Influence of a Maternal Dietary Yeast Supplement on Immunoglobulin Concentrations in Quarter Horse Foals from Birth to Four Months of Age

Thesis

Partial Fulfillment of Requirements for Undergraduate Research Distinction

By

Rachel Leimbach

The Ohio State University

2015

Project Advisor:

Dr. Kimberly Cole
ABSTRACT

There is limited information on the effect of maternal dietary yeast supplementation on immunoglobulin levels in foals. In this study eight, Quarter Horse mares (14.5 ± 7.5 yr) were randomly assigned to one of two groups: yeast or control. All mares received a basal diet of 0.5% BW of a 16% CP pelleted concentrate with water and mixed grass hay ad libitum. Mares in the yeast treatment group also received 1g/45.4 kg of BW/d of a live culture of \textit{Saccharomyces cerevisiae} from 250 d of gestation to 90 d post-foaling. All mares were vaccinated at d 300 of gestation against Eastern and Western equine encephalomyelitis, equine rhinopneumonitis (EHV-1 and EHV-4), equine influenza (type A2), tetanus, and West Nile virus. Blood samples were collected from the foals via jugular venipuncture immediately after parturition (d 0), at 12 and 24 hr and 30, 60, 90, and 120 d post-foaling. Sera samples were analyzed for total IgG including IgGa, IgGb, and IgG(T), as well as IgA, IgM, and IgE concentrations using commercial ELISA kits. Data were analyzed using PROC MIXED of SAS and a p-value of ≤ 0.05 was considered statistically significant. IgG(T) concentrations were significantly increased on d 60 post-foaling in foals born from mares fed the yeast supplement compared to controls. However, supplementing the maternal diet with live yeast did not influence foal IgGa, IgGb, IgA, IgM, or IgE concentrations.

KEY WORDS

Immunoglobulins, Horse, ELISA, Yeast
INTRODUCTION

Foals are born immunodeficient (Tizzard, 2000). Like most farm animals, there is no transfer of immunoglobulins (Ig) through the placenta during gestation. Foals receive immunoglobulins from the dam’s colostrum which can be absorbed through the foal’s gastrointestinal epithelium for the first 18 – 24 hr of life. If a foal does not absorb sufficient quantities of immunoglobulins, it could develop a condition referred to as failure of passive transfer (FPT) and be at increased risk of morbidity and mortality due to septicemia (Riley et al., 2007).

IgG is the most prevalent immunoglobulin in equine blood and colostrum (Keggan et al., 2013). Originally it was thought that there were five IgG subclasses but it has been discovered that there are actually seven subclasses (IgGa = IgG1; IgGb = IgG4 and IgG7; IgG(T) = IgG3 and IgG5; and IgGc = IgG6). In serum, the IgG isotypes rank from greatest to lowest concentration as follows: IgGb > IgG(T) > IgGa > IgGc (Keggan et al., 2013).

IgM is the first antibody to respond to a pathogen to the system (Tortora et al., 2013). If the pathogen is novel, IgM will be the most prevalent immunoglobulin at the beginning of the immune response while the body is initiating production of IgG. IgA is the primary immunoglobulin found in mucosal membranes and secretions and can also be found in the blood. It is the second most prevalent immunoglobulin next to IgG in colostrum. As the colostrum changes into milk, IgA becomes the most prevalent while IgG becomes second (Tizard, 2000). The main function of IgA is to prevent attachment of microbial pathogens to mucosal surfaces which help to protect suckling animals from gastrointestinal infections (Tortora et al., 2013). IgE is reported to have the lowest concentration in serum compared to the previously described immunoglobulins and is associated with allergic reactions (Keggan et al., 2013; Tortora et al., 2013). The function of IgE in foals is still being determined.
Probiotics have been used to stimulate the immune system in many animal species (Corcionivoschi et al., 2010). *Saccharomyces cerevisiae* has been shown to be a general immunostimulant in that it increases immunoglobulin concentration in horses and cattle (Krakowski et al., 1999; Emmanuel et al., 2007; Cakiroglu et al., 2010; Thorson et al., 2010). Due to yeast probiotic’s immunostimulatory effect, it is theoretically possible that an increased immunoglobulin level in the mare could potentially cascade down to the foal via the colostrum by passive transfer to increase foal serum immunoglobulin concentrations. The objective of this study was to determine if maternal dietary yeast supplementation during late gestation and early lactation affect serum immunoglobulin levels in their foals.

**MATERIALS AND METHODS**

*Horses and Supplementation* – Eight pregnant Quarter Horse mares (14.5 ± 7.5 yr) were used in a completely randomized design to evaluate the effect of dietary live yeast supplementation on foal serum immunoglobulin concentrations from birth to four months of age. Each mare received a basal diet consisting of 0.5% BW/d of a 12% CP pelleted concentrate until parturition with water and mixed grass hay *ad libitum*. After parturition, the basal diet consisted of approximately 1% BW / d of a 16% CP pelleted concentrate with water and mixed grass hay *ad libitum*. Mares were randomly assigned to one of two treatments from d 250 of gestation to 90 d (+/- 15d) post-parturition: the basal diet or the basal diet supplemented with a targeted dose of 1g (4.5 x 109 CFU) /45.4 kg of BW per day of a live culture of *Saccharomyces cerevisiae* yeast (Alltech; Nicholasville, KY). Prior to parturition, and within 7 d after parturition, mares were housed outdoors in paddocks with access to shelter. For parturition, mares were housed
in 3.7 x 7.3 m box stalls. By 28 d (+/- 14d) post-parturition, mares were acclimated to grass pasture. Throughout the study, foals did not have access to the mares’ concentrate. Starting at approximately 14 d of age, foals were creep fed with a 16 % CP pelleted concentrate at 1% of BW and increased to 3% of BW by weaning.

**Serum Collection**—Blood samples were collected from foals via jugular venipuncture immediately after foaling (d 0) and on d 0.5, 1, 30, 60, 90, and 120 post-foaling. Blood samples were centrifuged at 2,500 rpm for 10 min and serum decanted. Serum samples were then stored at -80°C until further analysis.

**ELISA Kits**—Serum samples were evaluated by the use of commercially available kits for equine serum: Horse IgG(T) ELISA Quantitation Set; (Bethyl Laboratories Cat. No. E70-105), Horse IgA ELISA Quantitation Set; (Bethyl Laboratories Cat. No. E70-116), Horse IgM ELISA Quantitation Set; (Bethyl Laboratories Cat. No. E70-114), Horse IgGa ELISA Quantitation Set; (Bethyl Laboratories Cat. No. E70-124), Horse IgGb ELISA Quantitation Set; (Bethyl Laboratories Cat. NO. E70-127), Horse IgE ELISA Quantitation Set; (ICL, Inc.), and four parameter logistics curves. Serum samples were diluted in order to fit the curve set by the standards. Dilutions for IgM and IgA was 1:10,000; IgG(T), IgGa, and IgGb was 1:50,000; and IgE was 1:100. Samples and standards were run in duplicate for each ELISA. All duplicate values were within 10% of each other. The intra-assay coefficient of variation was less than 7.2%, the inter-assay variation less than 3.9%. The minimal detectable concentration for IgA, IgM, and IgG(T) was 15.6 ng/ml, and for IgGa, IgGb, and IgE was 3.12 ng/ml.

**Data Analysis**—Data were analyzed using the MIXED procedure of SAS v 9.3 (SAS Institute Inc; Cary, N.C.).
RESULTS

Serum IgG(T) concentrations are shown in Figure 1. There were no differences in IgG(T) concentrations in foals due to maternal dietary yeast supplementation except on d 60 post-partum in which foals born from mares fed the yeast supplement had increased IgG(T) concentrations compared to control foals.

Serum IgGa concentrations are shown in Figure 2. Serum IgGa concentrations increased within 12 hr of birth and peaked at 90 d post-partum for foals in both treatment groups. There were no differences due to maternal dietary yeast supplementation at any time throughout the study.

Serum IgGb concentrations are shown in Figure 3. Similar to IgGa and IgGb concentrations increased within 12 hr of birth, regardless of treatment group. IgGb concentrations peaked at 12 hr post-partum for control foals compared to 24 hr for foals born from mares fed a live yeast supplement during late gestation and early lactation.

Total IgG concentrations were calculated by totaling IgGa, IgGb and IgG(T) concentrations and are shown in Figure 4. Total IgG concentrations peaked at 12 hr post-partum for control foals compared to 90 d post-partum for foals in the treatment group.

Serum IgA concentrations are shown in Figure 5. Low levels of IgA were detected in foals immediately after birth (d0) prior to the ingestion of colostrum. Peak IgA concentrations were observed at 12 hr post-partum. There were no differences due to treatment throughout the study.

Serum IgM concentrations are shown in Figure 6. Low levels of IgM were detected in foals immediately after birth (d0) prior to the ingestion of colostrum and increased with the age.
of the foal. Peak IgM concentrations were observed at 120 d of age. There were no differences in foal IgM concentrations due to maternal dietary yeast supplementation.

Serum IgE concentrations are shown in Figure 2. IgE concentrations peaked 12 hr post-partum and remained elevated at 24 hr for foals in both treatment groups. IgE concentrations decreased by 30 d post-partum and there were no differences in IgE concentrations due to maternal dietary yeast supplementation.

**DISCUSSION**

All of the serum immunoglobulins concentrations analyzed in the present study were consistent with normal ranges previously reported for foals (Jeffcott, 1974; Marti et al., 2009; Siciliano et al., 2009). Although it is widely recognized that there is no placental transfer of immunoglobulins in the horse (Jeffcott, 1974; Sedlinská, 2006; Wagner et al., 2006), low concentrations of immunoglobulins were detected prior to the ingestion of colostrum in the present study. These findings are supported by Tizard (2000) that found detectable amounts of serum IgM, IgG, and occasionally IgG(T) suggesting that there are some fetal immunoglobulins produced in utero that can be measured before colostrum uptake immediately. The initial increase in immunoglobulins from immediately after birth to 24 hr post-partum supports the absorption of maternal immunoglobulins via colostrum.

Maternal immunoglobulins in the foal start to decline at approximately three to four months of age. Holmes and Lunn (1991) determined that maternal IgG remained present in the foal up to approximately 90 days post-partum. In the present study, IgG(T), IgGa, total IgG and IgA concentrations increased, regardless of treatment, at 90 d post-partum but quickly declined by d 120 post-partum. In contrast, IgM concentrations increased with age of the foal and are similar
with previous research in horses demonstrating that IgM reaches adult levels by four to six months of age (Sedlinská et al., 2006).

While IgA did not differ between the control and yeast treatment groups, the foals in the yeast treatment group had consistently higher concentrations than the foals in the control group with the exception of d 0. IgA is the secretory immunoglobulin and is directly affected by the contents of the gut including the microflora population. By feeding a yeast supplement that is not part of the native equine microflora may have been a response of secretory IgA (Czerucka et al., 2007).

Several studies have shown that Saccharomyces cerevisiae will impact the immune system (Krakowski et al., 1999; Emmanuel et al., 2007; Corchonivoschi et al., 2010). When sows were supplemented with S. cerevisiae, varying effects were observed on the immunoglobulin concentrations of their piglets (Leszek et al., 2002; Jang et al., 2013). Krakowski et al., (1999) found that mares injected with 1,3/1,6 glucan, a component of yeast cell walls, had increased colostral IgG and IgG(T) concentrations and their foals had increased serum IgG(T) levels. Although immunoglobulin concentrations significantly differed over time in the present study, there were no differences in foal immunoglobulin concentrations from birth to four months of age due to the addition of S. cerevisiae to the maternal diet during late gestation and early lactation.

CONCLUSION

Overall, maternal dietary yeast supplementation during late gestation and early lactation did not influence foal serum immunoglobulin concentrations in this study.
REFERENCES

(Saccharomyces cerevisiae) on Humoral and Cellular Immunity of Jersey Cows in Early


Czerucka, D., T. Piche, and P. Rampal. 2007. Review article: Yeast as probiotics –

live cultures of Enterococcus faecium and Saccharomyces cerevisiae induces an


Jeffcott, L. B. 1974. Some Practical Aspects of the Transfer of Passive Immunity to Newborn

Effects of live yeast supplementation to gestation and lactation diets on reproductive

187-193.

immunostimulation of pregnant mares with 1,3/1,6 glucan and levamisole on the
immunoglobulins levels in colostrum, selected indices of nonspecific cellular and

nonspecific immunostimulation of pregnant sows on the immunological value of

transfer of IgE and subsequent development of IgE responses in the horse (Equus


FIGURES

**Figure 1:** Serum IgG(T) concentrations in mg/dL from d 0 through d 120 post-partum (n = 4 per time point per group). Bars represent least square mean ± SE. A * denotes the value is statistically significant (p = < 0.0001).

**Figure 2:** Serum IgG(a) concentrations in mg/dL from d 0 through d 120 post-partum (n = 4 per time point per group). Bars represent least square mean ± SE.
**Figure 3:** Serum IgG(b) concentrations in mg/dL from d 0 through d 120 post-partum (n = 4 per time point per group). Bars represent least square mean ± SE.

**Figure 4:** Serum IgG concentrations in mg/dL from d 0 through d 120 post-partum (n = 4 per time point per group). Bars represent least square mean ± SE.
**Figure 5:** Serum IgA concentrations in mg/dL from d 0 through d 120 post-partum (n = 4 per time point per group). Bars represent least square mean $\pm$ SE.

**Figure 6:** Serum IgM concentrations in mg/dL from d 0 through d 120 post-partum (n = 4 per time point per group). Bars represent least square mean $\pm$ SE.
Figure 6: Serum IgE concentrations in mg/dL from D 0 through D 120 post-partum (n = 4 per time point per group). Bars represent least square mean ± SE.