Research Article

Received: 6 March 2019 Revised: 12 June 2019 Accepted article published: 24 June 2019 Published online in Wiley Online Library: 27 July 2019

(wileyonlinelibrary.com) DOI 10.1002/jsfa.9885

Multi-strain yeast fraction product supplementation can alleviate weaning stress and improve performance and health of piglets raised under low sanitary conditions

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Abstract

BACKGROUND: This study was conducted to evaluate the health benefits to weaning pigs, raised under low sanitary conditions, of dietary supplementation with a multi-strain yeast fraction product (*Cyberlindnera jadinii* and *Saccharomyces cerevisiae*). In total, 160 weaning pigs (7.21 ± 1.05 kg) were randomly allotted to two dietary treatments in a 6-week feeding trial. The dietary treatments included a corn-soybean meal-based basal diet (CON) and CON + 2 g kg^{-1} multi-strain yeast fraction product (MsYF) during weeks 1–2 and 0.4 g kg^{-1} MsYF during weeks 3–6.

RESULTS: The MsYF supplementation increased ($P < 0.05$) body weight (BW) at day 42 and average daily gain (ADG) during days 1–14 and days 1–42 ($P < 0.05$) compared to CON. The total tract digestibility of dry matter (DM), fecal *Lactobacillus* counts, and serum immunoglobulin G (IgG) concentration at day 42 were higher ($P < 0.05$) in pigs fed a MsYF supplemented diet. The concentration of serum haptoglobin in pigs receiving a MsYF-supplemented diet was higher ($P < 0.05$) at days 7, 14, and 42 than those receiving CON. The mRNA expression for INF-γ and TNF-α genes were lower ($P < 0.05$) at days 14 and 7 respectively and the expression of IL-6 and TLR-2 genes was lower ($P < 0.01$) at days 7 and 14 in pigs fed an MsFY supplemented diet than those fed CON.

CONCLUSION: Supplementation with a multi-strain yeast fraction product had a positive effect on ADG during the early post-weaning period and led to better health in weaning pigs.
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Keywords: cytokines; *Cyberlindnera jadinii*; growth performance; *Saccharomyces cerevisiae*; yeast derivative

INTRODUCTION

The emergence of antibiotic-resistant bacterial strains, together with restrictions and bans on the use of growth-promoting antibiotics in animal feed, has led to significant interest in finding alternative products. Among several alternatives, the application of yeast products has gained momentum in recent years.[1]

Yeasts belong to the fungi kingdom and are single-cell, eukaryotic microorganisms.[2] They are cosmopolitan in distribution and are found in abundant quantities in the environment.[3] Various forms of yeast and yeast derivatives have been fed to animals for decades.[4] Some of the reported beneficial yeast species, which have a probiotic effect, include *Saccharomyces cerevisiae*, *Kluyveromyces marxianus,* and *Candida utilis*.[5] *Cyberlindnera jadinii* was recently described as an antagonist to the important human fungal pathogen *Candida albicans*, suggesting its use as a probiotic agent.[6]

The dietary supplementation of live yeast, yeast cultures, or yeast cell-wall products has positively influenced the performance and health of weaned piglets by mitigating negative effects associated with stress and disease.[6–8] Yeast or yeast-based product supplementation has also been demonstrated to boost feed intake, augment mucosal immunity, promote intestinal development, modulate gut microbiota, and reduce post-weaning diarrhea.[9–12] The components of yeast cell walls, which are made up of β-D-glucans and α-D-mannans, are considered to be responsible for their positive effects.[9,10] The presence of nucleotides in yeast cell extracts also makes them more effective in augmenting mucosal immunity. Although nucleotides are present in nearly all feed ingredients, and the requirement for nucleotides is typically met via endogenous synthesis,[11] the requirement for nucleotides may be increased under diseased conditions, high stress, or rapid growth.[13] There are several studies that have reported the use of *Saccharomyces cerevisiae* as a feed additive in ruminants,[15] pigs[8,16]

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and poultry.[17] *Cyberlindnera jadinii* has been used for flavoring food because it is rich in glutamic acid. However, the use of *Cyberlindnera jadinii* as a feed additive is limited. It was hypothesized that yeast fractions from different yeast strains might have synergistic effects.

Thus, the objective of the present study was to evaluate the dietary supplementation of inactivated yeast fractions derived from *Cyberlindnera jadinii* and *Saccharomyces cerevisiae* on performance and immunity in weaning pigs raised in a low-sanitary environment.

MATERIAL AND METHODS

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University, South Korea.

Source of inactivated yeast derivative

The product (YANG®) used for supplementation in this study was derived from two complementary species of *Saccharomyces cerevisiae* and *Cyberlindnera jadinii*, and was obtained from the commercial company Lallemand Animal Nutrition, Blagnac 31702 Midi-Pyrénées, France. The product is an association of fractions from specifically selected inactivated yeast strains grown on molasses, inactivated, fractionated, and spray dried. The presence of *Cyberlindnera jadinii* and *Saccharomyces cerevisiae* was confirmed by the presence of their restriction profiles identified using an internal transcribed spacer – polymerase chain reaction (ITS-PCR) followed by DNA digestion using restriction enzymes.

Animals, experimental design, and housing

In the present experiment, a total of 160 weaning pigs (Yorkshire × Landrace) × Duroc, 7.21 ± 1.05 kg, weaned at 24 days of age, were randomly allotted to two dietary treatments according to initial body weight and sex in a 6-week feeding trial. Piglets were allocated to two rooms with 16 pens / room and five piglets (mixed sexes) / pen (1.8 × 1 m). There was thus a total of 16 replicates per treatment, evenly distributed between both rooms. The dietary treatments included a basal diet (CON) and basal diet supplemented with 2 g kg^{-1} of a multi-strain yeast fraction product (MsYF) during weeks 1–2, and 0.4 g kg^{-1} MsYF during weeks 3–6. A two-phase nursery feeding program was employed in the experiment, with diets formulated to provide all of the nutrients to meet or exceed National Research Council (NRC)[18] requirements (Table 1). The MsYF was supplemented in the diet at the expense of corn. All pigs were housed in an environmentally controlled room with a slatted plastic floor. The temperature during week 1 was maintained at 30 °C and then gradually reduced by 1 °C every week. Each pen was equipped with a self-feeder and a nipple drinker to ensure *ad libitum* access to fresh water and feed throughout the experiment. To achieve the low sanitary environment, the pens and the floors were not cleaned from previous raising cycle. Throughout the experiment, the animals were handled carefully to minimize any discomfort. During housing, the animals were monitored twice daily for their health status.

Data, sample collection and measurement of parameters

Growth performance

Individual body weight (BW) and feed consumption were measured on a pen basis at days 0, 14, and 42 of the experiment to calculate the average daily gain (ADG), average daily feed intake (ADFI), and the feed conversion ratio (FCR).

Table 1. Ingredient composition of experimental diets (as-fed basis; g kg^{-1})

Items	Phase 1 (d 1–14)		Phase 2 (d 15–42)	
	Control	Yeast	Control	Yeast
Ingredients				
Corn	451.4	450.1	558.9	559.6
Corn gluten	30	30	–	–
SBM	186.1	185.8	205.5	205.2
SBM, dehulled	79	79	80	80
Soybean oil	33.5	32.9	17.6	16.8
Fish meal	34	34	–	–
Lactose	68	68	50	50
Whey	50	50	30	30
MCP	13.2	13.2	15	15
Limestone	12.3	12.3	9.5	9.5
Sugar	30	30	20	20
Methionine (99%)	0.6	0.6	1.5	1.5
Lysine (78%)	5	5	4.8	4.8
Threonine (99%)	1.9	2.1	2.2	2.2
Vitamin premix[a]	2	2	2	2
Mineral premix[b]	2	2	2	2
Yeast fraction	–	2	–	0.4
Choline (25%)	1	1	1	1
Chemical Composition, g kg^{-1}				
ME, MJ kg^{-1}	15.07	15.07	14.86	14.86
Crude protein	200	200	190	190
Lysine	15.6	15.6	14.5	14.5
Methionine	4	4	4.5	4.5
Calcium	9.7	9.7	7.5	7.5
Phosphorous	7.1	7.1	6.5	6.5
Lactose	101	101	69.4	69.4
Dry matter	875	876	875	876

[a] Provided per kilogram of complete diet: vitamin A, 10 000 IU; vitamin D_3, 2000 IU; vitamin E, 48 IU; vitamin K_3, 1.5 mg; riboflavin, 6 mg; niacin, 40 mg; d-pantothenic, 17 mg; biotin, 0.2 mg; folic acid, 2 mg; choline, 166 mg; vitamin B_6, 2 mg; and vitamin B_{12}, 28 g.
[b] Provided per kilogram of complete diet: Fe (as $FeSO_4 \cdot 7H_2O$), 90 mg; copper (as $CuSO_4 \cdot 5H_2O$), 15 mg; zinc (as $ZnSO_4$), 50 mg; Mn (as MnO_2), 54 mg; I (as KI), 0.99 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.25 mg.

Nutrient digestibility

To estimate the apparent total tract digestibility (ATTD) of dry matter (DM) and crude protein (CP), the experimental diets were supplemented with chromic oxide (2 g kg^{-1}) as an indigestible marker. During the last 2 days of the study, fecal samples were collected via rectal massage from two pigs (one gilt and one barrow) from ten randomly chosen replicate pens. The samples were pooled on a pen basis and mixed immediately, after which samples were stored at −20 °C until required for analysis. For chemical analysis, fecal samples were oven dried at 60 °C for 72 h, after which they were ground to pass through a 1 mm screen. Diets and fecal samples were analyzed, using AOAC[19] methods, for DM (method 930.15) and CP (method 984.13). Chromium was analyzed via UV absorption spectrophotometry (UV-1201, Shimadzu Corp., Kyoto, Japan), according to the method described by Williams *et al.*[20] The ATTD was calculated using the following formula:

$$\text{ATTD}\ (\%) = [1 - \{(\text{Nf} \times \text{Cd})/(\text{Nd} \times \text{Cf})\}] \times 100$$

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Table 2. Oligonucleotide primers used for a relative-quantitative real-time PCR analysis

Items	Primer forward (5′ → 3′)	Primer reverse (5′ → 3′)	References
TNF-α	ACTCGGAACCTCATGGACAG	AGGGGTGAGTCAGTGTGACC	Gabler *et al.*[43]
IL-6	GATGCTTCCAATCTGGGTTCA	CACAAGACCGGTGGTGATTCT	Chen *et al.*[44]
TLR2	TCACTTGTCTAACTTATCATCCTCTTG	TCAGCGAAGGTGTCATTATTGC	Yin *et al.*[45]
IL-1-R1	ACCCCCATATCAGCGGACCG	TTGCTTCCCCCGGAACGTAT	Pinteaux *et al.*[46]
IL-10	TGAGAACAGCTGCATCCACTTC	TCTGGTCCTTCGTTTGAAAGAAA	Royaee *et al.*[47]
IL-1β	GAGTCTGCCCTGTACCCCAAC	ACCAACTTTTCCAGTCCCCTT	Kuang *et al.*[48]
IL-12	GGAGTATAAGAAGTACAGAGTGG	CATTGATGCCATGGAGCTGTA	Yi *et al.*[49]
INF-γ	TGGTAGCTCTGGGAAACTGAATG	GGCTTTGCGCTGGATCTG	Royaee *et al.*[47]
GATA3	CCCGTCCTACTACGGAAAC	GTGGTGGATGGACGTCTTG	Renu *et al.*[50]

where Nf = nutrient concentration in feces (% DM), Nd = nutrient concentration in diet (% DM), Cd = chromium concentration in diet (% DM), and Cf = chromium concentration in feces (% DM).

Blood profile

At days 7, 14, and 42, three piglets from two pens per treatment were randomly selected ($n = 6$ per treatment per sampling time) and blood samples were collected from the jugular vein into a sterile syringe. A different series of two pens was used at each sampling time by carefully handling the animals. At the end of the trial, 36 piglets (18 piglets per treatment) were sampled. Blood samples were allowed to rest at room temperature for a few minutes and centrifuged at 1500×*g* at 4 °C for 20 min to obtain serum and then frozen at −20 °C until analysis. The glucose, in serum was analyzed with an automatic blood analyzer (Advia 120, Bayer, Tarrytown, NY, USA). Serum haptoglobin was determined, using an enzyme-linked immunosorbent assay kit (TP801; Tri-Delta Diagnostics, Morris Plains, NJ, USA). Serum immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM), were determined using an enzyme-linked immunosorbent assay kit (Rodent Cortisol ELISA kit, Endocrine Technologies, Minneapolis, USA).

Determination of the expression of mRNA encoding for cytokines using real-time PCR

After blood collection in each period, the same sampled piglets were electrically stunned, then immediately sacrificed by exsanguination, and the entire gastrointestinal tract was removed. Samples from the mid-jejunum were collected from the slaughtered piglets. All intestinal samples at each collection time were frozen in liquid nitrogen and stored at −80 °C. Total RNA was isolated using TRIzol reagent (Invitrogen). The RNA quality was assessed using an Agilent 2100 bioanalyzer with an RNA 6000 Nano Chip (Agilent Technologies, Amstelveen, Netherlands), and RNA quantification was performed using an ND-2000 spectrophotometer (Thermo Inc., Wilmington, DE, USA). For the quantitative reverse transcription – polymerase chain reaction (qRT-PCR), total RNA (1 μg) was used for complementary DNA synthesis with a Maxima First-strand cDNA Synthesis Kit (Life Technologies Corporation, Van Allen Way, Carlsbad, USA). The primer designs used in this study for qRT-PCR for each gene transcript (cytokines) were obtained from different studies, as shown in Table 2. The qRT-PCR was performed using a 7500 Fast Real-time PCR system (Applied Biosystems, Lincoln Centre Drive, Foster City, CA, USA) with the following conditions: 94 °C for 3 min, followed by 40 cycles at 94 °C for 30 s, 59–61 °C for 30 s, and 72 °C for 30 s. Melting-curve profiles were analyzed for the amplicons. The qRT-PCR data were normalized to the expression of glyceraldehMsYFe 3-phosphate dehMsYFrogenase (GAPDH), an endogenous control gene, and calculated using the $2 - \Delta\Delta Ct$ method, where $\Delta\Delta Ct$ (cycle threshold) = ΔCt (treated) − ΔCt (control) and ΔCt = Ct of the target gene − Ct of GAPDH (treated or control, respectively).

Fecal scores

Subjective fecal scores were recorded daily on a pen basis for clinical signs of diarrhea throughout the experiment by the same operator, using the five-grade scoring system described by Hu *et al.*[21] Briefly, 1 = hard, dry pellet; 2 = firm, formed stool; 3= soft, moist stool that retains shape; 4 = soft, unformed stool that assumes the shape of the container; 5 = watery liquid that can be poured. The values are reported as averages per week.

Fecal Lactobacillus *and* Escherichia coli *counts*

For fecal *Lactobacillus* and *E. coli* counts, fresh feces were taken at the end of the experiment from two pigs (one gilt and one barrow) from ten pens per treatment via rectal massage. They were the same piglets used for nutrient digestibility analysis. The samples were pooled on a pen basis, placed on ice, and immediately transported to the laboratory for microbial analysis. One gram of fecal sample (1 g) from each pen was diluted with 9 mL of 10 g kg^{-1} peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and homogenized. Then, tenfold dilutions of fecal samples were performed (ranging from 10^{-1} to 10^{-8}) and then cultivated onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) for the enumeration of *E. coli* and lactobacilli medium III agar plates (Medium 638; DSMZ, Braunschweig, Germany) for the enumeration of *Lactobacillus*. The lactobacilli medium III agar plates were then incubated for 48 h at 39 °C under anaerobic conditions, while the MacConkey agar plates were incubated for 24 h at 37 °C. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator values reported as $\log_{10}$ colony-forming units per gram.

Statistical analyses

All data were analyzed statistically as a randomized complete design using the Generalized linear model (GLM) procedure of SAS (SAS Inst. Inc., Cary, NC, USA), using treatment as main effect. The growth performance parameters were also analyzed by a mixed SAS procedure (The SAS Stat. v.9.3, Cary, NC, USA.) for repeated measurements at different time points in which

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Table 3. Effect of multi-strain yeast fraction product supplementation on growth performance of weaning pig measured at different time points[a]

				P-value		
Treat	CON	MsYF	SEM[b]	Time	Treatment	Treat × Day
Body weight (kg)						
Initial BW	7.21	7.21	0	–	0.997	–
Day 14	11.75	12.16	0.11	–	0.396	–
Day 42	26.29	27.37	0.28	–	0.032	–
Mean	15.08	15.58	0.13	0.001	0.255	0.237
ADG (g day^{-1})						
Day 1 – 14	324	354	8	–	0.025	–
Day 14 – 42	519	541	11	–	0.119	–
Day 1 – 42	454	480	7	–	0.003	–
Mean	422	447	4.619	0.013	0.099	0.666
ADFI (g day^{-1})						
Day 1 – 14	433	448	13		0.385	–
Day 14 – 42	818	825	15		0.709	–
Day 1 – 42	689	700	12		0.552	–
Mean	625	637	6.7	0.006	0.190	0.797
FCR						
Day 1 – 14	1.359	1.28	0.059	–	0.347	–
Day 14 – 42	1.583	1.532	0.038	–	0.359	–
Day 1 – 42	1.521	1.461	0.028	–	0.174	–
Mean	1.471	1.406	0.025	0.037	0.133	0.782

[a] Abbreviation: CON, Basal diet; MsYF, CON + 2 g kg^{-1} multi-strain yeast fraction product at Phase 1, CON + 0.4 g kg^{-1} multi strain yeast fraction product at Phase 2.
[b] Standard error of means.

the statistical model accounted for the main effects of treatment, and time, also considering their interaction. The experimental unit was the pen. Differences among the treatment means were determined by using Tukey's test, with $P \leq 0.01$ indicating a highly significant difference and $P < 0.05$ indicating a significant difference.

RESULTS

Growth performance and ATTD

The effect of MsYF supplementation on growth performance is presented in Table 3. The final BW was significantly higher ($P < 0.05$) in pigs fed the MsYF-supplemented diet than in those fed the CON diet. The ADG was higher during days 1 – 14 ($P < 0.05$) and over the entire experimental period (days 1 – 42) ($P < 0.01$) in pigs fed MsYF-supplemented diet than in those fed the CON diet, whereas ADFI and FCR were unaffected ($P > 0.05$) by dietary treatments. Moreover, during days 14 – 42, the ADG, ADFI, and FCR remained unaffected with the supplementation of MsYF in the diet. There were significant time effects ($P < 0.05$) for ADG, ADFI, and FCR. However, there were no interactions between time and treatment for any of the growth performance variables.

The apparent total tract digestibility of DM was higher ($P < 0.05$) in pigs fed a MsYF-supplemented diet than in those fed a CON diet but the digestibility of CP remained unaffected by dietary treatment (Table 4).

Table 4. Effect of multi-strain yeast fraction product supplementation on nutrient digestibility at day 42[a]

Item, %	CON	MsYF	SEM[b]	*P*-value
Dry matter	81.12	82.8	0.46	0.036
Crude protein	79.5	80.58	0.72	0.321

[a] Abbreviation: CON, Basal diet; MsYF, CON + 2 g kg^{-1} multi strain yeast fraction product at Phase 1, CON + 0.4 g kg^{-1} multi-strain yeast fraction product at Phase 2.
[b] Standard error of means.

Blood profiles

The effect of MsYF supplementation on blood profiles is presented in Table 5. The concentration of haptoglobin in pigs receiving a MsYF-supplemented diet was higher than in those receiving a CON diet at days 7 ($P < 0.01$), 14 ($P < 0.01$), and 42 ($P < 0.0001$). In addition, the concentration of IgG was higher ($P < 0.05$) at the end of the study but was not affected at days 7 and 14 in pigs fed a MsYF-supplemented diet. Other blood metabolites such as glucose or insulin concentrations were unaffected ($P > 0.05$) by dietary treatment.

mRNA expression of cytokine related genes extracted from mid-jejunum

The mRNA expressions of cytokine-related genes with significant effects are presented in Fig. 1. The mRNA expressions of INF-γ and TNF-α genes was lower ($P < 0.05$) at days 14 and 7, respectively, in pigs fed the MsYF-supplemented diet than in those fed the CON diet. In addition, the expressions of IL-6 and TLR-2 genes were significantly lower ($P < 0.01$) in pigs fed MsYF-supplemented diet at days 7 and 14. However, there were no significant differences in the expression of IL-1-R1, IL-10, IL-1B, IL12, and GATA3 genes between pigs fed the CON and MsYF-supplemented diets (data not shown) during different experiment phases.

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Table 5. Effect of multi-strain yeast fraction product supplementation on blood metabolite in weaning pig[a]

Items	CON	MsYF	SEM[b]	*P*-value
Day 7				
Glucose, mg dL^{-1}	112.3	114.0	3.10	0.719
IgG, mg dL^{-1}	197.3	206.7	11.25	0.583
IgA, mg dL^{-1}	27.8	29.3	0.83	0.258
Haptoglobin, mg dL^{-1}	5.5	9.5	0.80	0.016
Insulin, uU mL^{-1}	0.35	0.40	0.09	0.695
Day 14				
Glucose, mg dL^{-1}	124.0	127.2	6.96	0.761
IgG, mg dL^{-1}	173.7	178.2	11.43	0.792
IgA, mg dL^{-1}	27.3	28.5	0.22	0.411
Haptoglobin, mg dL^{-1}	12.5	15.7	0.22	<0.001
Insulin, uU mL^{-1}	0.40	0.62	0.10	0.195
Day 42				
Glucose, mg dL^{-1}	109.7	112.7	1.56	0.232
IgG, mg dL^{-1}	259.2	303.5	11.72	0.044
IgA, mg dL^{-1}	28.0	31.3	0.40	0.153
Haptoglobin, mg dL^{-1}	13.0	16.5	0.40	0.002
Insulin, uU mL^{-1}	0.75	0.88	0.20	0.657

[a] Abbreviation: CON, Basal diet; MsYF, CON + 2 g kg^{-1} multi-strain yeast fraction product at Phase 1, CON + 0.4 g kg^{-1} multi-strain yeast fraction product at Phase 2.
[b] Standard error of means.

Fecal scores

The supplementation of MsYF did not have significant effects on fecal scores of weaning pigs throughout the experiment ($P > 0.05$; Table 6).

Fecal *E.coli* and *Lactobacillus* counts

The inclusion of MsYF in the diet of weaning pigs increased ($P < 0.05$) the fecal *Lactobacillus* count but did not affect fecal *E.coli* counts compared to piglets fed the CON diet (Table 7).

DISCUSSION

It has been well documented that yeast-based products contain prebiotic compounds present in yeast cells and cell walls such as β-glucans and mannan-oligosaccharides (MOS), and that they also contain nucleotides that have generally been shown to improve animal growth performance and health. In the present study, a yeast derivative from *Saccharomyces cerevisiae* and *Cyberlindnera jadinii* was evaluated for its efficacy in improving growth performance, immune status, and incidence of diarrhea in weaning pigs reared under a low hygiene environment.

In a review by Spring *et al.*[22] it has been noted that dietary supplementation of MOS from yeast cells to various livestock species conferred positive effects in body weight gain and feed conversion. Several other studies also reported an improved rate of daily gain and feed efficiency in weaning pigs fed MOS.[10,23–26] In agreement with these findings, in the present study the supplementation of MsYF increased BW and ADG during the first phase of the experiment. No significant effects on performance parameters were observed during the second phase, suggesting that yeast-based products are more effective at a younger age when the animal is under physiological and environmental stress immediately after early weaning. However, several other studies demonstrated no benefits of dietary inclusion of MOS.[16,27] In another study, the improvements in growth performance in pigs with diets supplemented with yeast-based products were only observed in low-hygiene environments and not in high-health swine herds.[28] The beneficial effects of yeast derivatives may be associated with the binding of MOS in the gut lumen instead of the epithelia, and limiting the colonization of pathogens in the gastrointestinal tract, thereby maintaining intestinal integrity.[22] It has been suggested that the growth-enhancing effects of MOS from yeast cells are largely dependent on structure of MOS, dose, and culture conditions of yeast, stage of growth of animals, species, or feeding duration.[29,30] There was no interaction between time and treatment on growth performance indicating that the product has the same effect on performance regardless of measurements at different time points.

Yeast supplementation favors the digestibility of crude protein.[31] However, some animals may have difficulties digesting intact yeast cells, but the removal of the cell wall enhances the yeast extract's digestibility.[32]

A study by Shen *et al.*[10] reported that supplementation of yeast culture in diets to weanling piglets improved total tract digestibility of DM, CP, and GE. Nochta *et al.*[33] also reported that supplementation of a mannan-containing feed additive led to higher ileal digestibility of nutrients, particularly indispensable amino acids. In the current study, the digestibility of DM was increased but CP digestibility was unaffected by the supplementation of yeast derivative to weaning pigs. Some other studies revealed no effect or a decreased digestibility of nutrients in diets with yeast supplementation.[8,34,35] Czech *et al.*[36] suggested that the digestibility of yeast differs with production technology, type of yeast-based product, substrate, and strain.

Live yeast products and their derivatives are currently used in animal feed for animal health and wellbeing.[37] We assessed haptoglobin, IgA, IgG, insulin, and glucose at days 7, 14, and 42 to evaluate immune related markers. The amount of haptoglobin (an acute-phase protein) in blood is used as an indicator of physiological stress caused by weaning. It was reported that the inclusion of yeast derivatives in piglet feeds led to a significant reduction in haptoglobin concentration, indicating that multi-strain yeast fraction product supplementation to newly weaned piglets diet contributes to better health, healthier piglets, and better growth performance and efficiency.[38] However, in the current study, haptoglobin concentration was increased at days 7, 14, and 42. This may be explained by the current vaccination program in the farm, i.e. swine fever at weeks 2 and 4, and cholera at weeks 3 and 5. However, this increase does not support greater inflammation as suggested by lowered pro-inflammatory cytokine gene expression (TNF-α, INF-γ, IL-6, TLR2). Increased haptoglobin at day 7 seems to be a random issue due to group randomization. The significant increase in IgG concentration in the present study may indicate that the MsYF can improve the immune function of weaning pigs. The effect of yeast on the immune system has been investigated in many studies.[39] However, reports in the literature may not be consistent. It has been thought that the characteristics of a particular yeast strain, the physiologic stage of the animals, or the environment in which they are raised, is likely to be the cause.[40]

Recent advances show that nutrition can modulate intestinal cytokine levels. For instance, supplementation of prebiotic and probiotic substances has been suggested to alter the local production of cytokines.[41] In the present study, we observed a

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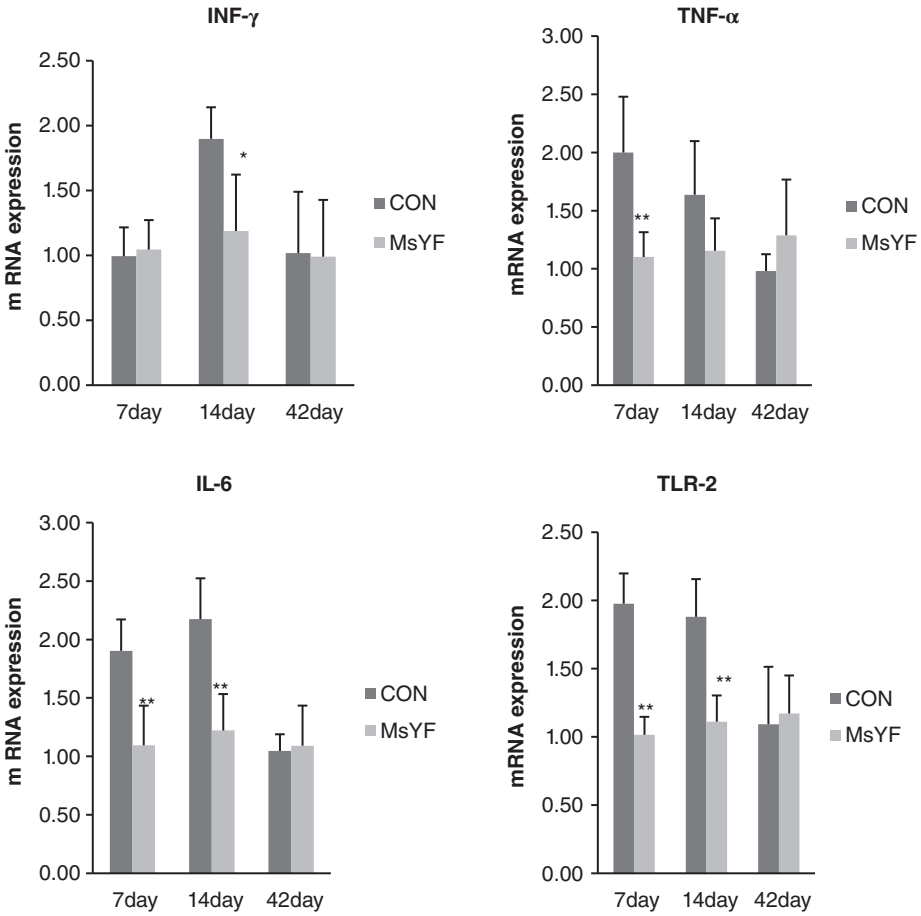

Figure 1. Quantitative gene expression of inflammatory cytokines INF-γ, TNF-α, IL-6, and TLR-2 in the gastro-intestinal tract (mid-jejunum) on days 7, 14, and 42 post-weaning of weaning pigs fed or not fed a multi-strain yeast fraction product. *$P < 0.05$; **$P < 0.01$. CON = Control, basal diet without yeast fraction; MsYF = multi-strain (*S. cerevisae* and *C. jadinii*) yeast fraction product supplemented basal diet.

Table 6. Effect of multi-strain yeast fraction product supplementation on fecal score in weaning pig[a]

Items	CON	MsYF	SEM[b]	*P*-value
Fecal score[c]				
Week 1	3.33	3.35	0.04	0.747
Week 2	3.30	3.28	0.03	0.666
Week 3	3.20	3.23	0.03	0.442
Week 4	3.24	3.22	0.03	0.688
Week 5	3.18	3.17	0.29	0.675
Week 6	3.13	3.14	0.16	0.462

[a] Abbreviation: CON, Basal diet; MsYF, CON + 2 g kg^{-1} multi-strain yeast fraction product at Phase 1, CON + 0.4 g kg^{-1} multi-strain yeast fraction product at Phase 2.
[b] Standard error of means.
[c] Fecal scores were determined using the following fecal scoring system: 1 = hard, dry pellet; 2 = firm, formed stool; 3 = soft, moist stool that retains shape; 4 = soft, unformed stool that assumes shape of container; 5 = watery liquid that can be poured.

Table 7. Effect of multi strain yeast fraction product supplementation on fresh fecal microbial counts in weaning pig at day 42[a]

Items, $\log_{10}$ cfu g^{-1}	CON	MsYF	SEM[b]	*P*-value
Lactobacillus	7.02	7.19	0.05	0.051
E. coli	5.74	5.66	0.04	0.289

[a] Abbreviation: CON, Basal diet; MsYF, CON + 2 g kg^{-1} multi-strain yeast fraction product at Phase 1, CON + 0.4 g kg^{-1} multi-strain yeast fraction product at Phase 2.
[b] Standard error of means.

reduction in mRNA encoding for inflammatory cytokines (TNF-α, INF-γ, IL-6 and TLR-2) in the mid-jejunum of weaned piglets fed diets supplemented with MsYF compared to control. Pié *et al.*[42] demonstrated that, during the post-weaning period, a transient increase in mRNA encoding for inflammatory cytokines such as IL-1, IL-6 and TNF-α concentrations was significantly increased and a late downregulation of IL-12 and IL-18 mRNA was observed. The downregulation of pro-inflammatory cytokines in the current study indicate that MsYF inhibited the inflammation of gastrointestinal tract in weaned piglets, which is evidenced by an increase in *Lactobacillus* counts.

CONCLUSION

In conclusion, supplementation with a multi-strain yeast fraction product improved ADG during the early phase of weaning and, overall, improved DM digestibility and serum IgG concentration, increased fecal *Lactobacillus* counts, and downregulated the

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mRNA expression of TNF-α, INF-γ, IL-6 and TLR-2, indicating that the weaning stress can be alleviated by the dietary supplementation of yeast fractions in weaning pigs raised under low sanitary environment.

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2016R1D1A1B03933206) and Lallemand (Danstar Ferment AG, Poststrasse 30, 6300 Zug, Switzerland).

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