Evaluation of *Rhizopus oligosporus* Yeast Supplementation on Growth Performance and Nutrient Digestibility in Nursery Pigs

A.S. Leaflet R2724

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Summary and Implications

The growth and performance of 24 nursery pigs fed a fungal cultivation of *Rhizopus oligosporus* (RO) was evaluated in a 28-day feeding and digestibility study. Nursery pigs average start weight of 5.62 ± 0.35 kg were provided ad libitum access to a corn-soybean diets containing three levels of RO, 0, 10, or 20%. Diets were formulated to be isocaloric and isolysinic, and contained the digestibility marker titanium dioxide. There was no difference in pig performance based on dietary inclusion of RO fungus. However, total tract DE was improved when feeding RO. Altogether, these data indicate that *Rhizopus oligosporus* cultivated on distillers stillage and bioproducts can be used in nursery swine diets with no negative effects on performance.

Introduction

Corn used in ethanol production competes directly with its use as animal feed. Corn ethanol plants in the US currently have a production capacity of 14 billion gallons of ethanol per year. The main byproduct, distillers dried grains (DDG), is now commonly used in livestock feed rations. However, DDG has shortcomings in its nutritional value. The dry-grind corn ethanol process grinds corn, adds water and enzymes to break starches down to sugars, and then ferments the sugars with yeasts to ethanol. The ethanol is recovered by distillation, but it leaves about 6 gallons leftovers per gallon ethanol after distillation – known as stillage. Part of this is recycled directly as backset, but most is centrifugally separated into distiller's grains and thin stillage. Thin stillage contains biodegradable organic compounds, sufficient micronutrients and is somewhat acidic, which makes thin stillage an ideal feedstock for fungal cultivation such as *Rhizopus oligosporus* (RO). *Rhizopus oligosporus* cultivation 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, rich in fat and protein, and specifically has a high content of the important amino acids lysine and methionine (Table 1). Additionally, this fungal biomass is rich in phosphorus, chitin, chitosan and β-glucans. These latter three compounds found in fungal biomass also provide desirable health benefits to animals via antioxidant and antimicrobial mechanisms. Thus, this value added byproduct may be a suitable feed ingredient for swine nutrition. Therefore, the objective of this study was to evaluate the use of RO supplementation in nursery pig diets on growth performance and total tract digestibility.

Materials and Methods

All procedures were approved by the Iowa State University Intuitional Animal Care and Use Committee. Twenty four gilts were selected based on body weight (5.62 ± 0.35 kg), separated into individual pens and randomly allotted to one of three dietary treatments. In a corn-soybean base diet, the treatments included: 1) 0% RO, 2) 10% RO or 3) 20% RO (n=8 pigs/trt). The diets were formulated (Table 2) to be isocaloric and isolysinic, and contained 0.5% titanium dioxide (digestibility marker) and were formulated to meet or exceed swine NRC nutrient requirements (1998). Each pig was allowed to acclimate to the pen and diet for one week. All pigs were fed ad libitum and had free access to water at all times. After the acclimation period, pigs and feeders were weighed weekly and weight of feed presented and feed disappearance recorded for each animal. Thereafter, pig body weight gain, feed intake and feed efficiency were calculated for 28 days. Total tract fecal samples were collected at week four over a two day period, pooled and homogenized within pig. Feed and fecal energy, nitrogen, protein and phosphorus were determined by proximate analysis. Apparent total tract digestibility coefficients (ATTD) and digestible energy (DE) was then calculated for each diet. Results were analyzed using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC).

Results and Discussion

Irrespective of dietary inclusion of RO, there was no difference in pig ADFI ($P=0.97$), ADG ($P=0.94$) or G:F ($P=0.55$) over the four week nursery period (Table 3). Proximate analysis of feed and fecal samples yielded the following apparent total tract digestibility coefficients for 0% RO, 10% RO or 20% RO, respectively (Table 4): Gross Energy, 85.6, 86.7 and 81.1% ($P<0.001$); P, 58.0, 59.2 and 46.5% ($P<0.01$); and N, 86.2, 84.3 and 82.8 ($P<0.05$). Total tract DE was improved feeding 10% RO (4.16 Mcal/kg), but not 20% RO (3.81 Mcal/kg) versus the control treatment (4.01 Mcal/kg). Altogether, these data indicate that *Rhizopus oligosporus* cultivated on distillers stillage and bioproducts can be used in phase 2 of nursery swine diets with no negative effects on performance. Fungal RO additives may be a suitable energy and amino acid substitute in swine diets. However, as the inclusion rates increase, nutrient digestibility may decrease due to the high fat content of the RO product. Further evaluation of RO inclusion in swine diets and its impact on intestinal and whole animal health are also warranted.

Table 1. Comparison of *Rhizopus oligosporus* (RO), Corn and DDGS ingredient composition.

	Corn	DDGSS	RO
Starch (%)	70	4	2.5
NDF (%)	9.5	33.5	21.1
Crude Fat (%)	4.2	13.4	26.00
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
Ash (%)	1.5	5.1	5.13
Phosphorus (%)	0.3	0.9	1.34

Table 2. Diet composition.

Item (%)	Control	10% MycoMeal	20% MycoMeal
Corn	65.35	59.54	53.42
SBM	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
Monocal (21P17Ca)	0.76	0.28	-
Limestone	0.66	0.87	1.05
Salt (NaCl)	0.35	0.35	0.35
Vitamin mix	0.30	0.30	0.30
L-Lysine HCL	0.19	0.13	0.07
Trace mineral mix	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
	100.00	100.00	100.00
Calculated			
ME (Kcal/kg)	3,880	3,888	3,894
Crude protein (%)	19.02	21.43	23.83
Lysine (SID. %)	1.11	1.11	1.11
Avail. phosphorus (%)	0.35	0.36	0.40

[1]Supplied per kilogram of diet: vitamin A, 6,600 IU; vitamin D_3, 880 IU; vitamin E, 44IU; vitamin K(menadione sodium bisulfate complex), 6.4 mg; thiamin, 4.0 mg; riboflavin, 8.8 mg; pyridoxine, 4.4 mg; vitamin B_{12}, 33 µg; folic acid, 1.3 mg; niacin, 44 mg; pantothenic acid, 22 mg; D-biotin, 0.22 mg.

[2] Supplied per kilogram 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 CaI_2O_6; Co, 0.23 mg as $CoCO_3$; Se, 0.28 mg as Na_2O_3Se.

Table 3. The effects of *Rhizopus oligosporus* (RO) supplementation on nursery pig performance.

Item	Diet[1]			Statistics	
	0% RO	10% RO	20% RO	SEM	P-value
Average daily feed intake (kg/d)	0.64	0.65	0.65	0.052	0.98
Average daily gain (kg/d)	0.39	0.40	0.39	0.033	0.94
Gain:Feed	0.62	0.62	0.60	0.013	0.55

[1]Diets equal mean of 8 pigs per treatment fed for four weeks.

Table 4. Apparent total tract digestibility coefficients of nursery pigs fed different inclusion rates of *Rhizopus oligosporus* (RO).

Item	Diet[1]			Statistics	
	0% RO	10% RO	20% RO	SEM	P-value
Fecal[2]					
Gross Energy	85.6[a]	86.7[a]	81.1[b]	1.02	<0.0001
Nitrogen	86.2[a]	84.3[ab]	82.8[a]	1.23	0.042
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 fecal grab samples pooled over a two day period after 26 days on feed.
[a,b,c] Means in the same row with different superscripts significantly differ ($P<0.05$)