Canadian Journal of Animal Science
Revue canadienne de science animale

Factors associated with serum vitamin A and E concentrations in beef calves from Alberta and Saskatchewan and the relationship between vitamin concentrations and calf health outcomes

Journal:	<i>Canadian Journal of Animal Science</i>
Manuscript ID	CJAS-2016-0055.R2
Manuscript Type:	Article
Date Submitted by the Author:	08-Jun-2016
Complete List of Authors:	Waldner, Cheryl; Western College of Veterinary Medicine, University of Saskatchewan, Large Animal Clinical Sciences Uehlinger, Fabienne; Western College of Veterinary Medicine, University of Saskatchewan, Large Animal Clinical Sciences
Keywords:	Beef, Forages, Health, Micronutrient, Vitamins/minerals

SCHOLARONE™
Manuscripts

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

Factors associated with serum vitamin A and E concentrations in beef calves from Alberta and Saskatchewan and the relationship between vitamin concentrations and calf health outcomes

Waldner, Cheryl L. *, Uehlinger, Fabienne D.

Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Dr.,
Saskatoon, SK, S7N 5B4

Address correspondence and reprint requests to:*

Cheryl Waldner, email: cheryl.waldner@usask.ca

General disclaimer: The use of trade names, proprietary produce or vendor does not imply endorsement by the authors, nor criticism of similar products not mentioned.

ABSTRACT

The study objectives were to identify factors associated with serum vitamin A and E concentrations in beef calves < 1 month old, and to examine associations between vitamin concentrations and health outcomes. Serum vitamin A concentrations were highest in: calves > 4 d old, with serum immunoglobulin G concentrations > 19 g/L, in calves from cows without perinatal health problems, and where precipitation in the previous growing season was ≥ 200 mm. Serum vitamin E was highest in: calves > 4 d old, born earlier in the calving season, not born to heifers, and that received selenium and vitamin E injections at or shortly after birth. After accounting for other risk factors, calves with serum vitamin A < 0.14 $\mu\text{g/mL}$ were 2.8 x more likely to die ($P = 0.02$), and calves with serum vitamin E less than adequate for their age (2 to 7 d old: < 0.8 $\mu\text{g/mL}$; > 7 d old: < 0.5 $\mu\text{g/mL}$) were 3.2 x more likely to be treated for enteritis than calves with higher concentrations ($P = 0.0001$). Drought conditions, dam peri-partum health problems, and inadequate colostrum intake contribute to low vitamin A and E concentrations and adverse health outcomes in beef calves.

KEYWORDS: vitamin A; vitamin E; beef calves; risk factors; health

ABBREVIATIONS: **IgG**, immunoglobulin G; **BCS**, body condition score; **RR**, relative risk, **IRR**, incidence rate ratio; **CI**, confidence interval; **SD**, standard deviation

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

Nutritional factors can dramatically affect calf health and productivity. Growth, reproduction, and the immune system all depend on adequate nutrition. Unpredictable weather conditions, such as the drought seen in many areas of western Canada in the summer of 2015, increase the challenges associated with ensuring optimal nutrition in cow-calf herds. In the summer of 2001 and 2002, western Canada also experienced drought conditions. Variations in cow body condition were associated with increased risks of pregnancy failure, abortion, dystocia, and stillbirth (Waldner and Garcia Guerra 2013; Waldner 2014a; Waldner 2014b). However, the drought conditions had an impact on productivity beyond that associated with changes in cow condition. For example, cows from regions with less than 200 mm precipitation during the 2001 growing season were more likely to have stillborn calves in spring of 2002 after accounting for differences in individual cow body condition score (Waldner 2014b).

In addition to stillbirths, the cow-calf industry also experiences losses of neonatal calves before they are moved to pasture. While diseases such as scours and pneumonia are commonly reported reasons for treatment and death loss in western Canada (Gow and Waldner 2009; Waldner et al. 2013), infectious disease does not explain all calf deaths. Postmortem examinations of calves that died in 200 herds from across western Canada in the spring of 2002 suggested noninfectious factors, including nutrition, were important causes of loss (Waldner et al. 2010). However, in contrast to abortions and stillbirths (Waldner 2014a; Waldner 2014b), poor cow body condition was not identified as an important contributor to neonatal calf mortality in these herds (Elghafghuf et al. 2014) raising questions about the role of other nutritional factors such as micronutrient deficiencies. For example, examination of trace mineral concentrations in cows at pregnancy testing identified low serum selenium concentrations as a risk factor for degenerative myopathy in the subsequent calf crop (Waldner and Van De Weyer 2011).

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

To follow up on questions regarding the role of micronutrient status in calf losses, liver samples from 221 of the calves that died from 106 of these herds were analyzed to better understand the importance of trace mineral and vitamin nutrition (Waldner and Blakley 2014). The two most commonly identified micronutrient deficiencies potentially associated with death in calves born alive were vitamin A and E. To better understand the importance of low vitamin A and E liver concentrations, serum samples were collected during calving season from neonatal calves in participating herds. The calf blood samples were also used to examine the importance of and risk factors for failure of passive transfer in this group of herds (Waldner and Rosengren 2009).

The primary objective of this study was to describe serum vitamin A and E concentrations in neonatal beef calves from Alberta and Saskatchewan and to identify risk factors associated with low serum concentrations. Specifically, our goal was to determine if calves that were born in the most extreme drought areas were more likely to have lower concentrations of serum vitamin A or E. The second objective was to investigate the relationship between serum vitamin A and E concentrations and treatment for infectious disease and mortality in neonatal beef calves. Given that individual animal treatment and mortality records were available in these herds, we also had an opportunity to describe serum concentrations in surviving calves with no history of treatment and compare values in this group of clinically normal calves with existing laboratory cut points. The third objective was to critically evaluate current definitions of adequate values in calves less than 1 mo of age.

MATERIALS AND METHODS

Herd and sample selection

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

The convenience sample of calves included in this study were from a volunteer subset of 203 producers that participated in an investigation of factors affecting productivity and health of cow-calf herds in western Canada (Waldner 2008). Private veterinary clinics across Alberta, Saskatchewan, and north-eastern British Columbia were asked to participate. Client herds were enrolled based on selection criteria that considered size (>50 cows), animal identification, calving records, and animal handling facilities. Only herds using a calving season in late winter and spring were enrolled in the study. Additional details on herd selection criteria and how the selected herds reflected the cow-calf industry in western Canada have been previously published (Waldner 2008). Project-employed veterinarians regularly visited each herd to collect samples and data, and to monitor the quality and consistency of on-farm records. All study protocols were consistent with the Canadian Council on Animal Care guidelines and were approved by the University Committee on Animal Care and Supply.

Samples were collected during a single herd visit between February and May 2002 from the first individually identified calves successfully restrained in the calving and nursery areas. The number of calf samples collected per herd was primarily determined by the number of accessible animals and was also limited by producer willingness to participate and available budget for sample collection and analysis. Blood samples were collected by jugular venipuncture using 10 ml draw Vacutainer[®] tubes. All samples were transported on ice to the Western College of Veterinary Medicine. The serum was separated immediately after arrival and stored at -80°C until analysis at a commercial laboratory (Prairie Diagnostic Laboratories, Saskatoon, SK). All samples were analyzed during the spring and summer of 2002. Only samples from calves 1 month of age or less were included in this investigation.

Radial immunodiffusion for serum antibody determination

Serum immunoglobulin G (IgG) concentrations were assessed to measure the success of passive transfer in each calf as previously described using radial immunodiffusion (Waldner and Rosengren 2009).

Serum micronutrient analysis

Retinol (Milne and Botmen 1986) and tocopherol (Catignani and Bieri 1983) concentrations were determined in serum by high pressure liquid chromatography (HPLC), as measures of vitamins A and E, respectively. Although extraction and analysis of individual vitamins was conducted separately, the procedure was identical. Briefly, 1 mL of serum was added to a 15 mL glass stoppered centrifuge tube along with 1 mL of a 1% solution of bovine serum albumin, 1.6 mL of ethanol, and 0.4 mL of internal standard. The sample was mixed using a vortex for 10 s, and a 4 mL aliquot of petroleum ether was added. The mixture was vortexed for another 45 s and centrifuged (International Refrigerated Centrifuge PR – 6, International Equipment Co., Needham, MA) at 550 x g (ca. 2500 rpm) for 5 min. The ether phase was transferred to a clean 12 x 75 mm glass tube and evaporated to dryness under air. The residue was dissolved in 500 µl filtered HPLC grade methanol, and vitamin was detected by HPLC using a 5 µm Ultrasphere™ ODS (4.6 mm x 15 cm) column (Beckman Coulter Canada Inc., Mississauga, ON) and a fluorescent detector at 325 or 285 nm for vitamins A and E, respectively. Samples and standards were protected from light at all times.

Reference concentrations for vitamin and trace mineral analysis

The reference concentrations that were used were adapted from Puls (1994). For calves from 2 to 7 d of age, 0.8 µg/mL was the minimum adequate concentration for vitamin E, while for calves greater than 7 d of age, 0.5 µg/mL was considered the minimum adequate concentration. The expected concentrations in adults are higher for vitamin E, where 3 µg/mL is considered the minimum adequate concentration in serum.

For vitamin A in calves 2 to 7 d of age, 0.2 µg/mL was considered the minimum adequate concentration and for calves greater than 7 d of age, 0.225 µg/mL was the minimum adequate concentration. The minimum adequate concentration for adults is only slightly higher at 0.25 µg/mL.

Risk factors, herd management, and reproduction data

Data were collected between 2001 Apr 1 and 2002 June 30. Participants were provided with standard forms to prospectively maintain records on animal inventory and health. Study veterinarians visited participating herds in the fall of 2001, before the calving season in 2002, and during calving season in 2002 to collect additional data on herd-level and cow-level risk factors potentially associated with nutritional and calf health status.

Study veterinarians conducted herd visits during the fall 2001 to collect pregnancy test results and score the body condition of cows using a 9-point scale (Rice 1991). Before calving season in 2002, each herd was visited again to condition score the cows, and collect data on feeding management. The third visit during the calving season was used to collect calf blood samples as well as data on risk factors for calf mortality and disease and data on post-calving feeding management. Calving records included: cow identification, date of calving, single or twin birth, calf sex, degree of assistance provided to the cow, and a description of post-calving

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

cow health problems. Owners also recorded the dates of all calf losses. Calf mortality was defined as a calf that died more than 1 h after birth and before June 30.

Records of calf treatments provided by the herd owners were to include any pharmaceutical, other than vaccinations, given to a calf for therapeutic or prophylactic indications between 1 h after birth to 2002 June 30. This cutoff date minimized data inaccuracies and incomplete records that occurred after calves were on summer pasture. The risk of treatment for any reason, diarrhea (scours/enteritis/colitis), and pneumonia was also determined based on herd owner records of reason for treatment.

Meteorological data and herd location within ecoregion

All land locations including pastures and wintering and calving grounds were plotted by quarter section (0.8 x 0.8 km, 65 ha) on an electronic map of western Canada. Information on total accumulated precipitation during the 2001 growing season was obtained from 2001 Apr 1 to Aug 31, from Drought Watch, which is maintained by Agriculture and AgriFood Canada and the herds were categorized into tertiles (<200 mm, 200 – 250 mm, >250 mm) based on existing categories established by Agriculture and AgriFood Canada. The pastures were also categorized into their appropriate ecoregions as defined by Environment Canada (Wiken 1986). Ecoregions are characterized by regional ecological factors such as vegetation, soil, climate, water, and fauna. The ecoregions included in this study were: Aspen Parkland, Boreal Transition, Fescue Grassland, Mixed Grassland, Moist Mixed Grassland, Northern Continental Divide, Peace Lowland, Western Alberta Upland and Western Boreal.

Data comparisons and statistical analysis

Calf- and herd-level factors were summarized using commercially available software (Microsoft Excel[®], Microsoft Corporation, Redmond, Washington, USA). The correlation between serum vitamin A and E concentrations was examined using Pearson's and Spearman's correlation coefficients. A *P*-value < 0.05 was considered statistically significant.

Generalized linear mixed models were used to estimate the association between potential risk factors and the outcomes of interest (Stata[®]/SE 14.0 for Windows, StataCorp LP, College Station, TX) while accounting for clustering of outcomes of interest within herd using random intercepts. Models that also included random intercepts for twin calves from the same cow were examined, but did not improve model fit based on examination of Akaike's Information Criteria and resulted in convergence failure in some cases.

Predictors of serum vitamin A and E concentrations were examined using linear mixed models. Models were adjusted for clustering by including a random intercept for each herd. Individual cow factors examined included calf gender, calf age at sampling, twin birth, history of assistance at birth, perinatal cow health problems, birth month, average ambient temperature on the calving date, body condition score (BCS) of cow at pregnancy detection and before calving, cow breed, and cow age. Herd level factors considered included: precipitation during the previous growing season, ecoregion as a proxy for geographic differences in feed sources and unmeasured local differences in herd management, use of vitamin E / selenium injections in calves, vitamin A injections to the cows, provision of commercial trace mineral or vitamin supplement to the cows, and 3 yr old stored forage used for winter feed. The proportion of variation in vitamin concentrations among herds explained by ecoregion and precipitation was calculated as the (variation among herds in the final model without the factor – variation among

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

herds with the factor in the model)/ (variation among herds in the final model without the factor in the model).

The association between serum vitamin A and E concentrations and four calf health outcomes were examined using generalized linear mixed models with a random intercept to adjust for clustering by herd, a Poisson distribution and log link function with robust variance estimation (Stata[®]/SE 14.0 for Windows, StataCorp LP, College Station, TX) (Zou 2004; Zou and Donner 2011). The associations were reported as incidence rate ratios ($IRR = \exp\beta$) for calf mortality, as only incident losses after sampling were included, or as risk ratios ($RR = \exp\beta$) for calf treatment with 95% confidence intervals (CI), as all calf treatments from birth were considered (Dohoo et al. 2009).

The health outcomes examined included: calf death, calf treatment for any reason, treatment for diarrhea, and treatment for pneumonia between birth and 2002 June 30. Time from calf birth date to either death or 2002 June 30, was included in the model as an exposure variable to account for variation across calves in time at risk of each health outcome. Calf serum vitamin concentrations were evaluated sequentially as continuous variables, categorized into quartiles, and then categorized as less than adequate or adequate for calf age based on published references (Puls 1994). Other risk factors for calf mortality and treatment considered based on causal diagrams developed from previously reported research included: cow age and breed, calf age at sampling, calf gender, calving month, and history of assistance at calving. Herd-level factors considered based on previous analysis of calf mortality in these herds included colostrum management, use of vitamin E / selenium injections in calves, the use of bedding and shelters in the calving facility, removal of calves from calving facility to nursery pasture within 48 h of

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

birth, and the purchase of foster calves (Gow and Waldner 2009; Waldner and Rosengren 2009; Elghafghuf et al. 2014).

All potential risk factors were screened based on unconditional analysis; factors with $P < 0.2$ were considered for inclusion in the final multivariable model. Continuous predictors were examined to determine if they were linearly associated with the outcome (vitamins A and E) or log of the disease outcome by adding the squared value of the continuous predictor to the unconditional model and evaluating the significance of the squared term. Manual step-wise backward selection was used to develop a main effects model, retaining only variables significant at $P < 0.05$ and, for health outcomes, serum vitamin concentrations of interest. Risk factors removed from the full model were then evaluated to see if they changed important effect estimates by more than 20%, and if so, were retained as confounders. Biologically plausible first-order interactions were considered and retained in the final model along with the main effects if $P < 0.05$ (Dohoo et al. 2009). Residuals of the final models were examined visually for outliers. Any extreme outliers were reported and no outliers were removed during the analysis. The model with the lowest Akaike's Information Criteria was chosen when more than one measure of vitamin status (continuous, quartiles, less than adequate for age) was a significant risk factor for a calf death or treatment outcome after accounting for other important risk factors.

RESULTS

Study population

Of the 899 calves for which blood samples were collected, the laboratory reported 887 vitamin A concentrations and 898 vitamin E concentrations. This represented an average of 6 calves per herd (median = 6, interquartile range = 5 to 7) from 150 herds; 110 in Alberta and 40

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

in Saskatchewan. No herds from British Columbia participated in this part of the study. Calves were born between 2002 Jan 6 and May 18, and sampled between 2002 Jan 14 and May 26. At sampling, calves ranged from less than one to 31 d of age (median = 6; interquartile range = 4 to 9). Seventy five (8.3%) of the selected calves had a twin, with 19 twin pairs included in the analysis.

The 899 calves were born to 736 cows (717 accounting for twins) and 163 heifers, which were predominantly continental breeds, British breeds, and crossbreds (Table 1). Most cows were in good (≥ 5) body condition at pregnancy testing and before calving. The proportion of cows or heifers that required assistance at calving or were diagnosed with post-calving health problems such as uterine prolapse, retained placenta or metritis were also summarized in Table 1. On the day of birth, the average ambient temperature ranged from $+13^{\circ}\text{C}$ to -32°C with a mean of -9.2°C ($s = 9.0^{\circ}\text{C}$). Serum IgG concentrations were less than 24 g/L in 37.9% (341/899) and less than 10 g/L in 9.7% (87/899) of calves.

Most calves were healthy from birth to the end of the reporting period on 2002 June 30. However, 164 (18%) calves with vitamin E results and 161 calves with vitamin A results were treated by the herd owner with therapeutic or prophylactic pharmaceuticals; 74 calves were treated for scours (enteritis/colitis) and 27 calves were treated for pneumonia. Thirteen calves were treated for more than one condition. Twenty-seven calves (3.0%) died after birth and before June 30, 2002; 16 of these had been treated prior to death.

Most producers (81%, 122/150) reported that they routinely ensured that calves received colostrum at a volume equal to 10% of their body weight within 12 h of birth, provided shelters for calves separate from cows and heifers (81%, 121/150), and moved cow-calf pairs to a nursery

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

pasture within 48 h of birth (71%, 107/150). Only 8.7% (13/150) of participants reported buying calves to replace those that died in the herd.

Approximately half of producers (56%, 84/150) injected calves with selenium and vitamin E at birth (Table 1). A smaller percentage of producers reported using injectable selenium (11%, 16/150) or vitamin A (22%, 33/150) in their cow herd. While only 17% (26/150) reported they did not provide any commercial trace mineral or vitamin supplement to their cow herd, just 21% (32/150) reported supplementing specifically with vitamin A. Other feeding practices of interest in evaluating vitamin A and E status in herds facing drought and feed shortages included feeding two year old forage (61%, 91/150), three year old forage (14%, 21/150), and feeding more straw than typically fed by the producer in past years (9%, 13/150).

Serum vitamin A and E concentrations in all calves

Serum vitamin A concentrations were classified as less than adequate for age (58.3%, 517/887) more frequently than vitamin E (12.9%, 116/898). For calves 1 wk of age or less, 60% (334/561) had less than the recommended adequate concentration of vitamin A (0.2 µg/mL), but only 15% (86/567) had less than the recommended adequate concentration of vitamin E (0.8 µg/mL). Similarly for calves between 1 wk and 1 mo of age, 56% (183/326) had less than the recommended adequate concentration of vitamin A (0.225 µg/mL), and 9% (30/331) had less than the recommended adequate concentration of vitamin E (0.5 µg/mL).

The mean concentrations of vitamin A (0.187 µg/mL, 95% CI 0.180 to 0.195) were below the recommended minimum adequate concentrations for all calves less than 1 mo of age; however, the mean concentrations of vitamin E (2.06 µg/mL, 95% CI 1.90 to 2.21) for all calves less than 1 mo of age were considered adequate.

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

There was a nonlinear, but monotonic increase in vitamin A with increasing vitamin E concentrations ($P < 0.0001$); however, the correlation between vitamin A and E was relatively weak for both individual samples ($r = 0.25$, $\rho = 0.33$; $n = 886$) and herd average concentrations ($r=0.21$, $\rho=0.32$; $n=148$).

Serum vitamin A concentrations in never treated, surviving calves compared to current diagnostic criteria

For the 461 calves < 1 wk of age with vitamin A results that were never treated and survived to the end of the follow-up period (clinically 'normal' calves), serum vitamin A concentrations averaged $0.187 \mu\text{g/mL}$ (standard deviation (SD) $0.070 \mu\text{g/mL}$) and had a median of $0.18 \mu\text{g/mL}$ (5th percentile $0.09 \mu\text{g/mL}$, 95th percentile $0.32 \mu\text{g/mL}$). The current recommended adequate serum concentration of $0.20 \mu\text{g/mL}$ for calves between 2 and 7 d of age falls at the 60th percentile of the reported values for untreated, surviving calves.

For the 254 calves with vitamin A results collected between 1 wk and 1 mo of age that were never treated and survived to the end of the follow-up period, serum vitamin A concentrations averaged $0.19 \mu\text{g/mL}$ (SD $0.059 \mu\text{g/mL}$) and had a median of $0.19 \mu\text{g/mL}$ (5th percentile $0.10 \mu\text{g/mL}$, 95th percentile $0.31 \mu\text{g/mL}$). The current recommended adequate serum concentration of $0.225 \mu\text{g/mL}$ for calves between 7 and 30 d of age falls at the 72nd percentile of the reported values for untreated, surviving calves.

Serum vitamin E concentrations in never treated, surviving calves compared to current diagnostic criteria

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

For the 466 untreated surviving calves with vitamin E results sampled at less than 1 wk of age, serum vitamin E concentrations averaged 1.97 $\mu\text{g/mL}$ (SD 1.28 $\mu\text{g/mL}$) with a median of 1.70 $\mu\text{g/mL}$ (5th percentile 0.54 $\mu\text{g/mL}$, 95th percentile 4.38 $\mu\text{g/mL}$). The current recommended adequate serum concentration of 0.8 $\mu\text{g/mL}$ for calves between 2 and 7 d of age falls at the 15th percentile of the reported values for untreated, surviving calves.

For the 257 untreated surviving calves with vitamin E results sampled between 1 wk and 1 mo of age, serum vitamin E concentrations averaged 2.25 $\mu\text{g/mL}$ (SD 1.45 $\mu\text{g/mL}$) and had a median of 2.01 $\mu\text{g/mL}$ (5th percentile 0.70 $\mu\text{g/mL}$, 95th percentile 4.77 $\mu\text{g/mL}$). The current recommended adequate serum concentration of 0.5 $\mu\text{g/mL}$ for calves between 7 and 30 d of age falls at the 2.5th percentile of the reported values for untreated, surviving calves.

Risk factors for serum vitamin A concentrations

In a series of unconditional models adjusting only for herd effects, serum vitamin A concentrations were lower ($P < 0.05$) in calves that were less than 4 d of age, where the dam was a heifer or British breed cow, or where the calf had IgG concentrations < 19 g/L. They were also lower where the calf was born into a herd that did not administer selenium with vitamin E at birth or where the herd's location received less than 200 mm of precipitation during the previous growing season. Concentrations of serum vitamin A also varied among ecoregions (Table 1).

When considered in a multivariable model, only calf age, IgG concentration, dam was a heifer, cow perinatal health problems, precipitation, and differences among ecoregions remained significant (Table 2).

Serum vitamin A was 16.9% lower in calves born in herds receiving less than 200 mm precipitation the previous growing season (0.17 $\mu\text{g/mL}$, 95% CI 0.16 to 0.18) than in herds

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

receiving more than 200 mm (0.20 µg/mL, 95% CI 0.19 to 0.21) after accounting for other risk factors including ecological region (Table 2). Precipitation explained 18.0% of the variation among herds in the final model.

Ecoregion explained 14.4% of the variation between herds remaining in the final model for serum vitamin A concentration after accounting for other risk factors. Serum vitamin A concentrations were highest in herds from the Boreal Transition ecoregion (0.20 µg/mL, 95% CI 0.18 to 0.22) and lowest in the western Boreal ecoregion (0.15 µg/mL, 95% CI 0.12 to 0.18) after accounting for other individual and herd level risk factors (Tables 2 and 3).

Risk factors for serum vitamin E concentrations

In a series of unconditional models adjusting only for herd effects, serum vitamin E concentrations were lower in calves up to 4 d of age, calves that were not twins, calves born to heifers and British breed cows, and calves born in April and May. Vitamin E concentrations were also lower in calves from herds that did not administer selenium with vitamin E at birth, and in calves from herds that received less than 200 mm precipitation the previous growing season. Concentrations of vitamin E also varied among ecoregions (Table 1).

When considered in a multivariable model, calf age, month of birth, whether the dam was a heifer, use of selenium with vitamin E at birth, and ecoregion remained significant (Table 4). Serum vitamin E concentrations were on average 28% lower in calves that did not receive selenium and vitamin E injections at birth (1.67 µg/mL, 95% CI 1.48 to 1.87) than in calves that did (2.33 µg/mL, 95% CI 2.16 to 2.51) after accounting for other risk factors.

Serum vitamin E concentrations were lowest in calves born in the western Alberta Upland (1.50 µg/mL, 95% CI 0.89 to 2.12) and highest in the Aspen Parkland (2.48 µg/mL, 95%

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

CI 2.23 to 2.73) after accounting for other risk factors (Tables 3 and 4). Ecoregion accounted for 22.1% of the variation between herds for serum vitamin E concentrations after accounting for other risk factors in the final model.

After accounting for other risk factors included in the final multivariable model (Table 4), precipitation in the previous growing season was not associated with the serum vitamin E concentrations whether ($P = 0.94$) or not ($P = 0.86$) ecoregion was included in the final model.

Association between serum vitamin A and E concentrations and calf health outcomes

Serum vitamin A concentrations in calves less than 1 mo of age were unconditionally associated with the risk of calf death after birth and before June 30 (Table 5). This association remained significant after accounting for calf age at sampling, serum IgG concentrations, month of calving, cow age at calving, cow body condition score before calving, whether the calf was assisted at birth, and if selenium with vitamin E was administered at birth (Table 6). The association between vitamin A concentrations and the log of the incidence of calf mortality was not linear. Calves with serum vitamin A concentrations in the lowest quartile were 2.8 times more likely to die during the follow up period than calves with higher vitamin A concentrations. Being classified as having less vitamin A than the currently reported age-appropriate cutoff was not associated with the risk of calf mortality (IRR 1.31, 95% CI 0.57 to 3.04, $P = 0.52$) after accounting for other risk factors.

Based on the unconditional analysis there was no association between calf death and vitamin E. There was, however, an unconditional association between low serum vitamin E concentrations in calves and the risk of enteritis (scours) (Table 5). The association between vitamin E concentrations and the log of the risk of treatment for diarrhea was not linear, but was

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

monotonic. Calves with less than adequate serum vitamin E concentrations were 3.1 times more likely to be treated for scours from birth to the end of the study period or the calf's death than calves with higher vitamin E concentrations after accounting for calf age at sampling, serum IgG concentrations, month of calving, cow age at calving, and whether the calf was assisted at birth (Table 7). To ensure this relationship was consistent when considering only disease that occurred after vitamin concentrations were measured as required to meet the temporality criteria for causal associations, this analysis was repeated considering only new cases of enteritis reported after the blood samples were collected and very similar results were obtained (IRR 2.5, 95% CI 1.2 to 5.6). Administration of selenium with vitamin E at birth was not associated with treatment for scours after accounting for vitamin E status and other risk factors (RR 0.68, 0.31 to 1.50, $P = 0.34$) listed in Table 7 or just for the other risk factors (RR 0.59, 0.27 to 1.29, $P = 0.19$).

Neither vitamin A nor vitamin E concentrations were associated with the risk of pneumonia or treatment for any reason during the follow up period either before (Table 5) or after accounting for other risk factors. Serum vitamin A concentrations were not significantly associated with the risk of pneumonia (RR per 0.1 $\mu\text{g/mL}$: 1.49, 95% CI 0.96 to 2.3, $P = 0.08$) after accounting for calf age at sampling, calving month, or cow age at calving. Similarly, less than adequate serum vitamin E concentrations for calf age were not associated with the risk of calf treatment for any reason (RR 1.44, 95% CI 0.96 to 2.16, $P = 0.08$) after adjusting for calf age at sampling, serum IgG, month of calving, and whether the calf was assisted at birth.

Finally, the risks of mortality or treatment were examined for the 10.1% (91/889) of calves that were in the lowest quartile for both vitamin A and E compared to calves that had better serum status for at least one of the vitamins. There was no association between having serum concentrations in the lowest quartile for both vitamin A and E and the risk of calf

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

treatment for pneumonia (RR 0.37, 95% CI 0.05 to 2.75, $P = 0.33$), treatment for enteritis (RR 1.63, 95% CI 0.78 to 3.41, $P = 0.19$), or treatment for any reason (RR 1.36, 95% CI 0.83 to 2.24, $P = 0.22$). Calves in the lowest quartile for both vitamin A and E were significantly more likely to die than calves that had higher serum concentrations for either one or both vitamins (RR 2.91, 95% CI 1.08 to 7.88, $P = 0.035$). This, however, was almost the same value estimated for low serum vitamin A concentration without considering vitamin E (Table 6), suggesting there was no substantial synergistic effect of also being low in vitamin E in this sample of calves.

DISCUSSION

The present study was successful in obtaining measures of serum vitamin A and E from a large sample of beef calves less than a month of age in Alberta and Saskatchewan in the midst of the 2001 and 2002 drought. All samples were collected during the late winter and spring of 2002 before the cows would have had access to green grass. More than 60% of serum vitamin A values were below the reported adequate concentrations and vitamin A was significantly lower in herds from areas receiving less than 200mm of rain in the previous growing season than in herds receiving higher levels of precipitation. In contrast, most serum vitamin E concentrations were at or above what has been reported as adequate concentrations.

Vitamin A is not present in plants. Therefore, cattle acquire vitamin A primarily through bioconversion of its precursor, β -carotene, present in forages. The intensity of green color in a plant is a good approximation of its carotene content and in a growing plant, all green parts are rich in carotene and, therefore, have a high vitamin A value (Frye et al. 1991). In contrast, yellow to orange pigmentation is an important indicator of carotenoid-rich and, therefore, vitamin A-rich food in human nutrition (Bauernfeind 1972). The amount of vitamin A in cows' colostrum and

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

milk depends on their intake during late gestation. Concentrates are a poor source of vitamin A and β -carotene, and vitamin A concentrations are higher in cattle after summer grazing periods compared to after winter feeding (Puls 1994; Nozière et al. 2006; Van de Weyer et al. 2010). This is at least in part due to the decrease in vitamin A precursor content and biological activity which occurs due to heat, light and moisture during harvesting for hay or silage, and dehydrating in preparation for storage (McDowell, 1989). Losses of more than 50% of vitamin A over the course of 1 year have occurred in stored commercial feed (Frye et al. 1991).

Primary hypovitaminosis A in pastured cattle may occur during periods of drought conditions when heat and sunlight contribute to oxidation of carotenoids thereby reducing their concentration in sun-dried pastures (Radostits et al. 2007; Hill et al. 2009). Reduced quality and quantity of forage resulting from drought conditions during the plant growing period decrease the availability of carotene for vitamin A production in the cow and, therefore, its transfer in the colostum. The finding that serum vitamin A concentrations in these calves were associated with precipitation during the previous growing season was therefore not surprising, particularly as low precipitation in the preceding growing season had previously been associated with decreases in neonatal liver vitamin A concentrations measured from a smaller group of calf death losses (Waldner and Blakley 2014).

Newborn calves must get most of their vitamin A from colostrum as they are born with very limited plasma β -carotene and retinol concentrations and little vitamin A reserves (Bondi 1987; Blum et al. 1997; Puvogel et al. 2008). Low vitamin A intake in the cow during pregnancy increases the risk of hypovitaminosis A in the calf. Calves are also born agammaglobulinemic and must rely on colostrum intake and absorption to acquire passive immunity (Weaver et al. 2000). Immunoglobulin G is the most abundant immunoglobulin in cow colostrum and

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

measurement of serum concentration of IgG in calves is a measure of the adequacy of transfer of passive immunity. In dairy calves, a serum IgG concentration of 10 g/L is generally considered as evidence of adequate passive transfer of immunity while in beef calves, a concentration of ≥ 24 g/L was associated with improved health and performance (Tyler et al. 1996; Dewell et al. 2006; Waldner and Rosengren 2009). Intake of decreased quality or quantity of colostrum may result in deficient serum concentrations of vitamin A, reflected in this study by the significantly lower vitamin A concentrations in the 25% of calves with IgG < 19 g/L. Furthermore, plasma β -carotene and retinol concentrations in calves with delayed colostrum administration are reduced, possibly because of reduced intestinal absorptive capacity or changed post-absorptive repartitioning (Blum et al. 1997; Zanker et al. 2000).

Vitamin A is essential for normal growth, differentiation of epithelial tissues, reproduction and adequate immune function. A lack of vitamin A can result in decreased disease resistance and increased mortality, particularly in growing animals (Frye et al. 1991; Puvogel et al. 2005; Moosavian et al. 2010). Increased susceptibility to bacterial infections, particularly mastitis and pneumonia, reproductive failure and post-partum diseases such as retained placenta and metritis have been associated with hypovitaminosis A (Chew et al. 1982; Frye et al. 1991; Michal et al. 1994; He et al. 2012). In this study, calves born to cows that experienced post-calving health problems such as uterine prolapse, retained placenta and metritis had a significantly lower vitamin A concentration compared to calves born to cows without reported post-partum health concerns. A number of reasons could have contributed to this result. The transition period puts cows at an increased risk of metabolic and infectious diseases due to peripartum immunosuppression (LeBlanc et al. 2004). Vitamin A (and E) concentrations decrease in cows around calving (Goff and Horst 1997). This may be compounded further by

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

reduced body vitamin A stores resulting from winter feeding and poor-quality forage and the post-calving health problems in these cows may have been at least in part due to vitamin A deficiency. Second, as previously described, a lack of vitamin A in the cow may result in low concentrations of vitamin A in the colostrum and milk. Reduced production of colostrum/milk is common in sick cows and may be caused by anorexia, decreased water intake, and pain. Increased recumbency in sick cows may also prevent calves from ingesting adequate amounts of colostrum or milk. In combination, these factors may have contributed to the significantly lower vitamin A concentration in calves from dams with post-partum health problems compared to dams without reported post-calving concerns and this finding highlights the importance of adequate vitamin A supplementation to pregnant beef cows.

Calves with vitamin A concentrations in the lowest quartile were 2.8 times more likely to die during the first month of life compared to calves with higher vitamin A concentrations. This association likely reflects the role that vitamin A plays to normal growth, development and metabolism. Hypovitaminosis A in growing calves has been associated with weakness, ataxia, blindness, xerophthalmia, ocular and cardiac deformities, inability to suckle normally, respiratory diseases and diarrhea, as well as convulsions and syncope (Jones et al. 1962; Divers et al. 1986; Mason et al. 2003; Millemann et al. 2007; Hill et al. 2009). In the present study, there was no association between treatment for enteritis or pneumonia and vitamin A concentrations in calves. While some literature links diarrhea and respiratory disease in calves to vitamin A deficiency, the evidence supporting these associations is lacking and few convincing studies are available (Jones et al. 1962; Frye et al. 1991). Most authors attribute a general increase in disease susceptibility to a decrease in immune function due to hypovitaminosis A (Bondi 1987; Chew 1987; McDowell 1989). Furthermore, the association between vitamin A concentrations and

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

enteritis or pneumonia in this study here depended on the quality of treatment records kept by producers. Gow and Waldner (2009) have previously reported that many beef producers in western Canada maintain less than satisfactory calf treatment records; inaccurate recording of calf treatments for enteritis or pneumonia may limited the power to detect associations with vitamin concentrations in this study.

Both vitamin A and E concentrations were lower in calves from heifers than in calves born to cows. Although heifers may have lower volume and quality of colostrum, this relationship is not immediately intuitive and in fact, a number of studies have demonstrated that the serum vitamin A, E and β -carotene concentrations in older cows were lower compared to younger cows (Katsoulos et al. 2005; van de Weyer et al. 2010). Similarly, calves from heifers had higher liver vitamin A concentrations compared to calves from cows in a study on micronutrient concentrations in beef calves that had died (Waldner and Blakley 2014). Due to the generally poorer mothering ability of heifers, calves born to heifers might have ingested colostrum later, ingested less colostrum or tended to absorb less colostrum compared to calves born to cows. This does not, however, appear to explain the observed differences. While serum vitamin A concentrations were associated with serum IgG in the present study, serum vitamin E concentrations were not. The difference between heifers and cows persisted whether or not the estimate was adjusted for serum IgG concentrations. There was also no associations between reported dystocia in this study and either vitamin A or E concentrations.

Forage type is considered to be the primary factor affecting β -carotene concentration in milk while parity and production level seem to have only a limited effect (Calderon et al. 2007; Cozma et al. 2014). It is, therefore, possible that feeding management differed between heifers and cows with heifers receiving feed of poorer quality compared to the cows. However, reported

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

herd supplementation practices were not associated with serum vitamin A or E concentrations and did not explain the differences between heifers and cows. The vitamin requirements of heifers may be greater compared to (non-lactating) cows due to growth and it is possible that despite identical feeding practices, heifers had reduced vitamin stores compared to cows.

While the level of precipitation was a significant predictor of vitamin A concentrations and explained more of the variation between herds than ecoregion, it did not remain significant in the multivariable models for vitamin E. However, ecoregion was a significant predictor of the concentrations of both vitamins. It is likely that unmeasured differences in soil type, hours of sunshine, plant species or other differences across geographic areas accounted for the differences in vitamin A and E concentrations seen in these calves. There could also be local unmeasured differences in herd management that might explain the spatial variations in vitamin A and E concentrations attributed to ecoregion. The areas with the highest and lowest vitamin A concentrations were not the same areas with the highest and lowest vitamin E concentrations. This suggests that there was no one underlying unmeasured climate or vegetation factor that had a consistent effect on these two fat soluble vitamins.

Similar to vitamin A, green grass is one of the most important sources of vitamin E for cattle. Although vitamin E tends to be less susceptible to destruction in stored feed (including premixes) compared to vitamin A, it will deplete over time and is sensitive to heat and moisture (Frye et al. 1991; Shurson et al. 2010). Most un-supplemented rations consisting of stored forages and cereals or processed feed are considered poor sources of vitamin E (McDowell 1989; Frye et al. 1991). Stage of maturity of grass at harvesting for hay, and the time from cutting to dehydration affect vitamin E concentration in forage with losses of up to 50% in forage stored for 1 month and up to 60% within 4 days during drying in the swath (McDowell 1989). In alfalfa

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

stored for 12 weeks, up to 73% of vitamin E losses were observed and ensiling of moist grain caused almost complete loss of vitamin E (McDowell 1989). In premixes, losses of 2% after 6 months have been reported and losses following processing may range from 10 to 15% (Charlton and Ewing 2007).

Also, in contrast to vitamin A, which is stored to 90 % in the liver and can serve as a vitamin A source to the animal for a long period of time, the liver is not a major storage site for vitamin E (McDowell 1989; Frye et al. 1991). Adipose tissues and muscles also store a significant amount of the total body vitamin E and body stores are generally more limited (Bondi 1987; Charlton and Ewing 2007). Although specific time frames for the duration of vitamin E persistence in the body have not been reported, small amounts of vitamin E can persist for a long time. The rate at which stores are depleted varies depending polyunsaturated fatty acids in feedstuffs and other factors increasing the need for antioxidants (McDowell 1989; Frye et al. 1991).

This might explain the lack of apparent association between the drought and vitamin E concentrations as compared to vitamin A. An association with the previous growing season would not be expected if vitamin E consumed during the growing season was not stored by the cow for prolonged periods. Vitamin E concentrations were lower in calves born later in the calving season, but vitamin A concentrations were not. As with vitamin A, the newborn calf depends largely on colostrum as a source of vitamin E at birth as placental transfer is minimal (Frye et al. 1991). Calves born in April and May most likely had lower vitamin E concentration compared to calves born earlier in the year because of reduced vitamin E concentration in dams fed forage stored for a longer period.

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

Vitamin E is an essential antioxidant that enhances neutrophil function by protecting them from oxidative damage and it has immunostimulatory functions (Krueger et al. 2014; Reddy et al. 1987). It is further essential for the integrity and optimum function of the reproductive, muscular, circulatory and nervous systems. Nutritional myodegeneration is probably the most recognized condition associated with vitamin E deficiency (Frye et al. 1991).

Vitamin E and selenium work synergistically as antioxidants to decrease free radicals that accumulate from physiologic metabolism. Therefore, it is not surprising that calves which received selenium and vitamin E at birth had on average 28% higher concentrations of vitamin E compared to calves that did not receive such supplementation. Other studies have demonstrated similar outcomes and supplementation is a principal preventive measure for white muscle disease in young calves (Radostits et al. 2007; Krueger et al. 2014).

The amount of vitamin E contained in the currently available commercial injectable products containing both selenium and vitamin E in Canada are, however, limited and these injections alone should not be relied on to address vitamin E deficiency (Maas and Valberg 2009). The product label recommended dose of Mu-Se[®] (Merck Animal Health, Intervet Canada Corp., Kirkland, QC) for a 45 kg calf contains 34 IU of vitamin E and the recommended doses of Dystosel[®] (Zoetis Canada Inc., Kirkland, QC) or Selon-E[®] (Vétoquinol N.-A. Inc., Lavaltrie, QC) contain 136 IU. There are, however, injectable vitamin E formulations without selenium licensed for use in cattle in the United States that have much higher concentrations. One product, Vitamin E-300 Injection[®] (VET ONE, MWI Veterinary Supply, Boise, ID) provides 1200 to 1600 IU of vitamin E to a newborn calf at the recommended dose.

Calves with low serum vitamin E concentrations had an increased risk of enteritis in the present study. This is in accordance with other studies, where calves with scours had lower

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

vitamin E concentrations or were not supplemented with vitamin E compared to calves that did not experience enteritis (Radostits et al. 1992; Krueger et al. 2014). As supported by the association between selenium and vitamin E injections and lower calf mortality in the current analysis accounting for the impact of serum vitamin A concentrations, the odds of death and treatment for any reason had also previously been reported to be higher in beef herds in Alberta and Saskatchewan which did not routinely administer selenium and vitamin E to calves or supplement the dams (Waldner and Rosengren 2009). Although injection with selenium and vitamin E was not associated with the risk of enteritis in the present study, these findings highlight the potential importance of vitamin E in the health of newborn calves.

For this study, age-specific reference values used were adapted from Puls (1994). The age categories of 2 to 7 d and 7 d to 1 mo recognize the importance of colostrum intake and absorption during the first few days of life. However, there are no clear guidelines on what constitutes a deficient concentration of vitamin A and E in calves during the first month of life, particularly for beef calves under western Canadian conditions. Others have tried to address this important missing set of data and concluded that vitamin E concentrations in beef calves at 1 mo of age may range from 1.8 to 4.19 $\mu\text{g/mL}$ and that calves from dams on dry feed may have concentrations below 2 $\mu\text{g/mL}$ (Maas et al. 2008). The concentration of both vitamins in calves depends on the amount and quality of colostrum ingested and pre-colostral assessment of vitamin status is not reliable. It is interesting that all but 13% of the calves in this study, regardless of whether they survived or were treated by the producer for health problems, had vitamin E concentrations at or above the reported reference range while more than 58% had vitamin A concentrations below the reported adequate age-specific reference range. However, similar values were also reported (12% and 57%) for those calves that survived to the end of the study

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

period and were never treated by the producer. These results may highlight the inadequacy of the existing reference ranges, particularly for vitamin A in this population of calves. The concentration at which vitamin A was associated with calf mortality was below the current lower boundary for the reference range.

It is important to consider that plasma concentrations of vitamin A may not provide an accurate indication of current vitamin A status. Liver biopsy samples have been reported to be more accurate (Hammell et al. 2000), but are not typically feasible in field situations or for large scale epidemiologic studies. In a dose-response assay in newborn dairy calves, Hammell et al. (2000) demonstrated that although plasma concentrations of vitamin A increased in supplemented calves, treated calves were classified as deficient based on the cutoff values used in that study whereas liver biopsy samples indicated adequate concentrations. This delay in equilibrating between liver and serum could be another possible explanation why younger calves (< 4 d) in this study had lower vitamin A concentrations compared to older calves. Younger calves also had lower vitamin E concentrations; however, vitamin E concentrations appeared to continue increasing up to 6 d of age.

In conclusion, results of this study emphasize the importance of adequate vitamin A and E concentrations to the health and well-being of beef calves and the detrimental effects that drought conditions may have on forage quality and subsequently on calf health. Drought conditions are projected to become more frequent in the future (Burke et al. 2006; Smith et al. 2007; World Meteorological Organization 2016) and beef producers in western Canada should be aware of the effects of drought, poor quality forage and winter feeding on the potential for vitamin deficiencies in their calf crop.

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

To reduce mortality and the need for treatment of common calf conditions, such as enteritis, due to low vitamin A and E concentrations, a number of recommendations can be made based on the findings of this study. Producers should consider supplementing their cows and particularly heifers in spring calving herds, particularly following drought years, because vitamins will have depleted in stored forage and liver vitamin A stores may be lower in cows and heifers that grazed pastures in drought conditions during the previous growing season. Herd health strategies aimed at preventing post-partum health problems in cows will further help reduce adverse health outcomes in calves due to low vitamin A concentrations. Injection of calves with selenium and vitamin E at birth is recommended, especially where selenium in feed is known to be deficient. Producers should have a colostrum management protocol in place to ensure that newborn calves receive adequate amounts of good quality colostrum in a timely manner.

Finally, this study highlights that the reference ranges currently used to determine adequate serum vitamin A concentrations in beef calves < 1 mo of age were not associated with disease outcomes or death loss in beef calf population in western Canada. These findings indicate the need to reevaluate the reference range for adequate serum vitamin A concentrations in this population.

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

ACKNOWLEDGEMENTS

The data in this paper were collected as part of the field research activities for the Western Canada Study of the Animal Health Effects Associated with Exposure to Emissions from Oil and Natural Gas Field Facilities. Funding support was provided through the Western Interprovincial Scientific Studies Association (WISSA). Additional support for laboratory analysis was provided by the Saskatchewan Cattle Marketing Deductions Fund and the Saskatchewan Horned Cattle Fund.

LITERATURE CITED

Agriculture and Agrifood Canada. Drought watch [online] Available: www.agr.gc.ca/drought
[Accessed 01Oct2014].

Bauernfeind, J. C. 1972. Carotenoid vitamin A precursors and analogs in foods and feeds. *J. Agr. Food Chem.* **20**: 455-473.

Blum, J. W., Hadorn, U., Sallmann, H. P. and Schuep, W. 1997. Delaying colostrum intake by one day impairs plasma lipid, essential fatty acid, carotene, retinol and alpha-tocopherol status in neonatal calves. *J. Nutr.* **127**: 2024-2029.

Bondi, A. A. 1987. Animal nutrition. J. Wiley and Sons Ltd., Chichester, UK.

Burke, E. J., Brown, S. J. and Christidis, N. 2006. Modeling the recent evolution of global drought and projections for the twenty-first century with the Hadley Centre Climate Model. *J Hydrometeor.* **7**: 1113-1125.

Calderon, F., Chauveau-Duriot, B., Pradel, P., Martin, B., Graulet, B., Doreau, M. and Nozière, P. 2007. Variations in carotenoids, vitamins A and E, and color in cow's plasma and milk following a shift from hay diet to diets containing increasing levels of carotenoids and vitamin E. *J. Dairy Sci.* **90**: 5651-5664.

Catignani, G. L. and Bieri, J. G. 1983. Simultaneous determination of retinol and alpha-tocopherol in serum or plasma by liquid chromatography. *Clin. Chem.* **29**: 708-712.

Charlton, S. J. and Ewing, W. N. 2007. 2nd ed. The vitamins directory. Context Products Ltd. Ashby de la Zouch, ENG.

Chew, B. P., Hollen, L. L., Hillers, J. K. and Herlugson, M. L. 1982. Relationship between vitamin A and beta-carotene in blood plasma and milk and mastitis in Holsteins. *J. Dairy Sci.* **65**: 2111-2118.

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

- Chew, B. P. 1987.** Vitamin A and β -carotene on host defense. *J. Dairy Sci.* **70**: 2732-2743.
- Cozma A., Miere, D., Filip, L., Banc, R., Stanciu, O., Andrei, S. and Loghin F. 2014.** Factors influencing the concentration of certain liposoluble components in cow and goat milk: a review. *Not. Sci. Biol.* **6**: 267-272.
- Dewell, R. D, Hungerford, L. L., Keen, J. E., Laegreid, W. W., Griffin, D., Rupp, G. P. and Grotelueschen, D. M. 2006.** Association of neonatal serum immunoglobulin G1 concentration with health and performance in beef calves. *J. Am. Vet. Med. Assoc.* **228**: 914-921.
- Divers, T. J., Blackmon, D. M., Martin, C. L. and Worrell, D. E. 1986.** Blindness and convulsions associated with vitamin A deficiency in feedlot steers. *J. Am. Vet. Med. Assoc.* **189**: 1579-1582.
- Dohoo, I., Martin, W. and Stryhn, H. 2009.** *Veterinary Epidemiologic Research*. 2nd ed. VER Inc., Charlottetown, PE.
- Elghafghuf, A., Stryhn, H., and Waldner, C. 2014.** A cross-classified and multiple membership Cox model applied to calf mortality data. *Prev. Vet. Med.* **115**: 29-38.
- Frye, T. M., Williams, S. N. and Graham, T. W. 1991.** Vitamin deficiencies in cattle. *Vet. Clin. North Am. Food Anim. Pract.* **7**: 217-275.
- Goff, J. P. and Horst, R.L. 1997.** Physiological changes at parturition and their relationship to metabolic disorders. *J. Dairy Sci.* **80**: 1260-1268.
- Gow, S. P. and Waldner, C. L. 2009.** Antimicrobial drug use and reason for treatment in 203 western Canadian cow-calf herds during calving season. *Prev. Vet. Med.* **90**: 55-65.

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

- Hammell, D. C., Franklin, S. T. and Nonnecke, B. J. 2000.** Use of the relative dose response (RDR) assay to determine vitamin A status of calves at birth and four weeks of age. *J. Dairy Sci.* **83**: 1256-1263.
- He, X., Li, Y., Li, M., Jia, G., Dong, H., Zhang, Y., He, C., Wang, C., Deng, L. and Yang, Y. 2012.** Hypovitaminosis A coupled to secondary bacterial infection in beef cattle. *BMC Vet. Res.* **8**: 222.
- Hill, B., Holroyd, R. and Sullivan, M. 2009.** Clinical and pathological findings associated with congenital hypovitaminosis A in extensively grazed beef cattle. *Aust. Vet. J.* **87**: 94-98.
- Jones, T. J., Hough, R. M. and Gerard, W. T. 1962.** Vitamin A Studies in the Dairy Calf. *Can. Vet. J.* **3**: 248-256.
- Katsoulos, P. D., Roubies, N., Panousis, N., Karatzanos, P. and Karatzias, H. 2005.** Long-term fluctuations and effect of age on serum concentrations of certain fat-soluble vitamins in dairy cows. *Vet. Clin. Pathol.* **34**: 362-367.
- Krueger, L. A., Beitz, D. C., Onda, K., Osman, M., O'Neil, M. R., Lei, S., Wattoo, F. H., Stuart, R. L., Tyler, H. D. and Nonnecke, B. 2014.** Effects of d-alpha-tocopherol and dietary energy on growth and health of preruminant dairy calves. *J. Dairy Sci.* **97**: 3715-3727.
- LeBlanc, S. J., Herdt, T. H., Seymour, W. M., Duffield, T. F. and Leslie, K.E. 2004.** Peripartum serum vitamin E, retinol, and beta-carotene in dairy cattle and their associations with disease. *J. Dairy Sci.* **87**: 609-619.
- Nozière, P., Graulet, B., Lucas, A., Martin, B., Grolier, P. and Doreau, M. 2006.** Carotenoids for ruminants: from forages to dairy products. *Anim. Sci. Feed Technol.* **131**: 418-450.

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

- Maas, J., Hoar, B. R., Myers, D. M., Tindall, J. and Puschner, B. 2008.** Vitamin E and selenium concentrations in month-old beef calves. *J. Vet. Diagn. Invest.* **20**: 86-89.
- Maas, J. and Valberg, S. J. 2009.** Nutritional and toxic rhabdomyolysis. Pages 1405 - 1408 *in* B. P. Smith, ed. Large Animal Internal Medicine. Mosby Elsevier, St. Louis, MO.
- Mason, C. S., Buxton, D. and Gartside, J. F. 2003.** Congenital ocular abnormalities in calves associated with maternal hypovitaminosis A. *Vet. Rec.* **153**: 213-214.
- McDowell, L. R. 1989.** Vitamins in animal nutrition. Cunha, T. J. (ed). Academic Press Inc. San Diego, CA.
- Michal, J. J., Heirman, L. R., Wong, T. S., Chew, B. P., Frigg, M. and Volker, L. 1994.** Modulatory effects of dietary beta-carotene on blood and mammary leukocyte function in periparturient dairy cows. *J. Dairy Sci.* **77**: 1408-1421.
- Millemann, Y., Benoit-Valiergue, H., Bonnin, J. P., Fontaine, J. J. and Maillard, R. 2007.** Ocular and cardiac malformations associated with maternal hypovitaminosis A in cattle. *Vet. Rec.* **160**: 441-443.
- Milne, D. B. and Botmen J. 1986.** Retinol, alpha tocopherol, lycopene and alpha- and beta-carotene simultaneously determined in plasma by isocratic liquid chromatography. *Clin. Chem.* **32**: 874-876.
- Moosavian, H. R., Mohri, M. and Seifi, H. A. 2010.** Effects of parenteral over-supplementation of vitamin A and iron on hematology, iron biochemistry, weight gain, and health of neonatal dairy calves. *Food Chem. Toxicol.* **48**: 1316-1320.
- Puls, R. 1994.** Vitamin levels in animal health. 2nd ed. Sherpa International, Clearbrook, BC.
- Puvogel, G., Baumrucker, C. R., Sauerwein, H., Ruhl, R., Ontsouka, E., Hammon, H. M. and Blum, J. W. 2005.** Effects of an enhanced vitamin A intake during the dry period on

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

retinoids, lactoferrin, IGF system, mammary gland epithelial cell apoptosis, and subsequent lactation in dairy cows. *J. Dairy Sci.* **88**: 1785-1800.

Puvogel, G., Baumrucker, C., Blum, J. W. 2008. Plasma vitamin A status in calves fed colostrum from cows that were fed vitamin A during late pregnancy. *J. Anim. Physiol. Anim. Nutr.* **92**: 614-620.

Reddy, P. G., Morrill, J. L., Minocha, H. C. and Stevenson, J. S. 1987. Vitamin E is immunostimulatory in calves. *J. Dairy Sci.* **70**: 993-999.

Radostits, R. M., Booker, C. W, and Shepherd, G. 1992. Fatal, non-responsive diarrhea in beef calves 6 to 8 weeks of age. Proceedings of the 24th annual conference of the American Association of Bovine Practitioners.

Radostits, O. M., Gay, C. C., Hinchcliff, K. W. and Contable, P. D. 2007. Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats and horses. 10th ed. Saunders Elsevier, Philadelphia, PA.

Rice, L. E. 1991. The effects of nutrition on reproductive performance of beef cattle. *Vet. Clin. North Am. Food Anim. Pract.* **7**: 1-26.

Shurson, G. C., Salzer, T. M., Koehler, D. D. and Whitney, M. H. 2010. Effect of metal specific amino acid complexes and inorganic trace minerals on vitamin stability in premixes. *Anim. Feed Sci. Technol.* **163**: 200-206.

Smith, D. M., Cusack, S., Colman, A. W., Folland, C. K., Harris, G. R. and Murphy, J. M. 2007. Improved surface temperature prediction for the coming decade from a global climate model. *Science.* **317**: 796-799.

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

- Tyler, J. W., Hancock, D. D., Parish, S. M., Rea, D. E., Besser, T. E., Sanders, S. G. and Wilson, L. K. 1996.** Evaluation of 3 assays for failure of passive transfer in calves. *J. Vet. Int. Med.* **10**: 304-307.
- Van De Weyer, L. M., Hendrick, S., and Waldner, C. L. 2010.** Serum micronutrient concentrations in beef cows before and after the summer grazing season. *Can. J. Anim. Sci.* **90**: 563-574.
- Waldner, C., Jelinski, M. D. and McIntyre-Zimmer, K. 2013.** Survey of western Canadian beef producers regarding calf-hood diseases, management practices, and veterinary service usage. *Can. Vet. J.* **54**: 559-564.
- Waldner, C. L. 2008.** Western Canada study of animal health effects associated with exposure to emissions from oil and natural gas field facilities. Study design and data collection I. Herd performance records and management. *Arch. Environ. Occup. Health*, **63**: 167-186.
- Waldner, C. L. 2014a.** Cow attributes, herd management, and reproductive history events associated with abortion in cow-calf herds from Western Canada. *Theriogenology*. **81**: 840-848.
- Waldner, C. 2014b.** Cow attributes, herd management and environmental factors associated with the risk of calf death at or within one hour of birth and the risk of dystocia in cow-calf herds in Western Canada. *Livest. Sci.* **163**: 126-139.
- Waldner, C. L. and Blakley, B. 2014.** Evaluating micronutrient concentrations in liver samples from abortions, stillbirths, and neonatal and postnatal losses in beef calves. *J. Vet. Diagn. Invest.* **26**: 376-389.

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

- Waldner, C. L. and Garcia Guerra, A. 2013.** Cow attributes, herd management, and reproductive history events associated with the risk of nonpregnancy in cow-calf herds in Western Canada. *Theriogenology*. **79**: 1083-1094.
- Waldner, C. L., Kennedy, R. I., Rosengren, L. B., Pollock, C. M. and Clark, E. T. 2010.** Gross postmortem and histologic examination findings from abortion losses and calf mortalities in western Canadian beef herds. *Can. Vet. J.* **51**: 1227-1238.
- Waldner, C. L. and Rosengren, L. B. 2009.** Factors associated with serum immunoglobulin levels in beef calves from Alberta and Saskatchewan and association between passive transfer and health outcomes. *Can. Vet. J.* **50**: 275-281.
- Waldner, C. L. and Van De Weyer, L. M. 2011.** Selenium status at the end of the grazing season, reproductive performance and degenerative myopathy in beef herds. *Can. Vet. J.* **52**: 1083-1088.
- Weaver, D. M., Tyler, J. W., VanMetre, D. C., Hostetler, D. E. and Barrington, G. M. 2000.** Passive transfer of colostral immunoglobulins in calves. *J. Vet. Intern. Med.* **14**: 569-577.
- Wiken, E. B. 1986.** Terrestrial ecozones of Canada. Ecological land classification series No. 19. Environment Canada. Hull, Quebec, 26 pp.
- World Meteorological Organization 2016.** World Meteorological Day: hotter, drier, wetter. Face the future. <http://public.wmo.int/en/media/press-release/state-of-climate-record-heat-and-weather-extremes> [2016 Mar 19].
- Zanker, I. A., Hammon, H. M. and Blum, J. W. 2000.** Beta-carotene, retinol and alpha-tocopherol status in calves fed the first colostrum at 0-2, 6-7, 12-13 or 24-25 hours after birth. *Int. J. Vitam. Nutr. Res.* **70**: 305-310.

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

Zou, G. 2004. A modified poisson regression approach to prospective studies with binary data.
Am. J. Epidemiol. **159**: 702-706.

Zou, G. and Donner, A. 2011. Extension of the modified Poisson regression model to
prospective studies with correlated binary data. Stat. Methods Med. Res. **22**: 661-670.

For Review Only

1
2

Table 1. Characteristics of calves sampled up to 1 month of age (n=899) and unconditional associations between each potential risk factor and serum A and E concentrations adjusted for similarities in concentrations in calves from the same herd (N=150)

		Calves sampled up to 1 month of age									
		Serum vitamin A (µg/mL)					Serum vitamin E (µg/mL)				
Variable		N	%	Mean	Standard deviation	P	N	%	Mean	Standard deviation	P
<i>All samples</i>		887	98.7	0.19	0.068	--	898	99.9	2.04	1.35	--
<i>Calf and cow attributes</i>											
Gender	Male	494	55.7	0.18	0.067	0.27	502	55.9	2.06	1.402	0.56
	Female	390	44.0	0.19	0.070		393	43.8	2.02	1.286	
	Not reported	3	0.3	0.18	0.031		3	0.3	1.64	0.786	
Age in days (quartiles)	0 to < 4	212	23.9	0.15	0.062	< 0.0001	217	24.2	1.62	1.152	< 0.0001
	4 to < 6	203	22.9	0.20	0.065		202	22.5	2.09	1.105	
	6 to < 9	213	24.0	0.20	0.070		217	24.2	2.23	1.505	
	9 to ≤ 31	259	29.2	0.19	0.065		262	29.2	2.19	1.468	
Twin	Yes	73	8.2	0.18	0.086	0.96	75	8.4	2.53	2.107	0.003
	No	814	91.8	0.19	0.066		823	91.6	1.99	1.251	
Assistance at birth	Yes	107	12.1	0.18	0.078	0.71	108	12.0	1.99	1.351	0.87
	No	780	87.9	0.19	0.067		790	88.0	2.05	1.351	
Average temperature day of birth	Above -25 C	860	97.0	0.19	0.066	0.09	870	96.9	2.04	1.358	0.59
	Below -25 C	27	3.0	0.22	0.108		28	3.1	2.14	1.087	
Cow age at	2 years old	160	18.0	0.18	0.067	0.33	163	18.2	1.77	1.258	0.009

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

calving	heifer										
	(1 st calf)										
	3 years old	140	15.8	0.18	0.068		139	15.5	1.99	1.082	
	4 years old	109	12.3	0.19	0.065		109	12.1	1.99	1.104	
	Mature cow										
	(5 to 9 years)	399	45.0	0.19	0.068		406	45.2	2.13	1.456	
	≥ 10 years										
Dam heifer	olds	56	6.3	0.18	0.077		57	6.3	2.28	1.476	
	Not reported	23	2.6	0.19	0.079		24	2.7	2.25	1.914	
	Yes	160	18.0	0.18	0.067	0.02	163	18.2	1.77	1.258	0.007
	No	727	82.0	0.19	0.069		735	81.8	2.10	1.363	
Cow breed type	British	430	48.5	0.17	0.060	0.02	432	48.1	1.76	1.133	0.02
	Continental	404	45.5	0.20	0.074		410	45.7	2.34	1.485	
	Other	46	5.2	0.19	0.064		49	5.5	2.02	1.394	
	Not reported	7	0.8	0.18	0.091		7	0.8	1.87	1.629	
Cow perinatal	Yes	18	2.0	0.16	0.059	0.07	18	2.0	2.78	3.030	0.12
	health problems	No	869	98.0	0.19	0.068		880	98.0	2.02	
Body condition	< 5	85	9.6	0.18	0.074	0.94	86	9.6	2.04	1.455	0.93
score (BCS) at	≥ 5	790	89.1	0.19	0.068		800	89.1	2.04	1.343	
pregnancy testing	Not reported	12	1.4	0.18	0.072		12	1.3	1.87	1.131	
Body condition	< 5	61	6.9	0.19	0.086	0.58	63	7.0	2.11	1.238	0.88
score (BCS)	≥ 5	683	77.0	0.19	0.067		689	76.7	2.00	1.371	
before calving	Not reported	143	16.1	0.19	0.066		146	16.3	2.18	1.296	
Month of calving	January	59	6.7	0.17	0.081	0.95	60	6.7	1.68	1.044	< 0.0001
	February	166	18.7	0.19	0.070		168	18.7	2.30	1.457	
	March	431	48.6	0.19	0.069		440	49.0	2.27	1.414	
	April	209	23.6	0.18	0.061		208	23.2	1.50	1.025	

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

	May	22	2.5	0.20	0.071		22	2.4	1.44	0.639	
Immunoglobulin	< 19	219	24.7	0.17	0.074	0.002	222	24.7	2.12	1.613	0.09
G Radial	19 to < 28.4	220	24.8	0.20	0.070		223	24.8	1.93	1.117	
immunodiffusion	28.4 to 38.1	222	25.0	0.19	0.065		224	24.9	2.13	1.286	
(quartiles) (g/L)	> 38.1	224	25.3	0.19	0.062		227	25.3	1.97	1.338	
<i>Herd attributes</i>											
Born into herd	Yes	469	52.9	0.20	0.072	0.003	476	53.0	2.38	1.503	< 0.0001
that administered											
selenium /	No	418	47.1	0.17	0.061		422	47.0	1.65	1.026	
vitamin E at birth											
Vitamin A	Yes	214	24.1	0.18	0.074	0.33	214	23.8	2.09	1.321	0.90
injections to cows	No	673	75.9	0.19	0.066		684	76.2	2.02	1.360	
Supplemented	Yes	188	21.2	0.18	0.063	0.47	191	21.3	2.06	1.255	0.87
vitamin A to cows											
before calving	No	699	78.8	0.19	0.070		707	78.7	2.03	1.376	
No commercial	Yes	149	16.8	0.18	0.069	0.57	150	16.7	1.90	1.133	0.68
trace minerals or											
vitamins to the	No										
cows		738	83.2	0.19	0.068		748	83.3	2.07	1.389	
3 year old forage	Yes	147	16.6	0.17	0.064	0.12	149	16.6	2.07	1.360	0.66
used for winter											
feed	No	734	82.8	0.19	0.069		743	82.7	2.04	1.352	
Precipitation											
during the	< 200 mm	385	43.4	0.16	0.058	< 0.0001	389	43.3	1.80	1.341	0.02
growing season											
	200 - 250 mm	264	29.8	0.21	0.070		267	29.7	2.36	1.411	

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

	> 250 mm	238	26.8	0.20	0.070	242	26.9	2.07	1.218	
Ecoregion	9 regions				< 0.0001					0.0001

- 3 **Note:** Samples were collected from 899 calves. Vitamin A results were missing for 12 calves (n=887) and vitamin E results were
- 4 missing for 1 calf (n=898).

For Review Only

Table 2. Final model describing the associations between calf and cow characteristics, herd location and serum vitamin A concentrations for calves sampled before 1 month of age (n=887)

		95% Confidence			
		Difference	interval		
Risk factor		(µg/mL) ^a	Lower	Upper	P
Calf age in days	0 to < 4	Reference category			< 0.0001 ^b
(categories based on quartiles)	4 to < 6	0.034	0.023	0.045	< 0.001
	6 to < 9	0.037	0.026	0.049	< 0.001
	9 to ≤ 31	0.037	0.026	0.049	< 0.001
Immunoglobulin G	< 19	Reference category			0.0002 ^b
radial	19 to < 28.4	0.020	0.009	0.031	< 0.001
immunodiffusion	28.4 to 38.1	0.018	0.007	0.029	0.001
(quartiles) (g/L)	> 38.1	0.023	0.012	0.035	< 0.001
Born to a heifer	Yes	-0.010	-0.021	0.0003	0.058
	No	Reference category			
Cow perinatal health problems	Yes	-0.030	-0.057	-0.001	0.036
	No	Reference category			
Precipitation during the previous growing season ≥ 200 mm	Yes	0.034	0.018	0.050	< 0.001
	No	Reference category			
Ecoregion					0.045 ^{b,c}

^aThe regression coefficient represents the average absolute difference in vitamin A concentrations between categories after accounting for other variables in the final model.

^bP-values represent the type 3 likelihood ratio test for multiple category variables in the model.

Waldner and Uehlinger, Vitamin A and E concentrations in beef calves

- 10 Predicted serum vitamin A concentrations for ecoregion reported in Table 3.

For Review Only

11 Table 3. Predicted mean serum vitamin A and vitamin E concentrations (µg/mL) for each ecoregion based on the results of the final
12 multivariable models (Table 2 and 4) and rank ordered for each vitamin

Ecoregions ranked based on serum vitamin concentrations							
	Vitamin A (µg/mL)				Vitamin E (µg/mL)		
	Mean	95% Confidence interval			Mean	95% Confidence interval	
		Lower	Upper			Lower	Upper
Western Boreal	0.147 ^a	0.117	0.176	Western Alberta Upland	1.505 ^{a,e}	0.889	2.121
Mixed Grassland	0.175 ^c	0.159	0.191	Mixed Grassland	1.650 ^{c,e}	1.331	1.969
Fescue Grassland	0.179	0.162	0.195	Peace Lowland	1.651 ^e	1.220	2.081
Western Alberta Upland	0.188	0.157	0.218	Fescue Grassland	1.926 ^e	1.579	2.274
Moist Mixed Grassland	0.190 ^b	0.171	0.208	Western Boreal	1.993	1.393	2.592
Aspen Parkland	0.192 ^b	0.180	0.204	Boreal Transition	2.055	1.664	2.446
Northern Continental Divide	0.196 ^b	0.163	0.230	Moist Mixed Grassland	2.130 ^d	1.769	2.491
Peace Lowland	0.198 ^b	0.177	0.220	Northern Continental Divide	2.446 ^{b,d}	1.768	3.124
Boreal Transition	0.202 ^d	0.183	0.220	Aspen Parkland	2.480 ^f	2.235	2.725

13 **Note:** *a, b, c, d*; and *e, f* within a column indicate pairings that are significantly different ($P < 0.05$) within each type of vitamin.

Uehlinger and Waldner, Vitamin A and E concentrations in beef calves

Table 4. Final model describing the associations between calf and cow characteristics, herd location and management and serum vitamin E concentrations for calves sampled before 1 month of age (n=898)

		95% Confidence			
		Difference	interval		
Risk factor		(µg/mL) ^a	Lower	Upper	P
Calf age in days	0 to < 4	Reference category			< 0.0001 ^b
(categories based on quartiles)	4 to < 6	0.38	0.16	0.60	0.001
	6 to < 9	0.63	0.41	0.85	< 0.001
	9 to ≤ 31	0.67	0.45	0.89	< 0.001
Month of calving	January	Reference category			0.0002 ^b
	February	0.15	-0.31	0.63	0.52
	March	0.06	-0.38	0.49	0.79
	April	-0.42	-0.88	0.04	0.07
	May	-1.05	-1.86	-0.23	0.01
Born to a heifer	Yes	-0.26	-0.46	-0.06	0.01
	No	Reference category			
Selenium / vitamin E administered	Yes	0.66	0.39	0.94	< 0.0001
at birth	No	Reference category			
Ecoregion					0.0007 ^{b,c}

^aThe regression coefficient represents the average absolute difference in vitamin E concentrations between categories after accounting for other variables in the final model.

^bP-values represent the type 3 likelihood ratio test for multiple category variables in the model.

^cPredicted serum vitamin E concentrations for ecoregion reported in Table 3.

Uehlinger and Waldner, Vitamin A and E concentrations in beef calves

21 Table 5. Unconditional or bivariate associations between serum vitamin A (n=887) and vitamin
22 E (n=898) and the risk of calf death, risk of calf treatment for pneumonia, scours, or treatment
23 with antibiotics for any reason at least one time between birth and June 30th adjusted for time at
24 risk and clustering by herd (N=150)

Outcome	Calf serum concentration	Risk ratio	95% Confidence interval		<i>P</i>
			Lower	Upper	
Calf death	Vitamin A (0.1 µg/mL) ^a	0.43	0.19	0.96	0.040
	Vitamin A (quartiles)				0.038 ^b
	< 0.14 µg/mL	2.90	1.04	8.11	0.043
	0.14 to < 0.18 µg/mL	1.04	0.39	2.78	0.94
	0.18 to ≤ 0.225 µg/mL	0.66	0.19	2.28	0.51
	> 0.225 µg/mL	Reference category			
	Vitamin A < adequate compared to ≥ adequate for calf age ^c	1.90	0.85	4.25	0.12
Calf death	Vitamin E (1 µg/mL) ^d	0.83	0.53	1.32	0.44
	Vitamin E (quartiles)				0.76 ^b
	< 1.09 µg/mL	1.55	0.49	4.91	0.46
	1.09 to < 1.80 µg/mL	1.20	0.39	3.73	0.75

Uehlinger and Waldner, Vitamin A and E concentrations in beef calves

	1.80 to $\leq$ 2.60	0.81	0.27	2.49	0.72
	$\mu\text{g/mL}$				
	$> 2.60 \mu\text{g/mL}$	Reference category			
Vitamin E < adequate		1.55	0.49	4.95	0.46
compared to $\geq$ adequate for					
calf age ^e					
Enteritis	Vitamin A (0.1 $\mu\text{g/mL}$) ^a	0.93	0.63	1.39	0.73
(scours)	Vitamin A (quartiles)				0.33 ^b
	$< 0.14 \mu\text{g/mL}$	1.21	0.65	2.23	0.55
	0.14 to < 0.18	0.94	0.50	1.79	0.86
	$\mu\text{g/mL}$				
	0.18 to ≤ 0.225	0.73	0.41	1.30	0.29
	$\mu\text{g/mL}$				
	$> 0.225 \mu\text{g/mL}$	Reference category			
Vitamin A < adequate		1.34	0.82	2.18	0.24
compared to $\geq$ adequate for					
calf age ^c					
Enteritis	Vitamin E (1 $\mu\text{g/mL}$) ^d	0.74	0.55	1.00	0.053
(scours)	Vitamin E (quartiles)				0.15 ^b
	$< 1.09 \mu\text{g/mL}$	2.32	1.07	5.01	0.045
	1.09 to < 1.80	1.68	0.84	3.34	0.22
	$\mu\text{g/mL}$				
	1.80 to ≤ 2.60	1.30	0.65	2.60	0.46

Uehlinger and Waldner, Vitamin A and E concentrations in beef calves

$\mu\text{g/mL}$					
$> 2.60 \mu\text{g/mL}$		Reference category			
Vitamin E $<$ adequate		2.71	1.55	4.74	0.01
compared to $\geq$ adequate for					
calf age ^e					
Pneumonia	Vitamin A (0.1 $\mu\text{g/mL}$) ^a	1.44	0.81	2.57	0.21
	Vitamin A (quartiles)				0.78 ^b
	$< 0.14 \mu\text{g/mL}$	0.82	0.33	2.07	0.68
	0.14 to < 0.18	0.75	0.27	2.04	0.57
	$\mu\text{g/mL}$				
	0.18 to ≤ 0.225	0.53	0.15	1.83	0.31
	$\mu\text{g/mL}$				
	$> 0.225 \mu\text{g/mL}$	Reference category			
	Vitamin A $<$ adequate	0.73	0.36	1.50	0.39
	compared to $\geq$ adequate for				
	calf age ^c				
Pneumonia	Vitamin E (1 $\mu\text{g/mL}$) ^d	0.87	0.66	1.14	0.31
	Vitamin E (quartiles)				0.54 ^b
	$< 1.09 \mu\text{g/mL}$	1.11	0.33	3.68	0.87
	1.09 to < 1.80	1.78	0.58	5.44	0.31
	$\mu\text{g/mL}$				
	1.80 to ≤ 2.60	1.86	0.69	5.00	0.22
$\mu\text{g/mL}$					

Uehlinger and Waldner, Vitamin A and E concentrations in beef calves

	> 2.60 µg/mL	Reference category			
	Vitamin E < adequate	0.57	0.18	1.78	0.33
	compared to ≥ adequate for				
	calf age ^e				
Treatment for	Vitamin A (0.1 µg/mL) ^a	0.92	0.70	1.23	0.59
any reason	Vitamin A (quartiles)				0.13 ^b
	< 0.14 µg/mL	1.35	0.87	2.08	0.18
	0.14 to < 0.18	0.81	0.52	1.25	0.34
	µg/mL				
	0.18 to ≤ 0.225	0.91	0.62	1.34	0.63
	µg/mL				
	> 0.225 µg/mL	Reference category			
	Vitamin A < adequate	1.11	0.81	1.52	0.53
	compared to ≥ adequate for				
	calf age ^e				
Treatment for	Vitamin E (1 µg/mL) ^d	0.95	0.82	1.09	0.45
any reason	Vitamin E (quartiles)				0.73 ^b
	< 1.09 µg/mL	1.28	0.83	1.96	0.27
	1.09 to < 1.80	1.11	0.73	1.69	0.63
	µg/mL				
	1.80 to ≤ 2.60	1.15	0.81	1.63	0.44
	µg/mL				
	> 2.60 µg/mL	Reference category			

Uehlinger and Waldner, Vitamin A and E concentrations in beef calves

Vitamin E < adequate	1.47	0.99	2.17	0.06
compared to $\geq$ adequate for				
calf age ^e				

^aThe risk ratio represents the average relative increase in the risk of the outcome for each 0.1 $\mu\text{g/mL}$ increase in vitamin A concentrations.

^b*P*-values represent the type 3 likelihood ratio test for multiple category variables in the model.

^cFor vitamin A in calves 2 to 7 days of age, 0.2 $\mu\text{g/mL}$ was considered the minimum adequate level and for calves greater than 7 days of age, 0.225 $\mu\text{g/mL}$ was the minimum adequate level.

^dThe risk ratio represents the average relative increase in the risk of the outcome for each 1 $\mu\text{g/mL}$ increase in vitamin E concentrations.

^eFor vitamin E in calves 2 to 7 days of age, 0.8 $\mu\text{g/mL}$ was considered the minimum adequate level and for calves greater than 7 days of age, 0.5 $\mu\text{g/mL}$ was the minimum adequate level.

Uehlinger and Waldner, Vitamin A and E concentrations in beef calves

34 Table 6. Final models describing the association between serum vitamin A concentrations and
 35 the incidence of calf death before June 30, 2002 (n=887, N=150)

Outcome	Risk factor		Risk ratio	95% Confidence interval		<i>P</i>
				Lower	Upper	
Calf death	Vitamin A	< 0.14 µg/mL	2.83 ^a	1.18	6.80	0.020
	($< 25^{\text{th}}$ percentile /	≥ 0.14 µg/mL	Reference category			
	$\geq 25^{\text{th}}$ percentile)					
	Calf age at sampling	Days	0.94 ^b	0.86	1.03	0.20
	Serum IgG	g/L	0.97	0.94	1.00	0.066
	concentration					
	Month of calving	January &	Reference category			
		February				
		March	16.9	2.08	137	0.008
		April & May	13.1	1.61	107	0.016
	Cow age at calving	Heifer	0.72	0.21	2.44	0.60
		(2 years)				
		3 years	0.20	0.03	1.19	0.08
		4 years	1.12	0.30	4.15	0.86
		Mature cow	Reference category			
		(5 to 9 years)				
		≥ 10 years	3.91	1.11	13.7	0.03
		Not reported	0.89	0.06	14.2	0.93

Uehlinger and Waldner, Vitamin A and E concentrations in beef calves

Body condition	< 5	3.57	0.75	17.1	0.11
score (BCS) before	≥ 5	Reference category			
calving	Not reported	1.86	0.61	5.71	0.27
Assistance at birth	Yes	2.59	0.36	18.9	0.35
	No	Reference category			
Selenium /	Yes	Reference category			
vitamin E	No	5.40	1.85	15.8	0.002
administered at birth					

^aThe risk ratio represents the average relative increase in the risk of calf mortality when comparing calves with serum vitamin A concentrations in the lowest quartile (<0.14 µg/mL) compared to calves with higher vitamin A concentrations. Calves with vitamin A concentrations <0.14 µg/mL were 2.83 times more likely to die than those with higher concentrations.

^bThe risk ratio represents the average relative increase in the risk of calf mortality for each additional day in calf age.

Uehlinger and Waldner, Vitamin A and E concentrations in beef calves

42 Table 7. Final models of the association between serum vitamin E concentrations and the risk of
 43 calf treatment for enteritis before June 30, 2002 (n=898, N=150)

Outcome	Risk factor		Risk ratio	95% Confidence interval		<i>P</i>
				Lower	Upper	
Calf enteritis (scours)	Vitamin E	Less than adequate	3.15	1.81	5.49	0.001
		for age ^{a,b}				
		Adequate for age	Reference category			
	Calf age at sampling	Days ^c	1.02	0.97	1.08	0.36
	Serum IgG concentration	g/L	1.00	0.99	1.02	0.56
	Month of calving	January & February	Reference category			
		March	2.23	0.88	5.64	0.09
		April & May	1.77	0.57	5.47	0.32
	Cow age at calving	Heifer (2 years)	0.93	0.56	1.55	0.79
		3 years	0.70	0.31	1.62	0.41
	4 years	1.40	0.84	2.33	0.20	
	Mature cow (5 to 9 years)	Reference category				

Uehlinger and Waldner, Vitamin A and E concentrations in beef calves

	≥ 10 years	1.09	0.36	3.26	0.88
	Not reported	0.60	0.13	2.72	0.51
Assistance at birth	Yes	1.89	0.69	5.18	0.22
	No	Reference category			

^aFor vitamin E in calves 2 to 7 days of age, 0.8 µg/mL was considered the minimum adequate level and for calves greater than 7 days of age, 0.5 µg/mL was the minimum adequate level.

^bThe risk ratio represents the average relative increase in the risk of calf treatment for diarrhea when comparing calves with serum vitamin E concentrations below the level considered to be adequate compared to calves with higher serum vitamin E concentrations. Calves with vitamin E concentrations that were less than adequate for their age were 3.15 times more likely to be treated for diarrhea at least once than those with higher concentrations.

^cThe risk ratio represents the average relative increase in the risk of calf mortality for each additional day in calf age.