Oxidative Stress, Antioxidants, and Animal Function

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ABSTRACT

Reactive oxygen metabolites generated during normal metabolism and metabolism stimulated by xenobiotics can enter into reactions that, when uncontrolled, can impair performance of dairy cows. Direct effects include peroxidative changes in membranes and other cellular components. Indirectly, competitive consumption of reducing equivalents can interfere with important metabolic functions and divert glucose from other pathways by inducing the monophosphate shunt. Normally, the body is protected by a wide range of antioxidant systems working in concert. Metal catalysts of oxidative reactions are removed in extracellular fluids by metal-binding macromolecules. Superoxide dismutases, glutathione peroxidase, and catalase within cells remove superoxide and peroxides before they react with metal catalysts to form more reactive species. Finally, peroxidative chain reactions initiated by reactive species that escaped enzymatic degradation are terminated by chain-breaking antioxidants, including water-soluble ascorbate, glutathione, and urate and lipid-soluble vitamin E, ubiquinone, and β-carotene. To optimize performance, oxidative stress in high producing cows must be controlled by supplying all known antioxidant nutrients and by minimizing effects of substances that stimulate reactive oxygen metabolites.

(**Key words**: free radicals, oxidative stress, antioxidants, dairy cows)

Abbreviation key: E_2 = 17β-estradiol, **GSH** = reduced glutathione, $1,25\text{-}(OH)_2D$ = 1,25-dihydroxy vitamin D, P_4 = progesterone, **ROM** = reactive oxygen metabolites, **SOD** = superoxide dismutase.

INTRODUCTION

Toxicity of oxygen, although oxygen is essential for all aerobic organisms, has been termed the oxygen paradox. Increasing interest has been focused on potentially harmful metabolites of oxygen in relation to human disease (22, 23). The term "reactive oxygen metabolites" (ROM) has been applied to oxygen-centered free radicals and their metabolites (40). Some ROM are produced endogenously by normal metabolic processes, but amounts may be increased markedly by exogenous factors, including solar radiation, fungal toxins, and pesticides (18, 31, 40, 47). Deficiencies of natural protective substances or excess exposure to stimulators of ROM production may result in oxidative stress, which occurs when prooxidants exceed the capacity of antioxidants. Involvement of oxidative stress in etiologies of certain disorders of dairy cattle is suggested by reductions in incidence of retained placenta (24) and mastitis (48) when the antioxidants, vitamin E and Se, are supplemented. Retained placenta, mastitis, and udder edema may share common causes, as suggested by analysis of records from over 61,000 cows (19). Evidence that oxidative stress also may contribute to udder edema and suboptimal reproductive performance is reviewed herein. Our objectives were to examine

possible relationships between ROM and the health of dairy cows and to explore how control of ROM with antioxidants improves performance.

ROM REACTIONS

Reactive oxygen metabolites are unavoidable products of normal metabolic processes (Figure 1) and are not always harmful. Superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are involved physiologically in the chemistry of several enzymes and are used by phagocytic cells to kill bacteria (22). Imbalance between production of ROM and their safe disposal, however, can initiate oxidative chain reactions and lipid peroxidation (Table 1).

Cytochrome P-450 enzymes may be an important source of ROM. The various P-450 isozymes can be divided into two broad categories according to whether exogenous (xenobiotic) or endogenous (physiological) substrates are metabolized (61). Xenobiotics in cattle feed that are likely to increase ROM production through induction of cytochrome P-450 include Maillard reaction products (68), mycotoxins (53), and endophyte-infected tall fescue (67). Many of the P-450 enzymes that metabolize endogenous substrates have important physiological functions, including production of cholesterol, sex hormones, glucocorticoids, mineral corticoids, and active forms of vitamin D (61). The relevance of ROM to steroid metabolism is discussed later.

Transition elements (under biological conditions, Fe is of great concern) that are "free" (also called "decompartmentalized", "ill placed", or "catalytic") may be involved in ROM reactions (22). Decompartmentalized Fe may stimulate production of the extremely reactive hydroxyl radical (·OH) from O_2^- and H_2O_2 in Fenton-type reactions (Table 1), catalyze transfer of electrons from NAD(P)H to oxygen in oxidation-reduction shuttles, and react with lipid hydroperoxides to produce more reactive species (47). Cells normally are protected against harmful effects of Fe by complexing it in large molecules that are compartmented away from sites susceptible to damage. Dietary imbalances, inflammation, infection, and environmental stresses all may contribute to "indiscriminate coordination" of transition

elements, particularly Fe (33), which increases likelihood of decompartmentalization. Potential sources of catalytic Fe include the low molecular weight pool involved in transfer of Fe from transferrin to ferritin (22), Fe released from ferritin by O_2^- or lipid peroxides (22), and Fe released from hemoglobin by peroxides (20).

OXIDATIVE STRESS AND ANIMAL HEALTH

When ROM are not effectively and safely removed, oxidative stress may impair health in dairy cows both directly and indirectly. Direct effects include peroxidative damage to important lipids and macromolecules. Indirectly, changes induced by ROM in cellular membranes and components can modify metabolic pathways, resulting in altered physiology and possibly pathology.

Oxidative Damage to Cells

The extremely reactive ·OH attacks lipids, proteins, polysaccharides, DNA, and other macromolecules. The nature of the damage depends on the location within the organism of metal complexes that promote ·OH formation (22). Oxidized molecules abstract electrons from other molecules, resulting in a chain reaction (Table 1). This reaction, if not controlled, can cause extensive tissue damage, which may affect membrane permeability, enzyme function, and even muscle tone. Relative deficiency of total antioxidants may contribute to impaired uterine contractibility, thereby decreasing transport of sperm to ova of ewes (42) and cows (43) unsupplemented with vitamin E and Se.

Metabolic Changes Mediated by ROM

Impairment of animal performance by ROM may involve altered metabolism as much or more than actual cell damage. Both antioxidant defense and reactions catalyzed by steroidogenic enzymes require reducing equivalents provided by NADPH (3, 46). Excessive consumption of reducing equivalents by severe free radical stress can lower $NADPH_2$ and increase NADP concentrations despite elevated activity of the monophosphate shunt, which generates the reduced form (18). Consumption
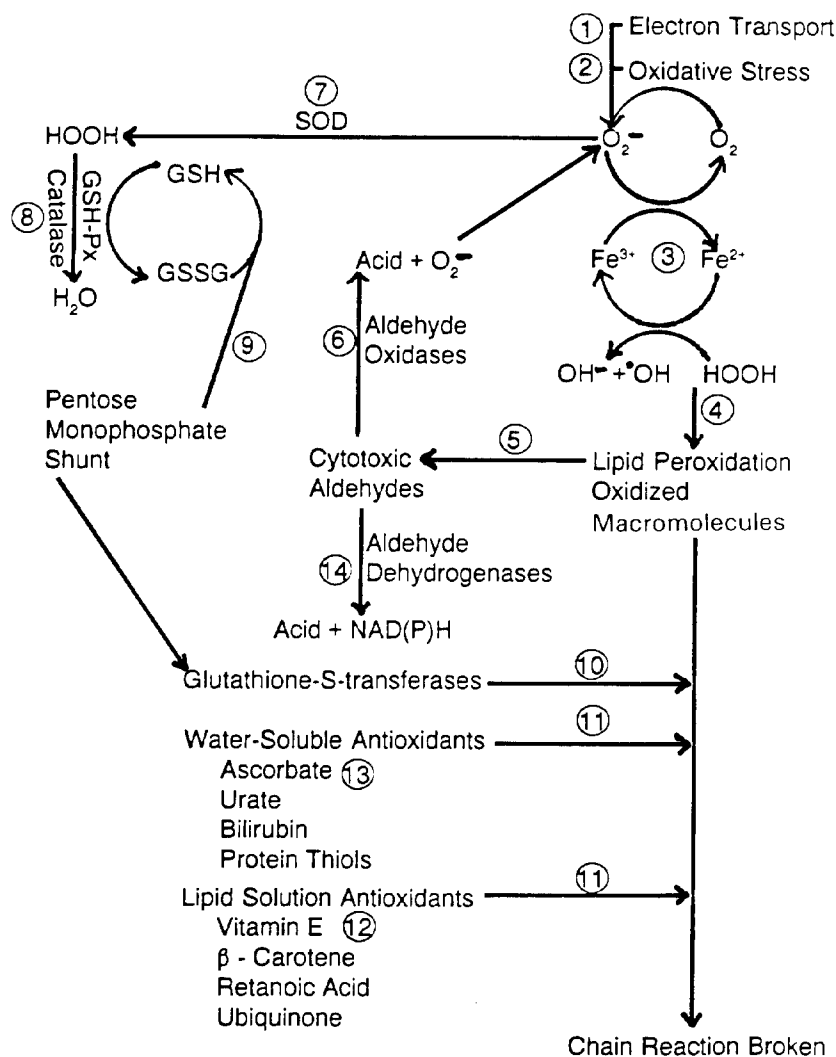
Figure 1. Systems for protection against reactive oxygen metabolites. 1) Superoxide is generated during normal metabolism (18, 22). 2) Exogenous contributors to oxidative stress include dietary imbalances, disease, environmental pollutants, and solar radiation (64). 3) Superoxide reduces Fe^{3+}, enabling it to enter into Fenton-type reactions (22, 64), which produce hydroxyl radical. 4) The extremely reactive hydroxyl radical attacks macromolecules and initiates peroxidative chain reactions (22). 5) Cytotoxic aldehydes are end products of lipid peroxidation (23). 6) When tissues are disrupted, aldehyde dehydrogenases are converted to aldehyde oxidases, which generate superoxide (23). 7) Superoxide dismutases (Mn, Cu, and Zn) convert superoxide to peroxides. This conversion retards reduction of Fe^{3+} to Fe^{2+}, which catalyzes formation of ·OH (22, 47, 64). 8) Catalase (Fe) and glutathione peroxidase (Se) convert peroxides to compounds that do not participate in Fenton-type reactions (22). Reduction of peroxides is accompanied by oxidation of reduced glutathione (64). 9) Reduced glutathione can be regenerated from glutathione disulfide (GSSG) by reducing equivalents from NADPH, which is generated by the pentose monophosphate shunt (18, 64). 10) Glutathione S-transferases conjugate glutathione with peroxy radicals (18). This pathway may be more active when it is deficient in Se or vitamin E. The resulting destruction of glutathione increases consumption of reducing equivalents, thus competing with other metabolic pathways that depended on NADPH. 11) Chain-breaking antioxidants interrupt peroxicative chains initiated by reactive oxygen metabolites that escaped enzymatic degradation. 12) Vitamin E serves as a chain-breaking antioxidant by reacting directly with free radicals (31, 64). Although vitamin E is consumed when free radicals are quenched (18), reducing equivalents are conserved in comparison with glutathione S-transferases serving as chain breakers. 13) Vitamin C, in addition to regenerating vitamin E and possibly also glutathione, can act in its own right as a water-soluble antioxidant (64). 14) Aldehyde dehydrogenases convert aldehydes to less toxic products (23).

TABLE 1. Initiation and propagation of reactive oxygen metabolites.[1]

Reaction	Product
O_2 + 1 electron $\rightarrow$ O_2^-	Superoxide
$2O_2^-$ + $2H^+$ $\rightarrow$ O_2 + HOOH	Hydrogen peroxide
O_2^- + Fe^+ $\rightarrow$ O_2 + Fe^{2+}	Reduced iron
HOOH + Fe^{2+} $\rightarrow$ Fe^{3+} O_2 + ·OH	Hydroxyl radical
·OH + RH or LH $\rightarrow$ H_2O + R· or L·	Fatty acid or other organic molecule oxidized
R· + LH $\rightarrow$ RH + L·	Oxidized fatty acid
L· or R· + O_2 $\rightarrow$ LO_2· or RO_2·	Peroxy radical
LO_2· + LH $\rightarrow$ L· + LOOH	Lipid peroxide

[1]From references (22, 23, 36).

of reducing equivalents by ROM reactions can diminish the supply of NADPH available for important physiological processes. Additionally, induction of the monophosphate shunt by increased ROM imbalance can divert glucose from other pathways. This possibility assumes greater importance when the requirement for glucose and the quantity available in the ruminant are considered.

Changes In Steroidogenesis Induced by ROM

Peroxidative inactivation of steroidogenic enzymes also can impair reproduction. Normal reproduction depends on suitable concentrations of progesterone (P_4) and estrogen at appropriate times. Normal conception in dairy cows depends on appropriate concentrations of P_4 before and after estrus (9, 14). Susceptibility of steroidogenic enzymes dependent on cytochrome P-450 to lipid peroxidation (49, 54) can limit synthesis of steroid hormones under oxidative stress. A partial list of these enzymes is in Figure 2.

Hydroxylases specific to cytochrome P-450 appear to differ in their vulnerability to ROM attack. When adrenal microsomes were depleted of vitamin E in vitro, 3β-hydroxysteroid dehydrogenase-isomerase and 21-hydroxylase were five to six times more resistant to inactivation than were 17α-hydroxylase and 17,20-lyase (54). Fetal adrenal cortisol, which increases markedly preceding parturition (5), acts on the placenta to increase activities of 17α-hydroxylase, 17,20-lyase, and aromatase. Androgens, estrogens, and cortisol

all are synthesized from pregnenolone by either of two pathways, both of which require 17α-hydroxylase (Figure 2). Because cortisol is produced by a 17α-hydroxylase pathway, ROM inactivation of this key enzyme could also inhibit synthesis of androgens and estrogens by the placenta. In addition, androstenedione and, thus, estrogen are not produced by either pathway without 17,20-lyase, which is as vulnerable to ROM attack as is 17α-hydroxylase (54).

Although speculative at this point, unequal vulnerability of specific steroidogenic enzymes to ROM damage (54) reasonably can contribute to problems in periparturient cows. Steroidogenesis proceeds by different pathways (Figure 2), and inadequacy of a key enzyme for one pathway may misdirect the reaction (3). For example, if 17α-hydroxylase and 17,20-lyase are damaged more than 21-hydroxylase by ROM, adrenal lipid peroxidation can be more inhibitory of cortisol, androgen, and estrogen than of mineral corticoid synthesis. Congenital deficiency of 17α-hydroxylase impairs production of sex hormones and increases plasma concentrations of 11-deoxycorticosterone and corticosterone as expected, but aldosterone is decreased (65). Sodium retention caused by 11-deoxycorticosterone and corticosterone expands blood volume, which in turn suppresses plasma renin and aldosterone secretion (65). Suppression of androgens and estrogens induced by oxidative stress accompanied by elevated corticosterone can impair reproduction but increase sodium and water retention, thereby contributing to udder edema.
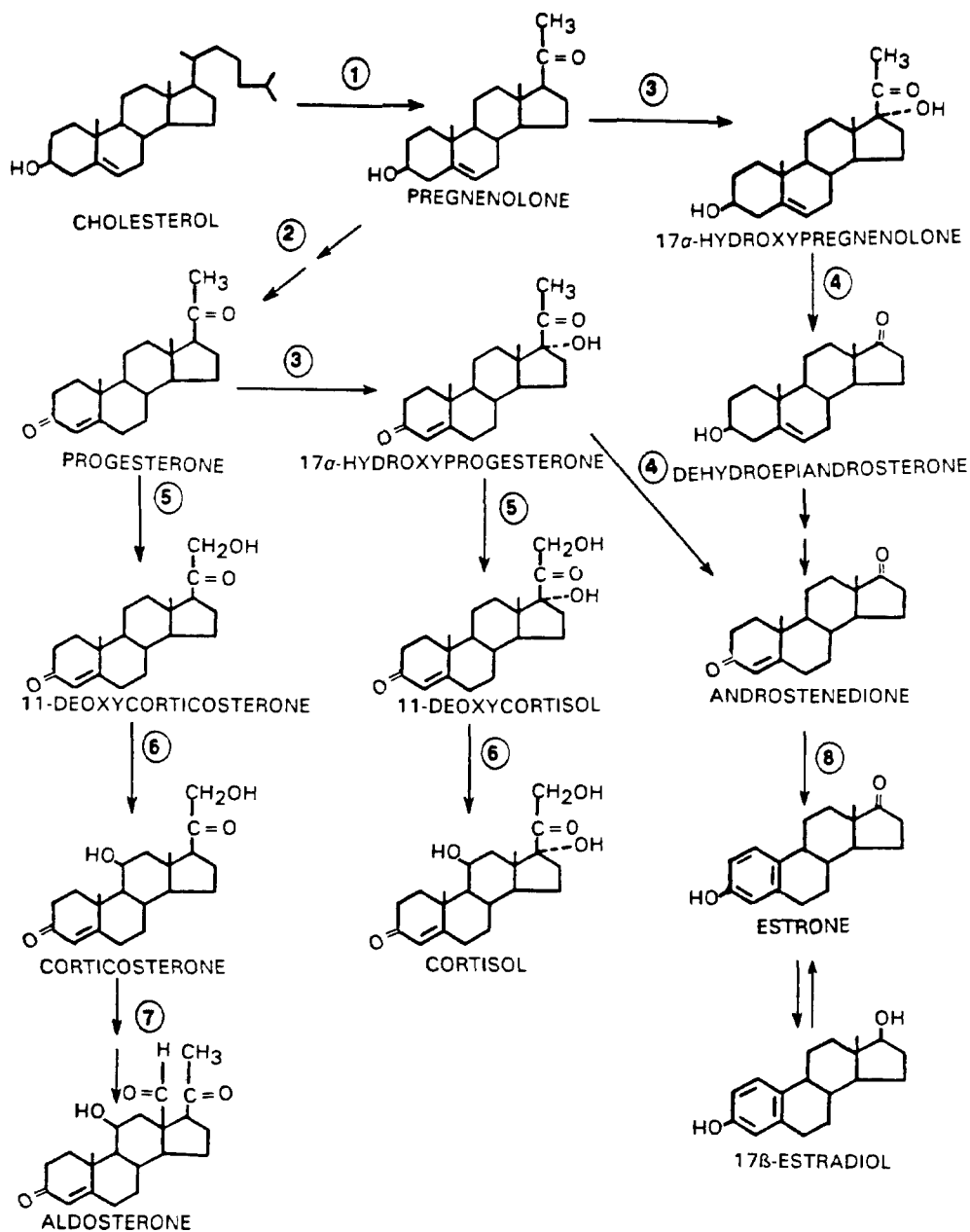
Figure 2. Synthesis of steroid hormones from cholesterol. Adapted from Bhagavan (3). Circled numbers correspond to steroidogenic enzymes for which deficiencies have been linked to steroid disorders. 1) Cytochrome P-450 side-chain cleavage enzyme: when lacking steroid hormone synthesis is blocked. 2) 3β-ol-Dehydrogenase: necessary for formation of progesterone from pregnenalone. 3) 17α-Hydroxylase: necessary for 17α-hydroxylation of pregnenalone and progesterone, either of which is a step in formation of cortisol, androgens, and estrogens. When this enzyme is inadequate, deficiencies of these hormones may be accompanied by excesses of corticosterone and aldosterone. 4) 17,20 Lyase: a deficiency limits production of androgens and estrogens. 5) 21-Hydroxylase: concentrations of androgens are elevated, and production of cortisol and aldosterone is decreased, when this enzyme is deficient. 6) 11β-Hydroxylase: when inadequate, depressed production of cortisol and aldosterone is accompanied by elevated concentrations of androgens. 7) 18α-Hydroxylase: a defect in aldosterone production results from a deficiency. 8) Aromatase: production of estrogens is limited by a deficiency.

DEFENSE AGAINST ROM

Normally, the body is protected against ROM and their toxic products by a wide range of known defense mechanisms (Figure 1). The components of this integrated system have been classified as preventive or chain breaking. Included among preventive systems are both metal-binding macromolecules and antioxidant enzymes. Metal catalysts of ROM reactions in extracellular fluids are removed by transferrin, ceruloplasmin, and albumin (22). Within cells, superoxide dismutases (SOD) (Mn, Cu, Zn), glutathione peroxidase (Se), and catalase (Fe) remove O_2^- and H_2O_2 before they approach available promoters of Fenton chemistry (22). Reduction of peroxide is accompanied by oxidation of reduced glutathione (GSH), which can be regenerated by reducing equivalents from $NADPH_2$ (18, 64). Despite these preventive enzymes, some O_2^- and H_2O_2 may escape and, in the presence of decompartmentalized Fe, may be catalyzed to more reactive ROM (22).

Chain-breaking antioxidants act after initiation of a chain reaction. This class of antioxidants includes lipid-soluble vitamin E, ubiquinone, and β-carotene and water-soluble ascorbate, GSH, and urate (31, 46). Retinoic acid has been listed with lipid-soluble antioxidants (46) although it does not have major chain-breaking activity. Vitamin E terminates peroxidative chains by reacting directly with a variety of organic peroxy radicals (31, 64). When vitamin E is inadequate, glutathione-S-transferases form GSH conjugates with peroxy radicals, resulting in net consumption of GSH (18). Vitamin E is oxidized when ROM are quenched (18), but it can be regenerated by vitamin C (64). Reducing equivalents are conserved when vitamin E, rather than GSH, serves as a chain breaker. In addition to regenerating vitamin E, vitamin C can act directly as a water-soluble antioxidant (64).

Cytotoxic aldehydes (e.g., malondialdehyde) remain after termination of lipid peroxidation (23). These aldehydes provide the basis for the thiobarbituric acid test for measuring lipid peroxidation end products in body fluids. A third line of defense against ROM involves aldehyde dehydrogenases, which oxidize cytotoxic aldehydes by a transfer of electrons to NAD^+ (7). An example is xanthine de-hydrogenase, which catalyzes, but is not specific to, production of urate and NADH from xanthine, NAD^+, and H_2O. Urate is an effective water-soluble antioxidant. As an additional benefit, xanthine dehydrogenase helps to keep Fe in the less reactive, oxidized form (12), thereby conserving vitamin E. Unfortunately, if cell respiration is impaired or if tissue integrity is disrupted, dehydrogenases often are converted to corresponding oxidases (52). These oxidases can transfer electrons to oxygen, subsequently producing O_2^- and increasing oxidative load (23).

Reactive oxygen metabolites thus are removed through a combination of systems. Several essential nutrients are involved in manufacture or structure of known components of antioxidant defense (46, 47). Metal chelators, ubiquinone, urate, GSH, and ascorbate may be of dietary or endogenous origin, but the diet also should contain adequate N, S, and energy. Dietary essential trace elements required for antioxidant enzymes include Mn, Cu, and Zn for SOD, Se for glutathione peroxidase, Fe for catalase, and Fe plus Mo for aldehyde dehydrogenases. Certain transition elements coordinated to low molecular weight organic ligands also may mimic SOD activity (J.R.J. Sorenson, 1992, personal communication). Proper function of enzymes and other pathways are major controls for ROM, and derangement of any component of the system may reduce effectiveness of ROM control.

OXIDATIVE STRESS, ANTIOXIDANTS, AND THE PERIPARTURIENT DAIRY COW

The periparturient period is especially important for health of dairy cattle. A survey including 551 cows and 1305 lactations (44) revealed that over one-half of total health costs resulting from mammary and reproductive problems occurred during the first 30 DIM. In addition to cost of treatment, udder edema (8, 60), retained placenta (29), and mastitis (48) can reduce milk production, market value, and the productive life of the cow and can cause indirect costs that are difficult to quantify. Delayed first estrus, delayed first breeding, and repeated breeding resulting from failure to conceive or from early embryonic death increase days open and prolong calving intervals. Additional expenses include treatment, repeated
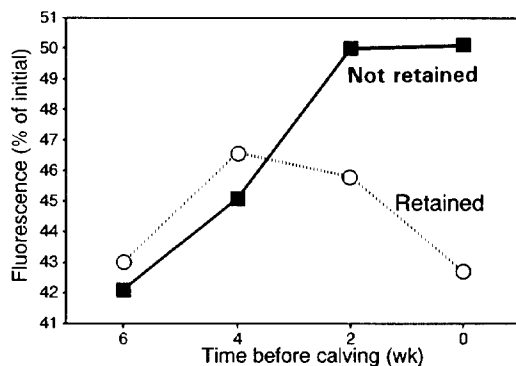
Figure 3. Total antioxidants in bovine plasma as measured by their protection of phycoerythrin fluorescence in vitro. Plasma from 48 apparently healthy cows protected phycoerythrin longer than plasma from 16 cows that failed to shed the placenta within 12 h after parturition, thus reflecting lower total antioxidant content in plasma of cows with periparturient disorders.

breeding, and culling of cows for failure to conceive.

Oxidant-Antioxidant Balance

An imbalance between production and safe disposal of ROM may contribute to periparturient disorders in dairy cows. We have employed a fluorescence procedure based on phycoerythrin for measurement of total antioxidant content of biological fluids (15) in research with cows and heifers (4, 50). In this procedure, the antioxidant content of plasma is assayed by its ability to quench free radicals that are generated in vitro, thus protecting phycoerythrin from degradation.

Plasma from eight apparently healthy heifers protected phycoerythrin from ROM attack longer than plasma from eight heifers with udder edema or two cows with retained placenta (50). More recently, we compared results from 48 cows that shed the placenta in < 12 h after calving with those from 16 cows that retained the placenta ≥ 12 h. Total antioxidant activity of plasma did not differ between the two groups at 6 or 4 wk before expected parturition (Figure 3). At 2 wk before and near calving, however, plasma from apparently healthy cows was superior ($P < .01$) to plasma from cows with retained placenta in protecting phycoerythrin from degradation. Lower total antioxidant activity in blood from affected

cows could have contributed to the disorders or have been a consequence of other free radical stress that lowered total antioxidant activity. In either case, diminished total antioxidant activity as a result of ROM-quenching reactions increases vulnerability to additional free radical stress.

The 64 cows in the comparison just described were fed in groups grass hay to appetite plus an average of 5 kg/d of commercial dairy concentrate, which supplied about 160 IU/d of vitamin E (4). One-half of the cows were each given 1000 IU/d of additional vitamin E as d,1 α-tocopherol acetate by capsule. Serum α-tocopherol decreased linearly during the last 6 wk of gestation in cows that were not supplemented with vitamin E and was lowest at calving. Supplementation with 1000 IU/d of vitamin E was accompanied by increased ($P < .01$) serum α-tocopherol (Figure 4A) and plasma total antioxidants (Figure 4B) and by decreased ($P < .01$) erythrocyte substances reactive to thiobarbituric acid (Figure 4C). Substances, reactive to thiobarbituric acid, an index of lipid peroxidation, were correlated ($P < .01$) negatively with plasma total antioxidants ($r = -.57$) and α-tocopherol ($r = -.27$), as expected when lipid peroxidation increases as antioxidant protection decreases.

Placental Retention, Udder Edema, and Reproductive Performance

Reproduction is likely to fail long before life is endangered by deficiency of any required nutrient (11). Supplementation of dairy cows with dietary antioxidants is especially critical during the periparturient period (63), when plasma α-tocopherol is lowest (17). As the major lipid-soluble antioxidant (31), vitamin E provides an important component of protection against synthesis and accumulation of lipoperoxides in tissues (66) and reduces tissue pathology that is due to accumulation of Fe (10). Vitamin E is concentrated in tissues producing steroid hormones, in which it protects highly sensitive steroidogenic activities of cytochrome P-450 against lipid peroxidation (49, 54).

Thomas et al. (55) compared serum concentrations of P_4 and 17β-estradiol (E_2) in cows that were supplemented with 1000 IU/d of vitamin E during the last 6 wk of gestation and in unsupplemented control cows. Cows that
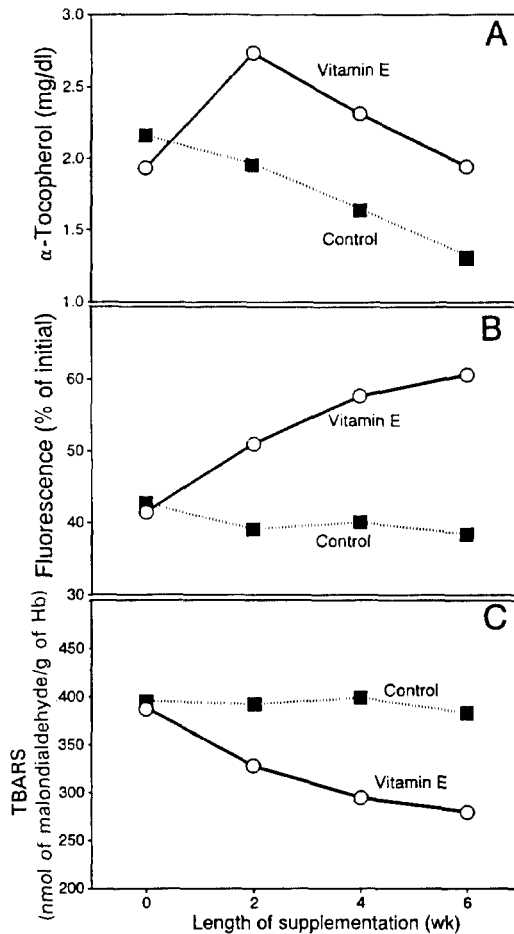
Figure 4. Comparison of cows supplemented or unsupplemented with 1000 IU/d of vitamin E during the last 6 wk of gestation: A) Serum α-tocopherol; B) total antioxidants in plasma; C) erythrocyte thiobarbituric acid reactive substances (TBARS).

TABLE 2. Incidence of placental retention in dairy cows fed diets contain >.12 ppm of Se with or without 1000 IU of supplemental vitamin E during the last 40 d of gestation.

Year	Reference	Treatment	
		Control	Vitamin E
		(% of group)	
1988	(39)	26.7	6.9*
1989	(38)	34.4	10.8**
1990	(56)	52.9	22.0*
1991	(4)	32.3	21.9

*$P < .05$.

**$P < .01$.

did not retain the placenta had higher E_2 and lower P_4 8 d before calving than cows that retained the placenta. Thereafter, P_4 declined faster for unsupplemented cows than for cows given vitamin E or for cows that retained the placenta compared with cows that shed the placenta before 12 h. Serum E_2 increased more rapidly during the 8 d before calving and was higher at calving for cows given 1000 IU/d of vitamin E than for unsupplemented cows. Whether ROM effects on steroidogenic enzymes in cows unsupplemented with vitamin E contributed to more rapidly declining P_4 dur-

ing the last 8 d of gestation and lower E_2 at calving was not determined.

Supplementation with vitamin E and Se has reduced incidence of retained placenta and improved reproduction of dairy cows in some (24, 38, 39, 56, 58, 59), but not all (21, 26, 28, 30, 41, 51), comparisons. In the majority of investigations, 680 IU of vitamin E plus 50 mg of Se were administered as a single intramuscular injection 3 wk before expected parturition. This amount of Se appears to be sufficient when vitamin E is adequate (24). However, 680 IU of vitamin E distributed over 3 wk (21, 28, 30, 41) or 500 IU/d fed throughout lactation, but not during the dry period (51), did not improve performance. Reproductive efficiency improved (24, 38, 39, 56) when 1000 IU of vitamin E were fed daily beginning at least 4 wk before expected calving.

Supplemental vitamin E appears to be less effective when Se is lacking and vice versa. Retained placenta and days to conception were not reduced when 1000 IU/d of vitamin E were fed to cows receiving diets containing < .06 ppm of Se unless cows also were injected intramuscularly with Se at .1 mg/kg of BW 3 wk before expected calving (24). Daily supplementation with 1000 IU of vitamin E reduced incidence of retained placenta in multiparous cows (Table 2) and severity of udder edema in primiparous cows (Table 3) when the diet contained at least .12 ppm of Se. Udder edema was not reduced by vitamin E supplementation when dietary Se was ≤ .07 ppm (Table 3). Because preventive and chain-breaking antioxidants work in concert, effec-

TABLE 3. Udder edema in primiparous cows at three Tennessee locations fed diets containing .06 to .15 ppm of Se with or without 1000 IU of supplemental vitamin E daily during the last 40 d of gestation.[1]

Location	Dietary Se	Reference	Day after calving and treatment					
			d 1		d 2		d 3	
			Control	Vitamin E	Control	Vitamin E	Control	Vitamin E
	(ppm)		(%)					
Knoxville	.15	(37)	15.4	22.5	16.6	24.2	16.8	24.3*
Knoxville	.15	(57)	12.4	25.3**	15.0	21.1*	18.5	26.2*
Lewisburg	.06	(36)	10.6	11.4	10.1	12.9	13.9	11.9
Martin	.07	(36)	21.6	15.9	23.7	21.6	27.4	22.8

[1]Percentage of decrease in udder floor area after removal of milk was used as an index of edema. The more edematous and rigid the udder, the less the udder floor area decreased after milking.

*$P < .05$.

**$P < .01$.

tiveness of the total system is impaired when one or more components is inadequate. Thus, supplementation with one nutrient is less beneficial if another nutrient is more limiting.

Immune Function and Mastitis

Impaired immunological response during oxidative stress is suggested by several observations. Diets high in unsaturated fats, which have long been recognized as contributing to oxidative stress, also reduce immunocompetence (34). Immunity is reduced by inadequacies of essential trace elements, including Cu, Fe, and Zn (45), and vitamins E (25), A, and C (2), all of which are among those required for antioxidant defense (Figure 1).

Infection and tissue repair are common even in well-managed dairy herds, and cows may experience some degree of immune response, especially after calving. Stress, disease, and induction of the immune response increase requirements for nutrients, including vitamins and essential trace elements (32, 33). Inadequacies of these nutrients required for both immunity and antioxidant defense could impair function of both systems.

Nutrient involvement in innate and acquired immunocompetence and, thus, resistance to mastitis has been discussed (62). Antioxidants in relation to the immune response and mastitis also are discussed in the present symposium (1, 6, 27) and thus are not pursued further herein.

Parturient Paresis

At parturition, a dairy cow commonly secretes six times more Ca into colostrum than

the amount contained in her extracellular pool (16). Prevention of parturient paresis, or milk fever, depends on rapid replacement of Ca that was lost from the extracellular pool. An important component of the homeostatic control that regulates extracellular Ca is 1,25-dihydroxy vitamin D [1,25-(OH)$_2$D]. Delayed or insufficient production of 1,25-(OH)$_2$D is thought to be a common cause of milk fever (16). Because hydroxylation of cholecalciferol in the 1 and 25 positions is dependent on cytochrome P-450 enzymes (61), ROM inactivation of these enzymes (49, 54) may inhibit 1,25-(OH)$_2$ production and have implications for milk fever. An association has been suggested between milk fever and retained placenta (13, 35).

CONCLUSIONS

The periparturient and early lactation periods are critical for the health of dairy cows. Udder edema, milk fever, retained placenta, mastitis, and suboptimal reproduction reduce profits for dairy producers. Oxidative stress may contribute to all of these disorders. Antioxidant requirements of high producing dairy cows may be higher than generally recognized, and intakes of antioxidants needed to control ROM balance effectively may exceed amounts supplied by average feeds. For this reason, supplementation with all known nutrients required for antioxidant defense in adequate and balanced amounts would be beneficial. Additional research is needed, however, to identify optimal amounts of each nutrient.

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