RESEARCH REPORT
ANIMAL SCIENCE

Title: Critical evaluation of a new novel bio-fuels byproduct "MycoMeal" on growth performance, body composition and intestinal health in nursery pigs – **NPB #12-112**

Investigator: Nicholas Gabler

Institution: Iowa State University

Date Submitted: July 16, 2013

Industry Summary. The thin stillage leftover from ethanol production contains biodegradable organic compounds and sufficient micronutrients that an ideal feedstock for fungal cultivation such as *Rhizopus microsporus* variant *oligosporus.* The fungus removes about 60% of the organic material, including the suspended solids and even more of some specific substances that are undesirable for recycling. Then the fungal pellets can easily be harvested as a food-grade organism (RO), which is rich in fat and protein (specifically the amino acids lysine and methionine). Thus, this value added byproduct may be a suitable feed ingredient for swine nutrition. Additionally, RO may also have some added health benefits. The objectives of the proposed research were: (1) evaluate the effects of RO on nursery pig growth performance, tissue accretion rates and total tract digestibility; and (2) evaluate potential intestinal health and function benefits that would facilitate long-term growth, caloric efficiency and pig viability. Each of these objectives was completed in nursery pigs. Please contact Nicholas Gabler at Iowa State University for further detail (ngabler@iastate.edu).

Keywords: Alternative feed stuff, Digestibility, *Rhizopus microsporus*, Tissue Accretion

Scientific Abstract. Fluctuations in feed prices have led pig producers to search for alternative feed ingredients. Our previous studies have suggested that the filamentous fungus *Rhizopus microsporus* variant *oligosporus* (RO), grown in the leftovers of ethanol production can potentially be used as a high quality source of dietary protein, fat, vitamins and minerals for growing pigs. The objective of this study was to evaluate the bioavailability of lysine (Lys) in RO for nursery pigs. A total of 32 gilts (initial BW (6.5±0.25 kg) were individually penned and assigned to 5 dietary treatments. A basal diet (n=5) formulated to contain 8 g of lysine/kg, but adequate in all other amino acids, was supplemented with 2 and 4 g of lysine/kg from either RO (n =14) or L-lysine-HCl (n =13). Diets were formulated to contain 15.4 MJ/kg of DE. Average daily gain (ADG) and feed intake (ADFI), gain to feed ratio (G:F) and lean growth (LG) were determined over a 7 week period. The latter was determined using dual-energy X-ray absorptiometry. Bioavailability of Lys was estimated using the slope ratios (RO:L-lysine-HCl) obtained from common-intercept multiple regression analyses. Fecal and ileal digesta samples were collected to determine ATTD & AID. Daily feed intake was similar among

treatment groups (P > *0.10*). Average daily gain, LG and G:F response to dietary Lys was linear regardless of Lys source (P < *0.01*). Results show no significant difference between Ref-1 and RO-1 for AID or ATTD except for Nitrogen ATTD (83.1 v. 81.7; P < 0.02). Results for Ref-2 and RO-2 show significant differences in all parameters, although AID differences are much higher. Specifically, lysine AID (86.9 v. 72.3; P <0.01) nitrogen AID (77.1 v. 62.5; P <0.02) and energy AID (81.6 v. 62.7; P <0.01) and nitrogen ATTD (81.8 v. 78.8; P <0.02). The bioavailability of Lys in RO for ADG, G: F and LG was 0.54, 0.61 and 0.69, respectively. Digestibility and bioavailability of the RO biomass may be affected by fungal chitin and chitosan concentrations. Altogether, these results suggest that RO can be used as a source of protein and energy in the diet of nursery pigs.

Introduction. Corn ethanol plants in the US currently have a production capacity of 13 billion gallons of ethanol per year (EIA, 2012). Corn used in ethanol production competes directly with its use as animal feed as ethanol production primarily relies on corn starch fermentation process. Once the ethanol is removed, the leftover protein, lipid and fiber solids can be sold as wet or dried distiller's grains. Likewise, the remaining liquid fraction, thin stillage, can be evaporated into a condensed distiller's syrup, which can be sold separately or added back to distiller's grains (DDGS). However, two of the shortfalls in ethanol production, outside of corn usage, are the large inputs of water and energy. The industry consumes about as much energy as is contained in the ethanol fuel and about 45 billion gallons of water in producing this amount of ethanol (Aden, 2007).

DDGS is used for livestock feed, but is low in essential amino acids, e.g., lysine, limiting its usage (NRC 2012), particularly for swine and poultry. A review (Stein and Shurson, 2009), showed that DDGS can be formulated up to 30% in weanling pig, and grow-finish diets, although gestating sows can be up to 50%. Further summarized, DDGS fed pigs may have lower dressing percentages, but primary carcass traits such as backfat thickness, and loin depth are not affected (Stein and Shurson, 2009). Belly firmness is much softer due to the higher percentages of unsaturated fat within DDGS that results in increased iodine levels for the carcass fat. To counter this, addition of conjugated linoleic acid in the diet before slaughter may partially lower the iodine value (White et al., 2009). Work by Xu (Xu et al., 2010) demonstrated lower iodine values when pigs were removed from DDGS at least 3 weeks prior to slaughter. Both options present ways for producers to mitigate lower costs on pigs sold to slaughter. Previous work (Whitney et al., 2006b) showed that 10% DDGS based diet decreased lesion length and prevalence in the ileum and colon, similar to antibiotic treated animals, when moderately challenged with *Lawsonia intracellularis*. However, when the challenge was more severe, there was no mediation by DDGS inclusion (Whitney et al., 2006a). So DDGS inclusion into the diet has a mixed effect on gut health in challenged conditions.

New technologies are emerging in which the water and thin stillage can be recycled back into the fermentation process. One technology is the use of fungus cultivars such as *Rhizopus oligosporus.* Thin stillage contains biodegradable organic compounds, sufficient micronutrients and is somewhat acidic, making it an ideal feedstock for fungal cultivation. The fungal organism, *Rhizopus microsporus* var. *oligosporus*, is a white rot fungus that breaks down fiber within the stillage and also metabolizes glycerol, acetic and lactic acid. The resulting residual biomass can easily be separated from the liquid fraction, dried and used as a value added byproduct. This fungal biomass (RO), which is commonly used in varieties of tempeh, is rich in fat and protein, but contains a higher amount of limiting amino acids (lysine, tryptophan, threonine and methionine) when compared to corn or DDGS (Table 1). The biomass is also rich in phosphorus and contains additional complex polysaccharides such as chitin, chitosan, and β-glucans (Rop et al., 2009), which may have health promoting benefits (Friedman and Juneja, 2010). This fungal product represents a possible alternative to other feed supplements currently on the market. However, it is first necessary to characterize the biomass to determine its effects on growing pigs and on intestinal health.

Table 1. Ingredient comparison, %

Item	Corn[1]	DDGS[1]	RO[2]
Crude Protein	9.4	30.1	36.1
Lysine	0.27	0.66	1.54
Tryptophan	0.06	0.25	0.27
Threonine	0.29	0.94	1.10
Methionine	0.17	0.50	0.21
NDF	9.5	33.5	21.1
Starch	70	4	2.5
Crude Fat	4.2	13.4	26.0
Ash	1.5	5.1	5.1
Phosphorus	0.3	0.9	1.3

[1]Based on Swine NRC (2012)
[2]Our own chemical analysis

Objectives. The overall objective of this proposal was to evaluate the nutritional value of fungus *Rhizopus microsporus* var. *oligosporus* grown on thin stillage (RO), from the ethanol process, as a novel swine feed ingredient, for growing pigs. We also aimed to understand the physiologically, the effects of RO on nursery pig health, nutrient and energy retention and tissue accretion rates. Additionally, we determined the lysine bioavailability of RO. This was addressed by completing the following two specific objectives:

Obj. #1. To evaluate the effects of feeding different levels of RO on apparent ileal and fecal digestibility of dietary nutrients, growth performance, and tissue accretion rates in nursery pigs.

Obj. #2. To evaluate the impact of RO on intestinal health and physiology.

Materials & Methods. *Study design and animals.* All animal procedures were approved by the Animal Care and Use Committee of Iowa State University (IACUC# 5-11-7152-S). To address our two specific aims, two nursery pig experiments were conducted.

Experiment 1
In experiment 1, a growth performance study using three inclusion rates of RO was conducted. A total of 24 gilts (5.62 ± 0.35 kg BW) were assigned to one of three treatments (8 pigs/trt). Treatments were based on a typical corn-soybean meal (SBM) diet with inclusion of RO, in place of corn-SBM, at 0, 10 and 20% (Table 2). All diets were formulated to contain the same amount of SID lysine and be iso-caloric. The inclusion of RO was at the expense of soybean meal, corn or soybean oil. The digestibility marker titanium dioxide was mixed in each diet for fecal digestibility analysis. After a four day acclimation to individual pens and diets, a four week growth performance and feed intake study was conducted. All pigs had free access to water and ad libitum feed. Weekly body weights and feed intakes were recorded and G:F calculated. During the fourth week and over three days, total feces from each pig were collected and pooled within pig for determining apparent total tract nutrient and energy digestibility of RO.

Proximate analysis was carried out on feed and pooled fecal samples as previously described (Harris et al., 2012; Patience et al., 2009). Titanium dioxide (Modified method of Leone, 1973) and phosphorus (AOAC 7.123) contents of diet and feces were determined using colorimetric assays. Dry matter (DM), Nitrogen (N, Kjeldahl method), ether extract (EE, Soxhlet method) and energy (DE, calorimetry) digestibility coefficients were calculated.

Experiment 2

The objective of this experiment was to measure tissue accretion rates and lysine bioavailability of RO in nursery pigs. Thirty eight gilts (6.5 ± 0.25 kg BW) were individually penned and assigned to one of five dietary treatments (Table 3). A basal diet (n=11) formulated to contain 8 g of lysine/kg, but adequate in all other amino acids, was supplemented with 2 and 4 g of lysine/kg from either RO (RO-1 n=7, RO-2 n=7, respectively) or L-lysine-HCl (Ref-1 n=6, Ref-2 n=7, respectively). Diets were formulated to contain 15.4 MJ/kg of DE (Table 3). All pigs had free access to water and were fed at 90-95% of ad libitum feed intake. After a four day acclimation period, weekly growth rates and feed intake were recorded for seven weeks and ADG, ADFI and G:F determined. Total tract fecal grab samples were taken in the final week for proximate and titanium dioxide analysis. At the end of the experiment, all pigs were euthanized via overdose of sodium pentobarbital and ileal contents were collected from the last meter of ileum prior to the ileal-cecal junction for apparent ileal dry matter, energy and amino acid analysis. Amino acid analysis was conducted by the University of Missouri Experimental Station and Chemical Laboratories. Feed, ileum contents and fecal samples were analyzed as described above in Experiment 1 for N, DM, titanium dioxide and energy.

Additionally at slaughter, freshly isolated ileum, 1 m from the distal region, and mid cecum were mounted in modified Ussing Chambers to assess ex vivo nutrient transport and intestinal barrier permeability. Barrier permeability was measured by transepithelial resistance (TER) and FITC-labeled dextran (4.4 kDa) permeability as previously described (Mani et al., 2013a; Mani et al., 2013b; Pearce et al., 2012). Formalin fixed ileum tissue was also assessed for villus height and crypt depth as described by Gabler et al., (2007).

To determine the effects of RO inclusion and lysine bioavailability on tissue accretion in our nursery pigs, a serial slaughter approach was used. Briefly, after the four day acclimation period, six initial slaughter group pigs (ISG) were selected from the basal diet and euthanized. The final slaughter group (FSG) consisted of the 32 pigs fed the basal, Ref-1, Ref-2, RO-1 and RO-2 diets minus 10 cm of ileum and cecum. All euthanized pigs where then body scanned using a Hologic Discovery A Dual X-ray Absorptiometry (DXA) machine to determine body composition. Scan data was then corrected using internally built calibration curves as described by Suster et al., (2003). The DXA scan data provided whole body lean, bone and fat mass. Tissue accretion was measured using the net change between the treatment FSG and basal ISG body composition divided by the days on test.

Growth assays using the slope-ratio methodology was used to determine the bioavailability of lysine in RO. Briefly, common-intercept, multiple linear regression and slope-ratio techniques (Finney, 1978) were used to estimate availability of lysine in the RO diets in Experiment 2. This analysis tested for linearity of the slopes and lack of curvature (essential requirement for statistical validity) and for intersection of responses to reference (L-lysine reference diets) and test (RO-L-lysine) diets at the basal response (basal diet).

Statistical analysis

Data was analyzed using MIXED procedure in SAS (SAS Inst. Inc., Cary, NC) and all results were expressed as LS means ± SEM. The model included fixed effect of treatment; with random effects of day and rep. Statistical significance of differences was determined by Tukey's range test for pair wise comparisons. Differences were deemed significant at $P \leq 0.05$ and tendencies at $P \leq 0.10$. Slope-ratios were determined using multiple regression analyses conducted in SAS 9.2. Average daily gain, lean tissue gain, G:F and lysine intake were partitioned to estimate the attribution of supplemental lysine from RO diets to lysine intake alone. A linear relationship was assumed between ADG, G:F, lean accretion and lysine intake for the pigs that received the

basal diet and references diets. Approximate standard errors of the bioavailability estimates were calculated as outlined by Littell et al., (1995).

Results

Experiment 1

Results from the growth performance data in which RO was formulated in nursery pig diets is shown in Table 4. There was no difference in ADFI, ADG, or G:F ratio between the three diets. These data indicated that inclusion of RO in the nursery diet up to 20% in place of corn, soybean meal and soy oil had no negative effect on pig performance in this four week study. However, ATTD coefficients (Table 4) for energy, nitrogen, fat and phosphorus were all reduced by the 20% RO inclusion compared to the control diet ($P < 0.05$). Digestible energy (DE) values were significantly improved when feeding 10% RO (4.16 Mcal/kg), but not 20% RO (3.81 Mcal/kg), versus the control treatment (4.01 Mcal/kg). The results of this first experiment demonstrated that RO may be a viable feed source for nursery diets at low inclusion rates with no adverse effects on pig performance.

Experiment 2

Experiment 2 was designed to assess the lysine bioavailability of RO biomass in nursery pig diets. When compared to the basal diet, increasing lysine concentrations by the addition of RO, treatments RO-1 and RO-2 increased ADG and end body weights of pigs (Table 5, $P < 0.05$). However, this performance was not equal to those observed in Ref-1 and Ref-2 ($P < 0.05$). There was no difference in starting BW ($P=0.82$) and ADFI ($P=0.45$) as pigs were feed restrictively by design during the project. Compared to the basal diet, nursery pig G:F was significantly improved with the Ref-1 and 2 diets ($P<0.01$). However, the RO fed pigs were less feed efficient than their Reference (Ref-1, 2) counterparts. The DXA predicted body composition and tissue accretion rates are shown in Table 6. These data yielded no treatment difference for bone mineral density ($P=0.26$). However, significant differences were reported for fat, lean and bone mineral content (BMC) mass between the diets at the end of the experimental period ($P<0.05$, Table 6). The final body composition bone mineral density was also higher in the Ref-2 treatment compared to the basal diet ($P=0.04$), but not different from Ref-1, RO-1 and RO-2. Interestingly, RO fed pigs had reduced fat mass and body fat percentage at either level compared to the basal and both Ref diets ($P<0.01$). As expected, whole body lean mass was increased with increasing dietary lysine intake (basal<Ref-1<Ref-2, $P<0.01$). However, whole body lean tissue and protein accretion rates were not different between the Ref-1, RO-1 and RO-2 treatments ($P>0.05$) and highest for the Ref-2 diet ($P<0.05$, Table 6).

These reductions in growth rates due to increasing RO inclusion can be attributed to decreased digestibility of nutrients and energy (Table 7). Apparent ileal digestibility (AID) showed numerical differences within each diet pair (RO-1/Ref-1 and RO-2/Ref-2). However, no statistical difference was noted between RO-1, and Ref-1, while there was a statistical difference was detected between RO-2 and Ref-2 treatments ($P<0.05$). In regards to specific lysine, threonine, methionine and tryptophan AID, there was no significant difference between basal, RO-1 and Ref-1, but RO-2 had an approximate 10-20 reduction in AID coefficients compared to Ref-2. These data were reaffirmed by the ATTD coefficients for nitrogen and energy (Table 7). Increasing RO inclusion resulted in lower nitrogen and energy digestibility coefficients compared to their reference diets ($P<0.05$).

The impact of RO inclusion on ileum and cecum morphology and ex vivo ileum nutrient transport and integrity is shown in Table 8. No difference in ileal nutrient transport for glucose ($P=0.86$), glutamate ($P=0.35$), and lysine ($P=0.65$) were observed in our modified Ussing chamber measures. Transepithelial resistance (TER), a sign of intestinal integrity, also showed no difference between RO-2 and Ref-2 ileum samples, although RO-1 did have a significantly lower TER ($P<0.01$). However, no differences were observed in cecal TER ($P=0.97$). Additionally, intestinal permeability assessed by the macromolecule flux of 4.4 kDa FITC-labeled dextran in both ileum and cecum was not different ($P>0.05$). Ileum morphology measures of villus height, crypt depth and villus:crypt ratio were not different with RO inclusion ($P>0.05$, Table 8).

Finally, using the growth performance and tissue accretion data, we were able to calculate the bioavailability of lysine within RO for ADG (Figure 1), G:F (Figure 2) and lean gain (LG, Figure 3) by slope-ratio methods. In response to dietary lysine, the slopes were all linear regardless of lysine source (synthetic Ref or RO, $P<0.01$). The bioavailability of lysine from RO inclusion for nursery pigs was estimated for ADG, G:F and LG to be 54, 61, and 69% respectively.

Discussion

This study assessed the use of RO biomass, grown from the conversion of corn millings waste stream, as a high value fungal protein and energy source in pig nursery diets. An attached growth fungal system was used to effectively convert corn wet-millings into a high value fungal biomass (Jasti et al., 2008). Compared to corn and DDGS, the resulting RO biomass ingredient is high in essential amino acids, fat, energy and vitamins and minerals (Table 1). However, its use in livestock and swine feed has not been evaluated. The data presented herein, showed that RO biomass inclusion on nursery pig diets has potential to be a good source of amino acids and energy in a nursery-growing pig diets. Our data showed that RO biomass inclusion did not affect feed intake, growth rates or feed efficiency when substituted for corn and SBM (Experiment 1, Table 4). However, performance, and particularly ADG, was affected when RO biomass provided the primary lysine source (Experiment 2, Table 5).

The relative bioavailability of lysine was estimated using slope-ratio methodology in which body weight gain, G:F and lean tissue accretion was regressed on lysine intake from L-lysine and RO biomass. The ratio of slopes indicated a relative lysine bioavailability for RO biomass of 54% for ADG (Figure 1). This is lower than what has been reported for soybean meal [(85-88%, (Adeola et al., 1994)], corn DDGS [67%, (Lumpkins and Batal, 2005)], fishmeal (80.3%) and spray-dried plasma (67.8%) as reported by Kim and Easter (2001). The estimated RO biomass lysine bioavailability was 61% for G:F (Figure 2) and 70% for lean tissue growth (Figure 3) compared against the synthetic L-lysine-HCl. This is 10-20% lower than that of soybean meal. In poultry the lysine bioavailability of corn has been determined at 73% in terms of gain and true ileal digestibility of 78% (Lewis and Bayley, 1995). The swine SID for lysine in corn has been shown to be 74%, 61% for corn DDGS and 86-90% for soybean meal (NRC, 2012). This suggests that lysine bioavailability in corn and DDGS is similar to that of the RO biomass, but lower than soybean meal.

The reduced lysine bioavailability of the RO biomass was also evident in the lower lean and adipose tissue accretion rates compared to Ref treatment accretion rates. Based on the digestibility and bioavailability data, these results would be expected as lysine is considered the first limiting amino acid. The reduction in AID and ATTD coefficients for RO is likely attributed to the presence of fungal chitin and chitosan derivatives in the fungal cell walls. Fermenting yeasts like *Saccharomyces cerevisiae*, used in ethanol fermentation, only have 1-2% of chitin in their cell walls, whereas filamentous fungi like *Rhizopus microsporus* may have closer to 15% chitin (Free, 2013). These complex polysaccharides cannot be easily digested by pigs. However, it has been reported that pigs may have the capacity for digesting chitin, because chitinase may be produced in considerable amounts by the microorganisms of the gastrointestinal tract (Fanimo et al., 2006). In particular, *Bacillus subtilis* in the large intestine is a known chitinase producer. Therefore, the lower digestibility (AID and ATTD) we observed in our experiments is likely the result of energy and amino acid digestibility being affected due to lower luminal RO degradation in the small intestines. We also report no negative effects of RO supplementation on intestinal morphology, integrity, health or active nutrient transport. Interestingly, it has been shown that chitosan, the partially deacetylated form of chitin, which is high in RO biomass as a component of fungal cell walls, can alter intestinal integrity via modification of intestinal tight junction proteins between cells and increasing permeability (Rosenthal et al., 2012; Yeh et al., 2011).

Altogether, as RO biomass inclusion rates increase in nursery pig diets, lysine bioavailability and nutrient and energy digestibility is reduced. This is likely due to the high content of chitin and chitosan in the RO biomass inhibiting digestion of nutrients and energy. Further research is needed to determine if exogenous enzymes could be fed to enhance digestion, absorption and bioavailability. However, feeding RO biomass in substitution

of corn and soybean meal has no detrimental effects on pig growth performance if energy and lysine is not limiting. The RO biomass may be a good source of amino acids and energy if the fungal cell walls can be more easily digested.

References.

Aden, A. 2007. Water Usage for Current and Future Ethanol Production Southwest Hydrology No. 6. p 2. SAHRA, Arizona.

Adeola, O., B. V. Lawrence, and T. R. Cline. 1994. Availability of Amino Acids for 10- to 20-Kilogram Pigs: Lysine and Threonine in Soybean Meal. J. Anim. Sci 72: 2061-2067.

EIA. 2012. Annual Energy Review 2011. In: U. S. E. I. Administration (ed.). p 287.

Fanimo, A. O., A. Susenbeth, and K.-H. Sudekum. 2006. Protein utilisation, lysine bioavailability and nutrient digestibility of shrimp meal in growing pigs. Animal Feed Science and Technology 129: 196-209.

Finney, D. J. 1978. Statistical Methods in Biological Assay. 3rd ed. Charles Griffin & Co., London.

Free, S. J. 2013. Fungal Cell Wall Organization and Biosynthesis. In: T. Friedmann, J. C. Dunlap and S. F. Goodwin (eds.) Advances in Genetics No. 81. p 33-82. Elsevier Inc.

Friedman, M., and V. K. Juneja. 2010. Review of Antimicrobial and Antioxidative Activities of Chitosans in Food. Journal of Food Protection 73: 1737-1761.

Gabler, N. K., J. D. Spencer, D. M. Webel, and M. E. Spurlock. 2007. In utero and postnatal exposure to long chain (n-3) PUFA enhances intestinal glucose absorption and energy stores in weanling pigs. J Nutr 137: 2351-2358.

Harris, A. J., J. F. Patience, S. M. Lonergan, J. C. M. Dekkers, and N. K. Gabler. 2012. Improved nutrient digestibility and retention partially explains feed efficiency gains in pigs selected for low residual feed intake. J Anim Sci 90: 164-166.

Jasti, N., S. K. Khanal, A. L. Pometto, 3rd, and J. H. van Leeuwen. 2008. Converting corn wet-milling effluent into high-value fungal biomass in a biofilm reactor. Biotechnology and bioengineering 101: 1223-1233.

Kim, S. W., and R. A. Easter. 2001. Nutritional value of fish meals in the diet for young pigs. J Anim Sci 79: 1829-1839.

Lewis, A. J., and H. S. Bayley. 1995. Amino Acid Availability. In: C. B. Ammerman, D. H. Baker and A. J. Lewis (eds.) Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. p 35-60. Academic Press, San Diego.

Littell, R. C., A. J. Lewis, and P. R. Henry. 1995. Statistical evaluation of bioavailability assays. In: C. B. Ammerman, D. H. Baker and A. J. Lewis (eds.) Bioavailability of Nutrients for
Animals: Amino Acids, Minerals, and Vitamins. p 5-33. Academic Press, New York.

Lumpkins, B. S., and A. B. Batal. 2005. The bioavailability of lysine and phosphorus in distillers dried grains with solubles. Poultry science 84: 581-586.

Mani, V., A. J. Harris, A. F. Keating, T. E. Weber, J. C. Dekkers, and N. K. Gabler. 2013a. Intestinal integrity, endotoxin transport and detoxification in pigs divergently selected for residual feed intake. J Anim Sci. 10.2527/jas.2012-6053.

Mani, V., J. H. Hollis, and N. K. Gabler. 2013b. Dietary oil composition differentially modulates intestinal endotoxin transport and postprandial endotoxemia. Nutrition & metabolism 10: 6.

NRC. 2012. Nutrient Requirements of Swine. 11th Ed. ed. Natl. Acad. Press, Washington, DC.

Patience, J. F., A. D. Beaulieu, and N. H. Williams. 2009. Response to dietary digestible energy concentration in growing pigs fed cereal grain-based diets. Journal of Animal Science 87: 965-976.

Pearce, S. C., V. Mani, R. L. Boddicker, J. S. Johnson, T. E. Weber, J. W. Ross, L. H. Baumgard, and N. K. Gabler. 2012. Heat stress reduces barrier function and alters intestinal metabolism in growing pigs. J Anim Sci 90 Suppl 4: 257-259.

Rop, O., J. Mleck, and T. Jurikova. 2009. Beta-glucans in higher fungi and their health effects. Nutrition Reviews 67: 624-631.
Rosenthal, R., D. Gunzel, C. Finger, S. M. Krug, J. F. Richter, J. Schulzke, M. Fromm, and S. Amasheh. 2012. The effect of chitosan on transcellular and paracellular mechanisms in the epithelial barrier. Biomaterials 33: 2791-2800.
Stein, H. H., and G. C. Shurson. 2009. The use and application of distillers dried grains with solubles in swine diets. J. Anim. Sci 87: 1292-1303.
Suster, D., B. J. Leury, E. Ostrowska, K. L. Butler, D. J. Kerton, J. D. Wark, and F. R. Dunshea. 2003. Accuracy of dual energy X-ray absorptiometry (DXA), weight and P2 back fat to predict whole body and carcass composition in pigs within and across experiments. Livestock Prod Sci 84: 231-242.
White, H. M., B. T. Richert, J. S. Radcliffe, A. P. Schinckel, J. R. Burgess, S. L. Koser, S. S. Donkin, and M. A. Latour. 2009. Feeding connugated linoleic aicd partially recovers carcass quality in pigs fed dried corn distillers grains with solubles. J. Anim. Sci 87: 157-166.
Whitney, M. H., G. C. Shurson, and R. C. Guedes. 2006a. Effect of dietary inclusion of distillers dried grains with solubles on the ability of growing pigs to resist a *Lawsonia intracellularis* challenge. J. Anim. Sci 84: 1860-1869.
Whitney, M. H., G. C. Shurson, and R. C. Guedes. 2006b. Effect of including distillers dried grains with solubles in the diet, with or without antimicrobial regimen, on the ability of growing pigs to resist a *Lawsonia intracellularis* challenge. J. Anim. Sci 84: 1870-1879.
Xu, G., S. K. Baidoo, L. J. Johnston, D. Bibus, J. E. Cannon, and G. C. Shurson. 2010. The effects of feeding diets containing corn distillers dried grains with solubles, and withdrawal period of distillers dried grains with solubles, on growth performance and pork quality in grower-finisher pigs. J. Anim. Sci 88: 1388-1397.
Yeh, T., L. Hsu, M. T. Tseng, P. Lee, K. Sonjae, Y. Ho., and H. Sung. 2011. Mechanism and consequence of chitosan-mediated reversible epithelial tight junction opening. Biomaterials 32: 6164-6173.

Table 2. Experiment #1 diet composition, as fed basis

Ingredient, %	**0%RO**	**10%RO**	**20%RO**
Corn	65.35	59.54	53.42
Soybean meal, 48% CP	20.70	18.70	16.71
Whey-dried	5.00	5.00	5.00
Soybean Oil	3.50	1.70	-
Fishmeal-mhdn	2.50	2.50	2.50
Monocalcium phosphate	0.76	0.28	-
Limestone	0.66	0.87	1.05
Sodium chloride	0.35	0.35	0.35
Vitamin premix[1]	0.30	0.30	0.30
L-Lysine HCl	0.19	0.13	0.07
Trace mineral premix[2]	0.10	0.10	0.10
L-Threonine	0.05	0.01	-
DL-Methionine	0.04	0.02	-
Rhizopus oligosporus (RO)	-	10.00	20.00
Titanium dioxide	0.50	0.50	0.50
Calculated composition			
Crude Protein, %	19.02	21.43	23.83
ME, kcal/kg	3880	3888	3894
NDF, %	8.54	9.59	10.62
Lysine (SID. %)	1.11	1.11	1.11
Available Phosphorus, %	0.32	0.32	0.36

[1]Supplied per kg of diet: vitamin A, 6,600 IU; vitamin D_3, 880 IU; vitamin E, 44 IU; vitamin K (menadione sodium bisulfate complex), 6.4 mg; thiamin, 4.0 mg; riboflavin, 8.8 mg; pyridoxine, 4.4 mg; vitamin B12, 33 µg; folic acid, 1.3 mg; niacin, 44 mg.

[2]Supplied per kg of diet: Zn, 131 mg as ZnO; Fe, 131 mg as $FeSO_4 \bullet H_2O$; Mn 45 mg, as MnO; Cu, 13 mg as $CuSO_4 \bullet 5H_2O$; I, 1.5 mg as $CaIO_6$; Co, 0.23 mg as $CoCO_3$; Se, 0.28 mg as Na_2O_3Se.

Table 3. Experiment #2 diet composition, as fed basis

Ingredients, %	**Basal**	**Ref-1**	**Ref-2**	**RO-1**	**RO-2**
Rhizopus oligosporus (RO)	0	0	0	10.5	21
Corn (NRC)	51	51	51	51	51
Cornstarch	15.9	15.7	15.5	8	0
Whey, dried	5	5	5	5	5
Soya oil	5	5	5	2.5	0
Fishmeal, herring	3	3	3	3	3
Soybean meal 48% CP	15	15	15	15	15
Lysine HCl	0.02	0.24	0.45	0.02	0.02
DL-Methionine	0.27	0.27	0.27	0.27	0.27
Threonine	0.28	0.28	0.28	0.28	0.28
Tryptophan	0.09	0.09	0.09	0.09	0.09
Isoleucine	0.15	0.15	0.15	0.15	0.15
Phenylalanine	0.18	0.18	0.18	0.18	0.18
Valine	0.27	0.27	0.27	0.27	0.27
Limestone	1.5	1.5	1.5	1.5	1.5
Salt	0.3	0.3	0.3	0.3	0.3
Monocalcium phosphate	1.49	1.49	1.49	1.49	1.49
Mineral premix[2]	0.15	0.15	0.15	0.15	0.15
Vitamin premix[1]	0.15	0.15	0.15	0.15	0.15
Marker, TiO2	0.25	0.25	0.25	0.25	0.25
Calculated nutrient contents, %					
Digestible energy, MJ/kg	15.40	15.41	15.43	15.38	15.36
Amino Acids total basis, %					
Lys	0.81	0.98	1.15	0.98	1.15
Met	0.52	0.52	0.52	0.55	0.57
Thr	0.83	0.83	0.83	0.94	1.06
Trp	0.25	0.25	0.25	0.28	0.31

[1]Supplied per kg of diet: vitamin A, 6,600 IU; vitamin D_3, 880 IU; vitamin E, 44 IU; vitamin K (menadione sodium bisulfate complex), 6.4 mg; thiamin, 4.0 mg; riboflavin, 8.8 mg; pyridoxine, 4.4 mg; vitamin B12, 33 µg; folic acid, 1.3 mg; niacin, 44 mg.

[2]Supplied per kg of diet: Zn, 131 mg as ZnO; Fe, 131 mg as $FeSO_4 \cdot H_2O$; Mn 45 mg, as MnO; Cu, 13 mg as $CuSO_4 \cdot 5H_2O$; I, 1.5 mg as $CaIO_6$; Co, 0.23 mg as $CoCO_3$; Se, 0.28 mg as Na_2O_3Se.

Table 4. Experiment 1 growth performance and apparent total tract digestibility coefficients of nursery pigs fed different inclusion rates of *Rhizopus oligosporus* (RO)

Item	Diet[1]			SEM	P-value
	0%RO	10%RO	20%RO		
Growth Performance					
ADG (kg/day)	0.39	0.40	0.39	0.063	0.94
ADFI (kg/day)	0.64	0.65	0.65	0.035	0.97
G:F	0.62	0.62	0.60	0.100	0.55
Digestibility coefficients[2]					
Energy	85.6[a]	86.7[a]	81.1[b]	1.02	<0.0001
Nitrogen	86.2[a]	84.3[ab]	82.8[b]	1.23	0.042
Fat	83.1[a]	80.4[ab]	77.1[b]	1.51	0.036
Phosphorus	58.0[a]	59.2[a]	46.5[b]	2.82	0.0003
Energy value					
DE (Mcal/kg)	4.01[b]	4.16[a]	3.81[c]	0.048	<0.001

[1]Diets equal mean of 8 pigs per treatment.
[2]Mean digestibility coefficients calculated based upon pooled fecal grab samples. [a,b,c] Means in the same row with different superscripts significantly differ (P<0.05).

Table 5. Experiment 2 growth performance

Parameters	ISG[2]	Diets[1]					SE	*P*-value
		Basal	Ref-1	Ref-2	RO-1	RO-2		
Starting BW, kg	7.3	7.2	7.5	7.5	7.3	7.3	0.23	0.82
Final BW, kg		25.0^{a}	27.7^{c}	29.4^{d}	26.7^{b}	27.3^{bc}	0.37	<0.01
ADFI (kg/day)		0.65^{a}	0.64^{a}	0.67^{a}	0.64^{a}	0.65^{a}	0.011	0.45
ADG (kg/day)		0.32^{a}	0.36^{b}	0.39^{c}	0.35^{b}	0.36^{b}	0.006	<0.01
G:F		0.50^{a}	0.56^{c}	0.59^{d}	0.54^{b}	0.55^{bc}	0.007	<0.01

[1]Diets equal means of 5, 6, 7, 7, and 7 respectively.
[2]Initial Slaughter Group used as baseline for tissue accretion evaluation
[a,b,c] Means in the same row with different superscripts significantly differ (P<0.05).

Table 6. Whole body composition and tissue accretion rates of pigs fed *Rhizopus oligosporus* (RO) in Experiment 2

Parameters	ISG[2]	Diets[1]					SE	*P*-value
		Basal	Ref-1	Ref-2	RO-1	RO-2		
Body Composition								
Dual X-ray Absorptiometry (DXA)								
BMC[3] (g)	146.3	487.2[a]	514.2[ab]	543.1[b]	516.7[ab]	513.7[ab]	13.03	0.04
Fat Mass (kg)	1.3	5.6[a]	5.9[a]	5.5[a]	5.2[b]	4.9[b]	0.13	<0.01
Lean Mass (kg)	5.4	18.9[a]	21.2[bc]	23.3[d]	20.9[b]	21.6[c]	0.31	<0.01
%Lean	78.4	75.6[a]	76.9[a]	79.3[bc]	78.6[ab]	80.1[bc]	1.01	0.03
%Fat	19.5	22.44[a]	21.2[b]	18.7[cd]	19.4[c]	18.0[d]	0.43	<0.01
BMD[3] (g/cm^2)	0.34	0.71	0.76	0.75	0.75	0.73	0.016	0.26
Tissue Accretion[4]								
DXA predicted, g/d								
BMC		6.2[a]	6.6[ab]	7.1[b]	6.6[ab]	6.6[ab]	0.20	0.05
Fat Mass		77[a]	81[a]	74[a]	68[b]	63[b]	2.20	<0.01
Lean Mass		240[a]	280[b]	320[c]	280[b]	290[b]	6	<0.01
Whole Body Protein[6]		56.2[a]	65.2[b]	73.5[c]	63.5[b]	66.7[b]	1.71	<0.01

[1]Diets equal means of 5, 6, 7, 7, and 7 respectively.
[2]Initial Slaughter Group used as baseline for tissue accretion evaluation
[3]Bone Mineral Content, Bone Mineral Density
[4]All values calculated at g/day, Tissue Accretion = (Diet - ISG)/Days on Test
[5]No data
[6]Whole Body Protein calculated using lean mass*0.23 from Appendix 1 (NRC, 1998)
[a,b,c] Means in the same row with different superscripts significantly differ (P<0.05).

Table 7. Experiment 2 apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) coefficients in nursery pigs fed *Rhizopus oligosporus* (RO)

Parameters	Diets[1]					SE	*P-value*
	Basal	**Ref-1**	**Ref-2**	**RO-1**	**RO-2**		
AID							
Nitrogen	74.2[a]	72.2[ab]	77.1[ab]	72.6[ab]	62.5[c]	3.32	0.014
Lysine	80.6[a]	85.0[ab]	86.9[b]	80.2[a]	72.3[c]	1.81	<0.001
Threonine	82.9[a]	81.4[ab]	81.9[ab]	79.6[ab]	71.9[c]	2.23	0.003
Methionine	93.0[a]	94.0[ab]	93.4[ab]	92.3[ab]	86.1[c]	0.76	<0.001
Tryptophan	90.3[a]	88.3[ab]	88.3[ab]	87.7[ab]	81.3[c]	2.27	0.036
Energy	74.7[a]	79.7[ab]	81.6[a]	73.6[ab]	62.7[c]	2.93	<0.001
ATTD							
Nitrogen	77.7[a]	83.1[c]	81.8[bc]	81.7[b]	78.8[a]	1.28	0.018
Energy	82.3[a]	86.3[b]	86.2[b]	84.3[ab]	80.3[a]	0.86	<.0001
Phosphorus	60.5[a]	65.4[b]	68.4[b]	70.7[b]	61.2[a]	1.36	<.0001

[1]Diets equal means of 5, 6, 7, 7, and 7 respectively.
[a,b,c] Means within row significantly differ $P < 0.05$.

Table 8. Intestinal nutrient transport, integrity and morphology of nursery pigs fed *Rhizopus oligosporus* (RO) from Experiment 2

Parameters	Diets[1]			SE	*P*-value
	Ref-2	RO-1	RO-2		
Ileum Nutrient Transport, ΔμA/cm^2					
Glucose	9.68	10.63	11.35	2.54	0.86
Lysine	1.01	1.18	0.93	0.30	0.65
Glutamate	1.79	2.96	2.29	0.74	0.35
TER, AU					
Ileum	1.00[a]	0.73[b]	1.00[a]	0.07	<0.01
Cecum	1.00	1.02	1.04	0.18	0.97
FITC-Dextran APP[3]					
Ileum	1.6	3.2	2.6	1.22	0.67
Cecum	3.8	3.5	2.6	1.59	0.63
Ileum Morphology					
Villus height, μm	312.8	341.1	317.4	13.29	0.29
Width, μm	138.5	134	143.7	4.76	0.38
Crypt Depth, μm	220.4	219.3	223.4	14.43	0.98
Villus:Crypt ratio	1.4	1.6	1.4	0.18	0.92

[1]Diets equal means of n=7/trt
[2]Transepethelial resistance (TER)
[3]Fluorescein isothiocyanate-dextran (4.4 kDa) permeability coefficient
[a,b,c] Means within row differ significantly P<0.05.

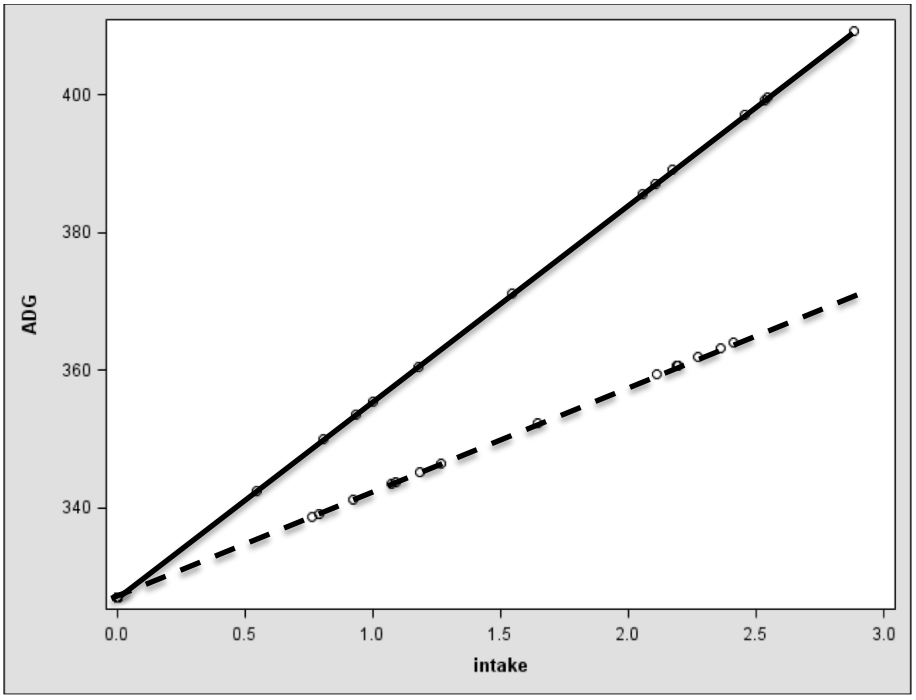

Figure 1. Common-intercept, multiple linear regression of ratio of the lysine intake (g/d) to ADG (g/d), Y, on nursery pigs fed supplemental *Rhizopus oligosporus* (RO). The Upper line (solid) represents Reference treatments (X_1), bottom line (dashed) represents RO treatments (X_2). Y= 327 (±3.69) + 28.6 (±2.68)X_1 + 15.4 (±2.95)X_2. Slope ratio = 0.54, availability = 54 %.

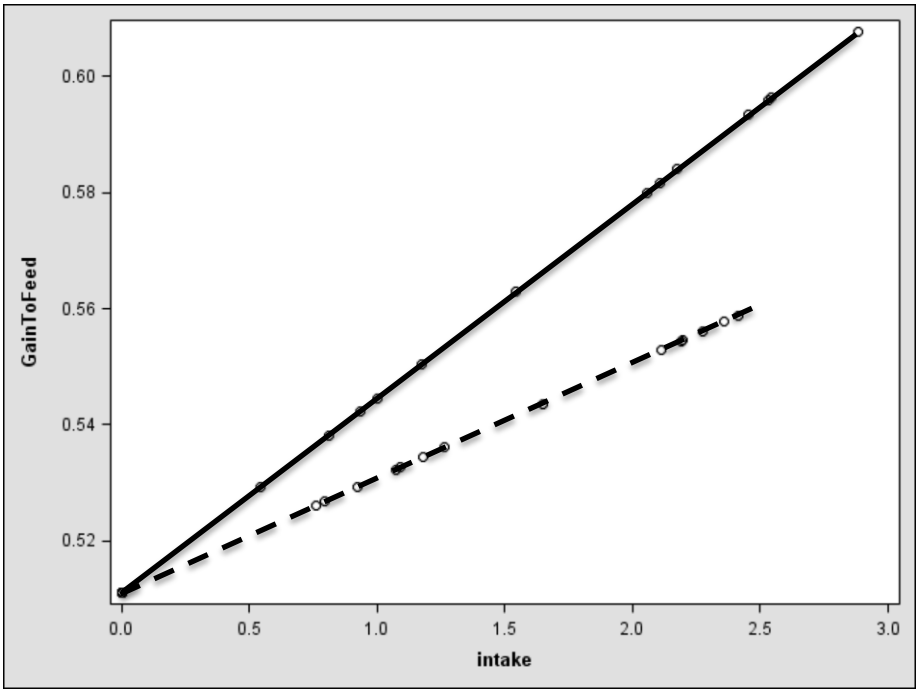

Figure 2. Common-intercept, multiple linear regression of ratio of the lysine intake (g/d) to G:F, Y, on nursery pigs fed supplemental *Rhizopus oligosporus* (RO). The Upper line (solid) represents Reference treatments (X_1), bottom line (dashed) represents RO treatments (X_2). Y= 0.51 (±0.0006) + 0.033 (±0.005)X_1 + 0.020 (±0.005)X_2. Slope ratio = 0.61, availability = 61 %.

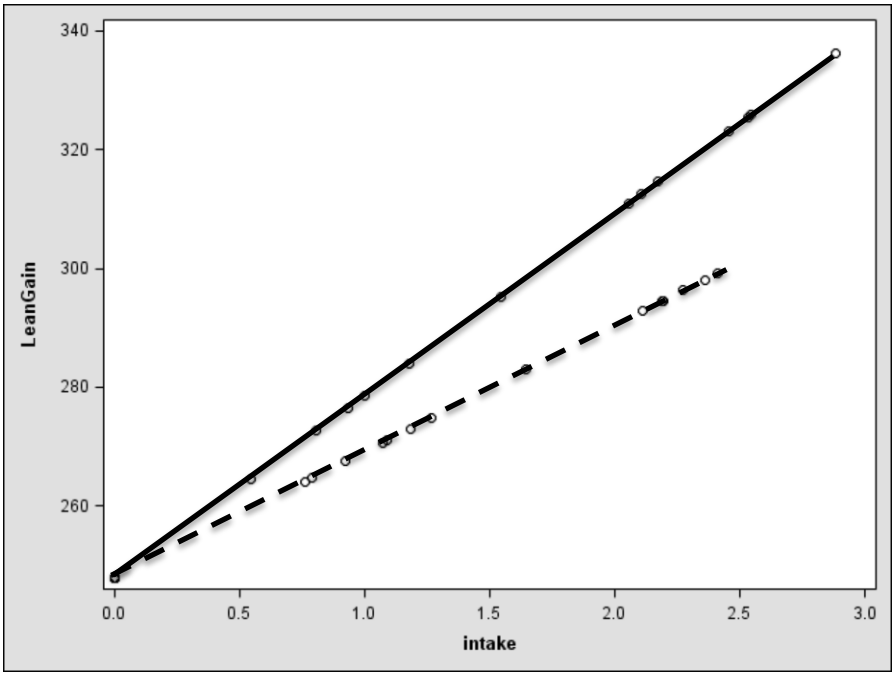

Figure 3. Common-intercept, multiple linear regression of ratio of the lysine intake (g/d) to lean tissue gain (g/d), Y, on nursery pigs fed supplemental *Rhizopus oligosporus* (RO). The Upper line (solid) represents Reference treatments (X_1), bottom line (dashed) represents RO treatments (X_2). Y Y= 248 (±3.5) + 30.7 (±2.53)X_1 21.3 (±2.79)X_2. Slope ratio = 0.695, availability = 69.5 %.