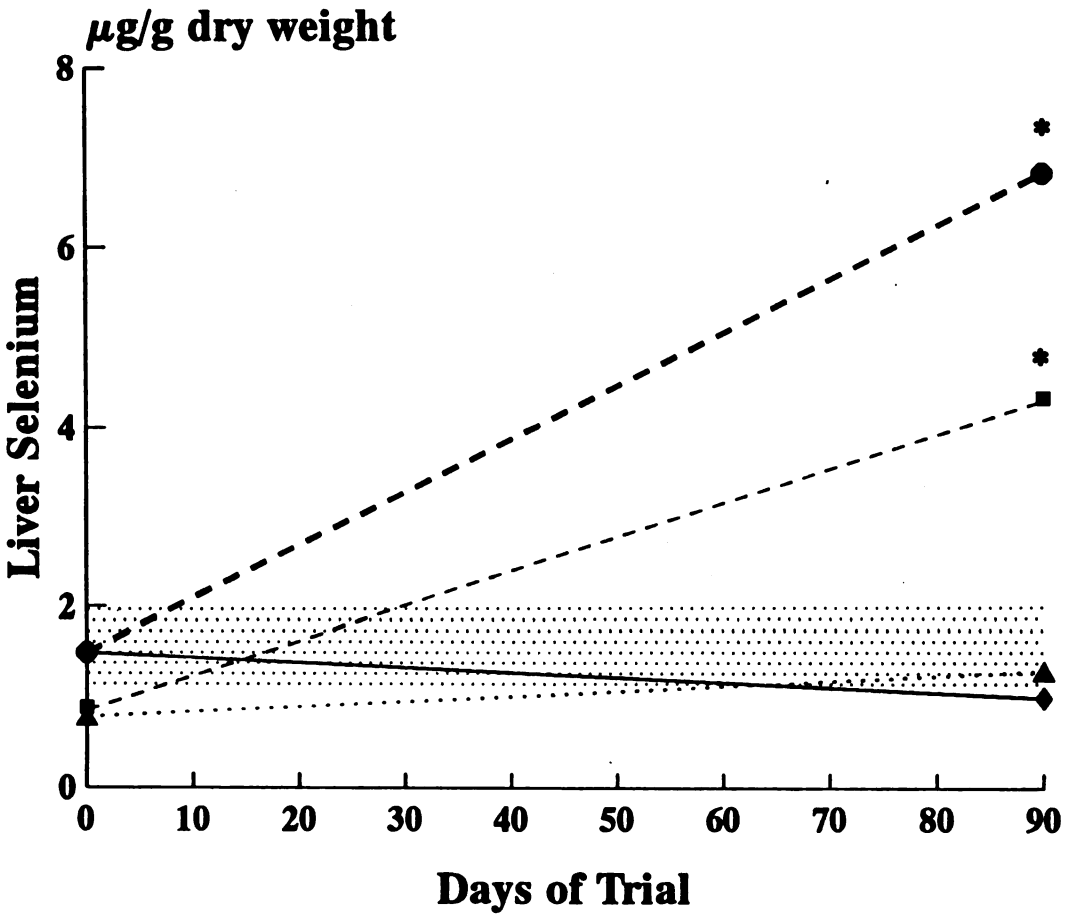

Figure 4. Mean selenium concentrations of liver biopsies obtained on days 0 and 90 in trial 1 for cows supplemented with 0 (control) ◆—◆, 3 ▲····▲, 20 ■---■ and 50 ●- - -● mg Se/head/day. |::::::::| reference range (1.2 - 2.0 μg/g dry wt.), * significantly different from control group ($P \leq .01$).

The mean concentrations of urinary and fecal selenium were significantly greater ($P < .05$) in the 20- and 50-mg groups when compared to the control at day 90 of the trial. There was no difference between the mean urine and fecal Se concentrations in the control and 3-mg groups at day 90 of the trial (Figures 5 and 6 and Table 4 and 5 of Appendix A).

Means for the hematologic variables measured, including packed cell volumes and concentrations of white blood cells, red blood cells and hemoglobin did not deviate from reference ranges or from controls ($P > .05$) (Tables 5-8 of Appendix A). Likewise, there was no deviation from reference values in the mean activities of serum enzymes which included AST, GGT, SDH and CPK (Tables 9-12 of Appendix A). No significant differences ($P \leq .05$), in the above variables were detected in any groups compared to controls.

The cows gained weight in all groups and maintained their body condition score throughout the trial (Tables 13-14 of Appendix A). No significant difference ($P \geq .05$) in hoof growth was observed between treatment groups, compared to the control (Table 15 of Appendix A).

Following vaccination there were no differences in rabies titers among groups. All rabies titers were negative initially in all groups and rose after the first and second vaccinations, showing comparable primary and secondary immune responses (Table 16 of Appendix A). Baseline lymphocyte blastogenesis, as well as the response to three non-specific mitogens, revealed no significant differences ($P > .05$) between supplemented groups and the controls (Tables 17-20 of Appendix A).

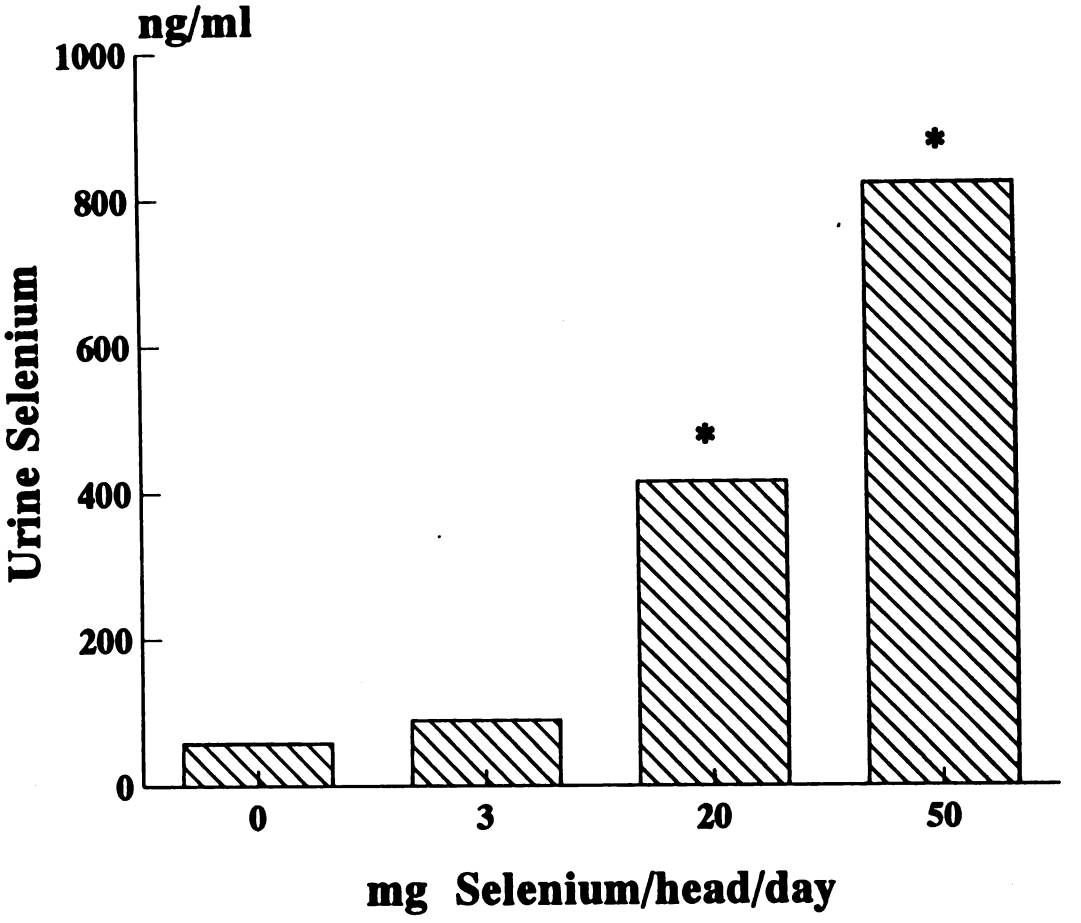

Figure 5. Mean urine selenium concentrations of cows in trial 1 on day 90, * significantly different from control group ($P \leq .01$).

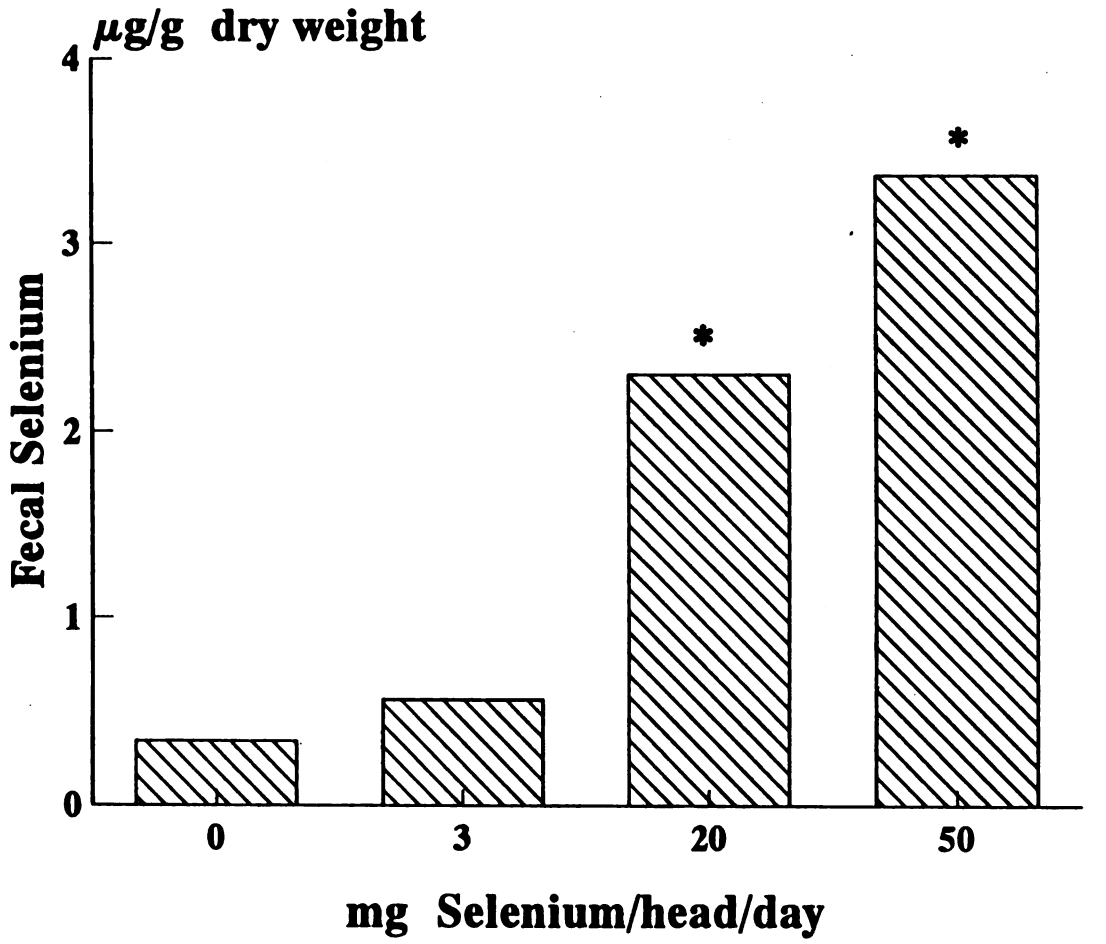

Figure 6. Mean fecal selenium concentrations of cows in trial 1 on day 90, * significantly different from control group ($P \leq .01$).

The other health variables monitored including attitude, body temperature, heart rate, respiratory rate showed no apparent deviation from reference ranges or differences between groups.

Trial Two:

Mean serum Se concentration increased very rapidly during the first ten days after Se intake was increased from 50 to 100 mg/head/day (100-mg group) and continued to increase for the next 18 days to a concentration about two and one half times reference concentrations (Figure 7. and Table 1 of Appendix B). When the Se supplementation was terminated at day 128, mean serum Se concentration fell very rapidly to approximately one half of the peak values by day 132 and continued to fall gradually, reaching the reference range by day 156 of the trial. The mean concentration declined below the reported reference concentrations by day 184.

The mean whole-blood Se concentration rose gradually throughout the 28 days of 100-mg Se/head/day supplementation reaching a peak mean concentration of 485 ng/ml (Figure 8 and Table 2 of Appendix B). After supplementation was terminated, mean whole-blood Se concentration also fell very rapidly to about four fifths peak concentration by day 132 and then very slowly decreased to about two times the reported reference concentrations and slightly above the predicted reference concentrations at day 184.

Mean liver Se concentration from liver biopsies at day 128 of 100-mg group was seven times higher than reference concentrations and two and one half times beginning concentrations (Figure 9 and Table 3 of Appendix B). After 56 days with no additional Se supplementation, mean liver Se concentration had returned to normal.

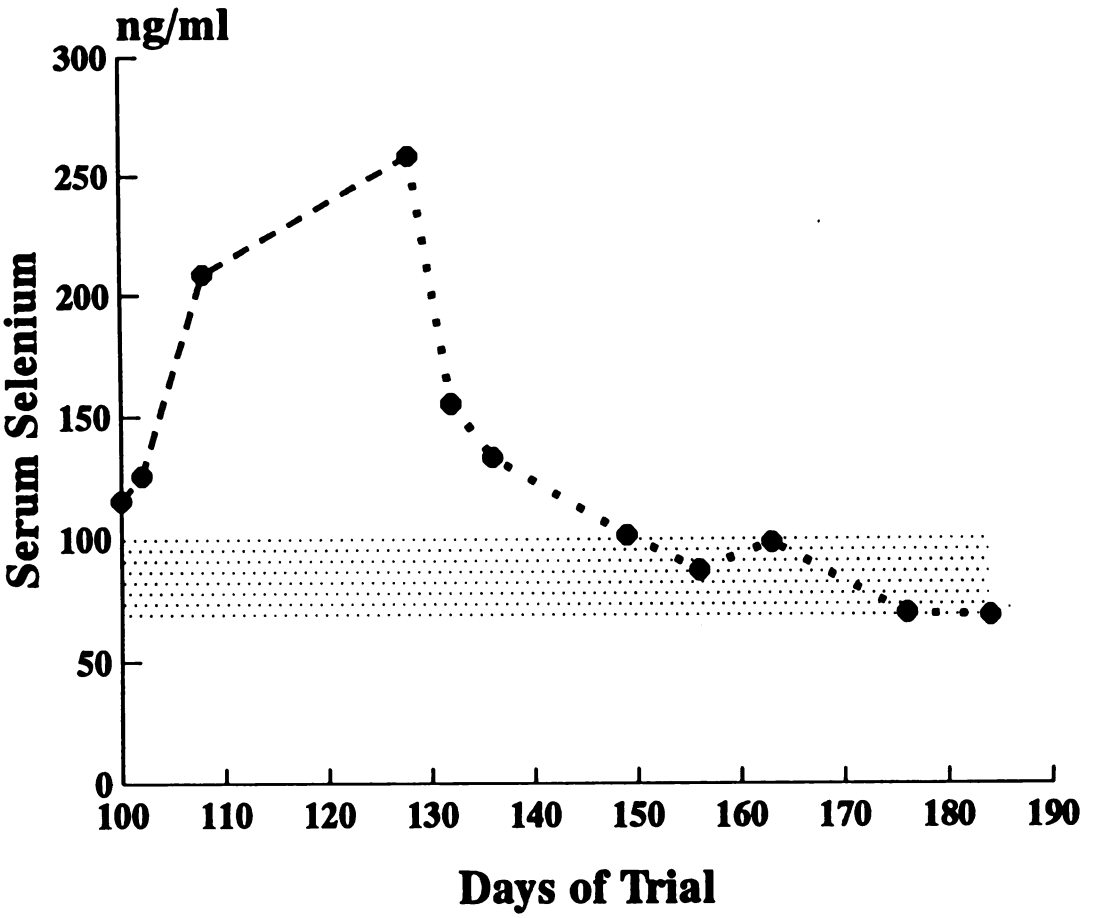

Figure 7. Mean serum selenium concentrations for cows previously fed 50 mg Se/head/day for 100 days in trial 1 and (in trial 2) supplemented with 100 mg Se/head/day for 28 days ●- -●, followed by no supplemental selenium for 56 days ●●●. |::::::::| reference range (70 - 100 ng/ml).

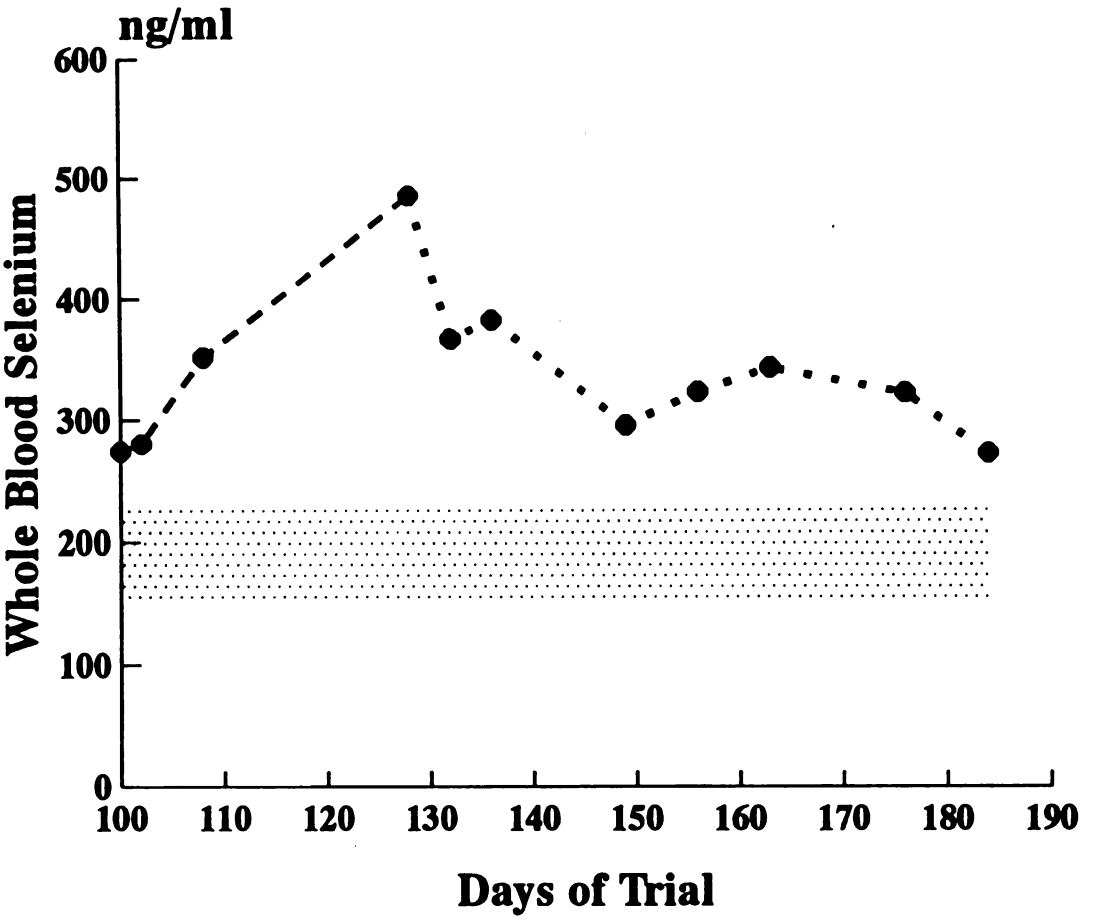

Figure 8. Mean whole blood selenium concentrations for cows previously fed 50 mg Se/head/day for 100 days in trial 1 and (in trial 2) supplemented with 100 mg Se/head/day for 28 days ●- -●, followed by no supplemental selenium for 56 days ●●●. |::::::::::| reference range (150 - 220 ng/ml).

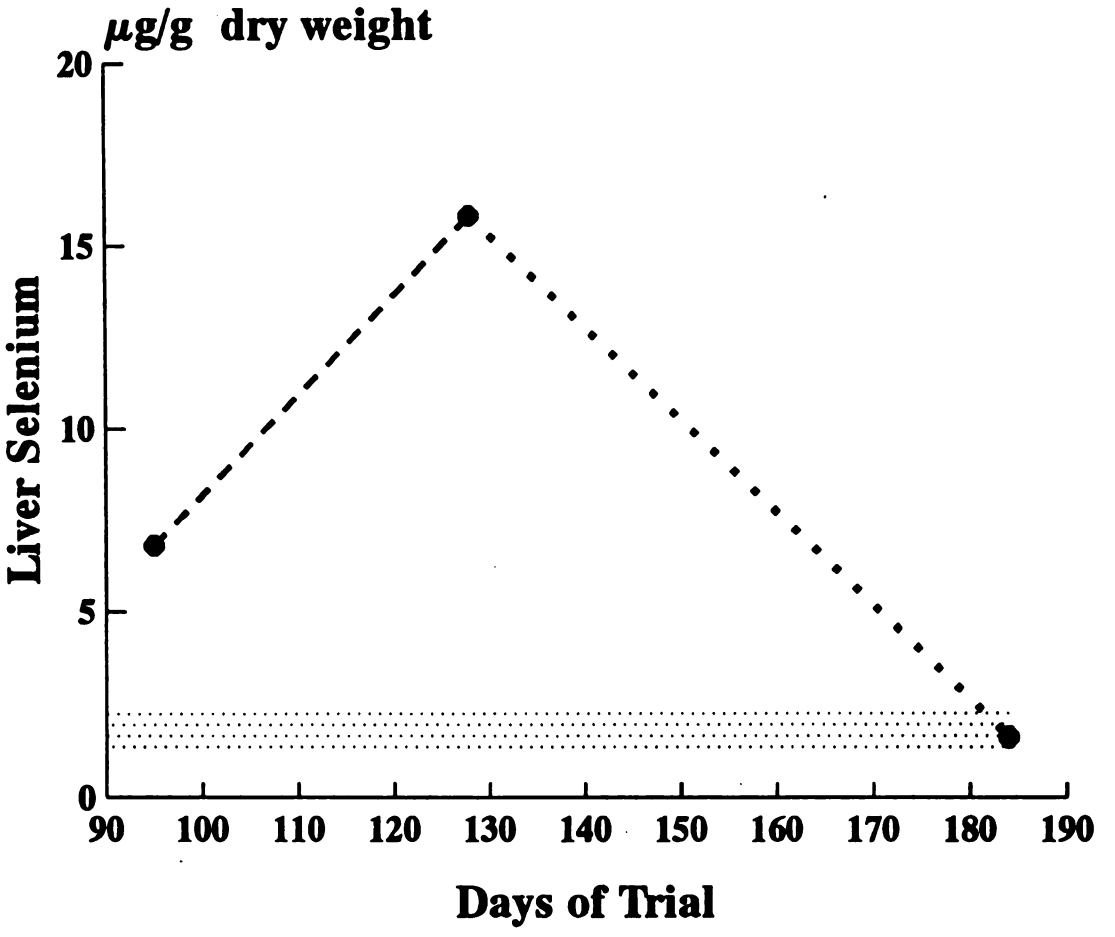

Figure 9. Mean selenium concentrations of liver biopsies from cows previously fed 50 mg Se/head/day for 100 days in trial 1 and (in trial 2) supplemented with 100 mg Se/head/day for 28 days ●- -●, followed by no supplemental selenium for 56 days ●●●. |::::::::::| reference range (1.2 - 2.0 μg/g dry wt).

The mean concentrations of urinary and fecal selenium was markedly increased at the end of supplementation. By the end of the trial, concentrations had returned to values comparable to the original control group on day 90 of trial one (Figures 10 and 11 and Tables 4 and 5 of Appendix B).

Means for the hematologic variables measured, including packed cell volumes and concentrations of white blood cells, red blood cells and hemoglobin, did not deviate from reference ranges (Tables 6-9 of Appendix B). Likewise the mean activities of serum enzymes, which included aspartate aminotransferase, gamma-glutamyl transferase, sorbitol dehydrogenase, and creatine phosphokinase, did not deviate from normal (Tables 10-13 of Appendix B). The cows gained weight and maintained their body condition throughout the trial (Table 14 and 15 of Appendix B).

Tests of lymphocyte responses to the three non-specific mitogens showed no differences at any time during the trial. (Tables 16-19 of Appendix B).

The other health variables monitored, including attitude, body temperature, heart rate, respiratory rate and mucus membrane color, showed no apparent deviation from normal ranges during the trial.

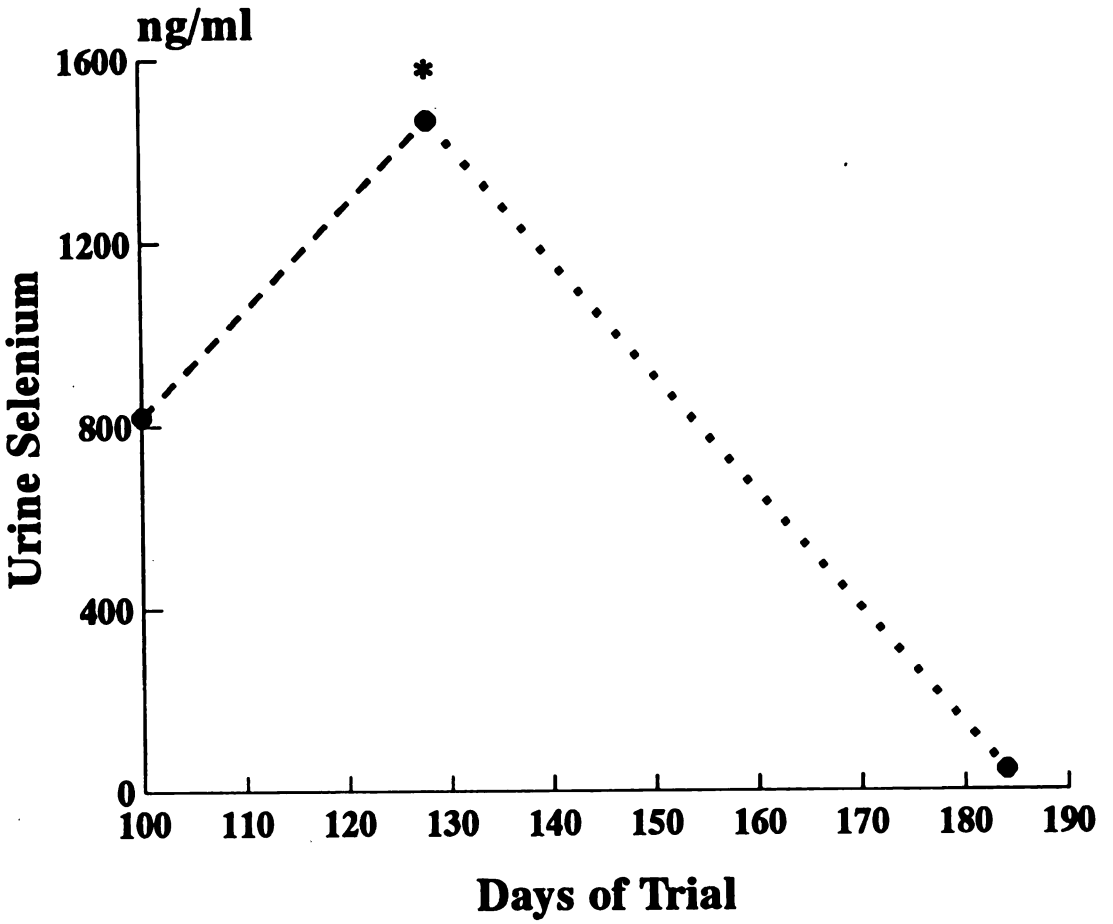

Figure 10. Mean urine selenium concentrations of cows previously fed 50 mg Se/head/day for 100 days in trial 1 and (in trial 2) supplemented with 100 mg Se/head/day for 28 days ●- -●, followed by no supplemental selenium for 56 days ●●●. * significant different from 50 mg group ($P < .01$).

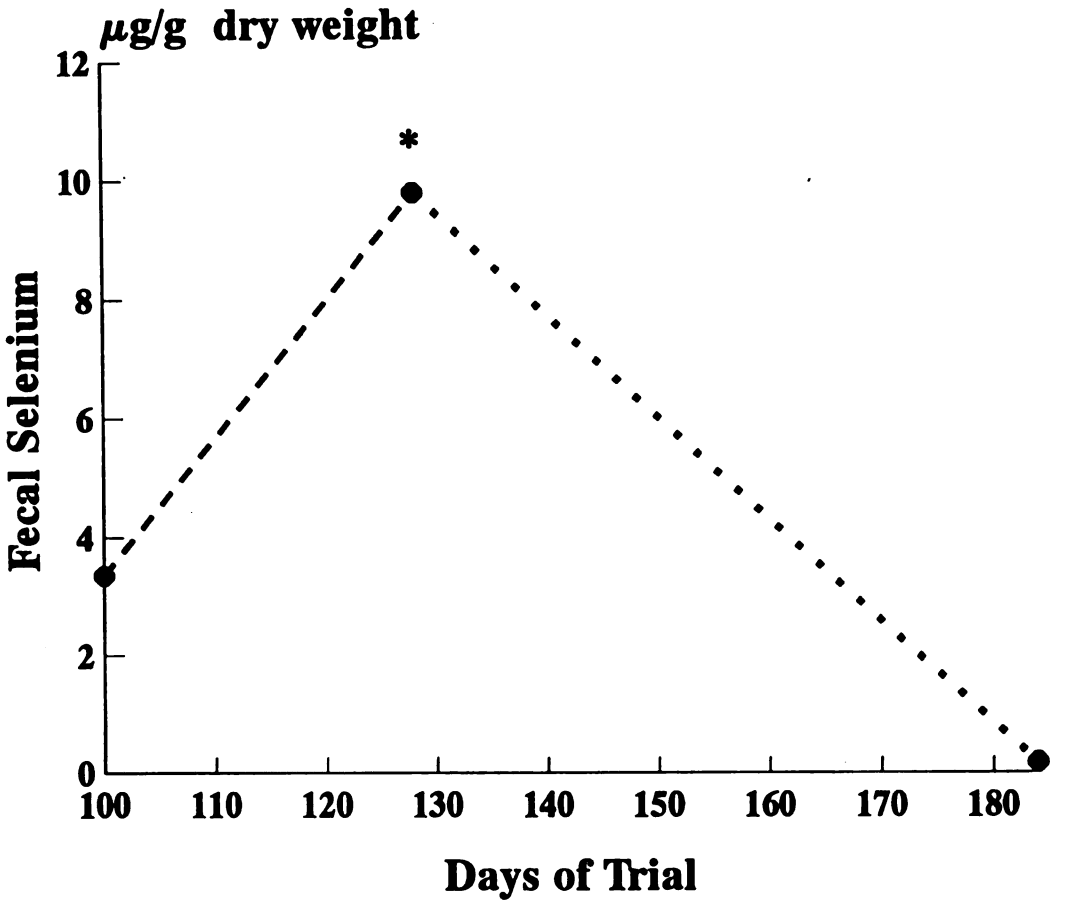

Figure 11. Mean fecal selenium concentrations of cows previously fed 50 mg Se/head/day for 100 days in trial 1 and (in trial 2 supplemented with 100 mg Se/head/day for 28 days ●- -●, followed by no supplemental selenium for 56 days ●●●. * significant different from 50 mg group ($P < .01$).

DISCUSSION

The major purpose of this experiment was to study clinical responses of Holstein cows to dietary Se concentrations which ranged from allowable (.3ppm) to excessive as might result accidentally in the preparation of diets. The responses were evaluated on the basis of changes over time in serum, whole-blood, liver, urine and fecal Se concentrations. From these observations, an effort was made to identify the most sensitive measure of Se status.

Serum Se concentrations changed much more rapidly in response to treatment than did whole-blood Se (Figure 2 and 3 and Table 1 and 2 Appendix A). These different rates of response have occurred due to differences in Se dynamics in serum, compared to red cells. Se in red cells is present almost entirely as glutathione peroxidase, an enzyme protein synthesized during development of the cell, prior to release into the circulation. Serum Se, on the other hand, consists of several protein and nonprotein bound forms of Se. Therefore, that portion of whole-blood Se that is contributed by the red cells, probably represents Se availability during development of the current red cell population. Because the red cell life span is 100-120 days, red cell Se may better reflect long-term changes in Se availability while serum Se reflects short term changes. This interpretation is consistent with the data of this experiment. It is interesting to note that whole-blood Se concentrations first became significantly different from controls after

about 10 days of Se supplementation in the 50-mg Se/head/day group versus 70 days in the 20-mg group after the beginning of supplementation (Figure 3 and table 2 of Appendix A). The more rapid increase in whole-blood Se concentrations in the 50-mg group compared to the 20-mg group probably reflects the proportionally higher Se concentration in the serum fraction of the whole-blood in the 50-mg group.

When the ratio of whole blood Se to serum Se for a given sample day is calculated, the overall mean ratio is approximately 2.2 (Figure 12). Reference concentrations for serum Se are reported to be 70 - 100 ng/ml.[24] If whole-blood Se is on average 2.2 times serum Se reference, concentrations the predicted whole blood Se reference concentrations should be 150 - 220 ng/ml. These reference concentrations are supported by various other investigations,[25,26,27] but are inconsistent with 80 - 120 ng/ml as proposed by others.[28] This observation is important when evaluating the Se status of cows when different methods of Se testing have been used.[29] In this experiment, mean whole-blood Se concentrations in all groups exceeded the lower (80-120 ng/ml) reported reference concentrations at all times.[30,31] However, mean whole-blood Se concentrations exceeded the higher (150-220 ng/ml) proposed reference concentrations only in the 50-mg group at the end of the trial.

As evident in this experiment, this 50-mg group of cows would be diagnosed as having a low Se status based on serum Se concentrations and an adequate Se status based on whole blood Se concentrations if the lower whole-blood Se reference concentrations were used(Figures 2 & 3 and table 1 & 2 of Appendix A). However, using the higher proposed whole blood Se reference, concentrations the cows would be diagnosed as having low Se status based upon both serum and whole-blood Se tests.

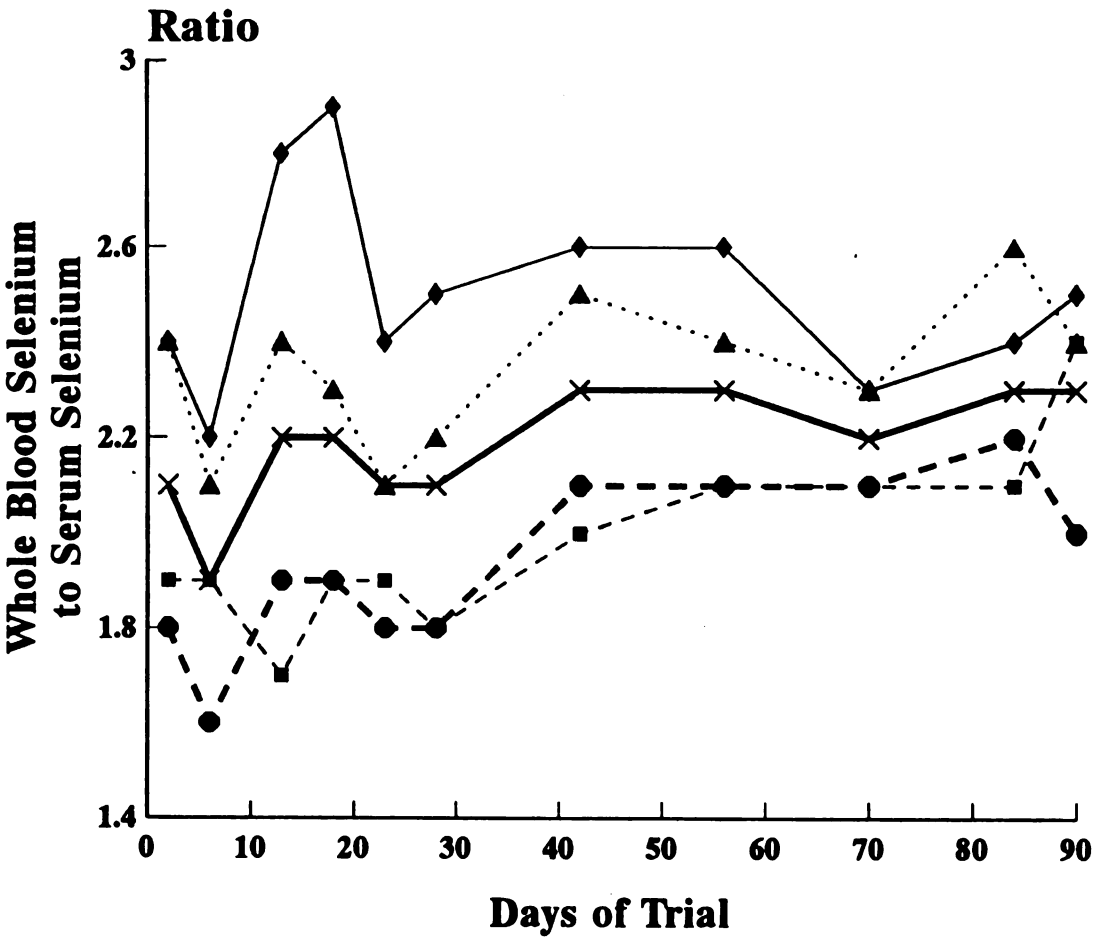

Figure 12. Mean ratio of whole blood selenium concentrations to serum selenium concentrations in cows fed 0 ◆—◆, 3 ▲····▲, 20 ■---■ and 50 ●- -● mg Se/head/day and the overall mean ratio ×_ _×.

The whole-blood Se concentration in cows, after being fed 100-mg Se for 28 days, was approximately five times the lower reported reference concentrations and only two times the higher proposed reference concentrations. This difference in reference concentrations could lead to an erroneous diagnosis of toxicity.

Because of the need for surgical intervention to obtain liver biopsy samples to measure liver Se concentration, liver biopsies were only collected at the beginning and end of each trial. Liver Se concentrations were at normal reference values[32] at the beginning of the trial in all four groups. There was no difference between the control and 3-mg group during the 90 days of feeding (Figure 4 and Table 3 of Appendix A). Liver Se concentrations in the 20- and 50-mg groups were significantly higher than the control group at the end of the first trial ($P < 0.01$). Liver Se concentrations in the 100-mg group, which initially were about 3 times the reference values, increased to 8 times reference values in the 28 day feeding period. Liver Se values declined to the reference range within 60 days after discontinuing the 100 mg Se/head/day supplementation (Figure 9 and Table 3 of Appendix B).

The concentrations of Se in the urine and feces were highest in cows fed the highest amounts of Se (Figures 5,6,10 & 11 and table 4 Appendix A and table 4 & 5 Appendix B). It is important to note that the urine and fecal Se concentrations were only from one-time catch samples and that 24-hour urine and fecal collections would be necessary to quantify, more accurately, the actual amount of Se excreted. Measurement of the ratio of urinary total Se and trimethylselenonium Se concentration has also been used in the rat and man to help establish guidelines for adequate supplementation.[33,34] This is an area that should receive attention in cows.

Assuming that a Holstein cow produces 4 kg of fecal dry matter and 20 liters of urine daily, the Se retained by the cows fed the four concentrations of supplemental Se was estimated. A regression line, (Figure 13) based on the estimated retention of Se on the four diets predicts the amount of supplemental Se necessary to maintain Se balance in cows receiving low Se forages. Based on the above assumptions, the requirement appears to be about 6 - 8 mg of Se daily. In areas where forages and grains are low in Se (< 0.05 mg Se /kg), a lactating Holstein cow consuming 20 Kg of dry matter supplemented at .3 ppm would obtain about 1 mg of Se naturally from forages and grains and would receive about 6 mg of Se from the supplementation. This would appear to be adequate to maintain Se balance in the lactating cow and is in agreement with other investigations.[35] However, a dry cow consuming 10 Kg of dry matter supplemented at 0.3 ppm would be obtaining only about 0.5 mg of Se from forages and grains and only about 3 mg from supplemental Se. This would not be adequate to maintain Se balance. This apparent Se imbalance could be particularly harmful during the period of rapid fetal growth during late gestation.[36] More extensive balance studies, including 24-hour collection of urine and feces, are needed to confirm these interpretations.

Selenium toxicity, while expected, was not observed at the amounts of Se supplementation in this experiment. This is probably due to at least two factors. Namely, the differences between the ruminant and monogastric digestive tracts, and the Se excretion rates via urine and feces. Proposed toxic concentrations for ruminants appear to have been extrapolated from data collected in monogastric animals. Studies have shown that monogastrics absorb as much as 2.5 times more Se than ruminants.[37] The lower absorption of Se by the ruminant is likely due to the reduction of dietary Se

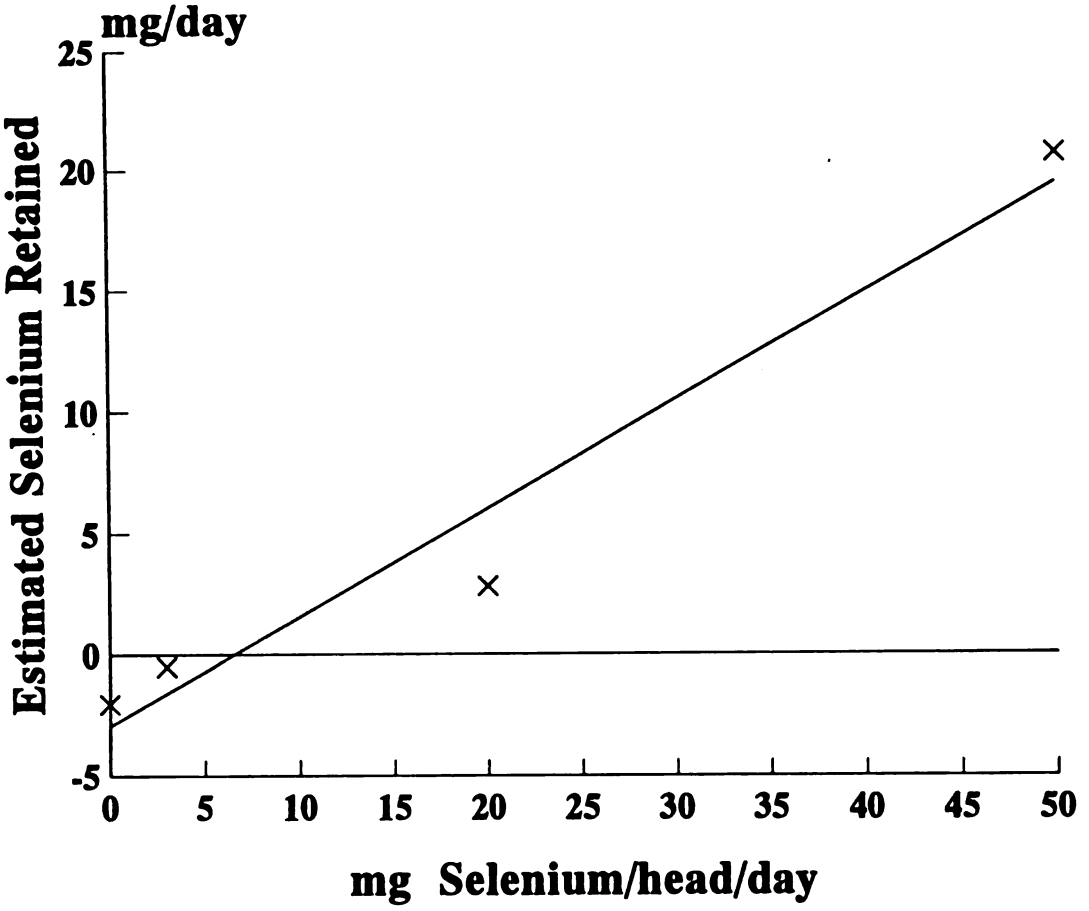

Figure 13. Regression of estimated daily fecal and urinary selenium losses by adult cows orally supplemented with selenium at 0, 3, 20 and 50 mg Se/head/day. R^2 =.958. Daily fecal dry matter and urine volumes were estimated at 4 kg and 20 L, respectively.

to less available forms, such as elemental Se, by rumen microbes prior to the opportunity for absorption in the small intestine. Biosynthesis of seleno-compounds from inorganic or unbound Se has been demonstrated in the rumen of sheep and in in vitro incubations of Se^{75} with rumen microbes.[38,39] Selenium absorption is related to whether Se is in bound or unbound forms. Unbound, inorganic Se forms are absorbed more completely than bound forms.[40] Inorganic Se in the soluble forms of selenate and selenite is reduced in the rumen to insoluble elemental Se.[41]

It is clear from the data presented that absorption and excretion increased with dietary intake. This is supported by the increases in all three measures of Se status and both urine and fecal Se concentrations in cows supplemented at higher concentrations. Reasons for the lack of toxicity therefore are likely related to both decreased absorption and increased excretion of Se.

The lack of any significant affect on the hematologic, serum enzyme and health variables measured, as well as the fact that animals maintained or gained weight, maintained body condition and remained in good health during the experiment, indicate that no apparent negative affects occurred at these excessive amounts of Se supplementation in Holstein cows. These observations indicate that there is a large difference between allowable and toxic supplemental Se concentrations in animal feeds. However, it is important to note that no apparent benefits to health or immunological variables were observed to be associated with high Se supplementation concentrations.

The addition of supplemental Se to cattle diets is a routine nutritional management practice in the United States. This is due to the convenience and modest cost of oral supplementation when compared to other methods of supplementation. A

common method of incorporating Se into grain mixes is to mix either 3 pounds of Se supplement (200 ppm) or 1 pound of Se supplement (600 ppm) per ton of grain, to create a final dietary Se concentration of .3ppm. A cow consuming 10 kilograms of the concentrate daily would then receive 3 mg of supplemental Se. A sixteen fold mistake in formulation for 90 days would be necessary to equal the Se intake in the 50-mg group in this experiment. A thirty three fold mistake for 28 days would be necessary to equal the Se intake rate of the 100-mg supplemental Se group.

A second method of supplementation is to top dress 200 ppm or 600 ppm Se premixes daily on the feed for each cow or alternatively incorporate supplements into a total-mixed ration. Commonly, 1 ounce of the 200 ppm Se premix is fed daily per head. This provides 5.5 mg of supplemental Se per head, an amount approximating the maximum allowed by FDA. Depending on the type of animal housing and the completeness of feed mixing, cows might get two to three times the expected amount of Se by consuming feed intended for other animals. A feeding or mixing error of about 9 times the target amount daily for 90 days would be required to equal the amount of Se provided to the experimental 50-mg group. An error of 19 times the daily targeted amount for 28 days would be necessary to equal the amount of Se provided to the 100-mg experimental group. Based on the results of this experiment, the above scenarios would not cause short term problems with Holstein cows. NRC guidelines suggest Se concentrations in excess of 2 ppm Se fed are toxic to cattle.[42] This guideline could lead to the incorrect, presumptive diagnosis of Se toxicity in cows consuming diets with Se concentrations of 2 ppm or greater.

Legally the concentration of .3 ppm Se of total dietary dry matter is not to be exceeded.[43] In this experiment cows fed the 3 mg supplemental Se/head/day would have consumed .3 ppm dietary Se, assuming a dry matter intake of 10 kg/day. Serum and whole blood Se concentrations in the 3 mg group were never significantly different ($P \leq .05$) from cows in the group receiving no supplemental Se (controls). The Se status of both the control and 3-mg group remained below the reference ranges for all three measures of Se status. This observation, combined with the absence of detectable signs of toxicity at the higher rates of supplementation, suggests that the current legal rate (0.3 ppm) of oral Se supplementation as sodium selenite is certainly in a very safe range and indeed may be lower than optimal. It is important to note that no benefits of feeding the excessive concentrations of Se used in this experiment were detected. This research indicates that in ruminants, the margin of safety between legal (.3 ppm) and toxic Se intake is greater than was previously thought. Therefore, Se supplementation within reasonable boundaries should not be avoided because of the concern for a low margin of safety and the production of accidental toxicity.[44]

APPENDIX A[a]

[a] Includes data (individual, mean, SD) for the respective variables from cows fed 0, 3, 20, and 50 mg supplemental Se/hd/d for 90 days. Unless otherwise indicated reference ranges are from the Animal Health Diagnostic and Clinical Pathology Laboratories at the College of Veterinary Medicine, Michigan State University.

APPENDIX A

Table 1. Serum selenium concentrations.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		ng/ml*											
0	1	26	45	46	49	54	60	63	59	48	50	56	61
	2	33	44	45	38	34	41	44	35	40	38	38	43
	3	35	51	53	44	57	56	60	57	72	58	52	64
	4	35	40	43	39	40	41	45	48	52	58	58	68
	5	73	72	70	58	52	59	55	46	47	50	46	57
	6	41	52	52	44	48	48	45	47	46	53	51	63
	Mean	40	51	52	45	48	51	52	49	51	51	50	59
	SD ±	15	10	9	7	8	8	8	8	10	7	7	8
3	7	24	37	48	59	59	60	73	60	75	60	47	69
	8	38	55	64	60	59	62	62	60	60	61	53	63
	9	41	48	56	47	53	56	61	49	55	56	54	64
	10	37	54	63	56	52	78	61	58	62	55	54	64
	11	40	55	57	50	84	53	64	56	56	52	55	62
	12	26	43	45	49	53	56	65	54	55	54	49	62
	Mean	34	49	56	54	60	61	64	56	60	56	52	64
	SD ±	7	7	7	5	11	8	4	4	7	3	3	2
20	13	28	53	70	79	71	72	80	67	64	65	70	85
	14	33	54	77	95	76	78	83	67	74	76	88	88
	15	40	63	60	87	89	77	85	71	76	78	81	83
	16	36	60	70	102	68	75	80	79	75	72	78	93
	17	36	79	77	66	70	77	78	76	76	76	78	85
	18	48	73	77	73	84	77	75	78	83	80	81	90
	Mean	37	64	72	84	76	76	80	73	75	74	79	87
	SD ±	6	10	6	12	8	2	3	5	6	5	5	3
50	19	32	74	92	107	130	114	117	92	89	96	112	102
	20	35	66	88	84	85	106	106	87	93	96	103	115
	21	44	86	102	82	101	110	110	88	108	93	107	129
	22	28	71	86	87	94	106	106	91	106	94	106	112
	23	48	66	97	86	102	117	117	95	106	99	112	104
	24	43	82	108	100	102	147	147	100	122	101	117	125
	Mean	38	74	96	91	102	99	117	92	104	96	110	114
	SD ±	7	8	8	9	14	9	14	4	11	3	5	10

* Reference range 70-100

APPENDIX A

Table 2. Whole blood selenium concentrations.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		ng/ml*											
0	1	63	59	62	74	82	81	84	94	85	81	87	114
	2	139	117	117	129	131	131	138	137	129	118	132	154
	3	145	123	122	127	150	133	140	137	156	138	133	144
	4	157	146	135	137	154	124	146	148	143	143	143	184
	5	153	174	156	171	190	160	170	152	169	142	135	175
	6	88	112	86	111	112	96	107	99	103	99	92	125
	Mean	124	122	113	129	136	121	131	128	131	120	120	149
	SD ±	36	35	31	29	34	26	28	23	29	24	22	25
3	7	148	56	65	90	100	92	91	102	112	105	102	139
	8	149	137	129	131	155	154	171	163	162	143	155	154
	9	158	148	140	145	152	150	147	139	144	125	124	144
	10	130	125	132	135	140	122	142	144	152	136	135	184
	11	154	140	144	147	158	132	157	158	167	145	143	175
	12	100	98	97	109	126	130	139	126	134	119	147	125
	Mean	140	117	118	126	138	130	141	139	145	129	134	154
	SD ±	20	32	28	20	20	20	25	20	18	14	17	20
20	13	106	99	101	128	143	150	137	132	150	142	151	199
	14	139	134	175	145	170	160	165	164	177	164	185	220
	15	109	107	119	115	124	120	132	132	141	136	169	206
	16	137	144	143	162	159	162	160	174	165	148	192	234
	17	115	106	113	129	131	136	142	147	164	170	160	194
	18	140	130	148	163	151	147	152	143	162	158	149	185
	Mean	124	120	133	140	146	146	148	149	160	153	168	206
	SD ±	15	17	25	18	16	14	12	16	12	12	16	16
50	19	115	106	142	161	165	169	173	167	174	170	179	223
	20	146	146	162	177	186	186	200	202	222	214	248	240
	21	155	150	167	190	201	180	216	199	215	203	241	235
	22	106	117	127	151	162	158	195	183	217	205	250	237
	23	139	124	148	164	202	175	220	184	233	194	244	193
	24	153	156	166	191	235	206	228	216	267	212	301	261
	Mean	136	133	152	172	192	179	205	192	221	200	244	232
	SD ±	19	18	14	15	25	15	18	16	28	15	35	21

* Reference range 150-220 (derived from Figure 12)

APPENDIX A

Table 3. Selenium concentrations of liver biopsies obtained on days 0 and 90.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		μg/g dry wt*											
0	1	0.69	.	.	.	.	.	.	.	.	.	.	0.96
	2	1.08	.	.	.	.	.	.	.	.	.	.	0.65
	3	1.54	.	.	.	.	.	.	.	.	.	.	1.39
	4	1.85	.	.	.	.	.	.	.	.	.	.	1.43
	5	2.04	.	.	.	.	.	.	.	.	.	.	0.77
	6	1.87	.	.	.	.	.	.	.	.	.	.	0.83
	Mean	1.51	.	.	.	.	.	.	.	.	.	.	1.01
	SD ±	0.48	.	.	.	.	.	.	.	.	.	.	0.30
3	7	0.50	.	.	.	.	.	.	.	.	.	.	1.38
	8	0.98	.	.	.	.	.	.	.	.	.	.	1.08
	9	1.36	.	.	.	.	.	.	.	.	.	.	1.56
	10	0.64	.	.	.	.	.	.	.	.	.	.	1.39
	11	0.64	.	.	.	.	.	.	.	.	.	.	1.34
	12	0.89	.	.	.	.	.	.	.	.	.	.	1.32
	Mean	0.84	.	.	.	.	.	.	.	.	.	.	1.35
	SD ±	0.29	.	.	.	.	.	.	.	.	.	.	0.14
20	13	0.41	.	.	.	.	.	.	.	.	.	.	6.22
	14	1.34	.	.	.	.	.	.	.	.	.	.	6.67
	15	0.70	.	.	.	.	.	.	.	.	.	.	4.62
	16	2.19	.	.	.	.	.	.	.	.	.	.	4.49
	17	0.40	.	.	.	.	.	.	.	.	.	.	1.63
	18	0.37	.	.	.	.	.	.	.	.	.	.	2.37
	Mean	0.90	.	.	.	.	.	.	.	.	.	.	4.33
	SD ±	0.67	.	.	.	.	.	.	.	.	.	.	1.84
50	19	1.33	.	.	.	.	.	.	.	.	.	.	4.56
	20	0.89	.	.	.	.	.	.	.	.	.	.	6.47
	21	1.97	.	.	.	.	.	.	.	.	.	.	4.47
	22	1.48	.	.	.	.	.	.	.	.	.	.	7.32
	23	2.31	.	.	.	.	.	.	.	.	.	.	9.39
	24	1.28	.	.	.	.	.	.	.	.	.	.	8.76
	Mean	1.54	.	.	.	.	.	.	.	.	.	.	6.83
	SD ±	0.47	.	.	.	.	.	.	.	.	.	.	1.89

* Reference range 1.2-2.0

APPENDIX A

Table 4. Urinary and fecal selenium concentrations.[1]

Se mg/d	Cow ID	Urine Selenium ng/ml	Fecal Selenium µg/g dry wt
0	1	36	0.28
	2	35	0.26
	3	58	0.33
	4	144	0.71
	5	52	0.26
	6	17	0.24
Mean		57	0.35
SD ±		41	0.16
3	7	107	0.84
	8	88	0.54
	9	105	0.44
	10	69	0.52
	11	68	0.45
	12	92	0.57
Mean		88	0.56
SD ±		15	0.13
20	13	100	2.78
	14	377	2.58
	15	390	1.81
	16	711	3.56
	17	402	1.37
	18	504	2.03
Mean		414	2.36
SD ±		181	0.71
50	19	672	2.32
	20	744	3.14
	21	954	4.02
	22	966	3.95
	23	577	3.20
	24	1002	3.58
Mean		819	3.37
SD ±		163	0.58

[1]Samples obtained on day 90

APPENDIX A

Table 5. White blood cell concentrations.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		cells/mm³ × 10³*											
0	1	7.7	.	.	.	7.8	.	.	.	8.0	.	.	6.7
	2	7.2	.	.	.	8.7	.	.	.	8.4	.	.	4.1
	3	10.4	.	.	.	6.8	.	.	.	6.0	.	.	3.9
	4	11.3	.	.	.	9.8	.	.	.	6.6	.	.	8.1
	5	8.7	.	.	.	9.3	.	.	.	9.2	.	.	6.0
	6	6.3	.	.	.	7.0	.	.	.	6.6	.	.	5.4
	Mean	8.6	.	.	.	8.2	.	.	.	7.5	.	.	5.7
	SD ±	1.8	.	.	.	1.1	.	.	.	1.1	.	.	1.5
3	7	6.9	.	.	.	6.7	.	.	.	6.4	.	.	4.8
	8	12.0	.	.	.	10.2	.	.	.	9.6	.	.	8.9
	9	7.3	.	.	.	6.8	.	.	.	8.1	.	.	6.0
	10	10.0	.	.	.	11.7	.	.	.	12.2	.	.	7.3
	11	10.3	.	.	.	9.3	.	.	.	10.9	.	.	8.3
	12	7.3	.	.	.	10.7	.	.	.	9.7	.	.	9.2
	Mean	9.0	.	.	.	9.2	.	.	.	9.5	.	.	7.4
	SD ±	1.9	.	.	.	1.9	.	.	.	1.9	.	.	1.6
20	13	9.2	.	.	.	10.9	.	.	.	9.6	.	.	8.5
	14	11.6	.	.	.	8.8	.	.	.	8.4	.	.	10.0
	15	4.8	.	.	.	6.5	.	.	.	5.6	.	.	5.2
	16	11.4	.	.	.	10.8	.	.	.	8.4	.	.	6.9
	17	3.9	.	.	.	6.7	.	.	.	8.1	.	.	5.8
	18	11.9	.	.	.	8.0	.	.	.	9.0	.	.	5.8
	Mean	8.8	.	.	.	8.6	.	.	.	8.2	.	.	7.0
	SD ±	3.3	.	.	.	1.8	.	.	.	1.3	.	.	1.7
50	19	5.9	.	.	.	7.2	.	.	.	7.7	.	.	7.5
	20	6.4	.	.	.	6.8	.	.	.	7.4	.	.	5.8
	21	9.0	.	.	.	8.9	.	.	.	9.1	.	.	6.3
	22	7.5	.	.	.	9.1	.	.	.	10.5	.	.	7.5
	23	8.6	.	.	.	5.4	.	.	.	7.4	.	.	7.7
	24	8.2	.	.	.	7.5	.	.	.	8.1	.	.	6.4
	Mean	7.6	.	.	.	7.5	.	.	.	8.4	.	.	6.9
	SD ±	1.1	.	.	.	1.3	.	.	.	1.1	.	.	0.7

* Reference range 4.7-11.5

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Table 6. Erythrocyte concentrations.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		cells/mm^3 × 10^6*											
0	1	6.46	.	.	.	5.92	.	.	.	6.39	.	.	6.23
	2	9.07	.	.	.	7.42	.	.	.	8.02	.	.	8.00
	3	10.40	.	.	.	6.75	.	.	.	7.10	.	.	6.85
	4	11.30	.	.	.	7.44	.	.	.	7.20	.	.	8.40
	5	6.36	.	.	.	6.23	.	.	.	7.03	.	.	6.40
	6	6.62	.	.	.	5.22	.	.	.	5.87	.	.	6.15
	Mean	8.37	.	.	.	6.50	.	.	.	6.94	.	.	7.01
	SD ±	2.00	.	.	.	0.80	.	.	.	0.67	.	.	0.88
3	7	5.92	.	.	.	5.09	.	.	.	5.71	.	.	5.80
	8	8.61	.	.	.	7.63	.	.	.	8.25	.	.	7.90
	9	6.78	.	.	.	5.61	.	.	.	6.09	.	.	6.00
	10	6.63	.	.	.	6.75	.	.	.	7.50	.	.	7.30
	11	7.64	.	.	.	6.74	.	.	.	7.47	.	.	6.90
	12	7.37	.	.	.	6.27	.	.	.	6.95	.	.	6.50
	Mean	7.16	.	.	.	6.35	.	.	.	7.00	.	.	6.73
	SD ±	0.85	.	.	.	0.83	.	.	.	0.87	.	.	0.73
20	13	7.17	.	.	.	6.04	.	.	.	6.70	.	.	6.23
	14	8.34	.	.	.	7.40	.	.	.	7.78	.	.	7.74
	15	6.12	.	.	.	4.68	.	.	.	5.93	.	.	5.26
	16	7.21	.	.	.	6.69	.	.	.	6.99	.	.	6.60
	17	6.24	.	.	.	5.27	.	.	.	6.20	.	.	5.40
	18	6.90	.	.	.	5.77	.	.	.	6.21	.	.	5.95
	Mean	7.00	.	.	.	5.98	.	.	.	6.64	.	.	6.20
	SD ±	0.73	.	.	.	0.89	.	.	.	0.62	.	.	0.83
50	19	8.14	.	.	.	8.16	.	.	.	8.28	.	.	7.88
	20	7.55	.	.	.	6.17	.	.	.	5.94	.	.	5.66
	21	7.49	.	.	.	6.39	.	.	.	7.02	.	.	6.36
	22	8.28	.	.	.	7.36	.	.	.	7.99	.	.	7.07
	23	6.72	.	.	.	5.89	.	.	.	6.68	.	.	6.98
	24	7.11	.	.	.	6.30	.	.	.	6.46	.	.	6.19
	Mean	7.55	.	.	.	6.71	.	.	.	7.06	.	.	6.69
	SD ±	0.54	.	.	.	0.79	.	.	.	0.83	.	.	0.71

* Reference range 5.29-9.19

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Table 7. Hemoglobin concentrations.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		g/dl*											
0	1	11.8	.	.	.	10.9	.	.	.	11.1	.	.	11.1
	2	14.3	.	.	.	11.8	.	.	.	12.3	.	.	12.3
	3	12.1	.	.	.	11.1	.	.	.	11.4	.	.	11.5
	4	14.0	.	.	.	12.8	.	.	.	12.2	.	.	14.6
	5	11.5	.	.	.	11.2	.	.	.	12.5	.	.	10.8
	6	13.0	.	.	.	10.4	.	.	.	11.5	.	.	12.0
	Mean	12.8	.	.	.	11.4	.	.	.	11.8	.	.	12.1
	SD ±	1.1	.	.	.	0.8	.	.	.	0.5	.	.	1.2
3	7	12.4	.	.	.	10.7	.	.	.	11.5	.	.	11.5
	8	11.1	.	.	.	10.2	.	.	.	11.1	.	.	11.0
	9	12.5	.	.	.	10.5	.	.	.	11.2	.	.	10.9
	10	9.6	.	.	.	9.8	.	.	.	10.7	.	.	10.6
	11	12.0	.	.	.	10.6	.	.	.	11.8	.	.	10.9
	12	12.9	.	.	.	10.9	.	.	.	11.6	.	.	11.0
	Mean	11.8	.	.	.	10.5	.	.	.	11.3	.	.	11.0
	SD ±	1.1	.	.	.	0.4	.	.	.	0.4	.	.	0.3
20	13	12.8	.	.	.	10.8	.	.	.	11.7	.	.	10.7
	14	11.5	.	.	.	10.2	.	.	.	10.6	.	.	10.6
	15	13.0	.	.	.	9.6	.	.	.	12.0	.	.	10.9
	16	13.1	.	.	.	12.0	.	.	.	12.0	.	.	10.2
	17	11.3	.	.	.	9.8	.	.	.	11.3	.	.	10.2
	18	12.1	.	.	.	10.4	.	.	.	10.9	.	.	10.5
	Mean	12.3	.	.	.	10.5	.	.	.	11.4	.	.	10.7
	SD ±	0.7	.	.	.	0.8	.	.	.	0.5	.	.	0.3
50	19	12.0	.	.	.	11.8	.	.	.	11.6	.	.	11.2
	20	14.7	.	.	.	12.0	.	.	.	11.3	.	.	11.0
	21	12.6	.	.	.	10.9	.	.	.	11.5	.	.	10.5
	22	13.0	.	.	.	11.5	.	.	.	12.5	.	.	11.3
	23	10.8	.	.	.	9.5	.	.	.	10.5	.	.	10.9
	24	11.3	.	.	.	10.2	.	.	.	10.6	.	.	10.5
	Mean	12.4	.	.	.	11.0	.	.	.	11.3	.	.	10.9
	SD ±	1.3	.	.	.	0.9	.	.	.	0.7	.	.	0.3

* Reference range 8.8-15.6

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Table 8. Packed cell volumes.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		% *											
0	1	31.4	.	.	.	28.3	.	.	.	29.7	.	.	28.5
	2	41.0	.	.	.	31.9	.	.	.	34.5	.	.	33.0
	3	32.7	.	.	.	28.8	.	.	.	31.4	.	.	29.1
	4	38.1	.	.	.	34.0	.	.	.	33.1	.	.	37.8
	5	31.1	.	.	.	30.1	.	.	.	33.8	.	.	28.5
	6	34.3	.	.	.	27.1	.	.	.	30.8	.	.	30.9
	Mean	34.8	.	.	.	30.0	.	.	.	32.2	.	.	31.3
	SD ±	3.6	.	.	.	2.3	.	.	.	1.7	.	.	3.3
3	7	33.3	.	.	.	27.9	.	.	.	30.7	.	.	29.8
	8	34.4	.	.	.	31.1	.	.	.	34	.	.	31.0
	9	32.9	.	.	.	27.3	.	.	.	29.5	.	.	27.8
	10	27.3	.	.	.	28	.	.	.	31.5	.	.	29.4
	11	33.4	.	.	.	29.1	.	.	.	32.5	.	.	28.3
	12	34.9	.	.	.	28.9	.	.	.	31.9	.	.	28.8
	Mean	32.7	.	.	.	28.7	.	.	.	31.7	.	.	29.2
	SD ±	2.5	.	.	.	1.2	.	.	.	1.4	.	.	1.0
20	13	35.1	.	.	.	28.6	.	.	.	30.8	.	.	27.2
	14	33.3	.	.	.	29.6	.	.	.	32.0	.	.	30.3
	15	34.5	.	.	.	25.4	.	.	.	31.7	.	.	27.8
	16	35.1	.	.	.	31.7	.	.	.	32.3	.	.	29.5
	17	29.2	.	.	.	24.9	.	.	.	29.8	.	.	26.0
	18	32.8	.	.	.	26.6	.	.	.	29.3	.	.	27.4
	Mean	33.3	.	.	.	27.8	.	.	.	31.0	.	.	28.0
	SD ±	2.0	.	.	.	2.4	.	.	.	1.1	.	.	1.4
50	19	34.3	.	.	.	40.7	.	.	.	34.2	.	.	30.5
	20	38.1	.	.	.	30.7	.	.	.	29.6	.	.	27.9
	21	34.5	.	.	.	28.2	.	.	.	31.2	.	.	28.4
	22	36.1	.	.	.	32.1	.	.	.	35.0	.	.	30.0
	23	29.1	.	.	.	25.3	.	.	.	28.8	.	.	29.2
	24	31.3	.	.	.	28.2	.	.	.	29.6	.	.	27.2
	Mean	33.9	.	.	.	30.9	.	.	.	31.4	.	.	28.9
	SD ±	3.0	.	.	.	4.9	.	.	.	2.4	.	.	1.2

* Reference range 23.7-41.4

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Table 9. Serum aspartate amino transferase activities.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		IU/L*											
0	1	58	.	.	.	60	.	.	.	52	.	.	57
	2	46	.	.	.	48	.	.	.	49	.	.	70
	3	64	.	.	.	48	.	.	.	45	.	.	48
	4	38	.	.	.	36	.	.	.	35	.	.	45
	5	54	.	.	.	49	.	.	.	49	.	.	48
	6	48	.	.	.	40	.	.	.	47	.	.	49
	Mean	51.3	.	.	.	46.8	.	.	.	46.2	.	.	52.8
	SD ±	8.5	.	.	.	7.6	.	.	.	5.4	.	.	8.5
3	7	39	.	.	.	46	.	.	.	41	.	.	39
	8	50	.	.	.	43	.	.	.	48	.	.	42
	9	48	.	.	.	45	.	.	.	53	.	.	49
	10	61	.	.	.	56	.	.	.	50	.	.	54
	11	41	.	.	.	38	.	.	.	49	.	.	37
	12	81	.	.	.	50	.	.	.	62	.	.	57
	Mean	53.3	.	.	.	46.3	.	.	.	50.5	.	.	46.3
	SD ±	14.3	.	.	.	5.6	.	.	.	6.3	.	.	7.5
20	13	53	.	.	.	44	.	.	.	51	.	.	49
	14	54	.	.	.	46	.	.	.	45	.	.	55
	15	41	.	.	.	39	.	.	.	45	.	.	39
	16	58	.	.	.	56	.	.	.	77	.	.	62
	17	45	.	.	.	40	.	.	.	42	.	.	48
	18	53	.	.	.	50	.	.	.	42	.	.	49
	Mean	50.7	.	.	.	45.8	.	.	.	50.3	.	.	50.3
	SD ±	5.8	.	.	.	5.8	.	.	.	12.3	.	.	7.0
50	19	63	.	.	.	64	.	.	.	48	.	.	48
	20	45	.	.	.	47	.	.	.	40	.	.	40
	21	45	.	.	.	49	.	.	.	58	.	.	50
	22	60	.	.	.	70	.	.	.	51	.	.	44
	23	44	.	.	.	46	.	.	.	42	.	.	58
	24	51	.	.	.	44	.	.	.	55	.	.	50
	Mean	51.3	.	.	.	53.3	.	.	.	49.0	.	.	48.3
	SD ±	7.6	.	.	.	9.9	.	.	.	6.5	.	.	5.6

* Reference range 48-109

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Table 10. Serum gamma-glutamyl transferase activities.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		IU/L*											
0	1	33	.	.	.	25	.	.	.	40	.	.	36
	2	38	.	.	.	36	.	.	.	36	.	.	47
	3	50	.	.	.	38	.	.	.	39	.	.	38
	4	42	.	.	.	25	.	.	.	31	.	.	38
	5	28	.	.	.	32	.	.	.	29	.	.	24
	6	37	.	.	.	32	.	.	.	32	.	.	43
	Mean	38.0	.	.	.	31.3	.	.	.	34.5	.	.	37.7
	SD ±	6.9	.	.	.	5.0	.	.	.	4.1	.	.	7.1
3	7	34	.	.	.	20	.	.	.	31	.	.	26
	8	49	.	.	.	35	.	.	.	33	.	.	31
	9	39	.	.	.	28	.	.	.	33	.	.	35
	10	25	.	.	.	23	.	.	.	29	.	.	33
	11	30	.	.	.	31	.	.	.	27	.	.	31
	12	28	.	.	.	23	.	.	.	26	.	.	29
	Mean	34.2	.	.	.	26.7	.	.	.	29.8	.	.	30.8
	SD ±	8.0	.	.	.	5.2	.	.	.	2.7	.	.	2.9
20	13	36	.	.	.	30	.	.	.	29	.	.	32
	14	41	.	.	.	24	.	.	.	29	.	.	27
	15	31	.	.	.	34	.	.	.	35	.	.	30
	16	41	.	.	.	40	.	.	.	38	.	.	46
	17	40	.	.	.	38	.	.	.	37	.	.	41
	18	46	.	.	.	35	.	.	.	38	.	.	36
	Mean	39.2	.	.	.	33.5	.	.	.	34.3	.	.	35.3
	SD ±	4.7	.	.	.	5.3	.	.	.	3.9	.	.	6.5
50	19	24	.	.	.	6	.	.	.	26	.	.	24
	20	24	.	.	.	13	.	.	.	22	.	.	20
	21	73	.	.	.	46	.	.	.	38	.	.	22
	22	27	.	.	.	17	.	.	.	27	.	.	19
	23	25	.	.	.	22	.	.	.	25	.	.	23
	24	40	.	.	.	34	.	.	.	31	.	.	23
	Mean	35.5	.	.	.	23.0	.	.	.	28.2	.	.	21.8
	SD ±	17.7	.	.	.	13.4	.	.	.	5.1	.	.	1.8

* Reference range 0-40

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Table 11. Serum sorbitol dehydrogenase activities.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		IU/L*											
0	1	27	.	.	.	22	.	.	.	14	.	.	32
	2	23	.	.	.	18	.	.	.	14	.	.	26
	3	29	.	.	.	15	.	.	.	14	.	.	21
	4	21	.	.	.	13	.	.	.	8	.	.	14
	5	19	.	.	.	27	.	.	.	19	.	.	11
	6	29	.	.	.	19	.	.	.	17	.	.	26
	Mean	24.7	.	.	.	19.0	.	.	.	14.3	.	.	21.7
	SD ±	3.9	.	.	.	4.6	.	.	.	3.4	.	.	7.3
3	7	22	.	.	.	17	.	.	.	28	.	.	15
	8	24	.	.	.	16	.	.	.	16	.	.	18
	9	19	.	.	.	12	.	.	.	10	.	.	14
	10	38	.	.	.	32	.	.	.	36	.	.	51
	11	17	.	.	.	14	.	.	.	44	.	.	13
	12	79	.	.	.	25	.	.	.	35	.	.	37
	Mean	33.2	.	.	.	19.3	.	.	.	28.2	.	.	24.7
	SD ±	21.6	.	.	.	7.0	.	.	.	11.8	.	.	14.3
20	13	33	.	.	.	22	.	.	.	22	.	.	25
	14	30	.	.	.	21	.	.	.	22	.	.	51
	15	24	.	.	.	28	.	.	.	31	.	.	17
	16	29	.	.	.	17	.	.	.	24	.	.	23
	17	24	.	.	.	17	.	.	.	17	.	.	17
	18	13	.	.	.	26	.	.	.	14	.	.	19
	Mean	25.5	.	.	.	21.8	.	.	.	21.7	.	.	25.3
	SD ±	6.4	.	.	.	4.1	.	.	.	5.4	.	.	11.9
50	19	33	.	.	.	26	.	.	.	25	.	.	33
	20	31	.	.	.	13	.	.	.	14	.	.	11
	21	43	.	.	.	22	.	.	.	23	.	.	16
	22	63	.	.	.	15	.	.	.	20	.	.	17
	23	27	.	.	.	18	.	.	.	20	.	.	25
	24	28	.	.	.	18	.	.	.	25	.	.	18
	Mean	37.5	.	.	.	18.7	.	.	.	21.2	.	.	20.0
	SD ±	12.5	.	.	.	4.3	.	.	.	3.8	.	.	7.1

* Reference range 24-42

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Table 12. Serum creatine phosphokinase activities.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		IU/L*											
0	1	62	.	.	.	46	.	.	.	105	.	.	102
	2	32	.	.	.	44	.	.	.	97	.	.	105
	3	232	.	.	.	42	.	.	.	94	.	.	86
	4	28	.	.	.	25	.	.	.	71	.	.	96
	5	36	.	.	.	37	.	.	.	115	.	.	158
	6	42	.	.	.	39	.	.	.	169	.	.	466
	Mean	72.0	.	.	.	38.8	.	.	.	108.5	.	.	168.8
	SD ±	72.4	.	.	.	6.9	.	.	.	30.2	.	.	134.9
3	7	23	.	.	.	30	.	.	.	78	.	.	75
	8	30	.	.	.	35	.	.	.	98	.	.	76
	9	15	.	.	.	19	.	.	.	48	.	.	54
	10	78	.	.	.	63	.	.	.	143	.	.	138
	11	26	.	.	.	34	.	.	.	93	.	.	65
	12	39	.	.	.	39	.	.	.	145	.	.	167
	Mean	35.2	.	.	.	36.7	.	.	.	100.8	.	.	95.8
	SD ±	20.5	.	.	.	13.3	.	.	.	34.4	.	.	41.6
20	13	303	.	.	.	32	.	.	.	120	.	.	76
	14	43	.	.	.	34	.	.	.	108	.	.	110
	15	27	.	.	.	14	.	.	.	78	.	.	61
	16	39	.	.	.	40	.	.	.	124	.	.	121
	17	37	.	.	.	37	.	.	.	105	.	.	93
	18	35	.	.	.	24	.	.	.	90	.	.	74
	Mean	80.7	.	.	.	30.2	.	.	.	104.2	.	.	89.2
	SD ±	99.5	.	.	.	8.8	.	.	.	16.0	.	.	21.1
50	19	33	.	.	.	41	.	.	.	191	.	.	92
	20	28	.	.	.	194	.	.	.	97	.	.	69
	21	21	.	.	.	43	.	.	.	96	.	.	114
	22	51	.	.	.	37	.	.	.	143	.	.	115
	23	45	.	.	.	102	.	.	.	63	.	.	63
	24	36	.	.	.	40	.	.	.	116	.	.	78
	Mean	35.7	.	.	.	76.2	.	.	.	117.7	.	.	88.5
	SD ±	10.0	.	.	.	57.3	.	.	.	40.6	.	.	20.4

* Reference range 23-118

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Table 13. Body weights.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		kg											
0	1	591	.	.	.	569	.	.	.	599	.	.	614
	2	742	.	.	.	673	.	.	.	694	.	.	694
	3	577	.	.	.	569	.	.	.	644	.	.	629
	4	562	.	.	.	584	.	.	.	562	.	.	569
	5	603	.	.	.	606	.	.	.	610	.	.	659
	6	518	.	.	.	547	.	.	.	569	.	.	606
	Mean	599	.	.	.	592	.	.	.	613	.	.	629
	SD ±	69	.	.	.	41	.	.	.	45	.	.	40
3	7	591	.	.	.	591	.	.	.	621	.	.	614
	8	462	.	.	.	456	.	.	.	497	.	.	489
	9	701	.	.	.	701	.	.	.	680	.	.	666
	10	429	.	.	.	429	.	.	.	448	.	.	476
	11	614	.	.	.	599	.	.	.	621	.	.	629
	12	435	.	.	.	448	.	.	.	469	.	.	497
	Mean	539	.	.	.	538	.	.	.	556	.	.	562
	SD ±	103	.	.	.	100	.	.	.	88	.	.	76
20	13	448	.	.	.	448	.	.	.	476	.	.	497
	14	396	.	.	.	409	.	.	.	448	.	.	448
	15	701	.	.	.	715	.	.	.	715	.	.	709
	16	483	.	.	.	504	.	.	.	555	.	.	547
	17	629	.	.	.	621	.	.	.	651	.	.	673
	18	533	.	.	.	555	.	.	.	577	.	.	606
	Mean	532	.	.	.	542	.	.	.	570	.	.	580
	SD ±	105	.	.	.	104	.	.	.	93	.	.	92
50	19	422	.	.	.	455	.	.	.	462	.	.	483
	20	635	.	.	.	701	.	.	.	660	.	.	673
	21	584	.	.	.	599	.	.	.	614	.	.	614
	22	422	.	.	.	469	.	.	.	483	.	.	511
	23	544	.	.	.	544	.	.	.	569	.	.	569
	24	533	.	.	.	544	.	.	.	533	.	.	599
	Mean	523	.	.	.	552	.	.	.	553	.	.	575
	SD ±	79	.	.	.	83	.	.	.	70	.	.	64

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Table 14. Body condition scores.[1]

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d													
0	1	3.5	.	.	.	3.0	.	.	.	3.0	.	.	3.0
	2	4.0	.	.	.	4.0	.	.	.	3.5	.	.	3.5
	3	3.5	.	.	.	3.5	.	.	.	3.5	.	.	4.0
	4	3.5	.	.	.	4.0	.	.	.	3.5	.	.	3.0
	5	4.0	.	.	.	4.0	.	.	.	4.0	.	.	4.0
	6	4.0	.	.	.	3.5	.	.	.	3.5	.	.	4.0
	Mean	3.8	.	.	.	3.7	.	.	.	3.5	.	.	3.6
	SD ±	0.3	.	.	.	0.4	.	.	.	0.3	.	.	0.4
3	7	4.5	.	.	.	4.5	.	.	.	4.0	.	.	4.0
	8	3.5	.	.	.	3.5	.	.	.	3.5	.	.	3.0
	9	4.0	.	.	.	3.5	.	.	.	3.5	.	.	3.5
	10	3.5	.	.	.	3.5	.	.	.	3.5	.	.	3.0
	11	4.0	.	.	.	4.0	.	.	.	4.0	.	.	3.5
	12	3.5	.	.	.	3.5	.	.	.	3.5	.	.	3.5
	Mean	3.8	.	.	.	3.8	.	.	.	3.7	.	.	3.4
	SD ±	0.4	.	.	.	0.4	.	.	.	0.2	.	.	0.3
20	13	3.0	.	.	.	3.0	.	.	.	3.5	.	.	3.0
	14	3.5	.	.	.	3.5	.	.	.	3.5	.	.	3.0
	15	4.0	.	.	.	4.0	.	.	.	4.0	.	.	4.0
	16	4.0	.	.	.	4.0	.	.	.	4.0	.	.	3.5
	17	3.5	.	.	.	3.0	.	.	.	3.5	.	.	3.5
	18	3.0	.	.	.	3.5	.	.	.	3.5	.	.	3.5
	Mean	3.5	.	.	.	3.5	.	.	.	3.7	.	.	3.4
	SD ±	0.4	.	.	.	0.4	.	.	.	0.2	.	.	0.3
50	19	3.5	.	.	.	3.0	.	.	.	3.0	.	.	3.0
	20	4.5	.	.	.	4.0	.	.	.	4.0	.	.	4.0
	21	3.5	.	.	.	3.5	.	.	.	3.0	.	.	3.5
	22	3.0	.	.	.	3.5	.	.	.	3.5	.	.	3.5
	23	3.0	.	.	.	3.0	.	.	.	3.0	.	.	3.0
	24	3.5	.	.	.	3.5	.	.	.	3.5	.	.	3.0
	Mean	3.5	.	.	.	3.4	.	.	.	3.3	.	.	3.3
	SD ±	0.5	.	.	.	0.3	.	.	.	0.4	.	.	0.4

[1]Range 1(thin) to 5(fat) (from Mulvany 1977).

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Table 15. Average daily hoof growth.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		mm/day											
0	1	0.0	.	.	.	.38	.	.	.	.17	.	.	.22
	2	0.0	.	.	.	.38	.	.	.	.16	.	.	.22
	3	0.0	.	.	.	.38	.	.	.	.18	.	.	.21
	4	0.0	.	.	.	.38	.	.	.	.20	.	.	.24
	5	0.0	.	.	.	.50	.	.	.	.20	.	.	.25
	6	0.0	.	.	.	.42	.	.	.	.17	.	.	.21
	Mean	0.0	.	.	.	.41	.	.	.	.18	.	.	.22
	SD ±	0.0	.	.	.	.04	.	.	.	.02	.	.	.01
3	7	0.0	.	.	.	.38	.	.	.	.18	.	.	.24
	8	0.0	.	.	.	.42	.	.	.	.16	.	.	.22
	9	0.0	.	.	.	.33	.	.	.	.18	.	.	.22
	10	0.0	.	.	.	.38	.	.	.	.20	.	.	.24
	11	0.0	.	.	.	.38	.	.	.	.20	.	.	.21
	12	0.0	.	.	.	.36	.	.	.	.20	.	.	.22
	Mean	0.0	.	.	.	.38	.	.	.	.19	.	.	.22
	SD ±	0.0	.	.	.	.03	.	.	.	.01	.	.	.01
20	13	0.0	.	.	.	.38	.	.	.	.21	.	.	.22
	14	0.0	.	.	.	.38	.	.	.	.18	.	.	.24
	15	0.0	.	.	.	.38	.	.	.	.21	.	.	.21
	16	0.0	.	.	.	.38	.	.	.	.20	.	.	.22
	17	0.0	.	.	.	.38	.	.	.	.18	.	.	.22
	18	0.0	.	.	.	.44	.	.	.	.20	.	.	.24
	Mean	0.0	.	.	.	.40	.	.	.	.20	.	.	.22
	SD ±	0.0	.	.	.	.02	.	.	.	.01	.	.	.01
50	19	0.0	.	.	.	.38	.	.	.	.20	.	.	.24
	20	0.0	.	.	.	.38	.	.	.	.16	.	.	.22
	21	0.0	.	.	.	.38	.	.	.	.18	.	.	.22
	22	0.0	.	.	.	.38	.	.	.	.20	.	.	.24
	23	0.0	.	.	.	.38	.	.	.	.18	.	.	.21
	24	0.0	.	.	.	.38	.	.	.	.21	.	.	.22
	Mean	0.0	.	.	.	.38	.	.	.	.19	.	.	.22
	SD ±	0.0	.	.	.	0.0	.	.	.	.02	.	.	.01

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Table 16. Rabies titers.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		IU/ml											
0	1	.	.	.	.	.	.	0	0	1.5	0.9	26.8	24.8
	2	.	.	.	.	.	.	0	0	1.1	0.3	1.2	3.9
	3	.	.	.	.	.	.	0	0.2	1.1	5.0	4.6	19.5
	4	.	.	.	.	.	.	0	0.2	4.6	0.6	29.1	26.9
	5	.	.	.	.	.	.	0	0	0.2	1.0	1.2	5.0
	6	.	.	.	.	.	.	0	0	1.9	1.0	29.1	24.8
	Mean	.	.	.	.	.	.	0	.067	1.73	1.46	15.3	17.48
	SD ±	.	.	.	.	.	.	0	.094	1.38	1.6	13.1	9.5
3	7	.	.	.	.	.	.	0	0	0.8	4.6	18.0	19.5
	8	.	.	.	.	.	.	0	0	4.2	1.0	27.0	24.8
	9	.	.	.	.	.	.	0	0	1.4	1.0	27.0	21.1
	10	.	.	.	.	.	.	0	0	1.1	1.0	19.5	22.9
	11	.	.	.	.	.	.	0	0	3.3	1.0	6.8	22.9
	12	.	.	.	.	.	.	0	0	3.3	2.4	8.0	8.0
	Mean	.	.	.	.	.	.	0	0	2.35	1.83	17.72	19.87
	SD ±	.	.	.	.	.	.	0	0	1.3	1.34	8.05	5.55
20	13	.	.	.	.	.	.	0	0	3.6	1.0	5.8	26.9
	14	.	.	.	.	.	.	0	0	4.2	1.0	5.8	6.8
	15	.	.	.	.	.	.	0	0	1.1	1.0	5.8	21.1
	16	.	.	.	.	.	.	0	0.2	1.1	1.0	11.1	12.0
	17	.	.	.	.	.	.	0	0	0.4	0.9	5.8	13.0
	18	.	.	.	.	.	.	0	0	0.9	0.7	5.8	17.9
	Mean	.	.	.	.	.	.	0	.03	1.88	.93	6.68	16.28
	SD ±	.	.	.	.	.	.	0	.075	1.21	.11	1.98	6.55
50	19	.	.	.	.	.	.	0	0	0.8	1.0	21.1	17.9
	20	.	.	.	.	.	.	0	0	3.6	0.4	9.4	11.0
	21	.	.	.	.	.	.	0	0	1.0	1.0	5.8	13.0
	22	.	.	.	.	.	.	0	0	1.1	1.0	8.7	15.3
	23	.	.	.	.	.	.	0	0	1.1	1.0	14.1	19.5
	24	.	.	.	.	.	.	0	0	1.1	1.0	29.1	26.9
	Mean	.	.	.	.	.	.	0	0	1.45	0.90	14.7	17.27
	SD ±	.	.	.	.	.	.	0	0	0.97	0.22	8.1	5.16

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Table 17. [^{3}H]-thymidine uptake of unstimulated lymphocytes.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		cpm (log_{10})[1]											
0	1	3.41	.	.	.	.	.	.	3.49	.	.	.	3.99
	2	3.81	.	.	.	.	.	.	4.13	.	.	.	4.37
	3	2.98	.	.	.	.	.	.	3.02	.	.	.	2.77
	4	3.41	.	.	.	.	.	.	3.70	.	.	.	3.64
	5	3.17	.	.	.	.	.	.	4.53	.	.	.	4.46
	6	3.72	.	.	.	.	.	.	3.23	.	.	.	4.12
	Mean	3.42	.	.	.	.	.	.	3.71	.	.	.	3.94
	SD ±	0.29	.	.	.	.	.	.	0.52	.	.	.	0.57
3	7	2.87	.	.	.	.	.	.	3.71	.	.	.	3.94
	8	3.46	.	.	.	.	.	.	4.05	.	.	.	4.58
	9	3.69	.	.	.	.	.	.	3.48	.	.	.	3.86
	10	4.67	.	.	.	.	.	.	3.51	.	.	.	4.57
	11	4.10	.	.	.	.	.	.	4.42	.	.	.	4.56
	12	3.62	.	.	.	.	.	.	3.70	.	.	.	3.58
	Mean	3.73	.	.	.	.	.	.	3.81	.	.	.	4.18
	SD ±	0.56	.	.	.	.	.	.	0.33	.	.	.	0.40
20	13	2.85	.	.	.	.	.	.	3.12	.	.	.	3.95
	14	3.72	.	.	.	.	.	.	4.11	.	.	.	3.88
	15	3.44	.	.	.	.	.	.	3.94	.	.	.	3.64
	16	3.60	.	.	.	.	.	.	3.61	.	.	.	4.45
	17	4.52	.	.	.	.	.	.	4.24	.	.	.	4.46
	18	3.10	.	.	.	.	.	.	3.43	.	.	.	3.72
	Mean	3.54	.	.	.	.	.	.	3.74	.	.	.	4.02
	SD ±	0.53	.	.	.	.	.	.	0.39	.	.	.	0.33
50	19	3.41	.	.	.	.	.	.	3.18	.	.	.	3.91
	20	2.91	.	.	.	.	.	.	3.62	.	.	.	3.71
	21	3.21	.	.	.	.	.	.	2.64	.	.	.	3.56
	22	4.49	.	.	.	.	.	.	3.08	.	.	.	3.79
	23	3.26	.	.	.	.	.	.	3.42	.	.	.	3.49
	24	3.60	.	.	.	.	.	.	3.31	.	.	.	4.08
	Mean	3.48	.	.	.	.	.	.	3.21	.	.	.	3.76
	SD ±	0.50	.	.	.	.	.	.	0.31	.	.	.	0.20

[1]Counts per minute

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Table 18. [^{3}H]-thymidine uptake of phytohemagglutinin-stimulated lymphocytes.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		cpm (log_{10})[1]											
0	1	4.99	.	.	.	.	.	.	3.94	.	.	.	5.42
	2	5.39	.	.	.	.	.	.	5.24	.	.	.	5.26
	3	5.17	.	.	.	.	.	.	3.92	.	.	.	5.29
	4	4.89	.	.	.	.	.	.	3.92	.	.	.	3.45
	5	5.00	.	.	.	.	.	.	4.83	.	.	.	5.43
	6	5.54	.	.	.	.	.	.	4.87	.	.	.	5.47
	Mean	5.16	.	.	.	.	.	.	4.67	.	.	.	5.05
	SD ±	0.23	.	.	.	.	.	.	0.55	.	.	.	0.72
3	7	4.61	.	.	.	.	.	.	4.04	.	.	.	5.41
	8	4.92	.	.	.	.	.	.	4.90	.	.	.	5.37
	9	4.92	.	.	.	.	.	.	5.20	.	.	.	5.38
	10	4.67	.	.	.	.	.	.	3.77	.	.	.	5.29
	11	4.83	.	.	.	.	.	.	5.31	.	.	.	5.35
	12	4.44	.	.	.	.	.	.	4.83	.	.	.	5.10
	Mean	4.73	.	.	.	.	.	.	4.68	.	.	.	5.32
	SD ±	0.17	.	.	.	.	.	.	0.57	.	.	.	0.11
20	13	5.26	.	.	.	.	.	.	4.37	.	.	.	5.48
	14	4.17	.	.	.	.	.	.	3.57	.	.	.	5.41
	15	4.72	.	.	.	.	.	.	4.37	.	.	.	5.10
	16	4.86	.	.	.	.	.	.	3.66	.	.	.	5.31
	17	5.10	.	.	.	.	.	.	4.36	.	.	.	5.15
	18	4.37	.	.	.	.	.	.	4.27	.	.	.	5.19
	Mean	4.75	.	.	.	.	.	.	4.10	.	.	.	5.27
	SD ±	0.38	.	.	.	.	.	.	0.34	.	.	.	0.14
50	19	3.91	.	.	.	.	.	.	4.07	.	.	.	5.05
	20	5.11	.	.	.	.	.	.	4.89	.	.	.	5.45
	21	5.41	.	.	.	.	.	.	4.16	.	.	.	5.37
	22	5.05	.	.	.	.	.	.	3.79	.	.	.	5.57
	23	4.88	.	.	.	.	.	.	5.00	.	.	.	5.12
	24	5.27	.	.	.	.	.	.	5.22	.	.	.	5.38
	Mean	4.94	.	.	.	.	.	.	4.52	.	.	.	5.32
	SD ±	0.49	.	.	.	.	.	.	0.53	.	.	.	0.18

[1]Counts per minute

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Table 19. [^{3}H]-thymidine uptake of concanavalin A-stimulated lymphocytes.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		cpm (log_{10})[1]											
0	1	4.98	.	.	.	.	.	.	4.61	.	.	.	5.56
	2	5.39	.	.	.	.	.	.	4.93	.	.	.	5.46
	3	4.93	.	.	.	.	.	.	4.69	.	.	.	5.47
	4	4.59	.	.	.	.	.	.	5.31	.	.	.	5.27
	5	5.10	.	.	.	.	.	.	5.15	.	.	.	5.48
	6	5.36	.	.	.	.	.	.	5.12	.	.	.	5.34
	Mean	5.06	.	.	.	.	.	.	4.97	.	.	.	5.43
	SD ±	0.27	.	.	.	.	.	.	0.25	.	.	.	0.10
3	7	4.27	.	.	.	.	.	.	3.14	.	.	.	5.45
	8	4.33	.	.	.	.	.	.	4.19	.	.	.	4.32
	9	4.74	.	.	.	.	.	.	5.40	.	.	.	5.22
	10	4.77	.	.	.	.	.	.	3.97	.	.	.	4.95
	11	4.71	.	.	.	.	.	.	5.30	.	.	.	5.36
	12	4.47	.	.	.	.	.	.	5.29	.	.	.	5.43
	Mean	4.55	.	.	.	.	.	.	4.55	.	.	.	5.12
	SD ±	0.20	.	.	.	.	.	.	0.85	.	.	.	0.39
20	13	4.95	.	.	.	.	.	.	4.83	.	.	.	5.43
	14	4.54	.	.	.	.	.	.	4.75	.	.	.	5.41
	15	4.85	.	.	.	.	.	.	5.07	.	.	.	5.58
	16	5.43	.	.	.	.	.	.	4.83	.	.	.	5.14
	17	5.25	.	.	.	.	.	.	4.93	.	.	.	5.24
	18	4.52	.	.	.	.	.	.	4.52	.	.	.	5.25
	Mean	4.92	.	.	.	.	.	.	4.82	.	.	.	5.34
	SD ±	0.34	.	.	.	.	.	.	0.17	.	.	.	0.15
50	19	3.98	.	.	.	.	.	.	4.49	.	.	.	5.23
	20	4.50	.	.	.	.	.	.	5.00	.	.	.	5.04
	21	5.03	.	.	.	.	.	.	4.83	.	.	.	5.38
	22	4.77	.	.	.	.	.	.	4.46	.	.	.	5.54
	23	4.63	.	.	.	.	.	.	5.14	.	.	.	4.44
	24	5.34	.	.	.	.	.	.	5.25	.	.	.	5.42
	Mean	4.71	.	.	.	.	.	.	4.86	.	.	.	5.18
	SD ±	0.43	.	.	.	.	.	.	0.30	.	.	.	0.36

[1]Counts per minute

APPENDIX A

Table 20. [^{3}H]-thymidine uptake of pokeweed-stimulated lymphocytes.

Se	Cow ID	Days of the Trial 0	2	6	13	18	23	28	42	56	70	84	90
mg/d		cpm (log_{10})[1]											
0	1	4.82	.	.	.	.	.	.	4.35	.	.	.	5.32
	2	5.22	.	.	.	.	.	.	5.38	.	.	.	5.17
	3	5.05	.	.	.	.	.	.	4.55	.	.	.	5.29
	4	5.16	.	.	.	.	.	.	5.41	.	.	.	4.77
	5	4.97	.	.	.	.	.	.	5.14	.	.	.	5.43
	6	5.36	.	.	.	.	.	.	5.19	.	.	.	5.29
	Mean	5.10	.	.	.	.	.	.	5.01	.	.	.	5.21
	SD ±	0.17	.	.	.	.	.	.	0.41	.	.	.	0.21
3	7	4.62	.	.	.	.	.	.	4.43	.	.	.	5.46
	8	4.67	.	.	.	.	.	.	4.94	.	.	.	5.16
	9	4.59	.	.	.	.	.	.	5.43	.	.	.	5.32
	10	5.24	.	.	.	.	.	.	4.19	.	.	.	5.31
	11	5.08	.	.	.	.	.	.	5.41	.	.	.	5.23
	12	4.42	.	.	.	.	.	.	5.32	.	.	.	5.29
	Mean	4.77	.	.	.	.	.	.	4.95	.	.	.	5.29
	SD ±	0.29	.	.	.	.	.	.	0.49	.	.	.	0.09
20	13	5.02	.	.	.	.	.	.	4.93	.	.	.	5.58
	14	4.50	.	.	.	.	.	.	4.79	.	.	.	5.58
	15	4.74	.	.	.	.	.	.	5.28	.	.	.	5.22
	16	4.83	.	.	.	.	.	.	4.67	.	.	.	5.37
	17	5.13	.	.	.	.	.	.	5.37	.	.	.	5.10
	18	4.40	.	.	.	.	.	.	4.30	.	.	.	5.24
	Mean	4.77	.	.	.	.	.	.	4.89	.	.	.	5.35
	SD ±	0.26	.	.	.	.	.	.	0.36	.	.	.	0.18
50	19	4.03	.	.	.	.	.	.	4.52	.	.	.	5.08
	20	4.68	.	.	.	.	.	.	4.95	.	.	.	5.53
	21	5.29	.	.	.	.	.	.	5.25	.	.	.	5.41
	22	4.62	.	.	.	.	.	.	4.02	.	.	.	5.38
	23	4.69	.	.	.	.	.	.	4.96	.	.	.	5.26
	24	4.70	.	.	.	.	.	.	5.29	.	.	.	5.62
	Mean	4.67	.	.	.	.	.	.	4.83	.	.	.	5.38
	SD ±	0.36	.	.	.	.	.	.	0.44	.	.	.	0.17

[1]Counts per minute

APPENDIX B[b]

[b] Includes data (individual, mean, SD) for the respective variables for cows which had comprised the 50 mg group in trial 1. These cows were subsequently fed (trial 2) 100 mg supplemental Se/hd/d for 28 days, followed by no supplemtal Se for 56 days. Unless otherwise indicated reference ranges are from the Animal Health Diagnostic and Clinical Pathology Laboratories at the College of Veterinary Medicine, Michigan State University.

APPENDIX B

Table 1. Serum selenium concentrations.

Cow ID	Days of the Trial 100	102	108	120	128	132	136	149	156	163	176	184
	ng/ml*											
19	91	99	131	300	175	132	100	94	77	83	56	55
20	98	113	196	333	289	154	128	94	87	M	64	71
21	138	108	177	232	186	116	117	76	70	86	65	66
22	140	144	255	435	311	176	152	116	93	105	75	73
23	97	136	191	342	250	151	127	107	90	105	78	73
24	130	154	302	425	338	202	168	120	106	111	82	75
Mean	116	126	209	344	258	155	132	101	87	82	70	69
SD ±	21	20	55	70	61	28	22	15	12	38	9	7

* Reference range 70-100

Table 2. Whole blood selenium concentrations.

Cow ID	Days of the Trial 100	102	108	120	128	132	136	149	156	163	176	184
	ng/ml*											
19	234	237	232	.	329	293	242	220	223	279	216	176
20	246	326	369	.	542	304	357	313	374	357	372	345
21	325	298	346	.	542	304	357	313	374	357	372	345
22	275	276	393	.	535	491	567	368	338	455	411	310
23	258	238	300	.	417	422	403	273	270	356	261	232
24	304	306	470	.	633	383	389	325	420	340	371	334
Mean	274	280	352	.	485	366	382.3	295	322	342	321	271
SD ±	32	34	74	.	98	74	97.5	47	65	62	69	62

* Reference range 150-220 (derived from Figure 12)

APPENDIX B

Table 3. Selenium concentrations of liver biopsies.

Cow ID	Days of the Trial 100	102	108	120	128	132	136	149	156	163	176	184
	μg/g dry wt*											
19	4.56	.	.	.	14.91	.	.	.	.	.	.	1.58
20	6.47	.	.	.	22.94	.	.	.	.	.	.	0.98
21	4.47	.	.	.	8.49	.	.	.	.	.	.	2.07
22	7.32	.	.	.	16.86	.	.	.	.	.	.	1.90
23	4.65	.	.	.	M	.	.	.	.	.	.	1.61
24	8.76	.	.	.	12.74	.	.	.	.	.	.	1.59
Mean	6.0	.	.	.	15.2	.	.	.	.	.	.	1.60
SD ±	1.6	.	.	.	4.8	.	.	.	.	.	.	0.30

* Reference range 1.2-2.0

Table 4. Urine selenium concentrations.

Cow ID	Days of the Trial 100	102	108	120	128	132	136	149	156	163	176	184
	ng/ml											
19	672	.	.	.	1400	.	.	.	.	.	.	40
20	744	.	.	.	1410	.	.	.	.	.	.	28
21	954	.	.	.	1280	.	.	.	.	.	.	47
22	966	.	.	.	1670	.	.	.	.	.	.	19
23	577	.	.	.	1560	.	.	.	.	.	.	64
24	1002	.	.	.	.	.	.	.	.	.	.	57
Mean	819	.	.	.	1464	.	.	.	.	.	.	42.5
SD ±	163	.	.	.	136	.	.	.	.	.	.	15.6

APPENDIX B

Table 5. Fecal selenium concentrations.

Cow ID	Days of the Trial											
	100	102	108	120	128	132	136	149	156	163	176	184
	μg/g dry wt											
19	2.32	.	.	.	7.75	.	.	.	.	.	.	0.15
20	3.14	.	.	.	11.04	.	.	.	.	.	.	0.16
21	4.02	.	.	.	9.02	.	.	.	.	.	.	0.15
22	3.95	.	.	.	9.82	.	.	.	.	.	.	0.16
23	3.20	.	.	.	10.99	.	.	.	.	.	.	0.21
24	3.58	.	.	.	9.99	.	.	.	.	.	.	0.19
Mean	3.37	.	.	.	9.77	.	.	.	.	.	.	0.17
SD ±	0.58	.	.	.	1.14	.	.	.	.	.	.	0.02

Table 6. White blood cell concentrations.

Cow ID	Days of the Trial											
	100	102	108	120	128	132	136	149	156	163	176	184
	cells/mm^3 × 10^3*											
19	7.5	.	.	.	6.8	.	.	.	.	5.63	.	7.7
20	5.8	.	.	.	6.06	.	.	.	.	6.0	.	7
21	6.3	.	.	.	8.20	.	.	.	.	6.08	.	10.9
22	7.5	.	.	.	6.41	.	.	.	.	6.84	.	8.7
23	7.7	.	.	.	7.25	.	.	.	.	6.4	.	8.2
24	6.4	.	.	.	5.91	.	.	.	.	5.24	.	6.6
Mean	6.9	.	.	.	6.8	.	.	.	.	6.0	.	8.2
SD ±	0.7	.	.	.	0.8	.	.	.	.	0.5	.	1.4

* Reference range 4.7-11.5

APPENDIX B

Table 7. Erythrocyte concentrations.

Cow ID	Days of the Trial 100	102	108	120	128	132	136	149	156	163	176	184
	cells/mm^3 × 10^{6}*											
19	7.9	.	.	.	6.9	.	.	.	.	8.2	.	8.03
20	5.7	.	.	.	6.2	.	.	.	.	6.4	.	6.5
21	6.4	.	.	.	7.0	.	.	.	.	6.0	.	6.28
22	7.1	.	.	.	6.0	.	.	.	.	7.0	.	6.97
23	7.0	.	.	.	8.3	.	.	.	.	6.8	.	6.25
24	6.2	.	.	.	6.4	.	.	.	.	5.7	.	5.63
Mean	6.7	.	.	.	6.8	.	.	.	.	6.7	.	6.60
SD ±	0.7	.	.	.	0.8	.	.	.	.	0.8	.	0.70

* Reference range 5.29-9.19 × 10^{6}

Table 8. Hemoglobin concentrations.

Cow ID	Days of the Trial 100	102	108	120	128	132	136	149	156	163	176	184
	g/dl*											
19	11.2	.	.	.	11.2	.	.	.	.	11.4	.	11.7
20	11.0	.	.	.	10.5	.	.	.	.	12.3	.	12.2
21	10.5	.	.	.	11.4	.	.	.	.	10.9	.	11.3
22	11.3	.	.	.	10.2	.	.	.	.	12.2	.	12.1
23	10.9	.	.	.	11.5	.	.	.	.	11.4	.	10.6
24	10.5	.	.	.	12.3	.	.	.	.	9.8	.	9.9
Mean	10.9	.	.	.	11.2	.	.	.	.	11.3	.	11.3
SD ±	0.3	.	.	.	0.7	.	.	.	.	0.8	.	0.8

* Reference range 8.8-15.6

APPENDIX B

Table 9. Packed cell volumes.

Cow ID	Days of the Trial											
	100	102	108	120	128	132	136	149	156	163	176	184
	%											
19	30.5	.	.	.	29.4	.	.	.	.	31.6	.	31.6
20	27.9	.	.	.	26.7	.	.	.	.	31.5	.	31.4
21	28.4	.	.	.	30.2	.	.	.	.	28.1	.	29.7
22	30.0	.	.	.	26.8	.	.	.	.	31.5	.	31.8
23	29.2	.	.	.	30.8	.	.	.	.	30.0	.	28.1
24	27.2	.	.	.	31.6	.	.	.	.	25.5	.	25.3
Mean	28.9	.	.	.	29.3	.	.	.	.	29.7	.	29.7
SD ±	1.2	.	.	.	1.9	.	.	.	.	2.3	.	2.3

* Reference range 23.7-41.4

Table 10. Serum aspartate aminotransferase activities.

Cow ID	Days of the Trial											
	100	102	108	120	128	132	136	149	156	163	176	184
	IU/L*											
19	48	.	.	.	51	.	.	.	.	54	.	75
20	40	.	.	.	57	.	.	.	.	48	.	49
21	50	.	.	.	63	.	.	.	.	62	.	66
22	44	.	.	.	50	.	.	.	.	47	.	59
23	58	.	.	.	70	.	.	.	.	81	.	58
24	50	.	.	.	49	.	.	.	.	52	.	55
Mean	48	.	.	.	57	.	.	.	.	57	.	60
SD ±	6	.	.	.	8	.	.	.	.	12	.	8

* Reference range 48-109

APPENDIX B

Table 11. Serum gamma-glutamyl transferase activities.

Cow ID	Days of the Trial											
	100	102	108	120	128	132	136	149	156	163	176	184
	IU/L*											
19	24	.	.	.	34	.	.	.	.	26	.	20
20	20	.	.	.	18	.	.	.	.	16	.	11
21	22	.	.	.	28	.	.	.	.	24	.	25
22	19	.	.	.	26	.	.	.	.	21	.	19
23	23	.	.	.	29	.	.	.	.	28	.	21
24	23	.	.	.	29	.	.	.	.	24	.	21
Mean	22	.	.	.	27	.	.	.	.	23	.	20
SD ±	2	.	.	.	5	.	.	.	.	4	.	4

* Reference range 0-40

Table 12. Serum sorbitol dehydrogenase activities.

Cow ID	Days of the Trial											
	100	102	108	120	128	132	136	149	156	163	176	184
	IU/L*											
19	33	.	.	.	25	.	.	.	.	24	.	64
20	11	.	.	.	14	.	.	.	.	12	.	13
21	16	.	.	.	19	.	.	.	.	30	.	25
22	17	.	.	.	20	.	.	.	.	16	.	26
23	25	.	.	.	42	.	.	.	.	54	.	20
24	18	.	.	.	14	.	.	.	.	16	.	14
Mean	20	.	.	.	22	.	.	.	.	25	.	27
SD ±	7	.	.	.	10	.	.	.	.	14	.	17

* Reference range 24-42

APPENDIX B

Table 13. Serum creatine phosphokinase activities.

Cow ID	Days of the Trial 100	102	108	120	128	132	136	149	156	163	176	184
	IU/L*											
19	92	.	.	.	109	.	.	.	.	103	.	146
20	69	.	.	.	129	.	.	.	.	85	.	87
21	114	.	.	.	145	.	.	.	.	97	.	228
22	115	.	.	.	108	.	.	.	.	138	.	145
23	63	.	.	.	80	.	.	.	.	68	.	86
24	78	.	.	.	73	.	.	.	.	96	.	105
Mean	88	.	.	.	107	.	.	.	.	98	.	133
SD ±	20	.	.	.	25	.	.	.	.	21	.	49

* Reference range 23-118

Table 14. Body weights.

Cow ID	Days of the Trial 100	102	108	120	128	132	136	149	156	163	176	184
	kg											
19	483	.	.	.	483	.	.	.	483	.	.	497
20	673	.	.	.	687	.	.	.	621*	.	.	621
21	614	.	.	.	629	.	.	.	636	.	.	636
22	511	.	.	.	525	.	.	.	540	.	.	533
23	569	.	.	.	577	.	.	.	591	.	.	591
24	599	.	.	.	584	.	.	.	569	.	.	622
Mean	575	.	.	.	581	.	.	.	573	.	.	583
SD ±	64	.	.	.	66	.	.	.	52	.	.	51

* Cow calved since previous body weight

APPENDIX B

Table 15. Body condition score.[1]

Cow ID	Days of the Trial 100	102	108	120	128	132	136	149	156	163	176	184
19	3	.	.	.	3	.	.	.	3	.	.	3
20	4	.	.	.	4	.	.	.	4	.	.	4
21	3.5	.	.	.	3	.	.	.	3	.	.	3
22	3.5	.	.	.	3.5	.	.	.	3.5	.	.	3.5
23	3	.	.	.	3	.	.	.	3.5	.	.	3
24	3	.	.	.	3.5	.	.	.	3.5	.	.	3.5
Mean	3.3	.	.	.	3.3	.	.	.	3.4	.	.	3.3
SD ±	0.4	.	.	.	0.4	.	.	.	0.3	.	.	0.4

[1]Range 1(thin) to 5(fat) (from Mulvany, 1977)

Table 16. [^{3}H]-thymidine uptake of unstimulated lymphocytes.

Cow ID	Days of the Trial 100	102	108	120	128	132	136	149	156	163	176	184
	cpm ($\log_{10}$)[1]											
19	3.91	.	.	.	3.62	.	.	.	.	.	.	.
20	3.71	.	.	.	3.66	.	.	.	.	.	.	.
21	3.56	.	.	.	3.75	.	.	.	.	.	.	.
22	3.79	.	.	.	3.82	.	.	.	.	.	.	.
23	3.49	.	.	.	3.77	.	.	.	.	.	.	.
24	4.08	.	.	.	3.74	.	.	.	.	.	.	.
Mean	3.76	.	.	.	3.73	.	.	.	.	.	.	.
SD ±	0.2	.	.	.	0.07	.	.	.	.	.	.	.

[1] Counts per minute

APPENDIX B

Table 17. [^{3}H]-thymidine uptakes of phytohemagglutinin-stimulated lymphocytes.

Cow ID	Days of the Trial											
	100	102	108	120	128	132	136	149	156	163	176	184
	cpm (log_{10})[1]											
19	5.05	.	.	.	4.97	.	.	.	.	.	.	.
20	5.45	.	.	.	5.19	.	.	.	.	.	.	.
21	5.37	.	.	.	5.45	.	.	.	.	.	.	.
22	5.57	.	.	.	5.40	.	.	.	.	.	.	.
23	5.12	.	.	.	5.49	.	.	.	.	.	.	.
24	5.38	.	.	.	5.59	.	.	.	.	.	.	.
Mean	5.32	.	.	.	5.35	.	.	.	.	.	.	.
SD ±	0.18	.	.	.	0.21	.	.	.	.	.	.	.

[1]Counts per minute

Table 18. [^{3}H]-thymidine uptakes of concanavalin A-stimulated lymphocytes.

Cow ID	Days of the Trial											
	100	102	108	120	128	132	136	149	156	163	176	184
	cpm (log_{10})[1]											
19	5.23	.	.	.	5.26	.	.	.	.	.	.	.
20	5.04	.	.	.	5.42	.	.	.	.	.	.	.
21	5.38	.	.	.	5.30	.	.	.	.	.	.	.
22	5.54	.	.	.	5.34	.	.	.	.	.	.	.
23	4.44	.	.	.	5.42	.	.	.	.	.	.	.
24	5.42	.	.	.	5.49	.	.	.	.	.	.	.
Mean	5.18	.	.	.	5.37	.	.	.	.	.	.	.
SD ±	0.36	.	.	.	0.08	.	.	.	.	.	.	.

[1]Counts per minute

APPENDIX B

Table 19. [^{3}H]-thymidine of pokeweed-stimulated lymphocytes.

Cow ID	Days of the Trial											
	100	102	108	120	128	132	136	149	156	163	176	184
	cpm (log_{10})[1]											
19	5.08	.	.	.	4.96	.	.	.	.	.	.	3.0
20	5.53	.	.	.	5.43	.	.	.	.	.	.	4.0
21	5.41	.	.	.	5.54	.	.	.	.	.	.	3.0
22	5.38	.	.	.	5.60	.	.	.	.	.	.	3.5
23	5.26	.	.	.	5.61	.	.	.	.	.	.	3.0
24	5.62	.	.	.	5.59	.	.	.	.	.	.	3.5
Mean	5.38	.	.	.	5.45	.	.	.	.	.	.	3.3
SD ±	0.18	.	.	.	0.23	.	.	.	.	.	.	0.4

[1]Counts per minute

BIBLIOGRAPHY

1. Franke KW, Potter WR. A new intoxicant occurring naturally in certain samples of plant foodstuffs. IX. Toxic effects of orally ingested selenium. J Nutr 1935;10:213.

2. Shortridge EH, Ohara PJ, Marshall PM. Acute selenium poisoning in cattle. N Z Vet J 1971;19:47.

3. Maag DD, Osborn JS, Clopton JR. The effect of sodium selenite on cattle. Am J Vet Res 1960;10:1049.

4. Miller WT, Williams KT. Minimum lethal doses of selenium, as sodium selenite, for horses, mules, cattle and swine. J Agric Res 1940;60:163.

5. Muth OH, Oldfield JE, Schubert JR, Remmert LF. White muscle disease (myopathy) in lambs and calves. VI. Effects of Selenium and Vitamin E on lambs. Am J Vet Res 1959;20:231.

6. Hartley WJ. Selenium and ewe fertility. Proc N Z Soc Ani Prod 1963;23:20.

7. Trinder N, Woodhouse CD, Rentan CP. The effect of vitamin E and selenium on the incidence of retained placentae in dairy cows. Vet Rec 1969;85:550.

8. Julien WE, Conrad HR, Jones JE, Moxon AL. Selenium and Vitamin E and the incidence of retained placenta in parturient dairy cows. J Dairy Sci 1976a;59:1954.

9. Harrison JH, Hancock DD, Conrad HR. Selenium deficiency and ovarian function in dairy cattle. Fed Proc 1982;41:786.

10. Yamini B, Mullaney TP. Vitamin E and selenium deficiency as a possible cause of abortion in food animals. Proc 28th An Meet Am Assoc Vet Lab Diag 1985:131-144.

11. Anderws ED, Hartley WJ, Grant AB. Selenium-responsive diseases of animals in New Zealand. N Z Vet J 1968;16:3.

12. Norman BB, Johnson W. Selenium responsive disease. Ani Nutr Health 1976;31:6.

13. Gyang EO, Stevens JB, Olson WG, Tsitsamis SD, Usen KEA. Effects of selenium-vitamin E injections on bovine polymorpho-nucleated leukocytes phagocytosis and killing of Staphylococcus aureus. Amer J Vet Res 1984;45:175.

14. Smith LK, Harrison JH, Hancock DD, Todhunter DA, Conrad HR. Effect of Vitamin E and Selenium Supplementation on the Incidence of Clinical Mastitis and Duration of Clinical Symptoms. J Dairy Sci 1984;67:1293-1300.

15. Rotruck JT, Pope AL, Ganther HE. Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973;179:588.

16. Combs, GF, Combs SB. The Role of Selenium in Nutrition. Academic Press, New York 1986:220.

17. Selenium regulations amended. FDA Vet. 1987:8.

18. Ames NE, deSilva RB, Whitehair CK. Instrumentation and technique for obtaining percutaneous liver biopsies suitable for chemical analysis in Cattle. Proc XIth International Congress on Diseases of Cattle. 1980; Tel Aviv, Israel.

19. Whetter PA, Ullrey DE. Improved flurometric method of determining selenium. J Assoc Off Anal Chem 1978;61:927.

20. SAS user's guide:statistics. Version 5 ed. Cary, NC: SAS Institute Inc, 1982.

21. Gill JL. Repeated Measurement: Sensitive Tests for Experiments with few Animals. J Anim Sci 1986;63:943-954.

22. Julien WE, Conrad HR, Moxon AL. Selenium and vitamin E and the incidence of retained placenta in parturient dairy cows. II. Prevention in commercial herds with prepartum treatment. J Dairy Sci 1976b;59:1960-1962.

23. Segerson EC, Riviere GJ, Dalton HL, Whitacre MD. Retained placenta of Holstein cows treated with selenium and vitamin E. J Dairy Sci 1981;64:1833-1836.

24. Stevens, JB. Serum selenium concentrations and Glutathione peroxidase activities in cattle grazing forages of various selenium concentrations. AJVR 1985;46:1556-1560.

25. Scholz RW, Hutchinson LJ. Distribution of glutathione peroxidase activity and selenium in the blood of dairy cows. AJVR 1979;40:245-49.

26. Erskine RJ, Eberhart RJ, Hutchinson LJ, et al. Blood selenium concentrations and glutathione peroxidase activities in dairy herds with high and low somatic cell counts. J Am Vet Med Assoc 1987;190:1417-1421

27. Smith LK, Hogan JS, Conrad HR. Selenium in dairy cattle: Its role in disease resistance. Vet Med 1988;83:72-78.

28. Backall BS, Scholz RW. Reference values for a field test to estimate inadequate glutathione peroxidase activity and selenium status in the blood of cattle. AJVR 1979;40:733-738.

29. Arthur JR. Nutritional Inter-Relationships between selenium and Vitamin E. Proc of a Meeting on "Selenium and Ruminant Health", Glenfield New South Wales, Australia 1985:1.1-1.2.

30. Koller LD, South PJ, Exon JH, Whitbeck GA. Selenium deficiency of beef cattle in Idaho and Washington and a practical means of prevention. Cornell Vet 1983;73:323-332.

31. Maas JP. Diagnosis and management of selenium-responsive diseases in cattle. Comp Cont Ed Pract Vet 1983;5:393-400.

32. Maas JP, Koller LD. Selenium deficiency in beef cattle and sheep: Diagnosis, treatment and prevention. In: Selenium responsive diseases in food animals. Proc Symposium Western States Vet Conf., Las Vegas, Nevada, Vet. Learning Sys. Co., Inc. 1985:20-25.

33. Nahapetian AT, Janghorbani M, Young, VR. Urinary Trimethylselenionium Excretion by the Rat: Effect of Level and Source of Selenium-75. J Nutr 1983;113:401-411.

34. Sun XF, Ting BTG, Janghorbani M. Excretion of Trimetylselenonium Ion in Human Urine. Ana Biochem 1987;167:304-311.

35. Stowe HD, Thomas JW, Johnson T, Marteniuk JV, Morrow DA, Ullrey DE. Responses of dairy cattle to Long-Term and Short-Term supplementation with oral selenium and vitamin E. J Dairy Sci 1988;71:1830-1839.

36. VanSaun RJ. Selenium and Vitamin E: Relationships between the pregnant dairy cow and fetus. Thesis, 1988.

37. Wright PL, Bell MC. Comparative metabolism of selenium and tellurium in sheep and swine. Am J Physi 1966;211:6-10.

38. Hidiroglou M, Heaney DP, Jenkins KJ. Metabolism of inorganic selenium in Rumen bacteria. Can J Physiol Pharm 1968;444.

39. Rosenfeld I. Biosyntesis of seleno-compound from inorganic selenium; Proceedings of the Society of Experimental Biology and Medicine. 1962;111:670.

40. Thomson CD, Robinson BA, Stewart RDH, Robinson MF. Metabolic studies of Se-75 selenocystine and Se-75 selenomethionine in the rat. Br J Nutr 1975;34:501.

41. Whanger PD, Wewig Ph, Muth OH. Metabolism of 75-Se-selenite and 75 Se-seleomethionine by rumen microorganisms. Federation Proceedings 1968;27:418.

42.National Research Council (NRC). Mineral Tolerance of Domestic Animals. Washington, DC:Natl Acad Sci 1980;400.

43. National Research Council (NRC). Nutrient Requirements of Dairy Cattle. 6th ed. Washington DC:Natl Acad Sci;1989;35-36.

44. Frost DV. The two faces of selenium-Can selenophobia be cured? Crit Rev Toxico 1972;1:467