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Impact of rare earth element-enriched yeast on growth performance, nutrient digestibility, blood profile, and fecal microflora in finishing pigs

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This study was conducted to evaluate effects of rare earth element-enriched yeast (RY) on growth performance, nutrient digestibility, blood profile, and fecal microbial flora in finishing pigs. A total of 100 finishing pigs [(Landrace × Yorkshire) × Duroc] were used in a 2-phase feeding trial. Pigs were randomly assigned to 5 treatments according to their initial body weight (5 pens per treatment, 2 gilts and 2 barrows per pen). Treatments were: basal diets, free of antibiotics [negative control (NC)]; NC + 500, 1000, and 1500 mg/kg, respectively, of RY; antibiotics diets, NC + 500 mg/kg of tiamulin [positive control (PC)]. Overall, average daily gain (ADG) and gain-to-feed ratio (G:F) increased linearly with increasing level of RY in diets. At the end of the experiment, blood lymphocyte concentration and apparent total tract digestibility of dry matter and gross energy increased linearly in pigs fed the increasing level of RY in diets. The better ADG, G:F, and fecal *Lactobacillus* counts were observed in both RY and PC groups compared with the NC group. In conclusion, diets with RY improved growth performance, digestibility, blood lymphocyte counts, and fecal *lactobacillus* counts in finishing pigs, also, RY may be considered as an alternative to antibiotics.

Running head: rare earth element-enriched yeast in finishing pigs

Key words: average daily gain, cerium, fecal microbial flora, lanthanum, *Pichia kudriavzevii* LA30

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; ATTD, apparent total tract digestibility; DDGS, distillers dried grains; DM, dry matter; GE, gross energy; G:F, gain-to-feed ratio; NC, negative control; PC, positive control; RBC, red blood cells;

REE, rare earth elements; **RY**, rare earth element-enriched yeast; **WBC**, white blood cells

For Review Only

Antibiotics have been supplemented to diets as a growth promoter in livestock for decades, but the usage of antibiotics in animal husbandry is restricted or forbidden in most countries, because it poses safety problem (e.g., residues and drug tolerance) (Dibner and Richards 2005; Milbradt et al. 2014). As a result, researchers have been attempting to find alternatives to antibiotics [e.g., rare earth elements (REE), direct-fed microbials] (Adu et al. 2009; Cai et al. 2015a).

According to the periodic table, the atomic radius of La and Ce is similar to that of Ca or other metallic ions, and may therefore potentially act as cofactors to replace Ca or other metallic ions in metabolism to influence the growth of livestock (Martin and Richardson 1979). Rare earth elements (mostly La and Ce) have been supplemented to swine or poultry diets via organic or inorganic forms as feed additives in the feeding experiments (Pagano et al. 2015). It is reported that organic REE (e.g., REE-citrate and REE-enriched yeast (RY)) inclusion in non-ruminant diets increased their growth performance, nutrient digestibility, and serum triiodothyronine concentration (Kraatz et al. 2006; Förster et al. 2008; Cai et al. 2015b, 2016), and it was also much more effective than the inorganic one (e.g., REE-chlorides) (He and Rambeck 2000; He et al. 2003, 2010). Yang et al. (2009) reported that supplemented of REE to *in vitro* rumen improved their microflora proportions. Thus, REE may be a potential alternative to antibiotics. Moreover, there was no residue or safety problems in non-ruminant animals supplemented with La or Ce less than 100 mg/kg of diets (Bruce et al. 1963; Wang and Xu 2003; Han and Thacker 2010; He et al. 2010). During fermentation, yeast can absorb REE and then form organic REE compounds (RY) (Andrès et al. 2003). However, no study

was conducted to assess the effects of RY in finishing pigs. Therefore, the objective of this study was to establish the effects of RY on growth performance, nutrient digestibility, blood profiles, and fecal microbial flora in finishing pigs, and to evaluate whether PY can be considered as an alternative to antibiotics in their diets.

MATERIALS AND METHODS

Preparation of RY

The RY (Biotopia Co., Ltd., Chuncheon-city, Gangwon-do, Korea) product used in this study was obtained through a fermentation process. *Pichia kudriavzevii* LA30 (KCCM 11262P) was inoculated into a YM broth (YM medium, Difco Laboratories, Detroit, MI, USA) at 30°C for 2 d in a shaking incubator (250 ×g). Then, 2% (v/v) of the culture was inoculated into an YM broth containing 320 mg/kg of La (purified above 95% La in the form of $\text{LaCl}_3 \cdot 5\text{H}_2\text{O}$) and 480 mg/kg of Ce (purified above 95% Ce in the form of $\text{CeCl}_3 \cdot 4\text{H}_2\text{O}$) at 30°C for 5 d in the fermenter. The culture broth was continuously centrifuged at 15,800 ×g (25 ± 5°C, flow rate: 1.3 L/min) using KS type super-high speed centrifugal separator (Open Type Centrifugal Separators, Kansai Centrifugal Separator Manufacturing, Japan) to separate the cultured cells and supernatant. The collected cells were washed 2 or 3 times with distilled water. The washed cells were crushed using a pulverizer (Avestin, model n.: EmuSiFlex-C160B, Canada) and then mixed with distillers dried grains (DDGS) as an excipient. The resulting mixture was air-dried, after that the product was made. A representative sample of the product was prepared by coning and quartering for the component analysis. The concentration of La and

Ce in the representative sample was measured by an Elan 9000DRCII ICP-MS (Perkin-Elmer, Waltham, MA, USA). The content of yeast was determined by a 20-mesh sieve to separate yeast and DDGS, then weighing. The DDGS content was calculated by deducting the weight of other three constituents (La, Ce, and yeast) from that of representative sample. The product of RY containing 2.82% La, 4.71% Ce, 40.3% DDGS, and 52.17% yeast was used in this study. The quality control assessment of RY product was checked by measuring the concentrations of each component (mainly La, Ce, yeast, DDGS) in the product, which should not range out of 5% of relative deviation among batches.

Animals, Experimental Design, and Housing

A total of 100 pigs [(Landrace × Yorkshire) × Duroc] with an initial body weight (BW) of 51.1 ± 2.12 kg were used in a 70-d study. Pigs were randomly assigned to 5 treatments with 5 pens per treatment and 4 pigs (2 gilts and 2 barrows) in each pen. Treatments were corn-soybean meal-based diets and included: 1) basal diet, free of antibiotics [negative control (NC)], 2) NC + 500 mg/kg of RY (La, 14.1 mg/kg; Ce, 23.6 mg/kg) (RY500), 3) NC + 1,000 mg/kg of RY (La, 28.2 mg/kg; Ce, 47.1 mg/kg) (RY1000), 4) NC + 1,500 mg/kg of RY (La, 42.3 mg/kg; Ce, 70.65 mg/kg) (RY1500), and 5) antibiotics diet, NC + 500 mg/kg of tiamulin [positive control (PC)]. The experiment was separated into 2 phases, d 0 to 35 and d 36 to 70. All diets were formulated in accordance with NRC (2012) recommendations to meet the nutrient requirements of finishing pigs (Table 1). All pigs were housed in an environmentally controlled room with a slatted plastic floor. Each pen (1.8 m width × 1.8 m length) was

equipped with a one-sided self-feeder and a nipple drinker. Pigs are allowed *ad libitum* access to feed and water throughout the experiment.

Sampling and Measurements

Feed consumption per pen and individually BW were monitored on d 0, 35 and 70 to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F).

From d 64 to 70, chromium oxide (0.20%) was added to the diet as an indigestible marker to determine the apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen, and gross energy (GE) (Fenton and Fenton 1979). On d 70, fresh fecal samples were collected from at least 2 pigs in each pen via rectal massage and then mixed well for each respective pen. All feed and fecal samples were stored immediately at -20°C until analysis.

Before chemical analysis, feed and fecal samples were dried at 70 °C for 72 h, after which they were finely ground to pass through a 1-mm screen for analyzing DM (method 934.01; AOAC 2000). Chromium levels were determined via ultraviolet absorption spectrophotometry (Shimadzu, UV-1201, Shimadzu, Kyoto, Japan) following the method described by Williams et al. (1962). Nitrogen was determined by a nitrogen analyzer (Kjeltec 2300; Foss Tecator AB, Hoeganaes, Sweden) (method 968.06; AOAC 2000) and crude protein was calculated as nitrogen $\times$ 6.25. The GE was determined by an oxygen bomb calorimeter (Mode 1241, Parr instrument Co., Moline, IL, USA). The ATTD of GE and nutrients was calculated according to Stein et al. (2006).

On d 35 and 70, 2 pigs (1 barrow and 1 gilt) were randomly chosen from each pen and bled

via jugular venipuncture to obtain blood samples. Blood samples (5 mL) were collected into vacuum tubes containing K₃EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) to obtain whole blood. The concentration of white blood cells (WBC), red blood cells (RBC), and lymphocyte in the whole blood sample were determined using an automatic blood analyzer (ADVIA 120, Bayer., New York, USA).

Fresh fecal samples collected on d 70 were pooled and placed on ice and transported to the laboratory, where analysis was immediately carried out. The composite fecal sample (1 g) from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson Co., Franklin Lakes, NJ, USA) and homogenized. Viable counts of bacteria in the fecal samples were then determined by plating serial 10-fold dilutions (in 1 g/l peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and *Lactobacillus* medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate *Escherichia coli* (*E. coli*) and *Lactobacillus*, respectively. The *Lactobacillus* medium III agar plates were then incubated for 48 h at 39 °C, and the MacConkey agar plates were incubated for 24 h at 37 °C under anaerobic conditions. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator.

Statistical Analysis

All the data were analyzed using the GLM procedure (SAS Institute, Cary, NC, USA). The effects of increasing dietary concentration of supplemental RY were evaluated by polynomial contrasts. The pen was considered as the experimental unit. Before carrying out statistical

analysis of the microbial counts, a logarithmic transformation of the data was done.

Differences among all treatments were analyzed by Duncan's multiple range test. Contrasts were also used for the effect of treatments: NC vs. PC. Variability in the data was expressed as standard error of mean. Results were considered significant at $P < 0.05$.

RESULTS

Growth Performance and Nutrient Digestibility

From d 0 to 35, ADG, ADFI, and G:F linearly increased ($P < 0.05$) with the increasing level of RY in the diet (Table 2). Gain-to-feed ratio increased ($P < 0.05$) in pigs fed RY1000, RY1500, and PC diets compared with the NC group. From d 36 to 70, the inclusion of graded levels of RY linearly enhanced ($P < 0.05$) ADG. The significant differences ($P < 0.05$) of ADG and G:F were observed between NC and PC treatments. Also, pigs fed with RY1500 had a higher ($P < 0.05$) ADG than the NC group. Overall, ADG and G:F enhanced linearly ($P < 0.05$) in pigs receiving diets supplemented with the increasing levels of RY. Pigs fed the RY1500 and antibiotics diet had better ($P < 0.05$) ADG and G:F compared with the NC group. A better ADG ($P < 0.05$) was found in pigs supplemented with RY1000 compared with the NC group. At the end of the feeding trial, diets supplemented with RY linearly increased ($P < 0.05$) DM and GE digestibility, and pigs fed with RY1500 had a higher ($P < 0.05$) ATTD of DM and GE than the NC group, but no difference of nitrogen digestibility was observed among treatments (Table 3).

Blood Profiles and Fecal Microflora

On d 35, no effect was observed on RBC, WBC, and lymphocytes in finishing pigs supplemented with RY. On d 70, lymphocyte counts increased linearly ($P < 0.05$) as the dietary RY was increased (Table 4). Also, lymphocyte counts were increased ($P < 0.05$) in pigs supplemented with antibiotics compared with the NC group. However, no significant differences were found in blood profiles among treatments. At the end of the feeding trial, the significant difference ($P < 0.05$) of fecal *E. coli* and *Lactobacillus* concentration was found in pigs fed PC diets compared with the NC group (Table 5). The PC group had a lower ($P < 0.05$) fecal *E. coli* counts compared with both NC and RY groups. Fecal *Lactobacillus* counts were higher ($P < 0.05$) in all RY groups compared with the NC groups.

DISCUSSION

The objective of the present study was to evaluate the feeding value of RY in finishing pigs. The RY containing 2.82% La, 4.71% Ce, 40.3% DDGS, and 52.17% yeast. The 2 elements, La and Ce, belong to the lanthanide whose atomic radius is similar to that of calcium. The similarity of atomic radius may have similar physical, chemical, and biological properties, which means that La and Ce may cooperate or compete with Ca to affect the metabolism. Yeast can absorb La and Ce and then form organic REE compounds during fermentation (Andrs et al. 2003). Moreover, in the previous studies, the inclusion levels of DDGS and yeast ranged from 50 to 500 g/kg and 1 to 50 g/kg, respectively, in diets of pigs cannot affect their growth performance, digestibility, blood profile, and fecal microflora (White et al. 2002;

Shen et al. 2009; Dahlen et al. 2011; Duttlinger et al. 2012; Agyekum et al. 2014). Thus, according to the amount of RY added to each treatments, the concentration of DDGS ranged from 0.2 to 0.6 g/kg of diets was too low to have effect on pigs. Also, the dose of yeast ranged from 261 to 782 mg/kg of diets was unable to have either increases or decreases in all parameters of the present study. Therefore, results of this study could be mainly attributed to REE instead of DDGS or yeast. The following discussion will be focus on the effect of La and Ce in pigs in this and the previous studies.

In the current study, ADG and G:F were improved in pigs fed RY diets, which is in accordance with Wang and Xu (2003), who observed an increase of ADG and G:F in finishing pigs supplemented with 104.5 mg/kg of La ($\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$). The results are also similar to the previous studies from Han and Thacker (2010) and He et al. (2001). Han and Thacker (2010) reported that ADG was increased in weanling pigs supplemented with 1,000 mg/kg of REE mineral-yeast (353 mg/kg of La oxide, 252 mg/kg of Ce oxide, and 102 mg/kg of Pr oxide) in diets. He et al. (2001) found that ADG and G:F were improved in growing pigs supplemented with REE mixture (La, 36 to 43 mg/kg; Ce, 49 to 57 mg/kg). In the present study, the improvement in growth performance may be explained by the following reasons. First, it may be attributed to enhanced digestibility of DM and GE in the current study. Second, La and Ce may influence some metallic ion channels to affect metabolic pathways in animal (e.g., Ca^{2+} is important in cellular metabolism and signal transduction; Mg^{2+} is an essential cofactor in tricarboxylic acid cycle) (Martin and Richardson 1979; Adu et al. 2009; Liu et al. 2004). Because the atomic radius of La and Ce is similar to that of some metallic ions (e.g., Ca^{2+}), it

is assumed that they may affect the switch of these metallic ion channels on plasma or organelle membrane resulting in the changed cell metabolism.

However, Kraatz et al. (2006) indicated that REE compounds decreased G:F in weanling pigs supplemented with 200 mg/kg of REE-citrate (La, 46.6 mg/kg; Ce, 136.4 mg/kg; Pr, 16 mg/kg; Nd, 0.8 mg/kg). Förster et al. (2008) also reported that piglets supplemented with 200 mg/kg of REE-citrate (60 mg/kg of La, 110 mg/kg of Ce, 10 mg/kg of Pr, and 20 mg/kg of Nd) showed a lower G:F. Interestingly, He and Rambeck (2000) found that 150 mg/kg of REE mixture (22.5 mg/kg of La as $\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$, 30.0 mg/kg of Ce as $\text{CeCl}_3 \cdot 6\text{H}_2\text{O}$, 1.8 mg/kg of Pr as $\text{PrCl}_3 \cdot 6\text{H}_2\text{O}$) cannot improve ADG and G:F when the weight of pigs was from 7.3 to 17.5 kg, but it worked when the weight of pigs was from 17.5 to 60 kg. The exact reasons for the differing results are largely unknown at this time. It is speculated that the difference may be attributed to the form and concentration of REE compound and the growth stage of pigs. Moreover, the overall ADFI was not affected by the RY diet in this study, which is in line with the study of Förster et al. (2008), whose diets were supplemented with REE-citrate ranged from 0 to 800 mg/kg of diets in piglets.

In the present study, the ATTD of DM and GE was increased in pigs fed the RY1500 diet, which is similar to Han and Thacker (2010) who reported that ATTD of DM and GE was increased in weanling pigs fed with rare earth mineral-yeast. However, Han and Thacker (2010) also found that digestibility of crude protein was increased in pigs fed with REE mineral-yeast compared with the control group, which is not in agreement with this study. The differing results may be attributed to the animal age and REE chemical forms.

Rare earth element-enriched yeast supplementation failed to affect the concentration of RBC and WBC, which is in line with the previous report from Adu et al. (2009), who reported that blood erythrocytes and leukocytes counts in rabbits were not affected by inclusion of La oxide (0, 100, 200, 300, and 400 mg/kg) in diets. But the results of the present study showed the linearly enhanced blood lymphocyte counts with increasing level of RY in diets at the end of feeding experiment, which may be due to the form of REE, proper dose gradient of RY, and the experiment animal.

Lactobacillus and *E. coli* are predominant organisms in the gut flora of normal pigs (Kaper 2005). Inclusion of RY in diets led to a higher fecal *Lactobacillus* counts compared with the NC group, but *E. coli* was not influenced by RY diets in the current study. However, Kraatz et al. (2006) observed that the fecal microbiota was not influenced in piglets supplemented with REE-citrate. The results of the present study may be explained by the following reasons. First, the modulation of REE to microbial activity is dose-dependent. Li et al. (2003) reported that La stimulated the endogenic and ectogenic metabolism of bacteria at concentration from 50 to 150 µg/mL. A stimulatory effect on the growth of *E. coli* was found in cultures added with Ce at concentrations below 350 µg/mL and an inhibitory effect at or above 400 µg/mL (Zhao et al. 2002). Moreover, in the present study, pigs fed the PC diet showed a lower *E. coli* counts and a greater *Lactobacillus* counts compared with the NC group. It may be attributed to the supplementation of antibiotics to the diets, which can eliminate harmful bacteria in the gut (Kohanski et al. 2010).

In conclusion, supplementation of antibiotics in diets had a positive influence on growth

performance, ATTD and fecal microflora in finishing pigs compared with the NC group, and the same effect also found in the RY group, thus indicating that RY may be considered as a new alternative to antibiotics in diets of finishing pigs. The best additive dosage of RY was 1,500 mg/kg in this study. But the further study needs to focus on the exact mechanism of RY in finishing pigs and find a method to measure the exact form of REE in the yeast.

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Table 1. Basal diet composition (as-fed basis)

Ingredient (% of diet)	Phase1 (d 0 to 35)	Phase2 (d 36 to 70)
Corn	64.44	71.64
Soybean meal	15.00	7.00
Distillers dried grains with soluble	4.39	4.39
Rapeseed meal	3.00	3.00
Wheat	6.00	6.00
Tallow	2.00	2.00
Molasses	2.70	3.50
Dicalcium phosphate	0.94	0.94
Limestone	0.72	0.72
Salt	0.30	0.30
L-Lysine-HCl	0.18	0.18
Trace mineral premix ^a	0.10	0.10
Vitamin premix ^b	0.20	0.20
Choline chloride	0.03	0.03
Calculated composition		
ME (kcal/kg)	3,285	3,284
Lysine	0.88	0.66

Calcium	0.57	0.54
Total phosphorus	0.53	0.50
Analyzed composition		
Gross energy (kcal/kg)	4,058	4,054
Crude protein	16.30	13.10
DM	87.20	87.10

^a Provided per kilogram of complete diet: 140 mg of Cu as copper sulfate; 145mg of Fe as ferrous sulfate; 179 mg of Zn as zine oxide; 12.5 mg of Mn as manganese oxide; 0.5 mg of I as potassium iodate; and 0.4mg ofSe assodium selenite.

^b Provided per kilogramof complete diet: 10,000 IU of vitamin A; 2,000 IU of vitamin D3; 42IU of vitamin E; 5 mg of vitamin K3; 9.6 mg of vitamin riboflavin; 2.45 mg of vitamin B6; 40 µg of vitamin B12; 49 mg of niacin; 27 mg of pantothenic acid; 0.05 mg of biotin.

Table 2. Effect of rare earth element-enriched yeast on growth performance in finishing pigs.^a

Item	Rare earth element-enriched yeast					SEM ^b	P-value ^c		
	NC	RY500	RY1000	RY1500	PC		Treatments effect	NC vs. PC	Linear
51 to 80 kg BW (d 0 to 35)									
ADG (g)	778	798	849	852	860	27	0.173	0.050	0.037
ADFI (g)	2,398	2,277	2,231	2,243	2,236	58	0.260	0.066	0.045
G:F	0.324 ^b	0.351 ^{ab}	0.383 ^a	0.381 ^a	0.385 ^a	0.015	0.036	0.009	0.006
80 to 110 kg BW (d 36 to 70)									
ADG (g)	812 ^b	832 ^{ab}	835 ^{ab}	891 ^a	903 ^a	23	0.043	0.011	0.030
ADFI (g)	2,599	2,540	2,616	2,572	2,600	54	0.868	0.988	0.980
G:F	0.313	0.328	0.320	0.347	0.347	0.010	0.108	0.035	0.070

51 to 110 kg BW (d 0 to 70)									
ADG (g)	805 ^b	805 ^{ab}	842 ^a	871 ^a	881 ^a	19	0.031	0.013	0.012
ADFI (g)	2,469	2,438	2,423	2,407	2,418	48	0.904	0.462	0.360
G:F	0.326 ^b	0.332 ^{ab}	0.351 ^{ab}	0.364 ^a	0.366 ^a	0.011	0.042	0.019	0.012

Note: Means within a row lacking a common superscript differ ($P < 0.05$).

^a Abbreviation: NC, basal diet (free of antibiotics); RY500, NC + 500 mg/kg of rare earth element-enriched yeast (Biotopia Co., Ltd., Chuncheon-city, Gangwon-do, Korea); RY1000, NC + 1,000 mg/kg of rare earth element-enriched yeast; RY1500, NC + 1,500 mg/kg of rare earth element-enriched yeast; PC, NC + 500 mg/kg of tiamulin; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain-to-feed ratio.

^b Standard error of mean.

^c No quadratic significance was observed.

Table 3. Effect of rare earth element-enriched yeast on nutrient digestibility in finishing pigs.^a

Item (%)	Rare earth element-enriched yeast					SEM ^b	P-value ^c		
	NC	RY500	RY1000	RY1500	PC		Treatments	NC vs. PC	Linear
Dry matter	73.1 <i>b</i>	74.8 <i>ab</i>	75.0 <i>ab</i>	78.9 <i>a</i>	75.9 <i>ab</i>	1.54	0.021	0.220	0.011
Nitrogen	73.6	75.7	73.6	77.1	74.7	1.73	0.178	0.654	0.284
Gross energy	73.4 <i>b</i>	75.3 <i>ab</i>	76.8 <i>ab</i>	78.2 <i>a</i>	76.3 <i>ab</i>	1.14	0.011	0.090	0.019

Note: Means within a row lacking a common superscript differ ($P < 0.05$).

^a Abbreviation: NC, basal diet (free of antibiotics); RY500, NC + 500 mg/kg of rare earth element-enriched yeast (Biotopia Co., Ltd., Chuncheon-city, Gangwon-do, Korea); RY1000, NC + 1,000 mg/kg of rare earth element-enriched yeast; RY1500, NC + 1,500 mg/kg of rare earth element-enriched yeast; PC, NC + 500 mg/kg of tiamulin.

^b Standard error of mean.

^c No quadratic significance was observed.

Table 4. Effect of rare earth element-enriched yeast on blood profiles in finishing pigs.^a

Item	Rare earth element-enriched yeast						P-value ^c		
	NC	RY500	RY1000	RY1500	PC	SEM ^b	Treatments	NC vs. PC	Linear
80 kg BW (d 35)									
RBC (10 ⁶ /μl)	6.18	6.33	6.24	6.16	6.26	0.146	0.939	0.732	0.829
WBC (10 ³ /μl)	17.63	18.67	17.66	18.44	17.28	1.269	0.926	0.845	0.766
Lymphocytes (% of whole blood)	54.74	54.70	59.60	59.52	59.44	2.206	0.272	0.151	0.086
110 kg BW (d 70)									
RBC (10 ⁶ /μl)	6.71	6.76	7.02	6.93	7.06	0.120	0.199	0.051	0.185
WBC (10 ³ /μl)	21.61	21.18	24.53	24.05	24.53	1.269	0.201	0.124	0.167
Lymphocytes (% of whole blood)	52.92	55.66	56.28	62.38	62.42	3.132	0.167	0.048	0.042

^a Abbreviation: NC, basal diet (free of antibiotics); RY500, NC + 500 mg/kg of rare earth element-enriched yeast (Biotopia Co., Ltd.,

Chuncheon-city, Gangwon-do, Korea); RY1000, NC + 1,000 mg/kg of rare earth element-enriched yeast; RY1500, NC + 1,500 mg/kg of rare earth element-enriched yeast; PC, NC + 500 mg/kg of tiamulin.

^b Standard error of mean.

^c No quadratic significance was observed.

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Table 5. Effect of rare earth element-enriched yeast on fecal microflora in finishing pigs.^a

Item (log10 cfu/g)	Rare earth element-enriched yeast						P-value			
	NC	RY500	RY1000	RY1500	PC	SEM ^b	treatments	NC vs. PC	Linear	Quadratic
<i>Escherichia coli</i>	6.7a	6.6a	6.6a	6.6a	6.4b	0.06	0.047	0.005	0.328	0.737
<i>Lactobacillus</i>	6.4b	7.3a	7.3a	7.1a	7.0a	0.15	0.002	0.012	0.033	0.019

Note: Means within a row lacking a common superscript differ ($P < 0.05$).

^a Abbreviation: NC, basal diet (free of antibiotics); RY500, NC + 500 mg/kg of rare earth element-enriched yeast (Biotopia Co., Ltd., Chuncheon-city, Gangwon-do, Korea); RY1000, NC + 1,000 mg/kg of rare earth element-enriched yeast; RY1500, NC + 1,500 mg/kg of rare earth element-enriched yeast; PC, NC + 500 mg/kg of tiamulin.

^b Standard error of mean.