Effects of Cordyceps Militaris and Cordyceps Sinesus on nursery pig performance

by

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Abstract

Cordyceps Militaris is a human therapeutic food with many bioactive compounds with antimicrobial and antiviral activities. There is little research in feeding this mushroom powder (MP) to pigs, to evaluate its potential various levels and combinations were fed to nursery pigs. Experiment one used one-hundred sixty crossbred pigs ((Duroc × (York × Landrace)) (19.4 d of age; initial BW 7.24 kg), to test a previously reported dose to pigs and a human equivalent dose. One ppm proved to be too low of inclusion with identical performance to the NC, while 300 ppm had numerically improved performance over the NC and matched Carbadox's performance in the final phase. Experiment two was a titration study to find the optimal level of inclusion for this Cordyceps product. As a potential alternative to Carbadox in nursery pig diets one-hundred sixty crossbred pigs (18.8 d of age; initial BW 5.94 kg) were used in a 35-day growth trial. At various points of the study, pigs fed the 300 ppm and the step-down mushroom powder treatments tended to have improved ($P < 0.10$) growth performance compared with those fed the NC diet. During Phase 4 of the study, pigs fed Carbadox had greater ADG ($P < 0.02$) and improved G:F ($P < 0.09$) over pigs fed the NC. However, overall (d 0-35) there were no differences among treatments ($P > 0.05$). Experiment three evaluated the independent and additive effects of Cordyceps MP and Carbadox to pharmacological copper+zinc. Two hundred-ten crossbred weanling pigs (19 d of age; initial BW 5.8 kg) were used in a 33 day growth trial. Pigs fed the PC, PC+MP and CuZn treatment had increased BW ($P<0.05$), ADG ($P<0.05$), ADFI ($P<0.10$) and G:F ($P<0.05$) over the NC for phases 1, 2, and 3, with MP treatment being intermediate. During Phase 4, pigs fed MP, PC, MP+PC, and CuZn diets all had increased ADG ($P<0.05$; 455, 476, 504, 487, 431 g/d) and ADFI ($P<0.05$) over the NC pigs. Experiment four utilized one-hundred thirty two weanling pigs (18.2 d of age; initial BW 5.77 kg) for a 35 day growth trial to

evaluate mushroom Beta-Glucans (BG) and MP. During Phase 1 (d 0-7) PC pigs had increased ADG, ADFI, and d7 BW ($P < 0.05$) over NC, pigs fed BG + MP also had increased ADFI in Phase 1 over the NC ($P < 0.05$). During Phase 2 and 3 (d 7-21) an illness affected the pigs. This has led to inconsistent performance because of pigs eating, but losing weight. In phase 4 there was a difference in BG and MP diets, with an interaction between source and dose of the MP and BG. The 300 level of MP improved feed efficiency, while the 300 level of BG reduced efficiency in phase 4. On average across all of the experiments the Cordyceps MP and BG improved nursery growth performance above NC and provided a portion of the antimicrobial treatment response.

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Dedication

I would like to dedicate this to my family, who supports me no matter what.

Chapter 1 - Literature Review

Introduction

Research to discover the relationship between gastrointestinal health and immunological response in farm animal production is an important and growing area of research (Spurlock et al., 1997; Didierlaurent et al., 2005; Zijlstra et al., 2010). Ultimately 'gut health' represents the outcome of the gastrointestinal tract in response to its capacity and ability to respond and adapt to the insults and challenges it encounters (Pluske et al., 2017). Research and innovation in the pigs early life is not a new frontier; however, economical and practical uses are needed in order to make any impact. Gut health in pigs can be compromised even in the absence of a disease challenge. Reduced feed intake after weaning (Dong and Pluske, 2007) for example means an absence of luminal nutrition (Diamond and Karasov, 1983). Stressors and challenges associated with weaning also cause changes to the structure and function of the GIT (Celi et al., 2017; Kim et al., 2012; Jayaraman and Nyachoti, 2017; Moeser et al., 2017; Pluske et al., 1997). Together the immediate post-weaning period in pigs not only causes structural but also functional changes to the small intestine (e.g., Camilleri et al., 1991; Pluske et al., 1996a,b), and also contributes to an intestinal inflammatory status that in turn compromises the villous-crypt architecture (e.g. McCracken et al., 1999; Spreeuwenberg et al., 2001; Pié et al., 2004), GIT barrier function (e.g., Camilleri et al., 2012; Kim et al., 2012; Moeser et al., 2017; Wijtten et al., 2011), and disruption of the microbiota (e.g., Fouhse et al., 2016; Gresse et al., 2017; Schachtschneider et al., 2013). Complex interactions occurring in the GIT between nutrition, the mucosa, and the microbiota impact gut health in early life (Pluske et al., 2017). Bischoff (2011) inferred the two most prominent keys to achieving a healthy GIT system are the microbiota and the GIT barrier function. The pork industries attention to these 2 factors has increased dramatically in the last 15

years with the changes in antibiotic growth promotants, and the use of heavy metals in nursery diets.

The intestinal microbiota is established with a compromise between helpful barrier function, synthesis of nutrients, improved energy usage from the diet, and the effects of inflammation and sub-clinical as well as clinical pathologies (Celi et al., 2017). The microbiota is also in constant communication, with other enteric bacteria and the host. Distribution of bacterial binding sites on the gut surface is key in determining the hosts susceptibility and in triggering immune responses, with this being especially pronounced in young animals (e.g., Kelly and King, 2001; Montagne et al., 2003; Celi et al., 2017). Microbiota disruption in the GIT is likely a key influence in postweaning diarrhea. Most studies conducted during weaning transition have shown a decrease in Lactobacillus spp. group and a loss of overall microbiota diversity, while Clostridium and E. Coli were increased(Gresse et al., 2017; Pluske et al., 2017). It is theorized that the use of antibiotic growth promotants cause disruption to the natural concentration of harmful and beneficial microbes due to their wide spectrum of activity (Gresse et al., 2017). Furthermore, it is possible the extended use of growth promotant antibiotics could increase the chances for pathogenic microorganisms to colonize and trigger a disease response (Fouhse et al., 2016). Dou et al. (2017) discovered postweaning diarrhea can be predicted as early as day 7 post-natal. At this point in life there is already a greater abundance of Prevotellaceae, Lachnospiraceae, Ruminocacaceae, and Lactobacillaceae in healthy pigs compared to pigs who later suffer from postweaning diarrhea.

Epithelial barrier function is compromised in the immediate postweaning period as weaning increases small intestine permeability (Spreeuwenberg et al., 2001; Moeser et al., 2007; Pohl et al., 2017). Inflammation of the intestine is associated with increased permeability that

can potentially lead to translocation of toxins, bacteria, viruses, and allergens. If these compunds cross or permeate and enter the lamina propria they can cause an inflammatory response. How these early life stresses impact the whole body system of the pig requires further understanding in order to discover new targets for management, and possibly new products to alleviate such responses in order for the newly weaned pigs to thrive.

Antibiotic alternatives

There are many feed additives being researched currently, however do any of them have a legitimate effectiveness to replace carbadox or other antimicrobials? Some of the most common include acidifiers, pharmacological zinc and copper, prebiotics, probiotics, yeast products, nucleotides, and many plants and plant extracts (Liu et al. 2017). Satisfying the consumer can come at certain costs, such as the current trend to go antibiotic free in production systems. However, removing antibiotics from productions systems leaves the door open to pathogenic threats. In newly weaned pigs removing growth promoting antibiotics results in increased disease pressure and reduced growth performance. Later in the pigs life, growth promotional antibiotics may not be as important (Wierup, 2001). As stated in the earlier portion of this review, early challenges can have life-long effects on the pig. If the pig is started in optimal conditions, odds are the pig will continue to thrive however, general industry conditions are often suboptimal. There are many methods to preventing disease, one possibility is preventing enteric pathogenic bacteria from colonizing in the intestines. In order to defend against pathogens, pigs need the support to eliminate the threat themselves through their immune system.

Acidifiers

Acidifiers are being researched as an alternative to antibiotic growth promoters. The concept behind acidifiers is to decrease the pH in the gastrointestinal tract to make it less suitable for invading microorganisms, and more suitable for beneficial microorganisms to thrive. Despite many years of research there is no consensus on the exact mode of action. There are a few suggestions such as a decreased or stabilized gastric system could lead to an increase in pepsin activity, alteration of the microbiota of the gut could lead to pathogenic bacteria being unable to thrive, and acidifiers could increase nutrient digestibility in the intestines (Kil et al., 2011; Papatsiros and Billinis, 2012).

Inorganic acids are the most popular of the acidifiers being researched, including fumaric acid, lactic acid, and citric acid. Formic, citric, and benzoic acid all improved growth performance and feed efficiency when fed to weanling pigs (Guggenbuhl et al., 2007; Halas et al., 2010; Papatsiros et al., 2011; Diao et al., 2016; Luise et al ., 2017) as well as in growing pigs (Giesting and Easter, 1985; Suryanarayana et al., 2012). The addition of citric acid to the diet of sows was shown to increase digestibility of protein, calcium, and phosphorus (Liu et al., 2014a,b). Benzoic acid has been shown to improve apparent digestibility of calcium and phosphorus in growing pigs (Sauer et al., 2009; Bühler et al., 2010; Xu et al., 2018) and digestibility of crude protein in weanling pigs (Guggenbuhl et al., 2007; Halas et al., 2010; Xu et al., 2018).

Recently the combination of feeding medium chain fatty acids and organic acids has been researched. The combined use shows the ability to reduce pathogenic activity (Zentek et al., 2013) as well as having positive impacts on the digestibility of nutrients and on growth performance (Upadhaya et al., 2014; Kuang et al., 2015; Long et al., 2018). The modes of

action behind this positive effect was the decreased expression of proinflammatory cytokines and increased lactobacillus populations (Kuang et al., 2015). In addition to the previous findings the pH of the stomach as well as the concentrations of pathogenic bacteria in the GI tract were reduced (Zentek et al., 2013). With pigs regularly not consuming feed postweaning, another alternative is to acidify the water the pigs drink. Walsh et al. (2007) found acidifying the water improved ADG in all diets and may have its greatest benefit the first few days postweaning when some pigs will drink but not eat.

Minerals

One of the most popular alternatives for growth promoting antibiotics is pharmacological levels of copper and zinc. However in the European Union zinc beyond nutritional requirements is being phased out, and only low levels of copper (150-100 ppm) are allowed. These rules were put into place due to environmental concerns of these heavy metals. Copper and zinc are the best examples of minerals that are required by the pig that also have antimicrobial properties. Zinc is used as an activator and component of several metalloenzymes, as well as playing a major role in production and secretion of hormones (Liu et al., 2018). Zinc also plays roles in wound healing and supporting various parts of the immune system (McDowell, 1992). Zinc's requirement in postweaning diets is 100 mg/kg, then reducing to 80 mg/kg of zinc (NRC, 2012), when deficient, zinc results in poor growth performance, skeletal abnormalities, loss of appetite, and parakeratosis (Ku et al., 1970; Prasad et al., 1971). Pharmacological levels of inorganic zinc are commonly fed in the form of zinc oxide. Pharmacological can range from 1500 to 4000 mg/kg of zinc, these levels have been shown to reduce postweaning diarrhea and improve growth performance (Poulsen, 1998; Smith et al., 1997; Hill et al., 2000; Hu et al., 2012). The mechanisms behind the improved growth

performance is possibly due to the improvements in intestinal morphology, such as increased villous height and villus height to crypt depth ratio (Carlson et al., 1998; Li et al., 2001; Li et al., 2006; Hu et al., 2013a; Xia et al., 2017; Zhu et al., 2017) and decreased the crypt depth in postweaning pigs (Li et al., 2001; Zhu et al., 2017). Zinc also assists in regeneration of damaged intestinal epithelial tissue (Alam et al., 1994), reduction of intestinal permeability (Zhang and Guo, 2009) and lymphocyte proliferation (van Heugten et al., 2003).

Copper is similar to zinc in several instances such as being used in metalloenzymes. Copper is also used in oxidation-reduction reactions, transport of oxygen and electrons, and protection against oxidative stress (Hill, 2013). Copper is involved in metabolic reactions, tissue pigmentation, hemoglobin formation, and connective tissue development (McDowell, 1992). Neonatal pigs require 5-6 mg/kg of copper for their normal metabolism (NRC, 2012). As pigs get older their requirements decreases. Pharmacological levels of copper in nursery diets have been common practice, usually in a concentration of 100-250 mg/kg. This is used to reduce postweaning diarrhea and improve postweaning performance (Ma et al., 2015). The growth promotion effects of added copper are a result of the copper's bacteriostatic and bactericidal properties (Stahly et al., 1980). In addition, supplemental copper also increases villous height and decreases crypt depth in the intestine (Zhao et al., 2007). Many experiments have concluded that there is a beneficial additive effect when feeding postweaning pigs zinc and copper in combination (Perez et al., 2011; Feldpausch et al., 2018;). It is theorized that zinc may modify the colonic microbial profile, while copper reduces the microbial diversity in the ileum and colon (Namkung et al., 2006).

Prebiotics and Probiotics

Recently research has been popular in the area of prebiotics and probiotics. Prebiotics are mainly non-digestible oligosaccharides that are defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson and Roberfroid, 1995). The main beneficial effects of these products are due to increased fermentability of the diet and the resultant shift in microbiome.

Probiotics, or also known as direct fed microbials, are beneficial microorganisms fed directly to pigs. Probiotics may improve gut health by modifying the gut microflora, which in turn may help control pathogens (Prescott et al., 2005). Probiotics also have the potential to improve immune response (Galdeano and Perdigon, 2006), improve overall health status, as well as improve growth performance (Kenny et al., 2011; Cromwell, 2013). Another theory is due to decreased energy usage by the immune system from being more effective and less stimulated, the energy normally utilized by the immune system is being redirected towards growth performance (Cho et al., 2011). The low pH of the pigs stomach has proven to be an issue in direct supplementation of microbes, as most are not stable at such a low pH. This leads to the microbes needing protective coatings, or using stable spores that are stable and dormant through the stomach and thrive in the higher pH intestinal tract segments. Research in this area has proved to be rather inconsistent. The swine industry as a whole is learning; and improvements are naturally being made in this area. Because of this, older papers may not be as accurate, as we have improved bacterial strains and discovered what strains are most effective. While growth data can be inconsistent we see other areas of gut health offer positive results; such as Lewis et al. (2013) discovered lactic-acid producing microbials up-regulated proteins associated with

epithelial tight junctions and reduced Immunoglobulin A in the intestinal mucosa, suggesting that these bacteria increased gut barrier function. In a study of wean-to-finish pigs using a 1:1 ratio of B. licheniformis and B. subtilis, looked at inclusion and duration effects of these probiotics. These microbes increased growth performance, as well as improved carcass characteristics as dose and duration increased (Alexopoulos et al., 2004). Grow-finish pigs supplemented with Bacillus spp. had increased fecal SCFA concentrations, which led to increased energy for growth performance leading to greater growth as well as greater loin eye area and higher fat-free percent lean (Jaworski et al., 2017). It should be noted that with these positive outcomes, probiotics are still inconsistent and warrant further and more comprehensive investigation.

Plant extracts

Plant extracts are secondary plant metabolites, which are responsible for the plants odor and color (Liu et al., 2018). There are hundreds of components to plant extracts, and can be provided in two primary forms; powder and oil. Most plant extract oils are water insoluble and referred to commonly as essential oils. Plant extracts are of interest because of their biological activity. Many can have antiviral, antimicrobial, antioxidant, and anti-inflammatory effects (Baydar et al., 2004; Sökmen et al., 2004; Dundar et al., 2008; Liu et al., 2012; 2013a, b; 2014a, b). Plant extracts have the possibility to replace antibiotics in animal production (Pettigrew, 2006; Stein and Kil, 2006). Plant extracts have the ability to improve animal health through a multitude of mechanisms, such as the suppression of microorganisms, alteration of microbial populations, and enhancement of immune function (Lee et al., 2004; Calsamiglia et al., 2007). The major bioactive compounds of plant extracts are polyphenols, and their structure and concentrations will vary from plant to plant. Many factors can effect this such as geographical

location, environmental factors, processing techniques, and storage conditions. With all of these factors it is difficult to get a consistent product with similar concentrations, and should be obtained with a certificate of analysis or perform compositional analysis on the product purchased.

With so many options and variables attributing to the bioactivity of all of these potential antibiotic replacements it appears as though we are only scratching the surface with these products. Much more research is needed, as in vivo experiments can vary greatly to in vitro experiments. With decreased antibiotic use for growth promotion and reduced uses mandated by consumers, it is our duty as animal research scientists to further these investigations to find suitable replacements and alternatives.

Cordyceps Mushroom

Medicinal mushrooms have been around for thousands of years, most of which produce biometabolites that can possibly treat diseases. One particular species of mushrooms, Cordyceps, has shown medical promise and has been used in parts of Asia for millennia (Gu et al., 2007). The two subspecies of particular interest in this review are the Cordyceps Militaris and Cordyceps Sinesus. Cordyceps Militaris belongs to the phylum Ascomycita classified in the order Hypocreales, as spores are produced internally in a sac referred to as ascus (Wang et al., 2008). It is an entomopathogenis fungus which has an annual growing season. This particular fungus typically grows parasitically on lepidopteron larvae and pupae of insects and spiders in the winter time, leading to the formation of fruiting bodies in the summer, (Tuli et al., 2014). Cordyceps Sinesus has been reported to have been used in westen Nepal to cure multiple diseases including, diarrhea, headache, cough, rheumatism, and liver disease. This mushroom is referred to as “Himalayan Gold” due to its broad clinical and commercial uses (Devkota, 2006).

There are many bioactive compounds in the Cordyceps family of mushrooms. However, the most popular appears to be Cordycepin because of its broad spectrum of activity. It is known to interfere with many biological and molecular processes such as purine biosynthesis (Overgaard, 1964; Rottman and Guarino, 1964), DNA/RNA synthesis (Holbein et al., 2009), and mTOR signaling transduction (Wong et al., 2010). With technology constantly advancing the ability to extract the bioactive compounds from the mushrooms has become feasible to produce products in powders and pill forms. Cordyceps and its compounds have a long track record of health effects such as hepatic, renal, cardiovascular, respiratory, nervous, sexual, immunological, as well as having anti-cancer, anti-oxidant, anti-inflammatory and anti-microbial activities (Zhou et al., 2008; Wang et al., 2011; Lee et al., 2011a, b; Zhang et al., 2012; Patel and Goyal, 2012; Yue et al., 2012).

The fruiting body of the Cordyceps is very small and blade like. Because of this it is difficult and labor intensive to harvest. Cordyceps can grow on several nutrient containing medias, but most popular for cultivation are insect larvae or cereal grains (Tuli et al., 2014). Cordyceps also has a wide range of nutrients present, with many essential amino acids, vitamins and carbohydrates. The most important of the metabolites are the biometabolites, which are cordycepin, cordycepic acid, adenosine, exo-polysaccharides, vitamins and enzymes.

There are three mechanisms in which cordycepin works, inhibition of purine biosynthesis pathways, RNA chain termination, and interfering with mTOR signal transduction. Once cordycepin is inside the cell, it is converted into 5’ mono-, di-, and tri-phosphates that inhibit the activity of enzymes such as ribose-phosphate pyrophosphokinase and 5-phosphoribosyl-1pyrophosphate amidotransferase which is used in de novo biosynthesis of purines (Klenow, 1963; Overgaard 1964; Rottman and Garino 1964; Tuli et al., 2014).

RNA chain termination works as Cordycepin lacks 3' hydroxyl group in its structure, which differs from adenosine (Figure 1.1). Adenosine is used in cells for synthesis of DNA and RNA, during the process some enzymes cannot differentiate the difference between Adenosine and cordycepin causing the termination of that strand. (Chen et al., 2008; Holbein et al., 2009).

Cordycepin interferes with mTOR signal transduction by shortening the poly A tail of mRNA, which further degrades its stability inside the cell. At high doses Cordycepin prevents cell attachment, and at even higher doses it inhibits the mTOR pathway. The mTOR pathway was discovered in part from the drug rapamycin, because this drug also inhibits the mTOR pathway. The mTOR pathway plays a crucial role in protein synthesis, but mTOR itself is regulated by many cellular signals like hormones and growth factors.

As mentioned above Cordyceps has shown many medicinal uses, however one in particular appears interesting to the animal science world. In 1993 at the National Chinese Games women athletes broke 9 world records. This group of women admitted to regularly taking Cordyceps. Upon further investigation Cordyceps was found to enhance physical stamina making it useful for elderly people and athletes. It was discovered that Cordyceps enhances cellular energy in the form of ATP, and upon hydrolysis energy is released to be used by the cell (Dai et al., 2001; Siu et al., 2004; Tuli et al., 2014). In theory increased cellular energy could be used to increase growth performance, which is critical in our field of study.

Alternative dosages of Cordyceps have been investigated; however depending on condition and health it may vary a bit. In patients with health issues 3-6 g/d were given to reduce clinical symptoms. However, there were no dosage recommendations given for humans to take as a preventative supplement. There is also no evidence of toxic effects at any dosages in

humans, and animal studies with levels as great as 80 g/kg of bodyweight per day found no fatalities in mice (Li et al., 2006).

Cordyceps militaris effect of piglet performance

Cheng et al. (2016) feed fermented Cordyceps militaris to weanling pigs with concentrations of 0 ug/kg, 500 ug/kg, 1000 ug/kg, or 1500 ug/kg. Cheng et al. (2016) discovered at the 1000 ug/kg level there was a statistical improvement in BW at d 28 when compared to the control treatment, at 19.9 vs 22.5 kg of BW, respectively. Overall ADG and F:G ratio of 500, 1000, and 1500 ug/kg were all significantly improved over the control treatment. Feed intake was significantly improved for the 1000 ug/kg and 1500 ug/kg treatments when compared to the control as well. There were significant decreases in serum Aspartate aminotransferase and Alanine aminotransferase of all 3 diet levels compared to the control treatment. Serum glucose and Triglyceride levels were significantly decreased in the 1000 and 1500 ug/kg levels when compared to the control treatments.

Cordyceps militaris also has the ability to modulate cytokine espression at the transcriptional level. Cordyceps diets significantly altered the the mRNA coding for Th1 cytokines in the spleen. The 1500 level showed 3.9 and 5.0 fold increases in IL-2 and IFN-γ when compared to the spleens of control pigs. This in theory would increase cellular immune response. Based on the authors findings it is feasible to say that supplementation of Cordyceps militaris improves growth performance and enhances cell-mediated immunity in nursery pigs.

Beta Glucans

Based on analysis of product used in our experiments Cordyceps mushrooms contain about 33% beta 1-3, 1-6 glucans. Beta-Glucans are another antibiotic alternative being investigated. Beta-Glucans are major structure components of yeast and fungal cells (Jorgenson

and Robertson, 1995). Beta-Glucans are also known to have antitumor (Ohno et al., 1987) and antimicrobial effects (Hetland et al., 2000) on the body by enhancing the hosts immune function. Beta glucans have possible beneficial effects on weanling pigs (Mowat, 1987; Stokes et al., 1987) because it increases nonspecific immunity, as well as increasing tolerance to oral antigens.

Beta-Glucans have shown trends for increasing ADG (Schoenherr et al., 1994; Dritz at al., 1995; Hahn et al., 2006). Hahn et al. (2006) found that as beta-glucan content increased there was a trend for a linear increase in ADG from 0% to 0.4% beta-glucan content. Improvements in ADG appeared to level out for the overall study, above 0.2% inclusion there were not additional effects on ADG. Similarly there was a slight numeric improvement in ADFI for pigs were fed the 0.2% inclusion level.

Based on the second experiment performed by Hahn et al. (2006), there appeared to be an increase in MHC-II lymphocytes at week 4 of the study, and CD4 cell numbers were greater in week 8 of the study. This seems to support the claim that Beta-Glucans play a role in the immune system.

Conclusion

In summary, there are many potential alternatives available for future research. The early life of the pig is critical to lifelong health and maximum productivity. As an industry it would be in our best interest to find what supplements give the young pigs the best chance to thrive. We have a start, however there is much more work to be done. Essential oils contain many bioactive compounds, however oxidation and feed delivery has been an issue. Not only do products have issues in the feed, but also in getting to the correct absorptive site. This is the common problem with probiotics, getting past the stomach which has such low pH it kills many bacteria. Acidification of the stomach can help from oral consumption threats, but is it feasible on a large

scale to include in an already expensive nursery diet? Cordyceps Mushrooms show many positive bioactive compounds. However; many of these compounds were researched in human, rat, and mice. There is very little research in the area of swine production and mushrooms. Mushrooms have a history of therapeutic potential that is waiting to be untapped by our industry.

Literature Cited

Alam A.N., S. A. Sarker, M. A. Wahed, M. Khatun, M. M. Rahaman. Enteric protein loss and intestinal permeability changes in children during acute shigellosis and after recovery: effect of zinc supplementation. Gut 1994;35:1707-11. Doi:10.1111/j.1439-0442.2004.00637.x.

Alexopoulos C., I. E. Georgoulakis, A. Tzivara, C. S. Kyriakis, A. Govaris, S. C. Kyriakis. Field evaluation of the effect of a probiotic-containing Bacillus licheniformis and Bacillus subtilis spores on the health status, performance, and carcass quality of grower and finisher pigs. J Vet Med A Physiol Pathol Clin Med 2004;51:306-12. Doi:10.1111/j.1439-0442.2004.00637.x.

Baydar N. G., G. Özkan, O. Sağdiç. Total phenolic contents and antibacterial activities of grape (Vitis vinifera L.) extracts. Food Control 2004;15:335-39. Doi:10.1016/S0956-7135(03)00083-5.

Bischoff S. C. 'Gut health': a new objective in medicine? BMC Med 2011;9:24.

Bühler K., A. Liesegang, B. Bucher, C. Wenk, J. Broz. Influence of benzoic acid and phytase in low-phosphorus diets on bone characteristics in growing-finishing pigs. J Anim Sci 2010;88:3363-71. Doi:10.2527/jas.2009-1940.

Calsamiglia S., M. Busquet, P.W. Cardozo, L. Castillejos, A. Ferret. Invited review: essential oils as modifiers of rumen microbial fermentation. J Dairy Sci 2007;90: 2580-95. Doi:10.3168/jds.2006-644.

Camilleri M, Madsen K, Spiller R, van Meerveld BG, Verne GN. Intestinal barrier function in health and gastrointestinal disease. Neurogastrol and Motil 2012;24:503-12. Doi:10.1111/j.1365-2982.2012.01921.x.

Camilleri, M., M. Vassallo. Small intestinal motility and transit in disease. Bailliere's Clinical Gastroenterology. 1991; 5:431-451. Doi:10.1016/0950-3528(91)90036-Z.

Carlson MS, Hoover SL, Hill GM, Link JE, Turk JR. Effect of pharmacological zinc on intestinal metallothionein concentration and morphology in the nursery pig. J Anim Sci 1998;76(Suppl. 1):57 [Abstr].

Celi P, Cowieson AJ, Fru-Nji F, Steinert RE, Kluenter A-M, Verlhac V. Gastrointestinal functionality in animal nutrition and health: new opportunities for sustainable animal production. Anim Feed Sci Technol 2017;234:88-100. Doi:10.1016/j.anifeedsci.2017.09.012.

Chen LS, Stellrecht CM, Gandhi V. RNA-directed agent, cordycepin, induces cell death in multiple myeloma cells. Brit J Haematol 2008; 140:391-682. Doi:10.1111/j.1365-2141.2007.06955.x.

Cheng, Y. H., Wen, C. M., Dybus, A., Proskura, W. S. Fermentation of Cordyceps militaris enhancement performance and modulate immune response of weaned piglets. South African J Anim Sci. 2016; 46:121-128. Doi:10.4314/sajas.v46i2.2.

Cho JH, Zhao PY, Kim IH. Probiotics as a dietary additive for pigs: a review. J Anim Vet Adv 2011;10:2127-34. Doi:10.3923/java.2011.2127.2134.

Cromwell GL. Feed additives in swine diets. In: Chiba LI, editor. Sustainable swine nutrition. 1st ed. Ames, IA: John Wiley & Sons, Inc; 2013. p. 302-4.

Dai G, Bao T, Xu C, Cooper R, Zhu JX. CordyMaxTM Cs-4 improves steady-state bioenergy status in mouse liver. J Altern Complem Med 2001; 7:231–240.

Devkota S. Yarsagumba [Cordyceps sinensis (Berk.) Sacc.]; traditional utilization in Dolpa district, western Nepal. Our Nat 2006; 4:48–55.

Diamond JM, Karasov WH. Trophic control of the intestinal mucosa. Nat (Lond) 1983;304-18.

Diao H. Effects of benzoic acid (VevoVitall(R)) on the performance and jejunal digestive physiology in young pigs. J Anim Sci Biotechnol 2016;7:32-8.

Didierlaurent, A., M. Simonet, J. C. Sirard. Intestinal epithelial barrier and mucosal immunity. Cell. Mol. Life Sci. 2005; 62: 1285. Doi:10.1007/s00018-005-5032-4.

Dong GZ, Pluske JR. The low feed intake in newly-weaned pigs: problems and possible solutions. Asian Aust J Anim Sci 2007;20:440-52. Doi:10.5713/ajas.2007.440.

Dou S, Gadonna-Widehem P, Rome V, Hamoudi D, Rhazi L, Lakhal L, T. Larcher, N. Bahi-Jaber, A. Pinon-Quintana, A. Guyonvarch, I. L. E. Huerou-Luron. Characterisation of early-life fecal microbiota in susceptible and healthy pigs to post-weaning diarrhoea. PLoS One 2017;12(1), e0169851. Doi:10.1371/journal.pone.0169851.

Dritz, S. S., J. Shi, T. L. Kielian, R. D. Goodband, J. L. Nelssen, M. D. Tokach, M. M. Chengappa, J. E. Smith, F. Blecha. Influence of dietary β-glucan on growth performance, nonspecific immunity and resistance to streptococcus suis infection in weanling pigs. J. Anim. Sci. 1995; 73:3341-3350. Doi:10.2527/1995.73113341x.

Dundar E, Olgun EG, Isiksoy S, Kurkcuoglu M, Baser KH, Bal C. The effects of intra-rectal and intra-peritoneal application of Origanum onites L. essential oil on 2,4,6-trinitrobenzenesulfonic acid-induced colitis in the rat. Exp Toxicol Pathol 2008;59:399-408. Doi:10.1016/j.etp.2007.11.009.

Everaert, N., Cruchten, S. V., Weström, B., Bailey, M., Ginneken, C. V., Thymann, T., Pieper, R. A review on early gut maturation and colonization in pigs, including biological and dietary factors affecting gut homeostasis. Anim Feed Sci Technol 2017; 233: 89-103. Doi:10.1016/j.anifeedsci.2017.06.011.

Feldpausch, J. A., R. G. Amachawadi. M. D. Tokach, H. M. Scott. S. S. Dritz, R. D. Goodband. J. C. Woodworth, J. M. DeRouchey. Transl. Anim. Sci. 2018; 2:62-73. Doi: 10.1093/tas/txx004.

Fouhse JM, Zijlstra RT, Willing BP. The role of gut microbiota in the health and disease of pigs. Anim Front 2016;6:30-36. Doi:10.2527/af.2016-0031.

Galdeano CM, Perdigon G. The probiotic bacterium Lactobacillus casei induces activation of the gut mucosal immune system through innate immunity. Clin Vaccine Immunol 2006;13:219-26. Doi:10.1128/CVI.13.2.219-226.2006.

Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 1995;125:1401-12. Doi:10.1093/jn/125.6.1401.

Giesting DW, Easter RA. Response of starter pigs to supplementation of corn- soybean meal diets with organic acids. J Anim Sci 1985;60:1288-94. Doi:10.2527/jas1985.6051288x.

Gresse R, Chaucheyras-Durand F, Fleury MA, van de Wiele T, Forano E, Blanquet- Diot S. Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends Microbiol 2017;25:851-73. Doi:10.1016/j.tim.2017.05.004.

Gu YX, Wang ZS, Li SX, Yuan, G.S. Effect of multiple factors on accumulation of nucleosides and bases in Cordyceps militaris. Food Chem 2007; 102:1304–1309. Doi:10.1016/j.foodchem.2006.07.018.

Guggenbuhl P, Séon A, Quintana AP, Nunes CS. Effects of dietary supplementation with benzoic acid (VevoVitall®) on the zootechnical performance, the gastro- intestinal microflora and the ileal digestibility of the young pig. Livest Sci 2007;108:218-21. Doi:10.1016/j.livsci.2007.01.068

Hahn, T. W., J. D. Lohakare, S. L. Lee, W. K. Moon, B. J. Chae. Effects of supplementation of β-glucans on growth performance, nutrient digestibility, and immunity in weanling pigs. J. Anim. Sci. 2006; 84: 1422-1428. Doi:10.2527/2006.8461422x.

Halas D, Hansen CF, Hampson DJ, Mullan BP, Kim JC, Wilson RH, J. R. Pluske. Dietary supplementation with benzoic acid improves apparent ileal digestibility of total nitrogen and increases villous height and caecal microbial diversity in weaner pigs. Anim Feed Sci Technol 2010;160:137-47. Doi:10.1016/j.anifeedsci.2010.07.001.

Hetland, G., N. Ohno, I. S. aaberge, and M. Lovik. Protective effect of β-glucan against Streptococus pneumoniae infection in mine. FEMS Immunol. Med. Microbiol. 2000; 27:111-116.

Hill GM, Cromwell GL, Crenshaw TD, Dove CR, Ewan RC, Knabe DA, Lewis, A. J., Libal, G. W., Mahan, D. C., Shurson, G. C., Southern, L.L., Veum, T.L. Growth promotion effects and plasma changes from feeding high dietary concentrations of zinc and copper to weanling pigs (regional study). J Anim Sci 2000;78: 1010-16. Doi:10.2527/2000.7841010x.

Hill GM. Minerals and mineral utilization in swine. In: Chiba LI, editor. Sustainable swine nutrition. Oxford, U.K.: Blackwell Publishing Ltd.; 2013. p. 186-89.

Holbein S, Wengi A, Decourty L, Freimoser FM, Jacquier A, DichtlrnaI B. Cordycepin interferes with 30 end formation in yeast independently of its potential to terminate RNA chain elongation. RNA 2009; 15:837–849. Doi:10.1261/rna.1458909.

Hu C, Song J, Li Y, Luan Z, Zhu K. Diosmetite-zinc oxide composite improves intestinal barrier function, modulates expression of pro-inflammatory cytokines and tight junction protein in early weaned pigs. Br J Nutr 2013a;110:681-88. Doi:10.1017/S0007114512005508.

Hu C, Song J, You Z, Luan Z, Li W. Zinc Oxide-Montmorillonite hybrid influences diarrhea, intestinal mucosal integrity, and digestive enzyme activity in weaned pigs. Biol Trace Elem Res 2012;149:190-96. Doi:10.1007/s12011-012-9422-9.

Jaworski NW, Owusu-Asiedu A, Walsh MC, McCann JC, Loor JJ, Stein HH. Effects of a 3 strain Bacillus-based direct-fed microbial and dietary fiber concentration on growth performance, intestinal concentrations of volatile fatty acids, and expression of genes related to absorption and metabolism of volatile fatty acids in weanling pigs. J Anim Sci 2017;95:308-19. Doi:10.2527/jas.2016.0557.

Jayaraman B, Nyachoti CM. Husbandry practices and gut health outcomes in weaned piglets: a review. Anim Nutr 2017;3:205-11. Doi:10.1016/j.aninu.2017.06.002.

Jorgensen, J.B., Robertsen, B. Yeast β-glucan stimulates respiratory burst activity of atlantic salmon (*salmo salae* L.) macrophages. Dev. Comp. Immunol. 1995; 19:43-57.

Kelly D, King TP. Luminal bacteria: regulation of gut function and immunity. In: Piva A, Bach Knudsen KE, Lindberg JE, editors. Gut environment of pigs. Loughborough, UK: Nottingham University Press; 2001. p. 113-31.

Kenny M, Smidt H, Mengheri E, Miller B. Probiotics - do they have a role in the pig industry? Animal 2011;5:462-70. Doi:10.1017/S175173111000193X.

Kil DY, Kwon WB, Kim BG. Dietary acidifiers in weanling pig diets: a review. Revista Colombiana de Ciencias Pecuarias 2011;24:231-47.

Kil DY, Kwon WB, Kim BG. Dietary acidifiers in weanling pig diets: a review. Rev Colombiana Ciencias Pecuarias 2011;24:231-47.

Kim JC, Hansen CF, Mullan BP, Pluske JR. Nutrition and pathology of weaner pigs: nutritional strategies to support barrier function in the gastrointestinal tract. Anim Feed Sci Technol 2012;173:3-16. Doi:10.1016/j.anifeedsci.2011.12.022.

Klenow H (1963) Formation of the mono-, di- and triphosphate of cordycepin in Ehrlich ascites-tumor cells in vitro. Biochim Biophys Acta 76:347–353. Doi:10.1016/0926-6550(63)90054-9.

Ku PK, Ullrey DE, Miller ER. In: Mills CF, editor. Zinc deficiency and tissue nucleic acid and protein concentration. Edinburgh, UK: E. & S. Livingstone; 1970. p. 158-64.

Kuang Y, Wang Y, Zhang Y, Song Y, Zhang X, Lin Y, Che, L. Xu, S. Wu, D. Xue, B. Fang, Z. Effects of dietary combi- nations of organic acids and medium chain fatty acids as a replacement of zinc oxide on growth, digestibility and immunity of weaned pigs. Anim Feed Sc Technol 2015;208:145-57. Doi:10.1016/j.anifeedsci.2015.07.010.

Lee B, Park J, Park J, Shin HJ, Kwon S, Yeom M, Sur B, Kim S, Kim M, Lee H, Yoon SH, Hahm DH. Cordyceps militaris improves neurite outgrowth in neuro2A cells and reverses memory impairment in rats. Food Sci Biotechnol 2011a; 20:1599–1608.

Lee JH, Hong SM, Yun JY, Myoung H, Kim MJ Anti-cancer effects of cordycepin on oral squamous cell carcinoma proliferation and apoptosis in vitro. J Cancer Ther 2011b; 2:224–234.

Lee KW, Everts H, Beynen AC. Essential oils in broiler nutrition. Int J Poult Sci 2004;3:738-52. doi:10.3923/ijps.2004.738.752.

Lewis MC, Patel DV, Fowler J, Duncker S, Zuercher AW, Mercenier A, Bailey, M. Dietary supplementation with Bifidobacterium lactis NCC2818 from weaning reduces local immunoglobulin production in lymphoid-associated tissues but increases systemic antibodies in healthy neonates. Br J Nutr 2013;110:1243-52. Doi:10.1017/S0007114513000251.

Li BT, van Kessel AG, Caine WR, Huang SX, Kirkwood RN. Small intestinal morphology and bacterial populations in ileal digesta and feces of newly weaned pigs receiving a high dietary level of zinc oxide. Can J Anim Sci 2001;81:511-16. Doi:10.4141/A01-043.

Li SP, Yang FQ, Tsim KW. Quality control of Cordyceps sinensis, a valued traditional Chinese medicine. J Pharm Biomed Anal 2006; 41:1571–1584.

Li X, Yin J, Li D, Chen X, Zang J, Zhou X. Dietary supplementation with zinc oxide increases Igf-I and Igf-I receptor gene expression in the small intestine of weanling piglets. J Nutr 2006;136:1786-91.

Liu ST, Hou WX, Cheng SY, Shi BM, Shan AS. Effects of dietary citric acid on performance, digestibility of calcium and phosphorus, milk composition and immunoglobulin in sows during late gestation and lactation. Anim Feed Sci Technol 2014a;191:67-75. Doi: 10.1016/j.anifeedsci.2014.01.017.

Liu ST, Hou WX, Cheng SY, Shi BM, Shan AS. Effects of dietary citric acid on performance, digestibility of calcium and phosphorus, milk composition and immunoglobulin in sows during late gestation and lactation. Anim Feed Sci Technol 2014a;191:67-75. Doi:10.1016/j.anifeedsci.2014.01.017.

Liu Y, Che TM, Song M, Lee JJ, Almeida JA, Bravo D, Van Alstine, W. G., Pettigrew, J. E. Dietary plant extracts improve immune responses and growth efficiency of pigs

experimentally infected with porcine reproductive and respiratory syndrome virus. J Anim Sci 2013a;91:5668-79. Doi:10.2527/jas2013-6495.

Liu Y, Song M, Che TM, Almeida JA, Lee JJ, Bravo D, Maddox, C. W., Pettigrew, J. E. Dietary plant extracts alleviate diarrhea and alter immune responses of weaned pigs experimentally infected with a pathogenic Escherichia coli. J Anim Sci 2013b;91:5294-306. Doi: 10.2527/jas.2012-6194.

Liu Y, Song M, Che TM, Lee JJ, Bravo D, Maddox CW, J. E. Pettigrew. Dietary plant extracts modulate gene expression profiles in ileal mucosa of weaned pigs after an Escherichia coli infection. J Anim Sci 2014b;92:2050-62. Doi:10.2527/jas2013-6422

Liu Y, Song M, Che TM, Lee JJ, Bravo D, Maddox CW, Pettigrew, J. E. Dietary plant extracts modulate gene expression profiles in ileal mucosa of weaned pigs after an Escherichia coli infection. J Anim Sci 2014b;92:2050-62. Doi: 10.2527/jas.2013-6422.

Liu Y,Song M,Che T M, Bravo D, Pettigrew JE. Anti-inflammatory effects of several plant extracts on porcine alveolar macrophages in vitro. J Anim Sci 2012;90:2774-83. Doi:10.2527/jas.2011-4304.

Liu, Y., Espinosa, C. D., Abelilla, J. J., Casas, G. A., Lagos, L. V., Lee, S. A., Kwon, W. B., Mathai, J. K., Navarro, D. M. D. L., Jaworski, N. W., Stein, H. H. Non-antibiotic feed additives in diets for pigs: a review. J Ani Nu 2018; 4:113-125. Doi:10.1016/j.aninu.2018.01.007.

Long SF, Xu YT, Pan L, Wang QQ, Wang CL, Wu, J.Y., Wu, Y.Y., Han, Y.M., Yun, C.H., Piao, X. S. Mixed organic acids as antibiotic substitutes improve performance, serum immunity, intestinal morphology and microbiota for weaned piglets. Anim Feed Sci Technol 2018;235:23-32. Doi:10.1016/j.anifeedsci.2017.08.018.

Luise D, Motta V, Salvarani C, Chiappelli M, Fusco L, Bertocchi M, M. Mazzoni, G. Maiorano, L. N. Costa, J. V. MIlgen, P. Bosi, P. Trevisi. Long-term administration of formic acid to weaners: influence on intestinal microbiota, immunity parameters and growth performance. Anim Feed Sci Technol 2017;232:160-8. Doi 10.1016/j.anifeedsci.2017.06.015

Ma YL, Zanton GI, Zhao J, Wedekind K, Escobar J, Vazquez-Anon M. Multitrial analysis of the effects of copper level and source on performance in nursery pigs. J Anim Sci 2015;93:606-14. Doi:10.2527/jas.2014-7796.

McCracken BA, Spurlock ME, Roos MA, Zuckerman FA, Gaskins HR. Weaning anorexia may contribute to local inflammation in the piglet small intestine. J Nutr 1999;129:613-19.

McDowell LR. Minerals in animal and human nutrition. San Diego, CA: Academic Press Inc.; 1992.

Moeser A.J., Vander Klok C., Ryan K.A., Wooten J.G., Little J.G., Cook V.L., Blikslager, A.T. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. Am J Physiol: Gastro Liver Physiol 2007;292:G173-81. Doi:10.1152/ajpgi.00197.2006.

Moeser AJ, Pohl CS, Rajput M. Weaning stress and gastrointestinal barrier devel- opment: implications for lifelong gut health in pigs. Anim Nutr 2017; 3:313-321. Doi:10.1016/j.aninu.2017.06.003.

Montagne L, Pluske JR, Hampson DJ. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim Feed Sci Technol 2003;108:95-117. Doi:10.1016/S0377-8401(03)00163-9.

Mowat, A. M. The regulation of immune responses to dietary protein antigens. Immunol. Today. 1987; 8:93-98.

Namkung H, Gong J, Yu H, de Lange CFM. Effect of pharmacological intakes of zinc and copper on growth performance, circulating cytokines and gut microbiota of newly weaned piglets challenged with coliform lipopolysaccharides. Can J Anim Sci 2006;86:511-22. Doi:10.4141/A05-075.

NRC. In: Nutrient requirements of swine. 11th rev. Washington, DC: Natl. Acad. Press; 2012.

Ohno, N., K. Kurachi, T. Yadomae. Anti-tumor activity of highly branched (1→3) beta-D-glucan, SSG, obtained from SClerotinia sclerotiorum IFO 9395. J. Pharmacobiodyn. 10:478-486.

Overgaard KH. The inhibition of 5-phosphoribosyl-1-pyrophosphate formation by Cordycepin triphosphate in extracts of Ehrlich ascites tumor cells. Biochim Biophys Acta 1964; 80:504–507. Doi:10.1016/0926-6550(64)90154-9.

Papatsiros VG, Billinis C. The prophylactic use of acidifiers as antibacterial agents in swine. In: Antimicrobial agents, Bobbarala V, editors. InTech, Rijeka, Croatia; 2012. p. 295-310

Papatsiros VG, Tassis PD, Tzika ED, Papaioannou DS, Petridou E, Alexopoulos C, S. C. Kyriakis. Effect of benzoic acid and combination of benzoic acid with a probiotic containing Bacillus cereus var. Toyoi in weaned pig nutrition. Pol J Vet Sci 2011;14:117-25. Doi:10.2478/v10181-011-0017-8.

Patel S, Goyal A. Recent developments in mushrooms as anti- cancer therapeutics: a review. 3 Biotech 2012; 2:1–15. Doi:10.1007/s13205-011-0036-2.

Perez VG, Waguespack AM, Bidner TD, Southern LL, Fakler TM, Ward TL, Steidinger, M., Pettigrew, J. E. Additivity of effects from dietary copper and zinc on growth performance and fecal microbiota of pigs after weaning. J Anim Sci 2011;89:414-25. Doi:10.2527/jas.2010-2839.

Pettigrew JE. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: dietary tools, Part 1. Anim Biotechnol 2006;17:207-15. Doi:10.1080/1049530600956946.

Pié S, Lallès JP, Blazy F, Laffitte J, Seve B, Oswald IP. Weaning is associated with an upregulation of expression if inflammatory cytokines in the intestine of piglets. J Nutr 2004;134:641-47.

Pluske JR, Hampson DJ, Williams IH. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest Prod Sci 1997;51: 215-36. Doi:10.1016/S0301-6226(97)00057-2.

Pluske JR, Williams IH, Aherne FX. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Anim Sci 1996a;62: 131-44. Doi:10.1017/S1357729800014417.

Pluske JR, Williams IH, Aherne FX. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. Anim Sci 1996b;62:145-58 doi:10.1017/S1357729800014429.

Pluske, J. R., Turpin, D. L., Kim, J. C. Gastrointestinal tract (gut) health in the young pig. An. Nutr. 2017; 1-10. Doi:10.1016/j.aninu.2017.12.004.

Pohl CS, Medland JE, Mackey E, Edwards LL, Bagley KD, DeWilde MP, K. J. Williams, A. J. Moeser. Early weaning stress induces chronic functional diarrhea, intestinal barrier defects, and increased mast cell activity in a porcine model of early life adversity. Neuro Gastroenterol Motil 2017. Doi:10.1111/nmo.13118.

Poulsen HD. Zinc and copper as feed additives, growth factors or unwanted envi- ronmental factors. J Anim Feed Sci 1998;7:135-42. Doi:10.22358/jafs/69961/1998.

Prasad AS, Oberleas D, Miller ER, Luecke RW. Biochemical effects of zinc difficiency: changes in activities of zinc-dependent enzymes and ribonucleic acid and deoxyribonucleic acid content of tissues. J Lab Clin Med 1971;77:144-52.

Prescott SL, Dunstan JA, Hale J, Breckler L, Lehmann H,Weston S, Richmond, P. Clinical effects of probiotics are associated with increased interferon-gamma responses in very young children with atopic dermatitis. Clin Exp Allergy 2005;35:1557-64. Doi:10.1111/j.1365-2222.2005.02376.x

Rottman F, Guarino AJ. The inhibition of phosphoribosyl- pyrophosphate amidotransferase activity by cordycepin mono phosphate. Biochim Biophys Acta 1964; 89:465–472. Doi:10.1016/0926-6569(64)90072-0.

Sauer W, Cervantes M, Yanez J, Araiza B, Murdoch G, Morales A, R. T. Zilstra. Effect of dietary inclusion of benzoic acid on mineral balance in growing pigs. Livest Sci 2009;122:162-8. Doi:10.1016/j.livsci.2008.08.008.

Schachtschneider KM, Yeoman CJ, Isaacson RE, White BA, Schook LB, Pieters M. Modulation of systemic immune responses through commensal gastrointestinal microbiota. PLoS One 2013;8(1):e53969. Doi:10.1371/ journal.pone.0053969.

Schoenherr, W. D., D. S. Pollmann, J. A. Coalson. Titration of Macro Gard TM-S on growth performance of nursery pigs. J. Anim. Sci. 1994; 72(Suppl. 2):57. (Abstr.)

Shen, H. S., S. Shao, J. C. Chen, T. Zhou. Antimicrobials from mushrooms for assuring food safety. Comprehensive Reviews in Food Science and Food Safety. 2017; 16. Doi: 10.1111/1541-4337.12255.

Siu KM, Mak HFD, Chiu PY, Poon KTM, Du Y, Ko KM. Pharmacological basis of 'Yin-nourishing' and 'Yang-invigorating' actions of Cordyceps, a Chinese tonifying herb. Life Sci 2004; 76:385–395. Doi:10.1016/jlfs.2004.07.014.

Smith, II, JW, Tokach MD, Goodband RD, Nelssen JL, Richert BT. Effects of the interrelationship between zinc oxide and copper sulfate on growth performance of early-weaned pigs. J Anim Sci 1997;75:1861-66. Doi:10.2527/1997.7571861x.

Spreeuwenberg MA, Verdonk JM, Gaskins HR, Verstegen MWA. Small intestine epithelial barrier function is compromised in pigs with low feed intake at weaning. J Nutr 2001;131:1520-27.

Spurlock, M. E., G. R. Frank, G. M. Willis, J. L. Kuske, S. G. Cornelius. Effect of dietary energy source and immunological challenge on growth performance and immunological variables in growing pigs. J. Anim. Sci. 1997; 74:720-726. Doi:10.2527/1997.753720x.

Stahly TS, Cromwell GL, Monegue HJ. Effects of the dietary inclusion of copper and (or) antibiotics on the performance of weanling pigs. J Anim Sci 1980;51: 1347-51. Doi:10.2527/jas1981.51611347x.

Stein HH, Kil DY. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: dietary tools, part 2. Anim Biotechnol 2006;17:217-31. Doi:10.1080/10495390600957191.

Stokes, C.R., B.G. Miller, M. Bailey, A. D. Wilson, F. J. Bourne. The immune response to dietary antigens and its influence on disease susceptibility in farm animals. Vet. Immunol. Immunopathol. 1987; 17:413-423.

Suryanarayana MVAN, Suresh J, Rajasekhar MV. Organic acids in swine feeding: a review. Agric Sci Res J 2012;2:523-33.

Sökmen M, Serkedjieva J, Daferera D, Gulluce M, Polissiou M, Tepe B, Akpulat, H. A., Sahin, F., Sokmen, A. In vitro antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of Origanum acutidens. J Agr Food Chem 2004;52:3309-12. Doi:10.1021/jf049859g.

Tuli, H. S., Sandhu, S. S., Sharma, A. K. Pharmacological and therapeutic potential of Cordyceps with special reference to Cordycepin. Biotech 2014; 4:1-12. Doi: 10.1007/s13205-013-0121-9.

Upadhaya SD, Lee KY, Kim IH. Protected organic Acid blends as an alternative to antibiotics in finishing pigs. Asian Australas J Anim Sci 2014;27:1600-7. Doi:10.5713/ajas.2014.14356.

van Heugten E, Spears JW, Kegley EB, Ward JD, Qureshi MA. Effects of organic forms of zinc on growth performance, tissue zinc distribution, and immune response of weanling pigs. J Anim Sci 2003;81:2063-71. Doi:10.2527/2003.8182063x.

Walsh, M. C., D. M. Sholly, R. B. Hinson, K. L. Saddoris, A. L. Sutton, J. S. Radcliffe, R. Odgaard, J. Murphy, B. T. Richert. Effects of water and diet acidification with and without antibiotics on weanling pig growth and microbial shedding. J. Anim. Sci. 2007; 85:1799-1808. Doi:10.2527/jas.2006-049.

Wang L, Zhang WM, Hu B, Chen YQ, Qu LH. Genetic variation of Cordyceps militaris and its allies based on phylogenetic analysis of rDNA ITS sequence data. Fungal Divers 2008; 31:147–156.

Wang ZM, Peng X, Lee KLD, Tang JCO, Cheung PCK, Wu JY. Structural characterisation and immunomodulatory property of an acidic polysaccharide from mycelial culture of Cordyceps sinensis fungus Cs-HK1. Food Chem 2011; 125:637–643.

Wierup M. The Swedish experience of the 1986 year ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. Microb Drug Resist 2001;7:183-90. https://doi.org/10.1089/10766290152045066

Wierup M. The Swedish experience of the 1986 year ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. Microb Drug Resist 2001;7:183-90. Doi:10.1089/10766290152045066.

Wijtten PJA, van der Meulen J, Verstegen MWA. Intestinal barrier function and absorption in pigs after weaning: a review. Br J Nutr 2011;105:967-81. Doi:10.1017/S0007114510005660.

Wong YY, Moon A, Duffin R, Barthet-Barateig A, Meijer HA, Clemens MJ, de Moor CH. Cordycepin inhibits protein synthesis and cell adhesion through effects on signal transduc- tion. J Biol Chem 2010; 285:2610–2621. Doi:10.1074/jbc.m109.071159.

Xia T, Lai W, Han M, Ma X, Zhang L. Dietary ZnO nanoparticles alters intestinal microbiota and inflammation response in weaned piglets. Oncotarget 2017;8: 64878-91. Doi:10.18632/oncotarget.17612.

Xu YT, Liu LI, Long SF, Piao XS. Effects of organic acids and essential oils on performance, intestinal health and digestive enzyme activities of weaned pigs. Anim Feed Sci Technol 2018;235:110-9. Doi:10.1016/j.anifeedsci.2017.10.012.

Yue K, Ye M, Zhou Z, Sun W, Lin X. The genus Cordyceps:a chemical and pharmacological review. J Pharm Pharmacol. 2012; doi: 10.1111/j.2042-7158.2012.01601.x.

Zentek J, Ferrara F, Pieper R, Tedin L, Meyer W, Vahjen W. Effects of dietary combinations of organic acids and medium chain fatty acids on the gastrointestinal microbial ecology and bacterial metabolites in the digestive tract of weaning piglets. J Anim Sci 2013;91:3200-10. Doi:10.2527/jas.2012-5673

Zhang B, Guo Y. Supplemental zinc reduced intestinal permeability by enhancing occludin and zonula occludens protein-1 (ZO-1) expression in weaning piglets. Br J Nutr 2009;102:687-93. Doi:10.1017/S0007114509289033.

Zhang XL, Cheng LB, Assaf SA, Phillips GO, Phillips AO (2012) Cordyceps sinensis decreases TGF-b1 dependent epithelial to mesenchymal transdifferentiation and attenuates renal fibrosis. Food Hydrocolloids 2012; 28:200–212.

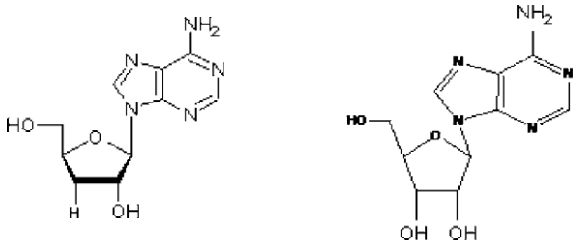
Zhao J, Harper AF, Estienne MJ, Webb Jr KE, McElroy AP, Denbow DM. Growth performance and intestinal morphology responses in early weaned pigs to supplementation of antibiotic-free diets with an organic copper complex and spray-dried plasma protein in sanitary and nonsanitary environments. J Anim Sci 2007;85:1302-10. Doi:10.2527/jas.2006-434.

Zhou X, Luo L, Dressel W,Shadier G,Krumbiegel D,Schmidtke P, Zepp F, Meyer CU. Cordycepin is an immunoregulatory active ingredient of Cordyceps sinensis. Am J Chin Med 2008; 36:967–980. Doi:10.1142/S0192415X09006387.

Zhu C, Lv H, Chen Z, Wang K, Wu X, Chen Z, Zhang, W., Liang, R., Jiang, Z. Dietary zinc oxide modulates antioxidant capacity, small intestinal development, and jejunal gene expression in weaned pigs. Biol Trace Elem Res 2017;175:331-38. Doi:10.1007/s12011-016-0767-3.

Zijlstra, R. T., A. Owusu-Asiedu, P. H. Simmins. Future of NSP-degrading enzymes to improve nutrient utilization of co-products and gut health in pigs. 2010; 134:255-257. Doi:10.1016/j.livsci.2010.07.017.

Figure 1.1



Cordycepin (3 deoxyadenosine) Adenosine

Chapter 2 - The interactive effect of Mushrooms, Stress Relief by ADM, and Carbadox on nursery growth performance

Abstract

One-hundred sixty crossbred pigs ((Duroc × (York × Landrace)) weaned at 19.4 d of age and weighing an average of 6.0 kg were used in a 39-day growth trial to evaluate ADM's Stress relief treatment and Cordyceps mushroom powder (MP) as potential alternative to carbadox (55 ppm) in nursery pig diets. Pigs were allotted by BW into 4 rooms consisted of 5 pigs per pen and one room consisting of 6 pigs per pen. There were 4 diets with 10 pens for negative and positive controls, Stress Relief treatment, the combination of Stress Relief and carbadox, and 9 pens for both mushroom treatments. Ad libitum access to feed and water was provided in each pen through a single hole wean-to-finish feeder and one nipple waterer. Pigs and feeders were weighed on day 0, 8, 15, 22, 29, 34, 39. The individual body weights and pen feed intake were recorded, to determine ADG, ADFI, and G:F. The data was analyzed using the GLM procedure of SAS. During Phase 1 (d 0 to 8) pigs fed carbadox tended to have increased ADG ($P = 0.09$), ADFI ($P = 0.11$) and d 8 BW ($P = 0.10$). Also pigs fed the 300 ppm MP tended to have greater ADFI ($P = 0.08$) than pigs fed 1 ppm MP. For Phase 2 (d 8 to 22), pigs fed carbadox had increased ($P < 0.05$) in ADG and ADFI, and tended to have increased feed efficiency ($P < 0.10$). There was also a trend for increased feed intake when comparing the 300 ppm MP treatment to the 1 ppm MP treatment ($P < 0.10$). However this increased ADFI led to a tendency ($P < 0.10$) for the 300 MP to have reduced feed efficiency compared to the NC. During Phase 3 (d 22 to 39) stress relief and carbadox both tended to have improved ADG compared to the NC ($P < 0.10$). The combination of Stress Relief and carbadox had numerically the greatest ADG and

ADFI for Phase 3, as well as having the greatest final BW at 21.50 kg. It is worth noting through Phase 3, 300 ppm MP did numerically outperform carbadox averaging 506 g/d ADG with carbadox averaging 499 g/d, however this was not statistically different. Overall (d 0-39) pigs fed carbadox had greater ($P < 0.05$) ADG, ADFI and feed efficiency compared to pigs fed the NC treatment. The 300 ppm MP pigs tended ($P = 0.10$) to have greater ADFI than the 1 ppm MP and NC treatments, but tended to have reduced feed efficiency ($P < 0.10$). Based on this data it appears mushroom powder has potential to be a replacement for carbadox.

Introduction

The global population is projected to reach 9 billion people by 2050. As the population increases so does the need for animal products. To feed this many people more crops and more livestock need to be raised. With the European union banning antibiotic growth promotants, and therapeutic copper and zinc in nursery diets, alternatives need to be found. Without antibiotics growth performance suffers in pigs and can have lifelong detrimental effects on the pigs gastrointestinal tract. One possible alternative is a Chinese herbal mushroom blend of Cordyceps militaris and Cordyceps sinensis (Shen et al. 2017). These mushrooms have long been used by the Chinese as a human health promoting additive. The compound cordycepin, found in these mushrooms is currently being studied as a possible anti-cancer agent by many research institutes. The mushroom itself has antimicrobial and antiviral characteristics (Zhou et al. 2008). Based on a previous study that had positive results with this mushroom (Cheng et al. 2016) we decided to attempt to reproduce their results with an added level similar to those used for human consumption.

Materials and methods:

Two hundred-eight gilts and barrows (19.4 d of age) weighing an average of 6.03 kg consisting of US purebred genetics (Duroc X (York X Landrace)) were put on test for a 39 day growth trial. Growth performance was analyzed using body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed (G:F) ratios. Pigs were allotted by BW into 4 rooms consisting of 5 pigs per pen and one room consisting of 6 pigs per pen. There were 4 diets with 10 pens for negative and positive controls, Stress Relief treatment, the combination of Stress Relief and carbadox, and 9 pens for both mushroom treatments. There were 52 pigs per diet with negative and positive control, Stress Relief, and the combination of Stress Relief and carbadox. There were 47 pigs assigned to the mushroom diets. Pigs were divided by weight, sex, litter, and assigned to BW blocks. Within BW blocks, sex ratios were constant in each pen. Each pen within a BW block was assigned a diet via a random number generator with pens that had the lowest random number being assigned diet 1 sequentially to diet 6 for the highest random number.

Pigs had ad libitum access to feed and water with each pen having a single hole wean-to-finish feeder and one nipple waterer. Feeders and waterers were checked daily, targeting partial pan coverage (40-50% coverage) while also minimizing feed wastage. Feeders were cleaned when feed became spoiled, and waterers adjusted to shoulder height of the pigs. Daily checks consisted of checking feeders, waterers, observations of the pigs, filling feeders if needed, treating pigs with antibiotics when signs of disease were detected, and completing treatment paperwork. Pigs and feeders were weighed on day 0, 8, 15, 22, 29, 34, and 39. Individual pig body weights and pen feed intake data were recorded. The GLM procedure of SAS (v9.4) was used for the statistical analysis. Individual degrees of freedom contrasts were used to test

significance between dietary treatments. The parameters measured were BW, ADG, ADFI and feed efficiency every week and summarized by dietary phase and overall. All procedures were approved by Purdue University's animal care and use committee (PACUC #1303000841).

Diets

There were six diets tested in this study. Diet A was the negative control containing a 0.5% corn premix, Diet B contained a commercial supplement (Stress Relief, ADM) at 0.15% (3 lbs/ton), Diet C contained a Cordyceps mushroom powder (MP) (Aloha Medicinals, Carson City, NV) at 1 ppm, Diet D contained MP at 300 ppm, Diet E contained carbadox (55 ppm), Diet F contained a combination of Stress Relief and carbadox.

The pigs were fed three dietary phases over a 39 day period, with all 3 Phases being fed in meal form. Phase 1 was d 0-8, Phase 2 was d 8-22, Phase 3 was d 22-39. Phase 1 was made with a basal diet which was split then remixed with the treatment premix added. Phase 2 and 3 were made as individual diet treatment batches. All Phase 1 diets contained 2991 ppm Zn and Phase 2 diets contained 2018 ppm Zn from zinc oxide. Phase 3 diets all contained 202 ppm Cu from copper sulfate.

Feed samples for each phase were collected and stored for future analysis at the Purdue University's Swine Nutrition Lab. Dietary CP, energy, dry matter, ash, and phosphorus concentrations were analyzed. Diets were ground through a 1 mm screen. Crude protein percent was determined by combustion using a Leco nitrogen analyzer (Leco Model 2000 CHN analyzer. Leco Corp., St. Joseph, MI, USA). Caloric content was determined via bomb calorimeter procedure (Parr 1261 bomb calorimeter; Parr instruments Co., Moline, IL, USA). Percent dry matter and ash was analyzed by weighing the crucible and sample on the same scale after being dried in the drying oven for 12 hours at 60 ° C, and ashed in the ashing oven for 6 hours at 500 °

C. Phosphorus was analyzed on the diet ash by the total phosphorus colormetric method (Murphy and Riley, 1962). All samples were analyzed in duplicates and adjusted for standards. If the values exceeded a 5% difference the samples will be repeated until values are within 5%.

Results

During the first 8 days of the study there was a trend for pigs fed carbadox to have increased ADG (P = 0.09), ADFI (P = 0.11) and d 8 BW (P = 0.08) compared to the pigs fed the NC diet. Pigs fed the 300 ppm treatment tended to have greater ADFI (P = 0.08) than pigs fed the 1 ppm MP. For the second period (d 8-15), carbadox increased ADG (P = 0.08), ADFI (P = 0.05), and 15d BW (P = 0.05) in comparison to the NC diet. Also, the 300 ppm mushroom treatment had greater ADFI (P = 0.03) that led to a tendency for increased d 15 BW (P = 0.11) over the 1 ppm mushroom treatment. Carbadox in the 3rd period (d 15-22) significantly increased ADG, ADFI, and BW from d 15-22 compared to the NC treatment (P < 0.02). The Stress Relief treatment decreased G:F compared to the NC (P < 0.05), and the 300 ppm mushroom also had decreased feed efficiency compared to the NC (P < 0.05). Overall for Phase 2 (d 8 to 22), pigs fed carbadox significantly increased ADG (P < 0.01) and ADFI (P = 0.02), and tended to have increased feed efficiency (P < 0.1) compared to the NC treatment. Pigs fed 300 ppm MP tended to have greater ADFI (P = 0.07) than pigs fed the NC treatment. There was also a trend for decreased feed efficiency when comparing the 300 ppm treatment to the 1 ppm treatment (P < 0.1). The only statistical difference from d 22 to 29, and d 29 to 34 was d 29 BW and d 34 BW, with carbadox pigs being the heavier (P < 0.05). At d 39, carbadox fed pigs were significantly heavier than the NC at 21.2 kg, and 20.1 kg, respectively. Stress relief pigs also tended to have increased ADFI in the final 5 days of the study (P = 0.07). Pigs fed MP300 tended to have greater ADFI (P = 0.06) than pigs fed the NC during d 34-39. During Phase 3 (d 22 to

39) stress relief and carbadox both tended to have improved ADG compared to the NC ($P < 0.1$). The combination of stress relief and carbadox had the numerically greatest ADG and ADFI for phase 3, as well as having the greatest ending BW at 21.5 kg. It is worth noting through Phase 3 the 300 ppm mushroom treatment numerically outperformed the carbadox treatment averaging 506 g/d ADG with carbadox averaging 499 g/d. Overall d 0-39 carbadox fed pigs had greater ADG ($P = 0.01$), ADFI ($P=0.04$), and feed efficiency ($P < 0.05$) than NC pigs. Pigs fed 300 ppm MP tended to have increased ADFI over 1 ppm MP ($P = 0.10$) and NC ($P = 0.10$) but reduced feed efficiency ($P < 0.10$) compared to the NC pigs.

Discussion

This was an initial investigative study on the potential effects of Cordyceps mushroom powder on nursery pig performance, as well as the effects of ADM's stress relief pack alone or in combination with carbadox. The mushroom powder is a blend of *Cordyceps militaris* and *Cordyceps sinesus*. The ADM stress relief pack is a unique blend of nutritional supplements to support physiological systems affected by stress.

There is one paper on a similar mushroom product being fed to nursery pigs. Based on this study we determined the 1 ppm was too low inclusion. Cheng et al. (2016) observed that the 1000 and 1500 µg/kg (1 and 1.5 ppm) were most effective dietary concentrations to improve growth performance. In our study, this concentration of MP did not improve pig growth performance. ADM's stress relief pack improved ADG compared to the NC in phase 3, and had an additive effect with carbadox. The combination of carbadox and stress relief had the heaviest pigs at the end of the 39 day trial indicating likely different modes of action. The 300 ppm mushroom concentration was calculated based on the Aloha Medicinals recommendation for human consumption, which appeared to be closer to an ideal concentration in the diet for nursery

pigs. During phase 3, the 300 ppm mushroom treatment numerically outperformed carbadox, and ended with heavier pigs than other treatments not containing antimicrobials. Based on a previous paper by Zhou et al. (2008) there are antimicrobial and anti-viral characteristics to this mushroom and this may be the mode of action.

In conclusion, 300 ppm mushroom numerically improved d 39 BW and tended to increase ADFI compared to the NC, and had similar ADG to carbadox in phase 3 (d 22-39), based on this studies results we decided to investigate the therapeutic potential of this mushroom powder further.

Literature Cited

Cheng, Y. H., Wen, C. M., Dybus, A., Proskura, W. S. Fermentation of Cordyceps militaris enhancement performance and modulate immune response of weaned piglets. South African J Anim Sci. 2016; 46:121-128. Doi:10.4314/sajas.v46i2.2.

Shen, H. S., S. Shao, J. C. Chen, T. Zhou. Antimicrobials from mushrooms for assuring food safety. Comprehensive Reviews in Food Science and Food Safety. 2017; 16. Doi: 10.1111/1541-4337.12255.

Zhou X, Luo L, Dressel W,Shadier G,Krumbiegel D,Schmidtke P, Zepp F, Meyer CU. Cordycepin is an immunoregulatory active ingredient of Cordyceps sinensis. Am J Chin Med 2008; 36:967–980. Doi:10.1142/S0192415X09006387.

Table 2-1 Basal diet formulation

Ingredient,%	**Phase 1**	**Phase 2**	**Phase 3**
Corn	36.985	45.960	52.640
SBM, CP 48%	15.600	20.750	30.080
DDGS, 7% Fat	0.000	5.000	10.000
Choice White Grease	0.000	0.000	3.000
Soybean Oil	4.000	3.000	0.000
Limestone NRC 12	0.760	0.910	1.360
MonoCal Phos. NRC 12	0.450	0.600	0.660
Vitamin Prx[1]	0.250	0.250	0.250
TM Prx[2]	0.150	0.150	0.150
Se Prx[3]	0.050	0.050	0.050
Phytase[4]	0.100	0.100	0.100
Salt	0.250	0.300	0.350
Plasma Protein	5.000	0.000	0.400
SD Blood Meal	1.000	1.000	0.000
Soy Conc.	4.000	4.000	0.000
Fish Meal	5.000	4.000	0.000
Dried Whey	25.000	12.500	0.000
Lysine-HCL	0.160	0.300	0.400
DL-Methionine	0.230	0.200	0.145
L-Threonine	0.080	0.130	0.125
L-Tryptophan	0.020	0.020	0.010
Carbadox – 10	0.000	0.000	0.000
Zinc Oxide	0.415	0.280	0.000
Banmith-48	0.000	0.000	0.100
Treatment Premix[5]	**0.500**	**0.500**	**0.500**
Total	100.00	100.00	100.00
Calculated Nutrients			
ME, Kcal/kg	3478.6	3414.8	3400.2
NE, Kcal/kg	2682.5	2572.4	2501.1
CP, %	24.38	23.00	21.89
Total Lys, %	1.73	1.56	1.43
SID Lys	1.55	1.40	1.25
SID Met:Lys	0.55	0.53	0.44
SID M+C:Lys	0.91	0.82	0.73
SID Thr:Lys	0.97	0.88	0.78
SID Tryp:Lys	0.29	0.26	0.23

SID Iso:Lys	0.86	0.82	0.77
SID Val:Lys	1.06	0.95	0.85
Ca,%	0.90	0.85	0.75
P, %	0.76	0.68	0.57
Avail. Phos., %	0.60	0.50	0.37

[1]Provided per kg of diet available minerals: iron, 121.3 mg; zinc, 121.2 mg; manganese, 15.0 mg; copper, 11.33 mg; iodine, and 0.46 mg.
[2]Provided per kg of diet: vitamin A, 6,614 IU; vitamin D3, 661 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 9 mg; pantothenic acid, 22 mg; niacin, 33 mg and B12 38.6 mg.
[3]Provided 0.3 ppm Se
[4]Provided 600 FTU per kg of the diet
[5] Diet A was the negative control containing a 0.5% corn premix, Diet B contained a commercial supplement (Stress Relief, ADM) at 0.15%, Diet C contained a Cordyceps mushroom powder (MP) (Aloha Medicinals, Carson City, NV) at 1 ppm, Diet D contained MP at 300 ppm, Diet E contained Carbadox (55 ppm), Diet F contained a combination of Stress Relief and Carbadox.

Table 2-2 Nursery Growth Performance

										Probability, P<	
Diets[1]	NC	SR	1ppm	300ppm	Carb	Carb&SR	SE	Carb	SR	Carb X SP	1 vs 300	300 vs NC
Pens/diet	10	10	9	9	10	10						
Initial Wt, kg	6.03	6.03	6.03	6.05	6.04	6.02	0.010	0.94	0.55	0.23	0.27	0.18
Day 0-8												
ADG, g	157	159	143	164	188	165	11.7	0.09	0.34	0.25	0.21	0.64
ADFI, g	176	176	168	194	199	185	10.2	0.11	0.45	0.46	0.08	0.22
G:F	0.878	0.910	0.853	0.846	0.943	0.894	0.0321	0.43	0.79	0.18	0.87	0.47
D8 BW, kg	7.28	7.30	7.18	7.36	7.55	7.34	0.094	0.09	0.32	0.20	0.17	0.54
Day 8-15												
ADG, g	190	208	168	206	219	243	19.1	0.08	0.26	0.88	0.17	0.54
ADFI, g	323	330	294	349	348	372	17.4	0.05	0.33	0.63	0.03	0.27
G:F	0.586	0.625	0.558	0.586	0.621	0.645	0.0341	0.39	0.32	0.81	0.57	0.99
D15 BW, kg	8.60	8.76	8.36	8.80	9.08	9.04	0.195	0.04	0.76	0.60	0.11	0.47
Day 15-22												
ADG, g	449	437	429	445	494	497	17.3	<0.01	0.79	0.65	0.52	0.86
ADFI, g	533	567	541	581	589	617	21.6	0.01	0.13	0.87	0.20	0.11
G:F	0.847	0.774	0.797	0.771	0.843	0.811	0.0209	0.42	0.01	0.31	0.37	0.01
D22 BW, kg	11.69	11.82	11.36	11.92	12.49	12.52	0.273	0.01	0.75	0.86	0.15	0.55
Day 8-22 (Phase 2)												
ADG, g	315	323	299	326	353	370	15.1	<0.01	0.39	0.73	0.21	0.62
ADFI, g	428	449	418	465	468	494	18.3	0.02	0.18	0.89	0.07	0.14
G:F	0.740	0.720	0.715	0.704	0.753	0.751	0.0137	0.09	0.38	0.49	0.55	0.06
Day 22-29												
ADG, g	413	433	414	442	432	464	18.7	0.16	0.15	0.73	0.28	0.26

ADFI, g	709	729	688	739	729	763	21.9	0.18	0.20	0.72	0.10	0.33
G:F	0.584	0.592	0.603	0.599	0.594	0.604	0.0154	0.44	0.52	0.92	0.85	0.48
D29 BW, kg	14.58	14.85	14.26	15.01	15.51	15.77	0.327	<0.01	0.40	0.99	0.11	0.34
Day 29-34												
ADG, g	481	502	488	489	494	529	24.4	0.39	0.23	0.74	0.99	0.84
ADFI, g	772	780	786	807	792	814	31.3	0.36	0.62	0.82	0.62	0.41
G:F	0.619	0.653	0.629	0.606	0.626	0.653	0.0213	0.89	0.14	0.86	0.45	0.64
D34 BW, kg	16.99	17.35	16.69	17.45	18.06	18.41	0.379	<0.01	0.32	0.99	0.15	0.38
Day 34-39												
ADG, g	583	594	621	613	581	616	17.3	0.52	0.17	0.46	0.72	0.22
ADFI, g	936	993	988	1009	980	1019	27.5	0.18	0.07	0.73	0.59	0.06
G:F	0.621	0.599	0.632	0.606	0.594	0.610	0.0150	0.56	0.82	0.18	0.22	0.47
D39 BW, kg	19.90	20.30	19.80	20.52	20.97	21.50	0.412	<0.01	0.23	0.85	0.22	0.28
Day 22-39 (Phase 3)												
ADG, g	483	499	497	506	499	528	14.1	0.09	0.09	0.60	0.64	0.24
ADFI, g	794	822	805	839	822	854	23.1	0.18	0.18	0.91	0.31	0.17
G:F	0.608	0.608	0.621	0.604	0.608	0.620	0.0088	0.47	0.46	0.45	0.18	0.75
Day 0-39												
ADG, g	355	365	352	371	383	397	10.6	<0.01	0.23	0.82	0.23	0.30
ADFI, g	536	555	535	572	567	587	15.9	0.04	0.19	0.98	0.10	0.10
G:F	0.665	0.661	0.663	0.650	0.676	0.678	0.0062	0.03	0.85	0.59	0.15	0.08

[1]Diets: NC = negative control; SR = Stress Relief product at 0.15%; 1 ppm = mushroom product at 1ppm; 300ppm = mushroom product at 300ppm; Carb = Carbadox at 55 ppm; Carb + SR = Carbadox at 55 ppm plus Stress Relief product at 0.15%.

Chapter 3 - Effects of Varying Concentrations of Cordyceps Mushroom Powder on Nursery Pig Performance

Summary

One-hundred sixty crossbred pigs ((Duroc × (York × Landrace)) weaned at 18.8 d of age and weighing an average of 5.94 kg were used in a 35-day growth trial to evaluate varying concentrations of Cordyceps mushroom powder as a potential alternative to carbadox in nursery pig diets. Pigs were alloted by weight, sex, litter, and assigned to body weight (BW) blocks. Within BW blocks, sex ratios were constant in each pen. Each pen within a BW block was randomly assigned a dietary treatment. Growth performance was analyzed using BW, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion as gain-to-feed (G:F). Pigs were blocked by BW with 5 or 6 pigs per pen with 6 pens per treatment. There were 5 diets used in the study: a negative control and a positive control (carbadox, 55 ppm); 300 or 600 ppm mushroom powder, and a step down treatment (900, 900, 450, 300, and 150 ppm mushroom powder during weeks 1, 2, 3, 4, and 5, respectively). At various points of the study, pigs fed the 300 ppm and the step-down mushroom powder treatments tended to have improved ($P < 0.10$) growth performance compared with those fed the negative control diet. During Phase 4 of the study, pigs fed carbadox had greater ADG ($P < 0.02$) and improved feed efficiency ($P < 0.09$) over pigs fed the negative control diet. However, overall, there were no statistical differences among treatments ($P > 0.05$). Based on PCR there was a trend for difference in Toll Like Receptor 2 when comparing the NC to the PC, with the NC having a 1 fold increase in TLR2

when compared to the housekeeping gene. IL6 was variable and not different among treatments, however there was a 13.38 fold increase in presence for the NC treatment, and a 13.10 fold increase in the Step-down treatment. Plasma TNF-α concentrations were taken at two time points during the study, at d 14 and d 34. There were no differences in d 14 levels of TNF-α, however numerically as the concentration of mushroom increased in the diet, so did the TNF-α concentrations and carbadox fed pigs had the lowest TNF-α. On d 34, there was a Quadradic mushroom effect, as 300 and the step down treatments decreased, but the 600 ppm level stayed relatively consistent in concentration similar to the NC. In summary, pigs fed 300 ppm mushroom powder or the step-down treatment had comparable performance to pigs fed carbadox. However, future research is needed under a greater disease pressure to show mushroom powder's full potential as an alternative to antibiotics.

Introduction

Swine production is reducing the use of antibiotics, following the trend of consumer desires. Antibiotics are an important aspect of swine production for disease prevention and treatment. Antimicrobials used in feed post-weaning, such as carbadox, are under heavy scrutiny due to the growing concerns of antibiotic resistant pathogens. Feeding carbadox to nursery pigs has traditionally shown improved growth performance and feed efficiency compared to pigs fed diets without antimicrobial agents. This has led to research in antibiotic alternatives due to industry concern over potential monetary losses. One of these possible alternatives is a Chinese herbal mushroom blend of Cordyceps militaris and Cordyceps sinensis. These mushrooms have long been used by the Chinese as a human health promoting additive. A particular compound, cordycepin, found in these mushrooms is currently being studied as a possible anti-cancer agent

by many research institutes. The mushroom products have antimicrobial and antiviral characteristics. Based on a previous study with this mushroom having positive results, we decided to perform a titration study to evaluate the optimal levels to feed to nursery pigs.

Materials and Methods

One-hundred sixty weanling pigs (18.8 d of age) weighing an average of 5.94 kg consisting of US purebred genetics (Duroc × (York × Landrace)) were used in a 35 day growth trial. Growth performance was analyzed using BW, ADG, ADFI, G:F, and F:G. Pigs were allotted by BW with 5 or 6 pigs per pen. There were 5 dietary treatments: a negative control diet without a feed antimicrobial; a positive control diet containing 55 ppm carbadox; 300 or 600 ppm mushroom powder (MP); and a step down treatment containing 900, 900, 450, 300, and 150 ppm mushroom powder for weeks 1, 2, 3, 4, and 5, respectively. The 300 ppm MP dose was equivalent to the daily recommended human dose by Aloha Medicinals (2300 Arrowhead Drive, Carson City, NV). Pigs were alloted by weight, sex, litter, and assigned to BW blocks. Within BW blocks sex ratios were constant in each pen. Each pen within a BW block was then assigned a diet via a random number generator with pens that had the lowest random number being assigned diet 1 ascending to diet 5 for the highest random number. The experiment was conducted at the Purdue University Swine Farm, West Lafayette, IN. All procedures were approved by Purdue University's animal care and use committee (PACUC #1303000841).

The pigs were fed four dietary phases over a 35-day period (Table 1). Phase 1 was d 0-7, Phase 2 was d 7-14, Phase 3 was d 14-21, Phase 4 was d 21-35. Phase 1 and 2 were made with a basal diet which was split then remixed with the treatment premixes added for each diet. Phase 3 and 4 were made as individual diet treatment batches. All diets were formulated to meet or

exceed the nutrient requirements based on the Swine NRC (2012). All phase 1, 2, and 3 diets contained 2700 ppm zinc from zinc oxide and all phase 4 diets contained 200 ppm copper from copper sulfate.

Pigs had ad libitum access to feed and water in each pen through a five-hole stainless steel feeder and one cup waterer. Feeders and waterers were checked daily, with a target of partial pan coverage (40 to 50% coverage) for feed flow while also minimizing feed wastage. Pigs were individually treated with injectable antibiotics when signs of disease were detected and recorded for later treatment rate analysis. Pigs and feeders were weighed on day 0, 7, 14, 21, 28, and 35. The individual body weights and pen feed intake were recorded, then transferred to Microsoft Excel (2016, Redmond, Washington) to determine pen ADG, ADFI, and G:F.

Growth data were analyzed as a randomized complete block design using the GLM procedure in SAS (9.4, SAS Institute, Inc., Cary, NC) with pen being the experimental unit. Individual single degree of freedom contrasts were used to test for linear, quadratic, and cubic responses of the mushroom product when appropriate, as well as the positive vs. negative control, and on the overall (d 0-35) data, the effect of the constant 300 ppm dose vs. the step-down mushroom dose. The parameters measured were BW, ADG, ADFI, and feed efficiency every week and summarized by dietary phase and overall. Differences among treatments were considered significantly different at $P \leq 0.05$ or a tendency for difference at $0.05 < P \leq 0.10$.

Feed samples for each phase were collected and stored for future analysis at the Purdue University Swine Nutrition Lab. Dietary crude protein, energy, dry matter, ash, and phosphorus concentrations were analyzed. Prior to analyzing the diets they were ground through a 1 mm screen. Crude protein percentage was determined by combustion using a Leco nitrogen analyzer (Leco Model 2000 CHN analyzer; Leco Corp., St. Joseph, MI). Caloric content was determined

via bomb calorimeter procedure (Parr 1261 bomb calorimeter; Parr instruments Co., Moline, IL, USA). Percent dry matter and ash were analyzed by weighing the crucible and sample on the same scale after being dried in the drying oven for 12 hours at 60°C, and ashed in the ashing oven for 6 hours at 500°C. Phosphorus was analyzed on the diet ash by the total phosphorus colormetric method (Murphy and Riley, 1962) . All samples were analyzed in duplicates and adjusted for standards. If the values exceeded a 5% difference the samples were repeated until values are within 5%.

VFA Analysis

A fecal sample from one medium barrow and gilt per pen were collected by rectal massage on d 14 and frozen at -20° C until later analyzed. Volatile fatty acid concentrations in fecal samples were determined by a gas chromatographic method (Erwin et al., 1961). Briefly, fecal samples were thawed and 4 ± 0.1 g samples were taken, diluted with 4 mL distilled water and 2 mL of 25% metaphosphoric acid, mixed (VWR Mini Vortexer MV1, IKA Works, Inc., Wilmington, NC 28405), and centrifuged at 15,000 g, 4°C, for 10 min (Beckman J-21C, Beckman Instruments, Inc., Palo Alto, CA 94304). After centrifugation, the supernatant was transferred into a 2-dram vial. The sample was re-centrifuged (15,000 g, 4°C, for 15 min) and the supernatant was filtered through a polyethersulphone membrane filter (0.25 mm, Whatman, UK) and 1.5 mL transferred into a DPID vial. The concentrations of VFA were determined by gas chromatography (Varian 3900, Varian, Inc., Walnut Creek, CA 94598). The least detectable limit for all VFA was 0.1 mmol/L.

Blood Sampling

Blood samples were collected from one medium weight barrow and gilt per pen in one EDTA and one ACD vacutainer tube using an 18 gauge, 3.81 cm length needle on study d 14 and 34. The tubes of blood were used to determine plasma TNF-α concentrations and mRNA extraction for PCR analysis.

Following blood collection, EDTA blood tubes were gently inverted 3 to 4 times to distribute EDTA and placed immediately on ice immediately. Once returned to campus blood was spun down in centrifuge at 4° C for 15 minutes at 2,000X G's, with plasma aliquoted into 3 samples and stored in the -20° C freezer. The ACD blood tubes were used for isolating leukocytes by hypotonic lysis (Eicher et al., 2010). The final cell pellet was resuspended in two aliquots of Trizol at a total volume of 1 mL and stored at -80° C for PCR analysis.

ELISA

Plasma TNF-α concentrations were determined using a solid-phase sandwich ELISA kits (Chaytor et al., 2011). All the recommendations of the manufacturing company were followed (R&D Systems Inc, McKinley Place NE Minneapolis, MN). The optical density (OD) value was read at 450 nm within 2 hours by an ELISA plate reader (Tecan Spark 10M, Tecan Group Ltd. Seestrasse 103, 8708 Männedorf, Switzerland). A standard curve of OD value versus TNF-α concentration was generated, and the plasma TNF-α concentration was then determined according to the standard curve.

Gene Expression

White blood cell RNA was isolated using a commercially available kit (PureLinkTM RNA Mini Kit; Aambion by Life Technologies, Carlsbad, CA, USA). Quantification of RNA was completed by measuring absorbance at 260 nm using a NanoDrop spectrophotometer (ND-100, NanoDrop Technologies, Rockland, DE). Purity was determined by using the ratio of absorbance at 260 nm and 280 nm. All samples had 260/280 nm ratios above 1.9 (Wilfinger, 1997). After RNA was quantified, each sample was diluted to 1 µg of RNA per 4.75 µL of nuclease free water. For PCR analysis samples were reverse transcribed using TacMan reverse-transcription reagents (Applied Biosystems Inc., Foster City, CA) at a volume of 100 µL, containing 50 U of MultiScribe reverse transcriptase/µL, 10 µL of TacMan RT buffer, 25 mM $MgCl_2$, 20 µL of deoxyribonucleotide triphosphates (dNTP), 2.5 µM random hexomers, 0.4 U of RNase inhibitor/µL, and 50 µM dNTP. The mixture was incubated in a Thermo Hybaid PCR Express thermal cycler (Midwest Scientific, St. Louis MO) at 25° C for 10 min, followed by 48° C for 30 min, and finally 95° C for 5 min. Samples were stored at -20° C until amplified. Two and a half microliters of the diluted cDNA sample (0.1 µg/mL) from reverse transcription was placed in a 96-well plate with 2.25 µL of 900 nM primers, 1.75 µL of 250nM TaqMan MGB probe, 12.5 µL of of PCR Master Mix (Applied Biosystems Inc.), and water to a final volume of 25 µL. Samples were incubated at 50°C for 2 min and then heated at 95°C for 10 min, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C. The samples and standards were analyzed using porcine-specific primers and probes to determine expression of TLR2, IL-6, IL-10 (Applied Biosystems Inc.). All qRT-PCR reactions were performed in duplicate using template from individual animals in each reaction. Relative standard curves were generated by serial dilution of a sample with abundant expression for each gene. Gene qualities were recorded as a ratio of the

gene of interest to that of the 18S internal standard (Applied Biosystems Inc.). The standard curve was constructed using the following dilutions of cDNA (in triplicate): 1, 0.5, 0.25, 0.125, 0.0625, and 0.0315. A single control sample that had the lowest CT value was selected to be used as the template for the standard curve. Quantitative RT-PCR was performed and analyzed using an ABI Prism 7000 sequence detection system (Applied Biosciences Inc.). Primer and probe sequences for qRTPCR were designed using Primer Express 1.1 software (Applied Biosystems Inc.) and synthesized by Applied Biosystems Inc. Probes were labeled with FAM fluorescent dye.

Results

During Phase 1 of the trial (d 0 to7) there was a tendency for a linear reduction in BW ($P < 0.07$) as the mushroom concentration increased in the diet (Table 3.2). During Phase 2 (d 7-14) there was a trend for a cubic mushroom response ($P < 0.07$) in ADFI and ADG ($P < 0.11$), resulting in a cubic mushroom response in BW at d 14 of age ($P < 0.04$). These cubic responses were caused by the pigs fed the 300 ppm dose having 8.7% greater ADG and 9.8% greater ADFI above the 0 ppm negative control pigs followed by the lowest ADG and ADFI at the 600 ppm level and the 900 ppm dose having a partial recovery of the pig growth performance. For Phase 3 (d 14-21) showed no statistical differences in performance among treatments ($P > 0.10$). During the first week of Phase 4 (d 21 to 28), pigs fed carbadox had increased ADG ($P < 0.04$) and increased feed efficiency ($P < 0.02$) over pigs fed the negative control. There was also a tendency for a linear increase in ADG ($P < 0.10$) and improvement in feed efficiency ($P < 0.08$) as mushroom concentration increased to 600 ppm. During the second week of Phase 4 (d 28 to 35), there was a tendency for cubic ADFI response ($P < 0.10$) for mushroom inclusion with a 13%

greater ADFI at the lowest 150 ppm inclusion followed by a 10.7% lower ADFI at the 300 ppm level and ADFI then increasing at the 600 ppm level close to the negative control. For the entire Phase 4 period, d 21-35, pigs fed carbadox had greater ADG ($P < 0.02$) and tended to have improved feed efficiency ($P < 0.09$). For the pigs fed the MP there was a tendency for ADG ($P < 0.11$) to linearly increase to 600 ppm while ADFI ($P < 0.10$) had a cubic response optimized at the lowest inclusion. Overall (d 0 to 35), pigs fed the 300 ppm diet tended ($P < 0.10$) to have better feed efficiency compared to those fed the step-down treatment. However, there were no other dietary treatment effects for the overall nursery study.

VFA concentrations did not differ among treatments other than a quadratic mushroom response in propionic acid, valeric, and total VFA concentrations ($P < 0.05$), and a tendency ($P < 0.10$) was observed in butyric, and valeric acids. In all VFA's concentrations from NC decreased at 300 ppm returned to control levels at 600 ppm and increased at 900 pm. Total VFA concentrations shifted numerically with carbadox and the Step-down treatment having the greatest total concentrations at 136.2 and 141.3 each respectively. As a percent of total VFA's acetic acid tended to quadratically increase at 300 ppm and then decrease at 900 ppm ($P < 0.08$) while propionic acid tended ($P < 0.08$) to do the quadratic inverse with lower values at 300 ppm and increased percentages at 900 ppm MP.

Gene expression of TLR-2 had a trend for difference when comparing the NC to the PC, with the NC having a 1 fold increase in TLR2 ($P < 0.08$) when compared to the housekeeping gene. The expression of IL6 was 13.38 fold greater for the NC treatment, and 13.10 fold greater in the Step-down treatment. With the variability observed for IL6, there was no statistical differences. There were not differences in presence of IL10 across treatments. Plasma concentrations of TNF-α were taken at two time points during the study, at d 14 and d 34. There

were no differences in d 14 levels of TNF-α, however as the concentration of mushroom increased in the diet, so did the TNF-α concentrations. Using Duncan's mean separation test pigs fed carbadox tended to have lower plasma TNF-α concentrations than the pigs fed mushroom 600 and 900 ppm. On d 34 there was a Quadradic mushroom effect ($P < 0.03$), as 300 and the step down treatments decreased, but the 600 ppm level stayed relatively similar to the NC in concentration.

Discussion

Carbadox has been well documented in its effects in post-weaning performance. Most industry professionals tend to use some form of antimicrobial in the post-weaning diet. In this current study, carbadox primarily only improved pig growth performance during Phase 4, the last 2 weeks of the study, resulting in about a 0.5 kg heavier pig over the negative control. It should also be noted that the growth performance of pigs in general was very good in this study and is likely related to the small response to the antimicrobial.

The heaviest pigs in this study (d 35) were fed the constant level of 300 ppm mushroom (18.9 kg) similar to the carbadox fed pigs (18.8 kg). As the mushroom level decreased to 150 ppm in the last week of the study, the greatest growth performance of all treatments was observed by pigs fed the step-down treatment. The poorer growth performance earlier in the study by pigs fed 600 and 900 ppm mushroom powder may indicate that these levels may have been too high and may require future evaluation with dietary concentrations below 300 ppm.

Pigs fed the step-down treatment had a 13 fold increase in abundance IL-6. IL-6 did not always amplify correctly during the PCR analysis, therefore this aspect of the study is questionable and warrants further investigation. Cheng et al. (2016) theorized Cordyceps

militaris enhances cell-mediated immunity, however because of cost constraints we could not investigate the same immune responses. Concentrations of TNF-α on day 14 tended to show a linear increase in concentration in correspondence with the increasing concentrations of mushroom powder, however this was not statistically significant (P = 0.22). Pigs fed the antimicrobial carbadox had the lowest d 14 TNF-α, possibly indicating less disease challenge. On day 34 interestingly there was a quadratic response showing the lower levels of mushroom powder late in the nursery had less abundance of TNF-α compared to the high dose of 600 ppm and NC pigs. Growth performance was not poor on the 600 ppm diet, however this may indicate 600 ppm was too high inclusion and was stimulating an immune response or gut inflammation. VFA results showed the carbadox and Step-down treatments having the greatest total values, suggesting that they have made the intestinal tract more acidic as well as having access to additional energy from the VFA's generated.

When considering the economics of feeding this human-grade mushroom product, the 300 ppm mushroom product is slightly more expensive at $35/ton compared $26/ton for the carbadox treatment. When feeding the 150 ppm level at the end of the study, feed costs were less than the carbadox positive control. In conclusion, the 300 ppm mushroom and step-down treatments were both comparable in results to carbadox. Therefore, mushroom powder could serve as a possible antimicrobial replacement to carbadox.

In conclusion, the mushroom step-down treatment starting at 900 ppm was clearly too high of concentration, as well as the 600 ppm. The step-down treatment improved late in the study as MP levels in the diet dropped, with the highest performance coming at 150 ppm in the final week. The 300 ppm treatment showed the most promising results again in this study,

however it appears a refined titration study could be beneficial with lower levels in the step-down.

Literature Cited

Cheng, Y. H., Wen, C. M., Dybus, A., Proskura, W. S. Fermentation of Cordyceps militaris enhancement performance and modulate immune response of weaned piglets. South African J Anim Sci. 2016; 46:121-128. Doi:10.4314/sajas.v46i2.2.

Shen, H. S., S. Shao, J. C. Chen, T. Zhou. Antimicrobials from mushrooms for assuring food safety. Comprehensive Reviews in Food Science and Food Safety. 2017; 16. Doi: 10.1111/1541-4337.12255.

Zhou X, Luo L, Dressel W,Shadier G,Krumbiegel D,Schmidtke P, Zepp F, Meyer CU. Cordycepin is an immunoregulatory active ingredient of Cordyceps sinensis. Am J Chin Med 2008; 36:967–980. Doi:10.1142/S0192415X09006387.

Table 3-1 Basal diet formulation

Ingredient, %	Phase 1	Phase 2	Phase 3	Phase 4
Corn	36.140	42.290	46.390	54.055
Soybean meal, 48% crude protein	14.000	16.950	26.500	28.925
Dried distillers grains with solubles, 7% fat	0.000	5.000	7.500	10.000
Choice white grease	0.000	0.000	0.000	3.000
Soybean oil	5.000	4.000	3.000	0.000
Limestone	0.650	0.810	0.890	1.415
Monocalcium phosphate, 21% P	0.480	0.530	0.180	0.560
Vitamin premix[1]	0.250	0.250	0.250	0.250
Trace mineral premix[2]	0.125	0.125	0.125	0.125
Selenium premix[3]	0.050	0.050	0.050	0.050
Phytase[4]	0.100	0.100	0.100	0.100
Salt	0.250	0.250	0.300	0.350
Plasma protein	5.000	2.500	0.000	0.000
Spray-dried blood meal	1.500	1.000	0.000	0.000
Soy concentrate	5.000	3.250	0.000	0.000
Fish meal	4.650	4.500	5.000	0.000
Dried whey	25.750	17.150	8.600	0.000
Lysine-HCL	0.130	0.275	0.300	0.435
DL-Methionine	0.230	0.210	0.160	0.150
L-Threonine	0.060	0.110	0.110	0.140
L-Tryptophan	0.010	0.025	0.015	0.015
Copper sulfate	0.000	0.000	0.000	0.08
Zinc oxide	0.375	0.375	0.375	0.000
Banmith-48	0.000	0.000	0.000	0.100
Treatment premix[5]	0.250	0.250	0.250	0.250
Total	100.00	100.00	100.00	100.00
Calculated nutrients				
Metabolizable energy, kcal/kg	3529	3472.9	3427.3	3401.5
Net energy, kcal/kg	2743.3	2658.4	2567.4	2516.8
Crude protein, %	24.46	23.22	23.12	21.53
Total lysine, %	1.727	1.62	1.52	1.43
Standardized ileal digestible amino acids, %				
Lysine	1.55	1.45	1.35	1.25
Methionine	0.54	0.53	0.50	0.44
Methionine+cystine	0.91	0.85	0.79	0.73

Threonine	0.97	0.90	0.84	0.78
Tryptophan	0.28	0.26	0.24	0.23
Isoleucine	0.86	0.81	0.82	0.75
Valine	1.08	0.97	0.91	0.83
Ca,%	0.85	0.85	0.80	0.75
P, %	0.76	0.72	0.62	0.55
Available P, %	0.55	0.50	0.38	0.28

[1]Provided per kg of diet available minerals: iron, 121.3 mg; zinc, 121.2 mg; manganese, 15.0 mg; copper, 11.33 mg; iodine, and 0.46 mg.

[2]Provided per kg of diet: vitamin A, 6,614 IU; vitamin D3, 661 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 9 mg; pantothenic acid, 22 mg; niacin, 33 mg and B12 38.6 mg.

[3]Provided 0.3 ppm Se

[4]Provided 600 FTU per kg of the diet

[5] There were 5 dietary treatments: a negative control diet without a feed antimicrobial; a positive control diet containing 55 ppm carbadox; 300 or 600 ppm mushroom powder; and a step down treatment containing 900, 900, 450, 300, and 150 ppm mushroom powder for weeks 1, 2, 3, 4, and 5, respectively.

Table 3-2 Mushroom Titration nursery growth performance

Diet	**Negative Control**	**300 ppm Mushroom**	**600 ppm mushroom**	**Step-down**	**Carbadox**	**Probability, P<**				
	A	B	C	D	E	SE	Lin Mush	Quad Mush	Cubic Mush	NC vs PC
Pens/diet	6	6	6	6	6					
Initial Wt, kg	5.95	5.93	5.90	5.91	5.94	0.228	0.143	0.6392	0.5993	0.7713
Day 0-7										
ADG, g	127	128	109	108	117	10.8	0.14	0.94	0.45	0.51
ADFI, g	133	129	115	121	136	12.7	0.40	0.71	0.62	0.88
G:F	0.983	1.002	0.88	0.892	0.859	0.0687	0.22	0.96	0.41	0.22
D7 BW, kg	6.84	6.83	6.57	6.66	6.75	0.089	0.07	0.58	0.15	0.53
Day 7-14										
ADG, g	313	342	284	300	316	20.9	0.31	0.75	0.10	0.94
ADFI, g	372	408	335	406	395	29.7	0.84	0.57	0.07	0.59
G:F	0.833	0.846	0.86	0.778	0.846	0.6008	0.60	0.45	0.72	0.87
D14 BW, kg	9.03	9.23	8.56	8.76	8.96	0.171	0.07	0.99	0.04	0.79
Day 14-21										
ADG, g	373	359	362	400	345	20.7	0.96	0.77	0.14	0.34
ADFI, g	580	597	548	613	569	32.7	0.73	0.23	0.46	0.82
G:F	0.645	0.608	0.663	0.652	0.604	0.02	0.46	0.13	0.30	0.16
D21 BW, kg	11.64	11.74	11.10	11.56	11.38	0.275	0.22	0.22	0.89	0.51

Day 21-28										
ADG, g	434	481	484	461	498	19.7	0.09	0.55	-	0.03
ADFI, g	721	761	747	763	740	21.9	0.41	0.21	-	0.54
G:F	0.607	0.633	0.652	0.603	0.676	0.0175	0.08	0.50	-	0.01
D28 BW, kg	14.68	15.11	14.48	14.79	14.86	0.314	0.66	0.26	-	0.68
Day 28-35										
ADG, g	521	539	552	567	564	20.6	0.51	0.51	0.20	0.15
ADFI, g	1103	982	1063	1249	1072	100.7	0.45	0.88	0.10	0.83
G:F	0.484	0.571	0.531	0.459	0.561	0.0478	0.31	0.56	0.19	0.27
D35 BW, kg	18.33	18.88	18.35	18.76	18.82	0.394	0.90	0.23	0.98	0.39
Day 21-35 (Phase 4)							Linear Mush	Quad Mush	Stepdown Vs 300	NC vs PC
ADG, g	478	510	518	514	531	14.4	0.06	0.49	0.86	0.02
ADFI, g	912	872	905	1006	906	53.2	0.92	0.58	0.90	0.94
G:F	0.527	0.595	0.575	0.511	0.604	0.030	0.27	0.25	0.63	0.09
Day 0-35										
Treatments; Contrasts							Linear Mush	Quad Mush	Stepdown Vs 300	NC vs PC
ADG, g	354	370	356	367	368	11.00	0.91	0.27	0.86	0.37
ADFI, g	582	576	562	623	582	26.1	0.58	0.91	0.21	0.99
G:F	0.609	0.644	0.635	0.59	0.643	0.0226	0.42	0.44	0.11	0.30

Table 3-3 Volatile Fatty Acids

Diet	Negative Control	300 ppm mushroom	600 ppm mushroom	Step-down	Carbadox		Probability, P <			
	A	B	C	D	E	SE	Lin Mush	Quad Mush	Cubic Mush	NC vs PC
A	69.56	62.31	70.82	72.33	68.57	3.186	0.25	0.19	0.13	0.83
P	32.06	26.26	30.39	36.59	32.33	2.190	0.09	0.01	0.43	0.93
iB	2.50	1.75	2.44	2.60	2.89	0.299	0.47	0.14	0.16	0.37
B	18.66	14.38	17.59	21.58	24.03	2.396	0.28	0.10	0.54	0.13
iV	2.87	1.99	2.93	3.39	3.85	0.480	0.26	0.18	0.30	0.17
V	3.88	2.57	3.66	4.79	4.51	0.617	0.18	0.06	0.41	0.48
Total	129.54	109.26	127.84	141.28	136.17	7.217	0.11	0.03	0.19	0.52

Table 3-4 Percent of Volatile Fatty Acids

Diet	Negative Control	300 ppm mushroom	600 ppm mushroom	Step-down	Carbadox		Probability, P <			
	A	B	C	D	E	SE	Lin Mush	Quad Mush	Cubic Mush	NC vs PC
A, %	54.40	56.84	55.26	51.39	50.19	1.703	0.18	0.08	0.82	0.10
P, %	24.74	23.98	23.70	25.91	23.71	0.778	0.37	0.07	0.57	0.36
iB, %	1.92	1.64	1.92	1.85	2.17	0.217	0.96	0.64	0.35	0.43
B, %	13.83	13.25	13.87	15.15	17.68	1.203	0.40	0.45	0.92	0.04
iV, %	2.20	1.89	2.35	2.39	2.90	0.366	0.53	0.64	0.48	0.19
V, %	2.91	2.40	2.90	3.31	3.35	0.371	0.32	0.23	0.52	0.41

Table 3-5 Blood TNF-α

							Probability, P<			
	Negative Control	**300 ppm**	**600 ppm**	**Step-Down**	**Carbadox**	**SE**	**Lin Mush**	**Quad Mush**	**Cubic Mush**	**NC vs PC**
TNF-α, d 14	71.1	75.3	78.9	82.6	58.8	6.69	0.22	0.97	0.98	0.21

							Lin Mush	**Quad Mush**	**300 vs Step-down**	**NC vs PC**
TNF-α, d 34	77.3	49.5	74.4	56.8	60.8	10.02	0.82	0.03	0.59	0.21

Table 3-6 White Blood Cell gene expression

							Probability, P<			
	Negative Control	**300 ppm**	**600 ppm**	**Step-Down**	**Carbadox**	**SE**	**Lin Mush**	**Quad Mush**	**Cubic Mush**	**NC vs PC**
TLR2	1.07	0.88	0.68	0.78	0.52	0.226	0.28	0.52	0.75	0.07
IL6	13.38	5.53	9.81	13.10	7.19	4.493	0.86	0.21	0.50	0.29
IL10	0.78	0.62	0.72	0.52	0.51	0.196	0.43	0.91	0.52	0.30

Chapter 4 - Cordyceps Mushroom Powder by Carbadox

Abstract

The objective was to evaluate the independent and additive effects of Cordyceps mushroom powder and carbadox to pharmacological copper+zinc in nursery pig diets. Two hundred-ten crossbred weanling pigs ((Duroc × (York × Landrace)) average of 19 d of age and 5.8 kg were used in a 33 day growth trial. Pigs were alloted by weight, sex, ancestry, and assigned to body weight (BW) blocks. Within BW blocks, sex ratios were constant in each pen. Pen was the experimental unit and growth performance was analyzed using BW, ADG, ADFI, and G:F. There were 7 pigs/pen and 6 pens/treatment. Treatments were: 1) a negative diet (NC); 2) positive control (PC; carbadox, 55 ppm); 3) NC+300 ppm Cordyceps mushroom powder (NC+MP); 4) PC +300 ppm mushroom(PC+MP); 5) supplemental copper sulfate (125 ppm) and zinc oxide (3000 ppm d 0-7, 2000 ppm d 7-35), CuZn. Dietary treatments were fed in a four-phase feeding program (d0-7, d7-14, d14-21, and d21-33). There were no interactions between MP and carbadox at any time point (P>0.10). Pigs fed the PC, PC+MP and CuZn treatment had increased BW (P<0.05), ADG (P<0.05), ADFI (P<0.10) and G:F (P<0.05) over the NC at the end of phases 1, 2, and 3, with no main effect of MP treatment. During Phase 4, pigs fed MP, PC, and CuZn diets all had increased ADG (P<0.05; 431, 477, 455, 505, 486 g/d, diet 1-5, respectively) and ADFI (P<0.05) over the NC fed pigs. Overall, d0-33, PC diets and CuZn supplemented pigs had increased ADG (P<0.05) and ADFI (P<0.05), with pigs fed MP tending to have increased ADFI (P<0.08) over NC fed pigs. Plasma TNF-α concentrations d 14 postweaning showed a trend for a carbadox main effect, as well as a mushroom by carbadox interaction (P < 0.10) for plasma TNF-a, with the 300 ppm mushroom having the numerically

highest value on the study, while the combination of carbadox and 300 ppm mushroom had the lowest concentration of TNF-α. Feeding nursery pigs pharmacological levels of Cu+Zn and carbadox have economical value to increase nursery pig performance with MP may increase pig ADFI and final BW through potentially complimentary modes of action.

Introduction

Swine production is reducing antibiotic use, following the trend of consumer desires. Antibiotics are an important aspect of swine production for disease prevention and treatment. Antimicrobials used in feed post-weaning, such as carbadox, are under heavy scrutiny due to the growing concerns of antibiotic resistant pathogens. The effects of feeding carbadox to nursery pigs has traditionally shown improved growth performance and feed efficiency compared to pigs fed diets without antimicrobial agents. This has led to research in alternatives to antibiotics due to industry concern over potential monetary losses. One of these possible alternatives is a Chinese herbal mushroom blend of Cordyceps militaris and Cordyceps sinensis (Shen et al. 2017). These mushrooms have long been used by the Chinese as a human health promoting additive. The particular compound, cordycepin, found in these mushrooms is currently being studied as a possible anti-cancer agent by many research institutes. The mushroom itself has antimicrobial and antiviral characteristics (Zhou et al. 2008). Based on a previous study with this mushroom (Cheng et al. 2016) with positive results, as well as our own research, we decided to perform a study to determine whether there is an additive effect when combining the mushroom and carbadox in the nursery phase.

Materials and Methods

One-hundred sixty gilts and barrows (18.4 d of age) weighing an average of 5.8 kg (Duroc X (York X Landrace)) were put on test for a 33 day growth trial. Growth performance was analyzed using body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion as feed-to-gain (F:G) and gain-to-feed (G:F) ratios. There were 5 diets, negative and positive (carbadox) controls, 300 ppm Mushroom, 3000 ppm Zn d 0-7 (2000 ppm Zn d 7-33) & 125 ppm Cu, and 300 ppm mushroom powder in combination with carbadox. There were 42 pigs per treatment. Pigs were divided by weight, sex, litter, and assigned to BW blocks with 7 pigs per pen. Within BW blocks sex ratios were constant in each pen. Each pen within a BW block was then randomly assigned a diet via a random number generator with pens that had the lowest random number being assigned diet 1 to diet 5 for the highest random number.

Diets

There were five diets tested in this study. Nursery diet A was the negative control containing a 0.5% corn premix, Diet B contained Cordyceps mushroom powder (Aloha Medicinals, Carson City, NV) at 300 ppm, Diet C contained 3000 ppm Zn from d0-7 declining to 2000 ppm from d 7-33 and 125 ppm Cu, Diet D contained carbadox at 55 ppm, Diet E contained carbadox (55 ppm) in combination with 300 ppm mushroom powder.

The pigs were fed four dietary phases over a 33 day period. Phase 1 was d 0-7, Phase 2 was d 7-14, Phase 3 was d 14-21, Phase 4 was d 21-33. Phase 1 and 2 were made with a basal diet which was split then remixed with the treatment premix added. Phase 3 and 4 were made as individual diet treatment batches.

Feed samples for each phase were collected and stored for future analysis at the Purdue University's Swine Nutrition Lab. Diet CP, energy, dry matter, ash, and phosphorus concentrations will be analyzed. The diets were ground through a 1 mm screen. Crude protein percent was determined by combustion using a Leco nitrogen analyzer (Leco Model 2000 CHN analyzer. Leco Corp., St. Joseph, MI, USA). Caloric content was determined via bomb calorimeter procedure (Parr 1261 bomb calorimeter; Parr instruments Co., Moline, IL, USA). Percent dry matter and ash was analyzed by weighing the crucible and sample on the same scale after being dried in the drying oven for 12 hours at 60 ° C, and ashed in the ashing oven for 6 hours at 500 ° C. Phosphorus was analyzed on the diet ash by the total phosphorus colormetric method (Murphy and Riley, 1962). All samples were analyzed in duplicates and adjusted for standards. If the values exceeded a 5% difference the samples were repeated until values are within 5%.

Blood and fecal sampling

Blood samples were collected from 1 median weight barrow and gilt in each pen in one EDTA vacutainer tube using an 18 gauge, 3.81 cm length needle on study d 14. Following blood collection, EDTA blood tubes were gently inverted 3 to 4 times to distribute EDTA and placed on ice immediately. Blood was spun down in centrifuge at 4 °C for 15 minutes at 2000 x g, with plasma aliquoted into 3 samples and stored in the -20 C freezer.

Fecal sampling occurred on day 32 of the study by rectal stimulation of 1 median weight barrow and gilt of each pen for fecal VFA analysis and future microbiome analysis.

ELISA

Plasma TNF-α concentrations were determined using a solid-phase sandwich ELISA kits (Chaytor et al., 2011). All the recommendations of the manufacturing company were followed

(R&D Systems Inc, McKinley Place NE Minneapolis, MN). The optical density (OD) value was read at 450 nm within 30 minutes by an ELISA plate reader (Tecan Spark 10M, Tecan Group Ltd. Seestrasse 103, 8708 Männedorf, Switzerland). A standard curve of OD value versus TNF-α concentration was generated, and the plasma TNF-α concentration was then determined according to the standard curve.

Volatile fatty acids Analyses

Volatile fatty acid concentrations in fecal samples (d 32) were determined by a gas chromatographic method (Erwin et al., 1961). Briefly, fecal samples were thawed and 4 ± 0.1 g samples were taken, diluted with 4 mL distilled water and 2 mL of 25% metaphosphoric acid, mixed (VWR Mini Vortexer MV1, IKA Works, Inc., Wilmington, NC 28405), and centrifuged at 15,000 x g, 4°C, for 10 min (Beckman J-21C, Beckman Instruments, Inc., Palo Alto, CA 94304). After centrifugation, the supernatant was transferred into a 2-dram vial. The sample was re-centrifuged (15,000 x g, 4°C, for 15 min) and the supernatant was filtered through a polyethersulphone membrane filter (0.25 mm, Whatman, UK) and 1.5 mL transferred into a DPID vial. The concentrations of VFA were determined by gas chromatography (Varian 3900, Varian, Inc., Walnut Creek, CA 94598). The least detectable limit for all VFA was 0.1 mmol/L.

Pigs had ad libitum access to feed and water with in each pen having a single hole wean-to-finish feeder and one nipple waterer. Feeders and waterers were checked daily, with the idea of having partial pan coverage (40-50% coverage) while also minimizing feed wastage. Feeders were cleaned when feed became spoiled, and waterers adjusted to shoulder height of the pigs. Daily checks consisted of checking feeders, waterers, observations of the pigs, filling feeders if needed, treating pigs with antibiotics when signs of disease were detected, and completing treatment paperwork. Pigs and feeders were weighed on day 0, 7, 14, 21, 27, 33. The individual

body weights and pen feed intake were recorded to determine pen ADG, ADFI, F:G and G:F before submitting the data to SAS (v9.4) for statistical analysis using the GLM procedure. The parameters measured were BW, ADG, ADFI and feed efficiency every week and summarized by dietary phase and overall.

Results

During phase 1 of the trial (d 0 to 7) there were significant increases in ADG, ADFI, feed efficiency, and d7 BW ($P < 0.05$) for CuZn, and carbadox had increases in ADG, feed efficiency, and d7 BW with a tendency for an increase in ADFI vs the NC. Mushroom x carbadox interaction had a tendency for difference in Phase 1 ADFI ($P <0.1$) with the MP300 treatment increasing ADFI over the NC treatment. However the combination of 300MP and carbadox consumed less feed than carbadox alone. For phases 2 and 3 CuZn was different in ADG, ADFI, G:F, and BW ($P < 0.05$) compared to the NC. Carbadox was different in ADG, ADFI, G:F, and BW in phase 2 ($P < 0.05$). In phase 3 carbadox was improved in ADG, ADFI, and BW ($P < 0.05$) vs the NC. In phase two the Mushroom diets had a significant difference in F:G ratio ($P < 0.05$), while MxC interaction had a significant difference in G:F ($P < 0.05$). Phase 3 had no statistical differences for MxC interaction or Mushroom effect. In Phase 4 Mushroom effect, and carbadox had a statistical difference in ADG and ADFI ($P < 0.05$). In Phase 4, CuZn fed pigs had improved in ADG, and ADFI ($P < 0.05$) over the NC. Overall, carbdox had greater ADG, ADFI, and G:F ($P<0.05$), while CuZn was different in ADG and ADFI ($P < 0.05$) compared to the NC. While CuZn increased ADG and ADFI dramatically, it did not improve feed efficiency over the negative control treatment. Pigs fed the CuZn diet averaged 88 g/d better ADG over the negative control, with similar feed efficiency to the negative control. The final BW for the CuZn treatment was 2.9 kg greater than the negative control. Carbadox has well documented positive

growth performance effects in the nursery, in this study these results were reproduced with carbadox significantly improved performance in every category over the Negative control, adding 1.7 kg of BW by the end of the study. The mushroom treatment appears to have a delayed effect, the pigs were slightly numerically improved over the negative control pigs throughout the study in ADG, but in the final week of Phase 4, matched carbadox performance in ADG and tended to increase overall ADFI ($P<0.08$). Additionally the treatment of carbadox + Mushroom had similar ADG throughout the study compared to carbadox until the second week of phase 4, where the treatment was gaining an additional 67 g/d compared to the carbadox alone treatment.

There were no differences in fecal acetic acid concentrations between treatments ($P >0.1$). Propionoic acid had a mushroom by carbadox effect ($P < 0.05$), with both treatments having elevated levels compared to the NC but reduced levels when combined. There was a tendency ($P<0.10$) for mushroom by carbadox interaction for isobuteric acid, butyric acid, and total VFA concentration with the individual treatments being elevated in concentration. There was an interaction also between isovaleric and valeric acid concentrations ($P < 0.05$). Interestingly, the 300 ppm mushroom and carbadox treatments had elevated VFA concentrations. However when the treatments were combined, the VFA reduce to levels resembled in the NC. There was also a main carbadox effect to decrease valeric acid concentrations ($P<0.03$). These effects tend to carry over when calculated as a percent of total VFA's. AS a percent of total VFA's, CuZn decreased propionic acid ($P<0.08$) and increased butyric acid ($P<0.05$) over the NC.

Plasma concentrations of TNF-α at d-14 postweaning had a trend ($P < 0.10$) for a carbadox main effect, as well as a mushroom by carbadox interaction. The 300 ppm mushroom

treatment had the numerically greatest value on the study, while the combination of carbadox and 300 ppm mushroom had the lowest TNF-α concentrations.

Discussion

With the previous studies performance analyzed it was decided to see what this mushroom product would do with a true negative control, with no added Zn or Cu. Based on ADM's stress relief product having an additive effect when fed with carbadox it was worth exploring to see if the mushroom powder also had an additive effect on growth performance.

Final BW for the CuZn treatment pigs were 2.9 kg greater than the negative control pigs. Carbadox significantly improved pig performance in every category over the Negative control, adding 1.7 kg of BW by the end of the study. Mushroom powder improved performance slightly early, but primarily had a delayed response. During the final week MP 300 matched carbadox in ADG, as well as had an additive effect in the final phase when added into the carbadox treatment. The Cu and Zn treatment outperformed every treatment other than the final week when the combination of carbadox and MP300 were the fastest gaining treatment. It also appears MP300 may not be as effective when combined with a true negative control. It appeared to perform better with Zn and Cu included in the diet.

Interestingly MP300 had the greatest total VFA concentrations out of any treatment, but the combination of carbadox and MP300 had lowest total VFA concentrations. In theory greater VFA concentrations should allow the pig to have access to more energy, but this did not translate into added growth performance. This is also the opposite of what the concentrations looked like in the titration study for the 300 ppm level, warranting further investigation as to what impact this level of mushroom has on VFA concentrations.

Plasma TNF-α concentrations were greatest in the 300 ppm mushroom treatment, which is again conflicting with the previous titration study. There was a tendency for reduced TNF-α concentrations in carbadox fed pigs. However in the combination of carbadox and 300 ppm mushroom powder the TNF-α levels decreased in concentration further than carbadox alone.

The Mushroom treatment could provide some benefit to pigs. However the response is late in the nursery phase. Based on previous results it is possible the level of the mushroom needs adjusted to different levels at certain time points, a refined titration study may be warranted. Further research is needed to investigate whether there is a possible carryover effect into the grow-finish stage of production. Further research is also needed on the composition of this mushroom product, to determine why the effect is delayed. Additional investigation into whether there is a compound in the mushroom that is reducing the pigs performance early in the nursery phase.

In conclusion, mushroom powder at 300 ppm appears to offer a delayed response in nursery pigs. It is worth exploring if 300 ppm is the optimal level, or if there are some compounds that are affecting it in the early nursery phase. Based on the fact it showed a late additive response suggests there are different mechanisms at work than carbadox.

References

Cheng, Y. H., Wen, C. M., Dybus, A., Proskura, W. S. Fermentation of Cordyceps militaris enhancement performance and modulate immune response of weaned piglets. South African J Anim Sci. 2016; 46:121-128. Doi:10.4314/sajas.v46i2.2.

Shen, H. S., S. Shao, J. C. Chen, T. Zhou. Antimicrobials from mushrooms for assuring food safety. Comprehensive Reviews in Food Science and Food Safety. 2017; 16. Doi: 10.1111/1541-4337.12255.

Zhou X, Luo L, Dressel W,Shadier G,Krumbiegel D,Schmidtke P, Zepp F, Meyer CU. Cordycepin is an immunoregulatory active ingredient of Cordyceps sinensis. Am J Chin Med 2008; 36:967–980. Doi:10.1142/S0192415X09006387.

Table 4-1 Basal Diet composition

Ingredient,%	**Phase 1**	**Phase 2**	**Phase 3**	**Phase 4**
Corn	36.265	42.415	46.420	53.885
SBM, CP 48%	14.000	16.950	26.500	28.925
DDGS, 7% Fat	0.000	5.000	7.500	10.000
Choice White Grease	0.000	0.000	0.000	3.000
Soybean Oil	5.000	4.000	3.000	0.000
Limestone	0.650	0.810	0.890	1.415
MonoCal Phos.	0.480	0.530	0.180	0.560
Vitamin Prx[1]	0.250	0.250	0.250	0.250
TM Prx[2]	0.125	0.125	0.125	0.125
Se Prx[3]	0.050	0.050	0.050	0.050
Phytase[4]	0.100	0.100	0.100	0.100
Salt	0.250	0.250	0.300	0.350
Plasma Protein	5.000	2.500	0.000	0.000
SD Blood Meal	1.500	1.000	0.000	0.000
Soy Conc.	5.000	3.250	0.000	0.000
Fish Meal	4.650	4.500	5.000	0.000
Dried Whey	25.750	17.150	8.600	0.000
Lysine-HCL	0.130	0.275	0.300	0.435
DL-Methionine	0.230	0.210	0.160	0.150
L-Threonine	0.060	0.110	0.110	0.140
L-Tryptophan	0.010	0.025	0.015	0.015
Banmith-48	0.000	0.000	0.000	0.100
Treatment Premix	**0.500**	**0.500**	**0.500**	**0.500**
Total	100.00	100.00	100.00	100.00
Calculated Nutrients				
ME, Kcal/kg	3541.8	3485.7	3436.8	3404.3
NE, Kcal/kg	2746.6	2661.7	2568.2	2512.3
CP, %	24.49	23.25	23.15	21.53
Total Lys, %	1.73	1.63	1.53	1.43
SID Lys	1.55	1.45	1.35	1.25
SID Met:Lys	0.55	0.53	0.50	0.44
SID M+C:Lys	0.91	0.85	0.79	0.73
SID Thr:Lys	0.97	0.91	0.84	0.78
SID Tryp:Lys	0.28	0.26	0.24	0.23
SID Iso:Lys	0.86	0.81	0.82	0.75
SID Val:Lys	1.08	0.98	0.91	0.83
Ca,%	0.85	0.85	0.80	0.75

P, %	0.76	0.72	0.62	0.55
Avail. Phos., %	0.55	0.50	0.39	0.28

[1]Provided per kg of diet available minerals: iron, 121.3 mg; zinc, 121.2 mg; manganese, 15.0 mg; copper, 11.33 mg; iodine, and 0.46 mg.
[2]Provided per kg of diet: vitamin A, 6,614 IU; vitamin D3, 661 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 9 mg; pantothenic acid, 22 mg; niacin, 33 mg and B12 38.6 mg.
[3]Provided 0.3 ppm Se
[4]Provided 600 FTU per kg of the diet

Table 4-2 Mushroom by Carbadox growth performance

Diet	NC	300 ppm Mushroom	Zinc + Copper	Carbadox	Carbadox + 300 ppm	SE	Probability, P< Mush Effect	Carb Effect	MXC Interaction	NC vs ZnCu
Pens/diet	6	6	6	6	6					
Initial Wt, kg	5.81	5.82	5.83	5.82	5.85	0.027	0.20	0.16	0.27	0.40
Day 0-7										
ADG, g	76	103	160	135	127	12.8	0.47	<0.01	0.20	<0.01
ADFI, g	113	144	187	154	145	11.3	0.35	0.08	0.10	<0.01
F:G	1.562	1.462	1.207	1.191	1.153	0.0809	0.40	<0.01	0.71	<0.01
G:F	0.670	0.700	0.842	0.857	0.873	0.0378	0.58	<0.01	0.88	<0.01
D7 BW, kg	6.35	6.53	6.94	6.76	6.74	0.095	0.39	<0.01	0.28	<0.01
Day 7-14										
ADG, g	107	95	246	162	165	14.8	0.76	<0.01	0.63	<0.01
ADFI, g	196	211	343	234	243	14.3	0.41	0.02	0.82	<0.01
F:G	1.868	2.422	1.403	1.520	1.535	0.1139	0.02	<0.01	0.03	<0.01
G:F	0.546	0.436	0.719	0.679	0.675	0.0354	0.12	<0.01	0.15	<0.01
D14 BW, kg	7.09	7.20	8.66	7.89	7.89	0.161	0.76	<0.01	0.74	<0.01

Day 14-21										
ADG, g	281	298	377	338	323	18.3	0.96	0.04	0.41	<0.01
ADFI, g	365	383	541	437	418	21.8	0.99	0.02	0.41	<0.01
F:G	1.301	1.291	1.449	1.291	1.299	0.0455	0.97	0.98	0.84	0.03
G:F	0.773	0.781	0.694	0.779	0.776	0.0259	0.91	0.98	0.84	0.04
D21 BW, kg	9.06	9.28	11.29	10.26	10.15	0.247	0.82	<0.01	0.52	<0.01
Day 21-27										
ADG, g	324	353	400	396	395	18.2	0.45	<0.01	0.41	<0.01
ADFI, g	557	603	707	645	650	18.6	0.19	<0.01	0.28	<0.01
F:G	1.719	1.726	1.779	1.632	1.651	0.0622	0.83	0.20	0.93	0.51
G:F	0.584	0.583	0.569	0.620	0.610	0.0218	0.80	0.17	0.85	0.63
D27 BW, kg	11.01	11.40	13.70	12.63	12.52	0.256	0.59	<0.01	0.33	<0.01
Day 27-33										
ADG, g	537	556	574	556	613	18.3	0.05	0.05	0.31	0.17
ADFI, g	727	758	868	772	840	15.5	<0.01	<0.01	0.26	<0.01
F:G	1.359	1.361	1.517	1.390	1.389	0.0384	0.98	0.46	0.97	<0.01
G:F	0.741	0.735	0.661	0.721	0.726	0.0209	0.98	0.50	0.79	0.01
D33 BW, kg	14.23	14.74	17.14	15.97	16.20	0.273	0.19	<0.01	0.62	<0.01
Day 21-33										

(Phase 4)

ADG, g	431	455	487	476	504	10.1	0.02	<0.01	0.85	<0.01
ADFI, g	641	680	787	709	744	14.9	0.02	<0.01	0.93	<0.01
F:G	1.487	1.498	1.615	1.484	1.481	0.0268	0.88	0.71	0.79	<0.01
G:F	0.674	0.668	0.620	0.675	0.677	0.0122	0.87	0.68	0.73	<0.01

Day 0-33

ADG, g	255	270	343	308	314	8.2	0.21	<0.01	0.58	<0.01
ADFI, g	433	463	572	492	507	12.0	0.08	<0.01	0.54	<0.01
F:G	1.696	1.715	1.670	1.599	1.616	0.0188	0.34	<0.01	0.95	0.35
G:F	0.590	0.584	0.599	0.626	0.619	0.0067	0.33	<0.01	0.98	0.37

Table 4-3 Volatile fatty acids

Diet	Negative Control	300 ppm mushroom	Carbadox	Carb + 300 Mush	Zinc + Copper	Probability, P <			
	A	B	D	E	C	SE	Mush Effect	Carb Effect	Mush X Carb	CuZn vs NC
A	75.64	78.93	77.57	74.11	71.30	3.715	0.98	0.70	0.36	0.40
P	31.97	36.64	35.23	31.56	27.71	1.914	0.79	0.63	0.04	0.12
iB	2.21	2.75	2.52	2.06	1.98	0.281	0.88	0.50	0.08	0.56
B	14.73	16.05	17.32	13.22	17.19	1.587	0.38	0.94	0.09	0.27
iV	2.58	3.60	3.34	2.45	2.36	0.438	0.88	0.65	0.04	0.72
V	3.40	4.69	3.56	2.56	2.69	0.420	0.73	0.03	0.01	0.23
Total	130.52	142.66	139.54	125.94	123.24	6.373	0.91	0.54	0.05	0.41

Table 4-4 Percent of volatile fatty acids

Diet	Negative Control	300 ppm mushroom	Carbadox	Carb + 300 Mush	Zinc + Copper	Probability, P <			
	A	B	D	E	C	SE	Mush Effect	Carb Effect	Mush X Carb	CuZn vs NC
A, %	58.21	55.22	55.71	58.86	57.96	1.466	0.96	0.71	0.05	0.91
P, %	24.45	25.78	25.26	25.04	22.48	0.804	0.49	0.97	0.33	0.09
iB, %	1.70	1.91	1.79	1.64	1.60	0.169	0.84	0.60	0.29	0.67
B, %	11.11	11.32	12.37	10.47	13.94	0.965	0.38	0.83	0.28	0.04
iV, %	1.98	2.49	2.35	1.95	1.90	0.266	0.84	0.74	0.10	0.82
V, %	2.55	3.29	2.54	2.04	2.13	0.246	0.62	0.02	0.02	0.22

Table 4-5 Blood TNF-α

	NC	300 ppm	Zn&Cu	Carbadox	Carbadox + 300	SE	Probability, P< Mush Effect	Carb Effect	MushXCarb Interaction	CuZn vs NC
TNF-α	67.41	82.66	70.11	65.93	59.47	6.588	0.48	0.06	0.08	0.76

Chapter 5 - The Effects of Cordyceps Mushroom Powder and Purified Beta-Glucan on Nursery Pig Performance

Summary

One-hundred thirty two gilts and barrows (18.2 d of age) weighing an average of 5.77 kg (Duroc X (York X Landrace)) were put on test for a 35 day growth trial. Growth performance was analyzed using body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion as feed-to-gain (F:G) and gain-to-feed (G:F) ratios. Pigs were allotted by BW and placed with 3 or 4 pigs per pen. There were 6 diets, negative and positive controls, 150 ppm beta-glucan, 150 ppm mushroom powder, 300 ppm beta-glucan, 300 ppm mushroom powder. Pigs were divided by weight, sex, litter, and assigned to BW blocks. Within BW blocks sex ratios were constant in each pen. Each pen within a BW block was then randomly assigned a treatment. Ad libitum access to feed and water was provided in each pen through a single hole wean-to-finish feeder and one nipple waterer. . Pigs and feeders were weighed on day 0, 7, 14, 21, 28, and 35. The individual body weights and pen feed intake were recorded to determine ADG, ADFI, and G:F.before submitting the data to SAS for statistical analysis using the GLM procedure. During Phase 1 (d 0-7), positive control had increased ADG, ADFI, and d7 BW ($P < 0.05$) compared to pigs fed the NC. BG + MP also differed from the NC in ADFI in Phase 1 ($P < 0.05$). During Phase 2 (d 7-14) an illness went through half of the pigs, with the other half getting the illness in Phase 3 (d 14-21). This has led to some odd values in performance because of the pigs eating, but losing weight. In phase 4 there was a statistical difference in Beta-Glucan and Mushroom powder diets, with there being an interaction between

source and dose for the MP and BG. The 300 level of mushroom improved feed efficiency, while the 300 level of Beta-glucans reduced efficiency in phase 4. Overall, there was a statistical improvement in F:G ratio in the Positive control when compared to the negative control. There was also an interaction between source and dose for ADFI ($P < 0.05$) between the BG and MP treatments. Overall there was a significant improvement in feed efficiency in MP and BG pigs compared to NC pigs ($P < 0.05$). On day 35 there were no differences in BW.

Introduction

Swine production today is reducing the use of antibiotics, following the trend of consumer desires. Antibiotics are an important aspect of swine production as disease treatment and prevention resulting in a growth promotion effect. Growth promoting antimicrobials used in feed post weaning such as carbadox are under heavy scrutiny in today's environment due to the growing concerns of antibiotic resistance. Feeding carbadox greatly improved growth performance and feed efficiency over diets containing no antimicrobial agents. This has led to research in effective alternatives to carbadox due to industry concern over potential monetary losses when attempting to produce pork under and antibiotic free program. One possible alternatives is a Chinese herbal mushroom blend of Cordyceps Militaris and Cordyceps Sinensis (Shen et al. 2017). These mushrooms have long been used by the Chinese as a health promoting additive. The mushroom itself has antimicrobial and antiviral characteristics (Zhou et al. 2008). Based on previous positive results with this product, we decided to perform an investigative study to determine if the high levels of beta-glucans present in the Cordyceps mushroom powder are responsible for a portion of the increased pig performance.

Materials and Methods

One hundred thirty two gilts and barrows (18.2 d of age) weighing an average of 5.77 kg consisting of US purebred genetics ((Duroc X (York X Landrace)) were put on test for a 35 day growth trial. Growth performance was analyzed using body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion as gain-to-feed (G:F) ratios. There were 6 diets, negative and positive controls, 150 ppm beta-glucan, 150 ppm mushroom powder, 300 ppm beta-glucan, 300 ppm mushroom powder. There were 22 pigs per diet with 3 or 4 pigs per pen. Pigs were divided by weight, sex, litter, and assigned to BW blocks. Within BW blocks sex ratios were constant in each pen. Each pen within a BW block was then randomly assigned a diet.

Pigs were provided ad libitum access to feed and water with a four or five hole nursery feeder and one nipple waterer. Feeders and waterers were checked daily, with a target of having partial pan coverage (40-50% coverage) while also minimizing feed wastage. Feeders were cleaned when feed became spoiled, and waterers adjusted to shoulder height of the pigs throughout the study. Due to cold weather and transport of the newly weaned pigs to the Segregated Early Weaning facility, all pigs were given a preventative shot of Excede. Daily checks consisted of checking feeders, waterers, observations of the pigs, filling feeders if needed, treating pigs with antibiotics when signs of disease were detected, and completing treatment records. Pigs and feeders were weighed on d 0, 7, 14, 21, 28, 35. The individual BWs and pen feed intake were recorded, data was then analyzed using the GLM procedure of SAS (SAS institute; Athens, Va.). The parameters measured were BW, ADG, ADFI and feed efficiency every week and summarized by dietary phase and overall. One blood sample per pen was taken on d 21 from the pig closest to the average pen weight.

Diets

There were six diets tested in this study. The negative control containing a 0.5% fine ground corn premix, which was then partially replaced with Cordyceps mushroom powder (Aloha Medicinals, Carson City, NV) at 150 ppm or 300 ppm, purified mushroom beta-glucan diets containing 150 ppm or 300 ppm to match the beta glucan concentration of the mushroom diets, and Positive control contained during Phase 1 and 2 38.5 ppm Tiamulin and 440 ppm Chlortetracycline. Phase 3 contained 440 ppm Chlortetracycline and Phase 4 was 110 ppm lincomycin.

The pigs were fed four dietary phases over a 35 day period. Phase 1 was d 0-7, Phase 2 was d 7-14, Phase 3 was d 14-21, Phase 4 was d 21-35. Phase 1 and 2 were made with a basal diet which was split then remixed with the treatment premix added. Phase 3 and 4 were made as individual diet treatment batches.

Feed samples for each phase were collected and store for future analysis at the Purdue University's Swine Nutrition Lab. Diet CP, energy, dry matter, ash, and phosphorus concentrations will be analyzed. Prior to analyzing the diets they were ground through a 1 mm screen. Crude protein percent will be determined by combustion using a Leco nitrogen analyzer (Leco Model 2000 CHN analyzer, Leco Corp., St. Joseph, MI, USA). Caloric content will be determined via bomb calorimeter procedure (Parr 1261 bomb calorimeter; Parr instruments Co., Moline, IL, USA). Percent dry matter and ash will be analyzed by weighing the crucible and sample on the same scale after being dried in the drying oven for 12 hours at 60 ° C, and ashed in the ashing oven for 6 hours at 500 ° C. Phosphorus will be analyzed on the diet ash by the total phosphorus colormetric method (Murphy and Riley, 1962). All samples will be analyzed in

duplicates and adjusted for standards. If the values exceeded a 5% difference the samples will be repeated until values are within 5%.

Results

During Phase 1 (d 0-7) Positive control differed from the negative control in ADG, ADFI, and d7 BW ($P < 0.05$). BG plus MP also differed from the NC in ADFI in Phase 1 ($P < 0.05$). During Phase 2 (d 7-14) an illness went through half of the pigs, with the other half getting the illness in Phase 3 (d 14-21). This has led to some odd values in performance because of the pigs eating, but losing weight. In phase 2 there was a statistical difference between the BG and MP diets in ADG, ADFI, and d 14 BW. Feed intake in Phase 2 was improved in BG, MP, and PC compared to the negative control ($P < 0.05$). There was a tendency for an interaction effect of Dose x Source of Beta-Glucans ($P < 0.10$). The Positive control increased ADG, Feed efficiency, and d 21 BW in phase 3 when compared to the NC fed pigs ($P < 0.05$). Pigs fed the Beta-Glucan and Mushroom powder diet had improved feed efficiency compared to the negative control ($P < 0.05$). In phase 4, there was a difference in Beta-Glucan and Mushroom powder diets ($P < 0.05$), with there being an interaction between source and dose for the MP and BG. The 300 ppm level of mushroom improved feed efficiency, while the 300 ppm level of beta-glucans reduced efficiency in phase 4. There was also an interaction between source and dose for ADFI ($P < 0.05$) between the BG and MP treatments. Overall MP and BG pigs had improved feed efficiency compared to NC pigs ($P < 0.05$). On day 35 there were no treatment differences for BW. For d 63 BW, BG pigs were 2.27 kg heavier at the 150 level, and 3.31 kg heavier at the 300 ppm level than the NC. There were also differences in ADG between BG and MP pigs ($P = <0.01$), as well as a trend for a difference between NC and BG & MP pigs ($P = 0.05$). For d 154 BW there was a difference in BG and MP pigs ($P = <0.01$) as well as BG & MP pigs when

compared to the NC (P = 0.03). Pigs fed the BG 300 treatment were 11.06 kg heavier than the NC, and 6.98 kg heavier than the PC. Because of this large difference in BW a difference in ADG occurred when comparing BG to MP as well as comparing BG & MP to NC from days 63 to 154 post weaning, and day 35 to 154 postweaning (P < 0.05).

With the illness affecting the pigs in this study it is difficult to draw conclusions from Phases 2 and 3 of this study. Phase 4 can also be questioned because it is unknown how much of this data is due to compensatory gain. During Phase 4 however the 300 level of BG and MP were the numerically greatest in gain at 405 and 434 g/d, respectively. The positive control pigs ended the study very poorly, this appears to coincide with the antibiotic switch from CTC to lincomix. This data indicates that the mushroom powder has a late nursery additive effect. More research needs to be conducted to determine if the mushroom powder is economical to feed in the first two weeks of the nursery phase, or if it can simply be a late nursery additive to improve performance. Due to a disease outbreak, data for this study may not be completely accurate. In Table 5-4 are the recorded treatments given during this study. Week one only had two pigs treated, so that data was not included. During week two there was a large outbreak in disease resulting in 37.2% of pigs being treated who were given the positive control treatment, up to 81.7% of pigs on the mushroom powder 150 ppm treatment requiring treatment. There was a decrease in pigs treated when comparing the BG diets compared to the mushroom powder diet (P = <0.01), as well as a source by dose interaction with the 300 concentration of each diet requiring less treatments compared to the 150 levels. During weeks 3 and 4 disease treatments were not as prevalent as week 2; however there were still a significant portion of pigs requiring treatment. In week 5 the treatments drop with no diet being above 10% in pigs treated. When looking at the overall data, there were no differences in percent of pigs treated. There were

trends for differences in total therapies given when comparing the NC and PC, as well as the BG and MP (P = 0.06, P = 0.08). There also was a trend for difference when looking at therapies per pig between the NC and PC (P = 0.08). The 300 ppm mushroom had 2.58 therapies given per pig and the lowest total of the treatments being 1.07 per pig for the positive control treatment.

Discussion

This study investigating whether the active compound of the Cordyceps mushroom powder is Beta-Glucans, or if there is something other than Beta-Glucans that is active in improving growth performance in nursery pigs. Unfortunately during this study and enteric disease broke out in these pigs, with the light block becoming sick first, then the next week the heavy block of pigs becoming sick. Due to this the data in weeks 2 and 3 are questionable at best, as well as questionable week 4 data possibly due to compensatory gain.

During this illness in week 2 ADG ranged from 54 g/d to 115 g/d, as well as feed intake and efficiency being variable, and even getting a negative feed efficiency as a result of the pigs gaining poorly. Pig performance improved in week 3. However, many pigs were still being treated. To quantify how sick these pigs were the number of treatments were recorded with 81% of mushroom 150 ppm level were treated. The positive control treatment had 37% of pigs treated during this period. The treatment records indicated that the 150 ppm treatment of both Beta-Glucan and Mushroom powder had greater treatment rates than their 300 ppm counterparts, suggesting that 300 ppm is a more effective dose at preventing and possibly recovering from illness. Treatments per pig for the positive control were 1 per pig, with the greatest number of 2.5 treatments per pig in the MP 300 diet.

Due to this illness and the nursery data being questionable, we decided to follow these pigs to market to observe whether there is a carry-over effect once out of the nursery. Pigs of the

BG 300 treatment had BW at d 154 6.98 kg greater than the positive control pigs. The positive control pigs were 4.08 kg heavier than the negative control pigs. With this illness it is worth considering this Beta-Glucan product promoted gut healing, and led to a more rapid full recovery than the other treatments.

In conclusion, the BG300 treatment increased final d 154 BW. However, due to the illness it is worth looking into repeating the study to determine if this is the response in healthy pigs. It is also worth investigating in an illness challenge to see if these results can be repeated. Purified beta-glucans from mushroom sources may have the potential to replace antibiotics in the nursery phase.

Table 5-1 Basal diet formulation

Ingredient,%	**Phase 1**	**Phase 2**	**Phase 3**	**Phase 4**
Corn	36.205	42.925	51.500	59.840
SBM, CP 48%	14.000	17.700	25.670	32.900
Choice White Grease	0.000	0.000	0.000	3.000
Soybean Oil	5.000	4.000	3.000	0.000
Limestone	0.650	0.770	0.860	1.290
MonoCal Phos.	0.480	0.640	0.490	0.800
Vitamin Prx[1]	0.250	0.250	0.250	0.250
TM Prx[2]	0.125	0.125	0.125	0.125
Se Prx[3]	0.050	0.050	0.050	0.050
Phytase[4]	0.100	0.100	0.100	0.100
Salt	0.250	0.250	0.300	0.350
Plasma Protein	5.000	2.500	0.000	0.000
SD Blood Meal	1.500	1.000	0.000	0.000
Soy Conc.	5.000	4.000	2.500	0.000
Fish Meal	4.650	4.000	4.000	0.000
Dried Whey	25.750	20.000	10.000	0.000
Lysine-HCL	0.130	0.240	0.280	0.350
DL-Methionine	0.230	0.220	0.180	0.170
L-Threonine	0.060	0.110	0.120	0.135
L-Tryptophan	0.010	0.020	0.015	0.000
Banmith-48	0.000	0.000	0.000	0.100
Copper Sulfate	0.060	0.600	0.060	0.040
Corn Premix	**0.500**	**0.500**	**0.500**	**0.500**
Total	100.00	100.00	100.00	100.00
Calculated Nutrients				
ME, Kcal/kg	3539.8	3478.6	3431.3	3400.6
NE, Kcal/kg	2745.0	2677.3	2587.8	2523.7
CP, %	24.49	22.86	22.34	21.21
Total Lys, %	1.73	1.61	1.50	1.40
SID Lys, %	1.55	1.45	1.35	1.25
SID Met:Lys	0.55	0.53	0.50	0.45
SID M+C:Lys	0.91	0.85	0.79	0.73
SID Thr:Lys	0.97	0.91	0.84	0.78
SID Tryp:Lys	0.28	0.27	0.25	0.23
SID Iso:Lys	0.86	0.82	0.83	0.77
SID Val:Lys	1.08	0.97	0.89	0.83
Ca,%	0.85	0.85	0.80	0.75

P, %	0.76	0.72	0.64	0.56
Avail. Phos., %	0.55	0.50	0.38	0.27

[1]Provided per kg of diet available minerals: iron, 121.3 mg; zinc, 121.2 mg; manganese, 15.0 mg; copper, 11.33 mg; iodine, and 0.46 mg.
[2]Provided per kg of diet: vitamin A, 6,614 IU; vitamin D3, 661 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 9 mg; pantothenic acid, 22 mg; niacin, 33 mg and B12 38.6 mg.
[3]Provided 0.3 ppm Se
[4]Provided 600 FTU per kg of the diet

Table 5-2 Beta-glucan and Mushroom powder nursery growth performance

Diet	NC	PC	BG150	BG300	MP150	MP300		Probability, P<				
	1	**2**	**3**	**4**	**5**	**6**	**SE**	**NC vs PC**	**BG vs MP**	**150 vs 300**	**Interaction Source X Dose**	**NC vs BG & MP**
Pens/diet	6	6	6	6	6	5						
Initial Wt, kg	6.08	6.09	6.05	6.06	6.06	6.06	0.630	0.92	0.94	0.89	0.91	0.68
Day 0-7												
ADG, g	107	167	106	137	105	102	15.7	<0.01	0.22	0.34	0.24	0.72
ADFI, g	154	201	185	205	189	187	13.9	0.02	0.59	0.50	0.40	0.01
F:G	1.460	1.226	1.962	1.554	1.893	2.211	0.266	0.49	0.24	0.85	0.15	0.11
G:F	0.775	0.832	0.562	0.676	0.551	0.549	0.0986	0.64	0.44	0.54	0.52	0.06
D7 BW, kg	6.83	7.26	6.78	7.02	6.79	6.76	0.595	0.02	0.32	0.41	0.29	0.94
Day 7-14												
ADG, g	100	106	109	116	85	54	27.4	0.82	0.02	0.50	0.31	0.63
ADFI, g	220	281	271	318	275	225	34.5	0.02	0.02	0.92	0.01	0.01
F:G	-1.142	4.235	2.785	3.230	5.384	-2.083	2.4556	0.10	0.56	0.14	0.10	0.18
G:F	0.493	0.376	0.388	0.361	0.290	0.123	0.1267	0.47	0.16	0.41	0.55	0.12

D14 BW, kg	7.53	8.00	7.55	7.82	7.38	7.14	0.483	0.09	0.04	0.94	0.19	0.79
Day 14-21												
ADG, g	130	233	202	186	179	161	32.6	0.02	0.43	0.58	0.97	0.13
ADFI, g	346	409	396	384	371	330	29.2	0.10	0.16	0.34	0.60	0.42
F:G	3.041	1.852	2.132	2.100	2.197	1.512	0.2951	<0.01	0.35	0.21	0.25	<0.01
G:F	0.355	0.563	0.496	0.482	0.473	0.464	0.0679	0.03	0.75	0.87	0.97	0.09
D21 BW, kg	8.44	9.63	8.97	9.13	8.64	8.25	0.551	0.02	0.11	0.77	0.46	0.44
Day 21-28												
ADG, g	261	221	317	276	255	285	43.1	0.39	0.44	0.87	0.31	0.56
ADFI, g	740	573	581	691	637	578	64.6	0.03	0.58	0.62	0.11	0.04
F:G	3.166	2.743	2.020	2.896	2.637	2.130	0.4602	0.41	0.84	0.62	0.07	0.07
G:F	0.372	0.388	0.547	0.411	0.409	0.509	0.0697	0.81	0.68	0.71	0.02	0.08
D28 BW, kg	10.27	11.18	11.18	11.06	10.42	10.24	0.731	0.18	0.11	0.75	0.95	0.39
Day 28-35												
ADG, g	464	428	462	535	521	583	41.3	0.50	0.18	0.09	0.88	0.15
ADFI, g	857	728	755	897	889	928	54.0	0.06	0.10	0.07	0.29	0.84
F:G	1.907	1.701	1.645	1.698	1.728	1.661	0.1277	0.22	0.85	0.95	0.62	0.10
G:F	0.541	0.591	0.613	0.600	0.591	0.629	0.0401	0.34	0.93	0.74	0.50	0.12

D35 BW, kg	13.52	14.17	14.42	14.81	14.07	14.31	0.867	0.40	0.46	0.59	0.89	0.16
Day 21-35 (Phase 4)												
ADG, g	363	325	390	406	388	434	34.9	0.35	0.64	0.30	0.62	0.20
ADFI, g	798	651	668	794	763	761	44.2	0.01	0.45	0.14	0.12	0.25
F:G	2.537	2.222	1.832	2.300	2.182	1.893	0.2436	0.26	0.90	0.66	0.07	0.04
G:F	0.457	0.489	0.580	0.505	0.500	0.571	0.0418	0.48	0.83	0.96	0.04	0.03
Day 0-35												
ADG, g	212	231	239	250	229	239	15.4	0.36	0.49	0.46	0.96	0.10
ADFI, g	463	438	427	499	472	452	26.1	0.44	0.97	0.27	0.06	0.98
F:G	2.208	1.911	1.830	2.012	2.069	1.921	0.1136	0.05	0.49	0.88	0.13	0.04
G:F	0.459	0.530	0.566	0.503	0.487	0.528	0.0338	0.11	0.40	0.74	0.11	0.08

Table 5-3 Beta-glucan and mushroom powder grow-finish growth performance when fed common diets

								Probability, P<				
	NC	**PC**	**BG150**	**BG300**	**MP150**	**MP300**	**SE**	**NC vs PC**	**BG vs MP**	**150 vs 300**	**Interaction Source X Dose**	**NC vs BG & MP**
Wt d 35, kg	13.85	14.07	13.76	14.59	13.90	13.42	0.689	0.81	0.45	0.80	0.33	0.93
Wt d 63, kg	30.53	31.13	32.97	34.00	30.68	30.69	1.171	0.71	0.02	0.65	0.66	0.24
Wt d 154, kg	112.46	116.54	121.13	123.52	113.68	115.04	2.397	0.23	<0.01	0.43	0.83	0.03
ADG d 35 to d 65, g	596	610	686	693	599	617	24.5	0.69	<0.01	0.61	0.83	0.05
ADG d 63 to d 154, g	899	939	969	984	912	929	19.5	0.14	<0.01	0.40	0.95	0.02
ADG d 35 to d 154	829	861	902	915	839	856	18.4	0.20	<0.01	0.37	0.90	0.02

Table 5-4 Beta-glucan and mushroom powder nursery treatments

								Probability, P<				
	NC	**PC**	**BG150**	**BG300**	**MP150**	**MP300**	**SE**	**NC vs PC**	**BG vs MP**	**150 vs 300**	**Interaction Source X Dose**	**NC vs BG & MP**
Week 2 Treatments, %	56.7	37.2	71.9	52.5	81.6	65.0	14.76	0.25	0.35	0.13	0.91	0.40
Week 3 Treatments, %	30.6	13.9	26.4	8.3	37.5	58.3	9.36	0.22	<0.01	0.88	0.05	0.84
Week 4 treatments, %	38.9	9.7	38.9	40.3	37.5	41.7	13.26	0.13	1.00	0.84	0.92	0.96
Week 5 treatments, %	8.3	4.2	5.6	0.00	0.00	4.2	3.94	0.46	0.86	0.86	0.23	0.19
Phase 4 treatments, %	47.2	13.9	38.9	40.3	37.5	41.7	12.89	0.08	1.00	0.83	0.91	0.60
Overall treatments, %	62.5	36.1	70.8	48.60	73.62	77.8	13.17	0.17	0.24	0.50	0.32	0.73
Total Therapies	6.1	2.5	6.0	3.8	6.8	7.8	1.50	0.06	0.08	0.66	0.24	0.98
Therapies/pig	2.1	1.1	2.2	1.4	2.3	2.6	0.48	0.08	0.10	0.51	0.19	0.85

Chapter 6 - Preliminary Mushroom nursery pig individually housed study

Abstract

Forty-eight weanling pigs were used for this preliminary study. Pigs were group housed by treatment and fed the Phase 1 diet for 4 days to get over the weaning stress before being isolated in individual pens. On Day 4 pigs were weighed and placed in their individual pen. Pigs were in the individual pens for 17 days. Pigs were fed a Phase 2 diet for 7 days and Phase 3 diet for 10 days (21 days total on the diets). Individual housing will provide 12 pigs per treatment for this preliminary data. Pigs were individually weighed and feed intake and feed efficiency data collected for each phase and overall. Individual pig therapeutic antibiotic treatments were recorded throughout the study. Diets included Negative Control, Oyster mushroom myceliated grain at 2.5%, oyster mushroom myceliated grain at 5%, and Positive control (carbadox at 55 ppm). Data were analyzed using the GLM procedure of SAS testing for linear and quadratic responses to the MG as well as comparing the NC to PC diets using single degree of freedom preplanned orthogonal contrasts. One pig per diet was removed from the final analysis for the NC, PC, and 5% MG treatments and 3 pigs from the 2.5% MG treatment due be outliers that had performance values greater than 2.5X standard deviation from the mean of that treatment. During group housing there was a trend for differences from day 0 to 4 between PC and NC. Once moved into individual pens, a disease broke. This made day 4-11 data unusable as some pigs lost weight and had negative feed efficiency. From day 11-21 there was a quadratic mushroom effect with the negative control and 5% myceliated grain diets outperforming the

2.5% diet in both ADG and ADFI ($P < 0.05$). This also carried the effect to the overall results, with a quadratic effect in ADG, ADFI, and G:F ($P < 0.05$). Carbadox also had a tendency for improved ADFI when compared to NC ($P = 0.085$). There was a difference in day 11 BW with the positive control pigs being heavier than the NC pigs ($P < 0.05$). There was a difference in day 21 BW with a quadratic mushroom effect, with the 2.5% diet being worse than the NC and 5% diet. Ending BW between PC and 5% Oyster mushroom were relatively similar at 10.37 and 10.18 kg each respectively.

Materials and Methods

Forty-eight pigs were weaned on Thursday June 28, 2018. Pigs were group housed by treatment and fed the Phase 1 diet for 4 days to get over the weaning stress before being isolated in individual pens. On Day 4 pigs were weighed and placed in their individual pen. Pigs were in the individual pens for 17 days. Pigs were fed a Phase 2 diet for 7 days and Phase 3 diet for 10 days (21 days total on the diets). Individual housing will provide 12 pigs per treatment for this preliminary data.

Pigs were individually weighed and feed intake and feed efficiency data collected for each phase and overall. Individual pig therapeutic antibiotic treatments were recorded throughout the study. Diets included Negative Control, Oyster mushroom myceliated grain at 2.5%, oyster mushroom myceliated grain at 5%, and Positive control (carbadox at 55 ppm).

Data were analyzed using the GLM procedure of SAS testing for linear and quadratic responses to the MG as well as comparing the NC to PC diets using single degree of freedom preplanned orthogonal contrasts. One pig per diet was removed from the final analysis for the NC, PC, and 5% MG treatments and 3 pigs from the 2.5% MG treatment due be outliers that had performance values greater than 2.5X standard deviation from the mean of that treatment.

Results

During group housing there was a trend for differences from day 0 to 4 between PC and NC. Once moved into individual pens, a disease broke. This made day 4-11 data unusable as some pigs lost weight and had negative feed efficiency. From day 11-21 there was a quadratic mushroom effect with the negative control and 5% myceliated grain diets outperforming the 2.5% diet in both ADG and ADFI ($P < 0.05$). This also carried the effect to the overall results, with a quadratic effect in ADG, ADFI, and G:F ($P < 0.05$). Carbadox also had a tendency for improved ADFI when compared to NC ($P = 0.09$). There was a difference in day 11 BW with the positive control pigs being heavier than the NC pigs ($P < 0.05$). There was a difference in day 21 BW with a quadratic mushroom effect, with the 2.5% diet being worse than the NC and 5% diet. Ending BW between PC and 5% Oyster mushroom were relatively similar at 10.37 and 10.18 kg each respectively.

Discussion

The purpose of this study was to determine if nursery pigs would eat this diet. This was a preliminary study for future work in feeding myceliated grains of various mushrooms to pigs. It appears the pigs will eat the diets, however with the early disease outbreak there is not much data on how they will perform on the diets. Myceliated grains are commonly just cereal grains that the mushrooms are grown on, and mushrooms lay some of their bioactive compounds in their root structure. If effective this could be an effective alternative to feeding the whole mushroom, which will have competition with humans for food.

In conclusion, pigs will eat these diets, however this area of research requires much more investigation to determine if it is feasible to feed on a large commercial scale, and if it is possibly a replacement for antibiotics.

Table 6-1 basal diet formulations

Ingredient, %	**Phase 1**	**Phase 2**	**Phase 3**
Duration	**4 d**	**7 d**	**10 d**
Corn NRC 2012	36.765	42.915	46.920
SBM NRC 12	14.000	16.950	26.500
DDGS - Rens. 7% fat	0.000	5.000	7.500
Choice White Grease	0.000	0.000	0.000
Soybean Oil	5.000	4.000	3.000
Limestone NRC 12	0.650	0.810	0.890
MonoCal NRC 12	0.480	0.530	0.180
Vitamin	0.250	0.250	0.250
TM	0.125	0.125	0.125
Selenium Premix	0.050	0.050	0.050
Phytase	0.100	0.100	0.100
Salt	0.250	0.250	0.300
Plasma Protein	5.000	2.500	0.000
SD Blood Meal	1.500	1.000	0.000
Soy Conc.	5.000	3.250	0.000
Fish Meal	4.650	4.500	5.000
Dried Whey	25.750	17.150	8.600
Lysine-HCL	0.130	0.275	0.300
DL-Methionine	0.230	0.210	0.160
L-Threonine	0.060	0.110	0.110
L-Tryptophan	0.010	0.025	0.015
Treatments of 2.5 and 5.0% replaced Corn	**0.000**	**0.000**	**0.000**
Total	100.000	100.000	100.000

Table 6-2 Oyster mushroom growth performance

Diet	Neg. Cont. (NC)	NC+2.5% MG	NC+ 5.0% MG	Pos. Control (PC)	SE	Linear Mush.	Quad. Mush.	NC vs PC
Pigs/Trt	11	9	11	11				
d0 BW, kg	6.09	6.20	6.06	6.05	0.053	0.67	0.06	0.47
d4 BW, kg	6.44	6.44	6.45	6.55	0.054	0.93	0.99	0.13
d11 BW, kg	6.50	6.18	6.47	7.18	0.191	0.91	0.18	0.01
d21 BW, kg	9.49	8.44	10.18	10.37	0.567	0.34	0.04	0.22
Day 0-4								
ADG, g/d	87	61	96	125	17.2	0.69	0.14	0.08
ADFI, g/d	71	75	83	83	--	--	--	--
Day 4-11								
ADG, g/d	9	-37	4	91	25.2	0.88	0.15	0.02
ADFI, g/d	163	112	159	232	17.7	0.85	0.02	<0.01
G:F	-0.060	-0.365	-0.013	0.340	0.1432	0.80	0.06	0.03
Day 0-11								
ADG, g/d	37	-2	37	103	18.9	0.99	0.09	0.01
ADFI, g/d	129.7	98.4	131.1	177.8	11.25	0.92	0.02	<0.01
G:F	0.212	-0.018	0.266	0.549	0.1356	0.74	0.12	0.05
Day 11-21								
ADG, g/d	299	226	371	319	43.1	0.19	0.04	0.71
ADFI, g/d	444	336	506	511	49.4	0.28	0.02	0.25
G:F	0.661	0.649	0.731	0.624	0.0613	0.37	0.52	0.63
Day 0-21								
ADG, g/d	162	107	196	206	27.6	0.33	0.03	0.21
ADFI, g/d	279	211	310	337	25.7	0.35	0.01	0.09
G:F	0.640	0.470	0.632	0.586	0.0526	0.94	0.02	0.47