

# Evaluation of Dietary Synbiotic Supplementation on Growth Performance, Muscle Antioxidant Ability and Mineral Accumulations, and Meat Quality in Late-finishing Pigs

Ye Fei CHENG<sup>1</sup> Yue Ping CHEN<sup>1</sup> Ming Fang DU<sup>1</sup> Chao WEN<sup>1</sup> Yan Min ZHOU<sup>1</sup> 

<sup>1</sup> College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, P. R. CHINA

Article Code: KVFD-2018-19713 Received: 06.03.2018 Accepted: 09.07.2018 Published Online: 10.07.2018

## How to Cite This Article

**Cheng YF, Chen YP, Du MF, Wen C, Zhou YM:** Evaluation of dietary synbiotic supplementation on growth performance, muscle antioxidant ability and mineral accumulations, and meat quality in late-finishing pigs. *Kafkas Univ Vet Fak Derg*, 24 (5): 673-679, 2018. DOI: 10.9775/kvfd.2018.19713

## Abstract

The present study aimed to investigate the effects of synbiotic supplementation on growth performance, muscle antioxidant capacity and mineral contents, and meat quality in late-finishing pigs. Fifty barrow pigs were randomly allocated into two treatments with five replicates each and fed a basal diet supplemented with or without 1 g/kg synbiotic consisted of prebiotics (yeast cell wall and xylooligosaccharide) and probiotics (*Clostridium butyricum*, *Bacillus licheniformis*, and *Bacillus subtilis*) for 21 days, respectively. Treatment did not affect growth performance in late-finishing pigs ( $P>0.05$ ). Compared with the control group, pigs in the synbiotic group exhibited a higher superoxide dismutase activity in the *Longissimus dorsi* (LD) muscle, whereas a lower malondialdehyde concentration in the gluteus muscle ( $P<0.05$ ). Additionally, dietary synbiotic inclusion decreased drip loss in the LD and gluteus muscles at 48 h post-mortem, and cooking loss in the LD muscle compared with the control group ( $P<0.05$ ). In contrast, dietary synbiotic supplementation numerically reduced total lead retention in the gluteus muscle ( $P<0.1$ ). The results suggested that dietary synbiotic supplementation to the diet of late-finishing pigs would enhance muscle antioxidant capacity, improve meat quality, whereas numerically reduce muscle lead retention.

**Keywords:** Synbiotic, Muscle antioxidant capacity, Meat quality, Mineral accumulations, Late-finishing pigs

## Domuzların Geç-Son Yetiştirme Döneminde Sinbiyotik İlavesinin Büyüme Performansı, Kas Antioksidan Kapasitesi, Mineral Birikimi ve Et Kalitesi Üzerine Etkilerinin Değerlendirilmesi

### Öz

Bu çalışmada domuzların geç-son yetiştirme döneminde sinbiyotik ilavesinin büyüme performansı, kas antioksidan kapasitesi, mineral birikimi ve et kalitesi üzerine etkilerinin araştırılması amaçlanmıştır. Çalışmada elli adet hadım edilmiş domuz 5 tekrar, iki uygulama grubu olarak rastgele ayrılmış, probiyotik (Mantar hücre duvarı ve Ksilooligosakkarit) ve probiyotikten (*Clostridium butyricum*, *Bacillus licheniformis* ve *Bacillus subtilis*) oluşan 1 g/kg sinbiyotik katılmış veya katılmamış bazal diyet ile 21 gün süresince beslendi. Sinbiyotik uygulaması geç-son yetiştirme dönemindeki domuzlarda büyüme performansına etki etmedi ( $P>0.05$ ). Kontrol grubu ile karşılaştırıldığında, sinbiyotik grubundaki domuzların *Longissimus dorsi* (LD) kasında süperoksit dismutaz aktivitesi daha yüksek gluteal kasında da malondialdehit konsantrasyonu daha düşüktü ( $P<0.05$ ). Diyetle sinbiyotik verilmesi, kontrol grubu ile karşılaştırıldığında LD ve gluteal kaslarında postmortem 48. saatte damla kaybında ve LD kasında pişirme kaybında azalmaya neden oldu ( $P<0.05$ ). Diyetle sinbiyotik verilmesi gluteal kasında sayısal olarak azalmış kurşun tutulmasına neden oldu ( $P<0.1$ ). Elde edilen sonuçlar domuzların geç-son yetiştirme döneminde diyetle sinbiyotik ilavesinin kas antioksidan kapasitesinde iyileşmeye ve et kalitesinde gelişmeye neden olurken sayısal olarak kas kurşun tutulumunda azalmaya neden olduğunu göstermiştir.

**Anahtar sözcükler:** Sinbiyotik, Kas antioksidan kapasitesi, Et kalitesi, Mineral birikimi, Geç-son yetiştirme dönemi domuzu

## INTRODUCTION

Sub-therapeutic dosages of antibiotics were usually used in commercial feed for the purpose of growth promotion in livestock production, which subsequently resulted in

the reduction of their therapeutic effectiveness against pathogenic microorganisms and development of antimicrobial resistance<sup>[1]</sup>. As a consequence, the European Union has forbidden the addition of antibiotics as growth promoters in the feed since 2006 due to the public



### İletişim (Correspondence)



+86 25 84396067 Fax: +86 25 84395314



zhouym6308@163.com

concern about the antibiotics resistance and food safety [2]. Accordingly, the search for alternatives to antibiotics has become more essential and emergent. Probiotic, prebiotic and/or their combination may represent potential alternatives approaches to replace antibiotics in animal production, and have received increasing attention due to their beneficial effects on livestock in recent years [3]. The term of synbiotic is defined as a combination of both probiotic and prebiotic, which could confer benefits on host animal by encouraging a better balance of gastrointestinal tract where living microorganisms are improved, and by selectively stimulating the favorable growth and/or activity of one or a limited number of health-promoting bacteria [3]. Previously published studies conducted on pigs have demonstrated that the supplementation of synbiotic with different components could replace antibiotics [4], promote body weight gain and feed conversion ratio [5], improve intestinal morphology [6], modulate intestinal microflora composition [5], regulate lipid metabolism [7], and enhance immune function [8]. Additionally, dietary synbiotic inclusion would also be an effective method to alleviate immune suppression in pigs induced by pathogenic microbe [9].

In addition to the regulatory function on animals, the effect of various synbiotics supplementation on meat quality in animals especially poultry has also been reported [10,11]. As active components of synbiotic, published papers have indicated that either probiotic (*Clostridium butyricum*, *Bacillus licheniformis*, and *Bacillus subtilis*) [11,12] or prebiotic (xylooligosaccharide and yeast cell wall) [12,13] could improve meat quality in animals including pigs and broilers. We recently manufactured a synbiotic that was composed of prebiotics (yeast cell wall and xylooligosaccharide) and probiotics (*Clostridium butyricum*, *Bacillus licheniformis*, and *Bacillus subtilis*), and demonstrated that the supplementation of which could promote growth performance, improve meat quality, whereas reduce muscle chromium (Cr) accumulation in broilers during a 42-day experiment [14]. We currently made a new synbiotic containing similar components to those used in our previous trial [14], and hypothesized that this type of synbiotic could exert beneficial consequences on late-finishing pigs. The aim of the present study was therefore conducted to evaluate the effects of dietary synbiotic supplementation on growth performance, muscle antioxidant ability and mineral accumulations, and meat quality in late-finishing pigs.

## MATERIAL and METHODS

All procedures describing management and care of animals in this experiment were conducted under approval by Institutional Animal Care and Use Committee of Nanjing Agricultural University (Protocol No. NJAU-CAST-2014-179).

### Animals, Diets, and Experimental Design

A total of fifty late-finishing barrow pigs [(Landrace ×

Yorkshire) × Duroc] with similar initial body weight ( $P > 0.05$ ) were randomly allocated into two treatments of five pens each, and fed a basal diet supplemented without or with 1 g/kg synbiotic for 21 days, respectively. The basal diet is formulated according to NRC (2012), and the ingredient and nutrient levels of which are presented in Table 1. The detailed components of synbiotic (1 g) were shown as following: yeast cell wall (500 mg), xylooligosaccharide (100 mg), *Clostridium butyricum* ( $3 \times 10^9$  CFU), *Bacillus licheniformis* ( $2 \times 10^9$  CFU), and *Bacillus subtilis* ( $3 \times 10^9$  CFU). Additionally, mineral contents in the diets were detected and showed in Table 2. Pigs were housed in the naturally ventilated shelter of 16 pens per shelter. Each shelter was designed with the solid concrete floor and large windows controlled by curtains. Additionally, relative humidity and temperature in the environment were recorded using data loggers. Pigs in each pen had *ad libitum* access to feed by the separate feeder and clean water by nipple waterer. At the end of the experiment, pigs were weighed after a 12-h fasting period, and feed consumption was recorded on a basis of the pen. All data were collected to calculate average daily gain, average daily feed intake, and feed/gain ratio.

**Table 1.** Composition and nutrient contents of the basal diet (g/kg, as feed basis)

Items	Content
<b>Ingredients</b>	
Corn	792.5
Soybean meal	171.0
Soybean oil	13.0
L-lysine	1.20
Choline chloride	1.40
Salt	4.20
Limestone	8.80
Monocalcium phosphate	4.60
Vitamin premix <sup>1</sup>	0.30
Mineral premix <sup>2</sup>	3.00
Total	1000
<b>Calculated chemical composition<sup>3</sup></b>	
Metabolizable energy, MJ/kg	14.23
Crude protein	137.6
Calcium	4.80
Available phosphorus	2.00
Lysine	6.80

<sup>1</sup> Provided per kg of complete diet: Vitamin A: 5.512 IU; Vitamin D<sub>3</sub>: 2.250 IU; Vitamin E 24 IU; Vitamin K<sub>3</sub>: 3 mg; Thiamin: 3 mg; Riboflavin 6 mg; Vitamin B<sub>6</sub>: 3 mg; Vitamin B<sub>12</sub>: 0.024 mg; Pantothenate: 15 mg; Folic acid: 1.2 mg; biotin: 0.15 mg

<sup>2</sup> Provided per kg of complete diet: 8 mg Cu (as copper sulfate); 60 mg Fe (as ferrous sulfate); 10 mg Mn (as manganese sulfate); 60 mg Zn (as zinc sulfate); 0.14 mg I (as potassium iodide); and 0.3 mg Se (as sodium selenite).

<sup>3</sup> Calculated according to tables of feed composition and nutritive values in China (2012)

**Table 2.** Mineral contents in the diets (mg/kg)

Item <sup>1,2</sup>	Control	Synbiotic
Zn	99.5±5.3	103±6
Fe	436±14	442±13
Mg	2504±110	2549±65
Mn	51.9±2.5	52.5±4.2
Cu	53.3±6.3	57.6±8.0
Cr	2.32±0.15	2.67±0.18
Pb	4.27±0.52	4.58±0.41
Cd	--	--

<sup>1</sup> Zn, zinc; Fe, iron; Mg, magnesium; Mn, manganese; Cu, copper; Cr, chromium; Pb, lead; Cd, cadmium  
<sup>2</sup> Values represent means and standard error of triplicate samples  
"--" indicates that mineral below the detection limit

### Samples Collection

At the end of the experiment, 20 late-finishing pigs (2 pigs per pen) were selected for the collection of muscle samples. In detail, pigs were transported to a commercial pork packing plant, electrically stunned (225 to 380 V, 0.5 A, for 5 to 6 s), exsanguinated and eviscerated according to standard commercial procedures, and split down the center of the vertebral column. Part of the left *Longissimus dorsi* (LD) and gluteus muscles were collected into self-sealing bags and immediately refrigerated at 4°C for subsequent determination of pH value, meat color, drip loss, and cooking loss. Meanwhile, part of the right LD and gluteus muscles were taken and quickly frozen at -20°C for the determination of antioxidant-related parameters and mineral contents.

### Determination of Antioxidant-Related Indexes

*Longissimus dorsi* and gluteus muscles samples were homogenized in 5 volumes (w/v) of 154 mmol/L ice-cold sterile sodium chloride solution using an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH, USA) at a high speed for 10 s. The homogenate was spun at 4450 × g for 15 min at 4°C. The collected supernatant was used to determine concentrations of malondialdehyde (MDA) and reduced glutathione (GSH), and activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). The related parameters were measured according to the manufacturer's recommended procedures using commercial chemistry assay kits (Nanjing Jiancheng Institute of Bioengineering, Jiangsu, P. R. China). Total protein content in the muscles was assayed following the description by our previous experiment [14]. Results for MDA and GSH contents were expressed as nmol per milligram protein, and mg per gram protein, respectively, and for GSH-Px and SOD activities were expressed as specific activity units per milligram protein of muscles in late-finishing pigs

### Meat Quality Assay

The pH value in muscles was measured (3 different places

around each muscle sample and averaged) both at 45 min and 24 h post-mortem using a portable pH meter (HI9125, HANNA Instruments, Italy), which was calibrated by 2-point method against standard buffer solutions with pH values of 4.0 and 7.0. Meat color in the muscles was estimated at 45 min after slaughter by three variables in triplicate using a hand-held colorimeter (Minolta CR-400, Konica Minolta, Tokyo, Japan) based on the CIELAB system (L\*=Lightness; a\*=Redness; b\*=Yellowness). The drip loss in the muscles at 24 h and 48 h post-mortem was assessed by calculating the difference between the initial and final weight of muscles. Briefly, LD and gluteus muscles were cut and suspended in sealed place at 4°C for 24 h and 48 h, respectively. The weight loss during 24- and 48-h hang time for each sample was recorded for the calculation of drip loss, which was expressed as weight loss (g)/initial weight (kg). The cooking loss in the muscles was determined in accordance with the methodology by our previous study [15]. In brief, roughly 15-mm-thick LD and gluteus muscles were weighed, placed into a plastic bag and cooked in a water bath until its internal temperature reached to 75°C, which was maintained for 20 min. The internal temperature was monitored during cooking using a hand-held temperature probe. Cooked muscle samples were allowed to cool to room temperature, blotted dry, and weighed. The difference of between pre- and post-cooking weight was recorded for cooking loss calculation, and it was expressed as weight loss (g)/initial weight (kg).

### Measurement of Muscle Mineral Contents

The mineral contents including Zn, Fe, Mn, Mg, Cu, Pb, and Cr in the muscles and diets were determined following the description as described by our previous studies [14,15]. In detail, each feed (around 0.5 g) and fresh muscle sample (approximately 2.0 g) was weighed and put in a digestion tube, added with 10 mL volume of mixture acid containing perchloric acid and nitric acid at a ratio of 1:4, which were stand for 12 h at room temperature for subsequent digestion. The digestion procedure was the same to that described by our previous study [15] using a heating block. Delaying digestion time if the digestive solution was not clear and adding appropriate mixture acid carefully if the digestive solution was rare during the digestion process. The mineral contents in the final solution were analyzed with an inductively coupled plasma mass spectrometry, which was selected, operated, and optimized in accordance with the methodology by Yang et al. [15].

### Statistical Analysis

All data were performed using SPSS 18.0 statistical software with pen as the experimental unit, and were analysed by independent samples t test. Statistical difference and trend between the two groups were considered if P<0.05 and 0.05<P<0.1, respectively. The means and standard errors of mean are presented.

## RESULTS

### Growth Performance

Compared with the control group (Table 3), dietary synbiotic supplementation did not affect growth performance in late-finishing pigs ( $P>0.05$ ).

### Muscle Antioxidant Ability

Pigs in the synbiotic group exhibited a higher SOD activity in the LD muscle (Table 4,  $P<0.05$ ), whereas a lower MDA concentration in the gluteus muscle ( $P<0.05$ ) compared with those in the control group. However, treatment did not alter remaining antioxidant parameters in the muscles ( $P>0.05$ ).

### Meat Quality

Dietary synbiotic supplementation did not affect meat color and pH value in the muscles (Table 5,  $P>0.05$ ). Drip loss at 48 h post-mortem in the LD and gluteus muscles ( $P<0.05$ ) and cooking loss in the LD muscle ( $P<0.05$ ) in the synbiotic group were lower than the control group.

**Table 3.** Dietary synbiotic supplementation on growth performance in late-finishing pigs

Items	Control	Synbiotic	SEM <sup>1</sup>	P-value
Initial body weight (kg)	98.6	100	2.6	0.771
Final body weight (kg)	119	120	2	0.786
Average daily gain (kg/d)	0.95	0.95	0.03	0.894
Average daily feed intake (kg/d)	3.67	3.61	0.08	0.772
Feed/gain ratio (kg/kg)	3.85	3.82	0.04	0.765

<sup>1</sup> SEM, standard errors of mean

**Table 4.** Dietary synbiotic supplementation on muscle antioxidant capacity in late-finishing pigs

Items <sup>1</sup>	Control	Synbiotic	SEM <sup>2</sup>	P-value
<b>Longissimus dorsi muscle</b>				
MDA (nmol/mg protein)	0.53	0.48	0.04	0.575
SOD (U/mg protein)	59.2	70.4	2.6	0.016
GSH (mg/g protein)	7.42	7.83	0.53	0.724
GSH-Px (U/mg protein)	23.6	28.8	2.5	0.328
<b>Gluteus muscle</b>				
MDA (nmol/mg protein)	0.64	0.50	0.03	0.004
SOD (U/mg protein)	64.1	69.7	3.1	0.387
GSH (mg/g protein)	9.00	8.90	0.73	0.950
GSH-Px (U/mg protein)	23.2	25.2	1.4	0.537

<sup>1</sup> MDA, malondialdehyde; SOD, superoxide dismutase; GSH, reduced glutathione; GSH-Px, glutathione peroxidase  
<sup>2</sup> SEM, standard errors of mean

**Table 5.** Dietary synbiotic supplementation on meat quality in late-finishing pigs

Items	Control	Synbiotic	SEM <sup>1</sup>	P-value
<b>Longissimus dorsi muscle</b>				
Lightness <sub>45 min</sub>	41.3	41.9	1.0	0.757
Redness <sub>45 min</sub>	6.93	8.20	0.36	0.128
Yellowness <sub>45 min</sub>	6.11	6.59	0.19	0.224
pH <sub>45 min</sub>	6.55	6.38	0.07	0.268
pH <sub>24 h</sub>	5.66	5.70	0.03	0.481
Drip loss <sub>24 h</sub> (g/kg)	47.0	41.5	2.8	0.353
Drip loss <sub>48 h</sub> (g/kg)	76.8	60.8	3.8	0.019
Cooking loss (g/kg)	274	224	11	0.014
<b>Gluteus muscle</b>				
Lightness <sub>45 min</sub>	36.1	38.2	1.0	0.336
Redness <sub>45 min</sub>	13.7	13.7	1.0	0.976
Yellowness <sub>45 min</sub>	6.19	6.71	0.33	0.465
pH <sub>45 min</sub>	6.57	6.57	0.05	0.967
pH <sub>24 h</sub>	5.79	5.72	0.04	0.414
Drip loss <sub>24 h</sub> (g/kg)	32.3	29.2	1.0	0.123
Drip loss <sub>48 h</sub> (g/kg)	63.2	52.8	2.3	0.044
Cooking loss (g/kg)	306	290	10	0.437

<sup>1</sup> SEM, standard errors of mean

### Muscle Mineral Contents

Total Pb accumulation in the gluteus muscle was numerically reduced with the dietary synbiotic inclusion when compared with the control group (Table 6,  $P<0.1$ ). However, the concentrations of muscle Zn, Fe, Mn, Mg, Cu, and Cr were similar between the two groups ( $P>0.05$ ).

## DISCUSSION

Probiotic, prebiotic and/or synbiotic represent potential alternatives to antibiotic that can promote growth performance in livestock. Shim et al.<sup>[5]</sup> reported that the supplementation of synbiotic (*Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and oligofructose) would increase average daily gain in suckling piglets. Meanwhile, as one component of synbiotic prepared in this study, yeast cell wall mainly consisted of  $\beta$ -glucans and manno oligosaccharides were demonstrated that it could promote growth performance in weanling pigs<sup>[16,17]</sup>. Inconsistent with those results above-mentioned, the inclusion of dietary synbiotic comprising prebiotics (yeast cell wall and xylooligosaccharide) and probiotics (*Clostridium butyricum*, *Bacillus licheniformis* and *Bacillus subtilis*) did not alter growth performance in late-finishing pigs in the present study, which was in agreement with the results by Liang et al.<sup>[7]</sup>, who found that the supplementation of synbiotic containing *Lactobacillus acidophilus* and fructooligosaccharide exerted no effect on



**Table 6.** Dietary synbiotic supplementation on muscle mineral accumulations in late-finishing pigs (mg/kg)

Items	Control	Synbiotic	SEM <sup>1</sup>	P-value
<b>Longissimus dorsi muscle</b>				
Zn	13.1	13.5	0.5	0.749
Fe	8.12	9.15	0.6	0.389
Mn	0.238	0.239	0.015	0.942
Mg	329	321	7	0.610
Cu	0.67	0.65	0.01	0.542
Pb	0.130	0.140	0.009	0.608
Cr	0.28	0.27	0.01	0.760
<b>Gluteus muscle</b>				
Zn	15.0	15.3	0.5	0.777
Fe	9.82	8.72	0.6	0.440
Mn	0.28	0.22	0.02	0.150
Mg	303	302	7	0.922
Cu	0.80	0.68	0.05	0.291
Pb	0.168	0.152	0.005	0.088
Cr	0.49	0.39	0.04	0.247

<sup>1</sup> SEM, standard errors of mean

growth rate, feed intake, and feed/gain ratio in growing-finishing pig. The unaffected growth performance by synbiotic supplementation in this study may result from the development of digestive system, immunity, and capacity as pigs becoming older, and it was supported by the findings of Alexopoulos et al.<sup>[12]</sup>. Further studies need to be conducted to evaluate the positive effect of this type of synbiotic on growth performance in pigs during early period. However, it has also been reported that probiotics (*Bacillus subtilis* and *Clostridium butyricum* endospores) inclusion could enhance growth performance in growing-finishing pigs<sup>[18]</sup>. Therefore, breed, management, and synbiotic variation and its dosage would be nonnegligible factors for growth discrepancy in pigs.

Enzymatic antioxidant system as a main antioxidant defence system plays a vital role in maintenance of redox balance. SOD is one of important components in enzymatic antioxidant system that can catalyze the dismutation of superoxide anions. Additionally, lipid peroxidation is a process that carbon-carbon double bond (s) of lipid is attacked by oxygen free radicals. MDA is decomposed product of lipid peroxidation, and the accumulation of which is an essential indicator for lipid peroxidation. In this study, dietary synbiotic enhanced SOD activity in the LD muscle, whereas reduced MDA concentration in the gluteus muscle. Similarly, decreased MDA accumulation in the thigh muscle of broilers has been observed with similar synbiotic inclusion in our previous trial<sup>[14]</sup>. Reduced MDA content in the muscle by the supplementation of synbiotic suggests a better oxidative stability of muscle, which may result from regulatory effect of probiotic, prebiotic and/or

synbiotic on lipid metabolism<sup>[18,19]</sup>. As active component of synbiotic used in the present study, it has been reported that *Clostridium butyricum*<sup>[19]</sup>, *Bacillus licheniformis*<sup>[20]</sup>, *Bacillus subtilis*<sup>[21]</sup>, yeast cell wall<sup>[22]</sup> or xylooligosaccharide<sup>[23]</sup> supplementation could improve antioxidant capacity (elevated antioxidant enzymes activities and/or reduced MDA content) in the tissues in animals and aquatic organism. Accordingly, a higher SOD activity in the LD muscle, but a lower MDA accumulation in the gluteus muscle with synbiotic inclusion in the present study may be related to the effect of synbiotic on improvement of body antioxidant capacity as well.

Previously published papers have demonstrated that the inclusion of probiotic or prebiotic, similar to the components of synbiotic appeared in the current study, could improve meat quality in broilers<sup>[13,24,25]</sup>. However, few literatures are available about the effect of this kind of probiotics, prebiotics and/or synbiotic on meat quality in finishing pigs. In this study, the supplementation of dietary synbiotic reduced drip loss at 48 h post-mortem in the muscles and cooking loss in gluteus muscle in late-finishing pigs, and it was consistent with the results by Yang et al.<sup>[25]</sup>, Park and Kim<sup>[24]</sup>, and Cho et al.<sup>[13]</sup> who reported that probiotic (*Clostridium butyricum* or *Bacillus subtilis*) or prebiotic ( $\beta$ -glucan) supplementation could decrease drip loss and/or cooking loss of raw muscle in broilers. Lipid peroxidation in meat usually results in the overproduction of free radicals and reduces water reservation among myofibrils, which would subsequently increase juice loss of meat<sup>[26]</sup>. Meat has endogenous antioxidant enzymes such as SOD and GSH-Px that play an important role in protecting against the damages induced by free radical including superoxide anion radical. Therefore, reduced drip loss in the both LD and gluteus muscles and cooking loss in the gluteus muscle in the synbiotic group in the current study may be related to the simultaneously increased SOD activity in LD muscle but decreased MDA accumulation in gluteus muscle.

Food safety such as heavy metal residue resulting from human activities has threatened human beings via food chain, and has also raised public attention due to the damage induced by heavy metal accumulation. The reduction of heavy metal accumulation from feed to animal meat mainly including pork and chicken meat would improve food safety in term of heavy metal retention. *In vitro* studies have indicated that *Bacillus* and fungal cell wall structure such as yeast cell wall used as biosorbents could effectively adsorb heavy metals as Pb, Cu, Cd and/or Cr from wastewater and/or soil contaminated with heavy metals<sup>[27,28]</sup>. In our recently published paper, synbiotic supplementation that was composed of yeast cell wall, xylooligosaccharide, *Clostridium butyricum*, *Bacillus licheniformis*, and *Bacillus subtilis* could significantly reduce total Cr retention in the thigh muscle of broilers<sup>[14]</sup>. In this study, significant decrease of total Pb and/or Cr accumulations

were not observed in the muscle, but numerical reduction of total Pb accumulation in the gluteus muscle was observed by the supplementation of dietary synbiotic. The discrepancy on muscle mineral accumulations and especially heavy metal retentions between the two experiments may be related to species, duration of synbiotic inclusion, management, and diets. Numerically reduction of total Pb retention in the gluteus muscle in the present study suggests a possibility that dietary synbiotic supplementation could reduce the risk of heavy metal accumulation from animals to human and its negative consequences on public health at some extent, which may be attributed to the strong adsorption ability of some components in synbiotic including yeast cell wall and *Bacillus* to heavy metals *in vitro*.

In conclusion, dietary synbiotic supplementation at the level of 1 g/kg to the diet of late-finishing pigs that was consisted of prebiotics (yeast cell wall and xylooligosaccharide) and probiotics (*Clostridium butyricum*, *Bacillus licheniformis*, and *Bacillus subtilis*) could enhance oxidative stability (increased SOD activity in the LD muscle, whereas decreased MDA concentration in the gluteus muscle), improve meat quality (reduced drip loss at 48 h post-mortem in the muscles and cooking loss in the LD muscle), and numerically lower total Pb retention in the gluteus muscle in late-finishing pigs.

## ACKNOWLEDGEMENTS

We are very grateful to our lab-mates for their technical assistance.

## CONFLICTS OF INTEREST

The authors declared that they have no conflict of interest.

## REFERENCES

- Tien YC, Li B, Zhang T, Scott A, Murray R, Sabourin L, Marti R, Topp E: Impact of dairy manure pre-application treatment on manure composition, soil dynamics of antibiotic resistance genes, and abundance of antibiotic-resistance genes on vegetables at harvest. *Sci Total Environ*, 581-582, 32-39, 2017. DOI: 10.1016/j.scitotenv.2016.12.138
- Lammie SL, Hughes JM: Antimicrobial resistance, food safety, and one health: The need for convergence. *Annu Rev Food Sci Technol*, 7 (1): 287-312, 2016. DOI: 10.1146/annurev-food-041715-033251
- Hume ME: Historic perspective: Prebiotics, probiotics, and other alternatives to antibiotics. *Poult Sci*, 90 (11): 2663-2669, 2011. DOI: 10.3382/ps.2010-01030
- Chu GM, Lee SJ, Jeong HS, Lee SS: Efficacy of probiotics from anaerobic microflora with prebiotics on growth performance and noxious gas emission in growing pigs. *Anim Sci J*, 82 (2): 282-290, 2011. DOI: 10.1111/j.1740-0929.2010.00828.x
- Shim SB, Verstegen MWA, Kim IH, Kwon OS, Verdonk JMAJ: Effects of feeding antibiotic-free creep feed supplemented with oligofructose, probiotics or synbiotics to suckling piglets increases the preweaning weight gain and composition of intestinal microbiota. *Arch Anim Nutr*, 59 (6): 419-427, 2005. DOI: 10.1080/17450390500353234
- Marinho MC, Pinho MA, Mascarenhas RD, Silva FC, Lordelo MM, Cunha LF, Freire JPB: Effect of prebiotic or probiotic supplementation and ileo rectal anastomosis on intestinal morphology of weaned piglets. *Livest Sci*, 108 (1-3): 240-243, 2007. DOI: 10.1016/j.livsci.2007.01.084
- Liong MT, Dunshea FR, Shah NP: Effects of a synbiotic containing *Lactobacillus acidophilus* ATCC 4962 on plasma lipid profiles and morphology of erythrocytes in hypercholesterolaemic pigs on high- and low-fat diets. *Br J Nutr*, 98 (4): 736-744, 2007. DOI: 10.1017/S0007114507747803
- Andrejčáková Z, Sopková D, Vlčková R, Kulichová L, Gancarčíková S, Almášiová V, Holovská K, Petrilla V, Krešáková L: Synbiotics suppress the release of lactate dehydrogenase, promote non-specific immunity and integrity of jejunum mucosa in piglets. *Anim Sci J*, 87 (9): 1157-1166, 2016. DOI: 10.1111/asj.12558
- Naqid IA, Owen JP, Maddison BC, Gardner DS, Foster N, Tchórzewska MA, La Ragione RM, Gough KC: Prebiotic and probiotic agents enhance antibody-based immune responses to *Salmonella Typhimurium* infection in pigs. *Anim Feed Sci Technol*, 201, 57-65, 2015. DOI: 10.1016/j.anifeeds.2014.12.005
- Maiorano G, Sobolewska A, Cianciullo D, Walasik K, Elminowska-Wenda G, Sławińska A, Tavaniello S, Żylińska J, Bardowski J, Bednarczyk M: Influence of *in ovo* prebiotic and synbiotic administration on meat quality of broiler chickens. *Poult Sci*, 91 (11): 2963-2969, 2012. DOI: 10.3382/ps.2012-02208
- Zhang ZF, Zhou TX, Ao X, Kim IH: Effects of  $\beta$ -glucan and *Bacillus subtilis* on growth performance, blood profiles, relative organ weight and meat quality in broilers fed maize-soybean meal based diets. *Livest Sci*, 150 (1-3): 419-424, 2012. DOI: 10.1016/j.livsci.2012.10.003
- Alexopoulos C, Georgoulakis IE, Tzivara A, Kyriakis CS, Govaris A, Kyriakis SC: Field evaluation of the effect of a probiotic-containing *Bacillus licheniformis* and *Bacillus subtilis* spores on the health status, performance, and carcass quality of grower and finisher pigs. *J Vet Med A Physiol Pathol Clin Med*, 51 (6): 306-312, 2004. DOI: 10.1111/j.1439-0442.2004.00637.x
- Cho JH, Zhang ZF, Kim IH: Effects of single or combined dietary supplementation of  $\beta$ -glucan and kefir on growth performance, blood characteristics and meat quality in broilers. *Br Poult Sci*, 54 (2): 216-221, 2013. DOI: 10.1080/00071668.2013.777691
- Cheng YF, Chen YP, Li XH, Yang WL, Wen C, Kang YR, Wang AQ, Zhou YM: Effects of synbiotic supplementation on growth performance, carcass characteristics, meat quality and muscular antioxidant capacity and mineral contents in broilers. *J Sci Food Agric*, 97 (11): 3699-3705, 2017. DOI: 10.1002/jsfa.8230
- Yang WL, Chen YP, Cheng YF, Li XH, Zhang RQ, Wen C, Zhou YM: An evaluation of zinc bearing palygorskite inclusion on the growth performance, mineral content, meat quality, and antioxidant status of broilers. *Poult Sci*, 95 (4): 878-885, 2016. DOI: 10.3382/ps/pev445
- Hahn TW, Lohakare JD, Lee SL, Moon WK, Chae BJ: Effects of supplementation of  $\beta$ -glucans on growth performance, nutrient digestibility, and immunity in weanling pigs. *J Anim Sci*, 84 (6): 1422-1428, 2006. DOI: 10.2527/2006.8461422x
- Che TM, Johnson RW, Kelley KW, Van Alstine WG, Dawson KA, Moran CA, Pettigrew JE: Mannan oligosaccharide improves immune responses and growth efficiency of nursery pigs experimentally infected with porcine reproductive and respiratory syndrome virus. *J Anim Sci*, 89 (8): 2592-2602, 2011. DOI: 10.2527/jas.2010-3208
- Meng QW, Yan L, Ao X, Zhou TX, Wang JP, Lee JH, Kim IH: Influence of probiotics in different energy and nutrient density diets on growth performance, nutrient digestibility, meat quality, and blood characteristics in growing-finishing pigs. *J Anim Sci*, 88 (10): 3320-3326, 2010. DOI: 10.2527/jas.2009-2308
- Liao XD, Wu RJ, Ma G, Zhao LM, Zheng ZJ, Zhang RJ: Effects of *Clostridium butyricum* on antioxidant properties, meat quality and fatty acid composition of broiler birds. *Lipids Health Dis*, 14, 36, 2015. DOI: 10.1186/s12944-015-0035-0
- Lei K, Li YL, Yu DY, Rajput IR, Li WF: Influence of dietary inclusion of *Bacillus licheniformis* on laying performance, egg quality, antioxidant enzyme activities, and intestinal barrier function of laying hens. *Poult Sci*, 92 (9): 2389-2395, 2013. DOI: 10.3382/ps.2012-02686

- 21. Shen WY, Fu LL, Li WF, Zhu YR:** Effect of dietary supplementation with *Bacillus subtilis* on the growth, performance, immune response and antioxidant activities of the shrimp (*Litopenaeus vannamei*). *Aquac Res*, 41 (11): 1691-1698, 2010. DOI: 10.1111/j.1365-2109.2010.02554.x
- 22. Li XH, Chen YP, Cheng YF, Yang WL, Wen C, Zhou YM:** Effect of yeast cell wall powder with different particle sizes on the growth performance, serum metabolites, immunity and oxidative status of broilers. *Anim Feed Sci Technol*, 212, 81-89, 2016. DOI: 10.1016/j.anifeedsci.2015.12.011
- 23. Wang J, Cao Y, Wang C, Sun B:** Wheat bran xylooligosaccharides improve blood lipid metabolism and antioxidant status in rats fed a high-fat diet. *Carbohydr Polym*, 86 (3): 1192-1197, 2011. DOI: 10.1016/j.carbpol.2011.06.014
- 24. Park JH, Kim IH:** Supplemental effect of probiotic *Bacillus subtilis* B2A on productivity, organ weight, intestinal *Salmonella* microflora, and breast meat quality of growing broiler chicks. *Poult Sci*, 93 (8): 2054-2059, 2014. DOI: 10.3382/ps.2013-03818
- 25. Yang X, Zhang B, Guo Y, Jiao P, Long F:** Effects of dietary lipids and *Clostridium butyricum* on fat deposition and meat quality of broiler chickens. *Poult Sci*, 89 (2): 254-260, 2010. DOI: 10.3382/ps.2009-00234
- 26. Mercier Y, Gatellier P, Renerre M:** Lipid and protein oxidation *in vitro*, and antioxidant potential in meat from *Charolais* cows finished on pasture or mixed diet. *Meat Sci*, 66 (2): 467-473, 2004. DOI: 10.1016/S0309-1740(03)00135-9
- 27. Brady D, Stoll AD, Starke L, Duncan JR:** Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of *Saccharomyces cerevisiae*. *Biotechnol Bioeng*, 44 (3): 297-302, 1994. DOI: 10.1002/bit.260440307
- 28. Brunetti G, Farrag K, Soler-Rovira P, Ferrara M, Nigro F, Senesi N:** The effect of compost and *Bacillus licheniformis* on the phytoextraction of Cr, Cu, Pb and Zn by three brassicaceae species from contaminated soils in the Apulia region, Southern Italy. *Geoderma*, 170, 322-330, 2012. DOI: 10.1016/j.geoderma.2011.11.029