Journal of Animal Science, 2023, **101**, 1–11
https://doi.org/10.1093/jas/skad087
Advance access publication 24 March 2023
Non ruminant nutrition

Torula yeast may improve intestinal health and immune function of weanling pigs

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Abstract

An experiment was conducted to test the hypothesis that inclusion of a conventional torula yeast or a torula yeast produced from forestry byproducts (i.e., woody torula yeast) in diets for weanling pigs instead of fish meal and plasma protein improves growth performance and intestinal health of pigs. A total of 120 weanling pigs (6.53 ± 0.78 kg) were allotted to three treatments with ten replicate pens per diet. Pigs were fed one of three diets from days 1 to 14 post-weaning (phase 1), whereas all pigs were fed a common diet in phase 2 (days 15 to 28). The three treatments in phase 1 included a control diet with 5% fish meal, 3.5% plasma protein, and no torula yeast. The second diet contained 1.5% fish meal, 14% woody torula yeast, and no plasma protein, whereas the third diet contained 1.5% fish meal, 14% conventional torula yeast, and no plasma protein. Fecal scores were assessed every other day. On day 7, one pig per pen was euthanized to collect ileal tissue and mucosa for determination of morphology and for ribonucleic acid (RNA) sequencing analysis. At the end of phases 1 and 2, blood samples were collected and concentrations of cytokines, plasma urea nitrogen (PUN), peptide YY, immunoglobulin G, total protein, and albumin were analyzed. Results indicated that both torula yeast sources could replace fish meal and plasma protein without affecting growth performance, intestinal morphology, or blood characteristics of pigs. Pigs fed a diet containing torula yeast had improved ($P < 0.05$) fecal scores during phase 1. Pigs fed the conventional torula yeast diet had greater ($P < 0.05$) concentration of interleukin-2 compared with pigs fed the control diet. On day 14, greater ($P < 0.05$) concentrations of interleukin-4 and interleukin-10 were observed in pigs fed the diet containing the woody torula yeast or conventional torula yeast compared with pigs fed the control diet. Results from the RNA sequencing indicated that 19 of 24 analyzed genes involved in digestion and absorption of protein and vitamins were downregulated in pigs fed the diet containing woody torula yeast compared with pigs fed the control diet. However, only two genes (i.e., ANKS4B and FAM54A) were downregulated in pigs fed the woody torula yeast diet compared with the conventional torula yeast diet. In conclusion, using woody or conventional torula yeast instead of fish meal and plasma protein in the phase 1 diet for weanling pigs may improve intestinal health without influencing growth performance of pigs.

Lay Summary

A torula yeast produced using forestry byproducts (i.e., woody torula yeast) had been demonstrated to have greater concentrations of digestible amino acids and phosphorus than fish meal, which indicates that the woody torula yeast can be used as a protein source for weanling pigs. However, information about effects of the woody torula yeast and conventional torula yeast on intestinal health and immune response are limited. Therefore, an experiment was conducted to test the hypothesis that the woody torula yeast improves intestinal health of pigs to a greater extent than conventional torula yeast. Results demonstrated that both woody torula yeast and conventional torula yeast could replace fish meal and plasma protein without negatively affecting growth performance, intestinal morphology, or blood characteristics of pigs. Regardless of source, torula yeast also improved fecal scores during the first 2 wk post-weaning and increased concentrations of anti-inflammatory cytokines in plasma of pigs. Therefore, dietary inclusion of torula yeast in diets for weanling pigs may represent a strategy to improve intestinal health of weanling pigs, but no differences between woody torula yeast and conventional torula yeast were observed.

Key words: immune response, intestinal health, growth performance, pigs, torula yeast

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; AEE, acid-hydrolyzed ether extract; FDR, false discovery rate; G:F, gain-to-feed ratio; IgG, immunoglobulin G; IL, interleukin; KEGG, Kyoto Encyclopedia of Genes and Genomes; RNA, ribonucleic acid; PUN, plasma urea nitrogen; PYY, peptide YY; TNF-α, tumor necrosis factor-α

Introduction

The concentration of soybean meal is usually restricted in diets for weanling pigs due to the presence of anti-nutritional factors such as trypsin inhibitors, lectins, antigens, and allergens, which negatively affect nutrient availability, immune response, and health of the animals (Smiricky-Tjardes et al., 2003; Palacios et al., 2004; Stein et al., 2008). Therefore, animal protein sources such as fish meal, milk products, plasma protein, or valued-added soybean meal are usually added to diets for weanling pigs to avoid the negative effects of soybean meal (Rojas and Stein, 2013). Yeast and yeast derivatives contain amino acids, mannans, β-glucans, and nucleotides (Kogan and Kocher, 2007; Mateo and Stein, 2007), and therefore, may be used at the expense of animal proteins to supplement soybean meal in diets for pigs (Cruz et al., 2020). An enhanced and sustainable torula yeast (i.e., woody torula yeast; Arbiom Inc., Durham, NC, USA) derived via fermentation of forestry and agricultural residues has been developed, and results from digestibility experiments indicated that this single-cell protein may be used as a source

Received January 22, 2023 Accepted March 21, 2023.

Table 1. Nutrient composition of the woody torula yeast, conventional torula yeast, and fish meal as-fed basis[1]

Item	Woody torula yeast	Conventional torula yeast	Fish meal[2]
Dry matter, %	91.08	92.96	91.45
Ash, %	8.31	8.40	19.61
Crude protein, %	56.50	53.15	64.77
Gross energy, kcal/kg	4,625	4,438	4,307
AEE[2], %	6.74	4.16	—
Indispensable amino acids, %			
Arg	2.56	2.65	3.60
His	1.19	0.77	1.17
Ile	1.76	1.77	2.55
Leu	3.21	3.37	4.22
Lys	3.41	3.34	4.51
Met	0.56	0.56	1.61
Phe	1.93	1.97	2.44
Thr	3.06	2.45	2.40
Trp	0.53	0.83	0.55
Val	2.29	2.31	2.94
Dispensable amino acids, %			
Ala	3.10	3.12	3.98
Asp	4.38	4.63	5.33
Cys	0.48	0.43	0.47
Glu	6.88	7.79	7.83
Gly	1.94	2.03	4.74
Pro	1.57	1.73	2.94
Ser	2.52	2.60	2.04
Tyr	1.89	1.83	1.68

[1]The woody torula yeast and conventional torula yeast were provided by Arbiom Inc., Durham, NC, USA.
[2]Adapted from Lagos and Stein (2020).
[3]AEE = acid-hydrolyzed ether extract.

of energy and digestible amino acids in diets for weanling pigs (Lagos and Stein, 2020). Indeed, it was demonstrated that woody torula yeast can replace plasma protein and fish meal in diets for weanling pigs without influencing protein utilization or growth performance (Espinosa et al., 2020), which is in agreement with results of experiments using other sources of torula yeast (Cruz et al., 2020). Espinosa et al. (2020) further demonstrated that increasing levels of the woody torula yeast resulted in increased feed efficiency of pigs, which is likely a result of a positive effect of torula yeast on digestive function and nutrient digestibility (Cruz et al., 2019). It is also hypothesized that inclusion of yeast in diets for weanling pigs may improve intestinal health and immune function (Broadway et al., 2015). There are, however, no data to demonstrate effects of the woody torula yeast on the immune response of pigs. Furthermore, differences in fermentation technique or substrates used during fermentation may contribute to differences in the nutritional composition of torula yeast sources (Øverland and Skrede, 2017), and it is not known if the woody torula yeast influences pig performance and intestinal health the same way as conventional torula yeast. Therefore, an experiment was conducted to test the hypothesis that both conventional and woody torula yeast improve growth performance and intestinal health of pigs compared with pigs fed a diet containing fish meal and protein plasma. The objective was to determine if pig growth performance, intestinal morphology, cytokine concentrations, and abundance of genes involved in different cellular pathways are changed if torula yeast replaces fish meal and plasma protein in phase 1 diets for weanling pigs.

Materials and Methods

The protocol for the experiment was submitted to the Institutional Animal Care and Use Committee at the University of Illinois (Urbana, IL, USA) and the protocol was approved prior to initiation of the experiment. Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used. A woody torula yeast and conventional torula yeast were procured (Table 1; Arbiom Inc., Durham, NC, USA). The woody torula yeast was produced using forestry byproducts (i.e., wood hydrolysate) as the carbon source, whereas the conventional torula yeast was produced using wheat dextrose as the carbon source.

Animals and dietary treatments

A total of 120 weanling pigs (21 d old; initial body weight: 6.53 ± 0.78 kg) were allotted to three dietary treatments in a randomized complete block design. Weaning weight was used as the blocking factor. There were four pigs per pen (i.e., two gilts and two barrows) and 10 replicate pens per diet. Pigs

were housed in pens that had fully slatted floors, a feeder, and a nipple drinker. The experiment was conducted for 28 d. A two-phase feeding program was used with days 1 to 14 as phase 1 and days 15 to 28 as phase 2. Pigs were fed one of three diets during phase 1, whereas all pigs were fed a common diet in phase 2 (Table 2). All diets in phases 1 and 2 were formulated to meet current estimates for nutrient requirements (NRC, 2012). The control phase 1 diet contained 5% fish meal, 3.5% plasma protein, and no torula yeast. The second phase 1 diet contained 14% of the woody torula yeast, 1.5% fish meal and no plasma protein, whereas the third phase 1 diet contained 14% conventional torula yeast, 1.5% fish meal, and no plasma protein.

Experimental procedure

Individual pig weights were recorded at the beginning of the experiment, on day 7, day 14, and day 28. Feed provision was recorded daily and the weight of feed left in the feeder was recorded on day 7, day 14, and day 28. Diarrhea scores were assessed visually per pen every other day using a subjective score ranging from 1 to 5 according to the method of Hu et al. (2012) and Espinosa et al. (2017): 1 = normal feces, 2 = moist feces, 3 = mild diarrhea, 4 = severe diarrhea, and 5 = watery diarrhea. Diarrhea frequency was obtained by totaling the number of pen days with diarrhea scores ≥3 divided by the total number of pen days multiplied by 100. At the conclusion of the experiment, data were summarized to calculate average daily feed intake (**ADFI**), average daily gain (**ADG**), and gain to feed ratio (**G:F**) within each pen and treatment group. Data were summarized for days 1 to 7, days 1 to 14, days 15 to 28, and for the entire experiment.

Sample collection

On day 7, the weight of all pigs was recorded, and one pig per pen (five gilts and five barrows per treatment) was euthanized via captive bolt stunning. The pig to be euthanized was the pig of the specified sex that had a body weight closest to the pen average. Ileal tissue samples between 2 and 3 cm long were collected approximately 80 cm from the ileal-cecal junction. Samples were cut and pinned with the serosa side down on a piece of cardboard. Samples were then fixed in 10% neutral buffered formalin until processing for staining and morphological evaluation. Ileal mucosa samples that were collected approximately 85 cm anterior to the ileocecal valve were scraped gently, snap frozen in liquid N, and stored at −80 °C until used for ribonucleic acid (**RNA**) sequencing analysis.

At the end of each phase (i.e., days 14 and 28), two blood samples were collected from one pig per pen via vena puncture. Within each treatment, the gilt with the body weight closest to the pen average was used in five of the pens, and the barrow with the body weight closest to the pen average was used in the other five pens. The same pig was used for blood collection at both sampling points. These samples were collected in vacutainers with heparin or with EDTA, and plasma was obtained by centrifuging blood samples at 4,000 × *g* at 4 °C for 13 min. Heparinized plasma and samples collected in EDTA containing tubes were frozen at −20 °C until analyzed.

Chemical analyses

All diet and ingredient samples (i.e., woody torula yeast and conventional torula yeast) were ground through a 1-mm screen hammermill (MM4; Schute Buffalo, NY, USA) prior to chemical analysis. Diets and ingredients were analyzed for dry matter (Method 930.15; AOAC Int., 2019) and ash (Method 942.05; AOAC Int., 2019), and gross energy was analyzed using bomb calorimetry (AAFCO, 2012; Model 6400; Parr Instruments, Moline, IL, USA). Acid-hydrolyzed ether extract was determined by acid hydrolysis using 3 N HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA; Method 2003.06.06; AOAC Int., 2019). Diets and ingredients were analyzed for N using the combustion procedure (Method 990.03; AOAC Int., 2019) on a LECO FP628 apparatus (LECO Corp., Saint Joseph, MI, USA). Crude protein was calculated as analyzed concentration of N multiplied by 6.25. Amino acids were analyzed on a Hitachi Amino Acid analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for postcolum derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6N HCl for 24 h at 110 °C [Method 982.30 E(a); AOAC Int., 20019]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [Method 982.30 E(b); AOAC Int., 2019]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C [Method 982.30 E(c); AOAC Int., 2019].

Blood profile analyses

Heparinized plasma samples were analyzed for plasma urea nitrogen (**PUN**), total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA). Plasma samples treated with EDTA were analyzed for the following cytokines: interleukin (**IL**)-1 beta, IL-2, IL-4, IL-8, IL-10, IL-12, and tumor necrosis factor-alpha (**TNF-α**) using a MILLIPLEX kit (EMD Millipore Corporation, Billercia, MA, USA) in a MagPix instrument with ProcartaPlex-Multiplex technology (R&D Systems, Inc., Minneapolis, MN, USA). Plasma samples treated with EDTA were also analyzed for peptide YY (**PYY**) and immunoglobulin G (**IgG**) using ELISA kits according to the recommendations from the manufacturer (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA; Bethyl Laboratories Inc., Montgomery, TX, USA, respectively).

Intestinal morphology

Each ileal sample was cut in 2 to 3 mm thick cross-sections and embedded in paraffin for slide preparation after fixation. From each sample, 3 to 4 transverse sections were stained with hematoxylin and eosin. Slides were then scanned using a 2.0-HT NanoZoomer (Hammatsu, Bridgewater, NJ, USA). Ten villi and the associated crypts were used to measure villus height, villus width, and crypt depth using NDP.View2 (Hammatsu, Bridgewater, NJ, USA). Villus height was measured from the villus tip to the crypt mouth and the crypts were measured from the crypt mouth to the top of the crypt valley. Villus width was measured at the middle of the villus.

Ribonucleid acid sequencing

Total RNA was extracted from 40 ± 0.2 mg of frozen ileal mucosa using β-mercaptoethanol according to the manufacturer's instructions of the RNeasy Mini Kit (Qiagen, Germantown, MD, USA). Total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The RNA quality was

Table 2. Composition of experimental diets[1]

Item	Phase 1 diets			Phase 2
	Control	Woody torula yeast	Conventional torula yeast	
Ingredient, %				
Corn	45.64	38.38	38.26	54.41
Soybean meal	25.00	25.00	25.00	26.00
Milk, Lactose	15.00	15.00	15.00	—
Whey powder	—	—	—	10.00
Woody torula yeast	—	14.00	—	—
Conventional torula yeast	—	—	14.00	—
Fish meal	5.00	1.50	1.50	3.50
Spray-dried protein plasma	3.50	—	—	—
Soybean oil	2.60	2.88	2.61	3.20
Limestone	0.82	1.75	1.40	0.90
Dicalcium phosphate	0.82	—	0.54	0.40
L-Lys HCL	0.35	0.24	0.35	0.36
DL-Met	0.16	0.22	0.26	0.13
L-Thr	0.11	0.03	0.04	0.10
L-Trp	—	—	0.01	—
L-Val	—	—	0.03	—
Sodium chloride	0.50	0.50	0.50	0.50
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50
Analyzed values				
Dry matter, %	90.64	91.54	89.61	89.77
Ash, %	4.89	5.60	5.24	5.00
Gross energy, kcal/kg	4,036	4,068	4,093	4,005
Crude protein, %	18.87	19.48	20.02	17.10
AEE[3], %	5.40	6.15	6.23	3.91
Amino acids, %				
Arg	1.33	1.33	1.25	1.13
His	0.56	0.50	0.51	0.47
Ile	0.88	0.97	0.94	0.80
Leu	1.81	1.72	1.73	1.58
Lys	1.67	1.59	1.66	1.38
Met	0.44	0.54	0.57	0.41
Phe	1.05	1.00	1.02	0.86
Thr	0.99	0.99	0.95	0.86
Trp	0.36	0.41	0.35	0.27
Val	1.05	1.12	1.07	0.87
Ala	1.12	1.22	1.21	0.97
Asp	2.11	2.12	2.10	1.87
Cys	0.35	0.29	0.29	0.30
Glu	3.60	3.66	3.63	3.24
Gly	0.99	0.97	0.95	0.81
Ser	0.98	0.94	0.95	0.81
Tyr	0.78	0.75	0.78	0.63

[1]The woody torula yeast and conventional torula yeast were provided by Arbiom Inc., Durham, NC, USA.
[2]The vitamin–mineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2,210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.
[3]AEE = acid-hydrolyzed ether extract.

determined using a Fragment Analyzer Automated CE System (Method DNF-471-33-SS Total RNA 15nt; Advanced Analytical, Ankeny, IA, USA) and RNA samples with an RNA quality number greater than 8 were used for library preparation. A portion of the RNA was analyzed for ultra-high-throughput sequencing of RNA using Illumina Novaseq 6000. Data from the library sequencing were then summarized using MultiQC version 1.9. The *Sus scrofa* transcriptome file from Annotation 106 from NCBI was used for quasi-mapping and count generation. Salmon version 1.4.0 was then used to quasi-map reads to the transcriptome and quantify the abundance of each transcript (Patro et al., 2017). Differential gene expression analysis was performed using the limma-trend method using a model of treatment after "remove unwanted variation" analysis (Ritchie et al., 2015). Hierarchical clustering was used to group genes with similar expression trends.

Statistical analysis

All data except data for RNA sequencing were analyzed using the Mixed Procedure of SAS (SAS Institute Inc., Cary, NC, USA) with the pen as the experimental unit. Homogeneity of the variances were confirmed using the UNIVARIATE procedure in SAS. Diet was the fixed effect. Block was considered the random effect. Treatment means were calculated and separated using the LSMEANS statement and the PDIFF option of PROC MIXED, respectively. The chi-squared test was used to analyze diarrhea frequency among dietary treatments. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

For RNA sequencing, the false discovery rate (**FDR**) threshold (adjusted *P*-value) of 0.05 and 0.15 was used, and three pairwise comparisons were pulled from the model (woody torula yeast vs. control, conventional torula yeast vs. control, and woody torula yeast vs. conventional torula yeast). The FDR threshold of 0.15 was used to visualize differentially expressed genes in a heatmap (Benjamini and Hochberg, 1995). More than 150 genes were visualized in a heatmap to visualize all patterns of change across all treatments. Over-representation analysis for Kyoto Encyclopedia of Genes and Genomes (**KEGG**) pathways using the top 500 most significant genes in each pairwise comparison was also performed (Kanehisa and Goto, 2000).

Results

Growth performance and fecal score

No differences were observed in any growth performance parameters (i.e., ADG, ADFI, G:F, final body weight) among treatments during phase 1, phase 2, or for the overall experimental period (Table 3). Pigs fed the phase 1 diet containing the woody torula yeast or the diet containing the conventional torula yeast had improved ($P < 0.05$) fecal scores and diarrhea frequency compared with pigs fed the control diet from days 1 to 7 and during the first phase of the experiment (Table 4). However, when all pigs were fed the common diet during the second phase of the experiment, no differences in fecal scores and diarrhea frequency of pigs were observed, but for the overall experimental period, frequency of diarrhea was reduced ($P < 0.05$) if pigs were fed phase 1 diets containing one of the two sources of torula yeast compared with pigs fed the control diet in phase 1.

Blood characteristics and intestinal morphology

On day 14, plasma concentrations of IL-1β, IL-8, IL-12, and TNF-α in pigs were not different among treatments (Table 5). On day 14, pigs fed the conventional torula yeast diet had greater ($P < 0.05$) concentration of IL-2 compared with pigs fed the control diet, and greater ($P < 0.05$) concentrations of IL-4 and IL-10 were observed in pigs fed the woody torula yeast or the conventional torula yeast compared with pigs fed the control diet. However, on day 28, no differences were observed in concentrations of plasma cytokines among treatments. PUN was less ($P < 0.01$) on day 14 of pigs fed the control diet compared with pigs fed a diet containing one of the two sources of torula yeast (Table 6). However, no difference was observed in PUN after pigs had been fed the common phase 2 diet from days 15 to 28. On days 14 and 28, no differences among treatments were observed in concentrations of total protein, albumin, IgG, or PYY. Likewise, on day 7, no differences among treatments were observed for villus height, villus width, crypth depth, or villus height:crypt depth ratio in the ileum (Table 7).

RNA sequencing

At the traditional FDR correction threshold of 0.05, the abundance of a few genes was among treatments. Most genes (19 of 24; i.e., ACE2, ATP1A1, ATP1B1, COL17A1, COL26A1, DPP4, KCNJ13, KCNK5, MEP1A, MME, SLC15A1, SLC1A1, SLC7A7, XPNPEP2, ABCC1, APOA1, SLC19A1, SLC23A1, SLC52A3) were downregulated in pigs fed the diet containing the woody torula yeast compared with pigs fed the control diet, whereas two genes (i.e., ANKS4B and FAM54A) were downregulated in pigs fed the woody torula yeast diet compared with pigs fed the diet containing conventional torula yeast (Table 8). If an FDR correction threshold of 0.15 was used, 138 genes were downregulated in pigs fed the diet containing the woody torula yeast diet compared with pigs fed the control diet, whereas five genes were downregulated in pigs fed the conventional torula yeast diet compared with pigs fed the control diet (Figure 1). Using KEGG pathway testing, the top two pathways that were influenced for the control vs. woody torula yeast diet comparison are "protein digestion and absorption" and "vitamin digestion and absorption" containing 14 and 5 genes, respectively. As was demonstrated in the heatmap illustration (Figure 2), all 19 genes from pigs fed the woody torula yeast diet were downregulated compared with pigs fed the control diet.

Discussion

Weaning is one of the most stressful periods that results in behavioral, immunological, and intestinal changes (Campbell et al., 2013). Therefore, strategies to prevent occurrence of infections, diseases, and intestinal villous atrophy have to be identified to reduce mortality during this period (Kil and Stein, 2010). The woody torula yeast that was used in this experiment has greater concentrations of digestible amino acids and P than fish meal (Lagos and Stein, 2020), and therefore, this torula yeast can replace fish meal and plasma protein in phase 1 diets for weanling pigs (Espinosa et al., 2020). Similar conclusions were reported from experiments with other sources of yeast (Cruz et al., 2019, 2020). However, data demonstrating effects of torula yeast on intestinal health and immune response are limited.

Table 3. Growth performance for pigs fed experimental diets[1]

Item	Control	Woody torula yeast	Conventional torula yeast	SEM	*P*-value
Days 1 to 7[2]					
Initial body weight, kg	6.645	6.676	6.629	0.266	0.473
ADG[3], kg	0.076	0.055	0.067	0.011	0.418
ADFI[3], kg	0.127	0.104	0.114	0.010	0.311
G:F[3]	0.597	0.521	0.582	0.072	0.680
Final body weight, kg	7.180	7.044	7.099	0.254	0.469
Days 8 to 14[4]					
Initial body weight, kg	7.121	7.071	7.128	0.267	0.889
ADG, kg	0.202	0.181	0.206	0.018	0.552
ADFI, kg	0.259	0.255	0.267	0.013	0.774
G:F	0.772	0.687	0.776	0.060	0.503
Final body weight, kg	8.536	8.327	8.570	0.289	0.543
Days 1 to 14 (phase 1)					
ADG, kg	0.135	0.119	0.134	0.011	0.422
ADFI, kg	0.184	0.169	0.179	0.010	0.554
G:F	0.740	0.686	0.775	0.055	0.487
Days 15 to 28 (phase 2)					
ADG, kg	0.392	0.399	0.378	0.023	0.693
ADFI, kg	0.582	0.577	0.546	0.028	0.315
G:F	0.674	0.696	0.689	0.017	0.665
Final body weight, kg	14.021	13.912	13.866	0.560	0.939
Days 1 to 28 (overall phase)					
ADG, kg	0.263	0.259	0.259	0.013	0.947
ADFI, kg	0.367	0.358	0.349	0.016	0.531
G:F	0.719	0.725	0.740	0.018	0.649

[1]Data are least squares means of 10 observations per treatment.
[2]All treatments had 4 pigs per pen from days 1 to 7.
[3]ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.
[4]All treatments had 3 pigs per pen from days 8 to 28.

The lack of differences among treatments for overall growth performance of pigs indicates that the woody torula yeast or conventional torula yeast can replace spray-dried plasma protein and fish meal in diets for weanling pigs without affecting growth performance of pigs. All diets were formulated to contain similar concentrations of metabolizable energy and standardized ileal digestible amino acids, which likely contributed to the lack of differences in growth performance. These results are in agreement with data indicating that inclusion of 14% to 20% torula yeast at the expense of fish meal, plasma protein, and soybean meal did not have negative effects on growth performance of weanling pigs (Cruz et al., 2019; Espinosa et al., 2020).

The first week of the post-weaning period is often associated with disruption and deterioration of the gastrointestinal structure and function in weanling pigs (Montagne et al., 2007). Therefore, the observed improvement in fecal scores of pigs fed the torula yeast diets during phase 1 indicates that either source of torula yeast may influence community structure of microorganisms in the intestinal tract of pigs. Soybean meal was included at 25% in all phase 1 diets to test the hypothesis that dysbiosis, due to high levels of soybean meal, can be alleviated by including torula yeast in the diet, and results for fecal scores indicated that the hypothesis was confirmed.

Anti-inflammatory cytokines, including IL-10, IL-2, and IL-4, contribute to protecting the intestinal barrier integrity from effects of interferon-ɤ and TNF-α in inducing barrier disruption (Al-Sadi et al., 2009). Anti-inflammatory cytokines also inhibit activation and effector function of T cells, monocytes, and macrophages (Moore et al., 2001). Therefore, the observed increase in concentrations of anti-inflammatory cytokines on day 14 upon inclusion of one of the sources of torula yeast in the phase 1 diet indicates that these feed ingredients influence the immune status of weanling pigs, which is in agreement with the observed reduction in fecal score of pigs. Yeast and yeast derivatives contain mannan oligosaccharides and β-glucans, which suppress activity of mycotoxins and reduce colonization of intestinal pathogenic bacteria (Kogan and Kocher, 2007). Therefore, the observed improvement in the immune response of pigs fed diets containing one of the sources of torula yeast may be a result of a positive influence of mannans and glucans on intestinal health of pigs.

PUN is often used as an indicator of the efficiency of amino acid utilization and N excretion in pigs (Coma et al., 1995). Therefore, the observed increase in the concentration of PUN on d 14 in pigs fed diets containing the woody torula yeast or conventional torula yeast indicates that torula yeast may reduce the efficiency of N utilization of pigs. This is in contrast

Table 4. Fecal score and frequency of diarrhea for pigs fed experimental diets[1]

Item	Control	Woody torula yeast	Conventional torula yeast	SEM	*P*-value
Fecal score[2]					
Days 1 to 7	1.95[a]	1.52[b]	1.52[b]	0.105	0.014
Days 8 to 14	2.00	1.80	1.73	0.172	0.504
Days 1 to 14 (phase 1)	1.97[a]	1.64[b]	1.61[b]	0.099	0.037
Days 15 to 28 (phase 2)	2.07	2.16	2.06	0.171	0.818
Days 1 to 28 (overall phase)	2.02	1.90	1.84	0.099	0.345
Frequency of diarrhea					
Days 1 to 7					
Pen days[3]	40	40	40		
Frequency[4]	32.50	10.00	12.50	—	0.017
Days 8 to 14					
Pen days	30	30	30		
Frequency	36.67	20.00	13.33	—	0.089
Days 1 to 14 (phase 1)					
Pen days	70	70	70		
Frequency	34.29	14.29	12.86	—	0.002
Days 15 to 28 (phase 2)					
Pen days	70	70	70		
Frequency	34.29	37.14	27.14	—	0.431
Days 1 to 28 (overall phase)					
Pen days	140	140	140		
Frequency	34.29	25.71	20.00	—	0.025

[a,b]Means within a row lacking a common letter are different ($P < 0.05$).
[1]Data are least squares means of 10 observations per treatment.
[2]Fecal score = 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; 5, watery diarrhea.
[3]Pen days = number of pens × the number of days assessing diarrhea scores.
[4]Frequency = (number of pen days with diarrhea scores ≥ 3/pen days) × 100.

with data indicating that inclusion of up to 26% torula yeast in diets does not affect PUN of weanling pigs (Espinosa et al., 2020). Nevertheless, the lack of differences in total protein, albumin, and PYY on days 14 and 28 indicates that overall, torula yeast does not influence hormone or nutrient metabolism in pigs.

Measurements for villus height and crypt depth are used as indicators for intestinal health of pigs. The lack of differences in villus height, villus width, and crypt depth among treatments indicates that replacing animal proteins in phase 1 diets for weanling pigs (i.e., plasma protein and fish meal) with one of the two sources of torula yeast does not influence intestinal development of pigs.

The observation that 19 genes (i.e., ACE2, ATP1A1, ATP1B1, COL17A1, COL26A1, DPP4, KCNJ13, KCNK5, MEP1A, MME, SLC15A1, SLC1A1, SLC7A7, XPNPEP2, ABCC1, APOA1, SLC19A1, SLC23A1, SLC52A3) from pigs fed the woody torula yeast diet were downregulated compared with pigs fed the control diet indicates that the woody torula yeast influences expression of a few genes involved in nutrient (i.e., mostly protein) digestion and absorption. The observed reduction in expression of these genes upon inclusion of the woody torula yeast indicates that pigs fed the woody torula yeast diet had earlier cell maturation and development at this period due to increased amino acid digestibility in the woody torula yeast (Lagos and Stein, 2020). It is also possible that pigs fed the control diet had increased inflammation, which subsequently resulted in upregulation of proteins as a mechanism to alleviate potential infection and rebuild tissues from intestinal damage (Limbach et al., 2021). However, further research is needed to confirm this hypothesis.

Conclusion

Results of this experiment indicated that replacement of fish meal and plasma protein in phase 1 diets for pigs with a woody or a conventional source of torula yeast did not influence pig growth performance, but reduced fecal scores during the initial 2-wk post-weaning. Concentrations of total protein, albumin, immunoglobulin G, and morphology in the ileum of pigs were not affected by using either source of torula yeast instead of fish meal or plasma protein. However, torula yeast increased concentrations of the anti-inflammatory cytokines (i.e., IL-4, IL-10) and downregulated abundance of a few genes involved in protein absorption. Overall, no difference was observed between the woody torula yeast and conventional torula yeast in influencing growth performance and intestinal health of pigs. Therefore, inclusion of torula yeast at the expense of fish meal and plasma protein in diets for weanling pigs may be a strategy to improve intestinal health without influencing growth performance of weanling pigs.

Table 5. Concentrations of cytokines in plasma of pigs fed experimental diets[1]

Item	Control	Woody torula yeast	Conventional torula yeast	SEM	*P*-value
Day 14					
IL[2]-1β, pg/mL	266.81	371.97	286.08	70.16	0.341
IL-2, pg/mL	60.44[b]	101.94[ab]	183.07[a]	34.67	0.025
IL-4, pg/mL	66.91[b]	333.82[a]	279.60[a]	74.07	0.046
IL-8, pg/mL	19.99	21.05	14.03	5.25	0.505
IL-10, pg/mL	151.35[b]	411.90[a]	387.11[a]	87.36	0.025
IL-12, pg/mL	1,279.33	1,173.90	1,207.48	119.95	0.817
TNF-α[2], pg/mL	51.58	64.91	39.34	10.58	0.126
Day 28					
IL-1β, pg/mL	84.30	49.27	37.15	28.66	0.511
IL-2, pg/mL	43.86	86.90	66.90	34.04	0.474
IL-4, pg/mL	35.39	93.03	49.43	32.77	0.123
IL-8, pg/mL	16.75	12.34	16.23	6.14	0.396
IL-10, pg/mL	117.41	163.13	175.62	48.70	0.535
IL-12, pg/mL	1,509.93	1,473.04	1,369.13	116.33	0.665
TNF-α, pg/mL	32.38	33.25	26.27	9.67	0.704

[a,b]Means within a row lacking a common letter are different ($P < 0.05$).
[1]Data are least squares means of 9 or 10 observations per treatment.
[2]IL = interleukin; TNF-α = tumor necrosis factor-α.

Table 6. Blood characteristics of pigs fed experimental diets[1]

Item	Control	Woody torula yeast	Conventional torula yeast	SEM	*P*-value
Day 14					
PUN[2], mg/dL	6.10[b]	10.56[a]	11.50[a]	0.87	<0.001
Total protein, g/dL	4.84	4.78	4.75	0.13	0.751
Albumin, g/dL	2.60	2.61	2.55	0.09	0.829
IgG[2], mg/mL	7.27	7.19	7.82	1.14	0.788
Peptide YY, ng/mL	1.97	2.18	2.41	0.44	0.780
Day 28					
PUN, mg/dL	6.31	5.90	5.10	0.53	0.173
Total protein, g/dL	4.74	4.87	4.81	0.12	0.391
Albumin, g/dL	2.63	2.87	2.76	0.12	0.159
IgG, mg/mL	6.53	6.55	6.63	0.37	0.982
Peptide YY, ng/mL	2.80	2.68	2.76	0.25	0.923

[a,b]Means within a row lacking a common letter are different ($P < 0.01$).
[1]Data are least squares means of 9 or 10 observations per treatment.
[2]PUN = plasma urea nitrogen; IgG = immunoglobulin G.

Table 7. Intestinal morphology in the ileum of pigs fed experimental diets[1]

Item	Control	Woody torula yeast	Conventional torula yeast	SEM	*P*-value
Day 7					
Villus height, μm	296.46	285.62	297.11	9.88	0.580
Villus width, μm	120.83	120.77	115.61	3.60	0.494
Crypt depth, μm	202.49	200.25	205.91	11.55	0.931
Villus height:crypt depth	1.54	1.51	1.54	0.07	0.907

[1]Data are least squares means of 9 or 10 observations per treatment.

Table 8. Number of differentially expressed genes using false discovery rate (FDR) at $P < 0.05$ and $P < 0.15$[1]

Item	Woody torula yeast vs. Control	Conventional torula yeast vs. Control	Woody torula yeast vs. Conventional torula yeast
FDR at $P < 0.05$			
Downregulated	19	0	2
Upregulated	5	0	1
Not significant	16,208	16,232	16,229
FDR at $P < 0.15$			
Downregulated	138	5	77
Upregulated	33	2	75
Not significant	16,061	16,232	16,080

[1]Data are least squares means of 9 or 10 observations per treatment.

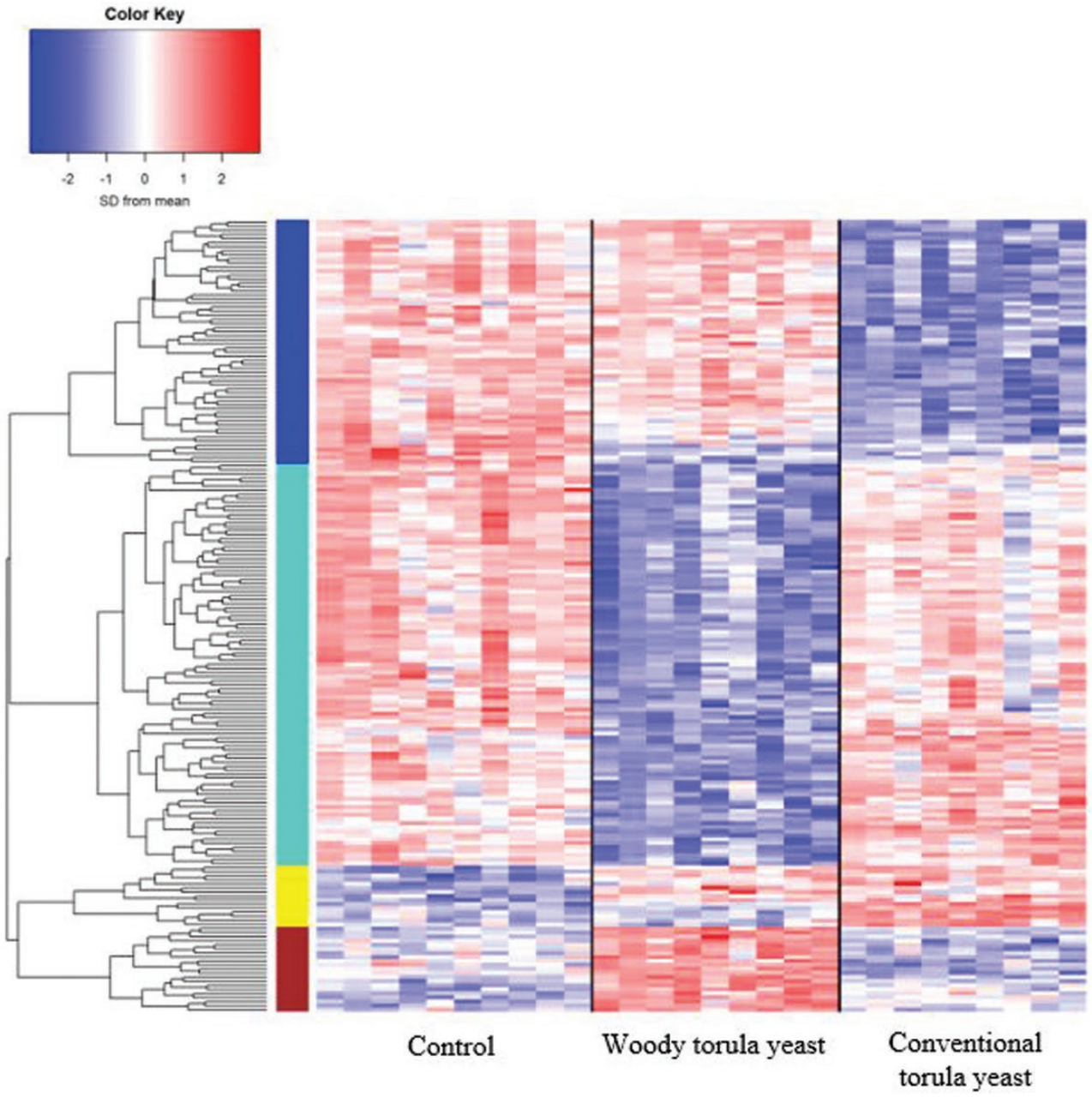

Figure 1. Heatmap of 195 genes using false discovery rate (FDR) at $P < 0.15$.

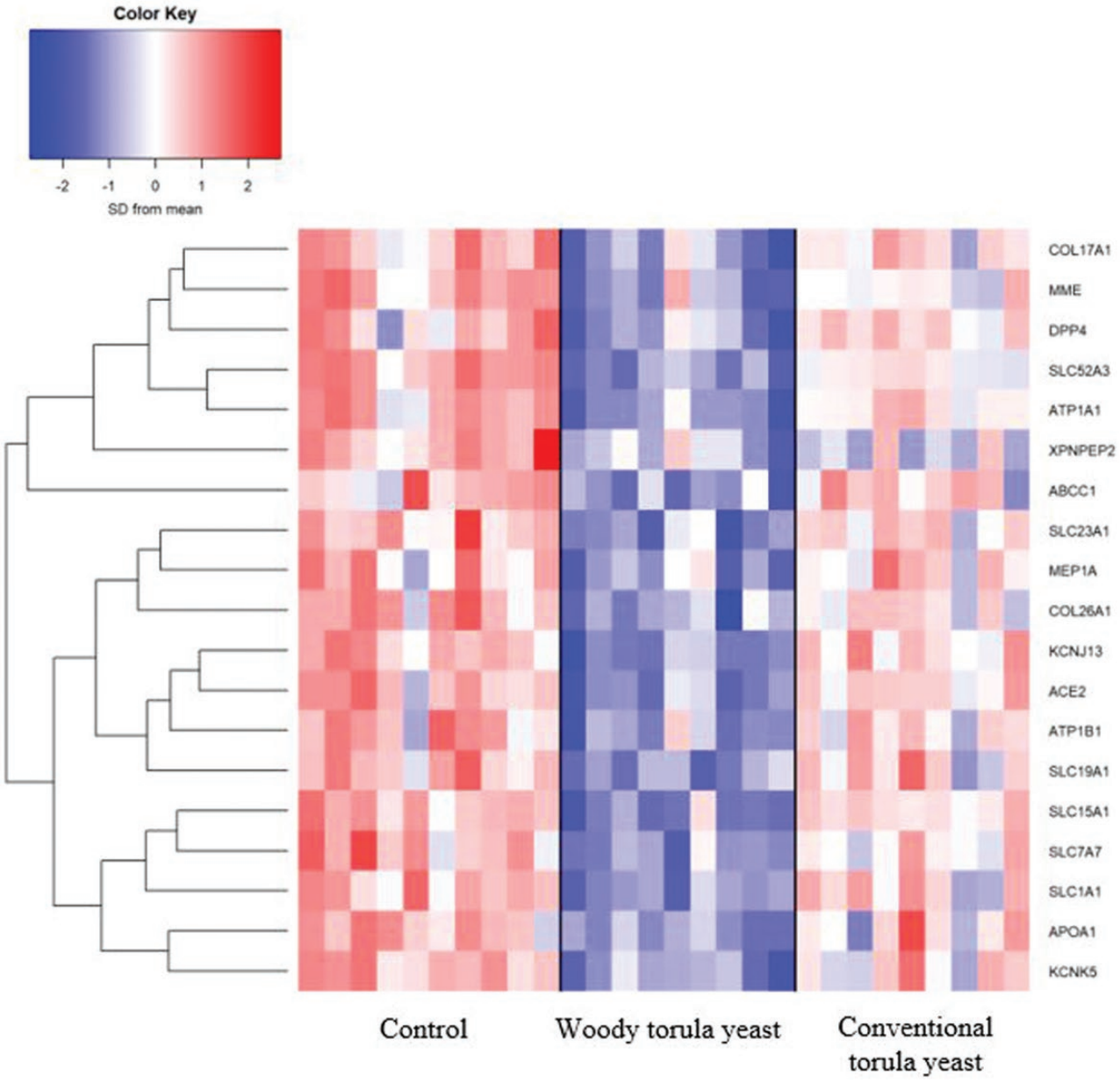

Figure 2. Heatmap of 19 genes involved in protein/vitamin digestion and absorption pathways.

Acknowledgments

Funding for this research by Arbiom Inc., Durham, NC, USA is greatly appreciated.

Conflict of interest statement

The authors have no real or perceived conflicts of interest.

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