Journal of Animal and Veterinary Advances 5 (10): 863-872, 2006

Vitamin E Nutrition and Immune Response in Dairy Cows with Peripartum Health Problems

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Abstract: The periparturient period is characterized by negative energy balance because feed intake gradually decreases prior to parturition and increases at a slower rate than milk production. This is one of the contributing factors to occurrence of other peripartum period health problems including mammary gland and reproductive tract infections. Especially high-producing dairy cows are prone to ketosis and infectious diseases, which adversely affect profitability of dairying. To maintain plasma concentration above 3 μg mL^{-1} at parturition, vitamin E supplementation during the transition period benefits to immune system via improving neutrophil activity and consequently reduces risk for mastitis and retained placenta. Ketone bodies also impair immune function. However, improvement in immune function in cows with ketosis in response to vitamin E supplementation remains to be investigated.

Key words: Peripartum cow, vitamin E, immune system, mastitis, uterine infection, ketosis

INTRODUCTION

Parturition is a tumultuous event and associated with a complex series of hormonal and metabolic changes[1]. Major changes during periparturient period include rapid growth of fetus, termination of gestation, activation of the mammary gland and involution of the uterus. These changes influence nutritional[2-5] and immune statuses[6,7] of dairy cows.

Transition from gestation to lactation is a tremendous stress in periparturient dairy cows partially due to occurrence of negative Energy Balance (EB) that leads to hepatic lipidosis and ketosis. Moreover, cows with these lipid-related metabolic disorders are susceptible to infectious diseases as a result of suppressed immune defense mechanisms. The objectives of this review study were to summarize the periparturient period with respect to metabolic profile and health and immune statuses of dairy cattle and elaborate the role of vitamin E in preventing peripartal health problems such as infections affecting uterine and mammary gland health and hyperketonemia.

Significance of the peripartum period: Selection of dairy cows for higher milk production is accompanied with increasing incidence of postpartum health problems[8]. It is a dilemma that incidences of postpartum metabolic and reproductive disorders have not decreased since 19th century despite significant advancements in knowledge of nutrition, reproduction and animal husbandry[9]. Incidences of metabolic disorders (milk fever, displaced abomasum and ketosis) and mammary gland (mastitis and mammary gland edema) and reproductive diseases (veterinary assisted dystocia, retained placenta and metritis) range from 7.8 to 16.8, from 2.8 to 12.6 and from 6.7 to 19.2%, respectively, in high producing dairy herds[10-12]. Postpartum diseases occur as a complex during the periparturient period[13-15]. Moreover, these health problems compromise lifetime milk yield and reproductive efficiency[16,17].

Without taking economic losses due to suppressed milk production and failed reproductive performance into account, health cost (veterinary and drug) was estimated to be five times greater during the periparturient period than during the mid- and late lactation periods[18]. This suggests that the periparturient period, three weeks prior to parturition through the first four weeks of subsequent lactation[19], is the most critical and potentially the most costly phase of a lactation cycle. Therefore, nutritional practices during the periparturient period have focused on improving EB and immune potency.

Nutritional and metabolic Status of peripartum dairy cows: As opposed to having considered as a resting and/or non-profitable phase in earlier[20,21], major dynamic changes occur during the dry period. Especially, the

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close-up period nutrition (the final three weeks of gestation) has carry-over effects on lactation, reproduction and health during postpartum[10,14,15]. Moreover, due to largely unknown reasons, a 20 to 40% exponential decline in feed intake as cows approach parturition initiate a negative EB[22-24]. Because milk production increases at a faster rate than feed intake, negative EB becomes more pronounced during early lactation[25,26]. Dairy cattle lose Body Weight (BW) and Body Condition Score (BCS), which promotes mobilization of body reserves in order to support energy deficit. Negative EB contributes to failure to overcome physiological changes and maintain immune potency.

Transition from gestation to parturition is associated with synchronized changes in metabolism through homeostasis and homeorhesis[1]. Homeostasis refers to a control mechanism that involves physiological equilibrium and homeorhesis refers to a control mechanism that involves coordination in metabolism of body tissues necessary to support physiological state. During late gestation and early lactation, flow of nutrients to fetus and mammary tissues are accorded a high degree of metabolic priority[25]. Therefore, lactogenesis is accompanied by increased lipolysis and decreased lipogenesis in adipose tissue, decreased glycogenesis and increased gluconeogenesis and glycogenolysis in the liver, decreased use of glucose and increased use of lipid as energy sources by body tissues[1,27,28]. The metabolic profile during early lactation includes low concentrations of serum insulin, plasma glucose and liver glycogen and high concentrations of serum glucagon, epinephrine and growth hormone, plasma β-hydroxybutyrate (BHBA), Nonesterified Fatty Acid (NEFA) and liver triglyceride[29,30]. This metabolic pattern affirms dominance of catabolic activities resulting from negative EB, because similar metabolic profile is also reported in cases of induced or spontaneous ketosis[31-35].

Vitamin E

Chemical forms and bioactivity of vitamin E: Vitamin E, discovered in 1922, is a lipophilic compound. It is also known as tocopherol, which is derived from Greek words tokos (offspring) and phero (to bear). Chemical structure of vitamin E (tocopherol) consists of a chromanol ring and isoprenoid side chain. Nomenclature and bioactivity of vitamin E depends on position(s) and number of methyl groups (Fig.1 and Table 1).

Table 1: Chemical forms of vitamin E

Side chains		
R_1	R_2	Nomenclature
CH_3	CH_3	α-tocopherol (5, 7, 8-trimethyl-tocol)
CH_3	H	β-tocopherol (5, 8-dimethyl-tocol)
H	CH_3	γ-tocopherol (7, 8-dimethyl-tocol)
H	H	δ-tocopherol (8-monomethyl-tocol)

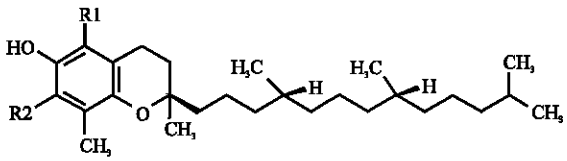

Fig. 1: Chemical structure of vitamin E

Depending upon side chains, vitamin E exists in various forms. However, all forms have different bioactivity (Table 2). The alpha form of tocopherol (α- tocopherol or 5, 7, 8-trimethyl-tocol; α-TOH) is the most active form of vitamin E. One mg of dl-α-tocopheryl-acetate is equivalent to one International Unit (IU) of vitamin E, which bases on 689 rats utilized in 12 experiments with median fertility dose of 0.986 mg dl-α-tocopheryl acetate. Several tests have been proposed for determining bioactivity of vitamin E, which are also known as biological function tests such as prevention of fetal resorption in rats, encephalomacia in chicks and prevention of erythrocyte hemolysis. The most commonly accepted assays for determination of bioactivity of vitamin E are gestation-resorption-sterility and hemolysis tests. Other forms of tocopherols have also been standardized according to resorption test (Table 3).

Metabolism of vitamin E: Active and total vitamin E concentrations of feedstuffs are quite variable (Table 4). In general, oils are rich in total vitamin E content. However, the proportion of the active form of vitamin E in oils varies. Forages are richer vitamin E sources than grains.

Once consumed, vitamin E is absorbed from the gastrointestinal tract and transported to circulation in a similar mechanism by which fats are absorbed (Fig. 2). That is, vitamin E esters are hydrolyzed to the alcohol in small intestine. After absorption, vitamin E is transported to the liver via the lymphatic system and then distributed to tissues. Up to the date, no specific carrier protein has been identified, but it is bound to the lipoprotein fraction of plasma.

Table 2: Relative bioactivity of tocopherol forms

Form	Resorption	Hemolysis
α-Tocopherol	100	100
β-Tocopherol	25-40	15-25
γ-Tocopherol	8-19	3-18
δ-Tocopherol	> 1	> 1

Table 3: Standardization of tocopherol activity*

Feedstuff	IU per mg	d-α-tocopherol equivalents/mg
d-α-tocopherol	1.49	1.00
d-α-tocopheryl acetate	1.36	0.91
dl-α-tocopherol	1.10	0.74
dl-α-tocopheryl acetate	1.00	0.67

*Based on gestation resorption sterility test

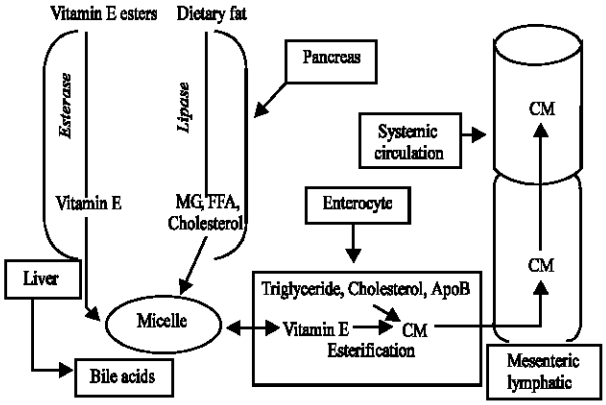

Fig. 2: Absorption and transport of vitamin E. MG = monoglyceride; FFA = free fatty acids; CM = chylomicrons

Table 4: Vitamin E concentration of feedstuffs (mg kg^{-1}, as-fed basis)

Feedstuff	Total tocopherol	α-tocopherol
Alfalfa, fresh	121	116
Alfalfa, hay	33	31
Corn, grain	37	4
Cottonseed meal	16	9
Sorghum, grain	10	2
Wheat, grain	35	18
Wheat, bran	62	6
Fat, animal	8	8
Sunflower oil	835	783
Corn oil	1148	324
Soybean oil	1233	107

Assessment of vitamin E status: There are several methods available to assess vitamin E requirement. These are briefly as follow:

- Direct measurement of vitamin E: This method involves in measuring vitamin E level in blood or other readily accessible tissues (e.g., liver storage). The ratio of α-TOH to plasma concentrations of lipids is better index of Vitamin E status[36].
- Direct measurements of vitamin E's homeostasis: Input and output of vitamin E are measured (e.g., consumption and excretion).
- Direct assessment of Vitamin E-dependent biochemical reactions: This method covers monitoring Vitamin E-dependent enzymatic reactions before and after adding excessive amount of vitamin E in assays.
- Indirect measurement of Vitamin E functions at tissue level: As discussed in later sections, this method involves in measurements of immune function and cell activity.
- Epidemiological relationship between Vitamin E intake and disease incidence: Involving a large scale of individuals, supplement trials (dose-response relationship) and dietary recalls are utilized to evaluate vitamin E status.

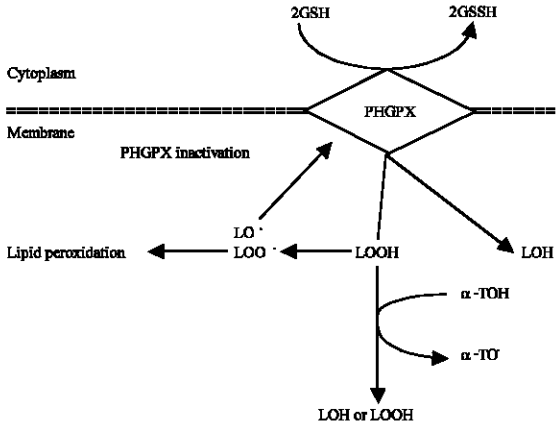

Fig. 3: Relationship between vitamin and Se. α-TOH = α-tocopherol; GSH = glutathione; GSSH = glutathione disulfide; LOOH = unsaturated fatty acid; PHGPX = phospholipid hydroperoxide glutathione peroxidase

Assay to measure vitamin E covers extraction by organic solvents, saponification to remove fat and purification via floridin chromatography, hydrogenation, freezing to remove sterols and followed by separation of tocopherols using chromatography (column, paper, thin layer, HPLC). Emmerie-Engle methods (reduction of ferric to ferrous iron by tocopherol), fluorometric method (activation at 294 nm, emission at 323 nm) and spectrophotometric method (absorption at 294 nm) are commonly used laboratory procedures.

Role of vitamin E: Vitamin E has numerous functions [37]. These are broadly a) tissue antioxidant, b) boosting immune systems, c) syntheses of coenzyme Q and vitamin C, d) involving in sulfur containing amino acid metabolism, e) interrelated with Se metabolism (Fig. 3), f) maintaining low peroxide levels in the cells, g) essential for intracellular respiration and h) maintaining structure of muscles, gastrointestinal and reproductive systems.

Free radicals, Reactive Oxygen Molecules (ROM), are not only generated in reduction of oxygen to water but also secreted by white blood cells in response to bacterial exposure as a means of killing bacteria. Free radicals are unstable compounds because they carry an unpair electron [Partially: 1) $O_2 + e^- \rightarrow O_2^-$ (Superoxide radical), 2) $O_2^- + e^- + 2H^+ \rightarrow H_2O_2$ (Hydrogen peroxide), 3) $H_2O_2 + e^- + H^+ \rightarrow H_2O + HO^-$ (Hydroxyl radical) and 4) $HO^- + e^- + H^+ \rightarrow H_2O$. Overall Reaction = $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$]. They prefer to donate or accept an electron to be in ground state. Polyunsaturated Fatty Acids (PUFA) in cell membranes are susceptible to damage by free radicals because the electrons of double bonds tend to be in pi

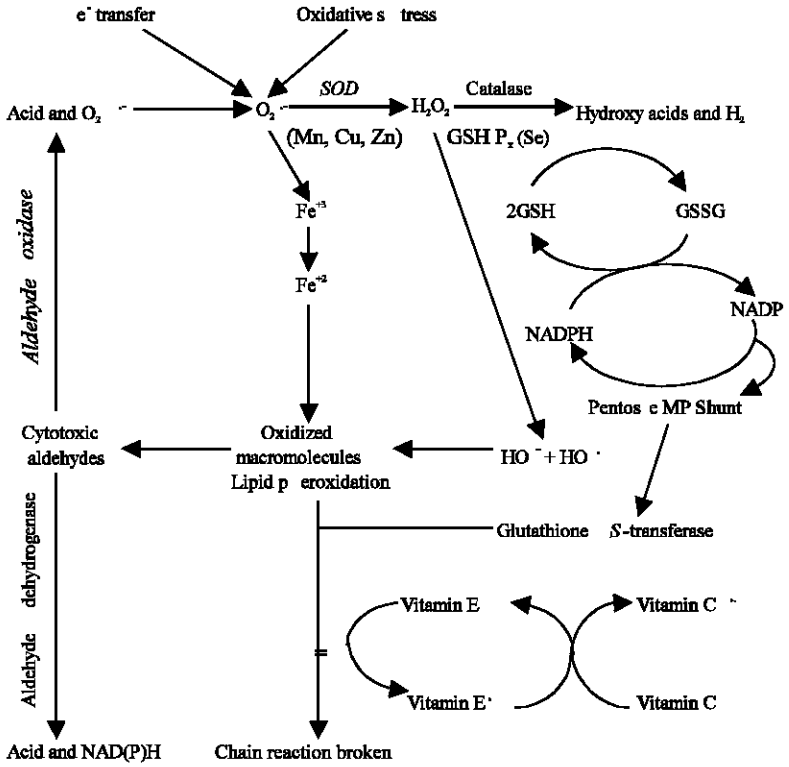

Fig. 4: The protective role of vitamin E against reactive oxygen molecules. GSH = glutathione; MP = monophosphate; SOD = superoxide dismutase. Oxidative stress, Lipid MP shunt

orbitals that form electron cloud around main carbon. That makes it easier to donate or accept an electron from the cloud using a free radical. Initial destruction of PUFA may follow destruction of another (propagation). Vitamin E reacts with free radicals by donation of a hydrogen atom from the hydroxyl group on the chromanol ring and prevents tissues from oxidative damage (autoxidation) (Fig. 4). Briefly, oxidation covers initiation (L-H + O_2 → L· + H^+ + O_2^-), propagation (L· + O_2 → LOO· and LOO· + L-H → LOOH + L·), branching (LOOH → LO· + HO· and 2LOOH → LOO· + LO· + H_2O) and termination (α-TOH + LOO· → α-TO· + LOOH) reactions.

Immune system: The body is protected by a variety of defense mechanisms, which include mainly nonspecific and specific immunities (Fig. 5). Nonspecific responses are always present and are mediated by neutrophils, natural killers and macrophages[38]. They are stimulated by pathogen (antigens) at the site of injury or infection. However, they are not augmented by repeated exposure. Of these, for instance, neutrophils are the predominant cell types in immune defense systems in the mammary gland. These nonspecific cells travel to the site of infection or injury in response inflammatory mediators. Neutrophils phagocytose and kill pathogens via exerting their bactericidal effects through a respiratory burst that produces ROM[37-38]. Specific immunity covers antigen-presenting cells and lymphocytes. Lymphocytes recognize antigens through membrane receptors that are specific for invading pathogens. There are two types of lymphocytes, T and B-lymphocytes. T lymphocytes are responsible for cell-mediated immune response via secreting cytokines and interferons, whereas B lymphocytes are responsible for humoral immune response via producing antibodies[38].

Peripartal health problems: In addition to negative EB, parturition stress also adversely affects immune potency, consequently results in high incidence of health problems in dairy cows[6]. Uterine and mammary gland infections are serious problems and compromise lifetime productivity of dairy cows[17]. According to National Mastitis Council, estimated annual losses caused by mastitis in USA is about $ 2 billions per year. Briefly, losses per case are due to decreased production ($ 116.1, 64%), discarded milk ($ 24.44, 14%), early cow replacement ($ 13.60, 8%), reduced cow sale value ($ 9.94, 5%), drugs ($ 9.68, 5%), veterinary services ($ 4.84, 3%) and labor ($ 2.42, 1%).

Ketosis is often a complication of hepatic lipidosis and is characterized by hypoglycemia, hypoinsulinemia, hyperketonemia, fatty liver, depleted hepatic glycogen,

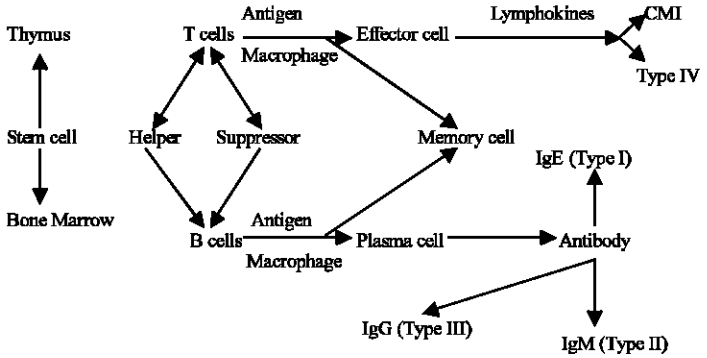

Fig. 5: General action of the immune system. CMI: cell-mediated immunity

hypophagia, decreased milk production, rapid loss of BW and BCS, lethargy and hyperexcitability[32,34,35]. Incidence of clinical ketosis varies from 3.5 to 15% in USA; estimated treatment cost is about $150 per case of clinical ketosis and annual treatment costs vary from $60 to 70 millions nationwide several years ago[40]. Ketosis typically occurs 21 to 28 days after parturition and increases the risk for displaced abomasum (53.5-fold), retained fetal membrane and metritis (16.4-fold), mastitis (15.3-fold) and milk fever (23.6-fold)[13,14]. Moreover, elevated plasma ketones (greater than >20 mg BHBA per deciliter) compromise immune potency through suppressing mitogenic response of lymphocytes in calves[41], heifers[42] and cows[43] *in vivo* and *in vitro* [44] and decreasing phagocytic activity of macrophages[45,46]. Suppression of interferon response in cows with subclinical and clinical ketosis has been demonstrated as well[47].

Infectious diseases and ketosis adversely influence the activity of leukocytes. Pathogens causing infectious diseases such as mastitis, retained placenta and metritis and elevated concentrations of plasma ketone bodies resulting from hyperketonemia interfere with the activity of leukocytes. Migration (chemotaxis) and ingestion are two stages of phagocytosis. The former process is measured via cytochrome C reduction and chemiluminescence tests. The latter process is measured via the iodination of halide ion of hydrogen peroxidase complex. Ingestion process depends on production and release of ROM and myeloperoxidase activity.

Cai[48] evaluated neutrophil functions including random migration, chemotaxis, ingestion, myeloperoxidase activity, superoxide production and antibody-dependent cell mediated cytotoxicity from 46 healthy cows, 20 cows with retained placenta, 18 cows with metritis and 13 cows with mastitis. Superoxide production and random migration and chemotaxis abilities by neutrophils from cows with reproductive problems and mastitis were lower than those measured from healthy cows. Moreover, decrease in superoxide production activity and chemotaxis ability by neutrophils from sick cows initiated before parturition. Myeloperoxidase activity and antibody-dependent cell-mediated cytotoxicity ability of neutrophils isolated from healthy cows were not different across treatments, but they were lower during early lactation than during late gestation. Also, there was a continuous increase in activity of neutrophils from healthy cows, but not those from cows with postpartum problems. These suggest that the host defense role of neutrophils gets worse during early lactation as compared to during late gestation and it is impaired in sick cows. A similar study conducted by Sato[43] to compare the effect of infectious diseases on lymphocyte blastogenesis (proliferation and/or mitogenesis) in 14 cows with metritis, 12 cows with mastitis and 26 healthy cows, as reflected by lymphocyte blastogenic activity (based on Glucose Consumption Index, GCI). Lymphocytes from cows with infectious diseases had lower GCI than those from healthy cows. Moreover, lymphocyte GCI was negatively correlated with plasma ammonia and α-TOH concentrations in cows with metritis and mastitis, respectively.

Unlike pathogens, ketone bodies are not antigens. Mitogenic response of lymphocytes were determined by measuring the incorporation of [^{3}H]-thymidine into DNA of lymphocytes or GCI, or using stimulators such as Concavalin A, phytohemagglutinin and pokeweed mitogen[41]. Concentrations of ketone bodies *in vivo* and *in vitro* studies causing suppression of mitogenic response of lymphocytes were: 6.3 and 7.4 mM for BHBA and acetoacetate (AcAc), respectively[42]; 2.02 and 0.15 mM for BHBA and AcAc, respectively[43]; 13 mg dL^{-1} for BHBA[41] and 10 and 50 mg/dl for BHBA and AcAc, respectively[44]. Concentrations of total ketone bodies causing a reduction of interferon response were reported to be 4.25, 1.25 and 0.60 mmol/l for cows with clinical ketosis and subclinical ketosis and healthy cows, respectively[47]. That is, low incorporation of [^{3}H]-thymidine into DNA of lymphocytes in animals with hyperketonemia causes impairment of lymphocyte blastogenesis and increases susceptibility to infectious diseases.

To clarify the mechanism by which physio-pathological concentrations of ketone bodies are related to suppression in blastogenesis of leukocytes, Salleh[49] incubated lymphocytes isolated from rat with ketone bodies, oleate and glucose. Replacing of glucose by ketone bodies (3 mM AcAc and 3 mM BHBA) failed to support lymphocyte proliferation, whereas addition of 0.5 mM oleate increased the rate of oxygen consumption following 48 h incubation. Moreover, ketone bodies decreased the rate of pyruvate oxidation and increased intracellular concentrations of hexose monophosphate and citrate, suggesting that lymphocytes are not ketogenic cells.

Based on knowledge that serine protease activity is essential for neutrophil chemotaxis, release of lysosomal enzyme and initiation and maintenance of superoxide production, Tsun[50] studied the effect of α-N-tosyl-L-phenylalanine (TPCK), a chloromethyl ketone derivative, on superoxide production by neutrophils. There were 18 and 49% reductions in superoxide production in cell medium added with 0.01 and 0.05 mM TPCK comparing to control cell medium. Recovery of superoxide production was achieved by addition of 0.2 mM reduced glutathione into cell medium. Moreover, increased TPCK concentration in medium was accompanied with inhibition of sulfhydryl groups (-SH), suggesting that inhibition of superoxide production by neutrophil due to ketone bodies is related to the adverse effect of ketone bodies on inhibition of sulfhydryl group of protease. A similar experiment was conducted by Sato[51] to determine the mechanism by which ketone bodies inhibit superoxide production. Leukocytes from healthy subjects were incubated with 0.1, 1 and 10 mM AcAc and BHBA to measure Luminol-Dependent Luminescence (LDCL) and Myeloperoxidase (MPO) activities and superoxide production. Superoxide production and LDCL activity decreased by 16 and 46% following the addition of 1.0 and 10.0 mM AcAc and BHBA, respectively. The pH was in physiological range; 7.38 and 7.39 for cell medium containing 10.0 mM AcAc and BHBA, respectively. Ketone bodies did not affect MPO activity. Therefore, reduction of leukocyte bactericidal activity in patients with ketoacidosis could be due to the reduction of superoxide production through decreased NADPH-oxidase activity because NADPH-oxidase exists in plasma membrane, whereas myeloperoxidase exists in azule granules.

Vitamin E supplementation and immune response: Free radicals generated by leukocytes in response to bacterial exposure can damage cellular membrane of leukocytes[52]. The antioxidant action of vitamin E[37] and its relation to enhancement of neutrophil functions in the mammary gland[36,52] are well documented. However, alteration in immune status of cows with ketosis in response to vitamin E supplementation has not been studied.

Migration of neutrophils to the site of injury or infection is the typical manifestation of an inflammatory response. During mastitis, neutrophils start to migrate by penetrating blood capillaries and accumulate on the surface of the epithelium. Therefore, rapid recruitment of neutrophils is a critical step in the mammary gland defense system. Politis[53] has shown that neutrophils in cell media prepared from cows supplemented with vitamin E (3000 IU/day) during dry period had greater chemotactic response to opsonized heat-killed *Staph. aureus* than those prepared from cows in control group (Fig. 6a and 6b), despite no change in superoxide production. An in vitro study conducted by Ndiweni and Finch[54] to investigate the effects of vitamin E on bovine neutrophil functions. In cell media supplemented at physiological concentration of α-TOH (3 μg mL), neutrophils showed greater migration (Fig. 7a) and phagocytic response (Fig. 7b) to *Staph. aureus* than those supplemented lower and higher α-TOH comparing to physiological range (Fig. 8).

Weiss[55] reported that plasma and neutrophil α- TOH concentrations were positively correlated with vitamin E intake and negatively correlated with incidence of intramammary infections (IMI) and rate of clinical mastitis (# of clinical cases/305 cow-d +0.001) (Table 5). Bulk tank SCC was negatively related to plasma Se concentration

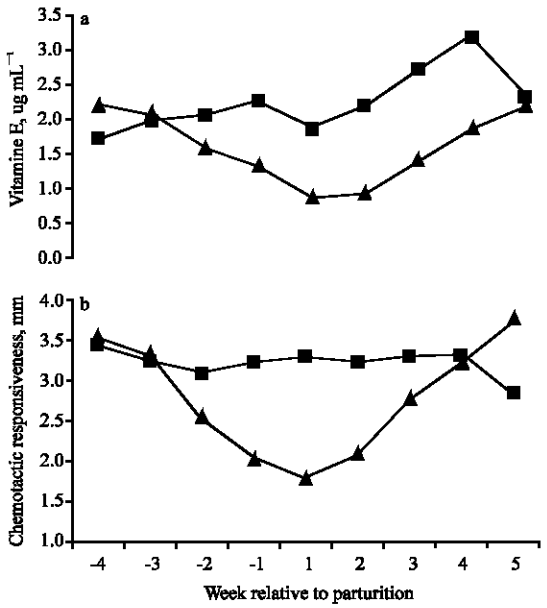

Fig. 6: Effects of vitamin E on serum tocopherol concentration (a) and chemotactic responsiveness of neutrophils to *Staph. Aureus* (b) (Vitamin E - 3000 IU/d via diet 5000 IU via injection a week prior to calving-, --■-- vs. control group, --▲--). Adapted from Politis[53]

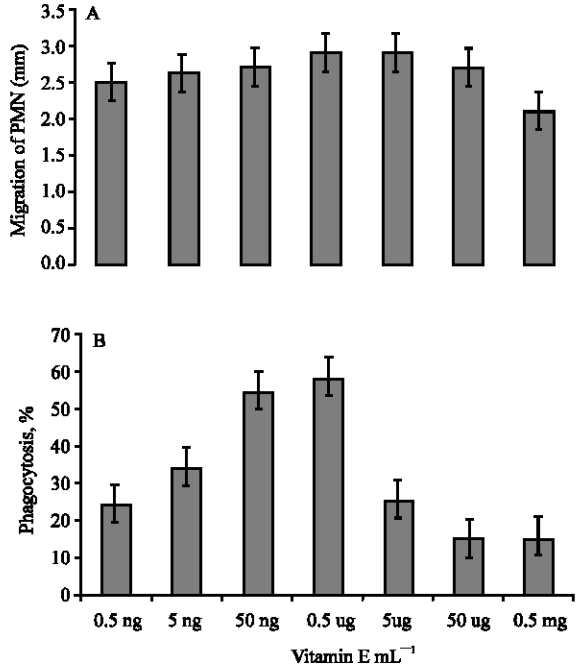

Fig. 7: Effects of vitamin E on migration (a) and phagocytic (b) responses of neutrophils to *Staph. aureus*. Adapted from Ndiweni and Finch[54]

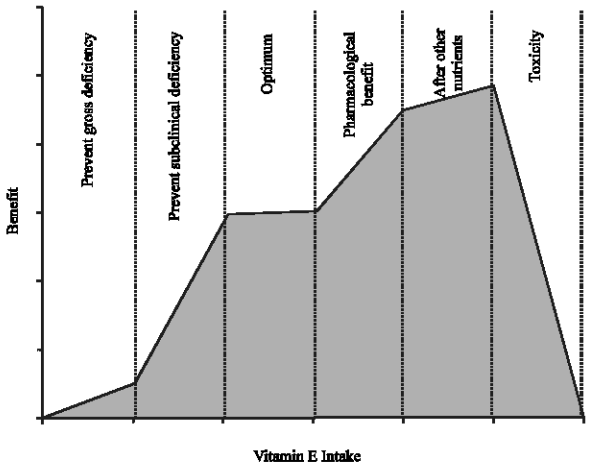

Fig. 8: Dose-Response relationship

Table 5: Effects of vitamin E supplementation on mammary gland health*

	Parity 1			Parity > 1		
	Vitamin E (IU/d)					
Health[1]	100	1000	4000	100	1000	4000
IMI	56.2[a]	57.2[a]	20.9[b]	17.9[a]	14.9[a]	10.0[b]
CM	37.4[a]	14.2[b]	0[c]	17.9[a]	17.9[a]	3.8[b]

*Adapted from Weiss[55], [a, b, c]Means in the same row within a parity group differ ($p<0.05$), [1]IMI = intramammary infections; CM = clinical mastitis. Infectious agents were staphylococci, streptococci. and coliforms

and glutathione peroxidase (GSH-Px) activity (μmol NADPH oxidized^{-1}). Hogan[36] fed 1040 IU vitamin E per day and injected 3000 IU vitamin E at 10 and 5 days prior

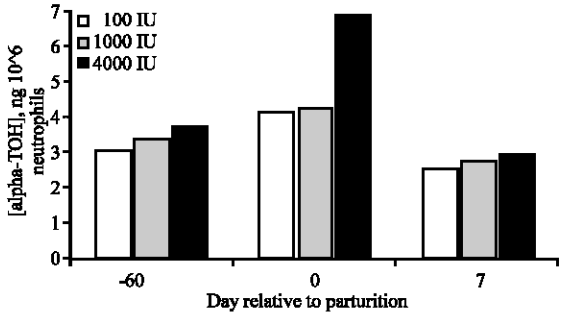

Fig. 9: Effects of vitamin E supplementation on plasma α-tocopherol concentration Adapted from Weiss[57]

Table 6: Effects of supplementation of vitamin E and Se on mammary gland health*

	Clinical cases per quarter			Clinical month[1]	
Group	Quarter	Case	Reduction, %	N	Reduction, %
Vitamin E + Se	84	22	37	14	42
Vitamin E	80	21	37	14	14
Se	76	27	12	12	27
Control	80	33	0	22	0

*Adapted from Smith[56]. Reduction, %=100- [(Experimental/Control)x100] [1]In quarters infected with Streptococci and/or coliform bacteria

Table 7: Relationship between plasma tocopherol concentration and likelihood of mastitis*

Item and group	Odds ratio	P
Plasma α-TOH concentration		
≤2 μg mL^{-1} vs. >2 μg mL^{-1}	2.6	0.07
≤2.5 μg mL^{-1} vs. >2.5 μg mL^{-1}	5.9	0.02
≤3 μg mL^{-1} vs. >3 μg mL^{-1}	9.4	0.02
α-TOH/mg Cholesterol		
≤ 5 μg mg^{-1} vs. >5 μg mg^{-1}	9.1	0.04

*Adapted from

to anticipated calving date. Plasma α-TOH concentration at calving was correlated with intracellular kill (number of dead phagocytosed bacteria)/(number of live and dead intracellular bacteria) and phagocytic index (average number of bacteria phagocytosed per neutrophil). These studies ascertain that decrease in concentration of plasma α-TOH around parturition might be related to high incidence of IMI and clinical mastitis and cows should be supplemented with vitamin E to maintain plasma α-TOH above 3 μg mL^{-1} is beneficial. Smith[56] fed vitamin E (0.74 g d^{-1}) plus Se (0.1 mg kg^{-1} BW), vitamin E (0.74 g d^{-1}) and Se (0.1 mg kg^{-1} BW) to cows from drying off to the third month of lactation and reported that % reduction in incidence of mastitis (clinical cases per quarter per lactation) in cows fed vitamin E alone and vitamin E plus Se. However, selenium alone was not as effective as (Table 6).

Weiss[57] performed a study to determine the effects of three levels of vitamin E supplementation (100, 1000

and 4000 IU d^{-1}) on plasma and neutrophil α-TOH concentrations and its impact on mammary gland health status. In response 4000 IU vitamin E supplementation per day, plasma and neutrophil α-TOH concentration became unchanged (Fig. 8) and IMI incidence at parturition decreased by 18% (Table 7). Vitamin E supplementation however did not affect concentration of Se and GSH-Px activity. It also appears that cows with plasma concentration of α-TOH < 3 μg mL^{-1} were 9.4 times more likely to have clinical mastitis during the first week of lactation. These results suggest that vitamin E supplementation during dry period increases plasma α-TOH concentration and plasma α-TOH concentration is related to bacteria killing ability of neutrophils at calving (Fig. 9).

CONCLUSION

Neutrophils are considered a primary defense mechanism against bacterial infections. Vitamin E minimizes the harmful effects of free radicals and increases phagocytic ability and mobilization of neutrophils during infections. Studies involving mammary gland confirm improvement in health status in cows supplemented with vitamin E during the transition period. Ketone bodies also deteriorate defense mechanisms. Influence of vitamin E on immune status in case of experimentally induced or spontaneous ketosis *in vivo* however remains to be elucidated.

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