Use of encapsulated Bacillus subtilis and essential oils to improve antioxidant and immune status of blood and production and hatching performance of laying hens

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ABSTRACT
The study was conducted to evaluate the effects of increasing inclusion levels of the mixture of Bacillus subtilis and essential oils (BSEO) on production, hatching performance, egg quality, serum antioxidant capacity, immune response, and hormone levels of laying hens. A total of 768 24-wk-old layers were randomly allotted into 4 treatments with 6 replicates of 32 birds each replicate. The experiment lasted for 84 d, and the birds were fed a basal diet (CON) or diets with BSEO at 300 (BSEO-1), 600 (BSEO-2), and 900 mg/kg (BSEO-3) in the other 3 groups, respectively. As the BSEO level increased, egg production (linear, \( p < .05 \)), yolk index (linear, \( p < .01 \)), glutathione peroxidase activity (linear, \( p < .01 \)), total antioxidant capacity (linear, \( p < .01 \)), oestradiol level (linear, \( p < .01 \)), the value luteinizing hormone/follicle-stimulating hormone (linear, \( p < .05 \)) increased in a linear manner. Hatchability (linear and quadratic, \( p < .01 \)), hatchability of fertile of eggs (linear and quadratic, \( p < .05 \)), avian influenza virus antibody level (linear, \( p < .01 \); quadratic, \( p < .05 \)), parathyroid hormone level (linear and quadratic, \( p < .01 \)) in the serum increased linearly and quadratically. Taken together, dietary with BSEO in laying hens could significantly improve egg production, hatchability, and hatchability of fertile eggs, which were associated with an enhancement of antioxidant capacity and the level of AIV-Ab in the serum. This study provided evidence of using BSEO as a potential feed additive for laying hens.

HIGHLIGHTS
- Dietary supplementation of BSEO decreased the feed conversion ratio and eggshell thickness of laying hens.
- Dietary supplementation of BSEO improved the egg weight, fertility, hatchability, hatchability of fertile eggs, and yolk index of laying hens.
- Dietary supplementation of BSEO enhanced the antioxidant capacity and the level of AIV-Ab in the serum.

Introduction
During the past decade, antibiotics have been widely used in the poultry industry, which can promote fast growth and prevent illness by using low doses. However, China banned the use of antibiotics as growth promoters in animal feed since 1 July 2020. With the demands for high-quality poultry products, it is imperative to exploit the effective and green feed additives to reduce economic losses caused by the prohibition (Attia and Al-Harthi 2015).

Bacillus subtilis (BS) is a commonly and widely used probiotic bacterium that can be incorporated in poultry diets for its beneficial advantages. A growing body of scientific studies reported that BS can improve the growth and laying performance (Neijat et al. 2019; Abramowicz et al. 2020; Hussein et al. 2020). Additionally, a noteworthy improvement of the activities oxidative stress and immune response in chicken (Abramowicz et al. 2019; Abudabos et al. 2019; Park et al. 2020), duck (Zhang et al. 2016), and quail (Abdel-Moneim et al. 2020) has been demonstrated. Furthermore, our previous studies also found that dietary supplementation of \( 9.0 \times 10^5 \) CFU/g BS effectively increased egg weight, fertility and...
hatchability, and improved egg quality in laying hens (Liu et al. 2019).

Essential oil (EO) are extracted from plant flowers, leaves, stems, roots, seeds or fruits by steam distillation, extrusion or solvent extraction, and are of a complex character with rather diverse effects. The major component of EO is phenolic compounds such as thymol, carvacrol, and eugenol (Attia et al. 2017). Generally, in vitro and in vivo studies have shown that EO has an effect on antibacterial, antimicrobial, antioxidant, and digestive stimulant properties (Barbarestani et al. 2020). Researches on laying hens found that diet supplemented with EO improved laying performance, immune response, and eggshell quality (Attia et al. 2019; Krauze et al. 2020; Lee et al. 2020). And, Hernández-Coronado et al. (2019) also reported that EO at 400 mg/L can serve as natural alternative additives in drinking water to improve broiler production and meat quality. Besides, there have been a number of studies about the use of BS or EO on piglet (Tian and Piao 2019), aquaculture (Jiang et al. 2019), beef (Alemu et al. 2019), and goat (Ma et al. 2020). Therefore, BS and EO are receiving increasing attention as potential antibiotic growth promotors and are already employed in many commercial applications.

Xuefeng black-bone chicken was originated from the Xuefeng mountain area in the southwest of Hunan Province and was included in the List of National Livestock and Poultry Genetic Resources Protection in China in January 2010. In recent years, it has been found that blends of EO and organic acids was effective in necrotic enteritis control in broiler chickens (Pham et al. 2020). Our previous study also showed that the combination of BS and montmorillonite can improve egg quality, antioxidant and immune status (Chen et al. 2019, 2020). To the best of our knowledge, manuscripts about the effects of combined use of BS and EO in poultry production are seldom studied. Thus, we hypothesise that appropriate dose of BSEO may exert antioxidant capacity, stimulate immune system, and hence improve growth performance in laying hens. Therefore, the aim of this study was to evaluate the different levels of BSEO on production, hatching performance, egg quality, serum antioxidant capacity, immune response, and hormone levels of laying hens, which provide a theoretical reference for the scientific use of encapsulated BS and EO on laying hens and the promotion of healthy breeding of native chickens.

Materials and methods

All the birds and experimental protocols in this study were approved by the Institutional Animal Care and Use Committee of Hunan Agricultural University, Hunan, China.

Experimental factor

The BSEO finnal product, Calsporin, was provided by Shanghai Naseco Products Company (Shanghai, China) and is composed of 10% BS (≥ 1 × 10^9 CFU/g), 18% EO (including 10% cinnamaldehyde, 5% tert-phenol, 5% thymol, 4.4% piperine), and 72% mineral elements.

Birds, diets, and experimental design

Briefly, 768 healthy Xuefeng black-bone laying hens and 60 healthy Xuefeng black-bone roosters at 24-wk of age were obtained from Hunan Yunfeifeng Agricultural Commercial Company (Hunan, China). 768 Laying hens randomly assigned to 4 experimental groups. Each group had 6 replicates and each replicate contained 32 hens. The dietary treatments were as follows: (1) basal diet (CON); (2) basal diet + 300 mg/kg BSEO (BSEO-1); (3) basal diet + 600 mg/kg BSEO (BSEO-2); (4) basal diet + 900 mg/kg BSEO (BSEO-3). The basal diet was formulated in accordance with the China Agricultural Standard (NY/T 33-2004) (Wen et al. 2004) to meet the nutrient requirements of laying hens (Table 1). All the experimental diets were prepared every week, packed in covered containers, and stored in a dry and well-ventilated storeroom.

This feeding experiment was conducted from March to June in 2019 at the original breeding farm of Hunan Yunfeifeng Agricultural Commercial Company. The hens were raised in a wire cage with 3 ladders, and 2 hens were raised in a cage (38 × 28 × 36 cm; length × width × height). The replications were allotted equally into the upper and middle cages to minimise the effects of the cage level. Hens were housed in an environmentally controlled room. During the experiment, the temperature and relative humidity in the room were 20.24 ± 1.59 °C (mean ± SD) and 68.21 ± 0.46% (mean ± SD), respectively. The hens were allowed a period of 1-wk to adapt to the environment. Then, all hens were fed the assigned experimental diets for 84 d. The hens were fed twice a day (08:00 h and 15:00 h) and given ad libitum access to water throughout the experiment. The lighting regimen used was a 16 h light and 8 h darkness cycle.
Assessment of production and hatching performance

During the experiment period, egg production, and egg weight were recorded daily by replicate, and feed consumption was recorded weekly by replicate to calculate egg production, feed intake, feed conversion ratio. A total of 60 pedigree roosters were fed at 06:00 am and the semen was collected at 3:00 pm. About 2.5 mL semen from 10 cocks was collected and slowly mixed with the same amount of diluent, and then fertilised 35 mL per hen as described by Liu et al. (2019). To keep the sperm vigour, the whole process was completed within 15 min. All qualified hatching eggs were collected and used to determine hatching performance. The number of eggs laid in the incubator, unfertilised eggs, infertile eggs, dead sperm eggs, dead embryos, and the sprout chicks was recorded daily by replicate to calculate the fertility, hatchability, and hatchability of fertile eggs.

Assessment of egg quality

During the experiment period, egg quality was measured on 5 eggs collected randomly from each replicate at 84 days. Eggshell-breaking strength was measured by an egg force reader (EFR-01, Orka Food Technology Ltd, Israel). Yolk colour and Haugh unit were measured by using an egg analyser (EA-01, Orka Food Technology Ltd, Ramat HaSharon, Israel). Eggshell thickness was measured by a digital micrometer (NFN380, FHK, Japan) at 3 different locations (bottom, middle, and top of the egg), and then was calculated the average shell thickness as described by Liu et al. (2017). The width and height of yolk were measured by using an electronic digital calliper (SH14100025, Shenhan, Shanghai, China). The yolk index was calculated by dividing yolk height by yolk width.

Blood sample collection

The immunisation program included vaccination against Newcastle disease (day 110, Intramuscular injection, 0.3 mL/per hen), and avian influenza disease (day 18, 85, and 210, Intramuscular injection, 0.5 mL/per) as described by Liu et al. (2019). At the end of the experiment, after 12 h of feed withdrawal, 2 hens were randomly selected from each replicate. Blood samples (about 6 mL/hen) were drawn from the wing vein using a disposable lancet, then immediately transferred into a heparinised tube. Blood samples were placed at room temperature for 1 h and then centrifuged at 2 500g for 10 min, stored in sterilised 1.5 mL Eppendorf tubes at −20°C for further analysis.

Serum antioxidant index, immune index, and hormone level measurement

Serum samples were individually used to measure the activities of glutathione peroxidase (GSH-Px), total superoxide dismutase (T-SOD), total antioxidant capacity (T-AOC), and concentration of malondialdehyde (MDA) using the assay kit (A005; A001-1-1; A015-1; Table 2.

Table 1.

| Ingredients a (%) | Content |
|------------------|---------|
| Corn             | 62.00   |
| Soybean meal     | 26.00   |
| Limestone        | 5.50    |
| CaHPO4           | 1.00    |
| Premix b         | 5.00    |
| Calculated composition |
| ME, MJ/kg        | 11.18   |
| CP, %            | 16.14   |
| Ca, %            | 2.99    |
| AP, %            | 0.38    |
| Salt, %          | 0.37    |
| Lys, %           | 0.95    |
| Met, %           | 0.51    |
| Met + Cys, %     | 0.82    |

aCaHPO4, Calcium hydrogen phosphate; ME, metabolisable energy; CP, crude protein; Ca, Calcium; AP, available phosphorus; Lys, lysine; Met, methionine; Met + Cys, methionine + cysteine; BSEO, mixture of Bacillus subtilis and essential oils.
bPremix provided per kilogram of diet: vitamin A, 170 000 IU; vitamin D3, 64 000 IU; vitamin E, 880 IU; vitamin B1, 48 mg; vitamin B2, 105 mg; vitamin B6, 48 mg; vitamin B12, 0.20 mg; vitamin K3, 48 mg; nicotinic acid, 380 mg; pantothenic acid, 270 mg; folic acid, 24 mg; Zn (from zinc sulfate), 1560 mg; Fe (from ferrous sulfate), 1100 mg; Mn (from manganese sulfate), 1800 mg; Cu (from copper sulfate), 240 mg; I (from potassium iodide) 23 mg; Se (from sodium selenite), 4.80 mg; Ca, 10.00 g; P, 2.60 g; NaCl, 3.70 g.

Table 2.

| Item                  | CON   | BSEO-1 | BSEO-2 | BSEO-3 | Pooled SEM | ANOVA | Linear | Quadratic |
|-----------------------|-------|--------|--------|--------|------------|--------|--------|-----------|
| Egg weight (g)        | 44.5  | 44.4   | 44.4   | 44.8   | 0.3         | .966   | .714   | .728      |
| Egg production (%)    | 65.3  | 65.6   | 66.0   | 68.0   | 0.4         | .076   | .020   | .278      |
| Feed intake (g/d per hen) | 88.8 | 87.9   | 88.1   | 88.0   | 1.0         | .989   | .818   | .832      |
| Feed conversion ratio (g of feed/g of e.g. g) | 3.48  | 3.21   | 3.18   | 3.04   | 0.06        | .079   | .014   | .607      |

Data are means of 6 replicates per treatment with 32 hens per replicate. **CON, BSEO-1, BSEO-2, BSEO-3 diets contained 0, 300, 600, 900 mg/kg of BSEO, respectively.

Means within a row with different superscripts differ significantly (p < .05).
A003-1; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) with a microplate reader (Multiskan GO, Thermo Fisher Scientific, Waltham, US) according to the instructions of the manufacturer. The method to determine antioxidant indicators using these kits were described by Qi et al. (2011). The levels of Newcastle disease virus antibody (NDV-Ab) and avian influenza virus antibody (AIV-Ab) were determined using enzyme-linked immunosorbent assay (ELISA) kits (E-80,133; E-1,92,022; R&D Systems, Minnesota, USA). The concentrations of immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), the levels of oestradiol (E2), progesterone (P), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and parathyroid hormone (PTH) were determined using ELISA kits (CSB-E11232Ch; CSB-EQ027259CH; CSB-E16200C; CSB-E12013C; CSB-E12012C; CSB-CF849533CH; CSB-CF009021CH; CSB-E11880Ch; Cusabio Biotech Co., Ltd, Wuhan, Hubei, China) with microplate reader according to the instructions of the manufacturer as described by OIE (2009).

Statistical analyses

All the data were statistically analysed by one-way analysis of variance (ANOVA) and linear and quadratic regression models using SPSS version 21.0 statistic software (SPSS Institute Inc., Chicago, Illinois). The analytical processing of results was performed by using Tukey’s multiple comparisons test using the replicate as the experimental unit. Data were expressed as mean±SEM, and p < .05 was considered statistically significant.

Table 3. Effects of increasing inclusion of dietary BSEO on hatching performance of laying hens*.  

| Item                      | Dietary treatment** | Pooled SEM | p-value   |
|---------------------------|---------------------|------------|-----------|
|                           | CON                 | BSEO-1     | BSEO-2    | BSEO-3    | ANOVA    | Linear | Quadratic |
| Fertility (%)             | 95.4                | 96.0       | 96.2      | 95.9      | 0.2      | .417    | .234  | .235 |
| Hatchability (%)          | 84.5b               | 87.3a      | 86.7a     | 87.0a     | 0.3      | .001    | .004  | .017 |
| Hatchability of fertile e.g. gs (%) | 88.7b               | 91.0a     | 90.3ab    | 90.7a     | 0.2      | .002    | .010  | .038 |

*Data are means of 6 replicates per treatment with 32 hens per replicate.
**CON, BSEO-1, BSEO-2, BSEO-3 diets contained 0, 300, 600, 900 mg/kg of BSEO, respectively.

Table 4. Effect of increasing inclusion of dietary BSEO on egg quality of laying hens*.  

| Item                      | Dietary treatment** | Pooled SEM | p-value   |
|---------------------------|---------------------|------------|-----------|
|                           | CON                 | BSEO-1     | BSEO-2    | BSEO-3    | ANOVA    | Linear | Quadratic |
| Eggshell-breaking strength (kgf) | 4.06               | 4.05       | 3.82      | 3.90      | 0.07     | .558    | .265  | .721 |
| Eggshell thickness (mm)   | 0.333               | 0.334      | 0.329     | 0.319     | 0.002    | .096    | .032  | .203 |
| Yolk index                | 0.377a              | 0.386ab    | 0.392ab   | 0.397a    | 0.002    | .007    | .001  | .476 |
| Yolk colour               | 5.55                | 5.30       | 5.29      | 5.75      | 0.16     | .576    | .623  | .200 |
| Haugh unit                | 60.5                | 60.9       | 61.0      | 61.5      | 0.7      | .971    | .656  | .988 |

*Data are means of 6 replicates per treatment with 32 hens per replicate.
**CON, BSEO-1, BSEO-2, BSEO-3 diets contained 0, 300, 600, 900 mg/kg of BSEO, respectively.
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Results

Production and hatching performance

The effects of BSEO on the production performance of laying hens were shown in Table 2. The egg production increased and the feed conversion ratio decreased with an increase in the concentration of BSEO in diets (linear, p < .05). The addition of BSEO to laying hens’ diets did not effect on the egg weight and feed intake. The results of hatching performance were shown in Table 3. The dietary BSEO significantly increased hatchability (ANOVA and linear, p < .01; quadratic, p < .05) and hatchability of fertile eggs (linear and quadratic, p < .05; ANOVA, p < .01). Fertility was not affected by dietary treatment.

Egg quality

The results of the egg quality of laying hens revealed that there were no differences in the eggshell-breaking strength, yolk colour, or Haugh unit among treatments. With an increased BSEO supplementation, the yolk index (ANOVA and linear, p < .01) increased, while the eggshell thickness (linear, p < .05) decreased (Table 4).

Serum antioxidant capacity and immune response

The serum antioxidant capacity of laying hens was shown in Table 5. With an increased BSEO supplementation, the activities of GSH-Px (ANOVA, p < .01; linear, p < .001) and T-AOC (ANOVA and linear, p < .001) in the serum increased. Immunoglobulin concentrations and antibody levels of laying hens were reported in
Table 5. Effect of increasing inclusion of dietary BSEO on serum antioxidant capacity of laying hens*.

| Item** | CON BSEO-1 BSEO-2 BSEO-3 Pooled SEM | ANOVA Linear Quadratic |
|----------------|-------------------------------------|------------------------|
| GSH-Px (U/mL) | 2271b 2580ab 2756a 2795a 58 | .002 <.001 .168 |
| T-SOD (U/mL)  | 228 235 238 234 1 | .220 .167 .115 |
| T-AOC (U/mL)  | 5.17c 6.26bc 7.67ab 7.76a 0.26 | <.001 <.001 .184 |
| MDA (nmol/mL) | 6.29 5.61 5.50 6.05 0.20 | .451 .639 .126 |

Table 6. Effects of increasing inclusion of dietary BSEO on serum immune indexes of laying hens*.

| Item** | CON BSEO-1 BSEO-2 BSEO-3 Pooled SEM | ANOVA Linear Quadratic |
|----------------|-------------------------------------|------------------------|
| IgA (g/L)     | 2.24 2.20 2.17 2.21 0.02 | .332 .318 .144 |
| IgG (g/L)     | 4.21 4.11 4.04 4.11 0.03 | .157 .133 .093 |
| IgM (g/L)     | 1.64 1.60 1.58 1.61 0.01 | .237 .284 .084 |
| NDV-Ab        | 1.14 1.21 1.28 1.10 0.05 | .680 .896 .240 |
| AIV-Ab        | 0.960b 1.24a 1.60c 1.22a 0.03 | .003 .004 .042 |

Table 7. Effect of increasing inclusion of dietary BSEO on serum hormone index of laying hens*.

| Item** | CON BSEO-1 BSEO-2 BSEO-3 Pooled SEM | ANOVA Linear Quadratic |
|----------------|-------------------------------------|------------------------|
| E2 (pg/mL)   | 16.0c 17.9b 16.0c 19.5a 0.3 | <.001 <.001 .059 |
| PTH (pg/mL)  | 144c 158b 163c 152ab 2 | <.001 .003 <.001 |
| P (ng/mL)    | 1.80 1.60 1.64 1.77 0.07 | .711 .970 .261 |
| LH/FSH       | 1.13bc 1.22ab 1.18ab 1.37a 0.03 | .047 .016 .393 |

*Data are means of 2 hens of 6 replicates per treatment. **GSH-Px, glutathione peroxidase; T-SOD, total superoxide dismutase; T-AOC, total antioxidant capacity; MDA, malondialdehyde. ***CON, BSEO-1, BSEO-2, BSEO-3 diets contained 0, 300, 600, 900 mg/kg of BSEO, respectively. *Means within a row with different superscripts differ significantly (p <.05).

Table 6. The concentrations of IgA, IgG, IgM, and the level of NDV-Ab in the serum were not affected by dietary treatment. The level of AIV-Ab (ANOVA and linear, p < .01; quadratic, p < .05) in the serum increased with an increased BSEO supplementation.

**Serum hormone index**

As shown in Table 7, compared with the control group, the level of E2 in BSEO-1 and BSEO-3 significantly increased (ANOVA and linear, p < .001), the value of LH/FSH in BSEO-3 significantly increased (ANOVA and linear, p < .05). The level of PTH in the serum increased (ANOVA and quadratic, p < .001; linear, p < .01) with an increased BSEO supplementation.

**Discussion**

In this study, we firstly evaluated the effects of increasing inclusion levels of the BSEO on production, hatching performance, egg quality, serum antioxidant capacity, immune response, and hormone levels of laying hens. Egg production was significantly elevated and feed conversion ratio lowered in a linear manner. As mentioned earlier, BS or EO supplementation of laying hen diet significantly decreased the feed conversion ratio and increased egg production rate (Bozkurt et al. 2012; Wang et al. 2018; Chen et al. 2019). This improvement could be related to various enzymes such as protease, amylase, and cellulase secreted by gastrointestinal tract, which were produced by BS or EO stimulation (Attia et al. 2015, 2017, 2019; Li et al. 2018; Teixeira et al. 2019; Krauze et al. 2020). Additionally, it has been shown that BS or EO supplementation can improve gut morphology, increase the population of beneficial gut microflora, thereby increasing the efficiency of nutrient utilisation (Mousavi et al. 2018). However, Abdel-Wareth (2016) reported that the effects of thymol and synbiotic on the performance of Hy-Line Brown hens and found that there was not a positive effect. Other researchers found that dietary BS or EO had minimal or no effects.
on the production performance of laying hens (Forte et al. 2016). Liu et al. (2019) and Bozkurt et al. (2009) found that dietary supplementation with BS or EO did not significantly influence on feed conversion ratio and egg production. The inconsistent results may be attributed to the different supplementation dosages, sources, diet composition, age, and species. However, more studies are needed to determine the effects of BSEO on growth performance in laying hens to verify the growth-stimulating effects.

Regarding egg quality, the Haugh unit and yolk index are important indicators to evaluate the freshness of an egg. The present study showed that the supplementation of BSEO increased yolk index linearly, whereas did not affect the Haugh unit. The Haugh unit was calculated using the height of the inner thick albumen and the weight of an egg. Ovomucin is responsible for the thick gel characteristics of liquid egg whites (Omana et al. 2010). The Haugh unit was influenced by the ovomucin content of the egg. Therefore, we assumed that BSEO have no significant effect on ovomucin content, and may be beneficial in extending the shelf life of the eggs via improving yolk index, this finding was similar to our previous study with result that dietary BS linearly increased yolk index (Liu et al. 2019). Unexpectedly, the supplementation of BSEO decreased eggshell thickness in a linear manner, which was contrary to the result of most researches (Abdel-Wareth 2016; Liu et al. 2019). And, the value of eggshell strength decreased with an increased BSEO supplementation. This decrease may be due to formation Ca-soaps which decreased Ca absorption (Attia et al. 2020). More notably, the level of PTH in the serum markedly elevated with the increase of BSEO concentrations in the diet. The main function of PTH is to regulate the metabolism of calcium and phosphorus vertebrates and to increase the level of blood calcium and decrease the level of blood phosphorus. The PTH is secreted when blood calcium level is low. It was speculated that the decrease in eggshell thickness may be because of the low level of blood calcium. Therefore, BSEO could improve the freshness of the egg to a certain extent, but it reduced the eggshell thickness and is not conducive to long-distance transportation.

It is noteworthy that hatchability and the hatchability of fertile eggs were linearly and quadratically increased in laying hens aged 24–36 weeks. To our knowledge, this is the first report of improvement in hatchability as a consequence of diet supplementation with a blend of BS and EO in poultry. In accordance with this background, previous studies reported that dietary supplementation with BS or EO significantly improved the hatchability of laying hens (Bozkurt et al. 2009; Mazanko et al. 2018). Supplementation of 0.25% thyme increased the hatchability of fertile eggs in native laying hens (Ali et al. 2007). Another study also found that thymol or isoeugenol supplemented groups improved the hatchability of quail adults by nearly 18.8% and 11.8%, respectively (Luna et al. 2012). Additionally, the level of E2 and LH/FSH in the serum significantly increased in our research. Wang et al. (2017) reported that dietary supplementation with BS significantly increased gonadotropin-releasing hormone levels in laying hens. Kim et al. (2018) found that dietary supplementation with BS significantly elevated levels of P and E2 in Korean Native Heifers. Therefore, the increase in reproductive hormones may contribute to improving hatching performance.

In addition, the antioxidant defense system is closely related to hatching performance (Zhu et al. 2015; Khaligh et al. 2018). Embryonic development of poultry in a closed system using nutrients that are incorporated into the egg before laying. An essential feature of avian embryonic metabolism is the use of yolk-derived fatty acids, and the energy for development is totally dependent on the β-oxidation pathway of fatty acids (Speake et al. 1998). Reactive oxygen species (ROS), constantly produced in aerobic metabolism, are normally removed by antioxidant enzymes. Surai (1999) reported that GPX activity in the liver of chick embryo increased throughout the time of development from day 10 of incubation to hatching. Wilaison and Mori (2009) found that selenium is important for embryonic development through antioxidant defense system by cellular glutathione peroxidase. In the present study, BSEO exerted antioxidant activities by improving the activities of GSH-Px and T-AOC on serum. The results of the present study were in agreement with previous findings showed that dietary supplementation with BS or EO significantly increased activity of GSH-Px and T-AOC and gene expression (Bai et al. 2017; Yu et al. 2018; Yang et al. 2019). Similarly, the addition of BS or EO had a positive response on antioxidant activity in serum of duck, goose, quail (Chen et al. 2013; Attia et al. 2017, 2019; Abdel-Moneim et al. 2020).

Our data show that BSEO supplementation significantly increased the serum AVI antibody titre, which indicates that the birds fed BSEO had a stronger immune response to AVI than did birds of the control group. Consistent with our result, Zhang et al. (2012) and Bozkurt et al. (2012) also demonstrated that dietary supplementation with BS or EO resulted in the
improvement of AIV-Ab levels. The bioactive compounds of essential oils might have been responsible for the elevated antibody titres against the experimental antigens. As reported previously, improved antibody titre might be due to the antioxidant properties of aromatic herbs and their effects on enhancing the proportions of systemic lymphocyte as an antioxidant producer (Attia and Al-Harthi 2015; Attia et al. 2017, 2019). However, there were no differences in immunoglobulin concentration in serum among all groups. As studies on the effect of BSEO on the immune response of laying hens are very little, further study is still needed to investigate its immune capacity.

Conclusions

In conclusion, the results demonstrate that supplementation of 900 mg/kg BSEO in laying hens have a positive effect on laying and hatching performance, which may be the result of enhancing antioxidant capacity and regulating reproductive hormones of Xuefeng black-bone chicken. Thus, the finding of this study can provide reference information on feed additives application of BSEO in the laying hens production.

Ethical approval statement

All animal research projects were sanctioned by the Hunan Agricultural University New Rural Development Research Institute Characteristics Industrial Base Project—Xuefeng Black-bone Chicken Special Industrial Base Construction Project (J16101). All the birds and experimental protocols in this study were approved by the Institutional Animal Care and Use Committee of Hunan Agricultural University, Hunan, China.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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