Effects of dietary synbiotic supplementation on growth performance, lipid metabolism, antioxidant status, and meat quality in Partridge shank chickens

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ABSTRACT
This experiment aimed to evaluate the effects of incorporating a synbiotic into diet on growth performance, lipid metabolism, antioxidant status, and meat quality in Partridge shank chickens. A total of 128 1-day-old male Partridge shank chicks were randomly divided into 2 groups, and each group consisted of 8 replicates with 8 birds each. Birds in the 2 treatments were fed a basal diet supplemented with or without 1.5 g of a synbiotic per kg of feed consisting of prebiotics (xylooligosaccharide and yeast cell wall) and probiotics (Bacillus licheniformis, Bacillus subtilis, and Clostridium butyricum) for 50 days. Compared with control group, supplementation with synbiotic significantly decreased feed to gain ratio during days 1–21, absolute and relative abdominal fat weight, and hepatic malondialdehyde concentration as well as plasma triglyceride concentration at 50 days of age. In addition, dietary supplemented with synbiotic had lower 24-h postmortem drip loss, cooking loss of breast muscle, whereas higher plasma high density lipoprotein cholesterol level, 24-h postmortem redness, and pH of breast muscle. Dietary synbiotic supplement could promote growth performance, regulate lipid metabolism, and improve antioxidant capacity and meat quality in Partridge shank chickens.

Introduction
In view of the development of antibiotic resistant bacteria and antibiotic residues in the body of the animal, the European Commission has banned the use of antibiotics as growth promoters in animal production since 2006 (EU Commission 2003). Therefore, much attention has been paid to find alternatives to antibiotic. The synbiotic, considered as an alternative, is a mix of probiotics and prebiotics. Probiotics (direct-fed microbial) are used as a live microorganism feed supplement that confer health to the host by improving microbial intestinal balance (Fuller 1989). On the other hand, prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activities of one or a limited number of beneficial bacteria (Gibson et al. 1995). The effects of synbiotic on growth performance, immunity and intestinal health in fast-growing commercial broilers (Arbor Acres and Ross broilers) have been reported in recent studies (Sohail et al. 2012; Hassanpour et al. 2013; Ghasemi et al. 2014; Naghi et al. 2016; Chen et al. 2018). In addition, dietary supplementation with probiotic and prebiotic is an effective measure to improve chicken meat quality (Sohail et al. 2012; Zhang et al. 2012).

In China, Partridge shank chickens is a characteristic local hybrid of broilers that have plenty of advantages such as improved adaptability and flavor, and therefore an increased customers’ attention is observed in recent years. The official data provided by China Animal Agriculture Association (2018) showed that the number of local chickens accounted for about 46.52% of broiler slaughter in China for the year 2017, and this percentage would increase according to available data from the first three quarters in 2018. In view of their huge market share and consumer preferences, it is therefore meaningful and imperative to improve growth performance, meat quality, and carcass characteristics of Partridge shank chickens. Previous studies have shown that several probiotics (Bacillus licheniformis, Bacillus subtilis, and Clostridium butyricum) and/or prebiotics (xylooligosaccharide and yeast cell wall) that are also used in the present study have been proved to regulate serum lipid metabolism and improve meat quality in animals (Cui et al. 2013; Zhao et al. 2013; Park and Kim 2014; Zhou et al. 2016; Abasubong et al. 2018). A recent study from our lab has demonstrated that a synbiotic could improve growth performance and meat quality in fast-growing commercial broilers (Cheng et al. 2017). However, little information is available in terms of synbiotic on meat quality and lipid metabolism in Partridge shank chickens. We therefore studied the effects of dietary supplementation with synbiotic consisting of probiotics (Bacillus licheniformis, Bacillus subtilis, and Clostridium butyricum), and prebiotics (xylooligosaccharide and yeast cell wall) on growth performance, lipid metabolism, antioxidant status and meat quality in Partridge shank chickens.

Materials and methods

Animals, diets, and experimental design

All procedures involving animals in the present study were approved by Nanjing Agricultural University Animal Care and Use Committee, Nanjing, People’s Republic of China.
hundred and twenty-eight 1-day-old male Partridge shank chicks obtained from a commercial hatchery were randomly divided into 2 treatments, and each treatment was composed of 8 replicates (cages) with 8 birds per replicate. All birds were housed in cages of identical size (120 cm × 60 cm × 50 cm). Birds in the two treatments were fed a basal diet supplemented with or without synbiotic (1.5 g of synbiotic per kg of diet) for 50 days. The birds had ad libitum access to water and mash feed. The composition and calculated analysis of the basal diet are shown in Table 1. Ambient temperature of the room was maintained at 32–34°C for the first 3 days, and reduced by 3°C per week to 22°C until the end of the experiment. Light was provided 23 h per day with 1 h of darkness. The synbiotic (1.5 g) contained 150 mg of xylooligosaccharide, 750 mg of yeast cell wall, 1 × 10⁹ colony-forming units (CFU) of Clostridium butyricum, 3 × 10⁸ CFU Bacillus licheniformis and 2 × 10¹⁰ CFU Bacillus subtilis. All birds were weighed by pen after their arriving at experimental farm (initial weight), and on day 21 as well as on day 50 (birds were weighted after a 12-h fasting). Then the feed intake was calculated from the difference between the offered and residual feed and was used to calculate average daily intake (ADFI), average daily gain (ADG), and feed/gain ratio (F/G).

Sample collection

On day 50, one bird per replicate close to the average body weight of the replicate (after a 12-h fasting period) was selected and weighed. Subsequently, about 5 mL of blood was taken from wing vein, and serum harvested by centrifugation at 3000 × g for 10 min was stored at −20°C for further analysis. After blood collection, birds were euthanized by cervical dislocation. The liver, pectoralis major muscle and abdominal fat (fat surrounding the cloaca and the gizzard) were excised and weighed, and the relative organ weight was calculated based on live body weight (g/kg). The thickness of subcutaneous fat and intermuscular fat width were measured using a vernier caliper. Subsequently, the pectoralis major muscles were stored at 4°C for the determination of meat colour, drip loss, pH values and cooking loss, and the liver samples were stored at −20°C for further analysis.

Measurement of lipid metabolites in the serum

The TC, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and LDL-C in serum were analysed according to the methods described by the kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, People’s Republic of China).

Determination of antioxidant parameters in the serum and liver

About 0.3 g of liver sample was homogenized (1:9, wt/vol) with ice-cold 154 mmol/L sterile sodium chloride solution using an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH, USA). The supernatant was harvested after centrifugation at 4450 × g for 15 min at 4°C, which was subsequently stored at −20°C for determination of antioxidant parameters. The activity of superoxide dismutase (SOD) and the content of malondialdehyde (MDA) in serum and liver were determined in accordance with methods provided by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, People’s Republic of China).

Meat quality in the breast muscle

The left pectoralis major muscle was used to determine pH, meat colour, and drip loss, and part of right breast muscle was used to measure cooking loss. The pH values were determined for three times at 45 min and 24 h postmortem in the breast of 1-cm depth via using a portable pH metre (H9125; HANNA Instruments, Padova, Italy). Meat colour was measured at 45 min and 24 h post-slaughter using a colorimeter (Minolta CR-400; Konica Minolta, Tokyo, Japan) based on the CIELAB system (L*, a*, and b* represent lightness, redness, and yellowness, respectively) at 3 different parts of the breast. The drip loss was determined in accordance with the methods conducted by Christensen (2003) and Wang et al. (2013). Namely, breast muscle samples were cut, weighed, then put in a plastic bag, sealed, stored at 4°C for 24 and 48 h and weighed again. The cooking loss was determined at 24 h after slaughter by the method of Wang et al. (2013). Part of the right pectoralis major muscle was cut, dried slightly using filter papers, weighed, placed in the sealed plastic bags, which were heated to 75°C in a water bath for 20 min. After cooling to ambient temperature, samples were dried using filter papers and weighed again (cooking loss is expressed as g/kg initial muscle weight).

Statistical analysis

All data were conducted using SPSS (Version 20.0, SPSS Inc., Chicago, IL, USA) with pen (cage) as the experimental unit.
Data were analysed using an independent samples t-test. The results were expressed as mean and total standard error of mean, and probability value less than 0.05 was considered significant.

**Results**

**Growth performance**

The effect of synbiotic on growth performance in Partridge shank chickens is shown in Table 2. Compared with the control group, supplementation with synbiotic reduced the F/G from days 1–21 (P < 0.05), while its supplementation did not affect growth performance during days 22–50 and days 1–50 (P > 0.05).

**Organ weight and fat deposition**

As shown in Table 3, chickens fed the basal diet supplemented with synbiotic had lower absolute and relative abdominal fat weight than those offered the basal diet (P < 0.05). However, dietary supplementation with synbiotic had no effect on liver and breast muscle weight, intermuscular fat width as well as subcutaneous fat thickness (P > 0.05).

**Lipid metabolites in the serum**

Chickens receiving the synbiotic supplemented diet (Table 4) had a higher concentration of HDL-C (P < 0.05), whereas a lower TG level (P < 0.05), when compared with those fed basal diet.

**Oxidative status in the serum and liver**

In the liver (Table 5), synbiotic dietary supplementation increased the activity of SOD (P < 0.05) whereas decreased MDA concentration compared to the control group (P < 0.05). However, the content of MDA and the activity of SOD in the serum were not affected by dietary synbiotic supplementation (P > 0.05).

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### Table 2. Effect of synbiotic supplementation on growth performance in Partridge shank chickens.

| Items | Control group | Synbiotic group | SEM | P-value |
|-------|---------------|-----------------|-----|---------|
| T–21 days | | | | |
| ADG (g/day) | 17.0 | 17.3 | 0.3 | 0.581 |
| ADFI (g/day) | 30.7 | 29.6 | 0.3 | 0.078 |
| F/G (g/1) | 1.82 | 1.71 | 0.02 | 0.001 |
| 22–50 days | | | | |
| ADG (g/day) | 45.4 | 44.2 | 0.7 | 0.353 |
| ADFI (g/day) | 109 | 105 | 2 | 0.323 |
| F/G (g/1) | 2.40 | 2.38 | 0.04 | 0.783 |
| 1–50 days | | | | |
| ADG (g/day) | 33.3 | 32.9 | 0.4 | 0.659 |
| ADFI (g/day) | 75.1 | 73.2 | 1.2 | 0.422 |
| F/G (g/1) | 2.26 | 2.22 | 0.06 | 0.610 |

*ADG, average daily gain; ADFI, average daily feed intake; F/G, feed/gain ratio.

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### Table 3. Effect of synbiotic supplementation on organ weights and fat deposition in Partridge shank chickens.

| Items | Control group | Synbiotic group | SEM | P-value |
|-------|---------------|-----------------|-----|---------|
| Absolute weights (g) | | | | |
| Liver | 41.8 | 43.1 | 2.0 | 0.757 |
| Breast muscle | 175 | 180 | 5 | 0.699 |
| Abdominal fat | 41.1 | 29.3 | 2.6 | 0.014 |
| Relative weights (g/kg) | | | | |
| Liver | 21.8 | 23.4 | 0.8 | 0.382 |
| Breast muscle | 91.2 | 101 | 2.9 | 0.102 |
| Abdominal fat | 21.1 | 15.8 | 1.1 | 0.007 |
| Subcutaneous fat thickness (cm) | 0.91 | 0.90 | 0.02 | 0.951 |
| SEM, standard errors of mean.

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### Table 4. Effect of synbiotic supplementation on serum lipids in Partridge shank chickens (mmol/L).

| Items | Control group | Synbiotic group | SEM | P-value |
|-------|---------------|-----------------|-----|---------|
| TC (mmol/mL) | 3.59 | 3.38 | 0.09 | 0.475 |
| TG (mmol/mL) | 0.68 | 0.59 | 0.02 | 0.021 |
| HDL-C (U/mL) | 3.10 | 4.73 | 0.3 | 0.001 |
| LDL-C (U/mL) | 0.83 | 0.68 | 0.04 | 0.071 |
| TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

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### Table 5. Effect of synbiotic supplementation on antioxidant ability in Partridge shank chickens.

| Items | Control group | Synbiotic group | SEM | P-value |
|-------|---------------|-----------------|-----|---------|
| MDA (nmol/mL) | 2.23 | 2.10 | 0.09 | 0.851 |
| MDA (nmol/mg protein) | 0.75 | 0.59 | 0.03 | 0.004 |
| SOD (U/mL) | 131 | 130 | 4 | 0.951 |
| SOD (U/mg protein) | 128 | 147 | 3 | 0.951 |

*Control and Synbiotic group, chickens were fed the basal diet supplemented with 0 and 1.5 g/kg synbiotic, respectively.

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### Table 6. Effect of synbiotic supplementation on breast meat quality in Partridge shank chickens.

| Items | Control group | Synbiotic group | SEM | P-value |
|-------|---------------|-----------------|-----|---------|
| pH 45 min | 6.33 | 6.32 | 0.06 | 0.906 |
| pH 24 h | 5.65 | 5.85 | 0.03 | 0.002 |
| Lightness (L°) 45 min | 45.9 | 46.3 | 0.6 | 0.749 |
| Redness (a°) 45 min | 7.68 | 7.81 | 0.34 | 0.857 |
| Yellowness (b°) 45 min | 14.7 | 15.6 | 0.4 | 0.190 |
| Lightness (L°) 24 h | 50.0 | 50.4 | 0.4 | 0.681 |
| Redness (a°) 24 h | 6.20 | 7.08 | 0.19 | 0.012 |
| Yellowness (b°) 24 h | 17.0 | 17.7 | 0.4 | 0.384 |
| Drip loss 24 h (g/kg) | 32.1 | 26.5 | 1.2 | 0.016 |
| Drip loss 48 h (g/kg) | 53.9 | 53.0 | 1.2 | 0.736 |
| Cooking loss (g/kg) | 223 | 196 | 6 | 0.025 |

*Control and Synbiotic group, chickens were fed the basal diet supplemented with 0 and 1.5 g/kg synbiotic, respectively.

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Breast muscle meat quality

The pH and redness values (Table 6) detected at 24 h postslaughter were found higher in broilers fed the basal diet supplemented with synbiotic compared with those offered the basal diet (P < 0.05). Compared with control group, the diet containing synbiotic significantly reduced meat cooking loss and 24-h drip loss postmortem (P < 0.05). However, the values of pH, lightness, redness and yellowness determined at 45 min postmortem did not differ between control and synbiotic group (P > 0.05).

Discussion

The present study indicated that the combination of prebiotics (xylooligosaccharide and yeast cell wall) and probiotics (Bacillus licheniformis, Bacillus subtilis, and Clostridium butyricum) could decrease F/G in Partridge shank chickens during days 1–21. Similar results were found by Cheng et al. (2017), who demonstrated that supplementation with symbiotic (1.5 g of a symbiotic per kg of diet) consisting of probiotics (Bacillus licheniformis, Bacillus subtilis, and Clostridium butyricum), and prebiotics (xylooligosaccharide and yeast cell wall) increased weight gain and gain to feed ratio in broilers from days 1–42. Chen et al. (2018) also found that dietary supplementation with 1.5 g of a symbiotic per kg of diet that was comprised of xylooligosaccharide, Clostridium butyricum and Bacillus subtilis increased the weight gain and feed utilization in broilers. In addition, Min et al. (2016) showed that supplementation with 2.15 g of a symbiotic per kg of feed consisting of Bacillus subtilis, xylooligosaccharide and mannan oligosaccharide could increase daily weight gain and feed conversion efficiency in broilers. Previous researches have demonstrated that dietary supplementation with symbiotic could improve intestinal integrity and barrier function, inhibit the colonization of pathogenic bacteria, stimulate the proliferation of beneficial bacteria in the intestine and modulate intestinal immunity (Madsen et al. 2001; Corthésy et al. 2007; Awad et al. 2008; Gaggia et al. 2010; Meng et al. 2010). The improved growth performance observed in this study may be therefore attributed to these beneficial activities of synbiotic products.

Cholesterol, insoluble in water, cannot be transported in blood on its own. The cholesterol interacts with certain proteins that function as transport vehicles carrying different types of lipids such as cholesterol and TG. These combinations of fats and protein are termed lipoproteins such as LDL and HDL. LDL transports LDL-C to extrahepatic tissue cells to cover their need for cholesterol, increasing the cholesterol level in extrahepatic tissue cells. On the contrary, HDL is the carrier for transporting HDL-C from arteries to liver, resulting in promoting the elimination of cholesterol in peripheral tissues. In this study, chickens receiving the symbiotic supplemented diet had lower absolute and relative abdominal fat weight, and concentrations of TG and LDL-C, whereas a higher HDL-C level in the serum. In consistency with our study, Ashayerizadeh et al. (2009) reported that dietary supplementation with 2.9 g of a symbiotic per kg of feed containing 0.9 g of probiotic (Primalac) plus 2 g of prebiotic (Biolex-MB) could reduce the abdominal fat yield in broilers. The reduced yield of abdominal fat may be related to the reduction of TG and LDL-C levels. Similarly, Naghi et al. (2016) showed that serum TC and LDL-C levels were significantly lower in the broilers fed diets containing symbiotic. In addition, the decreases in circulating TC and LDL-C after symbiotic administration were also observed by Ghasemi et al. (2016) in broilers and Liong et al. (2007) in pigs. The improved lipid profile observed in this study may be related to the microorganisms and oligosaccharides in the symbiotic, which deconjugate bile acids to produce free bile acids or dropping acetyl CoA carboxylase (as a limited enzyme in fatty acids synthesis) in the liver and adipose tissues (Ooi and Liong 2010; Velasco et al. 2010).

Both Min et al. (2016) and Anwar et al. (2012) demonstrated that broilers fed diet containing synbiotics exhibited a higher serum T-SOD activity whereas a lower serum MDA content. Similarly, Cheng et al. (2017) found that supplementation with symbiotic decreased the MDA accumulation in the thigh muscle of broilers. In the present study, Partridge shank chickens receiving the symbiotic supplemented diet had a lower MDA concentration whereas a higher activity of SOD in the liver, which was consistent with previous studies (Anwar et al. 2012; Min et al. 2016; Cheng et al. 2017). The enhanced hepatic antioxidant status observed in this study may be associated with the antioxidant characteristics of the components of symbiotic (Liao et al. 2016). Additionally, the improved lipid profile may also contribute to this beneficial consequence.

Probiotics, such as Clostridium butyricum and Bacillus subtilis, could improve meat quality by decreasing the drip loss and cooking loss of the breast muscle of broilers (Yang et al. 2010; Zhang et al. 2012). Studies also showed that a diet supplemented with Bacillus subtilis B2A (1.0 × 10⁶ CFU/g of feed) could improve water-holding capacity and reduce drip loss (Park and Kim 2014). Additionally, Cheng et al. (2017) showed that broilers fed the basal diet supplemented with symbiotic had a higher meat pH24 h value. In consistency with previous studies, we found that birds diet supplemented with symbiotic had a higher value of pH at 24 h post-mortem. pH is one of the most important post-slaughtering factors influencing water holding capacity of meat, and higher pH might lead to a greater water-holding capacity (Zhou et al. 2010). Lipid peroxidation is one of the most important causes of the deterioration of poultry meat quality, and the content of MDA is used to monitor lipid peroxidation in poultry meat (Salih et al. 1987). Meat oxidation could increase juice loss of meat by reducing hydrolysis sensitivity and water reservation among myofibrils. The decreased drip loss 24 h and cooking loss of breast muscle meat observed in this study might be associated with the reduced MDA levels.

Conclusions

In summary, the dietary supplementation with a symbiotic composed of prebiotic (xylooligosaccharide and yeast cell wall) and probiotic (Bacillus licheniformis, Bacillus subtilis, and Clostridium butyricum) can promote growth performance, regulate lipid metabolism, and improve oxidative status and meat quality in Partridge Shank chickens.

Disclosure statement

No potential conflict of interest was reported by the authors.
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