Feeding Supplemental Vitamin D_3 Improves Pork Longissimus Muscle Quality

B.S. Wilborn, C.R. Kerth, W.R. Jones, W.F. Owsley, and L.T. Frobish

Department of Animal Sciences, Auburn University, Auburn, AL 36849

Abstract

Duroc-cross pigs (n = 25) were assigned to one of three experimental finishing diets containing 0 (CON), 40,000 (40), or 80,000 (80) IU supplemental vitamin D_3/kg of feed to test the effects of vitamin D_3 on pork quality traits. Experimental diets were fed for 44 or 51 days prior to slaughter and days on feed were blocked in the experimental design. While the concentration of vitamin D_3 did not affect average daily gain (ADG; P= 0.08), a trend did exist for those receiving the highest concentration of vitamin supplementation to have a lower ADG (0.77 kg/day) compared to pigs fed either the 40 (0.88 kg/day) or CON (0.92 kg/day) diets. Diet did not affect (P > 0.10) fat thickness measured along the midline, 10th rib fat, longissimus (LD) cross-sectional area, or muscle score, but the pigs fed the 80 diet had lower (P > 0.003) hot carcass weights compared to pigs fed either CON or 40 diets. Longissimus pH in the diet main effect was higher (P < 0.05) for pigs on the 80 diet than those on the CON diet. Longissimus muscles from pigs fed either the 40 or 80 diet had lower (P < 0.05) L* values (less white) compared to those fed the CON diet. Trends existed (P > 0.10) for a* values to be higher for muscles taken from pigs fed the 80 diet compared to those fed the CON diet. Objective LD color scores were higher (P < 0.01) and firmness/wetness scores lower (P < 0.05) for pigs on the 80 diet as compared to those on the 40 or CON diets. Diet had no effect on Warner-Bratzler sheer force evaluation, percent cook loss or trained sensory panel evaluation for tenderness, juiciness and flavor. These data indicate that feeding supranutritional levels of vitamin D_3 for at least 44 days improves pork color and pH, but may retard growth if fed at 80,000 IU/kg of feed.

Introduction

Pork color, pH, and water holding capacity are all essential pork quality traits. It is estimated that pale soft and exudative (PSE) pork costs the pork industry 30 million dollars per year. Factors influencing pork quality such as diet, environment, handling and processing techniques all impact physiological processes which govern the determination of ultimate pork quality. Physiological factors such as pH decline and glycolytic potential are known to be closely related to vital pork quality traits such as color and water holding capacity (Huff-Lonergan et al. 2002). Muscle fiber types have also been correlated with similar pork quality traits (Kerth et al. 2001b). Recently, researchers have uncovered a metabolic pathway which plays a vital role in muscle fiber type expression. The calcineurin pathway, when up-regulated by high concentrations of cellular calcium over long periods of time, has been shown to increase the expression of genes linked to slow-twitch oxidative muscle fibers (Chin et al., 1998). The purpose of this study was to feed high concentrations of vitamin D_3 over a period of six weeks to increase cellular calcium and attempt to promote slow-twitch oxidative muscle fiber expression. By increasing the population of slow-twitch muscle fibers metabolic activity should become more oxidative in nature. By increasing oxidative metabolism and decreasing glycolytic metabolism the rate and extent of pH decline should be less thus improving pork color and water holding ability. The objective of this study was to test the effects of feeding pigs diets containing 0 (CON), 40,000 (40), or 80,000 (80) IU supplemental vitamin D_3/kg of feed to test the effects of vitamin D_3 on pork color, pH, water holding ability and muscle fiber type.

Materials and Methods

Twenty-five Duroc x Yorkshire, crossbred pigs were sorted by sire and sex then randomly assigned to one of three experimental treatment groups. Experimental diets were fortified corn-soybean meal formulations to meet or exceed NRC recommendations. Additional vitamin D_3 was added to supply 0 (CON), 40,000 (40), or 80,000 (80) IU/kg of supplemental vitamin D_3, and fed for either 44 or 51 days prior to slaughter. Time on test was blocked in the experimental design. Pigs were weighed weekly to calculate ADG. Blood was drawn from four pigs per pen at the onset of the experiment and every other week until the time of slaughter at which point blood samples were collected from all pigs on the slaughter floor. All blood samples were collected in heparinized tubes, the plasma was separated and stored at -80°C for subsequent analysis. Pigs were slaughtered at an average live weight of 102kg. Animals were electrically stunned, exsanguinated, scalded, and eviscerated. Carcasses were chilled for 24 hours at 1.8°C. Longissimus pH and temperature were measured at the last rib at 0.5, 1, 2, 3, 4, and 24h after stunning. Temperature and pH measurements were collected using a portable combination meter (IQ150 pH/mv/Temperature System) connected to a stainless probe (IQ part no. PH07-ss, IQ Scientific Instruments, Inc. San Diego, CA).

Carcass Measurements and Evaluation

After chilling for twenty-four hours, carcasses were ribbed between the 10th and 11th ribs and backfat at the first rib, tenth rib, last rib and last lumbar vertebrae, and loin eye area was measured. Subjective muscle score was also recorded. Longissimus muscles at the tenth rib were evaluated for objective color measurements with a Hunter Miniscan XE Plus (HunterLab, Reston, VA) for Hunter L*, a*, and b* values. The Miniscan utilized a D65 light source, a 10° viewing angle, and a 35mm viewing area. The Miniscan was calibrated according to manufacturer's recommendations. Additionally, subjective scores for color, firmness and marbling were evaluated by a trained observer using published visual standards (NPPC 1994). A section of the longissimus muscle was then removed from the 10th rib to the 4th rib. Starting at the 10th rib end, two chops, one-inch thick, were removed for sensory evaluation, two for shear force determination, one for water holding capacity and one for drip loss. Samples for water holding capacity and drip loss were analyzed fresh while chops to be used for sensory and shear evaluation were vacuum packaged and frozen for later analysis.

Warner-Bratzler Shear Force and Cooking Loss

Chops were tempered in vacuum package bags at 4°C for 24 h, removed from the bags, weighed and cooked to an internal temperature of 70°C on a clam-shell grill (model 25300 type ST09 grill, Hamilton Beach / Proctor Silex, Inc. Racine, WI.). Chops were removed from the grill, weighed and stored at 4°C for 24h on a metal pan covered with PVC overwrap. Three 1.27cm diameter cores were taken from each chop parallel with the direction of muscle fibers and sheared once perpendicular to the length of the core using a Warner-Bratzler shear device (1955 model, G-R Electric Manufacturing Co., Manhattan, KS). Peak force for each core was recorded in kg and six cores per animal were averaged (AMSA, 1995). Cook loss was measured as the percent of pre-cooked weight lost during cooking and averaged across the two chops for each pig.

Drip Loss and Water Holding Capacity

Briefly, samples taken for drip loss and water holding capacity were analyzed without freezing on the day of fabrication. Chops used for drip loss were trimmed free of fat and connective tissue, weighed, covered with a plastic bag and suspended on a hook at 4°C for eight days. Chops were weighed after two, four, and eight days of storage and total volume of moisture lost was measured on the last day. Drip loss was calculated as the percent of weight loss over time. Percent free water, bound water and immobilized water was determined using the Carver Press method (Grau and Hamm, 1953). A 0.5g sample of longissimus muscle was placed between two pieces of desiccated filter paper. The sample and filter paper were placed between two sheets of hard clear plastic and pressed at 500 psi for one minute. This procedure was duplicated for each sample. Then, two apparent rings, a meat ring and a moisture ring were traced and measured in square inches. Percent total moisture was determined by drying a five gram sample at 100°C for 24h. Total moisture for the pressed sample was then determined by multiplying the weight of the pressed sample by the determined percent moisture from drying. Percent free water was calculated as (area of water ring - area of meat ring) x (61.10) / (total moisture of sample). Bound water was calculated as 100 - percent free water, and immobilized water as percent bound water - percent free water.

Sensory Evaluation

Sensory evaluation of boneless top loin chops was conducted by a six member trained sensory panel made up of meat science faculty and graduate students according to guidelines set by AMSA (1995). Chops were prepared and cooked as previously described for shear force evaluation. After cooking, chops were cut into 1cm x 1cm x cooked chop thickness pieces and held in metal double stack poachers filled with sand and placed in a warming oven for a minimal period of time prior to sensory evaluation. Each panelist was given two pieces to evaluate on an eight point scale for initial and sustained tenderness, initial and sustained juiciness, flavor intensity and pork flavor (1 being extremely dry, extremely bland, and extremely uncharacteristic; 8 being extremely tender, extremely juicy, extremely intense, and extremely characteristic).

Calcium

Blood was collected in heparinized tubes. Serum was frozen at -80°C, packaged in dry ice and sent to Auburn University College of Veterinary Medicine Clinical Pathology laboratory for analysis using a commercially prepared kit (Roche Diagnostics Corp, Indianapolis, IN) for determination of serum chemistry using the cresophthaline complexone method. Muscle samples were frozen in liquid nitrogen, packaged in dry ice and sent to the Alabama C.S.R. Veterinary Diagnostic Laboratory for analysis using a variation of the AOAC, (1995) method.

Vitamin D_3, 25-Hydroxyvitamin D_3, and 1,25- dyhydroxyvitamin D_3 Determination

Plasma and muscle samples were deep frozen and sent to the U.S.D.A, A.R.S. National Animal Disease Center in Ames, Iowa for Vitamin D_3, 25-hydroxyvitamin D_3, and 1,25- dyhydroxyvitamin D_3 analysis. Vitamin D_3 and its metabolites were quantified by the method described by Montgomery et al. (2000).

Glycolytic Potential

Longissimus samples were taken at approximately 30 minutes postmortem from the the 10th rib (left side of carcass) and frozen in liquid nitrogen and held at -80°C until analysis. Glycolytic potential was determined using the method of Monin and Sellier (1985) and later described by Lonergan et al. (2001). Duplicate samples (0.5g) were held in an ice water bath and homogenized in 2.5mL cold perchloric acid (0.6 N) using a Tissue Teanor (model 985370 Biospec Products, Inc. Bartlesville, Oklahoma). Duplicate samples (200µL) were hydrolyzed with amyloglucosidase at 40°C for 120 minutes. Incubation was stopped by addition of 1mL of 0.6N perchloric acid, and samples were centrifuged to clarify. Clarified samples were used to determine total micromolar glucosyl units (free glucose, glucose-6-P and glucose from glycogen) using the glucose HK assay kit (Sigma Chemical Co.). Duplicate one-gram samples were extracted in 0.7N perchloric acid for the determination of lactate using a lactate kit (Sigma Chemical Co.). Glycolytic potential was calculated as 2(glycogen + glucose + glucose-6-P) + lactate.

Histochemical Analysis

Samples were taken from the the last rib (left side of carcass) at approximately thirty minutes postmortem. Duplicate muscle samples were cut into 0.5cm x 0.5cm x 1.5cm pieces and affixed to a 2cm x 2cm piece of thin cork board using tissue freezing media with the direction of the muscle fibers perpendicular to the flat surface of the cork board. The samples were frozen in 2-methyl butane submersed in liquid nitrogen, placed in plastic bags and stored at -80°C until analysis. Muscle samples were transported on dry ice, placed in a Reichert Jung Frigicut 2800N (currently serviced by Leica Microsystems, Wetzlar, German) instrument set at -20°C and allowed to equilibrate for at least 15 minutes. Slides were prepared by slicing samples perpendicular to the direction of the muscle fibers in 14µm slices. Slides were then stained for acid stable ATPase activity according to the methods of Solomon and Dunn (1988). Microscopic photographs were taken with a Nikon Eclipse E800 microscope fitted with a 100W mercury lamp illumination source, a polarizer, dark-field condenser, Nikon camera. The microscope and camera were linked to a Dell video monitor and computer equipped with Spot graphics program (Diagnostic Instruments, Inc., Sterling Heights, MI). Pictures were saved as uncompressed tiff. files and printed on Hewlett Packard high quality photo paper using an HP deskjet 645C. Muscle fibers were classified as β-red, α-red, or α-white and counted as a percentage of the total number of muscle fibers counted. At least 500 muscle fibers per animal were counted.

Statistical Analysis

The effect of vitamin D_3 on attributes measured was determined by analysis of variance for a randomized complete block design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Since time on feed was not part of the original objective, day of slaughter served as the block to remove any variation due to an extra seven days on test. Each measure was tested as a single mean for each treatment group. When differences (P < 0.05) were found, pairwise comparisons were made using Fisher's protected LSD. Plasma calcium, plasma vitamin D, and pH were analyzed as repeated measures.

Results

Vitamin D and Calcium in Plasma and Muscle

Feeding supplemental vitamin D_3 at the 40 level increased (P < 0.01) plasma vitamin D_3 compared to the CON diet after two weeks (Figure 3). Feeding the 80 diet increased (P < 0.01) plasma vitamin D_3 compared to the 40 diet. The same effect was observed for the fourth, sixth and seventh weeks of supplementation. 25-hydroxy vitamin D (25OHD), the primary metabolite of vitamin D_3, increased (P < 0.01) in plasma of pigs fed the 40 diet compared to the CON after 4 weeks of vitamin D_3 treatment (Figure 4). Feeding supplemental vitamin D_3 at the 80 level increased (P < 0.01) plasma 25OHD compared to the 40 diet at four weeks. Similar differences were found at six weeks. At seven weeks plasma 25OHD concentration was found to be higher (P < 0.05) in pigs fed the 80 diet as compared to the CON diet, but pigs fed the 40 diet did not differ (P > 0.05) from the CON pigs or the 80 pigs. Plasma calcium concentration was not different (P > 0.1) at zero, two, or four weeks (Figure 5). After six or seven weeks of supplementation, pigs fed the 80 diet had higher (P < 0.05) plasma calcium concentration than pigs receiving the 40 or CON diets.

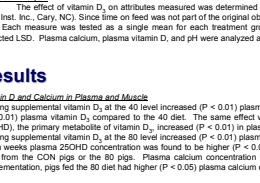

Figure 3. Effect of Feeding Vitamin D_3 to Finishing Pigs for Seven Weeks on Plasma Vitamin D_3 Concentration

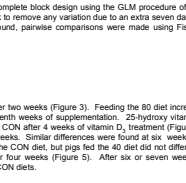

Figure 4. Effect of Feeding Vitamin D_3 to Finishing Pigs for Seven Weeks on Serum 25-Hydroxy Vitamin D Concentration

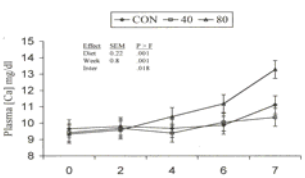

Figure 5. Effects of Feeding Vitamin D_3 to Finishing Pig for Seven Weeks on Serum Calcium Concentration

Carcass

Results for performance and carcass measurements can be found in Table 1. Feeding pigs supplemental vitamin D_3 at the 80 concentration tended to decrease(P = 0.08) average daily gain compared to the control. Supplemental vitamin D_3 did not affect hot carcass weight, fat thickness measured at the first rib, 10th rib, last rib, last lumbar vertebrae or the loin eye area. Muscle score was lower (P = 0.004) for pigs fed the 80 diet compared to those fed the 40 diet or the CON diet. Feeding pigs the 80 diet increased (P < 0.05) longissimus pH compared to pigs fed the CON or 40 diets across all times measured (Figure 1). The effect of diet on pH was independent of time postmortem (P = 0.64).

Table 1. Effects of Feeding Vitamin D_3 to Finishing Pigs on Performance and Carcass Measurements

	CON		40		80		
Item	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM	P > F
n	8		8		9		
ADG, kg/d	0.92	0.048	0.88	0.048	0.77	0.045	0.08
Hot carcass weight, kg	73.5	1.69	73.4	1.69	71.0	1.66	0.30
Fat thickness, cm							
First rib	3.0	0.31	3.6	0.31	3.8	0.29	0.31
10th rib	2.1	0.16	1.7	0.16	1.9	0.15	0.29
Last rib	2.2	0.15	1.9	0.13	2.1	0.12	0.42
Last lumbar	2.2	0.13	2.0	0.13	2.1	0.13	0.52
Loin eye area, cm^2	36.7	1.59	36.1	1.59	34.4	1.50	0.55
Muscle score	2.6[a]	0.04	2.6[a]	0.04	2.4[b]	0.04	0.004

[a,b]Means with the same superscript are not different (P > 0.05)

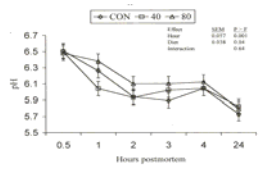

Figure 1. Effect of Feeding Vitamin D_3 to Finishing Pigs on Postmortem pH Values Over Time

Sensory and Shear Force Evaluation

Results for cook loss, sensory, and shear force evaluation can be found in Table 2. Vitamin D supplementation did not affect percent cook loss, Warner-Bratzler shear force values or trained sensory panel scores for tenderness, juiciness and flavor (P > 0.25).

Table 2. Effects of Feeding Vitamin D_3 to Finishing Pigs on Sensory Evaluation Shear Force and Cook Loss

	CON		40		80		
Item	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM	P > F
Percent cook loss	25.3	0.86	25.0	0.86	24.9	0.81	0.86
Warner Bratzler Shear, kg	3.61	0.320	3.48	0.320	3.90	0.302	0.62
Sensory							
Initial tenderness	5.7	0.30	5.6	0.30	5.8	0.30	0.87
Sustained tenderness	5.4	0.36	5.6	0.36	5.8	0.36	0.80
Initial juiciness	5.6	0.15	5.5	0.15	5.4	0.14	0.57
Sustained juiciness	6.1	0.17	5.7	0.17	5.9	0.17	0.27
Flavor intensity	5.9	0.11	5.9	0.11	6.0	0.10	0.64
Pork Flavor	6.0	0.12	6.2	0.12	6.2	0.11	0.53

Water Holding Objective and Color Evaluation

Feeding supplemental vitamin D_3 for a period of 44 days did not affect (P > 0.19) drip loss after two, four or eight days (Figure 2), or percent free water, percent bound water, or percent immobilized water (table 3). Longissimus muscle from pigs fed the 80 diet had lower (P = 0.02) Hunter L* values than CON pigs. No significant differences were found for Hunter a* or b* values between treatment groups (P > 0.24). Subjective color scores and firmness/ wetness scores for pigs fed the 80 diet were higher (P < 0.05) than those fed the 40 diet or CON diet. No significant differences were found for marbling (P < 0.05).

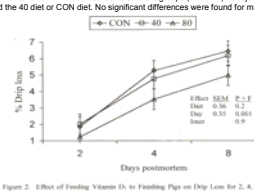

Figure 2. Effect of Feeding Vitamin D_3 to Finishing Pigs on Drip Loss for 2, 4, and 8 Days Postmortem

Table 3. Effects of Feeding Vitamin D_3 to Finishing Pigs on Water Holding Properties, Subjective Evaluation, and Objective Color Measurements

	CON		40		80		
Item	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM	P > F
Percent free water	2.36	0.126	2.45	0.126	2.18	0.119	0.30
Percent bound water	97.64	0.125	97.55	0.125	97.82	0.125	0.30
Percent immobilized water	95.28	0.251	95.10	0.251	95.64	0.237	0.30
Hunter color							
L	55.5[a]	0.81	53.2[b]	0.81	51.9[b]	0.77	0.02
a	5.8	0.41	6.7	0.41	6.7	0.39	0.25
b	14.8	0.35	14.9	0.35	14.5	0.33	0.66
Subjective evaluation							
Color	2.89[a]	0.129	2.89[a]	0.129	3.39[b]	0.122	0.01
Firmness	3.04[a]	0.102	3.01[a]	0.102	3.37[b]	0.096	0.03
Marbling	3.50	0.289	3.00	0.289	3.56	0.273	0.32

[a,b]Values with the same superscript are not different (P > 0.05)

Muscle Metabolism and Histology

No significant differences were found among diets for calcium concentration in muscle samples (P > 0.1, Table 4). Muscle vitamin D_3 concentration was increased (P < 0.01) by the 40 diet compared to the CON diet and by the 80 diet compared to the 40 diet. Likewise, feeding vitamin D_3 at the 40 level increased (P < 0.01) 25OHD in muscle compared to the control, and the 80 diet increased the 25OHD concentration compared to the 40 diet. 1,25-hydroxy vitamin D_3 was not detected in muscle or blood samples (data not shown). This is not uncommon considering its relatively low concentration in blood and muscle. Supranutritional concentrations of vitamin D_3 did tend to increase (P < 0.06) glycolytic potential of longissimus muscle. Additionally, there seemed to be a trend for vitamin D supplementation to increase (P < 0.13) lactate and total glucosyl units. Supplementation with vitamin D_3 did not affect (P > 0.3) the percent red, white or intermediate muscle fibers.

Table 4. Effects of Feeding Vitamin D_3 to Finishing Pigs on Muscle Calcium, Vitamin D_3 Concentration, Glycolytic Potential, and Muscle Fiber Types

	CON		40		80		
Item	LS Mean	SEM	LS Mean	SEM	LS Mean	SEM	P > F
Muscle calcium µmol/dl	3.93	0.248	4.10	0.248	4.13	0.234	0.84
Vitamin D_3 ng/g	13.9[a]	8.42	68.4[b]	7.85	153.1[c]	7.42	0.001
25-hydroxyvitamin D_3 ng/g	69.3[a]	107.14	324.2[b]	107.14	774.5[c]	101.24	0.001
TGU[a]	36.0	3.96	44.2	3.96	48.0	3.74	0.11
Lactate mM/g	30.2	1.76	35.3	1.76	34.0	1.67	0.13
Glycolytic potential[b]	102.3	8.19	123.6	8.19	130.1	7.74	0.06
Muscle fiber percentages							
Slow, oxidative, %	14.0	1.28	14.5	1.26	12.7	1.03	0.5
Fast, oxidative/glycolytic, %	8.3	1.24	10.5	1.22	10.0	1.00	0.4
Fast, glycolytic, %	77.7	1.65	75.1	1.62	77.4	1.33	0.5

[a,b,c]Values with the same superscript are not different (P > 0.05)
[a]Total Glucosyl Units Measures the combination of glycogen and free glucose in millimoles per gram of muscle
[b]Glycolytic Potential is calculated as (2*TGU)+Lactate and is reported as millimoles of potential lactate per gram of muscle

Discussion

Vitamin D is required for the regulation of plasma calcium and phosphorous concentrations necessary to maintain healthy bone structure in humans as well as pigs (Engstrom et al., 1986). Vitmamin D_3 is the product of UV irradiation of 7-dehydrocholesterol in animals. Most animals allowed access to direct sunlight are able to produce enough vitamin D_3 to meet their daily requirements. Animals not allowed direct access to sunlight, such as pigs raised on enclosed finishing floors, must be supplemented with dietary sources of vitamin D from either plant or animal sources. Pigs are reported to be able to use either the plant (vitamin D_2) or animal (vitamin D_3) sources (NRC 1998). Dietary recommendations for growing pigs are between 100 and 220 IU/kg of feed depending on stage of growth and confinement conditions (Engstrom et al., 1986). Dietary Vitamin D_3 metabolism as explained by Engstrom et al. (1986) starts with absorption in the small intestine. Vitamin D_3 is then transported to the liver where it is transformed to 25-hydroxy vitamin D_3 (25OHD). 25OHD is then available in the blood stream until it is converted to 1,25-hydroxy vitamin D_3 (1,25OHD) by the kidney. 1,25OHD is the most potent form of vitamin D and is found in the blood in transient concentrations and is regulated by Ca, P, parathyroid hormone (PTH), and vitamin D status. Vitamin D toxicity can be very harmful. Mild symptoms of vitamin D toxicity include depression, loss of appetite along with decreased gain, and more serious symptoms include vomiting, calcification of soft tissues such as the lungs, kidney, and heart and can lead to death. (Engstrom et al., 1986). Short term exposure to high concentrations of vitamin D can be very toxic. Toxicity in pigs has been reported after short term exposure to vitamin D_3 at concentrations of 473,000 IU/kg of feed (Long, 1984), and 825,000 IU/kg of feed (Engstrom et al., 1986). According to NRC (1998), the safe upper limit for dietary vitamin D_3 intake for pigs is 33,000 IU/kg for less than 60 days and 2,200 IU/kg for more than 60 days.

The intent of this study was to maximize plasma calcium concentrations for at least six weeks without jeopardizing live performance characteristics. Enright et al. (1998) fed low, moderate, and high concentrations (331IU/kg, 55,031 IU/kg, and 176,000 IU/kg of diet) of vitamin D_3 to finishing pigs for ten days prior to slaughter. While serum calcium was elevated in both the moderate and high treatment groups, vitamin D_3 treatment significantly reduced daily gain and feed intake. Treatment diets used in the present study were formulated to contain 0 IU/kg of added vitamin D_3 to the base diet (CON), 40,000 IU/kg of added vitamin D_3 (40), and 80,000 IU/kg of added vitamin D_3. Given the results presented by Enright et al. (1998), it is not surprising that the 80 treatment level tended (P = 0.08) to depress daily gain. The 40 treatment level, as expected, did not have an adverse affect on gain.

Researchers have found that feeding supranutritional levels of vitamin D_3 ranging from $0.5x10^6$ to $7.5x10^6$ IU/day to steers causes an increase in serum calcium concentration which peaked after 6d of administration (Montgomery et al., 2000, 2001). In the present study, an increase in plasma calcium concentration was not realized until the sixth week of treatment. The cause for the difference in speed of response is probably most likely attributable to the difference in the metabolism of vitamin D_3 between the two species and the level being fed.

It was hypothesized that increasing cellular calcium concentration would stimulate calcineurin activity and promote red oxidative muscle fiber types. Chin et al. (1998) demonstrated that changing calcineurin activity for six weeks caused a change in muscle fiber histology. While plasma calcium was increased in the present experiment, its effect on the calcineurin state of the animals is difficult to determine since the elevation in plasma calcium did not occur until the sixth week of the trial. Blood calcium concentration for pigs fed the 80 diet was elevated approximately 20% but only for one week prior to slaughter. This may explain why no significant differences were found in muscle fiber histology between pigs fed vitamin D_3 and the CON

Montgomery et al. (2001) found that feeding steers at least $0.5x10^6$ IU of vitamin D_3 per day increased muscle pH measured at 24 hours postmortem. It was suggested that increased pH was a result of increased cellular calcium and increased muscle metabolism as a result of increased calcium concentration. A similar affect was found in the present study in which the highest concentration of vitamin supplementation increased longissimus pH. It is also possible that the energy state of animals fed high concentrations of vitamin D_3 had an effect on pH decline. If feed intake was reduced, it is possible that energy reserves in the form of glycogen were not as plentiful in animals with a reduced appetite. This is probably not the case in the present study as glycolytic potential increased (P < 0.06) in animals fed the 80 diet as compared to the control. Researchers have found that glycolytic potential is very highly and negatively correlated with ultimate pH. This antagonistic relationship between glycolytic potential and ultimate pH does not exist in the present study. Van Laack and Kauffman (1999) did not find glycolytic potential to be the limiting factor in lactate production. According to Scopes (1974), ultimate pH is determined by substrate concentration, and other possible limiting factors such as AMP deaminase and glycogen phosphorylase. It is possible that hormone regulation of one of these enzymes was interrupted by vitamin D_3 or one of its metabolites.

While a change in muscle fiber type expression and an increase in oxidative metabolism was not detected, factors contributing to overall pork quality were improved. The results of this study are in agreement with those of Enright et al. (1998) who observed that feeding moderate and high concentrations of vitamin D_3 for ten days prior to slaughter increased subjective color and firmness scores while decreasing Hunter L and drip loss values. Similarly, the present study finds that Hunter L value is reduced while subjective color and firmness scores are increased by feeding pigs diets containing 80,000 IU/kg of feed.

Implications

While it is clear that vitamin D_3 supplementation has a beneficial impact on pork quality the mode of action is still unknown. Whether vitamin D_3 supplementation can impact muscle fiber type expression is still unclear. It seems that if a change in muscle fiber histology is possible, it may require higher levels of vitamin supplementation or longer duration on the supplement than is economically feasible given the adverse affects on feed intake and gain. A change in muscle fiber type may best be achieved by some other method.

References

AMSA. 1995. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. Am. Meat Sci. Assoc., Chicago, IL.
AOAC International. 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
Chin, E.R. Olson, J.A. Richardson, Q.Yang, C. Humphries, J.M. Shelton, H.Wu, W. Zhu, R. Bassel-Duby, R. Sanders Williams. 1998. A calcineurin-dependent transcriptional pathway controls skeletal muscle fiber type. Genes & Development 12:2499-2509
Engstrom, G.W., E.T. Littledike, Vitamin D metabolism in the pig. [Book chapter. Literature Surveys] Swine in biomedical research / edited by M.E. Tumbleson. New York: Plenum Press, c1986.ill. p. 1091-112
Enright, K.L., B.K. Anderson, M.Ellis, F.K. McKeith, L.L. Berger, D.H. Baker. 1998. The effects of feeding high levels of vitamin D_3 on pork quality. J. Anim. Sci. 76(suppl 1):149
Grau, R. and R. Hamm. 1953. Eine einfache Methode zur Bestimung der Wasserbindung im Muskel. Naturwissenschaften 40:29
Huff-Lonergan, E., T.J. Baas, M.Malek, J.C.M. Dekkers, K. Prusa, M.F. Rothchild. 2002. Correlations among selected pork quality traits. J. Anim. Sci. 80:617-627
Kerth, C.R., A.Ao- Helm, D.L. Kuhlers, L.B. Cagle, L.K. Blair-Kerth, W.R. Jones. 2001b. Relationship between muscle fiber type and pork quality traits of pigs selected for leanness and growth efficiency. Proc. 54th Annu. Rec. Meat Conf., Vol II
Larzul, C., L. Lefaucheur, P. Ecolan, J. Gogue, A. Talmant, P. Sellier, P. Le Roy, G. Monin. 1997. Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in Large White pigs. J. Anim. Sci. 75:3126-37
Lonergan, S.M., E. Huff-Lonergan, L.J. Rowe, D.L. Kuhlers, and S.B. Jungst. 2001. Selection for lean growth efficiency in Duroc pigs influences pork quality. J. Anim. Sci.79:2075-85
Long, G.G. 1984. Acute toxicosis in swine associated with excessive dietary intake of vitamin D. Journal of American Veterinary Medical Association. 184(2):164-170
Monin, G. and P. Sellier. 1985. Pork of low technological quality with normal rate of muscle pH fall in the immediate post-mortem period: The case of the Hampshire breed. Meat Science 13:49-63
Montgomery, J.L., F.C. Parrish, Jr., D.C. Beitz, R.L. Horst, E.J. Huff-Lonergan, A.H. Trenkle. 2000. The use of vitamin D_3 to improve beef tenderness. J. Anim. Sci. 78:2615-21
Montgomery, J.L., M.A. Carr, C.R. Kerth, G.G. Hilton, B.P. Price, M.L. Galyean, R.L. Horst, M.F. Miller. 2001. Effect of vitamin D_3 supplementation level on the postmortem tenderization of beef from steers. J. Anim. Sci. accepted
National Pork Producers Council Publication. 1994. Pork Quality Standards. Des Moines, IA.
National Research Council. 1998. Nutrient Requirements of Swine. 10th revised edition, National Academy of Science, Washington, DC.
Scopes, R.K. 1974. Studies with a reconstituted muscle glycolytic system. The rate and extent of glycolysis in simulated post mortem conditions. Biochem. J. 142:79-86
Solomon, M.B., and M.C. Dunn. 1988. Simultaneous histochemical determination of three fiber types in single sections of ovine, bovine, and porcine skeletal muscle. J. Anim. Sci. 66:255-64
Van Laack, R.L.J.M., and R.G. Kauffman. 1999. Glycolytic potential of red, soft, exudative pork longissimus muscle. J. Anim. Sci. v.77:2971-2973