

Effect of Selenium Supplementation from Various Dietary Sources on the Antioxidant and Selenium Status of Dairy Cows and Trace Element Status in Dairy Herds

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In the sweet memories of my loving brother

Abul'Aala Sultan Saeed

(May Allah shower His blessings upon him)
who always inspired me towards higher ideals in life

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ABBREVIATIONS

ADF	Acid detergent fiber
DTNB	5,5'-dithio-bis(2-nitrobenzoic acid)
EDTA	Ethylene-diamine-tetraacetic acid
FRAP	Ferric reducing ability of plasma
GSH	Reduced glutathione
GSHPx	Glutathione Peroxidase
GSSG	Glutathione disulfide
KS	Kolmogorov-Smirnov
NADPH	Nicotinamide adenine di nucleotide phosphate
NDF	Neutral detergent fibre
NK	Natural killer
ORAC	Oxygen radical absorption capacity
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SEM	Standard error of mean
SeY	Selenium yeast
Sel	Selenium inorganic (sodium selenite)
TEAC	Trolox equivalent antioxidant capacity
TMR	Total mixed ration
TRAP	Total peroxy radical trapping parameter
TROLOX	6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid

Reactive Species

H ₂ O ₂	Hydrogen peroxide
-OH	Hydroxyl radical
NO	Nitric oxide
ONOO-	Peroxynitrite
O ²⁻	Superoxide radical anion

Amino Acid Codes

Asp	Aspartate
Cys	Cysteine
Glu	Glutamate
Met	Methionine
SeMet	Selenomethionine
SeCys	Selenocysteine
Val	Valine

ZUSAMMENFASSUNG

Einleitung: Selen (Se) ist ein essenzielles Spurenelement in der Ernährung von Mensch und Tier. In der Tierernährung wird Milchkühen Selen über das Futter supplementiert, um einerseits die Tiere vor einem Mangel zu schützen und andererseits, um die Menge an Selen zu erhöhen, die aus dem Futter in die Milch übergeht. Mit einer fütterungsbedingten Anreicherung von Selen in der Milch eröffnen sich Möglichkeiten einer verbesserten Selenversorgung sowohl der Kälber als auch die Selenversorgung der Bevölkerung über den Konsum von Milch und Milchprodukten. Fragen der Qualität der erzeugten Milch und der daraus hergestellten Milchprodukte sowie Fragen der Lebensmittelsicherheit insgesamt haben dabei im Vordergrund der Betrachtungen zu stehen. Erreicht werden können diese Ziele nur dann, wenn eine hinreichende Balance zwischen Pro- und Antioxidantien bei der Nährstoffzufuhr gegeben ist. Selen kann durch seine biologische Rolle bei der genetischen Kodierung von Selenocystein (SeCys), welches als Bestandteil von Selenoproteinen vorkommt, als Antioxidations wirken. Ziel der Arbeit war herauszufinden, ob ein erwarteter Anstieg der Selenkonzentration in der Milch als Folge der Supplementierung der Futterrationen von Kühen mit Selenhefe mit einer Verbesserung des Status hinsichtlich der antioxidativen Kapazität sowie der Immunfunktionen bei den Milchkühen in der frühen Laktation verbunden ist. Es galt die Arbeitshypothese zu testen, nach der ein verbesserter Selenstatus der Milch auch zu einer Steigerung der Gesamtleistung der Antioxidantien beiträgt.

Material und Methoden: 16 Holstein-Friesian-Kühe erhielten drei unterschiedliche Futterrationen während des Zeitraums von sechs Wochen vor der Kalbung bis 15 Wochen *post partum*. Die tierexperimentellen Untersuchungen wurden durchgeführt auf dem Versuchsgut des Bundesinstitutes für Risikobewertung (BfR) in Berlin. Die Tiere der Kontrollgruppe (n = 5) erhielten dabei eine Basisration (verfüttert als Totale Mischration, TMR) mit einem mittleren Selengehalt von ~ 0.2 mg/kg Trockensubstanz, welches ausschließlich aus den Rationskomponenten stammte. Die Tiere der Versuchsgruppen erhielten die Basisration, allerdings supplementiert einerseits mit Natrium-Selenit (Sel) (n = 5) und andererseits mit Selenhefe (SeY) (n = 6), was zu mittleren Selengehalten in der TMR von 0,4 mg/kg TS bzw. 0,6 mg/kg TS

fürhte. Die Selensupplemente wurden individuell täglich jeweils vor dem Melken in Form von 20 bzw. 30 Gramm einer selenhaltigen Vormischung den Kühen verabreicht. Milch- und Serumproben wurden genommen am ersten Tag nach der Kalbung sowie in der 9., 12. und 15. Woche der Laktation. Die Selengehalte wurden ermittelt auf Basis der Hydrid-Atomabsorptionsspektrophotometrie, die Bestimmung der antioxidativen Kapazität (TEAC) erfolgte nach der Methode von Miller et al. (1996), adaptiert für ein Mikroplattenphotometer. Weiterhin wurde eine Untersuchung zum Spurenelementstatus in 11 großen Milchviehbetrieben in Sachsen durchgeführt, um den Spurenelementstatus anhand von Futter-, Blut- und Leberbioplatproben zu bestimmen.

Ergebnisse: Die mittleren Gehalte an Selen (\pm SEM) im Kolostrum der Tiere der Kontrollgruppe bzw. derjenigen Tiere, deren Futtermittel mit Natriumselenit (Sel) bzw. Selenhefe (SeY) supplementiert waren, beliefen sich auf $35,3 \pm 1,03 \mu\text{g/l}$, $39,1 \pm 2,56 \mu\text{g/l}$ bzw. $67,7 \pm 4,11 \mu\text{g/l}$. Die Selengehalte im Kolostrum der Tiere der Gruppe SeY unterschieden sich dabei ($P < 0,01$) von denjenigen der Tiere der beiden anderen Gruppen. Ebenso unterschieden sich auch die mittleren Konzentrationen an Selen in der Milch (\pm SEM) signifikant ($P < 0,01$) bei den Tieren, deren Ration mit Selenhefe supplementiert waren: $11,6 \pm 1,55 \mu\text{g/l}$ (Kontrolle), $15,4 \pm 3,24 \mu\text{g/l}$ (Sel) und $28,3 \pm 6,84 \mu\text{g/l}$ (SeY). Die Selengehalte in der Milch der Tiere der Kontrollgruppe und diejenigen der Kühe der Na-Selenit-Gruppe wiesen keine statistisch gesicherten Unterschiede auf ($P > 0,05$), jedoch zeigte sich bei den Tieren der Natriumselenit-Gruppe eine Erhöhung der mittleren Gehaltswerte um relativ 32%. Die TEAC-Werte für die Tiere der SeY-Gruppe unterschieden sich in allen drei Prüfzeiträumen ($P < 0,01$) sowohl von denjenigen der Tiere der Kontroll- sowie denen der Sel-Gruppe. Insgesamt zeigten sich geringgradige Unterschiede in den Gehaltswerten der Milch in Abhängigkeit vom Laktationsstadium. So beliefen sich die über die Beobachtungszeiträume gemittelten Milch-TEAC-Werte (mittlere Gehalte über den gesamten Versuchszeitraum \pm SEM) auf $586 \pm 0,95 \mu\text{Mol/l}$, $557 \pm 0,97 \mu\text{Mol/l}$ sowie $540 \pm 0,64 \mu\text{Mol/l}$ für die Tiere der SeY-Gruppe, der Sel-Gruppe bzw. der Kontrolltiere. Mit Blick auf die Gehaltswerte an Selen im Serum zeigten sich ähnliche Tendenzen wie bei den TEAC-Werten. Die Untersuchung des Spurenelementstatus von Milchkühen zeigte eine erhebliche Variabilität bei den Futterproben innerhalb und zwischen den Betrieben, die durch die tierbezogenen Proben (Plasma, Leber) nicht entsprechend reflektiert wurden. Die mittleren (\pm SEM)

Gehalte von Selen, Kupfer, Zink, Mangan und Eisen in den Leberbiopsien betragen $0.7 (\pm 0.04)$, $134.6 (\pm 6.81)$, $18.3 (\pm 0.9)$, $7.2 (\pm 0.71)$ und $89.2 (\pm 6.35)$ mg/kg.

Schlussfolgerung: Beim Einsatz von Selenhefe in der Fütterung von Milchkühen scheint ein Anstieg der antioxidativen Kapazität sowohl der Rindermilch als auch des Serums hervorgerufen zu werden. Weitere Studien sind angezeigt, um die Mechanismen aufzuklären, denen diese Effekte unterliegen.

Die Praxisstudie zeigte, dass viele Rationen für Milchkühe mehr Spurenelemente enthalten als die gegenwärtig empfohlenen Werte. Zwischen den verschiedenen Spurenelementen traten nach den Ergebnissen der Leberbiopsien Interaktionen auf, deren Ursachen und Konsequenzen unter praktischen Bedingungen weiter untersucht werden sollten.

ABSTRACT

Introduction: Selenium (Se) is an essential trace element for animal and human nutrition. Dietary selenium supplementation of dairy cows is practised to protect the animals from the risk of deficiency and to increase selenium transfer to the milk consequently benefiting the offspring and vulnerable human populations as milk and other dairy products make an important part of their diet. Milk quality and safety are both important. It cannot be ensured unless a proper balance between pro and antioxidant nutrients is maintained. Selenium, through its biological role by genetically encoded selenocysteine (SeCyS) residue in selenoproteins, can act as an antioxidant. The objective of this study was to find out whether the expected increase in milk selenium levels after supplementing the dairy rations with organic selenium yeast can affect the milk antioxidant status in the early lactating dairy cows. It was presumed that milk total antioxidant capacity might be boosted by the enhanced milk selenium status.

In addition to these experiments, a survey of the trace elements selenium, copper, zinc, iron and manganese was conducted in large dairy herds of Saxonia to determine their intake, bioavailability and interactions.

Materials and Methods: Sixteen Holstein-Friesian dairy cows were subjected to three dietary treatment groups from 6 weeks before calving to 15 weeks of lactation at the experimental station of the Bundesinstitut für Risikobewertung (BfR), Berlin, Germany. The control group (n=5) was maintained exclusively on the basal total mixed ration (TMR) containing ~ 0.2 mg/kg dietary DM selenium from the natural sources whereas sodium selenite (Sel) group (n=5) and selenium yeast (SeY) group (n=6) were supplemented with selenium at 0.4 mg/kg DM in the pre and 0.6 mg/kg DM in the post partum rations respectively. Each cow received the supplement individually in the form of 20 or 30 gram premix given before milking time. Samples were collected at one day after calving and at 9th, 12th and 15th week of lactation. Selenium content was analysed using the hydride generation atomic absorption spectrometry whereas Trolox equivalent antioxidant capacity (TEAC) was measured following the method of Miller et al. (1996) adapted for a microplate reader to accommodate the large number of samples in duplicates.

For trace elements survey, representative TMR samples and blood and liver samples from 11 selected farms were used to be analysed for trace element status.

Results: The mean (\pm SEM) selenium level in colostrum for the control, Sel and SeY groups was found to be 35.3 ± 1.03 $\mu\text{g/l}$, 39.1 ± 2.56 $\mu\text{g/l}$ and 67.7 ± 4.1 $\mu\text{g/l}$ respectively in this study. Selenium yeast group was different ($P < 0.01$) from both others. Average steady state milk (\pm SEM) selenium content was 11.6 ± 1.55 , 15.4 ± 3.24 and 28.3 ± 6.84 $\mu\text{g/l}$ for control, Sel and SeY groups respectively with SeY group differing ($P < 0.01$) from other groups. Control and Sel groups were not different ($P > 0.05$); however a relative increase of 32% was noted in Sel group. It has been noted that TEAC values for the SeY group were significantly different ($P < 0.01$) from that of control and Sel groups at all time points. However, negligible differences have been observed between different time points in all groups. Milk TEAC values of (mean of all time points \pm SEM) were 586 ± 0.95 $\mu\text{Mol/l}$, 557 ± 0.97 $\mu\text{Mol/l}$ and 540 ± 0.64 $\mu\text{Mol/l}$ for the SeY, Sel and control groups respectively. Similar trends in serum selenium and TEAC values have been noted. The investigation of the trace element concentrations in the total mixed rations of dairy cows indicated a huge variability within and between the farms that were not clearly reflected by the plasma and liver samples taken from the animals.

The mean (\pm SEM) concentrations of selenium, copper, zinc, manganese and iron in the fresh liver biopsy samples from Saxonian dairy herds were $0.7 (\pm 0.04)$, $134.6 (\pm 6.81)$, $18.3 (\pm 0.9)$, $7.2 (\pm 0.71)$ and $89.2 (\pm 6.35)$ mg/kg respectively.

Conclusion: This study reveals some sort of selenium-related increase in the total antioxidant capacity of bovine milk and serum. This can have implications for the health of the animals and public health concerns over milk safety. Further studies will help delineate the actual underlying mechanisms. Survey findings revealed that generally there is a trend of supplementing the dairy rations with trace elements above the requirements. Positive and negative interactions among the trace elements have been observed and will need further studies to explain effects under practical conditions.

1. INTRODUCTION

Efficient livestock and poultry production and the maintenance of normal health in animals require that essential nutrients be provided in appropriate amounts and in forms that are biologically utilizable. Deficiencies of certain nutrients occur in diets consisting of common feed ingredients and this has led to the common practice around the globe of supplementing the diets of farm animals with essential nutrients. Degree of the bioavailability of the nutrients does not only influence the dietary requirement but also the tolerance for a nutrient. Advances in the nutritional technologies have resulted in the development of innovative products to be used as animal feed supplements. These products must be designed to deliver the incremental nutrients in a safe and economical way in the food chain. Among various products used as animal feed supplements, amino acids, macro and micro minerals and enzymes are most important and popular. The trace element selenium (Se) has attracted substantial research efforts during the current and the last decade owing to its special place in the animal and human nutrition. Its essentiality and the toxicity are within narrow margins. Essentiality of this nutrient is based on its major role in the antioxidant defence system of the living cells.

Apart from being naturally found as sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3), selenium can be incorporated biologically in proteins containing methionine. Plants and yeast exposed to selenium salts accumulate the trace mineral in the form of selenomethionine (Se-Met). Sodium selenite and selenium enriched yeast are in common use as sources of selenium in farm animals. Although substantial amount of work has been carried out in the field of selenium nutrition of dairy cows, gaps still exist in the knowledge regarding comparative efficacy of supplementation from various sources. Moreover, some work in this regard has been done in Germany. It has been shown in several studies that dietary selenium yeast significantly increases selenium concentrations in blood, milk and other tissues as compared to inorganic selenium sources. Phenomenon of non-specific pooling of selenomethionine from selenium yeast into tissue proteins instead of methionine is accounted for this increase. However, Juniper et al. (2006), after conducting an experiment with selenium supplementation in the range of 0.27-0.4 mg/kg DM with

selenium yeast, reported that only 25-33% of total milk selenium increase could be attributed to selenomethionine and there are other selenoproteins in milk which might play a role as an antioxidant. Hence, the present study investigates the effect of selenium supplementation from sodium selenite and selenium yeast on the selenium status and Trolox equivalent antioxidant capacity (TEAC) in pregnant and lactating cows and their calves. It is hypothesized that increased selenium status in the supplemented cows' serum and milk will be reflected in the form of heightened antioxidant status. No such attempt has been made previously to get information regarding the effect of selenium from sodium selenite and selenium yeast on the total antioxidant capacity in dairy cows. This study provides basic information on the topic in addition to generate the data on selenium and revolves around the following objectives:

Investigations into selenium and antioxidant status on various time points of physiological importance during the periparturient and lactation stage

The assessment of selenium transfer into milk, risk assessment depending on the dietary level and source of selenium

Selenium transfer to calves and its impact on their health and well-being

Studies into the intake, bioavailability and interactions among essential trace elements in large dairy herds under practical conditions

2. REVIEW OF LITERATURE

This chapter is based on the review article “The Role of Dietary Selenium in the Bovine Mammary Gland Health and Immune Function” by Salman et al. (2009).

2.1 Selenium: From Toxicity to Essentiality

Selenium (Se, atomic number 34 and atomic weight 78.96) is placed in 4th period and 16th group of metalloids and non-metal chemical elements of the periodic table. Many of its chemical properties (outer valence electronic configuration, atomic size, bond energy, ionization potential and electronegativity) are similar to that of sulphur. Selenium occurs in oxidation states –II (selenide), 0 (elemental selenium), +IV (selenite) and +VI (selenate) forms. In isolated form, it is found like grey-black metallic cluster.

Discovered by Jöns Jacob Berzelius in 1817, the semi-metal selenium was named after the Greek Goddess of the moon, Selene (McKenzie et al. 1998). Dietary importance of selenium dates back in history when it was first reported to cause the toxic symptoms in the members of the caravan of the great adventurer, Marco Polo. Livestock disorder, commonly referred as alkali disease or blind stagger, was found endemic in areas with selenium rich soils. Similarly, symptoms of chronic selenium intoxication, depression and fatigue, and loss of hair and nails, were noticed in human beings living geographic in regions with high soil selenium before it was known to be the causative agent. That is why early scientists showed interest in selenium because of its toxic effects. However, the approach towards selenium research in life sciences began to change as early as 1916 when selenium was detected in normal human tissue samples. It was suggested “it may have a position in the organism which will without doubt be of the utmost significance in the study of life processes” (Gassmann 1916). The earliest evidence that selenium is involved in the immune function was found in 1957 with the observation that dogs injected with ⁷⁵Se incorporated the isotope into a leukocyte protein (now known to be the cytoplasmic glutathione peroxidase cGSHPx) (Schwarz and Foltz 1957). In sheep and humans, selenium is concentrated in tissues involved in the immune response such as spleen, liver and lymph nodes (Spallholz 1990). The question how this trace

element exerts its biochemical role was solved when it was discovered in 1973 to be the essential component of GSHPx and the cellular antioxidant defence system (Rotruck et al. 1973). The subsequent discoveries in rats about the fact that two thirds of the dietary selenium are not bound to this enzyme but are part of other compounds (Behne and Wolters 1983) led to the assumption that other selenoproteins may exist. Thus far 55 selenoproteins, including glutathione peroxidases (1-6), thioredoxin reductases (1-3) and iodothyronine deiodinase families of selenoenzymes have been reported. Consequently, dietary selenium deficiency has been known to cause various ailments in a number of animal species and humans. Keshan and Kashin-Beck diseases in humans, muscular dystrophy in sheep and cattle and exudative diathesis in poultry are notable among selenium deficiency disorders. This voyage of selenium from toxicity to essentiality is still in progress with revelation of new discoveries and facts about selenium and its related compounds and their role in diverse physiological functions of the body. The narrow margin of safety (average dietary intake for selenium and the tolerable upper intake level for both sexes has been reported by National Research Council (2001) as 113-220 μg and 400 $\mu\text{g}/\text{day}$ respectively for adult humans) is sufficient to stress its importance in the diets.

2.2 Selenium and Mechanism of Oxidative Stress

Oxygen is the prerequisite of life and ultimate source of energy for its sustainability. Animals, plants and many microorganisms rely on oxygen for efficient energy production. In doing so, free radicals capable of initiating further chain reactions are generated. These free radicals are capable of damaging the biologically relevant molecules such as DNA, proteins, lipids and carbohydrates. Superoxide (O_2^-) is the main free radical produced in biological systems during normal respiration in mitochondria and by autooxidation reactions at 37°C . It is notable that superoxide, by itself, is not extremely dangerous and does not rapidly cross the lipid membrane bilayer. However, it is a precursor of other more powerful free radicals collectively known as reactive oxygen species (**ROS**) and reactive nitrogen species (**RNS**). An imbalance in the production and accumulation of these highly reactive oxygen species (**ROS**) - activated derivatives of molecular oxygen, including singlet oxygen, O_2^- , H_2O_2 , hydroxyl radical, hypohalous acids and peroxyxynitrites - may lead to the most inevitable of the biological problems, the oxidative stress, because it derives

from the least-specific type of reaction: univalent electron transfer which can occur if the oxygen species come across with the redox cofactors at a lower potential than themselves. Reactions of this type (Figure 1) are responsible both for the formation of **ROS** and for their subsequent inactivation of various biomolecules. It has been experimentally manifested in the *E. coli* devoid of cytoplasmic superoxide dismutase (SOD) that these strains grew well anaerobically but exhibited a variety of aerobic growth defects that derived from endogenous O_2^- . Similarly, *E. coli* catalase/peroxidase mutants were poisoned by micromolar levels of H_2O_2 that accumulated inside the cell (Park et al. 2005). Both sets of mutants exhibited catabolic and biosynthetic defects that stem from the inactivation of a family of dehydratases.

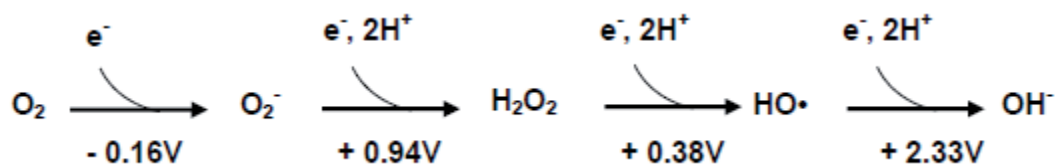


Figure 1 The redox states of oxygen with standard reduction potential (volts).
 Abbreviations: H_2O_2 , hydrogen peroxide; O_2^- , superoxide (Imlay 2008)

The other best-understood mechanisms of oxidative injury involve the oxidation and inactivation of exposed enzymic iron-sulphur clusters and the production of hydroxyl radicals within proteins and on the surface of DNA (Imlay 2008). Superoxides can also participate in the production of powerful radical ions by donating an electron and thereby reducing. It is speculated that basic biochemistry of the oxidative damage is likely shared by most cells, and most contemporary organisms have inherited from their ancestors a common set of strategies by which to defend themselves.

Although much remains to be understood about how cellular defences against the oxidative stress work, through its natural homeostatic balance the animal body must be able to keep free radicals in control. Defensive tactics revealed thus far include various free radical scavenger enzymes and isozymes for example superoxide dismutase, catalases, peroxidases and repair mechanisms. Inability or loss of oxidant-resistance strategies can be manifested in terms of many disease conditions in man and animals.

The transition period and early lactation in dairy cows is critically important for health, production and profitability (Drackley 1999). Dairy cows vigorous physiological activities during periparturient period concerning the rapid differentiation of secretory parenchyma, intense mammary gland growth and the onset of copious milk synthesis and secretion are accompanied by high energy demand and increased oxygen requirement . This increased oxygen demand can result in the augmented production of **ROS**, which are potential source of the cells and tissues injury, commonly referred as the oxidative stress leading to a high susceptibility of dairy cows to a variety of infections and metabolic disorders during the transition period. Vulnerability of the transition period in cattle is marked by reproductive problems and prevalence of mastitis. This can be ascribed to findings that various components of the host defence mechanisms, particularly the immune cells, are depressed during this period. It has been reported that functional capabilities of mammary macrophages decrease during the periparturient period and this alteration has been linked with an increased incidence of mastitis. Presence of neutrophils at the site is inversely correlated with the risk of mammary infections. In vitro efficacy of neutrophils obtained from selenium-deficient mice, rats and cattle in killing ingested microbes is significantly reduced as compared to that from selenium-sufficient animals. It is because of the reduced activity of the antioxidant enzyme Glutathione peroxidase (GSHPx), responsible to protect neutrophils to be damaged by their own superoxide-derived radicals, in selenium-deficient animals as selenium is an integral component of the enzyme. Supplementing the dairy rations with vitamin E and selenium has become a widely accepted practice throughout the world to address the issue of prooxidants and antioxidant balance. As being an essential component of the GSHPx, selenium is able not only to convert toxic hydrogen peroxides to water but also the lipid hydroperoxides to non reactive compounds participating in the antioxidant defence system of the body at initial and secondary levels of blocking the chain of reactions .

Selenium performs its biological role through the genetically encoded selenocysteine residue (**SeCys**) of selenoproteins. Selenium can affect three broad areas of cellular functions: antioxidant activities, thyroid hormone metabolism, and the regulation of redox-active protein activity. Out of 30-50 known selenoproteins (Köhrle 2000) at least 12 have been relatively well characterized as having wide-ranging implications

for immune function, malignancy and viral pathogenesis. The best-known selenoenzyme with respect to dairy cattle nutrition is glutathione peroxidase (GSHPx). Indeed, it is an essential component of the cellular antioxidant defence mechanism, which removes potentially damaging lipid hydro-peroxides and hydrogen peroxides and protects the immune cells from oxidative stress induced damage. A recent report describes that thioredoxin reductase (TrxR) may be an important antioxidant defence mechanism in peripheral blood mononuclear cells (PBMC) that is compromised during the periparturient period. Indeed the most of the functional capabilities of selenoproteins are related to their crucial role in regulating the ROS and redox status in nearly all tissues. However, some effects on the regulation of arachidonate metabolism in peripheral blood lymphocytes resulting in the partial reversal of proliferation have also been reported. New insight in the role of free radicals as signalling molecules and understanding the role of nutrients in gene expression have created new demands for further research related to the biological roles of selenium.

2.3 Metabolism of Selenium in Mammals

It is interesting to note that selenium is unique in its metabolism compared with typical essential trace elements such as copper and zinc. As with other dietary nutrients, selenium from organic and inorganic dietary sources has to be metabolized by the ruminal microorganisms before being absorbed by separate mechanisms in the small intestine of ruminants. Not much is known about selenium metabolism in the rumen. In sheep, ruminal absorption of ^{75}Se has been reported to be only 34% probably because of the conversion of dietary selenium to insoluble forms such as elemental selenium and selenide (Spears 2003). More recently, it has been demonstrated that inorganic selenium has a lower ruminal microbial uptake than organic selenium sources in dairy cows (Mainville et al. 2009). In the small intestine, amino acid derivatives of selenium (selenomethionine and selenocysteine), mainly found in the organic selenium sources such as selenium yeast, use the same carriers as their sulphur analogues methionine and cysteine (Glass et al. 1993), whereas selenate uses a sodium sulphate cotransporter for its absorption, which is driven by the activity of Na^+/K^+ -ATPase at the basolateral enterocyte membrane (Mehta et al. 2004). In the lumen of the small intestine, selenite partially reacts with glutathione or other thiols to selenotrisulfides, which are presumably taken up into the enterocytes

by amino acid transporters. Another part of selenite diffuses through the apical membrane and reacts with thiols in cytosol of enterocytes. Subsequently, selenium compounds are liberated in the blood stream at the basolateral enterocytes membrane and distributed to various peripheral tissues. The exact transport mechanism of various selenium compounds is not yet fully understood. Selenomethionine associates with hemoglobin while selenate and the remaining free selenite were found to be transported by α and γ -globulins (Beilstein and Whanger 1986b, a). Ionic selenium forms of selenite and selenate follow bicarbonate and phosphate, respectively, in their transport in the body because of similarity in their ionic forms (Suzuki 2005). In fact selenite ions are readily taken up by red blood cells (RBCs) through band three protein without being excreted into urine (Suzuki et al. 1998) while selenate ions are not taken up by RBCs but directly taken up by hepatocytes through transport system of phosphate and partly excreted directly into urine (Kobayashi et al. 2001). Selenite taken up by RBCs is readily reduced to selenide and then effluxed into the blood stream in the presence of albumin and transferred to liver in the form bound to albumin (Shiobara and Suzuki 1998). It can be concluded that selenide of selenite and selenate origin are taken up differently by the liver and utilized for the synthesis of selenoproteins. A surplus of inorganic selenium is stored in peripheral organs as “acid labile selenium”. This selenium fraction consists of selenium bound unspecifically to proteins presumably via the formation of selenium-sulphur bonds (Diplock et al. 1973; Ganther and Kraus 1984). The main excretion products of selenium detected in urine are the methylated metabolites monomethylselenol (MMS) and trimethylselenonium (TMS). Methylated selenium metabolites are formed from selenium reduced to the oxidation state $-II$ as well as from selenium stored unspecifically in proteins as selenomethionine and from acid labile selenium (Hassoun et al. 1995). Selenium exhalation as dimethylselenide only takes place when selenium is ingested in toxic doses. The metabolism and the fate of dietary selenium has been summarised demographically in the following representations (Figure 2 and Figure 3).

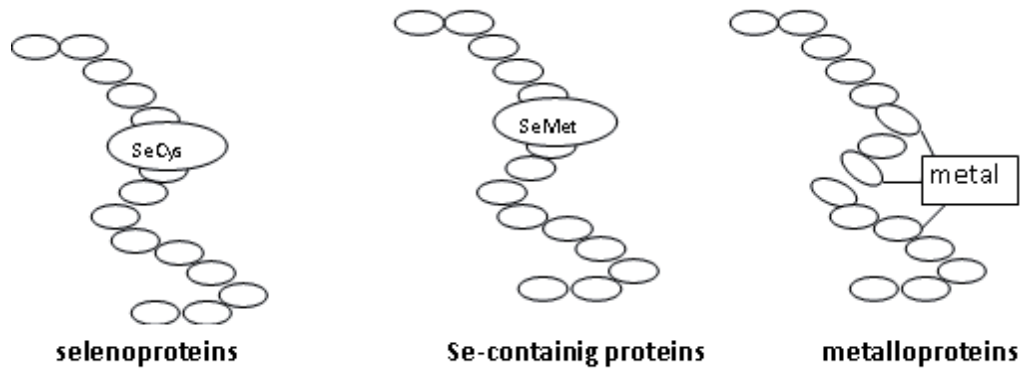


Figure 2 Selenium incorporation in proteins (Suzuki 2005)

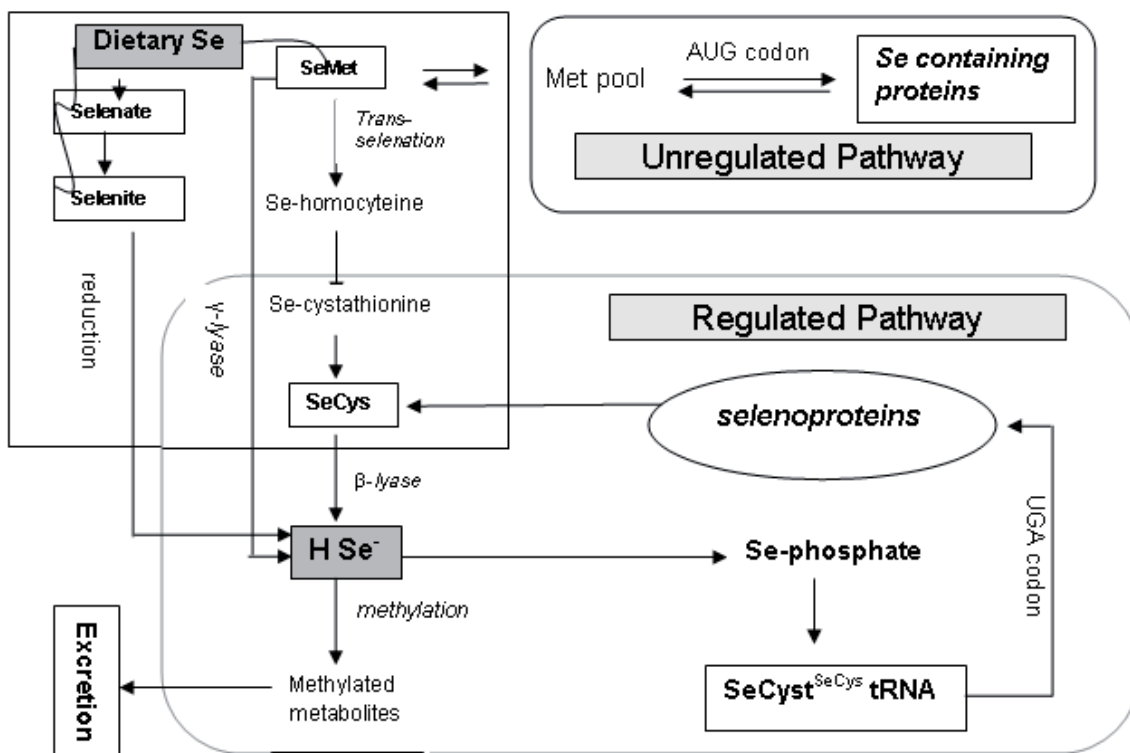


Figure 3 Selenium metabolism in mammals (Suzuki 2005)

2.4 Selenium Nutrition of Dairy Cows

The nutritional status of the animal is related to its overall health and its capacity to combat disease. The nutritionally modulated improvement of the immune system should culminate in increased resistance to disease. Research on micronutrients and their immunoregulatory role regarding udder health and bovine mastitis has focused mainly on selenium, vitamin A, vitamin E, β -carotene, copper and zinc. Among these,

selenium has been the most characterized trace element affecting bovine mammary gland health through its role in cell function.

Having been recognized as a dietary essential, selenium is being routinely supplemented in the rations of farm animals. In the United States, 0.1 mg selenium /kg dry matter (DM) is recommended for ruminant rations to correct symptoms of a selenium deficiency. However, owing to the beneficial effects of the additional selenium supplementation, the recommendation was increased to a level of 0.3 mg/kg DM (National Research Council 2001). The German Society for Nutritional Physiology (GfE) has recommended that selenium intake levels for dairy cattle should range from 0.2 mg/kg DM (GfE 2001) whereas the recommendations by the British authorities are 0.1 mg/kg DM (MAFF 1983). Supplementation of this nutrient to dairy animals can be one of the best options, not only to protect the animal from disease threats, but also to raise the selenium level in milk and subsequently transfer this essential element to the human population, many of whom are marginal deficient in selenium.

2.5 Biomarkers for Selenium Status

The scientific controversy regarding the identification of the best biomarker for selenium status assessment is still unresolved. In dairy cows, several approaches have been followed to assess the status of the herd or the individual animal. These approaches include the direct estimation of selenium in whole blood, serum or plasma, milk and others tissues of interest; and indirect measures such as the intra- and extra-cellular activity of the selenium containing enzyme, glutathione peroxidase (GSHPx) in whole blood, serum or plasma. A number of studies have shown that serum selenium or GSHPx activity represents the short-term selenium status, while parameters for the whole blood or erythrocytes reflect the long-term selenium status. Stowe and Herdt (1992) determined the reference range of serum selenium level of 70-100 ng/ml. This value has been described as an adequate level. Earlier reports (Maus et al. 1980; Detoledo and Perry 1985) suggested that an adequate selenium level in blood serum should be in the range of 40-120 ng/ml. Variations in these findings may be the result of dietary concentration and nutritional management practices. Gerloff (1992), on review of the data from various research groups, considered the value of 70-100 ng/ml for serum selenium as a consensus of opinion

regarding the adequacy of selenium, particularly when the dietary source is inorganic selenate or selenite.

With the discovery, that glutathione peroxidase (GSHPx) has selenocysteine as its essential component; the activity of this enzyme has been regarded as the pertinent parameter for the assessment of selenium status. Although numerous studies have associated the activity of GSHPx with the selenium status of the animal because of a linear response of GSHPx activity with selenium supplementation, GSHPx activity as the parameter of selenium status assessment has been criticized (Stowe and Herdt 1992). Inconsistency of units used in expressing the enzyme activities, difficulty in ensuring the proper storage conditions of samples, enzyme concentrations that reach a plateau while serum selenium concentrations continue to rise and delayed response to supplementation and different cellular and extra cellular forms are all points which need to be taken into account when considering GSHPx activity as a criterion for selenium status of the animal. On the other hand, the relationship between GSHPx and health is better explained than between plasma selenium concentration and health. Awadeh et al. (1998a) showed that only one-third of total selenium intake is incorporated into GSHPx, and that GSHPx activity is largely confined to the erythrocytes.

Milk selenium concentrations can potentially be used as a simple parameter for the selenium status assessment of dairy herds. In a study conducted with large dairy herds over several seasons, a sigmoid relationship with an adjusted R^2 value of .92 ($P < 0.0001$) was observed between the bulk tank milk selenium and mean serum selenium values (Wichtel et al. 2004). A plateau effect was noted in serum selenium concentrations when milk concentrations exceeded 20 $\mu\text{g/l}$. Tentative reference values for bulk tank milk selenium have been generated based on the relationship observed. Milk selenium concentrations less than 9.6 ng/ml are considered to indicate a deficiency, whilst a value of 21.8 ng selenium/ml appears to represent an adequate selenium supply. The value 15.7 ng/ml is the median between the marginal range of the low and high categories. However, it is notable that the source of the selenium has not been kept considered while making the bulk tank milk selenium as an accurate measure of the herd selenium status. Many studies have reported that milk selenium concentrations were significantly higher when diets were

supplemented with selenium yeast as compared to sodium selenite at the same level (Ortman and Pehrson 1999; Muniz-Naveiro et al. 2005; Juniper et al. 2006). Positive correlations, irrespective of the source of selenium supplementation, of 0.59, 0.64 and 0.68 have been observed between the cows' milk and their calves erythrocytes GSHPx activity, whole blood, and plasma selenium concentrations, respectively (Pehrson et al. 1999). A cautious estimate of the herd selenium status can be made by bulk tank milk selenium concentrations, keeping the source of selenium supplementation in mind.

The source and dietary level of the nutrient are important in determining the nutritional status of the animal. Different supplements of selenium are categorised based on organic and inorganic forms. Sodium selenite and sodium selenate are common inorganic forms whereas the organic form of selenium is produced from the yeast *Saccharomyces cerevisiae*, with almost 90% of the total selenium represented by selenomethionine (Muniz-Naveiro et al. 2005). As far as the bioavailability of selenium from organic versus inorganic sources is concerned, whole blood selenium concentration, GSHPx activity and milk selenium concentration in dairy cattle increase more efficiently after dietary selenium supplementation using organic sources compared to inorganic ones (Malbe et al. 1995; Awadeh et al. 1998b; Knowles et al. 1999; Ortman and Pehrson 1999; Gunter et al. 2003). However, selenium yeast and selenite follow a similar pattern of distribution among serum proteins (Awadeh et al. 1998b). Cattle fed selenium yeast have a higher percentage of selenium in whole blood (average 20%), milk (average 90%) and increased activity of GSHPx (16%) compared to cattle fed inorganic selenium (Weiss 2005). Previously, Knowles et al. (1999) had reported no difference in the blood GSHPx activity between cows fed selenite and those fed a selenium yeast compound, provided the cows consumed 4 mg/day of supplemental selenium (approximately 0.2 ppm). However, when cows were fed 2 mg/day, the GSHPx activity was 50% higher than when selenium yeast was used as source of dietary selenium. Comparative increases in milk and blood selenium levels after supplementing the diet of cows with a selenium yeast source have been largely attributed to non-specific incorporation of selenomethionine from the diet into the tissue proteins (Weiss 2005). However, Juniper et al. (2006), after conducting a study with selenium supplementation in the range of 0.27-to 0.4 ppm from a selenium-containing yeast source, reported that only

25-33% of total milk selenium increase could be attributed to selenomethionine and that there are other selenoproteins in milk, which might play a role as an antioxidant.

Interactions between the selenium status of dairy cows and the udder defence system have been explored. Parameters of milk somatic cells and microbial counts, incidence and duration of clinical mastitis cases in dairy herds, and controlled experiments with or without the experimental challenge of pathogenic microbes, have been the prime focus in this area. With the advent of selenium yeast products on the market, research is now focussing on safety and comparative efficacies. Based on the information cited above, it can be inferred that the selenium status of the animal is directly correlated with dietary level and source, and organic selenium sources tend to be comparatively more efficient in maintaining the selenium status of the animal than are inorganic sources.

2.6 Somatic Cell Count and Selenium Status

The somatic cell count (SCC) of milk is used as a benchmark parameter to estimate udder health and consequently milk quality. Cell concentration of the milk varies widely as a function of the lactation cycle. In healthy udder conditions, very few leukocytes should migrate into milk during full lactation. At cessation of milking, the SCC might increase owing to the intense physiological changes occurring in the udder. Milk from a healthy bovine udder should contain very few somatic cells (< 20,000/ml), and whenever the SCC rises above 20,000/ml, there has been histological evidence of inflammation in the udder (Schalm et al. 1971). Rainard and Riollet (2006) reported that the SCC in most uninfected and uninflamed quarters is considerably less than 100,000/ml, with a low portion of neutrophils, which can increase up to 40% near the drying off period. Somatic cell concentrations increase to reach $2-5 \times 10^6$ /ml during the first 7-10 days of the dry period. They then remain stabilized in the range of $1-3 \times 10^6$ /ml. After parturition, the SCC decreases to 10^5 /ml in the first 7-10 days after calving.

Higher SCC values in milk reflect a diseased udder making the milk less valuable. It is evident (Table 2) that milk SCC is negatively correlated to the selenium status of the animal. It was reported that the cow's udder is more prone to infection if GSHPx activity in the blood is below $3.3 \mu\text{kat/g}$ of haemoglobin (Malbe et al. 2003). Lack of

GSHPx activity causes oxidative damage to soft tissue, thus making the udder more vulnerable to mastitis pathogens. Consequently, infiltration of neutrophils in the udder tissue will cause the SCC to rise to higher levels. The effective role of neutrophils in combating the microbial threat is also dependent on GSHPx activity. Enhanced viability and vitality of neutrophils in response to optimum GSHPx activity could be a plausible explanation for the low SCC in the milk of cows having improved selenium status and consequent enhanced GSHPx activity.

Few studies failed to find a correlation (Grace et al. 1997; Wichtel et al. 2004) between the selenium status of cows and disease susceptibility. This has been attributed to the fact that the data involved the results of surveys conducted with herds having different management practices. Marginal bulk tank milk selenium levels of (0.018 µg/ml), and corresponding marginal serum selenium levels, could have been the reason why Wichtel et al. (2004) did not find any substantial relationships between bulk tank milk selenium levels and the general parameters used to assess udder health.

2.7 Mastitis Susceptibility and Selenium Status

Low selenium status is linked to increased susceptibility of dairy cows to intramammary infections (Table 2). Marked reduction (up to 60%) in infected mammary gland quarters has been observed in dairy cows after selenium supplementation for a period of 8 weeks at 0.2 ppm dietary level (Malbe et al. 1995; Ali-Vehmas et al. 1997). Duration of clinical mastitis was reduced by 46% in cows supplemented with selenium and by 62% in cows supplemented with selenium and vitamin E (Smith et al. 1984).

Supplementation with selenium and/or vitamin E at levels far above those required for growth and normal physiological function can result in the improvement of various components of the immune system and general animal health (Surai 2006). This is particularly important for cows infected with pathogens. In an experiment described by Hemingway (1999), 14 of 36 cows receiving intramammary antibiotic infusions at drying off needed extra treatment in the subsequent lactation whereas only 5 of 36 cows which received additionally 4 mg selenium at drying off needed such treatment. Udder health benefits have been attributed to antibacterial activities against *S.*

aureus in milk whey protein (Ali-Vehmas et al. 1997; Malbe et al. 2006). The underlying mechanism of this antibacterial activity is not well understood. However, it was proposed that impaired microbial growth rate in the whey fraction exhibiting high GSHPx activity may account for the results. The absence of both glutathione and GSHPx in bovine milk has been reported (Stagsted 2006). Therefore, further generation of more reactive radical oxygen species by phagocytes or the presence of other selenoproteins in milk may account for the results obtained. It can be concluded that selenium may affect mastitis susceptibility of the mammary gland by improving the phagocyte recruitment to the infected quarters, increasing their vitality and inducing unspecified antibacterial activity in milk whey against various pathogens.

2.8 Mammary Gland Immune System – Interactions with Selenium

The immune response is characterized by heterogeneity of reactive cells and their products, having specificity for the response and memory following subsequent antigen exposures. The bovine mammary gland produces colostrum which is rich in antibodies that can protect the newborn from infectious agents (Sordillo et al. 1997). The bovine mammary gland is itself protected by a variety of defence mechanisms, which can be separated into two distinct categories: innate immunity and adaptive immunity, each having sensing and effectors arms (Rainard and Riollot 2006). The innate and acquired immune systems interact closely in an attempt to provide protection against pathogens (Sordillo et al. 1997; Burvenich et al. 2003). The acquired immune response uses many innate immune effector mechanisms to eliminate microorganisms and its action frequently increases innate antimicrobial activity (Oviedo-Boyso et al. 2007). The efficacy of the adaptive immune response rests in its specificity, memory of the immune cells and also, to some extent, on the immune stimulus, which is augmented by repeated exposure to the antigen. On the other hand, innate immunity is non-antigen-specific, exists prior to the encounter with the pathogens, and is related to the processes of acute and chronic inflammation and sepsis (Finlay and Hancock 2004).

2.8.1 Physical Barriers

The first lines of defence against foreign molecules and invading pathogenic microorganisms are the natural physical barriers of the body. Mastitis can occur when bacteria gain entrance into the mammary gland via the teat canal. The teat end contains sphincter muscles that maintain tight closure between milkings and hinder bacterial penetration. Increased patency of these muscles is directly related to an increased incidence of mastitis (Murphy and Stuart 1953; Myllys et al. 1994). The teat canal is lined with keratin, which is crucial to the maintenance of the barrier function of the teat and removal of the keratin correlates with increased susceptibility to bacterial invasion and colonization (Capuco et al. 1994; Sordillo and Streicher 2002). Teat keratin is a waxy material derived from stratified squamous epithelium that traps invading bacteria and exhibits bactericidal properties (Hibbitt et al. 1969; Craven and Williams 1985). Esterified and non-esterified fatty acids (myristic, palmitoleic and linoleic) function as bacteriostatic agents, and are associated with keratin of the teat canal (Miller et al. 1992). More recently, it has been noted that certain cationic proteins associated with keratin can bind to pathogenic microorganisms, thus increasing their susceptibility to osmolarity changes leading to the lyses and death of the invading pathogens (Paulrud 2005). Because of the efficacy of the teat canal barrier, the intra-mammary lumen is an aseptic chamber to which the aseptic character of normal milk can be attributed. Thus, the teat canal is an important barrier against intra-mammary infections.

There may be a role for selenium in teat canal keratin function as it has been found that in mammalian spermatozoa phospholipid hydroperoxide glutathione peroxidase, a selenoprotein, is functionally associated with the cross linking of the structural elements of the cytoskeleton via the oxidation of high sulphur keratin-associated proteins (Maiorino et al. 2005a; Maiorino et al. 2005b). There is no direct evidence of the association of selenium with the bovine mammary gland teat canal.

2.8.2 Cellular Factors

Bacteria and other pathogens, upon entry into the body tissues, are only able to cause disease by overcoming the body's natural cellular defence mechanism. Different types of cells in combating the pathogens play a pivotal role. Cellular factors

of the bovine mammary gland immune system come from two main types: the mammary epithelial cells (MECs) and the immune cells comprising macrophages, neutrophils, Natural Killer (NK) and dendritic cells. Collectively these constitute the somatic cells of the milk.

Mammary epithelial cells (MEC) were previously considered the major cell type in milk (Schalm et al. 1971). However, a later study confirmed that MECs are rarely found in the milk and the major cell type of the tissue and secretion of the bovine mammary gland is the macrophages (McDonald and Anderson 1981). The presence of sub- and intra-epithelial leukocytes, and the repertoire and distribution of sensor receptors on MECs makes the immune system of the mammary gland peculiar, resembling the urinary tract system and differing from the intestine (Rainard and Riollot 2006). Mammary epithelial cells express mRNA for TLR 2, 4 and 9 and β -defensin 5, thus contributing positively towards the sensing of pathogens (Goldammer et al. 2004). Adhesion of bacteria and the interaction of bacterial toxins with the epithelial cells has been reported to induce the synthesis of tumour necrosis factor alpha (TNF- α), interleukin-6 (IL-6) and IL-8 (Rainard 2003).

Phagocyte Responses

Much of the uptake of foreign antigens is performed by macrophages, neutrophils and natural killer cells in the mammary gland. During the defence of the mammary gland against bacterial infection, tissue and milk macrophages recognise the invading pathogen and initiate the inflammatory response by releasing pro-inflammatory cytokines (TNF α - and IL-1 β), that induce neutrophils recruitment to the mammary gland (Bannerman et al. 2004).

Macrophages are the major cell type in milk, secretions of the involuted udder, and mammary tissue (Jensen and Eberhart 1981; McDonald and Anderson 1981). Although macrophages can ingest common mastitis pathogens, they are less active phagocytes than are milk neutrophils. Furthermore, both milk cell types are less efficient than their blood counterparts (Mullan et al. 1985). In addition to phagocytic activity, macrophages also play a role in antigen presentation (Politis et al. 1992) and are responsible for the removal of neutrophils following the elimination of bacterial pathogens. The functional capabilities of mammary macrophages decrease markedly

during periparturient periods and this alteration has been linked to an increased mastitis incidence (Waller 2000; Sordillo and Streicher 2002). Apart from the stress associated with parturition and the start of lactation, the underlying mechanism of the periparturient immunosuppression is still unclear.

Ndiweni and Finch (1995) worked with bovine mammary gland macrophages obtained from cows fed a selenium adequate diet. They investigated the effect of various doses of vitamin E, sodium selenite and combination of both on cellular functions in vitro. Sodium selenite supplementation in vitro from 1 nM-10 μ M to *S. aureus*-stimulated macrophages enhanced the production of chemotactic factors significantly ($P < 0.003$). Similar effects were recorded with vitamin E supplementation in the range from 5 ng/ml to 50 μ g/ml. There were no synergistic effects of both nutrients. Concentrations of selenium above 0.1 mM depressed chemotaxin production. It was suggested that the stimulatory effect of selenium might be attributed to its role as cofactor of LTB₄ synthase or hydase, as peritoneal macrophages from rats fed selenium-deficient diets are not able to produce a respiratory burst reaction and as a result, their antimicrobial function is compromised (Parnham et al. 1983).

Neutrophil numbers in normal milk from healthy bovine mammary gland are too low for efficient phagocytosis (Leijh et al. 1979). Pro-inflammatory cytokines released by macrophages and MECs activate the expression of cellular adhesion molecules by endothelial cells that cause the binding and subsequent migration of blood neutrophils from blood to the site of infection, or in the milk where they are further localised. Following bacterial entry into the mammary gland, neutrophils are the first cells that are recruited into the milk and represent the predominant cell type. Neutrophils recruitment from the circulation to the site of infection is essential in the defence of the mammary gland against invading bacteria. The promptness of the recruitment and the number of recruited neutrophils, which vary in intensity according to pathogen type and the cow, determines the outcome of the infection.

Neutrophil concentrations increase rapidly between 3-12 h post-challenge and can reach more than 10^7 /ml in milk following *E. coli* infusion in the mammary gland, whereas in the case of a *S. aureus* challenge, the recruitment is delayed (between

24-48 h and remains below 10^6 /ml (Riollet et al. 2000; Rainard and Riollet 2006). Recruited neutrophils at the site of infection phagocytose bacteria and produce reactive oxygen species, low molecular weight antibacterial peptides, and defensins, which eliminate a wide variety of pathogens (Mehrzhad et al. 2002; Paape et al. 2002; Sordillo and Streicher 2002; Paape et al. 2003). The increase in the concentration of milk neutrophils is in fact the origin of high SCC during mastitis and this is the reason why their presence is inversely correlated with the risk of intramammary infections (Burton and Erskine 2003).

The most important and widely investigated association between selenium and the immune function in dairy cows is the effect of this micronutrient on neutrophils function. Neutrophils perform their microbe killing function by producing super-oxide derived radicals. This type of process is a balance between sufficient radical production for microbial killing and the system that protects the neutrophils themselves from these radicals. This balance is attributed to the cytosolic glutathione peroxidase activity within the neutrophils, which is impaired in selenium deficiency, which permits neutrophils to be self-destroyed. The earliest evidence regarding the effect of selenium on neutrophils function was reported by Boyne and Arthur (1979). In that study, it was noted that the ability of neutrophils to phagocytose *Candida albicans* cells was not different ($P < 0.05$) between selenium-deficient and selenium-supplemented calves receiving 0.1 mg of dietary selenium/day. However, the number of neutrophils with the ability to kill phagocytosed *C. albicans* cells was about three times less for selenium-deficient animals having undetectable levels of blood GSHPx activity. On the other hand, both phagocytosis ($P < 0.05$) and killing ($P < 0.01$) of *S. aureus* by blood PMN leukocytes were higher ($P < 0.05$) when the dairy cows received between 10-17 mg selenium/day, along with an additional 350-1000 mg vitamin E/day for a period of 16 days (Gyang et al. 1984). However, phagocytosis by neutrophils from cattle supplemented with selenite or selenate at low levels (2 mg/day or 0.2 mg/kg DM, respectively) was not different from that of neutrophils from unsupplemented cows.

Direct and indirect measures of bacterial killing were higher ($P < 0.05$) in neutrophils isolated from selenium-supplemented cattle as compared to those from unsupplemented cows (Grasso et al. 1990; Hogan et al. 1990). In a survey

conducted by Cebra et al. (2003) higher blood selenium levels (> 300 ng/ml) were associated with enhanced neutrophils adhesion and intracellular kill by the neutrophils obtained from post parturient cows. With PMN cells isolated from the blood of selenium-adequate cows, it was found that in vitro supplementation of selenium (10 µM) had greater stimulatory effect (129%) on their random migration than did vitamin E (71%) and, at the highest concentration of selenite used (1 mM), random migration of PMN was inhibited (Ndiweni and Finch 1996). On the other hand, vitamin E enhanced phagocytosis of *S. aureus* to a greater extent than did sodium selenite after a 2 h incubation period (Ali-Vehmas et al. 1997). Both nutrients were not significantly different in their ability to stimulate PMN cells to produce superoxide. Enhanced recruitment of neutrophils at the site of infection in selenium-supplemented cows has also been reported previously (Ali-Vehmas et al. 1997).

Organic and inorganic sources of selenium at 0.3 mg/kg DM intake have been compared for their effect on the function of neutrophils obtained from the blood of lactating cows (Weiss and Hogan 2005). There were no significant differences regarding either the ability of neutrophils to phagocytise bacteria or the percentage of *E. coli* that were killed, although there was a slight increase in the percentage kill for the selenium yeast group. These observations agree with those of Malbe et al. (1995) regarding the effect of selenium source on bovine neutrophils' phagocytosis of *S. aureus*. A plausible explanation for this effect might be the non-specific pooling of selenomethionine from organic selenium sources into tissue proteins instead of methionine and the presence of 0.2% sulphur in the diets. However, it is difficult to interpret such data, as a negative control was not included. More recently, Mukherjee (2008) has reported an improvement ($P < 0.05$) in phagocytosis of *S. aureus* by milk neutrophils obtained from mastitic riverine buffaloes that had been injected with a selenium/vitamin E preparation containing sodium selenite and had been treated with enrofloxacin.

Lymphocyte Responses

Long-term cellular specific immunity is a function of both antigen-presenting cells and lymphocytes, which are the only cells of the immune system that recognize antigens by membrane receptors specific to invading pathogens. If the invading pathogens survive the activities of macrophages and neutrophils, T and B lymphocytes and

monocytes become the predominant cell type. Leitner et al. (2003) observed that lymphocytes were the most common infiltrating cell type within the two-layer epithelium lining the teat cistern; monocytes and macrophages were present in lower number. Nevertheless, neutrophils remain most important in chronic mastitis (Rainard and Riollot 2006). T lymphocytes are classified into two main groups: T $\alpha\beta$ and T $\gamma\delta$. T $\alpha\beta$ include CD4⁺ (helpers) and CD8⁺ (suppressors) cells. In healthy mammary glands CD8⁺ lymphocytes are the prevailing type, whereas in mastitis infected mammary glands CD4⁺ cells are predominantly activated by the formation of a molecular complex between the major histocompatibility complex class II (MHC II) and antigens presented by B lymphocytes and macrophages (Park et al. 2004). Through their ability to secrete certain cytokines, CD4⁺ cells help B lymphocytes to proliferate and secrete antibodies. CD4⁺ cells are mainly found in the inter-alveolar tissue of the mammary gland whereas CD8⁺ cells surround the alveoli (Leitner et al. 2003).

In contrast with the milk, cells obtained from blood exhibit a higher ratio of CD4⁺ to CD8⁺ cells; however, the functional significance of this elevated frequency has not been clearly established. CD8⁺ cells may be either cytotoxic or suppressor type. Post partum they are mainly of the cytotoxic type, whereas during mid and late lactation they are of the suppressor type (Sordillo et al. 1997). Cytotoxic T cells recognise and eliminate altered self cells via antigen presentation in conjunction with MHC I molecules. They act as the scavengers of old and damaged secretory cells and their secretions are related to the susceptibility of the bovine mammary gland to infections (Oviedo-Boyso et al. 2007). Although T $\gamma\delta$ cells are not well characterized, they are associated with the epithelial surface where they destroy damaged epithelial cells (Yamaguchi et al. 1999).

Natural Killer cells, B cells and dendritic cells are also part of the bovine mammary gland immune system. Natural Killer (NK) cells are large granular lymphocytes that have cytotoxic activity independent of MHC, through antibody-dependent cell mediated cytotoxicity. In contrast to neutrophils and macrophages, they are critical to the removal of intracellular pathogens. Bovine NK-like cells (CD2⁺ CD3⁻ T Lymphocytes), express bactericidal activity against *S. aureus* upon stimulation with IL-2 in a non-specific manner (Sordillo et al. 2005). These cells destroy both gram

positive and gram-negative bacteria and are fundamental to the prevention of bovine mammary gland infections (Sordillo and Streicher 2002). The primary role of B lymphocytes is to produce antibodies against invading pathogens. In doing so, they utilize their cell surface receptors to recognize specific pathogens and process the antigens. Processed antigens are thus presented to T helper cells, which secrete cytokine IL-2 that, in turn, induces the proliferation and differentiation of B lymphocytes into either plasma cells that produce antibodies or memory cells. Not much is known about the density and role of dendritic cells in the bovine mammary gland immune system. Normally they are associated with antigen presentation.

It has been suggested that selenium and vitamin E deficiencies affect T lymphocytes to a greater extent than B lymphocytes (Larsen et al. 1988). This was suggested to be the result of higher levels of polyunsaturated fatty acids in T lymphocytes and associated with higher membrane fluidity. Selenium and vitamin E deficiencies may affect both the maturation of specific lymphocyte subpopulations and proliferative capabilities of peripheral lymphocytes (Surai 2006). In an experiment with dairy cows fed either basal diet (~ 0.05 mg selenium/kg DM) or a diet supplemented with sodium selenite (~ 0.20 mg selenium/kg DM), it was noted that Con A stimulated lymphocyte proliferation was significantly higher in the selenium-supplemented group (Cao et al. 1992). Similar findings have been reported when bovine peripheral blood lymphocytes were supplemented with sodium selenite in vitro from 1 nM to 10 μ M concentrations (Ndiweni and Finch 1995).

Selenium supplementation or deficiency in mice altered the kinetics of IL-2 receptor expression (Roy et al. 1994). Supplementation in vitro or in vivo resulted in an earlier expression of high affinity IL-2 receptors, whereas selenium deficiency resulted in a delayed expression of receptors. This may explain the stimulatory role of selenium in the enhanced T cell function. In healthy aged humans, selenium supplementation (400 μ g/day, for 6 months) enhanced NK cell cytotoxicity over pre-treatment levels by 58% (Wood et al. 2000). There is no information on the effect of selenium on NK cell and dendritic cell function in dairy cows. It is interesting to note that enhanced immune cell function resulted from selenium supplementation levels, which are higher than normally recommended.

2.8.3 Soluble Factors

Soluble factors of the bovine mammary gland immune system are made up of various proteins that include complement proteins, cytokines and immunoglobulin. Each class performs its physiologically defined function with a high level of specificity.

The bovine complement system is a collection of proteins that is present in serum and milk, and has an important role in the defence of the mammary gland. Complement proteins are predominantly produced by hepatocytes, though they are also produced by monocytes and macrophages in different tissues. In the presence of antibodies, they lyse invading pathogens. Complement component C3b binds the antibody bacteria complex for efficient phagocytosis by neutrophils and macrophages (Paape et al. 2003) whereas C5a stimulates the recruitment of neutrophils, which augments their phagocytic and bactericidal activities (Rainard and Poutrel 2000).

Cytokines are produced by both immune and non-immune cells and are essential in almost all aspects of host defence. They regulate the activities of cells involved in the immune function. A variety of cytokines such as interleukins (IL) -1 β , -2, -6, -8, -12, colony stimulating factor (CSF), interferon gamma (IFN- γ) and TNF- α have been detected in healthy and infected bovine mammary glands (Sordillo and Streicher 2002; Alluwaimi 2004). TNF- α is the main cytokine produced by macrophages, neutrophils and epithelial cells during the early stage of infection and participate in the neutrophil chemotactic activity (Persson et al. 2003). CD4⁺ and CD8⁺ lymphocytes and NK cells in response to mitogenic and antigenic stimuli produce IFN- γ . Interferon- γ functions in activating the acquired immune response and phagocytic activity of neutrophils and is important in viral infections (Shtrichman and Samuel 2001). Monocytes, macrophages, and epithelial cells produce IL-1 β . During the inflammatory response, IL-1 β regulates the expression of adhesion molecules and neutrophils chemotaxis in *E. coli* infections (Yamanaka et al. 2000). IL-2, produced by CD4⁺ lymphocytes, regulates the acquired immune response by stimulating the growth and differentiation of B lymphocytes and the activation of NK and T cells. Alterations in IL-2 production cause a decrease in the mammary gland

immune response capacity, which facilitates mastitis (Sordillo et al. 1991; Sordillo and Streicher 2002).

Immunoglobulins (Ig) are synthesized by plasma cells that are differentiated from B lymphocytes upon activation by IL-2. In milk, immunoglobulin either are synthesized locally or originate from blood (Sordillo and Nickerson 1988). The role of antibodies in the natural defence mechanisms of the udder is to opsonise bacterial pathogens, thereby aiding the neutrophils and macrophages in phagocytosis.

Four classes of Ig are known to influence mammary gland defence against bacteria causing mastitis: IgG1, IgG2, IgA and IgM. Each of these classes differs in physicochemical and biological properties (Gershwin et al. 1995). The concentration of each immunoglobulin in the mammary secretion varies with the stage of lactation, increasing during dry periods and approaching peak concentrations during colostrumogenesis (Sordillo and Nickerson 1988). The largest part of the opsonic antibodies in adult serum and milk of cows is IgM (Williams and Hill 1982; Hill et al. 1983). The presence of IgM in cows, without a previous history of mastitis, suggests that they are mainly auto-antibodies directed against self-antigens and are poly-reactive in nature (Rainard and Riollot 2006). In this regard, the cow is not different from humans or rodents, who also have these types of antibodies in their blood (Saini et al. 1999).

Non-specific proteins such as lactoferrin, lysozyme, transferrin, xanthine oxidase and the lactoperoxidase system, exhibit bacteriostatic and bactericidal activities against common mastitis pathogens. In normal function, various components of the innate and adaptive immune system are coordinated to provide protection to animals against invasion by pathogens.

Improved selenium status of animals results in enhanced immunoglobulin titre in colostrum from cows receiving a high dose of selenium, administered by intramuscular injection pre-partum (Pavlata et al. 2004). Earlier studies reported lower ($P < 0.05$) concentrations of IgG and IgM in plasma and colostrum of beef cows and calves fed a free-choice salt/mineral mixture containing 20 ppm selenium as sodium selenite, compared to the cows and calves fed a salt mixture containing 60

ppm selenium in the form of selenium yeast compound, or 120 ppm sodium selenite. The source of selenium affects only the IgM concentration of plasma with higher concentrations ($P < 0.05$) when cows are fed a selenium yeast supplement (Awadeh et al. 1998b).

It was recommended that consideration should be given to the concentrations of T3 (thyroid hormone) and IgG whilst determining the nutritional requirement of cattle for selenium. Swecker et al. (1989) also confirmed higher concentrations of colostral IgG in beef cows fed higher selenium levels in free choice mineral mixtures. Enhanced proliferation of B lymphocytes in cell cultures containing 100 ng/ml selenium suggests a mechanism for the increased IgM production (Stabel et al. 1991). Contradictory observations of a no effect ($P < 0.05$) of selenium on the immunoglobulin have also been reported (Lacetera et al. 1996; Leyan et al. 2004). It is noteworthy that positive effects have been observed only when higher selenium doses were used. Depression in several leukocyte function parameters, including the forced antibody response, was noted in pre-parturient beef cows consuming 6 ppm or 12 ppm selenium as sodium selenite from their diets (Yaeger et al. 1998).

Studies on the interactions of selenium with the immune system of the mammary gland, general udder health and mastitis susceptibility are summarized in Table 1 and Table 2. There is little data on the effect of selenium on cytokines and other soluble factors in dairy cows available.

Table 1 Dairy cattle immune responses as affected by selenium

Reference	Study Type	Selenium Supplementation/Concentration	Selenium Source	Immune Response Studied	Matrix	Observations
Weiss and Hogan (2005)	Control Experiment with <i>E. Coli</i> challenge	0.3 mg/kg DM in diet	Selenium yeast and Sodium Selenite	Neutrophil function	Blood	Neither phagocytosis nor percentage kill was significantly affected by selenium source
Pavlata et al. (2004)	Control Experiment	44-88 mg IM injection	Sodium Selenite	Immunoglobulin	Colostrum	Significant increase ($P < 0.05$) in turbidity units was observed in supplemented group
Cebra et al. (2003)	Survey	> 300 ng/ml in blood	Sodium Selenite	Neutrophil function	Blood	Increased adhesion of neutrophils and increased intracellular kill along with higher milk production in cows with high selenium status
Panousis et al. (2001)	Control Experiment	IM injection of 0.1 mg/kg body weight	Sodium Selenite	Specific antibodies against <i>E. Coli</i>	Serum	Serum concentration of specific antibodies against <i>E. coli</i> increased ($P < 0.05$) in supplemented cows at day 63
Cao et al. (1992)	Control Experiment	0.05-0.2 mg/kg DM	Sodium Selenite	Lymphocytes	Blood	Significantly enhanced ($P < 0.05$) lymphocytes proliferation with Con A was observed in the cells from selenium supplemented cows during 48-96 hours
Grasso et al. (1990)	Control Experiment with <i>E. Coli</i> challenge	2 mg/day in diet (90 days)	Sodium Selenite	Neutrophils function	Milk	Phagocytosis remained unaffected but significant increase ($P < 0.05$) in killing of ingested bacteria was observed in supplemented cows

Table 2 Bovine udder health and mastitis susceptibility as affected by selenium

Reference	Study Type	Selenium Supplementation /Concentration	Selenium Source	Parameters Studied	Observations
Mukerjee R. (2008)	Control Experiment	1.5 mg/day in the form of intramuscular injection (5 days) 4 mg/day in diet (8 weeks)	Sodium Selenite	SCC GSHPx	SCC decreased significantly ($P < 0.05$) from 2961×10^3 to 630×10^3 in buffaloes screened positive for intra mammary infections whereas GSHPx activity increased significantly
Malbe et al. (2006)	Control Experiment	Se-yeast		Milk proteins antibacterial activity against <i>S. aureus</i> GSHPx	Selenium supplemented cows exhibited profound antibacterial activity in milk whey fractions when the activity of blood GSHPx increased significantly from $< 1.02 \mu\text{kat/g Hb}$ to $> 4 \mu\text{kat/g Hb}$
Kommisrud et al. (2005)	Survey	20-230 $\mu\text{g/l}$ in blood	Unspecified	SCC Mastitis Retained Placenta	Significantly low ($P = 0.03$) bulk milk SCC ($137 \times 10^3/\text{ml}$) was observed in herds with high blood selenium level as compared to $155 \times 10^3/\text{ml}$ in herds with low blood selenium level. Reduced incidences of disease treatment regarding mastitis and retained placenta were observed in animals with high blood selenium levels
Jukola et al. (1996)	Survey	191 $\mu\text{g/l}$ in blood	Unspecified	SCC Incidence of clinical mastitis	A 17.7% and 70.6% decrease in infections caused by <i>S. aureus</i> and <i>Corynebacterium</i> species respectively was found to be associated with high blood selenium level
Wichtel et al. (1994)	Control Experiment	6-12 mg/day (whole lactation)	Sodium Selenite	SCC	Significant decrease ($P < 0.02$) in SCC from the level of $235 \times 10^3/\text{ml}$ to $112 \times 10^3/\text{ml}$ in different herds with selenium supplementation was observed
Maddox et al. (1991)	Control Experiment with <i>E. coli</i> challenge	0.05-0.35 mg/kg DM	Sodium Selenite	Milk bacterial count	Milk bacterial count was significantly higher ($P < 0.05$) in selenium-deficient group and this group required therapeutic treatment where as supplemented group recovered without therapeutic intervention
Weiss et al. (1990)	Survey	70-90 $\mu\text{g/l}$ (herd mean plasma)	Unspecified	SCC Incidence of mastitis	Significant ($P < 0.05$) negative correlations ($-0.84, -0.68$) were observed between herd mean plasma selenium concentration and SCC in the range of $724-744 \times 10^3/\text{ml}$ and mastitis incidence during the whole lactation

2.9 Concluding Remarks

Survey findings and controlled studies with or without experimental challenge indicate a role for selenium in the immune function and improvement in bovine mammary gland health. Although selenium status has been noted to increase markedly as a result of the supplementation with selenium yeast as compared to inorganic sources, whether this increase is completely translated in terms of health benefits to the animal is not clear. Most recent findings have confirmed that selenium levels higher than those considered adequate can potentially enhance the natural defence mechanisms of the bovine mammary gland at maximum, especially the humoral responses.

There are limited studies on the clinical aspects of the health of the bovine mammary gland, as affected by organic versus inorganic selenium sources, or a combination of the two sources of selenium. Moreover, there are many gaps in our knowledge of the interactions of selenium with the immune function of the bovine mammary gland. Neutrophilic function has been the major point of focus of the research. Other aspects of the immune response, notably, the activity of Natural Killer (NK) cells as affected by selenium supplementation in combating both gram-positive and gram-negative mastitis pathogens, has not been studied. Furthermore, certain cytokines and mammary epithelial cells and lymphocyte proliferation response have great implications for mammary gland health and mastitis control. More work is required to delineate these interactions.

3. MATERIALS AND METHODS

3.1 Feeds and Animals

The experiment was performed with 16 pluriparous Holstein-Friesian cows maintained at the research station of German Federal Institute for Risk Assessment (BfR) in Marienfelde, Berlin (50°, 24.6', N; 13°, 22.1'). The research station is facilitated with the modern individual feeding chambers and milking parlour. The individual cow data were recorded using leg-band transponders fitted on the cows. All the experimental cows were in between their 1st and 3rd lactation and calved during June 2008 to February 2009. At drying off, cows were blocked based on parity and expected calving date into three groups (5, 5 and 6 cows) in a way to have minimum variation regarding the parity between different groups and then randomly allotted to receive additional supplementation. Each cow received either selenium supplement or placebo individually in addition to the basal diet (Table 3) containing 0.15-0.20 mg selenium/kg DM presuming a dry matter intake of 10 Kg daily and offered in the form of total mixed ration during the experimental period. Organic and inorganic selenium supplements were prepared by mixing the ground corn with Sel-Plex-1000 (Batch No. 71658-2, CNCM-I 3060) and sodium selenite (Na_2SeO_3) supplements obtained from the local feed company to give the final selenium content of 200 mg/kg in the product. All the cows were given a three months adaptation period with the basal diet before the start of the experiment. Each cow was fed 20 gram of either supplement or placebo at the time of morning milking during the pre partum period and 30 g during the post partum experimental period. Supplementation corresponded to an additional intake of 4 and 6 mg selenium/day during the prepartum and postpartum experimental phases respectively.

Table 3 Composition of total mixed ration (TMR) fed as basal diet during the feeding trial

Ingredients	%TMR	Selenium ($\mu\text{g}/\text{kg DM}$)	$\pm\text{SEM}$	n
Maize silage	75.5	12.2		2
Hay	4.4	20.6		2
Straw	4.4	27.0		2
Beet pulp	4.4	153.2	16.66	6
Soybean meal	2.2	227.4	15	5
Rapeseed meal	4.4	87.6	9	3
Vitamin-mineral mix ¹	4.4	1680.5	110.4	5
Milk concentrate ²		177.7	10.22	9
Nutrient composition				
Dry matter (%)	49.5		0.88	6
Crude protein (%DM)	10.7		0.005	6
Crude fat (%DM)	2.7		0.005	6
Crude ash (%DM)	5.3		0.015	6
Crude fiber (% DM)	17		0.15	6
Neutral detergent fiber (%DM)	53.1		4.05	4
Acid detergent fiber (%DM)	17.3		0.277	4

1 Contains 5.5% Ca, 1.5% P, 2.5% Mg, and 4.2 % Na, 460000 IU vitamin A, 33500 IU vitamin D3, 500 IU vitamin E and 490 mg CuSO₄.5H₂O per kg; 3.6 MJ NEL/kg

2 Contains 0.78% Ca, 0.5% P, 0.3% Na, and 10000 IU vitamin A, 800 IU vitamin D3, 90 IU vitamin E and 13 mg CuSO₄.5H₂O per kg; 7.0 MJ NEL/kg; offered extra as 1 kg for every 3 kg increase in milk production during the lactation

Table 4 Mineral composition (DM basis) of total mixed ration (TMR) fed as basal diet during the feeding trial (n=3)

Minerals	Mean	$\pm\text{SEM}$
Calcium (g/kg)	5.6	0.06
Phosphorus (g/kg)	3.4	0.02
Sodium (g/kg)	1.6	
Magnesium (g/kg)	2.2	
Potassium (g/kg)	10.7	0.07
Manganese (mg/kg)	87.0	0.73
Copper (mg/kg)	19.0	1.09
Cobalt (mg/kg)	0.7	0.06
Zinc (mg/kg)	136.0	0.54
Iron (mg/kg)	398.7	2.58
Selenium (mg/kg)	0.18	0.01

3.2 Sampling

All the procedures regarding the management and sampling from the animals were approved by the Landesamt für Gesundheit und Soziales (LAGeSo). Cows were sampled 6 and 3 weeks before anticipated calving, within 12 hours after the calving, and 1 and 12 weeks after calving for blood samples. Colostrum and milk samples were collected at day 1 after calving and 1, 9, 12, and 15 weeks after calving. Aliquots of milk samples were frozen at -80°C for subsequent analysis.

3.3 Chemicals and Instruments

Selenium standard solution (1000 mg selenium/l) and hydrochloric, nitric and perchloric acids were purchased from Merck (Darmstadt, Germany). Skim milk powder (Standard reference material, NIST 8435) was obtained from LGC Standards (Wesel, Germany) whereas, 2, 2'-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS), Trolox standard antioxidant (6 Hydroxy, 2, 5, 7, 8-tetramethylchroman 2-carboxylic acid) and activated manganese oxide were purchased from Sigma-Aldrich (Steinheim, Germany). The atomic absorption spectrometer (Vario 6 equipped with H52 hydride system and auto sampler) was made by Analytik Jena AG (Jena, Germany) whereas microplate reader (Sunrise TC) was from Tecan (Salzburg, Austria).

3.4 Estimation of Selenium

Total selenium in feeds, supplements and milk was estimated by the hydride generation atomic absorption spectrometry (HG-AAS). Samples were digested using a programmable electrically heated digestion block (Tecon, TZP-500). The digestion process was carried out in a mixture of nitric and perchloric acids by using the quartz digestion tubes. In the end stage of digestion process 6M hydrochloric acid was added in the tubes to reduce selenium (VI) to selenium (IV) for hydride generation in the system. The samples were diluted before measurement to a final volume of 40 ml. The method was standardised using whole milk powder (Standard reference material, NIST 8435). The analyses of the milk standard reference material resulted in $121.8 \pm 6.62 \mu\text{g}/\text{kg}$ (mean \pm SD, $n=15$) as compared to the reference range value of $131.0 \pm 14 \mu\text{g}/\text{kg}$. Reference standards were used for every twenty analyses. Ultra pure deionised water of $18.2 \text{ M}\Omega \text{ cm}$ (4 ppb TOC) obtained from Milli-Q apparatus was used for making dilutions and washing.

3.5 Estimation of Antioxidant Activity

Total antioxidant activity was measured using the Trolox Equivalent Antioxidant Capacity (TEAC) according to the method of Miller et al. (1996). However, the method was modified keeping in view the changes suggested by Wang et al. (2004) regarding the endpoint measurement and adapted to carry out large number of samples in the standard conditions using the microplate plate reader. The TEAC assay was originally based on the suppression of the absorbance of the radical cations of 2, 2'-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS) by antioxidants in the test sample when ABTS (Figure 4) incubates with peroxidase (metmyoglobin) and H₂O₂. The modified procedure requires the production of long living radical cation (ABTS^{•+}) by the action of ABTS and activated manganese oxide. Briefly, pure ABTS was dissolved in 5 mM PBS buffer with pH 7.4 to have a final solution of 5 mM ABTS. The solution was filtered through Wattmann filter paper across the activated manganese oxide while keeping it under light protection for 12-16 hours for efficient radical generation. The filtrate was finally passed through 0.2 μm syringe filter (VWR-cellulose acetate) and kept under light protection. Standard calibration curve was generated with the average of two values corresponding to blank, 50, 100, 150, 200 and 250 μM/l Trolox solution prepared from 97% Trolox standard antioxidant (6-hydroxy-2, 5, 7, 8-tetramethylchroman 2-carboxylic acid) for the each run. The absorbance was recorded at 620 nm after the inhibition period of 20 minutes in the 96-well micro plate containing 190 μl of ABTS^{•+} and 10 μl pre-diluted sample (milk).

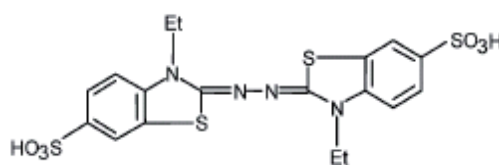


Figure 4 Chemical structure of ABTS molecule

3.6 Statistical Analysis

Data obtained was analysed statistically using SPSS 15 (Chicago, USA). Dunett's test of Post Hoc comparisons was performed for significance testing of the means of various groups in a multivariate ANOVA (Field 2005). This test was applied because it tests the significance of means of treatment groups in comparison with that of a control. Level of statistical significance was set as 0.05 during the data analysis. Correlations and regression equations were computed using the same software.

4. Results

4.1 Colostrum and Milk Selenium Status

The mean (\pm SEM) selenium level in colostrum for the control, Sel (Sodium Selenite) and SeY (selenium yeast) groups was 35.3 ± 1.03 $\mu\text{g/l}$, 39.1 ± 2.56 $\mu\text{g/l}$ and 67.7 ± 4.11 $\mu\text{g/l}$ respectively in this study. Statistical analysis has revealed that mean colostrum selenium content of the SeY group is different ($P = 0.032$) from that of the Sel and control group animals ($P = 0.018$). Furthermore, no difference ($P = 0.754$) has been observed between the Sel and control groups regarding colostrum selenium levels. In control group, colostrum selenium content ranged from 20.6 – 60.4 $\mu\text{g/l}$, whereas for Sel and SeY groups this range was found to be as 25.9 - 58.0 and 39.9 – 106.7 $\mu\text{g/l}$ respectively. The large variation in colostrum selenium content might be attributed to the genetic factors and to some extent to the health problems as the intake of the supplement and the placebo was not largely different in all groups.

In milk, a decrease of 60, 42 and 35 percent has been observed in selenium content after one week of calving for the control, Sel and SeY groups respectively. It can be assumed from the results of the present research that milk selenium content has a declining trend still the steady state is obtained after about 12 weeks of milking. The average steady state milk (\pm SEM) selenium content for the control, Sel and SeY groups has been noticed as 11.6 ± 1.55 , 15.4 ± 3.24 and 28.3 ± 6.84 $\mu\text{g/l}$, respectively. Statistical analysis of the data revealed that control and Sel groups milk selenium content at first week after calving was nearly different ($P = 0.072$) and after ninth weeks of calving it differed significantly ($P < 0.05$). No difference ($P > 0.05$) could be found between control and Sel groups after 12 and 15 weeks of the experimental period. It could also be observed that SeY group exhibited more variations in terms of standard deviations as compared to both others. The results have been shown graphically in the following figure 5.

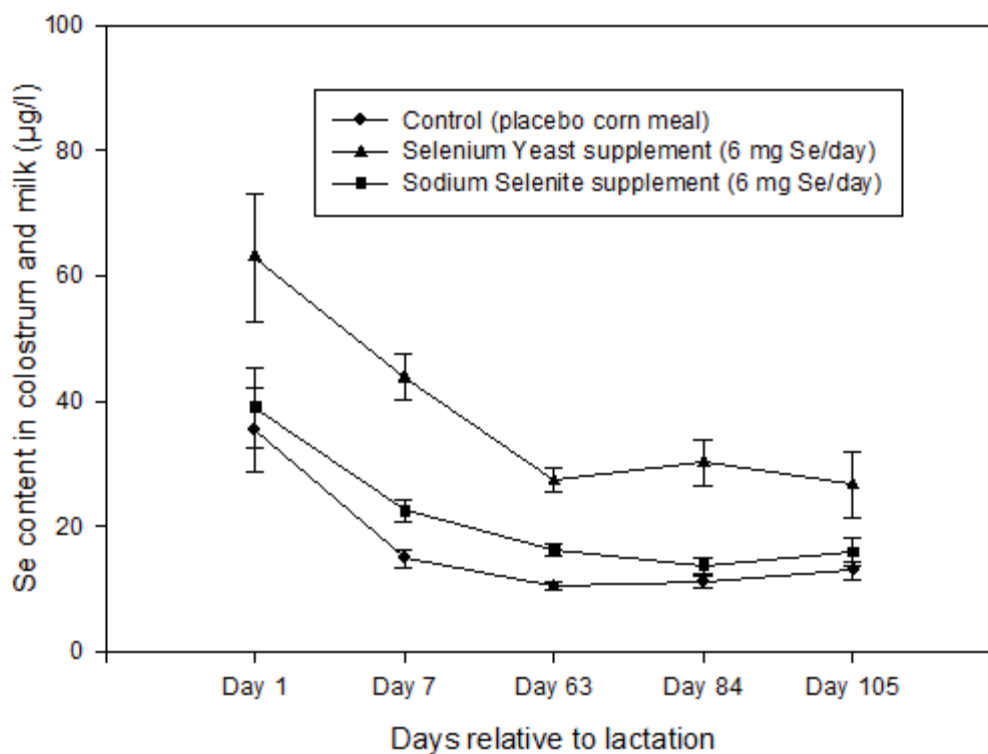


Figure 5 Colostrum and milk selenium concentrations in various treatment groups.

Each graphical symbol represents the mean \pm SEM of respective treatment groups. SeY group differs ($P < 0.05$) from other groups at all time points. Sel and control cows are not different ($P > 0.05$)

4.2 Milk Trolox Equivalent Antioxidant Capacity (TEAC)

Milk Trolox equivalent antioxidant capacity (TEAC) values for various treatment groups in the study have been graphically represented in Figure 6. The results are the mean of duplicate values measured for each sample on the specific time point regarding the day of lactation. Each group's samples have been measured on a separate 96 well microplate. Separate calibration curves for Trolox concentrations ranging from 0-250 $\mu\text{M/l}$ generated for each microplate. From the regression equation and the trend line it was evident that standard calibration curves exhibited good linearity ($R^2 = .998 - .999$) and were within the comparable absorption range. It has been noted that TEAC values for the SeY group are significantly different ($P < 0.001$) from that of control and Sel groups at all time points and Sel group differed ($P < 0.001$) from that of control in the same manner. However, negligible differences have been observed between different time points in all groups. Milk TEAC values of (mean of all time points \pm standard error) have been observed as $586 \pm 0.95 \mu\text{Mol/l}$,

557 ± 0.97 μMol/l and 540 ± 0.64 μMol/l for the SeY, Sel and control groups respectively.

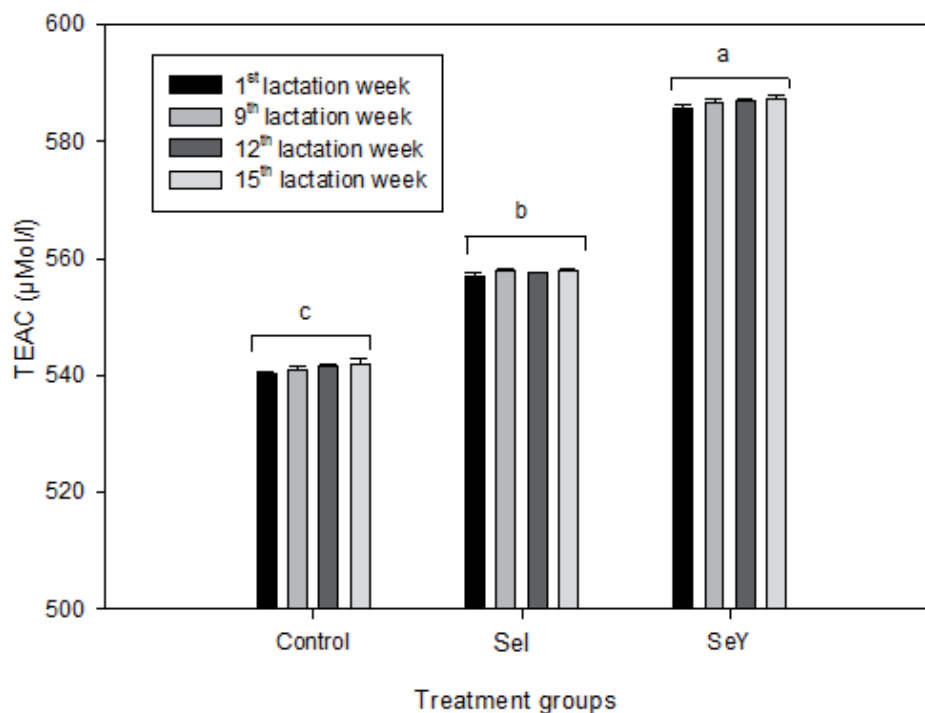


Figure 6 Milk TEAC values at various lactation stages in different groups.

All groups differ ($P < 0.01$) from each other. There is no difference within the groups ($P > 0.05$)

4.3 Milk Production

Milk production data has been presented in the Table 5. Milk yield was recorded digitally at the milking parlour during the milking process of the individual cows. Milk samples from each cow were taken fortnightly during the experimental period for subsequent analysis of the milk nutrients. No differences among the control and treatment groups have been observed regarding the milk and nutrient yield. Weiss and Hogan (2005) have reported the same previously. It can be assumed from the milk and nutrients yield data that experimental cows were normal producers.

Table 5 Milk and nutrients yield in different treatment groups during the feeding trial

	<i>n</i>	Milk		Fat		Protein			Dry Matter		Lactose	
		kg / day										
Control Cows	5	26.01 ±1.88	0.97 ±0.02	(3.72)	0.83 ±0.05	(3.2)	3.21 ±0.10	(12.36)	1.40 ±0.16	(5.4)		
SeI Cows	5	22.47 ±4.46	0.98 ±0.22	(4.36)	0.71 ±0.13	(3.18)	3.00 ±0.60	(13.36)	1.14 ±0.22	(5.09)		
SeY Cows	6	26.98 ±3.75	1.07 ±0.15	(3.96)	0.84 ±0.11	(3.12)	3.48 ±0.48	(12.9)	1.36 ±0.18	(5.07)		

* All values are represented as mean ± standard error of mean (n=7). Values in parentheses are %age nutrient content. Nutrient contents and milk yields are not different ($P > 0.05$) for various treatment groups

Table 6 demonstrates that treatment groups did not differ much regarding their reproductive and udder health. However, it can be noted that SeI group was less concerned whereas in both other groups' number of affected animals remained three during the experimental period. Seeing the total number of treatments, it can be observed that udder health disorders are not much different whereas SeY group suffered more (9) as compared to SeI (2) and control (5) with regard to reproductive health disorders.

Table 6 Health status of experimental cows during feeding trial

Cows	No. of times treated	
	Udder Health	Reproductive Health
Control	1 + 4 + 1	2 + 1 + 2
SeI	1 + 4	1 + 1
SeY	2 + 3 + 1	3 + 1 + 5

Each digit represents an animal and number of treatments it received during the feeding trial.

4.4 Serum Selenium in Cows

Figure 7 exhibits the profile of serum selenium concentration in various treatment groups on different sampling time points starting from 6 weeks before anticipated calving until 12th weeks after calving. Experimental cows were not different ($P > 0.05$) before supplementation started at 6th week before expected calving. Trend lines for the control and treatment groups follow different pattern. Control group cows serum selenium content declines 3 weeks before calving and at the calving from 47.9 ± 5.94 µg/l (Mean ± SEM) to 38.5 ± 4.67 µg/l and 30.6 ± 9.88 µg/l, respectively, after which it appears to plateau around 35.0 ± 6.56 µg/l. Trend in both the supplemented groups resembles showing a relative decrease of 8% and 16 % at calving for SeY and SeI

groups reactively. This decrease at calving in control group was found as 30%. It has been noted that both groups differ significantly ($P < 0.05$) from that of control except that Sel groups is not different ($P = 0.141$) at calving from both others. However, it is indicated that in spite of substantial relative increase, 8-34% at various time points in the serum selenium level of selenium yeast supplemented cows, Sel and SeY groups are not different ($P > 0.05$) in raising the selenium content of serum in dairy cows. However, P values were noted to be decreasing as 0.476, 0.385, 0.178 and 0.08 at three weeks before calving, at calving, one week after calving and 12 weeks after calving respectively.

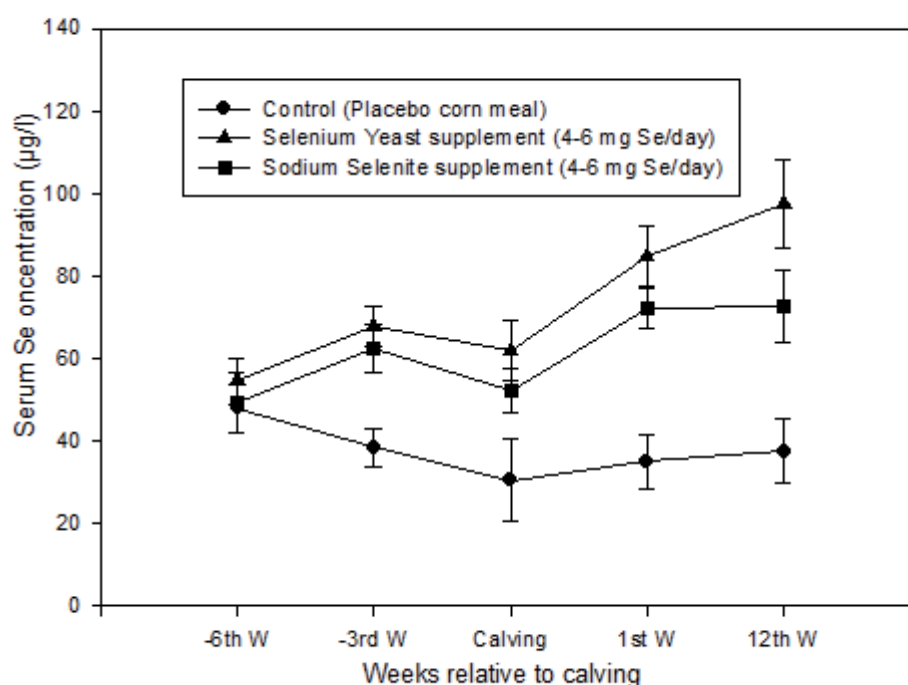


Figure 7 Serum selenium concentrations of dams in different groups during various physiological stages.

SeY and Sel groups differ ($P < 0.05$) from Control cows at all time points except at calving when only SeY group is different

4.5 Serum Selenium Level in Calves

Serum selenium content in calves from various treatment groups and at different time points has been shown in the following Figure 8. Some significant differences in groups and regarding time points have been observed. The control group was significantly different ($P < 0.05$) from SeY group at both the time point (within 12 hrs after calving and one week after calving). SeY group was also significantly different

($P < 0.05$) from Sel groups at both time points, however, Sel group calves were only different from control groups calves at calving in their serum selenium contents. Serum selenium content at calving for the control, Sel and SeY groups' calves has been noted to be (Mean \pm SEM) 23.5 ± 2.01 , 29.1 ± 3.5 , and 38.1 ± 0.96 $\mu\text{g/l}$ respectively. Whereas one week after calving respective contents increased for different groups as 28.4 ± 2.03 , 37.7 ± 1.23 and 47.7 ± 1.05 $\mu\text{g/l}$.

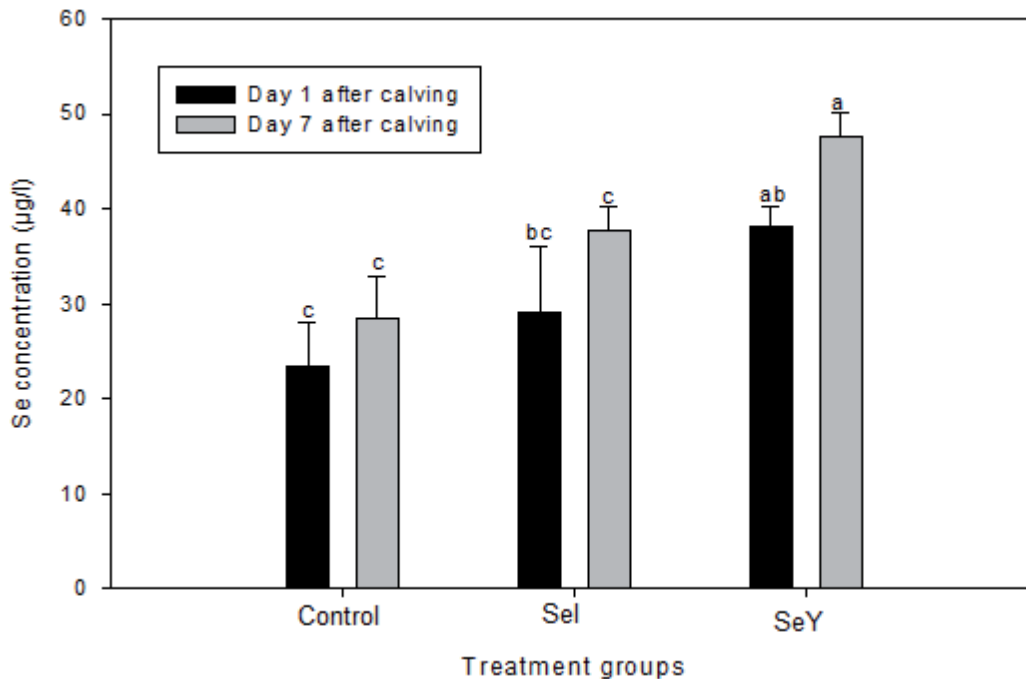


Figure 8 Serum selenium concentrations of calves borne to dams in different treatment groups.

Different superscripts within groups and time points denote significant differences ($P < 0.05$)

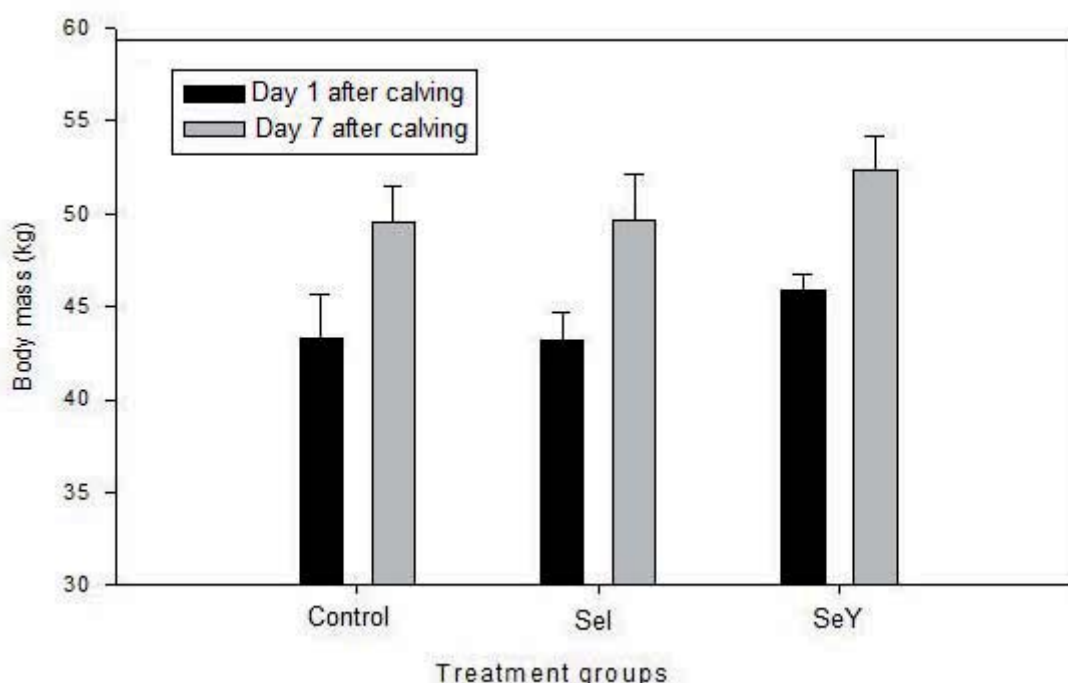
4.6 Body Mass of Calves

Body mass of dams and calves measured at various time points have been presented in Table 7. None of the values was different ($P > 0.05$). However, calves live body weight after one week of age was little higher (52 kg as compared to 50 kg) in SeY group. This can be attributed to the fact that 4 out of 5 calves in SeY group were male. Dams live body mass decreased at the end of the experimental period due to calves' birth.

Table 7 Body mass (kg) of cows and calves in various treatment groups

Groups	Cows (kg)		Calves (kg)		
	In the start	At the end	birth	After 1 week	
Control	654 ± 11	552 ± 47	43 ± 2	50 ± 2	$P > 0.05$
Sel	703 ± 19	633 ± 23	43 ± 1	50 ± 2 ¹	$P > 0.05$
SeY	672 ± 4 ²	644 ± 5	46 ± 1	52 ± 2	$P > 0.05$

1 n = 4 ; 2 n = 6 ; *All values are mean ± standard error rounded to nearest kg (n = 5). No difference among groups at a single time point ($P > 0.05$)

**Figure 9 Differences in body mass of calves at various time points**

No statistical difference among various groups could be noted.

4.7 Serum TEAC in Cows

Serum TEAC was measured in cows' samples obtained within 12 hours of calving, and one and twelve weeks after calving. The results of serum TEAC in dams have been presented in Figure 10. Although TEAC values were found within a narrow range (566 – 577 $\mu\text{Mol/l}$), statistical analysis revealed ($P < 0.001$) differences among the treatment groups. SeY group exhibited the highest values compared to other groups in the study. Slightly increased TEAC values at calving time suggest some sort of homeostatic mechanism to counteract the oxidative stress in this period. It has also been noted that SeY group is less different ($P = 0.056$) from other groups in

decrease in its TEAC values at 12 weeks of sampling time. No difference ($P > 0.05$) has been observed in TEAC at calving and one week after calving in all groups. The mean serum TEAC values (\pm SEM) for all time points in control, Sel and SeY groups have been found to be 566 ± 1.39 , 570 ± 0.61 and 577 ± 0.50 $\mu\text{Mol/l}$ respectively.

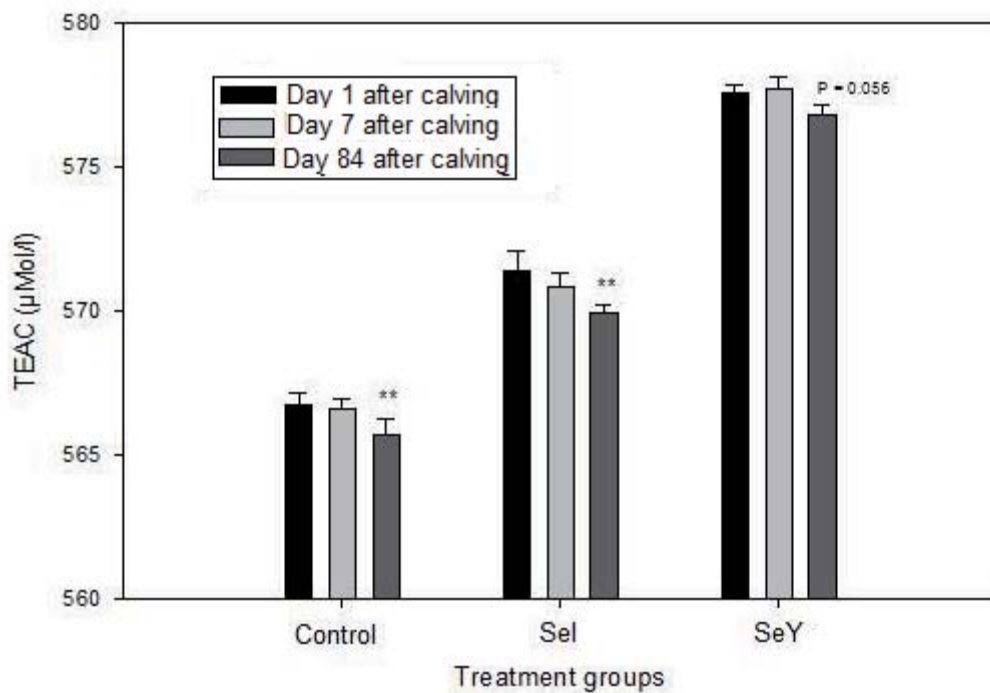


Figure 10 Serum TEAC values in cows of various treatment groups.

Groups differ with each other (** = $P < 0.01$)

4.8 Serum TEAC in Calves

Serum TEAC values were measured in calves within 12 hrs after birth and one week after calving. The results have been shown graphically in Figure 11. It is notable that TEAC values in calves' serum followed a decreasing trend as in dams regarding time after calving. However, in calves, TEAC values one week after calving were lower ($P < 0.01$) as compared to the values in prior samples obtained within 12 hrs after calving. This is also in contrast to their dams serum TEAC values, which were not found different ($P > 0.05$) one week after calving. The average TEAC values (Mean \pm SEM) for the control, Sel and SeY groups within 12 hrs after calving have been found to be 567 ± 0.25 , 571 ± 0.12 and 578 ± 0.5 $\mu\text{Mol/l}$ respectively. Whereas, one week after calving

TEAC values decreased in their respective groups as 566 ± 0.25 , 570 ± 0.16 and $577 \pm 0.15 \mu\text{Mol/l}$.

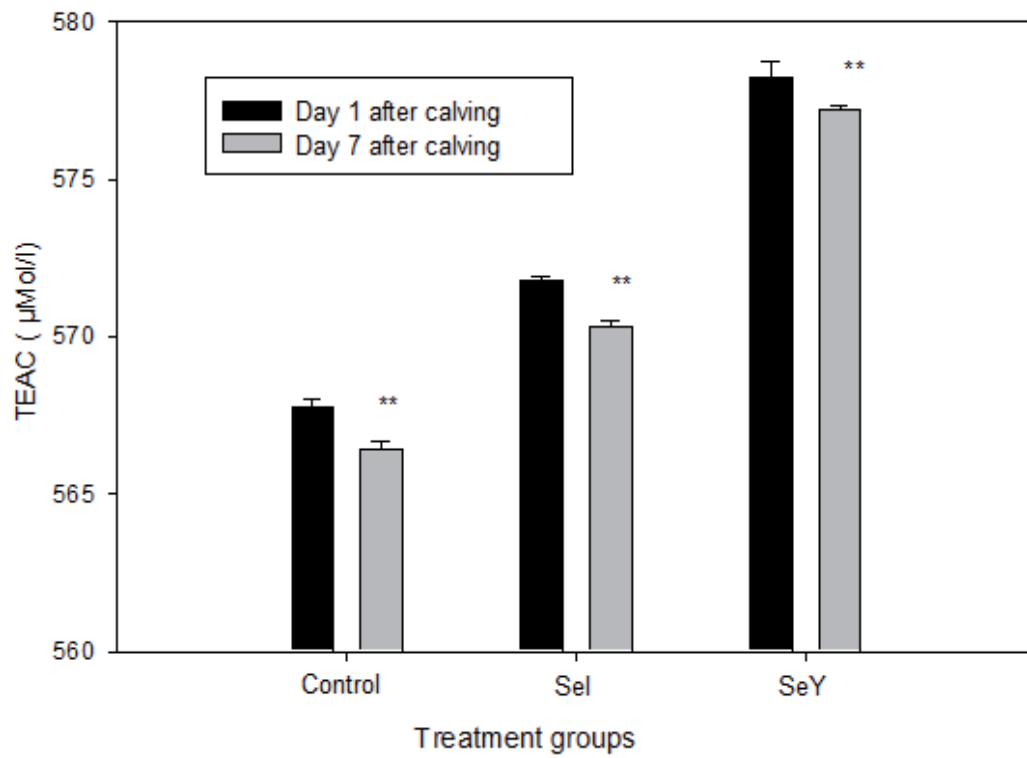


Figure 11 Serum TEAC in calves of various treatment groups.

Groups differ at $P < 0.01$ with each other

5. DISCUSSION

5.1 Colostrum and Milk Selenium Status

The mean colostrum selenium level in the SeY group has been found below the level (151 μ g/l) reported by Weiss and Hogan (2006) who supplemented the experimental cows rations at the level of 0.3 mg selenium/kg with selenium yeast and sodium selenite for a period of 60 days before the expected calving. This difference might be attributed to the daily intake and basal diet selenium concentrations as the relative increase in the colostrum selenium level of the selenium yeast group as compared to Sel group has been found exactly the same (1.73 times) in both the studies. It is important to note that no difference ($P = 0.754$) has been observed between the Sel and control groups regarding colostrum selenium levels. These findings are also in accordance with that of Awadeh et al. (1998a) who described no difference in colostrums selenium content among the cows consuming approximately 0.98, 3.3 and 7.3 mg selenium/day as Sel supplement. However, their reported values in colostrum (60-80 μ g/l) are much higher than those observed in this study are. This is probably because of the long duration of supplementation of one year before actually taking the colostrums samples for selenium analysis. Numerous researchers agreed that colostrum selenium content is much greater than normal milk (Abdelrahman and Kincaid 1995; Awadeh et al. 1998a; Ortman et al. 1999; Ortman and Pehrson 1999). It was found that selenium content in colostrum was 3.04, 2.4 and 2.54 times greater ($P < 0.05$) than the average milk selenium concentrations for the control, SeY and Sel groups respectively. These findings are in contrast with that of Weiss and Hogan (2005) who reported 3.8 times increase ($P < 0.01$) in colostrum selenium concentrations both in selenium yeast and inorganic selenium groups in their study.

In milk, a decrease of 60, 42 and 35 percent has been observed in selenium content after one week of calving for the control, Sel and SeY groups respectively. It can be assumed from the results of the present research that milk selenium content does not take a steady state after about one week of milking. A sharp decreasing trend until first week after calving is evident. However, prediction of the start of the plateau effect in milk selenium level from this study is difficult as the next sampling was done after ninth weeks of lactation. Up until ninth weeks after calving, the control and SeY groups seemed to attain a plateau level whereas Sel group was not harmonious with

other groups in this regard. The average milk selenium content for the control, Sel and SeY groups for all time points has been noticed as 11.6, 15.4 and 28.3 µg/l, respectively. Milk from SeY group cows differs significantly ($P < 0.05$) from that of control and Sel group cows at all time points considered in the study. On the relative percentile scale, selenium content of the milk obtained from cows supplemented with selenium yeast is 83 % higher than the milk from sodium selenite supplemented cows. This finding is overall in conformance with the results of studies reviewed by Weiss (2005), who cited a relative increase of 90%. Although milk selenium content of the Sel group has not been found significantly different ($P > 0.05$) from that of control cows, a relative increase of 32% has been noted in our study. These findings can be explained keeping in view the previous reports that supplementation with Sel increased milk selenium content when cows were fed rations low in naturally occurring selenium but there was less impact when cows were fed rations greater in naturally occurring selenium (Conrad and Moxon 1979). In addition, it was noted that an increase in selenium intake would not produce important increases in milk selenium content when cows were fed selenium adequate rations (Aspila 1991). A recent systematic review (Ceballos et al. 2009) of 42 studies regarding the effect of oral selenium supplementation on milk selenium concentrations in cattle has reported that in Americas, selenium supplementation of 6 mg/head per day in the form of selenium- yeast has resulted in cow's milk selenium content of 0.37 µmol/l (30 µg/l). Our study confirms this notion.

Higher levels of selenium in the milk of cows supplemented with selenium yeast can be explained with the proposition that selenomethionine in selenium yeast source replaces non-specifically methionine in the milk proteins following the genetic sequence for the incorporation of methionine in general proteins (Ortman et al. 1999; Weiss 2005). However, it has also been reported that only one third of total selenium is in the form of SeMet in the milk of cows fed selenium yeast as the supplement (Juniper et al. 2006) and therefore the possibility of presence of other selenoproteins with antioxidant properties cannot be ruled out. It can be concluded that more work is needed to delineate the incorporation of SeMet in different milk proteins and its effect on their functional properties. The knowledge regarding the dairy products quality made from the high selenium milk obtained after selenium yeast supplementation is still limited.

5.2 Total Antioxidant Capacity in Milk

Milk is a rapidly perishable food commodity and development of off-flavours due to the oxidation of various milk constituents is a major problem for the dairy industry. Therefore, it is important to study the complex interplay of the prooxidants and antioxidants in milk (Buettner 1993). Both fat soluble antioxidants and selenium compounds have been implied in the protection against development of milk off-flavour (Charmley et al. 1993; Jensen and Nielsen 1996). There is a scientific controversy whether glutathione peroxidase activity, which is the most important selenium-related antioxidant, is exhibited or not in milk (Chen et al. 2000; Stagsted 2006). In addition, there is lack of consensus among researchers regarding a standardized method for the milk total antioxidant capacity estimates. Trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) are two commonly used assays for the assessment of the antioxidant capacity of food components (Chen et al. 2003; Huang et al. 2005). We used the former method owing to its relative simplicity and for tailoring the large number of samples in the present study.

There is scarcity of the literature available on the topic of total antioxidant capacity as estimated by TEAC in bovine milk. Bovine milk TEAC values of 1246 and 4560 $\mu\text{Mol/l}$ measured after 10 minutes incubation time at different pH (Chen et al. 2003) 2649 $\mu\text{Mol/l}$ and ~ 5000 $\mu\text{Mol/l}$ measured after 3 and 20 minutes incubation time respectively (Zulueta et al. 2009) and ~ 4600 $\mu\text{Mol/l}$ measured after 60 minutes period of time (Clausen et al. 2009) have been reported. The same is the case with milk ORAC values, which have been reported between ~ 5000 -30000 $\mu\text{Mol/l}$. There might be many reasons for this non-conformity. It is evident that time factor is the most important consideration in these assays. Besides the variations in the measuring conditions, no information is available regarding feeding regime of cows from which milk was obtained. Milk samples analysed in this study are assumed to be low in their vitamin E content because the animals were fed below the normal dietary requirement for vitamin E in dairy cattle (National Research Council. 2001). One other reason for the comparatively low TEAC values might be the use of 620 nm wavelength in the present work. The absorption maxima for the ABTS radical have been reported as 414, 645, 734 and 815 nm (Re et al. 1999). In the present study,

we focussed on the issue of selenium source regarding their TEAC values in milk. It can be indicated that the differences obtained in milk TEAC values of various treatment groups might be attributed to their selenium content, which is significantly higher after supplementing the cows diet with selenium yeast. Although, the same trend has been observed in serum, more work is emphasized in this regard. This is implicated for maintaining the milk and dairy product quality. In addition, there is need to standardize the methods for total antioxidants capacity in milk.

5.3 Milk Selenium and TEAC Relationship

Highly significant Pearson correlation ($R^2 = .79$; $P < 0.001$) has been observed between milk TEAC values and selenium levels when data from all the groups was subjected to statistical analysis. Regression coefficients were calculated for the overall data and separately for the control and treatment groups (Figure 12 and Table 8). Although the overall model was found to be highly significant ($P < 0.001$), among the groups only Sel model was found statistically significant in explaining the positive correlations between TEAC and selenium level. These results further strengthen the idea that selenium may have an effect on the milk TEAC levels. Positivity of slopes in all models indicates that TEAC values are slightly increasing with the increase in selenium content. These observations are contrary to that found for the serum TEAC values, which decreased with the increase in serum selenium levels with the passage of time. A relatively blunt slope in SeY group might be attributed to the fact that selenium content approximately plateaus around 30 $\mu\text{g/l}$ in milk. Moreover, the mean selenium content in SeY group at 15th week of lactation was slightly less as compared to that of 12th week. Although this novel data indicates some sort of association between milk selenium and TEAC, further work will help delineate the mechanisms involved.

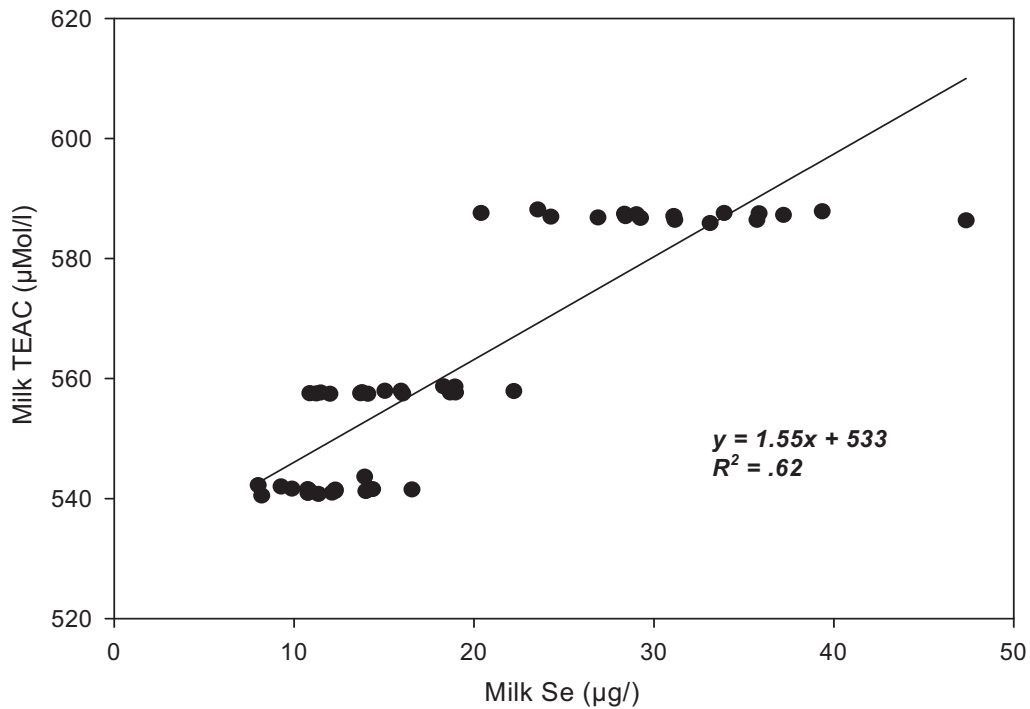


Figure 12 Overall milk selenium and TEAC regression model.

Each data point corresponds to individual cows data collected at 1, 9, 12 and 15 weeks after calving.

Table 8 Regression equations describing the relationship between milk TEAC and selenium levels in various treatment groups

Equation	Group	Dependent Var.	Independent Var.	n	R ²	Slope	SE	Intercept	SE	P <
1	Overall	Milk TEAC (µMol/l)	Milk Se (µg/l)	48	.62	1.55	0.17	533.56	3.87	0.001
2	Control	Milk TEAC (µMol/l)	Milk Se (µg/l)	15	.02	0.05	0.08	540.86	1.01	0.54
3	Sel	Milk TEAC (µMol/l)	Milk Se (µg/l)	15	.29	0.06	0.02	556.80	0.42	0.03
4	SeY	Milk TEAC (µMol/l)	Milk Se (µg/l)	18	.06	0.02	0.01	586.45	0.56	0.31

5.4 Milk Production

Milk production and nutrients yield data reveal that cows in experiment were normal producers and during the experimental period selenium supplementation had no profound effect on these parameters. No effect of selenium supplementation on the milk yields has also been previously reported (Weiss and Hogan 2005; Bourne et al. 2008). However, in a survey conducted in Prince Edward Island (Wichtel et al. 2004), it was noted that selenium-adequate herds had 7.6 % greater milk yield as compared to selenium-marginal herds.

5.5 Serum Selenium Content in Cows

Statistical analysis of the serum selenium data in dams has revealed that there is no significant difference between Sel and SeY groups. However, both treatment groups differ from the control cows. These findings seem to be in conformance with that of Juniper et al. (2006) who reported no difference ($P > 0.05$) in whole blood selenium levels of cows with total dietary selenium intake from selenium yeast and sodium selenite of 6.34 and 5.85 mg/day. The lack of significant differences between sodium selenite and selenium yeast in raising the blood or serum selenium levels can be associated with the dietary selenium intake. Significant difference was observed when cows were consuming 0.24-0.31 mg selenium/kg DM (Knowles et al. 1999; Ortman and Pehrson 1999). The relative increase in serum selenium levels of SeY group cows found in this study (8-34%) is in agreement with whole blood selenium levels in other studies reviewed (Weiss 2005). It is interesting to point out that serum selenium levels in the control cows are far below the reference values (70 $\mu\text{g/l}$) reported as indication of the adequate selenium status in dairy herds (Stowe and Herdt 1992) notwithstanding that dietary selenium intake from the basal diet (Table 3) was in accordance with the German recommendations (GfE 2001). These findings strengthen the results obtained previously in Germany (Gierus et al. 2002) reporting plasma selenium levels of 37.7 $\mu\text{g/l}$ with dietary selenium intakes up to 0.165 mg/kg DM. It can be speculated that cows might be at the risk of deficiency or at least vulnerability to disease threat. This provides a base for reconsideration of selenium dietary recommendation for dairy cows.

Decrease in the serum selenium levels at calving can be attributed to selenium transfer to calves. A relative increase of 30% has been noted in the calves born to dams of the SeY group as compared to ones in Sel group. This difference (30%) has not been found significant ($P = 0.33$) and is less (37%) than that observed by Weiss and Hogan (2005). However, one week after being fed on colostrums of their dams, a significant increase ($P < 0.05$) of 26 % has been noted in calves of SeY group when compared with those in Sel group. Again, this difference is comparatively less than reported previously regarding whole blood of calves (Awadeh et al. 1998a; Gunter et al. 2003). Possible reasons for this might be long supplementation duration in previous studies and high supplementation doses in the present investigation.

5.6 Selenium Transfer from Cows to Calves

Relationships between serum selenium levels of dams and their calves on the day of calving and one week after calving have been determined by regression analysis (Table 9). Overall regression model for all the groups has been presented in the Figure 13. It is evident that comparatively stronger relationship ($R^2 = .57$, $P < 0.01$) exists in the control group. This supports the idea that response to supplementation is weaker beyond the undefined threshold levels (Juniper et al. 2006). SeY group is also stronger ($R^2 = .41$, $P < 0.05$) than Sel group in transferring the dams selenium to calves.

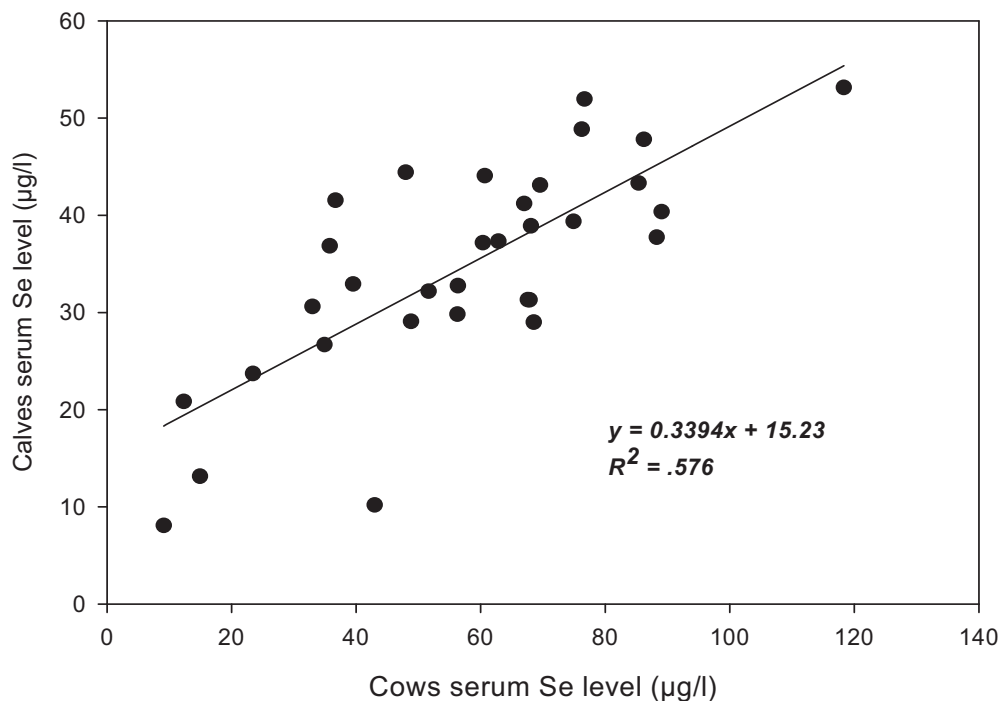


Figure 13 Regression model describing the relationship between dams and calves serum selenium levels.

Each data point corresponds to individual cows and calves data collected at calving and 1 week after calving.

Table 9 Regression equations describing the relationship between calves and dams serum selenium levels in various treatment groups

Equation	Group	Dependent Var.	Independent Var.	df	R ²	Slope	SE	Intercept	SE	P <
1	Overall	Calves serum Se (µg/l)	Cows serum Se (µg/l)	30	.57	0.33	0.05	15.23	3.30	0.0001
2	Control	Calves serum Se (µg/l)	Cows serum Se (µg/l)	8	.57	0.42	0.13	12.08	4.69	0.01
3	Sel	Calves serum Se (µg/l)	Cows serum Se (µg/l)	8	.19	0.35	0.19	11.62	11.31	0.207
4	SeY	Calves serum Se (µg/l)	Cows serum Se (µg/l)	10	.41	0.18	0.08	29.67	6.15	0.002

5.7 Serum Trolox Equivalent Antioxidant Capacity (TEAC) in Cows

The results of Trolox equivalent antioxidant capacity in serum have been found to act in accordance with those observed in the milk regarding the treatment effect. On the other hand, TEAC values in milk and serum differ with respect to the time point effect. Decreasing trend in TEAC values in serum, contrary to milk, has been noted. Regression analysis (Table 10) shows that slight negative correlations exist between the serum selenium and TEAC values within treatment groups, however, these models have been found non-significant with very low R² values in explaining the negativity of the slopes contrary to the overall model with stronger values (R² = .406; $P < 0.001$). Within the groups, SeY model seems to be comparatively stronger because selenium content in serum increased more sharply than other groups.

This data is novel as no previous report describes TEAC in serum of cows and calves. Although a strong indication of the effect of selenium on the total antioxidant capacity can be noted, further work in this regard can delineate the actual mechanism involved. It can be speculated from the contrasting findings in milk and serum regarding the effect of time on TEAC values, that different selenoproteins or selenium-containing proteins might be present in milk and serum.

Table 10 Regression equations describing the relationship between serum selenium and TEAC in dams

Equation	Group	n	R ²	Slope	SE	Intercept	SE	P <
1	Overall	48	.406	0.120	0.021	564.29	1.45	0.001
2	Control	15	.088	-0.044	0.039	567.23	1.51	0.282
3	Sel	15	.008	-0.004	0.013	571.02	0.93	0.749
4	SeY	15	.091	-0.011	0.007	578.64	0.54	0.117

Dependent variable = TEAC (μMol/l); Independent variable = selenium (μg/l)

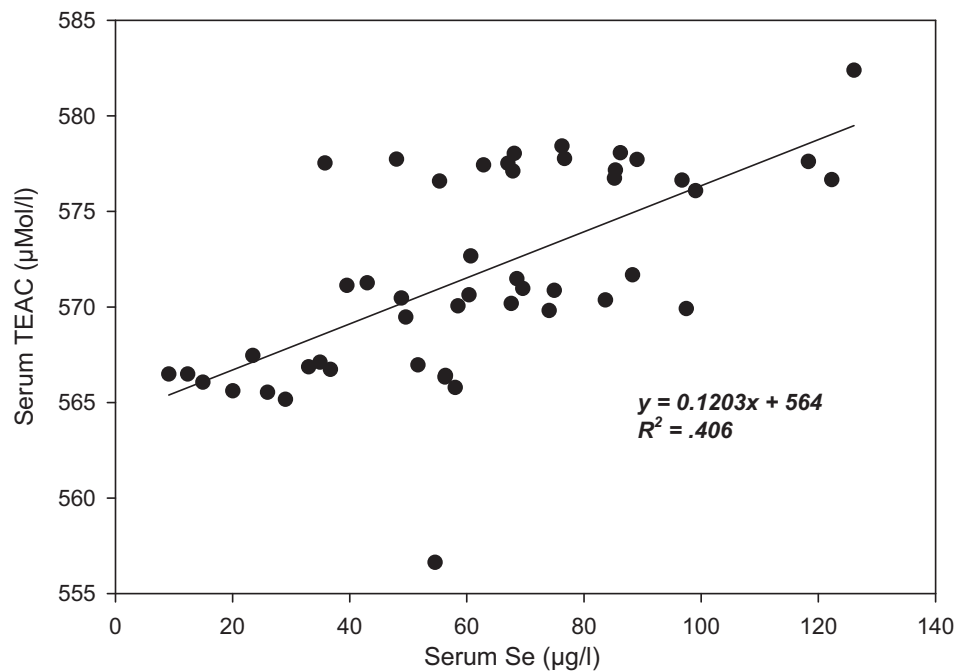


Figure 14 Regression model describing the relationship between serum selenium and TEAC in dams.

Each data point corresponds to individual cows' data collected at calving, 1 and 12 weeks after calving

5.8 Serum TEAC in Calves

Serum TEAC values in calves have been noted to follow a similar pattern regarding the treatment effect as noted in dams. Regression analysis (Figure 15) with selenium and TEAC values data has also generated a model quite similar to that for dams.

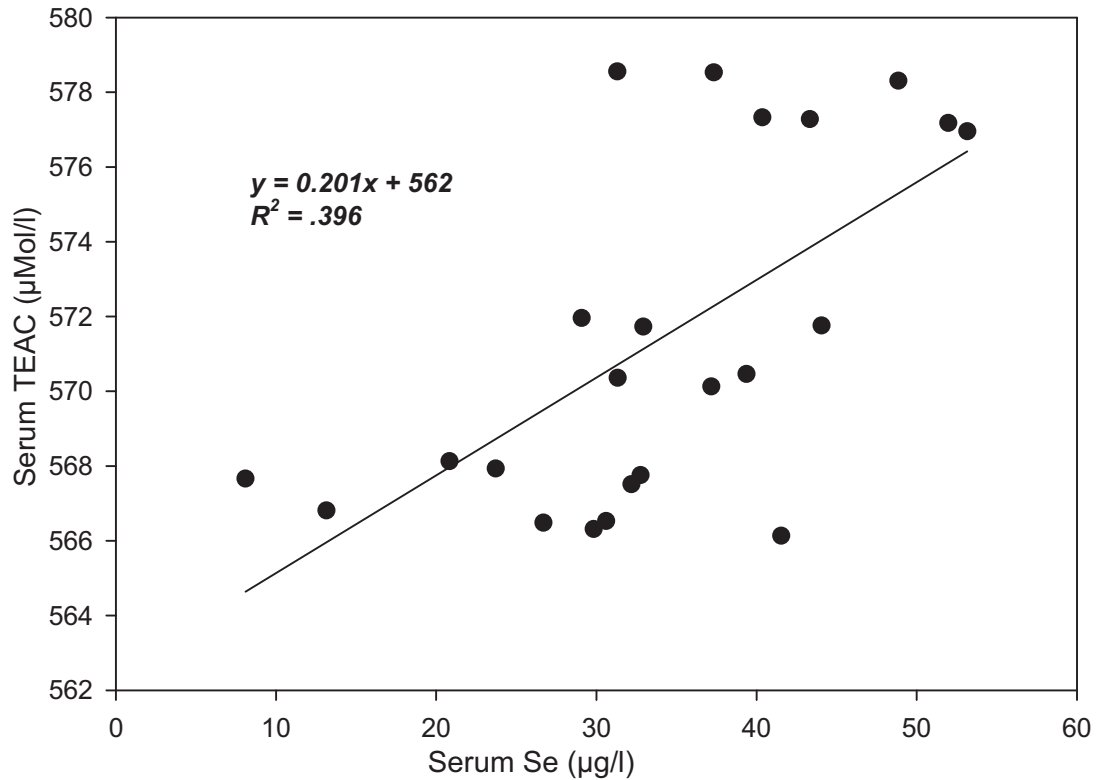


Figure 15 Regression model describing the relationship between calves' serum selenium and TEAC values.

Each data point corresponds to individual calves data collected within 12 hrs after calving and 1 week after calving

6. Trace Element Status in Large Dairy Herds

This work was supported by a grant from Sächsisches Landesamt für Umwelt, Landwirtschaft und Geologie. The author acknowledges the cooperation and support rendered by Dr. Steinhöfel and Mrs. Fröhlich.

6.1 Introduction

Scientific research over a long period has proved that many minerals are essential for the normal growth, physiological functioning and productivity of ruminants. Among these are included macro and micro minerals. Provision of adequate levels of trace metals in cattle diet is essential to promote growth and maintain animals in good health (Blanco-Penedo et al. 2009). Trace elements such as copper, zinc, manganese, iron and selenium are most important micro minerals for dairy cattle which are not only essential for the well being of the animals themselves but also have an importance for the public health owing to their transfer to them through the milk. As milk is an important component of the daily diets of human beings of all ages, deficiencies of these essential nutrients in dairy cows rations and consequently in the milk are likely to be reflected in the human populations. Moreover, trace metals that are included as mineral supplements may have toxic effects at supra-optimal concentrations (Underwood and Suttle 2002).

A number of ways can be applied to diagnose the possible deficiencies. Development of clinical symptoms and identification of post mortem tissue lesions can give some clue in this regard. However, differential diagnosis of any particular trace elements deficiency will be difficult because most of them do not show unique clinical signs or lesions for deficiency. In other instances, indirect proof of the deficiency can be provided by the positive response to supplementation of the suspected deficient mineral. This may not be incorrigible as time responsive effects of clinical signs might occur. It has been noted that trace elements are embedded in trace enzymes (Köhrle 2000). Although a difficult approach especially when large herds are concerned, the best way to establish the deficiency of a trace element is by testing for the unique functional deficit or the deficiency of the specific mineral containing protein or enzyme.

Estimation of the trace elements in various animal tissues of the representative samples can give an indication of the herds' mineral status. Usually the liver biopsy samples, whole blood or serum, milk, urine and hair samples can be used for the purpose. Liver biopsy is technically demanding in large populations of animals on the fields (Guyot et al. 2009). Plasma has been described as the most commonly used to assess copper and zinc status (Kincaid 2001). However, care must be taken about the feeding regimen, supplementation routine, disease condition and proper number of samples and standard procedures (Maas 2007) while making tissue analysis a criterion for the herd mineral status. Different antagonistic and positive correlations must also be kept in mind.

A survey of 11 selected farms concerning copper, zinc, manganese, iron and selenium was conducted to assess the trace element nutritional status of dairy cows and subsequent levels in liver and plasma samples. The objective was to study the intake, bioavailability and interactions among essential trace elements in large dairy herds under prevalent feeding practices. The impact on herd health was also a consideration.

6.2 Farms, Animals and Sampling

A survey of selected trace elements (copper, zinc, manganese, iron and selenium) was conducted for 11 large commercial dairy herds maintained in the state of Saxonia, Germany. The project was carried out with the support of Sächsisches Landesamt für Umwelt, Landwirtschaft und Geologie (Köllitsch). Liver biopsies and plasma samples were taken from 10 selected animals of each dairy farm. Twenty (20) samples of TMR were taken and analysed by the Landesamt for the whole nutrient composition and the data obtained were used for further statistical analysis. Liver biopsies and plasma samples were analysed for five selected trace elements composition at the Institute of Animal Nutrition laboratory, Freie Universität Berlin, using atomic absorption spectrometry (Vario 6 equipped with H52 hydride system and auto sampler, Analytik Jena AG, Germany). Analytical methods were standardised using reference standards (Atomic Spectroscopy Quality Control Standard 21, Perkin Elmer) and the results were within the permissible range (Figure 16). Plasma biochemistry was analysed by the Landeslabor Berlin-Brandenburg, Berlin.

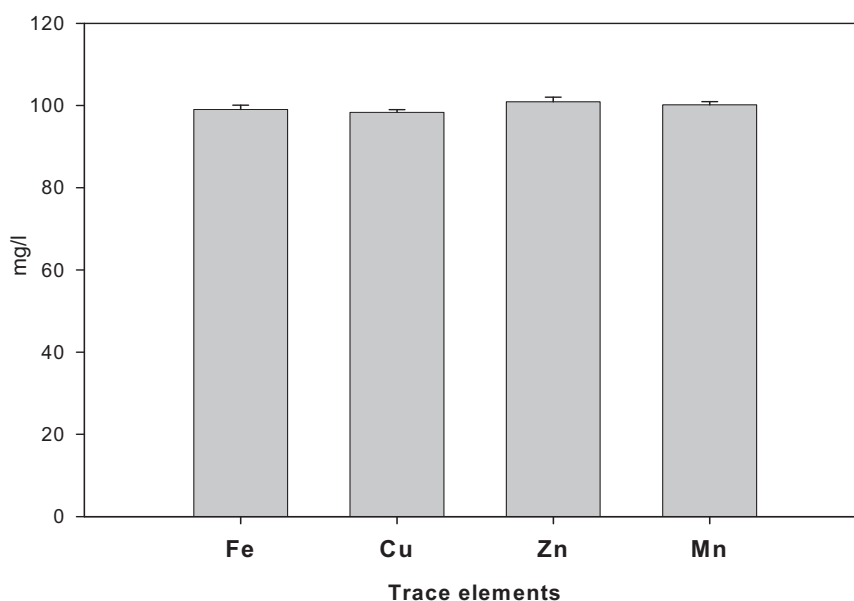


Figure 16 Trace elements results measured compared to a standard
value of 100 mg/l (n=3)

6.3 Statistical Analysis

Data were statistically analysed using SPSS 15 for descriptive statistics and various correlations. Multiple linear regression models were found out regarding the interaction of trace elements in feeds and liver tissue. Stepwise regression method was followed for multiple regression analyses in which feed trace elements, feed minerals and the remaining nutrient composition was added at consecutive stages. Level of significance was set as $P < 0.05$. Kolmogorov-Smirnov (KS) test was run to determine the normality of data.

6.4 Results and Discussion

Descriptive statistics for the data obtained on feed composition from 11 commercial dairy farms included in the survey has been summarized in the following Table 11. Proximate constituent data reveal measured nutrients mean values fall within the normal range according to the recommended dietary allowances. However, great farm-to-farm variation can be noted. This is attributable to different farm practices. The data seem to meet the assumption of normality when subjected to KS test. Macro minerals mean values have also been found to be little deviating from the recommended dietary allowances for dairy cattle. It can be noted that large variation in the sodium and chloride content has rendered the data non-normal. This might

have an effect on the dietary cation anion balance and subsequent productive performance in the concerned herds. Dietary cation anion balance calculated from the mean values for TMR is +17 milliequivalents that is quite less than the normal range (+ 20 to + 40 mEq/100 g dietary DM) described for rations for the lactating dairy cows (Beede 2005).

It is interesting to point out that all trace elements measured in TMR were found to be more than the recommended dietary allowances. Iron content of the ration has been noted to be exceptionally high. Overall variation in the trace element composition of the diet was also high (coefficient of variation, 22% - 43%). This indicates the high dosage use of mineral supplementation by the farmers or problems with mixing and homogeneity during the ration preparation could occur.

Table 11 Descriptive summary of feed composition (DM basis) data collected from 11 different farms in Saxonia (Germany)

Number	Nutrient	Mean*	Range	SEM	KS statistics **
<i>Proximate Constituents</i>					
1	Dry matter g/kg	414.1	320.7 - 487.5	2.9	.720
2	Crude ash g/kg	69.3	56.3 - 85.9	0.46	.294
3	Crude protein g/kg	170.4	137.4 - 213.9	1.04	.765
4	Crude fibre g/kg	165.6	141.2 - 204.0	0.91	.763
5	Crude fat g/kg	43.4	30.4 - 55.5	0.42	.941
6	Starch g/kg	228.3	159.3 - 276.2	1.82	.974
7	Sucrose g/kg	44.0	11.9 - 93.4	1.36	.055
8	Soluble organic matter g/kg	754.6	664.7 - 792.4	1.71	.43
<i>Mineral Constituents</i>					
9	Calcium g/kg	7.2	3.9 - 10.2	0.09	.238
10	Phosphorus g/kg	4.2	3.1 - 5.1	0.03	.833
11	Sodium g/kg	1.8	0.3 - 9.3	0.09	<.001
12	Magnesium g/kg	2.4	1.7 - 3.1	0.02	.008
13	Potassium g/kg	14.3	9.8 - 20.1	0.15	.743
14	Sulphur g/kg	2.1	1.7 - 2.7	0.01	.356
15	Chloride g/kg	4.6	1.9 - 18.9	0.18	.001
16	Copper mg/kg	23.9	9.4 - 44.5	0.56	.331
17	Zinc mg/kg	97.1	42.0 - 163.0	2.31	.227
18	Manganese mg/kg	71.9	32.4 - 119.1	1.63	.285
19	Iron mg/kg	374.6	208.1 - 655.4	6.17	.419
20	Selenium mg/kg	0.5	0.06 - 1.2	0.02	.696

*Mean values obtained after the analysis of 200 samples

**Kolmogorov-Smirnov (KS test) statistical values > 0.05 meet the assumption of the normality of the data

The whole feed data from 11 farms were subjected to Principal Component Analysis (PCA). The purpose of PCA is to express the main information contained in the initial variables in a lower number of variables, the so-called principal components (latent variables), which describe the main variations in the data. Practically PCA transforms a number of possibly correlated variables in a smaller number of uncorrelated variables or principal components. This statistics helps to perform the multiple regression analysis in situations where a large number of independent variables might have a cumulative effect on the dependent variable. By applying PCA on the feed composition data from the Saxonian dairy herds, it is observed that trace elements manganese, zinc, copper and selenium fall within the same principal component one. This means this component might have an effect as a group on the trace element concentrations in the liver or plasma or any other parameter of interest. Moreover, this also shows a trend of supplementation of these minerals. These latent variables generated could further be used in simple or multiple regression analysis. This is a novel result and application of this statistical tool to large sets of feed data should be further studied to find out interactions and relationships among various nutrients. Following table 12 and the related scree plot diagram shows a distinct group of trace elements with very high loadings in the component 1.

Table 12 Component matrix resulted from the principal component analysis of feed data of 11 dairy herds

	Feed Factors Extracted			
	1	2	3	4
Zinc	0.919			
Sodium	0.815			
Manganese	0.815			
Selenium	0.782			
Copper	0.777			
Sulphur		0.920		
Crude Protein		0.910		
Phosphorus	0.441	0.757		
Magnesium	0.527	0.613		
Potassium			0.901	
Starch			-0.804	
Crude Ash			0.787	
Dry Matter				0.889
Sucrose				0.726

Extractions method: Principal component analysis
 Rotations method: Varimax with Kaiser-Normalisation
 Kaiser Meyer Olkin = 0.70
 Bartlett test of sphericity $P < 0.001$, (df = 91)

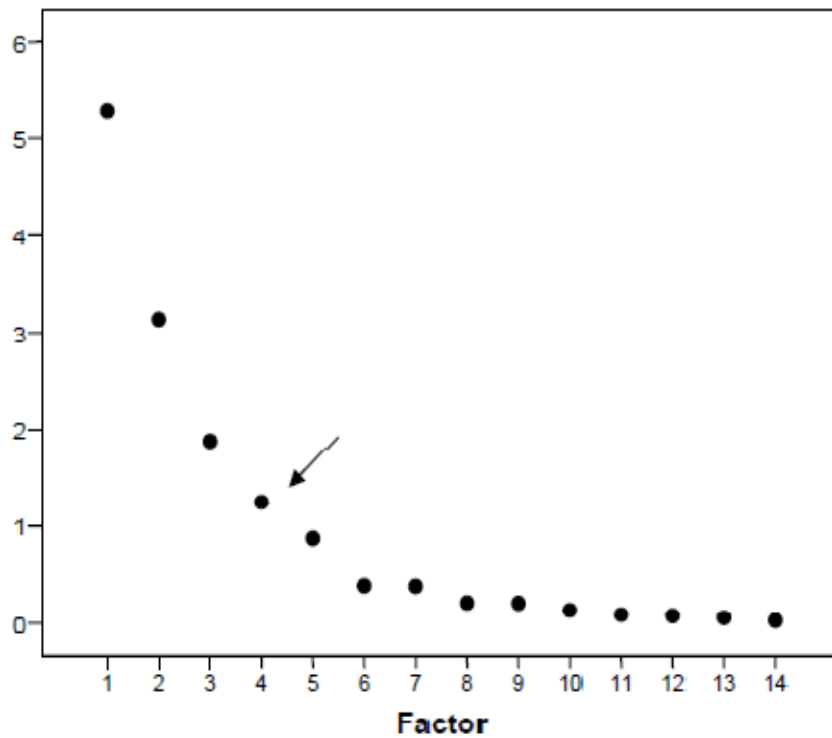


Figure 17 Screeplot diagram of the feed components

Milk production, plasma biochemistry and the liver trace elements data have been summarised in the following Table 13. It is evident that milk production data for two consecutive months did not much differ. Plasma biochemistry parameters reveal the cows to be healthy.

Liver tissue concentrations of trace elements are subjected to changes, depending on the age, production stage and disease condition of the animal and might exhibit large variations. Zinc liver content in this study has been found lower than recently reported (Nriagu et al. 2009) as 29.5 mg/kg (fresh weight) in grazing dairy cows. However, copper concentration in our study (134.5 mg/kg fresh weight) has been found quite high as compared to 20.4 mg/kg in the study of Nriagu et al. (2009). A previous report described a range of 1.4 – 134.5 mg/kg fresh weight in grazing cattle in Queensland, Australia (Kramer et al. 1983). Whereas, mean selenium concentration are relatively close to each other (0.43 mg/kg ~ 0.72 mg/kg). These findings indicate an expected lower content of trace element in the grazing cows, which can be due to the lower soil content. Contrary result regarding zinc might be associated with some particular antagonistic relationships.

Table 13 Descriptive summaries of various parameters measured in samples collected from 11 different farms in Saxonia (Germany)

Nutrient	Mean*	Range	SEM	KS statistics**
Milk November 2008				
Milk yield (kg)	38.02	20.60 – 59.30	0.69	.905
Fat (%)	3.76	2.0 – 5.75	0.07	.868
Protein (%)	3.37	2.65 – 4.14	0.03	.908
Milk urea (mg/l)	245.54	130 - 380	4.60	.230
Somatic cell count * 1000	229	9 - 3624	54.55	.000
Lactose (%)	4.80	4.22 – 5.17	0.02	.183
Days in milk	122	56 - 297	3.34	.383
Protein corrected milk (kg)	36.79	20.90 – 64.20	0.64	.765
Milk December 2008				
Milk yield (kg)	36.11	20.20 – 54.90	0.65	.924
Fat (%)	3.94	2.63 – 5.89	0.06	.998
Protein (%)	3.45	2.81 – 4.18	0.03	.946
Milk urea (mg/l)	257.72	140 - 430	4.86	.820
Somatic cell count * 1000	159	4.92 – 24.17	35.02	.000
Lactose (%)	4.77	4.22 – 5.28	0.02	.169
Days in milk	154	91 - 321	3.31	.326
Protein corrected milk (kg)	35.84	19.20 – 50.30	0.59	.940
Plasma Biochemistry				
ALAT (μ kat/l)	0.84	0.55 – 1.18	0.01	.423
ASAT (μ kat/l)	1.89	0.89 – 3.26	0.05	.059
Bilirubin (μ mol/l)	4.16	1.40 – 8.10	0.09	.065
Bilirubin indirect (μ mol/l)	4.02	0 – 7.80	0.01	.015
Bilirubin direct (μ mol/l)	0.13	0 – 0.50	0.10	.000
Ferritin (ng/l)	30.97	3.80 – 161.40	2.00	.047
Plasma Trace Elements				
Zinc (mg/l)	1.51 (n=94)	0.39 - 2.77	0.06	.195
Copper (mg/l)	1.78 (n=106)	0.18 - 8.09	0.15	.002
Manganese (mg/l)	0.80 (n=54)	0.02 - 4.20	0.13	.642
Selenium (mg/l)	0.101 (n=106)	0.05 – 0.17	1.85	.275
Iron (mg/l)	1.71 (n=66)	0.09 - 6.07	0.13	.355
Liver Trace Elements (Fresh matter basis)				
Zinc (mg/kg)	18.26 (n=106)	1.5 – 46.7	0.90	.185
Copper (mg/kg)	134.58 (n=105)	1.4 – 372	6.81	.85
Manganese (mg/kg)	7.22 (n=106)	0.29 – 52.5	0.71	.001
Iron (mg/kg)	89.17 (n=108)	3 – 278	6.35	.033
Selenium (mg/kg)	0.721 (n=104)	0.122 – 1.55	0.04	.272

*Mean values obtained after the analysis of 110 samples, for trace elements n is specified in brackets

**Kolmogorov-Smirnov statistical values > 0.05 meet the assumption of the normality of the data

Multiple correlations and regression models among trace elements in feeds and liver tissue have been sorted out. The results are presented in the Table 14 and Table 16.

Pearson correlation matrix among the liver trace elements (Table 14) clearly indicates strong positive and negative correlations among various trace minerals. zinc and copper have been shown to exhibit significant positive correlations with manganese and selenium liver contents and with each other. Strong positive correlation ($R = .35$, $P < .001$) between zinc and copper are comparatively higher than previously reported ($R = .19$, $P = .004$) by Nriagu et al. (2009).

It can be noted that high iron concentrations in the liver are having strong negative correlations with all other trace elements in the liver. iron, sulphur, molybdenum and stress have been described as the antagonists to copper, zinc and manganese bioavailability in dairy cows (Nockels et al. 1993).

Table 14 Pearson correlation matrix for various trace elements in liver tissues

	Zinc	Copper	Manganese	Selenium
Liver				
Zinc		$R = .353$ $P < 0.001$ $N = 104$	$R = .358$ $P < 0.001$ $N = 104$	$R = .229$ $P = 0.018$ $N = 106$
Copper	$R = .353$ $P < 0.001$ $N = 104$		$R = .227$ $P = 0.021$ $N = 102$	$R = .613$ $P < 0.001$ $N = 105$
Manganese	$R = .358$ $P = 0.001$ $N = 104$	$R = .227$ $P = 0.021$ $N = 102$		$R = .008$ $P = 0.937$ $N = 104$
Selenium	$R = .229$ $P = 0.018$ $N = 106$	$R = .613$ $P < 0.001$ $N = 105$	$R = .008$ $P = 0.937$ $N = 104$	
Iron	$R = -.433$ $P < 0.001$ $N = 105$	$R = -.503$ $P < 0.001$ $N = 104$	$R = -.257$ $P = 0.008$ $N = 105$	$R = -.266$ $P = 0.006$ $N = 106$

When all data on the feed composition and trace mineral in liver tissues were subjected to multiple linear regression analysis in a stepwise method, models were generated (Table 16). It is evident that trace elements included in the analysis have been found to be interacting with one and other in positive and negative relationships.

Selenium, copper and manganese in the feed have been found to increase their respective liver concentration whereas zinc and iron have negative relationship. Overall, it can be concluded that trace elements in feeds show antagonism towards one another of various magnitude. Possible reasons could be the chemical affinity of transition metals towards various biomolecules in the physiological system of ruminants. More work is ascertained in this regard.

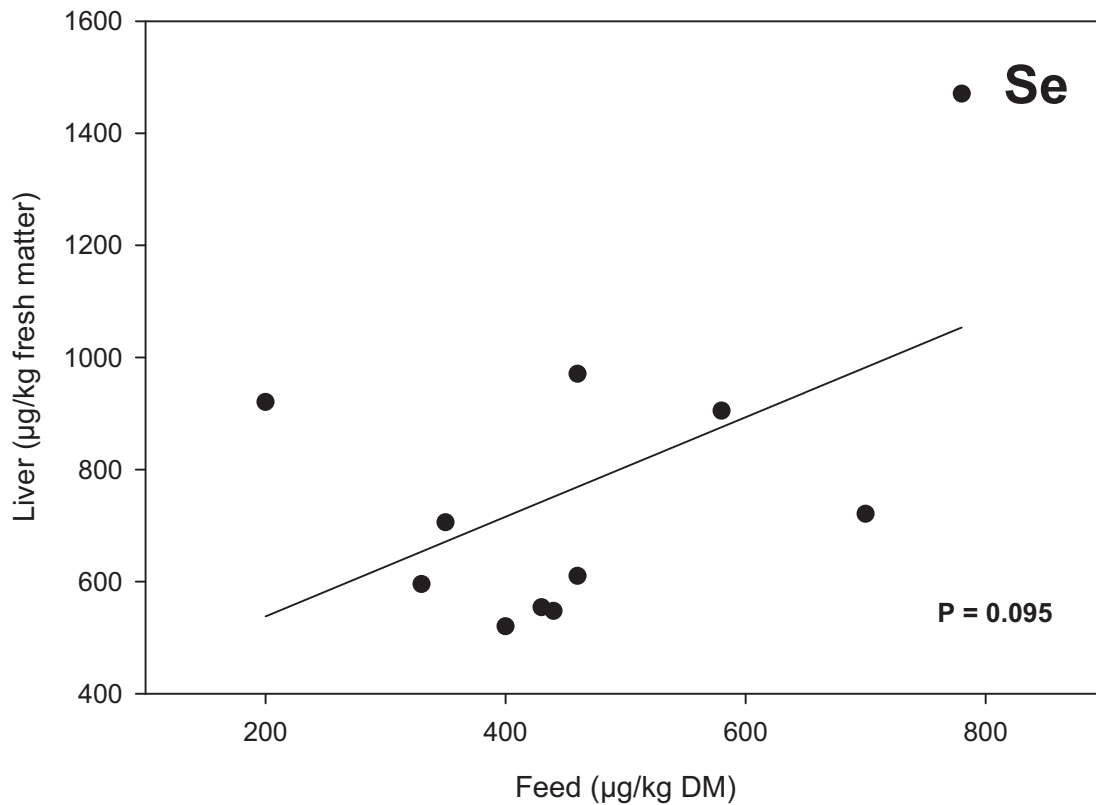


Figure 18 Feed and liver selenium relationship in Saxonian dairy farms

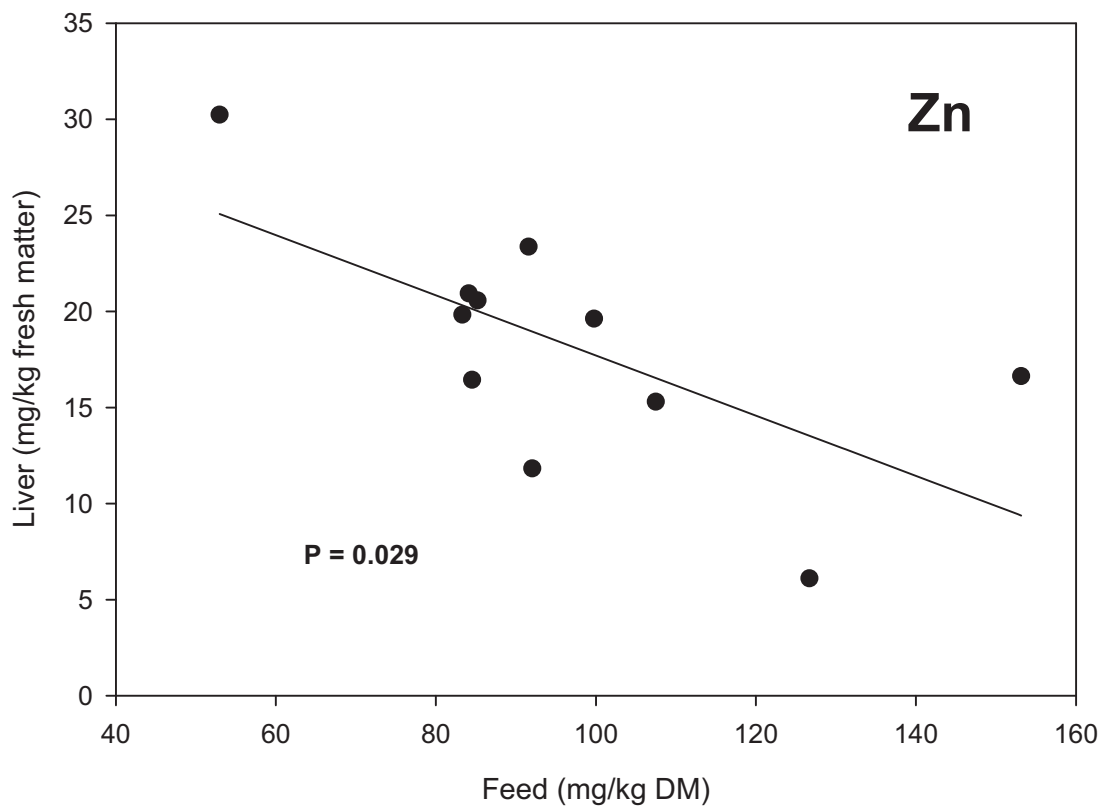


Figure 19 Feed and liver zinc relationship in Saxonian dairy farms

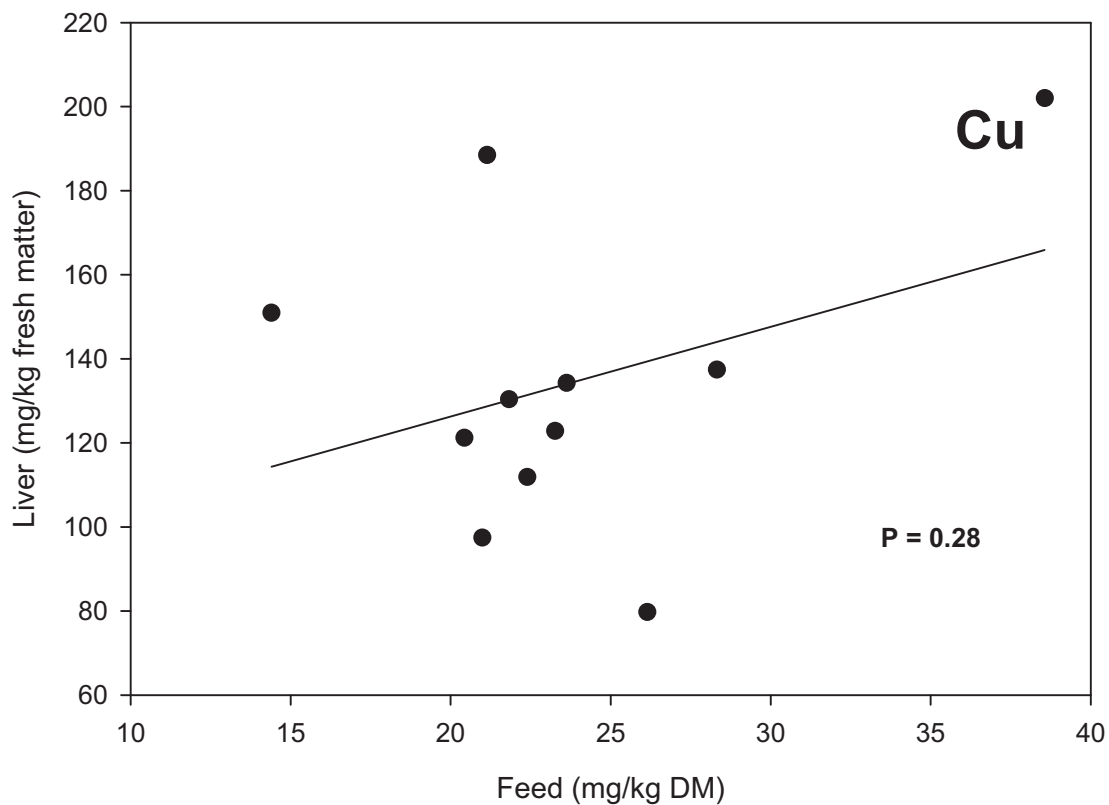


Figure 20 Feed and liver copper relationship in Saxonian dairy farms

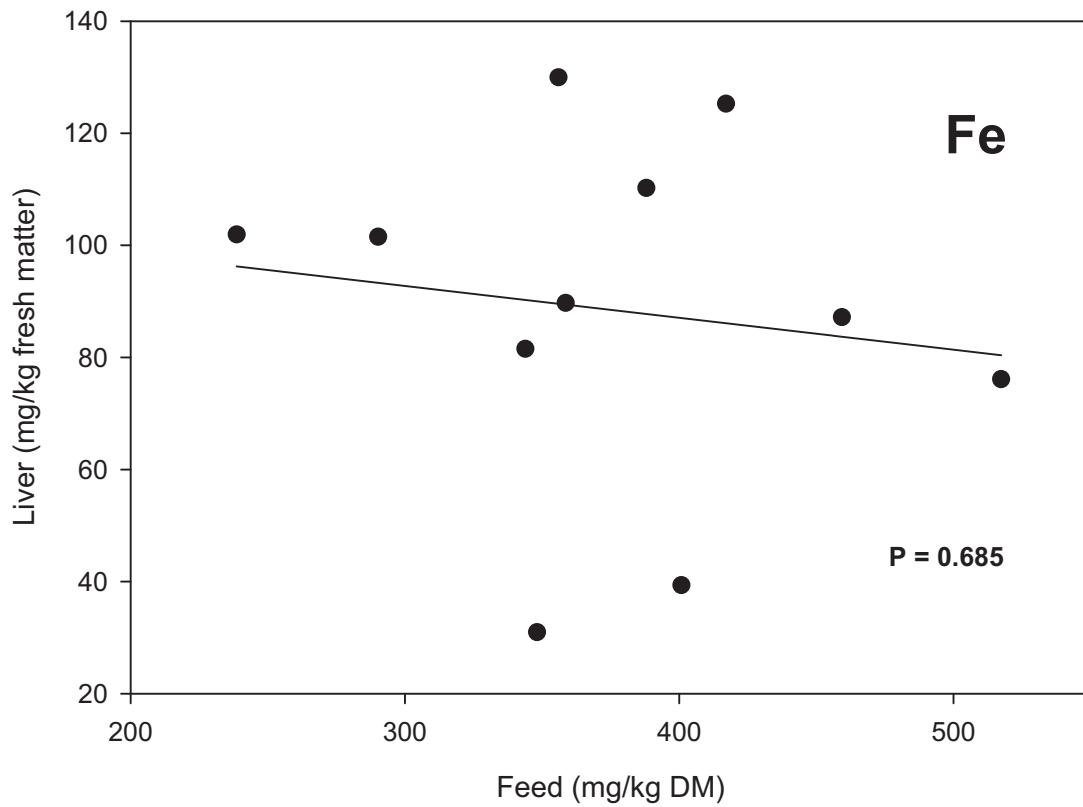


Figure 21 Feed and liver iron relationship in Saxonian dairy farms

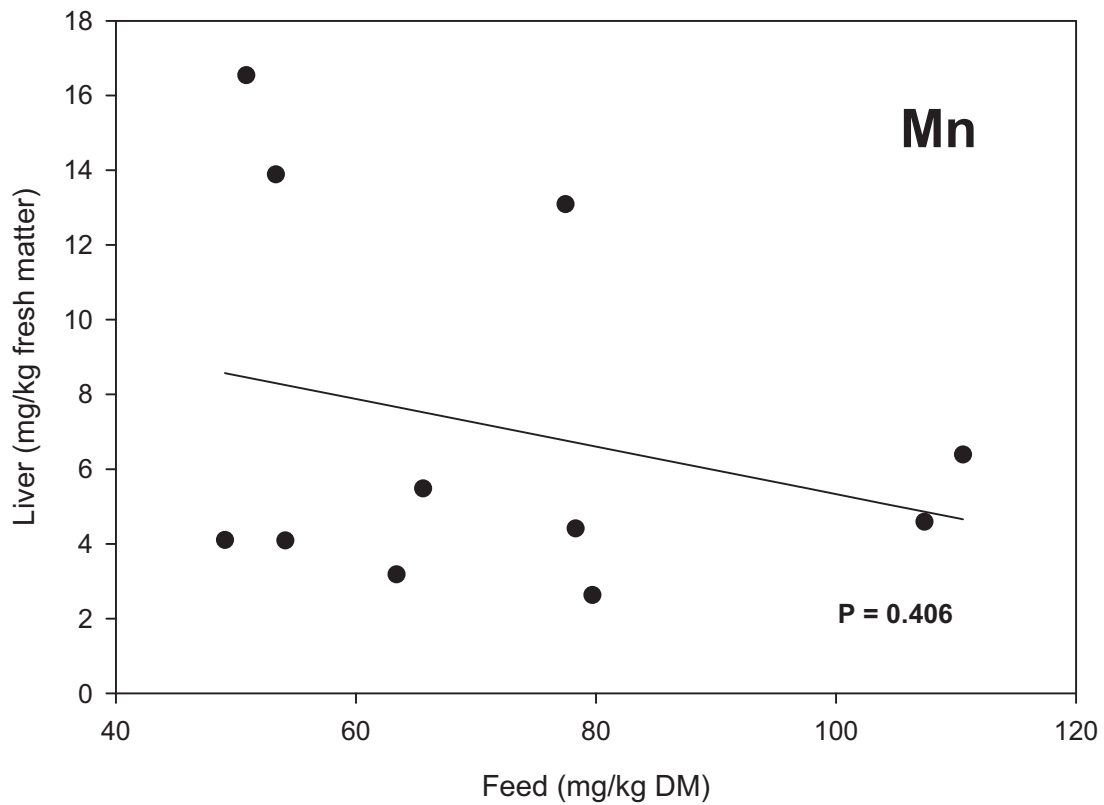


Figure 22 Feed and liver manganese relationship in Saxonian dairy farms

Table 15 Regression equations describing relationship between feed and liver tissues concentrations of various trace elements

Trace Element	R	R ²	F Ratio	Constant	Coefficient	t	p
Selenium	.524	.274	3.40	360.11	888.66	1.84	0.095
Zinc	.653	.427	6.70	33.36	-.15	-2.58	0.029
Copper	.358	.128	1.32	83.59	2.13	1.15	0.280
Manganese	.279	.078	0.76	11.69	-.06	-.87	0.406
Iron	.138	.019	.175	109.81	-.06	-.41	0.685

Table 16 Multiple linear regression models describing the relationship among liver trace elements (dependent) and feed composition (independent)

Dependent variable	Independent variables	R ²	F Ratio	p	Standardised Coefficient	t	p
Selenium		.960	12.03	0.033	-9.658	2.99	0.040
	Se				1.702	5.49	0.012
	Cu				0.118	0.47	0.688
	Zn				-0.773	-2.10	0.127
	Mn				-0.818	-2.46	0.091
	Fe				-0.280	-1.73	0.182
Zinc	ELOS				0.799	3.35	0.044
		.883	6.06	0.053	53.310	3.94	0.017
	Zn				-0.913	-1.78	0.149
	Cu				-0.309	-1.32	0.255
	Mn				-0.214	-0.70	0.523
	Fe				0.076	0.32	0.766
Copper	Se				0.494	1.10	0.333
		.960	11.86	0.034	-100.340	-1.67	0.193
	Cu				0.018	0.11	0.919
	Zn				-.673	-1.93	0.149
	Mn				-1.026	-4.15	0.025
	Fe				0.064	.39	0.723
Manganese	Se				0.731	2.23	0.111
	Crude Ash				0.959	6.22	0.008
		.984	30.49	0.009	-3.340	-0.46	0.676
	Mn				0.155	0.87	0.450
	Fe				0.189	1.42	0.251
	Se				-0.173	-0.89	0.435
Iron	Zn				-0.312	-1.25	0.297
	Cu				-0.471	-4.45	0.021
	K				0.818	6.86	0.006
		.998	176.91	0.006	258	14.23	0.005
	Fe				-0.535	-6.34	0.024
	Se				0.476	5.13	0.036
	Zn				-0.576	-4.59	0.044
	Cu				0.543	13.11	0.006
Iron	Mn				0.789	11.21	0.008
	K				-0.378	-4.16	0.053
	Crude Ash				-0.373	-5.03	0.037

Data on various parameters of milk production and trace element concentrations in liver were subjected to multiple linear regression analysis to find out the relationships among these variables. The results have been presented in Table 17. It can be noted that liver copper concentrations are having strong positive impact on the daily milk yield whereas zinc concentration are negatively correlated to milk yield. Somatic cell count has also been found to be negatively related with milk yield. This is according to Rainard and Riollet, (2006) who described that SCC decreased with the progress in lactation. Age of the cows has an effect on the SCC. With the increase in age, SCC in milk is also increased. This is logically attributable to the wear and tear in mammary gland tissues occurred with the increase in age. It is also evident from the strong positive correlation ($R = .365$; $P < 0.001$) noted between SCC and the age of the animal.

Table 17 Multiple linear regression models describing the relationship among liver trace elements and various parameters of herd performance

Dependent variable	Independent variables	R ²	F Ratio	P	Standardised Coefficient	T	P
Daily milk yield		.274	9.07	<0.001	30.680	15.78	<0.001
	Lactation number				0.487	4.41	<0.001
	Liver Cu				0.345	3.71	<0.001
	SCC				-0.273	-2.89	<0.001
SCC	Liver Zn				-0.247	-2.66	<0.001
		.188	11.34	<0.001	311.890	1.67	0.097
	Lactation number				0.450	4.67	<0.001
	Daily milk yield				-0.228	-2.36	0.02
Liver Cu	Liver Cu*				0.161	1.73	0.087
	Liver Mn*				-0.154	-1.70	0.092
		.051	5.50	0.021	52.270	1.46	0.146
Liver Se	Daily milk yield				0.225	2.34	0.021
	Age				-0.102	-1.02	0.302
SCC		.051	2.78	0.066	443.540	1.81	.072
	Age				-0.130	-1.31	0.193
	Daily milk yield				0.224	2.25	0.027
SCC		.166	10.16	<0.001	13.340	.071	0.943
	Age				0.413	4.52	<0.001
	Daily milk yield				-0.185	-2.03	.045

*Beta in (variables, which can be included in the original model with these values)

7. CONCLUSION

The research carried out thus far regarding the possible role of selenium in dairy cow nutrition has been primarily focused on the broad areas of effects of organic and inorganic selenium sources on selenium status, bioavailability and transfer to offspring and GSHPx activities and subsequent impact on the immune function. Still there are gaps in our present set of knowledge, which should be filled. The lower limit of 0.2 ppm of selenium in diets is generally regarded as a level below which the immune system might be vulnerable. Although, in our study no adverse health effects in the control group could be noted, the significantly lower selenium levels in serum, milk and selenium transfer to offspring compared to the other groups may pretend some sort of risk. Previous findings about significant increases in the milk and serum selenium levels as a result of the selenized yeast supplementation in the diets have been strengthened with the results of the present study. Although distribution of selenium in different milk and protein fractions has been worked out, our knowledge regarding the presence and characterization of selenoproteins in milk and their effects on dairy consumers and possible influence on technical properties of dairy products is still poor. Mechanisms behind the positive impact of selenium yeast on the total antioxidant capacity as observed in this study and the expected improvement in the milk quality are not fully elucidated. It can be argued that selenium might be interacting with the fatty acids present in the milk.

Survey findings have revealed a general trend of over supplementation for trace minerals in dairy rations. Minerals antagonistic relationships must be considered while formulating rations.

Important areas for further research and recommendations on the topic are following:

Dietary recommendations for selenium in dairy cows rations should take into consideration different bioavailability of selenium sources, however, more data are needed considering the biological function

Interactions with other micronutrients (copper, zinc, manganese and iron) and mammary gland trace element homeostasis should be studied further

Milk selenoproteins and selenium-containing proteins should be more extensively characterised

Standardised methods for total antioxidant capacity are needed to be established

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APPENDIX 1 Data on Feeding Trial with Dairy Cows Conducted at BfR

		Serum Se content ($\mu\text{g/l}$) in cows				
Cows		Weeks relative to calving				
Sel	Feeding	- 6 W	- 3 W	Birth	1 W	12 W
321	6.12.08	75.23	84.39	68.52	88.27	83.65
338	18.9.08	46.39	56.91	60.66	69.55	97.45
340	26.10.08	31.00	47.83	48.83	67.58	49.56
357	30.5.08	51.68	62.31	39.52	60.35	74.04
361	16.7.08	42.12	60.87	42.98	74.91	58.48
SeY						
322	26.1.2009	66.39	75.24	68.07	86.19	126.10
349	27.5.08	31.31	47.96	35.78	67.00	55.35
353	18.9.08	56.12	59.08	67.85	85.35	122.30
355	30.12.08	62.59	74.76	62.85	76.67	85.18
359	16.7.08	43.97	70.76	47.98	76.22	96.72
412	24.11.08	66.41	78.95	89.06	118.30	99.06
Control						
311	27.5.08	43.92	38.40	12.32	36.69	20.04
351	2.6.08	50.95	30.14	9.10	34.92	25.98
352	27.5.08	28.40	26.50	23.44	33.00	29.03
360	2.8.08	51.31	51.32	56.35	14.94	58.39
431	26.1.09	64.90	46.16	51.65	56.26	54.55

		Serum TEAC values ($\mu\text{Mol/l}$) in cows			
Cows		Weeks relative to calving			
Sel	Feeding	Birth	1 W	12 W	
321	6.12.08	571.48	571.68	570.36	
338	18.9.08	572.66	570.96	569.91	
340	26.10.08	570.46	570.18	569.46	
357	30.5.08	571.13	570.63	569.81	
361	16.7.08	571.26	570.86	570.06	
SeY					
322	26.1.2009	578.03	578.06	582.38	
349	27.5.08	577.53	577.51	576.58	
353	18.9.08	577.11	577.16	576.66	
355	30.12.08	577.43	577.76	576.73	
359	16.7.08	577.73	578.41	576.63	
412	24.11.08	577.71	577.61	576.08	
Control					
311	27.5.08	566.48	566.73	565.61	
351	2.6.08	566.48	567.11	565.53	
352	27.5.08	567.46	566.86	565.16	
360	2.8.08	566.41	566.06	565.78	
431	26.1.09	566.96	566.33	556.63	

Calves born to the experimental cows					
Calves	Sex	Serum Se ($\mu\text{g/l}$)		Serum TEAC ($\mu\text{Mol/l}$)	
		Birth	1 W	Birth	1 W
Sel					
321	F	44.06	43.10	571.76	570.13
338	M	32.92	37.17	571.73	570.36
340	F	29.08	31.32	571.96	570.46
357	F	10.20	39.37	561.21	561.21
361	dead				
SeY					
322	F	44.42	48.85	578.56	577.28
349	M	36.85	41.20	577.33	576.96
353	M	31.30	43.32	578.38	577.16
355	M	40.36	53.15	578.53	577.18
359	dead				
412	M	37.33	51.96	567.81	567.81
Control					
311	M	20.83	41.54	568.13	566.13
351	F	8.09	26.69	567.66	566.48
352	F	23.72	30.61	567.93	566.53
360	F	32.75	13.14	567.76	566.81
431	M	32.19	29.82	567.51	566.31

Milk TEAC values ($\mu\text{Mol/l}$) in cows				
Cows	Weeks relative to calving			
	1 W	9 W	12 W	15 W
Sel				
321	557.99	558.69	557.44	557.89
338	555.89	557.64	557.64	558.64
340	556.69	557.49	557.69	557.44
357	557.34	557.54	557.49	557.54
361	556.49	557.94	557.64	557.94
SeY				
322	585.82	586.97	587.25	586.72
349	586.07	586.42	586.62	586.00
353	586.25	587.05	587.37	587.42
355	584.17	586.82	587.05	588.15
359	585.62	585.87	586.42	587.57
412	586.32	587.57	587.50	587.85
Control				
311	540.20	541.55	541.47	542.00
351	540.80	540.90	542.22	543.62
352	539.65	540.47	540.75	540.97
360	540.57	541.27	541.57	541.50
431	540.02	541.22	541.62	541.20

APPENDIX 2 Saxonian Dairy Herds Data – Feed Composition

	Nutrients		N	Minimum	Maximum	Mean	SEM
	Farm 1	Dry matter	g/kg	19	388.10	465.35	427.87
Crude ash		g/kg DM	19	56.90	63.52	60.08	0.55
Crude protein			19	163.22	191.47	177.58	1.91
ELOS			19	595.12	775.76	733.67	12.24
Crude fibre			19	140.99	186.85	163.80	3.12
Crude fat			19	34.18	62.32	45.33	1.81
Starch			19	218.12	249.24	235.30	2.11
Sucrose			19	27.19	48.03	36.37	1.57
Calcium			19	4.52	8.61	6.34	0.27
Phosphorus			19	4.03	5.16	4.55	0.06
Sodium			19	0.64	1.61	1.10	0.07
Magnesium			19	2.10	3.17	2.62	0.08
Potassium			19	12.01	14.21	13.05	0.17
Sulphur			19	2.00	2.69	2.33	0.04
Chloride			19	1.79	3.49	2.49	0.11
Copper		mg/kg DM	19	9.66	39.70	20.99	2.01
Zinc			19	40.75	173.46	91.58	9.46
Manganese			19	32.03	71.37	49.09	2.85
Iron			19	249.03	374.65	290.27	6.75
Selenium			19	0.15	0.76	0.46	0.05
Farm 2	Dry matter	g/kg	21	367.71	483.75	412.97	5.27
	Crude ash	g/kg DM	21	58.56	77.37	68.45	0.91
	Crude protein		21	128.29	214.73	184.46	4.08
	ELOS		21	706.22	764.05	740.00	3.29
	Crude fibre		21	161.52	193.65	176.72	2.06
	Crude fat		21	31.13	43.74	38.95	0.64
	Starch		21	173.73	241.98	208.02	4.48
	Sucrose		21	43.85	61.64	51.26	1.23
	Calcium		21	5.37	10.55	8.01	0.30
	Phosphorus		21	3.25	4.95	4.29	0.08
	Sodium		21	0.29	1.17	0.75	0.06
	Magnesium		21	1.99	2.62	2.31	0.03
	Potassium		21	13.16	18.36	15.35	0.29
	Sulphur		21	1.99	2.73	2.37	0.04
	Chloride		21	1.79	4.26	3.07	0.14
	Copper	mg/kg DM	21	8.53	47.21	28.32	2.51
	Zinc		21	38.34	135.09	84.52	6.21
	Manganese		21	33.81	92.61	65.60	3.71
	Iron		21	285.90	472.28	387.97	9.46
	Selenium		21	0.07	0.81	0.44	0.05

	Nutrients		N	Minimum	Maximum	Mean	SEM
Farm 3	Dry matter	g/kg	20	433.27	485.59	468.90	3.66
	Crude ash	g/kg DM	20	72.68	83.37	77.32	0.75
	Crude protein		20	163.32	194.58	183.40	1.69
	ELOS		20	731.27	779.19	756.52	2.68
	Crude fibre		20	142.98	169.57	154.52	1.54
	Crude fat		20	36.61	42.58	40.47	0.36
	Starch		20	200.63	227.16	212.43	1.72
	Sucrose		20	75.49	95.74	86.94	1.30
	Calcium		20	6.62	8.62	7.86	0.11
	Phosphorus		20	4.16	4.97	4.62	0.05
	Sodium		20	1.00	1.43	1.25	0.03
	Magnesium		20	2.46	3.06	2.84	0.03
	Potassium		20	16.16	18.06	17.02	0.09
	Sulphur		20	2.13	2.58	2.40	0.03
	Chloride		20	3.60	5.02	4.10	0.08
	Copper		mg/kg DM	20	18.15	23.88	21.14
	Zinc	20		70.24	99.64	84.11	1.93
	Manganese	20		46.40	63.81	53.34	0.92
	Iron	20		269.32	559.43	348.17	14.03
	Selenium	20		0.21	0.50	0.35	0.02
Farm 4	Dry matter	g/kg	21	414.55	460.49	432.72	2.92
	Crude ash	g/kg DM	21	62.13	74.76	66.13	0.73
	Crude protein		21	144.36	177.73	159.17	1.92
	ELOS		21	760.37	796.01	781.68	2.41
	Crude fibre		21	133.19	169.67	150.09	1.99
	Crude fat		21	30.39	43.51	34.82	0.78
	Starch		21	206.68	283.57	248.65	4.06
	Sucrose		21	36.25	78.26	55.51	2.86
	Calcium		21	5.20	9.20	6.82	0.24
	Phosphorus		21	3.18	4.45	3.70	0.06
	Sodium		21	1.00	1.93	1.30	0.05
	Magnesium		21	1.70	2.55	2.03	0.04
	Potassium		21	11.68	17.76	14.63	0.33
	Sulphur		21	1.99	2.55	2.19	0.03
	Chloride		21	2.43	5.20	3.21	0.13
	Copper		mg/kg DM	21	11.19	23.97	14.40
	Zinc	21		40.28	87.59	52.97	2.52
	Manganese	21		39.33	76.88	50.87	1.80
	Iron	21		324.49	511.22	400.79	11.57
	Selenium	21		0.08	0.53	0.20	0.02

	Nutrients		N	Minimum	Maximum	Mean	SEM	
Farm 5	Dry matter	g/kg	20	314.91	452.45	380.41	7.78	
	Crude ash	g/kg DM	20	61.49	79.55	75.02	1.04	
	Crude protein		20	133.19	222.28	160.00	4.18	
	ELOS		20	706.78	811.92	751.72	5.26	
	Crude fibre		20	124.79	212.70	177.94	4.45	
	Crude fat		20	36.49	60.22	42.20	1.07	
	Starch		20	131.30	263.96	203.42	6.47	
	Sucrose		20	24.18	50.34	34.76	1.75	
	Calcium		20	5.41	7.43	6.58	0.11	
	Phosphorus		20	3.50	5.42	4.11	0.09	
	Sodium		20	0.88	1.88	1.47	0.05	
	Magnesium		20	1.85	2.55	2.21	0.04	
	Potassium		20	11.68	24.06	16.33	0.52	
	Sulphur		20	1.59	2.76	2.01	0.05	
	Chloride		20	2.72	4.24	3.73	0.10	
	Copper		mg/kg DM	20	16.54	29.55	23.62	0.78
	Zinc			20	60.98	109.06	83.30	2.85
	Manganese			20	59.17	98.24	77.46	2.17
Iron	20	283.40		444.14	358.61	10.94		
Selenium	20	0.22		0.62	0.40	0.03		
Farm 6	Dry matter	g/kg	20	369.78	400.68	383.49	1.93	
	Crude ash	g/kg DM	20	69.13	83.32	72.68	0.73	
	Crude protein		20	157.03	195.36	177.86	2.04	
	ELOS		20	706.53	751.72	733.42	2.26	
	Crude fibre		20	158.60	198.02	176.13	1.71	
	Crude fat		20	40.11	48.93	45.71	0.49	
	Starch		20	147.16	213.13	192.01	3.13	
	Sucrose		0					
	Calcium		20	6.69	7.85	7.30	0.07	
	Phosphorus		20	4.57	5.35	5.01	0.05	
	Sodium		20	2.34	3.04	2.72	0.04	
	Magnesium		20	2.76	3.29	3.01	0.03	
	Potassium		20	12.86	18.09	14.75	0.26	
	Sulphur		20	2.02	2.46	2.24	0.02	
	Chloride		20	5.31	7.13	6.22	0.10	
	Copper		mg/kg DM	20	34.41	42.62	38.56	0.49
	Zinc			20	133.71	165.36	153.14	1.91
	Manganese			20	91.65	118.76	107.37	1.49
Iron	20	314.43		420.41	343.90	5.89		
Selenium	20	0.33		0.55	0.46	0.02		

	Nutrients		N	Minimum	Maximum	Mean	SEM	
Farm 7	Dry matter	g/kg	22	338.14	383.33	364.73	2.88	
	Crude ash	g/kg DM	22	59.71	83.29	74.33	1.07	
	Crude protein		22	150.96	178.63	166.70	1.54	
	ELOS		22	760.25	786.45	772.20	1.39	
	Crude fibre		22	152.00	172.62	163.44	1.34	
	Crude fat		22	42.35	50.63	45.46	0.52	
	Starch		22	190.65	249.79	219.59	3.49	
	Sucrose		22	18.76	37.02	31.04	0.89	
	Calcium		22	6.11	9.19	7.71	0.13	
	Phosphorus		22	3.43	4.33	3.91	0.04	
	Sodium		22	1.13	1.95	1.64	0.04	
	Magnesium		22	2.14	2.75	2.41	0.03	
	Potassium		22	15.33	18.44	16.74	0.17	
	Sulphur		22	1.82	2.25	2.05	0.02	
	Chloride		22	4.72	6.75	5.88	0.09	
	Copper		mg/kg DM	22	20.48	30.89	26.14	0.55
	Zinc			22	91.77	156.56	126.67	3.12
	Manganese	22		87.05	123.93	110.58	2.06	
	Iron	22		294.80	403.26	355.99	6.72	
	Selenium	22		0.55	0.88	0.70	0.02	
Farm 8	Dry matter	g/kg	18	384.37	455.96	425.43	5.43	
	Crude ash	g/kg DM	18	59.40	77.39	66.54	1.28	
	Crude protein		18	164.83	215.36	180.37	2.97	
	ELOS		18	747.41	790.46	765.91	2.78	
	Crude fibre		18	139.60	169.19	156.89	1.79	
	Crude fat		18	35.42	43.62	39.98	0.68	
	Starch		18	233.01	282.87	256.73	3.37	
	Sucrose		18	41.32	65.13	55.30	1.49	
	Calcium		18	4.62	7.03	5.79	0.17	
	Phosphorus		18	3.69	4.96	4.24	0.06	
	Sodium		18	1.38	4.02	2.46	0.17	
	Magnesium		18	2.11	2.73	2.30	0.04	
	Potassium		18	11.55	15.12	13.17	0.26	
	Sulphur		18	1.99	2.95	2.27	0.05	
	Chloride		18	3.48	8.67	5.66	0.34	
	Copper		mg/kg DM	18	12.98	36.35	23.26	1.81
	Zinc			18	48.74	171.39	107.47	9.30
	Manganese	18		56.66	113.58	79.71	4.90	
	Iron	18		317.19	921.56	417.01	31.57	
	Selenium	18		0.33	1.32	0.78	0.07	

	Nutrients		N	Minimum	Maximum	Mean	SEM	
Farm 9	Dry matter	g/kg	20	307.22	375.96	356.26	4.54	
	Crude ash	g/kg DM	20	55.64	73.71	66.02	1.32	
	Crude protein		20	138.39	181.30	161.28	2.44	
	ELOS		20	722.05	774.15	749.16	3.19	
	Crude fibre		20	151.85	192.40	164.69	2.44	
	Crude fat		20	43.97	59.26	51.57	0.89	
	Starch		20	217.25	284.21	252.81	3.70	
	Sucrose		20	9.08	24.71	18.05	0.83	
	Calcium		20	3.81	11.30	8.44	0.50	
	Phosphorus		20	2.96	3.89	3.48	0.05	
	Sodium		20	0.32	1.24	0.84	0.07	
	Magnesium		20	1.58	2.31	2.00	0.05	
	Potassium		20	9.66	11.40	10.32	0.10	
	Sulphur		20	1.89	2.22	2.05	0.02	
	Chloride		20	2.22	4.18	3.30	0.14	
	Copper		mg/kg DM	20	9.08	29.96	20.43	1.58
	Zinc			20	40.13	123.56	85.21	6.49
	Manganese	20		32.37	86.54	63.39	4.47	
	Iron	20		429.62	657.99	517.34	14.81	
	Selenium	20		0.05	0.73	0.43	0.05	
Farm 10	Dry matter	g/kg	20	390.95	475.12	428.85	4.71	
	Crude ash	g/kg DM	20	56.07	86.16	70.99	1.99	
	Crude protein		20	155.83	197.23	174.23	1.99	
	ELOS		20	742.12	791.87	772.42	2.53	
	Crude fibre		20	156.77	189.82	168.27	1.87	
	Crude fat		20	38.16	61.20	47.79	1.54	
	Starch		20	200.04	247.60	234.08	2.36	
	Sucrose		20	31.53	47.19	39.54	0.82	
	Calcium		20	5.55	8.03	6.88	0.16	
	Phosphorus		20	4.05	5.45	4.48	0.09	
	Sodium		20	1.63	9.57	4.71	0.58	
	Magnesium		20	2.03	2.46	2.22	0.03	
	Potassium		20	11.34	13.56	12.41	0.15	
	Sulphur		20	1.92	2.34	2.08	0.02	
	Chloride		20	4.38	19.45	10.26	1.11	
	Copper		mg/kg DM	20	13.76	28.57	21.82	0.97
	Zinc			20	69.72	124.47	99.77	4.07
	Manganese	20		62.63	93.67	78.32	2.23	
	Iron	20		377.82	520.61	459.29	9.07	
	Selenium	20		0.29	0.85	0.58	0.03	

	Nutrients		N	Minimum	Maximum	Mean	SEM
Farm 11	Dry matter	g/kg	20	455.20	489.96	477.78	2.03
	Crude ash	g/kg DM	20	57.58	72.20	64.33	0.90
	Crude protein		20	137.96	159.39	151.91	1.26
	ELOS		20	711.37	769.80	746.57	3.39
	Crude fibre		20	153.37	180.19	165.47	1.99
	Crude fat		20	35.14	52.58	44.98	1.25
	Starch		20	220.99	271.67	253.04	3.35
	Sucrose		20	24.96	36.01	31.96	0.56
	Calcium		20	5.65	9.23	7.18	0.20
	Phosphorus		20	3.52	4.39	3.92	0.06
	Sodium		20	0.86	3.35	1.70	0.18
	Magnesium		20	2.03	2.65	2.34	0.04
	Potassium		20	11.97	15.25	13.55	0.16
	Sulphur		20	1.81	2.03	1.95	0.01
	Chloride		20	2.30	4.30	3.18	0.10
	Copper		mg/kg DM	20	9.94	39.05	22.39
	Zinc	20		45.61	145.38	92.04	5.59
	Manganese	20		31.80	82.40	54.12	2.80
	Iron	20		191.87	290.12	238.66	6.44
	Selenium	20		0.12	0.62	0.33	0.03

APPENDIX 3 Saxonian Dairy Herds Data – Trace Elements, Health and Production Parameters

	Parameters	N	Minimum	Maximum	Mean	SEM
Farm 1	Liver Trace Elements (Fresh matter basis)					
	Zinc (m/kg)	10	6.97	41.70	23.37	3.36
	Copper (m/kg)	10	16.30	199.00	97.47	19.19
	Manganese (m/kg)	10	0.29	13.80	4.10	1.21
	Iron (m/kg)	10	30.30	278.00	101.53	24.72
	Selenium (µg/kg)	10	248.30	887.50	609.86	65.04
	Plasma Trace Elements					
	Zinc (mg/l)	8	1.32	2.01	1.63	0.07
	Copper (mg/l)	10	0.18	1.13	0.65	0.08
	Manganese (mg/l)	0				
	Iron (m/l)	8	0.93	2.53	1.86	0.19
	Selenium (µg/l)	10	89.24	108.60	95.80	1.94
	Plasma Biochemistry					
	ALAT (µkat/l)	10	0.58	0.92	0.74	0.04
	ASAT (µkat/l)	10	1.20	3.20	1.75	0.19
	Bilirubin (µmol/l)	10	2.20	4.90	4.09	0.25
	Bilirubin-direct	10	0.00	0.20	0.13	0.02
	Bilirubin indirect	10	2.20	4.80	3.98	0.24
	Ferritin (ng/ml)	10	7.10	76.50	36.13	6.49
	Production Parameters December 2008					
	Milk yield (kg/day)	10	25.70	43.10	34.98	2.02
	Fat (%)	10	3.56	4.73	4.27	0.11
	Protein (%)	10	3.28	4.03	3.56	0.07
	Milk urea (mg/l)	10	270.00	430.00	331.00	17.41
	Somatic cell count (* 1000)	10	43.00	2162.00	318.70	205.94
	Lactose (%)	10	4.52	5.17	4.86	0.06
	Lactation number	10	1.00	6.00	3.10	0.53
	Days in milk	10	113.00	258.00	176.70	11.66
	Protein corrected milk (kg/day)	10	26.94	45.74	36.37	1.94
	Production Parameters November 2008					
	Milk yield (kg/day)	10	27.60	43.60	35.08	1.45
	Fat (%)	10	3.32	4.39	3.89	0.12
	Protein (%)	10	2.65	3.86	3.39	0.10
Milk urea (mg/l)	10	160.00	380.00	266.00	21.66	
Somatic cell count (* 1000)	10	26.00	3426.00	610.80	354.24	
Lactose (%)	10	4.43	5.17	4.83	0.06	
Lactation number	10	1.00	6.00	3.10	0.53	
Days in milk	10	78.00	223.00	141.70	11.66	
Protein corrected milk (kg/day)	10	26.60	39.85	34.57	1.33	

	Parameters	N	Minimum	Maximum	Mean	SEM
Farm 2	<i>Liver Trace Elements (Fresh matter basis)</i>					
	Zinc (m/kg)	9	11.60	25.70	16.44	1.64
	Copper (m/kg)	9	34.40	265.00	137.40	24.09
	Manganese (m/kg)	10	2.81	8.28	5.48	0.54
	Iron (m/kg)	10	2.85	250.00	110.21	22.85
	Selenium (µg/kg)	9	255.70	793.80	547.74	58.79
	<i>Plasma Trace Elements</i>					
	Zinc (mg/l)	10	1.63	2.77	2.00	0.10
	Copper (mg/l)	10	0.53	4.14	1.77	0.38
	Manganese (mg/l)	1	0.02	0.02	0.02	.
	Iron (m/l)	7	0.64	2.35	1.62	0.20
	Selenium (µg/l)	10	81.58	110.60	93.63	2.99
	<i>Plasma Biochemistry</i>					
	ALAT (µkat/l)	10	0.68	1.03	0.91	0.03
	ASAT (µkat/l)	10	1.36	2.16	1.79	0.08
	Bilirubin (µmol/l)	10	3.30	4.90	4.24	0.14
	Bilirubin-direct	10	0.00	0.20	0.10	0.02
	Bilirubin-indirect	10	3.30	4.80	4.14	0.14
	Ferritin (ng/ml)	10	13.80	64.40	36.65	6.26
	<i>Production Parameters December 2008</i>					
	Milk yield (kg/day)	10	32.40	44.50	37.81	1.17
	Fat (%)	10	3.86	4.68	4.21	0.09
	Protein (%)	10	3.16	3.78	3.43	0.06
	Milk urea (mg/l)	10	200.00	310.00	243.00	11.93
	Somatic cell count (* 1000)	10	26.00	127.00	62.20	8.76
	Lactose (%)	10	4.45	4.85	4.69	0.05
	Lactation number	10	2.00	3.00	2.40	0.16
	Days in milk	10	121.00	195.00	167.80	7.82
	Protein corrected milk (kg/day)	10	34.10	47.74	38.86	1.30
	<i>Production Parameters November 2008</i>					
	Milk yield (kg/day)	10	32.20	59.30	42.91	2.50
	Fat (%)	10	3.69	5.01	4.17	0.15
Protein (%)	10	2.90	3.79	3.26	0.07	
Milk urea (mg/l)	10	200.00	330.00	277.00	13.34	
Somatic cell count (* 1000)	10	36.00	2943.00	340.60	289.19	
Lactose (%)	10	4.22	4.93	4.76	0.07	
Lactation number	10	2.00	3.00	2.40	0.16	
Days in milk	10	72.00	146.00	118.80	7.82	
Protein corrected milk (kg/day)	10	35.29	64.20	43.36	2.68	

	Parameters	N	Minimum	Maximum	Mean	SEM
Farm 3	Liver Trace Elements (Fresh matter basis)					
	Zinc (m/kg)	10	11.60	23.20	20.94	1.08
	Copper (m/kg)	10	139.00	234.00	188.50	9.08
	Manganese (m/kg)	10	6.58	52.50	13.89	4.43
	Iron (m/kg)	9	4.34	56.10	30.97	5.37
	Selenium (µg/kg)	10	483.00	850.40	705.66	40.95
	Plasma Trace Elements					
	Zinc (mg/l)	9	0.69	2.43	1.71	0.21
	Copper (mg/l)	10	0.61	3.63	1.52	0.29
	Manganese (mg/l)	2	0.22	3.64	1.93	1.71
	Iron (m/l)	3	0.74	3.01	1.93	0.66
	Selenium (µg/l)	10	92.40	152.20	109.28	6.25
	Plasma Biochemistry					
	ALAT (µkat/l)	10	0.55	0.91	0.80	0.03
	ASAT (µkat/l)	10	1.00	3.03	2.00	0.19
	Bilirubin (µmol/l)	10	3.20	5.00	4.23	0.17
	Bilirubin-direct	10	0.00	0.30	0.11	0.03
	Bilirubin indirect	10	3.00	4.90	4.10	0.17
	Ferritin (ng/ml)	10	3.80	31.00	17.23	3.03
	Production Parameters December 2008					
	Milk yield (kg/day)	10	29.20	44.00	36.23	1.61
	Fat (%)	10	3.55	5.89	4.28	0.22
	Protein (%)	10	3.28	3.96	3.55	0.06
	Milk urea (mg/l)	10	250.00	320.00	281.00	6.57
	Somatic cell count (* 1000)	10	24.00	227.00	80.80	21.17
	Lactose (%)	10	4.22	5.06	4.64	0.08
	Lactation number	10	1.00	6.00	2.90	0.46
	Days in milk	10	160.00	192.00	167.50	2.85
	Protein corrected milk (kg/day)	10	31.37	43.40	37.51	1.18
	Production Parameters November 2008					
	Milk yield (kg/day)	10	27.00	45.00	38.08	1.66
	Fat (%)	10	3.65	5.23	4.30	0.17
	Protein (%)	10	3.21	3.95	3.49	0.07
Milk urea (mg/l)	10	210.00	330.00	257.00	12.21	
Somatic cell count (* 1000)	10	19.00	349.00	84.00	30.68	
Lactose (%)	10	4.35	4.94	4.69	0.07	
Lactation number	10	1.00	6.00	2.90	0.46	
Days in milk	10	132.00	164.00	139.50	2.85	
Protein corrected milk (kg/day)	10	29.02	44.62	39.42	1.32	

	Parameters	N	Minimum	Maximum	Mean	SEM
Farm 4	<i>Liver Trace Elements (Fresh matter basis)</i>					
	Zinc (m/kg)	10	16.00	46.70	30.24	3.07
	Copper (m/kg)	10	87.10	279.00	150.97	16.98
	Manganese (m/kg)	10	4.45	28.30	16.54	2.60
	Iron (m/kg)	10	3.13	84.60	39.34	9.09
	Selenium ($\mu\text{g/kg}$)	10	461.50	1380.00	920.31	108.12
	<i>Plasma Trace Elements</i>					
	Zinc (mg/l)	8	0.65	2.01	1.29	0.17
	Copper (mg/l)	10	0.47	4.45	1.85	0.37
	Manganese (mg/l)	9	0.02	2.32	1.20	0.27
	Iron (m/l)	7	0.50	2.98	1.61	0.35
	Selenium ($\mu\text{g/l}$)	10	101.80	136.90	114.75	3.88
	<i>Plasma Biochemistry</i>					
	ALAT ($\mu\text{kat/l}$)	10	0.60	1.14	0.85	0.06
	ASAT ($\mu\text{kat/l}$)	10	0.89	2.74	1.59	0.19
	Bilirubin ($\mu\text{mol/l}$)	10	3.60	5.60	4.12	0.21
	Bilirubin-direct	10	0.00	0.50	0.16	0.05
	Bilirubin indirect	10	3.30	5.10	3.98	0.19
	Ferritin (ng/ml)	10	12.40	41.40	28.73	3.27
	<i>Production Parameters December 2008</i>					
	Milk yield (kg/day)	10	25.50	47.40	33.43	2.28
	Fat (%)	10	3.33	5.10	3.79	0.17
	Protein (%)	10	3.30	3.99	3.63	0.07
	Milk urea (mg/l)	10	180.00	380.00	266.00	18.39
	Somatic cell count (* 1000)	10	9.00	234.00	59.20	20.78
	Lactose (%)	10	4.69	5.02	4.85	0.03
	Lactation number	10	1.00	2.00	1.40	0.16
	Days in milk	10	110.00	161.00	138.10	5.45
	Protein corrected milk (kg/day)	10	25.48	44.57	32.95	2.02
	<i>Production Parameters November 2008</i>					
	Milk yield (kg/day)	10	26.10	44.70	33.75	2.09
	Fat (%)	10	2.64	4.55	3.63	0.18
	Protein (%)	10	2.97	3.72	3.41	0.07
	Milk urea (mg/l)	10	200.00	380.00	267.00	20.00
Somatic cell count (* 1000)	10	9.00	919.00	120.20	89.01	
Lactose (%)	10	4.66	5.04	4.80	0.04	
Lactation number	10	1.00	2.00	1.40	0.16	
Days in milk	10	82.00	132.00	112.00	4.47	
Protein corrected milk (kg/day)	10	25.61	38.60	32.03	1.39	

	Parameters	N	Minimum	Maximum	Mean	SEM
Farm 5	Liver Trace Elements (Fresh matter basis)					
	Zinc (m/kg)	10	7.96	42.70	19.83	3.69
	Copper (m/kg)	9	1.39	211.00	134.28	20.87
	Manganese (m/kg)	10	6.52	23.50	13.09	1.83
	Iron (m/kg)	10	20.10	220.00	89.72	24.27
	Selenium ($\mu\text{g/kg}$)	10	202.70	857.50	520.04	63.06
	Plasma Trace Elements					
	Zinc (mg/l)	9	1.06	2.43	1.93	0.13
	Copper (mg/l)	10	0.61	3.35	1.61	0.26
	Manganese (mg/l)	4	0.05	0.43	0.19	0.09
	Iron (m/l)	7	0.72	2.22	1.51	0.19
	Selenium ($\mu\text{g/l}$)	10	59.40	98.17	82.45	4.14
	Plasma Biochemistry					
	ALAT ($\mu\text{kat/l}$)	10	0.62	1.04	0.88	0.04
	ASAT ($\mu\text{kat/l}$)	10	1.45	3.23	2.24	0.22
	Bilirubin ($\mu\text{mol/l}$)	10	2.20	5.80	4.26	0.33
	Bilirubin-direct	10	0.10	0.40	0.21	0.03
	Bilirubin indirect	10	2.00	5.60	4.03	0.32
	Ferritin (ng/ml)	10	14.20	161.40	43.00	13.79
	Production Parameters December 2008					
	Milk yield (kg/day)	10	23.00	43.90	34.63	2.35
	Fat (%)	10	2.96	5.37	3.98	0.25
	Protein (%)	10	3.14	4.18	3.54	0.10
	Milk urea (mg/l)	10	210.00	290.00	253.00	10.33
	Somatic cell count (* 1000)	10	19.00	437.00	105.00	40.50
	Lactose (%)	10	4.66	5.28	4.89	0.06
	Lactation number	10	1.00	3.00	1.90	0.31
	Days in milk	10	133.00	157.00	145.60	2.56
	Protein corrected milk (kg/day)	10	24.86	44.41	34.59	2.24
	Production Parameters November 2008					
	Milk yield (kg/day)	10	23.90	46.30	35.10	2.13
	Fat (%)	10	2.71	4.79	3.82	0.23
	Protein (%)	10	3.02	3.99	3.36	0.10
	Milk urea (mg/l)	10	200.00	290.00	241.00	10.48
	Somatic cell count (* 1000)	10	16.00	641.00	146.70	62.84
	Lactose (%)	10	4.49	5.13	4.85	0.06
	Lactation number	10	1.00	3.00	1.90	0.31
	Days in milk	10	98.00	122.00	110.60	2.56
	Protein corrected milk (kg/day)	10	26.17	41.75	33.92	1.69

Farm 6	Parameters	N	Minimum	Maximum	Mean	SEM
	Liver Trace Elements (Fresh matter basis)					
Zinc (m/kg)	9	9.28	23.00	16.63	1.80	
Copper (m/kg)	10	102.00	372.00	202.00	28.20	
Manganese (m/kg)	9	1.11	10.00	4.59	0.83	
Iron (m/kg)	10	10.60	255.00	81.54	28.93	
Selenium ($\mu\text{g/kg}$)	10	622.80	1566.00	970.57	113.82	
Plasma Trace Elements						
Zinc (mg/l)	9	0.41	1.91	0.91	0.14	
Copper (mg/l)	9	0.89	7.68	2.39	0.68	
Manganese (mg/l)	6	0.02	4.20	1.30	0.66	
Iron (m/l)	6	0.24	1.88	1.22	0.24	
Selenium ($\mu\text{g/l}$)	9	66.95	118.70	93.98	5.45	
Plasma Biochemistry						
ALAT ($\mu\text{kat/l}$)	10	0.69	0.89	0.78	0.02	
ASAT ($\mu\text{kat/l}$)	10	1.49	2.71	1.91	0.11	
Bilirubin ($\mu\text{mol/l}$)	10	1.60	5.70	3.77	0.37	
Bilirubin-direct	10	0.00	0.20	0.12	0.02	
Bilirubin indirect	10	0.00	5.40	3.48	0.47	
Ferritin (ng/ml)	10	8.40	45.70	23.45	4.43	
Production Parameters December 2008						
Milk yield (kg/day)	10	34.30	43.00	38.59	1.08	
Fat (%)	10	3.16	5.02	4.26	0.20	
Protein (%)	10	3.15	3.95	3.49	0.08	
Milk urea (mg/l)	10	250.00	300.00	277.00	5.78	
Somatic cell count (* 1000)	10	29.00	898.00	153.60	83.76	
Lactose (%)	10	4.67	4.93	4.82	0.03	
Lactation number	10	1.00	4.00	2.00	0.26	
Days in milk	10	91.00	197.00	133.80	11.55	
Protein corrected milk (kg/day)	10	32.83	46.04	39.89	1.11	
Production Parameters November 2008						
Milk yield (kg/day)	10	38.00	43.70	41.20	0.54	
Fat (%)	10	3.16	5.75	4.06	0.25	
Protein (%)	10	3.09	3.67	3.36	0.06	
Milk urea (mg/l)	10	200.00	310.00	246.00	9.09	
Somatic cell count (* 1000)	10	15.00	452.00	135.00	50.08	
Lactose (%)	10	4.74	5.03	4.89	0.03	
Lactation number	10	1.00	4.00	2.00	0.26	
Days in milk	10	56.00	162.00	98.80	11.55	
Protein corrected milk (kg/day)	10	34.78	52.87	41.46	1.59	

Farm 7	Parameters	N	Minimum	Maximum	Mean	SEM
	Liver Trace Elements (Fresh matter basis)					
	Zinc (m/kg)	10	2.40	9.72	6.11	0.76
	Copper (m/kg)	10	48.30	141.00	79.77	10.27
	Manganese (m/kg)	9	2.28	15.60	6.39	1.38
	Iron (m/kg)	10	52.70	187.00	129.98	14.71
	Selenium (µg/kg)	10	444.70	1049.00	720.80	58.44
Plasma Trace Elements						
	Zinc (mg/l)	10	0.57	2.05	1.36	0.19
	Copper (mg/l)	10	0.34	8.09	2.41	0.81
	Manganese (mg/l)	5	0.08	1.04	0.34	0.18
	Iron (m/l)	7	0.09	6.07	2.61	0.74
	Selenium (µg/l)	10	94.03	137.80	113.20	4.85
Plasma Biochemistry						
	ALAT (µkat/l)	10	0.84	1.18	0.93	0.03
	ASAT (µkat/l)	10	1.14	3.26	1.90	0.20
	Bilirubin (µmol/l)	10	2.70	6.70	4.36	0.34
	Bilirubin-direct	10	0.00	0.30	0.16	0.03
	Bilirubin indirect	10	2.60	6.40	4.21	0.31
	Ferritin (ng/ml)	10	12.10	88.40	38.02	8.44
Production Parameters December 2008						
	Milk yield (kg/day)	10	33.80	54.90	43.66	2.68
	Fat (%)	10	3.03	4.46	3.62	0.15
	Protein (%)	10	2.92	3.95	3.25	0.10
	Milk urea (mg/l)	10	200.00	320.00	263.00	12.12
	Somatic cell count (* 1000)	10	16.00	204.00	64.60	18.32
	Lactose (%)	10	4.40	5.08	4.83	0.06
	Lactation number	10	1.00	5.00	3.00	0.47
	Days in milk	10	102.00	173.00	134.80	7.87
	Protein corrected milk (kg/day)	10	31.01	50.26	41.07	2.09
Production Parameters November 2008						
	Milk yield (kg/day)	10	34.40	58.40	45.86	2.66
	Fat (%)	10	2.89	4.26	3.49	0.15
	Protein (%)	10	2.67	3.61	3.10	0.09
	Milk urea (mg/l)	10	180.00	290.00	245.00	10.25
	Somatic cell count (* 1000)	10	15.00	206.00	56.30	17.88
	Lactose (%)	10	4.34	5.09	4.83	0.06
	Lactation number	10	1.00	5.00	3.00	0.47
	Days in milk	10	76.00	157.00	117.20	8.60
	Protein corrected milk (kg/day)	10	31.66	50.71	42.12	2.29

	Parameters	N	Minimum	Maximum	Mean	SEM
Farm 8	<i>Liver Trace Elements (Fresh matter basis)</i>					
	Zinc (m/kg)	10	6.19	31.30	15.30	2.54
	Copper (m/kg)	10	35.20	348.00	122.84	32.09
	Manganese (m/kg)	10	0.38	8.75	2.63	0.82
	Iron (m/kg)	10	12.30	273.00	125.24	24.03
	Selenium ($\mu\text{g/kg}$)	10	472.10	3322.00	1470.47	295.86
	<i>Plasma Trace Elements</i>					
	Zinc (mg/l)	10	0.39	1.54	0.87	0.10
	Copper (mg/l)	10	0.80	4.26	2.13	0.37
	Manganese (mg/l)	8	0.03	1.21	0.59	0.15
	Iron (m/l)	6	0.46	2.40	0.97	0.32
	Selenium ($\mu\text{g/l}$)	10	99.67	167.60	118.76	6.22
	<i>Plasma Biochemistry</i>					
	ALAT ($\mu\text{kat/l}$)	10	0.62	1.01	0.82	0.04
	ASAT ($\mu\text{kat/l}$)	10	1.17	3.08	2.21	0.19
	Bilirubin ($\mu\text{mol/l}$)	10	1.40	6.00	4.34	0.41
	Bilirubin-direct	10	0.00	0.40	0.19	0.04
	Bilirubin indirect	10	1.30	5.80	4.11	0.40
	Ferritin (ng/ml)	10	16.20	38.50	26.52	2.62
	<i>Production Parameters December 2008</i>					
	Milk yield (kg/day)	10	20.20	48.90	35.66	2.99
	Fat (%)	10	2.63	4.76	3.53	0.22
	Protein (%)	10	3.01	3.90	3.48	0.08
	Milk urea (mg/l)	10	190.00	340.00	276.00	16.75
	Somatic cell count (* 1000)	10	29.00	277.00	118.10	27.51
	Lactose (%)	10	4.29	4.94	4.71	0.07
	Lactation number	10	1.00	4.00	1.90	0.38
	Days in milk	10	120.00	205.00	144.50	7.20
	Protein corrected milk (kg/day)	10	19.15	45.28	33.55	2.48
	<i>Production Parameters November 2008</i>					
	Milk yield (kg/day)	10	20.60	49.70	37.38	2.80
	Fat (%)	10	2.67	4.92	3.57	0.21
	Protein (%)	10	3.15	3.76	3.45	0.07
Milk urea (mg/l)	10	180.00	310.00	238.00	14.44	
Somatic cell count (* 1000)	10	16.00	3010.00	355.90	295.38	
Lactose (%)	10	4.35	5.08	4.84	0.07	
Lactation number	10	1.00	4.00	1.90	0.38	
Days in milk	10	85.00	170.00	109.50	7.20	
Protein corrected milk (kg/day)	10	20.93	49.19	35.44	2.64	

Farm 9	Parameters	N	Minimum	Maximum	Mean	SEM
	<i>Liver Trace Elements (Fresh matter basis)</i>					
	Zinc (m/kg)	9	13.00	32.00	20.57	2.16
	Copper (m/kg)	9	69.70	152.00	121.19	7.96
	Manganese (m/kg)	9	0.52	11.70	3.18	1.18
	Iron (m/kg)	9	21.50	150.89	76.10	15.49
	Selenium (µg/kg)	9	424.90	828.40	554.01	42.13
	<i>Plasma Trace Elements</i>					
	Zinc (mg/l)	9	0.60	2.01	1.47	0.17
	Copper (mg/l)	9	0.24	5.21	1.63	0.57
	Manganese (mg/l)	6	0.02	0.14	0.07	0.02
	Iron (m/l)	7	0.99	4.67	1.94	0.46
	Selenium (µg/l)	9	54.73	123.70	82.12	7.45
	<i>Plasma Biochemistry</i>					
	ALAT (µkat/l)	10	0.68	1.15	0.89	0.04
	ASAT (µkat/l)	10	1.15	2.37	1.82	0.14
	Bilirubin (µmol/l)	10	1.70	5.10	3.95	0.38
	Bilirubin-direct	10	0.00	0.20	0.08	0.02
	Bilirubin indirect	10	1.70	5.10	4.09	0.30
	Ferritin (ng/ml)	10	10.50	69.20	31.19	6.10
	<i>Production Parameters December 2008</i>					
	Milk yield (kg/day)	10	22.90	39.90	33.31	1.46
	Fat (%)	10	2.77	4.80	3.86	0.19
	Protein (%)	10	3.07	3.69	3.42	0.07
Milk urea (mg/l)	10	140.00	230.00	191.00	9.36	
Somatic cell count (* 1000)	10	21.00	235.00	84.60	24.54	
Lactose (%)	10	4.52	4.96	4.78	0.05	
Lactation number	10	1.00	5.00	2.50	0.43	
Days in milk	10	133.00	321.00	187.10	17.24	
Protein corrected milk (kg/day)	10	22.45	38.35	32.76	1.47	
<i>Production Parameters November 2008</i>						
Milk yield (kg/day)	10	24.90	42.10	32.51	1.49	
Fat (%)	10	2.93	4.73	3.97	0.18	
Protein (%)	10	3.22	3.70	3.44	0.05	
Milk urea (mg/l)	10	130.00	220.00	183.00	10.65	
Somatic cell count (* 1000)	10	21.00	221.00	100.90	21.67	
Lactose (%)	10	4.59	4.98	4.84	0.04	
Lactation number	10	1.00	5.00	2.50	0.43	
Days in milk	10	109.00	297.00	163.10	17.24	
Protein corrected milk (kg/day)	10	24.91	39.09	32.38	1.24	

	Parameters	N	Minimum	Maximum	Mean	SEM
Farm 10	<i>Liver Trace Elements (Fresh matter basis)</i>					
	Zinc (m/kg)	9	14.10	25.70	19.62	1.34
	Copper (m/kg)	9	66.60	227.00	130.36	18.68
	Manganese (m/kg)	10	2.55	12.50	4.41	0.93
	Iron (m/kg)	10	29.20	156.00	87.18	13.64
	Selenium (µg/kg)	9	576.90	1508.00	904.90	111.30
	<i>Plasma Trace Elements</i>					
	Zinc (mg/l)	5	1.17	2.54	1.79	0.23
	Copper (mg/l)	10	1.03	5.27	2.09	0.44
	Manganese (mg/l)	9	0.05	2.30	1.24	0.28
	Iron (m/l)	4	0.99	4.67	1.94	0.46
	Selenium (µg/l)	10	94.96	138.20	113.40	4.40
	<i>Plasma Biochemistry</i>					
	ALAT (µkat/l)	10	0.63	1.10	0.89	0.04
	ASAT (µkat/l)	10	1.12	2.01	1.51	0.07
	Bilirubin (µmol/l)	10	3.20	4.70	4.10	0.16
	Bilirubin-direct	10	0.00	0.20	0.11	0.02
	Bilirubin indirect	10	3.10	4.70	4.02	0.15
	Ferritin (ng/ml)	10	15.20	66.70	31.53	5.03
	<i>Production Parameters December 2008</i>					
	Milk yield (kg/day)	10	22.00	41.90	33.63	1.94
	Fat (%)	10	3.24	5.05	3.98	0.17
	Protein (%)	10	3.09	3.53	3.30	0.05
	Milk urea (mg/l)	10	180.00	270.00	236.00	7.77
	Somatic cell count (* 1000)	10	4.92	2417.00	388.69	236.80
	Lactose (%)	10	4.36	4.96	4.70	0.05
	Lactation number	10	1.00	6.00	2.90	0.62
	Days in milk	0				
	Protein corrected milk (kg/day)	10	24.65	42.70	33.23	1.83
	<i>Production Parameters November 2008</i>					
	Milk yield (kg/day)	10	26.10	46.30	36.12	2.19
	Fat (%)	10	2.83	3.91	3.49	0.12
	Protein (%)	10	3.17	3.82	3.47	0.06
Milk urea (mg/l)	10	190.00	300.00	258.00	11.23	
Somatic cell count (* 1000)	10	36.00	1689.00	399.10	195.76	
Lactose (%)	10	4.44	5.06	4.78	0.06	
Lactation number	10	1.00	6.00	2.90	0.62	
Days in milk	0					
Protein corrected milk (kg/day)	10	25.41	43.93	34.02	1.91	

	Parameters	N	Minimum	Maximum	Mean	SEM
Farm 11	<i>Liver Trace Elements (Fresh matter basis)</i>					
	Zinc (m/kg)	10	1.49	21.40	11.83	1.95
	Copper (m/kg)	9	21.90	205.00	111.87	21.89
	Manganese (m/kg)	9	1.46	9.19	4.09	0.75
	Iron (m/kg)	10	40.89	204.00	101.92	16.78
	Selenium ($\mu\text{g/kg}$)	10	122.60	1127.00	595.72	98.66
	<i>Plasma Trace Elements</i>					
	Zinc (mg/l)	7	1.53	2.33	1.91	0.11
	Copper (mg/l)	8	0.26	6.37	1.64	0.69
	Manganese (mg/l)	4	0.06	1.39	0.56	0.30
	Iron (m/l)	4	2.25	3.34	2.74	0.23
	Selenium ($\mu\text{g/l}$)	8	85.79	104.00	96.57	2.11
	<i>Plasma Biochemistry</i>					
	ALAT ($\mu\text{kat/l}$)	10	0.67	0.96	0.80	0.03
	ASAT ($\mu\text{kat/l}$)	10	1.34	2.54	2.08	0.13
	Bilirubin ($\mu\text{mol/l}$)	10	1.50	8.10	4.25	0.53
	Bilirubin-direct	10	0.00	0.20	0.08	0.02
	Bilirubin indirect	10	0.00	7.80	4.06	0.61
	Ferritin (ng/ml)	10	11.70	48.40	28.27	4.70
	<i>Production Parameters December 2008</i>					
	Milk yield (kg/day)	10	25.80	42.40	35.28	2.01
	Fat (%)	10	2.80	5.19	3.66	0.25
	Protein (%)	10	2.81	4.17	3.34	0.12
	Milk urea (mg/l)	10	160.00	270.00	218.00	10.52
	Somatic cell count (* 1000)	10	22.00	1980.00	313.70	193.12
	Lactose (%)	10	4.39	5.03	4.78	0.06
	Lactation number	10	1.00	4.00	2.20	0.33
	Days in milk	10	112.00	177.00	144.20	7.26
	Protein corrected milk (kg/day)	10	24.14	41.56	33.48	1.70
	<i>Production Parameters November 2008</i>					
	Milk yield (kg/day)	10	32.00	48.20	41.03	1.61
	Fat (%)	10	2.00	4.99	3.02	0.30
	Protein (%)	10	3.04	4.14	3.40	0.11
Milk urea (mg/l)	10	150.00	270.00	223.00	11.65	
Somatic cell count (* 1000)	10	13.00	1141.00	171.40	110.26	
Lactose (%)	10	4.48	5.04	4.81	0.06	
Lactation number	10	1.00	4.00	2.20	0.33	
Days in milk	10	84.00	149.00	112.70	7.67	
Protein corrected milk (kg/day)	10	31.46	41.40	36.03	1.17	