Evaluation of ewe and lamb immune responses when ewes are supplemented with Vitamin E
by John Todd Daniels

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
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Abstract:
Fifty-two Targhee twin-bearing ewes were used in a completely random design to investigate the role of supplemental vitamin E in immune function. Parainfluenza type 3 (PI3) vaccination was used to evoke an immune response. Ewes were randomly assigned to receive one of four treatment combinations in a 2 x 2 factorial arrangement. Treatments were 1) 400IU orally supplemented vitamin E and PI3 vaccination, 2) 400 IU orally supplemented vitamin E and no PI3 vaccination, 3) No supplemental vitamin E and PI3 vaccination, and 4) No supplemental vitamin E and no PI3 vaccination. Ewes receiving PI3 were vaccinated at 7 and 3 wk before the expected lambing date. Ewes receiving vitamin E were dosed daily, 28 to 0 d pre-lambing. Blood was collected from ewes prior to 7 wk vaccination and 4 h post partum. Blood was collected from lambs (n = 104) at 3 d post partum. Sera were analyzed for PI3, immunoglobulin G (IgG), and vitamin E concentrations. Colostrum was collected 4 h post partum and analyzed for IgG. The model for ewe and lamb analysis included the main effects of vitamin E and PI3 treatment, sex, and their interaction. No interactions were detected ($P > .20$) for ewe or lamb variables. Serum PI3 titers were greater ($P < .01$) in PI3 vaccinated ewes and their lambs than non-PI3 vaccinated ewes and their lambs. Serum vitamin E concentrations were greater ($P = .001$) in vitamin E supplemented ewes than ewes not receiving supplemental vitamin E. Colostral IgG concentrations and serum PI3 titers did not differ ($P > .20$) between ewes supplemented with vitamin E and ewes not receiving supplemental vitamin E. Serum IgG concentrations in vitamin E supplemented ewes and their lambs did not differ ($P > .10$) from concentrations in ewes not receiving supplemental vitamin E and their lambs. Serum IgG concentrations were greater ($P = .05$) in female lambs than male lambs. Serum vitamin E concentrations were greater ($P = .001$) in lambs reared by vitamin E supplemented ewes than in lambs reared by ewes not receiving supplemental vitamin E. Lamb PI3 titers did not differ ($P = .76$) between lambs reared by vitamin E supplemented ewes and lambs reared by ewes not receiving supplemental vitamin E. These results indicate that supplemental vitamin E to the ewe had no effect on humoral immunity in the ewe or passive immunity to the lamb. Research directed towards cell-mediated immune function and lamb immune system challenge may better address vitamin E's effect on sheep immune systems.

EVALUATION OF EWE AND LAMB IMMUNE RESPONSES WHEN EWES ARE SUPPLEMENTED WITH VITAMIN E

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This thesis has been read by each member of the graduate committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Fifty-two Targhee twin-bearing ewes were used in a completely random design to investigate the role of supplemental vitamin E in immune function. Parainfluenza type 3 (PI_3) vaccination was used to evoke an immune response. Ewes were randomly assigned to receive one of four treatment combinations in a 2 x 2 factorial arrangement. Treatments were 1) 400 IU orally supplemented vitamin E and PI_3 vaccination, 2) 400 IU orally supplemented vitamin E and no PI_3 vaccination, 3) No supplemental vitamin E and PI_3 vaccination, and 4) No supplemental vitamin E and no PI_3 vaccination. Ewes receiving PI_3 were vaccinated at 7 and 3 wk before the expected lambing date. Ewes receiving vitamin E were dosed daily, 28 to 0 d pre-lambing. Blood was collected from ewes prior to 7 wk vaccination and 4 h post partum. Blood was collected from lambs (n = 104) at 3 d post partum. Sera were analyzed for PI_3, immunoglobulin G (IgG), and vitamin E concentrations. Colostrum was collected 4 h post partum and analyzed for IgG. The model for ewe and lamb analysis included the main effects of vitamin E and PI_3 treatment, sex, and their interaction. No interactions were detected ($P > .20$) for ewe or lamb variables. Serum PI_3 titers were greater ($P < .01$) in PI_3 vaccinated ewes and their lambs than non-PI_3 vaccinated ewes and their lambs. Serum vitamin E concentrations were greater ($P = .001$) in vitamin E supplemented ewes than ewes not receiving supplemental vitamin E. Colostral IgG concentrations and serum PI_3 titers did not differ ($P > .20$) between ewes supplemented with vitamin E and ewes not receiving supplemental vitamin E. Serum IgG concentrations in vitamin E supplemented ewes and their lambs did not differ ($P > .10$) from concentrations in ewes not receiving supplemental vitamin E and their lambs. Serum IgG concentrations were greater ($P = .05$) in female lambs than male lambs. Serum vitamin E concentrations were greater ($P = .001$) in lambs reared by vitamin E supplemented ewes than in lambs reared by ewes not receiving supplemental vitamin E. Lamb PI_3 titers did not differ ($P = .76$) between lambs reared by vitamin E supplemented ewes and lambs reared by ewes not receiving supplemental vitamin E. These results indicate that supplemental vitamin E to the ewe had no effect on humoral immunity in the ewe or passive immunity to the lamb. Research directed towards cell-mediated immune function and lamb immune system challenge may better address vitamin E's effect on sheep immune systems.

INTRODUCTION

Lamb mortality is a major factor limiting profitability in sheep operations today. Recent estimates of pre-weaning lamb mortality vary from 15 to 51% (Rook, 1997; Bekele et al., 1992), with mortalities as high as 35% considered normal for large sheep operations (Rowland et al., 1990). Primary causes of lamb mortality are mismothering/ starvation, hypothermia, and pneumonia (Rook, 1997; Safford and Hoversland, 1960; Bekele et al., 1992).

Kott et al. (1998), in a 3-yr study, found oral supplementation of vitamin E to ewes in late gestation decreased lamb mortality by as much as 50% in the early part of the lambing season. Vitamin E given in an injection to the ewe has also shown to decrease lamb mortality (Gentry et al., 1992). However, Williamson et al. (1996) injected both lambs and ewes with vitamin E and found no effect of vitamin E on lamb mortality, but lambs born to ewes injected with vitamin E did receive higher vigor scores.

Research addressing vitamin E and immune function has given variable results. Rittacco et al. (1986) found lamb antibody titers to B. *ovis* increased with oral vitamin E supplementation. Reffet et al. (1988) supplemented lambs with vitamin E and found no effect of vitamin E on immunoglobulin G levels. However, Gentry et al. (1992) found lambs being born to ewes injected with vitamin E in late gestation had greater serum IgG levels compared to lambs born to ewes not injected with vitamin E. Bonnette et al. (1990) found no effect of vitamin E on immune response in pigs, however stress may have not been great enough to elicit an immune response. In examination of the vitamin E supplementation review by Finch and Turner (1989), these types of inconsistencies in

results between studies is rather prevalent. Therefore, our objective was to examine the effects of supplemental vitamin E to the pregnant ewe on humoral immunity in the ewe and passive immunity in the lamb in a lambing environment typical of western Montana sheep producers.

LITERATURE REVIEW

Lamb Mortality

Neonatal lamb mortality is a major factor reducing profitability in sheep operations. Bekele et al. (1992) found that neonatal lamb mortality was as high as 51.5%. Rook (1997), in a survey of Michigan sheep producers, concluded that 15 to 20% pre-weaning lamb losses are common in the sheep industry. Perinatal lamb mortality rates of 10 to 35% are considered normal and acceptable in large sheep operations (Rowland et al., 1990). Safford and Hoversland (1960) examined data recorded from a 3-yr study and found that pre-weaning death loss totaled 23.5%. Rowland et al. (1990) examined records from four large range flock operations over a 1-yr period and found overall mortality rates ranged from 8.2% to 12.2%, with 2/3 of the deaths considered preventable with improved management.

Reported causes of neonatal lamb mortality are similar in past literature. Rook (1997) found that hypothermia/starvation, stillbirth/dystocia, and pneumonia were the top three causes of death. Safford and Hoversland (1960), after examination of approximately 1000 lamb autopsies, noted pneumonia was the leading cause of death in neonatal lambs. Bekele et al. (1992) found the three top causes of death were starvation/mismothering, gastrointestinal parasites, and enteritis. They also indicated that

birth weight significantly affected lamb mortality and those lambs with a low birth weight tended to die from starvation/mismothering.

Male lambs are likely to be more susceptible to mortality than female lambs. Smith (1977) examined the factors affecting birth weight, dystocia, and preweaning survival using data from over 6,000 cross-bred and pure-bred lambs born over a 6 yr period. He found that male lambs weighed more than female lambs at birth and had a greater dystocia rate than female lambs. In addition to greater dystocia, neonatal male lamb mortality rate was also greater and more male lambs were classified as weak at birth than female lambs. Nash et al. (1996) examined extensive records on over 7,000 lambs born in a 6-yr period to investigate risk factors associated with mortality in lambs. Nash et al. (1996) found that male lambs had a greater risk of mortality than female lambs. Nash et al. (1996) also reported that lambs given better vigor scores than average had decreased mortality rates after the perinatal period.

The majority of lamb mortality occurs in the first few weeks of life. Rook (1997) examined flock information from Michigan sheep producers to determine at what time and what causes of death constitute the largest portion of lamb mortality. Mortality information from this study showed that 50% of lamb mortality occured within the first 3 d of life, independent of the production system. Rowland et al. (1990) found more than 50% of all lamb deaths occurred within 24 h of birth. Safford and Hoversland (1960) reported that the average age of death for lambs was 5.9 d and 56% of these deaths occurred in the first 3 d of life.

Neonatal lamb mortality rates and causes reported by Safford and Hoversland (1960) are similar to those reported by more recent research (Rowland et al., 1990; Bekele et al., 1992; Nash et al., 1996; Rook 1997). The results from these studies, spanning a period of more than 30 years, suggest that little improvement has been made in neonatal lamb mortality and continues to be a major problem for sheep producers.

Immune Function

Immunity

Immunity in an individual can be achieved by either passive or active immunization (Kuby, 1997). Passive immunity occurs when an individual is presented with preformed antibodies that are specific to an antigen. These antibodies are produced in another individual. Preformed antibodies can come from an injection from an immune individual to an unprotected, non-immune individual, or from a mother's colostrum to the neonate (Kuby, 1997). In sheep, transplacental crossing of preformed antibodies does not occur, leaving the neonatal lamb with an immature immune system and dependent upon consumption of colostrum for survival (Brambell, 1969). Passive immunization does not activate the recipient's immune system and does not provide for future protection (Kuby, 1997).

Active immunization is achieved by natural infection or artificially by giving a vaccine (Kuby, 1997). In this type of immunization the immune system is activated and memory cells are developed to protect against future infection. Activating and

developing memory cells can effectively protect an individual against an antigen for a longer period and at a greater level than with passive immunization (Kuby, 1997). The memory response can be demonstrated by measuring antibody levels in the blood after initial and subsequent exposure to an antigen (Tortora and Grabowski, 1996). After initial contact with an antigen there is a slow rise, over a 7 to 10 d period, in antibody levels, then a gradual decline over the next 7 to 10 d. This first rise and fall of antibodies is called the primary response (Tortora and Grabowski, 1996). The second time an individual comes in contact with this antigen there is very quick and more intense response in antibody levels in the blood which happens over about a 10 d period. This is called the secondary response (Tortora and Grabowski, 1996). This second group of antibodies has a greater affinity for the antigen and come from memory cells that were formed during the initial contact with the antigen. Active immune response in calves has been shown to develop quite early, with calves being able to respond to vaccines (having measurable antibody levels) as early as 1 to 3 wk of age (Perino and Rupp, 1994). Perino and Rupp (1994) stated that a response to vaccines in young calves was very dependent on the type of vaccine.

The Immune Response

Immune responses can be divided into two different types, the humoral immune response and the cell-mediated response. The humoral branch of the immune system involves B lymphocytes interacting with foreign materials (antigens; Kuby, 1997). The

humoral immune response also involves the proliferation and differentiation of B cells into antibody-secreting plasma cells. Antibodies are the effector cells of the humoral branch, binding to antigens and either neutralizing or preparing the antigen for elimination by phagocytic cells. Immunoglobulins (Ig) function as antibodies, of which there are five classes; IgG, IgM, IgD, IgE, and IgA. Immunoglobulin G makes up approximately 80% of the total serum immunoglobulin. Humoral or antibody mediated immunity is effective against antigens dissolved in body fluids and extracellular pathogens such as bacteria (Tortora and Grabowski, 1996).

The cell-mediated branch involves T lymphocytes, which are generated in response to an antigen. The effector cells of the cell-mediated branch are activated T helper cells (T_H) and cytotoxic T lymphocytes (CTL). Activated T_H cells can activate phagocytic cells to kill or phagocytize microorganisms more effectively (Kuby, 1997). This is especially important in protecting an individual against intracellular bacteria and protozoa. Cytotoxic T lymphocytes are involved in killing altered self-cells which is an important process in killing virus-infected cells and tumor cells (Kuby, 1997). The cell-mediated branch of the immune system relies on antigen specific and non-specific cells. Antigen specific cells include T_H cells and CTL cells. Antigen non-specific cells include macrophages, neutrophils, eosinophils, and natural killer cells (Tortora and Grabowski, 1996). Cell-mediated immunity is particularly effective against intracellular fungi, parasites, and viruses, some cancer cells, and foreign tissue transplants (Tortora and Grabowski, 1996).

Colostrum

Maidment and Thomas (1995) stated that colostrum is a substance with high concentrations of antibodies and is a good indicator of immunoglobulin G (IgG) passage to the neonate. The most important determinant of a calf's immunocompetence is the consumption of colostrum, since the newborn calf is essentially devoid of immunoglobulins (Perino and Rupp, 1994). Of the total antibodies available in colostrum, approximately 90% of the immunoglobulins are in the form of IgG (Maidment and Thomas, 1995). Reception of large amounts of IgG, via colostrum or artificial feeding, during the first 12 to 24 h is very important in keeping the neonatal calf alive (Perino and Rupp, 1994).

Reception of a sufficient amount of colostrum in the first 24 h of life can be challenging for twin lambs. Holst et al. (1996) collected colostrum samples immediately post-partum and found that the more viscous the colostrum was the longer the suckling bout. They also found that twin lambs suckled for longer periods than single born lambs. Holst et al. (1996) stated that since viscosity and volume are inversely related, the single born lambs may have gotten a more concentrated form of colostrum and twin lambs may have been short on volume, therefore suckling for a longer period. Holst et al. (1996) concluded that twin lamb survival depends partly on colostrum viscosity and availability, which maybe affected by pre-partum nutrition of the ewe.

According to Maidment and Thomas (1995) colostral immunoglobulin levels decrease by 50% with each successive milking, making it very important that the neonate receives colostrum as early as possible in life, as the ability to absorb antibodies after the first day is much reduced. By 48 h post partum the lamb's ability to absorb immunoglobulins is no longer functioning (Campbell et al., 1977). A process occurs known as 'closure' where the absorptive epithelium of the neonatal lamb's intestine is replaced by mature epithelium that is unable to absorb immunoglobulins. Closure of the gut and the decrease in immunoglobulin levels in the milk leave the neonate in a vulnerable state for the first 48 h of life.

Colostrum also contains leukocytes (white blood cells) which can influence the immune response of the calf (Maidment and Thomas, 1995). In a study where calves were fed colostral luekocytes isolated from heifers immunized with *Mycobacterium bovis*, Perino and Rupp (1994) stated that calves receiving colostral leukocytes from immunized heifers had increased lymphocyte blastogenesis to a purified protein derivative of *Mycobacterium bovis* compared to calves that were fed colostrum from non-immunized heifers.

Immunity of the neonate begins prior to parturition. Perino and Rupp (1994) reviewed immunization of the beef cow and its effect on the neonatal calf, and according to their report, fetal immunocompetence begins during gestation with lymphocytes being in the thymus as early as d 42 of gestation. At 75 to 80 d of gestation, these fetal lymphocytes have some suboptimal response capabilities to mitogens, and by d 120 have the same response as a normal adult bovine.

Vitamin E

Reactive Oxygen Species and Vitamin E

Vitamin E is required in the body for many functions. One major function it plays is that of an antioxidant, inhibiting reactions promoted by oxygen (Chow, 1979). Vitamin E can scavenge reactive oxygen species (ROS), molecules or atoms with an unpaired electron, produced through normal metabolism, sparing oxidation of cell membranes (Horton et al., 1996). Another important function of vitamin E is being a structural component of biological membranes. Vitamin E is also involved in blood clotting, and potentially in disease resistance through protection of membranes of immune system cells through antioxidant function.

During the reduction of oxygen to water several toxic intermediates can be produced, referred to as free radicals or ROS (Coelho, 1991). The ability of antioxidants such as vitamin E to scavenge and rid the system of ROS is important in the continuation of proper functioning of many systems including the reproductive, muscular, circulatory, immune, and nervous systems (Coelho, 1991). Reactive oxygen species create a potential threat to the integrity and function of all biomolecules, particularly proteins and lipids, due to their strong oxidizing ability. Vitamin E through itself being oxidized by ROS, can relieve the system of ROS thereby sparing surrounding cells from being damaged (Coelho, 1991; Chew, 1996).

In a review of antioxidant vitamins, Chew (1996) explained vitamin E's function in the body as a reducer of harmful lipid free radicals. This antioxidant activity by vitamin E is suggested to be one possible mechanism by which vitamin E enhances the immune system (Coelho, 1991). Sheffy and Schultz (1979) postulated, after examining many vitamin E and immune response studies, that vitamin E may have it's primary effect on the immune system by antagonizing the peroxidation of arachadonic acid and limiting prostaglandin production. Moriguchi et al. (1990) stated that because vitamin E acts as an antioxidant in cellular membranes, it is capable of being a free radical scavenger by blocking peroxidation of polyunsaturated fatty acids. Nockels (1996) in a review of the importance of antioxidants, stated that many reactive oxygen molecules are produced through normal metabolism and through the phagocytic action of neutrophils and macrophages. By having adequate antioxidants such as vitamin E, these reactive oxygen molecules can be reduced in number and lessen the potential of cells and cell membranes being damaged. Scott (1980) suggested that vitamin E in cellular and subcellular membranes is the "first line of defense" against phospholipid peroxidation, which produces harmful peroxides. Scott (1980) also suggested that with vitamin E located in the cell membrane protecting organelles such as mitochondria and endoplasmic reticulum, thus ensuring normal metabolism, the body might be less stressed during immune system responses.

Requirements and Deficiency

Dietary vitamin E requirements for ruminants are not clearly defined and range from 10 to 60 IU per kg of diet (NRC, 1984; NRC, 1985; NRC, 1989). Sheep requirements vary from 18 IU per d for a 70 kilogram ewe at maintenance to around 30 IU per d for a 70 kilogram ewe in the last 4 wk of gestation (NRC, 1985). Undernourishment of vitamin E, especially in neonates, is a frequent cause of immunodeficiency and supplementing the dam prior to parturition can be a preventative measure against deficiencies. Dreizen (1979) stated undernutrition affects humoral immunity, which is responsible for the production of antibodies including all five classes of immunoglobulins. Undernutrition also greatly affects cell-mediated immunity, which is responsible for protection against viral, protozoal, and fungal infections. Kelleher (1991) stated that a number of individual micronutrients, including vitamin E, have been reported to influence immune function. McDowell et al. (1996) stated that though levels of vitamin E cross the placenta, it is of little significance, and more importantly vitamin E is concentrated in colostrum. The fact that vitamin E does not cross the placenta in appreciable amounts make neonates highly susceptible to vitamin E deficiency (McDowell et al., 1996). Kelleher (1991) concluded, after reviewing vitamin E studies both in humans and animals, that vitamin E requirements would be greater if the requirement was based on lymphocyte proliferation, or more generally immune function, than on indicators of muscle degeneration which is traditionally used to estimate vitamin E requirements. For supplementation, to prevent decreased immune responses and

general vitamin E deficiencies, McDowell et al. (1996) suggested giving cows approximately 500 IU vitamin E 2 wk prior to parturition. Nockels (1986) suggested that vitamin E at 6 to 20 times the NRC recommended concentrations would improve the immune response of animals. Kott et al. (1998) reported increased lamb survivability when ewes were supplemented with approximately 10 times the NRC recommended concentration of vitamin E.

Vitamin E is intimately associated with selenium (Se); both play the role of an antioxidant and both have the ability to offset some of the deficiencies of the other. Scott (1980) stated that Se in the enzyme glutathione peroxidase plays a secondary defense role in destroying ROS that inevitably form. According to Scott (1980) Se spares vitamin E in three major ways. First by protecting the integrity of the pancreas allowing normal vitamin E digestion to take place, second by reducing the amount of peroxides attacking the cell membranes by way of glutathione peroxidase, and third by aiding in the retention of vitamin E in the blood.

Kelley and Bendich (1996) reviewed several studies concerning vitamin E and immunologic function. Studies reviewed showed that reducing fat content in the diet of humans increased the proliferation of peripheral blood lymphocytes. In addition, lowering fat content in the diet in other studies showed increased secretion of interleukin-1, increased natural killer cell activity, and increased lymphocyte proliferation (Barone et al., 1989; Kelley et al, 1989; Kelley et al., 1992). Kelley and Bendich (1996) stated those individuals consuming high-fat diets, with low antioxidant-nutrient status (such as

vitamin E), might be susceptible to a suppressed immune response. This statement is in agreement with Sheffy and Schultz (1979) who found that dogs deficient in vitamin E had significantly suppressed immune functions when fed a diet high in polyunsaturated fatty acids (PUFAs). Kelley and Bendich (1996) found that inhibition of lymphocyte proliferation caused by fish oil supplementation (high in PUFAs) could be overcome with increased intake of vitamin E.

Sheffy and Schultz (1979) showed that vitamin E and Se deficiencies in dogs suppressed immune system function. When the dogs were supplemented, oral supplementation of vitamin E had an immunostimulatory effect, however Se supplementation did not. Suppression of the immune system was most marked in dogs fed diets high in polyunsaturated fatty acids which would increase the level of lipid peroxidation, therefore causing damage to cell membranes and enzymes. Reddy et al. (1986), using serum creatine kinase as an indicator of tissue damage, found creatine kinase levels were reduced when calves were given vitamin E orally and as an injection. Nockels (1996) concluded that the level of nutrients needed for immunoenhancement is much greater than the amounts suggested by the NRC.

Serum Vitamin E

Serum vitamin E concentrations are good indicators of vitamin E status. Njeru et al. (1994) found that lamb serum concentrations of alpha-tocopherol increased linearly with increasing levels of supplemental vitamin E. Platelet alpha-tocopherol

concentrations also increased linearly with treatment levels and were found to be more sensitive to vitamin E supplementation than serum. Similar to Njeru et al. (1994), Daniels et al. (1998) found that lambs receiving two oral doses (782 IU) of vitamin E had greater serum vitamin E than single dosed (391 IU) lambs. The single dosed lambs had greater serum vitamin E than control lambs (no supplemental vitamin E). Njeru et al. (1994) stated that deficiency levels were unknown for platelet concentrations of alpha-tocopherol at the time of this study and serum alpha-tocopherol concentrations could be used as a reliable source for vitamin E status.

Forms and Availability of Vitamin E

Route of administration of vitamin E can effect uptake and level of serum and plasma vitamin E concentrations. Hidiroglou and Karpinski (1987) examined the route of administration of supplemental vitamin E and its effect on uptake of vitamin E by sheep. Oral administration of vitamin E, via gelatin capsules, showed decreased bioavailability when compared to either intramuscular or intravenous administration. Oral administration of vitamin E showed a lag time appearing in the serum, later than the other routes of administration, presumably due to the gelatin capsule having to dissolve. Fry et al. (1996a) found that oral supplementation and aqueous solutions given intramuscularly or subcutaneously of vitamin E were generally superior to oil-based vitamin E injections, with some sheep developing subclinical vitamin E deficiency symptoms when injected with oil-based vitamin E.

Vitamin E is available commercially in many different forms and tends to differ in bioavailability. Hidiroglou et al. (1992) examined the bioavailability of several forms of vitamin E and combinations of these forms. Supplementing lambs with D-α-tocopheryl acetate plus D-α-tocopheryl polyethylene glycol succinate resulted in greater serum vitamin E concentrations than any other form or combination of forms of vitamin E. Peak concentrations of serum vitamin E were observed between 15 and 21d after administration. Hidiroglou et al. (1992) concluded that the bioavailability of vitamin E is dependent upon the form administered, with D-α-tocopheryl acetate having the highest availability.

Vitamin E's Effect on Mortality or Production

Injecting ewes with vitamin E has been shown to influence lamb performance. Williamson et al. (1995) injected pregnant ewes with vitamin E 2 wk pre-partum and again at lambing. Half of the lambs born to vitamin E supplemented ewes were also injected with vitamin E at birth. Vigor score and average daily gain were greater when ewes were injected with vitamin E. Vitamin E injections to the ewe did not affect lamb birth weight and weaning weight. There was no significant effect of vitamin E injection on lamb mortality. Williamson et al. (1995) showed that average daily gain and vigor score were improved by vitamin E injections to the ewe, but kilograms of lamb weaned per ewe was not affected. Williamson et al. (1995) concluded that it was not economically beneficial to inject ewes or lambs with vitamin E. Gentry et al. (1992)

injected ewes with vitamin E and found that although vitamin E injections did not affect colostral IgG levels, they did increase serum IgG levels in lambs from treated ewes. Lamb mortality was not affected by vitamin E treatment. However, lamb weight gain was increased and ewes treated with vitamin E weaned heavier lambs. Gentry et al. (1992), in contrast to Williamson et al. (1995), concluded that providing ewes with supplemental vitamin E via injection was beneficial.

Vitamin E injections to the lamb soon after birth have been shown to be less beneficial than injecting the ewe prior to giving birth. Williamson et al. (1996) found that a single injection of vitamin E to lambs at birth did not affect lamb vigor, weight gain, or lamb death loss. Gentry et al. (1992) concluded that vitamin E injections to the lamb were less effective than injecting the ewe in terms of improving serum IgG and lamb weight gain.

Oral supplementation of vitamin E to the ewe prior to lambing may decrease lamb mortality. Four-hundred and seventy ewes were used in the first yr of a 3-yr study by Thomas et al. (1995) to determine the influence of feeding vitamin E in late pregnancy on lamb mortality, ewe body weight, ewe condition score, and number of live lambs born per ewe. Vitamin E was supplemented at a rate of 330 IU daily to 250 ewes for approximately 20 d prepartum. The remaining 220 ewes received no supplemental vitamin E. Supplemental vitamin E had no effect on ewe BW or condition score, or on number of live lambs born per ewe lambing. Lamb mortality from birth to turnout on summer range and mortality from birth to weaning were significantly lower for ewes supplemented with vitamin E that lambed early in the lambing season. Thomas et al.

(1995) suggested that lambs born early in the lambing season were environmentally stressed due to harsh weather conditions resulting in increased lamb mortality during this period. Thomas et al. (1995) showed an approximately 50 % decrease in lamb mortality in lambs born early to vitamin E supplemented ewes (first half of lambing season) compared to lambs from unsupplemented ewes (8.6 and 15.5% mortality, respectively). In a continuation of Thomas et al.'s (1995) study, Kott et al. (1998) continued supplementing ewes approximately 30 d prior to the expected lambing during the following two lambing seasons. Preweaning lamb mortality rates were significantly decreased by vitamin E supplementation over the 3 yr (Thomas et al., 1995; Kott et al., 1998). Those lambs born to vitamin E supplemented ewes had reduced mortality rates when born in the early part of the lambing season. Consequently, those supplemented ewes lambing in the early part of the lambing season weaned 2.6 kg more lamb than non-supplemented ewes. Lamb mortality rates were not affected by vitamin E supplementation when born during the late part of the lambing season, which may have been due to improved weather conditions.

Oral supplementation directly to the lamb soon after birth has shown to benefit male lambs. Daniels et al. (1998) orally dosed twin lambs with vitamin E to determine its effect on lamb survival, lamb body weight and serum vitamin E. Lambs received two doses (782 IU) of vitamin E, a single dose (391 IU) of vitamin E, or no vitamin E (control). Male lambs receiving two doses of vitamin had lower death loss than single dose or control lambs. Treatment with vitamin E did affect 30-d and 120-d weights when

dead lambs were given a 0 for weight. Daniels et al. (1998) concluded that two oral doses of vitamin E improved survival of male lambs.

Vitamin E's Effects on Mortality/Production in Other Species

Vitamin E supplementation has shown to positively affect mortality rates and levels of production in non-ruminant species. In a study comparing high-stress levels and low-stress levels in pigs and the effect of vitamin E on the pigs (BASF, 1997), researchers reported that pigs fed vitamin E performed as well in a high-stress environment as did pigs that received no vitamin E in a low-stress environment. Pig mortality was also reduced when their dams were fed 381 $IU \cdot sow^{-1} \cdot d^{-1}$ of supplemental vitamin E compared to when the dam was fed 109 $IU \cdot sow^{-1} \cdot d^{-1}$ supplemental vitamin E. Increasing sow intake of vitamin E from 109 $IU \cdot sow^{-1} \cdot d^{-1}$ to 381 $IU \cdot sow^{-1} \cdot d^{-1}$ also improved feed efficiency. Weaned pigs growing in a stressful environment and receiving supplemental vitamin E at 100 or 200 mg/kg of diet added to the industry average of 56 mg/kg of diet performed better, in terms of average daily gain, ending weight, and feed to gain ratio, than weaned pigs grown in a stressful environment that received vitamin E at industry standard. However, when these same vitamin E supplement levels were given to pigs grown in a low stress environment, performance was not substantially affected. The most important conclusion of this study was that pigs in a stressful environment have decreased production compared to pigs in a low stress environment, however, by increasing the dietary intake of vitamin E to 100 or 200 ppm over the industry standard, the negative impact of stress can be reduced to that of a low stress environment.

Tengerdy and Nockels (1975) immunized chicks with *Escheria coli* to examine the immunological effects of vitamin A or vitamin E either separately or in combination. Chicks were immunized with *Escheria coli* at 3 wk of age and again at 6 wk of age. Mortality rates due to *Escheria coli* infection were reduced by 35% when chicks received vitamin E as a dietary supplement compared to non supplemented chicks.

Peck and Alexander (1991) studied the effect of varying levels of vitamin E and vitamin C on guinea pigs infected with *Escheria coli* and *Staphylococcus aureus*. Three levels of each vitamin were given to the guinea pigs in their diet. The levels of vitamin were: 1 x the recommended daily allowance (RDA), 3 x RDA, and 9 x RDA. Peck and Alexander (1991) found that the group receiving the 3 x RDA amount of vitamin E had a lower mortality rate due to the infections compared to the 1 x RDA and 9 x RDA groups. Vitamin C had no effect on mortality rates due to infection.

Malick et al. (1978) subjected mice to radiation to determine the effects of vitamin E on their ability to survive. Mice were exposed to 800 Rads ^{60}Co gamma radiation (a lethal amount) and given, prior to and/or after exposure, one of three diets; a vitamin E deficient diet, vitamin E supplemented diet (50 IU of vitamin E added), or a diet with recommended amounts of vitamin E and one group of mice were injected with 1.25 IU of vitamin E immediately following exposure. Malick et al. (1978) found that dietary supplementation of vitamin E before or after radiation exposure had no effect on survival, however vitamin E injections given immediately after irradiation reduced mortality due to radiation.

Supplemental Vitamin E

Vitamin E and Immune Challenge

Challenging animals with an infectious agent can be useful in detecting treatment effects on immune system responses, especially when animals develop the disease or sickness associated with the infectious agent. Reffet et al. (1988) challenged lambs with a live parainfluenza type 3 virus (PI_3) to determine the effects of supplemental selenium and vitamin E on the primary and secondary immune responses. Lambs were fed a basal diet that was low, according to NRC (1985) recommendations, in selenium and vitamin E. Half of the lambs were then assigned to receive additional selenium and/or vitamin E at rates of .2 mg/kg and 20 mg/kg of the diet, respectively. The levels of selenium and vitamin E added to the basal diet provided levels at NRC (1985) recommendations. Lambs were housed in small plastic pens in a temperature-controlled room. All lambs were immunized with PI_3. Selenium-supplemented lambs had greater glutathione peroxidase (GSH-Px) activity, which may be associated with ridding the body of tissue-damaging oxygen radicals. Vitamin E supplementation did not affect GSH-Px activity, regardless of selenium status. Vitamin E supplemented lambs had greater immunoglobulin M (IgM) levels after the secondary challenge. Selenium supplemented lambs had greater IgM levels after both the primary and secondary challenges. Immunoglobulin G (IgG) levels were not affected by selenium or vitamin E. Supplemental selenium and vitamin E enhanced the immune response of lambs to PI_3, but a combined effect was not observed. Reffet et al. (1988) concluded, with results showing

increased intakes of Se and vitamin E providing beneficial increases in immune system responses, that it may be necessary to re-evaluate intake of nutrients that may be immunostimulatory.

Stephens et al. (1979) inoculated feeder lambs with chlamydia to determine the effects of vitamin E on infection and recovery. Half of the lambs were orally dosed with a single dose of 1000 IU vitamin E at the beginning of the study. After being on an alfalfa pellet diet for 23 d, all lambs were given a high concentrate pellet providing 300 IU vitamin E/kg pellets. Prior to inoculation, lambs received a total of 2182 IU vitamin E over a 15 d period. Eleven days after inoculation with chlamydia lambs were killed and complete necropsies were performed. Stephens et al. (1979) found that vitamin E supplemented lambs returned to pre-chlamydia inoculation food intakes 3 d faster than non-supplemented lambs. Supplemented lambs had significantly greater intake and weight gain than non-supplemented lambs. In post-mortem examination of the lungs, chlamydia was isolated from 4 of the 10 non-supplemented lambs, with no chlamydia being isolated from supplemented lambs. Vitamin E supplemented lambs also had less extensive pneumonia than non-supplemented lambs suggesting that vitamin E decreased the ability of the chlamydia to infect the animal.

Watson and Petro (1982) challenged mice fed a high vitamin E diet with *Listeria monocytogenes* to measure the effects of vitamin E on immune response, corticosteroid levels, and resistance to *Listeria monocytogenes*. Watson and Petro (1982) found that 4 wk after challenging mice with *Listeria monocytogenes*, those that received the high vitamin E diet had significantly reduced numbers of *Listeria monocytogenes* cells in the

peritoneal cavity. Mice supplemented with vitamin E also had significantly greater T lymphocyte mitogenisis when their spleen cells were stimulated with PHA. Vitamin E supplemented mice also had lower serum corticosterone levels, which may explain the increased T lymphocyte activity.

Vitamin E and Antigen Specific Antibody Responses: Ruminants

Measuring cell-mediated and humoral immune system responses in animals after injecting an antigen has been used as an alternative to an immune challenge when investigating vitamin E's potential effect on the immune system. This type of investigation does not lead to clinical symptoms in the animal but does elicit an immune response by the animal to the antigen. Bonnette et al. (1990) found no effect of vitamin E on humoral and cell-mediated immune responses in weaned pigs subjected to differing environmental temperatures. Bonnette et al. (1990) stated that there was no cell mediated response because the antigen used was injected with an adjuvant, which may overshadow any benefit due to the nutrient. In addition the environmental conditions, though altered, may not have created a stressful situation.

Reddy et al. (1987) supplemented calves with varying amounts of vitamin E from birth to 24 wk of age to measure its effect on lymphocyte proliferation using concanavilin-A, pokeweed mitogen, phytohaemaglutinin, and lipopolysaccarhide as mitogens. Reddy et al. (1987) also measured and antibody titer responses to *Bovine herpes*. The overall mean lymphocyte proliferation to all mitogens were significantly

greater when calves were supplemented with vitamin E, however increases in lymphocyte proliferation were not linearly associated with increasing levels of supplemental vitamin E. Calves were immunized with a commercial *Bovine herpes* modified live virus (BHV) at 7 wk of age and again at 21 wk of age. At 24 wk of age calves supplemented with 125 IU/d vitamin E had significantly greater BHV antibody titers.

Ritacco et al. (1986) supplemented 6-mo-old lambs with vitamins A and E to determine their effects on lambs' antibody responses to antigens. Lambs were orally dosed with 3000 mg vitamin E over a 3-d period. Four days after the lambs were given vitamin E, they were immunized with 15 mg keyhole limpet hemacyanin (KLH) and 1 ml *Brucella ovis* bacterin. Non- immunized lambs were given 2 ml phosphate buffered saline injection as a control. Twenty-one days later lambs were immunized again with identical doses. Anti-KLH and anti-*Brucella ovis* titers were determined by indirect ELISA. No significant differences in anti-KLH titers were observed between treatments. Antibody titers to *Brucella ovis* were significantly greater for vitamin E lambs after the secondary response. In a side study, lambs given vitamin E in the diet had significantly greater anti-KLH titers. This was explained by the fact that lambs receiving orally dosed vitamin E received approximately 24,000 mg less vitamin E than lambs given vitamin E in the diet.

Afzal et al. (1984) vaccinated rams with several different *Brucella ovis* vaccines to examine the effect of vitamin E as a vaccine adjuvant. Rams were vaccinated with their assigned vaccine and then infected with *Brucella ovis*. Rams that were given vitamin E adjuvant vaccine had an infectivity level of 22%. Control rams, which

received a commercial vaccine, had an infectivity level of 67%. Afzal et al. (1984) also noted that rams given a vitamin E placebo (no *Brucella ovis*) had a non-specific protecting effect, which suggests that there may have been factors other than the vitamin E that influencing the efficacy of the vaccines.

Tengerdy et al. (1983) supplemented lambs with vitamin E to determine its effect in lambs vaccinated with *Clostridium perfringens* type C and D toxoids on antibody levels and a subsequent immune challenge. Lambs were fed a dry vitamin E supplement, which increased antibody titers to *Clostridium perfringens* toxoid D compared to lambs not receiving supplemental vitamin E. In addition, vitamin E was used as an adjuvant in the vaccine on a small group of lambs and those lambs showed an even more profound increase in antibody titers compared to lambs receiving no supplemental vitamin E or lambs receiving supplemental vitamin E. Lambs were challenged with an intravenous injection of toxin D. When control lambs were challenged with the toxin all but one lamb died, therefore no correlation could be made to the vitamin E-enhanced antibody titers and increased protection (Tengerdy et al., 1983).

Vitamin E and Antigen Specific Antibody Responses: Non-Ruminants

Vitamin E supplementation has shown to be beneficial in laboratory animals and in avian species. Barber et al. (1977) injected guinea pigs with vitamin E to determine its effect on antibody titer response to a vaccine. Guinea pigs were given intramuscular injections of vitamin E before and after immunization with Venezuelan Equine Encephalomyelitis (VEE). Guinea pigs receiving injections of vitamin E had

significantly greater antibody titers to VEE than those that received no vitamin E. Antibody titers to VEE were not increased when guinea pigs were given vitamin E orally.

Jackson et al. (1977) supplemented hens with varying amounts of vitamin E to study its effect on passively acquired antibodies to *Brucella abortus*, via the yolk sac, in chicks. Antigen stimulation did not differ between vitamin E supplemented and non-vitamin E supplemented hens. Antibody levels in chicks specific for *Brucella abortus* were significantly increased when their dam was fed 150 or 450 ppm vitamin E per d. When dams were fed 90, 600, or 1200 ppm vitamin E, antibody levels in chicks were not significantly increased.

Tengerdy and Nockels (1975) immunized chicks with *Escheria coli* to examine the immunological effects of vitamin A and vitamin E used in combination or separately. Chicks were immunized with *Escheria coli* at 3 wk of age and again at 6 wk of age. Tengerdy and Nockels (1975) found that vitamin E alone had some protective effect according to hemagglutination titers, with those chicks receiving supplemental vitamin E having greater *Escheria coli* antibody titer levels compared to chicks not receiving supplemental vitamin E. However, the two vitamins in combination had no effect. Vitamin E or A alone did provide a somewhat quicker recovery rate from illness associated with the *Escheria coli* infection. Mortality rates of chicks due to *Escheria coli* infection were reduced by about 35% when dams received supplemental vitamin E compared to chicks whose dam received no supplemental vitamin E.

Schildknecht and Squibb (1979) experimentally infected turkeys with *Histomonas meleagridis* to examine the effects of supplemental vitamin E on weight gain, feed

conversion, internal lesions due to infection, and mortality. Supplemental vitamin E alone did not have an effect on any of the variables measured, however when ipronidazole (a low-level anti-histomonal agent) was added to the vitamin E the turkeys showed greater weight gain, reduced lesions, and reduced mortality rates. The effectiveness of the drug ipronidazole appeared to be enhanced by the vitamin E (Schildknecht and Squibb, 1979). This type of result is similar to Tengerdy et al. (1983) who found that when vitamin E is used as an adjuvant to a vaccine the vaccine's efficacy is increased.

In vitro and specific immune cell response to supplemental vitamin E

Vitamin E has been shown to increase lymphocyte proliferation in the presence of antigens such as concanavalin-A (Con-A), phytohaemaglutinin (PHA), KLH, and pokeweed mitogen (PWM; Finch and Turner, 1989; Pollock et al., 1994). Pollock et al. (1994) measured in vitro lymphocyte proliferation in fetal calf serum, autologous serum and pooled serum from four groups of calves. In fetal calf serum, lymphocyte proliferation responses to PWM were significantly enhanced in calves supplemented with vitamin E. In autologous serum, responses to KLH were significantly greater for calves supplemented with Se. Pooled sera from each group showed that calves supplemented with Se, in the presence of serum from calves supplemented with vitamin E, displayed enhanced lymphocyte proliferation to KLH. These results suggest that vitamin E and Se have an associative effect on lymphocyte responses to antigen. Finch and Turner (1989) isolated lymphocytes from lambs on a low Se, low vitamin E diet. Lymphocytes were stimulated with PHA along with varying doses of Se and/or vitamin E. Vitamin E added

to lymphocytes significantly increased lymphocyte responses to PHA. Lambs were also supplemented with vitamin E and lymphocytes collected from these lambs. Lymphocytes from lambs supplemented with vitamin E showed increased lymphocyte responses to Con-A and PHA.

Macrophages and neutrophils are important in inflammatory responses and have shown to increase in activity and efficiency from vitamin E supplementation (Eicher et al., 1994; Politis et al., 1995). Results from Politis et al. (1995) showed that vitamin E supplementation prevented neutrophil function suppression in the early post partum period in dairy cows. Those cows receiving no supplemental vitamin E had neutrophil function suppression, depression of interleukin I, and decreased major histocompatability complex (MHC) class-II antigen expression. Cows supplemented with vitamin E showed no depression of interleukin I and MHC class-II antigen expression, which are important in stimulating immune functions (Politis et al., 1995). Because vitamin E concentration decreases at parturition (Politis et al., 1995), these results indicate that supplemental vitamin E prior to parturition may be an important tool in improving the function of blood neutrophils and macrophages. Eicher et al. (1994) supplemented isolated neutrophils and pulmonary alveolar macrophages from dairy calves with vitamin E, vitamin A, and beta-carotene. Macrophage bactericidal activity was improved with supplementation of the combination of vitamins A and E compared with supplementation of the combination of beta-carotene and vitamin E or vitamin E. Neutrophil phagocytosis improved with supplementation of vitamin A, E, and the combination of vitamins A and E. The chemotactic function of neutrophils from 3-wk-old calves had less of a response

to supplemental vitamin E than neutrophils from the same calves at 6 wk of age. From this data, Eicher et al. (1994) suggests that optimal plasma concentrations of vitamins A and E exist for luekocyte function. After supplementing rats with varying levels of vitamin E, Moriguchi et al. (1990) found vitamin E to be immunostimulatory. Rat spleen weight was significantly increased by feeding supplemental vitamin E, presumably due to the increased number of splenocytes in the spleen. Those splenocytes from vitamin E supplemented rats also responded better to mitogenic stimulation when incubated with concanavalin-A and lipopolysaccharide. The number of alveolar macrophages was also significantly increased in rats that received supplemental vitamin E and the phagocytic abilities of those alveolar macrophages were also increased.

Colostral immunoglobulin levels are good indicators of passive immunity and sufficient transfer of immunoglobulins is vital to the survival of the neonate (Sawyer et al., 1977; Perino and Rupp, 1994; Maidment and Thomas, 1995). Bohn et al. (1995) reported no effect of feeding supplemental vitamin E to ewes prior to lambing on lamb serum IgG. Lambs were given a single dose of colostrum and isolated from the dam for 24 h, to ensure lambs received the same amount of colostrum. Isolating the lamb from the dam may have resulted in "gut closure" (Halliday, 1978), which Bohn et al. (1995) stated as the reason for no effect of supplemental vitamin E to the ewe on lamb serum IgG levels. Bohn et al. (1995) found that serum vitamin E concentrations in lambs peaked at 3 d post-partum. Hayek et al. (1989) injected sows in late gestation with vitamin E and/or Se to determine the effect on immunoglobulin transfer in the colostrum. Supplemental vitamin E and/or Se did not affect colostral immunoglobulin A (IgA) and

G (IgG) levels. Colostral immunoglobulin M (IgM) concentrations were significantly greater for sows injected with Se, but not for control group (injected with a saline solution), vitamin E, and combination of vitamin E and Se treatments. Serum IgM and IgG were increased at some time (14 d, 20 d, or 28 d) post-partum for all treatments. Lacetera et al. (1996) injected dairy cows with Se and vitamin E to examine the effects of these nutrients on colostral immunoglobulin concentrations, amount of colostrum produced, and plasma glutathione peroxidase activity (indicator of Se status). There were no differences in plasma immunoglobulin levels in the cows or in colostral immunoglobulin levels, however cows receiving Se and vitamin E produced 22% more colostrum than control cows in the first 36 h after parturition. Plasma immunoglobulin levels did not differ among the calves from treated and untreated cows.

Serum or plasma immunoglobulin levels are indicators of passive immunity to the lamb through consumption of colostrum and can be used as indicators of an animal's ability to mount an immune response (Sawyer et al., 1977; Besser and Gay, 1994). Hidiroglou et al. (1995) supplemented calves with vitamin E, vitamin C, or both to evaluate the vitamins' effect on immune status. None of the treatment groups showed a significant difference in IgG and IgM concentration, but supplemented calves tended to have greater Ig concentrations than control calves. There was no significant difference in response to KLH in any of the treatment groups. Nunn et al. (1995) injected cows and their calves with vitamin E to determine the effects of vitamin E on IgG and IgM levels and incidence of scours among calves. Vitamin E injections significantly increased plasma vitamin E levels. Vitamin E injections had no effect on immunoglobulin levels or

on the incidence of scours in calves. Tengerdy et al. (1973) investigated the humoral immune responses of mice to sheep red blood cells and tetanus toxoid to determine the effects of supplemental vitamin E in mouse diets. In this study Tengerdy et al. (1973) found that vitamin E increased the antibody levels to sheep red blood cells and tetanus toxoid. The IgG responses were more pronounced than the IgM responses, indicating that vitamin E may have more of an effect on the primary immune response rather than the secondary immune response. There was also a significant increase in spleen weight in mice that received supplemental vitamin E, indicating that vitamin E may have had an effect on increasing the number of antibody producing cells rather than increasing antibody secretion of antibody producing cells. Tengerdy et al. (1973) suggested that vitamin E's antioxidant characteristics alone could not enhance the immune system. Reddy et al. (1986) orally supplemented 24-h-old dairy calves with vitamin E to determine its effect on plasma protein, packed cell volume, serum immunoglobulin levels, and lymphocyte blastogenesis. Lymphocyte stimulation indices were increased by vitamin E supplementation *in vivo* but not *in vitro*. Serum vitamin E concentrations were increased with vitamin E supplementation, but serum IgG_1 and IgG_2 levels were not affected by vitamin E supplementation. Immunoglobulin M levels were increased only when high levels (2800 mg of vitamin E given orally once per wk for 12 wk) of vitamin E were given, but IgM levels did not differ from control animals when vitamin E was given as an injection (1400 mg of vitamin E given as injection once per wk for 12 wk). Plasma protein levels were similar across treatments, indicating that all calves received an adequate and similar amount of colostrum.

Meydani et al. (1990) in a double blind, placebo-controlled study showed that supplemental vitamin E given to healthy, elderly people enhanced some immune functions. The subjects supplemented with vitamin E showed a greater immune response with Delayed Type-Hypersensitivity (an in vivo measure of cell-mediated immunity). Supplemented individuals also showed increased IL-2 formation in response to Con-A, but not to PHA or the B-cell mitogen SAC, which is in agreement with results of vitamin E supplementation to aged mice (Meydani et al., 1990). These mitogens stimulate different T-cell populations, which may suggest that the vitamin E effect is specific to particular populations of immune response cells.

Batra et al. (1992) supplemented dairy cows in the diet with 1000 IU·cow^{-1}·d^{-1} from the dry-off period to the end of the first 3 mo of lactation when the amount of vitamin E was reduced to 500 IU·cow^{-1}·d^{-1} for the remainder of the lactation period. Batra et al. (1992) found that cows receiving vitamin E had lower somatic cell counts than control cows, however the number of clinical mastitis cases was not affected by treatment. This is in contrast with Smith et al. (1984) who used 80 cows to evaluate the effect of vitamin E and Se on clinical mastitis and duration of mastitis symptoms. Smith et al. (1984) found that supplementing cows with 1000 IU·cow^{-1}·d^{-1} of vitamin E reduced the incidence of clinical mastitis by as much as 37% over unsupplemented cows. Selenium and the combination of Se and vitamin E had no effect on incidence of mastitis. The duration of clinical symptoms was reduced by 62% when cows received a combination of supplemental vitamin E and Se compared to cows that received no supplemental vitamin E or Se.

Peplowski et al. (1981) gave dietary and injectible vitamin E and/or Se to determine the effects on weanling pigs' ability to mount an immune response to sheep red blood cells (SRBCs) and the effect on post-weaning performance. There were no differences in post-weaning performance among any treatments, but pigs receiving a diet deficient in Se and vitamin E tended to have lower gains and poorer feed conversions. Supplemental Se and vitamin E improved the level of titers to SRBCs when either Se or vitamin E was included in the diet or injected, but this improvement was only significant in pigs receiving supplemental Se. Giving both nutrients further increased titers to SRBCs, suggesting an additive effect. Peplowski et al. (1981) concluded that young weanling pigs that are marginally deficient in Se and/or vitamin E have suppressed production of humoral antibodies.

Corah (1996) examined many recent vitamin E studies when considering the justification of using vitamin E in cow-calf operations. Corah (1996) stated, based on the work reviewed, that although extensive research does not exist on feeding greater levels of vitamin E to beef cows, there appears to be adequate justification in implementing a strategic vitamin E supplementation program in beef cattle herds. Research conducted in Colorado, Kansas, and Canada show increased immunoglobulin levels, decreased incidence of scours, and significantly improved post-weaning gain, respectively, in calves either receiving vitamin E directly or born to cows receiving vitamin E pre-partum. Corah (1996) concluded that for vitamin E to be effective it needs to be given 50 to 60 d pre-partum, given at a rate of 500 to 1000 $IU \cdot cow^{-1} \cdot d^{-1}$, and to include adequate levels of selenium in the diet. Finch and Turner (1996) reviewed over 100 studies concerning the

effects of vitamin E and/or selenium on the immune responses of domestic animals. In this review, Tengerdy et al. (1983), Afzal et al. (1984), and Rittacco et al. (1986) showed increased antibody responses in sheep due to vitamin E supplementation. Larsen et al. (1988b) showed increased mitogenic responses to PHA and PMW in sheep due to supplementation of selenium and vitamin E. Finch and Turner (1996) suggested that basal levels of vitamin E are important in determining the effects of supplemental vitamin E.

Parainfluenza Type 3

Parainfluenza type 3 virus has been used in previous research to induce measurable immune responses in research animals. Reffet et al. (1988) challenged lambs with a live parainfluenza virus (PI_3) to determine the effects of supplemental selenium and vitamin E on the primary and secondary immune responses and found it to be effective in causing clinical symptoms of PI_3.

Two viruses are usually associated with mild respiratory disease in sheep. The first is PI_3 virus and the second is adeno-virus. Parainfluenza type 3 is the most commonly isolated respiratory virus in sheep according to Martin (1996). Martin (1996) suggests that the risk of respiratory infections increase with intensive management and with confinement. Cutlip and Lehmkuhl (1982), in a study of PI_3 infection of lambs, stated that respiratory tract disease is an important cause of economic loss in the sheep industry and many pneumonias of sheep are caused by PI_3. After infecting lambs with

PI_3, lambs were necropsied to observe the effect of the virus on the respiratory tract. Lesion consolidation was seen in all lobes of the lungs with a slight dominance in the ventral areas of the lungs. Lambs killed at 7d post-inoculation (PID) had similar lesions to those lambs killed at 3d PID. Cutlip and Lehmkuhl (1982) concluded that transtracheally administered PI_3 caused severe bronchiolitis, alveolitis, and interstitial pneumonitis. This epithelial destruction would provide an ideal environment for growth of secondary bacterial infections in the lungs.

In review of the literature we found that vitamin E supplementation has given variable results with some positive affects on lamb production and immune function being reported. Supplementation of vitamin E to the ewe prior to parturition has given more positive results than supplementing the lamb with vitamin E just after parturition. Therefore, the objective of this study was to examine the effects of orally supplemented vitamin E to the ewe in late gestation on ewe and lamb immune indicators in a production type environment.

EXPERIMENTAL PROCEDURE

Fifty-two mature (2 to 6 y of age) Targhee twin-bearing ewes were used to investigate the role of vitamin E in immune function. The study began February 27, 1998, (Day 1) approximately 45 d prior to lambing and concluded May 11, 1998, (Day 73) approximately 30 d after lambing. However, a final body weight on ewes and lambs was taken on June 1, 1998 and used in the data analysis.

Previous Management

Ewes were maintained prior to study at the Red Bluff Research Ranch near Norris, Montana. Elevation at this ranch ranges from 1402 to 1889 m and precipitation ranges from 35.5 to 43.1 cm, annually (Soder, 1993). Vegetation type is a foothill bunchgrass range (Soder, 1993). Beginning November 15, 1997 Targhee ewes were pen-mated to Targhee rams. After 20 d in pens, ewes were put out on range and mass-mated with black-face rams for an additional 20 d.

At the end of the breeding season, ewes grazed winter range from December 20, 1997 to March 11, 1998. Ewes received .30 kg of a barley based supplement (Tables 1 and 2) on alternate days. On February 6, 1998 twin-bearing Targhee ewes were selected for study based upon estimate of fetal numbers by real-time ultrasound scanning. The first 80 ewes that were identified as bearing twins were used for the study. Due to fetal number estimate error and ewe death 52 twin-bearing ewes were used in the final analysis of data for this study. Ewes were returned to winter range until March 11, 1998. Ewes

were then moved to the Fort Ellis Research Ranch east of Bozeman, Montana where they lambed and remained until May 11, 1998.

Ewes

Ewes were randomly assigned to receive one of four treatment combinations in a 2 x 2 factorial arrangement. Treatments were 1) 400 IU orally supplemented vitamin E and parainfluenza type 3 (PI_3) vaccination 2) 400 IU orally supplemented vitamin E and no PI_3 vaccination 3) No supplemental vitamin E and PI_3 vaccination 4) No supplemental vitamin E and no PI_3 vaccination. The number of ewes per treatment was 19, 20, 18, and 20, respectively. On Day 1,while ewes were at the Red Bluff Research Ranch, blood samples were collected in non-hepranized vacutainers (9.5 ml, Fisher Scientific, Santa Clara CA) from all ewes via jugular venipuncture and sera stored for later analysis. Ewes assigned to receive the PI_3 (Fort Dodge Laboratories, Fort Dodge IA) vaccination were then vaccinated. Four wk later these same ewes received a PI_3 booster vaccination. On Day 17 ewes assigned to receive supplemental vitamin E began receiving oral supplementation of vitamin E. Ewes were confined daily at 1600 and received 1 g of Rovimix E-40% (400 IU alpha-tocopherol acetate, Hoffman-LaRoche, Nutley NJ) in a gelatin capsule (1/8 oz size, M.W.I. Veterinary Supply, Nampa ID). Vitamin E supplementation for each ewe ended when she lambed.

Colostrum was collected from ewes (approximately 3ml), within 4 h post-partum, and stored frozen. Blood was collected from all ewes within 4 h post-partum and sera

stored frozen for later analysis. Ewes were weighed (no shrink) and condition scored on Day 13 of the study and on June 1, 1998. Body condition scores were based on a scale of 1 to 5, with 1 being an emaciated ewe and 5 being an obese ewe (Russel et. al., 1969).

Ewe Diet

After grazing winter range ewes were moved to Fort Ellis where they were given ad-libitum access alfalfa/grass mix hay and received .28 $kg^{-1}ewe^{-1}d$ of barley until 2 weeks prior to the expected lambing date (Day 34) when they began receiving .45 $kg^{-1}ewe^{-1}d$ of barley (Table 1). Ewes were confined with their newborn lambs in a $1.5m^2$ pen for approximately 24 h where ewes had ad libitum access to 80% alfalfa/20% barley pellets and water (Tables 1 and 2). After leaving the $1.5m^2$ pen ewes were given ad-libitum access to second-cutting alfalfa hay and received .45 $kg^{-1}ewe^{-1}d$ of barley. Samples were collected from hay, pellets, and barley for determination of protein, fiber, vitamin E, and selenium content. Ewes had ad-libitum access to water and a trace-mineralized salt before and after lambing (Table 3).

Lambs

Immediately after lambing, lambs were brought into a shed and confined with the dam in a $1.5m^2$ pen. Lamb birth weight, birth date, sex, and vigor score were recorded. Lamb vigor score was based on the following scale: 1 = no assistance, 2 = assistance, 3 =

treat and help nurse, and 4 = dead. Sixteen to eighteen hr after birth, lambs were ear tagged and tails banded, however male lambs were not castrated. Ewes and lambs remained in the shed for approximately 24 hr. Ewes and lambs were then moved to a 10 m x 5 m mixing pen until 3 d post-partum. At 3 d post-partum blood was collected from each lamb via jugular venipuncture using non-hepranized vacutainers (9.5 ml, Fisher Scientific, Santa Clara, CA) and sera frozen for later analysis. Lambs and ewes were then moved to a 50 m x 40 m pen where they remained until Day 73. Lambs were weighed June 1, 1998.

Serum Vitamin E Analysis

All ewe and lamb sera were analyzed for vitamin E concentration by the Wyoming State Veterinary Laboratory. Serum samples were diluted 1:3 with 2% ascorbate in ethanol then extracted twice with 4 ml of petroleum ether. The petroleum ether was evaporated with nitrogen at room temperature and the sample re-dissolved in 0.5 ml methanol. The methanolic samples were then chromatographed in 3% aqueous methanol (1 ml/min) on a 3 cm x 3 cm C_{18} column with 3% aqueous methanol and quantified by fluorometry (295 excitation, 325 emission, Shimadzu RF-535).

Colostral IgG Determination

Ewe colostrum was analyzed for IgG at the Animal Science Endocrinology Laboratory at New Mexico State University. Analysis was performed using a radioimmunoassay as described by Richards et al. (in review).

Serum Parainfluenza Type 3 Analysis

All ewe and lamb sera were analyzed for parainfluenza 3 titers at the Montana State Veterinary Diagnostic Laboratory by the hema-absorption method. Dilutions at 1:3 were made by adding 0.2 ml serum to 0.4 ml Eagles MEM in a sterile metal cap tube. These dilutions were inactivated for 30 minutes at 56°C in a waterbath. After removing from the water bath a small amount of Kaolin was added to each sample. Samples were then shaken and let stand for 30 minutes at room temperature. Next samples were slowly centrifuged at 100 rpm for 10 minutes. Supernatants from each sample were poured off into a new sterile metal cap tube and diluted 4 more times. Dilutions were made by taking 0.2 ml from each sample and adding 0.4 ml Eagles MEM, mixed, and 0.2 ml taken from this, put into a new tube and 0.4 ml Eagles MEM added to this new tube, etc. This was repeated until all dilutions were completed and the last 0.2 ml of sample was discarded. Next a parainfluenza type 3 stock virus at a dilution previously determined to each set of tubes per sample, with 0.2 ml in the first tube and 0.4 ml in all other tubes. Tubes were well shaken and let stand at room temperature for 1 hr. Culture tubes were labeled and 2 ml of fresh media was added to each tube. After a 1 hr incubation period the culture tubes were inoculated with 0.2 ml of the sample dilutions and 0.2 ml of virus control dilutions. Culture tubes were then corked with silicone stoppers and incubated for 4 d. Samples were then removed from incubator, unstopped, and rinsed with1 ml of saline. Saline was poured out and 0.2 ml of a 1:200 washed red blood cells, covered with plastic wrap, and refrigerated for 30 min. Samples were observed for hema-absorption.

Serum IgG Determination

To establish the levels of IgG in test samples (sheep serum) a capture ELISA was developed using a sheep IgG standard (Jackson ImmunoResearch, West Grove PA) with a total protein concentration of 28 mg/ml. The following method was used to determine the standard curve:

A ninety-six-well microtiter plate was coated with a 1:1 mix, 50 ul per well, of monoclonal antibodies, cell lines BIg501E and BIg43A (1 mg/ml concentration in PBS, VMRD Inc., Pullman WA), at a 1:3000 dilution in PBS (pH 7.2), giving a final concentration of .0003 mg/ml. The plate was then incubated overnight (16-18 h) at 4°C. After incubation, the plate was washed 3 times for 10 min per wash with 100 ul PBS .01% Tween 20 at room temperature. After washing, the plate was blocked for 1 h at room temperature with 50 ul per well of blocking solution [.5% casein (Sigma, St. Louis MO) in PBS .01% Tween 20]. After blocking, standard samples of a known concentration were immediately added to wells at the desired dilutions. Triplicates of standard samples, 50 ul per well, were diluted in .5% casein PBS .01% Tween 20 solution beginning with a 1:400 dilution. Doubling dilutions (i.e.; 1:400 wells 1, 2, 3; 1:800 wells 4, 5, 6; etc.) were performed for 2 rows. Standard samples were incubated over night (16-18 h) at 4°C. After incubation the plate was washed 3 times. After washing, a 1:1 mix of biotinylated monoclonal antibodies BIg501E and BIg43A (1 mg/ml concentration), 50 ul per well, diluted 1:6000 in .5% casein PBS .01% Tween 20 were added to the wells. After incubation of biotinylated monoclonal antibodies for 1 h at room temperature, the

plate was washed 3 times. Avidin-horseradish peroxidase (Vector Laboratories, Burlingame CA), at 50 ul per well, was diluted 1:6000 in .5% casein and PBS .01% Tween 20 and incubated for 1 h at room temperature. After incubation, the plate was again washed 3 times. An ABTS substrate/indicator solution (Kirkegaard and Perry, Gaithersburg MD) was immediately added at 100 ul per well. The absorbency was read at A_{405} (THERMOmax microtiter plate reader; Molecular Devices, Sunnyvale CA) and data recorded using SOFTmax software (Molecular Devices, Sunnyvale CA). The amount of IgG in the standard is proportional to the color at A_{405}. Optimal color development was at 30 min following the addition of ABTS, determined by the standard reaching an OD that correlated to the known concentration of IgG in the standard.

For control setup, each component was subtracted from a triplicate set. This resulted in having three triplicate sets with one component missing in each set. A set of wells was also included that contained only the capture monoclonal antibody and ABTS.

The standard curve was plotted using logarithmic transformation of the A_{405} associated with eight doubled dilutions beginning with 1:400 and ending with 1:51200, giving a sigmoid shaped curve. It was determined that the linearity of the curve occurred between 1.3 ug/ml and 28 ug/ml IgG (A_{405} = .500 and 1.000, respectively). The standard samples were replaced with test samples. Multiple dilutions of test samples were analyzed to ensure that these absorbencies fell somewhere along the standard curve. The final dilution for test samples used in this assay was 1:1600 and 1:3200. These dilutions were used due to their placement on the curve of approximately in the middle, which