Effects of a live yeast dietary supplement on fecal coliform counts and on peripheral blood CD4$^+$ and CD8$^+$ lymphocyte subpopulations in nursery pigs

Humberto Gustavo Monroy-Salazar, MSc; Luis Pérez-Sotelo, PhD; Yolanda González-Hernández, MSc; Gilberto Vaughan, PhD; Salvador Lagunas-Bernabé, MVZ; Jose Cuarón-Ibargüengoytia, PhD; Juan Antonio Montaño-Hirose, PhD; María Uxúa Alonso-Fresán, PhD; Pedro Pradal-Roa, PhD; Juan Carlos Vázquez-Chagoyán, PhD

Summary

Objective: To assess the effects of a dietary supplement, active dry yeast (*Saccharomyces cerevisiae* strain NCYC Sc47; Sc47), on CD4$^+$ and CD8$^+$ lymphocyte subpopulations and total fecal coliform counts in nursery pigs.

Materials and methods: Forty-eight nursery pigs were used in this experiment. At 35 days of age, pigs were randomly assigned to two treatments (control and yeast-supplemented groups) with 24 pigs per treatment. Blood and fecal samples were collected on study days 0, 7, 14, 21, 28, 35, and 42. Proportions of subpopulation of T lymphocytes

(CD4$^+$, CD4$^+$CD8$^+$, and CD8$^+$) were analyzed by flow cytometry, and fecal coliform counts were performed according to standard techniques.

Results: When active dry yeast was supplied as a probiotic at 0.3% in the diet of nursery pigs, total fecal coliform counts were lower and proportions of peripheral CD4$^+$, CD4$^+$CD8$^+$, and CD8$^+$ T lymphocyte subsets were higher ($P < .05$), when compared to those of the control group.

Implications: This study shows two pathways through which Sc47 may have a positive influence on pig health. Under the conditions of this study, numbers of peripheral

blood T-lymphocytes increase when Sc47 is included in the feed of nursery pigs, which may have a positive impact on animal health. Additionally, reduction of intestinal coliform numbers in nursery pigs receiving active dry yeast contributes to improving intestinal health and therefore to explaining why yeast as a feed additive may contribute to reducing the use of antibiotics as growth promoters or therapeutic agents.

Keywords: swine, coliforms, probiotic, *Saccharomyces cerevisiae* Sc47, lymphocytes.

Resumen - Efectos de un suplemento alimenticio de levadura viva en el conteo de coliformes fecales y en las subpoblaciones de linfocitos CD8$^+$ y CD4$^+$ de sangre periférica en cerdos de destete

Objetivo: Evaluar los efectos de un suplemento alimenticio, levadura seca activa (*Saccharomyces cerevisiae* cepa NCYC Sc47; Sc47), en subpoblaciones de linfocitos CD4$^+$ y CD8$^+$ y conteo total de coliformes fecales en cerdos de destete.

Materiales y métodos: En este experimento se utilizaron cuarenta y ocho cerdos de destete. A los 35 días de edad, los cerdos se asignaron al azar a dos tratamientos (grupo control y suplementado con levadura) con 24 cerdos por tratamiento. Se recolectaron muestras fecales y de sangre los días 0, 7, 14, 21, 28, 35, y 42 del estudio. Se analizaron las proporciones de subpoblación de linfocitos T (CD4$^+$, CD4$^+$CD8$^+$, y CD8$^+$) por medio de citometría de flujo, y se realizó conteo de coliformes fecales de acuerdo a las técnicas estándar.

Resultados: Cuando se suministró levadura seca activa como probiótico al 0.3% en la dieta de cerdos de destete, el conteo total de coliformes fecales fue más bajo y las proporciones de los subconjuntos de linfocitos T periféricos CD4$^+$, CD4$^+$CD8$^+$, y CD8$^+$, fue mayor ($P < .05$) cuando se compararon con los grupo control.

Implicaciones: Este estudio muestra dos caminos a través de los cuales el Sc47, puede tener una influencia positiva en la salud de los cerdos. Bajo las condiciones de este estudio, el número de linfocitos T de sangre periférica incrementaron cuando el Sc47 se incluyó en el alimento de los cerdos de destete, lo cual puede tener un impacto positivo en la salud del animal. Además, la reducción del número de coliformes intestinales en cerdos de granja que reciben levadura seca activa contribuye a mejorar la salud intestinal, y por lo tanto, a explicar por qué la levadura como aditivo en el alimento, contribuye a reducir el uso de antibióticos como promotores de crecimiento o agentes terapéuticos.

HGMS, LPS, SLB, MUAF, JCVC: Centro de Investigación y Estudios Avanzados en Salud Animal UAEM-FMVZ, Toluca, México.

YGH, GV: Instituto de Diagnóstico y Referencia Epidemiológicos, Secretaria de Salud, México City, México.

JAMH: Centro Nacional de Servicios de Diagnóstico en Salud Animal, México City, México.

PPR: Universidad Nacional Autónoma de México, México City, México.

JCI: CENID – Fisiología, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Querétaro, México.

Corresponding author: Dr Juan Carlos Vázquez-Chagoyán, Universidad Autónoma del Estado de México, Facultad de Medicina Veterinaria y Zootecnia, Centro de Investigación y Estudios Avanzados en Salud Animal, Toluca, México, Carretera Toluca – Atlacomulco Km. 15.5; Toluca, Estado de México Código Postal 50200; Tel: 01-722-1217227; Fax: 01 722-2 96 55 55; E-mail: jcvch@yahoo.com.

Résumé - Effets de l'ajout de levures vivantes à l'alimentation sur le dénombrement des coliformes fécaux et sur les sous-populations lymphocytaires CD4$^+$ et

CD8+ du sang périphérique de porcelets en pouponnière

Objectif: Évaluer les effets d'un supplément alimentaire, la levure active séchée (*Saccharomyces cerevisiae* souche NCYC Sc47; Sc47), sur les sous-populations lymphocytaires CD4+ et CD8+ et le dénombrement des coliformes fécaux totaux chez des porcelets en pouponnière.

Matériels et méthodes: Quarante-huit porcelets en pouponnière ont été utilisés dans la présente expérience. À 35 jours d'âge, les porcelets ont été répartis de manière aléatoire dans deux groupes de traitement (groupe témoin et groupe supplémenté avec la levure), avec 24 porcs par traitement. Des échantillons sanguins et de fèces ont été prélevés aux jours 0, 7, 14, 21, 28, 35, et 42. Les proportions des sous-populations de lymphocytes T (CD4+, CD4+CD8+, et CD8+) ont été analysées par cytométrie en flux, et le dénombrement des coliformes fécaux effectué selon une technique standardisée.

Résultats: Lorsque de la levure active était ajoutée à titre de probiotique à 0,3% dans l'alimentation de porcelets en pouponnière, les dénombrements de coliformes fécaux totaux étaient diminués et les proportions de lymphocytes T CD4+, CD4+CD8+, et CD8+ périphériques étaient plus élevées ($P < .05$), comparativement à ceux du groupe témoin.

Implications: Cette étude démontre deux avenues par lesquelles Sc47 peut avoir une influence positive sur la santé des porcelets. Dans les conditions expérimentales de l'étude, le nombre de lymphocytes T du sang périphérique a augmenté lorsque Sc47 est ajouté dans l'alimentation des porcelets en pouponnière, ce qui peut avoir un impact positif sur la santé des animaux. De plus, la réduction du nombre de coliformes intestinaux chez les porcelets en pouponnière recevant de la levure sèche active contribue à améliorer la santé intestinale, et ainsi, à expliquer pourquoi l'ajout de levures dans l'alimentation contribue à la réduction de l'utilisation des antibiotiques comme promoteur de croissance ou agent thérapeutique.

*S*accharomyces cerevisiae strain NCYC Sc47 (Sc47) has been used as a probiotic in animal feeds with favorable effects on productivity and resistance to infectious diseases in pigs.[1-6] Contact of the yeast with ileum M cells promotes migration of T lymphocytes from the blood stream to Peyer's patches[7-11] and stimulates production of IgG and IgA antibodies,[3,12] but there are no conclusive reports regarding how systemic lymphocyte subsets are affected when active dry yeast is used as a dietary supplement. In contrast, it has been reported that *Saccharomyces*-based products such as dry brewer's yeast and mannanoligosaccharides are able to affect gut microbiota, increasing lactobacilli and limiting coliform populations.[12-14] However, there are no conclusive data on the effects on enteric coliform populations when live yeast is used as a dietary supplement. Li et al[5] and Mathew et al[15] found no evidence that live *Saccharomyces* affected enteric microbiota, from which they concluded that different strains may produce different outcomes. Van Heugten et al[2] found that active dry yeast strain NCYC Sc47 reduced the number of enteric bacteria, but concluded that a more detailed analysis of specific microbial species should be conducted to fully understand the impact of yeast on gut microflora and on animal-health performance. Therefore, the objective of the present study was to assess the effects of a dietary supplement of active dry yeast (strain NCYC Sc47) on CD4+ and CD8+ lymphocyte subpopulations and on total fecal coliform counts in nursery pigs.

Materials and methods

The experimental protocols were approved by the institutional Bioethics and Animal Care Committee of the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Autónoma del Estado de México. Animal conditions complied with the recommendations of the Council for International Organizations of Medical Science[16] and NOM-062-Z00-1999.[17]

Animals, housing, and feeding

A total of 48 crossbred male (noncastrated) nursery pigs (Duroc-Landrace reciprocal breeding) were received at 21 days of age. A random-number generator was used to randomly assign animals to two 24 m² concrete pens with nonslatted floors (24 pigs per pen). Pens had roofed (18 m²) and non-roofed (6 m²) areas. Animals were restricted to the roofed area (0.75 m² per pig) to prevent pigs from experiencing open-air environmental conditions. The occupied area was heated with infrared 500-watt light bulbs, and temperature was maintained at 26°C to 30°C. Each pen was equipped with two 4-hole self-feeders and two water nipples, providing ad libitum access to feed and water. Pens were cleaned three times daily. Animals were allowed a 2-week acclimatization period to establish adequate intake of dry feed, which was a conventional lactose-rich starter diet (Phase 1 diet; Table 1). Previous to their arrival, pigs had never received feed additives, eg, prebiotics, probiotics, or antibiotics.

From Day 0 of the experiment, pigs were allowed access to the entire roofed area of the pen for a total available space per animal of 0.75 m². Average temperature was maintained at 18°C to 22°C. A lactose-free diet was fed, with a gradual change, beginning 3 days before Day 0, to a basal diet (Table 1) that served as an intestinal challenge. Feed was provided ad libitum.

On Day 0 of the study (at 35 days of age), pigs received either the basal diet (Control group) or the same diet supplemented with Sc47 (Sc47 group). Initial average weights were 11.87 kg and 11.71 kg in the Sc47 and Control groups, respectively. The basal diet was formulated to meet or exceed the pig's dietary nutrient requirements according to the recommendations of the National Research Council.[18] The Sc47 diet contained 0.3% *Saccharomyces cerevisiae* NCYC Sc47 (Table 1).

Individual health status of the pigs was assessed by physical appearance, average daily gain (ADG), and average body weight (BW). Pigs were individually weighed at weekly intervals, on the days when samples were collected.

Experimental design

A complete randomized design was used, in which pigs were allotted into two treatments with 24 pigs (experimental units) per treatment. Blood and individual fecal samples were collected from all pigs on Days 0, 7, 14, 21, 28, 35, and 42 of the experiment. Subpopulation proportions of T lymphocytes (CD4+, CD8+, and CD4+CD8+) were analyzed by flow cytometry, and fecal coliform counts were performed.

Blood sample collection and processing

All reagents used for flow cytometry protocols were purchased from Becton Dickinson (La Jolla, California) except antibodies, which were purchased from AbD Serotec (Raleigh, North Carolina). Blood samples were obtained by anterior vena cava venipuncture using commercial evacuated heparinized blood tubes. Lymphocytes were stained

Table 1: Dietary composition (as fed basis) for crossbred male nursery pigs weaned at 21 days of age and entered into a study in which the basal diet was supplemented or not with a live yeast product*

Ingredients	Phase 1 diet (kg/1000 kg)	Basal diet (kg/1000 kg)
Milk whey (sweet), whole	203.00	0.00
Maize, yellow	150.00	200.00
Soybean meal (47% CP)	140.00	240.00
Sorghum, low tannin	137.90	318.20
Wheat, hard	130.00	120.00
Fish meal (sardine, 63% CP)	70.00	0.00
Oats, whole	60.00	0.00
Safflower oil	59.00	44.00
Canola meal (36% CP)	20.00	40.00
L-lysine HCl	5.65	6.70
Limestone	5.60	9.50
Zinc oxide	4.20	0.00
Salt (NaCl)	4.00	3.60
Vitamin premix†	2.40	2.40
L–threonine	2.10	2.85
Mono and dicalcium phosphate	2.05	9.20
DL-methionine	1.40	2.00
Butyric acid (99% purity)	1.00	0.00
Trace mineral premix‡	1.00	1.00
L-tryptophan	0.50	0.35
Phytase (750 FTU/kg)	0.20	0.20
Total (kg)	1000.00	1000.00
Calculated composition (selected nutrients)		
ME (Mcal/kg)	3.46	3.39
NE (Mcal/kg)	2.55	2.50
Crude protein (%)	19.60	19.34
Lysine (%)	1.54	1.48
SID lysine (%)	1.35	1.28
Calcium (%)	0.74	0.64
Phosphorus (%)	0.62	0.53
Digestible phosphorus (%)	0.40	0.27
Lactose (%)	15.00	0.00

*   The Phase 1 diet was fed during the 2-week acclimatization period (between weaning and entry into the study at 35 days of age). The basal diet was fed throughout the study, either with active dry yeast additive (*Saccharomyces cerevisiae* strain Sc47; 0.3% w/w) or without yeast supplement.

†   The vitamin premix provided, per kg of diet, 8000 IU vitamin A, 1890 IU vitamin D3, 86 IU vitamin E, 2 mg vitamin K, 0.33 mg biotin, 804 mg choline, 2.17 mg folic acid, 33.56 mg niacin, 27.91 mg pantothenic acid, 12.26 mg riboflavin, 3.01 mg thiamine, 4.56 mg pyridoxine, and 0.04 mg vitamin B12.

‡   The trace mineral premix provided, per kg of diet, 24.05 mg copper from $CuSO_4 \cdot 5H_2O$, 109.52 mg iron from $FeSO_4 \cdot 7H_2O$, 37.49 mg manganese from $MnSO_4 \cdot H_2O$, 0.30 mg selenium from Na_2SeO_3, 125 mg zinc from $ZnSO_4 \cdot 7H_2O$, and 0.80 mg iodine from EDDI.

CP = crude protein; FTU = phytase unit; ME = metabolizable energy; NE = net energy; SID = standard ileal digestibility; EDDI: ethylenediamine dihydroiodide.

using a standard direct immunofluorescence method.[19,20] Briefly, all TCD4^{+} and TCD8^{+} pig lymphocytes were simultaneously stained using 100 μL of whole blood with 20 μL of anti-pig CD4a fluorescein isothiocyanate (FITC)-conjugated and CD8a R-phyco-erythrin-conjugated mouse antibodies and incubated 15 minutes in darkness at room temperature. Caprine anti-murine IgG FITC-conjugated antibodies (AbD Serotec) were used as background controls. After incubation, 300 μL of lysis buffer was added and incubated for 10 minutes in darkness at room temperature. Samples were then washed and the pellet resuspended in 300 μL of FACS-Flow buffer (Becton Dickinson). A FACSCalibur Flow Cytometer (Becton Dickinson) was used to read 10,000 events, and data were analyzed using CellQuest 3.1 software (Becton Dickinson).

Fecal sample collection and processing

Samples were collected by direct manual stimulation of the anal sphincter, and the sample was recovered using a sterile plastic bag. Samples were processed immediately after collection. Each fecal sample (10 g) was placed in a sterile glass container and homogenized with 90 mL phosphate buffer solution pH 7.4 (PBS; Gibco-Life Technology, Carlsbad, California). Tenfold dilutions (10^{-2} to 10^{-6}) were made using 10 mL of PBS, and 1 mL of each dilution was cultured in violet red bile agar (VRBA) containing ceftrioxone (Bioxon; Becton Dickinson, BD, Mexico) for evaluation. Coliform counts were performed according to standard techniques.[21,22] Briefly, after homogenization, 1 mL of each dilution was inoculated on the bottom of an empty sterile plate, and then 10 mL of warm VRBA was poured into the plate, thoroughly mixed, and allowed to solidify. An additional 4 mL of VRBA was poured on top, spread to cover the whole surface, allowed to solidify, and incubated at 35°C for 24 hours. Only plates with countable numbers of colonies were evaluated. The most suitable dilution for colony counting, 10^{-4}, was used to evaluate the samples. These results were converted to colony-forming units (CFU) per gram of feces and then to $\log_{10}$ values to perform statistical analysis.

Statistical analysis

Average daily gain (ADG) and phenotype of white blood cell subpopulations (least squares means; LSM) were analyzed in a completely randomized design for two treatments (n = 24 animals per group) in a linear mixed model with repeated measures. Data were analyzed establishing comparisons among individuals within and between groups. The statistical validation of the differences ($P < .05$) between groups was performed using a mixed model for variables with repeated observations over time (SAS version 8, SAS Institute Inc, Cary, North Carolina).[23,24] Coliform results ($\log_{10}$ values from CFU per g of feces) were analyzed using a Student t test with n-1 degrees of freedom ($P < .05$).

Results

Differences in ADG between Control and Sc47 groups ($P < .05$) are shown in Table 2.

The ranges for the lymphocyte subpopulations in counted cells were CD4^{+}, 928 to 1615; CD4^{+}CD8^{+}, 505 to 847; and CD8^{+}, 938 to 1441, in a total of 10,000 cells examined by flow cytometry (Table 3). Weekly average values for the Sc47 group were higher than those of the Control group on Days 7, 14, 21, 35, and 42 for lymphocyte subsets CD4^{+} and CD8^{+}, and on Days 7, 14, and 21 for CD4^{+}CD8^{+}. Additionally, total average values were higher for Sc47 than for the Control group for all three lymphocyte subsets ($P < .05$) (Table 3).

At Day 0, the $\log_{10}$ transformed coliform counts did not differ between treatment groups (Table 4). Values increased in both groups until Day 14, then colony counts decreased in both groups. Except for Day 0, coliform colony numbers were numerically lower for the Sc47 group in all weeks. Colony counts differed significantly between treatment groups on Days 7, 28, 35, and 42, but not on Days 0, 14, or 21 (Table 4).

Discussion

Several commercial formulations of *S cerevisiae* (Sc) or its derivatives are used as prebiotics or probiotics in swine diets. There are many reports about the impact of Sc yeast on the immune system and on enteric microbiota. However, reports in the literature may not agree, most likely due to the characteristics of a particular yeast strain and the physiologic stage of the animals or the environment in which they are raised. Therefore, it is difficult to make comparisons among experiments. For instance, dried yeast (dry nonfermentative yeast containing at least 40% crude protein) increased systemic IgG antibodies in weanling pigs and decreased colonization of total coliforms in the duodenum, jejunum, cecum, and colon.[12] Yeast cultures (dry yeast and the medium on which it was grown) altered total counts of intestinal *Lactobacillus* and *Streptococcus* species and *Escherichia coli*.[15] Kiarie et al[25] reported that bacterial variety and diversity in the ileal digesta were greater in pigs that received yeast fermentation products in the diet. These investigators also observed fewer

Table 2: Average daily gain (ADG)* in nursery pigs fed a diet supplemented with active dry yeast (*Saccharomyces cerevisiae* strain Sc47) or the same diet without supplementation

Day of study	Average daily gain (kg)†		Average body weight (kg)†	
	Sc47	Control	Sc47	Control
0	0.00	0.00	11.87	11.71
7	0.25[a]	0.40[b]	13.62[a]	14.58[b]
14	0.38	0.39	16.30	17.35
21	0.70[a]	0.57[b]	21.24	21.37
28	0.62[a]	0.57[b]	25.59[a]	25.42[b]
35	0.72[a]	0.57[b]	30.66[a]	29.45[b]
42	0.72	0.73	35.72[a]	34.58[b]
0-42	0.49	0.47	22.14	22.07

* Pigs were weaned at 21 days of age and were fed either the basal diet (Control) or the supplemented diet (Sc47) (Table 1) between 35 days of age (Day 0) and 77 days of age (Day 42), with 24 pigs per treatment group.

† Least squares means.

[ab] Values within a row with different superscripts differ ($P < .05$; mixed-model for variables with repeated observations over time).

Table 3: Subsets of peripheral blood lymphocytes (CD4^{+} and CD8^{+})* in nursery pigs fed a basal diet (Control) or the same diet supplemented with *Saccharomyces cerevisiae* strain Sc47 (Sc47)†

Day	CD4^{+}		CD4^{+}CD8^{+}		CD8^{+}	
	Sc47	Control	Sc47	Control	Sc47	Control
0	1032	1049	505	500	1067	1049
7	1123[a]	928[b]	606[a]	505[b]	1072[a]	987[b]
14	1306[a]	958[b]	756[a]	517[b]	1112[a]	938[b]
21	1615[a]	1145[b]	847[a]	656[b]	1204[a]	1068[b]
28	1324	1331	643	669	1121	1149
35	1275[a]	1201[b]	673	664	1214[a]	1111[b]
42	1294[a]	1114[b]	706	622	1442[a]	1070[b]
Total LSM	**1281[a]**	**1104[b]**	**667[a]**	**590[b]**	**1176[a]**	**1053[b]**

* Least squares means (LSM) of CD4^{+} and CD8^{+} phenotype cell counts/10,000 white blood cell counts performed using flow cytometry.

† Study described in Table 2. Blood samples were collected in evacuated heparinized tubes, and lymphocytes were separated and stained using standard methods.

ab Values within a subset and within a row with different superscripts differ ($P < .05$; mixed-model for variables with repeated observations over time), with 97.4, 80.5, and 91.4 degrees of freedom and standard error 35.2, 27.7, and 29.6 for CD4^{+}, CD4^{+}CD8^{+}, and CD8^{+}, respectively. For total LSM, standard error values were 13.3, 10.5, and 11.2, respectively.

Table 4: Mean fecal coliform counts (CFU/g of feces) in nursery pigs fed either a basal diet (Control) or the same diet supplemented with *Saccharomyces cerevisiae* strain Sc47 (Sc47)*

Day	0	7	14	21	28	35	42
Sc47	5.08	5.34[a]	6.48	6.38	5.08[a]	5.60[a]	5.04[a]
Control	5.00	6.65[b]	7.00	6.72	6.52[b]	6.46[b]	6.84[b]

* Study described in Table 2. Fecal samples were collected by direct stimulation of anal sphincter at weekly intervals beginning when the pigs were 35 days old (Day 0). Diluted fecal samples (10^{-4}) were cultured to determine CFU/g of feces. Values were transformed to $\log_{10}$ and data were analyzed using a Student *t* test with n-1 degrees of freedom.

ab Values with different superscripts within a column are different ($P < .05$).

CFU = colony forming units.

E coli strain K88 organisms adhering to the ileal mucosa after challenge in treated pigs than in negative controls, indicating that the intestinal tract environment was healthier in animals administered the yeast fermentation product. Price et al[26] found that a yeast fermentation product was able to modify the composition of the gastrointestinal microbial community in weanling pigs after an experimental *Salmonella* infection, resulting in larger populations of *Bacteroides* and *Lactobacillus* species. It has also been shown that when mannanoligosaccharides are added to the feed, enterobacteria counts in the jejunum are lower[14,27] and that active dry yeast (lyophilized live yeast cells) induces a decrease in total bacteria and lactobacilli.[5] The results of the present study support the idea that supplementary active dry yeast in the nursery pig's diet influences the intestinal microbiota. Here, we demonstrate that active dry yeast induces a reduction in coliform populations in fecal samples, which can be used as an indicator of the effects induced by the active dry Sc47 preparation in the colon microbiota. Previous reports have indicated that yeast is able to promote the growth of lactobacilli,[14] suggesting that the lower numbers of fecal coliforms in treated pigs in the present study could be interpreted as a positive effect of yeast on the enteric ecosystem, intestinal integrity, and health. Further studies should be conducted to confirm this.

Dietary yeast cultures and their derivatives exhibit beneficial effects on immune system regulation in pigs. For instance, Shen et al[28] reported that yeast-culture supplementation increased interferon γ (IFNγ) concentrations in the gut and reduced it in plasma. Collier et al[29] reported that in weaned pigs orally supplemented with *S cerevisiae boulardii* for 16 days or treated intravenously with *E coli* lipopolysaccharide (LPS), peak IFNγ in the blood was higher than in untreated controls. Additionally, these investigators found that pigs administered Sc produced an earlier blood tumor necrosis factor alpha (TNFα) peak after LPS challenge than did untreated controls. It has also been observed that ß-glucans increase expression of TNFα mRNA in the intestine, spleen, and liver, and expression of interleukin 1 receptor antagonist mRNA in the intestine.[30] The systemic antibody response to orally fed active dry yeast has been inferred from the increase in milk gamma globulin in sows.[3] Shin et al[31] provided further evidence concerning the effects of live yeast on the immune system. They demonstrated that oral vaccination of mice with live

yeast expressing an *Actinobacillus pleuropneumoniae* antigen induced a protective immune response against experimental challenge with a pathogenic strain of *A pleuropneumoniae,* both through IgA and IgG antibodies in the intestinal and lung mucosae and serum IgG.

The beneficial effects of Sc47 on swine growth performance has been previously reported.[2,3] These reports are partially supported by the findings of the present study. We observed that during the first week of supplementation, ADG was greater in the control group, and from the third week of the experiment on, with the exception of the last week of the study, ADG was higher in the Sc47 group. This was not reflected in the final mean BW, most likely because the study period was not long enough to make differences evident and because the number of animals used in the study was insufficient to assess this variable. These results suggest that there is a period of adaptation to the yeast during which the changes induced by the treatment have a negative impact on performance. Once the animals have adapted to the probiotic, there is an improvement in ADG that compensates for the loss during the adaptation period. The effect on ADG appears to parallel changes in the coliform population; however, further studies must be performed before any conclusion can be reached.

The effects of orally fed yeast on cells of the pig's immune system have not been completely elucidated, but this issue has been studied by Lessard et al,[32] who administered *S cerevisiae boulardii*, a yeast fermentation product, three times a week by gavage (10^9 CFU). The authors found no effects on CD8^{+} T cells in 18- or 24-day-old pigs, either in the ileal mucosa, mesenteric lymph nodes, or blood. Nevertheless, Shen et al[28] found that when yeast cultures were administered orally to nursery pigs, the proportion of TCD4^{+} lymphocytes in peripheral blood was lower. Our findings differ from the results of those studies. We found that when strain Sc47 was supplied as a probiotic in the diet of nursery pigs, proportions of CD4^{+}, CD4^{+}CD8^{+}, and CD8^{+} peripheral blood T lymphocytes were greater. The differences in results between this study and previous reports could be due to variations in strains, protocols, and yeast preparations used in each experiment. In our study, the three lymphocyte subpopulations studied did not differ between the Sc47 and Control groups on Day 0. These data suggest that the immunologic status of all animals was equivalent at the beginning of the experiment, and that

differences found later developed in response to treatment. Differences between treatment means for all three subpopulations of lymphocytes studied were evident on Days 7 and 14, and for CD4^{+} and CD8^{+} T cells on Days 35 and 42. Double-positive T cells (CD4^{+}CD8^{+}) differed only on Days 7, 14, and 21. Both treatment groups showed continuous immunological stimulation throughout the experiment, which suggests that all animals were immunologically active and that changes observed in the acquired immune response could be at least partially explained by the stimuli offered by intestinal microbiota. The higher proportions of CD4^{+}, CD8^{+}, and CD4^{+}CD8^{+} peripheral blood lymphocytes in the Sc47 group suggest that systemic cell-mediated immunity was activated by the changes induced by yeast or by the yeast itself. In pigs CD4^{+} and CD4^{+}CD8^{+} lymphocytes are T-helper cells. Activation of these subpopulations stimulates plasma cells for antibody producction.[33-35] Changes in these subpopulations might indicate a concurrent increase in plasma antibodies. However, evaluation of this variable should be considered for future studies.

The higher proportion of CD8^{+} cytotoxic T-cell lymphocytes observed in the Sc47 group on Days 7, 14, 21, 35, and 42 indicates that the cell-mediated immune system was also stimulated. Few reports describe the effects of yeast on CD8^{+} cytotoxic T-cell lymphocytes in pigs. However, studies performed in mice demonstrated that recombinant live Sc in a vaccine administered parenterally is able to stimulate the major histocompatibility complex (MHC) class I-restricted and class II-restricted antigen-specific T-cell responses.[36,37] Given the immunologic similarities among mammalian species, it is reasonable to hypothesize that Sc is able to induce this type of immune response in pigs if yeast is administered parenterally. It is possible that yeast administered orally also enhances stimulation of the cell-mediated immune system, since translocation of commensal bacteria[10,11,25] and brewers' yeast[7-9] to mesenteric lymph nodes has been clearly demonstrated and presumably explains development and activation of the intestinal immune system in response to orally administered yeast. Yeast mannanoligosaccharides and ß-glucan extracts supplied in the diet also stimulate the immune system.[38] Therefore, it may be inferred that changes in proportions of T-lymphocyte subsets in the present study were partially due to stimuli of M cells associated with Peyer's patches induced by the active dry yeast, as well as stimuli induced by naturally occurring bacteria.

In both groups of animals, the ratio of CD4^{+} to CD8^{+} cells remained within normal range.[39] In the yeast-supplemented group, CD4^{+} cells reached a peak on Day 21, and CD8^{+} cells on Day 42. Thus, the CD4^{+} to CD8^{+} cell ratio increased from 0.97 on Day 0 to 1.34 on Day 21, and then decreased to 0.9 on Day 42. Both cell subpopulations were higher in the Sc47 group than in the controls. Proliferation of CD4^{+} cells in pigs is associated with MHC class II, while CD8^{+} cell proliferation is associated with an MHC class I-restricted immune response.[40] Orally administered active dry yeast seems to stimulate both. The significance of these findings remain to be elucidated; however, the results of this study support the hypothesis that dietary supplementation of pigs with Sc contributes to modulation of the immune system, maintaining its activity, which in turn may help to explain why animals raised under suboptimal hygienic conditions benefit from oral yeast supplementation.

Implications

- Supplementing the feed of nursery pigs with *S cerevisiae* (strain NCYC Sc47) may have a positive influence in animals' health: peripheral T lymphocyte increments observed in animals fed active dry yeast are most likely related to the capacity of the yeast to modulate the immune response and to induce a positive impact on the animal's general well-being.

- Lower intestinal coliform numbers in pigs receiving Sc47 yeast may improve intestinal health; thus, supplementing with Sc47 yeast might reduce use of antibiotics as growth promoters or therapeutic agents.

Acknowledgements

References

1. Davis ME, Maxwell CV, Erf DC, Brown DC, Wistuba TJ. Dietary supplementation with phosphorylated mannans improves growth response and modulates immune function of weanling pigs. *J Anim Sci*. 2004;82:1882–1891.

2. Van Heugten E, Funderburke DW, Dorton KL. Growth performance, nutrient digestibility, and fecal microflora in weanling pigs fed live yeast. *J Anim Sci*. 2003;81:1004–1012.

3. Jurgens MH, Rikabi RA, Zimmerman DR. The effect of dietary active dry yeast supplement on performance of sows during gestation-lactation and their pigs. *J Anim Sci*. 1997;75:593–597.

4. Kornegay ET, Rhein-Welker D, Lindemann MD, Wood CM. Performance and nutrient digestibility in weanling pigs as influenced by yeast culture additions to starter diets containing dried whey or one of two fiber sources. *J Anim Sci*. 1995;73:1381–1389.

5. Li J, Li D, Gong L, Ma Y, He Y, Zhai H. Effects of live yeast on the performance, nutrient digestibility, gastrointestinal microbiota and concentration of volatile fatty acids in weanling pigs. *Arch Anim Nutr*. 2006;60:277–288.

6. Moore WEC, Moore LVH, Cato EP, Wilkins TD, Kornegay ET. Effect of high-fiber and high-oil diets on the fecal flora of swine. *Appl Environ Microbiol*. 1987;53:1638–1644.

7. Beier R, Gebert A. Kinetics of particle uptake in the domes of Peyer's patches. *Am J Physiol*. 1998;275:G130-G137.

8. Gebert A, Göke M, Rothkötter HJ, Dietrich CF. The mechanisms of antigen uptake in the small and large intestines: the role of the M cells for the initiation of immune responses [in German]. *Zeitschrift für Gastroenterologie*. 2000;38:855–872.

9. Gebert A, Steinmetz I, Fassbender S, Wendlandt KH. Antigen transport into Peyer's patches: increased uptake by constant numbers of M cells. *Am J Pathol*. 2004;164:65–72.

10. Perdigon G, Vintiñi E, Alvarez S, Medina M, Medici M. Study of the possible mechanisms involved in the mucosal immune system activation by lactic acid bacteria. *J Dairy Sci*. 1999;82:1108–1114.

11. Perdigon G, Medina M, Vintiñi E, Valdez JC. Intestinal pathway of internalization of lactic acid bacteria and gut mucosal immunostimulation. *Int J Immunopathol Pharmacol*. 2000;13:141–150.

12. White LA, Newman MC, Cromwell GL, Lindemann MD. Brewers dried yeast as a source of mannan oligosaccharides for weanling pigs. *J Anim Sci*. 2002;80:2619–2628.

13. Burkey TE, Dritz SS, Nietfeld JC, Johnson BJ, Minton JE. Effect of dietary mannanoligosaccharide and sodium chlorate on the growth performance, acute-phase response, and bacterial shedding of weaned pigs challenged with *Salmonella enterica* serotype Typhimurium. *J Anim Sci*. 2004;82:397–404.

14. Castillo M, Martín-Orúe SM, Taylor-Pickard JA, Pérez JF, Gasa J. Use of mannanoligosaccharides and zinc chelate as growth promoters and diarrhea preventative in weaning pigs: Effects on microbiota and gut function. *J Anim Sci*. 2008;86:94–101.

15. Mathew AG, Chattin SE, Robbins CM, Golden DA. Effects of a direct-fed yeast culture on enteric microbial populations, fermentation acids, and performance of weanling pigs. *J Anim Sci*. 1998;76:2138–2145.

16. Council for International Organizations of Medical Science. International Guiding Principles for Biomedical Research Involving Animals. World Health Organization. Geneva, Italy, 1985. Available at: http://cioms.ch/publications/guide-lines/1985_texts_of_guidelines.htm. Accessed 11 July 2012.

17. NOM-062-Z00–1999. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio [Technical specifications for the production, care and use of laboratory animals]. In: Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, ed. Diario Oficial de la Federación. México. 22–08–2001. Available at: http://www.senasica.gob.mx/?doc=743. Accessed 11 July 2012.

18. National Research Council. *Nutrient Requirements of Swine*. 10th rev ed. Washington, DC: National Academy Press; 1998:107–109. Available at: http://www.nap.edu/catalog/6016.html. Accessed 11 July 2012.

19. Givan AL. *Flow Cytometry: First Principles*. 2nd ed. New York, New York: John Wiley and Sons; 2001:81–108.

20. Shapiro MH. *Practical Flow Cytometry*. 4th ed. Hoboken, New Jersey: John Wiley and Sons; 2003:225–256.

21. US Food and Drug Administration. Bacteriological Analytical Manual Chapter 3. Aerobic Plate Count. 2001. Available at: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm063346.htm. Accessed 11 July 2012.

22. NOM-113-SSA1–1994. Método para la cuenta de microorganismos coliformes totales en placa [Total plate count method for coliforms organisms. Official Mexican Public Health Normativity, NOM-113-SSA1–1994]. In: Secretaria de Salubridad y Asistencia, ed. Diario Oficial de la Federación. 25–08–1995, México [published by the "Public Health and Assistance Ministry" in the official daily news paper in 25 August 1995, Mexico]. Available at: http://www.salud.gob.mx/unidades/cdi/nom/113ssa14.html. Accessed 10 September 2012.

23. Littell RC, Henry PR, Ammerman CB. Statistical analysis of repeated measures data using SAS procedures. *J Anim Sci*. 1998;76:1216–1231.

24. Gil JL. Comparación de los procedimientos GLM y MIXED del SAS®, para analizar diseños de parcelas divididas con bloques al azar [Comparison of GLM and MIXED procedures of SAS®, to analyze split-plot randomized block designs]. *Zootecnia Tropical*. 2001;19:43–58.

25. Kiarie E, Bhandari S, Scott M, Krauseand DO, Nyachoti CM. Growth performance and gastrointestinal microbial ecology responses of pigs receiving *Saccharomyces cerevisiae* fermentation products after an oral challenge with *Escherichia coli* (K88). *J Anim Sci*. 2011;89:1062–1078.

26. Price KL, Totty HR, Lee HB, Utt MD, Fitzner GE, Yoon I, Ponder MA, Escobar J. Use of *Saccharomyces cerevisiae* fermentation product on growth performance and microbiota of weaned pigs during *Salmonella* infection. *J Anim Sci*. 2010;88:3896–3908.

27. Corthesy B, Gaskins HR, Mercenier A. Cross-talk between probiotic bacteria and the host immune system. *J Nutr*. 2007;137(3)(suppl 2):781S-790S.

28. Shen YB, Piao XS, Kim SW, Wang L, Liu P, Yoon I, Zhen YG. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. *J Anim Sci*. 2009;87:2614–2624.

29. Collier CT, Carroll JA, Ballou MA, Starkey JD, Sparks JC. Oral administration of *Saccharomyces cerevisiae boulardii* reduces mortality associated with immune and cortisol responses to *Escherichia coli* endotoxin in pigs. *J Anim Sci*. 2011;89:52–58.

30. Eicher SD, McKee CA, Carroll JA, Pajor EA. Supplemental vitamin C and yeast cell wall beta-glucan as growth enhancers in newborn pigs and as immunomodulators after an endotoxin challenge after weaning. *J Anim Sci*. 2006;84:2352–2360.

31. Shin SJ, Shin SW, Kang ML, Lee DY, Yang MS, Jang YS, Yoo HS. Enhancement of protective immune responses by oral vaccination with *Saccharomyces cerevisiae* expressing recombinant *Actinobacillus pleuropneumoniae* ApxIA or ApxIIA in mice. *J Vet Sci*. 2007;8:383–392.

32. Lessard M, Dupuis M, Gagnon N, Nadeau É, Matte JJ, Goulet J, Fairbrother JM. Administration of *Pediococcus acidilactici* or *Saccharomyces cerevisiae boulardii* modulates development of porcine mucosal immunity and reduces intestinal bacterial translocation after *Escherichia coli* challenge. *J Anim Sci*. 2009;87:922–934.

33. Saalmüller A, Reddehase MJ, Buhring HJ, Jonjic S, Koszinowski UH. Simultaneous expression of CD4 and CD8 antigens by a substantial proportion of resting porcine T lymphocytes. *Eur J Immunol*. 1987;17:1297–1301.

34. Saalmüller A, Hirt W, Reddehase MJ. Porcine gamma/delta T lymphocyte subsets differing in their propensity to home to lymphoid tissue. *Eur J Immunol*. 1990;20:2343–2346.

35. Saalmüller A, Pauly T, Höhlich BJ, Pfaff E. Characterization of porcine T lymphocytes and their immune response against viral antigens. *J Biotechnol*. 1999;73:223–233.

36. Edwards AD, Manickasingham SP, Spörri R, Diebold SS, Schulz O, Sher A, Kaisho T, Akira S, Sousa CR. Microbial recognition via Toll-like receptor dependent and independent pathways determines the cytokine response of murine dendritic cell subsets to CD40 triggering. *J Immunol*. 2002;169:3652–3660.

37. Stubbs AC, Martin KS, Coeshott C, Skaates SV, Kuritzkes DR, Bellgrau D, Franzusoff A, Duke RC, Wilson CC. Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell-mediated immunity. *Nature Med*. 2001;7:625–629.

38. Kogan G, Kocher A. Role of yeast cell wall polysaccharides in pig nutrition and health protection. *Livest Sci*. 2007;109:161–165.

39. Appleyard GD, Furesz SE, Wilkie BN. Blood lymphocyte subsets in pigs vaccinated and challenged with *Actinobacillus pleuropneumoniae*. *Vet Immunol Immunopath*. 2002;86:221–228.

40. Saalmüller A. Antigen-specific immune response of porcine T lymphocytes to various pathogens. *Revue Scientifique et Technique*. 1998;17:71–73.