

Effects of Dietary Synbiotic Supplementation as an Alternative to Antibiotic on the Growth Performance, Carcass Characteristics, Meat Quality, Immunity, and Oxidative Status of Cherry Valley Ducks

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The aim of this study was to investigate the effects of synbiotic supplementation, a potential alternative to antibiotic, on growth performance, carcass characteristics, meat quality, immunity, and oxidative status of Cherry Valley ducks. In total, 540 1-day-old male Cherry Valley ducks were randomly subjected to 3 treatments, and each treatment consisted of 6 replicates with 30 birds each. Birds in the 3 treatments were fed a basal diet devoid of antibiotics (control group) or a basal diet supplemented with either 40 mg/kg zinc bacitracin or 1.5 g/kg synbiotic composed of xylooligosaccharide, Clostridium butyricum, and Bacillus subtilis for 42 days. Compared with the control group, dietary synbiotic and antibiotic supplementation decreased the feed/gain ratio of ducks (P=0.025) to a similar extent (P > 0.05). Birds in the antibiotic group exhibited a lower average daily feed intake (P = 0.024) whereas such an effect was not observed in the birds of the synbiotic group (P > 0.05). Synbiotic and antibiotic supplementation reduced abdominal fat yield (P=0.032) and drip loss of the breast muscle (P<0.001) to similar extents (P > 0.05). Additionally, synbiotic and antibiotic supplementation increased the relative weight of the bursa (P=0.005) and total superoxide dismutase activity in the ileal mucosa (P=0.025) to similar extents (P>0.05). Moreover, ileal malondial dehyde accumulation was reduced with the supplementation of synbiotic (P=0.028), but not antibiotic. The results indicated that dietary synbiotic supplementation was beneficial for growth performance, carcass compositions, meat quality, immune function, and antioxidant capacity of Cherry Valley ducks, and it could be used as an alternative to antibiotics in Cherry Valley ducks.

Key words: Cherry Valley duck, immunity, meat quality, oxidative status, synbiotic, carcass trait

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Introduction

Antibiotics have been used in animal production for approximately 60 years in many countries including European countries, United States, and China. Antibiotics serve as therapeutic agents to improve the health and welfare of animals, and they are especially administered for prophylactic purposes and to enhance the growth rate and feed conversion efficiency of animals (Huyghebaert *et al.*, 2011).

However, the use of antibiotics has resulted in the emergence of microbes resistant to antibiotics, and the transfer of antibiotic resistance genes from animal to human microbiota has been reported (Williams and Heymann, 1998; Wegener, 2003). The European Union has withdrawn the approval for antibiotics as growth promoters on January 1, 2006 (Marshall and Levy, 2011). Recently, the government of China has also prohibited the use of several antibiotics, including colistin sulfate, lomefloxacin, ofloxacin, norfloxacin, and pefloxacin, as growth promoters in food producing animals (Ministry of Agriculture of China, 2015, 2016). The withdrawal of antibiotics as growth promoters in animal production has been proven to induce animal growth problems, compromise feed conversion efficiency, and increase

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the incidence of animal diseases, such as subclinical necrotic enteritis (Wierup, 2001; Van Immerseel *et al.*, 2004; Dibner and Richards, 2005; Huyghebaert *et al.*, 2011). Therefore, alternatives to antibiotics in animal production are urgently needed.

Synbiotics are defined as a mixture of probiotics and prebiotics (Gibson and Roberfroid, 1995), and the use of synbiotics as alternatives to antibiotics could alleviate the negative consequences of phasing out antibiotics on the growth and health of animals (Gaggia et al., 2010; Huyghebaert et al., 2011). In chickens, previous studies have demonstrated that dietary supplementation of synbiotics could be used as an alternative to antibiotics (NaghiShokri et al., 2017), and it could improve gut microbiota composition (Erdoğan et al., 2010; Mookiah et al., 2014), regulate the immune system (Hassanpour et al., 2013; Min et al., 2016), and maintain intestinal integrity (Awad et al., 2009; Sohail et al., 2012; Ghasemiet al., 2014). Dietary synbiotics supplementation also improved antioxidant capacity, carcass characteristics, and meat quality of broiler chickens (Zhang et al., 2012; Ghasemi et al., 2016; Min et al., 2016). Rajput et al. (2012) reported that the supplementation of probiotic (*Bacillus subtilis*, 1.0×10^8 cfu per kilogram diet) was beneficial for digestive function, antioxidant capacity, and innate immunity of fowls (Shaoxing duck). Similarly, Xing et al. (2015) found that dietary supplementation with lysineyielding *Bacillus subtilis* $(5.0 \times 10^8$ and 5.0×10^{10} cfu per kilogram feed) improved gut morphology, increased the population of beneficial gut microbiota, and stimulated the intestinal immune response of Linwu ducks. Additionally, Tang *et al.* (2011) showed that a prebiotic, Sophy β -glucan, had regulatory or enhancing effects on the nonspecific cellular immunity of Peking ducks, whereas it did not improve their growth performance, carcass characteristics, and meat quality. However, studies that investigated the effects of synbiotics on ducks are scarce.

In this study, we prepared a new type of synbiotic that was composed of xylooligosaccharide, *Clostridium butyricum*, and *Bacillus subtilis*, and hypothesized that the supplementation of this synbiotic would exert beneficial effects in Cherry Valley ducks. Therefore, the present study evaluated the effects of synbiotic, used as an alternative to antibiotic, on the growth performance, carcass characteristics, meat quality, immunity, and oxidative status of Cherry Valley ducks in a 42-day assay.

Materials and Methods

Husbandry, Diets, and Experimental Design

All experiments were conducted in accordance with the guidelines approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University (Protocol No. NJAU-CAST-2014-179). In total, 540 1-day-old male Cherry Valley ducks were randomly distributed into 3 groups, with each group being composed of 6 replicates (pen) with 30 birds each. The 3 groups were the control group, antibiotic group, and synbiotic group, and the birds in these groups were fed a basal diet that was free from antibiotics, a

basal diet supplemented with an antibiotic (40 mg zinc bacitracin per kilogram of diet), and a basal diet supplemented with a synbiotic (1.5 g synbiotic per kilogram of diet), respectively, for 42 days. One and a half gram of the synbiotic contained 150 mg xylooligosaccharide (35%; Jiangsu Kangwei Biologic, Huaian, Jiangsu, China), 3×10^9 cfu C. butyricum (Yuanshan Biotech, Yancheng, Jiangsu, China), and 4.5×10^{10} cfu *B. subtilis* (Qingdao Vland Biotech, Qingdao, Shandong, China), using palygorskite as the carrier. The composition of the basal diet and its nutrient content are shown in Table 1. All ducks were housed in plastic-mesh pens $(4.5 \text{ m} \times 4.5 \text{ m} \times 60 \text{ cm})$ that were placed 0.4 m above the floor in a temperature-controlled and naturally ventilated room under a lighting schedule of 23 h of light and 1 h of darkness, with a light intensity of approximately 20 lux on average. The temperature in the room was maintained at 33-34°C for the first 5 days and then decreased by 1°C every other day until a final temperature of 20°C was reached. Mean relative humidity was kept around 70% for the first 3 days and maintained at 60-65% thereafter. Each pen (replicate) was equipped with a nipple drinker line (3 nipples/pen) and two hanging plastic pan feeders. The diameter and height of the panfeeder was 32 cm and 26 cm, respectively. Birds were allowed ad libitum access to water and pellet feed during the entire period of the experiment. Ducks (42-day-old) were weighed after a 12-h feed withdrawal, and feed consumption (spilled feed in the plastic trays set under each feeder was carefully removed and weighed, and was considered as wastage feed) was measured per pen (replicate) to determine average daily feed intake, average daily gain, and feed/gain ratio.

Sample Collection

At 42 days of age, 1 duck from each pen (6 ducks per treatment) was fasted for 12 h, and subsequently weighed and euthanized by cervical dislocation after bleeding. Following the sacrifice, intramuscular fat width and subcutaneous fat thickness were determined using a Vernier caliper. Carcass weight was measured after the removal of feathers, and eviscerated weight was determined by further removing all of the viscera except the lungs and kidneys, and abdominal fat. The carcass yield and eviscerated yield were calculated as g/kg live weight (body weight after a 12-h feed deprivation). Abdominal fat yield was calculated based on eviscerated weight (g/kg). Subsequently, the whole left breast muscle and thigh muscle were excised and weighed to determine the muscle yield, which was expressed as g/kg eviscerated weight. Next, samples of the left pectoralis major muscle were immediately taken and stored at 4°C for the measurement of meat color, pH, and drip loss. Parts of the right pectoralis major muscle samples were collected for the determination of cooking loss. Spleen, bursa, and thymus were excised and weighed to determine the relative organ weight that was expressed as g/kg of body weight. The jejunum (from the end of pancreatic loop to the Meckel's diverticulum) and ileum (from Meckel's diverticulum to the ileocecal junction) were excised to collect intestinal mucosa samples that were carefully and rapidly scratched using a

Ingredients	Day 1 to 21	Day 22 to 42
Corn	41.0	32.0
Corn germ meal	5.0	8.0
Wheat middling	10.0	18.0
Cottonseed meal	5.0	8.0
Soybean meal	23.2	6.0
Corn gluten meal	2.6	1.6
Soybean oil	1.5	2.5
Rice bran	7.0	20.0
L-Lysine · HCl	0.20	0.24
D,L-Methionine	0.14	0.07
Limestone	1.26	1.46
Dicalcium phosphate	1.8	0.83
Salt (NaCl)	0.3	0.3
Premix*	1.0	1.0
Calculated nutrient levels		
Apparent metabolizable energy (MJ/kg)	12.0	12.2
Crude protein	21.4	16.9
Calcium	1.02	0.84
Available phosphorus	0.42	0.30
Lysine	1.15	0.89
Methionine + Cysteine	0.80	0.64

 Table 1.
 Ingredient and nutrient composition of the basal diet (%, as-fed basis unless otherwise stated)

* Premix provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D₃, 2,000 IU; vitamin E, 20 IU; thiamin, 3 mg; riboflavin, 4 mg; nicotinamide, 60 mg; choline chloride, 600 mg; calcium pantothenate, 11 mg; pyridoxine HCl, 2.5 mg; biotin, 0.2 mg; folic acid, 0.6 mg; vitamin B₁₂ (cobalamin), 0.012 mg; Fe (ferrous sulfate), 80 mg; Mn (manganese sulfate), 80 mg; Zn (zinc sulfate), 60 mg; I (calcium iodate), 0.4 mg; Se (sodium selenite), 0.2 mg.

sterile glass microscope slide, immediately frozen in liquid nitrogen, and stored at -80° C until analysis.

Measurement of Meat Quality

At 45 min and 24 h postmortem, the pH was measured by direct insertion of an electrode (HI9125; Hanna Instruments, Padova, Italy) 2-cm deep into the breast muscle. Meat color was measured at 45 min after slaughter with a colorimeter (Minolta CR-400; Konica Minolta, Tokyo, Japan) using the CIELAB trichromatic system as lightness (L*), redness (a*), and yellowness (b*). The water holding capacity of meat was estimated by determining drip loss and cooking loss of the raw meat after storage. The breast muscles that were trimmed of adjacent fat and connective tissue were weighed and immediately placed in a plastic bag, hung from a hook, and stored at 4°C for 24 h. After hanging, the sample was wiped with absorbent paper and weighed again to measure drip loss that was calculated as gram of weight loss during 24 h per kilogram initial muscle weight. The cooking loss of meat was determined at 24 h postmortem after storage at 4°C as described by Wang et al. (2013).

Determination of Mucosal Parameters

Approximately 0.3-g mucosal samples were minced and placed in ice-cold 154 mmol/L sterile sodium chloride solution (1:9, wt/vol) containing protease inhibitors and then homogenized with an Ultra-Turrax homogenizer (Tekmar, Cincinnati, OH, USA) at a high speed for 30 s. Samples

were spun at $4450 \times g$ for 15 min at 4°C. The supernatant was aliquoted and stored at -80°C for analysis.

The activity of total superoxide dismutase (T-SOD) and the concentrations of total protein, malondialdehyde (MDA), and reduced glutathione (GSH) were quantified using colorimetry kits from the Nanjing Jiancheng Institute of Bioengineering (Nanjing, Jiangsu, China), as per the manufacturer's instructions. The contents of secretory immunoglobulin A (SIgA), immunoglobulin G (IgG), interleukin-1 β (IL-1 β), and tumor necrosis factor- α (TNF- α) in the mucosal samples were measured by enzyme-linked immunosorbent assay (Nanjing Jiancheng Bioengineering Institute). All results were normalized against total protein concentration in each sample for inter-sample comparison.

Statistical Analysis

Growth Performance

Data were analyzed by one-way analysis of variance (ANOVA) using SPSS 16.0 statistical software. Differences among treatments were examined using Tukey's post-hoc test for multiple comparisons, and they were considered significant at P < 0.05. Results are presented as means and total standard error of the means

Results

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Compared with the control group, dietary antibiotic and synbiotic supplementation decreased the feed/gain ratio of

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Items	Control	Antibiotic	Synbiotic	SEM [*]
Average daily gain (g/day)	68.0	67.2	70.4	0.7
Average daily feed intake (g/day)	134 ^a	126 ^b	133 ^a	2
Feed conversion ratio (g:g)	1.97 ^a	1.88 ^b	1.89 ^b	0.02

Table 2. Growth performance of Cherry Valley ducks

^{a, b} Means within a row with different superscripts are different at $P \le 0.05$.

* SEM, standard error of the mean (n=6).

Table 3.	Carcass traits and	breast meat qua	lity of Cherry	Valley ducks at	42 days of age
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Items	Control	Antibiotic	Synbiotic	SEM*
Carcass traits				
Carcass yield (g/kg)	885	886	886	2
Eviscerated yield (g/kg)	780	785	787	2
Breast muscle yield (g/kg)	130	121	131	4
Thigh muscle yield (g/kg)	111	112	113	3
Abdominal fat yield (g/kg)	18.1 ^a	14.7 ^b	13.8 ^b	0.8
Subcutaneous fat thickness (mm)	8.05	6.79	7.07	0.59
Intramuscular fat width (mm)	9.99	9.56	9.33	0.45
Breast meat quality				
pH _{45min}	5.98	5.99	6.16	0.04
pH _{24h}	5.75	5.73	5.71	0.01
Drip loss (g/kg)	61.4 ^a	47.1 ^b	52.3 ^b	2.1
Cooking loss (g/kg)	323	293	299	7
Lightness	39.2	36.4	38.5	0.6
Redness	20.0	20.2	18.0	0.6
Yellowness	7.74	8.41	6.71	0.51

^{a, b} Means within a row with different superscripts are different at P < 0.05.

* SEM, standard error of the mean (n=6).

Items	Control	Antibiotic	Synbiotic	SEM^*
Thymus	2.56	2.96	2.61	0.38
Spleen	0.682	0.619	0.695	0.049
Bursa	0.547^{b}	0.869^{a}	0.929^{a}	0.082

Table 4.Immune organ weight of Cherry Valleyducksat 42 days ofage (g/kg body weight)

^{a, b} Means within a row with different superscripts are different at $P \le 0.05$.

* SEM, standard error of the mean (n=6).

ducks (P=0.025) to a similar level. While birds fed the diet supplemented with antibiotic exhibited reduced average daily feed intake when compared with the control group (P=0.024), dietary synbiotic supplementation did not affect the average daily feed intake of ducks (P>0.05) (Table 2). The treatments did not alter average daily gain of ducks (P>0.05).

Carcass Characteristics and Meat Quality

The birds of the synbiotic group showed lower abdominal fat yield (P=0.032) and drip loss of breast muscle (P < 0.001) when compared with those of the control group, with the values of these two parameters being similar to those in the antibiotic group (P > 0.05) (Table 3). However, treat-

ments did not affect the other carcass-related parameters (carcass yield, eviscerated yield, muscle yield, subcutaneous fat thickness, and intramuscular fat width), pH, meat color, and cooking loss (P > 0.05).

Relative Immune Organ Weight

Compared with the control group, the synbiotic or antibiotic group showed an increase in the relative weight of the bursa (P=0.005) (Table 4). However, the relative weight of the bursa was similar between the synbiotic and antibiotic groups (P>0.05). Ducks exhibited similar relative weights of spleen and thymus among treatments (P>0.05).

Intestinal Oxidative Status and Immunity

Compared with the control group, the synbiotic group

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Items [†]	Control	Antibiotic	Synbiotic	SEM*
Jejunum				
T-SOD (U/mg protein)	34.3	31.3	40.8	2.2
MDA (nmol/mg protein)	0.585	0.378	0.489	0.045
GSH (mg/g protein)	15.2	15.6	15.2	0.8
SIgA (µg/mg protein)	0.98	1.05	1.05	0.05
IgG (µg/mg protein)	29.2	35.3	33.3	1.7
IL-1 β (ng/g protein)	2.91	3.30	2.76	0.12
TNF- α (ng/g protein)	14.0	17.2	12.4	1.0
Ileum				
T-SOD (U/mg protein)	28.7 ^b	46.3 ^a	41.6 ^a	3.2
MDA (nmol/mg protein)	0.539 ^a	0.542^{a}	0.421 ^b	0.022
GSH (mg/g protein)	19.7	24.1	21.7	3.0
SIgA (µg/mg protein)	0.83	1.25	1.02	0.07
IgG (µg/mg protein)	28.1	35.7	33.9	1.9
IL-1 β (ng/g protein)	2.89	3.54	2.90	0.19
TNF- α (ng/g protein)	14.0	15.8	11.7	1.0

Table 5. Intestinal immunity and oxidative status of 42-day-old Cherry Valley ducks

^{a, b} Means within a row with different superscripts are different at P < 0.05.

[†] IgG, immunoglobulin G; SIgA, secretory immunoglobulin A; IL-1 β , interleukin-1 β ; TNF- α , tumor ne-

crosis factor- α ; MDA, malondialdehyde; GSH, reduced glutathione; T-SOD, total superoxide dismutase. * SEM, standard error of the mean (n=6).

showed an increase in the ileal T-SOD activity to a level comparable with that of the antibiotic group (P=0.025) (Table 5). Additionally, the accumulation of ileal MDA was reduced by the supplementation of synbiotic (P=0.028), but not antibiotic. However, dietary treatments did not affect the concentrations of intestinal GSH and jejunal MDA, and T-SOD activity in the jejunum (P>0.05). Similarly, ducks exhibited similar contents of immunoglobulins (SIgA and IgG) and immune cytokines (IL-1 β and TNF- α) among groups (P > 0.05).

Discussion

Studies regarding the effects of synbiotic supplementation on the growth performance of meat ducks are rare. In the present study, the supplementation of a synbiotic composed of xylooligosaccharide, C. butyricum, and B. subtilis reduced the feed/gain ratio of Cherry Valley ducks to a similar extent as antibiotic supplementation, indicating that the synbiotic, like the antibiotic zinc bacitracin, improved feed conversion efficiency, and therefore it could potentially be used as an alternative for antibiotic growth promoters. Similar results in broilers were observed by Min et al. (2016), who recently reported that a synbiotic that consisted of B. subtilis, xylooligosaccharide, and mannanoligosaccharide, used as an alternative to xanthomycin, improved the feed efficiency of broilers in a 42-day assay. Beneficial effects of synbiotics on growth performance in broiler chickens were also reported by Mookiah et al. (2014) and NaghiShokri et al. (2017). The beneficial effect of synbiotics on growth performance appears to be related to their involvement in the modulation of intestinal microbial composition, regulation of immune system, and maintenance of intestinal integrity and barrier function (Tuohy et al., 2003; Saad et al., 2013).

Dietary probiotics and prebiotics can exhibit lipid-lowering effects on the lipid metabolism of animals, as summarized by Ooi and Liong (2010). Recently, Ghasemi et al. (2016) observed that the supplementation of synbiotic (a combination of the probiotic strain Enterococcus faecium and prebiotic fructo-oligosaccharides) reduced circulating cholesterol and low-density lipoprotein cholesterol in broilers. Ashayerizadeh et al. (2009) have reported that dietary synbiotic inclusion decreased abdominal fat yield in chickens. Accordingly, in this study, it was observed that synbiotic supplementation decreased abdominal fat yield in Cherry Valley ducks, and this may be owing to its lipidlowering function. In this study, dietary antibiotic supplementation (zinc bacitracin) also decreased abdominal fat yield in Cherry Valley ducks. In contrast, Tang et al. (2011) found that the inclusion of zinc bacitracin (5%) did not affect the abdominal fat yield of mixed-sex Pekin ducks. This discrepancy might be associated with sex, dosage of zinc bacitracin, and duck species.

In poultry, improvements in meat quality with the supplementation of prebiotic or probiotic have been observed (Zhang *et al.*, 2005; Liao *et al.*, 2015). Among the ingredients of the synbiotic used in the present study (xylooligosaccharide, *C. butyricum* and *B. subtilis*), dietary *C. butyricum* supplementation reduced drip loss in the breast muscle of broilers fed a diet containing fish oil (Yang *et al.*, 2010). Dietary supplementation of *B. subtilis* linearly decreased drip loss in the breast muscle of broiler chickens (Park and Kim, 2014). In this study, dietary inclusion of synbiotic also improved meat quality by reducing drip loss in the breast muscle of Cherry Valley ducks. Meat oxidation, including lipid peroxidation, would impair hydrolysis sensitivity, weaken protein degradation, and decrease water retention in myofibrils, which would consequently increase juice loss of meat (Wood *et al.*, 2004; Huff-Lonergan and Lonergan, 2005). Ghasemi *et al.* (2016) showed that thiobarbituric acid-reactive species in the meat after storage at 4°C linearly decreased as synbiotic concentrations in the diet increased. The reduced generation of thiobarbituric acid-reactive species resulting from synbiotic supplementation may therefore account for the decreased drip loss observed in this study. It is necessary to mention that dietary antibiotic supplementation also decreased drip loss of the breast muscle in this study. This may be because antibiotics can improve the utilization of various nutrients in the feed that are important for the maintenance of meat water-holding capacity.

The regulatory effects of probiotics, prebiotics, or their combination (synbiotic) on the growth and development of immune organs have been observed previously in broiler chickens (Ashayerizadeh et al., 2009; Chen et al., 2013; Zhang et al., 2014). In line with these findings, in this study, dietary synbiotic supplementation stimulated the growth and development of the bursa, a key immune organ for antibody production in poultry (Scott, 2004). The beneficial effects of synbiotic on the relative bursa weight might be related to the ingredients, which have immunoregulatory capacity (Murayama et al., 1995; Chen et al., 2013; Zhang et al., 2014). Consistent with the results of Ravindran et al. (2006) in broilers, the supplementation of zinc bacitracin also increased the relative bursa weight in Cherry Valley ducks, indicating that zinc bacitracin also has a modulatory effect on bursa growth and development.

Reactive oxygen species are generated by living organisms during normal cellular metabolism and as a result of various environmental factors, including toxins, heavy metals, and hazardous chemicals. However, the overproduction of reactive oxygen species can damage carbohydrates, nucleic acids, lipids, and proteins and impair their biological functions (Birben et al., 2012). Superoxide dismutases (SODs) are metal-containing proteins that catalyze the removal of the free radical, superoxide, generating water peroxide as a final product of the reaction (Limón-Pacheco and Gonsebatt, 2009). Lipid peroxidation can be described generally as a process under which oxidants, such as free radicals, attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids, and the accumulation of MDA is usually considered as a marker of lipid peroxidation (Ayala et al., 2014). In this study, dietary synbiotic supplementation increased the activity of T-SOD, whereas it reduced MDA accumulation in the ileum, suggesting that dietary synbiotic improves the intestinal antioxidant capacity of ducks. Similarly, Min et al. (2016) found that dietary synbiotic supplementation increased SOD activity, whereas it reduced MDA concentration in the serum of broilers. The improved intestinal oxidative status induced by synbiotic supplementation may be attributed to its ingredients that possess antioxidant capacity (Shen et al., 2010; Wang et al., 2011; Liao et al., 2015). Additionally, the protective effects of synbiotic on the intestinal microbial population, immunity, and integrity may also account for the improved oxidative

status in the intestine (Tuohy et al., 2003; Saad et al., 2013).

In conclusion, the supplementation of a new type of synbiotic composed of xylooligosaccharide, *C. butyricum*, and *B. subtilis*, used as a potential alternative to the antibiotic zinc bacitracin, can improve feed conversion efficiency, reduce abdominal fat yield and drip loss of breast meat, stimulate the growth and development of the bursa, and improve intestinal antioxidant status of Cherry Valley ducks.

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