The influence of vitamin E on immune function and response to vaccination in older horses

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ABSTRACT: Horses have an increased susceptibility to infection because of a decline in immune function with advancing age. Vitamin E has been found to play a key role in normal immune system function. The purpose of the study was to examine the effect of vitamin E supplementation on immune function and response to vaccination in older horses. Predominantly older horses (18.9 ± 1.3 yr, range 7 to 26 yr; 523 ± 38 kg of BW) were supplemented orally once daily for 16 wk with either all-rac-α-tocopheryl acetate (15 IU/kg of BW; n = 8) or a placebo (n = 8). One horse from each group was removed from the study for reasons not related to the study. Serum α-tocopherol concentration, neutrophil and monocyte bacterial killing ability, lysozyme activity, immunoglobulin concentration (IgGa, IgGb, IgGT, and IgM), and neutralizing antibody production to West Nile virus vaccination were determined. The overall serum α-tocopherol concentration of the vitamin E-supplemented horses was greater than that of placebo-supplemented horses (P < 0.001). Bacterial killing capacity of monocytes and neutrophils increased in the vitamin E-supplemented horses (P < 0.05). Vitamin E-supplemented horses had greater serum IgGs (P < 0.001) and IgGf (P = 0.003) concentrations but produced less serum IgGb (P = 0.023) than placebo-supplemented horses. There was no effect of vitamin E supplementation on IgM production. The neutralizing antibody response to vaccination against West Nile virus was unaffected by vitamin E supplementation. There was a continuous increase in serum lysozyme concentration in placebo-supplemented horses, whereas serum lysozyme concentration did not increase until wk 12 in vitamin E-supplemented horses. In conclusion, vitamin E supplementation of predominantly older horses differentially modulated general cell-mediated and humoral immune function. Further research is needed to fully understand the effect of vitamin E on the immune function of horses.

Key words: aging, horse, immune response, lysozyme activity, vaccination, vitamin E

INTRODUCTION

Aged horses (≥20 yr) constitute 15% of the US horse population (Siciliano, 2002). This population of horses is of particular concern because of increased susceptibility to infection, which comes with advancing age. A decline in immune function in the aged host has been well documented (Fermaglich and Horohov, 2002; Gay et al., 2006; Aw et al., 2007). Older horses have reduced antibody responses to influenza vaccine as compared with younger horses (Goto et al., 1993; Horohov et al., 1999; Muirhead et al., 2008). Vitamin E, recognized primarily for its role as a potent antioxidant, has been found to play a key role in the normal functioning of the immune system (Meydani and Beharka, 1996; Moriguchi and Muraga, 2000). Vitamin E supplementation has been shown to repair some of the age-related changes in the immune system (Meydani et al., 1997; Serafini, 2000).

Limited research on the effect of vitamin E supplementation on the immune system of the horse has been conducted. A greater antigen-specific antibody response to novel vaccine antigens, such as equine influenza A and tetanus toxoid, was found when mature horses were supplemented with vitamin E (78 IU/kg of
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DM) compared with control horses receiving 18 IU of vitamin E/kg of DM (Baalsrud and Overnes, 1986).

To our knowledge, no research to date has examined the effect of vitamin E supplementation on general cell-mediated and humoral immune function as well as the humoral immune response to vaccination in an older population of horses. The present study examined the effect of 16 wk of vitamin E supplementation on bacterial killing, immunoglobulin production, and lysozyme activity as well as the production of antigen-specific antibody production to West Nile virus (WNV) vaccination in an older population of horses.

MATERIALS AND METHODS

This study was conducted under the approval of and compliance with the Institutional Animal Care and Use Committee of the University of Rhode Island, according to the USDA Regulations and the Guide for the Care and Use of Laboratory Animals. All animals used in the study underwent yearly veterinary exams and were considered healthy at the beginning of the study.

Study Horses

Sixteen horses, 12 geldings and 4 mares from a local farm, ranging in age from 7 to 26 yr, were used for this study. The breeds represented in this study were as follows: 5 Quarter Horses, 2 Thoroughbreds, 2 Warmbloods, and 1 each of Appaloosa, Connemara, Percheron, Percheron Cross, Pinto, Shetland pony, and Welsh pony. All horses were housed at a local facility and had similar daily schedules. The exclusion criteria for study subjects were abnormal blood chemistry, suspected bleeding disorders, and horses with any systemic illnesses or infectious diseases that compromised general health. Body weights were estimated at the beginning and end of the study using a previously established (Hall, 1971) and validated (Carroll and Huntington, 1988) formula: BW (kg) = [girth² (cm) × length (cm)]/11,880.

Experimental Design

Horses were supplemented orally once daily for 16 wk with either 15 times the NRC (2007) requirement of vitamin E (15 IU of all-rac-α-tocopheryl acetate/kg of BW; n = 8) or an equivalent weight of placebo (wheat middlings; n = 8). Horses were fed mixed grass hay during the study at an estimated daily rate of 2% of BW and commercial concentrate. Daily access to heavily stocked paddocks, primarily dirt, was the same among horses. The vitamin E supplement and placebo (DSM Nutritional Products, Parsippany, NJ) were top-dressed onto grain at the morning feeding. Horses were paired according to BW (placebo: 518 ± 54 kg; vitamin E: 527 ± 56 kg) and age (placebo: 17.4 ± 1.4 yr; vitamin E: 20.5 ± 2.2 yr) and assigned randomly to treatments. There was no difference in age or BW between groups at the beginning of the study.

Blood was collected from all study animals via jugular venipuncture at wk 0, and at the beginning of wk 2, 4, 6, 8, 10, 12, 14, and 16. The yearly vaccination of all study horses was coordinated such that all horses were vaccinated against Eastern and Western equine encephalitis and tetanus (Equiloid Innovator, Fort Dodge Animal Health, Overland Park, KS), and rabies (IMRAB, Merial Inc., Duluth, GA) at wk 10 of the study and Potomac horse fever (PotomacGuard, Fort Dodge Animal Health) and strangles (Pinnacle, Fort Dodge Animal Health) at wk 13. Horses were vaccinated at wk 7 and 14 of the study with a recombinant WNV vaccine (Recombitek, Merial Inc.). All horses had been vaccinated against WNV the previous year with an inactivated virus vaccine (West Nile Innovator, Fort Dodge Animal Health).

Serum α-tocopherol concentration was determined at wk 0, 2, 4, 8, 12, and 16. The effect of vitamin E supplementation on innate immunity was assessed through measurement of lysozyme activity in serum every 2 wk and bacterial killing of Escherichia coli by monocytes and neutrophils (PMN) at wk 6 and 16. As a measure of adaptive immunity, serum IgG (IgGa, IgGb, IgGT) and IgM were quantified every 2 wk using ELISA. West Nile virus-specific antibodies in the serum were quantified by a WNV plaque reduction assay at wk 6 (1 wk before WNV vaccination), wk 12 (6 wk after the first vaccination and 2 wk before the booster vaccination), and wk 16 (end of the study).

Sampling and Analysis

Blood (16 mL) was collected into sterile serum separator Vacutainer tubes (Becton Dickinson and Co., Franklin Lakes, NJ), centrifuged (1,150 × g at room temperature for 20 min), and the serum was transferred into cryogenic vials and frozen at −80°C until analysis. Core samples of the hay fed to the horses were collected weekly. The amount of concentrate fed to the horses was recorded and sampled weekly.

Vitamin E Analysis. Vitamin E compounds in serum and feedstuffs were determined by reverse-phase HPLC using a C18 column with acetonitrile, methylene chloride, and methanol (70:20:10) as the mobile phase. Serum samples were analyzed by the Diagnostic Center for Population and Animal Health (Michigan State University, East Lansing), with intra- and inter-assay CV of 1.5 and 1.8%, respectively. Feedstuffs were analyzed by a commercial company (DSM Nutritional Products).

Lysozyme Activity. Lysozyme activity of serum samples was measured in triplicate using a fluorescence-based assay (EnzChek Lysozyme Assay, Molecular Probes, Carlsbad, CA). Fluorescence for each sample was measured with a fluorescence microplate reader (Synergy HT, Biotek Instruments, Winooski, VT) us-
ing the excitation/emission of 465/515 nm. The intra- and interassay CV were 4.3 and 12.4%, respectively.

**Bacterial Killing Capacity.** Bacterial killing capacity of monocytes and PMN was measured using a published procedure (Rosendal et al., 1987) with modifications. Briefly, an ampicillin (Amp)-resistant *E. coli* strain was streaked onto a Luria-Bertani (LB) + Amp agar plate and incubated overnight (37°C). Three colonies from the plate were inoculated into 5 mL of LB + Amp broth and incubated at 37°C until turbidity equaled the 0.5 McFarland standard. The optical density of the bacterial broth was confirmed with a spectrophotometer. The bacterial concentration was adjusted to 2.5 × 10^6 cfu/mL using Hank’s balanced salt solution (HBSS, Sigma-Aldrich Co., St. Louis, MO).

Horse blood monocytes and PMN were isolated as follows. Blood (7 mL) collected into sterile EDTA-containing (4.45 mM) Vacutainers (Becton Dickinson and Co.) was diluted 1:1 with PBS. Diluted blood was layered on top of a PBS-diluted Percoll (Sigma Chemical Co., St. Louis, MO) gradient (55:50:45). The tubes were centrifuged at 4°C for 5 min (400 × g), followed by centrifugation for 15 min (800 × g at 4°C), and the PMN + monocyte layer was collected from the interface below the red blood cell layer, transferred to a polystyrene tube, and placed on ice. The PMN + monocyte layer collected was confirmed using a hematology stain kit and then washed 5 times in physiological saline and centrifuged at 4°C for 10 min (800 × g), and the cell pellet was collected by removing the supernatant. The PMN + monocyte cell pellet was resuspended in HBSS (0.5 mL) and kept on ice. Trypan blue (Sigma-Aldrich Co.) was added to a cell suspension aliquot and placed on a hemocytometer to determine viable cell density. The PMN + monocyte concentration was adjusted to 2.5 × 10^5 cells/mL using HBSS.

The bacterial suspension (3 mL; 2.5 × 10^6 cfu/mL) was incubated with pooled equine serum (3%, vol/vol) for 20 min in a 37°C water bath for bacterial opsonization. The opsonized bacterial suspension (50 μL) was added to the PMN + monocyte suspension. At time = 0 and time = 120 min, samples of PMN + monocyte bacteria were taken and serial dilutions of each sample (full strength, 1:10, and 1:100) were plated onto LB-Amp agar in duplicate. The plates were incubated at 37°C overnight and the number of colonies was counted and averaged the next morning. Bacterial killing was expressed as the percentage of *E. coli* colonies killed [(colonies at 0 min − colonies at 120 min)/colonies at 0 min] × 100).

**Immunoglobulin Analysis.** Immunoglobulin G subtypes (IgGa, IgGb, and IgGT) and IgM concentrations were measured in triplicate using commercially available equine-specific ELISA quantification kits (IgGα: kit E70-124; IgGβ: kit E70-127; IgGT: kit E70-105; and IgM: kit E70-114; Bethyl Laboratories Inc., Montgomery, TX) following the instructions of the manufacturer. The inter- and intraassay CV were 7.6 and 10.2%, 7.7 and 5.9%, 10.7 and 5.1%, and 9.5 and 3.6% for IgGa, IgGb, IgGT, and IgM, respectively.

**WNV Antibody Analysis.** Horse serum samples were screened for WNV neutralizing antibodies (College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins) using a plaque reduction neutralization test. Briefly, serum samples were incubated at 37°C with 100 plaque-forming units of WNV. The serum-virus mixture was added to a layer of Vero cells. The first overlay of agar was placed on the cells. After incubation for 3 d, a second overlay containing a dye was put on the culture. The plate was further incubated, and then the virus plaques were counted (Davidson et al., 2005). Endpoint titers were assigned as the greatest dilution, in which ≥90% neutralization of the challenge virus was achieved. Titers ≥5 were considered positive (Minke et al., 2004).

**Statistical Analyses**

Serum α-tocopherol, lysozyme activity, antibody concentrations (IgGa, IgGb, IgGT, and IgM), and WNV plaque reduction neutralization data were analyzed using the mixed models procedure (SAS Inst. Inc., Cary, NC) with repeated measures. The serum α-tocopherol, lysozyme activity, antibody concentrations, and WNV plaque reduction neutralization data were all normally distributed. Therefore, a natural logarithm was applied to these data to normalize the variances before statistical analysis. Each multivariable model included the terms treatment, week, and treatment × week. Models for serum α-tocopherol, lysozyme activity, IgGT, and IgGb also included a term for baseline concentration (time 0) as a covariate to control for differences at wk 0. All terms were included in the model at the beginning and then removed one by one based on the order of least significance. The appropriate error structure was assessed and applied for each outcome variable. The final model for each analysis included only statistically significant terms. Once the final model was chosen, residuals were checked for homoscedasticity, outliers, leverage cases, and normal distribution. Significance was declared at *P* ≤ 0.05. Tukey’s adjusted *P*-values were calculated for each term to adjust for multiple 2-way comparisons. Serum α-tocopherol, lysozyme activity, antibody concentrations, and WNV plaque reduction neutralization least squares means and 95% confidence intervals were back-transformed to original units of measure for data presentation. The 95% confidence intervals were used to estimate the SE for each variable in original units of measure and, as such, are an approximation of the true SE (Stellflug, 2006). Student’s *t*-test was used to statistically analyze the difference in basal dietary vitamin E consumption between treatment groups. The bacterial killing assay was also statistically analyzed using a Student’s *t*-test at wk 6 and 16. Significance was declared at *P* ≤ 0.05.
RESULTS

One horse from each group did not complete the study for reasons not related to the study. Data from these horses were excluded from the analyses. The removal of these 2 horses resulted in a difference in age between treatment groups. The final BW distribution was 526 ± 62 kg (range 264 to 780 kg) and 489 ± 48 kg (range 263 to 636 kg) for the placebo- and vitamin E-supplemented group, respectively. The final age distribution was 17.6 ± 1.6 yr (range 12 to 24 yr) for the placebo group and 22.4 ± 1.2 yr (range 18 to 26 yr) for the vitamin E-supplemented group.

Body weight did not change (P = 0.391) over the course of the study. The basal tocopherol concentration of the commercial concentrate and mixed grass hay averaged 19.3 and 20 IU/kg, respectively. Dietary vitamin E intake from the commercial concentrate and hay did not differ between groups (vitamin E: 0.48 ± 0.01 IU/kg of BW per day; placebo: 0.51 ± 0.03 IU/kg of BW per day, P = 0.429).

Least squares means for treatment (across weeks) and time (across treatments) effects are shown in Tables 1 and 2, and Figure 1, respectively. Serum α-tocopherol was greater in vitamin E-supplemented horses than placebo-supplemented horses (P < 0.001; Table 1). When the effect of time was analyzed (across treatments), there was an increase (P < 0.001) in serum α-tocopherol concentration at wk 4 compared with wk 2 (Table 2). No treatment × week interaction (P = 0.063) was found in serum α-tocopherol concentration. Bacterial killing capacity in vitamin E-supplemented (84 ± 4%) and placebo-supplemented (76 ± 6%) horses measured at wk 6 did not differ (P = 0.315). By wk 16, however, blood cells from the vitamin E-supplemented horses (91 to 99%) exhibited increased bacterial killing capacity of PMN and macrophage cells compared with those of placebo-supplemented horses (79 to 88%; P < 0.001; Table 1). There was a treatment × week effect for lysozyme concentration (P < 0.05; Figure 1); however, all differences found were within treatment (placebo, wk 4 and 8 vs. 14 and 16; placebo, wk 12 vs. 16; and vitamin E, wk 4, 8, and 12 vs. 16). There was a continuous increase in serum lysozyme concentration in placebo-supplemented horses, whereas serum lysozyme concentration did not increase until wk 12 in vitamin E-supplemented horses.

Serum IgGa samples were missing for wk 2 and were therefore unable to be included in the analysis (Table 1). Horses supplemented with vitamin E produced greater quantities of serum IgGa (P < 0.001) and IgG T (P = 0.003) compared with placebo-supplemented horses (Table 1). By contrast, vitamin E-supplemented horses produced less serum IgGb (P = 0.023) than placebo-supplemented horses. There was no effect of vitamin E supplementation on IgM production (P = 0.195). Serum IgGα, IgG T, IgM (Figure 2), and WNV (Table 2) increased (P < 0.001) over time (across treatments). No treatment × week interactions were found for any of the tested immunoglobulins.

All horses were considered positive (≥5 titers) for neutralizing antibodies to WNV at the 90% plaque reduction titer before vaccination at wk 6 (20 ± 5/titer). The production of neutralizing antibodies in response to WNV vaccination did not differ (P = 0.538) between vitamin E- and placebo-supplemented horses. When differences were considered over time (across treatments), the neutralizing antibody titer increased (P < 0.001) from the baseline. There was a decrease (P = 0.004) in neutralizing antibody titer from wk 10 to 16 (Table 2).

DISCUSSION

Immunosenescence is described as the decline in immune function caused by the aging process (Lesourd et al., 1998; Gay et al., 2006; Aw et al., 2007). Advancing age in the equine is usually associated with an overall decline in fitness and general health, leaving these horses at an increased risk for infection (Ralston et al., 1988; Austin et al., 1995; Muirhead et al., 2008). Although the age-associated dysregulation of the immune system...
The system has been intensively researched in human and rodent models, a limited number of studies have examined immunocompetence in older horses (Malinowski et al., 1997, 2006; Horohov et al., 1999, 2002; Muirhead et al., 2008; Williams et al., 2008) and, to our knowledge, no studies to date have examined nutritional modulation of the immune system of an older population of horses through vitamin E supplementation.

The current study demonstrates that dietary all-rac-α-tocopheryl acetate supplementation at 15 times the current NRC (2007) recommendation improves several important components of innate and adaptive immune function in an older population of horses. Horses supplemented with vitamin E demonstrated increased bacterial killing ability as well as increased production of IgGα and IgGβ. By contrast, the production of serum IgGγ decreased in vitamin E-supplemented horses. There was no effect of vitamin E supplementation on IgM concentration or the production of neutralizing antibodies titers to WNV. There was a treatment × week effect on lysozyme activity.

Horses were assigned to treatment group based on BW and age. Despite complete randomization of horses to treatment groups, this did not result in equal baseline measurements for many of the collected variables. To control for these differences, baseline values were included in the statistical model as a covariate for these variables. In addition to BW and age, initial samples from each potential study animal should have been analyzed before the beginning of the study in an effort to stratify animals based on their baseline measurements for the variables of interest. This was not done for the current study because all samples were analyzed after the completion of the study.

Lysozyme activity can reflect the ability of the serum to lyse potential pathogens (Firth et al., 2005). Few studies have examined the effect of vitamin E supplementation on lysozyme activity. Lysozyme activity was shown to be responsive to vitamin E supplementation in piglets and rats (Babinszky et al., 1991; Jakubowski et al., 2004). In the current study, the lysozyme activity of the vitamin E-supplemented horses differed over time as compared with the placebo-supplemented horses. However, within each week, no differences in lysozyme activity were found between the 2 treatment groups. The biological significance of the different responses over the course of the study is unknown.

Bacterial killing ability by horse PMN and monocytes was enhanced by vitamin E supplementation. Blood cells from vitamin E-supplemented horses had a 12.4% increase in killing ability in response to E. coli after 16 wk of supplementation when compared with blood cells of placebo-supplemented horses. An increase in phagocytic function of macrophages and PMN attributed to vitamin E supplementation has also been demonstrated in mice and cattle (Gebremichael et al., 1984; Hogan et al., 1990; Eicher et al., 1994).

Conflicting reports exist regarding the relationship between age and innate immunity. Some studies indicated...

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<td>WNV-1/titer</td>
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<p>| Table 2. Least squares means (±SE) of serum variables measured over time across treatments in mature horses (n = 14) supplemented for 16 wk with vitamin E (15 IU α-tocopherol/kg of BW per day; n = 7) or placebo (n = 7). |
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α–cWithin a row, means without a common superscript differ (**P < 0.01, *P < 0.05).

*Two horses were removed from the study (1 from each group) for reasons not related to the study. Variables were log-transformed and analyzed. Least squares means and confidence intervals of the serum variables were changed back to their original units after analysis. An approximate SE for original units was estimated using the 95% confidence interval. Vitamin E was obtained from DSM Nutritional Products (Parsippany, NJ).

**Baseline values were used as a covariate for α-tocopherol.

†Within a row, means without a common superscript differ (**P < 0.01, *P < 0.05).

Within a cell, means without a common superscript differ (**P < 0.01, *P < 0.05).

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cate the innate immune system may be resistant to the effect of age (Franceschi et al., 2000) and others find measureable deficits, such as decreased phagocytic capability (Butcher et al., 2001; Lord et al., 2001). This study has demonstrated that 1 component of the innate immune system, bacterial killing ability, of an older population of horses benefited from the supplementation of vitamin E. Further studies in the horse are warranted to examine the effect of vitamin E supplementation on innate immunity over a wider range of ages.

The results of this study demonstrate that certain components of the immune system in an older population of horses are modulated by vitamin E supplementation, similar to previous studies in other species of animals (Hogan et al., 1993a; Hayek et al., 1997; Kandil and Abou-Zeina, 2005). As indicators of adaptive immunity, 3 isotypes of serum IgG, namely, IgGα (IgG1), IgGβ (IgG3), and IgGγ (IgG4), as well as IgM were analyzed in the current study. All horses were vaccinated against Eastern and Western equine encephalitis, tetanus, and rabies at wk 10 and Potomac horse fever and strangles at wk 13. In addition, the production of neutralizing antibodies to WNV in response to vaccination at wk 7 and 14 was measured.

The concentration of immunoglobulins measured in the current study are consistent with established values (Tizzard, 2004). With the exception of IgGβ, all immunoglobulins measured in this study increased over time in response to vaccination. There was an increase in IgGα and IgGγ in response to vaccinations occurring in all horses at wk 10 against Eastern and Western equine encephalitis, tetanus, and rabies, and in neutralizing antibodies produced in response to vaccination against WNV at wk 7.

In the current study, vitamin E-supplemented horses produced more IgGα and IgGγ but less IgGβ than placebo-supplemented horses. No difference was found in serum IgM concentration due to vitamin E supplementation. Immunoglobulin IgGγ was initially identified in response to tetanus toxoid immunization and was later found to be largely increased in horses used for tetanus antibody production (Lunn et al., 1995; Tizzard, 2004). Vitamin E-supplemented horses in the current study produced more IgGγ antibodies than did placebo-supplemented horses. Although tetanus-specific antibodies were not measured in the current study, we can reasonably assume that a portion of the IgGγ response would be reflective of tetanus-specific antibodies. It was reported that vitamin E supplementation of 600 mg/d (approximately 1 IU/kg of BW) of α-tocopherol acetate to horses enhanced the immune response to tetanus toxoid (Baaalsrud and Overnes, 1986).

Results of other studies on the effect of vitamin E supplementation on IgG and IgM production have been varied. Vitamin E supplementation at 1 IU of vitamin E/kg of BW (vs. 15 IU/kg of BW in the present study) did not affect serum IgG production in horses but did enhance the immune response to equine influenza (Baaalsrud and Overnes, 1986). Vitamin E supplementation has been shown to increase IgG in beef cattle (Rivera et al., 2002) and dogs (Kandil and Abou-Zeina, 2005), to increase both IgG and IgM in calves (Hidiroglou et al., 1992), and to increase serum IgM in dairy cows, but to have no effect on milk IgM or IgG in the serum and milk when added as a vaccine adjuvant (Hogan et al., 1993b).

We did not observe an effect of vitamin E supplementation on the response to WNV vaccination. All horses, however, were vaccinated the previous year against WNV and titers for all study animals were >5 before vaccination. This titer is considered to be protective in horses challenged with WNV after vaccination with a canarypox virus-based recombinant vaccine (Minke et al., 2004). Although it is possible that the other vaccinations given during the study may have confounded the titer response to WNV vaccination, it seems unlikely because the majority of horses had a strong vaccination response.

Serum α-tocopherol concentrations in vitamin E-supplemented horses in this study increased 1.55-fold over those of placebo-supplemented horses. Other
equine studies report a 1.4- to 3.7-fold increase in serum α-tocopherol (Ronéus et al., 1986; Petersson et al., 1991; Higgins et al., 2008) in horses supplemented with vitamin E at concentrations ranging from 1.5 to 20 IU/kg of BW. A previous study with horses has shown muscle, liver, and adipose stores of vitamin E to increase in response to supplementation at concentrations of 1.5 to 4.4 IU/kg of BW (Ronéus et al., 1986). Because the current study provided vitamin E supplementation at a dose greatly in excess of the current recommendations, it may be possible that smaller dosages of vitamin E may modulate the immune function of older horses to a similar degree.

It must be noted that the horses in this study were consuming a diet that is considered deficient in vitamin E according to the current recommendation (NRC, 2007) of 1 IU/kg of BW per day. The basal diet provided only one-half that amount. The privately owned horses used in this study were housed and fed at a local farm before the beginning of this study. During the study, the horses continued to receive their normal diet. However, the serum α-tocopherol concentrations of both the placebo- and vitamin E-supplemented horses at baseline were considered marginal and adequate, respectively (Craig et al., 1992). Because serum α-tocopherol concentrations may or may not accurately reflect the tissue stores of α-tocopherol of the horse, care must be taken in interpreting the effect of vitamin E supplementation in this study because the results may reflect changes from a deficient state in the placebo-supplemented animals rather than a state in which maintenance vitamin E requirements were being met.

Figure 2. Serum IgG and IgM concentrations measured over time across treatments in mature horses (n = 14) supplemented for 16 wk with vitamin E (DSM Nutritional Products, Parsippany, NJ; 15 IU of d-α-tocopherol/kg of BW per day; n = 7) or placebo (n = 7). Two horses were removed from the study (1 from each group) for reasons not related to the study, resulting in n = 7/group. Serum IgG samples were missing for wk 2 and were therefore unable to be included in the analysis. Variables were log-transformed and analyzed. Baseline values were used as a covariate for IgGb and IgGT. Values are least squares means ± SE. Least squares means and confidence intervals of the serum immunoglobulins were changed back to their original units after analysis. An approximate SE for original units was estimated using the 95% confidence intervals. A pound (#) indicates vs. wk 12, \( P \leq 0.05 \); a plus (+) indicates vs. wk 14, \( P \leq 0.05 \); an asterisk (*) indicates vs. wk 16, \( P \leq 0.05 \). Arrows indicate the time of vaccinations: West Nile virus (wk 7 and 14; Recombitek, Merial Inc., Duluth, GA); Eastern and Western equine encephalitis (Equiloid Innovator, Fort Dodge Animal Health, Overland Park, KS); rabies (IMRAB, Merial Inc.); and tetanus (wk 10; Equiloid Innovator, Fort Dodge Animal Health); and Potomac horse fever (PotomacGuard, Fort Dodge Animal Health) and strangles (wk 13; Pinnacle, Fort Dodge Animal Health).
Several studies have examined the vaccination response of the geriatric horse (Goto et al., 1993; Horohov et al., 1999; Muirhead et al., 2008). One published study indicated a negative correlation between the age of the horse and the antibody response to an influenza vaccine (Goto et al., 1993). Horohov et al. (1999) found decreased lymphocyte proliferation to mitogens, as well as a decreased antibody response to vaccination against equine influenza in aged horses. Most recently, a study investigated the effect of age on the antibody response of young and old horses to vaccination against rabies and influenza (Muirhead et al., 2008), and the primary antibody response to vaccination against rabies was similar between young and old horses that had not previously been vaccinated against rabies. However, a reduction in the anamnestic antibody response to the influenza vaccine was found in the older horses. Because the primary focus of the current study was to evaluate the effect of vitamin E supplementation on immune function in an older population of horses, it is imperative that studies be conducted in the future that specifically address the effect of age on the immune response of young and old horses supplemented with vitamin E.

In conclusion, these results show a promising response of supranutritional vitamin E supplementation in several key components of immune function. Vitamin E improved bacterial killing ability in supplemented horses. Furthermore, humoral immune response was enhanced, as evidenced by increases in particular subclasses of IgG concentrations in response to vitamin E supplementation. As a result, vitamin E supplementation may be an inexpensive, beneficial way of enhancing immune systems of older horses, potentially offering protection from disease and infection. Further research on the mechanism of action of vitamin E on the immune system of the aging horse as well as studies examining the immune response to specific pathogens is necessary.

LITERATURE CITED


