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Non Ruminant Nutrition

NON RUMINANT NUTRITION

Effect of torula yeast on growth performance, diarrhea incidence, and blood characteristics in weanling pigs

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Abstract

Two experiments were conducted to test the hypothesis that torula yeast may replace animal and plant proteins in diets for weanling pigs without negatively impacting growth performance or blood characteristics. In exp. 1, 128 weanling pigs (6.71 ± 0.76 kg) were allotted to four treatments with four pigs per pen and eight replicate pens per diet. Pigs were fed one of four diets from day 1 to 14 post-weaning (phase 1), whereas all pigs were fed a common diet in phase 2 (day 15 to 28). The four dietary treatments included a control diet with 5% fish meal, 2.5% plasma protein, and no torula yeast. The second diet contained 5% fish meal, 4.75% torula yeast, and no plasma protein. The third diet contained 2.5% plasma protein, 6% torula yeast, and no fish meal, and the last diet contained 10.75% torula yeast, no fish meal, and no plasma protein. The inclusion of torula yeast was calculated to replace the amount of digestible Lys provided by fish meal, plasma protein, or both fish meal and plasma protein in the control diet. During the initial 14 d, fecal scores were visually assessed. At the end of phase 1, blood samples were collected and tumor necrosis factor-α (**TNF-α**), blood urea nitrogen (**BUN**), peptide YY, immunoglobulin G, total protein, and albumin were analyzed. Results indicated that torula yeast could replace fish meal and plasma protein without affecting growth performance, fecal scores, or blood characteristics of pigs. In exp. 2, 160 weanling pigs (6.11 ± 0.62 kg) were allotted to one of four diets with five pigs per pen and eight replicate pens per diet. Phase 1 diets contained 0%, 10%, 18%, or 26% torula yeast, whereas phase 2 diets contained 0%, 8%, 14%, or 20% torula yeast. Torula yeast was included in diets at the expense of animal proteins and soybean meal. On days 14 and 28, blood samples were collected and concentrations of cytokines, BUN, total protein, and albumin were analyzed. Phase 2 gain-to-feed ratio (**G:F**) linearly increased ($P < 0.01$) as the concentration of torula yeast increased in the diets. The concentration of albumin on day 14 linearly increased ($P < 0.05$) and the concentration of TNF-α was linearly reduced ($P < 0.01$) as the concentration of torula yeast increased in the diets. In conclusion, under the conditions of this research, torula yeast could replace fish meal and plasma protein without affecting the growth performance of pigs, but inclusion of increasing levels of torula yeast improved G:F of pigs, which may be because of greater nutrient utilization.

Key words: blood characteristics, fecal score, growth performance, pigs, torula yeast, weaning

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
AEE	acid-hydrolyzed ether extract
BUN	blood urea nitrogen
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunoabsorbent assay
G:F	gain-to-feed ratio
IgG	immunoglobulin G
PYY	peptide YY
TNF-α	tumor necrosis factor-α

Introduction

Yeast and yeast derivatives contain significant quantities of amino acids, mannans, β-glucans, and nucleotides (Kogan and Kocher, 2007; Mateo and Stein, 2007) and are commonly used as feed additives to improve growth performance and immune response of weanling pigs (Shen et al., 2009; Jiang et al., 2015). However, yeast protein can also be included in diets for weanling and growing pigs as a source of amino acids at the expense of plasma protein and soybean meal (LeMieux et al., 2010; Mora et al., 2012). Torula yeast (currently classified as *Cyberlindnera jadinii*, but known classically as *Candida utilis*) is grown in sugars from molasses, alcohols, or wood and has been used in animal feeding for more than 60 yr (Bekatorou et al., 2006; Buerth et al., 2016). Torula yeast produced via fermentation of forestry and agricultural residues can be included in swine diets by up to 14.6% at the expense of both animal and plant protein sources without compromising the growth performance of pigs (Cruz et al., 2019, 2020). Unlike most protein sources included in diets for weanling pigs, torula yeast is a potential sustainable ingredient because of its ability to produce high-value substrates from nonfood biomass without depending on land, water, and climatic settings (Øverland and Skrede, 2017; Lagos and Stein, 2020).

A novel torula yeast derived from forestry byproducts has recently been developed (ARBIOM, 2018), and results from digestibility experiments indicate that this single-cell protein may be used as a source of energy and digestible amino acids in diets fed to weanling pigs (Lagos and Stein, 2020). Thus, it is believed that this source of torula yeast can be included in diets for weanling pigs at the expense of animal proteins and soybean meal, but there are currently no data to demonstrate this. Therefore, two experiments were conducted to test the hypothesis that torula yeast may replace animal and plant protein sources in diets for weanling pigs without compromising growth performance or blood characteristics. The objective of experiment 1 was to determine the effects on growth performance and immune response of weanling pigs of replacing fish meal and plasma protein by torula yeast. Experiment 2 was conducted to determine the effect of torula yeast, not only as a replacement for animal protein sources, but also as a substitute for soybean meal, in diets for weanling pigs.

Materials and Methods

Protocols for the two experiments were submitted to and approved by the Institutional Animal Care and Use Committee at the University of Illinois and followed protocols for animals as outlined by the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Societies 2010). Pigs were the offspring of PIC Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN).

Experiment 1: Replacing plasma protein and fish meal by torula yeast

Animals and treatments

A total of 128 weanling pigs (20 d old; initial body weight: 6.71 ± 0.76 kg) were allotted to four dietary treatments in a completely randomized design. There were four pigs per pen (two gilts and two castrates) and eight 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 4 wk. A two-phase feeding program was used with weeks 1 and 2 as phase 1 and weeks 3 and 4 as phase 2. Pigs were fed one of four diets during phase 1, whereas all pigs were fed a common diet in phase 2. Therefore, a total of five diets were formulated. The four dietary treatments were based on corn, soybean meal, and whey powder. In addition, torula yeast (SylPro, Arbiom Inc., Durham, NC), a commercial source of fish meal (Menhaden Select; Omega Protein, Houston, TX), and plasma protein (AP 920; APC Inc., Ankeny, IA) were used in the experiment as the main sources of amino acids. Neither antibiotic growth promoters nor pharmacological levels of Zn and Cu were used. Diets were formulated using data for metabolizable energy and digestibility of amino acids and P in torula yeast from the study of Lagos and Stein (2020; Table 1), whereas data for all other ingredients and current nutrient requirements for weanling pigs were from NRC (2012; Table 2). Inclusion of torula yeast in the diets was calculated to replace the amount of standardized ileal digestible Lys provided by fish meal, plasma protein, or both fish meal and

Table 1. Nutrient composition of torula yeast[1]

Item	Torula yeast
Metabolizable energy, kcal/kg	3,479
Dry matter, %	95.43
Crude protein, %	51.68
Ash, %	12.56
Ca, %	0.13
P[2], %	1.62
Indispensable amino acids[3], %	
Arg	2.21
His	0.83
Ile	2.08
Leu	3.13
Lys	3.16
Met	0.52
Phe	1.90
Thr	1.85
Trp	0.57
Val	2.41
Dispensable amino acids[3], %	
Ala	3.00
Asp	3.77
Cys	0.37
Glu	7.43
Gly	1.80
Ser	1.61
Tyr	1.50

[1]Torula yeast = SylPro, Arbiom Inc., Durham, NC; Adapted from Lagos and Stein (2020).
[2]Standardized total tract digestible P.
[3]Amino acids are indicated as standardized ileal digestible amino acids.

Table 2. Composition of experimental diets, exp. 1

	Phase 1				
Item	No torula yeast	No plasma	No fish meal	Torula yeast[1]	Phase 2
Ingredient, %					
Ground corn	52.11	49.71	50.11	47.72	53.18
Soybean meal	20.00	20.00	20.00	20.00	28.00
Whey powder	15.00	15.00	15.00	15.00	10.00
Torula yeast	—	4.75	6.00	10.75	—
Fish meal	5.00	5.00	—	—	3.00
Plasma protein	2.50	—	2.50	—	—
Soybean oil	3.00	3.20	3.42	3.62	3.50
Ground limestone	1.00	1.07	1.45	1.60	0.90
Dicalcium phosphate	0.15	—	0.25	—	0.25
L-Lys HCL	0.39	0.39	0.39	0.39	0.32
DL-Met	0.12	0.15	0.16	0.20	0.12
L-Thr	0.08	0.08	0.07	0.07	0.08
Sodium chloride	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral premix[2]	0.15	0.15	0.15	0.15	0.15
Analyzed values					
Gross energy, kcal/kg	3,934	4,022	4,014	4,057	4,028
Metabolizable energy[3], kcal/kg	3,469	3,480	3,472	3,486	3,464
Dry matter, %	88.56	88.93	88.89	89.31	88.49
Ash, %	5.89	6.54	6.28	6.51	5.61
Crude protein, %	19.14	20.33	19.91	22.57	20.20
AEE, %	6.87	6.56	6.30	6.86	7.09
Ca, %	0.81	0.84	0.73	0.82	0.69
P, %	0.52	0.59	0.64	0.55	0.53
Indispensable amino acids, %					
Arg	1.12	1.19	1.18	1.23	1.19
His	0.46	0.48	0.49	0.51	0.47
Ile	0.89	0.95	0.91	0.99	0.87
Leu	1.56	1.70	1.70	1.80	1.58
Lys	1.37	1.68	1.52	1.59	1.36
Met	0.41	0.43	0.43	0.45	0.38
Met + Cys	0.69	0.73	0.79	0.81	0.67
Phe	0.87	0.95	0.96	1.02	0.91
Thr	0.84	0.92	0.93	1.01	0.82
Trp	0.22	0.24	0.25	0.26	0.24
Val	0.93	1.04	1.06	1.16	0.95
Dispensable amino acids, %					
Ala	0.96	1.12	1.05	1.17	0.96
Asp	1.80	2.00	2.00	2.11	1.90
Cys	0.28	0.30	0.36	0.36	0.29
Glu	3.17	3.46	3.49	3.73	3.27
Gly	0.83	0.93	0.81	0.89	0.83
Pro	1.00	1.09	1.07	1.08	1.06
Ser	0.78	0.87	0.92	0.95	0.80
Tyr	0.62	0.68	0.72	0.76	0.64
Total amino acids, %	18.42	20.03	19.85	21.07	18.52

[1]Torula yeast = SylPro, Arbiom Inc., Durham, NC.
[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 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]Metabolizable energy = determined via calculation.

plasma protein in the control diet. Inclusion of crystalline amino acids was adjusted to maintain the ideal amino acid pattern.

Experimental procedures

Individual pig weights were recorded at the beginning of the experiment, on day 14, and on day 28. Feed addition was recorded daily, and the weight of feed left in the feeder was recorded on days 14 and 28. During the initial 14 d, fecal scores were assessed visually every other day by two independent observers 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 14, days 15 to 28, and for the entire experiment.

Blood collection and analyses

At the end of phase 1, two blood samples were collected from one pig per pen via vena puncture. These samples were collected in vacutainers without or with EDTA to yield blood serum and blood plasma, respectively. Serum and plasma samples were obtained by centrifuging blood samples at 1,500 × *g* at 4 °C for 15 min. Serum samples were analyzed for blood urea nitrogen (**BUN**), total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA). Plasma samples were analyzed for concentrations of tumor necrosis factor-α (**TNF-α**), immunoglobulin G (**IgG**), and peptide YY (**PYY**) using ELISA kits according to the recommendations from the manufacturer (R&D Systems, Inc., Minneapolis, MN; Bethyl Laboratories, Inc., Montgomery, TX; and Phoenix Pharmaceuticals, Inc., Burlingame, CA, respectively).

Diet analyses

All diet samples were ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) prior to chemical analysis. Diets were analyzed for dry matter (method 930.15; AOAC Int., 2007) and ash (method 942.05; AOAC Int., 2007), and gross energy was determined using bomb calorimetry (model 6400; Parr Instruments, Moline, IL). Acid-hydrolyzed ether extract (**AEE**) was analyzed by acid hydrolysis using 3 N HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY), and crude protein was determined using the combustion procedure (method 990.03; AOAC Int., 2007) on an FP628 protein analyzer (Leco Corporation, St. Joseph, MI). Amino acids were analyzed on a Hitachi Amino Acid Analyzer (model no. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard (method 982.30 E (a, b, c); AOAC Int., 2007). Calcium and P were analyzed by inductively coupled plasma optical emissions spectrometry (method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation [method 975.03 B(b); AOAC Int., 2007].

Statistical analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pen as the experimental unit. The normality of residuals and assumptions of the model were tested using PROC GPLOT and influence options of SAS (SAS Inst. Inc., Cary, NC). The statistical model included diet as the main effect, whereas replicate was the random effect. Treatment means were calculated using the LSMEANS statement and if significant, means were separated using the PDIFF statement with LSD test. The chi-squared test was used to analyze the frequency of diarrhea among treatments. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Experiment 2: Increasing levels of torula yeast in phase 1 and 2 diets

Animals and treatments

A total of 160 newly weaned pigs (20 d old; initial body weight: 6.11 ± 0.62 kg) were allotted to one of four diets. A two-phase feeding program was used with day 1 to 14 as phase 1 and day 15 to 28 as phase 2. There were five pigs per pen (three gilts and two castrates) and eight replicate pens per treatment. A total of eight diets were formulated and all diets in phases 1 and 2 were formulated to meet the current estimates for nutrient requirements (Table 3; NRC, 2012). In Phase 1, the control diet contained 5% fish meal, 2.5% plasma protein, and no torula yeast. Three additional diets contained 10%, 18%, or 26% torula yeast, but no fish meal or plasma protein. In phase 2, the control diet contained 3% fish meal and no plasma protein or torula yeast. Three additional diets were formulated to contain 8%, 14%, or 20% torula yeast and no fish meal or plasma protein. The torula yeast, plasma protein, and fish meal used in this experiment were sourced as explained for exp. 1, and neither antibiotic growth promoters nor pharmacological levels of Cu and Zn were used. The increasing levels of torula yeast in phases 1 and 2 diets were calculated to replace the amount of standardized ileal digestible amino acids provided by fish meal or both fish meal and some of the soybean meal in the control diet. Inclusion of soybean meal and crystalline amino acids were adjusted to maintain constant concentrations of standardized ileal digestible amino acids among diets.

Experimental procedure

Individual pig weights were recorded at the beginning of the experiment, on day 14, and on day 28. Feed addition was recorded daily and the weight of feed left in the feeder was recorded on days 14 and 28. Fecal scores were assessed visually per pen every other day and diarrhea frequency was calculated as explained for exp. 1. At the conclusion of the experiment, data were summarized to calculate ADFI, ADG, and G:F within each pen and treatment group. Data were summarized for days 1 to 14, days 15 to 28, and for the entire experiment.

Blood collection and analyses

At the end of each phase, two blood samples were collected from one pig per pen via vena puncture. Samples were collected in vacutainers with heparin or EDTA to yield blood plasma. Heparinized plasma samples were frozen at –20 °C and were analyzed for BUN, total protein, and albumin as explained for exp. 1. Heparinized plasma samples were also analyzed for interleukin-1β using an ELISA kit (R&D Systems, Inc., Minneapolis, MN). TNF-α, interleukin-10, and interleukin-6 were measured in plasma samples collected in EDTA-containing vacutainers using ELISA kits according to the recommendations from the manufacturer (Quantikine Porcine Immunoassays, R&D Systems, Inc., Minneapolis, MN).

Diet and statistical analyses

All diet samples were ground analyzed for dry matter, ash, gross energy, AEE, crude protein, amino acids, Ca, and P as explained for exp. 1. Normality of residuals was confirmed, and data were analyzed using the PROC MIXED procedure of SAS as described for exp. 1. The model included diet (or torula yeast inclusion rate) as a fixed effect and replicate as a random effect. Treatment means were calculated as explained for exp. 1. Linear and quadratic effects of increasing levels of torula yeast in phases 1

Table 3. Composition of experimental diets, exp. 2

	Phase 1 torula yeast[1], %				Phase 2 torula yeast, %			
Item	0	10	18	26	0	8	14	20
Ingredient, %								
Ground corn	50.71	47.00	45.34	43.53	55.56	55.30	54.46	53.62
Soybean meal	21.50	21.50	15.75	10.00	26.00	21.00	16.25	11.50
Whey powder	15.00	15.00	15.00	15.00	10.00	10.00	10.00	10.00
Fish meal	5.00	—	—	—	3.00	—	—	—
Plasma protein	2.50	—	—	—	—	—	—	—
Torula yeast	—	10.00	18.00	26.00	—	8.00	14.00	20.00
Choice white grease	3.00	3.65	3.25	2.95	3.00	3.00	2.72	2.45
Ground limestone	0.98	1.58	1.60	1.60	0.90	1.42	1.42	1.42
Dicalcium phosphate	0.15	—	—	—	0.27	—	—	—
L-Lys HCl	0.34	0.37	0.23	0.09	0.38	0.37	0.28	0.18
DL-Met	0.11	0.19	0.18	0.18	0.14	0.18	0.18	0.18
L-Thr	0.06	0.06	—	—	0.10	0.08	0.04	—
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral premix[2]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Analyzed values								
Gross energy, kcal/kg	4,035	4,120	4,130	4,145	4,041	4,051	4,087	4,098
Metabolizable energy[3], kcal/kg	3,471	3,471	3,470	3,472	3,442	3,441	3,441	3,442
Dry matter, %	89.98	90.53	91.07	91.46	87.72	89.78	90.10	90.37
Ash, %	6.14	6.57	6.26	6.67	5.44	5.37	5.22	5.36
Crude protein, %	21.34	21.23	22.23	24.75	19.28	19.47	20.00	20.66
AEE, %	5.18	5.60	5.62	5.07	5.69	5.49	5.50	5.22
Ca, %	0.77	0.82	0.82	0.88	0.69	0.69	0.70	0.62
P, %	0.52	0.51	0.62	0.67	0.49	0.48	0.53	0.57
Indispensable amino acids, %								
Arg	1.34	1.23	1.11	1.11	1.19	1.13	1.14	1.05
His	0.56	0.50	0.46	0.46	0.47	0.46	0.47	0.44
Ile	0.98	0.99	0.98	1.04	0.86	0.89	0.92	0.91
Leu	1.85	1.72	1.71	1.75	1.59	1.60	1.63	1.61
Lys	1.60	1.54	1.79	1.46	1.39	1.40	1.46	1.32
Met	0.48	0.53	0.41	0.49	0.39	0.47	0.47	0.45
Met + Cys	0.84	0.84	0.69	0.80	0.68	0.77	0.77	0.73
Phe	1.07	1.00	0.97	0.98	0.93	0.93	0.93	0.91
Thr	0.95	0.90	0.89	0.99	0.80	0.84	0.85	0.83
Trp	0.28	0.26	0.30	0.30	0.26	0.25	0.25	0.26
Val	1.15	1.09	1.12	1.22	0.93	0.99	1.05	1.06
Dispensable amino acids, %								
Ala	1.10	1.13	1.25	1.41	0.94	1.05	1.14	1.20
Asp	2.20	2.06	1.92	1.96	1.87	1.87	1.87	1.77
Cys	0.36	0.31	0.28	0.31	0.29	0.30	0.30	0.28
Glu	3.57	3.54	3.43	3.52	3.20	3.28	3.35	3.26
Gly	0.95	0.86	0.87	0.93	0.80	0.80	0.84	0.83
Pro	1.18	1.05	1.00	0.99	1.05	1.01	0.98	0.91
Ser	0.89	0.85	0.84	0.87	0.78	0.79	0.80	0.77
Tyr	0.76	0.72	0.71	0.75	0.66	0.67	0.68	0.68
Total amino acids, %	21.66	20.66	20.45	20.98	18.78	19.10	19.47	18.97

[1]Torula yeast = SylPro, Arbiom Inc., Durham, NC.
[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.
[3]Metabolizable energy = determined via calculation.

and 2 were determined using orthogonal CONTRAST statements. Contrast statements were used with coefficients for unequally spaced treatments being generated using the Proc Interactive Matrix Language statement in SAS. However, for the overall period, means were separated using the PDIFF statement and an LSD test. Statistical significance was considered at $P < 0.05$.

Results

Experiment 1: Replacing plasma protein and fish meal by torula yeast

All pigs consumed their diets without apparent problems; however, one pig fed the control diet died during phase 2 and

Table 4. Growth performance of pigs fed the experimental diets, exp. 1[1]

	Diet					
Item	No torula yeast	No plasma	No fish meal	Torula yeast[2]	SEM	P-value
Phase 1, day 1 to 14						
Initial body weight, kg	6.71	6.73	6.70	6.70	0.27	0.638
ADG, kg	0.097	0.097	0.118	0.119	0.01	0.192
ADFI, kg	0.176	0.156	0.178	0.176	0.01	0.440
G:F	0.542	0.656	0.654	0.689	0.06	0.274
Final body weight, kg	8.07	8.09	8.35	8.36	0.32	0.341
Phase 2, day 15 to 28						
ADG, kg	0.348	0.353	0.345	0.361	0.02	0.916
ADFI, kg	0.536	0.530	0.543	0.552	0.03	0.916
G:F	0.648	0.663	0.639	0.655	0.02	0.766
Final body weight, kg	12.59	12.67	12.83	13.06	0.56	0.752
Overall, day 1 to 28						
ADG, kg	0.217	0.220	0.227	0.236	0.01	0.709
ADFI, kg	0.349	0.336	0.354	0.357	0.02	0.804
G:F	0.621	0.654	0.642	0.663	0.02	0.294

[1]Data are least-squares means of eight observations (pen as the experimental unit; four pigs per pen) for all treatments.
[2]Torula yeast = SylPro, Arbiom Inc., Durham, NC.

Table 5. Fecal score and frequency of diarrhea of pigs fed phase 1 (day 1 to 14) experimental diets, exp. 1[1]

	Diet					
Item	No torula yeast	No plasma	No fish meal	Torula[2] yeast	SEM	P-value
Fecal score[3]	1.89	1.79	1.73	1.61	0.15	0.574
Frequency of diarrhea						
Pen days[4]	56	56	56	56		
Frequency[5]	33.93	23.21	21.43	17.86	—	0.222

[1]Data are least-squares means of eight observations (pen as the experimental unit; four pigs per pen) for all treatments.
[2]Torula yeast = SylPro, Arbiom Inc., Durham, NC.
[3]Fecal score = 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; 5, watery diarrhea.
[4]Pen days = number of pens × the number of days assessing diarrhea scores.
[5]Frequency = (number of pen days with diarrhea scores ≥ 3 ÷ pen days) × 100.

values for growth performance in the pen this pig was housed in were adjusted (Table 4). For final body weight, ADG, ADFI, or G:F, no differences among dietary treatments were observed in phase 1, phase 2, or for the overall experimental period. Likewise, there were no differences among the four dietary treatments for fecal score or frequency of diarrhea during phase 1 (Table 5). There was also no effect of diet on the concentrations of TNF-α, IgG, PYY, BUN, total protein, or albumin when torula yeast replaced plasma protein or fish meal, or both (Table 6).

Experiment 2: Increasing levels of torula yeast in phase 1 and 2 diets

There was no effect of increasing concentrations of torula yeast on final body weight, ADG, ADFI, or G:F of pigs from day 1 to 14 (Table 7). From day 15 to 28, ADFI of pigs linearly decreased ($P < 0.01$), whereas G:F linearly increased ($P < 0.01$), as the concentration of torula yeast increased in the diets. During the overall experimental period, ADFI was reduced ($P < 0.05$) if the concentration of torula yeast in the diet was 14% or greater. However, no differences among dietary treatments were observed for the overall experimental period for final BW, ADG, or G:F. Likewise, the level of torula yeast in the diets did not affect fecal scores or diarrhea frequency of pigs (Table 8). On day 14, albumin concentration of pigs linearly increased ($P < 0.05$), and the concentration of TNF-α was linearly reduced ($P < 0.01$), as the concentration of torula yeast increased in the diets (Table 9). A quadratic increase ($P < 0.05$) in the concentration of total protein was also observed on day 28 in pigs fed diets with increasing concentration of torula yeast.

Discussion

Weaning is a critical period in the life of pigs due to changes in diet form and composition, gastrointestinal function, and immune system activation (Pluske et al., 1997; Heo et al., 2013). At weaning, pigs have to cope with the abrupt withdrawal of sow milk, and this often causes diarrhea due to a lack of endogenous enzymes being produced by weanling pigs (Friesen et al., 1993). Weanling pigs are, therefore, susceptible to infections, diseases, and villous atrophy in the small intestine, which indicates that the intestinal barrier function is disturbed after weaning (Wijtten et al., 2011). Therefore, most diets for weanling pigs contain highly digestible animal proteins (i.e., plasma protein and fish meal) to improve the growth performance and intestinal health of pigs (Kim and Easter, 2001). However, yeast proteins are also available as potential protein sources in diets for pigs and may be used as alternatives to animal proteins (Cruz et al., 2019).

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

	Diet					
Item	No torula yeast	No plasma	No fish meal	Torula[2] yeast	SEM	P-value
TNF-α, pg/ml	141.41	145.40	128.67	118.76	8.97	0.167
PYY, ng/mL	2.25	2.06	2.21	2.38	0.23	0.794
IgG, mg/mL	4.57	5.84	6.39	5.10	0.61	0.135
BUN, mg/dL	7.88	11.75	10.50	10.63	1.09	0.101
Total protein, g/dL	4.17	4.18	4.34	4.28	0.10	0.450
Albumin, g/dL	2.39	2.39	2.41	2.48	0.09	0.852

[1]Data are least-squares mean of seven or eight observations (pen as the experimental unit; four pigs per pen) for all treatments.
[2]Torula yeast = SylPro, Arbiom Inc., Durham, NC.

Table 7. Growth performance of pigs fed diets containing increasing levels of torula yeast, exp. 2[1]

	Torula yeast inclusion[2], %					P-value	
Item	0/0	10/8	18/14	26/20	SEM	Linear	Quadratic
Day 1 to 14							
Initial body weight, kg	6.11	6.11	6.11	6.12	0.24	0.202	0.307
ADG, kg	0.115	0.104	0.095	0.099	0.01	0.105	0.502
ADFI, kg	0.186	0.181	0.180	0.171	0.02	0.185	0.608
G:F	0.618	0.574	0.527	0.578	0.04	0.231	0.235
Final body weight, kg	7.72	7.57	7.44	7.51	0.40	0.118	0.471
Day 15 to 28							
ADG, kg	0.419	0.415	0.423	0.395	0.03	0.431	0.496
ADFI, kg	0.639	0.622	0.580	0.547	0.04	0.005	0.534
G:F	0.657	0.670	0.728	0.718	0.02	0.006	0.766
Final body weight, kg	13.59	13.31	13.36	13.01	0.69	0.065	0.497
Day 1 to 28[3]							
ADG, kg	0.267	0.257	0.259	0.246	0.02	0.151	
ADFI, kg	0.410	0.398	0.380	0.359	0.03	0.020	
G:F	0.631	0.646	0.680	0.648	0.03	0.556	

[1]Data are least-squares means of eight observations (pen as the experimental unit; five pigs per pen) for all treatments.
[2]Torula yeast = SylPro, Arbiom, Durham, NC; phase 1/phase 2.
[3]For the overall period, means were separated using the PDIFF statement with LSD test.

The observation that growth performance, fecal scores, and blood characteristics of pigs in exp. 1 did not differ among dietary treatments indicates that immune response, protein utilization, and growth performance are not affected if plasma protein or fish meal is replaced by torula yeast. Torula yeast contains highly digestible amino acids (Lagos and Stein, 2020), vitamins, β-glucans, and glycoproteins (Alexandre and Guilloux-Benatier, 2006), and this may have supported the growth of pigs upon the absence of fish meal and plasma protein in the diets. These results are in agreement with data indicating that inclusion of up to 14.6% torula yeast (*C. jadinii*) at the expense of fish meal, soybean meal, potato protein concentrate, and rapeseed meal did not have negative effects on growth performance or fecal scores of weanling pigs (Cruz et al., 2019).

The observation in exp. 1 that inclusion of torula yeast in diets did not affect growth performance and blood characteristics of weanling pigs demonstrates that up to approximately 10% torula yeast may be included in diets at the expense of animal protein sources. However, because the maximum inclusion rate in exp. 1 was 10%, it was hypothesized that additional benefits in pig growth performance might be achieved if torula yeast was included in diets by more than 10%. Inclusion of soybean meal is also restricted in diets for weanling pigs, and thus, further investigation of torula yeast as a main protein source in diets is needed.

The lack of differences in growth performance, fecal score, and blood characteristics in exp. 2 between pigs fed the control diet and pigs fed the diet with 10% torula yeast and no plasma protein or fish meal during phase 1 confirms the results obtained in exp. 1. Thus, it appears that pigs grow at the same rate and with the same efficiency if approximately 10% torula yeast is included in diets at the expense of animal protein. The observation that there was no negative effect of increasing torula yeast on growth performance of pigs indicates that torula yeast can be included in phase 1 diets by up to 26%, but cost needs to be considered when replacing soybean meal with torula yeast.

The observation that the overall ADFI of pigs decreased if at least 20% torula yeast was included in the diet is in contrast with data indicating that the inclusion of yeast (*Kluyveromyces fragilis* grown on whey) in diets for weanling pigs resulted in an improvement in feed intake (Spark et al., 2005). However, the present results are in agreement with data indicating that the inclusion of high concentrations of dried brewers' yeast reduced feed intake of rainbow trouts (Rumsey et al., 1991). The observation that G:F of pigs linearly increased as the concentration of torula yeast increased in diets from day 15 to 28, agrees with data indicating that inclusion of hydrocarbon-grown yeast as a protein source in diets improved ADG and feed efficiency of growing pigs (Barber et al., 1971). Despite the observed reduction in feed intake, it appears that pigs digest and

Table 8. Fecal score and frequency of diarrhea of pigs fed diets containing increasing levels of torula yeast, exp. 2[1]

	Torula yeast inclusion[2], %					P-value	
Item	0/0	10/8	18/14	26/20	SEM	Linear	Quadratic
Fecal score[3]							
Day 1 to 14	2.25	2.48	2.43	2.36	0.153	0.606	0.262
Day 15 to 28	1.96	1.93	2.18	1.75	0.152	0.564	0.200
Day 1 to 28[4]	2.11	2.21	2.30	2.05	0.121	0.364	
Frequency of diarrhea							
Day 1 to 14							
Pen days[5]	56	56	56	56			
Frequency[6]	44.64	46.43	51.79	41.07	—	0.717	
Day 15 to 28							
Pen days	56	56	56	56			
Frequency	23.21	30.36	33.93	21.43	—	0.399	
Day 1 to 28							
Pen days	112	112	112	112			
Frequency	33.93	38.39	42.86	31.25	—	0.287	

[1]Data are least-squares means of eight observations (pen as the experimental unit; five pigs per pen) for all treatments.
[2]Torula yeast = SylPro, Arbiom, Durham, NC; phase 1/phase 2.
[3]Fecal score = 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; 5, watery diarrhea.
[4]For the overall period, means were separated using the PDIFF statement with LSD test.
[5]Pen days = number of pens × the number of days assessing diarrhea scores.
[6]Frequency = (number of pen days with diarrhea scores ≥ 3 ÷ pen days) × 100.

Table 9. Blood characteristics of pigs fed diets containing increasing levels of torula yeast, exp. 2[1]

	Torula yeast inclusion[2], %					P-value	
Item	0/0	10/8	18/14	26/20	SEM	Linear	Quadratic
Day 14							
BUN, mg/dL	12.88	12.38	14.63	13.63	1.81	0.504	0.945
Total protein, g/dL	4.44	4.60	4.55	4.74	0.11	0.075	0.880
Albumin, g/dL	2.34	2.46	2.46	2.64	0.11	0.047	0.748
TNF-α, pg/mL	138.23	149.52	123.36	90.58	16.22	0.009	0.075
Interleukin-6, pg/mL	14.27	14.17	15.47	15.35	0.65	0.133	0.895
Interleukin-1 β, pg/mL	6.58	7.71	5.81	6.00	1.18	0.452	0.568
Interleukin-10, pg/mL	7.21	8.14	7.45	6.99	1.07	0.768	0.389
Day 28							
BUN, mg/dL	9.50	9.38	9.88	9.32	1.18	0.997	0.871
Total protein, g/dL	4.50	4.73	4.79	4.59	0.10	0.366	0.040
Albumin, g/dL	2.61	2.66	2.68	2.58	0.10	0.862	0.466
TNF-α, pg/mL	120.94	104.16	109.91	113.15	16.95	0.779	0.553
Interleukin-6, pg/mL	16.99	17.12	17.03	16.68	1.09	0.825	0.798
Interleukin-1 β, pg/mL	8.85	8.03	8.57	9.11	1.04	0.809	0.475
Interleukin-10, pg/mL	16.00	15.08	15.24	14.91	0.90	0.344	0.718

[1]Data are least-squares means of eight observations (pen as the experimental unit; five pigs per pen) for all treatments.
[2]Torula yeast = SylPro, Arbiom, Durham, NC; phase 1/phase 2.

utilize nutrients more efficiently from diets containing torula yeast. It is possible that the increased G:F observed was a result of a positive effect of torula yeast on digestive function and nutrient digestibility (Cruz et al., 2019) due to improved digestive enzyme activity (Øverland and Skrede, 2017). However, because we did not determine those parameters in this experiment, we are unable to confirm the hypothesis that torula yeast influences digestive enzyme activity and thereby contributes to increased absorption of nutrients.

The observed linear increase in the concentration of albumin in pigs as the concentration of torula yeast increased in diets containing torula yeast indicates increased efficiency of transporting nutrients from the liver to peripheral tissues (Francis, 2010). Proteins in blood plasma mainly consist of albumin, globulin, and fibrinogen. Albumin constitutes approximately 60% of the total plasma protein and is involved in the binding and transport of fatty acids, amino acids, and metal ions (Quinlan et al., 2005), whereas globulin can modulate various immunological pathways, including inflammation, antigen presentation, and cell growth (Goncharova et al., 2017). The observed linear reduction in TNF-α concentration may be a result of the increase in total plasma protein concentration upon the inclusion of torula yeast in the diet. Globulin accounts for approximately 38% of the total plasma protein, and β-glucans from torula yeast may have increased globulin production resulting in a subsequent improvement in the immune function of pigs (Brown and Gordon, 2001).

Conclusions

Results from exp. 1 indicate that replacing plasma protein and fish meal by torula yeast as a protein source in diets does not affect immune response, protein utilization, or growth performance of weanling pigs. Inclusion of increasing levels of torula yeast resulted in a linear improvement in G:F of pigs during the second phase of exp. 2, and this may be a result of the positive effect of torula yeast on blood concentrations of indicators for nutrient utilization and inflammatory response. However, further research is needed to determine the mechanism of torula yeast in exerting positive effects on pig growth performance and blood parameters.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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