Effect of dietary nutrients on osteochondrosis lesions and cartilage properties in pigs

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Objective—To evaluate dietary ingredients involved in cartilage and bone metabolism and their influence on osteochondrosis lesions in swine.

Animals—80 crossbred gilts (mean initial weight, 39 kg).

Procedures—Pigs (10 pigs/treatment) were fed a corn–soybean meal basal (control) diet or the basal diet supplemented with additional minerals (copper and manganese or silicon), amino acids (proline and glycine; a combination of leucine, isoleucine, and valine; or methionine and threonine), or fatty acids (provided by fish oil) for 84 days. Pigs were then slaughtered and the distal portion of the left femur was collected for determination of osteochondrosis lesions at the femoral condyle. After evaluation of external joint surfaces, the distal portion of the femur was sectioned to evaluate lesions in the growth plate and articular cartilage. Additionally, a cartilage specimen was obtained from the patella for analysis.

Results—Pigs fed diets containing high amounts of methionine and threonine or the diet containing all additional ingredients had significantly lower total severity scores, compared with scores for pigs fed the control diet or a diet supplemented with fish oil. Pigs fed diets containing additional proline and glycine, copper and manganese, methionine and threonine, or all additional ingredients had significantly lower overall scores, compared with scores for pigs fed the control diet or a diet supplemented with fish oil.

Conclusions and Clinical Relevance—Dietary manipulation decreased the severity of osteochondrosis lesions, compared with results for pigs fed a control diet. However, additional research on optimal concentrations and combinations of dietary components is needed. (*Am J Vet Res* 2008;69:617–624)

Osteochondrosis, a failure in the endochondral ossification of cartilage to bone,[1,2] remains a common problem among growing swine in approximately 85% to 90% of all pigs.[3,4] Osteochondrosis lesions in pigs are similar to lesions in other animal species.[5] The main changes in cartilage that have been identified with osteochondrosis are a loss of proteoglycans and collagen type II, with increased chondrocyte necrosis.[6,7] Osteochondrosis can reduce sow longevity because of lameness[8] and negatively affects carcass meat-yield traits of finishing pigs.[9] It is believed by some that osteochondrosis results from a disruption in cartilage canal vessels that supply blood to the end of growing long bones,[10,11] but it is more likely that osteochondrosis results from focalized disruption of endochondral ossification by mechanical stress.[12] Fast growth rate has been cited as a factor in manifestation of osteochondrosis lesions.[13] Studies[14,15] have not detected a correlation between growth rate and incidence of osteochondrosis. Other factors, such as heredity[16] and trauma,[17] have also been implicated in the prevalence of osteochondrosis.

Abbreviations

MMP	Matrix metalloproteinase
SAMe	S-adenosylmethionine
TID	True ileal digestible
BCAA	Branched-chain amino acid
ME	Metabolizable energy

Several attempts have been made to determine the ability of dietary nutrients, such as protein, energy, calcium and phosphorus, vitamin D, and vitamin C, to influence the incidence and severity of osteochondrosis in swine, with little success.[15,18,19] The use of glycosaminoglycans (ie, glucosamine and chondroitin sulfate) has been suggested for treatment of animals with arthritic joint disorders[20] but has not been evaluated in swine with experimentally induced osteochondrosis. Evaluation of several minerals involved in collagen and proteoglycan synthesis has suggested the importance of Cu,[21] Mn,[22] or Si.[23–25] Dietary Cu plays a role in reducing the severity of osteochondrosis lesions in horses.[26,27] Glucosamine and n-3 fatty acids[28,29] are involved in minimizing cartilage degradation by blocking the production of MMPs; high concentrations of MMPs can excessively degrade cartilage components.[30,31] In addition, SAMe, a metabolite of methionine, can increase

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synthesis of collagen and proteoglycan[32] components of cartilage. Collagen contains a high concentration of the nonessential amino acids proline and glycine,[33] but the influence of feeding additional proline or glycine on osteochondrosis or cartilage synthesis has not been evaluated. Although the role of minerals (such as Cu, Mn, and Si) and n-3 fatty acids in cartilage and bone formation is relatively clearly defined, their ability to prevent or aid in repair of damage resulting from joint diseases, such as osteochondrosis, through dietary intervention has not been described. Therefore, the objective of the study reported here was to evaluate dietary ingredients involved in cartilage and bone metabolism for their influence on the frequency and severity of osteochondrosis lesions, other cartilage criteria, growth performance, and carcass characteristics in growing-finishing pigs.

Materials and Methods

Animals—Eighty gilts ([Hampshire × Pietrain] × [Large White × Landrace] crossbred gilts) with a mean initial body weight of 39 kg were used in the study. Gilts were blocked on the basis of body weight and allotted in a randomized manner to 1 of 8 dietary treatments. Experimental diets were fed in meal form for 84 days in three 28-day phases. The experiment was conducted at the Kansas State University Swine Research and Teaching Center finishing facility. The facility contained 80 pens with totally slatted concrete flooring. Each pen (1.52 × 1.52 m) was equipped with a 1-hole self-feeder[a] and nipple waterer to allow ad libitum access to feed and water. Each pen contained 1 pig for a total of 10 replicates (ie, pigs)/treatment. Procedures used in this experiment were approved by the Kansas State University Animal Care and Use Committee.

Treatments—Minimum TID amino acid ratios relative to lysine were maintained in all diets with minimum ratios set at 30% for methionine, 60% for methionine and cystine, 65% for threonine, and 16.5% for tryptophan. All essential nutrients were supplied at values equal to or greater than National Research Council estimates.[34] Diet samples were analyzed for amino acid concentration, and each diet contained amounts similar to calculated values.

Diets—Diets consisted of a basal diet (standard corn–soybean meal diet with 3.5% choice white grease [control diet]), fish oil diet (basal diet with fish oil [3.5%] substituted for the choice white grease), proline-glycine diet (proline and glycine at 300% and 200% of lysine, respectively), BCAA diet (leucine, isoleucine, and valine at 200%, 100%, and 100% of lysine, respectively), Si diet (1,000 µg of Si/g of diet), Cu-Mn diet (Cu and Mn at 250 and 100 µg/g of diet, respectively), methionine-threonine diet (methionine and threonine at 110% and 100% of lysine, respectively), and all ingredients diet (all ingredients in the aforementioned supplemented diets combined into 1 diet [**Appendix 1**]). The control diet contained various amounts of the amino acids proline (100% of lysine), glycine (65% of lysine), leucine (145% of lysine), isoleucine (69% of lysine), valine (76% of lysine), methionine (30% of lysine), and threonine (67% of lysine) with mineral concentrations of Cu (16.5 µg/g of diet [phase 1] and 14 µg/g of diet [phases 2 and 3]), Mn (40 µg/g of diet [phase 1] and 33 µg/g of diet [phases 2 and 3]), and Si (0 µg/g of diet). Diets for phase 1 were formulated to contain 1.07% TID lysine and 3,457 Mcal of ME (**Appendices 2 and 3**). Diets for phase 2 contained 0.94% TID lysine and 3,468 Mcal of ME, whereas diets for phase 3 contained 0.80% TID lysine and 3,463 Mcal of ME. In each phase, all essential amino acids other than those used in dietary treatments were provided at approximately 10% more than the requirement for pigs in these weight ranges, and the amount of added fat varied slightly to maintain isocaloric diets.

Growth performance and carcass data—Pigs and feeders were weighed every 14 days to determine average daily gain, average daily feed intake, and the gain-to-feed ratio. At the end of the study, each pig was weighed and identified with a distinctive tattoo before transport to the Kansas State University Meats Laboratory, where the pigs were slaughtered, carcass data were collected, and the left hind limb of each was collected for determination of osteochondrosis lesions. Pigs were loaded onto a trailer in small groups (18 to 20 pigs) and transported approximately 4 km to the Meats Laboratory.

At the start of the study, all gilts were ultrasonographically scanned to determine initial backfat depth and estimate the amount of fat-free lean. For carcass data, backfat depth at the 10th rib, longissimus muscle area, fat-free lean index, fat-free lean gain, and hot carcass weight were evaluated. Fat depth was measured with a ruler at the 10th rib (6 cm lateral to midline), whereas the longissimus muscle area was traced on translucent paper and calculated by use of a grid. Fat-free lean index was calculated in accordance with procedures of the National Pork Producers Council,[35] and fat-free lean gain per day was calculated as the final fat-free lean value minus the initial fat-free lean value, with the difference divided by number of days receiving feed.

Collection of cartilage data and osteochondrosis lesions scores—The left femur of each pig was removed, and the distal end of the femoral medial and lateral condyles was visually examined to determine the number of cartilage abnormalities and number of osteochondrosis lesions. Joints of the left hind limb were cleaned of excess tissue and then stored in neutral-buffered 10% formalin until evaluation. Joints were photographed to allow visual evaluation of the external surface, underlying articular cartilage, and subchondral bone interface. After external evaluation, the distal end of the femur was cut into sections (thickness of 3 mm) perpendicular to the long axis of the bone by use of a bandsaw.[16] This resulted in 12 faces (cut surfaces) for evaluation of lesions. Each joint was evaluated for the number of abnormalities of the external surface (fissures or defects in the cartilage surface) and number of osteochondrosis lesions at the articular and growth plate cartilages. Severity of lesions was scored on a scale of 0 to 4 (0 = normal, 1 = mild, 2 = moderate, 3 = severe, and 4 = osteochondrosis dissecans) on the basis of the extent of tissue involvement. This scoring system was similar to the system used in another study.[16] The number of abnormalities at the external surface and faces

or in sections with lesions at the articular cartilage and growth plate was also recorded. Each pig was also assigned a dichotomous outcome score (yes or no) for evidence of osteochondrosis lesions to determine the effect of treatment on frequency of osteochondrosis.

A cartilage sample was harvested from the patella of each pig and submitted for analysis to determine the effect of dietary nutrients on mechanical properties of the cartilage. This was similar to the process used in another study[36] (ie, the indenter system) but with force applied to the entire cartilage sample by a materials-testing machine.[b] Cartilage samples were weighed, thickness and length were measured with calipers, and samples were then tested for the ability to absorb compression or to resist shearing. Cartilage samples were placed between 2 flat surfaces of the machine to perform texture profile analysis; samples were compressed to half of their original thickness. A second procedure was conducted in which the cartilage was cut by use of a Warner-Bratzler shear blade to determine the ability of the cartilage to withstand shearing force. Compression values and shear values were adjusted per gram of cartilage weight to equalize for differences in actual cartilage weight.

Statistical analysis—Data were analyzed as a randomized complete block design by use of a generalized linear model,[c] with pig as the experimental unit. The response criteria of growth performance, carcass composition, cartilage compression and shearing, and number of abnormalities were tested. Although scored categorically, severity scores were analyzed via the generalized linear model because a small number of observations for some of the severity scores prevented categoric analysis. An overall score for the number of abnormalities at each location multiplied by the severity at each location and then a sum of the products was created to provide an overall severity score or indication of joint status. The yes-no comparison for evidence of osteochondrosis lesions was compared by use of the Fisher exact statistic. To evaluate the effect of amino acids or mineral-containing diets relative to the other dietary treatments, preplanned contrasts with 1 *df* were constructed.[37] Comparison of individual means was protected by significant *F* tests by use of a cutoff value of $P < 0.05$. Individual means and preplanned contrasts were compared by use of least significant difference tests with a cutoff value of $P < 0.05$.

Results

Growth and carcass data—Overall (84 days) growth performance and carcass characteristics were unaffected by dietary treatment (**Table 1**). Initial body weight of pigs was 39 kg, and mean final body weight was 131.5 kg.

Cartilage evaluation—Cartilage compression values were unaffected by dietary treatment (**Table 2**). However, pigs fed the fish oil diet had significantly lower shear energy values, compared with values for pigs fed the Cu-Mn diet. Pigs fed the fish oil diet had a higher compression-to-shear energy ratio, compared with the ratio for pigs fed the control diet, Cu-Mn diet, or Si diet.

Joint evaluation—The number of pigs with osteochondrosis did not differ significantly ($P = 0.58$) between dietary treatments (**Table 3**). Pigs fed the proline-glycine diet, Cu-Mn diet, methionine-threonine diet, or all ingredients diet had significantly lower overall scores, compared with scores for pigs fed the control diet or fish oil diet. Pigs fed the Si diet had a significantly lower overall score, compared with the score for pigs fed the fish oil diet. Analysis of contrast statements also revealed that pigs fed the diets containing additional minerals (ie, Si, Cu-Mn, and all ingredients diets) had significantly ($P = 0.02$) lower overall severity scores. Pigs fed diets containing additional amino acids (ie, proline-glycine, BCAA, methionine-threonine, and all ingredients diets) had a significantly lower number of external abnormalities or mean external abnormality severity score and total mean severity score, compared with results for pigs fed the other dietary treatments.

Table 1—Effect of diets supplemented with various nutrients on growth performance and carcass composition for 80 crossbred pigs fed the diets for 84 days (three 28-day feeding phases).

Variable	Control	Fish oil	Proline-glycine	BCAA	Si	Cu-Mn	Methionine-threonine	All ingredients*	SED
Growth									
ADG (kg)	1.09	1.06	1.08	1.12	1.11	1.13	1.03	1.04	0.047
ADFI (kg)	2.78	2.65	2.71	2.80	2.79	2.74	2.64	2.58	0.110
Gain-to-feed ratio	0.39	0.40	0.40	0.40	0.40	0.41	0.39	0.40	0.013
Final weight (kg)	133.6	128.0	132.2	133.1	135.5	130.3	128.9	129.5	4.64
Carcass data									
Initial backfat (mm)	5.3	5.4	5.1	5.5	5.1	5.1	5.4	5.1	0.02
Hot carcass weight (kg)†	95.2	93.4	93.0	94.9	96.5	93.8	91.5	89.4	3.28
Final backfat (mm)	15.6	15.7	15.1	14.3	14.2	15.5	15.9	16.1	0.06
LMA (cm^2)	49.5	51.2	48.3	48.8	48.4	49.0	53.5	47.1	2.24
Fat-free lean index‡	55.4	55.7	55.3	55.5	55.7	55.4	56.1	54.5	1.09
Fat-free lean gain (kg/d)§	0.436	0.432	0.428	0.454	0.450	0.431	0.424	0.400	0.020

Values reported are the mean of 10 replications (except for Cu-Mn, which had only 9 replications) with pigs that had an initial body weight of 39 kg and mean final body weight of 131.5 kg.

*Diet contained all additional ingredients. †Hot carcass weight was used as a covariate in analysis of carcass data, except for initial backfat and fat-free lean gain. ‡Fat-free lean index was calculated in accordance with procedures of the National Pork Producers Council.[35] §Calculated as the final fat-free lean minus initial fat-free lean, with the difference divided by number of days receiving feed.

SED = Standard error of the difference. ADG = Average daily gain. ADFI = Average daily feed intake. LMA = Longissimus muscle area.

Table 2—Effect of diets supplemented with various nutrients on cartilage properties in samples obtained from 80 crossbred pigs fed the diets for 84 days (three 28-day feeding phases).

Variable	Control	Fish oil	Proline-glycine	BCAA	Si	Cu-Mn	Methionine-threonine	All ingredients*	SED
Compression energy (N/g)†	85.4	126.9	144.7	102.5	86.0	59.8	116.4	110.7	39.22
Shear energy (N/g)‡	518.1[a,b]	371.4[a]	444.7[a,b]	491.8[a,b]	527.1[a,b]	601.5[b]	498.8[a,b]	540.9[a,b]	61.32
Total energy (N/g)[2]§	1,271.4	1,226.8	976.5	1,303.3	1,342.3	1,401.9	1,326.9	1,539.6	291.54
Compression energy-to-shear energy ratio‖	0.15[a]	0.41[b]	0.31[a,b]	0.25[a,b]	0.17[a]	0.15[a]	0.25[a,b]	0.21[a,b]	0.081

Values reported are the mean of 10 replications (except for Cu-Mn, which had only 9 replications) with pigs that had an initial body weight of 39 kg and mean final body weight of 131.5 kg.

†Amount of energy needed to compress the cartilage to half its original thickness. ‡Amount of peak energy needed to shear the cartilage into 2 pieces. §Total amount of energy needed to shear the cartilage into 2 pieces. ‖For this variable, lower values indicate more desirable characteristics of the cartilage.

[a,b]Within a row, values with different superscript letters differ significantly ($P < 0.05$).

See Table 1 for remainder of key.

Table 3—Effect of diets supplemented with various nutrients on incidence of osteochondrosis in 80 crossbred pigs fed the diets for 84 days (three 28-day feeding phases).

										Contrasts (*P* value)	
Variable	Control	Fish oil	Proline-glycine	BCAA	Si	Cu-Mn	Methionine-threonine	All ingredients*	SED	Mineral diets† vs all other diets	Amino acid diets‡ vs all other diets
No. of pigs/diet	10	10	10	10	10	9	10	10	—	—	—
No. of pigs with osteochondrosis lesions	9	9	9	9	6	7	7	7	—	—	—
External surface											
Abnormalities§	1.5	2.1	1.4	1.3	2.1	1.2	1.1	1.0	0.56	0.86	0.02
Severity score‖	1.9	1.7	1.6	1.2	1.8	1.3	1.1	1.0	0.48	0.46	0.01
Articular cartilage											
No. of faces¶	5.0	4.5	2.4	5.0	2.2	2.3	2.6	4.1	1.44	0.19	0.87
Severity score‖	2.0	1.3	1.2	1.6	0.7	0.8	0.7	1.3	0.48	0.08	0.98
Growth plate											
No. of faces¶	0.9	1.7	1.2	0.6	1.2	1.6	1.8	0.2	0.68	0.47	0.25
Severity score‖	0.6	1.1	0.9	0.6	0.8	0.8	1.0	0.1	0.43	0.21	0.43
Overall											
Total No. of faces#	5.9	6.2	3.6	5.6	3.4	3.9	4.4	4.3	1.81	0.17	0.68
Total No. of lesions**	7.8	8.8	5.6	7.0	5.9	5.7	5.7	5.7	1.89	0.21	0.27
Total severity††	4.7	4.9	4.0	3.6	3.9	3.4	3.0	2.8	0.85	0.12	0.05
Overall score‡‡	17.1[a]	15.0[a,b]	8.8[b,c]	12.4[a,b,c]	8.4[b,c]	6.4[c]	6.6[c]	7.0[c]	3.76	0.02	0.11

†Represents the mean of Si, Cu-Mn, and all ingredients diets. ‡Represents the mean of proline-glycine, BCAA, methionine-threonine, and all ingredients diets. §Mean number of abnormalities of the external surface per pig. ‖Lesion severity scored on a scale of 0 to 4 (0 = normal, 1 = mild, 2 = moderate, 3 = severe, and 4 = osteochondrosis dissecans). ¶Mean number of faces with lesions (evalution of 12 cut surfaces). #Sum of the mean values for articular cartilage and growth plate. **Sum of the mean value for abnormalities of the external surface and number of faces of the articular cartilage and growth plate. ††Sum of mean severity scores for external surface, articular cartilage, and growth plate. ‡‡Calculated as the number of abnormalities or faces multiplied by the severity for each location and then a sum of the products for each individual pig.

— = Not applicable. [a-c]Values with different superscript letters differ significantly ($P < 0.05$).

See Table 1 for remainder of key.

Discussion

The objective of the study reported here was to evaluate and identify nutrient strategies to reduce the severity or prevalence of osteochondrosis lesions in swine. The incidence of osteochondrosis is high in swine and is similar to that for osteochondrosis in other species.[3,5] One of the main concerns with osteochondrosis and lameness in swine is the negative effects it may have on sow longevity.[8,38] Turnover rates of > 50% for sow herds have been reported,[39] with the most common causes for turnover including reproductive failure and lameness.[40] In the study reported here, dietary ingredients were screened on the basis of structural and functional roles in cartilage and bone metabolism to determine their ability to impact the incidence and severity of osteochondrosis lesions. No differences in growth performance were detected. Growth performance in this study was similar to that in other experiments performed at our facility and slightly higher than growth rates measured for commercial facilities.

Analysis of the results from this study suggested that providing the minerals Cu and Mn in excess of requirements[34] may reduce the severity of osteochondrosis lesions by affecting the overall score. This data indicated that both Cu and Mn may reduce the severity of osteochondrosis lesions by maximizing lysyl oxidase activity,[41] stimulating proper collagen crosslinking,[42] improving the integrity of vascular walls of cartilage canal vessels, or increasing formation of proteoglycans.[34] Diets formulated for swine are often supplemented with Cu because of its beneficial effects on growth and

efficiency[22] and suspected antimicrobial activity. In a study[42] in which additional Cu was fed to rats, Cu-deficient diet or a diet with additional Cu did not affect the amount of lysyl oxidase mRNA, but additional dietary Cu supplementation increased activity of the enzyme. The addition of Cu to human articular chondrocytes results in a dose- and time-dependent increase in collagen synthesis, as determined by incorporation of [3H]-proline.[21] Similar benefits have been reported[43] in that the addition of Cu to cell cultures of porcine articular cartilage cells can prevent depletion of proteoglycans or stimulate proteoglycan synthesis. Diets supplemented with high amounts of zinc (5,000 µg/g of diet) have been proposed to inhibit Cu absorption and result in an increased incidence of osteochondrosis in swine.[44,45] In addition, sows fed additional Cu (100 µg/g of diet) have offspring with less severe osteochondrosis lesions, compared with the severity of lesions for sows fed diets containing Cu at 15 µg/g of diet.[d] Similar to results of other experiments, supplemental Cu decreased the severity of osteochondrosis. Additional research is required to elucidate the mechanism by which Cu exerts chondroprotective effects.

Manganese fed in combination with Cu reduced overall osteochondrosis score. Manganese is involved in proteoglycan metabolism through glycosyltransferases, which are abundant in cartilage,[46] and serves a structural role in linking chondroitin sulfate molecules. Manganese is also critically important in mitochondrial superoxide dismutase to control production of free radicals by oxidation reactions in the mitochondria.[47] Feeding rats a diet deficient in Mn results in a decrease in bone formation,[48] and such diets can negatively impact proteoglycan metabolism in chickens.[22] Studies on the influence of Mn on joint diseases are limited.

Feeding pigs a diet supplemented with Si, a mineral with no established requirement for swine,[34] reduced the overall osteochondrosis score. Silicon was supplied as zeolite A (silica acid), which can significantly increase serum concentrations of Si in horses.[49] It has been speculated that Si is required for proper cartilage and bone metabolism because of its role in collagen formation and bone mineralization,[50] and Si is found in relatively large quantities in the proteoglycan matrix.[23] Silicon is required for maximal prolyl hydroxylase enzyme activity in the synthesis of hydroxyproline, which is a rate-limiting step in collagen formation.[51] A deficiency of Si is associated with a decrease in collagen formation.[52] Supplementation of Si in diets fed to growing chickens results in a greater concentration of glycosaminoglycans and water content in the cartilage,[24] and there is a positive correlation between serum Si and collagen concentrations in bovine cartilage explants.[25] Increasing the dietary intake of Si can also increase bone mineral density in humans.[53] Thus, the positive results detected for osteochondrosis overall score in the study reported here may have been attributable to the beneficial role Si has in collagen formation and stabilization of the proteoglycan matrix.

Pigs fed high amounts of amino acids had reduced external abnormalities and severity scores, whereas the addition of high amounts of methionine-threonine reduced the overall osteochondrosis score. Results of another study[32] that involved a metabolite of methionine (ie, SAMe) indicated that SAMe has positive effects on collagen synthesis and proteoglycan formation. In addition, sulfur is required for the formation of proteoglycan chains that extend from the hyaluronic acid backbone and give cartilage its absorptive properties.[54] It has been proposed that protective effects on cartilage for nutrients that contain sulfur may be a result of overcoming a deficiency of sulfur in the extracellular matrix. This has emphasized the role of sulfur amino acids for glutathione formation (antioxidant) and in cartilage metabolism.[55] It is highly unlikely that the positive effects for the study reported here were attributable to the antioxidant or anti-inflammatory activity of methionine because fish oil and other anti-inflammatory or antioxidant strategies did not provide any benefit. Threonine can be metabolized by threonine dehydrogenase to form glycine, which is a component of collagen. Analysis of results has led us to believe that the effects are primarily attributable to methionine's role as SAMe or as a sulfur donor. The positive effects SAMe has on proteoglycan and collagen metabolism may help offset the loss of these 2 cartilage components during osteochondrosis.

Supplementing diets with additional fish oil did not affect the incidence or severity of osteochondrosis lesions in the pigs reported here. Conversely, fish oil added to the diet caused cartilage to require less energy to shear and resulted in the highest compression-to-shear ratio. This suggested that the cartilage samples from pigs fed fish oil required more energy to compress and concurrently were more brittle and easier to shear into 2 pieces, which indicated less ability to distribute mechanical forces and resist tearing. Fish oil may have prevented the proper turnover of cartilage components by inhibiting MMPs and resulted in cartilage with inferior mechanical properties. Excessive amounts of free radicals may signal increased activation of inflammatory cytokines that stimulate cartilage degradation by MMPs.[56] Increasing the amount of dietary n-3 fatty acids has yielded positive results in patients with arthritic joint disease because of their ability to reduce the production of inflammatory intermediates.[29,30,57] The inflammatory process involved in arthritic joint diseases may not be of major importance in the pathologic process that leads to osteochondrosis because arthritic conditions are often secondary to osteochondrosis in pigs.[5]

Feeding high amounts of proline and glycine had intermediary effects on overall severity of osteochondrosis lesions. Proline and glycine are 2 nonessential amino acids highly concentrated in collagen.[33] Supplementation of dietary proline and glycine has not been evaluated. We hypothesize that supplying large quantities of these amino acids may have a positive influence on collagen formation. The positive effect of these amino acids on osteochondrosis lesions may be a result of their increased availability, which would result in improved collagen formation. Additional studies will be required to elucidate the mechanism of action.

The study reported here raises the issue of whether dietary nutrients are preventing the formation of new osteochondrosis lesions or causing regression of exist-

ing lesions. Histologic and radiologic evidence of osteochondrosis lesions have been detected in boars as young as 25 days old.[58,59] In those studies, some lesions were already undergoing repair. Similarly, evidence of osteochondrosis-like changes were detected in another study[60] in pigs at 1 day of age. Radiographic evidence of osteochondrosis lesions at the humeral condyle is evident in pigs by 42 days of age and at the femoral condyle by 63 days of age.[61] In that study, regressing and progressive lesions were detected over time. In our study, pigs were fed treatment diets beginning at approximately 70 days of age. At this point, most pigs would have indications of osteochondrosis lesions, as determined on the basis of the aforementioned studies. Also, by the end of the treatment period, > 80% of the pigs still had lesions at the femoral condyle. Results of other studies indicate that it is possible to repair or regress existing lesions during development; however, there would be a point in development at which articular cartilage and bone lose the ability to repair or regress existing lesions. Because of the difficulty of monitoring changes in a single animal over time, it is challenging to determine the mode of action for such changes in growing pigs.

In the study reported here, feeding diets that contained ingredients involved in cartilage and bone metabolism reduced the severity of osteochondrosis lesions, but only fish oil negatively affected cartilage mechanical properties. The minerals Cu, Mn, and Si appeared to play a role in preventing cartilage matrix from degradation or increasing the ability of the tissue to repair lesions, particularly the articular-epiphyseal cartilage. Similarly, adding high amounts of methionine, threonine, proline, and glycine positively influenced cartilage metabolism and reduced the severity of osteochondrosis lesions. The limited effects of these nutrients on lesions in the growth plate may have been attributable to the fact that the lesions resolved before the pigs reached slaughter weight. Additional studies will be required to provide a better understanding of the influence these minerals and amino acids have on osteochondrosis and to evaluate various combinations of these dietary ingredients.

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Appendices appear on the next page

Appendix 1

Dietary treatments fed to crossbred pigs throughout three 28-day feeding phases.

Diet	Contents
Control	Standard corn–soybean meal diet.
Fish oil	3.5% fish oil substituted for choice white grease, which resulted in an n-6:n-3 ratio of 2:1.
Proline-glycine	L-Proline was added at 2.55% and L-glycine at 1.70% to create a proline-to-lysine ratio of 300% and a glycine-to-lysine ratio of 200%.
BCAA	L-Leucine was added at 0.60%, L-isoleucine at 0.35%, and L-valine at 0.29% to create a leucine-to-lysine ratio of 200%, isoleucine-to-lysine ratio of 100%, and valine-to-lysine ratio of 100%.
Si	Silicon (zeolite A) was added at 0.80% to create the Si diet (1,000 μg/g of diet).
Cu-Mn	Copper was added at 0.1% (250 μg/g of diet) and manganse was added at 0.02% (100 μg/g of diet).
Methionine-threonine	DL-Methionine was added at 1.05% to create a methionine-to-lysine ratio of 110%, whereas L-threonine was added at 0.45% to create a threonine-to-lysine ratio of 100%.
All ingredients	Contained all additional dietary components substituted to replace equivalent amounts of corn and choice white grease.

Appendix 2

Calculated values for diets fed to crossbred pigs throughout three 28-day feeding phases.

Calculated analysis	Phase 1	Phase 2	Phase 3
Total lysine (%)	1.20	1.05	0.90
TID amino acids			
Lysine (%)	1.07	0.94	0.80
Isoleucine-to-lysine ratio (%)	69	69	70
Leucine-to-lysine ratio (%)	145	154	164
Methionine-to-lysine ratio (%)	32	31	29
Methionine and cystine-to-lysine ratio (%)	60	60	60
Threonine-to-lysine ratio (%)	65	66	68
Tryptophan-to-lysine ratio (%)	20	19	19
Valine-to-lysine ratio (%)	76	78	80
ME (kcal/kg)	3,457	3,468	3,461
Crude protein (%)	19.5	17.4	15.4
Calcium (%)	0.80	0.72	0.72
Phosphorus (%)	0.70	0.62	0.62
Lysine-to-calorie ratio (g/Mcal)	3.47	3.03	2.60

Appendix 3

Diet composition (as-fed basis) fed to crossbred pigs throughout three 28-day feeding phases.

Ingredient	Phase 1	Phase 2	Phase 3
Corn	62.65	68.60	74.05
Soybean meal (46.5% crude protein)	30.45	24.95	19.50
Choice white grease*	3.50	3.50	3.50
Monocalcium phosphate (21% phosphorus)	1.50	1.25	1.25
Limestone	1.05	1.00	1.00
Salt	0.35	0.35	0.35
Vitamin premix†	0.15	0.13	0.13
Trace mineral premix‡	0.15	0.13	0.13
L-Lysine hydrochloride	0.15	0.15	0.15
DL-Methionine	0.06	0.03	0
L-Threonine	0.06	0.05	0.05
Total	**100.00**	**100.00**	**100.00**

Dietary treatments were created by substituting ingredients for corn or choice white grease. Laboratory analysis of samples of each diet revealed that amounts of lysine and other amino acids were similar to the calculated values.

*Choice white grease varied slightly among diets to maintain isocaloric diets. †Vitamin premix provided differing amounts to the diets (per kilogram of complete diet) during phase 1 (vitamin A, 6,613 units; vitamin D_3, 992 units; vitamin E, 26 units; vitamin K, 2.7 mg; vitamin B_{12}, 0.03 mg; riboflavin, 6 mg; pantothenic acid, 20 mg; and niacin, 33 mg) and phases 2 and 3 (vitamin A, 5,512 units; vitamin D_3, 827 units; vitamin E, 22 units; vitamin K, 2.2 mg; vitamin B_{12}, 0.02 mg; riboflavin, 5 mg; pantothenic acid, 16 mg; and niacin, 27 mg). ‡Trace mineral premix provided differing amounts to the diets (per kilogram of complete diet) during phase 1 (Cu [from Cu sulfate], 16.5 mg; iodine [from calcium iodate], 0.3 mg; iron [from ferrous sulfate], 165 mg; Mn [from Mn oxide], 40 mg; selenium [sodium selenite], 0.3 mg; and zinc [from zinc oxide], 165 mg) and phases 2 and 3 (Cu [from Cu sulfate], 14 mg; iodine [from calcium iodate], 0.25 mg; iron [from ferrous sulfate], 138 mg; Mn [from Mn oxide], 33 mg; selenium [sodium selenite], 0.25 mg; and zinc [from zinc oxide], 138 mg).