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A calcitonin receptor (CALCR) single nucleotide polymorphism is associated with growth performance and bone integrity in response to dietary phosphorus deficiency

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Abstract

Although concerns over the environmental impact of excess P in the excreta from pig production and governmental regulations have driven research toward reducing dietary supplementation of P to swine diets for over a decade, recent dramatic increases in feed costs have further motivated researchers to identify means to further reduce dietary P supplementation. We have demonstrated that genetic background impacts P utilization in young pigs and have identified genetic polymorphisms in several target genes related to mineral utilization. In this study, we examined the impact of a SNP in the calcitonin receptor gene (*CALCR*) on P utilization in growing pigs. In Exp. 1, 36 gilts representing the 3 genotypes identified by this *CALCR* SNP (11, 12, and 22) were fed a P-adequate (PA) or a marginally P-deficient (approximately 20% less available P; PD) diet for 14 wk. As expected, P deficiency reduced plasma P concentration, bone strength, and mineral content ($P < 0.05$). However, the dietary P deficiency was mild enough to not affect the growth performance of these pigs. A genotype × dietary P interaction ($P < 0.05$) was observed in measures of bone integrity and mineral content, with the greatest reduction in bone strength and mineral content due to dietary P deficiency being associated with the allele 1. In Exp. 2, 168 pigs from a control line and low residual feed intake (RFI) line were genotyped for the *CALCR* SNP and fed a PA diet. As expected, pigs from the low RFI line consumed less feed but also gained less BW when compared with the control line ($P < 0.05$). Although ADFI did not differ between genotypes, pigs having the 11 genotype gained less BW ($P < 0.05$) than pigs having the 12 or 22 genotypes. Pigs of the 11 and 12 genotypes had bones that tolerated greater load when compared with animals having the 22 genotype ($P < 0.05$). A similar trend was observed in bone modulus and ash % ($P < 0.10$). These data are supportive of the association of this *CALCR* SNP with bone integrity and its response to dietary P restriction. Although the allele 1 is associated with greater bone integrity and mineral content during adequate P nutrition, it is also associated with the greatest loss in bone integrity and mineral content in response to dietary P restriction. Understanding the underlying genetic mechanisms that regulate P utilization may lead to novel strategies to produce more environmentally friendly pigs.

Keywords

bone, genotype, phosphorus, pig

Disciplines

Agriculture | Animal Sciences | Genetics and Genomics

Comments

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ABSTRACT: Although concerns over the environmental impact of excess P in the excreta from pig production and governmental regulations have driven research toward reducing dietary supplementation of P to swine diets for over a decade, recent dramatic increases in feed costs have further motivated researchers to identify means to further reduce dietary P supplementation. We have demonstrated that genetic background impacts P utilization in young pigs and have identified genetic polymorphisms in several target genes related to mineral utilization. In this study, we examined the impact of a SNP in the calcitonin receptor gene (*CALCR*) on P utilization in growing pigs. In Exp. 1, 36 gilts representing the 3 genotypes identified by this *CALCR* SNP (11, 12, and 22) were fed a P-adequate (PA) or a marginally P-deficient (approximately 20% less available P; PD) diet for 14 wk. As expected, P deficiency reduced plasma P concentration, bone strength, and mineral content ($P < 0.05$). However, the dietary P deficiency was mild enough to not affect the growth performance of these pigs. A genotype × dietary P interaction ($P < 0.05$) was observed in measures of bone integrity and mineral content, with the greatest reduction in bone strength and mineral content due to dietary P deficiency being associated with the allele 1. In Exp. 2, 168 pigs from a control line and low residual feed intake (RFI) line were genotyped for the *CALCR* SNP and fed a PA diet. As expected, pigs from the low RFI line consumed less feed but also gained less BW when compared with the control line ($P < 0.05$). Although ADFI did not differ between genotypes, pigs having the 11 genotype gained less BW ($P < 0.05$) than pigs having the 12 or 22 genotypes. Pigs of the 11 and 12 genotypes had bones that tolerated greater load when compared with animals having the 22 genotype ($P < 0.05$). A similar trend was observed in bone modulus and ash % ($P < 0.10$). These data are supportive of the association of this *CALCR* SNP with bone integrity and its response to dietary P restriction. Although the allele 1 is associated with greater bone integrity and mineral content during adequate P nutrition, it is also associated with the greatest loss in bone integrity and mineral content in response to dietary P restriction. Understanding the underlying genetic mechanisms that regulate P utilization may lead to novel strategies to produce more environmentally friendly pigs.

Key words: bone, genotype, phosphorus, pig

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INTRODUCTION

Whereas Environmental Protection Agency regulations limiting the amount of P that can be applied per unit of land (Federal Register, 2003) have driven research toward finding ways to reduce the excretion of P by swine (Harper et al., 1997; Knowlton et al., 2004; Sutton and Richert, 2004), the dramatic increase in the cost of dietary inorganic P sources has further motivated producers to reduce the quantity of inorganic P added to swine diets. Although a great deal of P nutrition research in swine has focused on identifying requirements for optimizing production efficiency and minimizing P excretion (Harper et al., 1997; Hastad et al., 2004; Knowlton et al., 2004), few research efforts have examined the impact of genetics on P utilization. However, Hittmeier et al. (2006) demonstrated that the metabolic response to a severe dietary P deficiency was modulated by genetic background in young pigs. Among these animals, Hittmeier (2005) also identified a SNP in intron 12 of the calcitonin receptor gene (***CALCR***) that was associated with bone integrity. Although a SNP within the same intron of the *CALCR* has also been identified in humans (Wolfe et al., 2003), neither

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has been associated with bone integrity or P utilization in the human or pig model.

Increasing interest in furthering the reduction of the environmental impact of swine production has fueled the need for better understanding the impact of P deficiency and how genetics may mediate this impact. The objective of the first experiment was to examine the impact of a *CALCR* SNP on the effects of a long-term, subtle dietary P deficiency on growing pigs, whereas the second experiment sought to determine the impact of the *CALCR* SNP independent of any dietary restrictions.

MATERIALS AND METHODS

All animal protocols were approved by Institutional Animal Care and Use Committee of Iowa State University.

Animals

Exp. 1. Thirty-six young female pigs (8.1 ± 2.0 kg) were obtained by breeding sows (Camborough 22, Pig Improvement Corporation, Franklin, KY) with mixed semen from PIC337 boars. Before the beginning of the experiment, all animals were genotyped for the *BanII CALCR* polymorphism identified by Hittmeier (2005). Briefly, a 477-bp section of the *CALCR* gene spanning from intron 12 (from 7,085 bp) into exon 14 (GenBank: Z31356.1) was amplified by PCR, and digestion of this PCR product with *Ban*II (New England Biolabs, Ipswich, MA) resulted in fragments of 477 bp (allele 1) or 332 and 145 bp (allele 2), resulting in 2 homozygote genotypes (11 and 22) and the heterzyogote (12) (Figure 1). The allele 1 does not, although allele 2 does, contain the *Ban*II site in intron 12. Only litters that contained at least a gilt of each genotype (3 gilts per litter) were selected for use in this experiment. At 35 ± 2 d of age, piglets were housed 3 per pen, with 1 genotype represented in each pen, under controlled environmental conditions (23 to 26°C and 14 h of light/d). Pigs were assigned to 1 of 2 dietary treatment groups based on BW and litter. All animals had ad libitum access to water and a P-adequate (**PA**) or an approximately 20% P-deficient (**PD**) diet over a 14-wk trial period (Table 1). Diets were formulated based on the NRC (1998) recommendations and were formulated for 4 different growth periods over the course of the experiment to reflect the changing dietary requirements of the growing pig. The 20% dietary P restriction was based on published available P values of the feed ingredients (NRC, 1998). Blood samples (approximately 10 mL) were collected initially and monthly after an overnight fast by venipuncture using heparinized tubes (Vacutainer Plus BD Vacutainer, Franklin Lakes, NJ). Plasma was obtained by centrifugation at 3,500 × *g* and 4°C for 15 min and stored at −20°C until analysis. Body weight and feed intake were recorded monthly, immediately preceding blood collection, throughout the trial. At the completion of the experiment, all animals were processed under USDA inspection at the Iowa State University Meat Laboratory (Ames). Pigs were electrically stunned using a head-only electric stun tong apparatus (BTR 100 AVS, Freund Maschinenfabrik GmbH & Co. KG, Paderborn, Germany), subsequently exsanguinated, scalded, and mechanically dehaired in a scald/dehairing tank (Oscar Baumann GmbH Co. type BM 20, Pioneer Food Equipment, Pennsgrove, NJ), and then eviscerated and weighed. Metacarpals 3 and 4 from the left foot were collected and stored at 4°C for bone strength analysis and ash percentage, respectively.

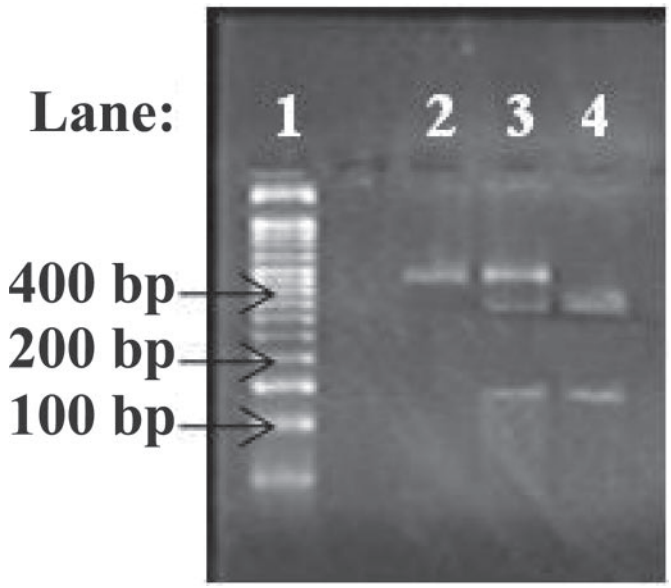

Figure 1. Identification of PCR-RFLP of calcitonin receptor gene (*CALCR*) SNP run in a 3% agarose gel. Lane 1: Step Ladder, 50 bp (Sigma, St. Louis, MO); lane 2: genotype 11 has a product length of 478 bp and is not cut by *Ban*II; lane 3: genotype 12, the heterozygote; and lane 4: genotype 22 has a 2-band product with the lengths of 332 and 145 bp.

Exp. 2. A total of 92 gilts from 27 litters from the fourth generation of a Yorkshire selection line for low residual feed intake and 76 gilts from 17 litters from a randomly selected control line were used for direct line comparison of growth and feed efficiency traits, as described by Cai et al. (2008). These animals were also used to determine the impact of the *CALCR* gene polymorphism on bone integrity, growth, and feed intake and efficiency. After standard rearing procedures, pigs from the 2 lines were mixed and allocated to 12 pens by BW and age when they were approximately 60 kg. All pigs were fed a diet that met or exceeded requirements of the NRC (1998). A split-plot design was used with line and *CALCR* genotype as factors. Litter was used as the main experimental unit to test for line differences and pig as the split-plot experimental unit to test for genotype differences. Individual feed intake was measured using electronic feeders (FIRE, Osborne Industries Inc., Osborne, KS). At the conclusion of the trial, pigs were slaughtered at a commercial abattoir (Hormel Foods, Austin, MN) in 3 groups with 1 group being slaughtered per day when a minimum BW of 102

Table 1. Composition of P-adequate (PA) and P-deficient (PD) diets by week of treatment for Exp. 1

	0 to 4 wk		4 to 8 wk		8 to 12 wk		12 to 14 wk	
Item	PA	PD	PA	PD	PA	PD	PA	PD
Ingredient, %								
Plasma, spray dried	1.5	1.5	0	0	0	0	0	0
Corn	64	64	70	70	74.2	74.2	79.4	79.4
Soybean meal, 48% CP	30	30	26	26	22	22	18	18
Dicalcium phosphate	1.30	1.10	0.95	0.70	0.75	0.48	0.58	0.35
Limestone	0.95	1.05	0.90	1.05	0.85	0.99	0.75	0.85
Corn oil	1	1	1	1	0	0	0	0
Vitamins[1]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Minerals[1]	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Se premix[2]	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Antibiotics[3]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calculated[4]								
CP, %	20.0	20.0	16.9	16.9	15.4	15.4	14.3	15.7
ME, kcal/g	3,287	3,287	3,357	3,357	3,281	3,281	3,324	3,324
$Calcium_{total}$, %	0.77	0.77	0.66	0.66	0.58	0.58	0.49	0.49
$Phosphorus_{total}$, %	0.63	0.59	0.53	0.49	0.48	0.43	0.43	0.39
$Phosphorus_{available}$, %	0.32	0.29	0.23	0.19	0.19	0.15	0.16	0.12

[1]Provided 6,614 IU of vitamin A, 1,653 IU of vitamin D_3, 33 IU of vitamin E, 0.03 mg of vitamin B_{12}, 9.9 mg of riboflavin, 49.6 mg of niacin, 26.45 mg of pantothenic acid, 105 mg of Fe from ferrous sulfate, 90 mg of Zn from zinc oxide, 36 mg of Mn from manganous oxide, 10.5 mg of Cu from copper oxide, and 1.2 mg of I from calcium iodate per kg of diet.

[2]Provided 0.25 mg of Se from sodium selenite per kg of diet.

[3]Provided 110 mg of oxytetracycline and 77 mg of neomycin sulfate (Anamix LLC, Juneau, WI) per kg of diet.

[4]Based on analysis of corn, soybean meal, dicalcium phosphate, and limestone (Eurofins, Des Moines, IA), and values provided by the suppliers of the other ingredients.

kg was reached. Further details are provided in Cai et al. (2008). Metacarpals 3 and 4 were collected from both front legs for analysis of bone strength and ash percentage, respectively.

Genotyping

Genomic DNA was isolated from pig tails before the beginning of each experiment (DNeasy Midi Kits, Qiagen, Valencia, CA) according to the manufacturer's instructions. A primer set (forward primer: TTCTCCTCGCCTGCCTTC and reverse primer: TCTGCCTGACACTGAACCAT) designed using a software (PrimerQuest, Integrated DNA Technologies, Coralville, IA) was used to amplify the region of the *CALCR* of interest. Thermocycling conditions for PCR included 1 cycle of melting at 94°C for 5 min followed by 30 cycles of melting at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 40 s. A final cycle included extension for 7 min at 72°C. After amplification, the *BanII* restriction enzyme (New England Biolabs) was used to digest the PCR product at 37°C for 4 h. After digestion, the restriction fragments were run on a 3% agarose gel for genotype determination (Figure 1).

Plasma Analysis

Plasma inorganic P (**PP**) and alkaline phosphatase (**ALP**) activity were determined for all plasma samples. Plasma inorganic P concentrations were determined by the method of Gomori (1942) modified for use with a microplate spectrophotometer (PowerWave HT Microplate Scanning Spectrophotometer, Bio-Tek, Winooski, VT). Briefly, plasma was deproteinated with 12.5% trichloroacetic acid and assayed using Elon solution (*p*-methylaminophenol sulfate). Alkaline phosphatase activity was assayed by the method of Bowers and McComb (1966), in which the rate of formation of yellow-colored structures by the hydrolysis of *p*-nitrophenol phosphate to *p*-nitrophenol is proportional to the level of ALP activity in the plasma. The rate of appearance of this yellow color was determined at 405 nm.

Bone Measures

The third metacarpals of all pigs were manually cleaned of all soft tissue and utilized for flexural testing with an instrument (Instron Universal Testing Machine Model 4502, Instron, Canton, MA) equipped with a 10 kN load cell and configured for 3-point bending tests. Load applied at failure was determined using the zero-slope method (Series IX, v. 8.08.00, Instron). For determination of bone modulus, the cross sectional moment of inertia was estimated using a formula for a perfectly elliptical cross-section (Turner et al., 1992). Before conducting the bend test, the lengths of the major and minor axis were determined at the point where the crosshead would come in contact with the bone. After completion of the bending test, 2 measurements of bone thickness at each axis, as close as possible to the point of breakage, were averaged and utilized for determination of the internal ellipse. Metacarpals were

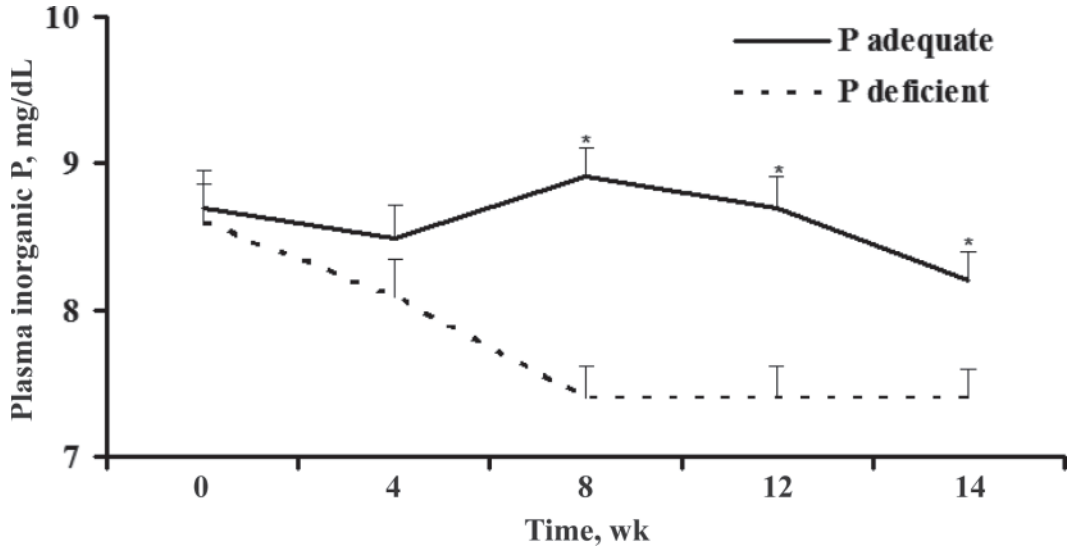

Figure 2. Effect of dietary P on plasma inorganic P concentrations (mg/dL; Exp. 1). Values presented are least squares means and SE. *Differences between dietary treatment groups were observed from 8 to 14 wk ($P < 0.05$). P adequate, n = 6; P deficient, n = 6.

placed on upright supports spaced 2 cm apart and the crosshead applied pressure to the bone equidistant between the 2 uprights. The crosshead speed was set at 50 mm/min. Mineral content of the fourth metacarpals were determined by drying at 110°C for 24 h followed by ashing at 600°C for 24 h.

Statistical Analysis

Using the pen as the experimental unit, data from individual animals in Exp. 1 were averaged for each pen and analyzed by a linear model using the MIXED procedure (SAS Inst. Inc., Cary, NC) with *CALCR* genotype, dietary treatment, and the interaction of the 2 considered as fixed effects. A standard *F*-test against the pooled residual variance was used to evaluate statistical significance of genotype, treatment, and their interaction. There were 3 pigs per pen with 1 pen per treatment for genotype 11, 2 pens per treatment for genotype 12, and 3 pens per treatment for genotype 22. Initial averaged BW for each was used as a covariate for growth performance data, whereas final average BW for each pen was utilized as a covariate for the bone biomechanical data. Growth performance and bone biomechanical data from animals in Exp. 2 were analyzed by a linear mixed model using the MIXED procedure of SAS, with on-test group (n = 2), slaughter day (n = 3), line, *CALCR* genotype, and the interaction of line and *CALCR* genotype as fixed effects. For growth performance, litter and pen within on-test group were considered random effects. On-test age was used as a covariate. Litter was considered as a random effect for bone biomechanical data, whereas off-test BW was used as a covariate (Cai et al., 2008). In Exp. 2, bone characteristics (length, load and displacement at failure, and modulus) of the left and right metacarpals were utilized for the determination of the correlation coefficient of these values.

RESULTS

Plasma

Because the animals in Exp. 2 were not subjected to any dietary treatments, PP concentrations were only determined for animals in Exp. 1. Initially, there was an effect of *CALCR* genotype on PP concentrations at 0 wk ($P < 0.05$), with animals of the 11 genotype having greater concentrations than animals of the 12 or 22 genotypes; however, this genotype effect was not seen at any of the other sampling times. There were no ($P > 0.1$) *CALCR* genotype × dietary treatment interactions throughout the experiment. After 4 wk, pigs receiving the PD tended ($P < 0.10$) to have less PP. This trend became statistically significant ($P < 0.05$) at 8 wk and remained for the duration of the trial (Figure 2). No effect ($P > 0.1$) of ALP activity was observed throughout the trial (data not shown).

Growth Performance

Exp. 1. The *CALCR* genotype, dietary treatment, or the interaction of the 2 did not affect ($P > 0.1$) growth performance of pigs in Exp. 1. Pigs in this experiment had ADG of 817 g and ADFI of 1.96 kg, which resulted in G:F of 0.417.

Exp. 2. There were effects of *CALCR* genotype, selection line, and *CALCR* genotype × selection line on ADG and ADFI in Exp. 2. As expected, animals selected for low RFI consumed less feed ($P < 0.01$), but also had slightly less BW gain ($P < 0.05$) when compared with those animals in the control line (Figure 3). Interactions between selection line and *CALCR* genotype were observed for ADG and ADFI (Figure 3). In the low RFI line, the effect of the *CALCR* genotype on ADG seemed to be additive, with the allele 1 resulting in less BW gain ($P < 0.05$). Pigs of the 11 genotype

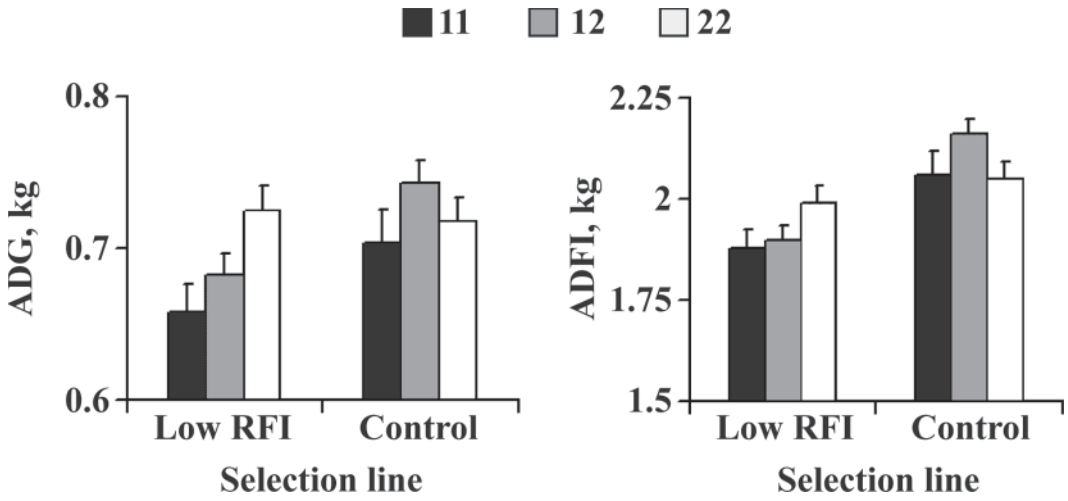

Figure 3. Effect of selection line and calcitonin receptor gene (*CALCR*) genotype on growth performance (kg/d; Exp. 2). Values presented are least squares means and SE. Differences between the selection lines [control (line 2) and line selected for low residual food intake (low RFI; line 1); $P < 0.05$], *CALCR* genotype ($P < 0.05$), and the interaction ($P < 0.05$) of the 2 were observed in ADG. An effect of selection line ($P < 0.01$) and selection line × *CALCR* genotype was noted in ADFI ($P < 0.05$). Line 1, n = 96; line 2, n = 72.

gained less BW than those of the 22 genotype, and the 12 genotype was intermediate and not different from the other genotypes. The effect of genotype in the low RFI line on ADFI was similar to its effect on ADG, but the differences were not statistically significant ($P > 0.1$; Figure 3). There were no differences ($P > 0.1$) in ADG or ADFI between genotypes in the control line, although heterozygotes tended ($P < 0.15$) to have greater ADG and ADFI than both homozygotes. It should also be noted that when comparing lines within genotype only animals having the 12 genotype differed in BW gain ($P < 0.01$) and feed consumption ($P < 0.01$).

Bone Characteristics

Exp. 1. Metacarpals of pigs fed the PD diet had reduced bone strength and mineral content ($P < 0.05$). Across genotypes, P deficiency reduced maximum load by 14%, bone modulus by 33%, and bone ash by 10% (Figure 4). Interactions between *CALCR* genotype and dietary P levels ($P < 0.05$) were observed in bone modulus and ash percentage (Figure 4). The metacarpals of pigs with the 11 genotype exhibited greater decreases in bone rigidity (modulus; $P < 0.05$) during P restriction when compared with pigs having the 12 or 22 genotype. The effect of the *CALCR* genotype on the loss of bone integrity due to dietary P deficiency seemed to be additive, with pigs of the 11 genotype having a greater loss of bone rigidity and mineral content ($P < 0.05$) than those having the 22 genotype, and the 12 genotype being intermediate and not different from the other genotypes. The allele 2 of this *CALCR* SNP was associated with less sensitivity to dietary P restriction, with the bones of the pigs homozygous for this allele not being affected by dietary P deficiency.

Exp. 2. Bone biomechanics data from the third metacarpals of the left and right legs were averaged for our analysis. Data from the left and right third metacarpal bones of each pigs were well correlated (length, r = 0.91; load at failure, r = 0.75; displacement at failure, r = 0.43; and bone modulus, r = 0.65). There were no effects ($P > 0.1$) of selection line or *CALCR* genotype × selection line on bone characteristics. Across the 2 lines, metacarpals from pigs having the 11 or 12 genotypes had 10 and 6% greater load bearing capability ($P < 0.05$), respectively, when compared with those animals having the 22 genotype (Figure 5). Metacarpals of animals having the 22 genotype tended to be less rigid ($P < 0.10$) and have the least mineral content ($P < 0.10$) when compared with the other genotypes. Conversely, the greatest rigidity ($P < 0.10$) and mineral content ($P < 0.10$) was observed in the bones of pigs having the 11 genotype.

DISCUSSION

In these experiments, we evaluated the association of a *CALCR* polymorphism with measures of bone integrity and their response to subtle dietary P deficiency in growing pigs. Although utilizing a subtle dietary P deficiency is not the most sensitive way to elucidate nutrient × genetic interactions, this mild deficiency was utilized to represent a plausible dietary situation in commercial pig production. With the dramatic increase in overall feed costs, as well as the cost of inorganic P sources, producers have reduced, if not removed, the safety margins for dietary P levels. The PD diet used in Exp. 1 was successful in achieving our desired level of P deficiency, as evidenced by the slightly less PP and reduced bone integrity and mineral content, while not affecting growth performance. Although the effects of dietary P deficiency in growing pigs have been described previously (Cromwell et al., 1995; Spencer et al., 2000; Veum et al., 2001; Jendza et al., 2005), very few studies have examined the effect of the interaction of genetics and dietary P utilization. To the best of our

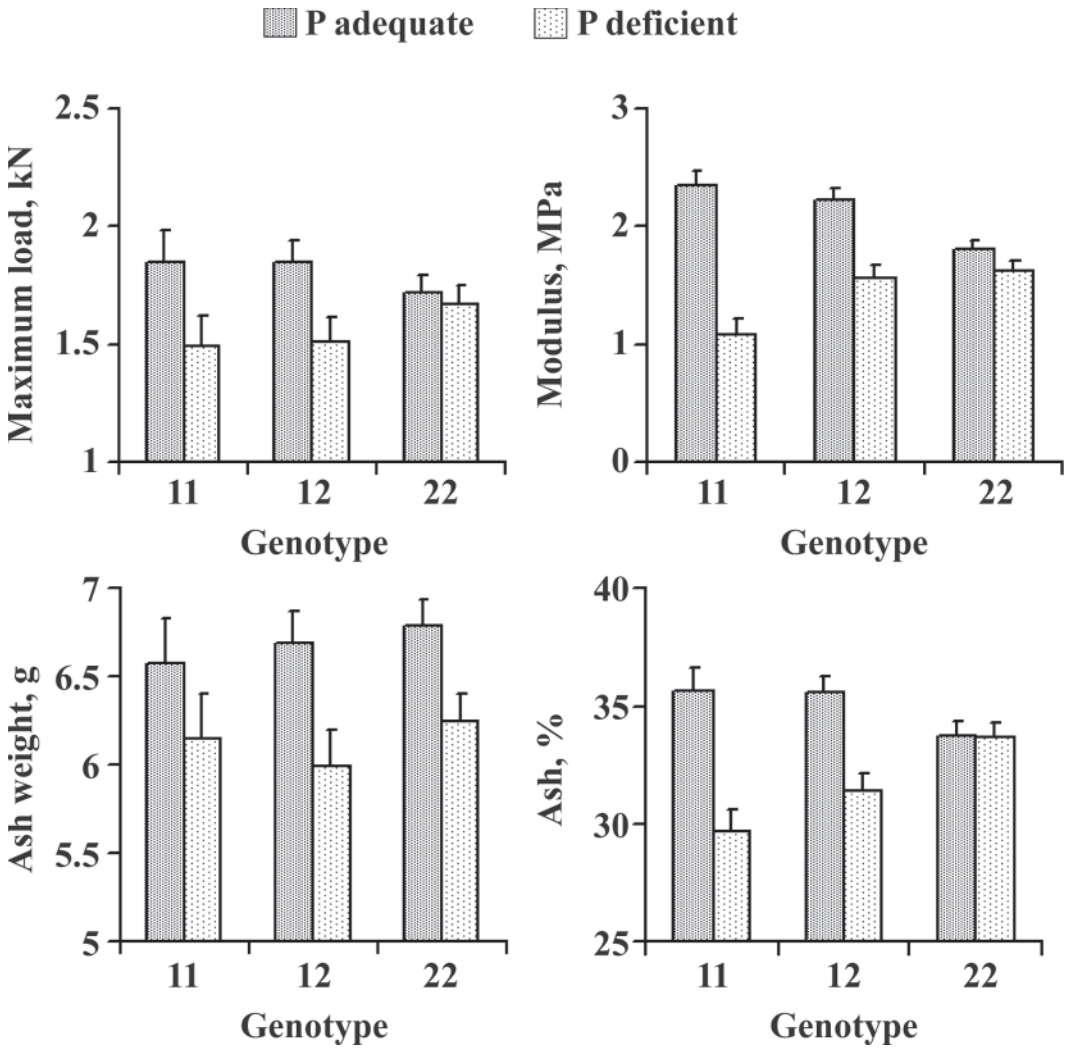

Figure 4. Effect of dietary P and genetic background interactions on bone integrity (Exp. 1). Values presented are least squares means and SE for the analysis of pen averages of 3 pigs per pen, with 1 pen per treatment for genotype 11, 2 pens per treatment for genotype 12, and 3 pens per treatment for genotype 22. Differences between dietary treatments ($P < 0.05$) were observed in maximum load and ash weight. An effect of dietary treatment ($P < 0.05$) and dietary treatment × calcitonin receptor gene (*CALCR*) genotype ($P < 0.05$) was noted in bone modulus and ash percentage.

knowledge, this is the first report demonstrating that a genetic polymorphism impacts the response to dietary P deficiency in swine.

A major focus for the discovery of polymorphisms in production animals has been to identify those associated with traits that are considered to be economically important such as lean muscle accretion, feed efficiency, and growth (Franco et al., 2005; de Oliveira Peixoto et al., 2006; Houston et al., 2006; Verner et al., 2007; Nie et al., 2008). Far fewer studies have identified polymorphisms associated with overall animal health, specifically as it relates to bone health. Although various gene polymorphisms associated with bone health have been identified in chickens (Li et al., 2003; Bennett et al., 2004; Zhou et al., 2005; Bennet et al., 2006), far fewer have been discovered in pigs (Kadarmideen, 2008). In these experiments, we have demonstrated that a *CALCR* SNP is associated with bone integrity, and of greater interest is that there is an interaction of this association with dietary P status.

Our analysis of bone integrity in Exp. 2 revealed an association of this *CALCR* SNP with load-bearing capability and mineral content among a low RFI line and control line (Figure 5) fed nutritionally adequate diets. Allele 1 was associated with greater bone integrity with the 11 animals tending to have the greatest bone strength and mineral content and the 22 animals having the poorest values. An association of this SNP with bone rigidity was also observed among the PA-fed pigs in Exp. 1; however, this SNP was not significantly associated with bone mineral content, which was not too surprising due to the dramatically smaller number of animal utilized in this feeding trial and the magnitude of the differences in bone mineral content observed in Exp. 2. However, when the nutritional needs of the animals were not being met, this SNP was associated with bone mineral content. The mild P deficiency imposed in Exp. 1 revealed an interaction between this *CALCR* SNP and nutritional status on bone integrity. The allele 1, which was associated with the greatest bone integrity

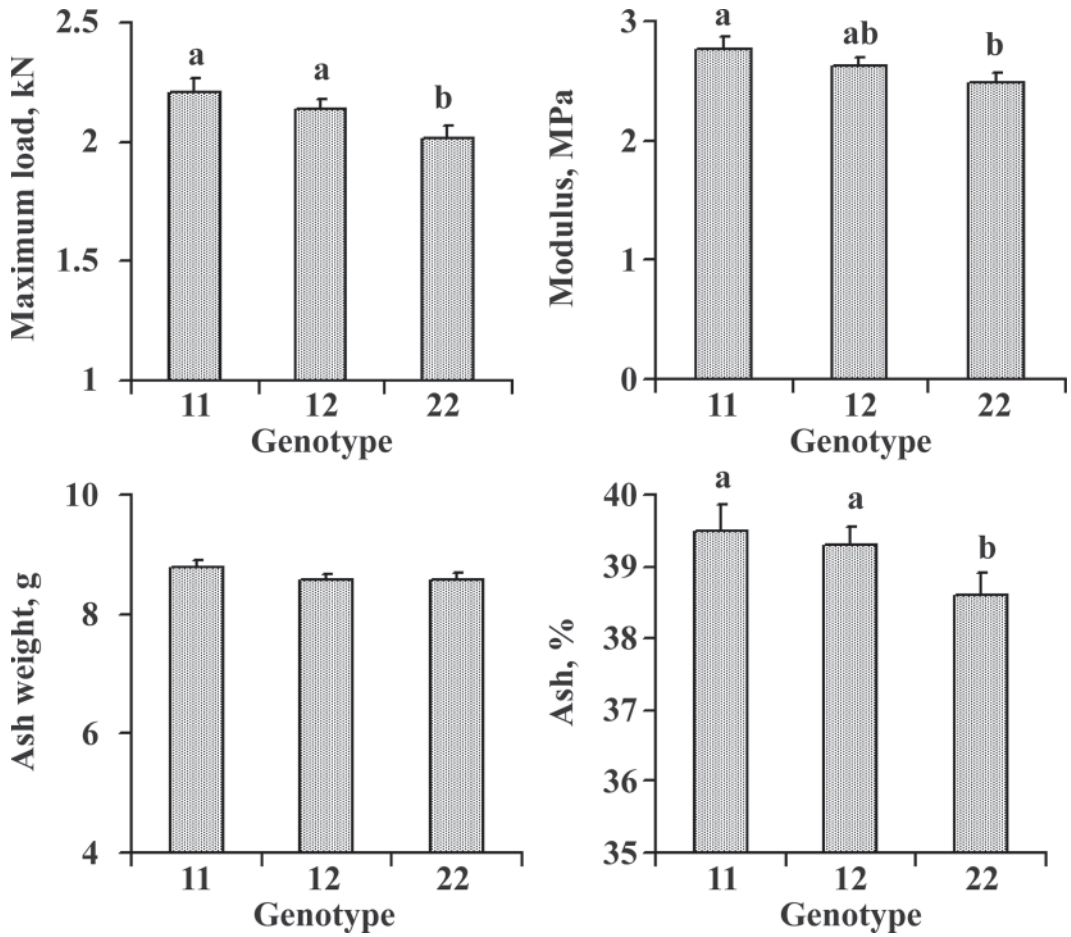

Figure 5. Effect of calcitonin receptor gene (*CALCR*) genotype on bone strength (Exp. 2). Values presented are least squares means and SE. A *CALCR* genotype effect ($P < 0.05$) was observed in load at yield, whereas similar trends existed for modulus ($P < 0.10$) and ash percentage ($P < 0.10$). Those sharing a common superscript are not different ($P > 0.1$). 11 genotype, n = 38; 12 genotype, n = 73; 22 genotype, n = 54.

during nutritional adequacy (Exp. 2), was associated with more dramatic reductions in bone integrity and mineral content during a mild P deficiency. During P deficiency, the metacarpals of those animals with the 22 genotype tended to have greater load capacity and significantly greater rigidity and ash percentage when compared with animals with the 11 genotype, whereas the bone characteristics of animals having the 12 genotype fell in between those of the homozygous animals. Whereas the classical reductions in bone mineral content and strength exhibited during dietary P deficiency were observed in animals of the 11 or 12 genotypes, they were not observed in gilts having the 22 genotype. Because there were no differences in growth performance between these genotypes during dietary P restriction but there were differences in measures of bone integrity, it is likely that pigs having the 22 genotype have altered homeorhetic control of P utilization and are not as responsive to marginal P restriction. Additionally, pigs with the 11 genotype are the most sensitive to dietary P restriction as demonstrated by greater decreases in metacarpal rigidity and ash % when compared with pigs with either of the other 2 genotypes.

The identification of genetic polymorphisms that interact with nutrients to affect utilization or bone integrity have not been previously identified in agriculturally relevant species; however, several polymorphisms associated with nutrient utilization and bone characteristics have been identified in humans. Harrington et al. (2004) found that Ca utilization during consumption of diets high in protein and sodium is altered based on the *Fok*I polymorphism in the *VDR* gene in women. Additionally, Ferrari et al. (2004) identified an effect of a polymorphism in the *IL-6* gene in modulating the impact of reduced dietary Ca on bone mineral density in men and women. With increasing pressure to improve the economic and environmental sustainability of swine production, more work is needed to identify genetic markers of nutrient utilization.

Breeding programs have greatly improved swine production efficiency by effectively reducing animal feed consumption while concurrently increasing the production of economically favorable traits. However, the selection for greater efficiency appears to carry the risk for greater health problems (Rauw et al., 1998). Selection for increased leanness and growth rate has been correlated with leg weakness in pigs (Rauw et al., 1998; Stalder et al., 2004), and leg weakness has been stated as the second most common reason for involuntary culling of sows (Stalder et al., 2004). Poor leg condition also results in increases in veterinary costs and reductions in efficiency (Kanis et al., 2005), neither of which

are economically beneficial to the producer. Because proper bone health plays a major role in the economic viability of swine production, utilization of our data may offer possible management strategies based on the interaction of the *CALCR* polymorphism and diet to improve sow longevity and gilt development. Although changing the genetics of a herd by selecting for a particular genotype may not be economically plausible, it may be beneficial to alter the diets fed to animals based on genotype to maximize muscle and skeletal growth potential. By defining P needs based on genetic background and developing genotype specific diets and novel breeding strategies, we will be able to produce more environmentally friendly pigs.

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