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Influence of short periods of incubation during egg storage on immune parameter of broiler chicken

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Abstract

This experiment investigated the impact of applying short periods of incubation during egg storage (SPIDES), with or without turning, on the immune responses of broiler hatching eggs subjected to extended storage. A total of 750 eggs obtained from 33.5-week-old broiler breeders were allocated into five treatment groups: T₁ (control, no SPIDES), T₂ (three SPIDES cycles without turning), T₃ (three SPIDES cycles with three turns per cycle), T₄ (four SPIDES cycles without turning), and T₅ (four SPIDES cycles with three turns per cycle). Eggs were maintained at 17 °C and 75% relative humidity, while SPIDES-treated groups were intermittently exposed to 37.7 °C for three hours at five-day intervals. All eggs were stored for 21 days and then incubated on day 22. Results demonstrated that SPIDES application significantly ($p \leq 0.01$) enhanced spleen weight; however, no notable effect was observed on Newcastle disease virus antibody titers at five weeks of age.

Keywords: NDV, SPIDES, lymphoid organs, antibody titers, broilers

1. Introduction

The quality of day-old chicks plays a crucial role in determining the overall growth performance and productivity of broilers throughout their lifecycle. The flock's performance is also affected by the birds' cellular and humoral immune competence, which contributes to their ability to combat pathogens and minimize productivity losses caused by disease or mortality (Kogut and Klasing, 2009; Korver, 2012) ^[6, 7]. The SPIDES (Short Periods of Incubation During Egg Storage) method represents a novel approach for preserving embryo viability by periodically exposing stored eggs to incubation temperatures during prolonged storage (Nicholson *et al.*, 2013; Dymond *et al.*, 2013) ^[8, 4]. After each warming period, eggs are returned to cool storage conditions to sustain their quality. Although prior research has investigated the effects of SPIDES on embryonic growth, hatchability, and subsequent performance, limited information is available regarding its influence on the immune function of broilers. Hence, the present study aimed to assess the impact of SPIDES application, with or without egg turning, on immune-related traits in broiler chickens.

2. Materials and Methods

A total of 750 Vencobb 430Y fertile eggs obtained from 33.5-week-old broiler breeders were used to evaluate the effects of Short Periods of Incubation During Egg Storage (SPIDES) on broiler immune parameters. The eggs were fumigated at three times the standard concentration for 20 minutes, then stored broad end up for 21 days before incubation commenced on day 22. The eggs were randomly distributed into five treatment groups (150 eggs per group): T₁ (control, no SPIDES), T₂ (three SPIDES cycles without turning), T₃ (three SPIDES cycles with three turns per cycle), T₄ (four SPIDES cycles without turning), and T₅ (four SPIDES cycles with three turns per cycle). Each treatment was further divided into three replicates of 50 eggs. Storage conditions were maintained at 17 °C and 75% relative humidity. SPIDES-treated groups (T₂-T₅) underwent periodic warming every five days, on days 5, 10, and 15 for T₂ and T₃, and on days 5, 10, 15, and 20 for T₄ and T₅. During each SPIDES session, eggs were exposed to 37.7 °C (100°F ± 3°F) and 55% relative humidity for three hours.

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For treatments involving turning (T_3 and T_5), eggs were rotated 45° to each side at hourly intervals. After 21 days of storage, chicks were removed from the hatcher once 95% had dried. From each treatment, 36 chicks were selected and

divided into three replicates, each containing six males and six females. Chicks were individually weighed, wing-banded, and housed in pens corresponding to their treatment groups. The experimental design is outlined in Table 1.

Table 1: Experimental design

S. No.	Treatment groups	Treatment	No of birds per treatment
1.	T_1	Control-Eggs storage without SPIDES	36
2.	T_2	3 SPIDES without turning	36
3.	T_3	3 SPIDES with turning	36
4.	T_4	4 SPIDES without turning	36
5.	T_5	4 SPIDES with turning	36
Total			180

During the five-week experimental period, all groups were reared under standard management conditions. Feed and water were supplied *ad libitum*, and a mash diet with consistent particle size was provided across all treatments. Broiler chicks received routine vaccinations: the B1 strain of Newcastle disease virus on day 7, the intermediate strain of Infectious Bursal Disease virus on day 14, and a Newcastle disease booster using the LaSota strain on day 21.

At the conclusion of the trial, one male and one female bird from each replicate (six birds per treatment) were randomly selected and slaughtered following the procedure described by Arumugam and Panda (1970) [1]. The weights of lymphoid organs including the bursa of Fabricius, thymus, and spleen were recorded. Blood samples were collected, and serum was separated to determine antibody titers against Newcastle

Disease Virus (NDV). The antibody titers were assessed using the hemagglutination (HA) and hemagglutination inhibition (HI) tests, following the micro-test method of Allan and Gouch (1974) [2]. Data on all measured parameters were analyzed using a Completely Randomized Design (CRD) according to the procedures outlined by Snedecor and Cochran (1989) [9]. Differences among treatment means were evaluated for statistical significance using Duncan's Multiple Range Test (Duncan, 1955) [3].

3. Results and Discussion

3.1 Lymphoid organs weight

The effect of short periods of incubation during egg storage on immune organ weight per cent of broilers is presented in Table 2.

Table 2: Mean ($\pm$ SE) lymphoid organs weight (% of live weight) of broilers as influenced by SPIDES with or without turning

Treatment	Spleen weight	Thymus weight	Bursa of Fabricius weight
T_1 (Control- without SPIDES)	0.12 ^b $\pm$ 0.01	0.07 $\pm$ 0.01	0.04 $\pm$ 0.00
T_2 (3 SPIDES without turning)	0.14 ^{ab} $\pm$ 0.01	0.10 $\pm$ 0.01	0.06 $\pm$ 0.00
T_3 (3 SPIDES with Turning)	0.16 ^{ab} $\pm$ 0.01	0.10 $\pm$ 0.03	0.05 $\pm$ 0.00
T_4 (4 SPIDES without turning)	0.14 ^{ab} $\pm$ 0.01	0.12 $\pm$ 0.03	0.04 $\pm$ 0.00
T_5 (4 SPIDES with Turning)	0.20 ^a $\pm$ 0.02	0.13 $\pm$ 0.01	0.06 $\pm$ 0.00

Mean within a column bearing different superscripts differ significantly ($p < 0.01$)

The findings indicated that treatment had a significant influence ($p < 0.01$) on spleen weight percentage among the experimental groups. Birds in the T_5 group recorded the highest spleen weight percentage (0.20%), which was significantly higher ($p < 0.01$) than that observed in the control group T_1 (0.12%). However, no significant differences were detected among the SPIDES-treated groups (T_2 - T_5) or between T_1 and T_4 . Conversely, the weights of the thymus and bursa of Fabricius did not differ significantly among treatments. Nonetheless, numerically, the T_5 group showed slightly higher thymus and bursa percentages (0.13% and 0.06%, respectively) compared with the other groups.

The findings for spleen weight in this study differ from those reported by Goliomytis *et al.* (2015) [3], who found that the size of lymphoid organs (spleen, thymus, and bursa of Fabricius) was not affected by the duration of egg storage. However, the thymus and bursa of Fabricius results observed here are consistent with the same study.

3.2 Antibody titers against Newcastle disease vaccine

The mean ($\pm$ S.E.) immune response against Newcastle disease virus in broiler chicken during the period of study from 0 to 35 days of age as influenced by the SPIDES programme is furnished in Table 3.

Table 3: Mean ($\pm$ SE) Antibody Titre Value against NDV in broilers as influenced by SPIDES with or without turning

Treatment	NDV (Log ₂) HI titer
T_1 (Control-without SPIDES)	1.67 $\pm$ 0.21
T_2 (3 SPIDES without turning)	2.33 $\pm$ 0.49
T_3 (3 SPIDES with Turning)	2.83 $\pm$ 1.24
T_4 (4 SPIDES without turning)	2.67 $\pm$ 0.61
T_5 (4 SPIDES with Turning)	3.50 $\pm$ 0.50

The analysis of data revealed no significant difference in log₂ HI titer against NDV among the treatment groups. The antibody titer value ranged from 1.67 (T_1) to 3.50 (T_5).

The antibody titer to NDV result of this study was in agreement with Goliomytis *et al.* (2015) as they reported that Newcastle disease vaccine immunizations were also not influenced by length of storage.

4. Conclusion

In the present study, the spleen weight was significantly ($p < 0.01$) increased in 4 SPIDES with 3 turning group than non- SPIDES control group and SPIDES had no effect on the antibody titer against NDV at five weeks of age in broilers.

Conflict of Interest

Not available

Financial Support

Not available

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