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I am submitting herewith a dissertation written by Markus Hugh Campbell entitled "Effects of Antioxidants Supplemented During the Periparturient Period of Lactating Dairy Cows." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

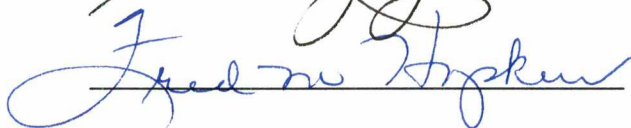

James K. Miller, Major Professor

We have read this dissertation
and recommend its acceptance:









Accepted for the Council:


Associate Vice Chancellor and
Dean of The Graduate School

EFFECTS OF ANTIOXIDANTS SUPPLEMENTED DURING
THE PERIPARTURIENT PERIOD
OF
LACTATING DAIRY COWS

A Dissertation
Presented for the
Doctor of Philosophy
Degree
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Markus Hugh Campbell

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DEDICATION

This dissertation is dedicated to my wife, son and daughter, Susan, Drew and Sarah Campbell. They are the people that have given the most and have the most to gain from the completion of this work.

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Abstract

Study 1. The hypothesis that incidence of retained fetal membranes can be reduced by limiting oxidative stress was evaluated with 56 Holstein and 8 Jersey primigravid heifers and 64 Holstein and 16 Jersey multiparous cows. Cows and heifers were supplemented with an antioxidant (vitamin E), a site specific damage retardant (Zn) or a potential prooxidant (Fe) in a 2 X 2 X 2 factorial arrangement during the last 42 d prepartum. Amounts of supplements per animal daily were 1,000 IU of vitamin E (as d,l α -tocopheryl acetate), 0.8 g of Zn (supplied half by Zn-methionine and half by ZnSO_4) and 12 g of Fe for Holsteins and 9 g of Fe for Jerseys (as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$). Incidence of retained fetal membranes was unaffected by supplementation of vitamin E or Zn. Although not directly affected by treatment, cows that retained fetal membranes had lower plasma α -tocopherol ($P = 0.03$). Similarly, cows that were not supplemented with vitamin E had lower plasma α -tocopherol ($P < 0.001$). Iron did not have any direct negative effects on reproductive performance. Cows receiving additional Fe, however, had lower unsaturated iron binding capacity ($P = 0.004$) which in turn was also lower ($P = 0.03$) in cows that retained fetal membranes. Supplementation with vitamin E or Zn reduced days to first estrus ($P < 0.005$). Vitamin E reduced days to first service

($P = 0.04$) and Zn tended to reduce days to first service ($P = 0.08$). Vitamin E tended to reduce days open ($P = 0.06$). Days open were not affected by supplementation of Zn. Services per conception or milk production were not affected by supplementation. Vitamin E and Zn were not directly effective at reducing the incidence of retained fetal membranes. However, vitamin E may be associated indirectly with retained fetal membranes through plasma α -tocopherol. Vitamin E and Zn were beneficial in the reduction of days to first estrus, indicating improved reproductive health in the early post partum period.

Study 2. The objectives of this research were: 1) to determine if organically complexed zinc, manganese, copper and cobalt would improve reproductive performance and(or) milk and milk component production in lactating dairy cows that started receiving bovine somatotropin in the ninth week of lactation and 2) if electronic estrous detection could replace blood analysis for estimating the onset of normal luteal activity in lactating dairy cows. Holsteins ($n = 50$) and Jerseys ($n = 10$) were blocked by breed, lactation number and incidence of retained fetal membranes. Two diets assigned within blocks and fed from parturition until 154 d of lactation were control or control supplemented with 359 mg Zn as zinc methionine, 199 mg Mn as manganese methionine, 125 mg Cu as copper lysine and 26 mg Co as cobalt glucoheptonate. The cows were fitted with electronic

pressure-sensing devices in the second wk of lactation. Ovarian structures were determined via transrectal ultrasonography at 7-d intervals from parturition until observation of the first corpus luteum. Blood samples were taken at 7-d intervals and analyzed for plasma concentration of progesterone, insulin and urea nitrogen. Onset of normal luteal activity was identified by progesterone concentrations ≥ 1 ng/mL. Retained fetal membranes increased days to first estrus (detected via electronic estrus detection) in the control cows (54.2 ± 10.2 vs. 81.0 ± 14.2 , $P = 0.05$) but not the supplemented cows (49.7 ± 11.0 vs. 44.0 ± 12.2 , $P = 0.64$). Furthermore, retained fetal membranes increased days to first luteal activity in the control cows (28.1 ± 2.7 vs. 41.8 ± 3.4 , $P < 0.001$) but not the supplemented cows (27.7 ± 2.8 vs. 30.0 ± 3.3 , $P = 0.36$). Retained fetal membranes also increased days to first corpus luteum in the control cows (31.3 ± 2.2 vs. 40.2 ± 3.8 , $P = 0.04$) but not the supplemented cows (29.8 ± 2.2 vs. 34.8 ± 3.6 , $P = 0.23$). Days to first service, days open, days from first service to conception, services per conception, milk production, milk components and somatic cell counts were similar for control and treatment cows. Mounts recorded by the electronic system were compared with increases in progesterone from values < 1 ng/mL as an index of luteal activity. Accuracies of detecting onset of normal luteal activity by ≥ 1 , ≥ 2 , ≥ 3 , or ≥ 4 mounts of ≥ 2 s duration

in 24 h, respectively, were $46.4\% \pm 0.06$ ($n = 26$ cows), $35.7\% \pm 0.06$ ($n = 20$ cows), $26.8\% \pm 0.06$ ($n = 15$ cows), and $23.2\% \pm 0.06$ ($n = 13$ cows), ($P = 0.04$). Accuracies of electronic estrus detection in determining estrus after 45 d postpartum by 1, 2, 3, ≥ 4 mounts were $30.1\% \pm 0.03$, $24.5\% \pm 0.03$, $44.8\% \pm 0.08$ and $73.9\% \pm 0.03$ ($P < 0.001$). Supplementation with complexed trace minerals effectively reduced days to first estrus. Although electronic estrous detection provides benefits in reproductive management, it is not an effective substitute for blood sampling in determining the onset of normal luteal activity in lactating dairy cattle.

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CHAPTER I

INTRODUCTION

The peripartum period is the most stressful time in the productive life of a dairy cow. The high veterinary cost during this period indicates the high degree of stress (Correa et al., 1993). Also, the peripartum period is when the cow's antioxidant defenses are at the lowest level (Goff and Stabel, 1990), most likely due to the reduced dry matter intake. Reduced dry matter intake complicates the negative energy balance generated by the initiation of lactation. Negative energy balance, along with other stressful events such as retained fetal membranes, metritis, and mastitis, as well as use of bovine somatotropin produce longer intervals from calving to first estrus and calving to conception.

To reduce the postpartum interval and thus calving interval, intensive estrus observation is critical. From a research standpoint, determination of the first estrus or onset of normal luteal activity is of value. The onset of normal luteal activity is determined through collection of blood samples at least weekly until the plasma progesterone reaches ≥ 1 ng/mL. If there is a high correlation between the onset of normal luteal activity and display and detection of estrus, then collection of blood samples could be eliminated. Elimination of blood collection would reduce

labor required and stress on the animal. Electronic estrous detection uses a pressure-sensitive transmitter which is placed in a patch and glued to the cows rump (Stevenson et al., 1996), approximately 13 cm posterior of the tail head on the midline. When the cow displaying estrus is mounted by another cow, a button on the transmitter is depressed and a signal is sent to the computer. The software maintains a record of cow identification mounting date, time and duration. The advantage of electronic estrus detection is 24 h surveillance.

Supplementation of antioxidants during the peripartum period could result in higher plasma levels. Due to reduced intake, antioxidant supplementation during the peripartum period may need to be greater than in later lactation. Supplementation of antioxidants have been shown to be beneficial in some (Trinder et al., 1969; Julien et al., 1976ab; Eger et al., 1985; Weiss et al., 1990), but not all cases (Gwazdauskas et al., 1979; Segerson et al., 1981; Hidiroglou et al., 1987). Research where antioxidants were not beneficial have a single injection during the prepartum period in common. Therefore, daily supplementation of antioxidants may be beneficial.

The objectives of this research were 1) determine if vitamin E and(or) Zn supplemented during the pre-partum period could reduce the incidence of retained fetal membranes and improve reproduction, 2) determine if complex

trace minerals known to have antioxidant function could improve reproductive performance and milk production and 3) determine if electronic estrous detection could replace the need for collecting blood samples to determine the onset of normal luteal activity.

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CHAPTER II

LITERATURE REVIEW

Oxygen and Oxidative Stress

Oxygen. Oxygen is an essential element for life of all aerobic animals. It is used to oxidize foodstuffs rich in carbon and hydrogen for energy. For example, glucose is oxidized by oxygen to produce carbon dioxide, water and energy. However, the above example is an over simplification of the conversion of foodstuffs to usable energy. Aerobic animals are very efficient in the conversion of glucose to useable energy. For example, complete oxidation of one molecule of glucose, through glycolysis, citric acid cycle and oxidative phosphorylation results in the production of 36 to 38 moles of adenosine triphosphate (ATP) compared to a net of 2 moles ATP produced under anaerobic conditions of glycolysis.

The process of oxidative phosphorylation produces energy through the transfer of electrons to oxygen and thus reducing it to water. This provides an opportunity for electrons to escape and produce partially reduced oxygen (superoxide). Oxygen requirement of oxidative phosphorylation is a paradox, in that electrons can escape in either the hypo or hyperoxia state (Levine and Kidd,

1985). For example, increased activity and metabolic rate results in excess oxygen which can escape partially reduced. In the event of oxygen deficiency, electrons can accumulate at the end of the oxidative phosphorylation chain and thus leak out.

Superoxide spontaneously dismutates to hydrogen peroxide. Neither superoxide nor hydrogen peroxide are highly reactive, although they are very mobile. When they come in contact with unbound Fe, the Fe catalyzes production of highly reactive hydroxyl radical. The hydroxyl radical is capable of initiating oxidative chain reactions and producing membrane damage. To prevent production of hydroxyl radical, a carefully balanced antioxidant system must be in place. Superoxide is converted to hydrogen peroxide by superoxide dismutase. Hydrogen peroxide is converted to water by glutathione peroxidase and the Fe must be bound to proteins such as albumin, ceruloplasmin and transferrin to prevent its involvement in reactions.

Oxidative stress. Ryan and Aust (1992) explained that "the simple fact that life exists in an oxygen-rich environment suggests that living organisms have developed efficient defense systems that prevent or correct uncontrolled oxidations." Although oxygen is essential, oxygen toxicity can occur when prooxidants (oxygen derived species) surpass the capacity of antioxidant defense systems (Miller et al., 1993; Gutteridge and Halliwell, 1994). The

balance can be lost due to over production of reactive oxygen metabolites (free radicals) that overcome the antioxidant defense or due to nutritional deficiency of nutrients such as vitamins and trace minerals which contribute to antioxidant defense (Machlin and Bendich, 1987). When production of reactive oxygen metabolites exceeds safe disposal, oxidative chain reactions can occur, for example lipid peroxidation.

Free Radicals

Definition. Free radicals are any substance which exists with one or more unpaired electrons (Johnson et al., 1992; Gutteridge and Halliwell, 1994) and are generated during normal cellular metabolism (Bendich, 1993). Since free radicals are unstable (Johnson et al., 1992), they cause cellular damage. For example, the hydroxyl radical will take an electron from the nearest molecule to satisfy it's outer electrons orbit. In the instance of lipid peroxidation, an electron is taken from the hydrocarbon chain of the phospholipid making up the cell membrane. With the addition of oxygen to hydrocarbon chains, the cell becomes more hydrophilic and thus more permeable to water. Emery (1991) called free radicals the "most powerful and destructive chemical agents that can occur in the body".

Sources. Environmental sources that increase the free

radical burden include ultraviolet light, tobacco smoke, organic solvents, pesticides, anesthetics, hyperoxia, certain drugs and other pollutants (Machlin and Bendich, 1987; Johnson et al., 1992; Bendich, 1993). Endogenous sources include autooxidants of flavins and thiols, the activity of electron transport chains, and enzymes such as oxidases, cyclooxygenases, lipoxygenases, dehydrogenases and peroxidases (Machlin and Bendich, 1987; Johnson et al., 1992). Free radicals can be generated in all cellular constituents (Machlin and Bendich, 1987) and in addition to normal metabolism can result from the metabolism of certain drugs or xenobiotics (Bendich, 1993). Oxygen species include the hydroxyl radical, which can result from the low level of radiation present in the atmosphere, and superoxide, which can result from leakage of the electron transport chain (Gutteridge and Halliwell, 1994). Willson (1987) stated that free radicals, such as the superoxide radical, the hydroxyl radical or the organic peroxy radicals generated in the body, are chemically and thermally identical to those generated by radiation. The hydroxyl radical is much more reactive than either hydrogen peroxide or the superoxide anion radical (Johnson et al., 1992) and will randomly initiate a surge of oxidizing free radicals reactions (Buettner, 1993).

Production of free radicals results in lipid peroxidation and in damage to membranes, enzymes, and

nucleic acids (DNA) (Halliwell, 1987; Machlin and Bendich, 1987; Bendich, 1993; Buettner, 1993). Buettner (1993) noted that polyunsaturated fatty acids (PUFA-H, H represents a very loosely bound bis-allylic hydrogen) are highly susceptible to peroxidation because of the easily oxidizable bis-allylic H. Machlin and Bendich (1987) reported that sulfur containing enzymes and other proteins were susceptible to free radical damage by inactivation, cross-linking, and denaturation.

In addition to the free radicals listed above, hydrogen peroxide and singlet oxygen are two other reactive substances which can cause similar damage but are not free radicals (Machlin and Bendich, 1987). Superoxide and hydrogen peroxide (reduced intermediates of molecular oxygen) are recognized as ubiquitous, inorganic products of normal aerobic metabolism (Gutteridge and Quinlan, 1993). However, superoxide and hydrogen peroxide are poorly reactive in aqueous solution and by themselves are not capable of oxidizing DNA, lipids or proteins. Transitional metal ions, particularly iron, are required to convert these into highly reactive and damaging forms of free radicals.

Iron and related free radicals. Iron, an essential trace element, has catalytic activity. Iron containing proteins have important roles in respiration, DNA synthesis, as well as regulation of the citric acid cycle (Ryan and Aust, 1992). However, the same properties that enable iron

to function as a cofactor in controlled redox chemistry also make it a potent toxin when oxidative susceptible biomolecules are not protected (Ryan and Aust, 1992). Free radical generation can be initiated by reactions (transfer of electrons) from transition metals such as iron to oxygen containing molecules (Halliwell, 1987; Machlin and Bendich, 1987; Beach and Giroux, 1992; Johnson et al., 1992). Buettner (1993) stated that the one electron reduction seems to be an especially dangerous route since not only will it result in dismutation of superoxide to hydrogen peroxide, superoxide can also reduce an Fe (III) complex to an Fe(II) complex, thus providing all the components for the Fenton reaction. For example, Fe(II) can react with hydrogen peroxide to produce the hydroxyl radical (Beach and Giroux, 1992; Harada and Mayberg, 1992; Johnson et al., 1992; Buettner, 1993) so hydrogen peroxide is considered a potential free radical.

Iron damage is believed to result from lipid peroxidation initiated through the generation of the hydroxyl radical (Morel et al., 1992). Johnson et al. (1992) postulated that iron overload causes hepatic injury by: 1) increased lysosomal membrane fragility, mediated perhaps by iron-induced lipid peroxidation; 2) peroxidative damage to mitochondria and microsomes, causing organelle dysfunction; and 3) the direct effect of iron on collagen biosynthesis. Hebbel (1986) stated that erythrocytes are

extremely susceptible to damage by active oxygen species because of their constant exposure to oxygen and high iron availability.

Many studies have shown the toxic effects of iron. Gutteridge and Quinlan (1993) demonstrated that plasma without any iron-binding and iron-oxidizing potential stimulates lipid peroxidation. Loss of membrane fluidity has been directly related to decreased ability of lymphocytes to respond to the demands of the immune system (Bendich, 1990). Chronic iron overload in humans is related to hepatocellular damage and fibrosis and leads to cirrhosis (Bassett et al., 1986). Lund and Aust (1990) observed a correlation between the extent of DNA damage and the iron content of asbestos, indicating that iron in asbestos, not the asbestos, was responsible for the DNA damage. Stevens et al. (1988) found that men in whom cancer developed showed a lower total iron-binding capacity and higher levels of transferrin saturation, implying that saturation of the iron-binding capacity, an indicator of iron overload, can be correlated to cancer in men.

Observations of animals and their diets have also shown the toxicity of iron. Kanner et al. (1990) found that lipid oxidation was reduced by 50% when iron supplementation was removed from turkey diets. Miller et al. (1994b) found non-heme iron was greater in pork from swine that consumed high iron diets and that iron levels in swine diets at or below

209 ppm decreased lipid oxidation. The deterioration of food was accelerated in the presence of iron (Willson, 1987; Miller et al., 1994a). Pitzten (1993) observed that when cows that were consuming water with high iron content were fed five pounds of roasted soybeans, they produced milk that had a rancid oxidized off-flavor, suggesting that the iron was involved in the lipid peroxidation of the milk.

Excessive supplementation of iron may well increase the risk of infection since bacteria, like most living organisms, require iron for growth (Reiter et al., 1975; Emery, 1991).

Not all free radicals are destructive to the body and some can benefit an organism, such as some reactive oxygen metabolites which are generated by normal metabolic processes (Buettner, 1993; Miller et al., 1993). For example, hydrogen peroxide (potential free radical) and superoxide are used by several enzymes and phagocytic cells to destroy bacteria, demonstrating their usefulness (Halliwell, 1987).

Antioxidants

Definition. Antioxidants can protect against free radical damage. An antioxidant is defined as any substance that prevents the transfer of electrons to and from molecular oxygen and organic molecules, stabilizes organic

free radicals, or terminates organic free radical reactions (Bray and Bettger, 1990).

Sources. Some substances, such as vitamin C, can act as either antioxidant or prooxidant (Beach and Giroux, 1992). Similarly, this property of vitamin C applies to vitamin E as well and has led Herbert (1994) to state "no supplement is a pure antioxidant" and question the advisability of supplementation with "so-called antioxidants". Rather, he advocates a diet naturally adequate in all nutrients required for antioxidant defense. Bendich (1992) states that both vitamin A and C are required for strong antioxidant effect, although vitamin A is less active, it is an antioxidant. Vitamin E is a potent antioxidant which protects membrane structure and function by protecting cells from lipid peroxidation (Bendich, 1993). Vitamin E and the selenium containing enzyme, glutathione peroxidase, are essential parts of the antioxidant system present in most mammalian cells (Hoekstra, 1975). The neutrophils of humans consuming vitamin E or selenium deficient diets have reduced bactericidal activity (Baehner et al., 1977; Boxer et al., 1979; Boxer, 1986). Vitamin E and selenium (as a component of glutathione peroxidase) are antioxidants that protect neutrophils from toxic oxygen molecules (Baker and Cohen, 1983) and their supplementation during the dry period reduced the incidence and duration of mastitis (Hogan et al., 1993). Vitamin E appears to stop

peroxidative chains by reacting with organic peroxy radicals (Machlin and Bendich, 1987; Willson, 1987) and provides protection against synthesis and accumulation of lipoperoxides in tissues (Yoshioka et al., 1987). Although not direct antioxidants, the trace minerals (copper, manganese and zinc) are important for enzyme functions. For example, copper, manganese and zinc are vital for the activity of superoxide dismutase (Bendich, 1993). Copper, iron and zinc are essential for proper immune activity (Sherman, 1992). Zinc functions as an antioxidant *in vitro* (Bray and Bettger, 1990). Unlike iron, zinc normally does not catalyze redox reactions which precludes generation of free radicals and reactive oxygen species (Cousins and Hempe, 1990).

Retained Fetal Membranes

Definition. Retention of the fetal membrane is commonly referred to as retained placenta. The fetus is attached by the umbilical cord to the placenta, an organ attached within the uterus by 70 or more cotyledons (McCormack, 1979). After calving, the placenta should be separated from the uterus and be expelled due to decreased blood flow to the cotyledons and increased uterine contractions. Incidence of retained fetal membranes is approximately 10% in dairy cattle (Erb et al., 1958; Muller

and Owens, 1974; Julien et al., 1976b; Gwazdauskas et al., 1979), however, occurrence can be as high as 50% in some areas known to be low in selenium (Muller and Owens 1974; Julien et al., 1976a; Julien et al., 1976b). Generally, retained placenta is suggested to occur if the fetal membranes have not been passed in a short period of time. The suggested time after which the cow is considered to be retained has varied in the opinions of different investigators. Some researchers considered that the fetal membranes are retained if the placenta is not passed within 24 h of parturition (Curtis et al., 1985; Holt et al., 1989; Schukken et al., 1989; Deluyker et al., 1991; Correa et al., 1993). Other investigators define retained fetal membranes as those which have not been eliminated within 12 h postpartum (McCormack, 1979; Heuwieser et al., 1993; Miller et al., 1993). Martin et al. (1986) used a range of time to describe the retained cow, stating that the placenta was retained if not expelled within 8 to 12 h of parturition.

McCormick (1979) stated that if the placenta is not separated, it is assumed that there is an infection present. However, this may not always be the case due to hormonal controls (Roche and Diskin, 1995) and nutritional status (Hogan et al., 1993) during the periparturient period.

Parturition. The events at parturition can have an effect on retention of fetal membranes. Allenstein (1978) suggests that mycotic or other non-specific infections in

the uterus, nutrition, condition of cow and even excitement during the calving process could result in retained placenta. Other events at parturition which can increase incidence of retained fetal membranes include stillbirth (Correa et al., 1993; Emanuelson et al., 1993), twins (Correa et al., 1993; Markusfeld, 1987) and dystocia (Correa et al., 1993; Emanuelson 1993). Curtis et al. (1985) stated that dystocias assisted by veterinarians did not increase the risk of retained placenta. However, it was noted that few veterinarian assisted calvings with dystocia were reported, possibly due to delivery of most calves by dairy operators themselves rather than by veterinarians. Sex of the offspring may be indirectly related to retention of fetal membranes through a higher incidence of dystocia if the calf is male (Correa et al., 1993) due to larger size of male calves. In contrast, Martin et al. (1986) reported that sex of the calf was not related to incidence of retained fetal membranes, however, dystocia was not considered.

Metabolic relationships. Numerous relationships between metabolic disorders and disease conditions have been reported. Cows that were hypocalcemic had a greater risk of retained placenta (Allenstein, 1978). Milk fever (hypocalcemia) increased the incidence of retained placenta (Curtis et al., 1985), and the incidence of retained fetal membranes increased the occurrence of ketosis (Curtis et

al., 1985; Bendixen et al., 1987; Markusfeld 1987).

Chassagne and Barnouin (1992) observed that cows which retained their placenta had lower plasma $\text{PGF}_{2\alpha}$ and glucose levels than cows that did not retain. The reduction of energy and plasma $\text{PGF}_{2\alpha}$ would explain why cows that retained fetal membranes would be slower to display estrus activity.

Related diseases. Retention of fetal membranes increased the occurrence of metritis (Erb et al., 1981; Bendixen et al., 1987; Werven et al., 1992; Emanuelson et al., 1993) and mastitis (Bendixen et al., 1988; Schukken et al., 1989; Werven et al., 1992; Correa et al., 1993; Emanuelson et al., 1993). Schukken et al. (1989) hypothesized that animals that retained fetal membranes have increased risk of clinical mastitis due to decreased peripheral leukocyte activity. Werven et al. (1992) reported an increase in displaced abomasum associated with an increase in the duration of retained placenta. This relationship between retained fetal membranes and displaced abomasum is probably due to suppressed appetite.

Milk production. Effects of periparturient disorders on milk production have varied in different reports. Dohoo et al. (1983) stated that diseases can reduce milk yield. After evaluating 388 lactations through 119 days in milk on a 500 cow Holstein dairy in the San Joaquin Valley, Deluyker et al. (1991) concluded retained fetal membranes decreased milk yield by 8.8% during the first 5 d and by a

nonsignificant ($P = 0.1$) 2.8% the first 21 d. Metritis decreased milk production by 9.6% the first 5 d, by 6.9% the first 21 d, and by 4.9% the first 119 d. Werven et al. (1992) compared cows which did not retain fetal membranes with cows which retained placentas increasing periods of time. In the first parity, percentage increases in total milk production in the first 100 d in comparison with cows which retained for longer periods were +7.5 for < 8 h, +11.0 for < 12 h, and +9.0 for < 23 h. In second and third parity cows, percentage declines in total 100 d milk yield with increasing retention times were -4.6 for 6 h, -5.5 for 8 h, -6.6 for 12 h, -6.2 for 23 h, -6.5 for 47 h and -7.0 for 71 h. In fourth and greater parity cows, percentage declines of total 100 d milk yield with increasing duration of retention were -3.9 for 6 h, -6.7 for 12 h, -7.8 for 23 h, -7.5 for 47 h and -6.7 for 71 h. These results suggest retained fetal membranes reduce subsequent milk production more in the first lactation than in later lactations. Reduction in milk yield also appeared to be greater after placental retention > 12 h but did not appear to increase thereafter.

In contrast to an earlier report (Dohoo et al., 1983), Dohoo and Martin (1984) found milk production was not related to a significant risk of any of the common disease conditions in 2875 lactations from 32 commercial herds near Guelph, Ontario. Martin et al. (1986) also reported milk

yield was not significantly different in cows with or without retained fetal membranes. In path model analysis, Erb et al. (1981) found the diseases discussed above were associated with increased breed class milk production. They discounted this increase, however, as due to delayed conception of cows with disease states and suggested the rise in milk production from cows with cystic ovaries was hormonally influenced. They also reported previous lactation is not important in developing reproductive disease but disease affects both current production and reproduction.

Reproductive performance. Disease can impair reproductive efficiency. Ovulatory dysfunction increases with metritis and ketosis (Emanuelson et al., 1993). Retained placenta and metritis are causes of cystic follicles and increased calving interval (Erb et al., 1981). Calving interval was increased 19.1 d by retained placenta and 4 wk by metritis. Holt et al. (1989) observed 60 Holstein cows, 20 of which retained placenta. Days in milk when corpus lutea were detected were 21 d for cows without retained placenta, 28 d for cows with uterine discharge, and 27 d for cows with retained fetal membranes. A normal corpus luteum duration of 12 to 16 d was found in 50% of the cows without retained placenta but in only 6.2% of the cows which retained fetal membranes. Retention of fetal membranes increased days to conception from 97 to 134 d and

services per conception from 1.6 to 2.5. Cows with retained placenta had four more days to first service, 19 more days open, and 0.2 more services per conception (Martin et al., 1986).

Effects of fetal membrane retention on reproductive performance may be influenced by parity. Werven et al. (1992) reported retained placenta did not affect reproductive performance in first through third parities. In fourth and greater parity, however, cows that retained the fetal membranes longer than 6 h had a 17 d increase to first service and an additional 26 d open. Diseases can reduce the reproductive life of the cow, thereby increasing replacement cost (Dohoo et al., 1983).

Condition and age. Cows in proper body condition, approximately 3.25 (scale 1 = thin, 5 = obese) at parturition, are less likely to retain fetal membranes (West, 1995). The reduced incidence of retained fetal membranes of cows in proper body condition suggests a relationship between nutritional status and retention of fetal membranes. Increased incidence of health and reproductive disorders can occur in cows that are either too thin or too fat (Gerhart et al., 1990). Loss of fat is also an indicator of disease susceptibility. Waltner et al. (1993) compared body condition with incidence of diseases and found no significant correlation between body condition score and incidence of pyometra, metritis, retained fetal

membranes, cystic ovarian disease, service per conception, days to first service or dystocia. Body condition varied little in the herd examined, however, with changes of only 0.3 for first lactation and 0.9 for lactations 2 through 4. Apparently body condition in this case was more a reflection of excellent management than of disease status. A relationship between body condition score, reproduction, and disease may be more evident in herds with a wider range of body condition scores (Waltner et al., 1993).

Incidence of retained placenta also appears to be influenced by age. As parity, an indicator of age increases, incidence of retained placenta also increases (Markusfeld, 1987). Effects of parity on retention of fetal membranes longer than 6 h were reported by Werven et al. (1992). Cows shedding the placenta within 6 h included 66% of the total herd, 73% of cows in first lactation, 67% for second and third parity and 59% for fourth parity.

Related cost. More veterinary cost and health problems are associated with the postpartum period than any other time (Correa et al., 1993). Shanks et al. (1981) reported that over 50% of medical costs occur in the first 30 d of lactation. It is generally agreed that additional days open cost the producer but costs per day vary for individual farms due to labor, production, and other cost. Grusenmeyer and Hillers (1989) estimate the range of annual costs from \$1.50 to \$5.00 per cow for each day open beyond 90 d. They

further stated that annual cost estimates are \$1.50 per cow in the herd for each 0.1 services per conception over 1.5. The cost of semen used in the operation would certainly affect the cost of additional services per conception. Grusenmeyer and Hillers (1989) estimated that there was a loss of production of 0.08 kg per day for each day over the average 150 d in milk.

Conclusion. Occurrence of one disorder increases risk of other disorders (Curtis et al., 1985) and many disease occurrences are not independent but are related (Dohoo and Martin, 1984). The occurrence of retained fetal membranes increases the incidence of other diseases and can cause a depression in milk production as well as reproductive failure which would increase the rate of involuntary culling. Although metritis that was developed due to retained placenta can be fatal (Trinder et al., 1969), it is generally controlled in today's commercial dairy herds. The indirect costs seem to outweigh the direct cost involved in treatment of retained fetal membranes. Efforts should concentrate on the condition and the nutrition of the cow to reduce the incidence of retained fetal membranes as well as improve reproduction. A high rate of retained fetal membranes occurred in an area known to have a history of white muscle disease in sheep (Trinder et al., 1969). These animals responded favorably to supplementation with vitamin E and selenium indicating that they may have been

experiencing oxidative stress. Additionally, the supplementation of selenium and vitamin E reduced the incidence of retained fetal membranes in some (Julien et al., 1976ab; Eger et al., 1985;) but not all studies (Gwazdauskas et al., 1979; Segerson et al., 1981). Supplementation as an injection of selenium and vitamin E once at approximately 20 d prepartum was similar in these experiments. Furthermore, it is known that a higher incidence of retained fetal membranes occur in geographical areas low in selenium (Segerson et al., 1981), which may explain the variable results reported.

Trace Elements and Reproduction

Supplementation. Two decades ago, ruminants diets were frequently deficient in trace elements which led to infertility through enzymatic dysfunction (Hidiroglou, 1979). Current commercial production of concentrates limit deficiencies through general supplementation of trace elements. However, there may be some benefits in reproduction when trace elements are supplemented beyond recommended daily allowances.

Cobalt. Cobalt deficiency results in poor reproductive performance, with reduced conception rate being the most common occurrence (Hidiroglou, 1979). Cows that were cycling normally had higher serum cobalt than cows that were

anestrous (Vhora et al., 1995). Similarly, sheep displayed less estrus activity per cycle when cobalt was deficient (Hidiroglou, 1979).

Copper. Early embryonic loss is the most common reproductive event when copper is deficient (Hidiroglou, 1979). In sheep, a copper deficient diet resulted in early embryonic loss (McChowell, 1968). Services per conception were reduced when cows were supplemented with copper (Hunter, 1977). Supplementation of equine diets with copper sulfate resulted in shorter postpartum interval (Fomina, 1970).

Manganese. Anestrus, reduced conception rate (Wilson, 1966) and increased embryonic mortality (Manspeaker, 1987) have been observed with manganese deficiency in cattle. Ewes consuming a low manganese diet had increased services per conception (Hidiroglou et al., 1978). Hurley and Keen (1987) stated that manganese deficiency only causes anestrous during the "acute stage of deficiency". However, DiCostanzo et al. (1986) reported a reduction in days to first estrus and days to conception when primiparous beef cows consumed a diet supplemented with manganese compared to cows that were provided an unsupplemented diet that was considered adequate in manganese.

Zinc. Early embryonic death resulted when ewes consumed a zinc deficient diet (Hidiroglou, 1979). Cows provided zinc supplement had an increased conception rate

(Nedyjlkov and Krustev, 1969). However, reproduction was unaffected when cows recieved 1000 or 2000 mg/kg zinc (Miller et al., 1988).

Combinations. Improvements in reproductive performance have resulted when combinations of trace elements have been supplemented. Days to first estrus and days to conception were reduced when cows were supplemented with copper, manganese and zinc when compared to cows that were recieving a diet considered adequate in trace elements (DiCostanzo et al., 1986). Additionally, chelated minerals have shown improvements in reproductive performance in beef cattle (Kropp, 1993) and dairy heifers (Manspeaker and Robl, 1993) over cows consuming diets that contained adequate levels of trace minerals.

Proper supplementation of adequate amounts of trace minerals and vitamins should be paramount during the dry period and early lactation since this is the time period when the majority of stressful events occur and also the period when plasma levels of these substances are at the lowest level (Goff and Stabel, 1990). Antioxidants would have a beneficial practical application if they successfully reduced the incidence of retained fetal membranes and(or) improved reproductive efficiency.

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CHAPTER III

EFFECT OF SUPPLEMENTAL VITAMIN E AND ZINC ON RETAINED FETAL MEMBRANES OF DAIRY COWS FED EXCESS IRON

Abstract

The hypothesis that incidence of retained fetal membranes can be reduced by limiting oxidative stress was evaluated with 56 Holstein and 8 Jersey primigravid heifers and 64 Holstein and 16 Jersey multiparous cows. Cows and heifers were supplemented with an antioxidant (vitamin E), a site specific damage retardant (Zn) or a potential prooxidant (Fe) in a 2 X 2 X 2 factorial arrangement during the last 42 d prepartum. Amounts of supplements per animal daily were 1,000 IU of vitamin E (as d,l α -tocopheryl acetate), 0.8 g of Zn (supplied half by Zn-methionine and half by ZnSO_4) and 12 g of Fe for Holsteins and 9 g of Fe for Jerseys (as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$). Incidence of retained fetal membranes was unaffected by supplementation of vitamin E or Zn. Although not directly affected by treatment, cows that retained fetal membranes had lower plasma α -tocopherol ($P = 0.03$). Similarly, cows that were not supplemented with vitamin E had lower plasma α -tocopherol ($P < 0.001$). Iron did not have any direct negative effects on reproductive performance. Cows receiving additional Fe, however, had

lower unsaturated iron binding capacity ($P = 0.004$) which in turn was also lower ($P = 0.03$) in cows that retained fetal membranes. Supplementation with vitamin E or Zn reduced days to first estrus ($P < 0.005$). Vitamin E reduced days to first service ($P = 0.04$) and Zn tended to reduce days to first service ($P = 0.08$). Vitamin E tended to reduce days open ($P = 0.06$). Days open were not affected by supplementation of Zn. Services per conception or milk production were not affected by supplementation. Vitamin E and Zn were not directly effective at reducing the incidence of retained fetal membranes. However, vitamin E may be associated indirectly with retained fetal membranes through plasma α -tocopherol. Vitamin E and Zn were beneficial in the reduction of days to first estrus, indicating improved reproductive health in the early post partum period.

Key words: Dairy Cattle Reproduction, Estrous Detection, Antioxidants

Introduction

The periparturient period is a critical time in the health of dairy cattle and can have a significant effect on the level of production for the entire lactation. More veterinary costs and health problems are associated with the early postpartum period than any other time (Correa et al.,

1993). Health disorders during this period involve cost of treatment as well as reductions in milk production, market value, and productive life of the cow (Miller et al., 1993).

Retained fetal membranes occur when the placenta has not been shed within a short time after parturition. Cows with retained fetal membranes have an increased risk of metritis (Correa et al., 1993; Emanuelson et al., 1993) and increased risk of developing mastitis during early lactation (Bendixen et al., 1988). Milk yield reportedly decreased 8.8% during the first 5 d of lactation by retained placenta and by 4.9% of the 119 d total by metritis (Deluyker et al., 1991). Other problems associated with retained placenta or metritis include cystic follicles and increased calving intervals (Erb et al., 1981), greater days to first service, more days open and increased services per conception (Martin et al., 1986). Annual cost estimates for each day open beyond 90 d from parturition range from \$1.50 to 5.00 per cow (Grusenmeyer and Hillers, 1989). This wide range of estimated cost for days open is due to variation in management costs of production. Still, even at the lower end of the range (\$1.50) an improvement in performance would be economically beneficial.

A high rate of retained fetal membranes in cattle has been observed in an area known to have a history of white muscle disease in sheep (Trinder et al., 1969). Favorable responses of these animals to supplementation with selenium

and vitamin E suggests they were experiencing oxidative stress. Oxidative stress occurs when reactive metabolites of oxygen are produced faster than they can be safely removed by antioxidant defense mechanisms (Gutteridge and Halliwell, 1994). These reactive oxygen species are produced during normal metabolism and serve some beneficial functions for the animal. However, they can be converted to the highly reactive hydroxyl radical in the presence of transition elements such as iron. The resulting hydroxyl radical can damage lipids, proteins, polysaccharides, DNA and other macromolecules (Halliwell, 1987) and randomly initiate expanding chains of oxidizing free radical reactions (Buettner, 1993). The potential toxicity of oxygen despite its requirement by all aerobic organisms has been termed "the oxygen paradox" (Miller et al., 1993).

To prevent damaging free radical events, antioxidants must be available. An antioxidant as usually defined is any substance which: 1) prevents transfer of electrons to and from molecular oxygen and organic molecules, 2) stabilizes organic free radicals, and 3) terminates free radical reactions (Bray and Bettger, 1990). Vitamin E functions as an antioxidant by protecting against synthesis and accumulation of lipoperoxides in tissues (Yoshioka et al., 1987) thereby maintaining membrane structure and function (Bendich, 1993). By inhibiting oxidation of polyunsaturated fatty acids in neutrophil membranes (Baker and Cohen, 1983),

vitamin E together with the selenium enzyme glutathione peroxidase protects neutrophils against toxic oxygen molecules (Hogan et al., 1992). Vitamin E and glutathione peroxidase are essential parts of the immune system present in most mammalian cells (Hoekstra, 1975). Tissue breakdown due to accumulation of iron and generation of hydroxyl radical is reduced by the Vitamin E chain breaking action (Dillard et al., 1983). Zinc, in contrast to vitamin E, does not function directly as a chain-breaking antioxidant apart from its role as an important component of enzymes with antioxidant activity (Bendich, 1993). Zinc may still have antioxidant functions, however, due to its ability to displace iron at sensitive sites on important molecules (Bray and Bettger, 1990). Unlike iron which can exist in the ferric or ferrous form, zinc does not display direct redox chemistry and generation of the hydroxyl radical and other free radicals capable of damaging DNA and other macromolecules are precluded.

Plasma concentration of both α -tocopherol (vitamin E) and zinc are lowest during the periparturient period (Goff and Stable, 1990). These decreases may be due to depressed dry matter intake (DMI), decreased transport capacity of the vitamin in plasma (Weiss et al., 1990), and secretion into colostrum (Goff and Stable, 1990) during the periparturient period. Supplementation with these nutrients may be beneficial during the dry period of dairy cows to compensate

for the low DMI that results in lower plasma levels of α -tocopherol and zinc. Recommended allowances of vitamin E are 150 IU/d for dry cows and 300 IU/d for lactating cows (NRC, 1989). However, Hogan et al. (1993) reported that both dry and lactating dairy cows should be supplemented with 1000 IU/d vitamin E for maximal health benefits. Additional zinc above the recommended 40 mg/kg of dry matter (NRC, 1989) may also be beneficial.

The occurrence of retained fetal membranes increases the incidence of other diseases and reduces reproduction and milk production. Thus, an increase in antioxidant defense with a reduction of retained fetal membranes would be beneficial. The objective of this experiment was to determine if supplementation of dairy cattle during the dry period with antioxidants (vitamin E and/or Zn) could reduce the incidence of retained fetal membranes and improve reproduction and milk production in the presence of two levels of Fe (prooxidant).

Materials and Methods

Holstein (n = 64 multiparous and 56 primigravid) and Jersey (n = 16 multiparous and 8 primigravid) cows were dosed daily with all combinations of vitamin E, Fe and Zn in gelatin capsules during the last 42 d ante partum in a 2 X 2 X 2 factorial arrangement. The treatments were control,

1000 IU of vitamin E (as 2 g of d,l α -tocopheryl acetate), 0.8 g of Zn (supplied half by 4 g of zinc methionine and half by 1.13 g of ZnSO_4) and 12 g of Fe for Holsteins and 9 g for Jerseys (as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$). Cows were provided 3.6 kg/d commercial concentrate (275 mg/kg Fe and 235 mg/kg Zn) and ad libitum grass hay (106 mg/kg Fe and 26 mg/kg Zn). Cows were maintained on a dry lot until -14 d of expected parturition at which time they were moved to the maternity lot for the remainder of the dry period. Cows in the maternity lot received 4.5 kg/d of commercial concentrate and ad libitum grass hay.

Blood samples were obtained via jugular vein at -42 d (prior to initiation of treatment), -28 d, -14 d and at parturition. Blood samples were maintained on ice during collection and transport to laboratory. Plasma α -tocopherol was measured by HPLC (Hidiroglou, 1989). Plasma α -tocopherol was measured to determine if supplementation of vitamin E (as d,l α -tocopheryl acetate) would result in increased plasma levels. Also, plasma α -tocopherol levels were used to determine if Zn would have a sparing effect or if Fe supplementation would result in reduced levels due to consuming reactions. Plasma Zn was measured by atomic absorption as a possible indicator of Zn status. Unsaturated iron-binding capacity measured by a commercial kit (Sigma Chemical Co., St. Louis, MO; Persijn, et al., 1971) was used as an indicator of iron overload.

Cows were observed at least every 4 h after parturition for the presence of fetal membranes. Cows that had not shed fetal membranes within 12 h of parturition were considered retained. After parturition, cows were moved in with the lactating herd. Cows were observed for estrus detection at 0700 and 1630. A voluntary waiting period of 55 d postpartum was observed for all cows before the initiation of first artificial insemination service. After the voluntary waiting period, cows were bred on the next observed estrus using an A.M./P.M. rule (cows that were in estrus in the A.M. were bred in the P.M. and cows that were in estrus in the P.M. were bred the following A.M.). Pregnancy was determined 40 to 47 d post breeding by transrectal ultrasonography.

Data were analyzed using a mixed model approach (SAS, 1989) with a randomized block design using the model $Y_{ijk} = \mu + T_i + B_j + P_k + e_{ijk}$ where Y_{ijk} = observation, μ = overall mean, T_i = effect of treatment (i = vitamin E, Fe, and(or) Zn), B_j = effect of breed (j = Holstein or Jersey), P_k = effect of parity (k = primigravid or multiparous) and e_{ijk} = residual. Cow and block were included in the model as random variables. Milk yield (kg/d) was analyzed as a repeated measure for wk 1 to 12. Results are reported as least squares means $\pm$ standard error.

Results

Dietary supplementation had no direct effect on the percentage of cows that retained fetal membranes. Incidence of retained fetal membranes was neither decreased by the antioxidants vitamin E or Zn nor increased by the potential prooxidant Fe. The least squares means of retained fetal membranes of the treatment groups are in Table 1.

Plasma α -tocopherol ($\mu\text{g/mL}$) measured at parturition, was higher for vitamin E supplemented cows (1.15 ± 0.06) than cows not supplemented (0.73 ± 0.07 , $P < 0.001$, Figure 1). It was not lowered by Fe (0.90 ± 0.06 vs. 0.99 ± 0.07 $\mu\text{g/mL}$, $P = 0.29$) or spared by Zn (0.96 ± 0.11 vs. 0.92 ± 0.11 $\mu\text{g/mL}$, $P = 0.68$). However, cows with retained fetal membranes had lower plasma α -tocopherol than cows that lost fetal membranes (Table 2).

Plasma Zn levels ($\mu\text{g/mL}$) at parturition were similar for cows supplemented with vitamin E (0.76 ± 0.03) and cows not supplemented with vitamin E (0.77 ± 0.03 , $P = 0.72$). Plasma Zn levels were similar for cows supplemented (0.75 ± 0.03) or not supplemented with Fe (0.78 ± 0.03 , $P = 0.55$) but were lower for cows supplemented with Zn (0.70 ± 0.03) than for cows not receiving Zn (0.82 ± 0.08 , $P = .002$, Figure 2). Plasma Zn was not lower in the cows that retained fetal membranes than cows that did not retain fetal membranes (Table 2).

Table 1. Least squares means of retained fetal membranes for cows supplemented or not supplemented with vitamin E, iron and zinc

Supplement	Not supplemented		Supplemented		P
	Mean	SE	Mean	SE	
	% of cows				
Vitamin E	16.6	5.8	16.0	5.5	0.93
Iron	17.6	5.8	15.1	5.5	0.70
Zinc	18.3	5.7	14.3	5.6	0.53

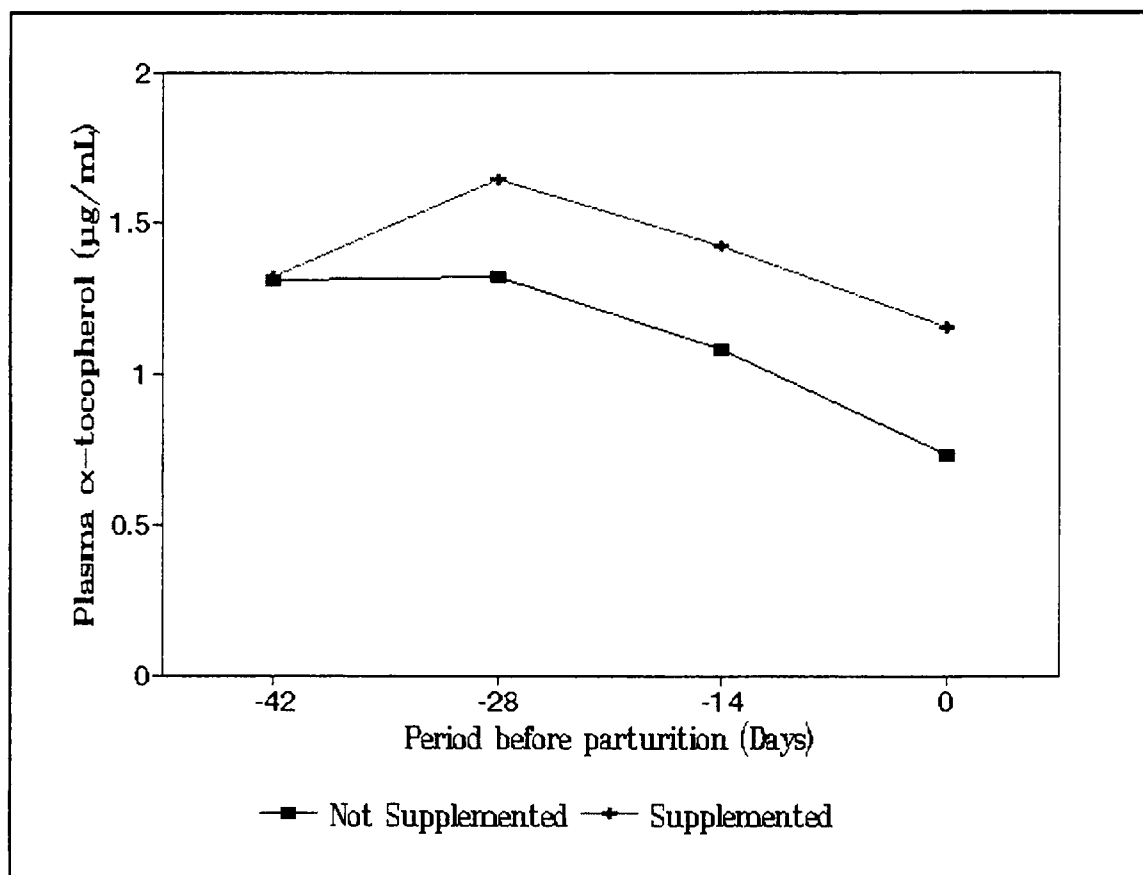


Figure 1. Least squares means of plasma α -tocopherol for cows supplemented or not supplemented with vitamin E. SE = 0.36.

Table 2. Least squares means of plasma α -tocopherol, serum unsaturated iron-binding capacity (UIBC) and plasma zinc for cows with absence or presence of retained fetal membranes

Measurement	Retained fetal membranes				P
	Absent		Present		
	Mean	SE	Mean	SE	
α -tocopherol ($\mu\text{g/mL}$)	1.02	0.04	0.83	0.08	0.03
UIBC ($\mu\text{g/dL}$)	183.5	3.7	158.5	10.8	0.03
UIBC ($\mu\text{g/dL}$, multiparous)	174.2	5.2	154.7	8.8	0.06
UIBC ($\mu\text{g/dL}$, primiparous)	192.8	5.3	162.4	19.7	0.14
Zinc ($\mu\text{g/mL}$)	0.76	0.02	0.70	0.04	0.12

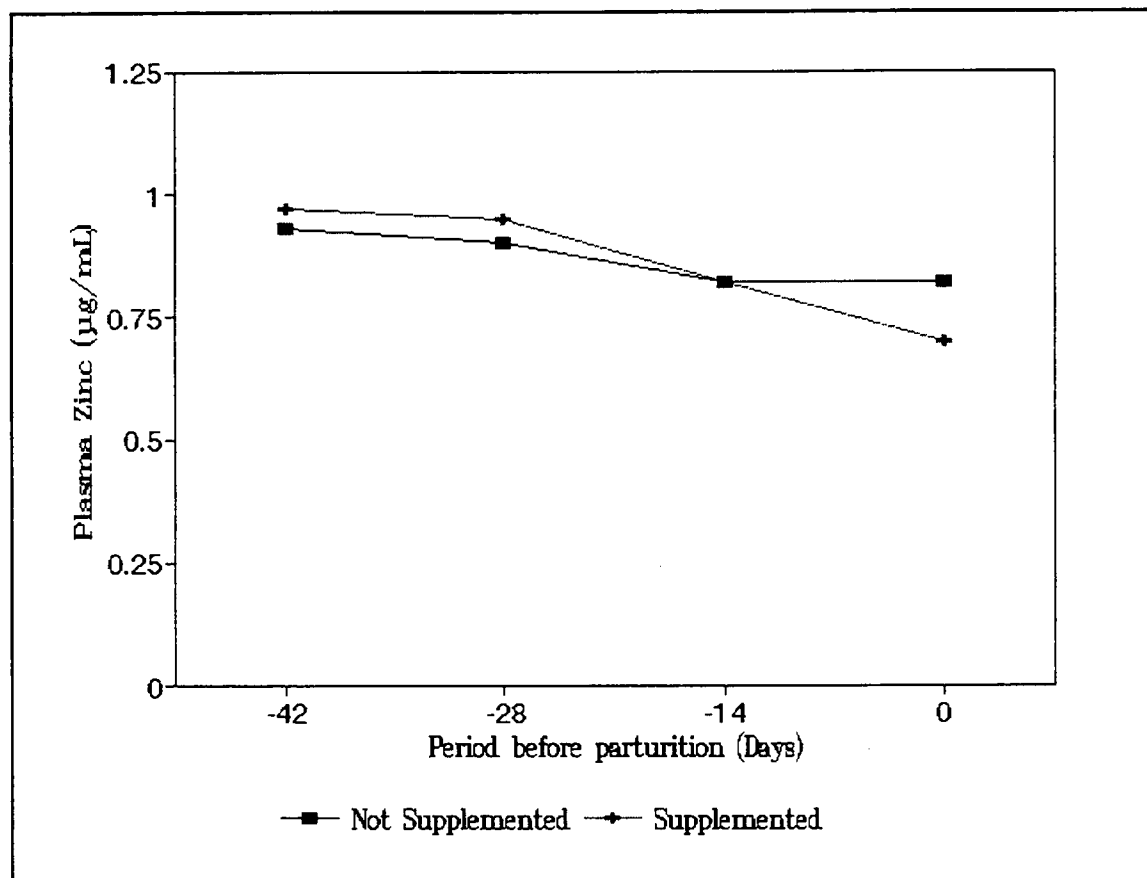


Figure 2. Least squares means of plasma zinc for cows supplemented or not supplemented with zinc. SE = 0.16.

Cows that retained fetal membranes had lower serum unsaturated iron-binding capacity than cows that did not retain fetal membranes (Table 2). Serum unsaturated iron-binding capacity was not affected by supplementation of vitamin E or Zn (Table 3). However, it was lower in cows receiving supplemental Fe than in cows not receiving additional Fe. Multiparous cows had lower unsaturated iron-binding capacity (175.8 ± 4.9 vs. 200.6 ± 5.6 $\mu\text{g/dL}$, $P < 0.001$), but higher incidence of retained fetal membranes (26.5 ± 5.5 vs. $6.1 \pm 5.8\%$, $P = 0.002$) than primiparous cows. Unsaturated iron-binding capacity was lower in primiparous cows and tended to be lower for multiparous with Fe supplementation (Table 3). There was only a tendency for unsaturated iron-binding capacity to be lower with presence of retained fetal membranes in multiparous cows and no difference in primiparous cows (Table 2).

Days to first estrus were lower for cows supplemented during the prepartum period with vitamin E, Fe or Zn in comparison with cows not receiving these supplements (Table 4). Data were then divided into two groups depending whether they were receiving additional Fe (Table 5). Cows receiving vitamin E had fewer days to first estrus regardless of Fe intake. However, cows supplemented with Zn only had fewer days to first estrus than cows not supplemented with Zn when additional Fe was not present. Days to first estrus were not different for cows

Table 3. Least squares means of serum unsaturated iron-binding capacity for cows supplemented or not supplemented with vitamin E, iron and zinc

Supplement	Not supplemented		Supplemented		<i>P</i>
	Mean	SE	Mean	SE	
	-----µg/dL-----				
Vitamin E	189.4	6.2	187.0	5.9	0.78
Iron	200.7	5.9	175.7	6.2	0.004
Iron (multiparous)	184.7	6.7	166.8	7.2	0.07
Iron (primiparous)	216.7	7.9	184.5	8.1	0.005
Zinc	188.3	6.1	188.1	5.9	0.99

Table 4. Least squares means of days to first estrus for cows supplemented or not supplemented with vitamin E, iron and zinc

Supplement	Not supplemented		Supplemented		P
	Mean	SE	Mean	SE	
	-----days-----				
Vitamin E	61.7	4.7	42.5	4.3	0.003
Iron	60.4	4.5	43.8	4.8	0.01
Zinc	61.3	4.5	42.9	4.5	0.004

Table 5. Least squares means of days to first estrus for cows supplemented or not supplemented with vitamin E and zinc in the absence (-Fe) or presence (+Fe) of additional iron

Supplement	Not supplemented		Supplemented		<i>P</i>
	Mean	SE	Mean	SE	
	-----days-----				
Vitamin E (-Fe)	70.5	6.6	50.4	6.0	0.03
Vitamin E (+Fe)	52.9	6.6	34.6	6.0	0.04
Zinc (-Fe)	73.8	6.5	47.1	6.1	0.004
Zinc (+Fe)	48.8	6.1	38.6	6.5	0.26

supplemented or not supplemented with Zn in the presence of additional Fe.

Cows supplemented with vitamin E had fewer days to first service than cows not supplemented with vitamin E (Table 6). Also, there was a tendency for days to first service to be reduced for cows supplemented with either Fe or Zn than for cows not supplemented with these elements. Again data were divided into two groups with regards to Fe supplementation (Table 7). Days to first service were similar for cows regardless of vitamin E status when no additional Fe was provided. However, cows supplemented with vitamin E tended to have fewer days to first service than cows not supplemented with vitamin E when additional Fe was supplemented. Cows supplemented with Zn had fewer days to first service than cows not receiving supplemental Zn when Fe was not provided. However when additional Fe was present, Zn supplementation did not affect days to first service.

Days open tended to be lower for cows supplemented with vitamin E or Fe than for cows not supplemented with vitamin E or Fe (Table 8). Days open were not different for cows supplemented with Zn and cows not receiving additional Zn. Cows supplemented with vitamin E tended to have fewer days open when additional Fe was not supplemented (Table 9). Cows supplemented with vitamin E had similar days open to cows not supplemented with vitamin E when additional Fe was

Table 6. Least squares means of days to first service for cows supplemented or not supplemented with vitamin E, iron and zinc

Supplement	Not supplemented		Supplemented		<i>P</i>
	Mean	SE	Mean	SE	
	-----days-----				
Vitamin E	71.5	10.4	62.4	10.4	0.04
Iron	70.6	10.4	63.3	10.3	0.10
Zinc	70.9	10.4	63.0	10.4	0.08

Table 7. Least squares means of days to first service for cows supplemented or not supplemented with vitamin E and zinc in the absence (-Fe) or presence (+Fe) of additional iron

Supplement	Not supplemented		Supplemented		<i>P</i>
	Mean	SE	Mean	SE	
	-----days-----				
Vitamin E (-Fe)	74.0	10.8	67.2	10.9	0.28
Vitamin E (+Fe)	69.1	10.9	57.6	10.7	0.07
Zinc (-Fe)	79.2	10.9	62.0	10.9	0.008
Zinc (+Fe)	62.6	10.8	64.0	10.8	0.82

Table 8. Least squares means of days open for cows supplemented or not supplemented with vitamin E, iron and zinc

Supplement	Not supplemented		Supplemented		<i>P</i>
	Mean	SE	Mean	SE	
	-----days-----				
Vitamin E	145.2	10.6	112.7	13.3	0.06
Iron	152.2	13.6	105.6	10.1	0.008
Zinc	134.1	12.0	123.8	12.0	0.55

Table 9. Least squares means of days open for cows supplemented or not supplemented with vitamin E and zinc in the absence (-Fe) or presence (+Fe) of additional iron

Supplement	Not supplemented		Supplemented		P
	Mean	SE	Mean	SE	
	-----days-----				
Vitamin E (-Fe)	178.1	15.3	126.4	22.7	0.06
Vitamin E (+Fe)	112.2	14.9	99.1	14.0	0.52
Zinc (-Fe)	163.9	19.5	140.5	19.3	0.40
Zinc (+Fe)	104.1	14.2	107.1	14.7	0.88

present. Cows supplemented with Zn had similar days open to cows not supplemented with Zn regardless of absence or presence of additional Fe.

Services per conception were similar for vitamin E, no vitamin E supplementation, Fe, no Fe supplementation, Zn and no Zn supplementation (Table 10). Milk production (kg/d) through the first 12 wk of lactation were similar for the all treatments (Table 11).

Discussion

Supplementation of vitamin E, Zn, or Fe did not directly affect the incidence of retained fetal membranes. Reports of vitamin E and selenium's affect on incidence of retained fetal membranes are contradictory. Some researchers have observed a reduction in the incidence of retained fetal membranes with a single injection of selenium and vitamin E given approximately 21 d prepartum (Julien et al., 1976a,b; Eger et al., 1985). In contrast others have found no benefit of vitamin E or selenium (Gwazdauskas et al., 1979; Segerson et al., 1981; Hidiroglou et al., 1987).

The incidence of retained fetal membranes can only be measured as an all or nothing situation. For this reason, an assay which would provide a more sensitive index of retained fetal membranes may provide a more useful indicator of treatment effects than presence or absence of this

Table 10. Least squares means of services per conception for cows supplemented or not supplemented with vitamin E, iron and zinc

Supplement	Not supplemented		Supplemented		P
	Mean	SE	Mean	SE	
	-----services/conception-----				
Vitamin E	1.9	0.5	2.0	0.5	0.53
Iron	2.0	0.5	2.0	0.5	0.98
Zinc	1.9	0.5	2.1	0.5	0.41

Table 11. Least squares means of milk production for cows supplemented or not supplemented with vitamin E, iron and zinc

Supplement	Not supplemented		Supplemented		<i>P</i>
	Mean	SE	Mean	SE	
	-----kg/d-----				
Vitamin E	29.1	1.0	28.3	0.9	0.60
Iron	29.4	1.0	27.9	1.0	0.28
Zinc	28.7	1.0	28.7	1.0	0.97

disorder. Blood assays chosen were plasma α -tocopherol, Zn and serum unsaturated iron-binding capacity. Although not directly related, supplementation of vitamin E can be linked to retained fetal membranes through plasma α -tocopherol. Supplementation of vitamin E increased plasma α -tocopherol concentration and cows that did not retain fetal membranes had higher plasma α -tocopherol than cows that retained fetal membranes. Plasma α -tocopherol at parturition was lower (1.15 $\mu\text{g/mL}$) in the present study when compared to other research in which plasma α -tocopherol ranged from approximately 2.3 to 2.9 $\mu\text{g/mL}$ (Weiss et al., 1992; Weiss et al., 1994; Hogan et al., 1996). However, these studies fed silage and Hidioglou et al. (1994) reported that cows receiving hay had lower (2.25 $\mu\text{g/mL}$) plasma α -tocopherol than cows consuming grass silage (3.41 $\mu\text{g/mL}$). In the present study, the cows were on a dry lot with no fresh forage and had free choice hay which would contribute to the lower plasma α -tocopherol.

Supplementation with Zn resulted in lower plasma Zn concentration contrary to expectation. Dufty et al. (1977) reported that the major cause of variation of plasma zinc was variation among individual animals. In the present study, plasma zinc at parturition ranged from 0.24 to 1.46 $\mu\text{g/mL}$. A lower plasma concentration of Zn may appear counterintuitive, however, in addition to improved absorption, the complexed form of Zn may be utilized more

efficiently after absorption (Wedekind et al., 1992). Furthermore, Zn in the control diet could have prevented any possible increases in plasma Zn due to the supplement. In the ruminant, Zn is almost evenly distributed throughout the body tissues (Miller, 1978) and plasma concentration may not be an accurate measurement of Zn status. Furthermore, plasma Zn was not different in cows that did or did not retain fetal membranes.

Unsaturated iron-binding capacity is an indication of the ability of serum to bind additional Fe. The lower the value, the fewer sites are available to bind Fe. It is important to bind Fe to prevent it from catalyzing the production of hydroxyl radicals. The free radical superoxide and the potential free radical hydrogen peroxide are not highly reactive and result in little damage themselves. In the presence of catalytic Fe, however, superoxide and hydrogen peroxide produce the highly reactive and destructive hydroxyl radical.

When both multiparous and primiparous cows are grouped together, those receiving supplemental Fe as well as those that retained fetal membranes had lower serum unsaturated iron-binding capacity than cattle without supplemental Fe or retained fetal membranes. However, lower unsaturated iron-binding capacity combined with higher incidence of retained fetal membranes in cows compared with heifers contributed to these results. Lower unsaturated iron-binding capacity in

multiparous cows than in heifers may result from greater accumulation of Fe over a longer time. One possible sequence of events could be Fe accumulation over a period of years, with more highly saturated iron-binding sites thus leading to increased availability of catalytic Fe which in turn contributed to a higher incidence of retained fetal membranes. Although treatment has not been shown to have a direct effect, vitamin E may be linked to reduced incidence of retained fetal membranes through plasma α -tocopherol. Likewise, Fe may be linked with an increased incidence of retained fetal membranes through serum unsaturated iron binding capacity.

Supplementation with vitamin E, Fe and Zn was discontinued at parturition but residual effects on reproduction performance were evident 2 to 3 mo later. Days to first estrus were reduced by all three of the supplements. Vitamin E reduced days to first estrus. In contrast to the present study, Hidioglou et al. (1987) reported no improvement in reproductive performance when a single injection of vitamin E and selenium was given approximately 21 d prior to parturition. Vitamin E reduced days to first estrus regardless of whether or not Fe was supplemented. However, Zn was only effective in reducing days to first estrus when Fe was not supplemented. Iron may increase the competition between Fe and Zn by masking binding sites (Bray and Bettger, 1990). Even with a 55 d

voluntary waiting period, days to first service were lower with supplementation of vitamin E and tended to be lower with supplementation of Fe and Zn. Vitamin E only tended to reduce days to first service when additional Fe was supplemented and did not affect days to first service when additional Fe was not supplemented. However, Zn reduced days to first service when no additional Fe was present. Days to first service were not affected by Zn supplementation when additional Fe was present. Days open were reduced for cows supplemented with Fe and tended to be reduced for cows supplemented with vitamin E when no supplemental Fe was present but was not affected by Zn supplementation. Services per conception were not different among treatments.

Whether differences in reproductive efficiency are treatment effects or due to an unusually high number of days to first estrus (82.0 ± 9.9) in the group receiving no supplement cannot be determined from the available data in the experiment. Under the present conditions, it can not be concluded with certainty that reproductive efficiency was improved by prepartum supplementation with vitamin E or Zn without also acknowledging it was improved by excess Fe. If effects of Zn are real, it is exceptional that addition of 800 mg of Zn to a diet already supplying approximately 1100 mg of Zn daily should improve days to first estrus. This would suggest improvement was due more likely to the

organically combined rather than the inorganic component of the Zn supplement.

Milk production during the first 12 wk of lactation was similar for groups receiving supplements. If oxidative stress had been reduced by vitamin E or Zn or increased by excess Fe during the supplementary period, residual carry over into lactation was insufficient to be reflected by milk production. Cost of vitamin E or Zn could thus not be recouped in increased milk production alone.

Implications

Although incidence of retained fetal membranes was not directly increased by excess Fe or decreased by supplemental vitamin E or Zn, indirect relationships were apparent. Plasma iron-binding sites tended to be more completely saturated when dietary Fe was excessive as evidenced by lower unsaturated iron-binding capacity which also tended to be lower in cows that retained fetal membranes. Supplemental vitamin E elevated plasma α -tocopherol which was elevated in cows which did not retain fetal membranes. Although supplementation was terminated at parturition, reproductive efficiency was improved in cows which had received vitamin E or Zn. Days to first estrus and first service were reduced and days open tended to be reduced by

vitamin E supplementation. Supplemental Zn also decreased days to first estrus and if the voluntary waiting period had been shorter, may have reduced days to first service and days open as well. Neither vitamin E or Zn improved conception rate. Iron supplementation reduced serum unsaturated iron-binding capacity which was linked to increased incidence of retained fetal membranes. Vitamin E and possibly Zn supplemented during the late gestation may have benefits which continue after parturition.

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CHAPTER IV

EFFECT OF ADDITIONAL ZINC, COPPER, MANGANESE, AND COBALT ON REPRODUCTION AND MILK PRODUCTION OF LACTATING DAIRY COWS RECEIVING BOVINE SOMATOTROPIN

Abstract

The objectives of this research were: 1) to determine if organically complexed zinc, manganese, copper and cobalt would improve reproductive performance and(or) milk and milk component production in lactating dairy cows that started receiving bovine somatotropin in the ninth week of lactation and 2) if electronic estrous detection could replace blood analysis for estimating the onset of normal luteal activity in lactating dairy cows. Holsteins (n = 50) and Jerseys (n = 10) were blocked by breed, lactation number and incidence of retained fetal membranes. Two diets assigned within blocks and fed from parturition until 154 d of lactation were control or control supplemented with 359 mg Zn as zinc methionine, 199 mg Mn as manganese methionine, 125 mg Cu as copper lysine and 26 mg Co as cobalt glucoheptonate. The cows were fitted with electronic pressure-sensing devices in the second wk of lactation. Ovarian structures were determined via transrectal ultrasonography at 7-d intervals

from parturition until observation of the first corpus luteum. Blood samples were taken at 7-d intervals and analyzed for plasma concentration of progesterone, insulin and urea nitrogen. Onset of normal luteal activity was identified by progesterone concentrations ≥ 1 ng/mL. Retained fetal membranes increased days to first estrus (detected via electronic estrus detection) in the control cows (54.2 ± 10.2 vs. 81.0 ± 14.2 , $P = 0.05$) but not the supplemented cows (49.7 ± 11.0 vs. 44.0 ± 12.2 , $P = 0.64$). Furthermore, retained fetal membranes increased days to first luteal activity in the control cows (28.1 ± 2.7 vs. 41.8 ± 3.4 , $P < 0.001$) but not the supplemented cows (27.7 ± 2.8 vs. 30.0 ± 3.3 , $P = 0.36$). Retained fetal membranes also increased days to first corpus luteum in the control cows (31.3 ± 2.2 vs. 40.2 ± 3.8 , $P = 0.04$) but not the supplemented cows (29.8 ± 2.2 vs. 34.8 ± 3.6 , $P = 0.23$). Days to first service, days open, days from first service to conception, services per conception, milk production, milk components and somatic cell counts were similar for control and treatment cows. Mounts recorded by the electronic system were compared with increases in progesterone from values < 1 ng/mL as an index of luteal activity. Accuracies of detecting onset of normal luteal activity by ≥ 1 , ≥ 2 , ≥ 3 , or ≥ 4 mounts of ≥ 2 s duration in 24 h, respectively, were $46.4\% \pm 0.06$ ($n = 26$ cows), $35.7\% \pm 0.06$ ($n = 20$ cows), $26.8\% \pm 0.06$ ($n = 15$ cows), and $23.2\% \pm 0.06$ ($n = 13$ cows).

cows), ($P = 0.04$). Accuracies of electronic estrus detection in determining estrus after 45 d postpartum by 1, 2, 3, ≥ 4 mounts were $30.1\% \pm 0.03$, $24.5\% \pm 0.03$, $44.8\% \pm 0.08$ and $73.9\% \pm 0.03$ ($P < 0.001$). Supplementation with complexed trace minerals effectively reduced days to first estrus. Although electronic estrous detection provides benefits in reproductive management, it is not an effective substitute for blood sampling in determining the onset of normal luteal activity in lactating dairy cattle.

Key words: Dairy cattle reproduction, Trace elements, Estrous detection

Introduction

The postpartum period is a stressful time for the dairy cow. A slow rise in dry matter intake accompanied with increased milk production results in a negative energy balance (Butler and Smith, 1989). This periparturient period is associated with the highest incidence of health disorders (Correa et al., 1993). Short and Adams (1988) suggested that reproduction follows basal metabolism, activity, growth, maintenance of basic energy reserves and lactation in the order of priority of energy partitioning. Thus, reproduction should be considered a luxury during this period when a cow is under stress of negative energy balance due to lactation. In addition to lactational stress,

recombinant bovine somatotropin can increase reproductive failures. Measures of reproductive performance that have indicated reproductive failure related to the supplementation of bovine somatotropin include increased incidence of anestrus (Morbeck et al., 1991; Esteban et al., 1994; Waterman et al., 1993), increased days to first service (Morbeck et al., 1991; Zhao et al., 1992), and reduced conception rate (Cole et al., 1992; Stanisiewski et al., 1992; Esteban et al., 1994; Hansen et al., 1994). Economic losses due to reproductive failure are second only to mastitis (Manspeaker and Robl, 1993).

Manganese deficiency delays estrus, reduces conception rate and increases embryonic mortality in beef cattle (Manspeaker, 1987). Increased display of estrus and conception rate have resulted when trace minerals were added to deficient diets (Kropp, 1993). The postpartum interval has been shortened 7.3 d with equine diets supplemented with copper sulfate (Fomina, 1970). Even when the basal diet contained adequate levels of trace minerals, chelated minerals have been shown to improve reproductive performance in beef cattle (Kropp, 1993) and dairy heifers (Manspeaker and Robl, 1993). In addition to improved reproductive performance, Kropp (1993) reported that 205 d weaning weights of calves from heifers receiving chelated minerals were greater, suggesting that milk production may have been higher.

An important measure of reproductive efficiency is days to first estrus, which indicates the female is cycling and preparing for conception. However, this can be a measurement of reproductive management quality rather than the actual response of the animal if estrus is displayed and not observed. Routine estrous detection is by visual observation consisting of two periods of 20 to 30 min duration. This leaves approximately 23 h when the animals are not observed strictly for estrus, although there will be some observation of animals in connection with other activities. Therefore, a method of 24 h estrous detection would measure response of animal without the effect of management. Also, reproductive efficiency could be increased by improving estrous detection since it is a critical part of the timing of artificial insemination (Grove et al., 1996). In respect to research, a tool to estimate first luteal activity would greatly reduce the amount of labor as well as the additional stress on the animal involved with blood sampling for the detection of progesterone.

The first objective of this study was to determine if complexed Co as cobalt glucoheptonate, Cu as copper lysine, Mn as manganese methionine, and Zn as zinc methionine added to diets adequate in these elements could improve reproductive performance and(or) milk and milk component production in lactating dairy cows before and after the

supplementation of recombinant bovine somatotropin. The second objective was to determine if electronic estrous detection could replace blood analysis for determining first normal luteal activity.

Materials and Methods

Lactating Holsteins (n = 50) and Jerseys (n = 10) were blocked according to lactation number (2 and 3, or ≥ 4), breed and whether fetal membranes had been retained > 12 h after present parturition. Cows within blocks were then assigned to either control or treatment diet at parturition until 154 d. A blended ration containing on DM basis 45% corn silage, 27% long stem alfalfa hay and 28% concentrate was provided to both groups for ad libitum intake at 0830 and 1630. Additional concentrates were supplied to meet or exceed NRC recommended allowances via computer feeder (Ration MasterTM; Alfa Laval Agri., Kansas City, MO). For the treatment group, part of the concentrate was fortified to supply 359 mg of Zn as Zn methionine, 199 mg of Mn as Mn methionine, 125 mg of Cu as Cu lysine and 26 mg of Co as Co glucoheptonate (4Plex; Zinpro Corp., Eden Prairie, MN). For mean DMI, calculated supplementation provided 14.9 mg/kg of Zn as Zn methionine, 8.2 mg/kg of Mn as Mn methionine, 5.2 mg/kg of Cu as Cu lysine and 1.06 mg/kg Co as Co glucoheptonate. Appropriate amounts of the fortified

concentrate from a separate bin on the computer feeder replaced corresponding amounts of the control concentrate for treatment cows. Composition of the concentrates is listed in Table 1.

Orts were removed daily at 0700. Feed samples were collected weekly and combined into 4-wk composites. Analysis was completed at a commercial laboratory (Northeast DHIA Forage Lab.; Ithaca, NY). Analyses of the diets are presented in Table 2. Cows were milked at 0130 and 1330 and milk weights were recorded electronically (Parlor MasterTM; Alfa Laval Agri., Kansas City, MO). Milk samples were taken monthly and analyzed (Dairy Herd Improvement Association Lab., Knoxville, TN) for fat and protein concentration as well as somatic cell count. Initiation of bovine somatotropin (500 mg sometribove zinc; Monsanto Co., St. Louis, MO) occurred in the ninth wk of lactation and continued at 14 d intervals thereafter.

Each cow was fitted with a pressure-sensitive heat detection device (HeatWatchTM; DDx, Inc., Boulder, Colorado) on the rump during the second wk of lactation. HeatWatch patches were observed twice daily and maintained on the cows until confirmation of pregnancy. Cows were given free access to the concrete lot and free stall area and were exercised on pasture lots 2 h/d when weather permitted. Estrus was determined when four or more mounts were recorded by the HeatWatch in a 24 h period or the cows were observed

Table 1. Composition of control and treatment concentrates

Ingredients	Control	Treatment
Soy hulls	23.55	23.55
Ground Corn	18.09	17.31
Distillers grain	17.80	17.80
Soy bean meal 48% CP as fed	12.50	12.50
Cotton seed with lint	10.00	10.00
Cane molasses	3.00	3.00
Animal fat	3.00	3.00
Meat and bone meal	2.50	2.50
Megalac	2.50	2.50
Limestone	1.90	1.90
Dicalcium phosphate	1.00	1.00
Blood meal	1.00	1.00
Sodium bicarbonate	0.96	0.96
Dynamate	0.80	0.80
Feed grade urea	0.70	0.70
Magnesium oxide	0.25	0.25
Salt (white)	0.25	0.25
Dyna-K	0.20	0.20
4Plex ¹	0	0.78

¹4Plex; Zinpro Corp.; Eden Prairie, MN.

Table 2. Chemical composition of control (CC) and treatment (TC) concentrate, blended ration (BR) and calculated mean composition of control (C) and treatment (T) diet

Item	CC	SE	TC	SE	BR	SE	C	T
DM, %	89.1	0.5	88.8	0.6	51.7	0.5	65.5	65.5
----- (% of DM) -----								
CP	23.1	0.4	22.2	0.5	14.0	0.5	17.3	17.3
ADF	21.5	1.4	21.6	1.7	36.9	1.6	31.2	31.2
NDF	32.7	1.1	33.2	1.3	50.7	1.2	44.1	44.1
CF	10.5	ND ¹	10.9	ND	3.6	ND	6.1	6.2
NE ₁ , Mcal/kg	1.69	0.04	1.75	0.05	1.40	0.05	1.54	1.54
Ca	1.71	0.11	1.83	0.12	0.92	0.11	1.20	1.20
P	0.65	0.02	0.65	0.02	0.34	0.02	0.50	0.50
Mg	0.50	0.01	0.57	0.02	0.24	0.01	0.30	0.30
K	1.49	0.06	1.57	0.07	2.10	0.06	1.9	1.9
Na	0.52	0.02	0.53	0.02	0.14	0.02	0.30	0.30
S	0.46	0.03	0.46	0.05	0.23	0.04	0.30	0.30
----- (mg/kg) -----								
Fe	355	46	444	55	238	50	281	286
Zn	106	18	366	21	39	19	64	78
Cu	28	5	112	6	12	5	17	22
Mn	71	14	218	17	45	16	55	63
Mo	2.5	0.24	2.2	0.29	1.4	0.29	1.8	1.8
Co	0.08	ND	3.49	ND	0.02	ND	0.04	0.23

¹Not determined.

in estrus. A pre-breeding voluntary waiting period of 60 d postpartum was observed for all cows. After the voluntary waiting period all cows were bred on the next recorded estrus following an a.m., p.m. rule (cows in estrus in a.m. were bred in the p.m. and cows in estrus in p.m. were bred the following a.m.). Pregnancy was determined by transrectal ultrasonography between 40 and 47 d post breeding.

Blood samples were taken at 7 d intervals from the first wk of lactation until confirmation of pregnancy. Feed was removed at 0700, 1 h before initiation of blood collection. A 15-mL sample of blood was collected by coccygeal venipuncture into heparinized evacuated tubes. Samples were placed on ice and transported to the laboratory. Plasma was separated after centrifugation (3000 x g) and then stored (-20°C) until analyzed for progesterone (Coat-A-Count Progesterone; Diagnostic Products Corp., Los Angeles, CA; Plata et al., 1990), insulin (Coat-A-Count Insulin; Diagnostic Products Corp.) and plasma urea nitrogen (Sigma Urea Nitrogen Kit 535; Sigma Chemical Co., St. Louis, MO; Crocker, 1967).

Progesterone was used to determine the onset of luteal activity. Insulin is involved in the production of progesterone by the corpus luteum (Gulyas et al., 1980; Ladenheim et al., 1984) and is required for the maintenance of pregnancy (Schrick et al., 1992). Infusion of insulin

resulted in increased production of progesterone of the corpus luteum in beef heifers (Harrison and Randel, 1986). When energy intake was increased, insulin level increased and previously anestrus beef cows began to cycle (Richards et al., 1989). Plasma urea nitrogen was negatively correlated with conception rate in dairy cows (Ferguson, et al., 1988). Therefore, plasma urea nitrogen is used in measuring the reproductive health status of cows.

Data were analyzed as a randomized block experimental design with diet, breed, lactation number, presence of retained fetal membranes, body condition score and appropriate interaction terms using a mixed model approach (SAS, 1989). Data for milk production, milk components, body condition scores, insulin and plasma urea nitrogen were analyzed as repeated measures. Level of significance was determined at $P < 0.05$ unless stated otherwise.

Results

Cows receiving the trace element supplemented diet consumed similar amounts of concentrate as the control group (Figure 1). Holsteins consumed more concentrates (10.7 ± 0.3 kg) than Jerseys (9.1 ± 0.6 , $P = 0.008$). However, there were no breed by treatment or treatment by week interactions.

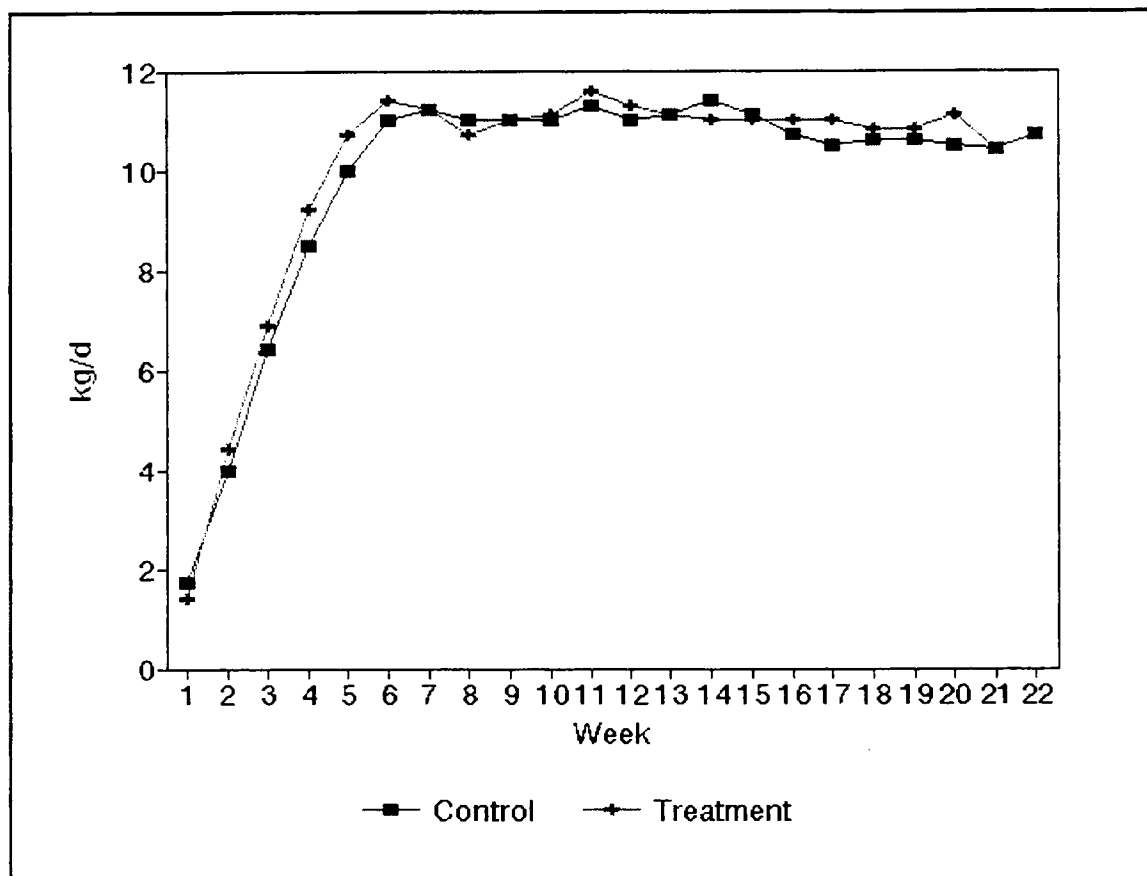


Figure 1. Least squares means of concentrate intake of control and treatment cows. SE = 0.70.

Days to first estrus were greater ($P = 0.02$) for the control cows than for the treatment cows (Table 3). No statistically significant treatment effects were evident for days to first service, days from first breeding to conception, days open, services per conception, days to first luteal activity or days to first corpus luteum (Table 3). Data were then divided into two groups according to whether cows had or had not retained fetal membranes after calving (Table 4). Retention of fetal membranes increased days to first estrus by almost half ($P = 0.05$) in control cows but had no effect in cows supplemented with complex trace elements. This effect of retained fetal membranes was also present when days to first luteal activity and first corpus luteum were analyzed. In the presence of retained fetal membranes, days to first luteal activity ($P < 0.001$) and days to first corpus luteum ($P = 0.04$) increased in control but not treatment cows (Table 4). These results suggest complex trace elements counteracted the stress imposed by retention of fetal membranes.

The presence of retained fetal membranes did not increase the days to first service for control or treatment cows (Table 4). Jerseys had fewer days to first estrus than Holsteins (24.5 ± 9.6 vs 53.0 ± 4.3 , $P = 0.009$), fewer days open (71.0 ± 12.5 vs. 100.9 ± 7.1 , $P = 0.04$), fewer days to first luteal activity (22.0 ± 0.9 vs. 41.8 ± 3.8 , $P < 0.001$) and days to first corpus lutea (27.5 ± 1.6 vs. 40.5 ± 2.5 , P

Table 3. Least squares means of reproductive data for cows supplemented or not supplemented with complexed trace elements

Item	Control		Trace elements		P
	Mean	SE	Mean	SE	
Days to first estrus	67.6	10.4	46.9	10.0	0.02
Days to first service	82.0	4.9	74.0	4.5	0.23
Days from first service to conception	14.2	4.7	10.7	5.2	0.62
Days open	91.7	10.4	80.2	10.8	0.45
Services per conception	1.4	0.2	1.4	0.2	0.89
Days to first luteal activity	35.0	2.8	28.8	2.8	0.13
Days to first corpus luteum	35.7	2.3	32.3	2.2	0.28

Table 4. Least squares means of reproductive data for cows with or without retained fetal membranes as affected by supplementation with complexed trace elements

Supplementation with complexed trace elements					
Item	Fetal membranes retained				P
	No ¹		Yes ²		
	Mean	SE	Mean	SE	
Days to first estrus					
Control	54.2	10.2	81.0	14.2	0.05
Trace elements	49.7	11.0	44.0	12.2	0.64
Days to first service					
Control	78.1	4.5	85.9	7.8	0.35
Trace elements	78.2	4.6	69.6	6.8	0.25
Days to first luteal activity					
Control	28.1	2.7	41.8	3.4	<0.001
Trace elements	27.7	2.8	30.0	3.3	0.36
Days to first corpus luteum					
Control	31.3	2.2	40.2	3.8	0.04
Trace elements	29.8	2.2	34.8	3.6	0.23

¹Control n = 24, Trace elements n = 23.

²Control n = 6, Trace elements n = 7.

< 0.001). However, there was no interaction between breed and treatment. The voluntary waiting period (60 d) could mask some of the reproductive results. However, the attempt to remove this effect by measuring days from first service to conception found no additional differences for the control and the treatment cows (Table 3). Furthermore, there was not a difference in services per conception for the control and the treatment cows.

Body condition scores (scale of 1 = thin and 5 = obese) were not different for control (2.7 ± 0.1) and treatment (2.6 ± 0.1) cows. Nor was there an interaction between treatment and week (Figure 2) for body condition scores.

Milk yield (kg/d) was higher for the Holsteins (40.6 ± 0.7) than the Jerseys (29.6 ± 1.5 , $P < 0.001$), however, there was not a breed by treatment interaction. Nor was there a treatment by week interaction (Figure 3) for milk yield. Similarly, four percent fat corrected milk yield (kg/d) was greater for Holsteins (37.7 ± 0.8) than for Jerseys (30.9 ± 1.7 , $P < 0.001$). Again there was neither a breed by treatment nor a treatment by week interaction (Figure 4) for four percent fat corrected milk yield. As expected milk fat percent was greater for Jersey (4.4 ± 0.1) cows than the Holstein cows (3.5 ± 0.1 , $P < 0.001$). However, fat yield (kg/d) only tended to be greater for Holsteins (1.4 ± 0.03) than for Jerseys (1.3 ± 0.1 , $P = 0.07$). Treatment had no effect on milk fat percent or yield

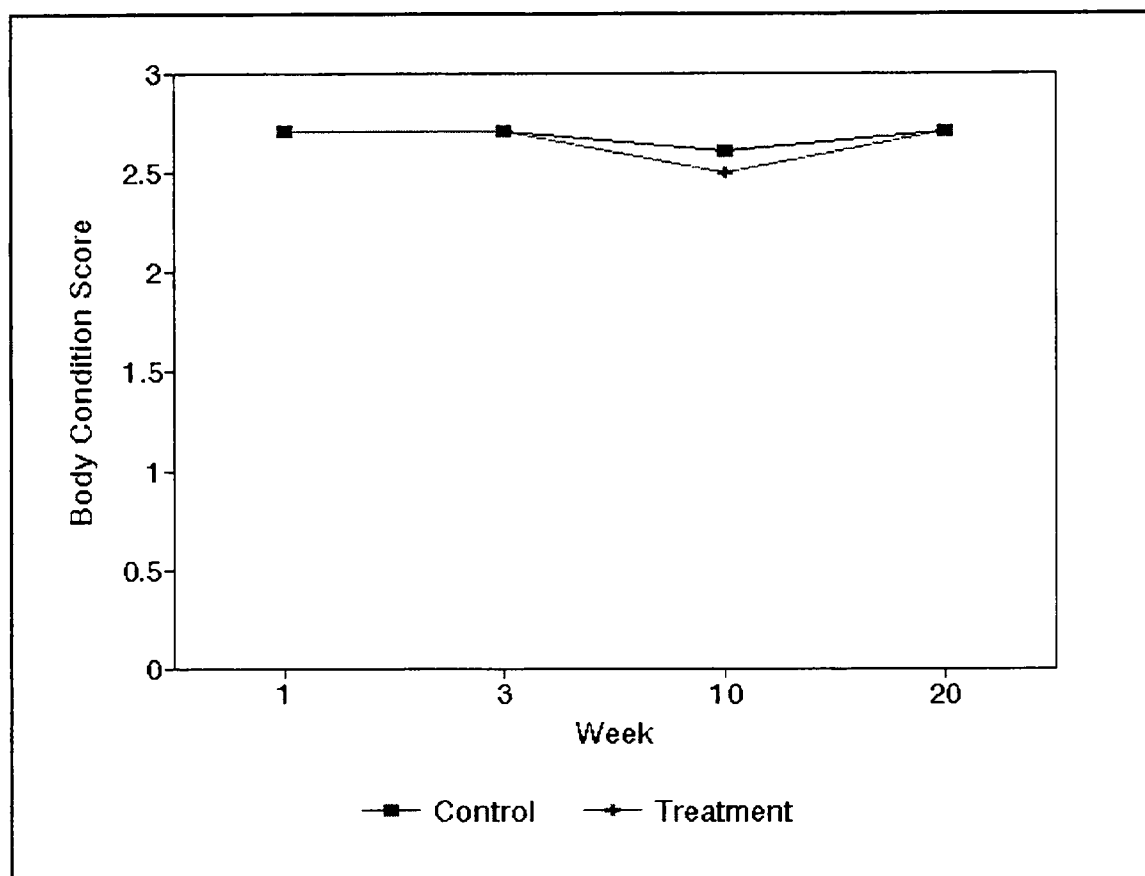


Figure 2. Least squares means of body condition scores of control and treatment cows. SE = 1.1.

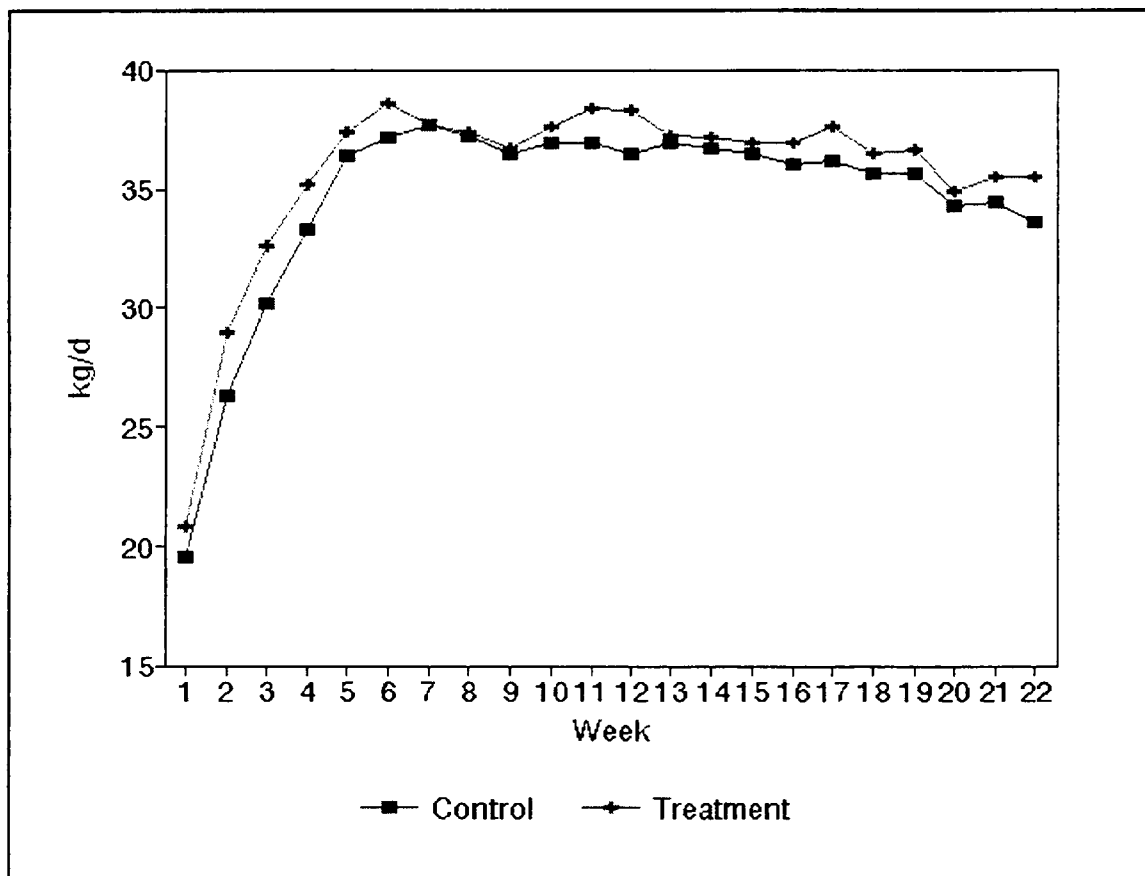


Figure 3. Least squares means of milk yield of control and treatment cows. SE = 0.74.

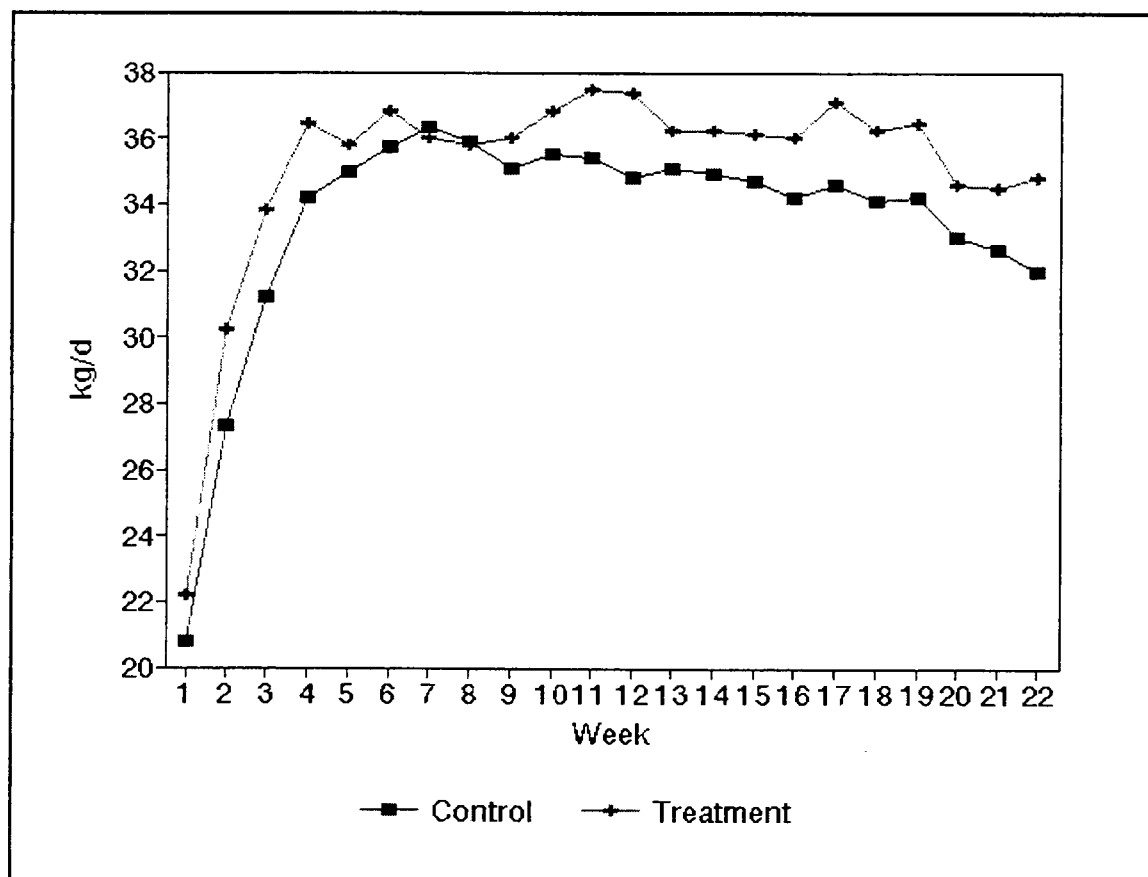


Figure 4. Least squares means of four percent fat corrected milk yield of control and treatment cows. SE = 0.45.

(Table 5). Neither was there a treatment by time interaction for either milk fat percent or yield. Milk protein percent was greater ($P < 0.001$) for Jerseys (3.5 ± 0.05) than Holsteins (3.0 ± 0.04), but the total yield of milk protein was higher ($P < 0.001$) for Holsteins (1.2 ± 0.02 kg/d) than for Jerseys (1.0 ± 0.05 kg/d). There were no treatment by breed interactions for either measurement. Nor was there an interaction between treatment and time for milk protein percent or yield. Somatic cell counts were not affected by treatment.

Plasma insulin (ng/mL) levels were similar for control and treatment cows (Table 6). However, there was an effect of week (of lactation) on plasma insulin level. As lactation progressed insulin level increased ($P < 0.001$, Figure 5). In an effort to evaluate the relationship between plasma insulin and reproduction, the data were sorted by increasing days to first estrus, first service, and days open. After sorting, the data were separated into three groups which contained the upper 25%, the medium 50% and the lower 25% and then compared to plasma insulin levels. The pregnancy status and services per conception were also compared to insulin levels. Insulin level had no effect on days to first estrus ($P = 0.6$), days to first service ($P = 0.4$) (Table 7) or services per conception ($P = 0.8$) (Table 8). However, the cows with the greatest number of days open had lower ($P < 0.002$) insulin than the cows

Table 5. Least squares means of milk yield and milk components for cows supplemented or not supplemented with complexed trace elements

Item	Control		Trace elements		P
	Mean	SE	Mean	SE	
Milk yield (kg/d)	34.6	1.1	35.7	1.1	0.38
4% fat corrected milk (kg/d)	33.5	1.3	35.1	1.3	0.38
Milk fat (%)	3.9	0.09	4.0	0.09	0.71
Milk fat yield (kg/d)	1.3	0.06	1.4	0.06	0.35
Milk protein (%)	3.2	0.05	3.3	0.05	0.33
Milk protein yield (kg/d)	1.1	0.04	1.1	0.04	0.19
Somatic cell counts (X1000/mL)	631	118	593	128	0.83

Table 6. Least squares means of insulin and plasma urea nitrogen of cows supplemented or not supplemented with complexed trace elements

Item	Control		Trace elements		<i>P</i>
	Mean	SE	Mean	SE	
Insulin	0.36	0.02	0.34	0.01	0.27
Plasma urea N	15.9	0.8	17.5	0.7	0.13

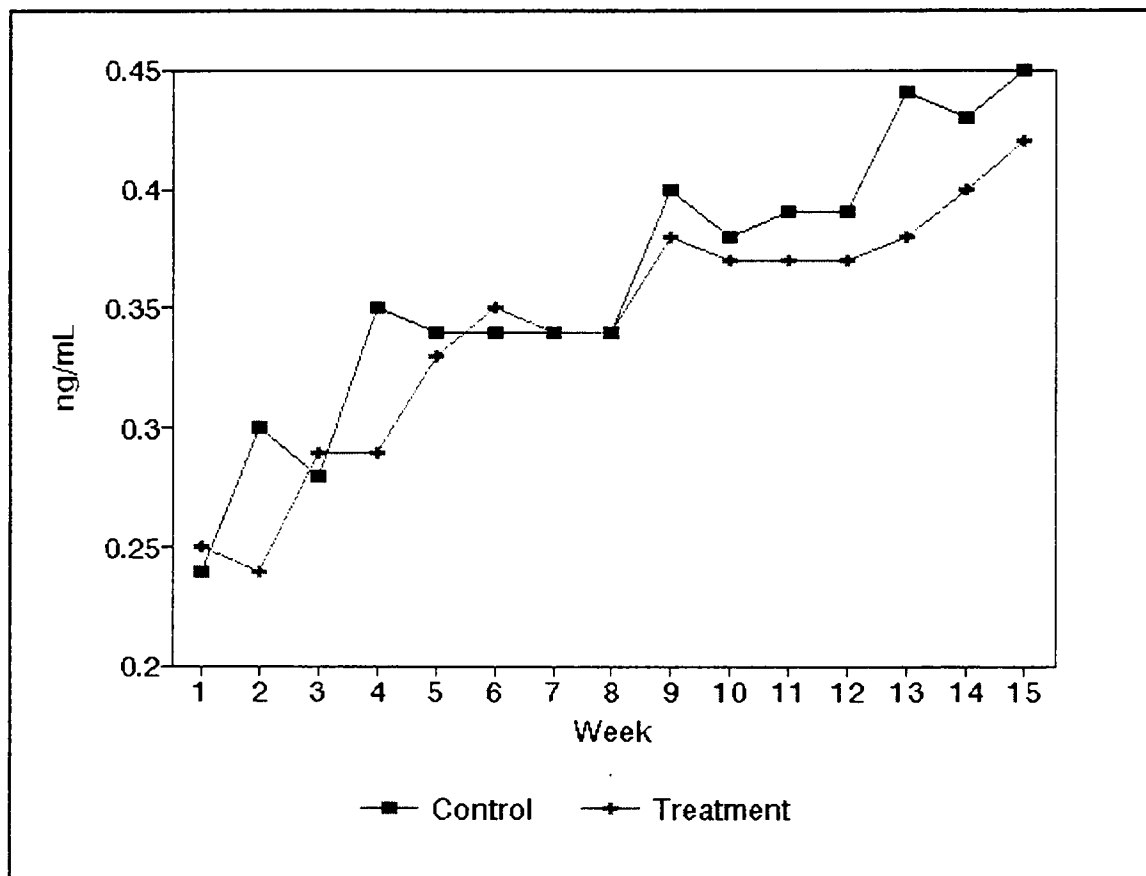


Figure 5. Least squares means of insulin (ng/mL) of the control and treatment cows. SE = 1.03.

Table 7. Relationships of plasma insulin and urea nitrogen to days to first estrus, days to first service and days open

Item	Relative rank						<i>P</i>
	Low		Medium		High		
	Mean	SE	Mean	SE	Mean	SE	
Days to first estrus							
Insulin (ng/mL)	0.35	0.01	0.34	0.01	0.34	0.01	0.60
Urea N (mg/dL)	16.6	0.8	16.8	0.6	15.4	0.7	0.24
Days to first service							
Insulin (ng/mL)	0.35	0.01	0.33	0.01	0.35	0.01	0.41
Urea N (mg/dL)	17.3	0.7	16.0	0.5	16.2	1.0	0.33
Days open							
Insulin (ng/mL)	0.36 ^a	0.01	0.36 ^a	0.01	0.30 ^b	0.01	0.002
Urea N (mg/dL)	15.9 ^a	0.5	16.2 ^a	0.5	19.0 ^b	0.9	0.01

^{ab}LSMeans within a row with different superscripts differ ($P < 0.01$).

Table 8. Relationships of plasma insulin and urea nitrogen to number of services per conception

	Service(s) per conception										
	1		2		3		4		5		
Mesurement	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P
Insulin	0.36	0.01	0.38	0.02	0.35	0.04	0.35	0.03	0.35	0.04	0.8
(ng/mL)											
Urea N	16.4	0.5	16.8	0.9	14.0	1.6	16.6	1.1	16.7	1.6	0.6
(mg/dL)											

that had medium or lowest days open. Similarly, the cows that conceived had higher ($P = 0.001$) insulin than the cows that remained open (Table 9).

Least squares means of plasma urea nitrogen (mg/dL, wk 1 to 17) of a sub-sample of cows ($n = 20$) were not different for the treatment and control cows (Table 6). Similar to the insulin, plasma urea N was not different for days to first estrus, days to first service (Table 7) or services per conception (Table 8). However, the cows with the greatest number of days open had higher ($P < 0.01$) levels of plasma urea N than the cows with the medium or lowest days open (Table 7). Also, the cows that conceived had a lower ($P = 0.002$) plasma urea N than the cows that remained open (Table 9).

When progesterone profiles were compared to mount(s) (duration ≥ 2 s) recorded, electronic estrous detection was not effective at predicting days to onset of normal luteal activity, as determined by progesterone ≥ 1 ng/mL. The accuracies of the mounting data are listed in Table 10. The mounting data were separated by mounts ≥ 1 , ≥ 2 , ≥ 3 or ≥ 4 mounts in a 24 h period, so that if a cow had > 4 mounts she would be included in all four groups. Accuracy of electronic estrous detection in determining estrus after 45 d postpartum (commonly used voluntary waiting period) increased from 30.1 to 73.9% for one to four mounts (Table 10). The incidence of mounts when progesterone (≥ 1 ng/mL)

Table 9. Relationships of plasma insulin and urea nitrogen to pregnancy status

Measurement	Pregnant		Open		P
	Mean	SE	Mean	SE	
Insulin (ng/mL)	0.36	0.01	0.31	0.01	0.001
Urea N (mg/dL)	16.1	0.4	19.0	0.9	0.002

Table 10. Least squares means of mounting data of electronic estrous detection

Accuracy	Mounts ¹								P
	1 ²	SE	2 ³	SE	3 ⁴	SE	4 ⁵	SE	
Detection of FNLA ⁶	46.4 ^{a7}	0.06	35.7 ^{ab}	0.06	26.8 ^b	0.06	23.2 ^b	0.06	0.04
Detection of estrous after 45 d	30.1 ^{ab}	0.03	24.5 ^a	0.03	44.8 ^b	0.08	73.9 ^c	0.03	0.03
Mounts after pregnancy	48		56		7		10		ND ⁸
Cows in each mount group ⁹	24		24		7		8		ND

¹Mounts = Mounts recorded by the HeatWatch system with duration ≥ 2 s.

²1 = ≥ 1 for detection of luteal activity, = 1 for detection of estrus, and mounts after pregnancy.

³2 = ≥ 2 for detection of luteal activity, = 2 for detection of estrus, and mounts after pregnancy.

⁴3 = ≥ 3 for detection of luteal activity, = 3 for detection of estrus, and mounts after pregnancy.

⁵4 = ≥ 4 for detection of luteal activity, estrus, and mounts after pregnancy.

⁶FNLA = First normal luteal activity.

⁷abcd = Least square means with different letters differ by $P < 0.05$.

⁸ND = Not determined.

⁹Group = Number of cows that displayed mounting activity in each group.

indicated anestrous and mounting activity after conception lowered the accuracy of electronic estrous detection.

Discussion

Cows consumed similar amounts of concentrates during this experiment. As expected Holsteins consumed more concentrate than the Jerseys, however, there was not a treatment effect within breed. Cobalt in the form of cobalt glucoheptonate has been suggested to help avoid appetite suppression. However, in the present experiment only concentrate intake was measured on the individual cow basis. The average dry matter intake was determined for the group.

With the added stress of retained fetal membranes, the control cows exhibited an increase in days to first estrus, however, the treatment group did not show an increase. In a study of Fresian cows ($n = 46$), Formigoni et al. (1993) observed days to first estrus after parturition to be similar for the chelated supplemented group and the control group. However, similar to the present experiment the levels of minerals reported in their ration were in excess of recommended daily allowance. Iron, which was already present in excess in their dairy ration, was included in their chelated mineral mix. Retained fetal membranes, in the present comparison had a similar effect on days to first luteal activity and days to first corpus lutea for the

control but not the treatment cows. This supports the accuracy of first estrus detection. Least squares means of first estrus were less than 63 d for all cases except for the control cows which had retained fetal membranes. Thus, supplementation of bovine ST beginning in the ninth week should not have affected the outcome of days to first estrus. A similar response to stress was observed when heifers that were supplemented with excess Fe responded favorably to the supplementation of zinc methionine with less udder edema (Cunningham et al., 1995). There was a voluntary waiting period of 60 d postpartum in the experimental herd which may make it impossible to detect some results in the reproductive data. Days to first service were not different, however, this probably is more a measure of management than a treatment effect. The least squares means for days to first service were within one estrous cycle indicating that both groups were reproductively active by the beginning of the breeding period. A similar response was observed by Formigoni et al. (1993) when cows which were supplemented with chelate minerals had similar days to first service as the controls. In an attempt to remove the management effect, days from first breeding to conception were compared and observed to be similar for both treatments. Services per conception were also identical for both treatments. This is in contrast with results reported by Kropp (1993) where beef cattle

supplemented with chelated minerals had a higher percent of conception on the first service. However, Formigoni et al. (1993) reported a similar services per conception for the chelated supplemented group and control group. Days open were similar for the control and treatment groups. This is a contrast to a study with Holstein heifers, where Manspeaker and Robl (1993) reported that heifers that received the chelated minerals had 45 fewer days to conception than the controls. Also Kellogg and Lane (1996) reported that cows supplemented with chelated minerals had 16 fewer days open. Formigoni et al. (1993) reported a nonsignificant reduction of days open. Days open can also be a measure of management since culling will affect total days open.

Body condition scores were not different among the control and treatment cows. Due to the slight change in body condition score throughout the experimental period, body condition score may be a measure of nutritional management rather than an attempt to measure differences among treatments. Waltner et al. (1993) reported that body condition scores had no significant correlation with reproductive measures when only a slight change in score was observed.

Treatment did not significantly affect milk yield. Formigoni et al. (1993) observed similar results when cows supplemented with chelated minerals did not produce more

milk than controls. However, Kropp (1993) reported that beef cows that were supplemented with chelated minerals weaned heavier calves than controls, indicating higher milk production. Furthermore, in a study where a large commercial herd was divided into two groups (Kellogg and Lane, 1996), an increase in average milk production (1.91 kg/d, $P < 0.01$) was observed.

Four percent fat corrected milk yield was not affected by supplementation of chelated minerals in the present study. Milk components were not affected by treatment, which is similar to results reported by Kellogg and Lane (1996). Although not significant in the present study, there was a numerically higher response of cows supplemented with complexed minerals to bovine ST. A similar response was observed by Kellogg and Lane (1996) in a large commercial herd. Somatic cell counts were not different for the complexed mineral supplemented group or the controls. This is in contrast with other studies that have shown a reduction in somatic cell counts when zinc methionine was supplemented in the diet.

Plasma insulin was measured as an indicator of stress. However, there was no difference in the plasma insulin levels of the complexed mineral supplemented cows compared to the control cows. Although there was not a treatment effect on plasma insulin levels, there was an effect of plasma insulin levels on days open. Furthermore, the cows

which conceived had higher insulin indicating that insulin can be used as an indicator of nutritional effect on reproduction.

Plasma urea nitrogen has been shown as an indicator of nutritional affects on reproduction such as high level of crude protein in the diet negatively affects reproduction (Ferguson et al., 1993). However, plasma urea nitrogen was similar in complexed mineral supplemented cows and control cows.

Although electronic estrus detection is effective in estrus detection, it was not effective in detection of first normal luteal activity. The accuracy of estrus detection was lower in the present study compared to other reports (Nebel et al., 1995; Walker et al., 1995; Borger et al., 1996; Greene and Borger, 1996; Whittier et al., 1996) which ranged from 87.5% to 100% accurate. The accuracy was lower due to mounts that were recorded after conception and mounts when progesterone indicated anestrus. However, electronic estrous detection was a useful reproductive management tool in indicating estrous activity.

Implications

Treatment cows had fewer days to first estrus than controls. The reduction of days to first estrus, days to first luteal activity and days to first corpus luteum, were

the only significant differences in this research. However, with the small number of animals that were available for this project it must be considered that type II errors are likely. The reproductive data showed slight improvements for days to first service and days from first breeding to conception for the treatment group. If the voluntary waiting period is reduced from 60 d to 45 d postpartum, then there probably would be an effect of treatment on days to first service, which could reduce the days open for the treatment group. Milk production or milk components were not different for the treatment and control groups. Plasma insulin was not a sensitive enough measure of stress for this research. However, plasma insulin may prove to be a useful indicator of nutritional status and ability to maintain pregnancy. Plasma urea nitrogen was not different between the two groups, which seems normal considering the similar reproductive results.

The electronic estrous detection was not a valid method of determining first normal luteal activity. However, it was an effective tool in reproductive management.

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