Research Article

Günnur Peşmen*

Effects of *in ovo* injection of black cumin (*Nigella sativa*) extract on hatching performance of broiler eggs

https://doi.org/10.1515/chem-2022-0256
received October 22, 2022; accepted November 14, 2022

Abstract: The objective of this research is to state the impact of black cumin (*Nigella sativa*) extract *in ovo* injected at different doses on the hatchability of Cobb 500 Broiler fertile eggs. Injected doses of black cumin were 3 and 6 mg, applied to the air sac of the eggs on the 17.5th day of incubation. It has been established that, black cumin extract given to fertile broiler eggs had a positive effect on chick weight and chick length, but did not have a significant impact on hatching power and chick quality.

Keywords: black cumin extract, *in ovo* injection, hatching power, chick length, chick quality

1 Introduction

The main purpose of poultry farming is to supply reliable, easily accessible and healthy food to the growing population. The provision of certain nutrients (protein, vitamins, minerals, hormones, etc.), vaccines, immunostimulants, phytochemicals *in ovo* has started to attract great attention worldwide in recent years to protect the productivity, resistance to diseases and good health of poultry. In addition to these advantages, *in ovo* feeding method is an alternative method to improve hatchability, nutritional status, chick quality, reduce the problems associated with infection and oxidative stress (decreased antioxidant level), and minimize the use of synthetic antibiotic growth promoters [1]. *In ovo* technology is based on the direct application of various nutrients and biological substances to poultry fertile eggs at any stage of the incubation period. Various studies have shown that *in ovo* nutrition application is more effective than post-hatching application in poultry production [2]. In addition, it becomes more important to give nutrients in the embryonic period in order to prevent the possible negative effects of errors and deficiencies in the feeding of breeders on hatchability and chick quality [3]. Chicks coming out of the hatching may not be able to consume feed and water for 2–3 days due to processes such as vaccination, sex separation and transfer to rearing houses. Since the chicks use only the nutrients in the yolk during this period, it causes delayed weight gain, delayed intestinal development, decreased pectoral muscle weight, and may reduce the profit of the breeder. In order to prevent this negative effect, nutrients (amino acids, carbohydrates, minerals, vitamins, etc.) can be given to the chicks by *in ovo* injection, which is an embryo feeding technique [3,4]. In addition, *in ovo* application has a long-term efficacy on the growth period after hatching and ensures efficient penetration of the injected material into the embryonic tissue [2].

Adequate concentrations of amino acids, vitamins and minerals are required, as the perinatal period is a crucial period when new muscle fibers are synthesized and matured and chicks grow and develop [2,5]. Early chick feeding is important and studies have shown that late access to chick feed results in reduced growth rate, reduced feed conversion ratios (FCR), increased long-term marketing body weight (BW) and mortality [2,6,7]. Improving the antioxidant status of developing embryos has positive effects, especially at the late stage of incubation. Therefore, the provision of antioxidant components to the embryo via *in ovo* can provide tremendous benefits to the embryo during the pre-hatch and post-hatch growth stages [8].

1.1 Black cumin – plant description

Plants are also used as natural food, forage and medicinal substance, and as well as for their role in the

* **Corresponding author: Günnur Peşmen,** Department of Laborant Veterinary Health, Şuhut Vocational School, Afyon Kocatepe University, Afyonkarahisar, Turkey, e-mail: gpesmen@aku.edu.tr

protection of the ecosystem [9]. Plant secondary metabolites and essential oils are biologically active compounds that can be used as feed additives in poultry production. They provide feed efficiency and reduce pathogenic load by increasing the production of digestive secretions and nutrient absorption [10].

In recent years, its use as growth promoters has attracted attention as a result of antioxidant compounds and various benefits derived from spices, herbs and their products [11]. *Nigella sativa* is an aromatic plant which is finely divided linear, and its seeds have been used worldwide for over 2,000 years for their pharmacological effects and medicinal properties. Its flowers are pale blue and white color with 5–10 leaves, and its fruits are in the form of capsules. Capsules separated into oval-shaped follicles contain many black cumin seeds with a diameter of approximately 1 mm [12]. These seeds contain pharmacologically active ingredients such as dithymoquinone, carvacrol, thymohydroquinone, thymol and thymoquinone [13]. Thymoquinone, an important phytochemical, has broad-spectrum medicinal properties such as antihistamine, antibacterial, anti-inflammatory and antioxidant [14–16]. Ahmad et al. [12] reported that the positive effects can be obtained from various forms of *N. sativa*, including seeds, powder and oil, and that more research is needed on the benefits of different forms. On the other hand, Bednarczyk et al. [17] reported that using *in ovo* prebiotics instead of antibiotic supplementation in water is a more beneficial and effective application. Although there are various studies in the literature on the inclusion of black cumin in the rations of chickens, the information on the effects of *in ovo* application is insufficient.

Studies induced that black cumin (6 mg) extract improved the antioxidant status and post-emergence performance of thermally challenged chickens [16]. Sulaiman and Tayeb [18] reported that *in ovo* injection of rosemary, olive, almond and black cumin oils can be used as an immunomodulatory agent to increase the resistance of birds against many diseases. It has also been reported that improvement in feed intake and feed efficiency is possible with *in ovo* application of rosemary oil and black cumin oil. Hussein et al. [19] reported that especially low virulence infectious bursal disease vaccines can be combined with black cumin oil and can be used safely in embryo vaccination. Khan et al. [20] revealed that black cumin extract has a strong immunotherapeutic effect against Newcastle Disease Virus infection in the histopathology of embryonated eggs.

Lika et al. [21] in a study they conducted on broiler chickens, concluded that adding 1, 2 and 3% black cumin to the ration improved the eggshell quality, Haugh unit and FCR ($P < 0.05$). In the same study, it was reported that herbal additives can expand the BW of chickens and egg producing of laying hens by about 7–10%, and increase the meat and egg quality by more than 25%. In another study, it was reported that the performance and carcass yields of broilers were increased by adding 1% black cumin, and black cumin could be used as a natural growth promoter in poultry ration [22].

Bizanov et al. [23] performed a work to evaluate the immunostimulatory activity of ten different herbal extracts from *Vitex agnus-castus*, *Vinca major*, *Aloe arborescens* and the polyherbal product. From this work it was found that the rabbits immunized with BSA in combination with either platinum nanoparticles or aluminum hydroxide had higher titers of BSA-specific IgA antibodies in their saliva at Day 56 of observation.

The main goal of this work is to state the impact of black cumin (*N. sativa*) extract *in ovo* injected at different doses on the hatchability of Cobb 500 Broiler fertile eggs.

2 Materials and methods

Fertile eggs from Cobb 500 line of broiler used in the study were obtained from a commercial farm. Eggs weighing 61.00–62.00 g were used in the study. The eggs were incubated in the hatchery at 56% relative humidity and 37.5°C. Incubation conditions (temperature, humidity, rotation and ventilation) are set automatically. On the tenth day, the eggs were checked for fertility and the eggs without embryo development were taken out from the trays. After that 400 fertile eggs were divided into four groups: control group (K), negative control group (NK; 0.5 mL deionized water), black cumin extract (BC3; including 3 mg black cumin extract in deionized water: 0.5 mL/egg) and black cumin extract (BC6; including 6 mg black cumin in deionized water: 0.5 mL/egg). Experimental groups were arranged as in Table 1.

On the 17.5th day of incubation, the injection site of the eggs removed from the machine was cleaned with 70% ethyl alcohol. The prepared black cumin extract solutions were injected into the air sac of the eggs with the help of a syringe [2]. After the injection process, it was closed with paraffin tape. The control group was kept outside the incubator for the same time without any treatment. *In ovo* injection application is shown in Figure 1.

Hatching power was calculated according to the formula below:.

Hatching power (%): count of live chicks/number of fertile eggs × 100 [24].

Table 1: Experimental groups

Groups	Applications	*n*
Control (K)	No solution was applied.	100
Negative control (NK)	0.5 mL saline/egg	100
Black cumin extract (BC3)	Including 3 mg black cumin extract in deionized water: 0.5 mL/egg	100
Black cumin extract (BC6)	Including 6 mg black cumin extract in deionized water: 0.5 mL/egg	100

Figure 1: *In ovo* injection application.

Chick was weighed with a digital scale with 0.01 g precision. The length of the chick was measured from the tip of the beak to tip of the finger with a ruler (Figure 2) [24,25].

Chick quality (%): Chick quality was evaluated and scored according to the chick quality scale of Tona et al. [26]. General activity, hair condition, appearance, remaining egg yolk, eyes, legs, umbilical region and remaining membrane parameters were evaluated in scoring. The quality assessment of the chicks was made according to Table 2 [26,27].

The data acquired in the study were analyzed using the SPSS 13.0 for Windows package program [28]. Chick weight, chick length and chick quality were analyzed using one-way analysis of variance to compare the groups. The significance level was taken as 0.05. Duncan test was used for pairwise comparison of the groups.

Figure 2: Measurement of chick length.

3 Results and discussions

The results of the analysis of variance for the comparison of the experimental groups in terms of chick weight are presented in Table 3. According to the results of analysis of variance, chick weights differed significantly between the groups ($P < 0.01$). When the mean values were investigated, it was determined that there was no significant difference between the control group and the other groups ($P > 0.05$), and the chick weights of the BC3 and BC6 groups were higher than the negative control group. Chick weights in K, NK, BC3 and BC6 groups, respectively, were 43.80, 43.41, 44.01 and 44.17 g. The highest chick weight was determined in the BC6 group and the lowest in the NK group. The effect of black cumin extract given *in ovo* on chick weight is given in Table 3.

The comparison of chick lengths by groups is given in Table 4. It was determined that there was a significant difference between the experiment groups in terms of chick length ($P < 0.01$). It was determined that the chick lengths of the BC3 and BC4 groups were higher than those of the K and NK groups. Chick length in K, NK, BC3 and BC6 groups, respectively, was found to be 17.90, 17.98, 18.41 and 18.22 cm. The highest chick length was determined in the BC3 group and the lowest in the K group.

Analysis of variance results for comparison of groups in terms of chick quality are presented in Table 5. It was determined that the chick quality did not show a significant difference in the experimental groups ($P > 0.05$). Chick quality in K, NK, BC3 and BC6 groups, respectively, was found to be 90.58, 91.00, 91.31 and 89.82. The highest chick quality was determined in the BC3 group and the lowest in the BC6 group.

Table 2: Parameter for determining chick quality in the Tona score method

Parameter	Characteristics	Scores
Activity	Good–weak	6–0
Down and appearance	Clean and dry–wet–dirty and wet	10–8–0
Retracted yolk	Body with normal swallowed yolk–body with swallowed large yolk and rather hard to touch	12–0
Eyes	Opened and bright–opened and not bright–closed eyes	16–8–0
Legs	Normal legs and toes–one infected leg–two infected legs	16–8–0
Navel area	Completely closed and clean–not completely closed and not discolored–not closed and discolored	12–6–0
Remaining membrane	No membrane–small membrane–large membrane–very large membrane	12–8–4–0
Remaining yolk	No yolk–small yolk–large yolk–very large yolk	16–12–8–0

Table 3: Comparison of groups in terms of chick weight ($P < 0.05$)

Groups	*n*	Min.	Max.	Mean	SD	*P*
K	96	38.10	47.50	43.80^{ab}	1.39	0.006^{*}
NK	94	40.30	47.40	43.41^{b}	1.31	
BC3	99	40.60	49.12	44.01^{a}	1.44	
BC6	94	34.50	47.96	44.17^{a}	1.99	

$^{*}P < 0.01$; ^{a,b}there is a significant difference between the groups containing different letters ($P < 0.05$).

Table 4: Comparison of groups in terms of chick length

Groups	*n*	Min.	Max.	Mean	SD	*P*
K	96	17.00	19.00	17.90^{b}	0.57	0.001^{*}
NK	94	16.50	19.00	17.98^{b}	0.64	
BC3	99	16.00	19.50	18.41^{a}	0.75	
BC6	94	16.50	19.80	18.22^{a}	0.72	

$^{*}P < 0.01$; ^{a,b}there is a significant difference between the groups containing different letters ($P < 0.05$).

The results obtained in the study are compatible with Hussein et al. [19] study, which reported that the use of black seed oil in eggs did not have a negative effect on hatchability. The same results were found in Khan et al. [28], which is consistent with the study that black cumin extract reduces embryo mortality [29]. Arslan et al. [15] reported that the improvement in hatchability was associated with the antioxidant properties of black cumin. Kumar et al. [30] stated that black cumin seeds can be used in the diet to improve the growth performance, nutrient utilization and immunity in broilers, and increase the antioxidant levels in blood and tissues.

This study showed that *in ovo* administration of 3 mg of black cumin extract make the incubation process better. These results are consistent with the study of Oke et al. [16] that reported that *in ovo* administration of black cumin extract had a positive effect on chick weight and chick length.

Usually, chick weight is used as an indicator of chick quality. Although daily chick weight is highly correlated with egg weight, it is not a good indicator of chick development [31]. Chick length is another method for assessing chick quality. The measurement of chick length is fast, reproducible and does not harm the chick [32]. When examined in studies, it has been suggested to use chick length to reveal the chick quality and predict the bird performance. In this study, the highest chick size and chick quality were found in the BC3 group consistent with the literature. Chick quality and prediction of the bird performance could be revealed by the chick length.

Sulaiman and Tayeb [18] stated that *in ovo* administration of black cumin oil had no effect on hatchability and chick weight. Tollba and Hassan [33] stated that the use of black cumin reduced the negative effects of thermal stress and Durrani et al. [34] reported that the use of black cumin in broiler rations has a significant effect on live weight gain and weight of different body organs.

In this study, the use of black cumin extract in fertilized eggs had a positive effect on hatching power, chick weight and chick length. In other words, *in ovo* use of

Table 5: Comparison of groups in terms of chick quality

Groups	*n*	Min.	Max.	Mean	SD	*P*
K	96	40.00	100.00	90.58	8.67	0.778
NK	94	76.00	100.00	91.00	5.80	
BC3	99	0.00	100.00	91.31	11.91	
BC6	94	18.00	100.00	89.82	13.38	

Hatching power ratios in K, NK, BC3 and BC6 groups, respectively, were found to be 96.00, 94.00, 99.00 and 94.00%. The lowest hatching power was found in the NK and BC6 group, and the highest in the BC3 group.

black cumin extract is a possible way to improve hatching power, chick weight and chick length.

4 Conclusion

In this study, there was no significant difference between the experimental groups in terms of hatchability and chick quality, but the best results were obtained from the group given 3 mg black cumin extract. In addition, the study clearly demonstrated the positive effect of in-egg feeding with 3 mg of black cumin extract on chick weight and chick length. Studies on the effect of BC extract on hatchability in poultry nutrition are very limited. More studies are needed to obtain clear information about *in ovo* injection site, time and injection dose.

Acknowledgments: The author would like to thank Gedik Piliç A.Ş. for providing the eggs and for the unwavering help during the study.

Funding information There is no funding for this article.

Author contributions: All works done by author herself.

Conflict of interest: The author declares no conflict of interest.

Institutional review board statement: Not applicable.

Informed consent statement: Not applicable.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: Derived data supporting the findings of this study are available from the corresponding author Günnur Peşmen on request.

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