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THE IMPACT OF SUPPLEMENT WITHDRAWAL AND WHEAT MIDDLING INCLUSION ON PORK NUTRIENT QUALITY AND BONE QUALITY

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THE IMPACT OF SUPPLEMENT WITHDRAWAL AND WHEAT MIDDLING INCLUSION ON PORK NUTRIENT CONTENT AND BONE QUALITY

By

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ABSTRACT

THE IMPACT OF SUPPLEMENT WITHDRAWAL AND WHEAT MIDDLING INCLUSION ON PORK NUTRIENT CONTENT AND BONE QUALITY

By

Daniel Towner Shaw

A study was conducted to determine if supplement withdrawal (omission of dietary vitamin and trace mineral premixes and 2/3 reduction of inorganic P) 28-d pre-slaughter and wheat middling inclusion affect the vitamin and mineral concentrations of the longissimus dorsi muscle (LDM), bone quality, and the incidence of bone fractures occurring at slaughter. Supplement withdrawal did not affect LDM thiamin, vitamin E, Ca, P, Zn, Fe, or Cu concentrations; however, riboflavin and niacin concentrations were decreased ($P < 0.01$). Supplement withdrawal increased serum osteocalcin and pyridinoline concentrations ($P < 0.05$), indicating an increase in bone turnover; consequently, bone mineral density, peak force, ultimate shear strength, and percent ash ($P < 0.01$) of the metacarpal bone was decreased. Dietary wheat middlings inclusion increased LDM thiamin, niacin, riboflavin, and vitamin E concentrations ($P < 0.04$), but did not alter bone quality. Neither supplement withdrawal nor wheat middlings affected the incidence of bone fractures at slaughter. The results of this study indicate that supplement withdrawal and dietary wheat middlings alter the nutrient content of pork. Additionally, supplement withdrawal increases bone metabolism and decreases bone quality.

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INTRODUCTION

Economic returns in the pork industry are the value of the pork products minus the costs expended to produce them. Production costs per unit of pork are generally lowest in pigs that are provided the amounts and types of nutrients that allow each biological process to proceed at the maximum rate. Deficient or excessive nutrient intakes relative to biological needs result in lower biological and economical efficiency of pork production. Furthermore, additions of dietary nutrients that only slightly enhance performance may not return the cost of the added nutrients.

An area where feed cost per unit of pork may be reduced is the late finishing period. Feed costs represent 60 to 70% of the total cost of production, and pigs consume approximately one-third of their total feed intake during the final four weeks of the finishing period (NRC, 1998). Therefore, even modest decreases in late-finishing feed costs that cause little or no hindrance to growth will likely decrease production cost per unit of pork.

The research described in this thesis evaluated the merits of the two methods of reducing ration costs: supplement withdrawal (removing dietary vitamin and trace mineral premixes and reducing inorganic P) 28 d prior to slaughter and wheat middling inclusion. The research provides a comprehensive review of these practices of interest to producers (growth performance, nutrient excretion), packers (carcass traits, bone fractures, blood splash), and consumers (nutrient content of pork).

Thesis Organization

The following thesis is organized as a literature review followed by two papers, which are in the style and format of the Journal of Animal Science, and will be submitted

to the Journal of Animal Science. The literature review is divided into two sections: the history of supplement withdrawal in the swine industry and the biological feeding value of wheat middlings for growing and finishing pigs. The research reported in the papers was conducted by Daniel T. Shaw under the direction of Dale W. Rozeboom, Gretchen M. Hill, Diana S. Rosenstein, Alden M. Booren, Michael W. Orth, and Jane E. Link. Included in the appendix is an explanation of blood splash as it relates to supplement withdrawal.

CHAPTER 1. HISTORY OF SUPPLEMENT WITHDRAWAL

Introduction

Nutrient concentrations in pig diets are typically based on minimum standards set by the National Research Council (1998), with sometimes generous safety margins to ensure against deficiencies. The suggested nutrient requirements for finishing pigs (NRC, 1998) were extrapolated from research trials where pigs were slaughtered at lighter weights than is common today. As pigs increase in age and size, their nutrient requirements as a percentage of the diet decrease. Therefore, dietary excesses of many nutrients are probably common in late-finishing diets.

Growth Studies

Omission of costly ingredients in late finishing diets has been studied for many years as a means of decreasing feed costs. Gall and associates (1982) replaced the complete finishing diet with ground corn three weeks prior to slaughter. This practice, while reducing the cost of the ration, also reduced ADG by 47% and gain/feed by 51%. Days to market increased by 19 d. An financial analysis identified that the costs associated with extended days to market and increased kg of total feed required to reach market weight exceeded the savings of the reduced ration cost, making this practice a nonviable economic alternative.

Subsequently, researchers in Brazil compared the effects of replacing a complete finishing ration with a corn-soybean meal mixture that provided adequate crude protein. Pigs fed the diet devoid of all supplemental vitamins and minerals for 21 days tended to experience a 6.5% reduction in ADG and an 8.6% reduction in ADFI relative pigs fed a complete and balanced ration. Replacing the complete ration with the corn-soybean meal

mixture for either 51 or 71 days prior to slaughter significantly reduced ADG and ADFI (Donzele et al., 1982).

The more modest approach of removing only vitamin and mineral premixes and/or reducing inorganic P additions may be a more viable alternative for reducing late-finishing ration costs. During recent years, several studies have established that removal of vitamin and mineral premixes from finishing diets 3 to 5 wk prior to slaughter does not affect growth performance as measured by ADG, ADFI, and gain/feed, or carcass quality as measured by backfat thickness, loin eye area, marbling, color, firmness, and tenderness (Patience and Gillis, 1995, 1996; Kim et al., 1997; Mavromichalis et al., 1999; McGlone, 2000).

Bone Quality

The dietary Ca and P concentrations needed to maximize growth performance in growing-finishing pigs are well defined (NRC, 1998). Maximum bone mineralization and bone strength requires higher dietary Ca and P concentrations than is required to maximize growth (Crenshaw et al., 1981; Maxson and Mahan, 1983; Combs et al., 1991). What remains unknown is whether the increased mineralization resulting from higher mineral supplementation reduces the likelihood of bone fractures.

In recent years, interest in minimizing inorganic P additions in finishing pigs diets to reduce nutrient excretion and feed costs has risen. Previous research has indicated that minimal dietary inorganic P additions are necessary during late finishing to maintain growth performance. Inorganic phosphorus can be deleted from barley-pea-based diets 75 days prior to slaughter without affecting growth performance if a Ca to total P ratio of 1.3:1 is maintained (Michal and Froseth, 1999). Furthermore, the authors observed a 40%

decrease in fecal P concentrations during the withdrawal period. O'Quinn and associates (1997) reported that removing inorganic P additions 48 d prior to slaughter from sorghum-soybean meal-based diets reduced fecal P excretion by 12% during the same period without affecting structural soundness, carcass leanness, and carcass characteristics.

During the withdrawal period, animals draw upon mineral body reserves found in bone and other tissues to support metabolic requirements that are not met by the diet. Consequently, bone strength is decreased (O'Quinn et al., 1997); however, it remains unknown if reducing dietary mineral additions prior to slaughter alters bone metabolism and decreases bone strength to the extent of increasing the incidence of bone fractures occurring at slaughter.

Dietary Influence on Pork Nutrient Content

The publishing date of the available research that evaluates the vitamin and mineral content of pork reflects the perceived value of the nutrient. Prior to World War II, meat, poultry, and fish were the primary sources of thiamin and niacin in the American food supply, and dairy products were the primary source of riboflavin. In the mid 1940's, flour was enriched with vitamins to ensure against deficiencies in the American population, making grains the leading dietary source of B-vitamins. Subsequently, the amount of research investigating the B-vitamin content of pork declined. Increasing the vitamin E content of pork is a current focus of research as it known to increase the shelf life and color stability of raw pork.

Because pigs lack a rumen and are unable to synthesize B-vitamins, they require that most nutrients be provided in the diet. Thus, there is a general relationship between

the vitamin concentrations of the diet and the muscle tissue (Tables 1 and 2). Miller and coworkers (1943) fed pigs diets containing 2.9, 7.6, and 12.7 mg/kg of thiamin. Increasing thiamin intake from 2.9 to 7.6 mg/kg and from 7.6 to 12.7 mg/kg increased loin thiamin concentrations by 110% and 15%, respectively. Ensminger and associates (1947) reported that the average loin thiamin concentration of gilts fed a thiamin-deficient diet was 0.09 mg/100g, while the average of gilts fed 23 mg/d of supplemental thiamin was 7.8 mg/100g.

Ittner and Hughes (1941) found that increasing dietary riboflavin supplementation from 0 to 6 mg/d increased loin riboflavin concentrations from 0.14 to 0.25 mg/100g. However, increasing supplementation to 12 mg/d did not further increase loin thiamin. Miller and coworkers (1943) observed no differences in loin riboflavin concentrations when feeding diets containing 3.68 to 5.44 mg/kg.

Christensen and associates (1943) showed that longissimus dorsi thiamin concentrations reflect the level of dietary niacin supplementation. In summary, this literature review shows that dietary B-vitamin supplementation influences the concentrations of thiamin, riboflavin, and niacin present in pork, though muscle riboflavin appears to reach saturation at moderate levels of supplementation.

Vitamin E acts as an antioxidant at the cellular level to prevent the peroxidation of polyunsaturated fatty acids. Feeding supranutritive amounts (100 to 700 mg/kg) of dietary α-tocopherol increases the muscle α-tocopherol concentrations (Table 3), decreasing lipid oxidation of raw pork (Monahan et al., 1990ab, 1992ab; Asghar et al., 1991ab; Cheah et al., 1995; Cannon et al., 1996; Jensen et al., 1997; O'Sullivan et al., 1997; Zanardi et al., 1998, 1999; Corino et al., 1999; Lauridsen et al., 2000).

Table 1. Effect of dietary thiamin supplementation on longissimus dorsi concentrations, fresh weight basis

Source	Total concentration in diet, mg/kg	Supplemented amount	Period of supplementation	Concentration, mg/100g
Miller et al., 1943	2.9		100 d	0.95 ± 0.21
	7.6			2.00 ± 0.44
	12.7			2.31 ± 0.51
Pence et al., 1945	unknown	0		0.98 ± 0.05
		50 mg/d	8 d	1.16 ± 0.04
		50 mg/d	15 d	1.52 ± 0.13
		50 mg/d	22 d	1.74 ± 0.06
		50 mg/d	35 d	2.48 ± 0.14
		50 mg/d	155 d	2.28 ± 0.10
Ensminger et al, 1947	0	0	140 d	0.09
		23 mg/d		0.68
Shaw et al., 2001	2.9	0	28 d	0.74 ± 0.05
	3.2	1 mg/kg		0.75 ± 0.05
	4.7	0		0.97 ± 0.05
	5.8	1 mg/kg		1.07 ± 0.05
Leonhardt et al., 1997	unknown	unknown	unknown	0.8 ± 0.2
USDA, 2001	unknown	unknown	unknown	0.99

Table 2. Effect of dietary riboflavin and niacin supplementation on longissimus dorsi concentrations, fresh weight basis

Vitamin	Source	Total concentration in diet, mg/kg	Supplemented amount	Period of supplementation	Concentration, mg/100g
Riboflavin	Ittner and Hughes, 1941	0	0 mg/d	77 d	0.14 ± 0.01
			2 mg/d		0.17 ± 0.01
			6 mg/d		0.25 ± 0.01
			12 mg/d		0.26 ± 0.01
	Miller et al., 1943	3.68		100d	0.22 ± 0.01
		3.97			0.23 ± 0.01
		5.44			0.22 ± 0.01
	Shaw et al., 2001	1.2	0	28 d	0.118 ± 0.006
		1.5	0		0.133 ± 0.006
		2.7	2.7 mg/kg		0.143 ± 0.006
		3.6	2.7 mg/kg		0.146 ± 0.006
	Leonhardt et al., 1997	unknown	unknown	unknown	0.16 ± 0.02
	USDA, 2001	unknown	unknown	unknown	0.267
Niacin	Christensen, 1943	unknown	0	113 to 138 d	4.66
			100 mg/d		7.35
	Shaw et al., 2001	22.8	0	28 d	4.08 ± 0.49
		31.5	10.9 mg/kg		5.80 ± 0.48
		65.6	0		6.57 ± 0.50
		71.9	10.9 mg/kg		8.70 ± 0.48
	USDA, 2001	unknown	unknown	unknown	4.91

Table 3. Effect of dietary α-tocopherol supplementation on longissimus dorsi α-tocopherol concentrations, fresh weight basis

Source	Form of α-tocopherol	Total concentration in diet, mg/kg	Supplemented amount, mg/kg	Period of supplementation	Concentration, μg/g
Monahan et al., 1990b	α-tocopheryl acetate	14.2	0	84 d	7.6 ± 0.5
		159.1	200		21.8 ± 2.5
Asgar et al., 1991b	all-rac-DL-α-tocopheryl acetate	unknown	10	98 d	0.5 ± 0.1
			100		2.6 ± 0.1
			200		4.7 ± 0.1
Monahan et al., 1992b	α-tocopheryl acetate	23.5	10	30-98 kg	0.78
		140.6	100		2.58
		214.7	200		4.07
Jensen et al., 1997	α-tocopheryl acetate	130	100	50-90 kg	5.1
		223	200		8.2
		742	700		11.1
Corino et al., 1999	all-rac-DL-α-tocopheryl acetate	unknown	25	60 d	5.6 ± 0.7
			100		4.9 ± 0.3
			300		8.4 ± 1.0
Lauridsen et al., 2000	all-rac-α-tocopheryl acetate	18	0	25-100 kg	3.2
			200		5.7
Shaw et al., 2001	DL-α-tocopheryl acetate	13.4	11	28 d	1.11 ± 0.16
		12.2	11		1.46 ± 0.16
		2.8	0		1.11 ± 0.15
		1.6	0		1.77 ± 0.15
USDA, 2001	unknown	unknown	unknown	unknown	2.90

The trace mineral content of pork is relatively consistent regardless of dietary levels (Leonhardt and Wenk, 1997). Muscle Zn concentrations are maintained during times of deficiency (Bentley and Grubb, 1991; O'Leary et al., 1979) Muscle Cu concentrations are not affected by dietary deficiencies (Ledoux et al., 1989) or excesses (Zanardi et al., 1998; Lauridsen et al., 2000). An exception to the lack of variation of the mineral content of meat may be Fe. Injecting growing pigs with 1600 mg of Fe IM from Fe-dextran during the nursery and growing phases increased ham Fe concentrations by 21% (Henry et al., 1961), and increasing dietary Fe from 62 to 209 mg/kg for 13 weeks increased loin Fe concentrations by 38% (Miller et al., 1994).

Supplement Withdrawal and Pork Nutrient Content

The impact of supplement withdrawal on pork nutrient content is not conclusive. Patience and Gillis (1996) removed vitamin supplements in wheat-barley-canola meal-based diets 35 d prior to slaughter. This resulted in a 20% decrease in longissimus dorsi (LDM) thiamin concentration, but did not affect the riboflavin, niacin, or pantothenic acid content. Rozeboom (personal communication), feeding a corn-soybean meal-based diet, removed vitamin and trace mineral supplements 31 d prior to slaughter and also observed no change in LDM thiamin content. However, supplement withdrawal reduced LDM iron concentrations by 12% and Zn concentrations by 17%.

Edmonds and Arentson (2001) fed diets devoid of supplemental vitamins and trace minerals for 6 to 12 wk prior to slaughter. Length of withdrawal did not affect growth performance or carcass traits, but reduced vitamin E concentrations of the LDM and ham muscles by 76 and 55%, respectively. Copper concentrations were reduced by

35% in the ham muscle, but not in the LDM. Muscle Zn and Fe concentrations were not affected by dietary treatment.

The purpose of this research was to identify the effects of supplement withdrawal of corn-soybean meal-based diets on pork nutrient content, pork oxidation, nutrient excretion, bone metabolism and quality, and skeletal integrity at slaughter. The results provide a comprehensive view of the practice from the perspective of the producer, the packer, and the consumer.

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CHAPTER 2. WHEAT MILLFEEDS IN SWINE DIETS

Introduction

Corn, without question, is the most common grain used in swine diets in the United States. However, other energy sources such as sorghum, barley, rye, tricticale, wheat, and by-product feeds are often cost effective substitutes for corn. In much of the Midwest, wheat middlings, a by-product of flour milling, is a favorite choice due to its availability and nutrient profile.

Wheat Milling

The milling of wheat flour for human use yields several by-products available for livestock feeding. A diagram of the wheat seed is shown in Figure 1. The endosperm, which is mainly starch, is covered by several fibrous layers that contain high protein and mineral concentrations. The ultimate purpose of wheat milling is to procure a maximum amount of flour from the endosperm with minimum foreign material; the normal flour extraction rate is 75 to 80 percent (Kent and Evers, 1994). Thus by-products, or millfeeds, represent 20 to 25 kilograms of every 100 kilograms of wheat processed. A flow diagram illustrating the milling process appears in Figure 2.

The first step of milling wheat is to remove contaminants from the wheat sample. The removal of wood, metal, straw, foreign seeds, dust and insect infested wheat kernels is accomplished by the use of differential sieving, magnetic separation, and aspiration (Posner and Hibbs, 1997). The removed products are referred to as "screenings" and are often ground and later mixed with the millfeed.

Figure 1. Diagram of Wheat Kernel

(Crop and Food Research Institute, 2001)

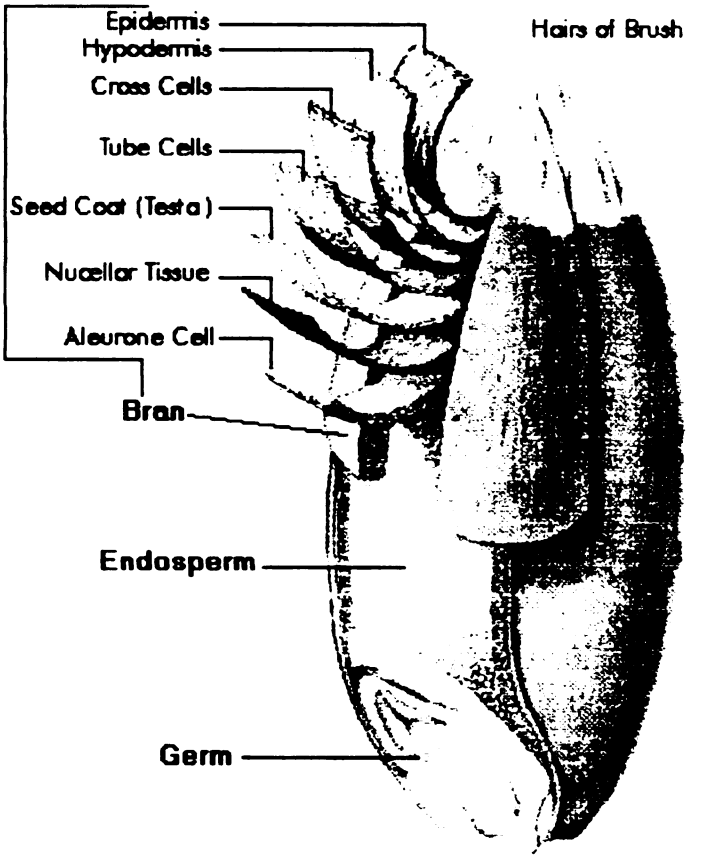
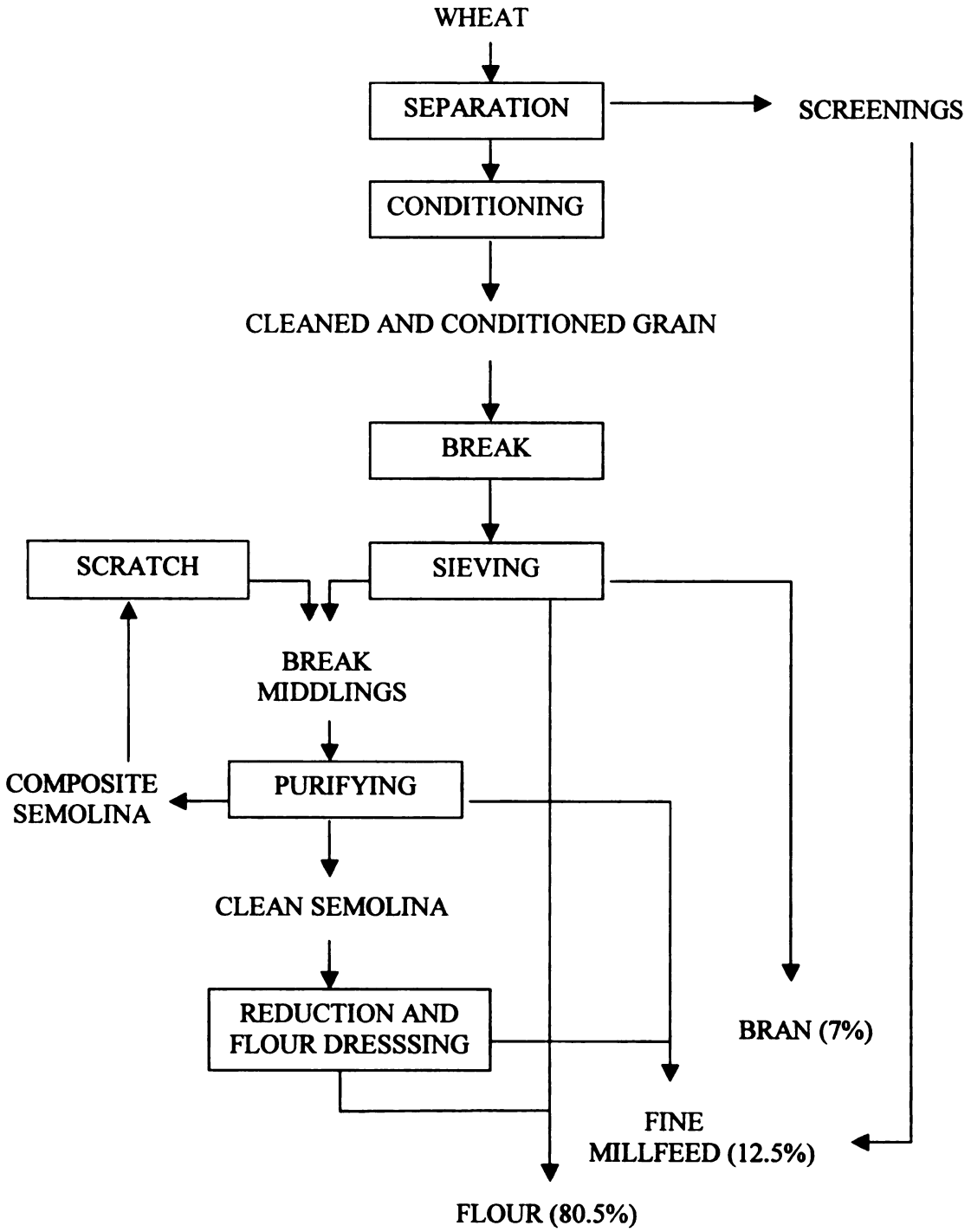


Figure 2. Flow diagram of milling process



The second step is tempering the kernels to facilitate optimal extraction. Water is added to the wheat, which was previously stored at low moisture content for maximum preservation, to raise the moisture content to between 14 and 17% (Posner and Hibbs, 1997). Wheat is stored at this elevated moisture content for 12 to 18 hr. Tempering toughens the bran and mellows the endosperm; thus when milled, the bran tends to remain in large particles from which the endosperm can be more efficiently separated. After tempering the wheat is dried to the desired milling moisture content.

The final phase is the actual milling. The wheat passes through a series of four or five break rolls, which consist of pairs of spirally fluted iron rollers that rotate in opposite directions (Posner and Hibbs, 1997). One of the rollers in each pair rotates faster than the other so a speed differential exists. As wheat progresses from the first break roll through last, the fluting becomes less coarse, the speed differential decreases, and the opening between the rollers diminishes. The ground material leaving the break rolls is sieved into three general streams based on particle size. The finest stream is pure flour. The very course stream consists of large bran particles. The intermediate stream, also known as break middlings, consists of shattered bran mixed with endosperm. Break middlings may either be marketed as millfeed or undergo further processing to separate additional flour.

Further processing of break middlings begins in purifiers that separate components according to size, shape and specific gravity (Kent and Evers, 1994). Purifiers consist of a series of screens enclosed in an airtight container. Break middlings enter at the head of the purifier and are conveyed to the tail. Screen apertures are graduated from very fine at the head to relatively coarse at the tail. As break middlings are conveyed across the screen, upward moving air passes through the screen. The less

dense material floats upwards with the moving air while the more dense middlings pass through the screen apertures. The material moved by aspiration, known as tailings, contains fine particles of fiber and is added to millfeeds. The material that passed through the coarse screen apertures, known as branny middlings, contains particles of bran and endosperm that are still stuck together. The branny middlings may either be marketed as millfeed or enter the scratch system where it passes through very finely fluted break rolls before reentering at the head of the purifier. The material that passes through the fine screen apertures of the purifiers is called clean middlings.

In the reduction system, clean middlings pass through a series of four to six non-fluted roller mills that further reduce the particle size (Kent and Evers, 1994). In the dressing stage, the material undergoes final sieving into its flour and millfeed fractions. Wheat germ may also be extracted during the flour dressing sieving.

Millfeeds. All non-flour fractions produced in the milling of wheat are collectively known as "millfeeds". Different mill streams may be combined to produce a variety of products of varying compositions. The following description of wheat milling by-products supplied to the feed industry comes from the AAFCO (1999) handbook.

(1) Wheat mill run (INF 4-05-206): consists of course wheat bran, fine particles of wheat bran, wheat shorts, wheat germ, wheat flour, and the offal from the "tail of the mill". This product must be obtained in the usual process of commercial flour milling and must not contain more than 9.5% crude fiber.

(2) Wheat bran (INF 4-05-190): the course outer covering on the wheat kernel as separated from cleaned and scoured wheat in the usual process of commercial milling.

(3) Wheat middlings (INF 4-05-205): consists of fine particles of wheat bran, wheat shorts, wheat germ, wheat flour, and some of the offal from the "tail of the mill". This product must be obtained in the usual process of commercial milling and must contain not more than 9.5% crude fiber.

(4) Wheat shorts (IFN 4-05-201): consists of fine particles of wheat bran, wheat germ, wheat flour and offal from the "tail of the mill". This product must be obtained in the usual process of commercial milling and must contain not more than 7% crude fiber.

(5) Wheat red dog (IFN 4-05-203): consists of the offal from the "tail of the mill" together with some fine particles of wheat bran, wheat germ, and wheat flour. This product must be obtained in the usual practice of commercial milling and must contain not more than 4% crude fiber.

Feeding Value of Wheat Middlings

Wheat middlings as an energy source. The organic matter of wheat middlings, like that of cereal grains, is largely carbohydrate. Wheat middlings are primarily composed of the intermediate layer of the wheat kernel between the endosperm and the outer bran covering. The cells of this layer, known as the aleurone layer, are the primary source of DE in wheat middlings. The non-ruminant is able to completely break down the aleurone layer including the cell walls, although a portion of the carbohydrate and about 10 percent of the protein are not absorbed and appear in the feces (Saunders et al., 1969; Saunders and Kohler, 1972).

Whole wheat grain contains about 82% of the white-starchy endosperm, which cannot possibly be separated exactly from the 18% of the bran, aleurone, and the embryo.

Thus, three to ten percent of the endosperm of whole wheat ends up in wheat middlings. The endosperm fraction of wheat middlings contains three percent of readily soluble monosaccharides, disaccharides, and oligosaccharides (Lineback and Rasper, 1988). Starch is the most abundant carbohydrate in the endosperm comprising 63 to 72 percent of the total organic matter (Pomerenz and MacMasters, 1968). The two components of starch, amylose and amylopectin, are essentially 100% digested in the small intestine of the non-ruminant (Lin et al., 1987).

Wheat middlings may contain a maximum of 9.5% CF. Fiber digestion, occurring primarily in the cecum and the large intestine, is a microbial fermentation process. Volatile fatty acids formed during fiber fermentation serve as a secondary energy source for pigs and may provide 14 to 16% of the energy requirements of the growing pig (Imoto and Namioka, 1978; Kass et el., 1980; Lin et al., 1987).

Wheat middlings contain 3,075 kcal/kg of DE and 3,025 kcal/kg of ME (NRC, 1998). While these values are somewhat lower than that of other grains (Table 4), wheat middlings is generally regarded as a satisfactory energy source in swine rations.

Wheat middlings as a protein source. Although grains are primarily considered as contributors of energy, the high levels at which they are used means they are incidentally suppliers of a considerable portion of the dietary protein. Consequently, CP and amino acid profile becomes an additional point of importance when fed to non-ruminants. Wheat middlings compares very favorably to other grains in regard to protein quality and quantity (Table 4).

Wheat middlings as a micro-nutrient source. The most expensive feed ingredients in swine rations are inorganic phosphorus and vitamin and mineral premixes. Including

Table 4: Nutrient Composition of Common Energy Feeds[a]

Nutrient	Corn	Wheat	Barley	Sorghum	Triticale	Rye	Wheat Middlings
DE, (kcal/kg)	3,525	3,365	3,050	3,380	3,320	3,270	3,075
ME, (kcal/kg)	3,420	3,210	2,910	3,340	3,180	3,060	3,025
CP, %	8.3	13.5	10.5	9.2	12.5	11.8	15.9
Lysine, %	.26	.34	.36	.22	.39	.38	.57
Threonine, %	.29	.37	.34	.31	.36	.32	.51
Methionine, %	.17	.20	.17	.17	.20	.17	.26
Ca, %	.03	.06	.06	.03	.05	.06	.12
Total P, %	.28	.37	.36	.29	.33	.33	.93
Avail. P, %	.04	.19	.11	.06	.15	—	.38
Mg, %	.12	.13	.12	.15	.10	.12	.41
Fe, mg/kg	29	39	88	45	31	60	84
Cu, mg/kg	3	6	8	5	8	7	10
Zn, mg/kg	18	40	15	15	32	31	92
Thiamin, mg/kg	3.5	4.5	4.0	3.0	—	3.6	16.5
Riboflavin, mg/kg	1.2	1.4	1.6	1.3	0.4	1.6	1.8
B_6, mg/kg	5.0	3.4	2.9	5.2	—	2.6	9.0
Vitamin E, mg/kg	8.3	11.6	7.4	5.0	1.7	9.0	20.1

[a]Based on NRC (1998) estimates

primary feed ingredients with high micronutrient concentrations in the ration formulation lessens the amount of supplementation necessary for maximum growth performance. Wheat middlings are an excellent source of P and most vitamins and minerals (Table 4).

Variability. Although wheat middlings are widely used throughout the world and can supply a considerable proportion of the protein, energy, and trace elements in swine diets, the variability in nutrient content is a concern. There are five primary classes of wheat produced in the United States: hard red spring, soft red winter, hard red winter, durum, and white; and within each class there are a number of species, each with a distinct nutrient profile (Oldfield, 1970). When provided with such a variety of inputs, there is no practical way for millers to produce flour to the buyers' specifications and also produce standardized millfeeds.

Wheat middlings are classified according to their bulk density as being "heavy" or "light". Heavy wheat middlings contain a high proportion of starchy endosperm attached to the bran. Light wheat middlings contain bran that is relatively free of endosperm. Heavy wheat middlings have a higher feeding value than light middlings for growing-finishing swine (Cromwell et al., 1992).

Wheat middlings have an average bulk density of 320 g/L, but the variation ranges from 289 to 365 g/L (Cromwell et al., 2001). Normal CP and P variation of wheat middlings are 14.6 to 17.8 percent and .70 to 1.19 percent, respectively, with the higher values associated with light middlings. Selenium content of wheat middlings varies by the region in which the wheat was raised.

Wheat Middlings in Swine Diets

Fiber in growing-finishing diets. The use of wheat middlings in growing-finishing diets is limited by its fiber content. Dietary fiber may dilute DE to the point that feed intake is restricted by physiological fill. Growing swine offered diets containing 6 to 8% CF are not able to consume enough feed to meet their energy needs (Hochstetler et al., 1959; Drewry, 1981). However, when DE does not decrease, incorporation of fibrous ingredients such as wheat bran, cellulose and ground corn cobs does not affect performance (Troelson and Bell, 1962). Likewise, when ME levels are held constant, dietary inclusion of cottonseed hulls, wheat bran, and rice mill feeds do not decrease performance (Baird et al., 1970).

Wheat middlings for growing-finishing pigs. A number of studies compared the biological value of wheat millfeeds to other cereal grains as a major energy source in growing-finishing swine rations. Generally these studies follow two patterns: either equivalent substitution of grains on a unit weight basis or dietary inclusion based on "least cost" ration formulation adjusted for lysine and/or energy. Experimental results most likely favor wheat middlings when rations are balanced for both lysine and energy.

Conrad and Harrington (1969) conducted a study in which wheat middlings replaced corn on a pound for pound basis. Their results showed a decrease in ADG and feed efficiency when growing and finishing pigs consumed diets containing more than 20% wheat middlings.

Researchers at the University of Guelph studied extensively the use of wheat shorts in market pig diets. Wheat shorts, like middlings, are comprised primarily of cells of the aleurone layer of the wheat kernel and contain a maximum of 7% CF. Young

(1980) reported two studies in which he evaluated the influence of wheat short inclusion rate on pig performance. In both trials, wheat shorts obtained from the milling of hard red spring wheat comprised either 0, 32.2, 64.4, or 96.6% of the diet; corn and soybean meal were allowed to float to maintain CP levels.

In trial 1, 64 pigs weighing 18 kg were fed the experimental diets for 8 weeks. Analyzed lysine and CP concentrations of all diets exceeded minimum recommended levels. However, the DE content of the diets containing wheat shorts was lower than recommended levels. Rate of gain was decreased with dietary levels of wheat shorts above 64.4%. There was also a linear decrease in gain:feed as level of wheat shorts increased.

In trial 2, 64 pigs weighing 21 kg were fed to approximately 90 kg or for 98 days, whichever came first. Animals were fed in 2 phases, from start to 50 kg and from 50 kg to slaughter. There was a quadratic decrease in ADG up to 50 kg with increasing levels of wheat shorts. From 50 kg to slaughter, ADG decreased in a linear manner as level of wheat shorts increased. Dietary level of shorts did not affect ADFI. The author attributed differences in performance between the two trials to differences in the wheat short bulk densities. The wheat shorts used in the second trial contained considerably more ADF than those used in the first (16.5 vs. 10.4%, respectively). Likewise, the estimated DE values of the shorts used in the first trial were appreciably greater than those used in the second (3.38 vs. 3.02 kcal/g, respectively).

Patience and coworkers (1977) at the University of Guelph conducted a growth trial evaluating the effect of level of wheat short inclusion in typical corn-soybean meal diets. Thirty-one pigs were allotted to receive a 15% CP diet containing either 0, 9.7,

19.3, 29.0, 38.6, 48.3, 58.0, 67.6, 77.3, 86.9, or 96.9% wheat shorts. Pigs were marketed when a carcass weight of about 70 kg was expected. Wheat short inclusion rates above 19.6% tended to depress ADG, though the trend was not significant. However, there was a linear depression in feed efficiency.

Erickson (1979) fed 96 crossbred pigs growing-finishing diets containing 0, 20, 40, or 60% wheat middlings milled from white winter wheat. Diets were pelleted and balanced for lysine and available P. Neutral detergent fiber varied from 38 to 41% in the grower diets and 33 to 37% in the finishing diets. Increasing the level of wheat middlings did not affect ADG or gain/feed. The author suggested that there may have been no difference because the ME values of the diets containing wheat middlings were very similar to the corn-soybean meal-based control diet. As level of wheat middlings inclusion increased, the amount of soybean meal and dicalcium phosphate required decreased, reducing the overall cost of the ration. The control diets also contained a binder to improve pellet quality. Removing the non-nutritive pellet binder in diets containing wheat middlings freed up 2.5% more nutritive space in the diet. Pellet quality did not deteriorate, rather pellet quality improved as the percentage of wheat middlings increased.

Erickson and coworkers (1985) evaluated the effects of increasing levels of wheat middlings on pig performance. In the first study, control diets were formulated to meet NRC requirements for starting, growing, and finishing pigs. Wheat middlings replaced corn on a pound for pound basis at 0, 10, 20 and 30% of the diet. One hundred sixty crossbred pigs were allotted to the four dietary treatment groups. In the starting phase, ADG and gain/feed linearly decreased and ADFI linearly increased as concentration of

dietary wheat middlings increased. However, pigs receiving 10 to 30% wheat middlings appeared to experience compensatory gain during grower period as they tended to have higher ADG than pigs consuming the control diets. Wheat middling inclusion in the finishing diets and overall did not affect ADG, but increased feed intake and decreased feed efficiency.

In the second study, the wheat middling inclusion rates in the growing and finishing diets were 0, 20, 40 and 60%. All diets were calculated to meet NRC requirements and were balanced for lysine. Feeding 96 crossbred pigs increasing amounts of wheat middlings linearly depressed ADG during both the growing and finishing phases. Wheat middling inclusion did not affect ADFI and linearly decreased feed efficiency in the finisher phase, but not in the grower phase. The authors attribute the lack of agreement in the grower pig performance of these two trials to the dietary lysine levels. In trial one, wheat middlings replaced corn on an equal weight basis. Thus, as the level of dietary wheat middlings increased from 0 to 30%, the calculated lysine level increased from .79 to .92%. In trial two, all diets were formulated to contain .75% lysine. The higher lysine levels in the diets containing wheat middlings of trial one may have prevented the decreased grower performance observed in trial two.

O'Hearn and Easter (1983) conducted two growth trials to evaluate the effect of various levels of wheat middlings inclusion on nursery pig performance. The average pig starting weight in both trials was 8.64 kg, and the estimated DE of the wheat middlings used in both trials was 2.89 kcal/g. In trial 1, a corn-soybean meal-based control diet was compared to diets containing 30% wheat middlings with and without tallow to determine if energy limited performance. The trial was 28 d in length. There were no differences in

ADG or ADFI due to the addition of wheat middlings with or without added tallow. Energy was not a limiting factor in using wheat middlings in the nursery diets, suggesting that greater wheat middlings levels may be used.

In trial 2 the researchers utilized increasing levels of wheat middlings (0, 28.9, 56.7, and 85.0%) in corn-soybean meal-based diets to determine at which inclusion rate growth performance was depressed in the nursery pig. Diets were fed for 28 days. As the level of middlings in the diets increased, ADG and gain/feed decreased. Although diets were formulated to be isonitrogenous, the diets containing wheat middlings contained a poorer overall amino acid profile than the control diet, which may have compromised performance. Protein quality may be a limiting factor in using wheat middlings in nursery diets.

Cromwell and associates (1992) evaluated the feeding value of two sources of wheat middlings in pigs weighing 37 to 100 kg. The bulk density of the Source A middlings was relatively low and the bulk density the Source B middlings was relatively high. Wheat middlings were added to the diet, on a lysine basis, at levels of 0, 10, 20, 40, and 60%. Feed intake was not affected by level of middling inclusion. Pigs fed Source A middlings grew slower and less efficiently as the level of middlings increased in the diet. For pigs fed Source B middlings, ADG and gain/feed were not affected with up to 20% inclusion, while growth performance was only slightly reduced with 40% inclusion in the diet.

Conclusion

Wheat middlings with a heavy bulk density can constitute up to 20% of swine growing-finishing rations without affecting performance. Light wheat middlings can

comprise 10 to 15% of growing-finishing diets. Wheat middlings can be used in nursery diets at an inclusion level of 5 to 10%, but care should be taken to maintain an adequate amino acid profile. Higher levels of wheat middlings may be incorporated into the rations of pigs with lower metabolic requirements (older, less lean gain potential). Furthermore, the moderately depressed growth performance that may accompany higher inclusion rates (greater than 30%) may be economically practical if the price of wheat middlings is sufficiently lower than that of the primary feed ingredients it replaces.

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CHAPTER 3. EFFECT OF SUPPLEMENT WITHDRAWAL AND WHEAT MIDDLING INCLUSION ON THE NUTRIENT CONTENT OF PORK, PORK OXIDATIVE STABILITY, AND NUTRIENT EXCRETION.[1]

A paper to be published in the *Journal of Animal Science*

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Abstract

A study was conducted to determine if supplement withdrawal (omission of vitamin and trace mineral premixes and 2/3 reduction of inorganic phosphorus) for 28 d pre-slaughter in corn-soybean meal-based diets containing either 0 or 30% added wheat middlings affects the nutrient content and oxidative stability of the longissimus dorsi muscle, growth performance, carcass characteristics, and nutrient excretion. Crossbred pigs (n = 62) were blocked by weight and assigned to one of four treatments in a 2 x 2 factorial design (with or without supplement withdrawal; 0 or 30% wheat middlings). Supplement withdrawal decreased longissimus dorsi riboflavin, niacin, and P concentrations ($P < 0.05$). Longissimus dorsi thiamin, vitamin E, Fe, Cu, Zn, and Ca concentrations were not altered. Inclusion of 30% wheat middlings increased longissimus dorsi thiamin, niacin, riboflavin, and vitamin E concentrations ($P < 0.04$) and decreased Cu concentrations ($P < 0.04$). However, wheat middling inclusion did not affect longissimus dorsi Ca, P, Fe, and Zn concentrations. Dietary treatment did not affect either Cu/Zn superoxide dismutase or glutathione peroxidase activity in the loin. In diets containing full vitamin and mineral supplementation, wheat middling inclusion decreased

[1] Appreciation is expressed to Roche Vitamins Inc. for vitamin E analysis

fecal Ca, Cu, Fe, and Zn concentrations ($P < 0.01$) and increased fecal Mn ($P < 0.01$). Supplement withdrawal decreased fecal Ca, P, Cu, Fe, Mn, Zn concentrations ($P < 0.01$). Supplement withdrawal did not influence ADG, ADFI, gain:feed, or carcass traits. The results from this study indicate that supplement withdrawal and dietary wheat middling inclusion alter pork nutrient content and nutrient excretion, but not the oxidative stability of pork.

Introduction

Removing dietary vitamin and mineral premixes from finishing diets 3 to 5 wk prior to slaughter does not affect pig growth and carcass traits (Patience and Gillis, 1995, 1996; Kim et al., 1997; Mavromichalis et al., 1999; McGlone, 2000). Deleting inorganic phosphorus from barley-pea-based diets 75 d prior to slaughter does not affecting growth performance and decreases fecal P by 40% during the removal period (Michal and Froseth, 1999). O'Quinn and associates (1997) demonstrated that removing inorganic P supplements 48 d prior to slaughter from sorghum-soybean meal-based diets reduced fecal P excretion by 12% without affecting structural soundness, carcass leanness, or carcass quality.

The effect of supplement withdrawal on pork's nutrient content is not conclusive. Patience and Gillis (1996) removed dietary vitamin supplements from wheat-barley-canola meal-based diets during the final 35 d prior to slaughter and found that the longissimus dorsi muscle (LDM) thiamin concentration was reduced by 20%. Longissimus riboflavin, niacin, or pantothenic acid concentrations were not affected. When Rozeboom (personal communication) removed vitamin and trace mineral supplements 31 d prior to slaughter from corn-soybean meal-based diets, LDM thiamin

and Mg concentrations were not changed, but Fe and Zn concentrations were reduced by 12 and 17%, respectively.

Edmonds and Arentson (2001) fed diets devoid of supplemental vitamins and trace minerals for 6 to 12 wk prior to slaughter. Length of withdrawal did not affect growth performance or carcass traits. However, vitamin E concentrations of the LDM and ham muscles were reduced by 76 and 55%, respectively. Copper concentrations were reduced by 35% in the ham muscle, but not in the LDM. Muscle zinc and iron concentrations were not affected by dietary treatment.

The objective of our research was to determine the effect of withdrawal of vitamin and trace mineral premixes and 2/3 reduction of inorganic phosphorus 28 d prior to slaughter on the nutrient content and oxidative stability of pork, growth performance, carcass characteristics, and nutrient excretion. Furthermore, this research evaluated the effect of dietary wheat middling inclusion on the same parameters.

Materials and Methods

Animal Use and Care. The experimental protocol used in this study was approved by the All-University Committee on Animal Use and Care at Michigan State University (AUF number: 01/00-030-00).

Experimental Design. Crossbred barrows [(Yorkshire x Landrace) x DRU or (Yorkshire x Musculor)] were blocked by weight and allotted to dietary treatments arranged in a 2 x 2 factorial design, replicated twice over time. The factors evaluated were the effects of supplement withdrawal (omitting vitamin and trace mineral premixes and 2/3 of the inorganic phosphorus) 28 d pre-slaughter in corn-soybean meal-based diets

containing either 0 or 30% added wheat middlings. Replication 1 was conducted from February to June, and replication 2 was conducted from July to November.

Sixty-two pigs with an average initial weight of 8.5 ± 0.7 kg were individually penned (1.51 x 1.58 m) in a totally enclosed, ventilated nursery-to-finish facility, with 23% solid-concrete and 77% slatted-concrete flooring. Pigs were fed 6 dietary phases to meet the changing physiological needs of the pigs in the nursery through early finishing periods. These diets met or exceeded NRC (1998) recommendations for all nutrients. Thirty pigs received typical corn-soybean meal-based (CSBM) diets. The remaining 32 pigs received corn-soybean meal-based diets with added wheat middlings (CSBM+WM) at the inclusion rates of 5% in nursery diets, 15% in the transition diet, and 30% in growing and early finishing diets. Diets were calculated to contain identical lysine, ME, Ca, and total P concentrations for each phase and had identical vitamin and trace mineral premixes additions. The compositions of the nursery and transition diets are presented in Table 5 and the growing and early finishing diets in Table 6.

Three vitamin premixes were utilized in this study, each prepared to provide the vitamins at or slightly above NRC (1998) recommendations for various stages of growth. The premixes were stored in air-tight containers at -20° C until diets were mixed. Complete feeds were mixed weekly and were stored at 5° C to maintain vitamin activity. Any feed that remained in the feeders more than 72 hr was weighed and discarded.

Dietary Treatments. Twenty-eight days prior to slaughter and at an average age of 114 d and weight of 79.0 kg, barrows were provided late finishing diets formulated to contain identical lysine and ME concentrations. Vitamin and trace mineral premixes and 2/3 of the inorganic P were removed from the diets of 15 pigs receiving CSBM diets and

Table 5. Percentage composition of nursery diets, as fed basis

	Wean to 11.3 kg		11.3 to 15.8 kg		15.8 to 28.1 kg	
Item	CSBM	CSBM+WM	CSBM	CSBM+WM	CSBM	CSBM+WM
Dent yellow corn	35.18	30.94	54.22	49.96	62.26	48.16
Soybean meal (44% CP)	24.75	23.7	23.36	22.32	29.35	27.62
Dried whey	20	20	10	10	–	–
Menhaden fish meal	7.5	7.5	5	5	–	–
Edible-grade lactose	6	6	–	–	–	–
Wheat middlings	–	5	–	5	–	15
Choice white grease	4	4.34	4	4.36	4	5.07
Vitamin premix[a]	1	1	1	1	1	1
Limestone	.41	.47	.7	.76	.99	1.17
Mineral premix[b]	.5	.5	.5	.5	.5	.5
Zinc oxide	.38	.38	–	–	–	–
Dicalcium phosphate, 21% P	.33	.2	.82	.69	1.36	.93
L-lysine·HCl, 78.8%	.15	.17	.15	.17	.2	.2
Salt	–	–	.35	.35	.35	.35
Calculated content						
CP, %	20.99	20.98	19.19	19.19	18.21	18.67
Lysine, %	1.45	1.45	1.25	1.25	1.15	1.15
Ca, %	.90	.90	.90	.9	.80	.8
P, total, %	.70	.70	.70	.7	.65	.65
P, available, %	.49	.48	.45	.44	.37	.33
ME, kcal/kg	3399	3399	3393	3393	3372	3372

[a]Provided per kg of complete diet: 1,920 IU vitamin A, 204 IU vitamin D_3, 13.5 IU vitamin E, .5 mg vitamin K_3, .05 mg biotin, .46 g choline, .3 mg folacin, 15 mg niacin, 10 mg pantothenic acid, 3.4 mg riboflavin, 1 mg thiamin, 1.5 mg vitamin B_6, and 17µg vitamin B_{12}.

[b]Provided per kg of complete diet: 100 mg Zn, 100 mg Fe, 10 Cu, 10 mg Mn, 150 µg I, and 300 µg of Se.

Table 6. Percentage composition of growing and early finishing diets, as fed basis

	28.1 to 47.2 kg		47.2 to 65.3 kg		65.3 to 79.0 kg	
Item	CSBM	CSBM+WM	CSBM	CSBM+WM	CSBM	CSBM+WM
Dent yellow corn	70.27	42.73	71.04	45.33	75.65	49.7
Soybean meal (44% CP)	24.2	19.83	23.53	17.31	19.57	13.4
Wheat middlings	–	30	–	30	–	30
Choice white grease	1.5	3.73	1.5	3.67	1.5	3.77
Dicalcium phosphate, 21% P	1.17	.56	1.18	.58	.78	.34
Vitamin premix[a]	1	1	1	1	1	1
Limestone	.9	1.16	.9	1.17	.76	.94
Mineral premix[b]	.5	.5	.5	.5	.5	.5
Salt	.35	.35	.35	.35	.25	.25
L-lysine·HCl, 78.8%	.11	.14	–	.09	–	.09
Calculated content						
CP, %	16.53	17.13	16.2	16.2	14.85	14.85
Lysine, %	.95	.95	.85	.85	.75	.75
Ca, %	.72	.72	.72	.72	.58	.58
P, total, %	.6	.65	.6	.64	.5	.58
P, available, %	.32	.29	.32	.29	.23	.23
ME, kcal/kg	3289	3289	3294	3294	3326	3326

[a]Provided per kg of complete diet: 1,570 IU vitamin A, 178 IU vitamin D_3, 11.5 IU vitamin E, .5 mg vitamin K_3, .05 mg biotin, .35 g choline, .3 mg folacin, 10.9 mg niacin, 8.4 mg pantothenic acid, 2.7 mg riboflavin, 1 mg thiamin, 1.2 mg vitamin B_6, and 12μg vitamin B_{12}.

[b]Provided per kg of complete diet: 100 mg Zn, 100 mg Fe, 10 Cu, 10 mg Mn, 150 μg I, and 300 μg of Se.

Table 7. Percentage composition of late finishing diets, as fed basis

Item	Full supplementation		Supplement withdrawal	
	CSBM	CSBM+WM	CSBM	CSBM+WM
Dent yellow corn	82.3	55.29	87.11	58.87
Soybean meal (44% CP)	12.74	7.67	11.79	7.34
Wheat middlings	–	30	–	30
Choice white grease	1.5	3.79	–	2.34
Dicalcium phosphate, 21% P	1	1	–	–
Vitamin premix[a]	.83	.38	.28	.13
Limestone	.79	.97	.46	.92
Mineral premix[b]	.5	.5	–	–
Salt	.25	.25	.25	.25
L-lysine·HCl, 78.8%	.1	.15	.11	.15
Calculated content				
CP, %	12.5	12.86	12.5	13.01
Lysine, %	.65	.65	.65	.65
Ca, %	.58	.58	.29	.45
P, total, %	.49	.56	.38	.52
P, available, %	.23	.23	.12	.18
ME, kcal/kg	3336	3336	3354	3336
Analyzed content				
Thiamin, IU/kg	3.2	5.7	2.9	4.7
Riboflavin, IU/kg	2.7	3.6	1.3	1.5
Niacin, IU/kg	31.5	72.1	22.8	65.6
D-L-α-tocopherol, IU/kg	13.4	12.4	2.8	1.6

[a]Provided per kg of complete diet: 1,570 IU vitamin A, 178 IU vitamin D_3, 11.5 IU vitamin E, .5 mg vitamin K_3, .05 mg biotin, .35 g choline, .3 mg folacin, 10.9 mg niacin, 8.4 mg pantothenic acid, 2.7 mg riboflavin, 1 mg thiamin, 1.2 mg vitamin B_6, and 12μg vitamin B_{12}.

[b]Provided per kg of complete diet: 100 mg Zn, 100 mg Fe, 10 Cu, 10 mg Mn, 150 μg I, and 300 μg of Se.

Table 8. Analyzed mineral composition of diets, as fed basis[a]

Diet	Ca, %	P, %	Fe, ppm	Zn, ppm	Cu, ppm	Mn, ppm
Nursery diets						
1 – CSBM	.978	.658	291.4	3596.7	16.9	30.3
1 – CSBM+WM	1.075	.641	295.7	3737.6	16.1	33.3
2 – CSBM	.990	.673	301.5	141.0	15.5	28.1
2 – CSBM+WM	.957	.662	275.8	130.6	16.0	32.1
3 – CSBM	.981	.643	363.5	158.7	15.4	35.7
3 – CSBM+WM	.988	.599	328.2	164.4	20.7	51.6
Growing diets						
1 – CSBM	.819	.554	294.0	140.1	13.9	29.4
1 – CSBM+WM	.875	.646	285.9	169.4	22.2	70.4
2 – CSBM	.812	.546	271.1	130.6	12.3	26.4
2 – CSBM+WM	.899	.663	283.2	171.7	21.5	66.2
Early finishing diets						
CSBM	.694	.427	260.8	156.8	14.4	34.0
CSBM+WM	.743	.577	245.6	176.6	20.0	67.4
Late finishing diets						
CSBM and full suppl.	.680	.450	246.7	146.6	11.5	28.3
CSBM+WM and full suppl.	.674	.554	225.8	162.8	18.3	64.3
CSBM and suppl. withdrawal	.381	.344	80.6	34.3	3.7	12.1
CSBM+WM and suppl. withdrawal	.613	.524	90.9	60.5	6.1	49.2
Wheat middlings	.100	1.015	131.5	118.7	12.5	137.9

[a]Nursery diets 1, 2, and 3 were fed from weaning to 11.3 kg, 11.3 to 15.8 kg, and 15.8 to 28.1 kg, respectively. Growing diets 1 and 2 were fed from 28.1 to 47.2 kg and 47.2 to 65.3 kg. Early finishing diets were fed from 65.3 to 70.9 kg. Late finishing diets were fed for final 28 d prior to slaughter.

16 pigs receiving CSBM+WM diets. Limestone additions were adjusted to maintain a Ca to available P ratio of 2.5:1.

Performance and Sample Collection. Pig weights and feed disappearance were determined for each dietary phase and were used to calculate ADG, ADFI, and gain/feed. Random samples of feed were placed in whirl-pac bags and frozen at –20° C until analyzed for thiamin, riboflavin, niacin, vitamin E, Ca, Cu, Fe, Mn, P, and Zn content. Ten random samples of wheat middlings were collected for bulk density measurement and were stored at –20° C until analyzed for Ca, Cu, Fe, Mn, P, and Zn.

To quantify fecal nutrient excretion during the early and late finishing periods, the top portion three freshly voided feces were obtained from each pig 48 hr prior to placement on the late finishing diet, and again 48 hr prior to slaughter. Fecal samples were weighed, freeze-dried, ground in a stainless steel blender, and frozen at -20° C until mineral analyses were performed.

Upon reaching an average live weight of 103.4 kg, all pigs were slaughtered at the Michigan State University Meat Laboratory according to standard operating procedures. Dressing percentages were calculated using hot carcass weights. Following a 24 h chill at 1° C, longissimus muscle area and backfat depth were recorded at the 10^{th} rib. Two 1-in.-thick loin chops were collected beginning at the tenth rib and proceeding anteriorly. The LDM was removed and frozen at -80° C until analyzed. Tissue from the initial chop was analyzed for thiamin by fluorometric method and riboflavin and niacin by microbial method (AOAC, 1995). The vitamin E content of the second chop was determined as DL-α-tocopherol by the method of Liu et al. (1996). The remaining tissue from the second

chop was analyzed for Cu/Zn superoxide dismutase activity (Cu/ZnSOD), glutathione peroxidase activity (GPX), and mineral concentrations.

Mineral Analyses. Feed, LDM, and fecal samples obtained in replication 1 were prepared for mineral analyses using nitric-perchloric acid wet digestion (Hill et al., 1983). Due to equipment failure, feed, LDM, and fecal samples obtained in replication 2 were prepared for mineral analyses using microwave digestion (Model HP-500Plus, CEM, Matthews, NC). For feed and fecal microwave digestion, 10 mL of nitric acid (70%) was added to either .5 g of feed sample or .4 g of fecal sample in a pressurized Telfon-lined digestion vessel. For LDM digestion, approximately .5 g of LDM samples was sliced from within the area of the frozen tissue and 5 mL of nitric acid and 2 mL of double distilled water were added. Samples were allowed to digest for 1 hr at room temperature. Vessels were then placed in the microwave digestor and power was intermittently applied for 25 min to gradually increase vessel pressure to 210 psi while maximum vessel temperature was 210° C. Vessels were maintained at 210 psi for 10 min, were allowed to cool for 10 min, and were vented. Two mL of hydrogen peroxide (30%) was added to the digested feed and fecal samples and 1 mL was added to the digested LDM. Digested samples were poured into volumetric flasks and brought to a uniform volume.

Calcium, copper, iron, manganese, and zinc analyses were conducted by flame atomic absorption spectrophotometry (Smith-Heiftje 4000, Thermo Jarrell Ash Corporation, Franklin, MA), and P concentrations were determined using the DU 7400 spectrophotometer (Beckman, Palo Alto, CA). Feed, LDM, and fecal mineral concentrations were reported on an as-fed, fresh, and DM basis, respectively.

Instrument accuracy for all mineral analyses was established using bovine liver standard (1577b; NIST: National Institute of Standards and Technology, Gaithersburg, MD). All glassware used in mineral analyses was washed in 30% nitric acid and rinsed with double deionized distilled water.

Superoxide Dismutase and Glutathione Peroxidase Activities. Longissimus dorsi muscle Cu/ZnSOD (EC 1.15.1.1) activity was determined with the method of Hill et al. (1999). Longissimus dorsi muscle samples (approximately 1 g) were sliced from within the area of the frozen tissue and homogenized with an Ultra Turrax T25 homogenizer (Tekmar-Dohrmann Corp., Cincinnati, OH) in 10 x volumes of ice-cold potassium phosphate buffer (pH 7.2, .05 M phosphate, .24 M sucrose) using a tissumizer probe (S25N-10G, 10mm diameter). The subsequent procedures were the same as for red blood cell hemolysates in the Hill et al. method. One unit of Cu/ZnSOD activity was defined as the amount of SOD necessary to inhibit the autoxidation of pyrogannol by 50%. Muscle GPX (EC 1.11.1.9) activity was determined with the method of Sunde and Hoekstra (1980). One GPX unit was defined as 1 μmol NADPH oxidized per minute, using the molar extinction coefficient of 6.22×10^3 for NADPH and the stoichiometry of reaction of 2 moles GPX formed per mole NADPH oxidized. Protein concentrations of the supernatant were determined by the method of Lowry et al. (1951), and units of Cu/ZnSOD and GPX activity were expressed per gram of protein.

During replication 2, two pigs receiving CSBM diets were removed from the experiment during the growing phase due to health considerations. One pig contracted a respiratory disease and the other a severe middle ear infection. One of the pigs would have received a supplement withdrawal diet in the late finishing phase.

Statistical Analysis. All data were analyzed by least squares ANOVA using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC) for a randomized complete block design. Pig served as the experimental unit. The model included the fixed effects of the factorial treatments, their interaction, replication, and block by initial weight. Litter within replication was specified as a random effect. All means presented are least square means. Differences were considered significant at the level of $P < 0.05$.

Results and Discussion

Nutrient Content of Pork. Dietary nutrient composition altered the B-vitamin content of the LDM (Table 9). Supplement withdrawal decreased the LDM riboflavin and niacin concentrations ($P < 0.01$), but not thiamin. Feeding 30% wheat middlings during the growing-finishing period increased LDM thiamin, riboflavin, and niacin ($P < 0.04$) concentrations.

Contrary to the findings of our study, Patience and Gillis (1996) fed wheat-barley-canola meal-based diets and observed that removing the vitamin premix 35 d prior to slaughter reduced LDM thiamin. Riboflavin and niacin concentrations were numerically but not significantly decreased by vitamin premix removal. Inconsistency of results may be attributed to barley grain and canola meal, which, unlike corn, are rich sources of riboflavin and available niacin, thus lessening the need to draw upon riboflavin and niacin muscle reserves to satisfy metabolic demands during supplement withdrawal. Patience and Gillis analyzed 15 LDM samples for vitamin content. We analyzed 62 LDM samples, increasing the power of the test and decreasing the probability of type II statistical error.

Table 8. Effects of supplement withdrawal and

Table 9. Effects of supplement withdrawal and wheat middling inclusion rate on pork vitamin and mineral concentrations and oxidative stability[ab]

	Full supplementation		Supplement withdrawal			P-values[c]		
Item	CSBM	Midds	CSBM	Midds	SE[d]	Withdrawal	Midds	Interaction
Vitamins								
Thiamin, mg/100g	.750	1.067	.741	.971	.0531	.23	.01	.31
Riboflavin, mg/100g	.143	.146	.118	.133	.0056	.01	.04	.13
Niacin, mg/100g	5.803	8.701	4.076	6.572	.4964	.01	.01	.62
DL-α-tocopherol, IU/kg	1.113	1.460	1.114	1.774	.1601	.30	.01	.30
Minerals								
Ca, ppm	31.359	31.678	30.270	32.786	1.8390	.99	.41	.52
Cu, ppm	.653	.450	.547	.485	.0545	.53	.03	.22
Fe, ppm	5.631	5.315	5.488	5.292	.2848	.72	.28	.80
P, mg/g	2.206	2.223	2.162	2.179	.0249	.05	.44	.99
Zn, ppm	12.999	12.914	13.132	12.700	.3781	.86	.38	.55
Enzyme activity								
Cu/ZnSOD, U/g protein	.556	.566	.572	.541	.0192	.79	.55	.23
GPX, U/g protein	3.484	3.107	3.297	2.842	.4071	.40	.12	.88

[a]Fresh weight basis.

[b]No. of animals by treatment for all variables except vitamin E and Cu: CSBM and full suppl. (n = 15), CSBM+WM and full suppl. (n = 16), CSBM and suppl. withdrawal (n = 15), CSBM+WM and suppl. withdrawal (n = 16). No. of animals by treatment for vitamin E: CSBM and full suppl. (n = 14), CSBM+WM and full suppl. (n = 15), CSBM and suppl. withdrawal (n = 14), CSBM+WM and suppl. withdrawal (n = 16). No. of animals by treatment for Cu: CSBM and full suppl. (n = 8), CSBM+WM and full suppl. (n = 8), CSBM and suppl. withdrawal (n = 8), CSBM+WM and suppl. withdrawal (n = 8).

[c]Indicates the probability of full supplementation vs supplement withdrawal (Withdrawal), 0 vs 30% wheat middling inclusion (Midds), and the interaction thereof (Interaction).

[d]Greatest SE among treatments.

Results from poultry studies indicate that removing supplemental riboflavin from broiler diets for 7 and 14 d decreases pectoralis major bioavailable riboflavin by 22 and 43%, respectively (Patel et al., 1997). A 21 d vitamin withdrawal period decreased p. major total thiamin and niacin concentrations by 45 and 31%, respectively (Deyhim et al., 1996). Standard practice is to slaughter broilers at 9 weeks of age. Broilers at six, seven, and eight weeks of age typically weigh 59, 73, and 87% of their slaughter weight, respectively (NRC, 1994). At the initiation of the supplement withdrawal period, the pigs in our study weighed 76% of their slaughter weight.

Previous swine research supports that there is a general relationship between the thiamin concentrations of the diet and skeletal muscle. Miller and coworkers (1943) fed diets containing 2.9, 7.6, and 12.7 mg/kg of thiamin for 100 d prior to slaughter. Increasing thiamin intake from 2.9 to 7.6 mg/kg and from 7.6 to 12.7 mg/kg increased loin thiamin concentrations by 110% (0.95 ± 0.21 to 2.00 ± 0.44 mg/100g) and 15% (2.00 ± 0.44 to 2.31 ± 0.51 mg/100g), respectively. Pence and associates (1945) supplemented finishing diets with 50 mg/d of thiamin for 8, 15, 22, 35 or 155 d prior to slaughter. Loin thiamin concentrations increased with lengthened periods of supplementation up to 35 d prior to slaughter. In our study, 30% wheat middling inclusion increased the thiamin content of the diet by 1.8 to 2.6 mg/kg. The late finishing vitamin premix provided 1 mg of thiamin/kg of feed. This explains why wheat middling inclusion significantly influenced LDM thiamin concentrations while supplement withdrawal did not.

Like thiamin, dietary niacin is strongly correlated to pork niacin concentrations. Christensen and associates (1943) increased the niacin content of pork from 4.66 to 7.35 mg/100g by feeding 100 mg/d of supplemental niacin to growing and finishing pigs. In

our study, the pigs receiving 30% wheat middlings received an additional 146 mg/d of dietary niacin, increasing LDM niacin concentration from 4.94 to 7.64 mg/100g. Supplement withdrawal decreased dietary niacin by 27 mg/d, decreasing LDM niacin from 7.25 to 5.32 mg/100g. Although NRC (1998) states that the niacin in wheat is totally unavailable, this data indicates that a large portion of the niacin in wheat middlings is bioavailable to the pig.

Dietary riboflavin concentrations influence muscle riboflavin content, but to a lesser extent than other B-vitamins. Ittner and Hughes (1941) found that increasing dietary riboflavin supplementation from 0 to 6 mg/d increased loin riboflavin concentrations from 0.14 to 0.25 mg/100g. However, when doubling supplementation to 12 mg/d, loin riboflavin remained at .26 mg/100g. Miller and coworkers (1943) observed no difference in loin riboflavin concentrations when feeding diets containing 3.68 to 5.44 mg/kg, confirming that the LDM approaches saturated storage capacity at 0.23 mg/100g. In our study, LDM riboflavin concentrations steadily increased as the dietary concentration increased from 1.2 to 3.6 mg/kg.

Supplement withdrawal did not affect the LDM Ca or trace mineral concentrations, but decreased LDM P ($P < 0.05$). Wheat middling inclusion decreased LDM concentrations of Cu ($P < 0.03$), but not Ca, Fe, P, or Zn. All late finishing diets contained Cu, Fe, Mn, and Zn in excess of the estimated requirements (NRC, 1998) for optimal growth with the exception of Zn in the CSBM withdrawal diet, which was approximately 30% below NRC (1998) recommendations.

Similarly, Edmonds and Arentson (2001) reported that removing vitamin and trace mineral premixes from finishing diets either 6 or 12 wk prior to slaughter did not

affect LDM Zn, Cu, or Fe concentrations. However, both 6 and 12 wk withdrawal reduced Cu concentrations in the ham. The trace mineral concentration of pork is relatively consistent regardless of dietary concentrations (Leonhardt and Wenk, 1997). Muscle Zn concentrations are maintained during times of deficiency (Bentley and Grubb, 1991; O'Leary et al., 1979). Muscle Cu concentrations are not affected by dietary deficiencies (Ledoux et al., 1989) or excesses (Zanardi et al., 1998; Lauridsen et al., 2000). An exception to the lack of variation of the mineral content of meat may be Fe. Injecting growing pigs with 1600 mg of Fe IM from Fe-dextran during the nursery and growing phases increased ham Fe concentrations by 21% (Henry et al., 1961), and increasing dietary Fe from 62 to 209 mg/kg for 13 weeks increased LDM Fe concentrations by 38% (Miller et al, 1994).

The reduced LDM P caused by supplement withdrawal in our study indicates that during periods of modest Ca and P deficiencies the pig draws upon muscle P reserves to increase serum P and meet metabolic needs. Nicodemo et al. (1998) fed pigs diets containing high supplementation (.86% Ca and .56% P), intermediate supplementation (6.0% Ca and .4% P), or low supplementation (3.9 % Ca and 2.5% P). After 56 d on trial, plasma Ca concentrations did not differ between dietary treatments, but low supplementation considerably reduced plasma P concentrations. Other studies found that serum and plasma P concentration are reduced during periods of extreme (Howe and Beecher, 1983; Koch and Mahan, 1985) but not moderate (Carter et al., 1996) dietary P deficiencies. Thus, during periods of mineral deficiencies the pig may rely upon secondary P reserves such as muscle to sustain adequate circulating P concentrations.

Pork Oxidative Stability. Supplement withdrawal did not decrease LDM vitamin E concentrations (Table 9). O'Sullivan et al. (1997) found that feeding 0 vs 20 mg DL-α-tocopheryl acetate/kg feed for 130 d prior to slaughter did not affect LDM vitamin E concentrations. In contrast, Dove and Ewan (1991) reported that deleting supplemental α-tocopheryl acetate from pig diets for 13 weeks prior to slaughter reduced LDM α-tocopherol concentrations by 82% (1.035 vs 0.185 μg/g). Likewise, Edmonds and Arentson (2001) reported that removing vitamin and trace minerals for 6 weeks pre-slaughter decreased LDM vitamin E concentrations by 77%. While withdrawal times and premix vitamin E concentrations differed between our study and the Edmonds and Arentson report, the vitamin E analyses were performed by the same laboratory using the same methods.

Supplementing 100 to 200 mg/kg of dietary α-tocopherol for extended periods of time has been reported to increase muscle α-tocopherol concentrations and decrease lipid oxidation as measured by thiobarbituric acid reactive substances (TBARS) (Monahan et al., 1990ab, 1992; Asghar et al., 1991ab; Cannon et al., 1996; Jensen et al., 1997; Lauridsen et al., 2000). In our study we did not conduct a TBARS assay because of the assay's high inherent variability. We anticipated that removing only 11 mg/kg of dietary α-tocopherol for 28 d would not sufficiently decrease LDM vitamin E concentrations to elicit a measurable increase of TBARS.

Wheat middling inclusion increased LDM vitamin E concentrations. However, laboratory analyses found that the diets containing wheat middlings had lower vitamin E concentrations than the diets not containing wheat middlings. Additional choice white grease was added to the CSBM+WM diets to balance diets for ME. This observation

suggests that the vitamin E in either the choice white grease or the wheat middlings may be more bioavailable than the supplemented vitamin E. Zanardi et al. (1998) reported that adding 6% dietary sunflower oil, though increasing total dietary vitamin E by 27 mg/kg, had a much greater influence on LDM vitamin E concentrations than 200 mg/kg of supplemental α-tocopheryl acetate.

Dietary treatment did not affect the activity of the LDM antioxidative enzymes Cu/ZnSOD and GPX. Previous research with 4 to 9 week-old weanling pigs shows that.3 mg/kg of supplemental dietary Se is necessary to maintain muscle GPX activity (Lei et al., 1998). Our study shows that this is not the case with finishing pigs. In agreement with our study, previous research has shown that removing supplemental Cu from the diets of growing pigs (Lauridsen et al., 1999) and rats (Paynter et al., 1979) does not affect Cu/ZnSOD activity in skeletal muscle. The analyzed concentrations of Cu of the withdrawal diets were 124 to 204% of the NRC (1998) estimated requirement, and the calculated Se concentrations of the withdrawal diets were 70 to 201% of the NRC estimated requirement.

Growth Performance and Carcass Characteristics. Supplement withdrawal did not affect growth performance as measured by ADG, ADFI, and gain:feed (Table 10), or carcass traits as measured by backfat depth, LEA, and dressing percentage (Table 11). These data agree with previous studies (Patience and Gillis, 1995, 1996; Kim et al., 1997; Mavromichalis et al., 1999; McGlone, 2000; Edmonds and Artentson, 2001) in which withdrawing vitamin and/or mineral supplementation for 17 to 45 d prior to slaughter did not affect growth and carcass traits.

Table 10. Effects of supplement withdrawal and wheat middling inclusion rate on growth performance[a]

Item	Full supplementation											

Table 10. Effects of supplement withdrawal and wheat middling inclusion rate on growth performance[a]

	Full supplementation		Supplement withdrawal			P-values[b]		
Item	CSBM	Midds	CSBM	Midds	SE[c]	Withdrawal	Midds	Interaction
Nursery								
Daily gain, kg	.563	.536	.528	.523	.0157	–	.27	–
Daily feed, kg	1.068	1.017	1.045	1.025	.0311	–	.15	–
Gain/feed, g/kg	525	535	513	519	12.9	–	.52	–
Grower								
Daily gain, kg	.990	.950	1.021	.953	.0167	–	.01	–
Daily feed, kg	2.344	2.269	2.316	2.264	.0576	–	.27	–
Gain/feed, g/kg	425	422	443	424	9.5	–	.28	–
Early finisher								
Daily gain, kg	1.021	.981	1.078	1.021	.0493	–	.32	–
Daily feed, kg	3.007	2.992	3.091	2.972	.1070	–	.53	–
Gain/feed, g/kg	337	331	350	348	12.3	–	.77	–
Late finisher								
Daily gain, kg	1.011	1.047	1.043	.987	.0402	.70	.76	.19
Daily feed, kg	3.520	3.530	3.582	3.499	.1087	.88	.71	.64
Gain/feed, g/kg	289	299	294	284	8.2	.55	.98	.22

[a]No. of animals by treatment for all variables: CSBM and full suppl. (n = 15), CSBM+WM and full suppl. (n = 16), CSBM and suppl. withdrawal (n = 15), CSBM+WM and suppl. withdrawal (n = 16).

[b]Indicates the probability of full supplementation vs supplement withdrawal (Withdrawal), 0 vs 30% wheat middling inclusion (Midds), and the interaction thereof (Interaction).

[c]Greatest SE among treatments.

Table 11. Effects of supplement withdrawal and wheat middling inclusion rate on carcass characteristics[a]

	Full supplementation		Supplement withdrawal			P-values[b]		
Item	CSBM	Midds	CSBM	Midds	SE[c]	Withdrawal	Midds	Interaction
Dressing percentage	75.62	75.44	75.51	74.61	.373	.18	.13	.31
Loin eye area, cm^2	38.39	39.44	39.19	37.94	1.214	.72	.91	.25
Backfat depth, cm	2.10	1.95	2.03	2.05	.123	.92	.54	.41

[a]No. of animals by treatment for all variables: CSBM and full suppl. (n = 15), CSBM+WM and full suppl. (n = 16), CSBM and suppl. withdrawal (n = 15), CSBM+WM and suppl. withdrawal (n = 16).

[b]Indicates the probability of full supplementation vs supplement withdrawal (Withdrawal), 0 vs 30% wheat middling inclusion (Midds), and the interaction thereof (Interaction).

[c]Greatest SE among treatments.

Table 12. Effects of supplement withdrawal and wheat middling inclusion rate on fecal nutrient excretion during the finishing phase[ab]

	Full supplementation		Supplement withdrawal			Effect, P-value		
Item	CSBM	Midds	CSBM	Midds	SE[d]	Withdrawal	Midds	Interaction
Early finishing								
Ca, mg/g	22.405	18.837			.6140		.01	
P, mg/g	16.880	15.832			.4343		.09	
Fe, mg/g	1.556	1.026			.0356		.01	
Cu, mg/kg	99.96	79.82			2.353		.01	
Mn, mg/kg	213.35	278.60			7.018		.01	
Zn, mg/kg	780.74	632.11			17.702		.01	
Late finishing								
Ca, mg/g	26.789	21.714	13.984	17.818	.9064	.01	.45	.01
P, mg/g	19.387	17.697	15.498	17.781	.6834	.01	.65	.01
Fe, mg/g	1.829	1.155	.601	.473	.03791	.01	.01	.01
Cu, mg/kg	98.00	79.64	28.20	30.03	1.712	.01	.01	.01
Mn, mg/kg	211.36	286.74	86.78	228.66	5.218	.01	.01	.01
Zn, mg/kg	800.58	674.95	177.96	209.53	16.345	.01	.01	.01

[a]Based on dry matter basis.

[b]No. of animals by treatment for all variables: CSBM and full suppl. (n = 15), CSBM+WM and full suppl.(n = 16), CSBM and suppl. withdrawal (n = 15), CSBM+WM and suppl. withdrawal (n = 16).

[c]Indicates the probability of full supplementation vs supplement withdrawal (Withdrawal), 0 vs 30% wheat middling inclusion (Midds), and the interaction thereof (Interaction).

[d]Greatest SE among treatments.

Wheat middling inclusion did not affect growth performance with the exception of ADG during the growing phase. Growth performance was not affected by lesser wheat middling inclusion rates (5 to 15%) during the nursery phase and 30% wheat middling inclusion during the early and late finishing phases.

Previous research (Patience et al., 1977) reported that a maximum of 20% wheat middlings can be included in growing diets before decreasing growth performance, while others found that inclusion rates of 30% (Young, 1980; Erickson et al., 1985) did not decrease growth. Cromwell et al. (1992) reported that when wheat middlings with a light bulk density are used, growth performance of growing-finishing pigs decreased linearly as the amount of wheat middlings increased. Wheat middlings with a heavy bulk density could constitute 20 to 40% of the ration without substantially affecting performance. The bulk density of the wheat middlings used in our study was 387.4 g/L, much heavier than the average wheat middling bulk density of 320 g/L as reported by Cromwell et al. (2001). The observed decrease in ADG during growing phase may reflect that these pigs had a higher lean gain potential than pigs used in previous research studies, thus requiring a more nutrient-dense ration.

Nutrient Excretion. As presented in Table 12, dietary mineral concentrations influenced nutrient excretion. There was a statistical interaction between the main effects of supplement withdrawal and wheat middling inclusion. In diets containing full vitamin and mineral supplementation, wheat middling inclusion reduced fecal Ca, Cu, Fe, and Zn concentrations ($P < 0.01$) and increased fecal Mn ($P < 0.01$).

Supplement withdrawal reduced fecal P excretion by 20% and trace mineral excretion by 59 to 78% ($P < 0.01$). These decreases are noteworthy when considering that

the market hog consumes approximately one-third of its total lifetime feed intake during the final 4 wk prior to slaughter, thus producing approximately one-third of its total fecal excretion. Michal and Froseth (1999) and O'Quinn et al. (1997) observed 40% and 12% decreases in P excretion, respectively, when deleting inorganic P from barley-pea and sorghum-soybean meal-based finishing diets, respectively.

Implications

The concentrations of many vitamins and minerals in pork are influenced by premix inclusion and the choice of primary feed ingredients utilized in ration formulation. Supplement withdrawal does not affect growth performance or carcass traits and decreases nutrient excretion, making it an attractive practice to pork producers. However, it also decreases the nutrient content of pork and may diminish consumer perception of the healthiness of pork.

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CHAPTER 4. EFFECTS OF SUPPLEMENT WITHDRAWAL AND WHEAT MIDDLING INCLUSION ON BONE METABOLISM, BONE STRENGTH, AND INCIDENCE OF BONE FRACTURES OCCURING AT SLAUGHTER.

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Abstract

The objective of this study was to determine if supplement withdrawal (omission of dietary vitamin and trace mineral premixes and 2/3 reduction of inorganic P) 28-d pre-slaughter affects bone metabolism, bone strength, and the incidence of bone fractures at slaughter. The effect of adding either 0 or 30% wheat middlings to corn-soybean meal-based growing and finishing diets was also evaluated. Crossbred pigs (n = 62) were assigned to one of four treatments in a 2 x 2 factorial design (with or without supplement withdrawal, 0 or 30% wheat middlings). Serum was collected on d 0, 14, and 27 of the withdrawal period to determine changes in the concentrations of osteocalcin, an indicator of osteoblast activity, and pyridinoline, an indicator of collagen degradation. The serum osteocalcin and pyridinoline concentrations on d 14 and d 27 were analyzed as change from the d 0 concentration. At slaughter, radiographs of the lumbar vertebrae and of the right and left femurs were taken to determine the incidence of bone fractures. Third metacarpal bones were analyzed for bone mineral density, peak load, ultimate shear stress, and percent ash. Removing dietary supplements increased the change of serum osteocalcin and pyridinoline concentrations, indicating an increase in osteoblast activity and bone resorption ($P < 0.05$). Supplement withdrawal decreased the bone mineral density, peak load, ultimate shear stress, and percent ash of the metacarpal bones ($P <$

0.01). Dietary wheat middling inclusion did not alter bone quality. Neither supplement withdrawal nor wheat middling inclusion affected the incidence of bone fractures at slaughter. The results of this study indicate that supplement withdrawal increases bone turnover and decreases bone quality.

Introduction

The dietary Ca and P concentrations necessary to maximize growth performance in growing-finishing pigs are well defined (NRC, 1998). However, maximum bone mineralization and bone strength require higher dietary Ca and P concentrations than is required to maximize growth performance (Crenshaw et al., 1981; Maxson and Mahan, 1983; Combs et al., 1991). It is not known if increased mineralization reduces the likelihood of bone fractures occurring during the slaughtering process.

In recent years, there has been increased interest in minimizing P in finishing pig diets to reduce nutrient excretion and feed costs. Previous research (O'Quinn et al., 1997; Mavromichalis et al., 1999; Shaw et al., 2001) has indicated that minimal dietary inorganic P additions are necessary during the late finishing phase to maintain growth performance and carcass quality. During this period, the animal may draw upon mineral body reserves found in the bone and other tissues to support metabolic requirements that are not met by the diet. Consequently, bone strength is decreased (O'Quinn et al., 1997). However, it is currently unknown if reducing dietary mineral additions prior to slaughter alters bone metabolism and decreases bone strength to the extent of increasing the incidence of bone fractures occurring at slaughter.

The objectives of this study were to determine the effect of supplement withdrawal (omission of vitamin and trace mineral premixes and 2/3 reduction of

inorganic phosphorus) for 28 d prior to slaughter on bone metabolism, bone strength, and incidences of bone fractures occurring at slaughter. Furthermore, this research evaluated the influence of dietary wheat middling inclusion on the same parameters.

Materials and Methods

The experimental design, dietary treatments, and management of the pigs utilized in this study have been reported previously (Shaw et al., 2001). In summary, 62 crossbred barrows were individually penned and fed corn-soybean meal-based grow-finish diets containing either 0 (CSBM) or 30% (CSBM+WM) wheat middlings. During the final 28 d before slaughter, 31 pigs were fed supplement withdrawal diets. The Ca to available P ratio was maintained at 2.5:1 in all late finishing diets.

On d 0, d 14, and d 27 of the withdrawal period, blood was collected from each pig by venapuncture from the anterior vena cava into 10 mL vacutainer tubes with 20 gauge, 1½ inch needles. Blood was centrifuged at 4° C, 3,000 x g, for 15 min (Beckman GS-6KR, Palo Alto, CA). Serum was collected into polypropylene tubes and stored at -80° C until osteocalcin and pyridinoline assays were performed.

Twenty-eight days after the initiation of the late finishing diets and at an average live weight of 103.4 kg, pigs were transported to the Michigan State University Meat Laboratory for slaughter. No visible indications of bone fractures were observed preslaughter. Pigs were electrically stunned (110 volts, 420 amps, 3 sec.), hoisted by chain from the right leg, and exsanguinated. Following scalding, pigs were mechanically dehaired (approximately 60 sec.) and the remaining hair and feet were manually removed. Pigs were eviscerated and the carcasses were split along the spinal column.

Radiographs. Following evisceration and prior to splitting the spine, a ventrodorsal projection radiograph of the lumbar vertebrae was taken (80 KVp, 17 mA, .18 sec., 30 cm focal film distance) of each carcass using a portable radiograph machine (Minxray, Northbrook, IL). Following the splitting of the spine, radiographs were taken (75 KVp, 17 mA, .12 sec., 30 cm focal film distance) of the right and left femurs, including the femoral heads. Radiographs were examined by a radiologist for incidence of bone fractures.

Computed Tomography. Bone mineral density of the third metacarpal of the right foot (MC III) was examined by x-ray-computed tomography (CT scan). Excised feet were placed on the CT table (CT 9800, GE Medical Systems, Milwaukee, WI) in palmar recumbency. A scout view was taken to determine mid-shaft location and a cross-sectional image was then acquired through the transverse plane. The pad on which each foot was placed contained three hydroxyapatite standards. The standards served as internal controls for each CT image to account for x-ray energy fluctuations that may occur between images. The bone mineral density of the MC III was determined by comparison of the x-ray linear attenuation coefficient of the bone to that of the hydroxyapatite standards. Total and cortical cross-sectional area were estimated by tracing the endosteal and periosteal margins of the MC III. Area within the outlined region and bone mineral density were estimated when recorded images were analyzed with a bone mineral density software package.

Bone characteristics. The MC III bones were cleaned of all muscle and connective tissue with a scalpel. Peak force and ultimate shear stress of the MC III was determined with an Instron Universal Testing Machine (Model 4202, Instron, Canton,

MA) fitted with a 20 kN load cell and that moved at a test speed of 5.0 mm/min according to the procedure described by Combs et al. (1991). The shape of the cross-sectional area of the bone tissue was assumed to be that of an elliptical quadrant and area was calculated using the following equation:

$$¼ * \pi * (B * D - b * d) + d * (B - b)/2 + b * (D - d)/2$$

Where B is the outside major diameter, b is the inside major diameter, D is the outside minor diameter, and d is the inside minor diameter. Ultimate shear stress was calculated according to the following equation (ASAE, 1999): $stress_s$ = Ultimate load/2 x Area.

The same bones that were mechanically tested were wrapped in cheesecloth and extracted with ethyl ether using a Soxhlet apparatus for 72 h. Bones were dried at 100°C for 12 h to determine the dry fat-free weight, placed in crucibles, then ashed in a muffle furnace for 16 h at 500°C. Percent ash was calculated as a percentage of the dry fat-free weight.

Mineral Analysis. The ashed MCIII bones were prepared for mineral analysis by nitric acid wet digestion. Samples were transferred to Phillips beakers and the crucibles were rinsed twice with 10 mL nitric acid (70%). Samples were digested in 30 mL of nitric acid with heat, and then rehydrated to 100 mL with .5% nitric acid. Calcium concentrations were determined by flame atomic absorption spectrophotometry (Smith-Heiftje 4000, Thermo Jarrell Ash Corporation, Franklin, MA), and P concentrations were determined using DU 7400 spectrophotometer (Beckman, Palo Alto, CA). Bone mineral concentrations were calculated as the percentage of the dry fat-free weight.

Osteocalcin Assay. Serum osteocalcin concentrations were determined in duplicate using commercially available ELISA tests (Novocalcin; Metra Biosystems,

Mountain View, CA) according to the manufacturer's instruction (Catalog number 8002, Metra Biosystems, Mountain View, CA). The antibody employed in the osteocalcin assay recognizes only the intact osteocalcin produced by osteoblasts and not the osteocalcin fragments from resorbed bone. Consequently, the analyzed serum osteocalcin concentrations are a reflection of osteoblast activity specifically, and not bone turnover.

Pyridinoline Assay. Serum pyridinoline concentrations were determined in duplicate using commercially available ELISA tests (Serum Pyd; Metra Biosystems, Mountain View, CA) according to the manufacturer's instruction (Catalog number 8019, Metra Biosystems, Mountain View, CA). This assay demonstrates high affinity for free pyridinoline and negligible binding to deoxypyridinoline and pyridinoline and deoxypyridinoline peptides, reducing variability in estimating collagen degradation.

Statistical Analysis. All data were analyzed by least squares ANOVA using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC) for a randomized complete block design. Pig served as the experimental unit. The model included the fixed effects of the factorial treatments, their interaction, replication, and block by initial weight. Litter within replication was specified as a random effect. All means presented are least square means. Differences were considered significant at the level of $P < 0.05$.

Results and Discussion

Analysis of Diets. The analyzed Ca concentrations of Diets 1 to 4 were .68, .67, .38, and .61%, and the total P concentrations were .45, .55, .34, and .52%, respectively. The resulting Ca to total P ratios were 1.51, 1.22, 1.12, and 1.17 for treatments 1 to 4, respectively. A suggested Ca to total P ratio for corn-soybean meal-based diets is between 1:1 and 1.25:1 (NRC, 1998).

Bone Metabolism. Because initial osteocalcin and pyridinoline concentrations were statistically different between dietary treatments, the d 14 and d 27 values were also analyzed as deviation from the d 0 concentration. Supplement withdrawal, but not wheat middling inclusion, increased serum osteocalcin concentrations on d 14 and d 27 of the withdrawal period (Table 13). Similar responses have been noted in other swine studies. Carter et al. (1996) observed that serum osteocalcin concentrations linearly decreased as dietary Ca decreased from 1.14 to .42% and dietary P decreased from .95 to .35% over a 30 d period. Eklou-Kalonji et al. (1999) found in a 30 d trial that reducing of dietary Ca from .90 to either .38 or .11% increased serum osteocalcin in growing and finishing pigs. Studies with rats have also concluded that serum osteocalcin concentrations are inversely correlated with dietary Ca and/or P content (Lian et al., 1987; Tanimoto et al., 1991, Hamalainen, 1994).

Serum osteocalcin, and therefore osteoblast activity, may be increased during dietary mineral deficiencies because of increased mechanical loading on the bone cells. Decreased mineralization of the bone surfaces increases the mechanical stress on individual cells of the bone matrix. Osteoblasts sense changes in matrix deformation and increase their activity.

Not all studies have concluded that decreased dietary mineral supplementation increases serum osteocalcin concentrations. Nicodemo et al. (1998) found that decreasing dietary Ca from .86 to .39% for 8 wk did not alter serum osteocalcin in growing pigs. Also, Hillman and coworkers (1993) did not observe differences in serum osteocalcin when neonatal pigs were fed diets containing excess, normal, or deficient Ca and P for 28 d.

Table 12. Effects of supplement withdrawal and wheat middling inclusion rate on fecal nutrient excretion during the finishing phase[ab]

	Full supplementation		Supplement withdrawal			Effect, P-value		
Item	CSBM	Midds	CSBM	Midds	SE[d]	Withdrawal	Midds	Interaction
Early finishing								
Ca, mg/g	22.405	18.837			.6140		.01	
P, mg/g	16.880	15.832			.4343		.09	
Fe, mg/g	1.556	1.026			.0356		.01	
Cu, mg/kg	99.96	79.82			2.353		.01	
Mn, mg/kg	213.35	278.60			7.018		.01	
Zn, mg/kg	780.74	632.11			17.702		.01	
Late finishing								
Ca, mg/g	26.789	21.714	13.984	17.818	.9064	.01	.45	.01
P, mg/g	19.387	17.697	15.498	17.781	.6834	.01	.65	.01
Fe, mg/g	1.829	1.155	.601	.473	.03791	.01	.01	.01
Cu, mg/kg	98.00	79.64	28.20	30.03	1.712	.01	.01	.01
Mn, mg/kg	211.36	286.74	86.78	228.66	5.218	.01	.01	.01
Zn, mg/kg	800.58	674.95	177.96	209.53	16.345	.01	.01	.01

[a]Based on dry matter basis.

[b]No. of animals by treatment for all variables: CSBM and full suppl. (n = 15), CSBM+WM and full suppl.(n = 16), CSBM and suppl. withdrawal (n = 15), CSBM+WM and suppl. withdrawal (n = 16).

[c]Indicates the probability of full supplementation vs supplement withdrawal (Withdrawal), 0 vs 30% wheat middling inclusion (Midds), and the interaction thereof (Interaction).

[d]Greatest SE among treatments.

In our study, supplement withdrawal, but not wheat middling inclusion, increased the change in serum pyridinoline concentration on d 14 and d 27 of the withdrawal period. Few published reports identify the relationship between serum pyridinoline and dietary mineral intake. Studies with rats (Egger et al., 1994) and humans (Garnero et al., 1994; Shapses et al., 1995; Shen et al., 1995) reported that urinary pyridinium excretion was inversely proportional to dietary calcium concentration.

Bone measurements. As shown in Table 14, supplement withdrawal decreased total bone mineral density, cortical bone mineral density, peak force, shear stress, percent bone ash, and the Ca and P concentrations of the MC III ($P < 0.04$). This indicates that bone resorption exceeded bone formation. Increased bone resorption caused by supplement withdrawal is reflected by the change of serum pyridinoline from d 0 to d 14 and from d 0 to d 27 rather than the analyzed d 14 and d 27 concentrations. Therefore, adjusting serum pyridinoline to reflect change over time appears to be an appropriate data modification.

Several nutrients that contribute to bone quality were decreased in diets where supplements were withdrawn. In swine studies, bone strength is decreased in pigs during moderate dietary restriction of Ca (O'Quinn et al., 1997; Nicodemo et al., 1998; Eklou-Kalonji et al., 1999) and P (Carter et al., 1996; Hall et al., 1991). Furthermore, dietary vitamin A (Zile et al., 1973), vitamin D (Sinha et al., 1988), vitamin K (Knapen et al., 1993), and Zn (Miller et al., 1968) are essential to maintain bone quality and were present below NRC (1998) suggested minimum concentrations in one or both of the supplement withdrawal diets.

Table 14. Effects of supplement withdrawal and wheat middling inclusion rate on metacarpal bone quality[ab]

	Full supplementation		Supplement withdrawal			P-values[c]		
Item	CSBM	Midds	CSBM	Midds	SE[d]	Withdrawal	Midds	Interaction
Physical characteristics								
Total BMD, mg/cm^3	367.8	378.3	323.9	341.2	11.49	.01	.23	.76
Cortical BMD, mg/cm^3	943.2	962.8	921.4	899.2	18.34	.01	.94	.22
Total CSA, cm^2	2.19	2.10	2.28	2.07	.055	.60	.01	.24
Cortical CSA, cm^2	1.09	1.07	1.06	1.01	.025	.07	.11	.57
Dry fat-free wt, g	16.23	14.82	14.89	14.57	.411	.04	.02	.15
Ash wt, g	8.82	8.17	7.98	7.70	.211	.01	.01	.32
Ash, %	54.78	55.35	53.76	53.23	.510	.01	.96	.26
Peak force, N	4290	4077	3539	3765	131.3	.01	.96	.10
Shear stress, MPa	17.34	17.14	15.20	16.80	.696	.08	.31	.20
Mineral concentrations								
Ca, mg/g	241.21	243.05	222.90	230.26	4.537	.01	.31	.54
P, mg/g	94.83	97.25	93.37	92.60	1.495	.04	.58	.29

[a]Based on dry fat-free weight.

[b]No. of animals by treatment for all variables: CSBM and full suppl. (n = 15), CSBM+WM and full suppl. (n = 16), CSBM and suppl. withdrawal (n = 15), CSBM+WM and suppl. withdrawal (n = 16).

[c]Indicates the probability of full supplementation vs supplement withdrawal (Withdrawal), 0 vs 30% wheat middling inclusion (Midds), and the interaction thereof (Interaction).

[d]Greatest SE among treatments.

Bone fractures. While supplement withdrawal decreased metacarpal bone quality, no femoral or vertebral fractures were observed in this study. It was observed that one pig, which received a fully supplemented CSBM diet, had a broken right tibia following hoisting by the chain.

Pond and others (1969) reported that the vertebrae and the proximal and distal ends of the femur are depleted of mineral before the mid-diaphyseal region of the long bones in young pigs. Crenshaw and associates (1981) reported that decreasing bone quality in the metacarpals parallels decreasing bone quality of the vertebrae and femoral bones in growing and finishing pigs. Although no bone fractures were observed in the radiographic films, the decreased bone mineralization of the MC III indicates that the pigs were at increased risk for vertebral and femoral fractures.

Dritz et al. (2000) described a case study in which a processor reported that the incidence of minor loin damage of pigs from a single farm was greater than twice that of pigs received from other producers. Most of the loin damage was reportedly caused by vertebral fractures occurring during the stunning process. Dietary available P of pigs weighing 95 to 109 kg and 109 kg to market was increased from approximately 4.4 and 3.2 g/d, respectively, to 5.7 and 4.8 g/d. After a two-month transition period, the incidences of minor loin damage dropped to that of pigs from other producers.

In the present study, 31 pigs were fed supplement withdrawal diets. Because wheat middlings are naturally high in P, only the 15 pigs fed CSBM supplement withdrawal diets received dietary Ca and P concentrations below the suggested NRC (1998) minimum requirements. Assuming that the industry average for loin damage caused by vertebral fractures approaches the .58% reported by Dritz and coworkers, it is

not surprising that we did not observe vertebral and femoral fractures in the present study. Furthermore, differences in livestock handling and slaughter procedures between commercial and university conditions decreased the likelihood bone fracture occurrence in this study.

Implications

Supplements should not be withdrawn from potential breeding animals so that sow longevity is maintained. Because of the relationship between dietary vitamins and minerals concentrations and bone metabolism, determination to practice supplement withdrawal should include consideration of the bone quality and its effect on carcass value. It still remains to be determined if supplement withdrawal increases the incidence of bone fractures occurring at slaughter.

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APPENDIX

APPENDIX A

Supplement Withdrawal Does Not Cause Blood Splash in Pork

Background

Blood hemorrhaging is a serious commercial problem and may occur in as much as 10% of pork carcasses (Swatland, 1984). Capillaries may rupture during slaughter causing small dark red areas to spread over the meat surface. During and after stunning, blood pressure escalates as the heart continues to pump; the muscles contract, constricting the capillaries, often causing the capillaries to rupture (Romans et al., 1994). Blood hemorrhage also occurs when violent muscular contractions tear the muscle tissue, resulting in hemorrhages that appear along muscle seams (Warrington, 1974).

The occurrence of blood hemorrhages can be reduced with proper stunning. Stunning pigs with either higher voltage or higher frequency electricity for a shorter period of time decreases the incidence of muscle hemorrhages (Warrington, 1974). Decreasing the time from stunning to sticking relieves blood pressure and also decreases the chance of capillary rupture (Romans et al., 1994).

Bone fractures are a third cause of muscle hemorrhages. Dritz et al. (2000) describe a case study of minor vertebral fractures causing minor loin damage. The authors proposed that inadequate dietary available P sufficiently decreased skeletal integrity to increase the incidences of loin damage. Dietary available P of pigs weighing 95-109 kg and 109 kg to market was increased from approximately 4.4 and 3.2 g/d, respectively, to 5.7 and 4.8 g/d. After a two-month transition period, the incidences of minor loin damage dropped to that of pigs from other producers.

Description

The experimental design, dietary treatments, and management of the pigs utilized in this study are presented in chapters 3 and 4. After fabricating the carcasses of the first replication of pigs, the Meat Lab staff notified the investigators that the frequency of intramuscular blood splash was 40 to 50%, much higher than that of previous pigs killed at the facility. This blood splash differs from the extramuscular loin damage reported by Dritz et al. (2000), which was likely caused by vertebral fractures. In the present study, no vertebral fractures were observable in the radiographic film. Therefore, we investigated if supplement withdrawal increases intramuscular blood splash by observing the carcasses of the pigs from the second replication.

Digital pictures were taken of the fabricated loin, shoulder, belly, and ham of each carcass. The pictures from each carcass were categorically scored based on the amount and the severity of blood splash as follows:

Score	Description
1 No BS	No signs
2 Slight BS	Splash at seams or tissues – 1 muscle in 1 to 2 cuts
3 Moderate BS	Splash at seams and tissues – multiple muscles in 1 to 2 cuts
4 Severe BS	Splash at seams and tissues – multiple muscles in 3+ cuts
5 Excessive BS	Splash throughout all tissues

There were insufficient carcasses (n = 30) for chi-square statistical analysis; therefore, mean averages are provided in Table 15.

Table 15. Effect of dietary treatment on carcass blood splash[ab]

	Full supplementation		Supplement withdrawal	
	CSBM	Midds	CSBM	Midds
Score				
1.0	1	1	3	2
1.5	2	0	2	2
2.0	3	6	0	2
2.5	1	0	1	1
3.0	0	1	1	1
Mean	1.78	2.29	1.44	1.81

[a]No. of animals by treatment: CSBM and full suppl. (n = 7), CSBM+WM and full suppl. (n = 8), CSBM and suppl. withdrawal (n = 7), CSBM+WM and suppl. withdrawal (n = 8).

[b]Indicates the number of animals assigned each categorical score.

Sixty percent of the carcasses contain at least slight blood splash, and 13% contained moderate blood splash. No severe or excessive blood splash was found. Neither supplement withdrawal nor wheat middling inclusion appeared to affect the incidences of blood splash. The high incidences of blood splash may be due to error of the electrical stunning mechanism or genetic disposition. These pigs were also the first offspring of the Musculor x York sire line slaughtered at the MSU Meat Lab. Overall, carcass quality was poor as the muscle was pale and watery. Similar carcass observations were noted in Musculor-sired pigs that were later slaughtered in the MSU Meat Lab. In addition to producing carcasses of poor quality, this genetic line may be susceptible to blood splash.

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