Limited effects of a commercial direct-fed microbial on weaning pig performance and gastrointestinal microbiology

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Summary

Objectives: To determine the effects of a direct-fed microbial (DFM) and a specific regimen of antibiotic administration (subtherapeutic dosages) on fecal *Escherichia coli* concentrations, protection against *Salmonella* and rotavirus infections, intestinal volatile fatty acid (VFA) concentrations, and growth of nursery pigs.

Methods: Parameters were compared in groups of pigs fed the DFM, the antibiotics, or a control diet under field conditions and after experimental challenge with *Salmonella enterica* serovar Typhimurium and rotavirus.

Results: In the field study, average daily gain of antibiotic-fed pigs was larger than that of DFM-fed pigs. Other growth-performance parameters, fecal *Escherichia coli* concentrations, and prevalence of *Salmonella* serovars were similar among treatment groups. Under experimental conditions, total fecal coliform concentration was significantly lower in the antibiotic-fed group than in the two other groups. Total VFA concentration in the DFM group was significantly higher than that in the antibiotic-fed group. Prevalence of *Salmonella* serovars and rotavirus following challenge was similar in all groups.

Implications: Under the conditions of this study, this DFM does not enhance growth of nursery pigs or protect against *Salmonella* or rotavirus infection. Effectiveness of a DFM should not be assumed solely on the basis of the genera of bacteria included. Each strain of bacteria in a DFM should be validated for effectiveness. Additional details concerning the mechanisms by which DFMs and subtherapeutic dosages of antibiotics modulate the ecological balance of bacterial flora in the gastrointestinal tract are required to understand how the beneficial effects associated with certain feed additives are mediated.

Keywords: swine, probiotics, *Salmonella enterica*, rotavirus, direct-fed microbial

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Resumen – Efectos limitados de un microbiano comercial alimentado directamente en el desempeño de cerdos de destete y en su microbiología gastrointestinal

Objetivos: Determinar los efectos de un microbiano alimentado directamente (DFM por sus siglas en inglés) y un régimen específico de administración de antibiótico (dosis subterapéuticas) en las concentraciones de *Escherichia coli* fecal, protección contra la infección por *Salmonella* y rotavirus, ácido graso volátil intestinal (VFA por sus siglas en inglés), y el crecimiento de cerdos de destete.

Métodos: Se compararon los parámetros en los grupos de cerdos alimentados con el DFM, los antibióticos, o una dieta control bajo condiciones de campo y después de un reto experimental con *Salmonella* serovar Typhimurium y rotavirus.

Resultados: En el estudio de campo, la ganancia diaria promedio de los cerdos alimentados con antibióticos fue mayor que la de los cerdos alimentados con el DFM. Otros parámetros de desempeño de crecimiento, concentraciones de *E coli* fecal, y la prevalencia de *Salmonella* serovars fueron similares entre los grupos de tratamiento. Bajo condiciones experimentales, la concentración de coliformes fecales total fue significativamente más baja en el grupo alimentado con antibióticos comparada con los otros dos grupos. La concentración de VFA total en el grupo de DFM fue significativamente más alta que la del grupo alimentado con antibióticos. La prevalencia de la *Salmonella* serovars y el rotavirus después del reto fue similar en todos los grupos.

Implicaciones: Bajo las condiciones de este estudio, este DFM no mejora el crecimiento de los cerdos de destete ni protege contra la infección por *Salmonella* o rotavirus. La eficacia de un DFM no debería suponerse únicamente con base en el género de la bacteria incluida. En un DFM, la eficacia de cada cepa bacteriana debería validarse. Se requieren detalles adicionales concernientes a los mecanismos mediante los cuáles los DFMs y las dosis subterapéuticas de antibióticos modulan el balance ecológico de la flora bacteriana en el tracto gastrointestinal para entender como se median los efectos benéficos asociados con ciertos aditivos de alimento.

Résumé – Effets limités d'une préparation commerciale de culture bactérienne vivante sur les performances de porcelets sevrés et la microbiologie gastro-intestinale

Objectifs: Déterminer les effets d'une préparation commerciale de culture bactérienne vivante (DFM) et d'un régime spécifique d'administration d'antibiotiques

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(dosages sub-thérapeutiques) sur les concentrations fécales d'*Escherichia coli*, sur la protection contre une infection par *Salmonella* et rotavirus, les concentrations intestinales d'acides gras volatils (VFA), et la croissance des porcs en maternité.

Méthodes: Les différents paramètres ont été comparés entre les groupes de porcs nourris avec le DFM, les antibiotiques, ou une diète contrôlée dans des conditions de terrain et après infection expérimentale avec *Salmonella* sérovar Typhimurium et rotavirus.

Résultats: Lors de l'étude dans les conditions de terrain, le gain moyen quotidien des porcs recevant des antibiotiques était plus élevé que celui des porcs nourris avec le DFM. Les autres paramètres de performance de croissance, les concentrations fécales de *E coli,* et la prévalence des sérovars de *Salmonella* étaient similaires entre les groupes de traitement. Sous conditions expérimentales, la concentration de coliformes totaux était significativement plus basse dans le groupe d'animaux recevant des antibiotiques comparativement aux deux autres groupes. La concentration totale de VFA dans le groupe DFM était significativement plus élevée que dans le groupe nourri avec des antibiotiques. La prévalence des sérovars de *Salmonella* et de rotavirus suite à une infection expérimentale était similaire pour tous les groupes.

Implications: Dans les conditions de cette étude, le DFM n'a pas favorisé la croissance des porcelets en pouponnière ou protégé contre une infection par *Salmonella* ou rotavirus. L'efficacité d'une préparation de DFM ne devrait pas être présumée uniquement en fonction des genres bactériens inclus et chaque souche bactérienne dans une DFM devrait être validée pour son efficacité. Des détails additionnels concernant les mécanismes par lesquels les DFM et l'administration de doses sub-thérapeutiques d'antibiotiques modulent la balance écologique de la flore gastro-intestinale sont nécessaires pour comprendre comment fonctionnent les effets bénéfiques associés avec certaines souches.

Due to potential and perceived threats to food safety and public health, there is mounting public, political, and producer desire to identify alternatives to use of antibiotics at subtherapeutic dosages in livestock production. One such option that has received increasing attention is use of direct-fed microbials (DFMs), defined as viable, nonpathogenic microorganisms that have beneficial effects in preventing or treating several enteric disease conditions.[1] The mechanism of action of DFMs remains unknown, but it is believed that they act by modifying the ecology of the intestinal microflora.[1] Many direct-fed products are commercially available for livestock. Previous research has also suggested a beneficial role of some bacteria (eg, *Lactobacillus* species, *Bifidobacterium* species, and *Streptococcus* species) in piglets, either in enhancing weight gain or protecting from bacterial infections such as *Salmonella enterica* serovars.[2,3] Furthermore, *Lactobacillus* species are reported to stimulate the gut immune response and, in human infants, accelerate recovery from rotavirus diarrhea.[4-6] The purpose of this study was to determine if a commercially available DFM product, administered to weaned pigs as a single initial oral dose followed by continuous in-feed administration, protects against intestinal colonization of a bacterial and a viral pathogen, enhances growth performance, or both, compared to feeding antibiotics at subtherapeutic dosages or an unsupplemented control diet.

Materials and methods

Effects of DFM and administration of subtherapeutic doses of antibiotics were measured in a longitudinal field trial conducted at an institutional swine facility. Protection against experimental challenge with *Salmonella* serovars and porcine rotavirus was determined under controlled laboratory conditions. All studies were approved and conducted in accordance with The Ohio State University Institutional Laboratory Animal Care and Use guidelines.

Field study

Animals, housing, and management. Research was conducted at the Ohio State University Agricultural Technical Institute swine facility, Wooster, Ohio, between September 2002 and October 2003. This facility has two nursery units, defined as hot and cold nurseries, maintained at 30°C and 25°C, respectively. In each building, nursery decks with tribar floors were divided into eight pens, each approximately 1.2 m × 2.4 m, with four pens separated by a central walkway. Two nipple drinkers and a single five-space hopper-style feeder (76 cm wide) are provided in each pen. Shortly after birth, all piglets were injected with iron supplement and had their tails docked, needle teeth clipped, and ears notched. The males were castrated. Piglets were weaned at 18 to 24 days of age. All piglets were vaccinated at weaning and 3 weeks post weaning against *Erysipelothrix rhusiopathiae*, *Bordetella bronchiseptica*, *Mycoplasma hyopneumoniae*, and *Pasteurella multocida*.

Experimental design. As space became available in the nursery, newly weaned piglets available for the study were sorted into heavy and light classes based upon the mean weight of all pigs weaned that week. Seven to 12 pigs in each weight class were randomly assigned to each pen. Each treatment was applied to eight pens of heavy pigs, eight pens of light pigs, and one pen of pigs not weight sorted due to the limited number of animals available during 1 week. After 3 weeks, pigs were moved from the hot nursery to the cold nursery with group integrity maintained. Pens were monitored daily for signs of illness or disease. Piglets that failed to thrive were removed from the study. Feed consumption was recorded daily on a per pen basis. Pigs were weighed at weaning and 3 and 6 weeks post weaning. Fresh feces were collected from four to six areas of the floor of each study pen and pooled each week, at intervals of 3 to 7 days. Samples were transported immediately to the laboratory and cultured for total coliforms, *Escherichia coli,* and salmonellae.

Treatments. The base ration contained 2.0% lysine for the first week on feed, 1.82% lysine during weeks two and three, and 1.56% lysine during the last 3 weeks of the study. Individual pens of pigs received the unsupplemented base ration or the same ration supplemented either with a DFM (Ultra Acidola Plus; Ultra Bio-Logics Inc, Montreal, Quebec, Canada) or with antibiotics routinely used in the facility.

Ultra Acidola Plus is labelled to "aid in the prevention and treatment of stress" and contains electrolytes and vitamins as well as the following bacteria: *Lactobacillus acidophilus*, 10^{10} colony-forming units (CFU)

per kg; and *Streptococcus fecalis, Bifidobacterium thermophilum,* and *Bifidobacterium pseudolongium,* each at 3.3×10^9 CFU per kg.[7] In the DFM treatment group, a single oral dose of 5 g of Ultra Acidola Plus was first administered to each pig. The DFM was then provided by continuous in-feed dosing at 550 mg per kg of feed in the hot nursery (first 3 weeks post weaning) and at 330 mg per kg of feed in the cold nursery.

Antibiotics selected were those historically used at the swine facility to control pneumonia and dysentery and to enhance growth. The feed additive CSP (Boehringer Ingelheim Vetmedica, St Joseph, Missouri) contains chlortetracycline, sulfathiazole, and penicillin. A feed formulation error occurred, resulting in only half of the desired dose of CSP being included in the feed. Thus, the complete mixed ration for the antibiotic group in the hot nursery contained chlortetracycline and sulfathiazole each at 55 mg per kg of feed and penicillin at 27.5 mg per kg of feed (chlortetracycline and sulfathiazole at approximately 2 mg per kg body weight and penicillin at approximately 1 mg per kg body weight daily). In the cold nursery, feed contained lincomycin (Akey Inc, Lewisburg, Ohio) at 220 mg per kg (approximately 11 mg per kg body weight daily). Feed and water were offered ad libitum.

Challenge study

Animals, housing, and management. On five separate occasions between February and April 2003, groups of 21 to 30 weaned pigs were transported from The Ohio State University Agricultural Technical Institute swine facility to isolation facilities at the Food Animal Health Research Program. Groups of seven to 10 pigs were housed in pens with solid floors (3 m × 4 m) that were cleaned daily. The 2% lysine base ration was provided twice daily on the floor and water was provided ad libitum from nipple drinkers. Challenged pigs did not receive lincomycin.

Experimental design. Piglets were randomly assigned to three treatment groups (rations) similar to those described for the field study, including the same dose of CSP that had been formulated in error. Piglets assigned to the DFM group were initially inoculated orally with 5 g of DFM product as in the field study. Piglets were allowed to adapt to the diets for 1 week prior to experimental challenge.

In Experiments One and Two, 5 mL of a broth culture containing 1.5×10^{10} CFU of a porcine-origin, multi-drug-resistant strain of *Salmonella* serovar Typhimurium was administered orally to one piglet in each replicated treatment group (Day 0). The challenged piglet was co-housed with the other piglets in the group and was allowed to co-mingle with them immediately after challenge.[8] Rectal swabs were used to collect fecal samples from each animal in each group on Days -3, 0, 1, 3, 7, and 10. Individual samples were cultured for salmonellae, coliforms, *E coli*, and lactic acid bacteria (LAB).

In Experiments Three to Five, each piglet was orally dosed with approximately 10^6 cell culture immunofluorescent foci of porcine rotavirus strain OSU (Day 0).[9] Rectal swabs were used to collect fecal samples from each animal on Days -3, 0, 1, 3, 7, and 10. Individual samples were cultured for salmonellae, coliforms, *E coli*, and LAB and were tested for rotaviruses. On Days 3, 7, and 10, two randomly selected piglets from each group were sacrificed and necropsied to detect lesions compatible with rotavirus infection and to obtain intestinal contents for determination of concentration of volatile fatty acids (VFAs) and detection of rotavirus.

Microbiological culture and laboratory analyses

The presence in the DFM of viable LAB, including *L acidophilus*, was determined by homogenising the product in buffered peptone water (BPW) and plating serial dilutions of this homogentate on ROGOSA agar (Becton Dickinson, Sparks, Maryland).[10] ROGOSA plates were incubated anaerobically at 37°C for 48 hours and colonies were counted. The same method was used to quantitatively culture LAB from fecal samples.

For detection and enumeration of coliforms, a 25-g sample of feces was added to 225 mL of BPW. Total *E coli* concentrations were determined by plating serial dilutions of this homogenate onto violet red bile agar containing 100 mg per mL 4-methylumbelliferyl-B-D-glucuronide (MUG).[11] After overnight incubation at 37°C, lactose-positive colonies (total coliforms) and lactose-positive, MUG-positive colonies (presumptive *E coli*) were enumerated, aided by ultraviolet illumination.

For detection of *Salmonella* serovars, BPW fecal homogenates were enriched overnight at 37°C. For each sample, 1 mL of homogenate was added to 9 mL of tetrathionate broth. After incubation overnight at 37°C, 0.1 mL of tetrathionate broth culture was transferred into 10 mL of Rappaport-Vassiliadis (RV) broth.[12] After overnight enrichment at 37°C, the RV broth culture was plated to xylosine-lysine Tergitol-4 (XLT4) agar. Black colonies appearing on XLT4 after 48 hours of incubation at 37°C were considered *Salmonella* suspects. This was confirmed by using biochemical reactions in triple sugar iron (TSI) and urea agar and agglutination with serogroup-specific antisera. In addition, serial dilutions of the BPW homogenates were plated on 150-mm XLT4 plates containing antibiotics and incubated overnight at 37°C to detect *Salmonella* Typhimurium inoculated in the challenge studies. Black colonies were enumerated and 10% of the suspect colonies were further verified as *Salmonella* on the basis of TSI and urea reactions.

Rotaviruses were detected by commercial ELISA kit (ImmunoCard STAT! Rotavirus; Meridian Bioscience Inc, Cincinnati, Ohio). Volatile fatty acid content of ingesta from the proximal colons of challenge-study pigs was determined using the method previously described by vanWinsen et al.[13]

Calculations and statistical analyses

In the field study, differences between treatment groups in prevalences of *Salmonella* serovars and rotavirus were compared using a chi-squared distribution test. Differences in shedding of salmonellae and rotavirus among treatment groups in the challenge studies were assessed using a repeated measures ANOVA procedure for nonparametric data and SAS software version 8.0 (SAS Institute, Carey, North Carolina).[14] In both the challenge and field studies, repeated measures ANOVA was used to compare LAB, coliform, and *E coli* concentrations between treatment groups. Average daily gain, feed consumption, and feed:gain ratios were calculated from pig weights and feed consumed, with pen as the unit of analysis. The analyses were not adjusted for differences in pig densities within pen. Generalized linear models followed by Tukey's test for multiple comparisons were used for the analysis of the growth performance parameters and the fecal VFA data from challenge studies. The dependant variables in the field study models were the pen-level

log-transformed bacterial counts modeled as a function of pig weight and treatment (diet) repeated on the week. A treatment-by-weight interaction effect was included in the model. In the challenge studies, the unit of observation for comparison was the pen-level prevalence of each pathogen. Bivariate analyses of correlation between fecal coliform, *E coli*, and LAB concentrations, fecal VFA concentrations, and performance parameters were performed using the Pearson product-moment correlation coefficient. Statistical significance for type I error was set at 0.05.[15]

Results

Field study

A total of 51 pens of animals were enrolled in this study, representing 17 pens per treatment (DFM, antibiotics, and control). During the 12 months of the study, a total of 23 pigs were removed from the study for failure to thrive: eight pigs from the DFM group, eight from the control group, and seven from the antibiotic group. No differences in growth parameters were observed among treatments during the hot nursery stage of the study (Table 1). During the cold nursery stage of production, pigs fed lincomycin consumed more fed and gained weight faster than pigs fed DFM; however, these growth parameters were not different from those of the control pigs (Table 1).

There were no significant differences between treatment groups for fecal coliforms, *E coli*, or LAB concentrations (Table 1). *Escherichia coli* and coliform concentrations in fecal samples were positively correlated (r = 0.86; $P < .001$). LAB concentrations were not correlated with either total coliform concentrations (r = 0.25; $P = .05$) or *E coli* concentrations (r = 0.02; $P = .02$). However, there were negative correlations between feed consumption and fecal coliform concentration (r = -0.45; $P < .01$) and between feed consumption and fecal *E coli* concentration (r = -0.36; $P = .01$). Consequently, there were also negative correlations between feed conversion and fecal coliform concentration (r = -0.50; $P < .001$) and between feed conversion and fecal *E coli* concentration (r = -0.43; $P < .01$).

Overall, salmonellae were detected in 16 of the 333 field samples cultured (4.8%): five of 114 samples from the antibiotic group (4.4%), seven of 110 samples from the control group (6.4%), and four of 109 samples from the DFM group (3.7%).

Table 1: Least squares means and standard error (SE) of growth, performance, and microbiological* parameters measured in a field study in nursery pigs fed either an unsupplemented basal diet (Control) or the same diet supplemented with a commercial direct-fed microbial product (DFM)† or with subtherapeutic dosages of antibiotics‡

	DFM n = 17	Antibiotic n = 17	Control n = 17	SE
Body weight (kg)				
Weaning	5.85	5.89	5.90	0.13
3 weeks post weaning	11.38	11.96	11.50	0.23
6 weeks post weaning	23.53[a]	25.29[b]	23.99[ab]	0.34
Hot nursery (weeks 0 to 3)				
ADG (kg)	0.26	0.29	0.27	0.01
ADFI (kg)§	0.35	0.38	0.36	0.01
Feed:gain (kg/kg)	1.35	1.33	1.37	0.02
Cold nursery (weeks 4 to 6)				
ADG (kg)	0.58[a]	0.63[b]	0.60[ab]	0.01
ADFI (kg)	0.85[a]	0.93[b]	0.89[ab]	0.01
Feed:gain (kg/kg)	1.48	1.47	1.49	0.01
Overall (weeks 0 to 6)				
ADG (kg)	0.42[a]	0.46[b]	0.43[ab]	0.01
ADFI (kg)	0.60	0.65	0.62	0.01
Feed:gain (kg/kg)	1.43	1.42	1.44	0.01
Total coliforms (CFU/g)§	6.46	6.29	6.50	0. 06
Escherichia coli (CFU/g)	6.13	5.62	6.04	0.10
LAB (CFU/g)§	10.59	10.57	10.56	0.04

* Averaged weekly counts per pen from pooled feces.

† Direct-fed microbial, Ultra Acidola Plus (Ultra Bio-Logics, Montreal, Quebec, Canada); 550 mg/kg of feed for weeks 0-3 and 330 mg/kg of feed weeks 4-6.

‡ Weeks 0 to 3 post weaning, chlortetracycline and sulfathiazole each at 55 mg/kg of feed and penicillin at 27.5 mg/kg of feed (CSP; Boehringer Ingelheim Vetmedica); weeks 4 to 6 post weaning, lincomycin (Akey Inc, Lewisburg, Ohio) at 220 mg/kg of feed.

§ ADFI: average daily feed intake; CFU = colony-forming units; LAB = lactic acid bacteria.

[ab] Values in a row with no common superscript are different ($P < .05$; Tukey's test).

Salmonella prevalence did not differ significantly among treatment groups ($P = .63$). Seven of 161 light-class piglets (4.4%) and eight of 144 heavy-class piglets (5.5%) were *Salmonella*-positive ($P = .83$). Four of 184 samples (2%) tested positive for rotavirus: three from the DFM treatment group and one from the antibiotic treatment group.

Experimental challenge

Experiments One and Two. Oral inoculation of a single piglet in each group with *Salmonella* Typhimurium resulted in dissemination of the pathogen to every pig in the pen. Prevalence of *Salmonella* in each pen varied daily and ranged between 12.5% and 100%. The overall number of *Salmonella*-positive samples was highest in the DFM group. *Salmonella* was isolated from 35 of 54 samples from the antibiotic treatment group, 31 of 49 samples from the control group, and 46 of 54 samples from the DFM group. Prevalence did not differ significantly among groups ($P = .71$) or days ($P = .23$). No day-by-treatment interaction was observed ($P = .42$).

Experiments Three, Four, and Five. Prevalence of rotavirus was greatest shortly after challenge (Days 2 to 4) and then dropped quickly. Prevalence varied among days ($P = .02$), but not among groups ($P = .73$).

No day-by-treatment interaction was observed ($P = .30$). Total coliform concentrations in fecal samples, expressed as base 10 logarithms ± SE, were lower in antibiotic-fed pigs (5.35 ± 0.17 CFU per g; n = 98) than in control (6.38 ± 0.18 CFU per g; n = 90) and DFM groups (6.77 ± 0.18 CFU per g; n = 97) ($P < .05$). Total VFA concentrations in ingesta were lower in antibiotic-fed pigs (104.6 ± 17.8 µmole per mL; n = 14) than in DFM pigs (128.6 ± 16.8 µmole per mL; n = 14) ($P < .05$), but did not differ in either group compared to the controls (113.9 ± 15.0 µmole per mL; n = 13) ($P > .05$). The partitioning of specific VFAs was similar in all groups (data not shown; $P > .05$). Coliform and *E coli* concentrations were significantly, but weakly, correlated (r = 0.21; $P < .001$). LAB concentrations were weakly correlated with total coliform concentrations (r = 0.20; $P = .02$), but no significant correlations between any bacterial parameter measured and the total VFA concentration were observed.

Discussion

Many commercial products are sold as DFMs with label claims to enhance growth and performance. The action of DFMs in modulating the ecology of the gastrointestinal tract is poorly understood. In the United States, several different bacterial species listed in the American Association of Feed Control Officials official publication[16] are considered safe and can be added to feed and sold without regulatory oversight as long as therapeutic claims are not made.The addition to the feed of some DFMs, especially LAB such as the ones included in Ultra Acidola Plus, provide beneficial effects, such as enhanced weight gain and feed conversion and protection from pathogen carriage in pigs and other animals.[17-20] On the other hand, other researchers have attempted to demonstrate beneficial effects of DFMs without success.[21-24] In one study, dogs actually excreted more *Salmonella enterica* and *Campylobacter* species. following treatment with DFMs.[25] Importantly, it is possible that most studies failing to identify beneficial effects subsequent to DFM administration are not being reported in the peer-reviewed scientific literature, so that the published literature is biased toward effectiveness of DFM feeding. Since we do not know how DFMs work, achieving favourable food safety, animal health, and performance effects by incorporating DFMs in the ration is a hit-or-miss approach until such time as predictive markers of effectiveness are identified. The low prevalence of rotavirus detected in this study precluded the possibility of drawing any conclusion about the effects of the treatments on rotavirus prevalence.

Sakata et al[26] hypothesized that the effects of LAB are modulated primarily through production of short-chain fatty acids or VFAs that have a detrimental effect on gram-negative bacterial flora (primarily coliforms and *E coli*). However, the results of this study suggest that this mechanistic view is oversimplified. LAB are known to produce VFAs.[26] In our experimental challenge study, VFA concentrations in the ingesta of DFM-fed pigs were high, but VFA concentrations were not correlated with either *E coli*, coliform, or LAB concentrations. These results agree with those of another recently published study conducted in piglets using a different DFM preparation.[27]

It is of interest to note that in the field study, *E coli* and coliform concentrations were significantly (negatively) correlated with feed consumption (ie, fewer *E coli* were cultured from pigs that ate more*).* From this study, it is not possible to determine whether high concentrations of *E coli* are a direct result of lower feed consumption and an unhealthy balance of microorganisms in the intestine or whether high *E coli* concentrations contribute to decreased appetite and feed conversion. The weak correlation between *E coli* and total coliform concentrations in the experimental study may be attributed to the extremely low (and frequently undetectable) concentrations of *E coli* observed in the young pigs during this study.

The absence of observable effects of antibiotic feeding on growth during the first 3 weeks post weaning in the field study may be attributed to the feed formulation error. Nevertheless, the appropriate dose of lincomycin did have a positive effect on ADG. The exact reasons that the DFM product failed to produce the desired effects are unknown. Storage and processing might cause a decrease in potency; however, counts of LAB were comparable to label claims for *L acidophilus*. It is possible that *Lactobacillus* bacteria recovered on the ROGOSA plates were not *L acidophilus,* or that the other bacteria claimed to be present in the product were nonviable.

The bacterial species included in the DFM product have been previously isolated from swine feces.[28,29] Nevertheless the species-of-origin, bacterial subspecies, and type of each strain present in the commercial product tested were not provided. It is possible that the DFM strains poorly colonized the pigs. Fecal isolates of LAB, streptococci, and *Bifidobacterium* organisms were not strain typed. Clearly, different bacterial strains have different abilities to adhere to and colonize the gut epithelium.[28] Furthermore, some bacteria commonly included in DFM products do not grow well in the presence of bile acids.[30,31] In one study, 83% and 62% of *Bifidobacterium* and *Lactobacillus* isolates, respectively, were bile sensitive.[31]

Other non-antibiotic approaches to modify the gastrointestinal microbial population of swine have also been attempted. For example, feeding fermented feed products,[10] grinding feed,[32] and adding organic acids to acidify the feed[33] are alternative approaches to lowering the pH of the gastrointestinal content and controlling growth of *E coli.* The rationale of feeding DFMs differs from these two approaches in that DFMs are expected to colonize the gut, reduce pH, and produce VFAs in vivo. Since the gastrointestinal microbiology of piglets may vary significantly depending upon environment, health, and other management factors, it is presently not possible to guarantee or predict the effectiveness of a particular direct-fed microbial treatment on an individual farm, even if it has been used successfully under different management conditions. Elucidating the mechanism of action by which DFMs enhance animal health will facilitate consistent selection of beneficial DFM strains.

Implications

- Under the conditions of these studies, a commercial direct-fed microbial formulation had no effect on piglet growth, fecal *E coli* concentrations, or *Salmonella enterica* prevalence.
- Each strain of bacteria present in a DFM should be validated for effectiveness.
- The effectiveness of a DFM should not be assumed solely on the basis of the genera of bacteria it contains.
- Additional details concerning the mechanisms by which DFMs and

subtherapeutic doses of antibiotics modulate the ecological balance of bacterial flora in the gastrointestinal tract are required to understand how the beneficial effects associated with certain feed additives are mediated.

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