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THE DETERMINATION OF ROLE OF THE YOLK STALK AS A PATHWAY BETWEEN THE YOLK SAC AND INTESTINE USING INDIA INK AS HISTOLOGICAL MARKER IN POST-HATCH BROILER CHICKS

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ABSTRACT

India ink was used to examine the role of the yolk stalk lumen as a distinct pathway between the yolk sac and gut through the first 5 d of post-hatch growth in broiler chicks. Two hundred and sixteen newly hatched broiler chicks were equally divided into three treatment groups; gavaged via the esophagus (TRT_1), injected in the yolk sac (TRT_2) with 0.2 mL of water-based black India ink and third group of chicks were used as untreated controls (TRT_0). Tissue samples of the small intestine, yolk sac, and yolk stalk were removed and fixed in 10% buffered neutral formalin and were processed routinely, embedded in paraffin, sectioned at 5 to 6 μm, and stained with hematoxylin and eosin. The relative concentration of India ink in each of the tissue preparations was detected with a light microscope and assigned a relative score between 0 and 3, with 0 indicating the lowest and 3 the highest amount of ink present. The results show India ink was able to pass from the yolk sac through the yolk stalk and into the intestine, but was not able to pass from the intestine into the yolk stalk or yolk sac. It was concluded that India ink was useful materials in establishing that the yolk stalk provides a direct one-way passage by which material in the yolk sac may move into the intestine of broiler chicks during the first 5-d after hatching.

Key words: chick, India ink, yolk sac, yolk stalk

INTRODUCTION

The efficiency of yolk uptake in post-hatch broiler chicken and its subsequent effects on their viability and growth performance are of major concerns in the poultry industry. In spite of extensive mobilization of yolk during incubation, a large portion of yolk remains unabsorbed at hatching and becomes incorporated into the body of the chick as part of a yolk sac which extends off the small intestine (Romanoff, 1960; Freeman & Vince, 1974). Approximately 85% of residual yolk from the yolk sac of chicks is absorbed by the 5^{th} d post-hatch (Noble & Ogunyemi, 1989). However, what the role of yolk stalk, particularly after hatching, in the yolk absorption has been a controversial.

There are two possible routes by which yolk may be absorbed in the developing bird. Yolk nutrients may be either absorbed directly across the yolk sac membrane into the blood stream or be taken directly into the gut by conductance through the yolk stalk, a thick-walled tubular peduncle, connected at the junction of the jejunum and ileum. Peripherally attached vitelline blood vessels enter the embryo along side the yolk stalk (Sturkie, 1976). The possibility of yolk stalk role because transverse sections of the yolk stalk of 5-day-old chicks show gross histological features similar to those of the intestine (Kar, 1947).

Materials such as phenol red (Nitsan *et al*., 1995), blue dextran (Noy *et al*., 1996), and 41C polyethylene glycol-4000 (Esteban *et al.*, 1991) have been used as markers in determining yolk sac absorption in previous studies. India ink has been demonstrated to be a useful tissue marker in chickens. Pelaez & Arteaga (1993) used gelatin-India ink labeling to investigate the time of appearance of the truncus arteriosus in White Leghorn embryos. Jeurissen *et al*. (1989) injected a large quantity of India ink into the jejunum of exsanguinated chickens in order to investigate the immunological function of the yolk stalk.

In the present study, water-based India ink served as a histological marker to indicate if yolk is absorbed directly through the yolk stalk and if the yolk stalk may transport material into the intestine from the yolk sac during the first 5 d of growth

in broiler chicks. The result of this study can be used as base information in order to use the more knowledge of yolk and yolk stalk role in enhancing viability and growth performance of chicks.

MATERIALS AND METHOD

Two hundred and sixteen newly hatched broiler chicks were equally divided into three treatment groups. At 1300 h on Day 0, chicks were either gavaged via the esophagus (TRT_1) or injected in the yolk sac (TRT_2) with 0.2 mL of non-toxic, undiluted, water-based black India ink. A third group of chicks were used as untreated controls (TRT_0). In TRT_1, an 18-gauge gavage tube was inserted at a depth of 1.5 cm into each bird's esophagus for India ink delivery. In TRT_1, chicks were held with gentle pressure in an inverted position and India ink was injected with a 25-gauge needle directly through the abdominal wall into the underlying yolk sac approximately 4 mm anterior to the umbilicus. Chicks were placed at random according to treatment in a preheated Petersime brooder battery that contained 12 pens. There were 18 chicks in each pen with four replicate pens assigned to each treatment. All chicks were exposed to continuous lighting and normal brooding temperatures throughout the experiment.

Chicks were offered starter diets that met or exceeded NRC (1984) recommendations. Feed and water were available for *ad lib.* consumption. Immediately and at 1300 h each day for 5 consecutive day after treatment, three chicks from each replicate were euthanatized by cervical dislocation. The birds were held so as to prevent abdominal pressure that might unnaturally force the yolk sac contents into the yolk stalk.

Each day, India ink was well dispersed throughout the yolk sac contents in all yolk sac injected birds. For each chick, total body, intestinal (small and large), liver, gallbladder, yolk sac, and yolk stalk weights were determined. Relative [(grams of organ per grams BW) x 100] weights of each organ were calculated prior to analysis.

Tissue samples of the small intestine, yolk sac, and yolk stalk were removed and fixed in 10% buffered neutral formalin. Intestinal samples were taken from similar areas proximal to the site of yolk stalk attachment. For each organ, two tissue samples per time period in TRT_1 and TRT_2 (24 total) were processed routinely, embedded in paraffin, sectioned at 5 to 6 μm, and stained with hematoxylin and eosin. The relative concentration of India ink in each of the tissue preparations was detected with a light microscope and assigned a relative score between 0 and 3, with 0 indicating the lowest and 3 the highest amount of ink present.

Total BW and relative intestinal, liver, gallbladder, yolk sac, and yolk stalk weights were subjected to ANOVA to determine the effects of age and treatment. Arc sine angular transformations were used after the conversion of organ weights to a percentage of total BW (Steel & Torrie, 1980). Fisher's Protected LSD procedure was used to separate means. For yolk sac, yolk stalk, and intestine tissue samples, chi-square tests of homogeneity were used to compare treatments across bird age with regard to the amount of ink present. Within each treatment, the amounts of ink in tissues were compared using McNemar's procedure (Conover, 1980). Exact combinatorical procedures were used (SAS Institute, 1988) to assess significance in the presence of sparse data in some cells. Statements of statistical significance were based on $P < 0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

The yolk sacs of birds injected with India ink were significantly larger than those of the gavaged and control birds. However, changes in BW and relative organ weights were similar among all treatments between 0 and 5 day. Body weight and relative weights of the intestine, liver, gallbladder, and yolk stalk progressively increased, whereas relative weight of the yolk sac decreased between 0 and 5 day (Table 1).

Table 1. Body weight (BDW), and relative intestine (RINW), liver (RLVW), gallbladder (RGBW), yolk sac (RYSW), and yolk stalk (RYTW) weights across treatment in chicks at Days 0, 1, 2, 3, 4 and 5 post-hatch.

DAY	BDW	RINW	RLVW	RGBW	RYSW	RYTW
	(g)	----------[(g/g BDW)*100]----------				
0	40.49^{f}	4.65^{e}	2.61^{e}	0.20^{ab}	9.01^{a}	0.0067^{c}
1	46.49^{e}	8.37^{d}	3.27^{d}	0.09^{d}	5.31^{b}	0.0068^{c}
2	54.51^{d}	11.46^{c}	3.64^{c}	0.15^{c}	2.25^{c}	0.0125^{ab}
3	65.08^{c}	13.69^{b}	4.09^{b}	0.25^{a}	1.46^{d}	0.0109^{b}
4	78.81^{b}	15.02^{a}	4.65^{a}	0.22^{a}	0.78^{e}	0.0142^{a}
5	90.83^{a}	14.59^{a}	4.83^{a}	0.18^{cb}	0.45^{f}	0.0149^{a}

$^{a\text{-}f}$For each variable, means within a column with no common superscript differ significantly
($P < 0.05$) by Fisher's Protected LSD.

Calculated percentages of India ink present in tissue preparations from the yolk sac, yolk stalk, and intestine were based on a relative scoring system between 0 and 3, and comparisons between treatments for each tissue and between tissues for each treatment were made (Table 2 and Table 3). Birds that were gavaged with India ink had significantly ($P < 0.001$) less ink in their yolk sacs than those that were injected in the yolk sac with ink (0% present vs 100% present). There was also significantly ($P \pm 0.001$) less ink in the yolk stalks of gavaged birds than those that were injected (0% present vs 90% present). However, the amounts present in the intestine did not differ ($P = 0.81$) between treatments (50% present vs 30% present). It should be noted that in yolk sac injected birds, ink was found in the intestine throughout the entire 5-d period. Within the gavaged birds, no ink was found in the yolk sac or stalk, but the amount found in the intestine was marginally greater than

the amount found in either the yolk sac or stalk (0% in yolk sac, 0% in yolk stalk, 50% in Intestine, P = 0.06). Additionally, within the yolk sac injected birds, the amount of ink in the yolk stalk exceeded that in the yolk sac 10% of the time, whereas the amount in the yolk sac exceeded that in the yolk stalk 50% of the time. However, this difference was not significant (P = 0.22). The amount of ink in the intestine never exceeded the amount in the yolk sac, but the amount in the yolk sac exceeded the amount in the intestine 90% of the time, with this difference being significant (P < 0.01). Similarly, the amount of ink in the intestine never exceeded that in the yolk stalk, whereas the amount in the stalk exceeded that in the intestine 80% of the time, which was also significant (P < 0.01).

Table 2. Ink concentration scores[1] of yolk sac(YLKSC),yolk stalk (YLKST), and intestine (INT) in ink gavaged (TRT_1) and ink injected (TRT_2) chicks at Days 0, 1, 2, 3, 4, and 5 post-hatch

DAY	INK CONCENTRATION					
	TRT_1			TRT_2		
	YLKSC	YLKST	INT	YLKSC	YLKST	INT
0	0	0	1.0	2.0	1.0	2.0
1	0	0	0.5	2.5	1.0	0.5
2	0	0	0.5	3.0	1.5	1.0
3	0	0	0.5	2.5	2.0	1.0
4	0	0	1.5	2.0	1.0	1.0
5	0	0	0	3.0	1.5	0.5
p	0%	0%	50%	94%	72%	50%

[1]Slide preparations were made on 20% of collected organ tissue samples. 0=no ink, 1=small amount of ink, 2=moderate amount of ink, and 3=large amount of ink

Table 3. Ranked tranformations of ink concentration scores of yolk sac (YLKSC), yolk stalk (YLKST), and intestine(INT) within ink gavaged (TRT_1), and ink injected(TRT_2) chicks across day of age

TRT	INK CONCENTRATION RANK		
	YLKSC	YLKST	INT
1	14.00^{B}	14.00^{B}	23.92^{A}
	8.50^{b}	8.50^{b}	17.22^{a}
2	22.37^{A}	13.06^{B}	9.28^{B}
	25.00^{a}	25.00^{a}	17.81^{a}

^{A,B} For each variable, means within a row with no common supercript differ significantly (P< 0.05) by Fisher's Protected LSD.
^{a,b} For each variable, means within a column with no common supercript differ significantly (P< 0.05) by Fisher's Protected LSD.

In all birds examined, the yolk stalk was present as a finger-shaped projection midway along the length of the intestine. The remnant of the yolk stalk in chickens, commonly referred to as Meckel's diverticulum, may be found at approximately the midpoint of the small intestine (Duke, 1986), and Branton *et al.* (1988) have confirmed that its position is constant regardless of the bird's sex.

The yolk stalk continues to grow after hatch and persists throughout the life of both sexes of the domestic fowl (Kar, 1947; Olah and Glick, 1984). The average weight of the yolk stalk in this study was 0.027 g or 0.01% of chick BW, and as relative yolk sac weight decreased during the first 5 d posthatch, relative yolk stalk weight peaked at Day 4. Histological examination of the serous and muscle layers lining the yolk stalk's lumen has revealed that it is similar to that of theintestine, with connective tissue stroma and mucosa lined by glandular epithelium, but unlike the intestine, the epithelium lacks villi (Kar, 1947).

There has been controversy in the literature with regard to the function of the yolk stalk as a passageway between the yolk sac and intestine (Romanoff, 1960). Nitsan *et al*. (1995) did not observe 1% phenol red in either the yolk stalk or

intestine after it was injected (0.5mL) into the residual yolk of chicks. In contrast, Kar (1947) has noted that some yolk may be absorbed by the epithelial lining of the yolk stalk. Noy *et al*. (1996) stated that blue dextran injected into the yolk sac of the newly hatched chick could be seen moving in pulses into the intestine at irregular intervals, but that the accumulation of lymphoid cells occluded passage at 3 d post-hatch. Moreover, Esteban *et al.* (1991) reported that reserves were able to pass from the yolk sac to the gastrointestinal tract during the first 2 d of life, but that transit decreased proportionally with chick age during that time.

Slide preparations (Fig. 1 - 4) indicated that when India ink was injected directly into the yolk sac, it became embedded in the epithelia of the yolk stalk and yolk sac membrane through 5d of age. Furthermore, intestinal ink scores for injected and gavaged birds were not statistically different. Passage of ink through the yolk stalk and into the intestine from the yolk sac evidently occurred despite possible significant phagocytic activity in the walls of the yolk sac (Olah and Glick, 1984) and yolk stalk. When India ink was gavaged, a small amount was retained in the intestines, whereas none entered the yolk stalk or passed into the yolk sac. India ink of intestinal origin was apparently unable to pass into the yolk stalk and then into the yolk sac. These findings may also suggest that the movement of material through the yolk stalk in post-hatch broiler chicks through 5 d of age is not bidirectional, but that passage is restricted to a one-way direction from the yolk sac to the intestine.

CONCLUSION

Body weight and relative intestine, liver, and yolk stalk weights increased during the first 5 days posthatch. Conversely, relative yolk sac weight decreased at each age period through day 5. Ink scores in examined tissues were not influenced by bird age. It appeared that India ink was able to pass from the yolk sac through the yolk stalk and then into the intestine; conversely, India ink of intestinal origin was apparently not able to pass into the yolk stalk and then into the yolk sac. These findings indicate that some yolk may be absorbed in and pass through the yolk stalk

and into the gut of the post-hatch chick. India ink was useful in establishing that the yolk stalk provides a direct one-way passage by which material in the yolk sac may move into the intestine of broiler chicks during the first 5-days after hatching.

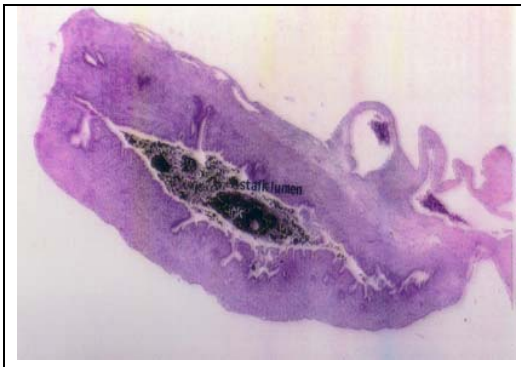

Figure 1. Yolk stalk lumen containing ink particles at day 1 in TRT_2

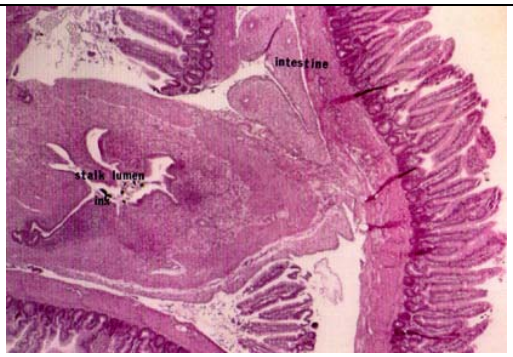

Figure 2. Cross section of yolk stalk lumen containing ink particles at day 5 in TRT_2

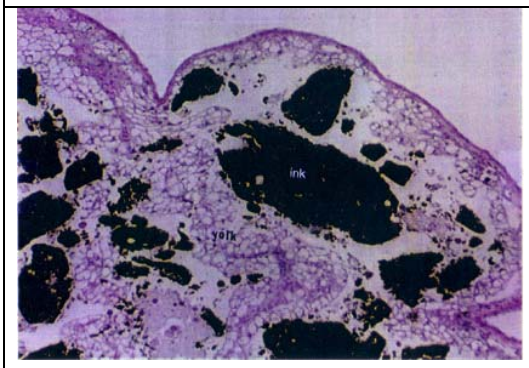

Figure 3. Cross section of yolk sac containing ink particle at day 1 in TRT_2

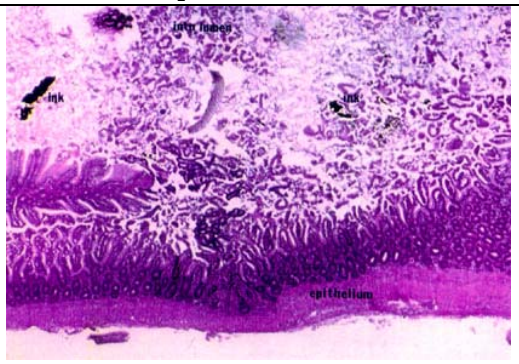

Figure 4. Intestinal lumen containing ink particle at day 5 in TRT_2

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