ABSTRACT

FRY, ROBERT SCOTT. Effect of Dietary Boron on Immune Function and Disease Resistance to Bovine Herpesvirus Type-1 in Growing Steers. (Under the direction of Dr. Jerry W. Spears).

Two experiments were conducted to examine the effects of dietary boron (B) on immune response and physiological responses to a viral disease challenge in growing steers. In experiment 1, 36 Angus and Angus × Simmental cross steers with an initial average body weight (BW) of 284 kg were fed one of three dietary treatments: 1) control (no supplemental B), 2) 5 mg supplemental B/kg DM, and 3) 15 mg supplemental B/kg DM from Na₂B₄O₇ for 47 d. The control diet analyzed 13.3 mg B/kg DM. On d 34 of the study steers were inoculated intranasally with bovine herpesvirus type-1 (BHV-1). Supplementation of dietary B increased ($P < 0.01$) plasma B concentration in a dose responsive manner. The change in DMI from d 0 (pre-inoculation) differed ($P < 0.05$) on d 1 and 2 among the control steers and those supplemented with 15 mg B/kg DM, due to an increase in DMI in control steers. The rectal temperature change from pre-inoculation values for steers supplemented with 15 mg B/kg DM tended ($P < 0.10$) to be less on d 6 and was less ($P < 0.01$) on d 7 and 8 post challenge than steers supplemented with 5 mg B/kg DM. On d 8 steers consuming the control diet had a greater decrease ($P < 0.05$) in rectal temperature change relative to d 0 when compared to steers supplemented with 5 mg B/kg DM. Inoculation of BHV-1 increased ($P < 0.01$) plasma concentrations of acute phase proteins by d 4 post challenge relative to d 0 values. Inoculation of BHV-1 decreased ($P < 0.01$) plasma IFN-γ concentrations on d 4, and increased ($P < 0.01$) plasma concentrations of TNF-α on d 2 post inoculation and d 4 TNF-α concentrations were lower ($P < 0.01$) than d 0 concentrations.
In experiment 2, 36 Angus and Angus × Simmental cross steers with an initial average body weight (BW) of 269 kg were used to determine the effect of dietary B on immune response. Steers were fed one of three dietary treatments: 1) control (no supplemental B), 2) 5 mg supplemental B/kg DM, and 3) 50 mg supplemental B/kg DM from sodium borate (Na₂B₄O₇·5H₂O) for 78 d. The control diet analyzed 10.2 mg B/kg DM. Supplementation of dietary B increased ($P < 0.01$) plasma B concentration in a dose responsive manner. Also, supplemental B tended ($P = 0.12$) to increase the blastogenic response of B-lymphocytes to pokeweed mitogen, but did not affect proliferation of T-lymphocytes when stimulated with concanavalin A or phytohaemagglutinin (PHA). Furthermore, specific anti-PRBC IgG titers tended to be affected by a treatment × day interaction ($P = 0.07$). Boron supplemented steers had greater ($P < 0.05$) IgG titers than controls on d 7 but not on d 14 or 21 post-injection. Specific anti-PRBC IgM titers were not affected by dietary B. Dietary B did not affect skinfold thickness following PHA injection. Boron supplemented steers tended ($P = 0.12$) to have greater ADFI than control steers. Average daily gain and G:F was not affected during the study.

Supplementation of dietary B had minimal effects on immune responses and disease resistance in growing steers. Lack of a significant effect of B was likely due to the B content in the basal diet. The basal diets containing 13.3 and 10.2 mg B/kg DM seemed to provide optimal physiological responses.
BIOGRAPHY

Robert Scott Fry was born in Searcy, Arkansas on March 6, 1983, to Eldon and Anne Fry of Quitman, Arkansas. He grew up on a cow/calf operation, later becoming a family owned stocker operation, where his father guided him in developing a passion for beef cattle production. Throughout his teenage years, Scott was actively involved in Future Farmers of America at Quitman High School, where he graduated in 2001. Upon graduation, he attended the University of Arkansas at Fayetteville receiving a Bachelor’s of Science in Animal Science. While in Fayetteville, not only did he obtain an excellent college education and experience, but he also met his future wife and best friend, Kayte Jo. After graduating from the U of A in 2005, Scott moved to North Carolina to attend North Carolina State University. Upon completion of his Master’s degree, Scott will continue his education at NCSU. This fall he will begin pursuit toward a Doctor of Philosophy in Animal Science, again under the direction of Dr. Jerry W. Spears.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Essentaility and Function of Boron in Plants</td>
<td>2</td>
</tr>
<tr>
<td>Boron in Human and Animal Nutrition</td>
<td>5</td>
</tr>
<tr>
<td>Metabolism of Boron</td>
<td>14</td>
</tr>
<tr>
<td>Boron and Immunity</td>
<td>15</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>23</td>
</tr>
<tr>
<td>CHAPTER 1. EFFECT OF DIETARY BORON ON PHYSIOLOGICAL RESPONSES IN GROWING STEERS CHALLENGED WITH BOVINE HERPESVIRUS TYPE-1</td>
<td>30</td>
</tr>
<tr>
<td>Introduction</td>
<td>31</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>32</td>
</tr>
<tr>
<td>Results</td>
<td>36</td>
</tr>
<tr>
<td>Discussion</td>
<td>40</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>45</td>
</tr>
<tr>
<td>CHAPTER 2. EFFECT OF DIETARY BORON ON IMMUNE FUNCTION IN GROWING STEERS</td>
<td>60</td>
</tr>
<tr>
<td>Introduction</td>
<td>61</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>62</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>67</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>72</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

## Chapter 1

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Composition of basal diet</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>Effect of dietary boron on performance of growing steers pre and post BHV-1</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>Effect of dietary boron and BHV-1 on plasma boron concentrations in growing</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td>Effect of dietary boron and BHV-1 on plasma interferon-gamma concentrations</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>Effect of dietary boron and BHV-1 on plasma tumor necrosis factor-alpha</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>Effect of dietary boron and BHV-1 on acute phase proteins in growing steers</td>
<td>54</td>
</tr>
<tr>
<td>7</td>
<td>Effect of dietary boron and BHV-1 on plasma haptoglobin concentration in</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>growing steers</td>
<td></td>
</tr>
</tbody>
</table>

## Chapter 2

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Composition of basal diet</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>Effect of dietary boron on performance of growing steers</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>Effect of dietary boron on plasma boron concentration in growing steers</td>
<td>77</td>
</tr>
<tr>
<td>4</td>
<td>Effect of dietary on lymphocyte blastogenesis in growing steers</td>
<td>78</td>
</tr>
<tr>
<td>5</td>
<td>Effect of dietary boron and lipopolysaccharide on tumor necrosis factor-alpha production from isolated peripheral bovine monocytes from growing steers</td>
<td>79</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

### Chapter 1

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of dietary boron and BHV-1 inoculation on change in dry matter intake in growing steers</td>
<td>56</td>
</tr>
<tr>
<td>2</td>
<td>Effect of dietary boron and BHV-1 inoculation on rectal temperature change in growing steers</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>Effect of BHV-1 inoculation on interferon-gamma and tumor necrosis factor-alpha concentration in growing steers</td>
<td>58</td>
</tr>
<tr>
<td>4</td>
<td>Effect of BHV-1 inoculation on acute phase proteins in growing steers</td>
<td>59</td>
</tr>
</tbody>
</table>

### Chapter 2

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of dietary boron on immunoglobulin G titer response to pig red blood cells in growing steers</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>Effect of dietary boron on immunoglobulin M titer response to pig red blood cells in growing steers</td>
<td>81</td>
</tr>
<tr>
<td>3</td>
<td>Effect of dietary boron on inflammatory response to phytohemagglutinin in growing steers</td>
<td>82</td>
</tr>
</tbody>
</table>
LITERATURE REVIEW

Chemistry of Boron

Boron (B) is a trivalent metalloid element that has an atomic mass of 10.811 g/mol. Elemental B can be found as either black monoclinic crystals or as a yellow-brown amorphous powder. On the other hand, boric acid (H$_2$BO$_3$), which is thought to be the most prevalent form of B in humans and animals, is colorless and odorless and is either present as a white granular powder or transparent crystalline form, which are easily solubilized in water (NRC, 2005).

In nature B is found either bound to oxygen or in the borate form. Organic compounds containing B bound to oxygen or nitrogen groups are referred to as organoboron compounds. These complexes can be found in plant, animal, and human tissues. There is experimental evidence to imply that the complexes are formed via interactions between B and the hydroxyl group of alcohols to produce ester compounds;

\[ B(OH)_3 + 3ROH \leftrightarrow B(OR)_3 + 3H_2O \]

Adjacent cis-hydroxyls form the best complex with B. Furthermore, compounds with two or more hydroxyl groups seem to react more strongly, thereby increasing the intensity of the reaction as the number of adjacent hydroxyl groups increase (Duggar, 1983). Some examples of biological compounds that contain adjacent cis-hydroxyl groups include riboflavin, adenosine 5-phosphate, pyridoxine, pyridine nucleotides, and sugar molecules (Hunt, 1998).

Boric acid is a Lewis acid (\(K_a = 6 \times 10^{-10}, pK_a \approx 9.1\)), in which it accepts a hydroxyl ion, not donating protons, to form the tetrahedral anion B(OH)$_4$; \(B(OH)_3 + H_2O \leftrightarrow B(OH)_4^{-} + H^+\). Of the species of B, only \(B(OH)_3\) and \(B(OH)_4^{-}\) are present at low
concentrations of B ($\leq 0.02 \, M$). Increasing concentrations of B coupled with a higher pH advocates formation of polynuclear ions. Furthermore, an increase in pH above 10 allows for selective production of $B(OH)_4^-$ (Power and Woods, 1997).

Of the non-metals, B has the lowest electronegativity, thus it is usually oxidized in biological reactions. Some of the most frequently used B compounds are anhydrous, pentahydrate and decahydrate forms of disodium tetraborate or borax (Na$_2$B$_4$O$_7$), boric acid (H$_3$BO$_3$), and monohydrate and tetrahydrate forms of sodium perborate (NaBO$_3$) (NRC, 2005). Uses of boric acid and borates are rather diverse, with these compounds found in heat-resistant borosilicate glass (e.g. Pyrex), preservatives for leathers and wood, detergents, insecticides, and fertilizers (Power and Woods, 1997; NRC, 2005). Elemental B and its carbides and nitrides are primarily used in steel-making and high-temperature abrasives, whereas, halides and hydrides of B are used catalytically and in fuels of jets and rockets (Larsen, 1988).

**Essentiality and Function of Boron in Plants**

Evidence of the essentiality of B in plants appeared in 1923 when Warrington found signs of B deficiency in several leguminous plants. Soon after Warrington’s report, Sommer and Lipman (1926) also reported that B was essential for the life cycle of numerous plants to be complete. It has been discovered that B easily forms complexes with a variety of sugars moieties and other biological compounds that contain cis-hydroxyl groups (Hunt, 1998).

The proposed role of B in plant cell wall structure was confirmed by the isolation, characterization, and *in vivo* action of rhamnogalacturonan-II-boron (RG-II-B)
complexes, which illustrated a B cross-link between apiose residues in pectin that also bind with calcium (Ca) (Bolanos et al., 2004). Boron binding and linking to hydroxyl-containing compounds, such as phosphoinositides, glycoproteins, and glycolipids is the foundation of evidence that B has a role in membrane composition. Extensive studies have been conducted that are supportive of the idea that B functions in plants at the cell membrane level. In 1983 Parr and Loughman made a rather convincing claim that deficiency of B results primarily in a disrupted membrane function. Accumulation of phenols and auxins, as well as an increase in the activity of RNAases are secondary symptoms that are associated with impaired membrane function that has been observed in B deficient plants (Robertson and Loughman, 1974; Dave and Kannan, 1980). Boron does not play a role in the synthesis of membrane constituents. There has not been any further evidence biochemically to explain any additional direct roles of boron in membrane integrity.

The uptake of numerous nutrients by the membrane has also been shown to be impaired by a B deficiency in plants, and can be reversed with rather small amounts of borate solution. An example to exemplify this mode of action was seen with leakage of potassium (K⁺) from B deficient sunflowers. The contrast between B-adequate and B-deficient plants resulted in a pronounced difference of a 35-fold increase in the amount of K⁺ leakage from the leaf (Cakmak et al., 1995).

Boron also has an effect on various enzymatic processes. The immobilization of the urease enzyme on the cell membrane is inhibited by boric acid, which is the only effective inhibitor of the enzyme (Zaborska, 1995). Alcohol dehydrogenase enzyme of yeast is also inhibited by boric acid. The enzyme inhibition is successful by B because B
resides in the active site thereby displacing the normal substrate (Power and Woods, 1997). Boron deficiency in plants disables the inhibition of the enzymes 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase resulting in an accumulation of phenols (Gomez-Rodriguez et al., 1987). This overactive mechanism causes an increase in the amount of substrate metabolized and causes an increase in phenolic compounds and subsequently causes cell death of plants in the subclass Dicotyledoneae (Shkol’nik and Il’inskaya, 1975).

In plants, it was discovered that B is required for plant signaling, specifically for nod-gene activation. Furthermore, for infection thread development and nodule invasion to occur B is required due to its role as a modulator of the interactions between plants derived infection thread matrix glycoproteins and the bacteria cell surface (Bolanos et al., 2004). In the presence and the absence of B in the cell, glycoproteins behave differently. While B is absent, the glycoproteins can attach to the cell surface of the rhizobia, thus the bacterium can be enclosed and interaction with the plant cell membrane is hindered resulting in the inhibition of the endocytosis process. However, in the presence of B, the in vitro bacteria-matrix glycoprotein attachment process is inhibited and rhizobial interaction with the plant membrane is now promoted. When Rhizobium reaches the interior of the cell, B promotes symbiosome development. Boron is specifically needed for pinpointing nodule-specific glycoproteins.

The discovery of autoinducer AI-2 is the most recent finding of a B-containing bacterial signal molecule having an unexpected role for B in quorum sensing (Chen et al., 2002). With this new role, bacterial populations are allowed to observe cell density via diffusible pheromones that are accumulated in the extracellular space as the cell
numbers increase (Bolanos et al., 2004). AI-2 is a novel-signaling molecule for both structure and functions and has been identified as a furanosyl borate diester (Bolanos et al., 2004). It is plausible that AI-2 could serve as both a universal bacterial signal and also serve as a B transporter (Chen et al., 2002). Additional research with AI-2 is needed to further understand its underlying mechanisms (Bolanos et al., 2004).

Boron interacts with calcium (Ca) in the signal transduction pathway. This interaction has been observed in pea nodule development and establishment under salt stress. Genes involved in the cell cycle, cell wall assembly, and ribosomes have been shown to be affected by B deficiency, and in some cases this could be reversed by Ca supplementation. The interaction between Ca and B has been observed in plants, bacteria, animals and humans, but the specific nature of the interaction is still under experimental investigation (Bolanos et al., 2004).

**Boron in Human and Animal Nutrition**

Research with B and animal nutrition was first published between the late 1930s and 1940s (Hunt, 1994). In these early studies, diets were either nutritionally inadequate in nutrients other than B, or were supplemented with high concentrations of B (100-2200 mg B/kg diet). These studies mostly hypothesized that supplemental B had a positive effect on animal performance (Hunt, 1994). When discussing the effects of supplemental B, it is important to take into account the basal level of B in the control diet. Animal studies with B have involved B supplementation to diets extremely low in B, as well as those evaluating B addition to more practical type diets containing a higher B concentration.
In 1981, Hunt and Nielsen evaluated the essentiality of B in chicks. In this study and in subsequent studies from their laboratory, diets low in B were prepared by acid-washing corn, to remove most of the B, and selecting other ingredients that were low in B. Protein was provided in these studies from casein, since animal protein is much lower in B than plant protein sources. Hunt and Nielsen (1981) reported an interrelationship between B and cholecalciferol, which reflected contrasting effects of dietary B on chicks that were deficient or adequate in cholecalciferol. When 0 or 3 mg B/kg diet was supplemented to diets containing 125 or 2500 IU/kg of cholecalciferol, there was an increase in growth by 38% in B-supplemented chicks compared to the B deprived chicks fed a cholecalciferol deficient diet. The non-boron supplemented diet used in this study contained only 0.28 $\mu$g B/g diet. Chicks receiving adequate cholecalciferol (2500 IU/kg) along with supplemental B, only grew 11% faster than B deprived chicks receiving adequate cholecalciferol (Hunt and Nielsen, 1981). They suggested that these differences in growth between the treatment groups could be due to the impact of B on energy substrate utilization via inhibition of glycolytic enzymes.

More recent evidence also suggests that B affects energy utilization in the chick (Hunt, 1994). Hunt and Nielsen (1987) conducted a study to evaluate the interaction between B, magnesium (Mg), and vitamin D$_3$. Dietary B contained in the respective diets was 3.04 mg B/kg diet compared to 0.04 mg B/kg diet. Chicks receiving the high dietary B coupled with a vitamin-D$_3$ deficiency experienced a decrease in the abnormally elevated plasma glucose concentrations by 29%; whereas, chicks receiving the same level of B, but with adequate supplementation of vitamin D$_3$, experienced a reduction of only 6% in glucose concentration (Hunt and Nielsen, 1987). Hunt (1989) further examined
this mode of action by B by evaluating it along with Mg and molybdenum (Mo) supplementation. Boron supplementation (3.47 vs. 0.05 mg/kg diet) coupled with inadequate or adequate Mg and in the presence or absence of supplemental Mo, decreased plasma glucose levels by similar percentages as those recorded previously. This response of dietary B has also been seen in postmenopausal women. Postmenopausal women consumed a low Mg diet containing either 3.23 or 0.23 mg B/day. Serum glucose concentration was approximately 6% lower in women consuming the high vs. low B level.

The strongest evidence of the essentiality of B is in zebrafish, trout, and *xenopus laevis*. Rowe and Eckhert (1999) evaluated the effect of varying levels of B as boric acid in water where zebrafish resided. Wild-type zebrafish were exposed to low B water (0.1 μmol B/L) or B supplemented water (45 μmol B/L) for six months prior to spawning. Fertilized eggs were removed from the tanks and placed in small cups containing the same media as their respective treatment groups. In the low B exposure group, 92% of the embryos died by 10 days post-fertilization compared with less than 10% mortality during this same period in the B-supplemented group. Transferring low B zygotes from their treatment group to B-supplemented water alleviated zygote death. After the B-deficient zygotes were exposed to B, there was an approximately 98% survival rate the first 24 hours in contrast to only a 19% survival rate in the low B group (Rowe and Eckhert, 1999).

Increasing water B concentration from 2 to 11 μmol B/L increased growth of trout during the embryonic and early larval stages by 8% (Eckhert, 1998). A follow-up study suggested that adequate B was more critical for growth during the embryonic than the
larval stage of development. Increasing the level of B up to 925 μmol B/L resulted in no morphological or swimming defects in embryos or larvae exposed to this high level (Eckhert, 1998).

Fort et al. (1999) provided evidence of the essentiality of B in *xenopus laevis* for reproduction. Adult frogs were given one of three dietary treatments: a non-purified beef liver and lung diet (BLL; 310 μg B/kg), a purified diet low in B (45μg B/kg) or the purified diet supplemented with B (1850 μg B/kg) for 120 days. Boron supplemented frogs and those fed the non-purified diet produced less necrotic eggs compared to frogs receiving the low B diet (12.2 and 11.3% vs. 54%). Also adults fed low B diets possessed less embryos during collections as opposed to those receiving supplemental B. Frogs consuming low B diets possessed embryos with a higher frequency of malformations than those fed the non-purified diet or the B supplemented diet. In the repletion phase of the experiment, 10 μmol B/L was added to low B embryos in an attempt to reverse the effects of low B exposure; however, the adverse effects of low B were not reversed contrasting results observed in zebrafish when zygotes from B-depleted water were transferred to B-supplemented water (Rowe and Eckhert, 1999).

Recent isolation of a B transport protein provides further evidence for B essentiality in animals. Park and coworkers (2004) isolated the transport protein, NaBC1 (initially called BTR1) that is ubiquitously expressed and can be located in the basolateral membrane of salivary gland acinar cells, kidney tubules, pancreas, liver and spleen. This robust transporter, a homolog of AtBor1, (found in plants by Takano et al., 2002) conducts Na⁺ and OH⁻ transport in the absence of borate, and while in the presence of
borate it serves as an electronegative Na⁺-coupled borate cotransporter (Park et al., 2004). Further characterizing the function of the transporter, researchers reported that arsenic is not taken up by NaBC₁. Boron and arsenic are metalloids that share similar pka values (9.25 vs. 9.23, respectively), making it plausible for both to be taken up through the ion exchange channels. NaBC₁ did not transport arsenic at concentrations as high as 20 mM (Park et al., 2004).

A number of studies have also evaluated B supplementation of practical corn-soybean meal based diets in poultry. The addition of 5 mg B/kg to diets of broilers from 1 day until 21 days of age increased body weights in males but not in females (Rossi et al., 1993). The practical corn-soybean meal diet used in the study contained 9.4 mg B/kg. Broilers (regardless of sex) supplemented with 5 mg B/kg also had a greater tibia breaking load and tibia weight at 21 days of age compared to controls. Higher additions of B (40 to 120 mg/kg diet) in this study did not significantly affect body weights or bone characteristics relative to controls. In a subsequent study from the same laboratory, the addition of 60 mg B/kg to a control diet containing 22.3 mg B/kg did not affect body weights or bone characteristics in broilers (Rossi et al., 1994). Kurtoglu et al. (2001) reported that additional B at levels of 5 and 25 mg B/kg to diets containing inadequate vitamin D₃ (250 IU kg) resulted in increased body weight gains on days 14 and 28 in broilers compared to no additional B coupled with inadequate vitamin D₃. Broilers consuming 25 mg B/kg and 2500 IU kg were more efficient during the first 28 days of the study when compared to the other treatment groups (Kurtoglu et al., 2001).

Wilson and Ruszler (1997) evaluated B supplementation in growing pullets beginning at 1 day of age. The control diet contained 14.7 mg B/kg and B was
supplemented at 0, 50, 100, 200 or 400 mg B/kg diet. Boron addition at 50 or 100 mg/kg diet improved the strength of the tibia at 14 weeks of age. The two higher concentrations of supplemental B did not affect bone strength and birds fed 400 mg B/kg diet had reduced body weight gains. Wilson and Ruszler (1998) supplemented B to one-day-old White Leghorn chicks at levels of 0, 50, 100, 200, and 400 mg B/kg diet as boric acid. The control birds received a basal diet containing 3 mg B/kg diet. By 32 weeks of age, the shear force of the tibia was higher in birds supplemented with B (regardless of level of B) than in controls. However, by 52 weeks of age this effect had ceased. The shear fracture energy also decreased from week 32 to 72, thereby making the bone become more brittle over time. In a follow-up study they evaluated 0, 50, 100, 200, and 400 mg B/kg, but began the birds on the treatments at 32 weeks of age. Interestingly, when birds were started on 200 mg B/kg diet at 32 weeks of age the birds experienced an increase in their shear fracture energy of the tibia and radius at 72 weeks. Birds supplemented with 400 mg B/kg experienced a decrease in feed consumption. Boron supplementation increased bone strength by approximately 45% compared to those birds on the control diet. These data are indicative that supplementation of 200 mg B/kg diet starting at 32 weeks age will significantly decrease brittle characteristics at the end of egg production (Wilson and Ruszler, 1998).

The effect of B on bone metabolism has also been evaluated in rodent models. Chapin et al. (1997) fed B to rats (60-70 days of age initially) as boric acid at levels of 525, 788, 1050, and 1575 mg B/kg diet for 9 weeks. No performance variables were affected by boric acid up to 1050 mg B/kg diet. However, animals receiving 1575 mg B/kg diet experienced an 11% decrease in feed consumption and they ended the study
weighing 16% less than the control animals. Strength characteristics of the tibia and femur were not affected by dietary B. In a second experiment, young adult rats were fed dietary B at levels of 0, 35, 175, 525, or 1575 mg B/kg diet boric acid for 12 weeks. Strength characteristics of the tibia and femur bone were not affected by dietary B. However, supplemental B concentrations up to 525 mg B/kg diet increased bone strength measurements (yield load, break load, yield stress, and break stress) of the vertebra (Chapin et al., 1997).

Boron has recently been studied in swine nutrition. Armstrong et al. (2000) evaluated supplemental B as sodium borate at levels of 0, 5, and 15 mg B/kg diet in both a natural ingredient (6.7 mg B/kg diet) and a semi-purified diet (0.98 mg B/kg diet) for weanling pigs (21 days of age). Supplementation of B to the natural ingredient diet had no effect on ADG, ADFI, G:F, plasma minerals or metabolites, and bone characteristics during the 40 day study. In experiment 2, feed efficiency was improved when 5 mg B/kg diet was added to the semi-purified diets of the weanling pigs compared to the pigs receiving the control diet and 15 mg B/kg diet. In this experiment, male pigs supplemented with B possessed lower bone lipid concentrations compared to the control pigs with no effect seen in female pigs. Plasma cholesterol concentrations were higher in pigs supplemented with 5 mg B/kg diet while pigs receiving 15 mg B/kg diet had higher plasma triglyceride concentrations than the controls. Concentration of plasma triglycerides and cholesterol did not differ among pigs receiving 5 and 15 mg B/kg diet. Furthermore, the weight and length of the femurs of both sexes were not affected by supplemental B in pigs fed the semi-purified diet (Armstrong et al., 2000).
Armstrong and Spears (2001) also evaluated the effect of sodium borate on growth, Ca and P metabolism, and bone mechanical properties in growing barrows. The basal diet contained 1.9 mg B/kg and 2.2 mg B/kg diet during the nursery and growing phases, respectively. Boron was supplemented to the basal at 0, 5, and 15 mg B/kg of diet. Barrows receiving additional B during the nursery and growing phase had increased ADG and ADFI compared to barrows receiving no additional B with no effect on G:F. There were no differences in ADG or ADFI among barrows receiving 5 or 15 mg B/kg diet. Addition of B to the basal diet increased bone P and ultimate shear force of the fibula bone was greater in pigs fed 15 mg B/kg than in those given 5 mg B/kg. However, B did not affect bone stress, cross-sectional area moment of inertia or bending of the femur (Armstrong and Spears, 2001).

Armstrong et al. (2001, 2002) investigated the long-term effects of dietary B on performance, reproductive characteristics and bone mechanical properties in gilts. Pigs were supplemented with 0 or 5 mg B/kg diet from weaning through sexual maturity, breeding, gestation, and lactation. The nursery, growing-finishing, gestation and lactation basal diets analyzed at 0.98 mg B/kg, 2.1 mg B/kg, 2.1 mg B/kg, and 2.9 mg B/kg diet, respectively. Gilts receiving supplemental B had higher body weight gains than controls throughout the finishing and growing-finishing phases (0.79 kg/d vs. 0.83 kg/d; 0.79 kg/d vs. 0.82 kg/d, respectively). Boron supplementation tended to increase ADFI during the growing-finishing phase when compared to non-boron supplemented gilts (2.01 kg/d vs. 2.12 kg/d). Boron supplemented gilts had numerically fewer dead embryos and had numerically higher litter weaning weights that averaged 8.0 kg heavier than non-supplemental gilts. The concentration of B in the embryos were increased
approximately threefold in B-supplemented gilts compared to the controls (1271 mg B/kg vs. 452 mg B/kg) (Armstrong et al., 2002). Additionally, Armstrong et al. (2001) showed that 5 mg B/kg diet added to the basal diet containing 2.1 mg B/kg diet reduced serum concentrations of T₃ and T₄ in gilts during the growing phase. Serum osteocalcin concentrations seemed to increase in those pigs receiving B, which may be indicative of increased osteoblast activity or overall bone turnover.

In 2003, Armstrong and Spears obtained offspring from sows that were receiving 2.2 or 5 mg B/kg diet prior to parturition and during lactation. At 21 days of age, pigs began to consume a basal diet containing 2.2 mg B/kg or an additional 5 mg B/kg. At the end of the nursery phase, pigs receiving additional B had higher body weight gains (0.47 kg/d vs. 0.36 kg/d) and also maintained higher weights throughout the growing phase than the control pigs (1.00 kg/d vs. 0.85 kg/d). The increased ADG during the growing phase was due to the increase in ADFI in B-supplemented pigs (2.48 kg/d vs. 2.16 kg/d). There were no differences reported in feed efficiency in either treatment group (Armstrong and Spears, 2003).

Boron has received little attention in ruminant nutrition. Green and Weeth (1977) reported that Hereford heifers (initial weight 288 ± 22.7 kg) consuming drinking water containing 150 and 300 mg B/L exhibited swelling and irritation in their legs and around the dew claws. Heifers consuming 300 mg B/L exhibited slight diarrhea and were lethargic when compared to other treatment animals. Even though heifers experienced abnormal biological functions, they were capable of walking, but consumed less hay and experienced decreased body weight gains compared to control heifers consuming 0.8 mg
B/L. The symptoms associated with high levels of B were not permanent (Green and Weeth, 1977).

Brown et al. (1989) evaluated the effects of dietary B on mineral balance in sheep. The basal diet supplied 30 mg B/hd/d. Boron supplemented wethers consumed either 75 or 170 mg or supplemental B/hd/d. Feed intake was not affected by dietary B in this study. Throughout the duration of the trial increasing levels of dietary B tended to improve Ca retention. Magnesium balance was improved only when comparing those wethers receiving supplemental B at 170 mg B/hd/d compared to control animals receiving 30 mg B/hd/d. These data suggest that B could have a practical role in the prevention of metabolic disorders such as grass tetany that occurs in cattle (Brown et al., 1989). Small et al. (1996) evaluated the effect of serum mineral levels and fertility in beef heifers and cows on pasture. Researchers discovered that serum B concentrations on day 21 of the study analyzed higher in early conceiving animals.

Results of the effects of dietary B on performance of animals and humans have been variable. Data suggests that age, species, and more importantly the basal level of B in the diet are important variables affecting the overall response of dietary B.

Metabolism of Boron

The mechanism of B absorption is not well understood mainly due to no useable radioisotopes of B. Sodium borate and boric acid, as well as food B are rapidly absorbed (DRI, 2001). Studies with animals and human subjects suggest that approximately 90 percent of B is absorbed when consuming a normal daily intake, and excretion occurs primarily via the urine. As B is absorbed, it is suggested that it is hydrolyzed in the gut and converted to B(OH)₃ which can be easily absorbed. Boron absorption likely occurs
via a passive, non-mediated diffusion involving B(OH)$_3$. Boron is found primarily as free B(OH)$_3$ in blood (DRI, 2001).

Both human and animal studies have been conducted indicating that B is rapidly absorbed and excreted in the urine. Postmenopausal women consuming 0.36 mg B/d from food excreted 89 percent of consumed B via urine and 3 percent in feces (Hunt and Stoecker, 1996). Furthermore, Sutherland et al. (1998) reported that men consuming an average B intake of 3.73 mg/d over a 42 day period excreted 3.20 mg/d B via urine. In a rodent study, 20 μg of $^{10}$B isotope was fed to rats, and 95 percent of the isotope was discovered in urine and 4 percent in feces (Nielsen, 1997).

**Boron and Immunity**

Research from a number of animal studies indicates that B is of nutritional importance and affects various immune processes (Nielsen, 2002; Hunt and Idso, 1999; Armstrong et al., 2001). The immune system consists of a complex array of cellular responses originating from bone marrow and the lymphatic and mucosal systems (Janeway et al., 2005). It is essential to understand the types of responses that the immune system elicits. Upon stimulation of the immune system, the innate immune response occurs first, rapidly responding, recruiting cells to the site of invasion of foreign substances in the host; this response occurs with short duration. During this process, inflammation occurs due to cellular communication via secretion of cytokines and chemokines from macrophages. The adaptive immune response occurs over a longer period of time after microbial invasion, but with a more substantiated response, in which lymphocytes proliferate and specific antibodies are formed that are directed to the foreign
invader. Throughout the adaptive immune response, immunological memory is formed via memory T and B-lymphocytes (Janeway et al., 2005).

*Boron and Inflammation*

The inflammatory response is a protective mechanism of tissues in the presence of microbial invasion or injury and is a key element to the innate immune response. The inflammatory process is important in localizing innate immune cells, such as neutrophils and monocytes as well as complement proteins, to the site of infection (Janeway et al., 2005). During the inflammatory response blood vessels become dilated and more permeable, resulting in an increased blood flow and fluid leakage that causes heat, redness of skin, and swelling at the site of infection or injury. Dilation of blood vessels allows the infiltration of phagocytes and complement components to enter and combat foreign substances. The complement system consists of plasma proteins that are responsible for coating the surfaces of antigenic and microbial cell surfaces that are recognized by phagocytes for phagocytosis (Janeway et al., 2005).

Although inflammation is important to the host defense mechanism, an excessive amount of inflammation can result in a number of inflammatory diseases, such as osteoarthritis. Both humans and animals have experienced major complications associated with this disease. In a double-blind study, Travers Fracp (1990) reported that after an 8-week treatment period, 71% of the subjects consuming 6 mg of B per day experienced improvements in subjective measurements of arthritis compared to only a 12% improvement in the placebo subjects. Newnham (2002) also reported that in some cases humans receiving supplemental B experienced a decrease in arthritic symptoms.
There are studies with animals indicating that dietary B is advantageous in reducing the inflammatory response (Hunt and Idso, 1999; Armstrong et al., 2001; Armstrong and Spears 2003). Dietary B decreased paw swelling in rats with adjuvant-induced arthritis suggesting that B has an effect on the inflammatory response (Hunt and Idso, 1999). Armstrong et al. (2001) reported that 5 mg B/kg added to a basal diet containing 2.2 mg B/kg reduced skinfold thickness during the growing-finishing phase in gilts at hours 6 and 12, with numerical differences at 24 and 48 hours post-injection of phytohematogluttin (PHA). Armstrong and Spears (2003) reported that pigs receiving 5 mg B/kg in addition to a basal diet containing 2.2 mg B/kg had a reduced inflammatory response at the site of PHA injection at hours 6, 12, 24, 48 post-injection of PHA. Research has shown that PHA injection into chicks stimulates the infiltration of neutrophils and macrophages resulting in increased inflammation (McCorkle et al., 1980).

It is pertinent to understand the role of macrophages during microbial invasion and at the site of infection. An adaptive immune response specific to the foreign invader does not occur if the macrophage is able to combat invasion without additional help from T-lymphocytes. However, macrophages that binds or ingests foreign material, but fail to eliminate the invader, possesses a role in the adaptive immune response acting as an antigen-presenting cell (Janeway et al., 2005). Macrophages not only engulf foreign particles but also phagocytose damaged cells and tissues. However, macrophages are also responsible for releasing angiogenic factors (e.g. TNF-α and VEGF) and growth factors that activate and promote fibroblast proliferation subsequently causing the synthesisization and secretion of collagen and proteoglycans.
In a study with nude mice, it was reported that B, as boric acid (3%), stimulated and potentiated the release of angiogenesis factors (TNF-α and VEGF), as well as hsp-70. Sponges containing boric acid or HCl were placed under the skin on the backs of the nude mice. In the process of wound healing, these data suggested that B enhances the formation of blood vessels as well as the synthesis of hsp 70, which provides the cell protection from stress (Dousset et al., 2000). In cell culture supernatant from pelvic cartilage of chick embryo, boric acid increased concentrations of TNF-α, collagen and proteoglycans (Benderdour et al., 1997). Furthermore, boric acid addition to human fibroblast resulted in increased secretion of TNF-α and TNF-α mRNA as well as collagen and proteoglycans into culture medium (Benderdour et al., 1998).

Modification of the inflammatory response by dietary B is not well understood. There are a number of possible modes of action in which B may alter inflammation that have been suggested based primarily on indirect research findings (Hunt and Idso, 1999). As discussed previously, the inflammatory response recruits phagocytic cells, such as neutrophils and macrophages to the site of infection to combat invasion of microorganisms and eliminate damaged tissues and cells. During this process, reactive oxygen (superoxide radical, hydrogen peroxide, hydroxyl radical) and nitrogen species (nitric oxide) are produced that are toxic to the foreign invader. Conversely, the generation of these reactive species also leads to tissue destruction resulting in chronic inflammation (Janeway et al., 2005). Furthermore, phagocytes release superoxide in large quantities. The dominant electron donor for the reduction of oxygen in the respiratory burst process is NADPH. This reaction is shown below and is catalyzed by NADPH oxidase (Hunt, 1998): $NADPH + 2O_2 \rightarrow NADP^+ + 2O_2^- + H^+$. It has been
suggested that B may be important in regulating the respiratory burst, given that B regulates the pentose-phosphate pathway in plants via inhibition of enzymes such as 6-phosphoglucomutase (Hunt, 1998).

Some evidence suggests that B may also reduce tissue damage from inflammation by hastening the destruction of reactive oxygen species via increasing activities of key antioxidant enzymes (Hunt and Idso, 1999). Pawa and Ali (2004) reported data supporting this hypothesis. In this study, rats were administered orally 0.07 or 0.45 mg B/kg body weight for 3 days prior to injection of thioacetamide (TAA). Thioacetamide is a compound containing liver damaging properties that induces liver necrosis and acute liver failure. The oral administration of B reduced liver damage in rats that were given TAA. The antioxidant enzyme activity of glutathione peroxidase and catalase were increased in rats with B post-administration of TAA; however, there were no effects of B in rats not administered TAA (Pawa and Ali, 2004). These data suggest that B advocates the protection of tissue upon cellular challenge by increasing protective antioxidant enzymes.

*Boron and Cytokines*

Cytokines are proteins that are released from phagocytes in response to infection and serve as a communication mechanism among various cell types. These proteins are associated with inflammation, as well as fever and anorexia.

Armstrong and Spears (2003) studied the effects of dietary B on the release of cytokines in pigs following injection of E. coli lipopolysaccharide (LPS). Lipopolysaccharide is a component of the cell wall of gram-negative bacteria and small amounts can elicit a pronounced immune response (Janeway et al., 2005). Injection of
LPS resulted in an increase in TNF-α production when compared to saline injected pigs. The addition of 5 mg B/kg to a basal diet containing 2.2 mg B/kg in experiment 1 increased serum TNF-α concentration at 2 hours and tended to increase at 6 and 24 hours post-injection of 100 μg/kg BW LPS. In experiment 2, administering 25 μg/kg BW LPS resulted in numerical increases in TNF-α concentrations in pigs consuming additional B compared to non-supplemented pigs. Isolated peripheral blood monocytes from B-supplemented pigs (not injected with LPS) produced numerically higher concentrations of TNF-α upon in vitro stimulation of LPS when compared to controls. Dietary B supplementation increased serum IFN-γ concentrations at 6 hours and a numerical increase was present at 24 hours post-injection of LPS when compared to saline injected pigs in experiment 1. In experiment 2, IFN-γ production was similar to the results of TNF-α, as there were only numerical tendencies across sampling times. Media containing 0.25% boric acid resulted in a greater release of TNF-α in the medium of cultured normal human fibroblast (Benderdour et al., 1998) and in chick embryos (Benderdour et al., 1997).

Cell receptors that recognize LPS are found on dendritic cells and macrophages. These cells are associated with TLR-4 (Toll-like receptor 4), which is a stimulus of the transcription factor NFκB that subsequently induces the expression of TNF-α (Janeway et al., 2005). Tumor necrosis factor- alpha is a proinflammatory cytokine that induces production of other anti-inflammatory cytokines. It is also a stimulus in the initiation of the acute phase response. During the acute phase response, the concentration of various plasma proteins goes down while others markedly increase. These increased plasma proteins...
proteins are known as acute phase proteins, and are stimulated by the proinflammatory cytokines TNF-\(\alpha\), IL-6, and IL-1 (Janeway et al., 2005). Acute phase proteins play a role in scavenging oxygen radicals and in the enhancement of wound healing.

Interferon gamma is responsible for innate and adaptive immune responses and is predominantly produced by macrophages, cytotoxic T-lymphocytes, and natural killer cells. Production of IFN-\(\gamma\) activates macrophages with subsequent production of nitric oxide that has antiviral activity.

An increase in core body temperature occurs in response to the invasion of foreign substances into the body and is thought to be mediated by cytokines (Blatteis and Sehic, 1998). Limited research exists evaluating the effects of dietary B on regulation of core body temperature. Experimental evaluation of body temperature is often conducted using LPS. Armstrong and Spears (2003) rectally measured core body temperature in LPS-challenged (100 \(\mu\)g/kg BW, experiment 1; 25 \(\mu\)g/kg BW, experiment 2) pigs consuming either 2.2 mg B/kg or an additional 5 mg B/kg. In experiment 1, at 2 and 6 hours following LPS injection, control pigs had lower rectal temperatures than the B-supplemented pigs. However, in experiment 2 rectal temperatures were not affected by dietary B (Armstrong and Spears, 2003).

**Boron and Adaptive Immunity**

At the present time there is limited research on the effect of dietary B on the adaptive immune response. Bai and coworkers (1997) reported that rats consuming a control diet containing 0.15 mg B/kg had lower concentrations of anti-typhoid IgM and IgG when compared to rats consuming 1.75 mg B/kg following injection of typhoid vaccine. However, in pigs, the addition of 5 mg B/kg to the basal diet containing 2.1 mg
B/kg resulted in no effect on specific IgM or IgG production following injection of sheep red blood cells (Armstrong et al., 2001). The differences in responses to B supplementation in these studies are likely due to the differences in dietary B concentration in the control diets (0.15 mg B/kg vs. 2.1 mg B/kg). Furthermore, additional B did not affect the in vitro blastogenic response of isolated porcine lymphocytes exposed to mitogenic stimulation (Armstrong et al., 2001).
Literature Cited


Washington, D.C.


Chapter 1

Effect of dietary boron on physiological responses in growing steers challenged with bovine herpesvirus type-1\textsuperscript{1,2}

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Introduction

Boron (B) was first reported to be essential in the completion of the lifecycle in several leguminous plants (Warrington, 1923; Sommer and Lipman, 1926). More recently B has been shown to be essential for zebrafish (Rowe and Eckhert, 1999) and frogs (Fort et al., 1999). Substantial evidence also suggests that B is required by mammals, although a specific biochemical role for B has not been established (NRC, 2005).

Recent studies indicate that dietary B may affect immune responses (Hunt and Idso, 1999; Armstrong et al., 2001; Nielsen, 2002). Supplementation of 2 mg B/kg to a low B diet (0.1 mg B/kg) decreased paw swelling in rats with adjuvant-induced arthritis, suggesting B has an affect on the inflammatory response (Hunt and Idso, 1999). Armstrong et al. (2001) reported that addition of 5 mg B/kg DM to a basal diet, containing 2.2 mg B/kg DM, reduced skinfold thickness in pigs following an intradermal injection of phytohemagglutinin. Increasing dietary B from 2.2 mg B/kg DM to 7.2 mg B/kg DM also potentiated an increase in TNF-α production following lipopolysaccharide administration to pigs (Armstrong and Spears, 2003).

Boron has received little attention in ruminant nutrition. Studies have indicated that B (provided in the drinking water) has a low order of toxicity in cattle (Green and Weeth 1977). Small et al. (1996) reported a positive relationship between plasma B concentration and conception rates in beef heifers and cows grazing pasture. The present study was conducted to determine if dietary B modifies physiological responses of steers to inoculation with bovine herpesvirus type-1 (BHV-1).
Materials and Methods

Animal Care and Handling

Care, handling, and sampling of the steers were approved by the North Carolina State University Animal Care and Use Committee. Thirty-six Angus (n = 12) and Angus × Simmental (n = 24) steers with an initial body weight of 284 kg were obtained from the North Carolina State University cow-calf facility. Steers were vaccinated two months prior to weaning with Vision 7® Clostridium (Intervet; Millsboro, DE). Steers were weaned and moved the same day approximately 2 km to our feedlot facility. Steers were blocked by weight within breed and randomly assigned to one of 18 covered, slotted floor pens. Pens were randomly assigned within a block to dietary treatments. Treatments consisted of 1) control (0 supplemental B) 2) 5 mg supplemental B/kg DM, and 3) 15 mg supplemental B/kg DM. Boron was supplemented as sodium borate. Steers were bunk fed a corn-cottonseed hull-based diet (Table 1) once daily in amounts that would allow ad libitum access to feed and feed intakes were recorded daily. The basal diet was formulated to meet the nutritional requirements of growing steers (NRC, 1996). The basal diet analyzed 13.3 mg B/kg DM. Steers were weighed at d 0, 14, 28, 34, 41, and 47 of the study.

BHV-1 Inoculation

On d 34 of the study steers were inoculated intranasally with a 2 ml (1 ml/nostril) dose of BHV-1 containing a mean cell culture infective dose (CCID₅₀) of $2.24 \times 10^{11}$ viral units/mL. Steers remained on their dietary treatment for 13 d following BHV-1 inoculation. Rectal temperatures were taken daily at 0900 h on d 0 prior to inoculation.
and on d 1 through 8 post-infection. Blood samples were obtained in heparinized tubes via jugular venipuncture on d 0, 2 and 4 following the BHV-1 challenge and immediately placed on ice. Samples were then transported to the laboratory and centrifuged at 1200 × g for 25 min at 4 °C. Plasma was obtained from whole blood for evaluation of plasma B concentration, acute phase proteins (haptoglobin (Hp), serum amyloid A (SAA), and ceruloplasmin (Cp)) and cytokines (interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α)). Aliquots for acute phase proteins and cytokines were frozen at -80 °C. Plasma aliquots for B concentration were frozen at -20 °C. Day 0 plasma samples were also assayed for BHV-1 specific antibody titers to determine if any of the calves had previously been exposed to the virus.

Boron Analysis

Boron concentration in feed samples and plasma was determined via inductively coupled plasma optical emission spectroscopy (Perkin Elmer, Optima 2000 DV). Polypropylene tubes were used in the digestion procedure to avoid possible B contamination from glass. All polypropylene tubes used for feed digestion were acid washed prior to use as described by Hunt (1997). Approximately 0.5 g of feed was added to a 50 mL conical tube and digested in 5 mL of trace mineral grade nitric acid (Fisher Scientific) overnight at room temperature prior to an open vessel microwave digestion (CEM, MARS5). Following microwave digestion, sample volume was increased to 25 mL using deionized H₂O. One milliliter of plasma was added to a 15 mL conical tube followed by the addition of 1 mL of trace mineral grade nitric acid for an overnight digestion at room temperature. Following the overnight digestion, samples were digested
in an open vessel microwave. Sample volume was then increased to 6 mL with the addition of deionized water to achieve a 2.6 N solution.

**Plasma Metabolites**

Haptoglobin and SAA, were quantified by a colorimetric bovine sandwich ELISA per manufacturer’s instructions (Life Diagnostics, West Chester, PA & Tridelta Diagnostics, Morris Plains, NJ). Quantification was determined by a linear standard curve generated by known analyte concentrations. Plasma Cp concentration was determined colorimetrically from the absorbance at 525 nm (Spectronic 1001, Bausch and Lomb, Rochester, NY) as described by Houchin (1958). Interferon-gamma (BioSource™; Carlsbad, CA) and TNF-α (Endogen, USA; validated for cell culture supernatant) were analyzed by a colorimetric bovine sandwich ELISA per manufacturer’s instructions. Polymerbase, black, optical, flat bottom 96 well plates (NUNC, Nalge Nunc International) were used to eliminate possible “cross-talk” between wells. Modifications to the TNF-α bovine sandwich ELISA were determined via linearity of dilutions and spike and recovery methods. Standards provided by manufacturer were reconstituted in stripped fetal calf serum. The stripped fetal calf serum was also used as a negative control. Prior to the assay samples were heavily (10 sec) vortexed followed by a brief (30 sec) centrifugation to eliminate the possibly of aspirating fibrinogen or other protein complexes that could possibly interfere with quantification. All other steps of the protocol were followed per manufacturer’s instructions. Interferon-γ was determined by a semi-quantitative colorimetric bovine sandwich ELISA that was modified to generate a linear standard curve by using the highest control supplied by the kit. As a result, the
values expressed are as a percent of the positive control. This modification was followed by the guidance of the protocol and technical support.

Statistical Analysis

Statistical analysis of data was performed by analysis of variance for a randomized complete block design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Pen was the experimental unit for animal performance data. Animal was considered the experimental unit for plasma B concentration, acute phase proteins, cytokine concentrations, and core body temperature. Plasma B concentration, all plasma metabolites, rectal temperature, and change in dry matter intake following BHV-1 challenge were analyzed as repeated measures. The model contained time, treatment, and treatment × time interaction. Differences among treatments and days were determined using the LSD test.
Results

Steers supplemented with 15 mg B/kg DM tended to have higher \( (P = 0.10) \) average daily gains (ADG) during the first 14 d compared to steers supplemented with 5 mg B/kg DM (Table 2). However, ADG did not differ between control steers and those supplemented with 5 or 15 mg B/kg DM. Steers supplemented with 15 mg B/kg DM tended to have higher \( (P = 0.07) \) dry matter intakes (DMI) compared to steers supplemented with 5 mg B/kg DM during the first 14 d. Dry matter intake of steers consuming the control diet did not differ from steers fed supplemental B. Boron supplementation did not affect gain:feed (G:F) ratio during the first 14 d, nor did supplementation of B affect ADG, DMI, and G:F from d 15 to 34 of the study.

Average daily gain, DMI, and G:F ratio were not affected by B supplementation following BHV-1 inoculation from d 0 to 8 or d 9 to 13 (Table 2). All steers were seronegative to BHV-1 prior to inoculation. However, DMI of all steers decreased by d 4 following inoculation and began to increase by d 6 reaching initial intake by d 10 (Figure 1). Furthermore, the change in DMI from d 0 was affected by B supplementation. On d 1 and 2 following BHV-1 inoculation DMI decreased relative to d 0 values in steers supplemented with 15 mg B/kg DM but not in the controls or steers receiving 5 mg B/kg DM. As a result, the change in DMI from d 0 differed \( (P < 0.05) \) between control steers and those supplemented with 15 mg B/kg DM on d 1 and 2. Steers consuming the control diet did not differ from steers supplemented with 5 mg B/kg DM during this period. The change in DMI was not affected by dietary B supplementation for the duration of the post BHV-1 inoculation period.
Mean rectal temperatures prior to inoculation were similar across treatments (39.1, 38.9, and 39.2 °C for control, 5 mg B/kg DM, and 15 mg B/kg DM treatments, respectively). Inoculation with BHV-1 increased rectal temperatures by d 2 and temperatures began to decrease by d 5 reaching at or below initial values by d 8 post inoculation (Figure 2). Change in rectal temperatures were affected by time ($P < 0.01$) and tended to be affected by a treatment × time interaction ($P = 0.09$). Change in rectal temperature from d 0 values did not differ among treatment groups on d 1 through 5 following inoculation. However, the rectal temperature change from d 0 in steers supplemented with 15 mg B/kg DM tended ($P < 0.10$) to be less (0.38 vs. 0.84 °C) on d 6 and was less ($P < 0.01$) on d 7 (-0.41 vs. 0.21 °C) and 8 (-0.62 vs. –0.10° C) post challenge than steers supplemented with 5 mg B/kg DM. On d 8 steers consuming the control diet had a greater ($P < 0.05$) decrease in rectal temperature change relative to d 0 when compared to steers supplemented with 5 mg B/kg DM (-0.45 vs. –0.10 °C).

Change in rectal temperature did not differ between control steers and steers supplemented with 15 mg B/kg DM.

Plasma B concentration was affected by treatment ($P < 0.01$), time ($P < 0.01$) and a treatment × time interaction ($P < 0.01$) (Table 3). On d 0, 2, and 4 following BHV-1 inoculation steers supplemented with 5 mg B/kg DM tended ($P < 0.10$) to have higher plasma B concentrations compared to the control steers. Steers supplemented with 15 mg B/kg DM had higher ($P < 0.01$) plasma B concentrations than the control steers and steers supplemented with 5 mg B/kg DM on all sampling days. Overall plasma B concentration was lower ($P < 0.01$) on d 2 (1.22 mg/L) and d 4 (0.87 mg/L) compared to d 0 (1.46
mg/L). The treatment × time interaction was due to plasma B concentration decreasing over time (d 0 vs. d 2, \( P < 0.01 \); d 2 vs. d 4, \( P < 0.01 \)) in steers supplemented with 15 mg B/kg DM. However, plasma B concentrations did not differ significantly across sampling days in steers fed the control or 5 mg B/kg diet. Feed intake decreased slightly by d 2 following the BHV-1 inoculation in steers supplemented with 15 mg B/kg DM and feed intake was greatly reduced in all treatment groups by d 4. The lower feed intakes following BHV-1 inoculation also would have reduced B intake per day, and can explain the decrease in plasma B concentrations with time. Plasma B concentration was correlated \((P < 0.01; R^2 = 0.79)\) to absolute dietary B intake (mg B/kg DM). Actual mg of B consumed per day was reduced to a greater extent following the viral challenge in steers fed 15 mg B/kg DM because of the higher dose of B supplemented. This may explain why the decrease in plasma B over time was greater in steers supplemented with 15 mg B/kg DM.

BHV-1 inoculation decreased plasma IFN-\( \gamma \) concentration over time (Figure 3). Plasma IFN-\( \gamma \) was lower \((P < 0.05)\) on d 2 compared to d 0 (18.6 vs. 25.1 %PC) and was further reduced \((P < 0.05)\) by d 4 (11.9 %PC). Steers supplemented with 5 mg B/kg DM tended \((P < 0.10)\) to have higher concentrations of IFN-\( \gamma \) compared to the control steers on d 4 following BHV-1 challenge (Table 4). Plasma IFN-\( \gamma \) in steers supplemented with 15 mg B/kg DM did not differ from controls or steers receiving 5 mg B/kg DM on any of the sampling days.

Plasma concentrations of TNF-\( \alpha \) were increased \((P < 0.05)\) on d 2 relative to pre-BHV-1 inoculation values (Figure 3). However, by d 4 plasma TNF-\( \alpha \) concentrations
had decreased ($P < 0.05$) below d 0 values. Supplemental B did not affect plasma TNF-α concentrations on d 0 or d 4 (Table 5). However, on d 2 post challenge steers supplemented with 15 mg B/kg DM tended ($P < 0.10$) to have lower concentrations of TNF-α than the control steers (Table 5). Control steers did not differ from steers supplemented with 5 mg B/kg DM nor did steers supplemented with 15 mg B/kg DM differ from steers supplemented with 5 mg B/kg DM.

Inoculation with BHV-1 affected acute phase protein concentrations (Figure 4). Plasma Cp concentrations tended ($P < 0.10$) to be increased by d 2 and were increased by d 4 following BHV-1 inoculation. Plasma SAA and Hp concentrations were also significantly increased ($P < 0.01$) by BHV-1 on d 4 compared to d 0 and 2. However, concentrations of both SAA and Hp were not different between d 0 and 2. Dietary B did not affect Cp and SAA concentrations post challenge (Table 6). On d 0 and 4 post challenge steers supplemented with 15 mg B/kg DM tended ($P < 0.10$) to have lower plasma concentrations of Hp compared to the control steers (Table 7). Boron supplemented steers did not differ between treatment groups and steers supplemented with 5 mg B/kg DM did not differ from the control steers.
Discussion

Effects of Dietary Boron

Boron supplementation did not affect performance of steers during the 34 d pre-challenge phase. In contrast, B supplementation at 5 or 15 mg B/kg DM increased ADG and DMI in pigs fed a control diet containing 2.2 mg B/kg DM (Armstrong and Spears, 2001). Lack of a performance response to B supplementation in the present study can probably be explained by the relatively high concentration (13.3 mg B/kg DM) of B in the control diet due to the presence of soybean meal and cottonseed hulls.

Dietary B had minimal effects on physiological responses of steers to BHV-1 inoculation. It is unclear why control steers had greater DMI on d 1 and 2 following inoculation than those supplemented with 15 mg B/kg DM. There was also a tendency for steers supplemented with 15 mg B/kg DM to have lower plasma concentrations of TNF-α on d 2 and Hp on d 4 than controls.

Previous studies have indicated that B addition to diets low in B can increase the humoral immune response (Bai et al., 1997) and reduce the inflammatory response (Hunt and Idso, 1999; Armstrong and Spears, 2003). It has also been reported that B affects cytokine release in cell culture supernatant as well as in pigs challenged with lipopolysaccharide (LPS). Boric acid addition to human fibroblasts resulted in increased secretion of TNF-α and TNF-α mRNA in culture medium (Benderdour et al., 1998). The addition of 5 mg B/kg DM to a basal diet containing 2.2 mg B/kg DM increased serum TNF-α and IFN-γ concentrations compared to non-supplemented pigs when challenged with LPS (Armstrong and Spears, 2003). The minimal responses to supplemental B following BHV-1 inoculation suggest that increasing dietary B did not enhance immune
responsiveness in the present study. It is likely that 13.3 mg B/kg DM supplied from the basal diet was adequate to maximize the immune response, and thus supplemental B did not further enhance immunity. If the control diet had been lower in B, perhaps B supplementation would have altered animal response to BHV-1 inoculation.

Little is known about the metabolism and bioavailability of B in ruminants. However, studies with animals and human subjects suggest that approximately 90% of sodium borate is absorbed rapidly and excreted in the urine (NRC, 2005). The main effect of dietary B supplementation on plasma B concentration is consistent with data from pigs. Supplementation of B increased plasma B concentration in a dose-responsive manner (Armstrong et al., 2000).

Effects of BHV-1

The increase in rectal temperatures associated with inoculation of BHV-1 is consistent with previous studies from our laboratory (Gengelbach et al., 1997; Kegley et al., 1997; Engle et al., 1999). Temperatures peaked at d 4 and then began to decrease with temperatures reaching pre-inoculation values by d 7. Dry matter intake decreased on d 4 post BHV-1 inoculation and did not return to pre-challenge values until approximately d 10. As a result of the decrease in DMI due to BHV-1, plasma B concentration also decreased from d 0 to 4 post challenge.

The acute phase response occurs in the blood during the onset of infection. As a result acute phase proteins are produced as a predominant host defense mechanism acting in scavenging oxygen radicals and enhancing the wound healing process (Janeway et al., 2005). The increase in plasma Cp concentration in the present study agrees with previous studies in cattle inoculated with BHV-1. Cusack et al. (2005) inoculated ruminating
heifer calves with BHV-1 and reported an increase in serum Cp concentration from week 4 (initial sampling) to week 6 of sampling.

Haptoglobin and SAA were increased due to viral inoculation as reported in previous studies in cattle (Heegaard et al., 2000; Ganheim et al., 2003). However, our sampling period (4 d) was of shorter duration than previous studies with viral infections in cattle, reporting Hp and SAA concentrations to peak further from the onset of infection. Therefore, acute phase proteins may not have reached peak concentrations by d 4 in the present study. Heegaard et al. (2000) reported Hp reached peak concentrations at approximately d 6 to 7 post bovine respiratory syncital virus (BRSV) inoculation and SAA to peak between d 5 to 8 post inoculation. Furthermore, Grell et al. (2005) reported that Hp was strongly induced by approximately d 5 to 6 and reached its peak concentration from d 7 to 9 post BRSV challenge. Ganheim et al. (2003) reported that Hp concentrations began to increase from approximately d 4 to 8 post bovine viral diarrhea virus (BVDV) inoculation and peaked between approximately d 8 and 9 post challenge, with SAA concentrations beginning to increase between d 2 to 4 post inoculation and peaking at d 8 to 9. Serum amyloid A closely depicted the change in Hp concentrations but at a lower response which is consistent with data from cattle inoculated with BRSV (Heegaard et al., 2000). However, in the present study SAA did not respond with greater sensitivity than Hp to BHV-1 inoculation, which contrasts responses of SAA to BRSV reported by Heegaard et al. (2000).

Interferon-gamma plays an active role in macrophage activation and increases expression of MHC molecules, as well as suppressing the T_{H2} response (Janeway et al., 2005). In the present study, the decreased IFN-\(\gamma\) concentration on d 2 and 4 following
BHV-1 inoculation can possibly be explained by its ineffectiveness to diminish BHV-1 activity combined with its brief antiviral activity. It has been reported that IFN-γ does not have an efficient antiviral activity to BHV-1 (Babiuk et al., 1991). Furthermore, BHV-1 can impede on activated T cells, thereby alleviating their function without viral replication occurring in the cell (Babiuk et al., 1991). Data from the present study is inconsistent with data from cattle inoculated with BRSV. Grell et al., (2005) reported an increase in IFN-γ mRNA production between d 1 and 3 post inoculation.

Plasma TNF-α concentrations increased on d 2 but decreased below pre-inoculation values at 4 d post BHV-1 inoculation. We hypothesize that the decrease in plasma TNF-α concentration on d 4 post BHV-1 can be partially explained due to localization of the infection. Tumor necrosis factor-alpha is produced by macrophages and T cells and localizes at the site of inflammation (Janeway et al., 2005). At 4 d following inoculation, signs of BHV-1 infection were most pronounced, suggesting that it is very likely the majority of TNF-α would have been localized in the lungs. A previous study with BRSV inoculated calves reported that high concentrations of TNF-α were found in lungs of infected calves compared to uninfected calves at necropsy (Røntved et al., 2000). Furthermore, bovine peripheral blood mononuclear cells infected in vitro with BHV-1 reported no significant increases in TNF-α mRNA from 24 to 72 hr following inoculation (Leite et al., 2004).

In the present study supplementing 5 or 15 mg B/kg DM to a basal diet containing 13.3 mg B/kg DM did not affect performance pre or post BHV-1 challenge. Plasma B concentration was increased by dietary B in a dose responsive manner which is consistent with studies in pigs (Armstrong et al., 2000). Inoculating steers with BHV-1 increased
rectal temperatures and acute phase protein concentrations and decreased DMI and plasma concentrations of IFN-γ and TNF-α by d 4 following viral challenge. Lack of a substantial affect of B on the immune response to an immunosuppressor is likely due to the relatively high B content of the basal diet.
Literature Cited


haemolytica leukotoxin with bovine peripheral blood mononuclear cells in vitro.


<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, ground</td>
<td>46.40</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>40.00</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>9.00</td>
</tr>
<tr>
<td>Urea</td>
<td>1.00</td>
</tr>
<tr>
<td>Corn-boron supplement, ground</td>
<td>2.00</td>
</tr>
<tr>
<td>Calcium sulfate</td>
<td>0.40</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.49</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.35</td>
</tr>
<tr>
<td>White salt</td>
<td>0.26</td>
</tr>
<tr>
<td>Vitamin mix(^3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Trace mineral mix(^4)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

\(^1\) Basal diet contained 13.3 mg B/kg DM.

\(^2\) Provided the supplemental B as Na\(_2\)B\(_4\)O\(_7\).

\(^3\) Contained per kg of premix: 6,600,000 IU of vitamin A, 1,520,000 IU of vitamin D, and 6,600 IU of vitamin E.

\(^4\) Provided per kg of diet: 30 mg of Zn as ZnSO\(_4\); 10 mg of Cu as CuSO\(_4\); 20 mg of Mn as MnSO\(_4\); 0.10 mg of Co as CoCO\(_3\); 0.10 mg of Se as Na\(_2\)SeO\(_3\) (1%); 0.5 mg of I as Ca(IO\(_3\))\(_2\) H\(_2\)O.
Table 2. Effect of dietary B on performance of growing steers pre and post BHV-1 challenge

<table>
<thead>
<tr>
<th>Supplemental B, mg/kg DM</th>
<th>0</th>
<th>5</th>
<th>15</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre Challenge</strong>¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0-14</td>
<td>2.1ᵃᵇ</td>
<td>1.6ᵃ</td>
<td>2.3ᵇ</td>
<td>0.269</td>
</tr>
<tr>
<td>d 15-34</td>
<td>1.8</td>
<td>2.1</td>
<td>1.7</td>
<td>0.257</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0-14</td>
<td>7.5ᶜᵈ</td>
<td>6.8ᶜ</td>
<td>7.7ᵈ</td>
<td>0.299</td>
</tr>
<tr>
<td>d 15-34</td>
<td>9.3</td>
<td>9.1</td>
<td>9.1</td>
<td>0.630</td>
</tr>
<tr>
<td>G:F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0-14</td>
<td>0.14</td>
<td>0.11</td>
<td>0.15</td>
<td>0.017</td>
</tr>
<tr>
<td>d 15-34</td>
<td>0.09</td>
<td>0.11</td>
<td>0.10</td>
<td>0.013</td>
</tr>
<tr>
<td><strong>Post Challenge</strong>²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0-8</td>
<td>-1.8</td>
<td>-1.9</td>
<td>-2.1</td>
<td>0.426</td>
</tr>
<tr>
<td>d 9-13</td>
<td>3.3</td>
<td>2.5</td>
<td>3.5</td>
<td>0.923</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0-8</td>
<td>7.9</td>
<td>7.5</td>
<td>8.2</td>
<td>0.426</td>
</tr>
<tr>
<td>d 9-13</td>
<td>9.1</td>
<td>8.3</td>
<td>9.2</td>
<td>0.753</td>
</tr>
<tr>
<td>G:F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0-8</td>
<td>-0.14</td>
<td>-0.15</td>
<td>-0.12</td>
<td>0.038</td>
</tr>
<tr>
<td>d 9-13</td>
<td>0.15</td>
<td>0.08</td>
<td>0.16</td>
<td>0.054</td>
</tr>
</tbody>
</table>

¹Values are means (n = 6) and pooled SEM for calves pre BHV-1 challenge.

²Values are means (n = 6) and pooled SEM for calves post BHV-1 challenge.

ᵃᵇMeans in a row without a common superscript differ, (P = 0.10)

ᶜᵈMeans in a row without a common superscript differ, (P < 0.10)
Table 3. Effect of dietary B and BHV-1 on plasma B concentrations in growing steers<sup>1</sup>

<table>
<thead>
<tr>
<th>Supplemental B, mg/kg DM</th>
<th>0</th>
<th>5</th>
<th>15</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall&lt;sup&gt;a,b,c,d&lt;/sup&gt;</td>
<td>0.58</td>
<td>0.84</td>
<td>2.15</td>
<td>0.055</td>
</tr>
<tr>
<td>Day 0</td>
<td>0.65&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.96&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.78&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.117</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.60&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.87&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.19&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.097</td>
</tr>
<tr>
<td>Day 4</td>
<td>0.47&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.68&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.47&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.073</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are means (n = 12) and pooled SEM.

<sup>a</sup>Means across all sampling times expressed as mg/L.

<sup>b</sup>Time ($P < 0.01$)

<sup>c</sup>Treatment ($P < 0.01$)

<sup>d</sup>Treatment × Time ($P < 0.01$)

<sup>e,f</sup>Means in a row without a common superscript differ ($P < 0.01$).
Table 4. Effect of dietary B and BHV-1 on plasma interferon-gamma (IFN-γ) concentrations in growing steers\(^1\,^2\,^3\)

<table>
<thead>
<tr>
<th>Supplemental B, mg/kg DM</th>
<th>0</th>
<th>5</th>
<th>15</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>24.0</td>
<td>28.3</td>
<td>23.1</td>
<td>3.809</td>
</tr>
<tr>
<td>Day 2</td>
<td>17.3</td>
<td>22.4</td>
<td>16.0</td>
<td>3.619</td>
</tr>
<tr>
<td>Day 4</td>
<td>9.9(^a)</td>
<td>15.6(^b)</td>
<td>10.3(^{a,b})</td>
<td>2.435</td>
</tr>
</tbody>
</table>

\(^1\)Values are means (n = 12) and pooled SEM.

\(^2\)Time = \((P < 0.01)\)

\(^3\)Means are expressed as a percentage of the positive control.

\(^{a,b}\)Means in a row without a common superscript differ, \((P = 0.10)\).
Table 5. Effect of dietary B and BHV-1 on plasma tumor necrosis factor-alpha (TNF-α) concentrations in growing steers$^{1,2}$

<table>
<thead>
<tr>
<th>Supplemental B, mg/kg DM</th>
<th>0</th>
<th>5</th>
<th>15</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>416.72</td>
<td>527.52</td>
<td>366.55</td>
<td>82.733</td>
</tr>
<tr>
<td>Day 2</td>
<td>667.39$^a$</td>
<td>583.39$^{ab}$</td>
<td>466.53$^b$</td>
<td>83.592</td>
</tr>
<tr>
<td>Day 4</td>
<td>315.39 $^a$</td>
<td>358.95</td>
<td>245.06</td>
<td>54.159</td>
</tr>
</tbody>
</table>

$^1$Values are means (n = 12) and pooled SEM.

$^2$Time = ($P < 0.01$)

$^{ab}$Means in a row without common superscript differ, ($P = 0.10$).
Table 6. Main effect of dietary B and BHV-1 on acute phase proteins in growing steers<sup>1</sup>

<table>
<thead>
<tr>
<th>Supplemental B, mg/kg DM</th>
<th>0</th>
<th>5</th>
<th>15</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haptoglobin, ng/ml</td>
<td>26,329.55</td>
<td>25,719.21</td>
<td>11,054.46</td>
<td>8290.83</td>
</tr>
<tr>
<td>Serum Amyloid A, ng/ml</td>
<td>16.14</td>
<td>14.42</td>
<td>15.82</td>
<td>4.03</td>
</tr>
<tr>
<td>Ceruloplasmin, ng/100 ml</td>
<td>29.46</td>
<td>30.78</td>
<td>30.75</td>
<td>2.00</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are means (n = 12) and pooled SEM.
Table 7. Effect of dietary B and BHV-1 on plasma haptoglobin (Hp) concentration in growing steers

<table>
<thead>
<tr>
<th></th>
<th>Supplemental B, mg/kg</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>ng/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>7829.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6715.94&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>6137.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>621.35</td>
</tr>
<tr>
<td>Day 2</td>
<td>20024.55</td>
<td>9971.54</td>
<td>3432.17</td>
<td>10216.64</td>
</tr>
<tr>
<td>Day 4</td>
<td>51134.19&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>60470.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23593.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14034.49</td>
</tr>
</tbody>
</table>

<sup>1</sup>Means is a row without a common superscript differ <sup>a,b</sup>(P < 0.10). Individual animal was the experimental unit, and there were no treatment × time interactions (P > 0.10).

<sup>2</sup>Values are means (n = 12) and pooled SEM.

<sup>3</sup>Time = (P < 0.01)
Figure 1. Effect of dietary boron and bovine herpes virus-type 1 (BHV-1) inoculation (2.24 × 10^{11}) on change in dry matter intake (DMI) in growing steers. Pooled SEM 1.450. *P < 0.05, treatment effect. Time, P < 0.01. Treatment × time, P > 0.10.
Figure 2. Effect of dietary boron and bovine herpes virus-type 1 (BHV-1) inoculation \((2.24 \times 10^{11})\) on rectal temperature change in growing steers. Pooled SEM 0.265. *\(P < 0.10\), treatment effect. **\(P < 0.01\), treatment effect. Time effect, \(P < 0.01\). Treatment × time interaction \(P = 0.09\).
Figure 3. Effect of bovine herpesvirus type-1 (BHV-1) inoculation (2.24 × 10^{11}) on interferon-gamma (IFN-\(\gamma\)) and tumor necrosis factor-alpha (TNF-\(\alpha\)) concentration in growing steers. Values are means (n = 36) for IFN-\(\gamma\) and TNF-\(\alpha\) pooled across all treatment groups each day. \(^{a,b,c}\)Means without a common superscript differ \((P < 0.05)\).
Figure 4. Effect of bovine herpesvirus type-1 (BHV-1) inoculation (2.24 × 10^{11}) on acute phase proteins in growing steers. Values are means (n = 36) for each acute phase protein pooled across all sampling days. Dashed lines (---) represent acute phase proteins with concentrations on left y-axis. Solid line (—) represents acute phase protein with concentration on right y-axis. Time effect, \( P < 0.01 \). a,b Means without a common superscript differ, \( P < 0.01 \), and is representative of Hp and SAA. c,d Means without a common superscript differ, \( P < 0.05 \), and is representative of Cp.
Chapter 2

Effect of dietary boron on immune function in growing steers\(^1,\text{\textsuperscript{2}}\)

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\(^1\)Use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service or criticism of similar products not mentioned.

\(^2\)Appreciation is extended to Greg Shaeffer, Joey Dickerson, Jay Woodlief, and Andrew Collicutt for their assistance in sampling and animal care.

\(^3\)Correspondence: Campus Box 7621; North Carolina State University, Raleigh, NC 27695-7621; (Phone: 919-515-4008; Fax 919-515-4463; e-mail: Jerry_Spears@ncsu.edu).
Introduction

The essentiality of boron (B) in higher plants is widely accepted (Bolanos et al., 2004). Research from a number of animal studies indicates that B is of nutritional importance and affects various physiological processes (Hunt and Idso, 1999; Armstrong et al., 2001; Nielsen, 2002), but the underlying biochemical function of B remains unclear.

Recent studies indicate that dietary B may reduce the inflammatory response (Hunt and Idso, 1999; Armstrong et al., 2001; Armstrong and Spears, 2003). In pigs, supplementation of 5 mg B/kg DM to a control diet, containing 2.2 mg B/kg DM, reduced skinfold thickness compared to pigs consuming the control diet following intradermal injection of phytohemagglutinin (Armstrong et al., 2001).

Limited research exists evaluating the effects of dietary B on the adaptive immune response. Rats consuming a control diet containing 0.15 mg B/kg had lower concentrations of anti-typhoid IgM and IgG when compared to rats consuming 1.75 mg B/kg following injection of typhoid vaccine (Bai et al., 1997). However, in pigs, addition of 5 mg B/kg DM to a control diet, containing 2.1 mg B/kg DM, did not affect antibody responses to administration of sheep red blood cells (Armstrong et al., 2001). Furthermore, dietary B did not affect blastogenic response of isolated porcine lymphocytes to mitogen stimulation (Armstrong et al., 2001).

Boron research in ruminants is limited. Studies have indicated that B has a low order of toxicity (when present in drinking water) in cattle (Green and Weeth, 1977). The objective of the present study was to evaluate the effect of dietary B on immune responses of cattle.
Materials and Methods

Animal Care and Handling

Care, handling, and sampling of steers were approved by the North Carolina State University Animal Care and Use Committee. Thirty-six Angus (n = 16) and Angus × Simmental (n = 20) steers with an average initial body weight (BW) of 269 kg were obtained from the North Carolina State University cow-calf facility. Steers were vaccinated two months prior to weaning with Vision 7® Clostridium (Intervet; Millsboro, DE). Steers were weaned and placed on pasture for approximately 2 months. Steers were then moved approximately 2 km to our feedlot facility on d -1. Steers were weighed on two consecutive days to obtain an initial BW on days -1 and 0 and a final BW on days 69 and 70. Interim weights were taken on 14 d intervals. Steers were blocked by weight within breed and randomly assigned to one of 18 covered, slotted floor pens. Pens within a block were randomly assigned to dietary treatments. Treatments consisted of 1) 0 (control) 2) 5, and 3) 50 mg supplemental B/kg DM. Steers were bunk fed a corn silage-based diet (Table 1) once daily to allow for ad libitum access to feed. The basal diet analyzed 10.2 mg B/kg DM, and was formulated to meet or exceed nutritional requirements for growing steers (NRC, 1996). Supplemental B was supplied from sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 5\text{H}_2\text{O}$).

Blood Sampling and Immune Measurements

Jugular blood was obtained in heparinized tubes prior to feeding on d 28, 63, and 77 for plasma B analysis. To evaluate lymphocyte proliferation and cytokine production from monocytes, blood samples were obtained in heparinized tubes from steers prior to feeding on d 42 and 44 of the study. Equal numbers of steers per treatment were sampled.
on each day. Percentage of isolated peripheral mononuclear cells was determined by methylene blue-stained cytospin smears and was greater than 90%. The blastogenic response of isolated peripheral lymphocytes (resuspended density of $2.5 \times 10^6$) was evaluated following mitogenic stimulation with concanavalin A (Con A), phytohemagglutinin (PHA), and pokeweed mitogen (PWM) by measuring incorporation of $[^{3}H]$ thymidine into lymphocytes (Engle et al., 1999). All mitogens were added at a concentration of 10 μg/ml. Peripheral monocytes (resuspended density of $6.0 \times 10^6$) were isolated and stimulated in vitro using lipopolysaccharide (LPS) (10 μg/ml) as described by Gengelbach et al. (1998). Production of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) by stimulated monocytes were quantified by a sandwich ELISA per manufacturer’s instructions (Endogen, USA).

On d 49 of the study humoral immunity was assessed by injecting steers intramuscularly (i.m.) with 10 mL of a 25% pig red blood cell (PRBC) suspension. Red blood cells collected for the injection and hemaglutination assay were obtained from a Large White Landrace sow. Blood was collected one day prior to injection in Alsever solution as described by Kuhlman et al. (1988). The PRBC were washed with 0.01 M sterile PBS (pH 7.4) three times at 960 × g for 15 min at 4 °C. Blood samples were obtained via jugular venipuncture from steers immediately prior to injection and on d 7, 14, and 21 following injection. Blood samples were immediately placed on ice and transported to the laboratory and centrifuged at 1200 × g for 25 min at 23 °C. Serum was obtained and frozen at -20 °C until analysis of hemagglutination titers.

Hemagglutination titers to PRBC were determined using a mercaptoethanol
(ME)-PBS microtitration technique as described by Droke et al. (1993). Serum was heat inactivated at 56 ºC in a water bath for 30 min prior to the assay. Total Ig titers (PBS titers) were assayed in clear, v-shaped bottom 96 well plates (Corning Scientific). Fifty microliters of PBS (pH 7.4) was added to the first row of wells, with the subsequent addition of 50 μL of heat inactivated test serum. Plates were then incubated for 30 min at 37 ºC. Immediately following the incubation, 50 μL of PBS was added to the remaining wells followed by serial dilutions via a microdiluter. Fifty microliters of 2% PRBC suspension was added followed by a 30 min incubation period at 37 ºC. Plates were read immediately following incubation on a test reading mirror for determination of hemaglutination. Mercaptoethanol titers (IgG titers) were determined by adding 25 μL of PBS and 25 μL of a 0.2 M ME solution in the first row of wells. Fifty microliters of heat inactivated serum was added to wells containing PBS and ME. Plates were then covered with a plastic plate sealer and incubated for 30 min at 37 ºC. After incubation, plates were removed from the incubator and 50 μL of PBS was added to the remaining wells. Samples were then diluted via a microdiluter, and subsequently 50 μL of a 2.0% PRBC suspension was added to all wells and incubated for an additional 30 min at 37 ºC. Following the incubation plates were read on a testing mirror for hemaglutination. Titors were recorded as ME-resistant (ME-R) titers. The ME-sensitive (ME-S) titers were calculated as the difference between the total Ig and ME-R recordings. Mercaptoethanol-sensitive titers are associated with IgM and ME-R is associated with IgG levels.

On d 77 and 78 of the study an in vivo evaluation of the inflammatory response was conducted. Hair was clipped on each side in the scapularly region. Steers were injected intradermally with 150 μg in 0.1 mL of PHA in one region while the other side
was injected with 0.1 ml of PBS to determine skinfold difference. Measurements were taken with calipers prior to injection and at 4, 8, and 24 h following PHA injection as described by Engle et al. (1999).

**Boron Analysis**

Boron concentrations in feed samples and plasma were determined via inductively coupled plasma optical emission spectroscopy (Perkin-Elmer, Optima 2000 DV). Polypropylene tubes were used in the digestion procedure to avoid possible B contamination from glass. All polypropylene tubes used for feed digestion were acid washed prior to use as described by Hunt (1997). Approximately 0.5 g of feed was added to a 50 mL conical tube and digested in 5 mL of trace mineral grade nitric acid (Fisher Scientific) overnight prior to an open vessel microwave digestion (CEM, MARS5). Following microwave digestion sample volume was increased to 25 mL using deionized H$_2$O. One milliliter of plasma was added to a 15 mL conical tube followed by the addition of 1 mL of trace mineral grade nitric acid for an overnight digestion at room temperature. Following the overnight digestion, samples were digested in an open vessel microwave. Sample volume was then increased to 6 mL with the addition of deionized water to achieve a 2.6 N solution.

**Statistical Analyses**

Statistical analysis of data was performed by analysis of variance for a randomized complete block design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Pen was the experimental unit for animal performance data. Animal was considered the experimental unit for plasma B concentration, lymphocyte proliferation, in vitro cytokine production, skinfold thickness, and antibody titer response to PRBC
injection. Plasma B concentration, antibody titers, and skinfold thickness responses were analyzed as repeated measures. The model for repeated measures variables included treatment, day, and treatment × day. Treatment effects were broken down into two single degree of freedom contrasts; 1) control vs. B (5 and 50 mg B/kg treatments) and 2) 5 mg B/kg vs. 50 mg B/kg. Significance was declared at a probability value P ≤ 0.05.
Results and Discussion

Performance

Boron supplementation did not affect average daily gain (ADG) or gain:feed ratio (G:F) during the 70 d study (Table 2). However, average daily feed intake (ADFI) of B supplemented steers were numerically ($P = 0.12$) higher than the control steers. Steers supplemented with low and high dietary B did not differ. The increase in ADFI in the present study is consistent with previous studies in pigs where B supplementation to a control diet containing 2.1 mg B/kg DM increased ADFI and ADG (Armstrong et al., 2001; Armstrong and Spears, 2001; Armstrong and Spears, 2003). However, supplementing 5 or 15 mg B/kg to a diet containing 6.7 mg B/kg DM did not affect ADG or ADFI in weanling pigs (Armstrong et al., 2000).

Plasma B Concentration

Plasma B concentrations were affected by treatment ($P < 0.01$), time ($P < 0.01$), and a treatment × time interaction ($P < 0.01$; Table 3). Boron supplemented steers had higher ($P < 0.01$) plasma B concentrations than controls on all sampling days. Steers supplemented with 50 mg B/kg DM also had higher plasma B concentrations than those receiving 5 mg B/kg DM at all dates. In pigs serum B concentrations also increased in a dose responsive manner when B was supplemented at 5 and 15 mg B/kg DM to a semi-purified diet containing 0.98 mg B/kg DM (Armstrong et al., 2000).

Plasma B concentrations decreased with time on feed in the present study (Table 3.) Although plasma B decreased numerically over time in all treatment groups, the decrease with time was only significant in steers supplemented with 50 mg B/kg DM. This resulted in a treatment × time interaction ($P < 0.01$). Based on studies with animals
and humans, dietary B is rapidly absorbed and excreted in the urine (NRC, 2005).

Plasma B concentrations were correlated \( (P < 0.01; R^2 = 0.95) \) to daily B intake (mg/d) in the present study. Previous results in pigs indicate that plasma B is closely related to B intake (Armstrong et al., 2000). In the present study, ADFI was lower on d 63 (6.8 kg/d) than d 28 (8.3 kg/d) and was further decreased on d 77 (6.0 kg/d). Thus, daily intake of B decreased over time. The change in B intake per head (mg/d) from d 28 to 77 for the control, 5 mg B/kg, and 50 mg B/kg DM treatments were approximately 20.4, 27.3, and 144.5 mg B/d, respectively. The greater decrease in B intake with time in steers supplemented with the high level of B may explain why plasma B decreased to a greater extent over time in this group.

*Immune Measurements*

Response of isolated peripheral blood lymphocytes from B-supplemented steers to stimulation with PHA and ConA did not differ from the controls (Table 4). Blastogenic response to PHA and ConA stimulation was numerically \( (P = 0.19) \) higher for lymphocytes from steers supplemented with 50 compared to 5 mg B/kg DM. Lymphocyte blastogenic response to PWM did not differ among control and B-supplemented steers. However, lymphocytes from steers supplemented with 50 mg B/kg DM had a numerically \( (P = 0.12) \) greater response to PWM stimulation than cells from steers supplemented with 5 mg B/kg DM. Armstrong et al. (2001) reported that addition of 5 or 15 mg B/kg DM to a diet containing 2.1 mg B/kg DM had no affect on blastogenic response of isolated porcine lymphocytes to mitogenic stimulation. The tendencies in the present study for lymphocytes isolated from steers receiving 50 mg B/kg DM to have a greater mitogen stimulation than lymphocytes from animals
supplemented with 5 mg B/kg DM, may be due to a pharmacological rather than a physiological response to dietary B.

Interferon-γ production from isolated peripheral blood monocytes was undetected. Tumor necrosis factor-α concentration is cultured supernatant elevated \((P < 0.01)\) due to \textit{in vitro} stimulation with LPS (Table 5). However, B supplementation did not affect TNF-α production from isolated peripheral blood monocytes. Cytokines are proteins that are released from phagocytic cells in response to infection. Furthermore, LPS is a component of the cell wall of gram-negative bacteria and small amounts can elicit a pronounced increase in cytokine production (Janeway et al., 2005). In pigs, supplementation of 5 mg B/kg DM increased \textit{in vitro} TNF-α production compared to controls receiving 2.2 mg B/kg following LPS stimulation of isolated peripheral monocytes (Armstrong and Spears, 2003). Boric acid addition to the medium of cultured normal human fibroblasts (Benderdour et al., 1998) and in chick embryos (Benderdour et al., 1997) also increased TNF-α release.

Immunoglobulin G (IgG) titer response to PRBC administration was affected by time \((P < 0.01)\) and tended to be affected by a treatment \(\times\) time interaction \((P = 0.07)\) (Figure 1). Anti-PRBC titers were not different among dietary treatments on d 0 prior to injection. However, on d 7 post-injection B-supplemented steers had higher \((P < 0.05)\) anti-PRBC IgG titers than control steers (5.8 vs. 5.0 log₂ titers). On d 14 and 21 following injection, there was no longer a difference in IgG titers between B supplemented steers and controls. Serum IgG titers did not differ among steers fed 5 and those supplemented with 50 mg B/kg DM on any of the sampling days. Dietary B did not
affect IgM titer response to PRBC administration (Figure 2). Bai et al. (1997) reported that rats consuming a control diet containing 0.15 mg B/kg had lower concentrations of anti-typhoid IgM and IgG when compared to rats consuming 1.75 mg B/kg following injection of typhoid vaccine. However, in pigs, the addition of 5 mg B/kg DM to a basal diet containing 2.1 mg B/kg DM did not affect specific IgM, IgG, or total Ig production following injection of sheep red blood cells (Armstrong et al., 2001).

The inflammatory response to intradermal injection of PHA in the present study was not affected by dietary B supplementation at 0, 4, 8, and 24 h post injection (Figure 3). In contrast, Hunt and Idso (1999) reported that supplementation of 2 mg B/kg to a control diet containing 0.1 mg B/kg decreased paw swelling in rats with adjuvant-induced arthritis. Addition of 5 mg B/kg DM to a control diet containing 2.2 mg B/kg DM also reduced the inflammatory response in pigs based on reduced skinfold thickness following an intradermal injection of PHA (Armstrong et al., 2001). It is unclear how B may affect the inflammatory response. However, some evidence suggests that B may reduce tissue damage from inflammation by hastening destructive reactive oxygen species via increasing activities of key antioxidant enzymes (Hunt and Idso, 1999). Lack of an effect of supplemental B on the inflammatory response in the present study may relate to the relatively high B content of the control diet (10.2 mg B/kg DM).

In the present study B supplementation of a corn silage-based diet increased plasma B concentrations but had little affect on immune responses. Steers supplemented with B had greater anti-PRBC IgG titers on d 7 following administration of PRBC, suggesting that B enhanced the development of IgG production. However, dietary B did not affect Ig titers on d 14 or 21, lymphocyte responses to mitogen stimulation or
inflammatory response. The control diet in the present study analyzed 10.2 mg B/kg DM. The minimal effects of dietary B observed on immune variables in this study may be attributed to the relatively high B content of the control diet. Previous studies that have reported increased humoral immune response (Bai et al., 1997) or reduced inflammatory response (Hunt and Idso, 1999; Armstrong et al., 2001) to increasing dietary B have used control diets that contained 2.2 mg B/kg DM or less.
Literature Cited


<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>86.00</td>
</tr>
<tr>
<td>Corn, ground</td>
<td>6.31</td>
</tr>
<tr>
<td>Corn-boron supplement(^2)</td>
<td>2.00</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>3.00</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.84</td>
</tr>
<tr>
<td>Urea</td>
<td>1.32</td>
</tr>
<tr>
<td>Trace mineral salt(^3)</td>
<td>0.50</td>
</tr>
<tr>
<td>Vitamin A,D, &amp; E premix(^4)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

\(^1\) Analyzed 10.2 mg B/kg DM.

\(^2\) Provided the supplemental B as Na\(_2\)B\(_4\)O\(_7\) \cdot 5\text{H}_2\text{O}.

\(^3\) Contained as percent of mineral: NaCl, 98.5 max., 94 min.; Zn, 0.350; Fe, 0.20; Mn, 0.20; Cu, 0.03; I, 0.007; Co, 0.005.

\(^4\) Contained per kg of premix: 6,600,000 IU of vitamin A, 1,520,000 IU of vitamin D, and 6,600 IU of vitamin E.
Table 2. Effect of dietary B on performance of growing steers$^1$

<table>
<thead>
<tr>
<th>Supplemental B, mg B/kg</th>
<th>0</th>
<th>5</th>
<th>50</th>
<th>SEM</th>
<th>0 vs. B P-value</th>
<th>5 vs. 50 P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG, kg</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>0.048</td>
<td>0.71</td>
<td>0.59</td>
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<tr>
<td>ADFI, kg</td>
<td>7.4</td>
<td>7.8</td>
<td>7.6</td>
<td>0.138</td>
<td>0.12</td>
<td>0.46</td>
</tr>
<tr>
<td>G:F</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.003</td>
<td>0.60</td>
<td>1.00</td>
</tr>
</tbody>
</table>

$^1$n = 6 replicate pens/treatment
Table 3. Effect of dietary B on plasma B concentration in growing steers

<table>
<thead>
<tr>
<th>Supplemental B, mg/kg</th>
<th>0</th>
<th>5</th>
<th>50</th>
<th>SEM</th>
<th>0 vs. B P-value</th>
<th>5 vs. 50 P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall¹²³⁴</td>
<td>0.58</td>
<td>1.17</td>
<td>5.70</td>
<td>0.065</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Day 28</td>
<td>0.65</td>
<td>1.23</td>
<td>6.20ᵃ</td>
<td>0.070</td>
<td>0.01</td>
<td>0.01</td>
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<tr>
<td>Day 63</td>
<td>0.58</td>
<td>1.19</td>
<td>5.64ᵇ</td>
<td>0.122</td>
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<td>Day 77</td>
<td>0.52</td>
<td>1.10</td>
<td>5.27ᶜ</td>
<td>0.120</td>
<td>0.01</td>
<td>0.01</td>
</tr>
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</table>

¹Means (n = 12) across all sampling times expressed as mg/L.

²Treatment (P < 0.01)

³Time (P < 0.01)

⁴Treatment × Time (P < 0.01)

ᵃ⁻ᶜMeans in a column with different superscript differ, P < 0.05
Table 4. Effect of dietary B on lymphocyte blastogenesis in growing steers\(^1\)

<table>
<thead>
<tr>
<th>Mitogen</th>
<th>Supplemental B, mg/kg</th>
<th>Contrasts</th>
<th>0 vs. B P-value</th>
<th>5 vs. 50 P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
<td>50</td>
<td>SEM</td>
</tr>
<tr>
<td>ConA(^2)</td>
<td>45.25</td>
<td>41.89</td>
<td>63.83</td>
<td>11.60</td>
</tr>
<tr>
<td>PHA(^2)</td>
<td>35.16</td>
<td>28.41</td>
<td>48.22</td>
<td>10.51</td>
</tr>
<tr>
<td>PWM(^2)</td>
<td>8.12</td>
<td>7.53</td>
<td>12.46</td>
<td>2.15</td>
</tr>
</tbody>
</table>

\(^1\)Values are expressed as a stimulation index (SI); n = 12.

\(^2\)Added at a concentration of 10 \(\mu g/ml\).
Table 5. Effect of dietary B and lipopolysaccharide (LPS, 10 μg/ml) on tumor necrosis factor-α production from isolated peripheral bovine monocytes from growing steers

<table>
<thead>
<tr>
<th>Supplemental B, mg/kg</th>
<th>Contrasts</th>
<th>0 vs. B</th>
<th>5 vs. 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+LPS</td>
<td>1.81</td>
<td>0.30</td>
<td>0.28</td>
</tr>
<tr>
<td>LPS</td>
<td>2.12</td>
<td>0.21</td>
<td>0.28</td>
</tr>
</tbody>
</table>

TNF-α, pg/ml

1 LPS effect, *P* < 0.01; n = 12
Figure 1. Effect of dietary B on immunoglobulin G (IgG) titer response to pig red blood cells in growing steers. Pooled SEM 0.129. Time, \( P < 0.01 \). Treatment × time interaction, \( P = 0.07 \). Control vs. B, \( P < 0.05 \) on d 7.
Figure 2. Effect of dietary B on immunoglobulin M (IgM) titer response to pig red blood cells in growing steers. Pooled SEM 0.124.
Figure 3. Effects of dietary B on inflammatory response to phytohemagglutinin in growing steers. Pooled SEM 0.500.