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ORIGINAL ARTICLE

The effects of dietary additives on faecal levels of *Lactobacillus* spp., coliforms, and *Escherichia coli*, and faecal prevalence of *Salmonella* spp. and *Campylobacter* spp. in US production nursery swine[1]

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[1]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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Abstract

Aims: In the United States, carbadox and copper sulfate are growth promoters commonly used in combination in nursery swine diets. Our aim was to determine how selected dietary additives affect selected bacterial populations and pathogens in nursery swine, and compare to larch extract, which contains potential antibacterial activities.

Methods and Results: Piglets were weaned and sorted into one of the four treatments: (i) basal diet without antimicrobials; (ii) basal diet with carbadox + copper sulfate; (iii) basal diet + 1000 ppm larch extract; or (iv) basal diet + 2000 ppm larch extract. Diets were fed for a 4-week period after weaning. In both trials, the carbadox + copper sulfate group consumed more feed over the 4-week period relative to the other three diet groups ($P < 0{\cdot}05$), but did not gain significantly more weight. Faecal shedding of *Salmonella* spp. was not affected by dietary supplement in either trial, but faecal shedding of *Campylobacter* spp. was the lowest for the carbadox + copper sulfate diet. In faecal samples collected at the end of each trial, *Lactobacillus* spp. cell counts for the basal and larch extract diets were nearly 1·0 $\log_{10}$ g^{-1} faeces greater ($P < 0{\cdot}05$) than the carbadox + copper sulfate group, whereas the coliforms and *Escherichia coli* were nearly 1·0 $\log_{10}$ g^{-1} faeces lower ($P < 0{\cdot}05$).

Conclusions: Compared to basal fed animals, supplementation with carbadox + copper sulfate significantly altered faecal *E. coli*, coliform bacteria and *Lactobacillus* spp. Larch extract has no benefit up to 0·2% of diet in regard to pathogen shedding, whereas carbadox + copper sulfate decreased faecal shedding of *Campylobacter* spp.

Significance and Impact of the Study: Current swine management practices in the United States may be beneficial to managing *Campylobacter* spp. shedding in nursery swine, but also result in significant changes in the resident gastrointestinal microflora.

Introduction

Most rations formulated in the United States for young swine contain antibiotic(s) and elimination of these antimicrobials could negatively impact health and production of these animals (Hayes *et al.* 2002; Mathews 2005). Carbadox is not an antibiotic *per se*, but is a synthetic growth-promoting feed additive with putative antimicrobial properties and is predominantly used in diets of nursery swine in the United States (Dewey *et al.* 1999). Copper sulfate at levels of 100–250 ppm is also a growth-promoting feed additive, and its effects may be additive with dietary antibiotics/antimicrobials in young swine (Stahly *et al.* 1980; Edmonds *et al.* 1985). However, tissue

residue and environmental contamination concerns with dietary supplementation of carbadox, carbadox metabolites (Yoshimura 2002; Hutchinson *et al.* 2005) and copper sulfate (Armstrong *et al.* 2004) have been reported.

Alternative natural antimicrobial products such as plant essential oils typically have a high phenolic content, and these compounds may increase animal performance in lieu of synthetic antimicrobials (Amrik and Bilkei 2004; Allan and Bilkei 2005; Penalver *et al.* 2005). Alternatively, nondigestible, microbial, fermentable products (prebiotics) may select for beneficial bacteria in the gastrointestinal system (Gibson and Roberfroid 1995). Larch extract is a by-product from processing of the larch tree, and this extract is predominantly an arabinogalactan polymer (US patent no. 6,210,678). Larch extract also contains a significant fraction of taxifolin, and this compound, like many small polyphenols, has putative antimicrobial activities (Hara-Kudo *et al.* 2004; Taguri *et al.* 2004). Few comparative studies have examined animal production, faecal flora and faecal pathogen shedding with feed additives. We designed a study to determine the impact of the dietary amendments on performance, selected faecal microbial flora and important zoonotic pathogens in nursery piglets and to examine the potential for larch tree extract as a feed grade additive compared to some industry practices in the United States.

Materials and methods

Animals, diets and management

The experimental protocol was reviewed and approved by the Animal Care and Use Committee of the US Meat Animal Research Center. The pigs used in the experiments were the composite offspring of the Duroc, Landrace, and Large White breeds. In trial 1 (July–August, 2004), 192 weaned piglets (age = 17·8 ± 0·1 days; weight = 5·0 ± 0·05 kg) were blocked into six weight groups and randomly assigned to a treatment group with each weight group represented in all treatments. In trial 2 (February–March, 2005), 256 weaned piglets (age = 17·5 ± 0·1 days; weight = 5·5 ± 0·08 kg) were blocked into eight weight groups and randomly assigned to a pen from each weight group such that all pens had an equal average weight. Some adjustments were made to distribute littermates across different treatments. Piglets were held in the farrowing facilities 5–7 days after weaning before transfer to the nursery. No feed additive antimicrobials were fed to the lactating sows or pigs (as creep) prior to experimental treatments. Animals were housed eight pigs per pen in environmentally regulated rooms, with six pens per treatment split evenly between two rooms in the nursery facility. Each pen measured 0·9 × 2·4 m and had a slotted concrete floor. Water was provided with an automatic nipple waterer, and pigs were allowed to consume weighed feed *ad libitum* from a five-hole feeder housed in the pen. Individual animals and pen feeders were weighed on weeks 0, 2 and 4 in the nursery for each trial.

The four dietary treatments were (i) basal diet, (ii) basal + 55 ppm carbadox and 250 ppm copper (sulfate), (iii) basal + 1000 ppm larch extract and (iv) basal + 2000 ppm larch extract. The 20·57% CP basal diet was a corn–soyabean meal diet with dry skim milk and steam-rolled oats, and fortified with crystalline AA, minerals and vitamins. Diets were formulated to meet or exceed NRC-suggested dietary requirements (NRC, 1998). For carbadox + copper sulfate, a premix in corn containing 0·55% carbadox was added at 1% level to yield 55 ppm carbadox, and copper sulfate pentahydrate (25% copper) was added at 0·1% level to yield 250 ppm copper to the diet. For the diets supplemented with larch extract, the commercial larch tree extract (LaraFeed; Larex, Inc., St Paul, MN, USA) was added to the basal diet in dry form premixed in corn. Palatability of larch tree extract in swine was unknown, but the levels tested were 0·1% (1000 ppm) and 0·2% (2000 ppm) at the advice of manufacturer. All diets were prepared <4 weeks before each trial. In trial 1, diets were fed as a ground meal, and in trial 2, diets were fed as a pellet.

Microbial analyses

Rectal swabs for faecal pathogens were taken at weeks 0, 2 and 4 in both trial 1 (*Salmonella* spp. only) and trial 2 (*Salmonella* spp. or *Campylobacter* spp.) using sterile cotton-tipped applicators. Each individual swab was immediately placed into a sterile 15 ml conical tube. Faecal samples were collected at week 4 for both trials 1 and 2 by rectal massage using a clean glove with each animal. Gloves with sample were inverted and placed into a labelled bag and sealed. Following collection, samples were transported to the laboratory and processed on the same day for microbial counts.

Salmonella were enriched from rectal swab with 13 ml Tetrathionate broth (Difco) in a sterile 15 ml loosely capped conical tube. Tubes were incubated for 48 h at 37°C, and a 20 μl aliquot was transferred to 10 ml Rappaport–Vassiliadis Soya peptone broth (RVS; Oxoid Ltd, Hampshire, UK) tube. Inoculated RVS tubes were incubated at 42°C for 24 h and then 25 μl was plated onto Hektoen-Enteric (HE; Difco brand, B-D Microbiological Systems, Sparks, MD, USA) agar. Black colonies were picked and plated onto a fresh HE plate. Isolated colonies were grown on Tryptic Soy broth (Difco) and confirmed to be presumptive *Salmonella* by characteristics

(Rose 1998) in triple sugar iron agar and lysine iron agar slants (Difco) and on CHROM *Salmonella* agar (Difco). All isolates were confirmed by PCR for serotype (Gillespie *et al.* 2003) or *inv*A (Ziemer and Steadham 2003).

Campylobacter was enriched from 1 g of faeces (trial 1) or rectal swab (trial 2) using 13 ml Bolton broth with supplement (Oxoid) and lysed horse blood cells (Lampire Biological Labs, Pipersville, PA, USA). Tubes were gently mixed, capped tightly and incubated 4 h at 37°C followed by 44 h at 42°C. A 10 μl aliquot was plated onto Campy–Cephex agar (Stern *et al.* 1992) and grown using MicroAero Packs in AnaeroPack System (Mitsubishi Gas Chemical, New York, NY, USA) for 48 h at 42°C. Colonies were verified by agglutination (*Campylobacter* test kit, Oxoid Ltd.) and tested by PCR for *lpx*A (Klena *et al.* 2004).

Selected bacterial populations were determined using direct plating of diluted faeces at week 4. A 1 g portion of each faecal sample was mixed by vortex with 9 ml of buffered peptone water (BPW; Difco) for 30 s. An aliquot (representing a 10^{-1} dilution) was taken from the BPW-faecal sample and used for 10× serial dilutions to 10^{-6} in a 96-well, deep-dish plates using BPW. Aliquots (1 ml) of dilutions 10^{-3}, 10^{-4} and 10^{-5} were transferred to Petrifilms (3M Microbiology Products, St Paul, MN, USA) to determine CFUs of *Enterobacteriaceae*, coliforms and generic *Escherichia coli*, and 100 μl of 10^{-5} and 10^{-6} were spread over modified lactobacilli deMan–Rogosa–Sharpe (MRS; Difco; pH 5·4 with acetic acid) agar plates to determine CFUs of *Lactobacillus* spp. (Wells *et al.* 2005). The modified MRS plates were sealed in anaerobic boxes (Mitsubishi Gas Chemical America) with AneroPak environment generators (Mitsubishi Gas Chemical America). Petrifilms and plates were incubated overnight at 37°C, and CFUs were counted.

Statistical analyses

Trials were analysed separately because polyphenol activity may have been affected by the form of diet. For both trials, data from each 2-week feeding period and the entire 4-week feeding period were analysed using the GLM procedure in SAS (SAS Inst., Cary, NC, USA) with pen as the experimental unit and initial weight as a covariant. The model included the effects of room, dietary treatments, room *vs* dietary treatment and initial weight. Faecal microbial numbers were transformed to $\log_{10}$ and also analysed using GLM with individual animal as the experimental unit. The model included the effects of room, dietary treatment and room *vs* dietary treatment. Pathogen prevalence with treatments within each trial was analysed individually against the basal diet and other treatments using the Fisher's exact probability test, and the *P* value reported is for the same or stronger association (Uitenbroek 1997). Pen prevalence, as measured by percent of pathogen positive animals in pen, was analysed using GLM with pen as the experimental unit, and the model included the effects of trial, room, dietary treatments, week of sample and the interactions. Effects of pathogens on gain were analysed using GLM with individual animal as the experimental unit and initial weight as a covariant. The model included the effects of room, pathogens, room *vs* pathogen, treatment, and initial weight, and dietary treatment was tested for significance with pen (dietary treatment) as error term. For all statistical analyses, differences were considered significant when *P* values were <0·05.

Results

Animal performance

Feed was provided to the piglets in trial 1 as a ground meal and in trial 2 as a pellet. In both trials 1 and 2, pigs gained weight better with carbadox + copper sulfate diet during the first 2 weeks (data not shown), but no difference ($P > 0{\cdot}24$) was observed in animal gain for the full 4 weeks of this nursery study for any of the four diets (Table 1). In both trials, feed intake (measured as is) was 9–12% higher ($P < 0{\cdot}01$) and gain : feed proportion was 4–6% lower ($P < 0{\cdot}05$) for piglets fed carbadox + copper sulfate diet compared to basal diet. In trial 1, animals fed the diet with 1000 ppm larch extract did not differ in feed intake or gain : feed proportion from basal ($P > 0{\cdot}05$), but in trial 2 when diets were provided in pelleted form piglets feed intake decreased by 5% ($P < 0{\cdot}05$), and gain : feed proportion increased by 5% ($P < 0{\cdot}05$) compared to basal diet. In each trial, the 2000 ppm larch extract did not significantly alter feed intake or gain : feed proportion relative to the basal diet ($P > 0{\cdot}05$). In each trial, diets with larch extract had lower feed intakes, but only the diet with 1000 ppm larch extract had improved gain : feed proportion compared to the diets with carbadox + copper sulfate.

Microbial analyses

Bacterial counts for all populations after 4 weeks on respective diets were lower in trial 1 compared to trial 2 (Fig. 1). In trial 1 when animals were fed each diet as a ground meal, the faecal *Lactobacillus* microbial counts at week 4 were between 8·40 and 8·70 $\log_{10}$ CFU g^{-1} faeces for pigs fed the basal and both larch extract treatments, but not different between these treatments. The faecal *Lactobacillus* CFUs per g faeces were nearly tenfold lower in pigs treated with carbadox + copper sulfate than pigs fed the other three treatments ($P < 0{\cdot}01$). In trial 2 when

Table 1 Effect of dietary supplement on means for pen gain, feed consumption (measured as is) and gain-to-feed ratio

	Basal	Basal + carbadox + copper sulfate	Basal + 0·1% LE	Basal + 0·2% LE	SEM	*P**
Initial pen weight (kg)						
Trial 1†	58·5 ± 3·0	59·0 ± 2·8	57·2 ± 3·2	56·8 ± 2·9		>0·1
Trial 2‡	66·6 ± 0·8	67·2 ± 0·8	67·7 ± 0·8	66·8 ± 0·8		>0·1
Pen gain (kg)						
Trial 1	104·08[a]	109·06[a]	105·25[a]	101·55[a]	2·58	0·267
Trial 2	116·30[a]	121·45[a]	117·27[a]	115·12[a]	2·27	0·246
Pen feed (kg)						
Trial 1	179·13[a]	199·79[b]	182·22[a]	181·78[a]	4·00	0·009
Trial 2	183·43[a]	200·37[b]	174·32[c]	186·22[a]	2·81	<0·001
Gain to feed						
Trial 1	0·582[a]	0·546[b]	0·578[a]	0·558[a,b]	0·0090	0·042
Trial 2	0·634[a]	0·606[b]	0·673[c]	0·618[a,b]	0·0076	<0·001

LE, larch extract.
*Test of treatment effect.
†Trial 1 was with ground meal diets.
‡Trial 2 was with pelleted diets.
[a,b,c]Within a row, means without a common superscript letter differ ($P < 0·05$).

the diet was pelleted, the faecal *Lactobacillus* microbial counts were highest for the piglets fed the diet with 0·1% larch extract ($P < 0·05$), and lowest for pigs fed the diet with carbadox + copper sulfate ($P < 0·01$).

In faeces collected from individual pigs fed basal diet in trial 1, the $\log_{10}$ CFU g^{-1} faeces for coliform bacteria and *E. coli* were 4·91 and 4·90, respectively, and these bacterial counts were similar to faecal counts observed from pigs fed both the 1000 and 2000 ppm larch extract treatments. Pigs fed diets supplemented with carbadox +copper sulfate had more than tenfold higher CFUs per g faeces compared to pigs fed the basal or either larch extract diet ($P < 0·01$). In trial 2, pigs fed basal diet had levels of coliform bacteria and *E. coli* of 5·51 and 5·40 $\log_{10}$ CFU g^{-1} faeces. As observed in trial 1, larch extract treatments did not significantly alter these levels, but animals fed diets with carbadox and copper sulfate had more than fivefold higher CFUs per g faeces compared to pigs fed the other treatments ($P < 0·01$). For both trials, pen had no apparent effect on the bacterial counts, and results did not change when analysed with pen as the experimental unit.

Faecal prevalence for *Salmonella* spp. at the start (day 0) of each trial was approximately 16% of the pigs in trial 1 and <5% in trial 2. Over the course of trial 1, each pen had at least one pig test positive for faecal *Salmonella* and 18·4% of the individual pigs sampled at weeks 2 or 4 tested positive for *Salmonella* spp. During trial 2, the prevalence of *Salmonella* spp. was sporadic but detected in all treatment groups and averaged 6·8% of the animals. None of the dietary treatments significantly altered the faecal prevalence of *Salmonella* spp. in either trial compared to basal diet alone when analysed using Fisher's exact test ($P > 0·1$), and the pooled results for both studies are shown in Fig. 2. When prevalence was analysed by pen as percent positives for both trials, the average pen prevalence was 7·4 ± 2·0% and not significantly affected by treatment ($P > 0·11$). All *Salmonella* spp. isolates were positive by latex test and for presence in *inv*A gene.

In trial 1, *Campylobacter* spp. was only assayed at the end of the study using the faecal samples collected for bacterial enumerations. In the limited data from trial 1, *Campylobacter* spp. was found in 16·7% of animals fed the basal diets and none of the other diets ($P < 0·05$, data not shown). In trail 2, we assayed for *Campylobacter* spp. over the entire study using rectal swabs. Prior to receiving experimental diets for trial 2, more than 50% of the individual animals were shedding *Campylobacter* spp. As in trial 1, individual pigs were sampled at weeks 2 and 4, and all pens had at least one pig test positive for faecal *Campylobacter* during this trial. Over the course of the trial, more than 65% of the pigs were found to be shedding *Campylobacter* spp., and more than 96% of the *Campylobacter* isolates were *Campylobacter coli*. Nearly, 80% of the pigs fed basal or supplemented with the larch tree extract were found to shed *Campylobacter* spp. (Fig. 2), but <15% of the carbadox + copper sulfate-supplemented pigs were found to be shedding *Campylobacter* spp. ($P = 0·02$ by Fisher's exact test). Persistent shedding (i.e. positive for both samples collected during the trial) of *Campylobacter* was detected in 50% of the individual animals for the 0·1% larch extract-fed pigs but none of the carbadox + copper sulfate-fed pigs. When prevalence was analysed by pen as percent positives, the average

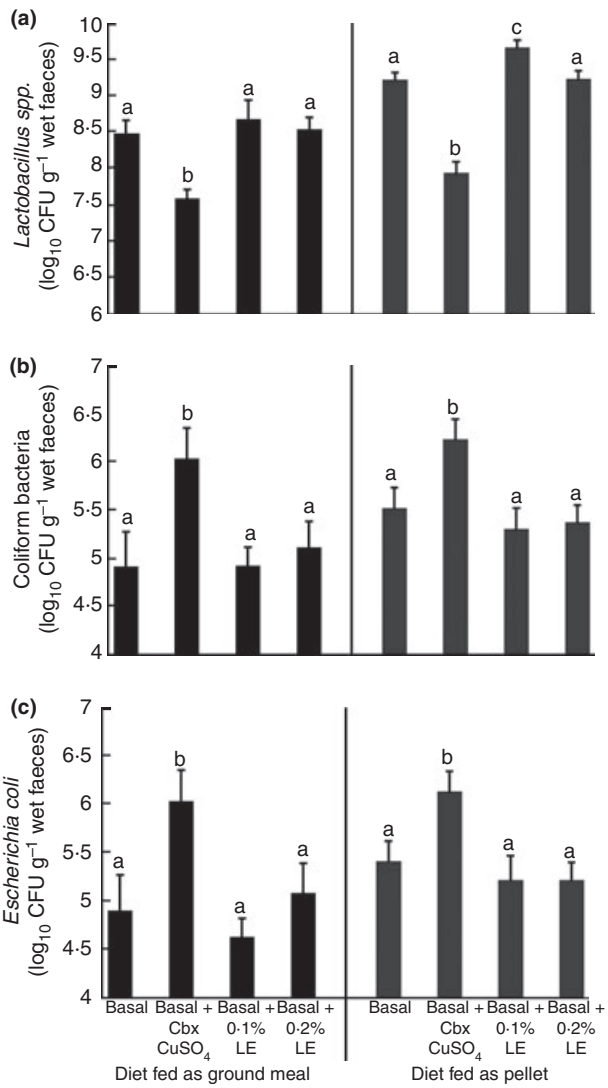

Figure 1 Effects of diet on bacterial counts for *Lactobacillus* spp. (a) Coliform bacteria (b) and *Escherichia coli* (c) at the end of the 4 weeks. The results for trial 1 (12 animals per treatment) with a ground meal diet are shown in black and on the left side of the figure; the results for trial 2 (16 animals per treatment) with a pelleted meal diet are shown in grey and on the right side of the figure. Different letters above each bar denote significant differences ($P < 0{\cdot}05$) for that measurement across the diets within each trial.

prevalence across all pens was 46 ± 5·0%. The carbadox + copper sulfate treatment significantly reduced pen prevalence for *Campylobacter* compared to the other treatments (7% *vs* 60%, $P < 0{\cdot}001$), but no other treatment effects were observed ($P > 0{\cdot}05$).

In pigs fed basal or either larch extract-supplemented diets, animal gains and pathogen prevalence were similar. When these groups were pooled in trial 2 where we had the most observed positives for pathogen shedding, the individual pig gain was affected ($P = 0{\cdot}030$) by persistence

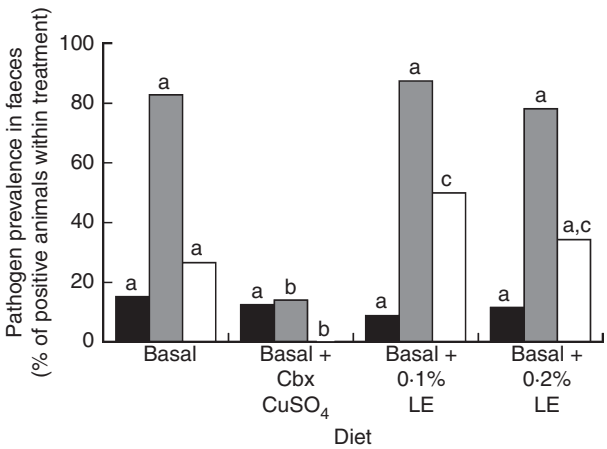

Figure 2 Effects of diet on pathogen prevalence in faecal samples collected from individual animals. The prevalence of *Salmonella* spp. (■) was compiled data from both trials with 112 animals per treatment. The prevalence of *Campylobacter* spp. (▩) was only determined in trial 2 with 64 animals per treatment. Prevalence of chronic *Campylobacter* shedders (□) represents animals in trial 2 that were positive for both samples collected during the trial. Different letters above each bar denote significant differences ($P < 0{\cdot}05$) for that measurement across the diets.

Table 2 Effect of pathogen-shedding frequency (persistence) on means for individual animal gain for piglets fed diets without carbadox + copper sulfate in trial 2*

	Shedding frequency			
	0	1	2	*P*†
Pathogens	Gain (kg)			
Campylobacter + *Salmonella*	15·16 ± 0·34[a]	14·62 ± 0·19[b]	14·62 ± 0·20[b]	0·030
Campylobacter	14·90 ± 0·31[a]	14·71 ± 0·19[a]	14·17 ± 0·21[b]	0·073

*Frequency is the number of pathogen-positive samples collected for individual animals over the two sampling times.
†Test of shedding frequency effect.
[a,b]Within a row, means without a common superscript letter differ ($P < 0{\cdot}05$).

of shedding of *Salmonella* spp. and/or *Campylobacter* spp. in faeces (Table 2). In this trial, only two pigs persistently shed *Salmonella* spp., but *Campylobacter* was shed persistently by 37% of the piglets, and individual gain tended to be lower if the pig was persistently shedding *Campylobacter* spp. ($P = 0{\cdot}073$). Dietary treatment had no statistical effect on these observations.

Discussion

Feed-grade additives have been fed to production animals at low levels since the 1950s to suppress gastrointestinal micro-organisms and to improve animal performance.

These antimicrobials, including many antibiotics, provide benefits to swine producers, particularly in young swine where antimicrobial use is highest (Dewey *et al.* 1999). However, a growing concern about antibiotic resistances being associated with bacteria from animal production systems and potential transfer to humans may change the use of antimicrobials in animal agriculture (Barton 2000; Wegener and Frimodt-Moller 2000). In addition, zoonotic pathogens can be shed in the faeces of production animals, and the potential transmission of these pathogens to the humans is a growing concern for regulatory agencies (Rose *et al.* 1999; Sobsey *et al.* 2001). Specifically, swine are known carriers of *Salmonella* spp. and *Campylobacter* spp. in faeces (Gray *et al.* 1996; Young *et al.* 2000). As a consequence, studies designed to identify alternatives to current feed grade antimicrobials need to examine how changes in swine ration formulations may alter pathogen shedding.

In this study, we document the potential effects of selected nonantibiotic dietary amendments on animal performance, selected faecal microbial flora and pathogen shedding in nursery swine. Responses in animal performance were similar across the two trials we conducted. In both trials, feed intakes were 9–12% higher in pigs supplemented with carbadox + copper sulfate diet. Previous studies with young swine have observed increases in feed intake when basal diets were supplemented with carbadox and/or copper sulfate (Stahly *et al.* 1980; Roof and Mahan 1982; Yen and Pond 1993). Body weight gains for pigs fed the diet with carbadox + copper sulfate were better for the first 2-week period, but were not significantly different for the entire 4-week experiment in either trial compared to the other diets. As a consequence, feed efficiency was poorer for carbadox + copper sulfate-fed pigs in both trials.

In contrast to results from diets with added carbadox + copper sulfate, supplementation of the basal diet with larch extract did not always increase feed intake. In both trials, pigs consuming 1000 ppm larch extract had a numerically higher gain-to-feed ratio than the other treatments. In trial 2, we did detect a significant reduction in feed intake and, as a consequence, a significant increase in the gain-to-feed ratio. The diets fed in trial 1 were provided as a ground meal, whereas the diets fed in trial 2 were pelleted. Many polyphenols have antioxidant activity (Nakayama 1994; Kravchenko *et al.* 2003), and it is possible that difference in seasons or pelleting of the diet may have protected this activity after diets were prepared and fed over the course of the study. In both trials, the diets with 2000 ppm larch extract reduced animal gain-to-feed ratio. In other studies, arabinogalactan purified from larch extract appears to have been tolerated well at higher doses in humans (Robinson *et al.* 2001) and dogs (Grieshop *et al.* 2002); therefore, the negative impact on performance in pigs fed diets with 2000 ppm larch extract may be an effect of the polyphenols. Previous reports suggest that higher dietary intake for polyphenols and other plant phenolics may limit intake and impair digestion and absorption in monogastric animals (Glick 1981; Chung *et al.* 1998).

In our trials, dietary treatments significantly altered faecal bacterial populations. Carbadox and copper sulfate are used in the United States as growth-promoting feed additives with antimicrobial activities. In particular, the combination of carbadox + copper sulfate significantly decreased *Lactobacillus* spp. bacterial counts in faeces in the current study. Previous work with older swine has shown copper sulfate to inhibit Gram-positive ureolytic bacteria, in particular *Streptococcus* spp., in the faeces (Varel *et al.* 1987), and recent work found fewer bacterial counts of both lactobacilli with 175 ppm copper as copper sulfate added to the diets (Hojberg *et al.* 2005). Carbadox alone at 55 ppm in swine nursery diets did not significantly affect any of the lactobacilli populations measured (White *et al.* 2002).

In each of our trials, the combination of carbadox + copper sulfate increased *Enterobacteriaceae*, coliforms, and *E. coli* bacterial counts in the faeces more than tenfold. These increases in coliform bacteria and *E. coli* are significant and were unexpected considering that carbadox at 55 ppm had no effect (White *et al.* 2002) whereas the carbadox analogs, olaquinox or cyadox, at 100 ppm (Ding *et al.* 2006) reduced the levels of these bacteria in animal faeces. Copper sulfate at 175 ppm in the diet has been shown to reduce faecal coliforms and *E. coli* (Hojberg *et al.* 2005), but other work has shown that many *E. coli* isolates were resistant to copper at >16 mmol l^{-1} or 450 ppm (Aarestrup and Hasman 2004). Our observed increases in coliforms and *E. coli* levels in faeces of animals fed diets with carbadox + copper sulfate may have been a consequence of reduced *Lactobacillus* spp. in these same animals. Although coliforms and commensal *E. coli* are not considered pathogenic, the lipopolysaccharide forming the outer membrane can be an endotoxin and may reduce animal performance when present at high levels (Ding *et al.* 2006). This may explain the reduced feed efficiency (as measured by gain-to-feed ratio) we observed in animals fed diets with carbadox + copper sulfate. In addition, these micro-organisms are typically indicator organisms for water quality and faecal contamination. The tenfold increased concentrations observed in the faeces in pigs fed carbadox + copper sulfate may pose problems for manure management if either population is used as a surrogate for faecal pathogen contamination of water from concentrated animal feeding operations.

Under typical US swine management systems as operated at USMARC, animals are transferred from the nursery to growing barns between 7 and 9 weeks of age, and diets, as well as the antimicrobial growth promoters, are changed for each new phase of production. In previous work with 10-week-old pigs at USMARC swine production facilities, we observed bacterial counts for *Lactobacillus* spp. >9·0 $\log_{10}$ g^{-1} faeces, and counts for coliform bacteria and *E. coli* of approximately 7·0 $\log_{10}$ g^{-1} faeces (Wells *et al.* 2005). Over the course of the next 8 weeks with production diets that included chlortetracycline, we observed more than a 2-$\log_{10}$ reduction in faecal counts for *E. coli* and a concomitant reduction in faecal prevalence for *Salmonella* spp from 60% to 0%. In this previous study, changes in dietary antimicrobials at the end of production were associated with changes in the faecal flora, in particular *E. coli*, and increased shedding of *Salmonella* and *Campylobacter*. In hindsight, it is interesting to note from our current studies with 8-week-old pigs that nursery diets with carbadox + copper sulfate resulted in low levels of *Lactobacillus* spp. and high *E. coli* in faeces, and it should be noted that the changes in diets and antimicrobials may help explain the high *Salmonella* prevalence in the 10-week-old pigs until a stable gastrointestinal flora was established.

Overall in our nursery studies, pathogen shedding differed with the two trials, with *Salmonella* spp. shedding in the faeces nearly threefold higher and *Campylobacter* spp. shedding 16-fold less in trial 1 than in trial 2 (data not shown). This difference may be because of different seasons (summer *vs* winter) or farrowing group. The higher shedding observed when pelleted feed was provided is unlikely to be caused by the form of diet (ground *vs* pellet), because previous research has indicated that the higher prevalence would be expected with a pelleted ration (Davies *et al.* 2004). Nonetheless, we did not observe a significant benefit with supplementation of either carbadox + copper sulfate or larch extract on faecal shedding of *Salmonella* spp., but supplementation with carbadox + copper sulfate did reduce shedding of *Campylobacter* spp. The possible benefit of carbadox and/or copper sulfate is of particular interest because *Campylobacter* spp. may account for most of the bacterial foodborne sicknesses in the United States (Allos 2001), and *Campylobacter* spp. has been shown to have a high prevalence in swine populations (Young *et al.* 2000). This is the first report of any dietary treatment significantly reducing *Campylobacter* spp. in swine, but the exact component generating this effect in the carbadox + copper sulfate treatment needs to be determined.

Typically, many zoonotic pathogens are considered benign to the animal host, and no problems with loose stools nor additional medical needs were recorded by animal care technicians in either study. However, possible relationships with animal performance are rare if ever reported. In trial 2, we had a high prevalence for *Campylobacter* spp. shedding over the 4 weeks of the experiment in pigs fed the basal and both larch extract-supplemented diets, and weight gains of pigs fed any of the three dietary treatments were similar. Analysis of this pooled dataset indicated a tendency for persistent shedders (those positive both at weeks 2 and 4) of *Campylobacter* spp. to have lower body weight (BW) gains and a significant effect for persistent shedders of both *Campylobacter* spp. and *Salmonella* spp. to decrease BW gain (Table 2). We did not have enough observations of *Campylobacter* spp. shedding to see these relationships in trial 1. Zoonotic pathogen control in the nursery pig would not only reduce the pathogen load early in the production system, but may also benefit pig performance in young swine and needs to be studied in greater detail.

In conclusion, we have examined the effects of selected dietary supplements in nursery swine diets in two separate trials with diets provided in different forms. Larch extract supplementation appeared to be most effective in a pelleted feed and increased feed efficiency, but this compound did not decrease faecal shedding of *Campylobacter* spp. or *Salmonella* spp. In comparison, carbadox + copper sulfate supplementations increased feed intake and decreased *Campylobacter* spp. shedding, but this combination in our trials had no effect on gain, decreased feed efficiency and increased faecal coliforms. Supplementation of nonantibiotic compounds to nursery swine can alter animal performances as measured by feed intake, gain, or gain-to-feed ratio. However, supplementation can alter the faecal flora and pathogens shed, and thus, affect manure management. Future work will need to discriminate the impact of carbadox and copper sulfate individually on faecal flora and pathogen shedding.

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