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Effect of short periods of incubation during egg storage on intestinal histomorphometry of broiler chicken

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Abstract
This experiment examined how applying short periods of incubation during egg storage (SPIDES), with or without egg turning, influenced the intestinal histomorphometry of broiler chickens hatched from eggs stored for extended periods. A total of 750 hatching eggs from 33.5-week-old broiler breeders were allotted into five treatments: T_1 (Control), T_2 (3 SPIDES without turning), T_3 (3 SPIDES with 3 turns during each SPIDES), T_4 (4 SPIDES without turning), and T_5 (4 SPIDES with 3 turns during each SPIDES). All eggs were fumigated and stored for 21 days at 17°C and 75% relative humidity. During storage, eggs in the SPIDES groups were exposed to 37.7°C for three hours every five days. In treatments with turning, eggs were rotated 45° hourly during the heat application. Following storage, eggs were incubated under standard conditions (99.8°F and 55% RH from day 1 to 18). After hatching, 36 chicks per treatment (6 males and 6 females per replicate) were reared under deep litter management and fed according to BIS (2007) standards.
Histomorphometry assessments were performed at five weeks of age. The results indicated that duodenal crypt depth was significantly higher ($P<0.05$) in SPIDES-treated groups compared with the control. Moreover, the T5 group exhibited the greatest ileal villus length. These outcomes demonstrate that SPIDES treatment, particularly when combined with turning, enhances intestinal development and nutrient absorption capacity in broilers originating from long-term stored eggs.

Keywords: SPIDES, prolonged egg storage, intestinal morphology, villi length, crypt depth

1. Introduction
The poultry industry plays a vital role in India's food sector, serving as a leading source of affordable meat and eggs, with broilers providing a significant share of the nation's protein supply. The productivity of broiler chickens is closely linked to the quality of day-old chicks, which is primarily determined by the condition and handling of hatching eggs. In commercial hatcheries, eggs are frequently stored for prolonged durations due to fluctuations in demand and operational logistics. However, extended storage can negatively affect embryonic development, resulting in poor intestinal growth and reduced nutrient absorption in the resulting chicks. To mitigate these issues, short periods of incubation during egg storage (SPIDES) have been introduced as a technique to intermittently warm stored eggs, thereby reactivating embryonic metabolism and minimizing developmental arrest. Incorporating egg turning during SPIDES may further promote uniform embryonic growth and reduce structural abnormalities. Although SPIDES has been documented to improve hatchability and post-hatch performance, its specific effects on intestinal histomorphometry remain insufficiently explored. Since traits like villus height and crypt depth are critical indicators of gut health and nutrient utilization efficiency, studying these parameters can provide insight into optimizing early intestinal development. Hence, the present research was designed to assess the influence of SPIDES, with or without turning, on the intestinal morphology of broilers hatched from long-term stored eggs.

2. Materials and methods
A total of 750 hatching eggs from Vencobb 430Y broiler breeders aged 33.5 weeks were used to investigate the effects of Short Periods of Incubation During Egg Storage (SPIDES)

on the immune parameters of broiler chickens. The eggs were fumigated using a 3× concentration for 20 minutes, stored for 21 days with the broad end facing upward, and transferred for incubation on the 22nd day. The eggs were randomly distributed into five treatment groups of 150 eggs each: T_1 (Control - without SPIDES), T_2 (3 SPIDES without turning), T_3 (3 SPIDES with three turns during each SPIDES), T_4 (4 SPIDES without turning), and T_5 (4 SPIDES with three turns during each SPIDES). Each treatment was further divided into three replicates of 50 eggs. All eggs were stored at 17°C and 75% relative humidity. Eggs in the SPIDES groups (T_2 to T_5) were exposed to heat treatment every five days. Specifically, T_2 and T_3 were heated on the 5th, 10th, and 15th days of storage, whereas T_4 and T_5 received heating on the 5th, 10th, 15th, and 20th days. During each SPIDES cycle, eggs were subjected to a temperature of 100°F ± 3°F and 55% relative humidity for three hours. In the turning treatments (T_3 and T_5), eggs were rotated at a 45° angle to either side at hourly intervals during the heating period. After 21 days of incubation, chicks were removed from the hatcher once 95% of them were completely dry. From each treatment group, 36 chicks (six males and six females per replicate) were selected, weighed, wing-banded, and placed in their respective experimental partitions. The experimental design layout is presented 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

Throughout the five-week experimental period, all treatment groups were maintained under uniform and standard management practices. Feed and water were supplied ad libitum for the entire duration of the study. The diets for all experimental groups were formulated in mash form, ensuring a consistent particle size across treatments. The broiler chicks were vaccinated against Newcastle disease on the 7th day using the B1 strain, followed by vaccination against Infectious Bursal Disease (IBD) on the 14th day with an intermediate strain. A booster dose for Newcastle disease was administered on the 21st day using the LaSota strain.

At the conclusion of the trial, one male and one female bird from each replicate (a total of six birds per treatment) were randomly selected and humanely slaughtered following the procedure described by Arumugam and Panda (1970) [1]. Samples of approximately 2 cm length were collected from the duodenum, jejunum, and ileum for histomorphometric evaluation at five weeks of age. The duodenal segment was taken from the duodenal loop, the jejunal segment from the portion between the duodenal loop and Meckel's diverticulum, and the ileal segment from the region between Meckel's diverticulum and the ileocecal junction, as outlined by Miller (2007) [3]. Tissue samples were preserved in 10% neutral buffered formalin, processed through paraffin embedding, and sectioned at 5 µm thickness using a microtome. The tissue sections were mounted on glass slides and stained with haematoxylin and eosin for microscopic examination. Measurements of villus height and crypt depth were recorded and expressed in micrometers (µm). The villus height-to-crypt depth ratio was calculated by dividing the villus height by the corresponding crypt depth.

The data collected on various parameters were subjected to statistical analysis in Completely Randomized Design (CRD) as per the methods suggested by Snedecor and Cochran (1989) [4] and the means of different treatment groups were tested for statistical significance by Duncan's multiple range test (Duncan, 1955) [2].

3. Result and discussion

3.1 Effect of short periods of incubation during egg storage on gut health of broiler chicken

3.1.1Histomorphometric study of small intestine

The result of SPIDES with or without turning on histomorphometry of small intestine of broiler chicken at five weeks of age is presented in Table 2.

At the end of five weeks, there was no significant difference recorded in duodenal villi length and villi length and crypt depth ratio between treatment groups. Duodenal crypt depth was significantly (P<0.05) higher in SPIDES treatment groups (138.83 to 162.00 µm) than control group (130.50 µm). Numerically, T_5 (1674.33 µm) had the highest duodenal villi length and T_1 (1534.60 µm) had the lowest duodenal villi length. T_5 had the highest duodenal villi length and crypt depth ratio (11.79 µm) and T_4 had the lowest duodenal villi length and crypt depth ratio (10.28 µm). The villi length, crypt depth and villi length and crypt depth ratio of jejunum had no significant difference between treatment groups.

The ileal villi length at five weeks of age differed significantly (P<0.05) between treatment groups. Significantly the longest ileal villi recorded in T_5 (1131.33 µm) followed by T_4 (1061.52 µm) and T_3 (1038.24 µm). No significant difference was noticed in ileal villi length between 4 SPIDES groups and 3 SPIDES with turning group (T_5, T_4 and T_3) and also between 3 SPIDES without turning and control groups (T_1 and T_2). There was no significant difference in ileal crypt depth and ileal villi length and crypt depth ratio between treatment groups.

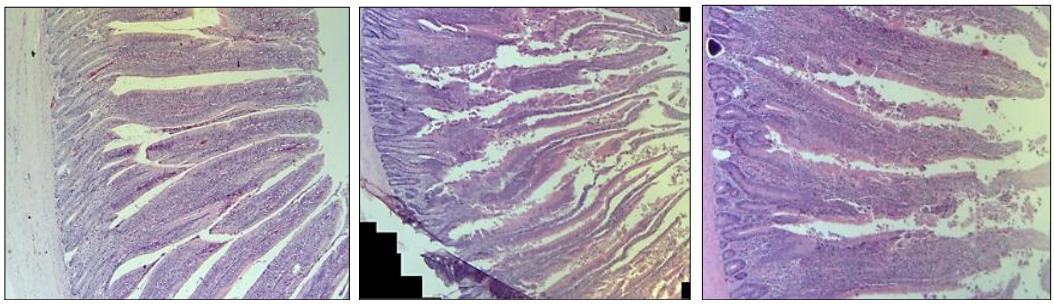
The result showed that 4 SPIDES with turning group had higher ileal villi length and duodenal crypt depth that may increase absorption and utilization of nutrients and in turn improve feed efficiency and body weight gain of birds.The literatures related to the effect of SPIDES on Histomorphometric study of small intestine were not traceable.

Table 2: Mean (± S.E.) duodenal, jejunal and ileal histomorphometry (µm) of broiler at fifth week of age as influenced by SPIDES with or without turning

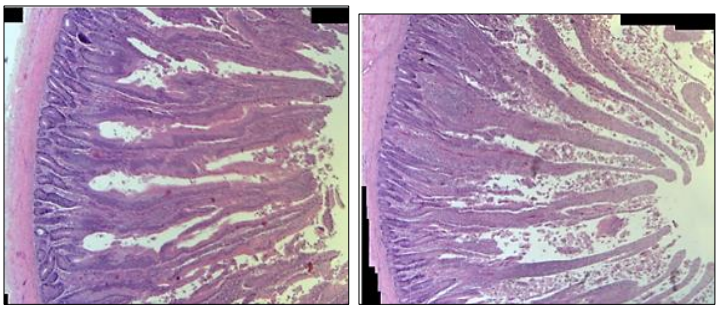
Treatment	Duodenal histomorphometry			Jejunal histomorphometry			Ileal histomorphometry		
	Villi length (µm)	Crypt depth (µm)	Villi length: Crypt depth (µm)	Villi length (µm)	Crypt depth (µm)	Villi length: Crypt depth (µm)	Villi length (µm)	Crypt depth (µm)	Villi length: Crypt depth (µm)
T_1 (Control)	1534.60 ±77.02	130.50^{b} ±5.90	11.75 ±0.82	1340.02 ±73.96	125.92 ±7.87	10.64 ±0.73	1006.75^{b} ±23.06	128.00 ±2.08	7.86±0.24
T_2 (3 SPIDES without turning)	1603.66 ±126.30	138.83^{ab} ±11.71	11.55 ±1.67	1481.91 ±59.77	163.50 ±16.86	9.06 ±1.60	1012.15^{b} ±29.43	130.33 ±3.01	7.76±0.18
T_3 (3 SPIDES with Turning)	1615.26 ±65.99	148.17^{ab} ±7.63	10.90 ±1.09	1412.76 ±144.20	148.00 ±12.13	9.54 ±1.17	1038.24^{ab} ±47.25	122.67 ±6.42	8.46 ±0.73
T_4 (4 SPIDES without turning)	1666.07 ±88.13	162.00^{a} ±11.78	10.28 ±0.93	1519.36 ±108.01	128.50 ±8.80	11.82 ±1.14	1061.52^{ab} ±62.76	122.83 ±6.97	8.64±0.79
T_5 (4 SPIDES with Turning)	1674.33 ±70.32	142.00^{ab} ±9.45	11.79 ±0.95	1332.10 ±127.51	134.33 ±11.56	9.91 ±0.66	1131.33^{a} ±42.67	137.33 ±3.58	8.23±0.14
Significance	NS	*	NS	NS	NS	NS	*	NS	NS
F value	0.40	1.50	0.34	0.60	1.73	0.74	2.25	1.59	1.13

Value given in each cell is the mean of 6 observations Mean within a column bearing different superscripts differ significantly
NS-Non-significant, *Significant (P<0.05)

Plate1: Duodenal histomorphometry as influenced by SPIDES with or without turning in broiler chicken. Representative light micrograph of duodenal villi length and crypt depth at 5 weeks of age

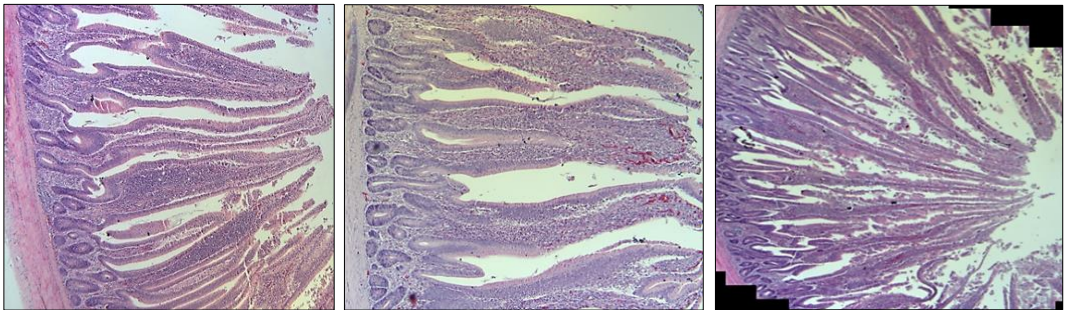


Control (T_1) 3 SPIDES without turning (T_2) 3 SPIDES with turning (T_3)

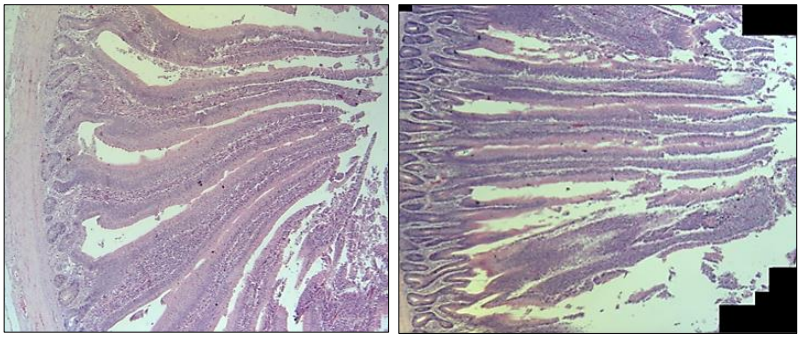


4 SPIDES without turning (T_4) 4 SPIDES with turning (T_5)

Plate 2: Jejunal histomorphometry as influenced by SPIDES with or without turning in broiler chicken. Representative light micrograph of jejunal villi length and crypt depth at 5 weeks of age

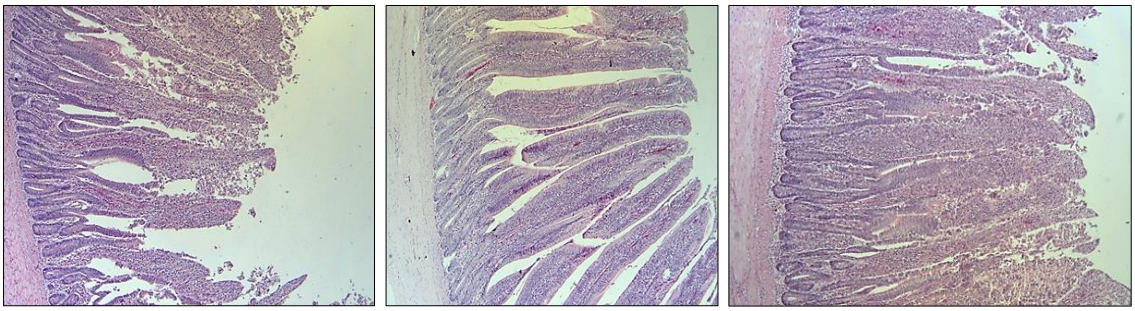


Control (T_1) 3 SPIDES without turning 3 SPIDES with turning (T_3)

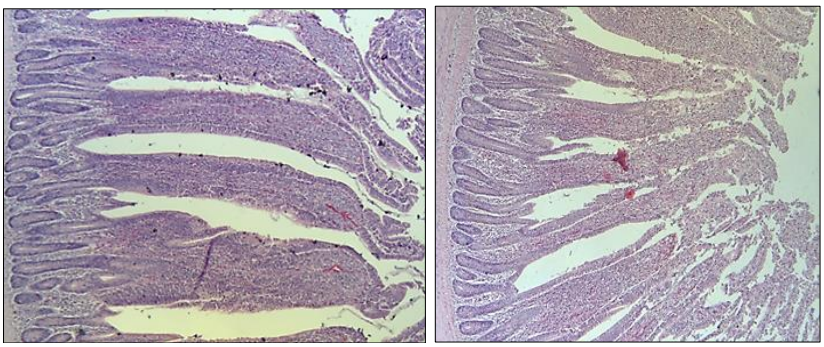


4 SPIDES without turning 4 SPIDES with turning (T_5)

Plate 3: Ileal histomorphometry as influenced by SPIDES with or without turning in broiler chicken. Representative light micrograph of ileal villi length and crypt depth at 5 weeks of age



Control (T_1) 3 SPIDES without turning (T_2) 3 SPIDES with turning (T_3)



4 SPIDES without turning (T_4) 4 SPIDES with turning (T_5)

Conclusion

At the end of the fifth week, duodenal crypt depth was significantly higher in SPIDES groups compared to the control. T_5 (4 SPIDES with turning) recorded the longest ileal villus length. Overall, 4 SPIDES without turning improved duodenal crypt depth, while turning enhanced ileal villus length, indicating a positive effect of SPIDES on intestinal development.

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