EFFECTS OF PORCINE CIRCOVIRUS TYPE 2 VACCINATION, BIOFUEL CO-PRODUCTS, AND DIETARY ENZYMES ON FINISHING PIG PERFORMANCE UNDER FIELD CONDITIONS

by

JAY YANORIA JACELA

D.V.M., University of the Philippines Los Baños, 1999

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine/Pathobiology
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Manhattan, Kansas

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Abstract

A total of 9,979 pigs were used in 11 experiments to quantify production responses under field conditions in growing pigs to PCV2 vaccination, biofuel co-products and dietary supplemental enzymes. Experiments 1 and 2 were conducted to determine the efficacy of a commercial 2-dose Porcine Circovirus Type 2 (PCV2) vaccine. Growth performance and mortality ($P < 0.05$) of vaccinated pigs improved compared to non-vaccinated pigs in both experiments with the vaccine causing a greater increase in ADG in vaccinated barrows than vaccinated gilts in Exp. 2. Experiment 3 compared the efficacy of 1-dose and 2-dose commercial PCV2 vaccines, where vaccinated pigs had greater ADG ($P < 0.05$) than vaccinated pigs regardless of vaccine type. The 2-dose group was heavier ($P < 0.05$) than the control group while the 1-dose group was intermediate. Therefore, PCV2 vaccines were efficacious under field conditions. Experiments 4, 5, and 6 were conducted to evaluate de-oiled corn dried distillers grains with solubles (dDGS) in grow-finish pigs. In Exp. 4, analyzed CP and AA content were higher, but lysine digestibility and energy content were lower in dDGS than traditional dried distillers grains with solubles (DDGS). In Exp. 5, 0 to 30% dDGS in nursery diets did not affect growth performance ($P > 0.52$). In Exp. 6, 0 to 30% dDGS reduced (linear; $P < 0.01$) ADG and ADFI, tended to improve (linear; $P > 0.07$) G:F, decreased (linear; $P < 0.01$) carcass yield, and increased (linear; $P < 0.01$) fat iodine values. Experiment 7 was conducted to determine the AA digestibility and energy concentration of novel high-CP distillers co-products from corn (HPC-DDG) and sorghum (HPS-DDGS). Digestibility of AA was higher for HPC-DDG but lower in HPS-DDGS than traditional DDGS. Both co-products had lower energy than traditional DDGS. Finally, Exp. 8, 9, 10, and 11 were used in a meta-analysis to evaluate supplementary dietary

enzymes in pigs. Supplemental enzymes, alone or in combination, did not improve grow-finish pig performance ($P > 0.58$) regardless of dietary DDGS level. In conclusion, these experiments provide important empirical data to quantify production responses of various interventions and dietary ingredients under actual field conditions.

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Approved by:	Approved by:
Co-Major Professor	Co-Major Professor
Steve S. Dritz	Joel M. DeRouchey

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Dedication

To my family: Nanay, Ate Ningning, Kuya Arden, Kuya Bobby, Kuya Dennis, Kuya Elmer, Ate Verna, Kuya Henry, Kuya Des, Zaza, Bien, and Orox

To my wife Ama

To Tatay and Kuya Russell

CHAPTER 1 - Feed Additives for Swine: Fact sheets

Introduction

Feed additives are non-nutritive products used in swine diets to improve production efficiency and performance. If chosen carefully and used properly, feed additives can be effective and can help increase the profitability of pig production. Not all feed additives are the same or provide a beneficial response, and, therefore, choosing a product will depend on the farm's specific situation and needs.

This series of fact sheets lists some of the major classifications of products used as feed additives. Every effort has been made to ensure that all the information in every fact sheet is current and based on the latest scientific publications available at the time of writing. The objective of these fact sheets is to discuss some of the basic concepts to help producers improve their understanding of these products. They also aim to promote more responsible and judicious use of feed additives.

Feed-additive products used in swine diets include natural and synthetic substances and have been grouped in this series of fact sheets according to the following classifications:

1. acidifiers
2. anthelmintics (dewormers)
3. in-feed antibiotics
4. carbohydrate-degrading enzymes and proteases
5. carcass modifiers
6. high dietary levels of copper and zinc for growing pigs

7. flavors
8. mold inhibitors, mycotoxin binders, and antioxidants
9. phytase
10. phytogenic feed additives (phytobiotics/botanicals)
11. Probiotics and prebiotics

Each group of feed additives is discussed in a separate fact sheet, with special emphasis on some of the common questions that producers might have for each product. Feed additives offer a variety of potential benefits. However, they add to total production cost and should be evaluated carefully. Because their use in pig diets is to improve performance and profitability, an effective feed-additive product must be able to pay for itself. It must be able to provide an improvement in productivity that is, at minimum, equivalent to the added cost of the feed-additive product. This highlights the value of scientific data from well-designed experiments as the basis for evaluating such products. Having access to such information is critical in determining if one product's claims are actually possible and repeatable in commercial settings. Producers must always try to verify that the data for a particular product came from controlled, unbiased experiments with supporting statistical data. When choosing between feed-additive products, priority for using a specific product should be given to those that have been shown to provide consistent results in research trials.

Acidifiers

Beneficial claims from dietary inclusions of acidifiers include control of bacterial growth in feed, increased growth performance, improvement in nutrient digestibility, and control of harmful bacteria in the gut.

Fast facts

Acidifiers used in pig diets are in organic or inorganic forms.

Acidifiers appear to be most effective in newly weaned pigs and in less complex nursery diets.

Growth-promoting effects of acidifiers in pig diets need to be further investigated to justify them as suitable replacements for antimicrobials.

What are acidifiers?

Acidifiers are compounds that have acidic properties: they may be organic or inorganic acids. Organic acids that have shown positive effects on growth performance in weaned pigs include citric, formic, fumaric, and propionic acids. In studiesinvolving inorganic acids,[1-3] positive growth responses have been reported with the use of phosphoric acid. However, research evaluating other inorganic acids, such as sulfuric acid, reported negative growth performance.[4] Thus, phosphoric acid is the most commonly utilized inorganic acid in swine diets.

Compared to organic acids, inorganic acids are typically lower in cost. Organic and inorganic acid combinations are often used in commercially available acidifiers. Mixed acid products generally have shown a better response than single acid addition.[5] This is apparently due to dissociation properties of these acids at various locations in the pig's digestive tract.

What are the benefits of using acidifiers?

A recent report[2] summarizing several studies on acidifiers indicated that, in general, they appear to improve pig growth performance. However, the magnitude and consistency of the response may vary, depending on inclusion rate and other dietary factors. The exact mode of action of acidifiers has not been fully elucidated. However, acidifiers are commonly marketed as growth-promoting products and as alternatives for in-feed antibiotics. Unfortunately, due to the lack of consistent results, acidifier use as a replacement for antibiotic growth promoters is still not justified. Acidifiers are believed to enhance growth by improving gut health through reduction of pH and buffering capacity of diets, improvement of pancreatic secretions that increase nutrient digestibility, or promotion of beneficial bacterial growth while inhibiting growth of pathogenic microbes.[2,6] Also, there is limited data indicating that acidifiers can act synergistically with phytase to improve phosphorus and magnesium digestibility.[7,8]

What factors affect the response to acidifiers?

Research suggests that age of pigs can affect the response to acidifiers, with newly weaned pigs showing the greatest response.[4,9] Acidifiers are most beneficial in diets for young pigs, particularly during the first few days after weaning. The stomach of a weaned pig is not yet physiologically mature and may not be able to secrete a sufficient amount of acid to aid in digestion of solid food or inhibit the proliferation of detrimental bacteria. However, the exact mechanism of the response to acidifiers is not clear.

Diet composition also may affect the response to acidifiers. It appears that greater responses to acidifiers are seen when simple diets are fed rather than complex diets containing milk products.[10] This is presumably due to the conversion of lactose from the milk products to lactic

acid by *Lactobacillus* species in the stomach, thus creating an acidic environment and reducing the need for dietary supplementation with acidifiers.

Disadvantages of acidifiers?

Corrosiveness is one disadvantage of using some acidifiers. This may pose some handling and equipment maintenance issues to the feed manufacturer. Salts of organic acids are generally odorless and not as corrosive as are their acid forms, which can be an advantage. Thus, the salt forms are easier to handle in the feed manufacturing process. In addition, acidifiers may negatively affect diet palatability when added at excessive levels, which can result in lowered feed intake. There may also be some legal restrictions with the use of some acids. For example, pure formic acid is not legal for use in the United States, but salts of formic acid are available for use in feeds.

Summary

Acidifiers added to pig diets may potentially help improve growth performance by improving digestive processes through several mechanisms. However, a clear mode of action has yet to be described. The use of acidifiers appears to be most beneficial in the early period after weaning. Thus, acidifier use is typically limited to diets for pigs weighing less than 6.75 kg (15 lb).

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Anthelmintics

Aside from viruses and bacteria, parasite control must be part of a comprehensive herd-health program in every swine-production system. Gastrointestinal worm infections may result in significant economic losses. Signs of infection are general and not readily apparent, since worm infections rarely cause elevated mortality levels. Some worms that are commonly found in pigs are roundworms (*Ascari suum*), nodular worms (O*esphagostomum spp.*), intestinal threadworms (*Strongyloides ransomi*), whipworms (*Trichuris suis*), kidney worms (*Stephanurus dentatus*), and lungworms (*Metastrongylus spp.*). Anthelmintics or "dewormers" are chemical substances that can be added to pig diets to control gastrointestinal worms.

Fast facts

Worm infections can negatively affect growth performance and decrease carcass value.

In-feed anthelmintics can be used for a successful deworming program.

Anthelmintics vary in efficacy and spectrum of activity.

An effective control program will depend on the specific worm problem and the stage of production and type of production system.

Anthelmintics are classified as drugs and their use is regulated by the FDA.

What are the consequences of worm infection?

Worms are parasites that deprive the pig of nutrients, negatively affecting pig growth and feed efficiency. Heavy infestation in some cases can lead to condemnation and loss of carcass value. An example is liver condemnation due to larval migration of *Ascaris suum* (pig roundworms). At some point in their development, the larval forms of this worm pass through the liver and create white scars known as "milk spots."

What products are available for use as anthelmintics in swine feed?

Dichlorvos. Dichlorvos is indicated for use to remove and control mature and immature forms of most common pig worms. However, it is relatively ineffective in controlling early larval forms of roundworms. Two consecutive days of feeding is recommended when using dichlorvos in pig diets. There is no withdrawal time required when using this product at the approved dose.

Fenbendazole. Fenbendazole is an anthelmintic with a relatively broad spectrum of activity. It is effective against mature and immature forms of common worms that infect pigs. However, fenbendazole has a higher activity when given at low doses for several days (9 mg/kg BW divided over 3 to 12 days) than when single-dosed. No withdrawal time is required for this product when used at the recommended dose.

Ivermectin. Ivermectin is highly effective against immature and adult forms of most gastrointestinal roundworms as well as pig external parasites such as lice and mange mites. Aside from the premix form, ivermectin is also available in an injectable preparation. The premix product is labeled to be fed for 7 consecutive days. A withdrawal time of 5 days is needed when using this product in feed.

Levamisole. Levamisole is effective against mature roundworms but only moderately effective against nodular worms. This anthelmintic is not well-liked by pigs and has a negative effect on diet palatability. Thus, it is more commonly available as a product administered through drinking water which is the best route to insure intake. When using levamisole in pig diets, withdrawing regular feed overnight is recommended prior to administering the medicated diet the following morning. Treated pigs should be fed the regular diet once the medicated diet is completely consumed. This anthelmintic requires a 3-day withdrawal time.

Piperazine. Piperazine is a dewormer with a relatively narrow spectrum of activity. It has good efficacy against roundworms, moderate efficacy against nodular worms, and is ineffective against other types of pig worms. This drug is more commonly available commercially as a water soluble product, but it is also FDA-approved for use as a feed additive. The main advantage of piperazine is that it is relatively inexpensive and is administered as a 1-day single treatment. However, it requires a withdrawal period of 21 days.

Pyrantel tartrate. Pyrantel tartrate is fed for 3 consecutive days to remove large roundworms or continuously to prevent migration and establishment of roundworms and nodular worms. This drug is photodegradable and, hence, must be used immediately upon opening the package. It also should not be mixed in diets containing bentonite. This product requires a 24-hour withdrawal time.

Additional detailed information on wormers approved for swine can be found in the Feed Additive Compendium[1] or on the Food and Drug Administration website[2].

When is it necessary to treat pigs with anthelmintics to control worms?

Worm infections occur more frequently in pigs raised in outdoor lots than in conventional confinement facilities. Therefore, production design is one consideration in terms of determining how frequently pigs should be fed anthelmintics. Breeding stock should be given anthelmintics after arrival at the farm and before introduction to the herd. Also, sows are a common source of worm eggs for piglets and should be dewormed several days before farrowing and before moving to the farrowing room. Scrubbing the sow to remove the worm eggs attached to her body before transfer to the farrowing barn also can reduce exposure of baby pigs to worm eggs.

Knowledge of the specific parasites present in the herd and their life cycle is helpful in establishing an effective control program. Prepatent period (Table 1-1)[3] refers to the period

between the time when the infection occurs and when the adult worms begin shedding eggs. Some worms produce eggs several days after infection, while others take months to begin producing eggs. Most anthelmintics are not able to destroy the egg and larval forms that develop into adults after several days. The interval for repeating deworming can be determined on the basis of the prepatent periods. Deworming must be repeated before the minimum prepatent period to kill the adult forms and prevent them from laying eggs.

Choosing the appropriate anthelmintic

Anthelmintics have different modes of action and vary in their effectiveness against different species of pig worms. Therefore, choosing the proper anthelmintic to be used in the feed will depend on the specific worm problem. The relative effectiveness and spectrum of activity of common anthelmintics are listed in Table 1-2[4]. Brand names of products available in the US are also enumerated in Table 1-3.It should be noted that anthelmintics, like antibiotics, may require specific withdrawal periods (Table 1-4)[1].

Summary

Worm control is an important component of every herd-health program. Many anthelmintics are effective against different types of worms. Therefore, selection of an appropriate anthelmintic will depend on the type of worm that needs to be controlled. Also, use of anthelmintics must not be relied on as the sole approach in controlling worms, but must be combined with good sanitation and production practices to be successful.

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Table 1-1: Prepatent period of common pig worms*

Type of Worm	Prepatent period (days)
Kidney worm (*Stephanurus dentatus*)	180 -270
Lungworm (*Metastrongylus spp*.)	30
Nodular worm (*Oesophagostomum spp*.)	23 -60
Red stomach worm (*Hyostrongylus rubidus*)	20
Roundworm (*Ascaris suum*)	42-56
Threadworm (*Strongyloides ransomi*)	3 - 8
Whipworm (*Trichuris suis*)	40

* Adapted from Myers, 1988.[3]

Table 1-2: Effectiveness (% of adult worms killed) and relative costs of in-feed anthelmintics against common pig worms*

Anthelmintic	Roundworm	Nodular worm	Whipworm	Lungworm	Threadworm	Kidney worm	Relative cost
Dichlorvos	99-100	95-100	90-100	0	60-80	0	++
Fenbendazole	92-100	99-100	94-100	97-99	Variable	100	++++
Ivermectin†	90-100	86-100	Variable	99-100	99-100	100	+++++
Levamisole	99-100	80-100	60-80	90-100	80-95	80-100	+++
Piperazine	75-100	50	0	0	0	0	+
Pyrantel tartrate	96-100	88-100	0	0	0	0	+

*Adapted from Myer and Brendemuhl, 2009[4].

†Also highly effective against external parasites (mange and lice).

Table 1-3 Registered brand names of FDA-approved anthelmintic products*

Anthelmintic	Brand name	Manufacturer	Address
Dichlorvos	Atgard® C Swine Wormer	Boehringer Ingelheim Vetmedica, Inc.	St. Joseph, MO
Fenbendazole	Safe-Guard®	Intervet, Inc.	Millsboro, DE
Ivermectin	Ivomec®	Merial	Duluth, GA
Levamisole	Tramisol®	Fort Dodge Animal Health	Fort Dodge, IA
Piperazine	Wazine® Pig Wormer	Fleming Laboratories, Inc.	Charlotte, NC
Pyrantel tartrate	Banminth® 48	Phibro Animal Health	Ridgefield Park, NJ
	Worm-Ban®	North American Nutrition Co., Inc.	Lewisburg, OH
	Purina® Ban Worm	Virbac AH, Inc.	Ft. Worth, TX

*Food and Drug Administration Center for Veterinary Medicine.[2]

Table 1-4: Withdrawal periods of FDA-approved anthelmintics*

Anthelmintic	Withdrawal period (days)
Dichlorvos	0
Fenbendazole	0
Ivermectin	5
Levamisole	3
Piperazine	21
Pyrantel tartrate	1 (24 hours)

* 2008 Feed Additive Compendium.[1]

In-feed Antibiotics

Antimicrobial agents, such as antibiotics, have been used in pig production for over 50 years. Early studies indicated significant improvements in pig growth performance when antibiotics were fed. With the improvements in production practices and health status of pig herds, positive responses to in-feed antibiotics may not be as large in today's modern facilities. Additionally, the magnitude of response differs with the stage of pig growth. Use of antibiotics as feed additives is subject to regulatory policies to prevent residues and enhance public health. It is therefore important to be aware of the current information available concerning the effects of commonly used in-feed antibiotics in pig production.

Fast facts

Use of in-feed antibiotics in pigs is regulated by the FDA and they must be used only as approved.

No extra-label usage is allowed for in-feed antibiotics.

The best responses in growth performance are seen in nursery pigs.

Magnitude of responses may differ depending on herd-health status and sanitation.

Concerns are increasing about the negative consequences of antibiotic use in food animals.

How do antibiotics enhance growth?

Antibiotics are non-nutritive feed additives, which means that they do not provide further nourishment to the pig, and their absence in a well-balanced diet will not result in nutritional deficiency. Antibiotics are included in swine feed for their therapeutic potential as well as their ability to promote growth. Some of the proposed possible mechanisms by which antibiotics improve growth include: inhibition of subclinical pathogenic bacterial infections; reduction of microbial metabolism products that may negatively affect pig growth; inhibition of microbial growth thereby increasing nutrients available to the pig; and an increase in uptake and utilization of nutrients through the intestinal wall.[1]

Efficacy of in-feed antibiotics

Studies[2] on the effects of antibiotic feed additives have indicated significant improvements in growth rate and feed efficiency (Table 1-5). These studies, however, were conducted more than two decades ago, when disease pressures in pig farms were relatively greater than in today's facilities. With numerous improvements such as multi-site pig production, nutrition, biosecurity, and overall pig husbandry practices in the last two decades, responses may not be as great as formerly. A more recent study[3] on the use of in-feed antibiotics in modern production systems showed that such additives are still effective in improving growth in nursery pigs, although the magnitude of the response is less (Table 1-6). However, in finishing pigs, no improvement is noted. Many factors can affect the efficacy of antibiotic feed additives, including nutrition,

management practices, and health status. When these factors are optimal, less or almost no response to antibiotics can be expected, especially with excellent sanitation practices and lack of bacterial disease pressure. The data on feeding antimicrobials in sow diets, however, is much more limited than that in growing pigs. Antibiotic in sow diets may also improve reproductive performance of sows in herds with high incidence of reproductive problems due to higher disease challenge.[4,5] Thus, herds experiencing problems with conception rates and litter size associated with bacterial infections may benefit from the addition of antibiotics to sow diets. Chlortetracycline and oxytetracycline, the two in-feed antibiotics approved for use in sow diets, are indicated to reduce the incidence of abortion due to *Leptospira interrogans* serovars and reduce shedding of these organisms. However, routine feeding of antibiotics to the breeding herd is discouraged.

Choosing the proper antibiotic

There are a number of important things to consider when choosing the antibiotic appropriate for a specific herd, for example, the possible disease organisms present in the herd. Knowing what type of disease challenge the pigs will be exposed to will be helpful in selecting the most suitable antibiotic. Certain antibiotics may be more efficacious in treating respiratory problems, while others may be more effective against enteric pathogens. Stage of production and withdrawal period also will determine the specific antibiotic of choice. While in-feed antibiotic use is most prevalent in nursery diets, it is sometimes necessary to use antibiotics in grow-finish diets, eg. during outbreaks of bacterial disease. Observing the proper withdrawal time for an in-feed antibiotic is important to avoid residues in the meat. Improper consideration of withdrawal time may result in delays in marketing pigs. The product also must be one that is approved for use in swine, as no extra-label usage is allowed for in-feed antimicrobials. Ultimately, choosing the

proper in-feed antibiotic depends on the benefit in production efficiency compared to cost and risk of residue.

Proper use of in-feed antibiotics

While most in-feed antibiotics are available without veterinary supervision, they should not be used indiscriminately. They should be used only for purposes specified on the labels. A good reference for the list of drugs that can be used as feed additives is the *Feed Additive Compendium,*[6] which is updated regularly to provide up-to-date information and provides guidelines on the proper use of antibiotics in feed. Each country has its own regulatory policies regarding use of feed additives in pigs. Thus, the recommendations in this fact sheet may not apply outside of the United States. It is, therefore, important for US producers to be aware of which antibiotics are forbidden in countries that import pork from the United States.

Which antibiotics are approved for use as feed additives in pig diets in the United States?

Antibiotics and combinations approved for use in swine diets, including withdrawal times, are listed in Table 1-7. Florfenicol and tilmicosin, which are classified as Veterinary Feed Directive (VFD) drugs, are also included in the list. Veterinary Feed Directive drugs can be used only under the order and professional supervision of an appropriately licensed veterinarian.[7] Before a VFD drug can be used, the producer must first contact the veterinarian to diagnose and treat the existing health problem. A VFD order can be written only by a veterinarian for drugs that are approved for that swine category and under a valid client-patient relationship.[7] This is accomplished by filling out a form in a format approved by the Food and Drug Administration Center for Veterinary Medicine. All pertinent information must be provided by the veterinarian

when filling the form. The veterinarian, producer, and feed miller must all follow the responsibilities outlined by the Food and Drug Administration Center for Veterinary Medicine when using VFD drugs. Issued VFDs for florfenicol and tilmicosin have an expiration period of 90 days.

Summary

Increased productivity, efficiency, and profitability are the goals of every swine production business. Antibiotics have been used in swine diets for several decades to improve growth performance, as well as to control and treat diseases. Because of the improvements made in housing, nutrition, production, and health-management practices over the years, the impact of antibiotics on growth performance may not be as large or as consistent in response as those observed during the early years of antibiotic use. In-feed antibiotics remain an effective tool in improving production efficiency, but are not a substitute for good production management. Also, these products must be used properly and responsibly.

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Table 1-5: Effectiveness of in-feed antibiotics on production responses in pigs

Parameter	Control	Antibiotic	Difference (%)
Starter phase (15 to 55 lb)			
ADG (lb)	0.86	0.99	16.4
F:G	2.28	2.13	6.9
Grower phase (37 to 108 lb)			
ADG (lb)	1.30	1.45	10.6
F:G	2.91	2.78	4.5
Grow-finish phase (53 to 196 lb)			
ADG (lb)	1.52	1.59	4.2
F:G	3.30	3.23	2.2

* Adapted from Cromwell (2002)[2] as adapted from Hays VW (*Effectiveness of Feed Additive Usage of Antibacterial Agents in Swine and Poultry Production*. Washington, DC: Office of Technology Assessment, US Congress; 1977) and Zimmerman DR [Role of subtherapeutic antimicrobials in animal production. *J Anim Sci*. 1986;62(Suppl3):6]. Data from 453, 298, and 443 experiments, involving 13,632, 5783, and 13, 140 pigs for the three phases, respectively. ADG = average daily gain; F:G = feed to gain ratio.

Table 1-6: Effectiveness of in-feed antibiotics in nursery

Item	Control	Antibiotic†
Nursery phase		
ADG (lb)	0.96†	1.01†
F:G	1.44	1.42
Grow-finish phase		
ADG (lb)	1.72	1.72
F:G	2.90	2.90

* Adapted from Dritz et al, 2002.[3] Data from five and four experiments, involving 3648 and 2660 pigs, for the nursery and grow-finish phases, respectively.
† ADG was greater (5.0% difference) in nursery pigs treated with antibiotics than in controls (ANOVA; $P < .05$).

Table 1-7: Withdrawal periods for FDA-approved in-feed antibiotics and combinations*

Antibiotic	Indication	Inclusion rate (g/ton)	Withdrawal period (days)
Bacitracin methylene disalicylate (BMD)	Increased ADG and feed efficiency	10-30	0
	Grow-finish: control of swine dysentery	250	0
	Sows: control of clostridial enteritis in suckling piglets	250	0
BMD + Chlortetracycline	Increased ADG and feed efficiency	BMD: 10-30	0
	Treatment of bacterial enteritis and bacterial pneumonia	CTC: 400‡	
Bacitracin zinc	Increased ADG and feed efficiency	10-50	0
Bambermycin	Increased ADG and feed efficiency	2-4	0
Carbadox	Increased ADG and feed efficiency	10-25	42
	Control of swine dysentery and salmonellosis	50	42
Chlortetracycline	Increased ADG and feed efficiency	10-50	VW†
	Reduction of jowl abscesses	50-100	VW†
	Control of leptospirosis in sows	400‡	VW†
	Control of proliferative enteropathies (ileitis)	10 mg/lb BW/day‡	VW†

continued

Table 1-7: Withdrawal periods for FDA-approved in-feed antibiotics and combinations*

Antibiotic	Indication	Inclusion rate (g/ton)	Withdrawal period (days)
CTC + sulfathiazole + penicillin	Reduction of abscesses; treatment of bacterial enteritis; maintenance of weight gain in the presence of rhinitis	100 CTC;100 Sulfa; 50 Penicillin	7
Florfenicol‡	Control of bacterial respiratory disease	182	13
Neomycin	Treatment and control of bacterial enteritis	10 mg/lb BW/day for 24-48 hrs beyond remission of symptoms, not to exceed 14 consecutive days	3
Neomycin/oxytetracyline	Increased ADG and feed efficiency	10-50	5
	Treatment of bacterial enteritis and bacterial pneumonia	10 mg/lb BW/day, 7-14 days	5
	Control and treatment of leptospirosis in breeders	10 mg/lb BW/day, 7-14 days	5
Lincomycin	Increased ADG and feed efficiency	20	0
	Control of swine dysentery and ileitis	40-100	0
	Reduce severity of mycoplasmal pneumonia	200	0

continued

Table 1-7: Withdrawal periods for FDA-approved in-feed antibiotics and combinations*

Antibiotic	Indication	Inclusion rate (g/ton)	Withdrawal period (days)
Oxytetracycline	Increased ADG and feed efficiency	10-50	0
	Treatment of bacterial enteritis and bacterial pneumonia	BW dosage: 10 mg/lb daily, 7-14 days	0
	Control of leptospirosis in sows	BW dosage: 10 mg/lb daily, 7-14 days	0
Oxytetracycline + carbadox	Treatment of bacterial enteritis and bacterial pneumonia	10-25 carbadox; oxytetracycline BW dosage: 10 mg/lb daily	42
Oxytetracycline + neomycin	Prevention or treatment of bacterial enteritis and dysentery; maintenance of weight gain in the presence of atrophic rhinitis	50-150 oxytetracycline; neomycin BW dosage: 35-140 mg/lb	10
Tiamulin	Control of dysentery and ileitis	35	2
	Treatment of dysentery	200	7
Tiamulin + chlortetracycline	Control of dysentery; treatment of bacterial enteritis and bacterial pneumonia	35 tiamulin + 400 chlortetracycline (10 mg/lb BW/day)	2
Tilmicosin‡	Control of bacterial respiratory disease	181-363	7

continued

Table 1-7: Withdrawal periods for FDA-approved in-feed antibiotics and combinations*

Antibiotic	Indication	Inclusion rate (g/ton)	Withdrawal period (days)
Tylosin	Increased ADG and feed efficiency in finishers	10-20	0
	Increased ADG and feed efficiency in growers	20-40	0
	Increased ADG and feed efficiency in nursery pigs	20-100	0
	Control of swine dysentery	40-100	0
	Control of dysentery and ileitis	100	0
Virginiamycin	Increased ADG and feed efficiency	5-10	0
	Control of swine dysentery	25	0
	Treatment of swine dysentery	100	0

* Source: 2008 Feed Additive Compendium[6] and Food and Drug Administration Center for Veterinary Medicine[7].
†Limitations: Feed continuously for not more than 14 days. Feed approximately 400 grams per ton of feed, varying with body weight and feed consumption to provide 10 milligrams per pound of body weight per day.
‡ Voluntary withdrawal to meet residue limits of certain export markets.
¶ Veterinary Feed Directive drug.

Carbohydrate-degrading enzymes and proteases

Swine diets are composed mostly of plant-based ingredients. Nutrients contained in these feedstuffs need to be broken down by the pig into simpler forms that will be used to support maintenance, growth, and reproduction. This poses a problem to the pig because, unlike ruminants, they do not have the ability to efficiently digest plant components that have relatively high fiber content. Pigs lack specific enzymes needed to break down fiber. For this reason, supplementing diets with exogenous carbohydrate-degrading enzymes that break down fiber have become increasingly popular to potentially improve availability of nutrients from these ingredients.

Fast facts

Carbohydrases and proteases can increase the nutrient digestibility in plant-derived feedstuffs. Enhanced nutrient digestibility does not necessarily translate to improvement in performance. More research is needed to support the claimed effects of enzyme supplementation on growth performance.

What are enzymes?

Enzymes are proteins that accelerate chemical reactions that would otherwise proceed at a very slow rate under normal conditions. They are used in swine nutrition as a feed additive to improve digestion and utilization of nutrients from feedstuffs. Based on this premise, enzyme supplementation may potentially lead to better growth performance and less nutrients being excreted as waste. Most enzymes, especially those used as feed additives, are characterized by names with the suffix “ase” (eg, xylanase). Carbohydrate-degrading enzymes or carbohydrases act on starches and indigestible cell wall components. Carbohydrases commonly used in swine

diets include β-glucanase and xylanase, as well as α-amylase, and cellulase. Proteases, on the other hand, are enzymes that break down protein molecules into simpler forms that can be absorbed in the gut. They can also act on protein-based anti-nutritional factors (ANF) to neutralize their effects.

What are the enzymes mode of action?

Plant-based ingredients contain varying amounts of ANF, such as non-starch polysaccharides in cereal grains and trypsin inhibitors in soybean meal. Their anti-nutritive effect is due to their resistance to the pig's digestive enzymes and may interfere with digestion and negatively affect performance. The proposed mode of action and role of exogenous enzymes[1] include:

- Degradation of feed components resistant to endogenous enzymes.
- Inactivating ANF to increase the efficacy of endogenous enzymes.
- Supplementing endogenous enzymes that are otherwise present in insufficient amounts within the animal, such as proteases in the case of young pigs.

Enzymes are highly specific and therefore must match the specific substrates present in feedstuffs included in the diets. It is, therefore, necessary to carefully evaluate the active enzymes present in a product and the level of enzyme activity present. If possible, feedstuffs must be analyzed for the type of substrates present to better match the enzyme product.

What are the expected benefits from using enzymes?

While carbohydrases and proteases have been used in poultry quite successfully, this has not been the case in pigs. A number of studies have shown that exogenous enzymes can improve the digestibility of nutrients in feedstuffs commonly used in pig diets,[2-5] though the positive increases in digestibility have not consistently translated into improvements in growth

performance especially in diets based on corn and soybean meal[6-9]. One of the supposed effects of enzymes is the increased availability of energy from fibrous plant materials. Increasing the availability of energy from feed ingredients should improve feed efficiency. Published scientific data,[6,7,10-13] on the other hand, show mixed results and are inconclusive. One theory for the differences in digestibility data and production responses is that the enzymes are increasing the digestibility of feed ingredients in the large intestine. However, most of the absorption of nutrients occurs in the small intestine of pigs. Thus, the absence or limited beneficial effect of enzyme in pigs may be because the increases in digestibility are occurring at a location in the gastrointestinal tract where the pigs are unable to use the increased energy to influence growth rate or feed efficiency.

Use of enzymes in DDGS-containing diets

Dried distillers grains with solubles (DDGS) have relatively higher fiber content compared to traditional feed ingredients like corn and soybean meal. As more DDGS is used in swine diets, there also has been an increasing interest in adding enzymes in such diets to improve their energy value. However, data from recent studies[5,8,14] have not shown significant improvements in growth performance of pigs fed enzyme-supplemented diets. Even at very high levels of DDGS (60%), addition of commercial enzymes did not result in performance improvements.

How should I choose the enzyme product appropriate for my diets?

Choosing the appropriate enzyme product will depend on the chemical composition of the diets which is determined by the feedstuffs that were added in the diets. For example, diets based on wheat will probably respond more to xylanase addition, while barley will respond more to β-glucanase. It will be very important to ask from suppliers for published data on the enzymes

present in the commercial product and not just their own research data. This will be helpful in evaluating the cost benefit of using the product. In other parts of the world, it is not uncommon to find many enzyme products from unreliable traders. Thus, procure products only from companies with proven track records and that are well-known in the industry.

Do multi-enzyme combinations (cocktail) work better than single-enzyme preparations?

As discussed earlier, enzymes are highly specific and thus a combination of different enzymes may work better than a single enzyme. However, several studies[4,6,14,15] have not been able to support this assumption. Still, the use of multi-enzyme products is widely practiced in other countries where a variety of byproducts can be found in a single diet and where, theoretically, more significant response to enzyme may be seen.

Withdrawal period

Just like any other protein, enzymes are broken down in the digestive tract, so no metabolites are absorbed or residues excreted through the feces that would require a withdrawal period.

Summary

Carbohydrate-degrading enzymes, proteases, and their combination have been shown to improve nutrient digestibility of feedstuffs in pigs.[2,10,16] However, there is still a lack of scientific data that would support commercial enzyme use in pig diets as research data have failed to consistently show benefits in performance.[5-7,12,14] Therefore, enzyme cost relative to the benefits is not justifiable at this time for regular inclusion in swine diets.

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Carcass modifiers

There is increasing consumer demand for leaner and healthier pork products. Improvements in genetics, new technologies, and increased understanding of nutrition have become instrumental in helping producers meet this demand. Continued research also has led to the development of products that can be included in swine diets as carcass modifiers. A dietary carcass modifier is broadly defined as any component of the diet that alters the resulting carcass composition of pigs. Generally, their mechanism of action is aimed at increasing protein and muscle deposition while reducing fat deposition. These products vary in the mechanisms by which they modify carcass quality. In addition, not all carcass modifiers are approved for use in pig diets for public-health reasons. Understanding their mode of actions and differences between these products is important for safe and effective use.

Fast facts

Carcass modifiers, which are feed additives included in swine diets to improve carcass quality include, chromium, betaine, carnitine, conjugated linoleic acid, and ractopamine HCL.

Ractopamine HCl, which has shown the most consistent results among the carcass modifiers, acts as a repartitioning agent by redirecting nutrients away from adipose tissue and towards muscle growth.

Amino acid levels need to be adjusted to meet the increased requirement for protein deposition with ractopamine supplementation.

Growth response to ractopamine HCl decreases over time.

More research is needed to validate the beneficial effects of the other carcass modifiers.

What compounds are commonly used as carcass modifiers?

Carcass modifiers available for use in swine include chromium, betaine, carnitine, conjugated linoleic acid, and ractopamine.

Chromium. Chromium is an element essential for growth and development in animals. It plays an important function in metabolic processes involved in the regulation of glucose, proteins, lipids, and cholesterol. Chromium from organic complexes like chromium picolinate and chromium nicotinate is more readily absorbed than other inorganic forms, like chromium chloride. A number of studies,[1-3] mostly utilizing chromium picolinate, have shown that adding chromium to pig diets during the growing-finishing period can improve growth performance or lean meat yield. However, the responses have not been consistently observed in all studies.[4-7] The exact physiological action of chromium that leads to increased carcass leanness is not clear. One possible mechanism is improved insulin sensitivity of tissue that leads to enhanced deposition of dietary protein and carbohydrate in the muscle cells

Betaine. Betaine is a byproduct of molasses production from the sugar beet and plays a role in metabolic processes as a methyl donor. Interest in this product increased after studies indicated that it can increase carcass leanness and improve feed efficiency when added in finishing diets.[8,9] However, results were not consistently repeated in other studies indicating unreliability of the responses.[10,11]

Carnitine. Carnitine is a vitamin-like compound essential for fatty-acid transport across the mitochondrial membrane. While results from earlier research[12] were inconsistent, more recent studies[13-15] have provided further evidence that the addition of carnitine in finishing diets results in leaner carcass and thinner backfat. This has been attributed to the increased ability of the pig

to more efficiently use fat for energy, divert carbon toward amino-acid synthesis, and spare branched-chain amino acids for protein synthesis.

Conjugated linoleic acid. Conjugated linoleic acid is a feed additive that has been shown to reduce whole-body fat accretion by repartitioning fat and lean tissue.[16,17] The use of conjugated linoleic acid in pig diets also influences fat quality by lowering its iodine value. Lower iodine value is an indication of a more saturated (firm) fat. However, the high cost of conjugated linoleic acid limits it practical use in swine diets.

Ractopamine HCl. Among the substances considered as carcass modifiers, ractopamine HCl is the one that has received the greatest amount of attention. Ractopamine HCl belongs to a group of compounds called β-agonists that include zilpaterol, cimaterol, clenbuterol, and salbutamol. However, only ractopamine HCl is approved for use in pigs in the United States. It is also legal for use in swine diets in more than 20 countries, but not in some other parts of the world. This product is recommended to be fed at concentrations of 5 to 10 ppm in the diet.

How does ractopamine improve the quality of pig carcass?

Ractopamine HCl, like the rest of the β-agonists, acts as a repartitioning agent by redirecting nutrients away from adipose tissue and towards muscle growth. It modifies the metabolic signals within muscle and fat cells to direct more nutrients to lean growth. Pigs fed diets supplemented with ractopamine HCl also exhibit an increase in daily gain accompanied, in many instances, by a slight decrease in feed intake. Efficiency of gain also is improved because it takes less energy to deposit lean than fat. These improvements in growth performance have been consistently shown in many experiments.[18] However, it should be noted that the use of this feed additive in pig diets can also have potentially negative consequence. Ractopamine HCl has been shown to affect behavior and stress hormone profiles of finishing pigs which made them more difficult to

handle. [19] This potentially could lead to difficulty in handling as well as increasing their susceptibility to transport stress at the time of marketing.

Do diet formulations need to be modified when adding ractopamine HCl?

Appropriate nutritional adjustments in finishing diet formulations need to be made to capture the maximum benefits of ractopamine HCl. This is due to the increased requirement for nutrients to support the higher rate of muscle deposition that results with dietary ractopamine HCl use. It is labeled for diets to have at least 16% crude protein when adding this product in swine diets. However, because swine do not have a requirement for crude protein, but rather requirements for amino acids, it is important that the appropriate amino acid levels be fed when using ractopamine HCl. The lysine requirement, in particular, is increased in ractopamine HCl-fed pigs. It is recommended that ractopamine HCl-supplemented diets should have a standardized ileal digestible lysine level that is 0.3% higher than that required by the pig of equal weight fed diets without ractopamine HCl.

At what stage of production should ractopamine be fed to pigs and for how long?

Ractopamine HCl is labeled to be fed continuously for up to the last 90 lb before marketing. It is important to note that the greatest response to the growth-promoting ability of ractopamine HCl occurs during the first 2 weeks of feeding and progressively decreases over time. This is due to the down-regulation or desensitization of β-receptors that results from chronic administration of β-agonists. Therefore, feeding ractopamine HCl-supplemented diets longer than recommended will not translate to further improvement in performance. Also, pigs must be continuously fed ractopamine HCl-supplemented diets until market. Beneficial effects on performance will be lost

once ractopamine HCl is removed from the diet before market. This beneficial effect can be lost with removal for as little as 7 days prior to market.

Is pork from a pig that was fed a diet containing ractopamine HCl safe for human consumption?

The use of ractopamine HCl as a feed additive in swine diets has been extensively studied for many years prior to its Food and Drug Administration approval in 1999. These studies have shown that pork from pigs that were fed ractopamine HCl-containing diets is safe for human consumption.[20] There is no withdrawal time required for ractopamine HCl. A major limitation to the acceptance of β-agonists such as ractopamine HCl in animal production in other countries is the risk associated with drug residues in the meat products. This is especially true for clenbuterol, which has been shown to have rather long elimination time (greater than 21 days)[21] from the animal body and thus, may cause unsafe drug residues in meat and meat products.[22] Consumption of pork containing clenbuterol residues can have adverse effects in humans.[20] For this reason, clenbuterol and other related products have been banned for use as repartitioning agent in many parts of the world including the United States.

Summary

Carcass modifiers are feed additives that can be used to increase lean growth rates and improve efficiency. Among these, ractopamine HCl has shown the most consistent results. However, optimal results for ractopamine HCl use will depend on the dose, duration of treatment, and nutrient levels in the diet.

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Flavors

Under the conditions of modern swine production, pigs need to be fed a balanced diet that meets their daily nutritional requirement for maintenance, growth, and reproduction. However, nutrient intake is largely determined by voluntary feed intake, which is greatly influenced by the chemical senses of olfaction and taste. Thus, it is essential to make sure that diets being offered to pigs are highly palatable to ensure high feed intake. This is especially important during times when pigs have decreased appetite, such as the first few days post weaning. Therefore, it is believed that enhancement of taste or smell through the use of flavors may help to improve the palatability of diets and, consequently, feed intake.

Fast facts

Flavors are feed additives that attempt to enhance the taste and smell of feed to stimulate feed intake.

Pigs show preference to certain flavors when given a choice.

Flavors do not improve feed intake when pigs are not given a choice.

Factors affecting feed intake

A number of factors have been identified that affect feed intake in pigs. In most cases, feed intake is influenced by the interaction between some or all of these factors, which include the thermal environment, social factors (eg, stocking density), animal factors eg, genotype), and dietary factors (eg, energy density and palatability).[1] Palatability of a diet refers to the acceptability features of the diet, including taste, smell, and texture, that the pig senses before feed is swallowed.

How can palatability of diets be improved?

Palatability of a diet may be measured by comparing the amount of that diet a pig consumes relative to the amounts consumed of other diets. Palatability can be improved by using ingredients preferred by pigs or by using feed additives, such as flavors, that make the diet more acceptable and encourage higher feed intake. Taste buds in pigs have been found to be at least three times the number of those found in humans,[2] suggesting that their sense of taste may be more developed and thus may be more responsive to varying tastes and flavors in their food.

What are flavors?

Flavors are feed additives that attempt to enhance the taste and smell of feed to stimulate feed intake. Taste and smell are the senses associated with feed intake. Because smell is the first sensation detected by the pig, aroma of the diet becomes the initial stimulus that drives the pig to eat.[3] Flavors also mask ingredients that are unpleasant to pigs.

In countries where small-scale pig production is still widespread and producers buy commercially available feed products from distributors, flavors are used by feed manufacturers mainly as a marketing tool to attract feed buyers. Farm owners tend to believe that feed products that smell good to them will also be acceptable to their pigs;[4] however, this is may not be the case as large differences in sense of taste are known to exist between species.[2,5]

At what stage of production are flavors applied or used in pig diets?

Flavors and aromas are primarily used at stages when feed intake is expected to be lower, such as in the post-weaning period. During and immediately after weaning, the pig is subjected to significant stress brought about by a number of physical, physiological, and behavioral changes. These include separation from the sow, new environment, and dietary transition from sow's milk

to a completely solid food. Identifying and adjusting to the new diet takes some time and further contributes to the growth lag experienced by a young pig. Flavors may help improve the performance of pigs during this stage through increased feed consumption by making the feed more attractive and highly palatable.

This same principle applies to the use of flavors and aromas in creep feed and milk replacers. Some products claim to enhance palatability of creep feed by mimicking the taste of sow's milk. Suckling pigs are stimulated and learn to eat solid food earlier, making the transition to completely solid food during weaning less stressful.

Most sows consume less feed than needed to support the demands during lactation. Thus, flavors may have some use to help increase sow feed intake. However, there is no research data to support this claim.

What flavors are included in commercially available swine feed additive?

A number of studies[5-9] have been conducted using a wide variety of flavors to identify those most preferred by the pig. Most of these studies reported a preference for a sweet taste. That is why most of the products added to the feed as flavoring agents include sweeteners such as saccharin and talin. Others include vanilla and milky or fruity flavors or a combination of these.[4] Acceptability of these flavors to pigs was identified using preference studies[7] wherein pigs were simultaneously offered different diets with different tastes. Taste preference was identified in terms of which diet with a particular flavor was consumed the most relative to the total feed intake of the test diets commonly expressed as the percentage of the total amount of feed consumed.[3,10]

Does the addition of flavors translate to improvement in performance?

Preference for a certain flavor does not necessarily mean that feeding it will result in improvement in feed intake and performance. While a number of studies[6,8,9] have shown that pigs prefer certain flavors when given a choice, using these same flavors in performance studies did not necessarily show positive effects when pigs were not given a choice.[6,11] Growth-performance experiments[12-15] also have had varying results, with most improvement in feed intake being observed during the first week after weaning.[8] In one recent study,[16] the addition of an enhanced flavor to creep feed given 3 days before weaning did not affect litter feed intake, the proportion of piglets consuming creep feed, or preweaning performance. However, exposure to the same enhanced-flavor product led to greater postweaning daily gain of pigs fed complex diets supplemented with the same flavor, but did not influence performance of pigs fed simple diets.

Summary

Feeding pigs with a well-balanced diet that is highly palatable is essential for optimal growth performance and production efficiency. While the use of flavors may be a useful tool to improve palatability and feed intake under certain conditions, the effectiveness of these feed additives has not been consistently observed in different experiments. In addition, feed intake is regulated by multiple factors, not just taste. Therefore, careful evaluation of commercially available products and consultation with a nutritionist is recommended before a flavor is added to a swine diet.

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High dietary levels of copper and zinc for growing pigs

Copper and zinc play important roles in many physiological processes. Dietary copper levels of 5 to 10 ppm and zinc levels of 50 to 125 ppm in the diet are generally enough to meet the pig's nutrient requirement for these processes. However, when supplied at high concentrations (100 to 250 ppm for copper and 2000 to 3000 ppm for zinc), these two minerals are known to exert positive influences on growth rate.

The improvement in growth performance when supplementing high levels of dietary copper is similar in magnitude to when in-feed antimicrobials are fed to nursery pigs. In addition, copper is efficacious even when antibiotics also are included in the diets. This suggests that the response to copper is additive to that of antimicrobials. Response to these high levels of dietary copper decreases as the pig grows older and when fed in longer durations.

Zinc fed at high dietary levels (2000 to 3000 ppm) has been shown to reduce incidence of diarrhea and increase weight gain in newly weaned pigs. Feeding duration should be limited to approximately 3 weeks after weaning when using high dietary zinc levels. Additive effects are usually not observed in weaned pigs when copper and zinc are added together at high levels. However, the data is conflicting and needs to be further investigated.

Fast facts

Copper and zinc are classified as trace minerals because they are required by pigs at relatively low levels for normal growth.

When added at high dietary levels, copper (100 to 250 ppm) and zinc (2000 to 3000 ppm) can increase the growth performance of young pigs.

The mechanism by which high levels of copper and zinc improve growth rate in pigs is still unclear.

Mode of action for growth promotion at high dietary levels

The mechanism by which high levels of copper and zinc improve growth rate in pigs is still unclear. Both are known to have some antibacterial property, which may explain the growth-promoting effect, but there is a lack of scientific evidence to understand the exact mode of action. Even though copper has antibacterial properties, growth rate is stimulated in an additive manner when adding in-feed antimicrobials and high levels of copper in nursery pig diets. Thus, it appears that the growth-promoting properties of high copper are in addition to its antimicrobial effect.

What are the sources used for growth-promoting levels of copper and zinc?

Most of the research with high levels of dietary copper has been done using copper sulfate. Increased growth rate has been demonstrated with the tri-basic chloride form as well. Limited information is available using other sources.

Increased growth rates from feeding high levels of zinc in the early period after weaning have been most consistently demonstrated with zinc provided as zinc oxide in the diet. Other sources such as zinc sulfate and zinc methionine have not consistently demonstrated positive effects.

What are the potential problems with adding copper or zinc at these very high levels?

Copper toxicity may occur when dietary levels exceed 250 ppm a longer period of time. Jaundice (yellow discoloration of the skin) may result due to excessive accumulation of copper in the liver. Toxic effects of zinc, on the other hand, may be indicated by depressed pigs, arthritis,

gastritis, and death. Zinc toxicity has been reported when highly absorbable zinc sources, such as zinc carbonate, are added up to 4000 ppm for an extended period of time. To avoid these problems, it is important to use only the recommended dietary levels for copper and zinc for growth promotion at specific growing periods (Table 1-8).

It is also important to keep in mind that as more of these nutrients are added in the diets, the amount that will be excreted by the animal can also increase. Thus, the addition of these elements at their growth-promoting levels can have negative implications in the environment because of high levels of these minerals being excreted through the feces. This can result to excessive accumulation of these minerals in the soils where the manure is applied. Another negative effect of high levels of copper is that it increases the amount of unsaturated fat which results in a softer pork fat. Thus, reducing dosages and eliminating feeding high levels in finishing diets can minimize these negative impacts.

Summary

Copper and zinc are important trace minerals needed by the pig for numerous metabolic functions. Inclusions in the diet of these trace minerals at high dietary levels increases growth performance, especially in young pigs.

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Table 1-8: Recommended dietary levels of zinc and copper for growing pigs

Production phase	Zinc (ppm)	Copper (ppm)
Nursery		
< 11 lb	3000	5*
11 - 15 lb	3000	5*
15 to 25 lb	2000	5*
25 to 50 lb	100	100-250
Grower (50 to 120 lb)	50*	50-100
Finisher (> 120 lb)	50*	3-3.5*

* Based on National Research Council minimum daily requirement.

Mold inhibitors, mycotoxin binders, and antioxidants

In providing a high quality diet to pigs, it is important to ensure that it contains the correct amount and balance of nutrients for optimal productivity, is highly palatable, safe to the animal, and free of any substances that may negatively affect their performance. Thus, the addition of feed additives to prolong shelf-life, prevent mold development, or bind mycotoxins present in the feed may be required in certain situations.

Fast facts

Feed ingredients such as grains are prone to mold growth and mycotoxin contamination.

Mold inhibitors such as organic acids are used in diets to prevent mold growth, but they are not effective against mycotoxins.

Mycotoxin binders are added in the diets to prevent pigs from absorbing the toxins from contaminated feed.

Common mycotoxin binders are effective against aflatoxins, but have limited activity against other mycotoxins.

Antioxidants may be feasible to use in diets containing ingredients that easily gets rancid due to high fat content.

What are mycotoxins?

Mycotoxins are chemical compounds produced by actively growing molds (fungi) as secondary metabolites that can negatively affect pig performance. While not all molds produce toxins, over 300 types of mycotoxins are known to be produced by molds, with aflatoxin, vomitoxin, zearalenone, fumonisin, and ochratoxin generally regarded to be the most significant mycotoxins

affecting livestock production (Table 1.9).[1] Young and breeding animals are generally more susceptible to mycotoxins.

The molds that produce the common mycotoxins found in livestock diets belong to the genera *Aspergillus*, *Claviceps*, *Fusarium*, and *Penicillium*.[2] Feedstuffs can get contaminated before harvest of the main plant source, during post-harvest handling and storage, and during processing into animal feed products. Grains such as corn, wheat, and barley are some of the feedstuffs that can get easily contaminated with molds. Molds or fungi are categorized into field and storage fungi. Field fungi are those that grow in grains before being harvested. These commonly include fungi belonging to *Fusarium* species, that produce the toxins vomitoxin, zearalenone, and fumonisin.[2] Storage fungi, which include molds under the genera *Aspergillus* and *Penicillium*, are significant producers of mycotoxins that commonly affect pigs such as aflatoxin and ochratoxin.[2] They have the ability to grow even at very low moisture levels, unlike the species of field fungi. One species of storage fungi, *Aspergillus flavus*, is known to produce high concentrations of aflatoxin in grains even before harvest. It is important to distinguish between field and storage fungi, since this affects the distribution of mycotoxins. When conditions are favorable for field fungi to produce mycotoxins, grain from a geographic location is expected to be widely affected. Thus, large quantities of grain may be affected. In contrast, storage fungi should have a more localized distribution due to specific conditions during storage. In fact, not all grain may be affected evenly within a storage bin. Thus, storage mycotoxins may be difficult to detect without extensive sampling.

With the increase in the availability of distillers grains, due to increasing ethanol production, the use of distillers grains in swine diets have also increased. Corn is the major grain product used to produce ethanol. Because most of the starch in the corn is consumed during fermentation, the

resulting distillers grains co-product is more concentrated in other proximate components such as fiber, protein, and fat compared to corn. However if the corn grain used for fermentation has been contaminated with mycotoxins, it can result to a distillers grains product that may have as much as three times the concentration of mycotoxins as the source corn.[3]

Mycotoxicosis refers to poisoning due to the ingestion of mycotoxins. This condition can lead to lower resistance to diseases, increased sensitivity to stress, and damage to vital organs, such as the liver and kidney. Ultimately, this may lead to mortalities and poor production performance.

What are mold inhibitors?

Mold inhibitors are feed additives used to minimize mold contamination and prevent mold growth, thereby minimizing the risk of having mycotoxin-producing molds proliferate in grain or feed. Commonly used feed additives for this purpose include propionic acids and other organic acids. However, even if mold growth has been prevented, mycotoxins may still be present, because mold inhibitors have no effect on mycotoxins already present in contaminated feed.

What are mycotoxin binders?

Mycotoxin binders or adsorbents are substances that bind to mycotoxins and prevent the toxins from being absorbed through the gut and into the blood circulation. When other preventive measures against molds and mycotoxins have failed, the use of mycotoxin binders can be valuable. There also may be instances when feeds and feedstuffs cannot be checked for mycotoxins on a regular basis. Mycotoxin binders in such cases are routinely added for safety measures and as some form of assurance to customers. There are a variety of substances known to have the ability to bind mycotoxins. The most commonly used and most researched mycotoxin binding agents are the aluminosilicates – clays and zeolites. These are natural adsorbents that

include hydrated sodium calcium aluminosilicates (HSCAS), bentonite, and zeolite (Table 1.10).[4,5] Most of these products are efficient binders of aflatoxins. However, they have limited or no activity against other types of mycotoxins. Other substances found to have toxin-binding capability include cell-wall components of yeasts. Some studies have shown that the cell-wall fraction β-glucan of yeasts such as *Saccharomyces cereviceae* can be effective in binding a wide range of mycotoxins.[6] Unlike clays, they can be added at low levels and are biodegradable. However, research in pigs documenting their efficacy in mitigating the effects of mycotoxins is limited and has shown inconsistent results.[7-10]

Choosing the appropriate product

In general, the following must be considered when choosing either mold-inhibitor or adsorbent products: efficacy in adsorbing the mycotoxin or inhibiting the mold of interest; safety to the animal, the handler, and to pork consumers; highly stable and able to withstand varying conditions during feed mixing; and cost effectiveness. Caution also must be exercised when using clays, because they can limit the bioavailability of minerals due to its high adsorptive capacity. This is most important when diets contain marginal levels of trace minerals. There may also be some risk of dioxin contamination associated with the use of natural clays and this needs to be considered.[6] It is important to know the source of clay products that will be used in swine diets. Dioxins are mainly by-products of industrial processes. Contamination of clay sources can be due to improper disposal or accidental leakage of these by-products into the environment.

What is an antioxidant?

An antioxidant is a product added to animal feeds to prevent oxidation of fat or vitamins.[11] Antioxidants found in commercial products include ethoxyquin, butylated hydroxytoluene

(BHT), butylated hydroxyanisole, (BHA), and propyl gallate. Combinations of these antioxidants are normally found in commercially available products. This is to take advantage of the different properties of each antioxidant. For instance, an antioxidant product combination may contain propyl gallate to provide a high level of initial protection, and BHA for longer follow-through effect. These antioxidants have also been shown to have inhibitory effect on mold growth in grains under laboratory settings [12,13] and may have some use as a mold inhibitor in pig diets in the future.

When is it advisable to use these products?

The use of mold inhibitors in swine diets may be advisable in geographic areas that are highly conducive for mold growth in grains and where incidence of mycotoxin contamination is more likely. Mycotoxin binders should be used when feed ingredients are suspected to be contaminated with mycotoxins at levels deemed unsafe for pigs (Table 1-9). The use of these products become more important in situations when moisture content of grains to be used for pig diets are greater than 14% and when storage conditions have a relative humidity that is higher than 85% and a temperature greater than 55°F.[14] Thus, the use of mold inhibitors or mycotoxin binders may also be needed when diets have to be stored for a relatively longer period of time. Antioxidants are highly applicable in areas with warm climate and when adding high levels of fat in the diet. Antioxidants are widely used in areas where byproducts that are high in unsaturated fat such as fish meal are often used. Oxidation of unsaturated fatty acids can produce substances that can cause off-flavors and toxic substances that can lead to rancidity. These substances can also lead to destruction of nutrients like the fat-soluble vitamins.[14] Adding an antioxidant will minimize fat oxidation, keep the diet highly palatable, and help prolong the shelf life of the feeds. It should be noted that antioxidants only delays but cannot prevent fatty acid oxidation.[15]

Summary

Some species of molds have the ability to produce mycotoxins. Mycotoxin contamination of diets can result in production and financial losses. Mold inhibitors and mycotoxin binders can be effective tools in controlling mold and mycotoxin problems. Antioxidants, on the other hand, can help preserve palatability of feed ingredients or complete diets.

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Table 1-9: Regulatory limits for the 5 major mycotoxins in feedstuffs used in swine diets*

Age group	Aflatoxin, ppb†	Fumonisin, ppm‡	Vomitoxin, ppm§	Ochratoxin A¶	Zearalenone¶
Young	20	20 (10)	5 (1.0)	-	-
Finishing (> 100 lb BW)	200	20 (10)	5 (1.0)	-	-
Breeders	100	20 (10)	5 (1.0)	-	-

*Source: Food and Drug Administration Center for Veterinary Medicine.[1]

†Indicate action levels. Level indicated for young pigs applies to complete diet and ingredient.
‡Indicate guidance levels. Not to exceed 50% of the diet as indicated by values in parentheses.
§Indicate advisory levels. Not to exceed 20% of the diet as indicated by values in parentheses.
¶No FDA action, advisory or guidance levels established in US feed. It should be noted that a minimum of 0.20 ppm ochratoxin A can cause a reduction in weight gain and mild renal lesions in finishing pigs at slaughter, and a minimum of 1 ppm zearalenone can cause vulvovaginitis and prolapse in prepubertal gilts.[2]

Table 1-10: Performance of weanling pigs fed aflatoxin-contaminated diets with either bentonite or HSCAS*

		Aflatoxin corn		
Parameter	Normal corn	No additive	Bentonite added†	HSCAS added†
ADG (kg)	0.63	0.52	0.60	0.61
ADFI (kg)	1.29	1.02	1.24	1.2
G;F	0.49	0.51	0.49	0.49

* Adapted from Schell et al, 1993.[5] Data are means from 3 pens of 3 pigs per pen.
†Added at a level 0.5% of the diet in place of corn.
ADG = average daily gain; ADFI: average daily feed intake; G:F = gain-to-feed ratio; HSCAS = hydrated sodium calcium aluminosilicate.

Phytase

Pigs need dietary phosphorus for normal body maintenance and growth. It is an essential element that is required in many physiological processes in the pig's body and thus, sufficient amounts must be included in the diet. This element is abundant in most grains found in swine diets. However, only a small amount of phosphorus is utilized from grains because the majority of the phosphorus exists in a form (phytate) that is not digestible in swine. The digestibility of phytate phosphorus can be increased when supplemental phytase is included in the diet.

Fast facts

Phytase is an enzyme that increases availability of phosphorusin pig diets.

Phytase lowers the level of supplemental phosphorus added to the diet.

Because of improved dietary phosphorus utilization, less phosphorus is excreted in the manure.

Phytase is susceptible to degradation with extended storage periods.

What is phytate?

Phytate or phytic acid is the main storage form of phosphorus in grains and oil seeds. Pigs are unable to digest phytate due to the lack of digestive enzymes that break down phytate. As a result, there is a substantial amount of phosphorus excreted as waste with approximately only 15% of the total phosphorus absorbed from corn and up to 50% in wheat. Because phosphorus is an essential element; inorganic phosphorus, which is highly available, is typically supplemented in the diet to meet the pig's requirement. Phytate also has other anti-nutritive effects as it is known to reduce the availability and utilization of other nutrients.

Phytase

Phytase is an enzyme that specifically acts on phytate and breaks it down to release phosphorus in a form that is available to the animal. This greatly reduces the need for supplemental inorganic phosphorus and improves the nutritional value of feedstuffs. Phytase activity is expressed as phytase units or FTU. One FTU is the activity of phytase required to liberate 1µmol of inorganic P per minute, at pH 5.5 from an excess of 15 M sodium phytate at 37 ^{0}C. Unlike the non-starch polysaccharide-degrading enzymes, phytase is the only exogenous enzyme that has consistently shown to be highly beneficial to pigs. The proven efficacy of phytase has resulted in worldwide acceptance and use in pig production.

Where is phytase derived from?

Some ingredients posses intrinsic phytase activity but varies greatly between plant species. Corn and soybean meal contain negligible levels of phytase activity compared to wheat, which contains considerably high levels of intrinsic phytase. Majority of phytase activity in feedstuffs is found in the bran. However, this may be lost when these ingredients are subjected to higher temperatures such as during the pelleting process. Commercially available exogenous phytases are derived from either fungi or bacteria, such as *Aspergillus niger* and *E. coli* but are expressed in yeasts for production purposes.

How much phytase should be added to the diets?

The amount of phytase needed in a diet depends on the dietary ingredients used and enzyme activity for the product to be used. However, it is important to note that differences in laboratory assays as well as differences between company products exist so the 1 FTU of one product may not be equivalent to 1 FTU of the other. It is important to obtain from the supplier the actual

amount of phosphorus release based on their claimed enzyme activity for accurate diet formulation. In general, recommended levels by manufacturers of commercially available phytases can replace inorganic phosphorus levels by 0.12% in pig diets. Phosphorus release with increasing phytase levels follows a curvilinear response. This means that maximum phosphorus release can only be reached at an optimal phytase level after which it will result to diminishing returns. It should also be noted that the intrinsic phytase activity of feedstuffs such as wheat (50% bioavailable phosphorus) must also be taken into account for better accuracy of diet formulation.

What affects the efficacy of phytase?

Several factors can influence the efficacy of phytase including: 1) the amount of phytate in the diet; 2) the amount of phytase that was added to the diet; and 3) the type of phytase used. Studies have shown greater responses to phytase in pigs fed diets that contain higher amounts of phytate. Phytase derived from the *E. coli* bacteria has also been shown to be more efficacious than the fungal phytases according to published data. However, other research suggests that the higher phosphorus release obtained from the *E. coli*–derived phytase was due to the underestimated actual phosphorus-release for every phytase unit as determined using a standard assay procedure. Thus, depending on the assay used, different results of phytase activity may be reported.

Because phytase enzyme is a protein, it is susceptible to denaturation when subjected to excessive heat, such as during pelleting. This may be addressed by spraying a liquid phytase to the cooled pellets to maintain the stability of the enzyme. Also, heat-stable phytases are available. It is important to bear in mind during storage that phytase, aside from high temperature, is sensitive to the presence of high moisture. Hence, proper storage procedures must

be practiced. Phytase products should only be stored in cool, dark, and dry areas. Manufacturer's recommendations should always be followed especially when phytase is included in vitamins and trace mineral premixes.

Another factor that may affect the magnitude of response to phytase is the calcium-to-phosphorus ratio. Studies have shown that wider calcium-to- phosphorus ratio can result to decreased absorption of phosphorus. Thus, a range of 1:1 to 1:1.25 calcium-to-phosphorus ratio is recommended.

Non-phosphorus effect of phytase

In some studies where pigs were fed non-phosphorus-deficient diets, improvements in growth performance were observed leading to the conclusion of a positive effect of phytase on other nutrients, such as increased digestibility of energy and amino acids. However, results from other studies are inconsistent to justify assigning a nutrient value (energy or amino acid digestibility) other than phosphorus release to phytase. Phytase does improve availability of calcium and other minerals in the diet. It should be noted that the addition of phytase will also result in an overall increase in energy value of the diet. This is due to the amount of inorganic phosphorus, which has no energy value, being replaced by corn in the diet.

Summary

Phytase is an enzyme that increases the digestibility of phytate phosphorus which improves the overall availability of dietary phosphorus. The use of this enzyme as a feed additive in swine diets reduces the level of inorganic phosphorus supplementation in swine diets. Less inorganic phosphorus leads to increased levels of corn or other grain sources in diet formulation thereby increasing the diet's overall energy content. Also, improved phosphorus utilization and reduced

inorganic phosphorus in the diet result in less phosphorus excretion from pigs. Other than phosphorus, phytase also can increase availability of other minerals such as calcium. Phytase is sensitive to high temperature and humidity, thus, proper storage and handling procedures should be followed to maintain the efficacy of the product. Heat stability of the product must also be considered when diets are pelleted.

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Phytogenic feed additives (Phytobiotics/Botanicals)

The restriction on the use of in-feed antibiotics in many countries has fueled the interest in alternative products. A group of natural products known as phytogenics have been the focus of several studies in recent years. Also, referred to as phytobiotics or botanicals, phytogenics are plant-derived products used in feed to potentially improve pig performance. Aside from having antimicrobial activity, these products potentially provide antioxidative effects, enhance palatability, improve gut functions, or promote growth. However, there is little research validating their potential benefits for pigs.

Fast facts

Phytogenic feed additives are substances derived from plants.

The potential benefits with the use of phytogenics in pigs have not been fully substantiated based on available research.

Current research data show that growth responses to phytogenic feed additives are still inadequate compared to responses obtained with the use of in-feed antimicrobials.

What products are being used as phytogenic feed additives?

Phytogenics comprise a wide range of substances and thus, have been further classified according to botanical origin, processing, and composition. A phytogenic feed additive may be herbs which are flowering, non-woody, and non-persistent plants; spices which are herbs with intensive smell or taste and are commonly added to human food; essential oils which are lipophilic compounds derived by cold expression or steam or alcohol distillation; or oleoresins which are extracts derived by non-aqueous solvents from plant material. Some examples that

have been evaluated in swine include the spices oregano, thyme, and sage and oregano oil extracts.

How do phytogenic feed additives exert their claimed effects?

The mode of action of most phytogenic feed additives is still not fully understood. However, the following are some of the potential mechanisms by which they may improve performance:

Increased feed intake. The stimulatory effect of phytogenics on feed intake is due to the claimed improvement in palatability of the diet resulting from the enhanced flavor and odor especially with the use of essential oils. However, the effect of adding essential oils in pig diets in feed intake is highly variable. In some phytogenic feed additive studies, the increased feed intake was found to be also influenced by the antibiotic supplemented in the diet. Other studies reported decreased feed intake with increasing inclusion levels of the phytogenic substance used. In a recent study the addition of a commercial phytogenic feed additive did not improve feed intake but improved feed efficiency. Increased palatability of the diets associated with the addition of phytogenics also may be due to the anti-oxidative effects of some phytogenic substances which could contribute in preserving the desired organoleptic qualities of the diet.

Improved gut function. Improvement in gut function is mainly attributed to the possible stimulatory effect of phytogenic substances on digestive secretions such as digestive enzymes, bile, and mucus. However, little evidence exists that support this hypothesis and is generally based on experiences derived in human nutrition with the use of spices. The use of phytogenic substances in humans also have shown that certain herbs, spices, and their extracts have

pharmacologic actions within the digestive tract as evidenced by their laxative and spasmolytic effects.

Anti-oxidative effects. Anti-oxidative properties of some phytogenic substances have been attributed to the phenolic terpenes in the essential oils. Essential oils of the Labiatae plant have been widely used as anti-oxidant in human foods with high fat content. Plants that are high in terpenes include rosemary, oregano, and thyme. However, whether they can be added in amounts enough to replace the effects of commonly used anti-oxidants such as α-tocopherols remains to be seen.

Antimicrobial effect. Antimicrobial properties of substances derived from different herbs and spices have been well-known for many centuries. This property is mainly attributed to the essential oils of these plants. Oregano and thyme are among those which have received a great deal of interest in this aspect. These plants contain the monoterpenes carvacrol and thymol, respectively, and have demonstrated high efficacy in vitro against several pathogens found in the intestinal tract of pigs. Currently, available research data appear to be insufficient to support the claimed beneficial effects on health and pig performance. In a recent study, a commercial product containing a proprietary blend of phytogenic substances improved postweaning growth performance in nursery pigs compared to control. However, pigs fed diets containing antibiotics had better growth performance than those fed the phytogenic test diets. In another study that evaluated the effects of oregano oil on nursery pig performance, growth performance of pigs fed diets with oregano oil did not improve in contrast to pigs that were fed diets containing antibiotics.

Is it possible that they may interact with other substances or compounds being added to the diets?

Most studies that evaluated the use of phytogenic feed additives in swine did not indicate any negative interaction to other supplements in the diets such as antibiotics or organic acids. However, negative interaction of phytogenic substances having astringent properties with proteinaceous feed additives have been reported due to partial denaturation.

Are phytogenic feed additives totally safe?

Even though a product is said to be of natural origin, it doesn't necessarily mean that it is better or safer than antibiotics or other synthetic feed additives. It is important to note that various antibiotics also are of natural origin. The fact that some herbs and spices also exhibit antimicrobial properties suggests that it may also pose similar risks to producers and meat consumers. Similarly, potential overdose that may be harmful to the pig also is possible. All of these warrant further investigation as to the safety of these products both for humans and animals.

Summary

Most of the beneficial effects claimed from using phytogenic feed additives are based on experience from the field of human medicine. Phytogenic feed additives, based on current research, will not replace the response observed with in-feed antibiotics during the nursery phase. Additionally, responses to feeding phytogenic additives have not been consistent among trials. Hence, more evidence is needed to confirm the apparent beneficial effects on pig performance before adding to swine diets on a regular basis. Finally, although these additives are

considered “natural” products, they need to be carefully evaluated for potential interactions with other ingredients or other potentially negative effects.

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Probiotics and Prebiotics

There is increasing pressure for livestock producers to minimize the use of antibiotics as growth promoters in food animals. Supplementing beneficial microorganisms in the gastrointestinal tract is one potential alternative. A diverse population of beneficial and potentially harmful microorganisms exists in the gastrointestinal tract of pigs. In a healthy animal, a delicate balance between these two groups of organisms is maintained. However, during times of stress, such as during weaning in the case of piglets, this balance may be affected and can lead to a rapid growth of harmful microorganisms. This may result in poor performance or disease. Thus, probiotics and prebiotics have been the subject of much research over the years as potential replacement for antibiotic growth promoters in pigs.

Fast facts

Prebiotics are non-digestible food substances that selectively stimulate the growth of favorable species of bacteria in the gut, thereby benefitting the host.

Probiotics are live cultures of beneficial organisms.

Results of growth performance trials, however, have been inconsistent.

More studies are needed to justify their use in pig diets.

What are prebiotics?

Prebiotics have been described as non-digestible food substances that selectively stimulate the growth of favorable species of bacteria in the gut, thereby benefitting the host. These substances are primarily derived from non-digestible oligosaccharides. Because they are not digested by the pig, they provide readily available substrates for the normal bacteria to grow. Oligofructose, fructooligosaccharide (FOS) and inulin are examples that have been used as prebiotics. However,

consistent beneficial effects on pig growth performance are yet to be demonstrated with prebiotics.

What are probiotics?

Probiotics are live cultures of beneficial organisms. Organisms used for this purpose can be bacteria belonging to the genera *Bacillus,* lactic-acid producing bacteria, and yeast. As a feed additive, they are supplemented in diets to improve the balance of bacteria in the gut. To be effective, a probiotic must have the following traits:

- Stability and ability to survive in feed.
- Ability to replicate after passage through the stomach.
- Ability to block the effects of harmful microorganisms or excrete metabolites that can inhibit growth of microorganisms.

The proposed benefits from probiotics are improved digestion, stimulation of the gastrointestinal immunity, and increased resistance to infectious diseases of the gut. Another possible mechanism by which probiotics may exert its beneficial effect is through its effect on the permeability of the gut which may increase nutrient uptake leading to improved growth performance. Unfortunately, research results have failed to consistently demonstrate beneficial effects.

What organisms are used as probiotics?

Some of the organisms commonly used include *Lactobacillus acidophilus, Enterococci faecium, Bacillus sp., and Bifidobacterium bifidum* including the yeast *Saccharomyces cerevisiae.*

What are synbiotics?

The combination of a prebiotic and probiotic is referred to as synbiotics. They have been proposed to be strategically beneficial for the pig by improving the survival rate and colonization

of the introduced probiotic microorganisms in the gastrointestinal tract. At the same time, the presence of prebiotics provides a readily available substrate for their growth and may promote the metabolism of the beneficial bacteria. However, similar to the use of probiotics or prebiotics alone, synbiotics have not been proven to provide consistent results in research trials.

What could be the reasons behind the inconsistency in results from research on prebiotics and probiotics?

The variability in responses suggests several possibilities. The fact that they were shown to improve pig performance in some studies but not in others indicates the influence of environment and production practices which may differ from one setting to another. It also may be possible that the number of viable organism was insufficient to be able to survive and establish them in the gastrointestinal tract. Another factor that may contribute to the variability in results might be that the microorganisms included in the probiotic product were not isolated from pigs but from other animal species.

Summary

Probiotics and prebiotics do not provide essential nutrients for normal growth. There are potential advantages to using probiotics and prebiotics from a health and growth promotion standpoint, including partial replacement of antibiotic growth promoters. However, studies with more consistent results are needed to justify pre- and probiotic use as additives to pig diets. For all the claimed beneficial effects and studies conducted, a consensus has yet to be made by the scientific community that they will consistently provide these benefits in commercial settings. Moreover, their addition in the diet entails additional cost and thus, must be evaluated thoroughly.

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CHAPTER 2 - Field evaluation of a porcine circovirus type 2 vaccine on finishing pig growth performance and mortality rate in a herd with a history of porcine circovirus disease

Summary

Objective: To evaluate the effects of a 2-dose commercial porcine circovirus type 2 (PCV2) vaccine on growth performance, and mortality rate of grow-finish pigs in a commercial finishing facility with a history of porcine circoviral disease (PCVD).

Materials and method: Two experiments were conducted using a total of 1,300 pigs in Exp. 1 and 1,253pigs in Exp. 2 using a 2 × 2 factorial completely randomized design. Pigs were commercial, cross-bred pigs (PIC L337 × C22). Pigs in Exp. 1 were vaccinated at 9 and 11 wk of age while pigs in Exp. 2 were vaccinated earlier at 5 and 7 wk of age. In Exp. 1; 1,300 pigs were individually weighed and the vaccine treatment administered 15 and 1 d before being placed on test in the finisher. Blood samples were collected from 10 randomly selected pigs from each treatment group for serology and PCR to detect the presence of infection and antibodies against PCV2 and other common co-infections. In Exp. 2; 1,253 pigs were used and randomly allotted based on nursery pen average pig weight and the vaccine treatment administered 41 and 27 d before being placed on test in the finisher. Pen weights were obtained on d 0 and every 2 weeks until the end of the trial. Feed intake was recorded on a pen basis.

Results: In Exp. 1; average daily gain (ADG), average daily feed intake (ADFI), gain-to-feed ratio (G:F), and mortality were improved ($P < 0.05$) in vaccinated pigs compared to unvaccinated pigs. In Exp. 2, there were vaccine-by-gender interactions for ADG ($P < 0.01$) and final weight

($P < 0.05$). These were the result of the vaccine increasing ADG to a greater extent in barrows than in gilts. Also, vaccination against PCV2 resulted in heavier weights among vaccinated barrows at market compared to non-vaccinated barrows but did not lead to weight improvement in vaccinated gilts. Overall, the improvement in ADG resulted in vaccinated pigs being 2.9 kg heavier ($P < 0.005$) than the non-vaccinated pigs at market. Mortality rates among vaccinated pigs were 2.8 and 6.2 percentage units lower than in non-vaccinated pigs in Exp. 1 and 2, respectively.

Implications: The commercial PCV2 vaccine used in this study was effective at reducing mortality and increasing growth performance in finishing pigs from a commercial herd with a history of PCVD.

Keywords: growth, porcine circovirus type 2, swine, vaccine

Porcine Circovirus Disease (PCVD) principally affects finishing pigs and was first described in Canadian herds in 1996.[1] It has since been identified in almost every country involved in pig production and has become one of the most economically important diseases affecting pigs worldwide.[2] The disease is caused by porcine circovirus type 2 (PCV2), a circular, single-stranded DNA virus.[3] Before the introduction of commercial vaccines, PCV2 was difficult to control[4] because of the virus' resistance to inactivation at a low pH, stability at high temperatures,[5] and resistance to common disinfectants[6,7]. In addition, the virus is highly transmissible via direct contact,[8] feces, and oro-nasal secretions.[9] Recent research has shown that the virus also may be spread through sow's milk,[10] potentially through semen,[11] and even through fresh pig tissues such as muscle.[12] Once infected, the pig can either appear normal or

show various clinical syndromes involving different body systems; hence, the more general term PCVD is used. The appearance of different clinical syndromes can be attributed to the immunosuppressive properties of PCV2, which predispose the infected animal to other infections.[13] Among the different clinical presentations of PCVD, postweaning multisystemic wasting syndrome (PMWS), characterized by progressive weight loss, respiratory signs, and enlargement of lymph nodes in growing-finishing pigs, is the most common.[14] Clinical disease can lead to high death loss and increased cull rates in growing and finishing pigs, resulting in huge losses in income.[4] Also, PCV2 was originally not thought to greatly affect growth performance in pigs with subclinical infection.

Approaches for PCVD control have focused on improving production practices and minimizing coinfections, but results can still remain unsatisfactory.[4] Initial results in the early stage of experimental PCV2 vaccine development have shown positive results in terms of reducing incidence of PMWS.[15] Beginning in 2006, PCV2 vaccines for growing-finishing pigs became commercially available in the United States. Several studies have since been conducted to evaluate the efficacy of these vaccines by using various criteria including mortality rate, viremia, coinfections, and growth rate.[16-20] Because of PMWS and possible subclinical effects of PCVD on pig growth, quantifying the impact of PCV2 vaccination on finishing pig performance under field conditions is also needed to justify the cost of vaccination. Therefore, these trials were conducted to evaluate the effects of a commercial PCV2 vaccine on growth performance, feed efficiency, and mortality in a commercial finishing facility.

Materials and Methods

Herd

Two experiments were conducted in a commercial swine research wean-to-finish facility in southwestern Minnesota with documented cases of PCVD. The herd is a porcine reproductive and respiratory syndrome (PRRS)-positive herd and had a historical finishing mortality rate of approximately 6% before the implementation of PCV2 vaccination.

Pigs and management

Experimental procedures used in the experiments were approved by the Kansas State University Institutional Animal Care and Use Committee. A total of 1,300 pigs, initially 24.3 kg, in groups of 27 pigs per pen were used in experiment one and a total of 1,253 pigs, initially 5.5 kg, in groups of 28 pigs per pen were used in experiment two. All pigs (PIC 337 × 1050) included in the study were evaluated physically before each experiment to ensure that only pigs free of any physical defect were included.

Pigs in both experiments were housed in pens measuring 5.5 × 3.0 m. The barns follow an all-in-all-out system and were double curtain sided with completely slatted flooring and a deep pit for manure storage. Each pen contained one self-feeder and one cup waterer. All pigs in each of the two experiments were fed similar diets based on corn and soybean meal in a phase feeding scheme based on body weight and formulated to meet or exceed NRC[21] recommendations for swine. Ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN) was added to the diet for the last 14 days in experiment two. Each barn at the research site used a robotic feeding system capable of delivering feed and providing data on feed amount delivered on an individual

pen basis (FeedPro; Feedlogic Corp., Willmar, MN). Pigs were weighed every 2 weeks during the course of the experiments. Pens were observed daily to ensure feeders and waterers were working properly and to check the health status of pigs as determined by physical appearance, pen activity, and absence or presence of abnormal clinical signs. Pigs with compromised welfare due to being sick or lagging behind in growth without evidence of recovery were removed from the study. Weights of pigs removed from the study (died or removed) were recorded at the time of removal. Seven days before the end of the test period, pigs visually identified as the heaviest in the pen (three per pen in experiment one, two per pen in experiment two) were weighed and marketed in accordance with the normal marketing procedures of the farm.

PCV2 vaccine

A commercially available killed, bacculovirus-expressed, capsid protein-derived vaccine (Circumvent; Intervet Inc., Millsboro, DE) was administered according to label dosage (2 mL per dose). Pigs in experiment one were vaccinated at 9 and 11 weeks of age; pigs in experiment two were vaccinated earlier at 5 and 7 weeks of age. The timing of vaccination in the first experiment was due to vaccine availability.

Experimental design

Experiments in this study were conducted using a completely randomized design.

In experiment one, pigs (648 barrows and 643 gilts) were individually weighed and ear tagged for identification. Pigs were then ranked within gender on the basis of body weight and randomly allotted within weight rank pairs to one of two treatments (control and vaccinated). Thus,

average weight was identical between control and vaccinated pigs before vaccination. Control groups were left unvaccinated, whereas pigs assigned to the vaccinated group were administered two doses of the vaccine (2 mL per dose at 9 and 11 weeks of age). The PCV2 vaccine was administered 15 days and 1 day before pigs were placed on test for the 96-day finishing period.

In experiment two, gilts and barrows were allocated in separate pens in the nursery. Pens were then ranked within gender on the basis of body weight and randomly allotted within weight rank pairs to one of two treatments (control and vaccinated) to ensure the same starting average pig weight for both treatments. Two doses of the vaccine (2 mL per dose) were administered to pigs from the vaccinated groups at 5 and 7 weeks of age (day 41 and 27 before pigs were placed on test), whereas pigs from the control groups were left unvaccinated. Vaccination occurred in the nursery phase, after which pigs were moved to the commercial research finishing site, commingled within vaccination group and gender, and gate-cut into their respective pens. Finisher pens were randomly assigned to treatment and gender. The on-test period was the last 105 days of the finishing period. Vaccinated and non-vaccinated pigs in each of the experiments were housed in the same barn throughout the study.

In both experiments, pen weights were obtained every 2 weeks during the on-test period to determine average daily gain (ADG; calculated by dividing weight gain by the number of pig days on test). Data from the feed delivery report generated by the automated feeding system were used to determine average daily feed intake (ADFI; calculated by dividing the total feed consumption per pen by the number of pig days on test). Gain-to-feed ratio (G:F) was calculated by dividing ADG by ADFI. On-test pigs that died during the finishing phase were recorded, and

mortality rate was calculated as the number of deaths divided by the initial number of pigs placed on test. Samples of clinically affected pigs with symptoms indicative of PCVD were necropsied, and tissue samples were submitted to a diagnostic laboratory to document PCVD-associated lesions and PCV2 infection. At the end of experiment two, pigs were individually tattooed according to pen number to allow for carcass data collection at the packing plant and data retrieval by pen. Pigs were transported to JBS Swift and Company (Worthington, MN) for processing and carcass data collection. Standard carcass criteria of loin and backfat depth, hot carcass weight, percentage lean, and yield were collected.

Serology

In experiment one, blood samples were collected from 10 randomly selected pigs (one pig per pen) from each treatment group on days -9, 14, 42, and 75 to determine serological status of pigs relative to PCV2, *Mycoplasma hyopneumoniae* (Mhyo), PRRS virus (PRRSV), and H1N1 and H3N2 swine influenza virus (SIV). Serum samples were then submitted to the Iowa State University Veterinary Diagnostic Laboratory and analyzed for the presence of antibodies by using an enzyme-linked immunosorbent assay (ELISA) for PCV2, Mhyo, and PRRS virus and a hemagglutination inhibition assay for SIV (H3N2 and H1N1). Five samples from the 10 serum samples for each treatment group were combined to create two pooled samples per treatment for detection of PCV2 nucleic acids with PCR. For ELISA results, sample-to-positive ratios of ≥ 0.3, > 0.4, and ≥ 0.4 were considered positive for antibodies against PCV2, Mhyo, and PRRSV, respectively. Titers > 3.2 ($\log_2$ transformed) were considered positive for antibodies against H1N1 and H3N2 SIV.

Statistical analysis

Growth performance and carcass data were analyzed as a completely randomized design. Analysis of variance was conducted on all data by using the GLIMMIX procedure of SAS version 9.1 (SAS Institute, Inc., Cary, NC) with the pen as the experimental unit in both experiments. The fixed effects of the statistical model were the effects of PCV2 vaccination (non-vaccinated or vaccinated), gender (barrow or gilt), and their interaction. Serological and PCR data were analyzed by repeated measures ANOVA using the MIXED procedure of SAS. Values are presented as least squares means, and all standard errors reported are pooled standard errors of the mean. Alpha level was set at 0.05 to assess significance among least squares means.

Results

Postmortem examination

Clinical signs and histopathologic lesions consistent with PCVD were found in necropsied pigs from both experiments.

Serology and PCR

Both treatment groups were seropositive for PCV2, and there was no difference ($P = 0.22$) in PCV2 antibody titers 9 days before pigs were put on test in the finisher (Figure 2-1). There was a vaccine-by-day interaction ($P < 0.01$) detected for PCV2; vaccinated pigs had increasing antibody titers as the trial progressed, whereas control pigs showed a decrease in antibody titers that reached negative levels on day 14 and then increased on days 42 and 75 on test. Antibody titers against PCV2 were substantially higher ($P = 0.02$) in vaccinated pigs on days 14 and 42 compared with non-vaccinated pigs. Both treatment groups tested positive for Mhyo and exhibited similar ($P = 0.52$) and constant ($P = 0.35$) antibody titers at all time points (Figure 2-

2). Both treatment groups tested positive for PRRS, and there was no difference between treatment groups (Figure 2-3). There were no H1N1 SIV antibodies detected (data not shown). Several pigs tested positive for H3N2 at day -9. However, the average number of antibody titers for both groups at day -9 was below the cutoff point to be considered positive because of a few pigs that had zero antibody titers (Figure 2-4). Both groups seroconverted on day 14 before antibody titers decreased to negative levels in succeeding sampling time points. Results of PCR showed that all pools tested were positive for PRRSV at days -9 and 14 and negative on days 42 and 75. All pools were positive for PCV2 at all time points, except for one negative pool on day -9 and one negative pool on day 75 (Table 2-1).

Mortality

In experiment one, there was no vaccine-by-gender interaction ($P > 0.37$) for mortality rate. However, mortality rate was lower ($P = 0.05$) in the vaccinated group (3.1%) than in the non-vaccinated group (5.9%; Table 2-2). Barrow mortality rate was numerically but not significantly higher ($P = 0.28$) than gilt mortality rate. In experiment two, there also was no vaccine-by-gender interaction ($P = 0.12$) detected, but the vaccinated group had a lower ($P < 0.001$) mortality rate (3.0%) than the non-vaccinated group (9.2%; Table 2-3). As shown in Figure 2-5, cumulative mortality rate among the non-vaccinated groups showed a sudden increase in mortality rate after week 4 and continued increasing until week 12. Similar to the results in experiment one, mortality rate was higher ($P = 0.04$) in barrows than in gilts (7.7% vs. 4.5%).

Growth performance

For experiment one, there were no vaccine-by-gender interactions for any of the growth criteria ($P > 0.85$; Table 2-2). However, PCV2 vaccination had a significant effect on growth; vaccinated pigs exhibited greater ADG (952 g; $P < 0.001$) than non-vaccinated pigs (920 g). This was due to vaccinated pigs having greater ($P = 0.005$) ADFI and better G:F (2.40 kg and 0.396, respectively) than non-vaccinated pigs (2.36 kg and 0.390, respectively). As expected, barrows exhibited greater ADG (952 g vs. 920 g; $P < 0.001$) and ADFI (2.47 kg vs. 2.30 kg; $P < 0.001$) than gilts. Also, as expected, feed efficiency was poorer ($P < 0.01$) in barrows than in gilts (0.386 vs. 0.401).

For experiment two, there was a vaccine-by-gender interaction ($P = 0.01$) for ADG as PCV2 vaccination improved ($P = 0.01$) ADG in barrows but not in gilts (Table 2-3). Overall, vaccinated pigs exhibited greater ($P < 0.0001$) ADG than non-vaccinated pigs (920 g vs. 887 g). The decrease in ADG among non-vaccinated pigs appeared to have occurred between 2 and 6 weeks on test (Figure 2-6). There were no significant differences in ADFI ($P = 0.28$) and G:F ($P = 0.14$) between vaccinated and non-vaccinated groups. Similar to experiment one, barrows had greater feed intake ($P < 0.001$; 2.35 vs. 2.19) but poorer G:F ($P = 0.014$; 0.39 vs. 0.41) than gilts.

Weight

In experiment one, there were no vaccine-by-gender interactions for average weight ($P > 0.52$), but barrows were 2.5 kg heavier ($P < 0.01$) than gilts at the end of the trial. However, vaccinated pigs tended ($P > 0.06$) to be 1.5 kg and 1.3 kg heavier than control pigs at day 89 (118.4 vs. 117.0 kg) and at market (119.2 vs. 117.9 kg), respectively. This is worth noting because

vaccinated pigs were 0.8 kg lighter ($P = 0.02$) than control pigs when placed on test in the finisher just after the second dose of vaccine was administered.

In experiment two, there were vaccine-by-gender interactions ($P < 0.05$) for weight on day 98 on test and at market. Vaccination against PCV2 resulted in heavier weights among vaccinated barrows at day 98 on test and at market compared with non-vaccinated barrows, but this was not seen in vaccinated vs. non-vaccinated gilts. Overall, the improvement in ADG resulted in vaccinated pigs being 2.7 kg and 2.9 kg heavier ($P < 0.005$) than non-vaccinated pigs at day 98 and at market. Because of the heavier average body weight, vaccinated pigs also had heavier ($P = 0.03$) carcass weights (92.4 vs. 90.9 kg) than non-vaccinated pigs, but percentage yield was equal ($P = 0.22$) between the two groups. After adjusting to a common carcass weight, there were no differences ($P > 0.37$) detected for percentage lean, loin depth, and backfat.

Discussion

Since the commercial introduction of PCV2 vaccines in 2006, several studies have been published that documented these vaccines' efficacy in reducing mortality due to PCVD.[16-20,22-25] However, production data measuring growth rate and feed efficiency of PCV2-vaccinated pigs in those studies was limited. The killed, bacculovirus-expressed, capsid protein-derived vaccine used in this study proved effective at minimizing the negative effects of PCVD as indicated by the reduction in mortality and improved growth performance and feed efficiency of vaccinated pigs. Vaccination against PCV2 resulted in a reduction in mortality of 47% (5.9 vs. 3.1%) in experiment one and 67% (9.2 vs. 3.0%) in experiment two. The reduced mortality rate in vaccinated pigs was consistent with that reported in previous studies.[16-18] Although interactions between vaccine and gender were not significant for mortality rate, barrows had a numerically

higher mortality rate than gilts in both experiments. Previous research reported similar results, wherein gilts showed a relative risk reduction of 76%, compared with only 46% for barrows, for finishing mortality when vaccinated against PCV2.[16] This suggests that even though the vaccine was able to reduce the negative effects of PCVD, barrows remained more susceptible to the disease than gilts. However, in contrast to Horlen et al.,[16] a greater reduction in barrow mortality rate was observed in our study and was consistent across the two experiments.

Porcine circovirus type 2 is now widely accepted as the main causative agent of PCVD, but evidence from previous research indicates that the presence of coinfections is needed to trigger development of clinical disease.[26-29] The specific roles of other infectious agents in PCVD have yet to be explained. However, it is clear that these agents, when not controlled, can exacerbate PCVD-associated conditions in the same way that other disease syndromes can be magnified by the presence of PCV2, such as in cases of respiratory diseases.[30] A study on the effect of PCV2 vaccination in pigs with porcine respiratory disease showed a numeric reduction in mortality rate among vaccinated pigs compared with placebo-treated pigs (6.6 vs. 8.7%).[17] The authors suggested the reduced mortality rate could be due to reduced viral burden brought about by PCV2 vaccination, which possibly led to a reduction in clinical effects of porcine respiratory disease. The authors noted, however, that according to the results of their study, PCV2 vaccination can help reduce clinical disease but not completely prevent other respiratory pathogens. In other research, a 53% (7.5% vs. 3.5%) reduction in mortality rate was observed for vaccinated pigs compared with the placebo group.[18] These researchers also observed that the quantity of coinfecting agents recovered from tissues of placebo-treated animals was higher than that recovered from vaccinated pigs. This suggests PCV2 vaccination is effective at minimizing

coinfections. This observation further supports the theory that PCV2, when present and in the absence of vaccination, weakens the immune system and predisposes the pig to other infectious agents. In the present study, we did not observe significant differences in antibody titers between controls and vaccinates for Mhyo, PRRS, and SIV, indicating that coinfections were not reduced after PCV2 vaccination. This agrees with the results of Fachinger et al.[17] However, despite having similar levels of coinfections, vaccinated pigs had a lower mortality rate than non-vaccinated pigs.

Results of our study are further confirmation of the major role PCV2 plays in causing PCVD and support findings from recent studies on the direct relationship between PCV2 and PCVD. In the present study, there was a marked increase in PCV2 antibodies from 4 to 8 weeks (days 14 and 42 on test) among vaccinated pigs following vaccination. Thus, the vaccine was successful at stimulating an immune response and inducing production of antibodies against PCV2. Fachinger et al.,[17] noted an increase in antibody titers in vaccinated pigs at 4 and 8 weeks after vaccination that provided protection for at least 15 weeks. They also noted that efficacy of the vaccine was not affected by the relatively high level of maternal antibodies present at the time of vaccination; this is similar to the observations made by Kixmoller et al.[18] and Opriessnig et al.[24], who used bacculovirus-expressed PCV2 ORF2 and PCV 1-2 chimeric vaccines, respectively. In the present study, non-vaccinated pigs had antibodies against PCV2 9 days before the start of the trial. These were possibly maternal antibodies as evidenced by the declining titer when non-vaccinated pigs were sampled at the subsequent time point (23 days later). However, antibody levels in non-vaccinated pigs increased at the succeeding sampling points, suggesting that pigs were actively getting infected during those times. In the case of vaccinated pigs, no further increase in antibody

titers occurred after day 42. This agrees with a previous PCV2 vaccine study[17] in which an increase in the antibody titer after vaccination resulted in a decrease in serologic response in vaccinated animals after exposure to PCV2. The observed increase in antibody titers in vaccinated pigs 4 weeks after vaccination (day 14 on test) suggests the vaccine used in this study was effective even in the possible presence of maternal antibodies.

Among syndromes associated with PCV2, PMWS is considered the most economically important.[2] One objective of the present study, aside from the effect of PCV2 on mortality, was to evaluate vaccine efficacy in terms of eliminating or minimizing PMWS and improving growth performance of susceptible pigs. We measured ADG and feed efficiency of pigs as indicators of vaccine efficacy. Vaccinating against PCV2 improved ADG, and barrows showing greater improvement in ADG than gilts. Thus, aside from being more protective in barrows than gilts on the basis of mortality rate, PCV2 vaccination appears to be more beneficial in barrows than gilts from a growth performance standpoint.

Horlen et al.[16] reported a 9.3% increase in ADG among vaccinated pigs that resulted in an 8.8 kg difference at market between vaccinated and non-vaccinated pigs. Another study reported an 18 g/day improvement in ADG among PCV2-vaccinated pigs compared with placebo-treated pigs which reduced days to market by 5.6 days.[17] In a third study, use of a single-dose PCV2 vaccine resulted in a 4.70 kg greater weight gain in vaccinated pigs compared with placebo-treated pigs.[18] In the present study, vaccinated pigs were 1.3 and 2.9 kg heavier at market than non-vaccinated pigs in experiments one and two, respectively. These results indicate the consistent efficacy of PCV2 vaccination in terms of growth performance improvement.

The effect of vaccine on ADG over time is shown in Figure 2. Growth rate differences between control and vaccinated pigs peaked between the second and sixth week on test. The decreased ADG in unvaccinated pigs preceded the observed rise in mortality, and the greatest difference in cumulative mortality between vaccinated and unvaccinated pigs was noted between the sixth and 12th week on test (Figure 1). The period in which increased mortality occurred in this trial was consistent with the study of Horlen et al.[16], who observed an increased mortality rate between the sixth and 14th week of the finishing phase.

Ractopamine HCl was added to the diets of both treatment groups 3 weeks before market, which explains the observed increase in ADG from day 84 to 98. Ractopamine HCl is a β-adrenergic agonist commonly used as a feed additive in pig diets during the last 3 to 4 weeks before market to improve pig growth performance and carcass leanness.[31]

Feed efficiency is another production parameter that could be used to evaluate improvement in overall herd health because feed intake and growth rate of pigs is negatively affected during disease conditions.[32,33] The significant improvement in feed efficiency exhibited by vaccinated pigs in experiment one was a clear indicator of improved health status of the vaccinated group. Although not statistically significant, the same magnitude of improvement in G:F was also observed in vaccinated pigs compared with controls in experiment two. Chronic infection with pathogenic microorganisms causes negative metabolic effects that can lead to reduced feed intake, inefficient utilization of nutrients, and, ultimately, poor growth performance.[34,35] It is possible that the protection conferred by vaccination may have led to a more efficient use of

energy for growth and lower amounts of energy and nutrients being spent on eliminating infectious agents and repairing tissue damage.

Implications

- To our knowledge, this study was the first to use ADG and G:F in addition to mortality rate to evaluate the efficacy of a commercially available vaccine under actual field conditions.
- The PCV2 vaccine used in this study is effective in reducing mortality rate and improving growth performance of pigs in a PCV2-infected herd as indicated by heavier weights and improved feed efficiency in the vaccinated pigs.
- The positive effects of PCV2 vaccination on growth performance observed in this study further validate the role of PCV2 as the main pathogenic organism in PCVD.

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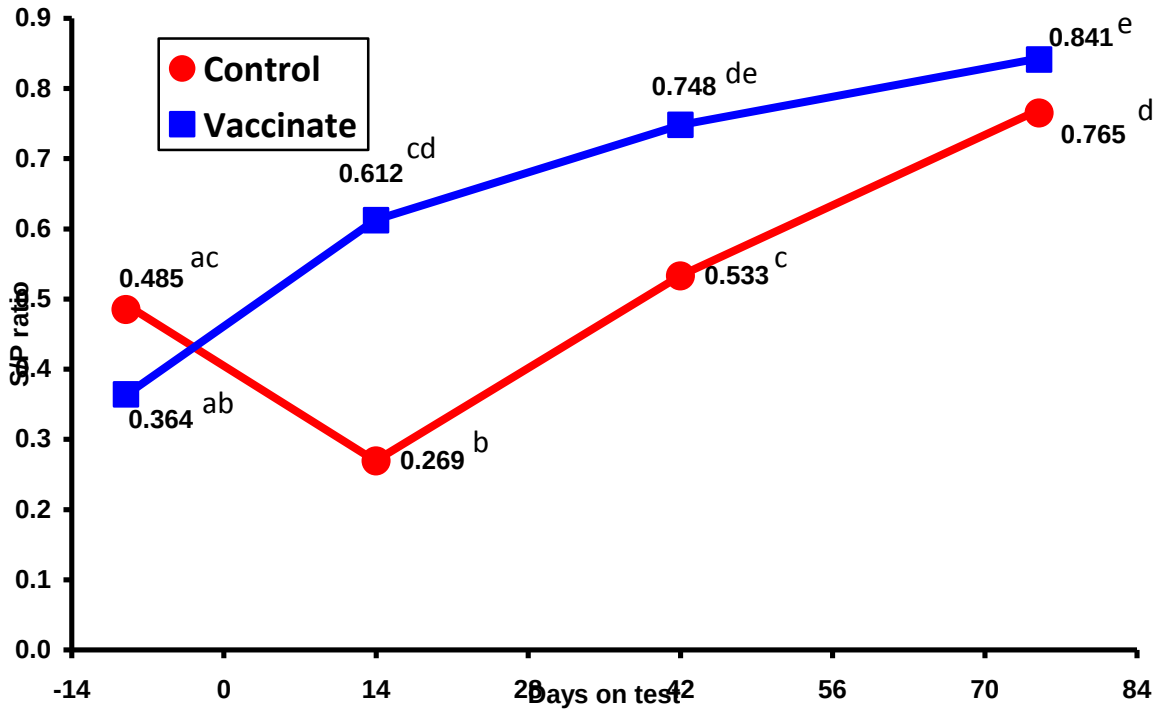

Figure 2-1: Antibody titers of porcine circovirus type 2-vaccinated and non-vaccinated pigs against PCV2 as determined by enzyme-linked immunosorbent assay. Samples with S/P ratio ≥ 0.3 were considered positive for antibody against PCV2. Each mean is the average S/P ratio from 10 pigs for each day on test. Vaccine-by-day interaction, $P < 0.01$. Values with different superscripts differ ($P < 0.05$).

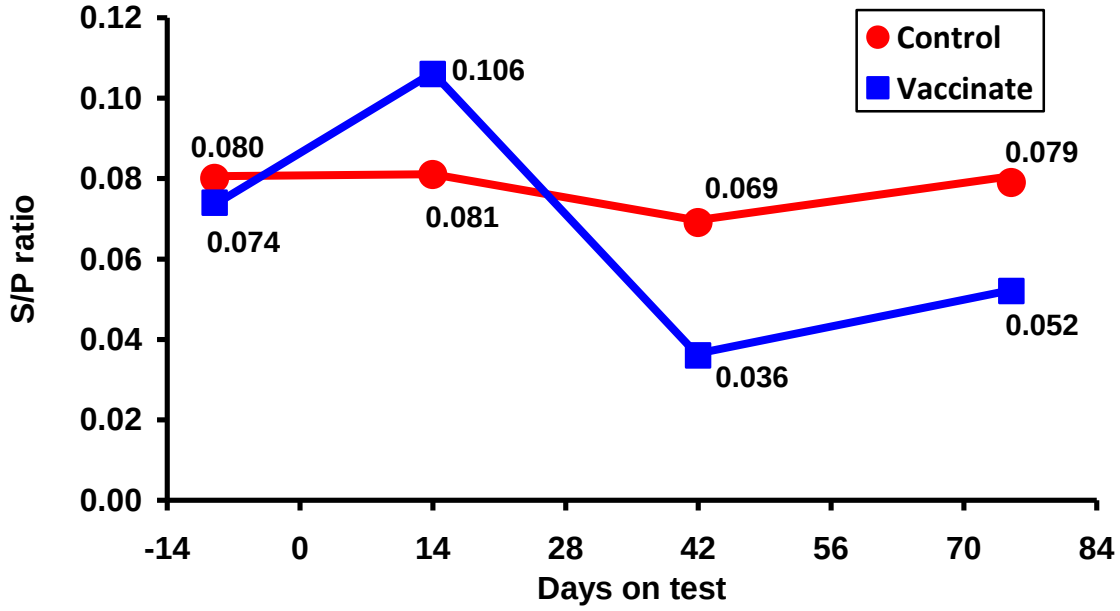

Figure 2-2: Antibody titers of porcine circovirus type 2-vaccinated and non-vaccinated pigs against *M. hyopneumoniae* as determined by enzyme-linked immunosorbent assay. Samples with S/P ratio > 0.4 were considered positive for antibody against *M. hyopneumoniae*. Each mean is the average S/P ratio from 10 pigs for each day on test. No significant vaccine-by-day interaction or vaccine or day effects ($P > 0.35$).

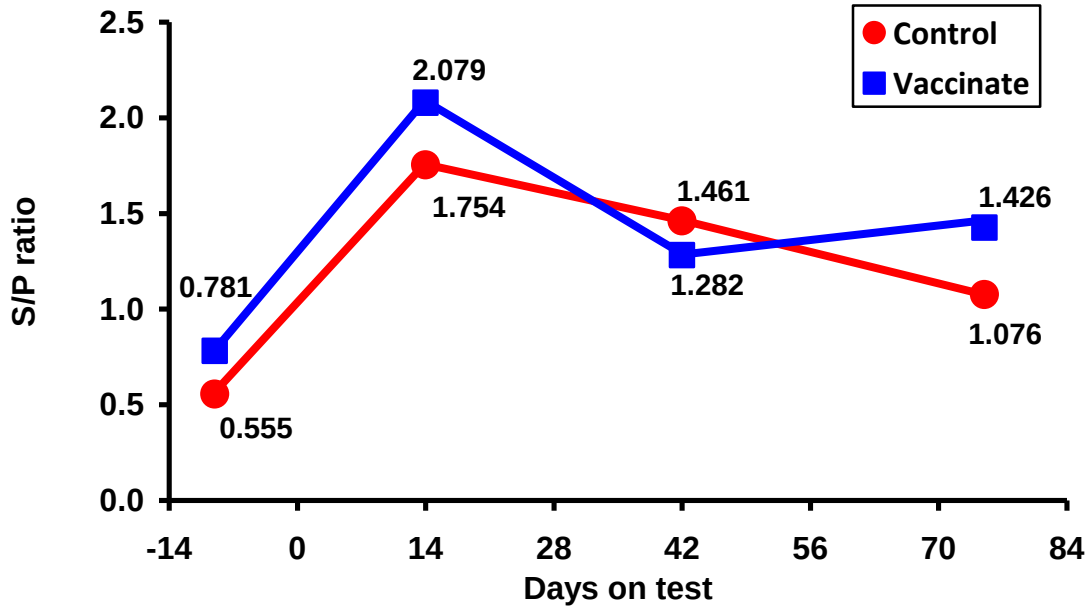

Figure 2-3: Antibody titers of porcine circovirus type 2-vaccinated and non-vaccinated pigs against PRRSV as determined by enzyme-linked immunosorbent assay. Samples with S/P ratio ≥ 0.4 were considered positive for antibody against PRRSV. Each mean is the average S/P ratio from 10 pigs for each day on test. No significant vaccine-by-day or vaccine main effects ($P > 0.43$). Significant day effect ($P < 0.001$).

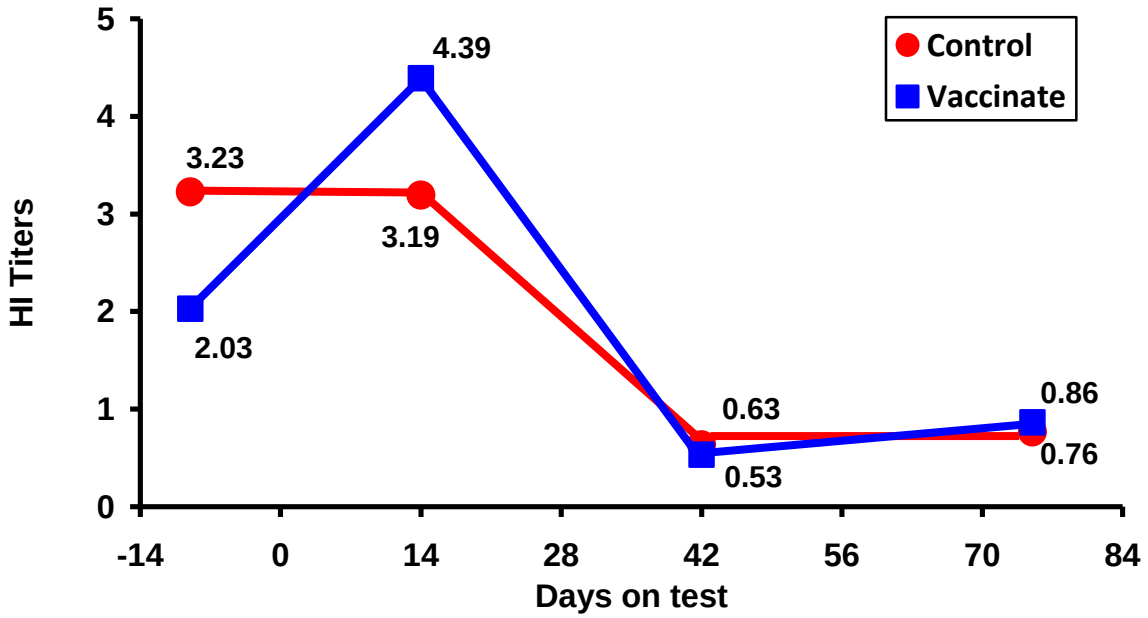

Figure 2-4: Antibody titers (log_2 transformed) of porcine circovirus type 2-vaccinated and non-vaccinated pigs against H3N2 swine influenza virus (SIV) as determined by hemagglutination inhibition test. Titers > 3.3 were considered positive for antibody against H3N2 SIV. Each mean is the average S/P ratio from 10 pigs for each day on test. No significant vaccine-by-day or vaccine main effects ($P > 0.46$). Significant day effect ($P < 0.001$). No H1N1 SIV antibodies were detected.

Table 2-1: Porcine circovirus type 2 and porcine reproductive and respiratory syndrome status of vaccinates and controls as determined by PCR*

			Test result			
Serum pool†	**PCR**	**Treatment**	**Day -9**	**Day 14**	**Day 42**	**Day 75**
1	PRRS	Vaccinate	+	+	-	-
2	PRRS	Vaccinate	+	+	-	-
3	PRRS	Control	+	+	-	-
4	PRRS	Control	+	+	-	-
1	PCV2	Vaccinate	-	+	+	+
2	PCV2	Vaccinate	+	+	+	-
3	PCV2	Control	+	+	+	+
4	PCV2	Control	+	+	+	+

*Serum samples were collected from 10 pigs and analyzed for the presence of PCV2 and PRRS nucleic acids by PCR.

†Five samples were pooled to form two pools of serum for each treatment group.

Table 2-2: Effects of gender on the efficacy of a porcine circovirus type 2 vaccine (Exp. 1)*†

	Barrow		Gilt			*P* values	
Item	**Control**	**Vaccine**	**Control**	**Vaccine**	**SEM**	**Vaccine**	**Gender**
Mortality, %	7.4	3.1	4.3	3.2	1.36	0.05	0.28
Day 0 to 96							
ADG, g	937	968	904	935	6.9	<0.001	<0.001
ADFI, kg	2.45	2.49	2.27	2.32	0.02	0.005	<0.001
G:F	0.383	0.389	0.398	0.404	0.002	0.01	<0.0001
Weight, kg							
Day 0	35.9	34.8	35.8	35.1	0.4	0.02	0.76
Day 89	118.1	119.9	115.8	116.9	0.8	0.06	0.001
Market‡	119.1	120.6	116.7	117.8	0.7	0.07	<0.001

*Commercial PCV2 vaccine (Circumvent; Intervet Inc., Millsboro, DE; 2 mL per dose) administered at 9 and 11 weeks of age.
†A total of 1,300 pigs were randomly assigned to one of the two treatments within barrows and gilts. Data were analyzed as a completely randomized design by ANOVA using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. The fixed effects of the statistical model were the effects of PCV2 vaccination (non-vaccinated or vaccinated), gender (barrow or gilt), and their interaction. There was no significant vaccine-by-gender interaction detected for any of the measured criteria ($P > 0.05$).
‡Market weight was the average weight of pigs topped 7 days before the end (day 89) of the trial and the pigs remaining at the end of the trial (day 96).

Table 2-3: Effects of gender on the efficacy of a porcine circovirus type 2 vaccine (Exp. 2)*†

	Barrow		Gilt			*P* value		
Item	**Control**	**Vaccine**	**Control**	**Vaccine**	**SEM**	**Vaccine × Gender**	**Vaccine**	**Gender**
Mortality rate, %	12.0	3.4	6.5	2.6	1.58	0.12	0.0002	0.04
Day 0 to 105								
ADG, g	894[a]	944[b]	881[a]	895[a]	7.6	0.01	<0.0001	0.0001
ADFI, kg	2.31	2.38	2.19	2.19	0.033	0.27	0.28	<.0001
G:F	0.387	0.397	0.403	0.411	0.387	0.87	0.14	0.014
Weight, kg								
Day 0	26.1	25.6	26.2	25.7	0.49	0.96	0.28	0.84
Day 98	113.7[a]	118.1[b]	112.6[a]	113.4[a]	0.94	0.05	0.005	0.003
Market‡	119.9[d]	124.7[b]	118.8[a]	119.7[a]	0.99	0.04	0.004	0.002
Carcass traits								
Carcass weight, kg	92.0	94.3	89.8	90.5	0.75	0.28	0.03	<0.0001
Yield, %	75.3	76.2	76.0	76.1	0.38	0.29	0.22	0.40
Backfat, cm§	1.73	1.74	1.51	1.50	0.028	0.73	0.93	<0.0001
Lean, %§	55.7	55.5	56.9	57.1	0.25	0.52	0.95	<0.0001
Loin, cm§	6.14	6.14	6.28	6.40	0.066	0.37	0.37	0.01

*Commercial PCV2 vaccine (Circumvent; Intervet Inc., Millsboro, DE; 2 mL per dose) administered at 5 and 7 weeks of age to the vaccine treatment (41 and 27 days before being placed on test in the finisher).

†A total of 1,253 pigs (initially 5.5 kg) were assigned randomly by nursery pen average weight to one of the two treatments within barrows and gilts before administration of the first vaccine dose. Data were analyzed as a completely randomized design by ANOVA using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with the pen as the experimental unit. The fixed effects of the statistical model were the effects of PCV2 vaccination (non-vaccinated or vaccinated), gender (barrow or gilt), and their interaction.

‡Market weight was the average weight of pigs topped 7 days before the end (day 98) of the trial and the pigs remaining at the end of the trial (day 105).

§Values were adjusted to a common carcass weight by using carcass weight as a covariate in the model.

[a,b]Values within a column with different superscripts differ significantly ($P < 0.05$).

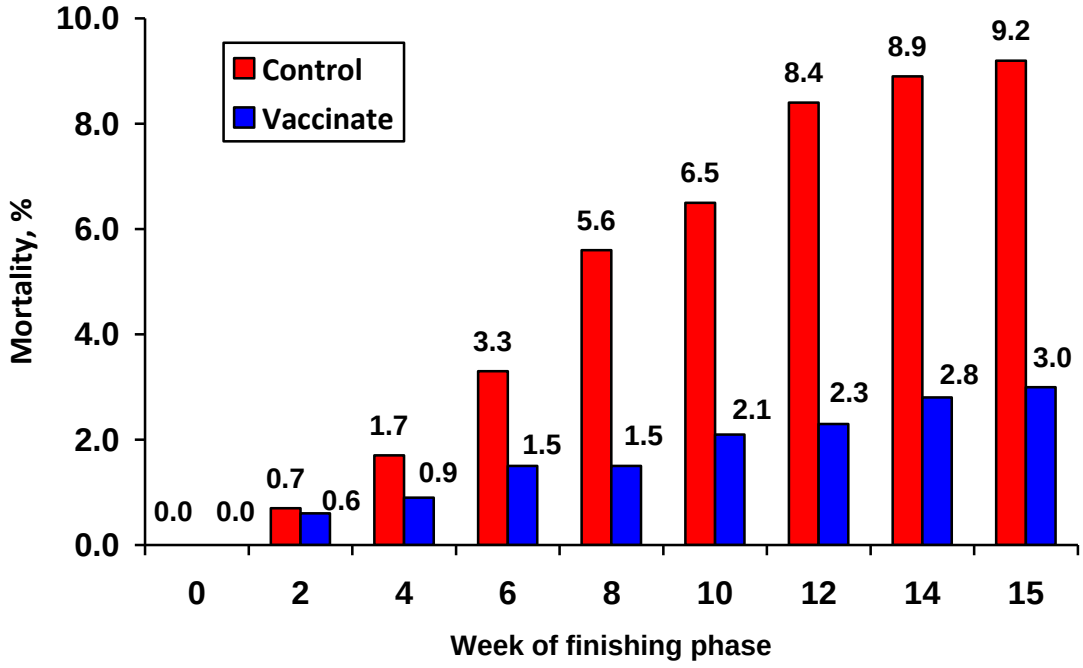

Figure 2-5: Effect of porcine circovirus type 2 vaccination on cumulative mortality rate in non-vaccinated and vaccinated pigs from day 0 to 105 on test (Exp. 2).

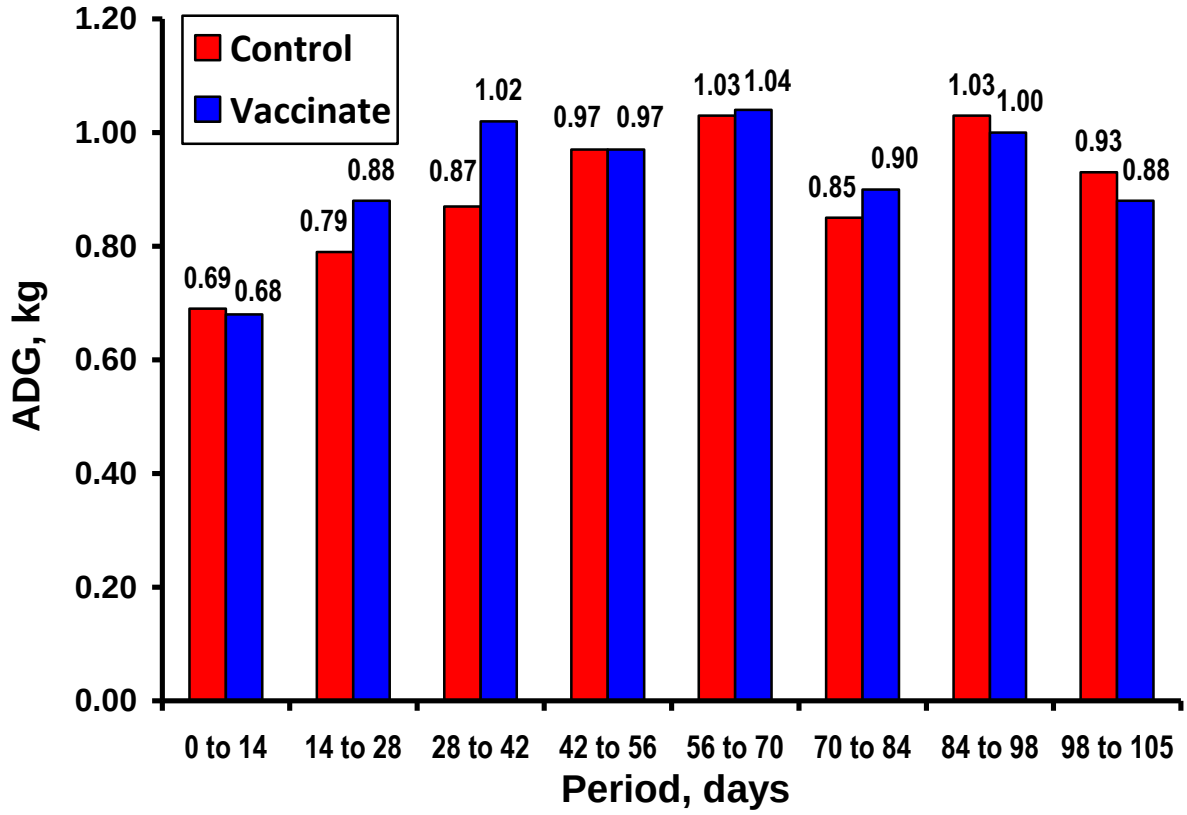

Figure 2-6: Growth rate during each period for unvaccinated and PCV2-vaccinated pigs over time (Exp. 2; day 0 to 105).

CHAPTER 3 - Comparison of two porcine circovirus type 2 (PCV2) vaccines on growth and mortality rate in a PCV2-positive commercial swine herd

Summary

Objective: To compare the effects of two commercially available porcine circovirus type 2 (PCV2) vaccines (one- or two-dose) on growth and mortality rate of pigs in a commercial facility with a history of porcine circovirus disease (PCVD).

Materials and Methods: A total of 1,470 crossbred pigs (825 barrows and 645 gilts; TR4 × PIC C22) were used in a randomized complete block design with a 2 × 3 factorial arrangement of treatments. Pigs (initially 8.8 kg BW) were allotted randomly to the control or one of the two vaccine treatments. The first vaccine was administered 1 week after weaning (one-dose; Suvaxyn PCV2 One Dose; Fort Dodge Animal Health, Fort Dodge, IA). The second was administered at weaning and 3 weeks later (two-dose; Circumvent PCV; Intervet, Inc., Millsboro, DE). Pigs were weighed individually on days 0 (weaning), 113, and 143 and at off test. Pigs from the three treatments were commingled within pens and received similar diets throughout the experiment. A subsample of pigs was submitted to a veterinary diagnostic laboratory for necropsy and histopathological examination.

Results: Histopathologic lesions associated with PCVD were observed in the necropsied pigs. There was no significant difference in mortality rate between treatments, but each vaccinated group had numerically lower mortality than control pigs (7.8% for the one-dose and 7.7% for the two-dose group vs. 11.0% for the control group). On day 113, the two-dose group was heavier (*P*

< 0.05) than the control group (86.5 vs. 82.5 kg), and the one-dose group was intermediate (85.4 kg). On day 143, the one- and two-dose groups were 3.4 and 4.6 kg heavier ($P < 0.05$) than the control pigs, respectively, but there was no difference ($P = 0.33$) between the two vaccinated groups. At off test, the two-dose group was heavier ($P < 0.05$) than the control group (120.2 vs. 116.4 kg), and the one-dose group was intermediate (118.8 kg). The one- and two-dose groups had greater average daily gain (ADG; $P < 0.05$) than the control group from day 0 to 113 (0.676 and 0.689 vs. 0.653 kg), day 0 to 143 (0.717 and 0.726 vs. 0.694 kg), and day 0 to off test (0.726 and 0.735 vs. 0.703 kg). From day 113 to 143 and at off test, ADG between groups was not different, suggesting that the increase in growth rate in vaccinated pigs occurred from day 0 to 113.

Implications: In the presence of natural infection, the PCV2 vaccines evaluated in this study were effective at improving the growth rate of pigs from weaning to finishing as shown by the greater ADG and heavier body weights of the vaccinated groups.

Keywords: growth, porcine circovirus type 2, swine, vaccine

Porcine Circovirus Disease (PCVD) is of major economic importance in the swine industry mainly because it can cause high death loss and poor growth performance.[1] The disease is caused by porcine circovirus type 2 (PCV2); thus, the condition is not responsive to antibiotic treatment. Clinical signs of the disease include poor body condition with various degrees of muscle wasting, labored breathing, and enlarged lymph nodes.[2,3] The absence of effective

therapeutic treatment coupled with the highly stable nature[4] and ability of PCV2 to resist inactivation[5,6] in the pig's environment make the disease difficult to control.

Since it was first described in Canadian herds in 1996,[7] PCVD has become widespread in swine herds throughout the world.[8] In the United States, reports of PCVD cases rapidly increased in 2005. Mortality rates as high as 20% were documented.[9,10] In the absence of any effective tool to control the disease, PCVD spread readily throughout the country. In 2006, PCV2 vaccines for growing pigs became commercially available in the United States. Early studies reported the vaccines were effective against PCVD. However, most of these studies were designed to evaluate the vaccines in terms of mortality reduction. Because commercial vaccines vary in antigen and adjuvant composition, differences in efficacy between vaccines may exist. Therefore, the objective of this trial was to compare the effects of two commercially available PCV2 vaccines (one- or two-dose) on growth rate and mortality.

Materials and Methods

Herd history

The experiment was conducted in a commercial farrow-to-finish swine operation with previously documented cases of PCVD. The swine operation is located in northeast Kansas and has a multi-site all-in-all-out production system. The herd is positive for porcine reproductive and respiratory syndrome (PRRS) virus, *Mycoplasma hyopneumoniae,* and *Actinobacillus pleuropneumoniae.* Pigs are weaned at 21 days of age and transferred to nursery barns at a separate location, where they are kept for 8 weeks before being transferred to finishing barns at a third location, where they are grown until marketing.

Pigs and management

Experimental procedures used in the experiment were approved by the Kansas State University Institutional Animal Care and Use Committee. A total of 1,470 pigs (825 barrows and 645 gilts; TR4 × PIC C22) from three weaning groups were transferred from the farrowing facility to three separate rooms in a common nursery barn. These pigs were physically evaluated and appeared healthy and free of any physical defect before the start of the experiment. The nursery rooms were mechanically ventilated and had perforated plastic flooring. Pens were equipped with a self-feeder and waterer. Pigs were kept in the nursery rooms for 8 weeks before being transferred to the finishing site. The finishing barns were double curtain sided, naturally ventilated buildings with slatted flooring. Each building had four rooms and a total capacity of 500 pigs per room. Pigs were fed similar diets based on corn or sorghum and soybean meal. Pens were observed daily to perform routine health checks and monitor the overall condition of the pigs in the barn. Pigs with compromised welfare due to being sick or lagging behind in growth without evidence of recovery were removed from the study. Weights of pigs removed from the study (died or removed) were recorded at the time of removal.

PCV2 vaccine

Two commercially available vaccines (one- and two-dose) were used and administered according to label recommendations. The one-dose vaccine was an inactivated chimeric PCV2 vaccine (Suvaxyn PCV2 One Dose; Fort Dodge Animal Health, Fort Dodge, IA). The two-dose vaccine was a killed, bacculovirus-expressed, capsid protein-derived vaccine (Circumvent PCV; Intervet Inc., Millsboro, DE). Products were selected for use on the basis of their commercial availability at the time of study.

Experimental design

A total of 1,470 weaned pigs (825 barrows and 645 gilts) were weighed individually in groups of 3 within each gender and assigned to one of the 3 treatments and sequentially allocated as the heavy pig assigned to the control treatment, the medium weight pig to the one dose, and the light weight pig to the two dose treatment. The subsequent group of three pigs was then assigned with the heaviest pigs assigned to the one dose treatment, the medium weight pig to the two dose treatment, and the light weight pig to the control treatment. The next group then had the heaviest pig assigned to the two dose treatment, the medium weight pig to the control treatment and the light weight pig assigned to the one dose treatment. This process was then repeated and started again with the assignments for the first three pigs. During allotment, each pig was individually identified using color-coded ear tags that correspond to each treatment. Barn staff members that provided daily pig care were blinded to the treatment classification of ear tag color. Thus, all the pigs were equally represented in each treatment group and gender. Pigs were placed on test from three different weaning groups, and weaning group was considered a block. The three treatments were a negative control (non-vaccinated) and one- and two-dose-vaccinated groups. One-dose pigs were vaccinated 1 week after weaning; two-dose pigs were vaccinated at weaning and again 3 weeks later.

Each weaning group initially was housed in three separate mechanically ventilated nursery rooms and was then transferred to open-sided, naturally ventilated buildings during the growing to finishing phase. Pigs were weighed individually on days 0, 113, and 143 and at off test (just before market) to determine average daily gain (ADG). Weighing of pigs just before market was

done in several batches for each group as part of the normal marketing procedure (topping) of the farm. Thus, heavier pigs were weighed earlier than the rest of the pigs if they weighed a minimum of 122 kg before the scheduled final weigh date for each block. Average daily gain was calculated by dividing the total weight gain over the number of pig days. Only the ADG of pigs that were marketed and weighed at off test were included in data analysis for growth rate. On-test pigs that died were recorded, and mortality rate was calculated as number of deaths divided by the initial number of pigs placed on test. A total of 15 pigs (5 nursery and 10 finishing) with clinical signs indicative of PCVD were submitted to the Kansas State University Diagnostic Laboratory for necropsy and histopathological examination to confirm the presence of PCV2 infection.

Statistical analysis

Data were analyzed as a 3 × 2 factorial randomized complete block design using the MIXED procedure of SAS. The fixed effects were vaccine treatment (control, one-dose, and two-dose) and gender (barrow or gilt), and the random effect was wean group (block). Individual pig was the experimental unit. Values are presented as least squares means, and all standard errors reported are pooled standard errors of the mean. Alpha level was set at 0.05 to assess significance among means.

Results

Postmortem examination

Histopathologic lesions consistent with PCV2 infection were noted in pigs necropsied from each of the three weaning groups.

Mortality

There was no vaccine-by-gender interaction detected for mortality rate ($P = 0.47$). Although there was no difference (P = 0.42; Table 3-1) between treatment groups, mortality rate was numerically lower in the one- (7.8%) and two-dose (7.7%) vaccinated groups compared with the controls (11.0%). Also, there was no difference ($P = 0.86$) in mortality rate between gilts and barrows.

Weight

There was no significant interaction between vaccination treatment and gender ($P > 0.61$) on all weigh days for body weight. However, average weight of two-dose pigs was greater ($P < 0.05$; 86.5 vs. 82.4 kg) than that of the control pigs at mid-finishing (day 113) but not different from one-dose pigs, which were intermediate (85.4 kg). On day 143, there was no significant difference in average pig weight between the two vaccinated groups. However, one-dose pigs were 3.4 kg heavier and two-dose pigs were 4.6 kg heavier ($P < 0.03$; 111.0 and 112.2 vs. 107.6 kg) than the control group. This was demonstrated by the significant shift to a heavier weight distribution (Figure 3-1) for the vaccinated groups relative to the control. A greater number of pigs in the vaccinated groups weighed ≥120 kg compared with the control group on day 143. Pigs on the two-dose treatment had heavier ($P < 0.05$; 120.2 vs. 116.4 kg) off-test weights than non-vaccinated pigs, and one-dose pigs were intermediate (118.8 kg). Barrows were heavier ($P < 0.05$) than gilts for all weigh dates after d 113.

Growth rate

There were no vaccine-by-gender interactions ($P > 0.19$) for ADG at any weigh period. The one- and two-dose pigs had similar ADG in all periods of the experiment. However, both vaccinated groups had greater ADG ($P < 0.05$) than the control group from day 0 to 113 (0.677 and 0.689 vs. 0.651 kg), on day 143 (0.716 and 0.727 vs. 0.693 kg), and at off test (0.726 and 0.737 vs. 0.705 kg). There were no differences in ADG ($P > 0.25$) between vaccination treatments from day 113 to 143 and at off test. Barrows had greater ($P < 0.02$) ADG than gilts from day 0 to 113 (0.686 vs. 0.659 kg), on day 143 (0.730 vs. 0.694 kg), and at off test (0.740 vs. 0.704 kg).

Discussion

Results of the present study (i.e., improvements in growth among vaccinated pigs and numeric reduction in mortality rate) confirmed the efficacy of these two vaccines in actual field conditions. Porcine circovirus type 2 vaccines have become valuable tools in controlling mortality rates associated with PCVD. Prior to introduction of the vaccines, finishing mortality rates from 4% to 20% were reported.[9-11] Use of vaccines in PCV2-infected herds has resulted in significant mortality rate reductions regardless of the commercial product used.[12-14] However, use of commercial PCV2 vaccines may not always reduce mortality rates to normal or pre-PCVD levels[15], suggesting that results may vary among vaccines. Response to vaccines may be influenced by a number of factors including proper vaccination protocols, correct dose, age, and health status of the animals. In the present study, differences in mortality rate between vaccinated and non-vaccinated pigs were numerical but not statistically significant. We theorize that the absence of statistical difference among the treatments was due to greater variability as a result of a respiratory disease outbreak during the trial. A clinical outbreak of bacterial disease due to *Haemophilus parasuis* was noted in two groups during the nursery phase. Additionally, an

outbreak of respiratory disease due to *Actinobacillus pleuropneumoniae* was noted in one finisher group.

Direct comparisons between commercially available PCV2 vaccines in terms of the duration of immunity have been reported previously. Opriessnig et al.[16] found a greater reduction in viremia with two-dose vaccines compared with one-dose vaccines (97% vs. 79%). However, they noted that the differences were driven largely by one of the one-dose PCV2 vaccine brands and that there was no difference in viremia between one- and two-dose preparations that came from the same manufacturer. In the present study, we evaluated efficacy of the PCV2 vaccines on the basis of growth rate improvement and mortality rate reduction under commercial production conditions in a PCV2-positive herd with a history of other bacterial and viral diseases commonly found in PCVD cases. It is widely accepted that other cofactors or infectious agents are necessary for PCVD to develop or manifest in the herd[17-20]. The presence of coinfections may also exacerbate PCVD.[21] These agents include common swine pathogens like PRRS virus, *M. hyopneumoniae*, and *A.pleuropneumoniae*, which were all present on the trial farm. In the present study, PCV2 vaccination resulted in significant improvement in growth rates under these conditions. The one- and two-dose PCV2 vaccines resulted in a 3.6% and 5.3% improvement, respectively, in ADG from day 0 to market. Previous studies have reported 2.8% to 9.3% improvements in ADG with the use of various PCV2 vaccines.[12-14] In the present study, improvements in growth rate for vaccinated pigs occurred between 0 and 113 days after weaning as there were no differences in ADG observed from day 113 to market. Thus, vaccinated pigs had heavier average weights at day 113 than non-vaccinated pigs. Although ADG was similar between treatment groups from day 113 to off test, the increase in ADG from day 0 to 113 was

enough to cause an increase in weights at day 143 for both vaccinated groups and at off test for the two-dose group compared with the non-vaccinated group. This period, 3 to 19 weeks of age, corresponds with the time of active infection (17 weeks of age) reported by Horlen et al.[12] Other studies also have indicated a strong association between ADG and level of viremia. One study[13] reported that differences in ADG between vaccinated and placebo-treated animals became more pronounced during the period of increasing viremia from 15 to 20 weeks of age and less pronounced after 20 weeks of age. A more recent study[14] showed that differences in weight gain between vaccinated and non-vaccinated pigs occurred much earlier (10 to 15 weeks of age). Therefore, it can be assumed that in our study, active infection or a higher level of viremia was present in non-vaccinated pigs between day 0 and 113, hence the observed depression in growth.

Improvements in ADG resulted in heavier body weights in vaccinated animals. However, weight differences between the vaccinated groups and the control group were noticeably smaller at off test compared with differences on day 143. This may be explained by the fact that pigs from each treatment group that reached market weight (top) before the expected market date were weighed and taken off test several days earlier. Because a greater number of vaccinated pigs reached market weight and were removed from test earlier compared with non-vaccinated pigs, more non-vaccinated pigs remained at the end of the experiment. Thus, on the average, the control group was on test longer ($P = 0.08$) than the two vaccinated groups, which allowed the control group to gain more weight and decrease the weight difference. Similar to a previous PCV2 vaccine study,[12] the distribution of pig weights for vaccinated pigs were shifted toward the heavier side, indicating that improvements in growth occurred throughout the whole population of vaccinated pigs, including the lighter weight pigs. The improvement in weight in the

vaccinated pig population appears to indicate that PCV2 infection causes decreases in growth rates even in pigs without clinical appearance of disease. The significant economic implication of the increased growth rate is the total potential revenue obtained by marketing vaccinated pigs earlier but at heavier weights in addition to reducing losses associated with increased mortality due to PCVD.

Implications

- The commercial PCV2 vaccines evaluated in this study were effective at improving the growth rate of pigs from weaning to finishing as shown by the greater ADG and heavier body weights of the vaccinated groups.
- Overall, both PCV2 vaccines were effective at mitigating effects of PCVD.
- Results of this study add to the increasing evidence of the negative impact of PCV2 infection on growth (as shown by the reduction in growth rate in non-vaccinated pigs compared with vaccinated pigs) and further confirm the efficacy of PCV2 vaccination under commercial conditions in the presence of natural infection.

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Table 3-1: Effects of porcine circovirus type 2 vaccine and gender on mortality, growth rate, and weight of growing-finishing pigs*

	Barrow			Gilt				*P* values	
Item	**Control**	**One-dose†**	**Two-dose‡**	**Control**	**One-dose**	**Two-dose**	**SEM**	**Vaccine**	**Gender**
Mortality, %	9.7	7.6	8.7	12.2	8.1	6.6	2.31	0.42	0.86
Weight, kg									
day 0	8.6	8.9	8.7	8.7	8.9	8.8	0.46	0.24	0.50
day 113	83.6	86.7	88.5	81.2	84	84.5	2.28	0.04	<0.001
day 143	109.5	113.9	115	105.7	108.2	109.5	2.87	0.03	<0.001
Off test	117.8	120.9	122.2	115	116.6	118.2	2.85	0.05	<0.001
Days on test	152.2	150.87	150.5	154.1	152.7	153.2	1.5	0.08	<0.001
ADG, kg									
day 0 to 113	0.661	0.689	0.707	0.640	0.665	0.670	0.016	0.02	<0.001
day 0 to 143	0.707	0.736	0.747	0.679	0.695	0.707	0.015	0.02	<0.001
day 0 to Off test	0.719	0.745	0.757	0.691	0.707	0.716	0.012	0.02	<0.001
day 113 to 143	0.887	0.919	0.909	0.829	0.815	0.853	0.021	0.39	<0.001
day 113 to Off test	0.889	0.918	0.917	0.828	0.829	0.852	0.019	0.25	<0.001

*A total of 1,470 weaned pigs (825 barrows and 645 gilts) were assigned randomly to one of three treatments within barrows and gilts before administration of the first vaccine dose. Data were analyzed as a randomized complete block design by ANOVA using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) with the pen as the experimental unit. The fixed effects of the statistical model were the effects of PCV2 vaccination (non-vaccinated, one dose, or two-dose), gender (barrow or gilt), and their interaction with the random effect of weaning group.

†Commercial one-dose PCV2 vaccine (Suvaxyn PCV2 One Dose; Fort Dodge Animal Health, Fort Dodge, IA) administered at 4 weeks of age.

‡Commercial two-dose PCV2 vaccine (Circumvent PCV; Intervet Inc., Millsboro, DE) administered at 3 and 6 weeks of age.

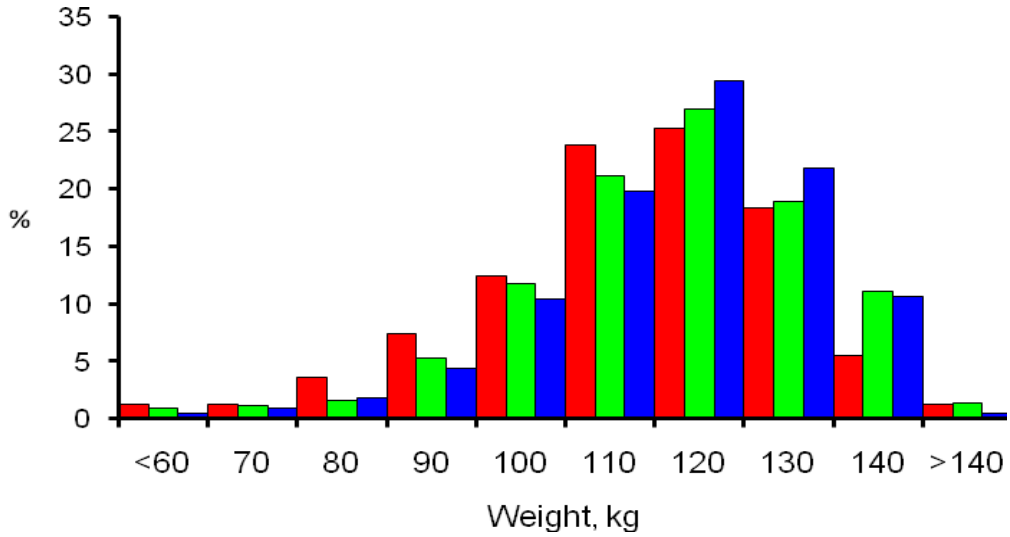

Figure 3-1: Effect of PCV2 vaccination on weight distribution within non-vaccinated ( ), one-dose vaccinated ( ), and two-dose vaccinated group of pigs ( ) at day 143 on test

CHAPTER 4 - Amino acid digestibility and energy content of de-oiled corn dried distillers grains with solubles, solvent extracted, for swine and its effects on growth performance and carcass characteristics

ABSTRACT: Three experiments were conducted to determine the AA digestibility and energy concentration of deoiled corn dried distillers grains with solubles, solvent extracted (dDGS) and evaluate its effect in diets for nursery and growing-finishing pigs. In Exp. 1, five growing barrows (initially 30.8 kg BW) were fitted with a T-cannula in the distal ileum and allotted in a crossover design at 68.0 kg BW to 1 of 2 treatments: (1) diet with dDGS as the sole protein source or (2) an N-free diet for determining basal endogenous AA losses. Apparent (AID) and standardized (SID) ileal digestibility of AA and energy concentration of dDGS were determined. In Exp. 2, a total of 210 pigs (initial BW 9.9 kg) were used in a 28-d study to evaluate the effect of dDGS on nursery pig performance. Pigs were allotted to 5 dietary treatments (0, 5, 10, 20, or 30% dDGS) formulated to contain equal ME (increasing levels of added fat with increasing dDGS) and SID Lys concentrations based on the values obtained from Exp. 1. In Exp. 3, a total of 1,215 pigs (initially 29.6 kg BW) were used in a 99-d trial to determine the effect of dDGS on growth and carcass characteristics of finishing pigs. Pigs were allotted to dietary treatments similar to those used in Exp. 2 and were fed in 4 phases. The analyzed chemical composition of dDGS in Exp. 1 was 35.6% CP, 5.29% ash, 4.6% fat, 18.4% ADF, and 39.5% NDF on a DM basis. Apparent ileal digestibilities of Lys, Met, and Thr in dDGS were 47.2, 79.4, and 64.1%, respectively, and SID were 50.4, 80.4, and 68.9%, respectively. The analyzed GE and DE and calculated ME and NE values of dDGS were 5,098; 3,100; 2,858; and 2,045 kcal/kg DM, respectively. In Exp. 2, nursery pig ADG ($P > 0.52$), ADFI ($P > 0.95$), and G:F ($P > 0.55$) were

similar between treatments regardless of the level of dDGS in the diet. In Exp. 3, increasing dDGS reduced (linear; $P < 0.01$) ADG and ADFI but tended to improve (linear; $P > 0.07$) G:F. Carcass weight and yield were reduced (linear; $P < 0.01$), loin depth tended to decrease (linear; $P < 0.09$), and iodine values of carcass fat increased (linear; $P < 0.01$) as dDGS was increased. There was no difference in backfat ($P > 0.26$), percentage lean ($P > 0.16$), or fat-free lean index ($P > 0.20$). In conclusion, dDGS has higher CP and AA levels than traditional DDGS but lower energy values and slightly lower Lys digestibility. When dietary fat was added to diets to offset the lower ME content, adding up to 30% dDGS did not affect growth performance of nursery pigs but negatively affected ADG, ADFI, and carcass fat quality of finishing pigs.

Key words: amino acid digestibility, carcass, deoiled dried distillers grains with solubles, energy, growth, pigs

INTRODUCTION

The Energy Independence and Security Act of 2007 mandates a total annual renewable fuel production in the United States to be 136 billion L by 2022 (U.S. Congress, 2007). This mandate has led to large-scale ethanol production and increased availability of ethanol coproducts like dried distillers grains with solubles (DDGS). This is a co-product that remains after ethanol is removed from fermented corn mash and contains higher levels of nutrients than corn. The increased production of DDGS in recent years has triggered numerous research studies that led to its widespread use in swine feeding (Stein and Shurson, 2009).

As the biofuel industry has evolved, additional coproducts have been developed. One such product is deoiled corn distillers dried grains with solubles, solvent extracted (dDGS; Verasun Energy Inc., Brookings, SD), which is traditional DDGS with the oil removed by

solvent extraction. When a majority of the oil is removed, CP, fiber, and mineral concentrations increase proportionately in the remaining coproduct. The extracted oil can then be used in the human food industry or for biodiesel production. Removal of oil may also improve the flowability of the co-product and address some of the handling issues normally encountered with traditional DDGS (Ganesan et al., 2009). In pig production, the increased content of CP and other nutrients potentially increases the value of dDGS relative to traditional DDGS. However, no data are available on the actual AA digestibility and energy concentration of this coproduct. Nutrient digestibility must be determined to accurately formulate and value dDGS in swine diets. Thus, objectives of this study were to: (1) determine the apparent (AID) and standardized ileal digestibility (SID) of AA, (2) determine the DE and calculated ME and NE for dDGS, and (3) use these values in diet formulation to determine the effect of dDGS on growth performance, carcass characteristics, and carcass fat quality of dDGS-fed pigs.

MATERIALS AND METHODS

This experiment consisted of 3 trials. The first was a digestibility trial conducted at the Kansas State University Swine Teaching and Research Center metabolism barn. The second was a nursery experiment conducted at the South Dakota State University Swine Unit. The third was a growing-finishing growth performance experiment in a commercial swine research facility in southwest Minnesota. The Institutional Animal Care and Use Committees at Kansas State University and South Dakota State University approved protocols used in these experiments.

Experiment 1. Amino Acid Digestibility and Energy Concentration

This experiment was done concurrently with another digestibility study on 2 other feed ingredients utilizing the same animals. Five growing barrows (initially 30.8 kg) were fitted with

a T-cannula on their right flank approximately 15 cm anterior to the ileocecal valve as described by Knabe et al. (1989). Pigs were housed individually in stainless steel metabolism crates (1.5 × 0.6 m) in an environmentally controlled building after surgery and fed a standard corn-soybean meal-based diet for 10 d during the recovery period. After the recovery period, pigs were used in a separate metabolism study for 5 wk and then fed a common corn-soybean meal diet for 7 d. Pigs then were allotted randomly in a balanced crossover design with an initial starting weight of 68.0 kg. Two diets were used for this experiment: the first diet was formulated to contain the dDGS, and the second diet was formulated to be N-free to determine the basal AA endogenous losses (Tables 4-1 and 4-2). Both diets contained 0.25% chromic oxide as an indigestible marker.

Each feeding period consisted of 7 d with the first 4 d used as an adaptation period to the diet. Feces were collected on d 5 and 6 in the morning (between 0600 and 1200 h), and ileal digesta were collected on d 6 and 7 between 0700 and 1700 h. Pigs were weighed at the beginning of each period to determine the amount of feed to be fed each day. Feed was given at a daily level of 3 times the estimated maintenance requirement for energy. Feeding was done twice daily at 0600 and 1800 h with the allocated daily amount divided into 2 equal meals. At the end of each period, all pigs were taken off feed overnight before the next experimental diet was fed the following morning. Pigs were given free access to water through a nipple waterer throughout the duration of the experiment.

Diets and dDGS samples were sent to Ward Laboratories, Inc. (Kearney, NE) for proximate analysis. Ileal digesta were collected by attaching a latex balloon to the cannula. Balloons were removed periodically or when filled with digesta and emptied in a collection container that was stored in a freezer until further analysis. After the collection phase of the experiment, ileal samples from each period from each animal were thawed and homogenized. A

subsample was taken from each homogenized sample, freeze-dried, and ground for AA analysis at the University of Missouri – Columbia Agricultural Experiment Station Chemical Laboratories (AOAC Official Method 982.30 E (a,b,c), chp. 45.3.05, 2006). Grab samples of feces collected on d 5 and 6 were stored and frozen until further analysis. Fecal samples were then thawed at the conclusion of the collection phase and homogenized within each pig and diet. A subsample was dried at 50ºC in a forced-air oven and ground for analysis. Energy concentrations in diets, dDGS, and fecal samples were determined with an adiabatic bomb calorimeter (Parr Instruments, Moline, IL). Diets, ground digesta, and ground fecal samples were analyzed for chromium concentration with an atomic absorption spectrometer (Perkin Elmer 3110; Waltham, MA) according to procedures described by Williams et al. (1962) for calculation of AA and energy digestibility values. The AID and SID values for AA were calculated with analytical values obtained from these analyses. The AID for AA in the dDGS diet was calculated as (Fan et al., 1995):

$$AID = [1 - (AAd/AAf) \times (Crf/Crd)] \times 100\%$$

where AID is the apparent ileal digestibility of an AA (%), AAd is the concentration of that AA in the ileal digesta (g/kg of DM), AAf is the concentration of that AA in the diets (g/kg of DM), Crf is the chromium concentration in the diet (g/kg of DM), and Crd is the chromium concentration in the ileal digesta (g/kg of DM).

The basal endogenous loss of each amino acid at the ileum was determined from the digesta samples obtained after feeding the N-free diet with the equation (Moughan et al., 1992):

IAAend = [AAd × (Crf/Crd)]

where IAAend is the basal ileal endogenous loss of an AA (g/kg of DMI).

The SID value for each AA was calculated as (Jondreville et al., 1995):

SID = [AID + (IAAend/AAf)]

where SID is the standardized ileal digestibility of an AA (%).

Digestible energy value of the dDGS diet was calculated with the equation for AID to determine the apparent total tract digestibility of energy. This value was then multiplied by the analyzed concentration of GE in the diets to determine the DE of the diet. Digestible energy of dDGS was calculated by subtracting 33% of the N-free DE from the DE of the dDGS diet using the difference procedure (Adeola, 2001). The DE value for dDGS was used in the following equations to calculate ME and NE:

ME = 1 × DE – 0.68 × CP (R^2 = 0.99; Noblet and Perez, 1993)

NE = (0.87 × ME) – 442 (R^2 = 0.94; Noblet et al., 1994)

Experiment 2. Nursery Pig Growth Performance

A total of 210 pigs (initially 9.9 kg) were allotted randomly to 1 of 5 dietary treatments balanced by average BW. There were 7 pens per treatment and 6 pigs per pen. Barrows and gilts were housed in separate mechanically ventilated barns. Both barns had completely slatted flooring. Barrows were housed in 1.2 × 1.2-m pens, gilts in 1.2 × 1.5-m pens. Each pen was equipped with nipple waterers and 3-hole feeders. All pigs were fed similar starter diets until the

start of the experiment. Metabolizable energy and SID AA values obtained in Exp. 1 were used in diet formulation. The 5 dietary treatments contained dDGS at 0, 5, 10, 20, or 30% (Table 4-3). All diets were formulated to contain equal ME and SID Lys concentrations. Soybean oil was added to the dDGS diets as an energy source to equalize dietary ME levels of the 5 treatments. Pigs were weighed and feed disappearance was determined on d 0, 14, and 28 to determine ADG, ADFI and G:F.

Experiment 3. Growing-finishing Performance, Carcass Traits, and Carcass Fat Quality

A total of 1,215 pigs (initially 29.6 kg BW) were balanced by average BW and randomly allotted to 1 of 5 dietary treatments fed in meal form. There were 27 pigs in each pen (5.5 × 3.0 m). The barns were double curtain sided with completely slatted flooring and deep pits for manure storage. Each pen contained 1 self-feeder and 1 cup waterer. A robotic feeding system (FeedPro; Feedlogic Corp., Willmar, MN) capable of providing and measuring feed amounts on an individual pen basis was used. The 5 treatments consisted of diets containing 0, 5, 10, 20, or 30% dDGS. Pigs were fed in 4 phases from approximately 29 to 54, 54 to 77, 77 to 100, and 100 to 120 kg BW for phases 1 to 4, respectively (Tables 4-4 and 4-5). Diets were formulated to contain 0.94, 0.80, 0.69, and 0.95% SID Lys and to maintain available P concentrations of at least 0.27, 0.24, 0.22, and 0.21% for phases 1 to 4, respectively. All dietary treatments were formulated to contain similar dietary ME and SID Lys concentrations within each phase. Choice white grease was added in increasing amounts as dDGS increased in the diet to maintain uniform dietary ME levels. Ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN) was added in all phase 4 diets. Hence, the increased SID Lys levels in the last dietary phase.

Pigs were weighed by pen and feed disappearance was measured on d 0; every 14 d until d 70; and on d 78, 93, and 99 to determine ADG, ADFI, and G:F. Feed intake and G:F were determined from feed delivery data generated through the automated feeding system and the amount of feed remaining in each pen's feeder on each weigh date. Two pigs representative of the average weight from each pen were selected, tattooed by pen, and transported to the JBS Swift & Company processing plant (Worthington, MN) on d 93 to collect jowl fat, belly fat, and backfat samples for fatty acid analysis and iodine value determination. Briefly, pigs were processed and carcasses were sent through deep chill chambers (approximately -40^{o}C) for approximately 90 minutes. Approximately 2 hours after taken out of deep chill chambers, the right side jowl was removed with a perpendicular cut flush with the carcass shoulder. A small (approximately 20 g) sample of backfat was removed from the 10th rib area off the carcass midline. Jowl and backfat samples were placed in a vacuum bag, vacuum sealed, and stored at approximately 4^{o}C. Carcasses were allowed to chill overnight. At approximately 18 hours post-stick, a belly strip approximately 5 cm wide and 70 cm long) was removed from the scribe side of each belly. Belly strips were vacuum-packaged and stored at 4^{o}C. All fat samples were transported to the Nutrition Wellness Research Center – Campus location at Iowa State University and were frozen at -18^{o}C before transfer to Kansas State University. After samples were transported to Kansas State University, fat samples were stored in a freezer until further analysis. At the end of the experiment (d 99), the remaining pigs were individually tattooed with pen numbers to allow carcass data collection by pen and transported to the JBS Swift & Company processing plant (Worthington, MN). Standard carcass criteria of loin and backfat depth, HCW, percentage lean, and yield were collected.

Fat samples collected on d 93 were processed at the Kansas State University Swine Laboratory and analyzed for fatty acids according to procedures described by Sukhija and Palmquist (1988). Briefly, 50 mg of fat was prepared in screw-cap tubes with polytetrafluoroethylene-lined

caps. Two milliliters of an internal standard (2 mg/mL of methyl heptadecanoic acid (C17:0) in benzene) and 3 mL of methanolic-HCl were then added into the tube containing the fat sample. The tubes were placed in a water bath for 120 min at 70°C for transmethylation. After the tubes cooled to room temperature, 5 mL of 6% K_2CO_3 and 2 mL of benzene were added to allow the methyl esters to be extracted and transferred to a vial for subsequent quantification of the methylated fatty acids by gas chromatography. Fatty acids from each fat sample were expressed as a percentage of the total fatty acids. Iodine value, expressed as g/100 g of fat, was then calculated on the basis of the fatty acid profile of each sample according to the following equation (AOCS, 1998):

$$IV = [C16:1] \times 0.95 + [C18:1] \times 0.86 + [C18:2] \times 1.732 + [C18:3] \times 2.616 + [C20:1] \times 0.785 + [C22:1] \times 0.723$$

where the brackets imply concentration (percentage) of the fatty acid.

Statistical Analysis

Data were analyzed as a completely randomized design by ANOVA using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit in Exp. 2 and 3. Linear and polynomial contrasts were used to determine the effects of increasing dDGS. Contrast coefficients were determined for unequally spaced treatments by using the IML procedure of SAS. In Exp. 3, backfat, loin depth, and percentage lean were adjusted to a common carcass weight.

RESULTS AND DISCUSSION

Experiment 1. Amino Acid Digestibility and Energy Value

Nutrient composition of dDGS used in Exp. 1 is reported in Table 4-6. The CP of dDGS was 35.58% (DM-basis), which, as expected, was higher than the published CP content in traditional DDGS (30.9, 26.5, and 31.1%, DM-basis; Stein et al., 2006; Pahm et al., 2008; Urriola et al., 2009). This value was slightly higher than the 34.0% CP (DM-basis) content of a dDGS reported by Saunders and Rosentrater (2009). However, the difference in CP content between the product evaluated in this study and that of Saunders and Rosentrater (2009) may be considered normal even though both were dDGS products. Variation in nutrient values has been reported to exist in samples of traditional DDGS between plants that use similar manufacturing technology (Spiehs et al., 2002; Pedersen et al., 2007). This variability in nutrient content may also apply to dDGS products. As expected, the ADF and NDF fractions as well as the P content in dDGS were also higher relative to traditional DDGS (Stein, 2007). Because P in DDGS is highly available, including DDGS in swine diets minimizes the need for inorganic P supplementation (Pedersen et al., 2007).

As expected, the fat level of dDGS was lower than that of traditional DDGS as a result of the oil separation used to produce dDGS. However, it was almost double the 2.7% fat content (DM basis) reported by Saunders and Rosentrater (2009) for a similar oil-extracted DDGS product. These differences may be a reflection of differences in efficiency of the fat extraction procedures used to make each coproduct.

Analyzed concentrations of almost all AA, with the exception of Trp, increased in dDGS relative to traditional DDGS. For AA digestibility, Lys, Met, and Thr in dDGS had AID values of 47.2, 79.4, and 64.1%, respectively (Table 4-7). The AID value of Lys was lower than

published values for traditional DDGS, but most other AA AID values were higher than published values for DDGS (Stein and Shurson, 2009). Standardized ileal digestibility values were 50.4% for Lys, 80.4% for Met, and 66.3% for Thr. Although Trp was present at a lower concentration, it had a higher digestibility value in dDGS than in traditional DDGS (78 vs. 70%; Stein and Shurson, 2009). Like AID, the SID value of Lys was lower than most published values for traditional DDGS (Stein and Shurson, 2009). The lower SID value for Lys for dDGS indicates the product may have been subjected to too much heat during drying. Lysine has consistently been found to have a lower SID value in traditional DDGS than in corn (Spiehs et al., 2002; Fastinger and Mahan, 2006; Stein et al., 2006; Pahm et al., 2008; Stein and Shurson, 2009). This can be attributed to the Maillard reaction that occurs when DDGS is subjected to excessive heat, wherein reducing sugars bind to Lys and render it unavailable to the animal (Adrian, 1974). It has been suggested that the ratio of Lys to CP can serve as a guideline for identifying DDGS with a greater degree of heat damage and that DDGS with a Lys-to-CP ratio of greater than 2.8 is ideal (Stein, 2007). The ratio for the dDGS tested in this study was 2.8 even though the SID value was at least 10 percentage points lower than the summarized values for SID Lys for traditional corn DDGS reported by Stein and Shurson (2009).

The analyzed GE and DE and calculated ME and NE values of dDGS were 5,098; 3,100; 2,858; and 2,045 kcal/kg DM, respectively (Table 4-8). These values were lower than traditional DDGS energy values, which was expected because of the removal of majority of the oil. The analyzed DE for dDGS represents only 61% of its GE. In a review of studies on traditional DDGS, Stein and Shurson (2009) reported averages of 5,434 and 4,140 kcal/kg DM for GE and DE values, respectively, of DDGS. Thus, DE in DDGS represented 76% of the GE value. The

lower digestibility of energy in dDGS can be attributed to its lower crude fat content and the energy dilution effect of its higher fiber content.

Experiment 2. Nursery Pig Growth Performance

Overall (d 0 to 28), nursery pigs fed increasing dDGS had similar ($P > 0.52$) ADG, ADFI, and G:F (Table 4-9). These data indicate that increasing dietary dDGS up to 30% did not affect growth performance for nursery pigs weighing 10 to 23 kg when diets were balanced for both SID AA and ME. Early research by Combs and Wallace (1969) on feeding traditional DDGS in nursery pigs showed that adding 20% DDGS in the diets did not affect growth performance. However, they also reported that digestibility of DM, CP, and ether extract was reduced when pigs were fed 20% DDGS diets compared with diets containing 10 or 0% DDGS. Using traditional DDGS from new-generation ethanol plants, Whitney and Shurson (2004) reported that DDGS can be fed at levels up to 25% without negatively affecting growth performance. Recent studies have further supported this conclusion; other researchers have not found differences in ADG between DDGS-fed and non-DDGS-fed nursery pigs (Gaines et al., 2006; Linneen et al., 2006; Spencer et al., 2007; Barbosa et al., 2008), and some researchers also reported improvement in G:F (Gaines et al., 2006; Spencer et al., 2007; and Barbosa et al., 2008). In the present study, dDGS was added at levels up to 30%, and the resulting growth performance in nursery pigs was similar to that of pigs fed diets without dDGS when diets were balanced for digestible AA and ME content.

Experiment 3. Growing-finishing Performance and Carcass Traits

Overall (d 0 to 99), ADG and ADFI decreased (linear, $P < 0.01$; Table 4-10) and G:F tended to increase (linear; $P > 0.07$) with increasing dDGS in the diet. Results from this trial are

similar to previous research on traditional DDGS in which feed intake was reduced when DDGS was fed at more than 20% of the diet (Fu et al., 2004; Xu et al., 2007; Linneen et al., 2008). Although in some other studies, feeding up to 30% traditional DDGS did not affect growth performance of finishing pigs (Cook et al., 2005; DeDecker et al., 2005; Xu et al., 2007). The addition of dDGS to growing-finishing diets appears to negatively affect palatability, but reasons for the decrease in feed intake are not clear.

Carcass weight and percentage yield decreased (linear, $P < 0.01$) and loin depth tended to decrease (linear, $P < 0.09$) as dDGS increased. However, there were no differences in backfat ($P > 0.25$), percentage lean ($P > 0.16$), or fat-free lean index ($P > 0.19$). The reduction in carcass weight can be attributed to the decreased ADG and yield as pigs were fed increasing dDGS. The decrease in carcass weight in pigs fed 30% dDGS compared with pigs not fed dDGS (4.8 kg) observed in this study is similar to the 5.1-kg decrease in carcass weight reported in an earlier study for pigs fed traditional DDGS (Whitney et al., 2006). The reduction in carcass yield was not unexpected as this effect has been reported consistently in finishing pigs fed traditional DDGS (Fu et al., 2004; Cook et al., 2005; Whitney et al., 2006; Xu et al., 2007; Linneen et al., 2008; Weimer et al., 2008). We hypothesize that the reduction in percentage yield is related to the high fiber content of the dDGS diets. Diets containing high levels of fiber have been suggested to increase basal metabolic rate (Pond et al., 1988), which could account for the lower percentage yield in pigs fed diets containing dDGS. Previous studies also have shown that diets high in fiber increase rate of passage in the gastrointestinal tract, resulting in increased gut cell proliferation and intestinal growth (Jin et al., 1994; Gill et al., 2000). The increased fiber content in dDGS could have led to a greater volume of intestinal fluid and volume of digesta (Pluske et al., 2003) and, along with the increased protein content of dDGS, increased weight of intestines

and other visceral organs (Kass et al., 1980; Stanogias and Pearce, 1985; Pond et al., 1989). Because visceral organs are excluded from the carcass, percentage yield is negatively affected in pigs fed dDGS diets because of the higher volume and weight of entrails removed during slaughter. In addition, the majority of the energy required for maintenance is used by visceral organs such as the liver and the gastrointestinal tract (Johnson et al., 1990; Mahr-un-nisa and Feroz, 1999). Thus, the resulting increase in weight of the visceral organs could have resulted in a further increase in maintenance requirement and diverted nutrient utilization away from the production of carcass lean and adipose tissue.

Like other nutrients, the fat content in traditional DDGS is increased approximately three-fold compared with corn and is rich in unsaturated fatty acids. Thus, feeding DDGS in pigs results in increased iodine value, a measure of degree of unsaturation, in carcass fat (Whitney et al., 2006; Feoli et al., 2007; Benz, 2008; White et al., 2009). The reduced fat content of dDGS would reduce the total amount of unsaturated fatty acids in the diet containing dDGS compared to traditional DDGS. Furthermore, the reduced oil content of dDGS also may improve some of the handling issues commonly encountered with traditional DDGS through increased flowability. Ganesan et al. (2009) reported that a low-oil DDGS product had a slight improvement in flowability compared with regular DDGS.

As dDGS increased in the diets, total saturated fatty acids decreased (linear; $P < 0.01$) and linoleic acid, MUFA and PUFA, and iodine value increased (linear; $P < 0.01$) in all fat depots (Table 4-11). Because dDGS was not completely devoid of corn oil, the diets still provided an increasing source of unsaturated fatty acids. Among the fatty acids, linoleic acid is considered to have the greatest effect on fat firmness (Berschauer, 1984). Linoleic acid is unsaturated, therefore, the higher the percentage of linoleic acid, the higher the degree of

unsaturation in the resulting fat. High concentration of unsaturated fatty acids has been shown to negatively affect bacon quality and cause processing difficulties (Person et al., 2005). It can also result in rapid oxidation, which decreases shelf life of pork (Wood, 2003).

Although dDGS contains less oil than traditional DDGS, the increase in fat iodine value was expected because of the increasing choice white grease in diets with dDGS. Iodine values from the 3 fat stores increased between 5.0 and 6.6 g/100 g in pigs fed 30% dDGS in the diet compared with control pigs. This translates into an approximate 1.7 to 2.2 g/100 g increase for every 10% inclusion of dDGS in the diet when fed in combination with choice white grease. This was similar to the rate of increase in fat iodine value reported by Whitney et al. (2006) for pigs fed 0, 10, 20, and 30% DDGS and by Benz (2008) in pigs fed 0, 5, 10, 15, and 20% DDGS. The increase in iodine value in this study, however, would not be expected to be as large without the increase in added choice white grease needed to maintain isocaloric diets within each phase. In addition, the lower iodine value ($P < 0.01$) for barrows compared with gilts was in agreement with reports of Averette-Gatlin et al. (2002) and Benz (2008).

In summary, AA and energy digestibility values were established for dDGS in this study and validated through growth performance experiments in nursery and growing-finishing pigs. This coproduct of the ethanol and fat extraction industries has increased CP and AA levels but lower energy and slightly lower Lys digestibility compared with traditional DDGS. Results of the growth experiments showed that up to 30% dDGS can be added to nursery diets for pigs weighing 10 to 23 kg without negatively affecting growth performance provided fat is added to the diets to offset the decreased ME content of dDGS. These data validate the accuracy of the previously determined ME (2,858 kcal/kg DM) and SID AA values for dDGS because there were no changes in G:F when dDGS was fed at increasing levels in the diet. However, increasing

levels of dDGS in growing-finishing diets resulted in reduced growth performance and had negative effects on carcass and fat quality, especially at levels greater than 20%. Thus, factors that may affect economics, such as feed ingredient prices, finishing space, and packer specifications, must be considered when using dDGS at higher levels in growing-finishing pig diets.

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Table 4-1. Ingredient composition of experimental diets (as-fed basis), Exp. 1

Ingredient, %	dDGS	N-Free
dDGS[1]	66.70	---
Corn starch	27.05	81.15
Soybean oil	1.00	3.00
Sucrose	3.00	9.00
Solka floc[2]	---	3.00
Limestone	1.25	0.40
Monocalcium phosphate, 21% P	---	1.75
Vitamin premix[3]	0.25	0.25
Salt	0.35	0.45
Trace mineral premix[4]	0.15	0.15
Potassium chloride	---	0.50
Magnessium oxide	---	0.10
Chromic oxide	0.25	0.25

[1]Deoiled dried distillers grains with solubles, solvent extracted (Verasun Energy Inc., Brookings, SD).
[2]Fiber Sales and Development Corp., Urbana, OH.
[3]Provided per kg of diet: 11,023 IU vitamin A;1,653 IU vitamin D3; 44 IU vitamin E; 4 mg vitamin K; 8 mg riboflavin; 28 mg pantothenic acid; 50 mg niacin; 0.04 mg vitamin B12.
[4]Provided per kg of complete diet: 39.7 mg Mn from manganese oxide, 165 mg Fe from iron sulfate, 165 mg Zn from zinc oxide, 16.5 mg Cu from copper sulfate, 0.298 mg I from calcium iodate, and 0.30 mg Se from sodium selenite.

Table 4-2. Analyzed nutrient composition (%) of experimental diets (as-fed basis)

	Diet	
Item	dDGS[1]	N-free
DM	90.2	93.2
CP	20.5	0.1
Indispensable AA		
Arg	0.85	0.00
His	0.53	0.00
Ile	0.77	0.00
Leu	2.36	0.00
Lys	0.56	0.00
Met	0.38	0.00
Phe	1.05	0.00
Thr	0.75	0.00
Trp	0.12	<0.04
Val	0.99	0.00
Total indispensable AA	8.34	0.00
Dispensable AA		
Ala	1.41	0.00
Asp	1.29	0.00
Cys	0.36	0.00
Glu	3.01	0.00
Gly	0.78	0.00
Pro	1.44	0.00
Ser	0.86	0.00
Tyr	0.71	0.00
Total dispensable AA	9.85	0.00

[1]Deoiled dried distillers grains with solubles, solvent extracted (Verasun Energy Inc., Brookings, SD).

Table 4-3. Diet composition (as-fed basis), Exp. 2[1]

	dDGS, %[2]				
Item	0	5	10	20	30
Ingredient, %					
Corn	63.56	58.88	54.26	44.98	35.68
Soybean meal, 46.5% CP	32.57	31.39	30.21	27.84	25.48
dDGS	---	5.00	10.00	20.00	30.00
Soybean oil	---	0.90	1.75	3.50	5.25
Monocalcium P (21% P)	1.65	1.50	1.40	1.15	0.90
Limestone	0.95	1.05	1.10	1.23	1.38
Salt	0.35	0.35	0.35	0.35	0.35
Vitamin premix[3]	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[4]	0.15	0.15	0.15	0.15	0.15
Lys HCl	0.30	0.33	0.35	0.40	0.45
DL-Met	0.12	0.11	0.10	0.08	0.06
L-Thr	0.10	0.10	0.09	0.08	0.06
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
Standardized ileal digestible AA[5]					
Lys, %	1.25	1.25	1.25	1.25	1.25
Met:Lys, %	33	33	33	33	33
Met and Cys:Lys, %	58	58	58	58	58
Thr:Lys, %	62	62	62	62	62
Trp:Lys, %	18	18	18	17	17
Total Lys, %	1.38	1.40	1.42	1.45	1.48
CP, %	21.0	21.6	22.2	23.5	24.7
SID Lys:ME, g/Mcal	3.79	3.79	3.79	3.79	3.79
ME, kcal/kg	3,298	3,299	3,298	3,299	3,299
Ca, %	0.80	0.80	0.80	0.80	0.80
P, %	0.75	0.73	0.73	0.71	0.69
Available P, %	0.42	0.42	0.42	0.42	0.42

[1]Dietary treatments fed in meal form from approximately 10 to 23 kg BW.
[2]Deoiled corn distillers dried grains with solubles, solvent extracted (Verasun Energy Inc., Brookings, SD).
[3]Provided per kg of diet: 11,023 IU vitamin A;1,653 IU vitamin D3; 44 IU vitamin E; 4 mg vitamin K; 8 mg riboflavin; 28 mg pantothenic acid; 50 mg niacin;0.04 mg vitamin B12.
[4]Provided per kg of complete diet: 39.7 mg Mn from manganese oxide, 165 mg Fe from iron sulfate, 165 mg Zn from zinc oxide, 16.5 mg Cu from copper sulfate, 0.298 mg I from calcium iodate, and 0.298 mg Se from sodium selenite.
[5]Based on standardized ileal digestible AA values determined from Exp. 1.

Table 4-4. Phase 1 and 2 diet composition (as-fed basis), Exp. 3[1]

	Phase 1, dDGS[2]					Phase 2, dDGS				
Item	0%	5%	10%	20%	30%	0%	5%	10%	20%	30%
Ingredient, %										
Corn	73.11	68.36	63.61	54.13	44.50	78.78	74.06	69.28	59.81	50.09
Soybean meal, 46.5% CP	24.79	23.62	22.44	20.09	17.75	19.22	18.04	16.87	14.52	12.18
dDGS	---	5.00	10.00	20.00	30.00	---	5.00	10.00	20.00	30.00
Choice white grease	---	0.95	1.93	3.80	5.75	---	0.95	1.93	3.80	5.80
Monocalcium phosphate, 21% P	0.60	0.48	0.35	0.13	0.00	0.50	0.35	0.25	0.00	0.00
Limestone	0.85	0.93	0.98	1.10	1.20	0.85	0.93	0.98	1.13	1.13
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix with phytase[3]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Trace mineral premix[4]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
L-Lys HCl	0.15	0.18	0.20	0.25	0.30	0.15	0.18	0.20	0.25	0.30
Total	100	100	100	100	100	100	100	100	100	100
Calculated analysis										
Standardized ileal digestible AA[5]										
Lys, %	0.94	0.94	0.94	0.94	0.94	0.80	0.80	0.80	0.80	0.80
Met:Lys, %	28	29	30	32	34	30	31	32	34	36
Met and Cys:Lys, %	58	59	60	62	64	61	62	64	66	68
Thr:Lys, %	61	62	62	64	65	62	63	64	65	67
Trp:Lys, %	19	19	19	19	18	19	19	19	18	18
Total Lys, %	1.06	1.07	1.09	1.12	1.15	0.90	0.92	0.93	0.96	0.99
CP, %	17.89	18.52	19.15	20.42	21.68	15.78	16.41	17.04	18.31	19.57
SID Lys:ME, g/Mcal	2.81	2.81	2.81	2.81	2.81	2.39	2.39	2.39	2.39	2.39
ME, kcal/kg	3,344	3,344	3,344	3,344	3,344	3,351	3,351	3,351	3,351	3,351
Ca, %	0.54	0.54	0.54	0.54	0.54	0.50	0.50	0.50	0.50	0.50
P, %	0.5	0.49	0.48	0.47	0.48	0.46	0.44	0.44	0.42	0.45
Available P, %[6]	0.27	0.27	0.27	0.27	0.30	0.24	0.24	0.24	0.24	0.29
Dietary fat iodine value, g/100g	121.4	108.4	97.8	98.1	88.4	117.2	108.0	100.9	94.2	88.7

Iodine value product[7]	25.5	36.8	42.1	55.9	69.8	16.4	30.2	39.3	57.5	70.9

[1]Dietary treatments fed in meal form from 29 to 54 kg BW for phase 1 and 54 to 77 kg BW for phase 2.
[2]Deoiled corn distillers dried grains with solubles, solvent extracted (Verasun Energy Inc., Brookings, SD).
[3]Provided per kg of diet: 3,527 IU vitamin A; 529 IU vitamin D3; 14 IU vitamin E; 1.4 mg vitamin K; 2.6 mg riboflavin; 8.8 mg pantothenic acid; 15.9 mg niacin; 0.01 mg vitamin B12; and 450 phytase units/kg phytase.
[4]Provided per kg of complete diet: 21.2 mg Mn from manganese oxide, 88.2 mg Fe from iron sulfate, 88.2 mg Zn from zinc oxide, 8.8 mg Cu from copper sulfate, 0.159 mg I from calcium iodate, and 0.159 mg Se from sodium selenite.
[5]Based on standardized ileal digestible AA values determined from Exp. 1.
[6]Includes expected phytate P release of 0.08% from phytase.
[7]Iodine value of diet oil × % diet oil × 0.10.

Table 4-5. Phase 3 and 4 diet composition (as-fed basis), Exp. 3[1]

	Phase 3, dDGS[2]					Phase 4, dDGS				
Item	0%	5%	10%	20%	30%	0%	5%	10%	20%	30%
Ingredient, %										
Corn	83.21	78.47	73.71	64.21	54.49	73.03	68.26	63.53	53.93	44.07
Soybean meal, 46.5% CP	14.84	13.66	12.49	10.14	7.81	25.17	23.99	22.82	20.47	18.15
dDGS[2]	---	5.00	10.00	20.00	30.00	---	5.00	10.00	20.00	30.00
Choice white grease	---	0.95	1.90	3.80	5.80	---	0.98	1.90	3.85	5.90
Monocalcium phosphate, 21% P	0.45	0.34	0.23	0.00	0.00	0.35	0.23	0.10	0.00	0.00
Limestone	0.88	0.93	1.00	1.13	1.13	0.80	0.88	0.95	1.00	1.08
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix with phytase[3]	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Trace mineral premix[4]	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
L-Lys HCl	0.15	0.18	0.20	0.25	0.30	0.15	0.18	0.20	0.25	0.30
Ractopamine HCl, 20 g/kg[5]	-	-	-	-	-	0.025	0.025	0.025	0.025	0.025
Total	100	100	100	100	100	100	100	100	100	100
Calculated analysis										
Standardized ileal digestible AA[6]										
Lys, %	0.69	0.69	0.69	0.69	0.69	0.95	0.95	0.95	0.95	0.95
Met:lys, %	32	33	34	37	39	28	29	30	32	33
Met and cys:lys, %	65	66	68	70	73	58	58	59	61	63
Thr:lys, %	63	64	65	67	69	61	62	62	64	65
Trp:lys, %	19	18	18	18	17	19	19	19	19	18
Total lys, %	0.78	0.80	0.81	0.84	0.87	1.07	1.08	1.10	1.13	1.16
CP, %	14.12	14.75	15.38	16.65	17.91	18.05	18.69	19.32	20.58	21.83
SID Lys:ME, g/Mcal	2.06	2.06	2.06	2.06	2.06	2.83	2.83	2.83	2.83	2.83
ME, kcal/kg	3,353	3,353	3,353	3,353	3,353	3,355	3,355	3,355	3,355	3,355
Ca, %	0.49	0.49	0.49	0.49	0.49	0.48	0.48	0.48	0.48	0.50
P, %	0.43	0.42	0.42	0.40	0.43	0.45	0.44	0.43	0.44	0.48
Available P, %[7]	0.22	0.22	0.22	0.23	0.28	0.21	0.21	0.21	0.24	0.29
Dietary fat iodine value, g/100g	116.1	107.5	100.8	94.2	89.4	121.6	107.8	100.2	94.7	88.2

Iodine value product[8]	19.7	33.3	41.3	59.3	69.8	26.8	31.3	40.1	54.9	67.9

[1]Dietary treatments fed in meal form from 170 to 220 kg BW for phase 3 and 220 to 265 kg BW for phase 4.

[2]Deoiled corn distillers dried grains with solubles, solvent extracted (Verasun Energy Inc., Brookings, SD).

[3]Provided per kg of diet: 2,646 IU vitamin A; 397 IU vitamin D3; 11 IU vitamin E; 1.1 mg vitamin K; 2.0 mg riboflavin; 6.6 mg pantothenic acid; 11.9 mg niacin; 0.01 mg vitamin B12; and 375 phytase units/kg phytase.

[4]Provided per kg of complete diet: 15.9 mg Mn from manganese oxide, 66.1 mg Fe from iron sulfate, 66.1 mg Zn from zinc oxide, 6.6 mg Cu from copper sulfate, 0.119 mg I from calcium iodate, and 0.119 mg Se from sodium selenite.

[5]Ractopamine HCl (Paylean, Elanco Animal Health, Greenfield, IN).

[6]Based on standardized ileal digestible AA values determined in Exp. 1

[7]Includes expected phytate P release of 0.07% from phytase.

[8]Iodine value of diet oil × % diet oil × 0.10.

Table 4-6. Analyzed nutrient composition of dDGS (%)[1]

Item	DM basis	As-fed basis
DM	100.00	87.69
CP	35.58	31.20
Crude fat	4.56	4.00
NDF	39.46	34.60
ADF	18.36	16.1
Ash	5.29	4.64
Ca	0.06	0.05
P	0.87	0.76
Indispensable AA		
Arg	1.50	1.31
His	0.93	0.82
Ile	1.38	1.21
Leu	4.15	3.64
Lys	0.99	0.87
Met	0.67	0.58
Phe	1.92	1.69
Thr	1.26	1.10
Trp	0.22	0.19
Val	1.75	1.54
Dispensable AA		
Ala	2.43	2.13
Asp	2.10	1.84
Cys	0.62	0.54
Glu	4.85	4.26
Gly	1.35	1.18
Pro	2.41	2.11
Ser	1.48	1.30
Tyr	1.29	1.13

[1]Deoiled dried distillers grains with solubles, solvent extracted (Verasun Energy Inc., Brookings, SD).

Table 4-7. Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of AA in dDGS[1] by growing pigs[2]

Item	SID, %[3]	AID, %
Indispensable AA		
Arg	82.7 (1.7)	79.7 (1.4)
His	74.6 (2.8)	72.8 (2.8)
Ile	74.5 (2.0)	72.5 (2.0)
Leu	83.8 (1.8)	82.7 (1.7)
Lys	50.4 (1.9)	47.2 (2.0)
Met	80.4 (1.6)	79.4 (1.5)
Phe	80.8 (1.6)	79.4 (1.6)
Thr	68.9 (2.3)	64.1 (2.2)
Trp	78.0 (1.6)	73.7 (1.5)
Val	73.8 (2.1)	71.8 (2.1)
Dispensable AA		
Ala	79.1 (2.1)	77.2 (2.0)
Asp	64.6 (2.5)	61.3 (2.5)
Cys	66.9 (4.0)	64.1 (3.8)
Glu	79.0 (2.5)	77.5 (2.4)
Gly	64.6 (3.0)	52.7 (3.7)
Pro	87.8 (6.6)	73.4 (4.2)
Ser	76.9 (2.1)	73.2 (1.9)
Tyr	82.4 (1.6)	80.6 (1.6)

[1]Deoiled corn dried distillers grains with solubles, solvent extracted (Verasun Energy Inc., Brookings, SD).
[2]Values are means of 5 observations per treatment. Standard deviation for each digestibility value is shown in parentheses.
[3]The SID represents the corrected AID accounting for basal endogenous loss of an AA. Calculated basal endogenous losses after feeding the N-free diet were (g/kg of DMI): Arg, 0.29; His, 0.11; Ile, 0.17; Leu, 0.29; Lys, 0.19; Met, 0.04; Phe, 0.16; Thr, 0.40; Trp, 0.06; Val, 0.22; Ala, 0.29; Asp, 0.46; Cys, 0.11; Glu, 0.52; Gly, 1.02; Pro, 2.28; Ser, 0.35; Tyr, 0.14.

Table 4-8. Energy values of dDGS for growing pigs[1,2]

Item	Value, kcal/kg DM
GE	5,098
DE	3,100
ME[3]	2,858
NE[4]	2,045

[1]Deoiled corn dried distillers grains with solubles, solvent extracted (Verasun Energy Inc., Brookings, SD).
[2]Values are means of 5 observations per treatment.
[3]The ME value of dDGS was calculated as: ME = 1 × DE – 0.68 × CP (R^2 = 0.99; Noblet and Perez, 1993).
[4]The NE value of dDGS was calculated as: NE = (0.87 × ME) – 442 (R^2 = 0.99; Noblet et al., 1994).

Table 4-9. Effects of deoiled corn dried distillers grains with solubles, solvent extracted, (dDGS) on nursery growth performance, Exp. 2[1]

	dDGS, %						Probability, *P* <	
Item	0	5	10	20	30	SEM	Linear	Quadratic
Weight, kg								
d 0	10.0	10.0	9.6	9.9	9.9	0.47	0.94	0.70
d 28	22.7	22.8	22.2	22.4	22.3	0.56	0.56	0.77
d 0 to 28								
ADG, kg	0.455	0.459	0.452	0.445	0.442	0.0197	0.52	0.98
ADFI, kg	0.749	0.771	0.760	0.751	0.761	0.009	0.95	0.86
G:F	0.609	0.595	0.594	0.593	0.582	0.028	0.55	0.94

[1]A total of 210 pigs (initially 9.9 kg BW) were used with 6 pigs per pen and 7 replicate pens per treatment.

Table 4-10. Effects of deoiled corn dried distillers grains with solubles, solvent extracted (dDGS) on growth performance and carcass characteristics of growing-finishing pigs , Exp. 3[1]

	dDGS, %						*P* - value	
Item	0	5	10	20	30	SE	Linear	Quadratic
Weight, kg								
d 0	29.6	29.6	29.6	29.6	29.6	0.45	0.94	0.99
d 99	121.4	119.3	118.8	118.2	116.2	0.94	0.001	0.68
d 0 to 99								
ADG, kg	0.909	0.893	0.887	0.887	0.873	0.0083	0.01	0.61
ADFI, kg	2.16	2.17	2.11	2.11	2.04	0.031	0.003	0.72
G:F	0.420	0.413	0.422	0.421	0.431	0.0055	0.07	0.42
Carcass wt, kg	91.1	89.0	89.1	87.7	86.3	0.83	<0.001	0.66
Yield, %	75.50	75.00	75.00	74.67	74.33	0.003	0.01	0.73
Backfat, mm^2	16.46	16.53	16.53	16.38	16.96	0.247	0.26	0.25
Loin depth, mm^2	2.50	2.45	2.46	2.48	2.39	0.033	0.09	0.55
Lean, %[2]	56.48	55.91	56.30	56.43	55.78	0.202	0.16	0.28

[1]A total of 1,215 pigs (initially, 29.6 kg BW) were used with 27 pigs per pen and 9 pens per treatment.
[2]Values are adjusted to a common carcass weight.

Table 4-11. Effects of deoiled corn dried distillers dried grains with solubles, solvent extracted, (dDGS) on fat quality

	dDGS, %[1]						Gender[2]			*P* - value		
Item	0	5	10	20	30	SEM	Barrow	Gilt	SEM	Linear	Quadratic	Gender
C 18:2 fatty acids, %												
Jowl fat	13.6	13.7	14.7	15.9	17.1	0.31	14.5	15.4	0.20	<0.001	0.75	0.002
Backfat	16.5	16.3	17.0	18.9	18.4	0.43	16.5	18.3	0.29	<0.001	0.40	<0.001
Belly fat	15.3	15.4	16.3	17.8	18.2	0.39	15.7	17.5	0.26	<0.001	0.50	<0.001
Total SFA, %[3]												
Jowl fat	35.7	35.1	35.0	33.8	32.3	0.34	34.9	33.9	0.22	<0.001	0.34	0.001
Backfat	37.6	37.5	37.2	34.8	33.6	0.39	36.7	35.5	0.26	<0.001	0.34	0.001
Belly fat	37.9	36.9	36.5	34.3	33.0	0.40	36.4	35.0	0.26	<0.001	0.85	0.0003
Total MUFA, %[4]												
Jowl fat	48.9	49.3	48.4	48.2	48.3	0.40	48.6	48.7	0.26	0.09	0.55	0.80
Backfat	43.9	44.4	43.8	44.1	45.8	0.43	44.8	44.0	0.29	0.01	0.07	0.07
Belly fat	44.8	45.7	45.2	45.6	46.6	0.41	45.9	45.3	0.27	0.01	0.54	0.152
Total PUFA, %[5]												
Jowl fat	15.4	15.6	16.6	18.0	19.4	0.33	16.5	17.5	0.22	<0.001	0.79	0.002
Backfat	18.5	18.2	19.0	21.1	20.6	0.47	18.5	20.4	0.31	<0.001	0.38	<0.001
Belly fat	17.2	17.4	18.4	20.1	20.4	0.42	17.7	19.7	0.28	<0.001	0.44	<0.001
Iodine value, g/100g[6]												
Jowl fat	67.5	68.1	69.0	71.1	73.3	0.45	68.9	70.7	0.30	<0.001	0.41	<0.001
Backfat	68.5	68.4	69.2	73.0	73.5	0.63	69.2	71.9	0.42	<0.001	0.99	<0.001
Belly fat	67.1	68.0	69.1	72.4	73.7	0.60	68.7	71.5	0.40	<0.001	0.64	<0.001

[1]Values are means of 18 observations per treatment.

[2]Values are means of 45 observations per treatment.

[3]Total SFA = {[C8:0] + [C10:0] + [C12:0] + [C14:0] + [C16:0] + [C17:0] + [C18:0] + [C20:0] + [C22:0] + [C24:0]}, where the brackets indicate concentration.

[4]Total MUFA = {[C14:1] + [C16:1] + [C18:1c9] + [C18:1n7] + [C20:1] + [C24:1]}, where the brackets indicate concentration.

[5]Total PUFA = {[C18:2n6] + [C18:3n3] + [C18:3n6] + [C20:2] + [C20:4n6]}, where the brackets indicate concentration.

[6]Calculated as: [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785+ [C22:1] × 0.723, where the brackets indicate concentration (AOCS, 1998).

CHAPTER 5 - Amino acid digestibility and energy concentration of a high protein corn dried distillers grains and a high protein sorghum dried distillers grains with solubles for swine

ABSTRACT: A study was conducted to determine the digestibility of AA and energy concentration of a specialized high-protein corn distillers dried grains (HPC-DDG) and a high-protein sorghum dried distillers grains with solubles (HPS-DDGS). Six growing barrows (BW = 22.7 kg) were surgically fitted with T-cannulas at the terminal ileum and randomly allotted to 3 treatments in a crossover design with 3 periods. The treatment diets were: 1) 67% HPC-DDG; 2) 50% HPS-DDGS as the sole protein sources; and 3) a N-free diet for determining basal endogenous AA loss. All diets contained 0.25% chromic oxide as an inert marker. Digesta and fecal samples were collected and analyzed for AA and energy concentrations. After chemical analysis, standardized and apparent ileal digestible (SID and AID, respectively) AA and GE were determined for each co-product. Also, DE, ME, and NE values for HPC-DDG and HPS-DDGS were calculated. The chemical composition of HPC-DDG and HPS-DDGS on a DM basis was 40.8% CP, 5.4% fat, 22.9% ADF, 36.6% NDF, 0.04% Ca, and 0.42% P; and 48.2% CP, 3.1% fat, 17.5% ADF, 20.4% NDF, 0.13% Ca, and 0.82% P; respectively. The DM content was 89.50% and 91.88% for HPC-DDG and HPS-DDGS. Analyzed AA content of HPC-DDG was higher than in traditional corn DDGS. The Lys content of HPC-DDG was 1.36% (DM basis) resulting to a Lys-to-CP ratio of 3.2%. In HPS-DDGS, most of the AA were present in higher proportions than in HPC-DDG or conventional sorghum DDGS. The HPS-DDGS Lys content was 1.7% on (DM basis) equivalent to a lys:CP ratio of 3.5%. In HPC-DDG, the AID for Lys, Met, Thr, and Trp were 65.9, 87.0, 72.8, and 76.2%, respectively while the SID values were 67.8, 87.5, 75.0, and 78.6%, respectively. For HPS-DDGS, AID for Lys, Met, Thr, and Trp were

51.9, 73.0, 60.6, and 71.7%, respectively. Values for SID were 53.7, 73.8, 63.0, and 73.8% for the same AA, respectively. The GE, DE, and calculated ME and NE values were 5,293; 3,703; 3,426; and 2,131 kcal/kg DM respectively for HPC-DDG. For HPS-DDGS, the GE, DE, ME, and NE values were 5,108; 3,878; 3,549; and 2,256 kcal/kg of DM, respectively. In conclusion, both coproducts can now be utilized in swine diet formulation using actual AA digestibility values and calculated energy concentrations.

Key words: amino acid digestibility, dried distillers grains, energy, pigs

INTRODUCTION

As the biofuel industry continues to evolve and mature, improvements and new technologies in ethanol fuel production are being adapted to increase efficiency and profitability. This has resulted in production of more types of co-products with increased attention paid to their quality. For example, a low-oil DDGS can now be produced through solvent extraction (Singh and Cheryan, 1998) which yields a co-product that has a high CP concentration due to removal of oil (Jacela et al., 2008; Saunders and Rosentrater, 2009). Another relatively new method is fractionation wherein the endosperm, germ, and bran components of corn are separated and removed before fermentation (Murthy et al., 2006). This optimizes the use of various corn fractions and allows for more efficient use of starch for ethanol production while the other components can then be used in other industries. Because majority of the bran (fiber) and germ (oil-rich) fractions have been removed, it yields a coproduct that has increased CP and reduced fiber and fat content compared to traditional DDGS (Widmer et al., 2007; Kim et al., 2009).

LifeLine Foods (St. Joseph, MO) is a company that, in addition to food products for human consumption, produces ethanol from corn by utilizing the dry defractionation method. The process differs from traditional fractionation methods in that instead of using direct heat, the distillers grains that remains after fermentation is dried with steam resulting in a high-CP, low-oil product that may have increased quality and digestibility. Aside from corn, sorghum is also a viable feedstock for ethanol production (Wang et al., 2008). Because sorghum has greater CP content than corn, DDGS derived from sorghum also has higher CP content than corn DDGS (Feoli, 2008; Urriola et al., 2009). White Energy Inc., through its ethanol plant in Russell, KS, utilizes a method called post-fermentation fractionation to produce a sorghum-based DDGS with increased CP content for use in feeding livestock.

Because HPC-DDG and HPS-DDGS are relatively new co-products of ethanol production, limited or no AA digestibility values and energy estimates are available. Thus, the objective of this experiment was to establish the AA digestibility and calculated energy of HPC-DDG and HPS-DDGS for swine.

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MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved protocols used in this experiment.

Six growing barrows (initially 22.7 kg BW; PIC, Hendersonville, TN) were surgically fitted with a T-cannula on their right flank approximately 15 cm anterior to the ileocecal valve using the procedures described by Knabe et al. (1989). The pigs were allowed to recover from surgery and were then placed in individual stainless steel metabolism (1.5 × 0.6 m) cages in an environmentally controlled building. Each cage was equipped with a feeder and a nipple drinker

for ad libitum access to water. During the first 9 d post-surgery (recovery period), the pigs were fed a common diet. On d 10 post-surgery, the pigs were randomly allocated to 1 of 3 dietary treatments in a crossover design. The treatments were: 1) 67% corn HPC-DDG; 2) 50% sorghum HPS-DDGS; and 3) a diet formulated to be N-free to allow for the determination of basal AA endogenous losses (Table 5-1 and 5-2). The corn HPC-DDG and sorghum HPS-DDGS were analyzed for AA content (Table 5-3). Chromic oxide was added in all diets at 0.25% as an indigestible marker. There were 3 periods in the experiment; each period consisted of 7 d. The first 4 d of each period was used to allow for adaptation to the dietary treatment. On d 5 and 6, grab-samples of feces were collected between 0600 and 1200 h each day while ileal digesta was collected on d 6 and 7 over a 10-h period (between 0700 and 1700 h each day). Pig weights were determined at the start of each period before switching to the next diet to determine the daily feed allocation which was given at a daily level of 3 times the estimated maintenance requirement for energy. Feed allocation was divided into 2 equal amounts and given twice daily at 0600 and 1800 h. No feed was given at the end of each period before the next experimental diet was fed the following morning.

During collection days, each pig's cannula was opened to allow the digesta to flow out of the ileum and ileal digesta was collected by attaching a latex balloon to the cannula. The balloons were checked for the level of fill and removed every 30 min or as soon as they were full. The contents of the balloons were then transferred to a 1-L plastic container and stored in a freezer (-20°C) until further chemical analyses were conducted. After the collection phase of the experiment, digesta samples from each period from each animal were thawed and homogenized. A subsample from each homogenized ileal digesta was then transferred in a 3.2-cm. × 15.2-cm. × 21.6-cm. aluminum pan, freeze-dried and ground for AA analysis. Fecal samples collected

during d 5 and 6 were stored and frozen. At the end of the collection phase, the frozen fecal samples were thawed and homogenized within each pig and diet. Subsamples of homogenized feces from each pig from each period were taken and dried in a forced air oven at 50ºC, and ground for chemical analysis.

Adiabatic bomb calorimetry (Parr Instruments, Moline, IL) was utilized to determine the energy content in the diets, HPC-DDG, HPS-DDGS, and fecal samples. The concentration of chromic oxide in the diets, digesta, and fecal samples was determined using atomic absorption spectroscopy (Perkin Elmer 3110, Waltham, MA) according to the procedures described by Williams et al. (1962). Chromic oxide served as the indigestible marker for calculating AA digestibility values. Amino acid analysis for the diets, HPC-DDG, HPS-DDGS, and ileal digesta samples was conducted at the University of Missouri—Columbia Agriculture Experiment Station Chemical Laboratories (AOAC Official Method 982.30 E (a,b,c), chp. 45.3.05, 2006). The test diets, HPC-DDG, and HPS-DDGS were submitted for proximate analysis at Ward Laboratories, Inc. (Kearney, NE).

The AID for AA (%) in the HPC-DDG and HPS-DDGS diets were calculated using the equation (Fan et al., 1995):

$$AID = [1 - (AAd/AAf) \times (Crf/Crd)] \times 100\%,$$

where AAd is the concentration of the AA in the ileal digesta (g/kg of DM), AAf is the concentration of the AA in the diets (g/kg of DM), Crf is the chromium concentration in the diet (g/kg of DM), and Crd is the chromium concentration in the ileal digesta (g/kg of DM).

The basal endogenous loss of each AA (g/kg DMI) at the ileum was determined on the basis of the digesta samples obtained when the pigs were fed with the N-free diet by using the equation (Moughan et al., 1992):

IAA_{end} = [AAd × (Crf/Crd)]

By using the values for AID and IAA_{end}, SID value for each AA (%) was then calculated as (Jondreville et al., 1995):

SID = [AID + (IAA_{end}/AAf)]

The apparent total tract digestibility (ATTD) of energy of the HPC-DDG diet was calculated using the same equation for AID of AA. This value was then multiplied by the analyzed concentration of GE in the diets to get the DE of the diet. Using the difference procedure (Adeola, 2001), DE of the HPC-DDG was calculated by subtracting 33% of the N-free DE from the DE of corn HPC-DDG diet and then dividing this amount by the percentage of corn HPC-DDG (67%) in the diet.

Digestible energy in HPS-DDGS was determined using a prediction equation based on the concentration of various nutrients:

DE = -174 + (0.848 × GE) + {2 × [100 - (CP + EE + Ash + NDF)]} - (16 × % ADF)

(R^2 = 0.87; Ewan, 1989).

Metabolizable and NE values for HPC-DDG and HPS-DDGS were calculated using the following equations:

ME = 1 × DE – 0.68 × CP (R^2 = 0.99; Noblet and Perez, 1993).

NE = (.726 × ME) + (13.3 × EE) + (3.9 × starch) - (6.7 × CP) - (8.7 × ADF) (R^2 = 0.97; Noblet et al., 1994)..

RESULTS AND DISCUSSION

Chemical Composition

The nutrient compositions of HPC-DDG and HPS-DDGS are reported in Table 5-3. For HPC-DDG, the CP content was 40.8% (DM basis) which higher than the published CP values in

traditional corn DDGS (Stein et al., 2006; Pahm et al., 2008; Urriola, 2009) but lower than previously reported by Widmer et al. (2007) and Kim et al. (2009) for other high-CP corn distillers grains products. The higher CP content of HPC-DDG resulted in every AA being higher than in traditional corn DDGS (Stein and Shurson, 2009). However, a majority of the AA were lower in HPC-DDG compared to those reported for other high-protein dried distillers grains (Widmer et al., 2007; Kim et al., 2009) which can be attributed to the higher CP content of those two co-products. This shows that nutrient composition may vary greatly between co-products of various fractionation processes used for ethanol production. Different fractionation techniques used in ethanol production have been previously reported to increase the variation in composition of coproducts (Martinez-Amezcua et al., 2007). The Lys content of HPC-DDG was 1.36% (DM basis) resulting to a high Lys-to-CP ratio of 3.2%. A Lys-to-CP ratio of not less than 2.8% is the recommended value when evaluating the quality of DDGS for use in swine diets (Stein, 2007). Stein (2007) suggested that higher Lys-to-CP ratio may indicate that the product is less heat-damaged and has good AA digestibility. Thus, the high ratio found in HPC-DDG may be an indication that the co-product has high Lys digestibility.

The fat content of the HPC-DDG was 5.4% (DM basis), which, as expected, is less than half of the average amount typically found in traditional corn DDGS. The lower fat content found in the HPC-DDG was a result of the mechanical separation of the germ portion from the rest of the corn kernel components during defractionation. On the other hand, although the ADF and NDF for the HPC-DDG were expected to be lower due to the separation of the bran during the defractionation process, the values were higher (22.9 and 36.6% of DM, respectively) than previously reported (Widmer et al., 2007) for another high-CP dried distillers grains product. However, these values were similar to the co-product evaluated by Kim et al. (2009).

The amount of Ca in HPC-DDG was similar to traditional corn DDGS at 0.04% (DM basis) and was slightly higher than Widmer et al. (2007). Similar to Widmer et al. (2007), P content was relatively low in HPC-DDG at only 0.42% (DM basis) compared to traditional corn DDGS. However, this was higher than Kim et al. (2009). The lower P content in HPC-DDG in comparison to traditional DDGS was expected because the distillers solubles, which contains most of the P (Knott et al., 2004), was not added back into the final distillers product. In addition, germ was removed during fractionation which contains majority of the phytate P (Odell et al., 1972) hence, the lower P concentration in HPC-DDG.

The other co-product evaluated in the present study which was derived from sorghum is a product of post-fermentation where a traditional sorghum DDGS co-product has undergone a second processing procedure to remove the majority of fiber and oil. This process leaves a high-protein sorghum DDGS which is also higher in concentration of other nutrients. The analyzed CP for HPS-DDGS was 48.2% (DM basis) which was higher than previously reported for conventional sorghum DDGS (Feoli, 2008; Urriola et al., 2009) as expected. Compared to HPC-DDG, CP in HPS-DDGS was higher by 7.5 percentage units. This was expected because conventional sorghum DDGS contains higher CP (Urriola et al., 2009) compared to traditional corn DDGS (Stein and Shurson, 2009). Amino acid analysis of HPS-DDGS product showed that most of the AA were present in higher proportions than in HPC-DDG or conventional sorghum DDGS as a result of the higher CP value. The Lys content of the product was 1.7% on DM basis which translates to a Lys:CP ratio of 3.5%.

The crude fat concentration was only 3.1% (DM basis), which is lower than the reported values for conventional sorghum DDGS (Urriola et al., 2009). The lower fat content was expected due to the removal of majority of the fat during post-fermentation fractionation. Also,

as a result of the removal of fiber during post-fermentation fractionation, the ADF and NDF values (17.5% and 20.4% of DM, respectively) for HPS-DDGS were also lower than reported for conventional sorghum DDGS (27.7 and 38.0%, respectively) by Urriola et al. (2009). In addition, both Ca and P were present in higher concentrations in HPS-DDGS when compared to traditional corn DDGS or to HPC-DDG.

Amino Acid Digestibility

Swine diets are ideally formulated based on the digestibility of the nutrients found in each ingredient. More specifically, these diets should be formulated based on SID AA. This study was conducted with the aim of establishing digestibility coefficients for AA values for HPC-DDG and HPS-DDGS.

In HPC-DDG, the AID for Lys, Met, Thr, and Trp were 65.9, 87.0, 72.8, and 76.2%, respectively (Table 5-4). These values were higher than previously reported AID values for traditional corn DDGS (Fastinger and Mahan, 2006; Stein et al., 2006; Pahm et al., 2008) and the high-protein dried distillers grains evaluated by Widmer et al. (2007). However, HPC-DDG had lower AID for Lys and Trp but higher AID for Met and Thr when compared to results of Kim et al. (2009). After correcting the AID values for basal ileal endogenous amino acid loss, the SID values were calculated to be 67.8, 87.5, 75.0, and 78.6% for Lys, Met, Thr, and Trp, respectively. Similar to AA AID in HPC-DDG, these SID values were also higher than those found in traditional DDGS (Stein and Shurson, 2009).

The greater digestibility of AA in HPC-DDG can be attributed to a number of factors such as the use of steam in drying instead of direct heat, which may have minimized Maillard reaction, and the absence of solubles in the final product. Pahm et al. (2008) observed that AA digestibility was greater in dried distillers grains compared to DDGS and suggested that the

solubles in DDGS are lower in AA digestibility. The low digestibility of AA in solubles may be attributed to its high concentration of reducing sugars, thus increasing the potential substrates for Maillard reaction (Wu, 1994).

However, when HPC-DDG and other high-protein dried distillers grains products were compared, AA SID was highly variable between coproducts. Values for SID in HPC-DDG were higher in the majority of AA compared to Kim et al. (2009) but lower in most AA compared to Widmer et al. (2007). These results suggest that differences in AA digestibility exist between high-protein dried distillers grains products similar to the variability commonly observed with traditional corn DDGS (Pahm et al., 2008). This may also be partly attributed to the different processing techniques which are known to influence nutrient composition of co-products (Martinez-Amezcua et al., 2007). In most cases, Lys would be expected to vary among AA digestibility values due to the heating process involved in the production of conventional DDGS. It is interesting to note that, together with Arg, Lys was the AA that was least variable (SD = 1.7) in digestibility among the AA in HPC-DDG. The high amount of lys in HPC-DDG and its high digestibility values increase its overall amino acid value in swine diets compared to traditional corn DDGS.

For HPS-DDGS, AID for Lys, Met, Thr, and Trp were 51.9, 73.0, 60.6, and 71.7%, respectively (Table 5-4). Values for SID were calculated to be 53.7, 73.8, 63.0, and 73.8% for Lys, Met, Thr, and Trp, respectively. These values were lower than those found in conventional sorghum DDGS with the exception of Trp (Urriola et al., 2009). Compared to HPC-DDG, all of the AA in HPS-DDGS, have lower SID values indicating that the method used in the production of HPS-DDGS may have subjected the AA to excessive heat. This also suggests that even though HPS-DDGS has a very high Lys-to-CP ratio, it is not a reliable indicator of the quality for

sorghum-based DDGS in terms of AA digestibility and suggests that other factors are reducing the digestibility of AA in HPS-DDGS. One factor could be the grain source itself. Digestibility of AA in sorghum is lower compared to corn (NRC, 1999; Jondreville et al., 2001) which agrees with an earlier study that reported a lower N digestibility in sorghum grain compared to corn grain (Healy et al., 1994). This was further supported by a later study comparing 2 types of DDGS which reported a lower digestibility of N in sorghum-based DDGS compared to a corn-based DDGS (Feoli, 2008). Urriola et al. (2009) suggested that, based on the study by Cervantes-Pahm and Stein (2008), higher oil in corn compared to sorghum may also contribute to increased AA digestibility in corn-based DDGS.

Energy Concentration

The analyzed and calculated energy values for the HPC-DDG and HPS-DDGS are presented in Table 5-5. The GE and calculated DE, ME, and NE for HPC-DDG were 5,293; 3,703; 3,426; and 2,131 kcal/kg of DM, respectively, which were lower than values for traditional DDGS and those reported for other HP-DDG (Widmer et al., 2007; Kim et al., 2009). The DE value for HPC-DDG represents only 70% of the GE in contrast to 88% for other HP-DDG products (Widmer et al., 2007; Kim et al., 2009). Widmer et al. (2007) explained that the high energy value of the HP-DDG they evaluated was due to the lower proportions of ADF and NDF in the co-product they tested. However, this was not the case for HPC-DDG which had higher fiber content and closely resembles the ADF and NDF profile of the HP-DDG that Kim et al. (2009) evaluated. Kim et al. (2009) suggested that the high DE in their product could be due to its high CP content.

The GE and calculated DE, ME, and NE for HPS-DDGS were 5,108; 3,878; 3,549; and 2,256 kcal/kg of DM, respectively. The DE for this DDGS product was, as expected, lower than

the DE in traditional corn DDGS (4,140 kcal/kg DM; Pedersen et al., 2007) because of its low fat content. However, the DE for HPS-DDG was substantially higher than previously reported for sorghum DDGS (Feoli, 2008). Compared to HPC-DDG, DE, ME, and NE were higher in HPS-DDGS.

In summary, this experiment has established the values for the digestibility coefficients of AA and energy for two distinct high-protein distillers grains co-products for use in swine diet formulation. Compared to corn and sorghum DDGS, both coproducts tested in this study had greater concentration of AA. However, AA digestibility was higher in HPC-DDG but lower in HPS- DDGS compared to traditional DDGS. Also, both have lower energy values than previously reported for conventional corn DDGS. However, HPS-DDGS has higher DE than conventional sorghum DDGS. Also, the phosphorus content was lower in HPC-DDG compared to traditional corn DDGS. Therefore, both coproducts appear to be well-suited for use in swine diets and actual digestibility of AA and energy can now be used in valuing these co-products.

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Table 5-1. Composition of test diets (as-fed basis)

	Diet		
Ingredient, %	High-protein corn distillers dried grains	High-protein sorghum dried distillers grains with solubles	N-free
HPC-DDG [1]	66.70	---	---
HPS-DDGS [2]	---	50.00	---
Corn starch	26.20	43.40	80.90
Soybean oil	1.00	1.00	3.00
Sucrose	3.00	3.00	9.00
Solka floc[3]	---	---	3.00
Limestone	0.80	1.35	0.40
Monocalcium phosphate, 21% P	1.05	0.00	1.75
Vitamin premix[4]	0.50	0.50	0.50
Trace mineral premix[5]	0.15	0.15	0.15
Salt	0.35	0.35	0.45
Potassium chloride	---	---	0.50
Magnessium oxide	---	---	0.10
Chromic oxide	0.25	0.25	0.25

[1]High-protein corn dried distillers grains (LifeLine Foods, LLC, St. Joseph, MO).
[2]High-protein sorghum dried distillers grains with solubles (White Energy, Inc., Russell, KS).
[3]Fiber Sales and Development Corp., Urbana, OH.
[4]Provided per kg of complete diet: 11,023 IU of vitamin A;1,653 IU of vitamin D3; 44 IU of vitamin E; 4 mg of vitamin K; 8 mg of riboflavin; 28 mg of pantothenic acid; 50 mg of niacin; 0.04 mg of vitamin B12; 551,150 mg of choline; 220 mg of biotin; 1,654 mg of folic acid; and 4,960 mg of pyridoxine.
[5]Provided per kg of complete diet: 39.7 mg Mn from manganese oxide, 165 mg Fe from iron sulfate, 165 mg Zn from zinc oxide, 16.5 mg Cu from copper sulfate, 0.298 mg I from calcium iodate, and 0.298 mg Se from sodium selenite.

Table 5-2. Analyzed nutrient composition of experimental diets (%; as-fed basis)

	Diet		
Item	High-protein corn distillers dried grains[1]	High-protein sorghum dried distillers grains with solubles[2]	N-free
DM	90.14	91.62	90.53
CP	24.50	25.20	0.50
Indispensable AA			
Arg	1.25	1.02	0.01
His	0.79	0.61	0.01
Ile	1.14	1.19	0.01
Leu	3.82	3.29	0.00
Lys	0.94	0.97	0.02
Met	0.60	0.46	0.00
Phe	1.53	1.38	0.02
Thr	1.05	0.97	0.01
Trp	0.23	0.26	< 0.04
Val	1.45	1.45	0.02
Total indispensable AA	12.81	11.59	< 0.11
Dispensable AA			
Ala	2.20	2.19	0.02
Asp	1.87	1.93	0.02
Cys	0.58	0.43	0.01
Glu	5.16	4.66	0.14
Gly	1.08	0.93	0.01
Pro	2.33	1.89	0.03
Ser	1.31	1.09	0.01
Tyr	1.09	0.97	0.02
Total dispensable AA	15.61	14.09	0.28

[1]High-protein corn dried distillers grains (LifeLine Foods, LLC, St. Joseph, MO).
[2]High-protein sorghum dried distillers grains with solubles (White Energy, Inc., Russell, KS).

Table 5-3. Analyzed nutrient composition of high-protein corn dried distillers grains and high-protein sorghum dried distillers grains with solubles

	HPC-DDG[1]		HPS-DDGS[2]	
Nutrient, %	DM-basis	As-is	DM-basis	As-is
DM	100	89.54	100	92.29
CP	40.76	36.5	48.22	44.5
Crude fat	5.36	4.8	3.14	2.9
ADF	22.9	20.5	17.5	16.1
NDF	36.6	32.8	20.4	18.8
Ca	0.04	0.04	0.13	0.12
P	0.42	0.38	0.82	0.76
Ash	1.84	1.65	5.01	4.62
Indispensable AA				
Arg	1.84	1.65	1.85	1.71
His	1.16	1.04	1.11	1.02
Ile	1.69	1.51	2.18	2.01
Leu	5.45	4.88	5.89	5.44
Lys	1.36	1.22	1.73	1.60
Met	0.88	0.79	0.85	0.78
Phe	2.14	1.92	2.47	2.28
Thr	1.45	1.30	1.79	1.65
Trp	0.26	0.23	0.39	0.36
Val	2.21	1.98	2.63	2.43
Dispensable AA				
Ala	3.06	2.74	3.86	3.56
Asp	2.66	2.38	3.48	3.21
Cys	0.85	0.76	0.80	0.74
Glu	6.99	6.26	7.68	7.09
Gly	1.49	1.33	1.64	1.51
Pro	3.17	2.84	3.11	2.87
Ser	1.72	1.54	1.96	1.81
Tyr	1.66	1.49	1.87	1.73

[1]High-protein corn dried distillers grains (LifeLine Foods, LLC St. Joseph, MO).
[2]High-protein sorghum dried distillers grains with solubles (White Energy, Inc., Russell, KS).

Table 5-4. Apparent (AID) and standardized (SID) ileal digestibility of AA in high-protein corn dried distillers grains and high-protein sorghum dried distillers grains with solubles[1]

	AID, %		SID, %[4]	
Amino acid	HPC-DDG[2]	HPS-DDGS[3]	HPC-DDG	HPS-DDGS
Indispensable AA				
Arg	83.79 (1.8)	76.08 (3.6)	85.32 (1.7)	77.97 (3.3)
His	79.04 (2.9)	61.38 (4.4)	80.00 (3.0)	62.62 (4.1)
Ile	80.23 (2.5)	68.64 (3.0)	81.35 (2.6)	69.71 (2.8)
Leu	88.31 (1.9)	73.09 (3.1)	88.87 (1.9)	73.74 (3.0)
Lys	65.91 (1.7)	51.86 (5.3)	67.82 (1.7)	53.71 (4.9)
Met	86.96 (1.9)	73.04 (3.1)	87.53 (1.9)	73.78 (3.0)
Phe	85.24 (2.0)	71.89 (3.0)	86.10 (2.1)	72.85 (2.8)
Thr	72.75 (3.4)	60.57 (5.3)	75.00 (3.5)	63.01 (4.9)
Trp	76.23 (3.5)	71.72 (3.4)	78.61 (3.7)	73.84 (2.8)
Val	78.13 (2.5)	66.52 (3.3)	79.70 (2.6)	68.08 (3.2)
Dispensable AA				
Ala	83.40 (1.9)	67.42 (3.9)	84.37 (2.0)	68.39 (3.8)
Asp	72.13 (3.5)	62.02 (4.8)	73.83 (3.7)	63.67 (4.5)
Cys	75.51 (5.0)	63.7 (5.4)	76.84 (5.1)	65.51 (5.0)
Glu	84.94 (2.3)	68.73 (3.8)	85.73 (2.4)	69.6 (3.6)
Gly	61.34 (3.4)	40.10 (8.0)	66.69 (3.7)	46.31 (7.1)
Pro	74.52 (9.5)	54.27 (10.1)	79.12 (8.2)	59.95 (10.8)
Ser	81.24 (2.8)	68.76 (3.6)	82.87 (2.9)	70.72 (3.3)
Tyr	85.10 (1.9)	70.46 (3.4)	86.07 (2.0)	71.56 (3.2)

[1]Values are means of 6 observations per treatment. Standard deviation for each digestibility value is shown in parentheses.

[2]High-protein corn dried distillers grains (LifeLine Foods, LLC St. Joseph, MO).

[3]High-protein sorghum dried distillers grains with solubles (White Energy, Inc., Russell, KS).

[4]The SID represents the corrected AID accounting for basal endogenous loss of an AA. Calculated basal endogenous losses after feeding the N-free diet were (g/kg of DMI): Arg, 0.19; His, 0.08; Ile, 0.13; Leu, 0.22; Lys, 0.18; Met, 0.03; Phe, 0.13; Thr, 0.24; Trp, 0.06; Val, 0.23; Ala, 0.21; Asp, 0.32; Cys, 0.08; Glu, 0.40; Gly, .58; Pro, 1.07; Ser, 0.21; Tyr, 0.11.

Table 5-5. Energy values (g/kg DM) of high-protein corn dried distillers grains and high-protein sorghum dried distillers grains with solubles[1]

Item	HPC-DDG[2]	HPS-DDGS[3]
GE	5,293	5,108
DE[4]	3,703	3,878
ME[5]	3,426	3,549
NE[6]	2,131	2,256

[1]Values are means of 6 observations per treatment.
[2]High-protein corn dried distillers grains (LifeLine Foods, LLC St. Joseph, MO).
[3]High-protein sorghum dried distillers grains with solubles (White Energy, Inc., Russell, KS).
[4]The DE value of HPC-DDG was determined using the difference procedure (Adeola, 2001). The DE value of HPS-DDGS was calculated by using the equation: DE = -174 + (0.848 × GE) + {2 × [100 - (CP + EE + Ash + NDF)]} - (16 × % ADF) (R^2 = 0.87; Ewan, 1989).
[5]The ME value of high protein corn DDG was calculated using the equation: ME = 1 × DE – 0.68 × CP (R^2 = 0.99; Noblet and Perez, 1993).
[6]The NE value of high protein corn DDG was calculated by using the equation: NE = (.726 × ME) + (13.3 × EE) + (3.9 × starch) - (6.7 × CP) - (8.7 × ADF) (R^2 = 0.97; Noblet et al., 1994).

CHAPTER 6 - A meta-analysis of supplemental enzyme studies in growing-finishing pigs fed diets containing dried distillers grains with solubles: effects on growth performance

ABSTRACT: A meta-analysis of 4 experiments involving 4,506 pigs was conducted to determine the effects of different commercial enzymes on the growth performance of grow-finish pigs fed dried distillers grains with solubles (DDGS). Experiments 1 and 2 utilized corn-soybean meal-based diets with 15% DDGS. A β-mannanase enzyme (Hemicell®; ChemGen Corp., Gaithersburg, Maryland) was used in Exp. 1 while a blend of enzymes that contained β-glucanase, cellulase, and protease activities (Agri-king REAP®; Agri-King, Inc., Fulton, IL) were used in Exp. 2. In Exp. 3, diets containing 45 and 60% DDGS were fed with or without 2 commercial proprietary enzyme products designed for use in diets containing DDGS. In Exp. 4, an enzyme product with bacterial endo-1,4-β-xylanase was evaluated in diets containing 30% DDGS. All the enzyme treatments in each experiment were pooled in a meta-analysis to compare the responses to diets with or without enzyme addition regardless of the other factors tested in each trial. All experiments were conducted in the same commercial swine research facility. There were no differences in ADG ($P > 0.80$), ADFI ($P > 0.99$), G:F ($P > 0.58$), and final weight ($P > 0.67$) among pigs that were fed diets with added enzyme compared to pigs fed diets without enzyme in any of the 4 experiments or in the pooled data. In conclusion, based on the combined results from the 4 experiments evaluated in this meta-analysis, adding dietary enzymes in diets containing DDGS was not beneficial in grow-finish pigs.

Key words: dried distillers grains with solubles, enzyme, growth, swine

INTRODUCTION

The use of carbohydrate- and protein-degrading enzymes in livestock diets has received a great deal of attention over the past decade as an aid to improve nutrient utilization from plant-based ingredients. Studies conducted in poultry have consistently shown favorable results with the use of exogenous enzymes (Wu et al., 2004; Cowieson and Adeola. 2005; Olukosi et al., 2007; Cowieson and Ravindran, 2008) but this has not been the case in pigs (Danicke et al., 1999; Partridge, 2000). Some experiments have reported beneficial effects of enzyme supplementation to diets on pig performance but in general results have been inconsistent. Nevertheless, in view of the potential benefits of improved feed efficiency with high feed cost, there is renewed interest in adding exogenous enzymes in swine diets.

The increased interest in dietary enzyme use can be attributed to the increasing use of less expensive alternative feed ingredients, most notably dried distillers grains with solubles (DDGS). Dried distillers grains with solubles have a higher fiber content and lower digestibility then corn when fed to swine (Stein and Shurson, 2009). Hence, there may be the potential to increase DDGS's nutritional value by using exogenous enzymes to aid in breaking down its fiber components and increase the availability of nutrients to the pig. Experimental results suggest that DDGS can be fed to pigs up to 30% in the diets before a decrease in performance is observed (Cook et al., 2005; Xu et al., 2007; Augspurger et al., 2008). With growing knowledge about enzyme technology, the use of fiber-degrading enzymes provides an opportunity to maximize the value of DDGS for swine by improving its nutrient digestibility. This, in turn, may potentially allow for higher inclusion rates of DDGS in swine diets than typically recommended. Therefore, we conducted a meta-analysis combining data from 4 different experiments utilizing various commercial enzyme products to determine their effects on the growth performance of grow-finish pigs fed varying amounts of DDGS.

MATERIALS AND METHODS

The procedures used in the experiments were approved by the KSU Institutional Animal Care and Use Committee. The meta-analysis conducted involved 4 different experiments using a total of 4,506 pigs of the same genetics (L337 × 1050; PIC, Hendersonville, TN). The first trial (Exp. 1) started on October 24, 2007 and the last trial (Exp. 4) ended on April 30, 2009. All experiments were conducted in the same commercial swine research facility located in southwestern Minnesota. The barns were naturally ventilated and double curtain sided. Pens were 5.5 × 3.0 m with completely slatted flooring and deep pits for manure storage. Each pen is equipped with a single sided self-feeder and a cup waterer. Each barn had an automated feeding system (FeedPro^{TM}; Feedlogic Corp., Willmar, MN) capable of delivering and recording data on feed amounts added on an individual pen basis.

Information regarding the 4 trials is shown in Table 6-1. In Exp. 1, a total of 1,269 pigs were assigned to treatments in a 2 × 2 × 2 factorial arrangement. The factors were Porcine Circovirus Type 2 vaccine dose (half or full), enzyme (with or without), and gender (barrow or gilt). Pigs were assigned to pens in the wean-to-finish facility, identified by treatment using colored ear tags, and vaccinated according to the vaccine treatment twice in 2-wk intervals. The enzyme used was a commercially available β-mannanase (Hemicell®, ChemGen Corp., Gaithersburg, Maryland; Table 6-2) added at 0.05% of the diet starting at d 20. In Exp. 2, a total of 1,129 pigs were assigned to treatments in a 2 × 3 factorial arrangement. The dietary treatments were increasing levels of fat (0, 2.5, and 5.0%) with or without added enzyme (0.05% or 0% Agri-king REAP®; Agri-King, Inc., Fulton, IL). The commercial enzyme used was a proprietary blend of enzymes that had β-glucanase, cellulase, and protease activities. Diet samples were analyzed and confirmed by the manufacturer to have the appropriate enzyme activities. Diets were fed in 3 phases with Phase 1 fed from 34 to 50 kg, Phase 2 fed from 50 to 73 kg, and Phase

3 fed from 73 to 91 kg BW (Table 6-3). Similar to Exp. 1, diets were based on corn and soybean meal with 15% added DDGS and were balanced to a constant lys-to-calorie ratio (2.98, 2.68. and 2.38 g SID Lys/Mcal ME for phases 1, 2, and 3; respectively) within diet phase.

In Exp. 3, a total of 1,032 pigs were allotted to a control treatment (approximately 30% DDGS) and 6 additional treatments in a 2 × 3 factorial arrangement based on DDGS level (45 or 60%) and enzyme used (none, Allzyme, or enzyme A). Allzyme is a commercial enzyme blend containing protease, amylase, xylanase, β-glucanase, pectinase, cellulase, and phytase (Allzyme® SSF; Alltech Inc., Nicholasville, KY) while enzyme A is a proprietary multi-enzyme product. We were unaware of the specific activity of the enzymes included in enzyme A. However, the manufacturer indicated the enzyme blend was designed for use in diets that contained DDGS as was Allzyme. Diets were fed in 4 phases from approximately 45 to 58 kg, 58 to 84 kg, 84 to 104 kg, and 104 to 123 kg BW for phases 1 to 4, respectively (Table 6-4). During the first 2 wk of the experiment (Phase 1), the 60% DDGS treatments contained only 45% DDGS to allow for an adjustment period to higher levels of DDGS in the diets. Regardless of treatment, levels of DDGS were reduced to 20% in all diets during the last 12 d of the experiment. This adjustment was done to help alleviate the decreased carcass yield impact when pigs are fed high levels of DDGS prior to market (Linneen et al., 2008).

In Exp. 4, a total of 1,076 pigs were randomly allotted to 1 of 3 treatments balanced by average BW within gender. A diet with 3% added fat (Control diet) was formulated using NRC (1998) values for ME of corn and soybean meal (3,420 and 3,380 kcal ME/lb, respectively; Tables 6-5 and 6-6). In this experiment, note that for DDGS, the NRC (1998) ME value for corn was used as the ME value for DDGS (3,420 kcal/kg). As directed by the manufacturer of the enzyme product tested in the study, an increased ME value was calculated for corn, soybean

meal, and DDGS to account for the expected increase in ME with the addition of enzyme (Table 6-5). This was based on the assumption that the addition of enzyme will increase the energy value of the ingredients. Using the calculated increased ME values, dietary fat was removed proportionately in the second dietary treatment with added enzyme (Nutrase) so that the dietary energy value was similar to the Control diet. The enzyme evaluated in the experiment was a commercial product containing bacterial endo-1,4-beta-xylanase (Nutrase; Nutrex, Lille, Belgium) added at the expense of corn. The third diet was similar in composition to the enzyme diet but without added enzyme (low energy). Thus, the calculated dietary energy content was lower than the control and enzyme diets (Table 6-6). Diets were corn-soybean meal-based containing DDGS fed in 3 phases (39 to 59 kg, 59 to 84 kg, and 84 to 95 kg BW, respectively). Thirty percent DDGS were included in diets from 39 to 84 kg and 15% included in the last phase from 84 to 95 kg. All diets were formulated to contain 0.94, 0.80, and 0.69% SID Lys from phase 1 to 3. Thus, the control and enzyme dietary treatments were balanced to a constant SID Lys-to-calorie ratio at 2.69, 2.29, and 1.97 g SID Lys/Mcal ME, respectively while the low energy dietary treatment had calculated Lys-to-calorie ratios of 2.73, 2.32, and 2.00 g SID Lys/Mcal ME for phases 1, 2, and 3, respectively.

Samples of DDGS from 5 different batches, were analyzed for non-starch polysaccharide (NSP) content using gas chromatography (Table 6-7 and 6-8; Englyst and Cummings, 1984). All the DDGS used in the four experiments was sourced from the same ethanol plant (Valero Energy Corp., Aurora, SD).

Statistical Analysis

Initially, within each trial the effects of feeding the various enzymes were evaluated with separate statistical models. In general these models included the effects of enzyme treatment and

for factorial studies the interaction between the enzyme treatments and the other factor or factors. With the exception of Exp. 3 which was analyzed as randomized complete block design, data from each experiment were analyzed as a completely randomized design with pen as the experimental unit. In Exp. 1, the main effects of PCV2 vaccine dose, enzyme, and gender were analyzed as well as the two- and three-way interactions of these factors. In Exp. 2, the main effects of enzyme and added fat were analyzed. Linear and polynomial contrasts were used to determine the main effects of increasing added fat levels. One pen on the 5% added fat with enzyme treatment was excluded from data analysis as an outlier due to ADG and G:F values that fell over two SD from the means during the last treatment period. In Exp. 3, linear and polynomial contrasts were used to determine the main effects of increasing DDGS. The main effects of enzyme and DDGS were determined using single degree of freedom contrast and estimate statements. In Exp. 4, the main effects of dietary treatment and gender as well as their interactions were tested. Since there were no significant effects of enzyme treatment detected within an individual experiment, the data from the four experiments were pooled to perform the metaanalysis in order to increase the sensitivity of detecting an enzyme response. A secondary analysis was performed by pooling the data from all 4 experiments. In each experiment, all the enzyme treatments were pooled into one treatment (yes) to compare the responses to treatments without enzyme (no). Pen was the experimental unit in all trials. Data from the 4 experiments were then pooled and statistical analysis was performed by ANOVA using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) with the fixed effect of enzyme (yes vs. no) and with the random effects of trial and sex.

RESULTS

Samples of DDGS that were analyzed for various NSP fractions were found to contain high amounts of arabinoxylans (Table 6-7 and 6-8).

In Exp. 1, there was neither ($P > 0.18$) a gender-by-vaccine-by-enzyme interaction nor gender-by-enzyme or vaccine-by-enzyme interaction for ADG, ADFI, and G:F (Table 6-9). As expected, there were gender effect ($P < 0.0001$) with barrows exhibiting greater ADG and ADFI but poorer G:F compared to gilts. There was, however, no effect ($P > 0.41$) of enzyme detected on growth performance.

In Exp. 2, there were no significant interactions ($P > 0.33$) between the addition of enzyme and added fat for ADG, ADFI, and G:F (Table 6-10). There was no difference ($P > 0.54$) in ADG, ADFI, or G:F between pigs fed diets with and without added enzyme. However, ADG and G:F increased (linear; $P < 0.01$) as added fat was increased in the diets.

In Exp. 3, there were no enzyme-by-DDGS level interactions ($P > 0.18$) for any variable measured (Table 6-11). Enzyme supplementation did not affect ($P > 0.24$) ADG, ADFI, or G:F. However, increasing DDGS led to ADFI reduction (linear; $P < 0.04$) but did not affect ($P > 0.17$) ADG, or G:F.

In Exp. 4, there were no treatment-by-gender interactions ($P > 0.25$) observed for any response criteria (Table 6-12). Similar to Exp. 1, barrows had greater ($P < 0.01$) ADG and ADFI but poorer G:F compared to gilts. Overall, there were no differences between Low energy, Control, and enzyme treatments for ADG ($P = 0.86$), ADFI ($P = 0.93$), G:F ($P = 0.65$) and final BW ($P = 0.88$)

To obtain increased power to detect potential differences of added dietary enzymes, data from the 4 experiments were pooled. A meta-analysis was used to take into account the variability within each of the experiments. There were no differences in ADG ($P > 0.80$), ADFI

($P > 0.99$), F/G ($P > 0.58$), and final weight ($P > 0.67$) among pigs that were fed diets with or without added enzyme in any of the 4 experiments or in the pooled data (Table 6-13).

DISCUSSION

The results of these trials and the pooled analysis are similar to a number of other experiments in pigs which did not find any significant impact of enzyme supplementation on growth performance of pigs fed diets containing DDGS both in the nursery (Jones, 2009) and finishing stages (Gaines et al., 2007; Feoli, 2008). Note that when the data were pooled across the 4 experiments, the standard error of the difference (SED) was reduced compared to the within trial SEDs for each of the response criteria. This reduction should lead to an increased sensitivity to detect a significant difference. However, no significant differences were detected even with the combined data.

In the first experiment, a commercially available enzyme with β-mannanase activity was used in corn-soybean meal-based diets with 15 % added DDGS. This enzyme specifically targets the hemicellulose galactomannan, a NSP found in relatively lower proportions in corn. Previous studies utilizing this enzyme in nursery and finishing pig diets have shown improvements in growth performance (Hahn et al., 1995; Smiricky et al., 2000; O'Quinn et al., 2002; Pettey et al., 2002). However, this was not the case in the first study included in this meta-analysis as no impact of β-mannanase was detected based on pig performance. This agrees with other studies with this enzyme which also did not find a significant effect on growth (Schneider et al., 2003; Lenehan et al., 2004). It is important to note that β-mannans are more abundant in soybean meal compared to corn or DDGS. As DDGS is added in a corn-soybean meal-based diet, it also replaces a significant proportion of soybean meal in the diet because it also has a relatively high AA content. Thus, the opportunity to find a significant β-mannanase effect would be less likely

due to the reduction in soybean meal as there was a decreased amount of potential substrates for β-mannanase to act on. This may be one plausible explanation to the absence of any response seen in Exp. 1.

Because of carbohydrate composition variability in swine diets based on ingredients utilized, and dietary enzymes act on specific substrates, a combination of several enzymes that can act on various substrates present in DDGS-containing diets might be a more logical approach. Using the same level of DDGS as in Exp. 1, a commercial enzyme blend designed to act on and break down various carbohydrate fractions was used in Exp. 2. Similar to the results obtained in Exp. 1, no significant improvement in growth performance was observed with the addition of the commercial enzyme product. It can be concluded, therefore, that the enzyme products used in Exp. 2 was not effective when used in diets containing 15% DDGS and fed to growing pigs. Ji et al. (2008) utilized the same enzyme product in their study to investigate its effects on nutrient digestibility in growing pigs. They found that hindgut digestibility of nutrients was increased. However, because majority of nutrient absorption occurs in the small intestine and not in the large intestine, the increased digestibility of nutrients described by Ji et al. (2008) may not be significant at all because the pig is not being able to utilize the digested nutrients. This may explain the lack of response in Exp. 2.

Some researchers (Danicke, 1999; Olukosi et al., 2007) offered a number of possible explanations as to why results from enzyme supplementation experiments in pig diets have been contradictory including diet composition (i.e., amount of substrate). Based on this, it may be possible that the 15% DDGS inclusion in the first 2 experiments may be insufficient to provide high enough levels of NSP in the diets to negatively influence the digestibility of nutrients in the diets. In the commercial research facility where the 4 experiments were conducted, diets

containing 30% DDGS fed to grow-finish pigs have resulted in growth performance comparable to corn-soybean meal-based diets without DDGS (Jacela et al., 2009). Thus, we tested the effect of feeding higher levels of DDGS (45 to 60%) and if enzyme supplementation, using two multi-enzyme preparations designed for use in DDGS-containing diets will help alleviate the negative effects of high levels of DDGS on growth performance. The use of these levels of dietary DDGS would significantly increase the amount of possible substrates for the enzymes to act on. However, there was no significant effect of enzyme supplementation on growth performance of grow-finish pigs even with high levels of DDGS that should have provided adequate substrate. In contrast, Emiola et al. (2009) reported a different finding using a wheat DDGS. They observed that the enzyme blend used in their study was more effective in improving nutrient digestibility when higher inclusions of wheat DDGS were used, apparently as a result of greater availability of substrate. The results from Exp. 3, unlike in Emiola et al. (2009), rule out substrate availability as a possible limiting factor in finding an enzyme effect in our study and would suggest that the commercial enzymes tested were not efficacious in diets containing DDGS derived from corn.

Substrate specificity is another factor that can influence the effect of enzyme. Corn DDGS contains appreciable amounts of arabinoxylans, a major NSP found in most grains (Ward et al., 2008b) such as corn, which is the main grain source of traditional DDGS in the U.S. (Ward et al., 2008a). The DDGS samples from the ethanol plant where all the DDGS for all the experiments in this meta-analysis were sourced were confirmed to contain significant amounts of arabinoxylan. Thus, an enzyme containing xylanase activity that can degrade arabinoxylans may aid in improving the digestibility of nutrients in corn DDGS. Available energy also can be potentially increased with supplementation of the appropriate enzyme. Energy source ingredients

such as added fat can be reduced in the diets and still meet the targeted energy level of the diet because of the expected uplift in energy value resulting from the addition of enzyme. This also can have a significant impact on economics by reducing the overall diet cost. Thus, using a product bearing xylanase activity can potentially increase the energy value of DDGS. In Exp. 4, we investigated the effect of a bacterial endo-1,4-β-xylanase on growth performance of pigs fed diets containing 30% DDGS. However, similar to the first 3 experiments, we did not observe any significant impact of enzyme supplementation on the growth performance of grow-finish pigs. Inconsistent results on growth performance from the use of xylanases have also been reported in other studies using other feedstuffs such as wheat (Mavromichalis et al., 2000; Jones, 2009) and diets containing other fibrous by-products such as wheat middlings (Feoli et al., 2006).

Previous research on the use of carbohydrases in pig diets have indicated mixed results. The use of single or combination of enzymes that target NSPs in fibrous feedstuffs improved the digestibility of nutrients in a number of studies (Li et al., 1996; Barrera et al., 2004; Nortey et al., 2007b; Nortey et al., 2007a; Sterk et al., 2007) but not in others (Zijlstra et al., 2004; Diebold et al., 2005). The reported increase in nutrient digestibility in pig diets also resulted in improved growth performance in some studies but was not consistent across all experiments (Inborr et al., 1993; Officer, 1995; Olukosi et al., 2007).

Historically, enzymes in pig diets have failed to show consistent positive results unlike in poultry (Danicke et al., 1999; Partridge, 2000). The differences in responses between pig and poultry had been attributed to a number of factors. Most of the beneficial effects of enzyme in poultry have been associated with the reduction in viscosity of the digesta leading to more efficient digestion of nutrients and better growth performance (Choct and Annison, 1992). However, the same cannot be said in pigs as the anti-nutritive effect of NSP in pigs does not

appear to be due to its viscosity-increasing effect on the digesta (Hogberg; Lindberg, 2004; Zijlstra et al., 2004) but may rather be attributed to its role as a physical barrier that traps the nutrients within feedstuffs and keeps them hidden from enzyme activity (Grieshop et al., 2001).

It has been recommended that in conducting experiments for evaluating enzymes in pigs, diets containing the enzyme must be formulated in such a way that it is deficient in a particular nutrient (e.g. energy) that will potentially be increased with the addition of enzyme (Bedford, 2002). According to Bedford (2002), feeding an animal with a diet that completely satisfies its nutrient requirements leaves no room for an enzyme to exert a comparable and measurable effect. To satisfy this requirement, a lower energy diet (negative control) was used in Exp. 4. The reduction in energy in the low energy diet was equivalent to the increase in ME recommended from the addition of enzyme according to the uplift value provided by the enzyme manufacturer. However, no significant improvement in growth performance was detected in pigs fed the enzyme containing diet that would have indicated increased energy availability. Even though pigs fed diets with enzyme supplementation performed similarly to the pigs fed the control diets with 3% added fat, we are unable to conclude that the addition of enzyme was able to increase the energy value of the diets because pigs fed the low energy diets also performed similarly to the control diet-fed pigs. In the study by Olukosi et al. (2007), they fed xylanase-supplemented diets that met or exceeded NRC nutrient recommendations to both growing pigs and broiler chicks. They found significant improvements in growth performance in broiler chicks when fed diets containing xylanase, but not in pigs. Based on these results, they suggested that whether a diet was nutritionally marginal or not, is not the main reason for the lack of enzyme effect on growth performance but whether anti-nutritional factors were causing enough deleterious effect for the enzymes to counteract. Another possibility that they raised was that NSPs in the

feedstuffs in the diets without enzymes were present in minimal amounts to have a negative impact on pig performance. However, in the third study included in this meta-analysis where we used 60% DDGS in pig diet, there should be very high amounts of NSPs present in the diets but no effects were seen using enzymes that were specifically intended for diets with added DDGS. The study by Olukosi et al. (2007) and the absence of positive response from the experiments in this meta-analysis, give further support to the fact that species of the animal could indeed be the major factor that affects the response to supplemental enzymes. In addition, because the very high inclusion levels of DDGS in Exp. 3 did not impact performance, it seems likely that NSPs found in corn DDGS are not detrimental to the pig, at least at the 60% inclusion that was used in the study. Thus, no further improvement in performance can be realized and this makes possible enzyme effect in pig diets insignificant.

Age of the animal may also influence the response to enzyme supplementation as younger pigs tend to show more response (Li et al., 1996; Diebold et al., 2004; Olukosi et al., 2007). This may be attributed to a more developed digestive tract and enzyme system as the pig matures. It was observed in a previous study that 20-kg pigs showed greater response to enzyme supplementation compared to 60 to 95-kg pigs (Graham et al., 1988). Olukosi et al. (2007) reported improved performance in 10-kg but not in 23 kg pigs fed corn-soybean meal-based diets with added multi-enzyme. In the studies included in this meta-analysis, the youngest pig started on a trial was almost 30-kg which may have limited the potential effect of enzymes. However, a lack of improvement in nutrient digestibility with the use of enzymes in nursery pig diets have also been reported previously (Diebold et al., 2005). This agrees with a later study by Jones et al. (2009) in nursery pig fed diets based on corn and soybean meal with added DDGS which showed that enzyme supplementation had no effect on growth performance.

Another factor that may possibly influence the potential effect of enzyme is particle size. One of the modes of action of supplemental enzymes in pig diets that have been proposed was it aids in breaking down the feedstuffs in smaller fractions, allowing the pig's endogenous enzymes to reach its substrate and exert their effect. However, studies have also shown that grinding cereal grains into smaller particle size results in better digestibility resulting in improved performance (Wondra et al., 1995; Mavromichalis et al., 2000; Kim et al., 2005). Particle size reduction has been shown to increase nutrient digestibility more than the addition of enzyme in the diet (Oryschak et al., 2002). In the production system where these experiments were conducted, smaller particle has been a major point of emphasis in preparing their diets. Thus, it seems likely that the potential effect of exogenous enzymes, if there is any, may have been limited by the already increased exposure of nutrients to the pig's exogenous enzymes due to breaking down of cell wall components during milling.

In conclusion, adding the enzymes used in these 4 studies withdiets containing DDGS as a means to improve nutrient and energy utilization, does not appear to be beneficial in pigs as measured by growth performance. Even with some of the factors that affect enzyme efficacy addressed in the 4 experiments such as substrate specificity and level of DDGS, the enzyme products used did not have any positive effect on growth performance. It appears, at this point, that the exogenous enzymes tested in the present trials are not justified for use in corn-soybean meal-based swine diets containing DDGS as a means to improve pig performance.

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Table 6-1. Details of individual experiments included in the study[1]

Trial No.	Duration, d	Experimental units, n	Initial BW, kg	DDGS, %	Enzyme activity of product used
1	92	47	29.6	15	β-mannanase
2	56	42	34.4	15	β-glucanase, cellulase, and protease
3	90	42	46.0	45 and 60	A product containing protease, amylase, xylanase, β-glucanase, pectinase, cellulose, and phytase activities, and a product containing proprietary blend of enzymes
4	66	39	39.6	30	Bacterial endo-1,4-beta-xylanase

[1]Data from 4 experiments involving 4,506 pigs.

Table 6-2. Diet composition (as-fed basis), Exp. 1[1,2]

	Phase			
Item	1	2	3	4
Ingredient, %				
Corn	54.92	58.62	57.93	62.12
Soybean meal, 46.5% CP	23.30	19.85	13.20	17.10
Dried distillers grains with solubles	15.00	15.00	20.00	12.00
Bakery by-product	5.00	5.00	5.00	5.00
Choice white grease	---	---	2.25	2.20
Limestone	0.90	0.80	0.80	0.75
Salt	0.25	0.25	0.25	0.25
Copper sulfate	0.10	---	---	---
Vitamin premix[3]	0.08	0.06	0.06	0.04
Trace mineral premix[4]	0.08	0.07	0.07	0.03
L-Lys HCl	0.29	0.27	0.35	0.37
DL-Met	0.01	---	---	0.03
L-Thr	0.02	0.03	0.03	0.08
Ractopamine HCl[5]	---	---	---	0.025
Virginiamycin[6]	0.05	0.05	0.05	---
Phytase[7]	0.01	0.01	0.01	0.01
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Standardized ileal digestible AA				
Lys, %	0.94	0.80	0.72	0.95
Met:Lys, %	31	34	36	30
Met and Cys:Lys, %	64	71	74	58
Thr:Lys, %	66	73	73	61
Trp:Lys, %	19	20	18	15
Total Lys, %	1.16	1.06	0.97	1.04
CP, %	19.8	18.6	17.0	17.0
SID Lys:ME, g/Mcal	2.82	2.39	2.08	2.75
ME, kcal/kg	3,331	3,347	3,465	3,459
Ca, %	0.44	0.40	0.38	0.37
P, %	0.42	0.41	0.40	0.38
Available P, %	0.28	0.26	0.28	0.23

[1]Phases 1, 2, 3, and 4 fed from approximately 41 to 59 kg, 59 to 82 kg, 82 to 104 kg., and 104 to 120 kg BW, respectively.

[2]A commercially available β-mannanase (Hemicell; ChemGen Corp., Gaithersburg, Maryland) replaced corn in each dietary phase at 0.50 kg/ton to make the enzyme treatment.

[3]Provided per kg of diet: 3,527 IU of vitamin A; 529 IU of vitamin D3; 14 IU of vitamin E; 1 mg of vitamin K; 3 mg of riboflavin; 9 mg of pantothenic acid; 16 mg of niacin; and 0.01 mg of vitamin B12 for phase 1; 2,645 IU of vitamin A; 397 IU of vitamin D3; 11 IU of vitamin E; 1 mg of vitamin K; 2 mg of riboflavin; 7 mg of pantothenic acid; 12 mg of niacin; and 0.01 mg of vitamin B12 for phase 2 and 3; 1,764 IU of vitamin A; 265 IU of vitamin D3; 7 IU of vitamin E; 0.7 mg of vitamin K; 1.3 mg of riboflavin; 4 mg of pantothenic acid; 8 mg of niacin; and 0.01 mg of vitamin B12 for phase 4
[4]Provided per kg of complete diet: 21.2 mg Mn from Mn oxide, 88 mg Fe from Fe sulfate, 88 mg Zn from Zn oxide, 8.8 mg Cu from Cu sulfate, 0.159 mg I from Ca Iodate, and 0.159 mg Se from Na selenite.
[5]Paylean, Elanco Animal Health, Greenfield, IN.
[6]Stafac 20, Phibro Animal Health, Ridgefield Park, NJ.
[7]OptiPhos 2000 (Enzyvia, LLC, Sheridan, IN); provided 400 phytase units per kg of diet in all phases.

Table 6-3. Diet Composition (as-fed basis), Exp. 2[1, 2]

	Phase 1			Phase 2			Phase 3		
Item Fat, %:	0	2.5	5.0	0	2.5	5.0	0	2.5	5.0
Ingredient, %									
Corn	60.59	56.54	52.45	64.73	60.82	56.92	68.76	64.98	61.21
Soybean meal, 46.5% CP	22.36	23.86	25.40	18.37	19.78	21.18	14.39	15.67	16.94
DDGS [3]	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Choice white grease	---	2.50	5.00	---	2.50	5.00	---	2.50	5.00
Monocalcium P, 21% P	0.15	0.20	0.25	---	---	---	---	---	---
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix with phytase[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.13	0.13	0.13
Trace mineral premix[5]	0.15	0.15	0.15	0.15	0.15	0.15	0.13	0.13	0.13
L-Lys HCl	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis									
Standardized ileal digestible (SID) AA									
Lys, %	1.00	1.03	1.07	0.90	0.93	0.96	0.80	0.83	0.85
Met:lys, %	30	29	28	31	30	30	33	32	31
Met and cys:lys, %	61	59	58	63	62	60	67	65	63
Thr:lys, %	62	61	61	62	62	61	63	63	62
Trp:lys, %	18	18	18	18	18	18	18	18	18
Total lys, %	1.15	1.18	1.22	1.04	1.07	1.10	0.93	0.96	0.98
CP, %	19.9	20.2	20.6	18.4	18.7	19.0	16.9	17.1	17.4
SID Lys:ME, g/Mcal	2.98	2.98	2.98	2.68	2.68	2.68	2.38	2.38	2.38
ME, kcal/kg	3,351	3,463	3,574	3,358	3,472	3,585	3,362	3,474	3,587
Ca, %	0.51	0.52	0.53	0.47	0.47	0.47	0.45	0.46	0.46
P, %	0.46	0.47	0.48	0.41	0.41	0.41	0.40	0.40	0.39

Available P, %	0.31	0.32	0.33	0.27	0.27	0.27	0.25	0.25	0.25

[1]Phase 1 fed from 34 to 50 kg, Phase 2 fed from 50 to 73 kg, and Phase 3 fed from 73 to 91 kg BW.
[2]Agriking REAP® (Agri-King Inc., Fulton, Illinois) added at 0.05% in all phases at increasing levels of fat to make the enzyme treatments.

[3]Distillers dried grains with solubles.
[4]Provided per kg of diet: 6,614 IU of vitamin A; 992 IU of vitamin D3; 26 IU of vitamin E; 4 mg of vitamin K; 5 mg of riboflavin; 17 mg of pantothenic acid; 30 mg of niacin;0.02 mg of vitamin B12. Also provided 898 phytase units with an expected phytate P release of 0.14% for phase 1; 898 phytase units with an expected phytate P release of 0.13% for phase 2; and 748 phytase units with an expected phytate P release of 0.12% for phase 3.
[5]Provided per kg of complete diet: 39.7 mg Mn from Mn oxide, 165 mg Fe from Fe sulfate, 165 mg Zn from Zn oxide, 16.5 mg Cu from Cu sulfate, 0.298 mg I from Ca Iodate, and 0.298 mg Se from Na selenite.

Table 6-4. Diet composition (as-fed basis), Exp. 3[1]

	Phase 1			Phase 2			Phase 3			Phase 4[3]		
Ingredient[2], %	30% DDGS	45% DDGS	60% DDGS	30% DDGS	45% DDGS	60% DDGS	30% DDGS	45% DDGS	60% DDGS	30% DDGS	45% DDGS	60% DDGS
Corn	46.23	29.50	29.50	49.03	32.43	17.61	52.14	37.66	22.85	60.10	60.10	60.10
Soybean meal (46.5% CP)	16.61	15.40	15.40	14.12	12.84	11.69	10.85	9.68	8.53	11.63	11.63	11.63
DDGS[4]	28.10	45.00	45.00	28.17	45.00	60.00	30.00	45.00	60.00	20.00	20.00	20.00
Bakery product	6.90	6.90	6.90	6.83	6.83	6.83	5.00	5.00	5.00	6.83	6.83	6.83
Choice white grease	0.24	1.19	1.19	0.00	0.95	1.80	0.00	0.74	1.59	---	---	---
Limestone	0.91	1.07	1.07	0.91	1.07	1.21	1.10	1.06	1.21	0.82	0.82	0.82
Salt	0.21	0.16	0.16	0.21	0.17	0.13	0.28	0.20	0.16	0.24	0.24	0.24
L-Lys HCl	0.61	0.61	0.61	0.56	0.57	0.58	0.50	0.51	0.51	0.27	0.27	0.27
L-Thr	0.03	---	---	0.01	---	---	---	---	---	0.03	0.03	0.03
Stafac	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	---	---	---
Phytase[5]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin and trace mineral premix[6]	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.08	0.08	0.08	0.08	0.08
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis												
Standardized ileal digestible (SID) AA												
Lys, %	1.06	1.08	1.08	0.97	0.99	1.01	0.86	0.87	0.89	0.71	0.71	0.71
Met:lys, %	29	33	33	31	34	38	34	37	41	37	37	37
Met and cys:lys, %	60	66	66	63	70	76	69	76	83	76	76	76
Thr:lys, %	60	62	62	60	65	69	63	68	74	70	70	70
Trp:lys, %	16	16	16	16	16	17	16	17	17	19	19	19
Total lys, %	1.22	1.28	1.28	1.13	1.18	1.23	1.01	1.06	1.11	0.84	0.84	0.84
CP, %	20.1	22.7	22.7	19.1	21.7	24	18.2	20.5	22.8	16.7	16.7	16.7
SID Lys:ME, g/Mcal	3.14	3.16	3.16	2.89	2.91	2.92	2.55	2.57	2.59	2.10	2.10	2.10
ME, kcal/kg	3,380	3,419	3,419	3,371	3,411	3,446	3,360	3,397	3,433	3,384	3,384	3,384

Ca, %	0.43	0.49	0.49	0.42	0.48	0.53	0.48	0.47	0.52	0.38	0.38	0.38
P, %	0.46	0.53	0.53	0.45	0.52	0.57	0.45	0.5	0.56	0.41	0.41	0.41
Available P, %	0.30	0.38	0.38	0.30	0.38	0.46	0.30	0.38	0.45	0.25	0.25	0.25

[1]Phases 1, 2, 3, and 4 fed from approximately 45 to 58 kg, 58 to 84 kg, 84 to 104 kg, and 104 to 123 kg BW, respectively.
[2]A commercial enzyme blend containing protease, amylase, xylanase, β-glucanase, pectinase, cellulose, and phytase (Allzyme® SSF; 200 g/ton; Alltech Inc., Nicholasville, KY) or an experimental proprietary blend of enzymes selected to have maximum activity for the non-starch polysaccharides in DDGS (Product B; 500 g/ton) was added in diets containing 45 and 60% DDGS in place of corn.
[3]Ractopamine HCl (Paylean®, Elanco Animal Health, Greenfield, IN) at 4.5 g/ton was added at the expense of corn.
[4]Diets in the 60% DDGS treatment contained only 45% DDGS during Phase 1 (d 0 to 14).
[5]OptiPhos 2000 (Enzyvia, LLC, Sheridan, IN); provided 400 phytase units per kg of diet in all phases.
[6]Provided per kg of diet: 3,968 IU of vitamin A; 595 IU of vitamin D3; 16 IU of vitamin E; 2 mg of vitamin K; 3 mg of riboflavin; 10 mg of pantothenic acid; 18 mg of niacin;0.01 mg of vitamin B12, 24 mg Mn from Mn oxide, 99 mg Fe from Fe sulfate, 99 mg Zn from Zn oxide, 9.9 mg Cu from Cu sulfate, 0.179 mg I from Ca Iodate, and 0.179 mg Se from Na selenite.

Table 6-5. Metabolizeable energy (kcal/kg DM) values used for diet formulation, Exp. 4

Ingredient	Control[1]	Nutrase[2]
Corn	3,420	3,474
Soybean meal	3,380	3,408
Dried distillers grains with solubles	3,420	3,474

[1]Based on NRC values except for DDGS which was assigned an ME value similar to corn NRC value.
[2]Calculated uplift values for ME as recommended by the manufacturer based on arabinoxylan content.

Table 6-6. Diet composition (as-fed basis), Exp. 4[1,2]

	Phase 1		Phase 2		Phase 3	
Ingredient	Control	Low energy	Control	Low energy	Control	Low energy
Corn	49.42	50.60	53.82	55.00	70.47	71.60
Soybean meal (46.5%)	15.60	15.50	11.22	11.15	9.72	9.65
Dried distillers grains with solubles	30.00	30.00	30.00	30.00	15.00	15.00
Choice white grease	3.00	1.92	3.00	1.92	3.00	1.92
Limestone	1.08	1.08	1.10	1.10	1.00	1.00
Salt	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin and trace mineral premix[3]	0.10	0.10	0.10	0.10	0.10	0.10
Phytase [4]	0.01	0.01	0.01	0.01	0.01	0.01
L-Lys HCl	0.45	0.45	0.40	0.40	0.35	0.35
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis:						
Standardized ileal digestible (SID) AA, %						
Lys, %	0.94	0.94	0.80	0.80	0.69	0.69
Met:lys, %	33	33	36	36	34	34
Met and cys:lys, %	66	66	73	73	70	70
Thr:lys, %	63	63	67	67	63	63
Trp:lys, %	17	17	17	17	17	17
Total lys, %	1.10	1.10	0.95	0.95	0.80	0.80
ME, kcal/kg	3,496	3,448	3,499	3,450	3,503	3,452
SID Lys:ME, g/Mcal	2.69	2.73	2.29	2.32	1.97	2.00
Ca, %	0.49	0.49	0.48	0.48	0.44	0.44
P, %	0.46	0.46	0.44	0.44	0.37	0.37
Available P, %	0.29	0.29	0.27	0.27	0.23	0.23

[1]Phases 1, 2, and 3 fed from approximately 39 to 59 kg, 59 to 84 kg, and 84 to 95 kg BW, respectively.
[2]A commercial enzyme product containing bacterial endo-1,4-beta-xylanase (Nutrase; Nutrex, Lille, Belgium) replaced corn in the Low Energy diet at 0.55 kg/ton to make the third dietary treatment.
[3]Provided per kg of diet: 4,409 IU of vitamin A; 661 IU of vitamin D3; 18 IU of vitamin E; 2 mg of vitamin K; 3 mg of riboflavin; 11 mg of pantothenic acid; 20 mg of niacin;0.02 mg of vitamin B12, 26 mg Mn from Mn oxide, 110 mg Fe from Fe sulfate, 110 mg Zn from Zn oxide, 11 mg Cu from Cu sulfate, 0.198 mg I from Ca Iodate, and 0.198 mg Se from Na selenite.
[4]OptiPhos 2000 (Enzyvia, LLC, Sheridan, IN); provided 300, 240, and 500 phytase units per kg of diet in Phases 1, 2, and 3, respectively.

Table 6-7. Analyzed total non-starch polysaccharide composition of dried distillers grains with solubles[1]

	Non-starch polysaccharide, % DM				
Sample	Arabinose	Xylose	Mannose	Galactose	Arabinoxylan
1	5.42	7.50	0.97	1.64	11.37
2	5.18	7.31	1.01	1.60	10.99
3	4.98	6.84	1.14	1.55	10.40
4	5.25	7.49	1.20	1.65	11.21
5	5.38	7.64	1.18	1.68	11.46

[1]Analyzed values from 5 samples of dried distillers grains with solubles (Valero Energy Corp., Aurora, SD) as determined by gas chromatography.

Table 6-8. Analyzed water-soluble non-starch polysaccharide (NSP) composition in five samples of dried distillers grains with solubles[1,2]

	NSP, % DM				
Sample	Arabinose	Xylose	Mannose	Galactose	Arabinoxylan
1	0.27	0.13	0.19	0.28	0.35
2	0.28	0.14	0.20	0.27	0.37
3	0.27	0.12	0.21	0.26	0.34
4	0.28	0.13	0.22	0.27	0.36
5	0.29	0.14	0.22	0.28	0.38

[1]Analyzed values from 5 samples of dried distillers grains with solubles (Valero Energy Corp., Aurora, SD) as determined by gas chromatography.
[2]The water insoluble values are calculated as the mathematical difference between the the total and water insoluble NSP values.

Table 6-9. Effect of vaccine dose, commercial enzyme product, and gender on performance of growing pigs, Exp. 1[1,2,3]

	Barrow				Gilt							
Vaccine:	Full dose		Half dose		Full dose		Half dose			*P* - value[4]		
Enzyme:	No	Yes	No	Yes	No	Yes	No	Yes	SEM	Enzyme	Vaccine dose	Gender
Weight, kg												
d 0	30.1	29.1	29.8	29.7	29.2	29.5	29.7	29.7	0.58	0.54	0.54	0.74
d 20	49.7	49.0	50.1	50.6	49.2	49.5	49.0	49.0	0.93	1.00	0.57	0.27
d 92	123.8	120.9	124.4	125.3	117.8	117.9	118.1	119.8	1.16	0.99	0.02	<0.001
D 0 to 20												
ADG, kg	0.976	0.994	1.013	1.045	0.997	0.995	0.965	0.963	0.0307	0.57	0.76	0.18
ADFI, kg	1.75	1.76	1.81	1.86	1.69	1.75	1.74	1.72	0.034	0.24	0.05	0.004
G:F	0.56	0.56	0.56	0.56	0.59	0.57	0.56	0.56	0.017	0.76	0.34	0.52
D 20 to 92												
ADG, kg	1.034	1.022	1.042	1.052	0.970	0.968	0.985	0.991	0.0137	0.94	0.04	<0.001
ADFI, kg	2.75	2.75	2.81	2.87	2.52	2.53	2.57	2.57	0.040	0.51	0.01	<0.001
G:F	0.38	0.37	0.37	0.37	0.39	0.38	0.38	0.39	0.005	0.64	0.52	0.001
D 0 to 92												
ADG, kg	1.020	1.016	1.037	1.053	0.975	0.975	0.980	0.987	0.0105	0.48	0.01	<0.001
ADFI, kg	2.53	2.53	2.58	2.64	2.33	2.35	2.39	2.38	0.036	0.42	0.01	<0.001
G:F	0.40	0.40	0.40	0.40	0.42	0.41	0.41	0.41	0.004	0.57	0.19	<0.001

[1]A total of 1,269 pigs (initially, 29.6 kg BW) were used with 27 pigs per pen and 6 pens per treatment.

[2]Commercial porcine circovirus type 2 vaccine (Circumvent, Intervet Inc., Millsboro, DE) administered twice at 2-wk interval at 2 mL (full dose) or 1 mL (half dose) per injection.

[3]A commercially available β-mannanase (Hemicell; ChemGen Corp., Gaithersburg, MD) added at 0.05% of the diet starting at d 20.

[4]No significant two- or three-way interactions between enzyme, vaccine dose, and gender were detected ($P > 0.05$).

Table 6-10. Effect of a commercial enzyme blend and increasing levels of added fat on growth performance, Exp. 2[1,2,3]

	Fat, %							*P* - value		
	0		2.5		5.0				Fat	
Item	No enzyme	Enzyme	No enzyme	Enzyme	No enzyme	Enzyme	SEM	Enzyme × fat	Linear	Quadratic
BW, kg										
d 0	34.4	34.3	34.3	34.5	34.3	33.8	0.81	0.93	0.69	0.81
d 56	86.8	86.6	87.9	87.2	87.4	87.6	1.16	0.94	0.47	0.62
D 0 to 56										
ADG, kg	0.933	0.927	0.951	0.941	0.946	0.96	0.008	0.33	0.01	0.54
ADFI, kg	2.3	2.26	2.24	2.24	2.17	2.21	0.035	0.58	0.01	0.84
G:F	0.41	0.41	0.43	0.42	0.44	0.44	0.006	0.69	<.0001	0.96

[1]A total of 1,129 pigs, initially 34.4 kg, with 27 pigs per pen were used with 7 replications per treatment.
[2]A blend of enzyme containing β-glucanase, cellulase, and protease (Agriking REAP; Agri-King Inc., Fulton, Illinois) added at 0.05% in all dietary phases at increasing levels of fat to make the enzyme treatments.
[3]One pen on the 5% added fat with enzyme treatment was excluded from data analysis as an outlier due to ADG and G:F values that fell over two SD from the means during the last treatment period..

Table 6-11. Effects of enzyme supplementation in diets containing high levels of dried distillers grains with solubles (DDGS) on growth performance and carcass characteristics of grow-finish pigs, Exp. 3[1]

	Treatment								
	30 % DDGS	45% DDGS			60% DDGS				
Item	No	No	Allzyme[2]	Enzyme A[3]	No	Allzyme	Enzyme A	SEM	*P* - value
Weight, kg									
d 0	46.1	46.1	46.1	45.9	46.0	46.0	45.9	1.26	1.00
d 78 (Tops)[4]	124.7	123.8	123.5	125.7	121.4	124.7	123.6	2.27	0.81
d 78 (After topping)	110.9	110.3	110.1	108.0	108.4	109.5	109.7	1.95	0.94
D 90	122.9	122.6	122.9	120.3	121.1	122.3	122.4	1.99	0.96
D 0 to 78									
ADG, kg	0.856	0.837	0.839	0.822	0.812	0.836	0.829	0.0136	0.33
ADFI, kg	2.32	2.23	2.22	2.16	2.17	2.24	2.19	0.034	0.03
G:F	0.37	0.38	0.38	0.38	0.38	0.37	0.38	0.004	0.79
D 78 to 90									
ADG, kg	0.976	1.007	1.039	1.018	1.044	1.045	1.025	0.0381	0.83
ADFI, kg	2.77	3.00	3.04	2.90	2.95	3.01	2.98	0.094	0.47
G:F	0.35	0.33	0.34	0.35	0.36	0.35	0.34	0.012	0.82
D 0 to 90									
ADG, kg	0.869	0.854	0.862	0.845	0.839	0.859	0.850	0.0131	0.64
ADFI, kg	2.38	2.31	2.32	2.24	2.26	2.33	2.28	0.036	0.18
G:F	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.004	0.88

[1]A total of 1,032 pigs, initially 46.0 kg, with 24 pigs per pen were used in this study with 6 replications per treatment.
[2]A commercial enzyme blend containing protease, amylase, xylanase, β-glucanase, pectinase cullulase, and phytase (Allzyme SSF; Alltech, Inc., Nicholasville, KY) added at 0.02% of the diet.
[3]A proprietary multi-enzyme product.
[4]On d 78, four of the heaviest pigs from each pen were marketed (top) and DDGS in all dietary treatments were reduced to 20%.

Table 6-12. Effect of a commercial enzyme product and gender on performance of growing pigs, Exp. 4[1,2]

	Treatment				Gender			*P* - value[3]	
Item	Low Control	High Control	Enzyme	SEM	Barrows	Gilts	SEM	Sex	Treat-ment
Weight, kg									
D 0	39.6	39.7	39.6	0.94	39.8	39.4	0.80	0.71	0.99
D 28	62.6	62.9	62.4	1.20	63.3	61.9	1.02	0.33	0.97
D 66	95.1	95.4	94.4	1.47	97.0	92.9	1.25	0.02	0.88
D 0 to 28									
ADG, kg	0.820	0.825	0.812	0.014	0.838	0.8000	0.012	0.03	0.81
ADFI, kg	1.77	1.76	1.79	0.04	1.84	1.71	0.03	0.005	0.75
G:F	0.47[a]	0.47[a]	0.45[b]	0.004	0.46	0.47	0.003	0.002	0.01
D 28 to 66									
ADG, kg	0.830	0.811	0.823	0.014	0.850	0.7928	0.012	0.002	0.66
ADFI, kg	2.38	2.35	2.35	0.04	2.49	2.23	0.03	<.0001	0.81
G:F	0.35	0.35	0.35	0.004	0.34	0.36	0.004	0.01	0.82
D 0 to 66									
ADG, kg	0.825	0.817	0.817	0.012	0.844	0.7950	0.010	0.001	0.86
ADFI, kg	2.12	2.10	2.11	0.03	2.21	2.01	0.03	<.0001	0.93
G:F	0.39	0.39	0.39	0.003	0.38	0.40	0.003	0.0004	0.65

[1]A total of 1,076 pigs (PIC 337 × C22), initially 39.6 kg, with 27 pigs per pen were used in this study with 13 replications per treatment.
[2]Bacterial endo-1,4-beta-xylanase (Nutrase; Nutrex, Lille, Belgium).
[3]Treatment × gender interactions for all criteria were not significant ($P > 0.05$).
[ab]Means within a row without a common superscript differ ($P < 0.05$).

Table 6-13. Effect of enzyme addition to diets containing dried distillers grains with solubles on growth performance of growing-finishing pigs[1]

Experiment	Start wt,, kg			Final wt., kg			ADG, kg			ADFI, kg			G:F		
	Control	Enzyme	SED[2]	Control	Enzyme	SED	Control	Enzyme	SED	Control	Enzyme	SED	Control	Enzyme	SED
1	29.7	29.5	0.37	120.9	120.9	1.12	1.00	1.01	0.011	2.45	2.47	0.040	0.408	0.408	0.0034
2	34.4	34.4	0.64	87.4	87.2	0.90	0.94	0.94	0.007	2.23	2.24	0.030	0.424	0.421	0.0059
3	46.1	46.0	0.90	122.2	122.0	1.45	0.86	0.85	0.009	2.32	2.29	0.028	0.370	0.373	0.0028
4	39.7	39.6	1.09	95.4	94.5	1.85	0.82	0.82	0.016	2.12	2.12	0.054	0.391	0.387	0.0048
Average	37.4	37.4	0.38	106.5	106.2	0.61	0.91	0.91	0.005	2.28	2.28	0.015	0.399	0.397	0.0020

[1]Data from 4 experiments involving 4,506 pigs. In each experiment, pigs fed enzyme-supplemented diets were compared to pigs that were fed diets with or without enzyme regardless of other factors being tested in the experiment. There was no significant difference ($P > 0.33$) between control and enzyme supplementation for any response criteria either within individual experiments or overall when data from all experiments were pooled together.
[2]Standard error of the difference.

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Karen Richardson
Publications Manager JSHAP
Population Medicine
University of Guelph
Guelph, Ontario
Canada N1G 2W1
FAX: 519-763-3117
Phone: 519-856-2089
Email: pub_mgr@aasv.org

October 28, 2009

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I-102 Mosier Hall,
1800 Denison Ave,
Manhattan, KS 66506
USA

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