Blood thiamine (vitamin B₁), ascorbic acid (vitamin C), and cortisol concentrations in healthy and ill neonatal foals

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Abstract

Background: Sepsis is common in foals and several treatments are used to facilitate recovery. Evidence in people suggests an association between low blood concentrations of thiamine, ascorbic acid, and cortisol and sepsis, with further evidence suggesting that administration of hydrocortisone, thiamine, and ascorbic acid may improve outcome. No information is available with regard to these treatments in foals.

Hypothesis/Objectives: To compare blood concentrations of thiamine, ascorbic acid, and cortisol in healthy and ill foals.

Animals: Fifteen healthy and 27 ill (septic and sick-nonseptic [SNS]) foals were evaluated at admission. Fewer healthy and ill foals were available for sampling at 72 and 120 hours.

Methods: Prospective study. Blood was collected from healthy foals at 12 (n = 15), 72 (n = 11), and 120 (n = 9) hours of age and from ill foals <48 hours old at admission (n = 27), 72 (n = 8), and 120 (n = 8) hours after presentation. Thiamine, ascorbic acid, and cortisol concentrations were measured in blood samples and compared between groups of foals.

Results: Blood concentrations of thiamine were significantly lower in septic compared to healthy foals at 72 (median, 1.72 ng/mL; P = .02) and 120 (median, 2.0 ng/mL; P = .04) hours after admission; blood concentrations of ascorbic acid also were significantly lower in septic compared to healthy foals at 72 (median, 4.4 μg/mL; P = .02) and 120 hours (median, 4.8 μg/mL; P = .03). Blood concentrations of ascorbic acid were lower in SNS compared to healthy foals at 72 (median, 6.9 μg/mL; P = .03) and 120 (median, 6.4 μg/mL; P = .04) hours after admission. Serum cortisol concentrations were significantly higher at admission in septic (median, 4.23 μg/dL) compared to SNS (median, 1.8 μg/dL; P = .01) and healthy (median, 2.2 μg/dL; P = .002) foals.

Conclusions and Clinical Importance: A potential association exists between illness in foals and lower blood concentrations of thiamine and ascorbic acid during...
hospitalization. Additional studies are needed to examine a larger population of foals and determine the clinical impact of low vitamin concentrations, if any, on morbidity and mortality.

**KEYWORDS**
hydrocortisone, hypovitaminosis, sepsis, treatment

### 1 | INTRODUCTION

Neonatal sepsis remains a prominent disease and frequent cause of morbidity and mortality in foals. Cornerstones of treatment include antimicrobial administration, maintenance of hydration and blood pressure, and supportive care. In addition, numerous pharmacological agents, designed to combat sepsis and improve survival, have been tested over the last several decades. Despite advances in the treatment of sepsis, efforts for more effective treatments continue.

Some studies have identified benefit from administration of a combination of 3 readily available medications, thiamine (vitamin B₁), ascorbic acid (vitamin C), and hydrocortisone, in the adjunctive treatment of sepsis in people. The endogenous dynamics of these 3 components have been investigated in critically ill patients. For example, hospital mortality was significantly lower (8.5%) in people with severe sepsis and septic shock who received thiamine, ascorbic acid, and hydrocortisone as compared to the control group (40.4%). In that study, mean baseline serum ascorbic acid concentration was 2.48 ± 2.07 μg/mL (normal, 7.0-10.6 μg/mL) in individuals from the treatment group. In another study, mean plasma ascorbic acid concentrations were significantly lower in people with septic shock, as compared to nonseptic patients, and were associated with increased vasoppressor requirements, kidney injury, multiple organ dysfunction, and increased mortality. Decreased blood concentrations of thiamine also have been identified in septic patients and were associated with increased mortality. Conversely, a multicenter study in people with sepsis and acute respiratory distress syndrome found no benefit in organ dysfunction scores, markers of inflammation, or vascular injury in patients who received ascorbic acid compared with placebo.

Inappropriately low cortisol concentrations also have been described in critical illness, including sepsis, a condition referred to as critical illness-related corticosteroid insufficiency. Low cortisol concentrations have been associated with dysregulation of the hypothalamic-pituitary-adrenal axis, altered cortisol metabolism, and tissue resistance to corticosteroids. Studies have examined serum cortisol concentrations in healthy and ill foals, but minimal information is available in regard to blood concentrations of thiamine and ascorbic acid. Our objectives were to measure blood concentrations of thiamine, ascorbic acid, and cortisol in healthy neonatal foals, and compare these variables between healthy and ill foals, as well as between survivors and nonsurvivors. Our hypothesis was that septic foals and nonsurvivors would have significantly lower blood concentrations of thiamine, ascorbic acid, and cortisol when compared to healthy foals and survivors.

### 2 | MATERIALS AND METHODS

#### 2.1 | Healthy foals

Fifteen healthy neonatal foals were examined within 24 hours of birth. Foals were considered healthy if they had a normal physical examination, serum IgG concentration >800 mg/dL and CBC results within age-specific reference intervals. Not all healthy foals were available for subsequent sampling because they were discharged before sampling time points; specifically at 72 and 120 hours, 11 and 9 foals, respectively, were available for serial sampling. The study was approved by Iowa State University’s Institutional Animal Care and Use Committee.

#### 2.2 | Ill foals

Twenty-seven ill foals presented at <48 hours old to the Lloyd Veterinary Medical Center’s Intensive Care Unit were included. Blood for culture was collected aseptically by direct venipuncture or immediately after an IV catheter was placed. Blood culture was performed using routine methods with microorganisms identified using standard identification methods. Ill foals were divided into septic and sick-nonseptic (SNS) groups. A foal was classified as septic if it fulfilled any or all of the following criteria: (a) positive blood culture, (b) clinical course consistent with sepsis (ie, infection of multiple organ systems), (c) sepsis score ≥12, or (d) necropsy evidence of disseminated septic processes. If a foal did not meet any of these criteria, it was considered SNS.

#### 2.3 | Study design

In healthy foals, blood (10 mL) was collected at ≤24 (n = 15), 72 (n = 11), and 120 (n = 9) hours of age by direct venipuncture; samples were split between EDTA and clot tubes, placed in an ice bath, and protected from light. Within 20 minutes, 5 mL of whole blood (EDTA) was transferred to a polypropylene tube for measurement of thiamine. Plasma was harvested from EDTA tubes after 5 minutes of centrifugation and placed in polypropylene tubes for measurement of ascorbic acid. Clot tubes were centrifuged for 5 minutes and serum collected and transferred to polypropylene tubes for measurement of cortisol. Samples were frozen at −80°C until analysis.

Blood (10 mL) was collected from ill foals at admission (baseline sample) either by venipuncture or immediately after placement of a jugular vein catheter, processed as described above, and used for...
measurement of thiamine, ascorbic acid, and cortisol. Subsequent blood samples were collected in a similar fashion 72 and 120 hours after admission in 8 ill, randomly available foals. Informed owner consent was obtained before sample collection.

2.4 Measurement of thiamine, ascorbic acid, and cortisol

Thiamine and ascorbic acid: 50 μL of plasma from each foal was precipitated using either 200 μL liquid chromatography-mass spectrometry (LC-MS) grade acetonitrile containing 50 μL 0.1 M ZnSO₄ and 100 ng/mL ¹³C₃-labeled thiamine or 1 μg/mL ¹³C₆-labeled ascorbic acid. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used to quantify ascorbic acid (Agilent 6490 mass spectrometer; Agilent 1290 HPLC, Santa Clara, California). A water/methanol reverse phase gradient was used to elute thiamine or ascorbic acid from a C18 column. Free thiamine was analyzed using LC-MS/MS. Eluate was analyzed using a precursor m/z of 265 Da and 2 fragment m/z of 144 Da and 122 Da for thiamine. Intraassay coefficient of variability (CV) ranged from 0.77% to 1.16% and interassay CV was 2.37%. Accuracy was ≥95.6%, with an average accuracy of 98.5%. For ascorbic acid, eluate was analyzed using a precursor m/z of 175 Da and a fragment m/z of 115 Da. Intraassay CV ranged from 1.56% to 3.26% and interassay CV was 2.46%, with an accuracy of ≥91% and an average accuracy of 96.4%.

Cortisol: Serum cortisol concentrations were measured (baseline sample only) using a human-specific radioimmunoassay validated for horses according to the manufacturer’s instructions (MP Biomedicals, Solon, Ohio).¹⁶

2.5 Statistical analysis

A commercial statistics software program (IBM SPSS Statistics version 24, IBM Corp, New York, and Graph Pad Prism version 8, GraphPad Software, California) was used. Power analysis based on previous studies in horses suggested that 16 horses per group afforded satisfactory power (>0.8, α = 0.05) to discriminate minimum differences in ascorbic acid concentration (mean group 1 = 4.7 μg/mL; mean group 2 = 3.4 μg/mL and SD of 1.3 μg/mL).¹⁹ Data sets were tested for normality using the Shapiro-Wilk test, which determined that thiamine concentrations at all 3 time points and ascorbic acid and cortisol concentrations on admission were not normally distributed. Median and ranges were calculated for continuous variables. Kruskal-Wallis statistics with Dunn’s post hoc test were used to compare the 3 groups of foals and a Mann-Whitney test was used to compare 2 groups. Differences in thiamine and ascorbic acid concentrations were analyzed by 2-way (time and group of foals) mixed model repeated measures analysis of variance (ANOVA) analysis with Tukey post hoc test for multiple comparisons in ill and healthy foals. Hypovitaminosis B₁ (<2 ng/mL) and C (<7 μg/dL) were defined as blood concentrations below the lowest limit of the 95% confidence interval (CI) from healthy foals. The relationship between categorical variables was analyzed using Fisher’s exact test.

3 RESULTS

Study population: Of the ill foals (n = 27), 48% (13/27) were classified as SNS and 52% (14/27) as septic. Median admission age was 12 hours (range, 1-48 hours) for all ill foals with a median age of 12 hours for SNS and 12 hours for septic foals.

Table 1: Blood thiamine, ascorbic acid, and cortisol concentrations in healthy, septic, and sick-nonseptic (SNS) foals measured at admission and at 72 and 120 hours after admission. Results reported as median and range

| Foal group      | Time points | Thiamine (ng/mL) | Ascorbic acid (μg /mL) | Cortisol (μg/dL) |
|-----------------|-------------|------------------|------------------------|-----------------|
| Healthy foals   | Admission (n = 15) | 2.2 (1.25-11.2) | 8.9 (1.8-15.5) | 2.2 (0.3-6) |
|                 | 72 hours (n = 11) | 4 (2.6-16.2)     | 11.6 (9-17)          |                 |
|                 | 120 hours (n = 9) | 4 (2.2-20.3)     | 14 (1.2-15.3)        |                 |
| Septic foals    | Admission (n = 14) | 1.4 (0.54-40)    | 6.8 (2.5-22.8)       | 4.23 (2.9-25.4)**,*** |
|                 | 72 hours (n = 4)  | 1.72 (0.95-2)*   | 4.4 (3.6-14)*        |                 |
|                 | 120 hours (n = 4) | 2.0 (1.8-5)*     | 4.8 (2.8-8.4)*       |                 |
| Sick-nonseptic  | Admission (n = 13) | 4.4 (1-30.2)     | 9.7 (3.1-19.3)       | 1.8 (0.3-5)    |
|                 | 72 hours (n = 4)  | 7.34 (1-18.4)    | 6.9 (4.9-9.2)*       |                 |
|                 | 120 hours (n = 4) | 3.25 (2.8-15.6)  | 6.4 (4.5-7.9)*       |                 |
| Ill foals       | Admission (n = 27) | 2.5 (0.54-40)    | 8.5 (2.5-22.8)       | 3.9 (0.3-25.4)* |
|                 | 72 hours (n = 8)  | 2.0 (0.95-18)    | 5.2 (3.6-14) **      |                 |
|                 | 120 hours (n = 8) | 2.8 (1.8-15.6)   | 5.4 (2.8-8.4)**      |                 |

*P < .05 compared to healthy at the same time point. **P < .05 compared to healthy foals at the same time point. ***P < .05 compared to SNS at the same time point.
range, 1-48 hours) for septic and 4 hours (range, 1-24 hours) for SNS foals. The median age for the first sample collection in healthy foals was 12 hours (range, 5-24 hours) old. Age was not significantly different between groups (P = .08). In ill foals, 19% (5/27) were non-survivors with 29% (4/14) of the septic foals and 7% (1/13) of the SNS foals not surviving (P = .3).

### 3.1 Thiamine, ascorbic acid, and cortisol concentrations in ill (septic and SNS) and healthy foals at admission and 72 and 120 hours after admission

Blood concentrations of thiamine, ascorbic acid, and cortisol in healthy, septic and SNS foals at admission and 72 and 120 hours after admission are presented in Table 1 and Figures 1 and 2. Plasma ascorbic acid concentrations were significantly lower in ill foals compared to healthy foals at 72 (P = .03) and 120 (P = .04) hours; ascorbic acid also was significantly lower in septic foals at 72 (P = .02) and 120 (P = .02) hours. Septic foals had significantly lower thiamine concentrations compared to healthy foals at 72 (P = .02) and 120 hours (P = .05; Figure 2B). At admission, cortisol concentrations were significantly higher in septic foals compared to healthy (P = .002) and SNS (P = .02) foals. Cortisol concentrations also were significantly higher in ill foals as compared with healthy foals at admission (P = .02).

### 3.2 Occurrence of hypovitaminosis B₁ and C in ill foals on admission

Hypovitaminosis B₁ was noted in 37% (10/27) of all ill foals and was significantly (P = .04) more common in septic foals (57%; 8/14) compared with SNS foals (15%; 2/13). In ill foals, hypovitaminosis C was noted in 37% (10/27) and was not significantly (P = .2) different between septic (50%; 7/14) and SNS (23%; 3/13) foals.
3.3 | Thiamine, ascorbic acid, and cortisol concentrations in surviving and nonsurviving foals at admission

Blood concentrations of thiamine, ascorbic acid, and cortisol in foals that survived until discharge from the hospital and died are presented in Table 2. The occurrence of hypovitaminosis B1 was 60% (3/5) in nonsurviving foals and 32% (7/22) in surviving foals (P = .24) and similarly the occurrence of hypovitaminosis C was 60% (3/5) in nonsurviving foals as compared with 32% (7/22) in surviving foals (P = .24). No difference was found for any of these variables between survivors and nonsurvivors.

4 | DISCUSSION

In our study, thiamine and ascorbic acid concentrations were significantly lower and serum cortisol concentrations significantly higher in hospitalized foals compared with healthy foals. Specifically, both blood thiamine and ascorbic acid concentrations were significantly lower in septic foals at 72 and 120 hours after admission, whereas only plasma ascorbic acid concentrations were significantly lower in SNS foals at 72 and 120 hours after admission compared with healthy foals. The occurrence of hypovitaminosis B2 was significantly more common in septic foals as compared with SNS foals.

Thiamine is a cofactor for multiple cellular enzymes essential for aerobic carbohydrate energy production. In people, hypovitaminosis B1 is linked to cardiac failure, neurodegeneration and refeeding syndrome, with other studies documenting deficiency in 10% to 25% of septic people. In our study, hypovitaminosis B2 was significantly more common in septic compared with SNS foals and was significantly lower in septic foals when compared with healthy foals. This finding suggests that thiamine concentrations can become abnormally low over time in septic foals. Thiamine deficiency has been noted in critically ill people, with increased incidence observed during hospitalization. This finding is exemplified by a study in septic people in which 10% of patients had hypovitaminosis B1 at admission with an additional 10% developing hypovitaminosis B1 over the next 72 hours.

The cause of low blood thiamine concentrations in the septic foals of our study is unknown, but the most plausible reason is increased demand for thiamine (and other micronutrients) secondary to hypermetabolism in critically ill or septic patients, paired with the systemic inflammatory response. Foals have a higher metabolic rate compared with adult horses and are in a rapid growth phase. Coupled with this is the fact that inflammation is associated with increased intermediate metabolism, both of these variables contribute to higher consumption of the cofactors involved in carbohydrate metabolism. Another potential cause might simply relate to decreased consumption of thiamine. Thiamine is a water-soluble essential nutrient the supply of which depends almost entirely on dietary intake (ie, the dam’s milk) and absorption from the proximal small intestine. Low blood concentrations of thiamine in ill foals might result from inadequate amounts of consumed or administered milk. Endogenous synthesis of thiamine does not occur, although some forms of bacteria in the intestine can produce small amounts. Neonatal foals still are establishing intestinal microflora, and thus the lack of production of thiamine by intestinal microflora might contribute, in a minor way, to hypovitaminosis B1. Other causes include intestinal bacteria capable of producing thiaminases, thiamine deficient milk or dam, and genetic defects in thiamine transport and metabolism. Although our study involved only a small number of ill foals, clinicians might consider measuring blood thiamine concentrations and possible supplementation with thiamine in foals that are hospitalized.

Ascorbic acid has been credited with a multitude of beneficial effects including scavenging reactive oxygen species (ROS), restoring cellular antioxidants and serving as a cofactor for enzymes. Ascorbic acid has been credited with a multitude of beneficial effects including scavenging reactive oxygen species (ROS), restoring cellular antioxidants and serving as a cofactor for enzymes. For example, in sepsis and associated systemic inflammatory response, endothelial cells and neutrophils are activated and release ROS. Inadequate dietary (milk) intake or enhanced renal excretions are other possible causes of hypovitaminosis C in ill foals. Insufficient endogenous synthesis also may contribute to low concentrations because, unlike people, horses have the ability to synthesize ascorbic acid from D-glucose or D-galactose via the gluconic pathway in the liver. As with thiamine, clinicians might consider measuring ascorbic acid and supplementation with ascorbic acid as necessary in foals that are hospitalized.

Cortisol plays a vital role in a number of physiologic processes in the neonatal foal including electrolyte and fluid balance, cardiovascular homeostasis, carbohydrate metabolism, and immune function. Transient cortisol deficiency can occur in critically ill and septic neonatal foals and is associated with higher mortality. In our study, admission cortisol concentrations were significantly higher in septic and SNS foals compared to healthy foals, but no association with mortality was identified. In addition, no differences in the concentrations of blood thiamine, ascorbic acid, or serum cortisol were noted between surviving and nonsurviving foals.

Our study had some limitations. Sample size was small and the study was conducted at a single center. Power analysis suggested that 16 horses per group would provide satisfactory power. Although the sample size of foals at admission was adequate, subsequent (72 and 120 hour) sampling was much lower. This emphasizes a critical
shortcoming of the study in that foals were frequently unavailable at later time points during hospitalization for various reasons (eg, death, discharge from hospital). Because so few samples were evaluated at later time points, our findings must be regarded as exploratory, with larger multi-institutional studies necessary to substantiate our findings. However, the fact that a difference in vitamin concentrations was noted in such small group sizes implies that altered concentrations of these vitamins might be clinically relevant in ill foals and should be investigated further. In summary, our findings suggest an association between low thiamine and ascorbic acid concentrations and hospitalized critically ill and septic foals.

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CONFLICT OF INTEREST DECLARATION
The authors declared no potential conflicts of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION
Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION
Approved by the IACUC of Iowa State University.

HUMAN ETHICS APPROVAL DECLARATION
Authors declare human ethics approval was not needed for this study.

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