

**I. Project Title: Assessment of the nutritional and immunological properties of yeast cells derived from fuel ethanol production in diets for baby pigs**

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**II. Abstract**

Growth performance, gut morphology, organ weights, intestinal lengths, acute phase protein levels, and circulating insulin-like growth factor 1 (IGF-1) concentrations were measured to determine the effectiveness of corn distiller's solubles by-products as potential replacements for carbadox and spray dried porcine plasma in diets for early-weaned pigs. In the past, corn condensed distiller's solubles (CDS), a by-product of dry-mill ethanol plants, has not been extensively fed to pigs because of its high moisture content (70%) and difficulty to dry. However, we have developed a new process for spray drying CDS and separating CDS into new fractions: yeast cream (YC) and residual solubles (RS).

In trial 1, 560 crossbred barrows (5.06 kg) were weaned at 17-d of age and used in a 6-wk trial. Pigs were fed diets containing each of three distiller's solubles by-products (DS, YC, RS), a control diet (NC), NC + 55 ppm carbadox (AB), NC + 6% spray dried porcine plasma (PP), and NC + 55 ppm carbadox + 6% spray dried porcine plasma (PC) for the first 10-d post-weaning (phase 1). Common phase 2 and phase 3 diets were fed to all pigs for the remainder of the trial. On d 10, one pig from each pen was sacrificed to determine the effects of dietary treatment on gut morphology measurements at 25, 50, and 75% of the small intestine length, intestinal length, and organ weights. Blood samples were collected on d 0, 3, 7, 10, 14, 21, 28, 35, and 42, and used to measure acute phase proteins and insulin like growth factor-1 concentrations. In trial 2, 441 crossbred barrows and gilts (6.19 kg) were used in a 5-wk growth performance trial and were fed the same dietary treatments used in trial 1. In trial 1, pigs fed the PC diet had higher ADG ( $P < 0.05$ ) for the first 10-d postweaning compared to pigs fed the NC, DS, YC, RS, and AB diets. However, although ADG was not affected by dietary treatment during phase 2, phase 3, and overall, pigs fed the DS and RS diets tended to have a greater increase in growth rate ( $P = 0.09$ ) during the subsequent phases of growth, compared to the other dietary treatments. There were no effects of dietary treatment on ADFI or G:F for phase 1, phase 2, phase 3, or overall, but there was no effect of dietary treatment on the relative changes in ADFI, or G:F during phase 2 or phase 3. In trial 2, overall ADG was not affected by dietary treatment. However, during phase 1, pigs fed the PP and PC diets had higher ADG ( $P < 0.05$ ) compared to pigs fed the other dietary treatments. Average daily feed intake was higher for pigs fed the PP and PC treatments ( $P < 0.05$ ) during phase 1, and pigs consuming the PC diets consumed more feed ( $P < 0.05$ ) per day than pigs fed the NC, DS, YC, and RS treatments during the overall 5-wk feeding period. Overall G:F was not affected by dietary treatment ( $P > 0.05$ ). Pigs fed the PP and PC diets had lower relative change in ADG during phase 2 compared to the other dietary treatments. However, during phase 3, pigs fed the NC had a lower relative change in ADG ( $P < 0.05$ ) compared to pigs fed the PP diet. There was no effect of dietary

treatment on the relative change of ADFI or G:F. Pigs fed the RS and PC diets had longer villi ( $P < 0.05$ ) and greater villi height:crypt depth ratio (VCR;  $P < 0.05$ ) in the upper 25% of the small intestine compared to pigs fed the NC, DS, YC, and AB diets. There was no effect of dietary treatment on villi height, crypt depth or VCR in the 50% and 75% portions of the small intestine, or overall. Organ weights and intestinal tract lengths were not affected by dietary treatment. Serum  $\alpha_1$ -acid glycoprotein (AGP) concentration was not affected by dietary treatment on d 3 or d 10. However, serum haptoglobin (Hp) concentrations were lower ( $P < 0.05$ ) on d 10 for pigs fed the AB and PC diets indicating a lower immune system activation. Circulating IGF-1 levels were not affected by dietary treatment. These results suggest that feeding diets containing spray dried DS, YC, and RS fractions provides similar performance to pigs fed diets containing carbadox, but lower ADG and ADFI compared to pigs fed diets containing porcine plasma. Based upon the greater relative increase in ADG in pigs during phase 2 after being fed the DS and RS diets during phase 1, there may be a beneficial, subsequent growth rate benefit from feeding diets containing these ingredients. Additionally, the RS and PC diets promoted greater villi height and VCR compared to pigs fed diets containing carbadox. However, pigs fed carbadox appeared to have lower immune system activation as measured by acute phase protein serum concentrations compared to diets that did not contain carbadox.

### **III. Introduction**

The animal feed industry continues to be challenged by many issues including transmissible spongiform encephalopathies (TSE), dioxins, bioterrorism, biosecurity, genetically modified organisms (GMO), food safety, and trade and regulatory issues. Two issues that have received considerable attention recently, are the use of subtherapeutic levels of feed grade antibiotics and the use of animal derived protein ingredients in livestock feeds. Antimicrobial resistant microorganisms and drug residues have caused health officials to closely scrutinize the use and application of antimicrobials in animal diets. Recent concerns regarding the potential spread of bovine spongiform encephalopathy (BSE) has government regulatory officials imposing bans on feeding some animal by-products to food producing animals, especially those of ruminant origin. Because of these scientific and government regulatory agency driven concerns, there is a need to identify feed ingredients that provide nutritional value and health benefits comparable to antimicrobials and specialized animal derived protein ingredients.

During the past decade, U.S. ethanol production has increased dramatically. This increase in ethanol production has allowed nutritionists to use an increasing supply of a variety of distiller's by-products in livestock and poultry feeds. The distiller's by-products produced by the dry-milling process include distiller's dried grains with solubles (DDGS), dried distiller's grains (DDG) and condensed distiller's solubles (CDS). Distiller's dried grains with solubles is the primary distiller's by-product used in swine diets because corn CDS has been difficult to dry and DDG is lower in nutritional value compared to DDGS and CDS. However, new manufacturing techniques for drying CDS and fractions of CDS have been developed at the University of Minnesota (Figure 1).



Corn distiller's by-products contain yeast and residual yeast metabolites. Ingeldew (1999) estimated that up to 3.9% of DDGS weight is contributed by yeast biomass. Previous research has suggested that CDS may contain "unidentified growth factors" that improve growth performance and stimulate feed intake in pigs and poultry. However, the growth factors have never been defined. The unidentified growth factors may be attributed to the yeast component of condensed distiller's solubles. Yeast cells have many biologically, and potentially immunologically important compounds that are found in either the yeast cell wall, or the yeast cell contents (yeast extracts).

Many research trials in recent years, have been conducted at the University of Minnesota to evaluate the effects of feeding DDGS, produced from "New Generation" ethanol plants, to swine and poultry. Recently, Whitney et al. (2003) showed that feeding a diet containing 10% DDGS to growing pigs subjected to a moderate *Lawsonia intracellularis* challenge had reduced length, severity and prevalence of intestinal lesions compared to challenged pigs fed a corn-soybean meal diet. However, compared to DDGS research, there has been little research conducted to determine the effects of feeding corn condensed distiller's dried solubles (CDS) to swine. Therefore, it is worthwhile to evaluate CDS as a source of unidentified growth factors in diets for early weaned pigs.

#### **IV. Objectives**

To determine the effects of feeding diets containing spray dried distiller's solubles, and distiller's solubles fractions to early-weaned pigs on growth performance, gut morphology (intestinal health), acute phase proteins (immune system responses), and insulin like growth factor-1 (a growth factor) concentrations compared to pigs fed diets containing antimicrobials, porcine plasma or a combination of antimicrobials and porcine plasma.

#### **V. Procedures**

Two trials were conducted to evaluate the effects of feeding diets containing corn distiller's solubles by-products to early-weaned pigs. In trial 1, 560 crossbred barrows were weaned at 17-d of age with an initial weight of  $5.06 \pm 0.99$  kg and housed in 56 pens of 10 pigs per pen. Each dietary treatment was replicated eight times during the 6-wk trial. In trial 2, 441 crossbred barrows and gilts weaned at 17-d of age (initial weight of  $6.19 \pm 0.91$  kg) were utilized and housed in 49 pens of 9 pigs per pen (5 barrows, 4 gilts). Each of the seven dietary treatments were replicated seven times during the 5-wk trial.

Dietary treatments (Table 1) included: 1) negative control (NC), 2) 15% spray dried distiller's solubles (DS), 3) 7.5% spray dried yeast cream (YC), 4) 15% spray dried residual solubles (RS), 5) 55 ppm carbadox (AB) 6), 6% spray dried porcine plasma, and 7) 55 ppm carbadox + 6% spray dried porcine plasma positive control (PC).

All diets contained corn, soybean meal, 20% lactose, 12.5% oat groats, and 11% fishmeal. The NC, DS, YC, RS, and AB diets contained 22.5% soybean meal, while the PP and PC diets contained only 12.5% soybean meal. All dietary treatments were formulated to provide 1.60% total lysine, 3440 kcal/kg of metabolizable energy, 0.87% Ca and 0.80% P. The AB and PC treatments contained 55 ppm carbadox as the antimicrobial. The PP and PC diets contained 6% spray dried porcine plasma, which reduced the amount of soybean meal and crude protein content in the diet. Diets were mixed and pelleted (0.794 cm). All dietary treatments were supplemented with adequate vitamin and mineral levels to meet or exceed NRC (1998) recommended requirements.

In trial 1, pigs were weighed on d-0 and then on d-7, 10, 14, 21, 28, 35, and 42. Feed disappearance was measured on d-7, 10, 14, 21, 28, 35, and 42. Average daily gain, ADFI and G:F were calculated for each dietary phase and overall. Additionally, the relative change in ADG, ADFI, and G:F was calculated for phase 2 and phase 3 to determine the effects of dietary treatment fed during phase 1 on subsequent growth rate, feed intake, or feed efficiency during subsequent phases.

In trial 2, pigs were weighed initially and on d-10, 21, and 35, which corresponded with dietary phase breaks. The same dietary treatments that were fed in trial 1 were fed in trial 2. Feed disappearance was measured on d-10, 21, and 35. Average daily gain ADG, ADFI and G:F were calculated for each dietary phase and for the overall trial.

**Table 1. Diet composition and calculated nutrient levels of experimental diets.**

<b>Ingredient</b>	<b>NC</b>	<b>DS</b>	<b>YC</b>	<b>RS</b>	<b>PP</b>	<b>AB</b>	<b>PC</b>
Corn, %	26.54	13.53	23.68	12.57	36.37	26.03	35.87
Soybean Meal, 46% CP, %	22.50	22.50	22.50	22.50	7.49	22.50	7.49
Lactose, %	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Oat Groats, %	12.50	12.50	12.50	12.50	12.50	12.50	12.50
Fish Meal, IPC 790, %	11.00	11.00	11.00	11.00	11.00	11.00	11.00
Porcine Plasma, %	-	-	-	-	6.00	-	6.00
SD Distiller's Solubles, %	-	15.00	-	-	-	-	-
SD Yeast Cream, %	-	-	7.52	-	-	-	-
SD Residual Solubles, %	-	-	-	15.00	-	-	-
Choice White Grease, %	4.62	2.84	-	3.73	3.87	4.62	3.87
Dicalcium Phosphate 18.5/21, %	1.48	1.10	1.38	1.18	1.32	1.48	1.32
Limestone, %	0.09	0.28	0.14	0.25	0.24	0.09	0.24
Salt, %	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine HCl, %	0.19	0.17	0.19	0.18	0.22	0.19	0.22
DL-Methionine, %	0.13	0.13	0.13	0.14	0.09	0.13	0.09
L-Threonine, %	0.12	0.12	0.13	0.12	0.07	0.12	0.07
L-Tryptophan, %	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Mecadox 10, %	-	-	-	-	-	0.50	0.50
VTM Premix <sup>a</sup> , %	0.50	0.50	0.50	0.50	0.50	0.50	0.50
<b>Calculated Nutrient Levels</b>							
ME, Kcal/kg	3440	3440	3440	3440	3440	3440	3440
Crude Protein, %	22.57	22.87	22.56	22.70	21.19	22.57	21.19
Lysine, %	1.60	1.60	1.60	1.60	1.60	1.60	1.6
Methionine + Cystine, %	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Tryptophan, %	0.29	0.29	0.29	0.29	0.29	0.29	0.29
Threonine, %	1.03	1.03	1.03	1.03	1.03	1.03	1.03
Crude Fat, %	7.48	6.20	6.61	6.15	7.12	7.48	7.12
Calcium, %	0.87	0.87	0.87	0.87	0.87	0.87	0.87
Phosphorus, %	0.80	0.80	0.80	0.80	0.80	0.80	0.80

NC = negative control

DS = 15% spray dried distiller's solubles

YC = 7.5% spray dried yeast cream

RS = 15% spray dried residual solubles

AB = 55 ppm carbadox

PP = 6% spray dried porcine plasma

PC = 55 ppm carbadox + 6% spray dried porcine plasma

<sup>a</sup>Vitamin Trace Mineral Premix, provided the following amounts per kg of complete feed: 11,025 IU vitamin A; 2,756 IU vitamin D<sub>3</sub>; 55 IU vitamin E; 4.41 mg vitamin K; 10 mg riboflavin; 55 mg niacin; 33 mg pantothenic acid; 0.06 mg vitamin B<sub>12</sub>; 2.21 mg iodine; 0.30 mg selenium; 90 mg zinc; 54 mg iron; 18 mg manganese; 5.40 mg copper.

On d-10 in trial 1, one pig from each pen (N=56) was euthanized, and the intestinal tract was removed. A 6-cm section of the small intestine was collected at 25, 50, and 75% of the small intestine length. The 10 longest and most regular villi, and their associated crypts from each section, were used to determine length measurements. Additionally, the villus height and associated crypt depth were used to calculate villus height:crypt depth ratio (VCR).

Also in trial 1, prior to consumption of the experimental diets (d 0), blood was collected from 4 randomly selected pigs in each pen. Subsequent blood samples were collected from the same pigs on d-3, 7, 10, 14, 21, 28, and 42. Acute phase proteins specific for swine (porcine haptoglobin; Hp and porcine  $\alpha_1$ -acid glycoprotein; AGP) were used to determine the effects of dietary treatments on the immune system on d 3 and d 10. Insulin-like growth factor 1 (IGF-1) analysis was performed using procedures according to Frey et al. (1994). The relative change in IGF-1 concentrations was calculated for phase 1, phase 2, and phase 3.

## VI. Results

### *Growth performance – Trial 1*

Pigs fed the PC diet had higher ADG ( $P < 0.05$ ) compared to pigs fed the NC, DS, YC, RS and AB diets during phase 1, while pigs consuming the PP diet had intermediate growth rates (Table 2). There were no differences among dietary treatments for ADFI or G:F during the initial 10 d feeding period.

**Table 2. Effects of dietary treatment on ADG, ADFI, and G:F during phase 1 (0-10 d postweaning; Trial 1).**

	NC	DS	YC	RS	AB	PP	PC	Pooled SE	Trt P-value
<b>ADG, g/d</b>	49.04 <sup>a</sup>	52.59 <sup>a</sup>	59.22 <sup>a</sup>	60.71 <sup>a</sup>	57.48 <sup>a</sup>	76.99 <sup>ab</sup>	96.56 <sup>b</sup>	9.73	0.05
<b>ADFI, g/d</b>	89.06	89.00	91.50	93.89	87.58	97.44	110.27	6.26	0.31
<b>G:F</b>	0.548	0.591	0.651	0.670	0.587	0.772	0.874	0.02	0.38

<sup>ab</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ )

Growth rates during phase 2 and phase 3 were not different ( $P > 0.05$ ) among treatments (Table 3). Average daily gain increased with time ( $P < 0.0001$ ) at each successive dietary phase, with no time by treatment interaction ( $P > 0.05$ ). During all phases of growth, there was a relatively wide range in daily feed consumption (Table 4). As pigs progressed through each successive phase, ADFI increased ( $P < 0.0001$ ), but there was no time by treatment interaction. Pigs were the most efficient in converting feed into body weight during phase 2 (Table 5). There was no time effect ( $P > 0.05$ ) or time by treatment interaction ( $P > 0.05$ ) for G:F.

**Table 3. Effects of dietary treatment on phase 2 and phase 3 ADG (Trial 1).**

	NC	DS	YC	RS	AB	PP	PC
Phase 2, g/d	289.1	294.3	287.5	293.6	300.2	306.9	303.5
Phase 3, g/d	468.6	504.0	473.7	496.6	477.6	493.8	485.8
Trt P-value = 0.89 Pooled SE = 18.36							
Time P-value = 0.0005 Pooled SE = 9.02							
Time x Trt P-value = 0.58							

**Table 4. Effects of dietary treatment on phase 2 and phase 3 ADFI (Trial 1).**

	NC	DS	YC	RS	AB	PP	PC
Phase 2, g/d	346.57	360.27	360.37	353.26	362.05	382.04	379.26
Phase 3, g/d	760.46	819.85	758.11	797.39	763.33	823.08	802.99
Trt P-value = 0.43 Pooled SE = 23.53							
Time P-value = 0.0001 Pooled SE = 11.57							
Time x Trt P-value = 0.14							

**Table 5. Effects of dietary treatment on phase 2 and phase 3 G:F (Trial 1).**

	NC	DS	YC	RS	AB	PP	PC
Phase 2	0.833	0.816	0.797	0.833	0.831	0.81	0.805
Phase 3	0.616	0.615	0.625	0.622	0.63	0.601	0.606
Trt P-value = 0.72 Pooled SE = 0.02							
Time P-value = 0.0001 Pooled SE = 0.02							
Time x Trt P-value = 0.19							

The relative change in ADG, ADFI, and G:F were calculated using absolute mean differences between growth performance levels at the end of phase 1 and phase 2, and the end of phase 2 and phase 3 (Table 6). Pigs fed the DS and RS diets tended to have a greater relative increase in growth rate ( $P = 0.09$ ) during phase 3. There was no effect of time ( $P = 0.77$ ) on the relative change in ADG, and no time by treatment interaction ( $P = 0.91$ ) was observed.

**Table 6. Effect of dietary treatment fed during phase 1 (0-10 d postweaning) on subsequent relative ADG change during phase 2 (11-21 d postweaning) and phase 3 (22-42 d postweaning; Trial 1).**

	NC	DS	YC	RS	AB	PP	PC
Phase 2, g/d	240.03	241.68	228.25	232.94	242.69	229.86	206.93
Phase 3, g/d	179.54	209.73	186.28	202.95	177.45	186.97	182.33
Trt P-value = 0.09 Pooled SE = 8.59							
Time P-value = 0.77 Pooled SE = 5.25							
Time x Trt P-value = 0.91							

There was no effect of dietary treatment fed during phase 1 on the relative ADFI change in phase 2 or phase 3 (Table 7). There was a time effect ( $P = 0.0006$ ) indicating that ADFI increased during the subsequent phases of growth. However, no time by treatment interaction was observed.

**Table 7. Effect of dietary treatment fed during phase 1 (0-10 d postweaning) on subsequent relative ADFI change during phase 2 (11-21 d postweaning) and phase 3 (22-42 d postweaning; Trial 1).**

	NC	DS	YC	RS	AB	PP	PC
<b>Phase 2, g/d</b>	257.51	271.27	268.87	259.37	274.47	284.60	268.99
<b>Phase 3, g/d</b>	413.89	459.58	397.74	444.13	401.28	441.04	423.73
Trt P-value = 0.33 Pooled SE = 15.20							
Time P-value = 0.0006 Pooled SE = 13.51							
Time x Trt P-value = 0.23							

Relative change in subsequent gain:feed was not affected by dietary treatment fed during phase 1 (Table 8). There was a time effect ( $P = 0.02$ ), which indicates that as pigs progressed through the dietary phases, the relative change in G:F decreased as pig age increased. There was no time by treatment interaction ( $P = 0.40$ ).

**Table 8. Effect of dietary treatment fed during phase 1 (0-10 d postweaning) on subsequent relative G:F change during phase 2 (11-21 d postweaning) and phase 3 (22-42 d postweaning; Trial 1).**

	NC	DS	YC	RS	AB	PP	PC
<b>Phase 2</b>	0.284	0.225	0.146	0.163	0.244	0.038	-0.069
<b>Phase 3</b>	-0.216	-0.201	-0.172	-0.211	-0.201	-0.209	-0.198
Trt P-value = 0.35 Pooled SE = 0.07							
Time P-value = 0.02 Pooled SE = 0.08							
Time x Trt P-value = 0.40							

Overall, there were no differences ( $P = 0.71$ ) in ADG among pigs fed different dietary treatments (Table 9). Furthermore, there were no differences ( $P > 0.60$ ) in ADFI or G:F ( $P > 0.70$ ) for pigs fed any of the dietary treatments.

**Table 9. Effects of dietary treatment on overall (0-42 d postweaning) ADG, ADFI, and G:F (Trial 1).**

Overall	NC	DS	YC	RS	AB	PP	PC	Pooled SE	Trt P-value
<b>ADG, g/d</b>	321.69	341.59	326.26	339.66	331.11	345.62	345.39	11.93	0.71
<b>ADFI, g/d</b>	475.83	504.61	481.97	493.04	480.02	507.09	515.76	17.14	0.60
<b>G:F</b>	0.676	0.678	0.677	0.691	0.694	0.685	0.671	0.01	0.71



*Growth performance Trial 2*

Pigs fed the PC and PP diets had higher ADG ( $P < 0.05$ ) compared to pigs fed the NC, DS, YC, RS and AB diets during phase 1 (Table 10). Pigs fed the AB diets had higher ADG ( $P < 0.05$ ) compared to pigs fed the NC and YC diets. During phase 1, pigs fed the PC diet had higher ADFI ( $P < 0.05$ ) compared to pigs fed the NC, DS, YC, RS and AB dietary treatments. The pigs fed the PP diet consumed more feed per day ( $P < 0.05$ ) than pigs fed the NC, DS, YC, and RS diets. During phase 1, pigs fed the PC and PP treatments had higher G:F ( $P < 0.05$ ) compared to pigs fed the NC, DS, and YC dietary treatments. Gain:feed of pigs fed the RS and AB dietary treatments were intermediate.

**Table 10. Effects of dietary treatment on ADG, ADFI, and G:F during phase 1 (0-10 d postweaning; Trial 2).**

	NC	DS	YC	RS	AB	PP	PC	Pooled SE	Trt P-value
<b>ADG, g/d</b>	61.22 <sup>a</sup>	73.64 <sup>ab</sup>	61.55 <sup>a</sup>	80.77 <sup>ab</sup>	89.43 <sup>b</sup>	116.38 <sup>c</sup>	136.82 <sup>c</sup>	8.67	0.0001
<b>ADFI, g/d</b>	119.69 <sup>a</sup>	118.49 <sup>a</sup>	114.39 <sup>a</sup>	123.74 <sup>a</sup>	129.68 <sup>ab</sup>	144.93 <sup>bc</sup>	163.14 <sup>c</sup>	6.54	0.0001
<b>G:F, g/d</b>	0.484 <sup>a</sup>	0.620 <sup>ac</sup>	0.537 <sup>ac</sup>	0.657 <sup>acd</sup>	0.677 <sup>bc</sup>	0.807 <sup>bd</sup>	0.846 <sup>b</sup>	0.063	0.003

<sup>abc</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ )

Average daily gain increased with time ( $P < 0.0001$ ), but no time by treatment interaction was observed (Table 11). Likewise, ADFI increased ( $P = 0.0001$ ) as pigs progressed through the dietary phases, but there was no effect of dietary treatment fed during phase 1 on phase 2 or phase 3 ADFI (Table 12). No time by treatment interaction for ADFI was observed. During phase 2 and phase 3, there was a significant effect of diet fed during phase 1 on G:F (Table 13). During phase 2, pigs fed the PP and PC diets had lower G:F ( $P < 0.05$ ) compared to pigs fed the NC, DS, and YC treatments. During phase 3, pigs fed the YC diet had higher G:F ( $P < 0.05$ ) compared to pigs fed the other dietary treatments. There was a significant time effect ( $P = 0.0001$ ) and time by treatment interaction ( $P = 0.0001$ ).

**Table 11. Effects of dietary treatment on phase 2 and phase 3 ADG (Trial 2).**

	NC	DS	YC	RS	AB	PP	PC
<b>Phase 2, g/d</b>	282.63	271.05	288.59	267.81	282.28	254.31	269.27
<b>Phase 3, g/d</b>	408.69	427.67	454.66	420.51	451.95	440.21	439.91
Trt P-value = 0.47 Pooled SE = 15.7							
Time P-value = 0.0001 Pooled SE = 12.3							
Time x Trt P-value = 0.40							

The relative change in ADG, ADFI, and G:F were calculated using absolute mean differences between growth performance levels at the end of phase 1 and phase 2, and the end of phase 2 and phase 3. There was a significant effect of diet fed to pigs during phase 1 on the relative change in ADG during phase 2 and phase 3 (Table 14). Additionally, there was a significant time effect ( $P < 0.01$ ) and time by treatment interaction ( $P < 0.05$ ). The interaction was explained by the slower increase in ADG of pigs fed the PP and PC diets during phase 2, and a concomitant greater increase in the

growth rate for pigs fed the NC and YC diets from phase 1 to phase 2. Pigs fed the NC, DS, YC, RS, and AB treatments during phase 1 had a higher relative increase in ADG ( $P < 0.05$ ) during phase 2 of the trial compared to pigs fed the PP and PC diets. However, during phase 3, pigs fed the NC diet had a lower relative increase ( $P < 0.05$ ) in ADG compared to pigs fed the PP diet.

**Table 12. Effects of dietary treatment on phase 2 and phase 3 ADFI (Trial 2).**

	NC	DS	YC	RS	AB	PP	PC
Phase 2, g/d	368.81	267.57	373.87	373.41	387.72	383.74	395.66
Phase 3, g/d	717.08	763.49	721.21	721.76	769.44	761.44	775.63
Trt P-value = 0.22 Pooled SE = 19.47							
Time P-value = 0.0001 Pooled SE = 15.32							
Time x Trt P-value = 0.56							

**Table 13. Effects of dietary treatment on phase 2 and phase 3 G:F (Trial 2).**

	NC	DS	YC	RS	AB	PP	PC
Phase 2	0.767 <sup>a</sup>	0.737 <sup>a</sup>	0.765 <sup>a</sup>	0.718 <sup>ab</sup>	0.730 <sup>ac</sup>	0.664 <sup>b</sup>	0.683 <sup>bc</sup>
Phase 3	0.569 <sup>a</sup>	0.562 <sup>a</sup>	0.629 <sup>b</sup>	0.579 <sup>a</sup>	0.586 <sup>a</sup>	0.577 <sup>a</sup>	0.565 <sup>a</sup>
Trt P-value = 0.01 Pooled SE = 0.02							
Time P-value = 0.0001 Pooled SE = 0.01							
Time x Trt P-value = 0.003							

**Table 14. Effects of dietary treatment fed during phase 1 (0-10 d postweaning) on subsequent relative ADG change during phase 2 (11-21 d postweaning) and phase 3 (22-35 d postweaning; Trial 2).**

	NC	DS	YC	RS	AB	PP	PC
Phase 2, g/d	218.51 <sup>a</sup>	196.96 <sup>a</sup>	226.60 <sup>a</sup>	186.59 <sup>a</sup>	192.40 <sup>a</sup>	137.49 <sup>b</sup>	132.00 <sup>b</sup>
Phase 3, g/d	124.21 <sup>a</sup>	155.35 <sup>ab</sup>	164.79 <sup>ab</sup>	151.44 <sup>ab</sup>	168.41 <sup>ab</sup>	184.63 <sup>b</sup>	169.37 <sup>ab</sup>
Trt P-value = 0.007 Pooled SE = 10.93							
Time P-value = 0.004 Pooled SE = 21.11							
Time x Trt P-value = 0.02							

There was no effect of phase 1 dietary treatment on the relative change in ADFI during phase 2 and phase 3 (Table 15). Average daily feed intake of pigs increased with time ( $P < 0.05$ ), but no time by treatment interaction was observed.

**Table 15. Effects of dietary treatment fed during phase 1 (0-10 d postweaning) on subsequent relative ADFI change during phase 2 (11-21 d postweaning) and phase 3 (22-35 d postweaning; Trial 2).**

	NC	DS	YC	RS	AB	PP	PC
<b>Phase 2</b>	247.39	248.66	259.07	249.25	257.62	238.38	232.11
<b>Phase 3</b>	344.37	394.46	345.87	346.88	380.18	376.24	378.49
Trt P-value = 0.50 Pooled SE = 14.41							
Time P-value = 0.03 Pooled SE = 19.55							
Time x Trt P-value = 0.56							

Pigs fed the PP and PC diets during phase 1 had a poorer ( $P < 0.05$ ) relative change in feed conversion during phase 2 compared to pigs fed the NC, DS, and YC treatments during phase 1 (Table 16). During phase 3, pigs fed the PP and PC diets continued to have less efficient feed conversion, but the rate of decrease was less compared to pigs fed the NC and DS treatments. There tended to be a time effect and there was a significant time by treatment interaction ( $P < 0.05$ ) for relative change in G:F.

**Table 16. Effects of dietary treatment fed during phase 1 (0-10 d postweaning) on subsequent relative gain:feed (G:F) change during phase 2 (11-21 d postweaning) and phase 3 (22-35 d postweaning; Trial 2).**

	NC	DS	YC	RS	AB	PP	PC
<b>Phase 2</b>	0.278 <sup>a</sup>	0.115 <sup>ac</sup>	0.226 <sup>ac</sup>	0.059 <sup>bc</sup>	0.051 <sup>bc</sup>	-0.144 <sup>b</sup>	-0.166 <sup>b</sup>
<b>Phase 3</b>	-0.197 <sup>a</sup>	-0.176 <sup>a</sup>	-0.137 <sup>ac</sup>	-0.140 <sup>ac</sup>	-0.145 <sup>ac</sup>	-0.088 <sup>bc</sup>	-0.118 <sup>bc</sup>
Trt P-value = 0.002 Pooled SE = 0.05							
Time P-value = 0.08 Pooled SE = 0.06							
Time x Trt P-value = 0.0005							

There were no effects of dietary treatment on overall ADG ( $P > 0.20$ ; Table 17). Pigs fed the PC diet during phase 1 had the greatest overall ADFI ( $P < 0.05$ ) compared to pigs fed the NC, DS, YC, and RS diets. Pigs fed the AB and PP diets during phase 1 had intermediate overall ADFI. Overall G:F was not affected by dietary treatment ( $P > 0.05$ ).

**Table 17. Effects of dietary treatment on overall (0-35 d postweaning) ADG, ADFI, and G:F (Trial 2).**

	NC	DS	YC	RS	AB	PP	PC	Pooled SE	Trt P-value
<b>ADG, g/d</b>	267.37	274.89	287.72	273.06	292.75	287.39	297.39	9.29	0.24
<b>ADFI, g/d</b>	434.15 <sup>a</sup>	443.22 <sup>a</sup>	435.21 <sup>a</sup>	438.97 <sup>a</sup>	460.53 <sup>ac</sup>	463.92 <sup>ac</sup>	479.57 <sup>bc</sup>	10.39	0.03
<b>G:F</b>	0.615	0.621	0.661	0.621	0.635	0.621	0.620	0.01	0.26

<sup>abcd</sup> Within a row, means without a common superscript letter differ ( $P < 0.05$ )

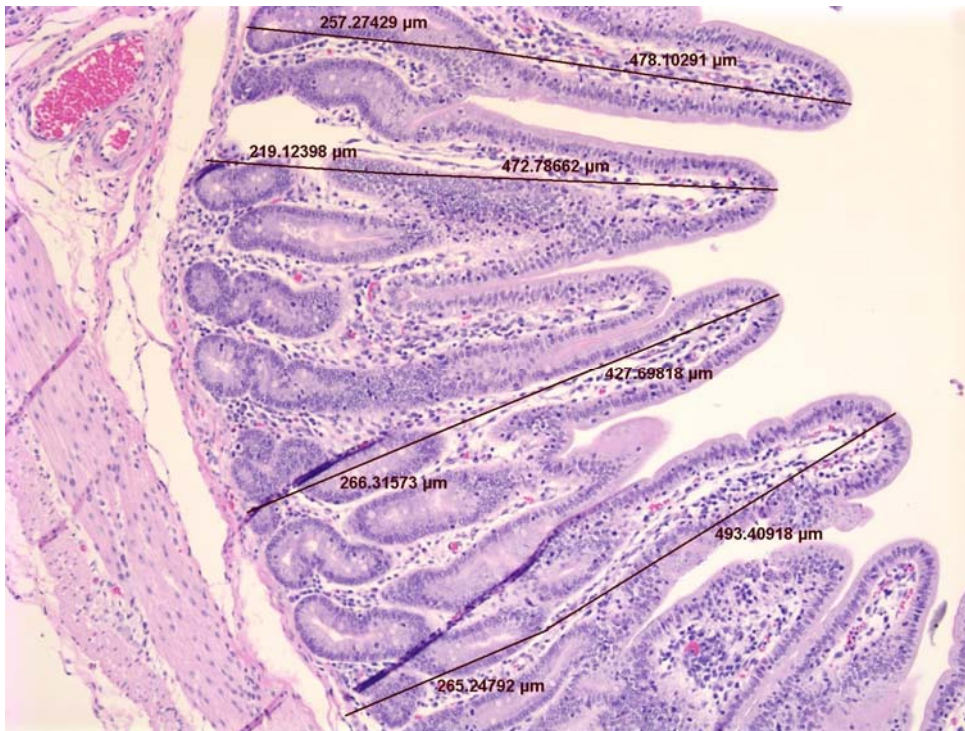
*Gut morphology*

Pigs fed the RS and PC diets had significantly longer villi ( $P < 0.05$ ) compared to pigs fed the NC, DS, YC, and AB diets in the upper 25% of the small intestine (Table 18). The villi height of pigs fed the PP diets was intermediate. Figures 2 and 3 show the differences in villi height and shape in the upper 25% of the small intestine between pigs fed the RS diet and the AB diet. There were no differences among dietary treatments for crypt depth ( $P > 0.05$ ). Using villi height and crypt depth measurements, a villus height to crypt depth ratio (VCR) was calculated. Pigs fed the RS and PC diets had greater ( $P < 0.05$ ) VCR in the upper 25% of the small intestine compared to pigs fed the NC, DS, and AB treatments. When villi height, crypt depth, and VCR were measured at 50% and 75% of the small intestine length, there were no differences ( $P > 0.05$ ) among pigs fed any of the dietary treatments.

**Table 18. Effects of dietary treatment on villi height and crypt depth in the upper 25% of the small intestine (d 10 postweaning).**

	NC	DS	YC	RS	AB	PP	PC	Pooled SE	Trt P-Value
Villi height, $\mu\text{m}$	335.4 <sup>a</sup>	319.1 <sup>a</sup>	346.2 <sup>a</sup>	427.6 <sup>b</sup>	323.1 <sup>a</sup>	376.2 <sup>ab</sup>	429.9 <sup>b</sup>	27.7	0.02
Crypt depth, $\mu\text{m}$	248.0	227.2	221.4	236.5	226.3	243.6	225.1	12.4	0.78
VCR <sup>c</sup>	1.41 <sup>a</sup>	1.48 <sup>a</sup>	1.66 <sup>ab</sup>	1.88 <sup>b</sup>	1.51 <sup>a</sup>	1.65 <sup>ab</sup>	1.98 <sup>b</sup>	0.12	0.02

<sup>ab</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ )



**Figure 2. Villi Measurements from the Upper 25% of the Small Intestine from a Pig Fed the Residual Solubles Diet (10X)**



**Figure 3. Villi Measurements from the Upper 25% of the Small Intestine from a Pig Fed the Carbadox Diet (10X)**

There tended to be a significant effect ( $P = 0.06$ ) of segment location (25%, 50%, or 75%) of the small intestine on villi height. The villi in the upper 25% of the small intestine ( $365.36 \mu\text{m}$ ) tended to be longer ( $P = 0.06$ ) than villi in the lower 25% of the small intestine ( $340.36 \mu\text{m}$ ). Additionally, there tended ( $P = 0.07$ ) to be a treatment by intestinal segment interaction. There was no effect of intestinal segment on crypt depth or VCR ( $P > 0.15$ ) and no treatment by segment interactions.

#### *Acute phase proteins*

Serum AGP concentrations did not differ ( $P > 0.05$ ) among dietary treatments on d-0, d-3, and d-10 (Table 19). Serum AGP concentrations decreased from d-0 to d-3, and increased from d-3 to d-10 ( $P < 0.0001$ ). However, there was no time by treatment interaction ( $P = 0.95$ ).

Initial and d-3 serum Hp concentration did not differ among dietary treatments ( $P > 0.05$ ; Table 20). However, on d-10, pigs fed the AB and PC diets had lower ( $P < 0.05$ ) serum Hp concentrations compared to pigs fed the NC, DS, YC, and RS diets. Pigs fed the PP diet had higher ( $P < 0.05$ ) serum Hp concentrations on d 10 compared to pigs fed the PC diet, but had intermediate concentrations compared to the other dietary treatments. There tended ( $P = 0.06$ ) to be an increase in serum Hp concentrations from d-0 to d-10. A significant time by treatment interaction ( $P = 0.001$ ) was observed due to the significant decrease of serum Hp concentrations of pigs fed the AB and PC treatments over time, while the serum Hp concentrations from pigs fed the other dietary treatments increased

with time. When the relative change of Hp concentrations were analyzed, there was an effect of dietary treatment ( $P = 0.001$ ), but there was no time by treatment interaction or a time effect observed. Pigs fed the AB and PC diets had lower ( $P < 0.05$ ) relative changes in serum Hp concentrations compared to pigs fed the other dietary treatments (Table 21).

**Table 19. Effects of dietary treatment on  $\alpha_1$ -acid glycoprotein (AGP) on d-0,d-3, and d-10 postweaning.**

	NC	DS	YC	RS	AB	PP	PC
Day 0, $\mu\text{g/ml}$	734.9	664.6	728.4	721.7	704.4	810.1	734.3
Day 3, $\mu\text{g/ml}$	640.2	550.4	661.5	654.7	566.6	624.2	626.6
Day 10, $\mu\text{g/ml}$	766.5	751.3	761.5	796.9	765.9	814.6	847
Trt P-value = 0.68 Pooled SE = 68.97							
Time P = 0.0001 Pooled SE = 44.69							
Time x Treatment P = 0.95							

**Table 20. Effects of dietary treatment on serum haptoglobin (Hp) concentration on d-0, d-3, and d-10 postweaning.**

	NC	DS	YC	RS	AB	PP	PC
Day 0, $\mu\text{g/ml}$	718.9	852.5	691.8	744.7	717.7	638.3	860.7
Day 3, $\mu\text{g/ml}$	1014.6	782.6	741.7	850.5	804.3	740.3	808.8
Day 10, $\mu\text{g/ml}$	937.5 <sup>a</sup>	1044.8 <sup>a</sup>	998.9 <sup>a</sup>	1024.7 <sup>a</sup>	589.9 <sup>bc</sup>	758.7 <sup>ac</sup>	555.9 <sup>b</sup>
Trt P-value = 0.26 Pooled SE = 124.3							
Time P = 0.06 Pooled SE = 78.2							
Time x Treatment P = 0.001							

**Table 21. Effects of dietary treatment on relative serum haptoglobin (Hp) concentration change on d-3 and d-10 postweaning.**

	NC <sup>a</sup>	DS <sup>a</sup>	YC <sup>a</sup>	RS <sup>a</sup>	AB <sup>b</sup>	PP <sup>a</sup>	PC <sup>b</sup>
Day 3, $\mu\text{g/ml}$	295.7	-69.9	49.9	105.8	71	102	-51.9
Day 10, $\mu\text{g/ml}$	-77.1	262.1	257.2	174.2	-206.5	18.4	-252.9
Trt P-value = 0.001 Pooled SE = 63.8							
Time P-value = 0.52 Pooled SE = 134.8							
Time x Trt P-value = 0.12							

<sup>ab</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ )

#### *Serum IGF-1 concentrations*

Initial IGF-1 on d-0 was not different among pigs assigned to different dietary treatments ( $P > 0.05$ ; Table 22). Subsequent serum concentrations of IGF-1 on d-3, 7, 10, 14, 21, 28, 35, and 42 were not affected by dietary treatment ( $P > 0.05$ ). Serum concentrations of

IGF-1 decreased and remained low during the first 10 d postweaning for all dietary treatments. At 28 d postweaning, serum IGF-1 concentrations appeared to decrease from d-21 concentrations. Serum IGF-1 concentrations increased from d-28 to d-35 and from d-35 to d-42. There was an overall time effect ( $P = 0.0001$ ), indicating that serum IGF-1 concentrations increased over the duration of the feeding period. However, there was no time by treatment interaction ( $P > 0.05$ ).

**Table 22. Effects of dietary treatment on serum insulin like growth factor-1 (IGF-1) concentrations on d-0, 3, 7, 10, 14, 21, 28, 35, and 42 postweaning.**

	NC	DS	YC	RS	AB	PP	PC
Day 0, ng/ml	105	108	108	107	107	105	90
Day 3, ng/ml	54	52	62	72	56	63	64
Day 7, ng/ml	68	63	67	63	68	61	69
Day 10, ng/ml	72	84	82	96	83	89	105
Day 14, ng/ml	208	182	223	252	211	234	205
Day 21, ng/ml	420	379	379	392	337	410	408
Day 28, ng/ml	325	335	266	327	283	337	286
Day 35, ng/ml	411	502	503	663	471	472	515
Day 42, ng/ml	652	675	680	728	583	581	687
Trt P-value = 0.38 Pooled SE = 48.9							
Time P = 0.0001 Pooled SE = 30.1							
Time x Treatment P = 0.24							

## Conclusions

Feeding the diet containing the residual solubles fraction of distiller's solubles to early weaned pigs during the first 10 days postweaning, increased villi height and VCR in the upper 25% of the small intestine, which suggests greater intestinal health and potentially greater nutrient absorptive capacity compared to pigs fed the diet containing carbadox. This suggests that that the distiller's residual solubles fraction may contain significant amounts of "unidentified growth factors". However, this improvement in gut health did not result in improvements in immune response, as measured by acute phase proteins, IGF-1, or growth performance compared to pigs fed diets containing porcine plasma or carbadox and porcine plasma. The spray dried distiller's solubles and associated fractions evaluated in these studies can be used effectively in phase 1 starter diets.