Effect of Bee Pollen on Reproductive Performance of Broiler Breeders

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Abstract **[Objective]** The paper was to verify whether chestnut bee pollen can be used as feed for breeding chickens and its appropriate addition proportion. **[Method]** A total of 924 44-week-old Ross 308 broiler breeders of parent generation with a male to female ratio of 1:10 were randomly divided into 4 groups, with 3 replicates in each group and 7 cocks and 70 hens in each replicate. The broiler breeders in the control group were fed with the basal diet, and those in experimental groups were fed with the diets supplemented with 0.5%, 1.0% and 1.5% chestnut bee pollen. The feeding trial lasted 35 d. **[Result]** Addition of chestnut bee pollen at different proportions improved the laying rate of breeder hens in different degrees. The experimental group supplemented with 1.5% chestnut bee pollen increased the laying rate by 9.1%, reduced the rate of unqualified eggs (broken eggs, cracked eggs, thin shelled eggs and hard shelled eggs) by 71.4%, increased the fertilization rate of hatching eggs and healthy chick rate by 4.4% and 5.2%, and improved the concentrations of testosterone (T), follicle stimulating hormone (FSH) and luteinizing hormone (LH) in breeder cocks by 60.8%, 32.3% and 61.4%, respectively. Statistical analysis showed that the results were significantly different. **[Conclusion]** Addition of chestnut bee pollen at certain concentration can improve the reproductive performance of breeder cocks and breeder hens, and improve the body immunity.

Keywords Bee pollen; Broiler breeder; Reproduction; Laying rate; Healthy chick rate; Reproductive hormone

Broiler breeder is a kind of grain saving poultry and has broad development prospect in China. However, there are some problems in breeding chickens industry such as low egg production and low fertilization rate of hatching eggs. Improving the reproductive performance of breeding chickens becomes a problem to be solved in the development of chicken breeding industry in China.

Chestnut bee pollen is the pollen mass brought back by bees when they collect chestnut nectar. Except for a small amount of nectar and bee saliva, it is mainly composed of chestnut pollen. Chestnut bee pollen is rich in nutrition, including protein, sugar, fat, a variety of trace elements, flavonoids, organic acids, enzymes and coenzymes, plant auxin, plant sex hormone estradiol, *etc.*[1]. Estradiol in chestnut bee pollen is the most abundant among all pollens, and the content of estradiol determined by HPLC is 144.4 pg/g[2]. Estradiol can promote the function of sex hormones. In recent years, it has been used clinically to treat male and female infertility and female climacteric syndrome, or as a feed additive to improve animal reproductive performance, and has achieved certain effects[3]. In-depth study on the application of chestnut bee pollen will have a positive effect on improving the reproductive performance of poultry.

A comparative experiment was conducted by adding bee pollen to Ross 308 parent broiler breeders, and the effects of bee pollen on reproductive performance and blood reproductive hormones of broiler breeders were studied, so as to provide an effective way to rationally utilize bee pollen resources and to improve reproductive performance of breeding chickens.

1 Materials and Methods

1.1 Chestnut pollen source The chestnut bee pollen was purchased from Teng Yongguang, a beekeeper in Houliangjia Village, Jiudian Town, Pingdu City, Shandong Province. Chestnut bee pollen is the pollinium collected from current-year chestnut flowers by Italian bees raised by beekeepers and processed by pollen trap. It is light yellow in color, dry, without agglomeration, mildew or worm damage. The main component is chestnut pollen, as well as a very small amount of bee saliva and nectar. The pollen source plant is the local chestnut forest around Daze Mountain.

1.2 Grouping of breeding chickens The experiment was carried out in Yikangbao Breeder Farm in Dongge Sub-district Office, Pingdu City, Shandong Province. A total of 924 44-week-old Ross 308 parent broiler breeders (male to female ratio 1:10) were randomly selected according to body weight and divided into 4 groups. Each group was registered according to the anklet worn during epidemic prevention. There were 3 replicates in control group, test group I, test group II and test group III, and each replicate had 70 hens and 7 cocks. The chickens in the control group were fed with the basal diet, and

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those in experimental groups were fed with the diets supplemented with 0.5%, 1.0% and 1.5% chestnut bee pollen. The pre-trial lasted 7 d and the formal trial lasted 35 d. During the experiment, all the chickens were allowed *ad libitum* to feed and water.

1.3 Diet The chickens in the control group were fed with the basal diet according to the nutritional needs, while those in experimental groups were fed with artificially mixed complete feed (supplemented with 0.5%, 1.0% and 1.5% chestnut bee pollen). The feed dosage should be estimated according to the feed intake of the last week, and the feed dosage should be reduced by 1 g per day per week compared with that of the last week to avoid obesity of breeding chickens. Bee pollen was not pulverized by micro-particle pulverizer, and chestnut bee pollen was directly mixed with basal diet to make pellet feed. The basic nutritional compositions of dried chestnut bee pollen were measured by Qingdao Feed and Veterinary Drug Testing Station, and the contents of nutritional compositions are shown in Tab. 1. The basal diet composition of the control group is shown in Tab.2.

1.4 Feeding and management All the chickens were raised in completely enclosed chicken house with 12 columns. Each group had 3 replicates, and each replicate had 77 chickens in a column, including 7 cocks and 70 hens. All the chickens were exposed to 16 h light per day, and the light intensity was 60 Lux. Two high and one low ground feeding was adopted, and the floor was laid with rice husk as bedding material. The house was equipped with trough and egg house. Full-time keepers were responsible for feeding and daily management, and the troughs were washed once a week. All the chickens were immunized according to the routine procedure.

Tab. 1 Nutrient composition of chestnut pollen %

Crude protein	Total amino acid	Saccharide	Lipid	Nucleic acid	Estradiol	Total flavone	Growth hormone	Vitamin A	Vitamin C
21.01	17.64	31.70	6.14	2.00	9.72×10^{-2}	0.090 8	0.78×10^{-6}	0.001 692	0.001 5

Tab.2 Formula and nutrient composition of basal diet

Feed Ingredients	Proportion//%
Corn	53.50
Flour	15.00
Bran	3.00
Soybean meal (43%)	16.00
Soybean oil	2.50
Limestone (granular)	5.00
Limestone	2.50
Monocalcium phosphate	0.90
Salt	0.20
Trace elements	0.20
Vitamin	0.20
Sodium bicarbonate	0.20
Choline chloride	0.20
Microbe	0.25
DL-methionine	0.15
L-threonine	0.05
Additive	0.15
Metabolic energy//MJ/kg	11.74
Crude protein//%	13.00
Crude fiber//%	6.00
Calcium//%	3.45
Available phosphorus//%	0.05
Lysine	0.65

Note: The 0.15% additives include 0.03% *Bacillus subtilis*, 0.03% phytase, 0.03% safmannan, 0.03% enzyme preparation, 0.03% Lysoforte soybean phospholipid.

1.5 Determination of reproductive performance of breeding hens Eggs were picked 4 times a day, and each egg was numbered, weighed, and recorded 4 times, with 52–68 g as qualified eggs. The eggs should be disinfected for 20 min on the same day after being picked up and fumigated with 3 times the amount of potassium permanganate and formalin, that is, 21 g of potassium permanganate and 42 mL of formalin should be used for per m^3 of disinfection space. The temperature of the fumigating room should be kept above 24℃ and the humidity should be kept at 65%. Afterwards, the eggs were placed undisturbed in the egg bank for 3 d at temperature 15–21 ℃ and humidity 75%–80% before incubation. Each incubator was regarded as a replicate, with the blunt end facing upwards to avoid adhesion during incubation. The fertilization rate and healthy chick rate were recorded.

1.6 Determination of serum samples from breeding cocks Five cocks were selected from each replicate, with a total of 70 cocks. Before the first morning feeding after the end of the experiment, 3 mL of blood was collected from the wing vein of each male, and 2 g/L heparin sodium was added for anticoagulation. After standing, the serum was obtained by centrifuging at 3 000 r/min for 10 min. The testosterone (T), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the serum were measured by radioimmunoassay (H^3-T, I^{125}-LH and I^{125}-FSH radioimmunoassay). All operations followed the instructions. The kit was produced by Beijing Furui Bioengineering Co., Ltd.

1.7 Data processing The database was established by Excel. The mean and standard deviation of experimental data were statistically analyzed by SPSS software, and the differences among the groups were compared by *t*-test.

2 Results and Analysis

2.1 Health condition The body condition of chickens in the control group was normal. The breeding chickens in the experimental groups showed strong appetite, fast feeding, bright yellow chicken feet, white and smooth feathers, normal feces, less disease and strong disease resistance.

Tab. 3 Effects of chestnut bee pollen on laying performance and hatching indexes of hens

Group	Number of eggs	Total weight of eggs// g	Weight of individual egg// g	Laying rate// %	Rate of unqualified eggs// %	Fertilization rate of hatching eggs// %	Healthy chick rate// %
Control group	2 038	109 441	53.7±5.4	83.2±4.8	6.3±2.4	95.0±8.5	94.2±5.1
Experimental group I	2 154 a	118 901 a	55.2±4.6	87.9±5.1 a	3.9±1.8 a	97.6±3.1 a	97.4±4.6 a
Experimental group II	2 190 a	121 983 a	55.7±3.9	89.4±4.6 a	2.1±1.4 a	98.7±2.3 a	98.5±2.2 a
Experimental group III	2 225 ab	124 822 ab	56.1±3.7	90.8±3.9 ab	1.8±1.0 ab	99.2±1.0 ab	99.1±1.3 ab

Note: Different lowercase letters in the same column represent significant difference at 0.05 level.

Tab. 4 Effects of chestnut bee pollen on blood reproductive hormones of cocks

Group	T// ng/mL	FSH// mIU/mL	LH // mIU/mL
Control group	3.24±0.22	1.36±0.18	2.36±0.19
Experimental group I	3.50±0.16	1.42±0.19	2.46±0.22
Experimental group II	4.62±0.28 a	1.54±0.16 a	3.20±0.16 a
Experimental group III	5.21±0.24 ab	1.80±0.15 a	3.81±0.17 ab

Note: Different lowercase letters in the same column represent significant difference at 0.05 level.

2.2 Effects of chestnut bee pollen on laying performance and hatching indexes of hens The results showed that the body condition could be improved by adding chestnut bee pollen to the diet. As shown in Tab.3, adding 1.5% chestnut bee pollen received the most significant effect, and the laying rate of hens was increased by 9.1%. There was no significant difference between experimental groups I and II. The total weight of eggs was increased by 14.1%, and the weight of eggs in experimental group III was significantly higher than those in other groups. Chestnut bee pollen had no significant effect on the weight of individual egg. In experimental group III, the rate of unqualified eggs was reduced by 60.0%, the fertilization rate of hatching eggs was increased by 4.4%, and the healthy chick rate was increased by 5.2%.

2.3 Effects of chestnut bee pollen on blood biochemical indexes of cocks As shown in Tab.4, addition of chestnut bee pollen in the diet significantly improved the reproductive performance of cocks, and the improvement effect was the most significant in experimental group III, including 60.8% increase in testosterone (T), 32.4% increase in follicle-stimulating hormone (FSH), and 61.4% increase in luteinizing hormone (LH). However, the concentration of FSH in blood in experimental group I was increased by 8.0%, and the difference was not significant, indicating that the concentration of reproductive hormone could be improved only when the content of chestnut bee pollen reached a certain level.

3 Discussion

(1) Chestnut bee pollen is safe for chickens[4-5]. After adding chestnut bee pollen into chicken feed, the feeding observation showed that the chickens grew well, and there were no abnormalities in appetite, excretion and disease, indicating that chestnut pollen was safe and reliable for chickens. At the same time, the experimental chickens fed with bee pollen were robust, responsive and had fewer diseases, which was related to the fact that pollen polysaccharide can activate the phagocytic activity of macrophages and improve the disease resistance of chickens[6]. Bee pollen can promote the early growth of bursa of fabricius and thymus, reduce the early degeneration of cloaca and bursa of fabricius, stimulate the immune response of spleen, delay the degeneration of cloacal bursa, and enhance the function of immune system, probably because vitamins and flavonoids in bee pollen stimulate the development of immune system jointly[7].

(2) At present, the research on the active components of bee pollen is still in its initial stage. Our research results suggested that bee pollen improved the egg laying performance and egg hatching performance of hens. Wang *et al.*[8] put forward that bee pollen stimulated the activity of thyroid gland, promoted the development and maturation of follicles, inhibited the occurrence of atretic follicles, and promoted ovulation, which may be related to the regulation of hypothalamic-pituitary-gonadal axis by hormones in bee pollen. Moreover, when the addition of chestnut pollen reached a certain proportion, the reproductive hormones in the blood, including the testosterone of cocks, were of varying degrees of improvement, probably attributed to certain amount of plant reproductive hormones in chestnut pollen, or to nucleic acid or enzymes and other biological active substances in pollen, or to mutual synergy results of other active substances and reproductive hormones. Chestnut bee pollen is rich in nutrients, which contains not only high content of protein, but also more free amino acids such as arginine, methionine and a variety of active enzymes, and can supplement and balance other essential nutrients in the feed, such as trace elements Zn, Mn and lipid soluble VA, VD, VE, *etc*. Chestnut bee pollen also contains more nucleic acids related to cell activation, of which DNA accounts for about 0.5% and RNA accounts

for about 1.0%, higher than the proportion of fish and shrimp that are used to improve appetite. It is worth noting that chestnut bee pollen contains sex hormones and gonadotropins, although the content is small, the purified substance can be used to treat infertility. The Second People's Hospital of Lianyungang, Jiangsu Province, treated 156 cases of male semen abnormal infertility with pollen, and the total effective rate was 84% for oligospermia, 93% for asthenozoospermia and 89% for mixed syndrome of oligospermia and asthenozoospermia[9], indicating that bee pollen had a significant effect on improving reproductive performance. Addition of bee pollen to the diet of breeding cocks can effectively increase the semen volume and sperm density, improve sperm motility, reduce sperm malformation rate, and improve the breeding performance of breeding cocks. However, the mechanism of bee pollen improving reproductive performance of cocks has been rarely studied. It is preliminary concluded that the hormones and carotene in bee pollen may promote the gonad development of cocks and increase the secretion of testosterone, thus improving the reproductive performance of the body. The specific hormones involved still need to be further studied.

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