NPPC Final Research Grant Report

I. **Project Title**: Effect of dietary boron supplementation on phosphorus and nitrogen excretion in the growing pig

NPPC Project identification Number: NPPC 00-011

Principal Investigator: Jerry W. Spears, Ph.D.

Institution: North Carolina State University

II. **Abstract:**

An experiment was conducted to evaluate the effects of dietary boron (B) on growth performance, bone mechanical properties, and calcium, phosphorus and nitrogen metabolism in pigs. Thirty-six barrows were weaned at approximately 21 days of age and randomly assigned to one of the following treatments: 1) control diet, 2) 5 mg B/kg diet or 3) 15 mg B/kg diet. Barrows remained on their respective experiment diets throughout the nursery (35 days) and growing (30 days) phases of production. Following the 30-day growing period, 8 barrows per treatment were transferred to stainless steel metabolism crates. Barrows had an adjustment period of 7 days, followed by a 7-day total collection of urine and feces. At the end of the collection period, barrows were harvested and femurs and fibulas were obtained for assessment of bone mechanical properties.

During the nursery phase, gain and feed intake were increased by 5 and 15 mg supplemental B/kg diet. During the growing phase, gain and feed intake were increased by 15 mg supplemental B/kg diet. Barrows fed 5 mg supplemental B/kg diet had decreased serum concentrations of triiodothyronine during the growing phase. Supplementation of 5 mg B/kg diet decreased urinary calcium and increased urinary phosphorus excretion. Apparent absorption and retention of calcium, phosphorus and nitrogen were not affected by dietary B. Boron did not

affect bone ash percentage, but supplementation of 15 mg B/kg diet increased bone phosphorus and increased bone stress of the femur and ultimate shear force of the fibula. Results of this study indicate that B supplementation of pig diets can increase growth and bone strength, without greatly affecting calcium and phosphorus metabolism.

III. **Introduction:**

Boron (B)is an essential element for plant growth and recent research indicates that B may have a physiological role in animal nutrition. Previous research at North Carolina State University indicated that B supplementation of a low B diet increased measures of bone mechanics in barrows. Boron also appears to affect the metabolism of calcium (Ca) and phosphorus (P). Supplementation of B decreased urinary Ca and P excretion in humans (Nielsen et al., 1987) Cattle consuming B supplemented drinking water had decreased urinary phosphate excretion (Green and Weeth, 1977). Dietary B supplementation increased apparent absorption of Ca in sheep (Brown et al., 1987) and increased apparent absorption and retention of Ca and P in rats (Hegsted et al., 1991).

Increasing dietary B may result in more P being available for physiological functions and thus, decreased the amount of phosphorus excreted in waste. Therefore, the present study was conducted to determine if supplemental B affects growth or Ca, P, and nitrogen metabolism in growing pigs.

IV. **Objectives:**

1) To determine if supplemental boron has a positive effect upon phosphorus, nitrogen, and calcium balance in growing barrows

2) To investigate the effect of boron on the mechanical properties of bone in growing barrows

3) To assess the effect of supplemental boron on growth performance and serum metabolites in weanling and growing barrows

V. **Procedures:**

Thirty-six weanling barrows were randomly allotted to one of three dietary treatments in two replicates (replicate 1, n=18; replicate 2, n=18). The dietary treatments consisted of 1) control basal diet; 2) basal diet +5 mg boron/kg diet; and 3) basal diet +15 mg boron/kg diet. Boron was supplemented as sodium borate. The basal diet was formulated to contain 20.0% crude protein and 1.4% lysine. Animals were housed with two barrows per pen, with each dietary treatment being replicated three times in each replicate. The nursery phase lasted for 35 days, and on day 35, average daily gain, average daily feed intake, and feed efficiency were determined on a pen basis. In addition, venous blood samples were obtained from the jugular vein. Plasma was analyzed for Ca, magnesium (Mg), and P concentrations. Serum was analyzed for alkaline phosphatase activity, total cholesterol, triglyceride, thyroid hormones (T_3 and T_4), and osteocalcin concentrations.

At the completion of the nursery phase, 15 barrows per replicate (five barrows/treatment/replicate) were transferred to individual pens. The barrows remained on their respective dietary treatments; however, the basal diet was altered to meet or exceed the requirements of growing barrows. The basal diet was formulated to contain 17.2% crude protein and 1.1% lysine. These dietary treatments were fed for one month, at which time barrows were weighed and feed disappearance recorded for the determination of animal performance characteristics. Blood samples were obtained from each barrow via jugular venapuncture at the completion of the one-month period. Plasma and serum were analyzed for the same metabolites as previously described for blood samples obtained at the end of the nursery period.

Following this one-month period, 12 barrows per replicate (4 barrows/treatment/replicate) were transferred to metabolism crates. Each animal was housed individually and remained on the same dietary treatments as fed in the previous one-month period. Feed intake was restricted to 90% of the previous ad libitum intake calculated during one-month grower phase. Barrows were permitted to adjust to the metabolism crates for one week, after which a total collection of urine and feces occurred for one week. Feed and urine were analyzed for boron, nitrogen, calcium, and phosphorus concentrations. Feces were analyzed for nitrogen, calcium, and phosphorus concentrations. After the one-week collection period, each barrow was euthanized and the entire right leg removed. The right femurs and fibulas were harvested for the determination of bone mechanical properties.

VI. **Results:**

Objective 1. Apparent absorption and retention of Ca and P are shown in Table 1. Supplementation of 5 mg B/kg diet increased ($P < 0.10$) urinary excretion of P and decreased ($P < 0.10$) urinary excretion of Ca compared to pigs fed the control diet. Boron did not affect absorption or retention of Ca or P. Apparent absorption and retention of nitrogen was also not affected by boron.

Plasma concentrations of Ca, P, and Mg were determined at the end of the nursery phase and on day 30 of the growing phase (Table 2). Plasma Ca and Mg were not affected by B at the end of the nursery phase; however, plasma P concentrations were increased ($P < 0.05$) 5 and 15 mg supplemental B/kg diet at the end of the nursery phase. This effect of B upon plasma P was not evident on day 30 of the growing phase. Boron supplementation did not affect plasma Ca during the growing phase in trial 1, but 5 and 15 mg supplemental B/kg diet decreased ($P < 0.05$) plasma Ca in trial 2.

Fat-free ash percentage of the femur was not affected by B supplementation (Table 3). Boron supplementation did not affect Ca concentrations of the fat-free bone ash, but 15 mg supplemental B/kg diet increased ($P < 0.01$) P concentrations of the fat-free bone ash compared with controls (Table 3). Boron did not affect the fat-free ash percentage of bone; therefore, the increase in bone ash P by B is not clear. It is possible that the concentrations of other minerals, such as Mg, copper, or zinc were decreased by B in the bone ash. The percent lipid of the femur was also not affected by B supplementation.

Osteocalcin is the most abundant noncollagenous protein in bone, and serum concentrations of osteocalcin can provide a measure of osteoblast activity or bone remodeling. Serum osteocalcin concentrations were decreased ($P < 0.07$) in barrows consuming diets supplemented with 5 mg B/kg diet compared to control barrows at the end of the nursery phase (Table 3). However, serum osteocalcin concentrations in barrows consuming diets supplemented with 15 mg B/kg diet were not different from the other two dietary treatments during the nursery phase. The effect upon serum osteocalcin at the end of the 35 d nursery phase would not appear to be of physiological importance, because the bone is actively remodeling and growing at this time. Also, at the end of the 30 d growing period, serum osteocalcin concentrations were not affected by B, and B increased bone stress (Table 4) at the completion of the balance period in pigs supplemented with 15 mg B/kg diet.

Objective 2. Measures of bone mechanical properties are shown in Table 4. Barrows consuming diets supplemented with 15 mg B/kg diet had an increased ($P < 0.10$) stress of the femur, compared with pigs supplemented with 0 or 5 mg B/kg diet, following a bending test. Ultimate shear force of the fibula bone was increased ($P < 0.10$) in barrows supplemented with 15 mg B/kg diet compared with pigs supplemented with 0 or 5 mg B/kg diet. Supplementing the diet with 5 mg B/kg did not affect bone mechanical properties. Boron supplementation did not affect maximum load, bending moment, or cross-sectional area moment of inertia of the femur.

Objective 3. Pig performance during the nursery and growing phase is presented in Table 5. Boron supplementation during the nursery phase resulted in increased ($P < 0.05$) gain and feed intake. Barrows that consumed the diet supplemented with 15 mg B/kg diet had higher ($P < 0.05$) gain and feed intake during the growing phase than control pigs. Gain and feed intake did not differ between pigs fed 5 and those supplemented with 15 mg B/kg diet. Feed efficiency was not affected by treatment in either phase, indicating that the increased gain was due to higher feed intake.

Serum metabolites measured are shown in Table 6. Serum concentrations of T_3 and T_4 were not affected by B during the nursery phase, but in the growing phase barrows consuming diets supplemented with 5 mg B/kg diet had decreased ($P < 0.05$) serum concentrations of T_3 compared to control barrows. Serum concentrations of T_3 and T_4 were not different between the control and the 15 mg B treatment. Boron supplementation at both levels decreased ($P < 0.05$) serum concentrations of triglycerides during the nursery phase, but not during the growing phase. Serum cholesterol concentrations and alkaline phosphatase activity were not affected by treatment.

Summary. Results from this study indicate that supplementing low levels of boron may improve growth rate and feed intake in nursery and growing barrows. Supplementing 15 mg of boron per kg of diet tended to improve bone strength, but absorption and retention of phosphorus and calcium were not affected by boron. The diet used in the present study was adequate in phosphorus. If the diet had been low or marginally deficient in phosphorus, boron supplementation may have affected phosphorus absorption or retention.

Table 1. Effect of boron supplementation on apparent phosphorus and calcium balance of growing barrows

	Supplemental B, mg/kg			
	0	5	15	SEM
Phosphorus				
P intake, g/d	6.79	6.79	6.79	--
Fecal P, g/d	2.87	3.05	3.00	0.11
Urine P, g/d	0.31 [a]	0.48 [b]	0.36 [ab]	0.07
P absorbed, %	57.7	55.1	55.8	1.3
P retained, g/d	3.61	3.26	3.43	0.16
Calcium				
Ca intake, g/d	8.54	8.54	8.54	--
Fecal Ca, g/d	4.12	4.78	4.27	0.29
Urine Ca, g/d	0.19 [a]	0.14 [b]	0.17 [ab]	0.02
Ca absorbed, %	51.8	44.0	50.0	6.4
Ca retained, g/d	4.23	3.62	4.10	0.29

[a,b] Means in a row without a common superscript are different ($P < 0.10$).

Table 2. Plasma mineral concentrations of barrows fed various dietary concentrations of supplemental boron

	Supplemental B, mg/kg			
	0	5	15	SEM
Nursery (35 d)				
Calcium, mg/dL	9.25	8.92	9.09	0.18
Magnesium, mg/dL	1.48	1.61	2.37	0.44
Phosphorus, mg/dL	6.79 [b]	7.77 [c]	7.60 [c]	0.25
Growing (30 d)				
Calcium, mg/dL [a]	12.78 [b]	12.17 [c]	12.15 [c]	0.21
Trial 1	13.55	13.69	13.75	0.28
Trial 2	12.00 [b]	10.65 [c]	10.54 [c]	0.31
Magnesium, mg/dL	1.94	1.90	1.97	0.05
Phosphorus, mg/dL	9.73	10.14	9.65	0.22

[a] Treatment x trial interaction ($P < 0.05$).

[b,c] Means in a row without a common superscript are different ($P < 0.05$).

Table 3. Effect of boron supplementation on femur chemical properties and serum osteocalcin concentrations

	Supplemental B, mg/kg			
	0	5	15	SEM
Percent lipid	3.3	3.6	4.1	0.84
Fat-free ash percentage	63.4	63.9	63.7	0.39
Bone Ca, x 10^4 mg/kg fat-free ash	39.1	39.3	39.1	0.36
Bone P, x 10^4 mg/kg fat-free ash	17.8 [c]	18.9 [cd]	19.9 [d]	0.57
Serum osteocalcin, ng/mL				
Nursery [a]	453.6 [e]	384.7 [f]	422.4 [ef]	25.7
Growing [b]	343.0	303.2	312.5	20.0

[a] Samples obtained at the end of the 35 d nursery phase.

[b] Samples obtained at the end of the 30 d growing phase.

[c,d] Means in a row without a common superscript are different ($P < 0.01$).

[e,f] Means in a row without a common superscript are different ($P < 0.07$).

Table 4. Effects of boron supplementation on mechanical properties of the femur following a three-point bending test and the fibula following a shear test in growing barrows

	Supplemental B, mg/kg			
	0	5	15	SEM
Three-point bending (femur)				
Maximum load, kN	2.89	3.11	3.15	0.13
Bending moment, kN-mm	50.7	54.4	55.1	2.2
Stress, MPa	60.6 [a]	62.6 [a]	66.7 [b]	2.4
Moment of inertia, mm^4	8896	9398	8905	322
Shear (fibula)				
Ultimate shear force, kN	1.93 [a]	1.85 [a]	2.16 [b]	0.08

[a,b] Means in a row without a common superscript are different ($P < 0.10$).

Table 5. Effect of boron supplementation and concentration on growth performance of barrows

	Supplemental B, mg/kg			
	0	5	15	SEM
Nursery (0 - 35 d)				
Gain, kg	0.39 [a]	0.44 [b]	0.44 [b]	0.015
Intake, kg	0.67 [a]	0.77 [b]	0.80 [b]	0.024
Gain:feed	0.58	0.57	0.55	0.013
Growing (36 - 66 d)				
Gain, kg	0.89 [a]	0.96 [ab]	1.00 [b]	0.032
Intake, kg	1.71 [a]	1.88 [ab]	1.93 [b]	0.071
Gain:feed	0.52	0.51	0.52	0.010

[a,b] Means in a row without a common superscript are different ($P < 0.05$).

Table 6. Serum metabolite concentrations of barrows fed various dietary concentrations of supplemental boron

	Supplemental B, mg/kg			
	0	5	15	SEM
Nursery (35 d)				
ALP, U/L	279	242	242	18
Cholesterol, mg/dL	115.7	113.7	112.2	3.3
Triglycerides, mg/dL	64.4 [a]	49.3 [b]	46.6 [b]	4.5
T_3, ng/dL	141.3	130.3	137.7	6.2
T_4, µg/dL	5.7	5.6	6.1	0.36
Growing (30 d)				
ALP, U/L	156	162	142	10
Cholesterol, mg/dL	109.8	106.4	102.1	4.9
Triglycerides, mg/dL	39.4	41.1	32.7	4.3
T_3, ng/dL	126.8 [a]	108.6 [b]	120.1 [ab]	6.2
T_4, µg/dL	6.1	6.0	6.2	0.33

[a,b] Means in a row without a common superscript are different ($P < 0.05$).