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EFFECT OF SURGICAL REMOVAL OF THE RESIDUAL YOLK SAC ON THE DEVELOPMENT OF THE DIGESTIVE SYSTEM AND IMMUNE RESPONSE IN BROILER CHICKS DURING EARLY DAYS POST-HATCH

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ABSTRACT: *This study was designed to investigate the effects of the residual yolk-sac on the development of the digestive system and immune response of broiler chicks. Two experiments were conducted in this study. In the first one, 60 day-old broiler chicks (Lohmann) were allocated into three experimental groups according to the status of the residual yolk sac; deutectomized (surgically-removed residual yolk sac), sham operated and intact chicks. Five chicks from each experimental groups were randomly selected at days 2, 4, 6 and 8, weighed, euthanized and the different parts of their digestive tract and liver were weighed. The body weight of deutectomizd chicks at day 2, 4 and 6 post-hatch was significantly (P<0.05) lower compared to that of sham operated and intact chicks. At day 8, the body weights of all experimental groups did not significantly (P<0.05) differ from each other. The liver weight in deutectomizd chicks was significantly (P<0.05) lower at days 2, 4 and 6 post-hatch compared to that of the other experimental groups. At day 8 the liver weights in the different experimental groups did not show any significant difference. The weights of the different parts of the digestive tract (crop, gizzard, provenriculus, intestine), somehow, in deutectomizd chicks were significantly (P<0.05) lower at day 2, 4 and 6 compared to that of sham operated and intact ones. In the second experiment, 60 day-old broiler chicks (Lohmann) were allocated to the above mentioned experimental groups; 20 chicks per each. Thereafter, they were challenged with 10% sheep RBC suspension at day 2 and day 12 post-hatch. Ten chicks were randomly selected from each experimental group at day 12 and day 20 post-hatching, killed and their lymphoid organs (spleen, thymus and bursa of fabricius) were incised and sera were harvested from blood samples. The lymphoid organs were significantly (P<0.05) lower in deutectomized chicks compared to the two other experimental groups. The geometric mean titers (GMT) of antibodies against 10% sheep RBC suspension for primary and secondary immune responses in deutectomized chicks, were lower than that of sham operated and intact chicks. The results of this study revealed that the residual yolk sac is essential for the development of the digestive system and immune response in broiler chicks.*

Key words: *Broiler Chicks, Deutectomy, Digestive system, lymphoid organs, Immune response.*

ORIGINAL ARTICLE

INTRODUCTION

The avian species depend on the egg constituents to meet their requirements for growth and energy during embryonic life. Nevertheless, the significance of the yolk constituents extends beyond the embryonic life to cover the early days post-hatching. Its effects *(as residual yolk sac, RYS)* on growth of broiler chicks have been investigated by many studies (Chamblee et al., 1992; Turro et al., 1994 and Ali et al, 2007). Deutectomy has been shown to delay the growth of broiler chicks by 2 days (Chamblee et al., 1992), however, the chick can compensate for this delay by the end of the first week post-hatch (Chamblee et al., 1992 and Ali et al., 2007). Moreover, surgical removal of the residual yolk sac significantly decreases the liver total lipids during the first six days post-hatch in broiler chicks, which an effect lasts by the 8th post-hatch (Ali et al., 2011).

Feeding chicks immediately after hatching enhances the efficiency of yolk sac utilization (Bhanja et al., 2009). Noteworthy, broiler and layer chicks, subjected to feed and water deprivation showed striking differences in their efficiency of yolk utilization; it is significantly higher during the first two days post-hatch in broiler chicks (Malik et al., 2011).

Other biological functions of RYS have been also demonstrated; Olah and Glick (1984) reported that the RYS transforms to lymphoid tissues, exhibiting myelpoietic activity after complete absorption of the yolk contents.

The current study was designed to reflect more light on the physiological roles of RYS on post-hatch development of the digestive system and immune response of broiler chicks.

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MATERIALS AND METHODS

Experimental Birds, Housing and Feeding

One hundred twenty commercial unsexed Lohman, day-old broiler chicks were employed in this study (60 chicks per each experiment). They were kept in a brooder house, at the Department of Physiology, Faculty of Veterinary Medicine, University of Khartoum, where water and feed were provided *ad libitum*. Artificial and natural light was provided 24 hours a day.

Experimental plan

The chicks were assigned into 3 groups according to the status of the residual yolk sac; deutectomized, sham operated and intact chicks as follows:

a) ***Deutectomized chicks***: The RYS was removed surgically within 4 hours post-hatching. Deutectomy was carried out according to a surgical technique described by Turo et al. (1994) with some modifications done to make the operation easier and more comfortable to the chick.

b) ***Sham operated chicks***: In this group of experimental chicks, 5 mm surgical incision was made in the abdomen within 4 hours post-hatching. The incision was made in the same level of the umbilicus and just to the right side of it, and then it was sutured using silk.

c) ***Intact chicks:*** No incision or surgical operation was performed in this group of animals and they were kept as control.

Experiment 1

Sixty day-old broiler chicks were equally assigned into the 3 mentioned experimental groups. Five chicks were randomly selected from each group at day 2, 4, 6, and 8, weighed and euthanized by cervical dislocation. The alimentary tract was removed and its different parts were excised, emptied and weighed.

Experiment 2

After been assigned into the 3 mentioned experimental groups, the chicks were challenged with 1 ml of 10% sheep red blood cell suspension (SRBC, I/V) at day 2 and day 12 post-hatch. At day 12 (primary immune response) and day 20 (secondary immune response), 10 chicks from each group were randomly selected, weighed and euthanized. Blood samples were collected and sera were harvested and frozen for subsequent measurement of antibody titers against SRBC. This was performed using haemagglutination test as described by Singh and Dhawedkar (1993). The lymphoid organs (spleen, thymus and bursa of fabricius) were excised and weighed.

RESULTS AND DISCUSSION

The early days post-hatch is very critical for the subsequent development of chick; during which the body undergoes many metabolic changes. As it has been previously reported (Nitsan et al., 1991; Nir et al., 1993), the development of the digestive system and enzymes show continuous changes during this period. The residual yolk sac is the only extra-embryonic membrane exists during this transitional period and it has been shown, in addition to exogenous feed, to be critical for these changes (Gonzales et al., 2008; Ali et al., 2011).

Deutectomized chicks exhibited a significant ($P<0.05$) decrease in the body weight at days 4 and 6, however, this effect was compensated at the 8th day post-hatch (Table 1). Previous findings showed that deutectomy did not influence the dietary energy use, lipids, and the carcass composition, but delayed the growth by 2 days behind the control chicks until the end of the first week, after which the chick can compensate for this delay (Murakami et al., 1992; Ali et al., 2007).

The current findings showed that deutectomy significantly ($P<0.05$) decreased the weight of the liver (Table 2). Since deutectomy does not significantly affect serum total lipids (Ali et al., 2007) and free fatty acids (Baranyiova and Standara, 1980), it seems that the deutectomized chicks depends on the liver lipids stored during embryonic life. Noteworthy, the liver total lipids content is significantly affected by the interaction between days post-hatch and surgical ablation of residual yolk sac (Ali et al., 2007), which a finding support this assumption. Hence, considerable amount of fats might be drawn from the liver to the circulation to meet the energy needs of the deutectomized chicks. This might lead to a decrease in the liver weight exhibited by dutectomized chicks.

Deutectomy resulted in initial decrease in the weight of the different parts of the digestive tract, but this was compensated for by the 8th day post-hatch, at which the development of the digestive system was parallel to that of intact chicks (Tables 3-6).

The broiler chicks are characterized by high growth rate during the early days post-hatch due to a marked increase in the weight of the gastrointestinal tract (Nitsan et al., 1991), which is much higher as compared to that of the rest of the body (Nir et al., 1993). Post-hatch starvation results in a decrease in carcass lipid content but did not modify the disappearance rate of yolk in the abdomen (Murakami et al., 1992; Malik et al., 2011). Immediately after hatching, most energy and proteins are used for intestinal growth. This preferential growth occurs regardless of feed presence (Noy and Sklan, 1999; Maiorka et al., 2000). When these nutrients are not supplied by feed, newly hatched chicks use for intestinal growth the energy and protein from yolk sac. 20% of the residual protein of yolk

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sac consists of maternal immunoglobulin, and the residual lipids of yolk sac are basically triglycerides, phospholipids, and cholesterol (Dibner et al., 1998). These components are used as intact macromolecules without metabolism, and the phospholipids and cholesterol are used for cell membrane formation (Maiorka et al., 2006). Nevertheless, it has been reported that nutrient supply from yolk is less crucial for the development of the digestive tract than withholding feed (Uni et al., 1998). Noteworthy, removal of the yolk sac reduces the activity of amylase, trypsin, and chymotrypsin in the intestinal chyme (Nitsan et al., 1995). Intubation with yolk increases enzyme activity (amylase excepted) in the pancreas or intestinal chyme only in chicks that had their yolk sacs removed (Nitsan et al., 1995).

The geometric mean titer (GMT) of antibodies against 10% sheep red blood cell suspension for primary and secondary immune response in deutectomized chicks was less than that of sham operated and intact chicks (Table 7). Moreover, surgical removal of the residual yolk sac significantly ($P<0.05$) reduced the weight of the lymphoid organs, (spleen, thymus and bursa of Fabricius), and this extended even after the completion of the course of the residual yolk absorption. By the 20th day post-hatch, the lymphoid organs weight in the deutectomized chicks were significantly ($P<0.05$) lesser compared to that of the other groups. These results are partially compatible with the findings of Thaxton (1984), who suggested that the yolk sac may play a role in clearance of antigens. Nevertheless, there are no previous findings supporting the direct effect of the residual yolk sac on the subsequent development of the lymphoid organs pos-thatching.

Table 1 - Effect of deutectomy on the body weight (gm) of broiler chicks during first week post-hatch (Mean SEM)

Days post-hatch	Status of the residual yolk sac		
	Intact	Sham operated	Deutectomized
2	47.82 ± 3.33 ^{a}	46.02 ± 1.92 ^{a}	42.42 ± 4.00 ^{b}
4	51.00 ± 4.30 ^{a}	53.80 ± 4.12 ^{a}	47.12 ± 3.00 ^{b}
6	58.42 ± 5.91 ^{a}	60.89 ± 5.78 ^{a}	52.68 ± 3.46 ^{b}
8	68.18 ± 6.82 ^{a}	71.50 ± 5.34 ^{a}	69.33 ± 4.89 ^{a}

Table 2 - Effect of deutectomy on the liver weight (gm) of broiler chicks during first week post-hatch

Days post-hatch	Status of the residual yolk sac		
	Intact	Sham operated	Deutectomized
2	2.59 ± 0.12 ^{a}	2.47 ± 0.21 ^{a}	2.21 ± 0.23 ^{b}
4	2.73 ± 0.22 ^{a}	2.72 ± 0.31 ^{a}	2.16 ± 0.15 ^{b}
6	3.13 ± 0.42 ^{a}	3.04 ± 0.36 ^{a}	2.60 ± 0.15 ^{b}
8	3.04 ± 0.19 ^{a}	2.99 ± 0.34 ^{a}	3.17 ± 0.14 ^{a}

Table 3 - Effect of deutectomy on the crop weight (gm) of broiler chicks during first week post-hatch

Days post-hatch	Status of the residual yolk sac		
	Intact	Sham operated	Deutectomized
2	0.76 ± 0.04^{a}	0.79 ± 0.04 ^{a}	0.72 ± 0.07 ^{b}
4	0.80 ± 0.03 ^{a}	0.77 ± 0.05 ^{ab}	0.68 ± 0.04 ^{b}
6	0.80 ± 0.04 ^{a}	0.75 ± 0.08 ^{a}	0.76 ± 0.03 ^{a}
8	0.73 ± 0.04 ^{a}	0.80 ± 0.03 ^{a}	0.63 ± 0.06 ^{c}

Table 4 - Effect of deutectomy on the proventriculus weight (gm) of broiler chicks during first week post-hatch.

Days post-hatch	Status of the residual yolk sac		
	Intact	Sham operated	Deutectomized
2	0.81 ± 0.06 ^{a}	0.70 ± 0.03 ^{b}	0.74 ± 0.05 ^{b}
4	0.81 ± 0.04 ^{a}	0.87 ± 0.09 ^{a}	0.72 ± 0.04 ^{b}
6	0.94 ± 0.09 ^{a}	0.86 ± 0.04 ^{a}	0.73 ± 0.07 ^{b}
8	0.88 ± 0.11 ^{a}	0.89 ± 0.05 ^{a}	0.90 ± 0.04 ^{a}

Table 5 - Effect of deutectomy on the gizzard weight (gm) of broiler chicks during first week post-hatch.

Days post-hatch	Status of the residual yolk sac		
	Intact	Sham operated	Deutectomized
2	3.26 ± 0.19 ^{a}	3.06 ± 0.09 ^{a}	2.68 ± 0.20 ^{b}
4	3.70 ± 0.28 ^{a}	3.35 ± 0.17 ^{a}	2.87± 0.12 ^{b}
6	3.56 ± 0.26 ^{a}	3.41 ± 0.21 ^{a}	2.96 ± 0.22 ^{a}
8	3.54 ± 0.39 ^{a}	3.59 ± 0.26 ^{a}	3.93 ± 0.29 ^{a}

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Table 6 - Effect of deutectomy on the weight of empty intestine (gm) of broiler chicks during first week post-hatch.

Days post-hatch	Status of the residual yolk sac		
	Intact	Sham operated	Deutectomized
2	2.80 ± 0.30 [a]	2.95 ± 0.22 [a]	3.05 ± 0.30 [a]
4	4.78 ± 0.27 [a]	4.71 ± 0.50 [a]	3.48 ± 0.34 [b]
6	4.97 ± 0.49 [a]	4.98 ± 0.25 [a]	4.45 ± 0.50 [b]
8	6.44 ± 0.39 [ab]	6.19 ± 0.26 [b]	6.74 ± 0.68 [a]

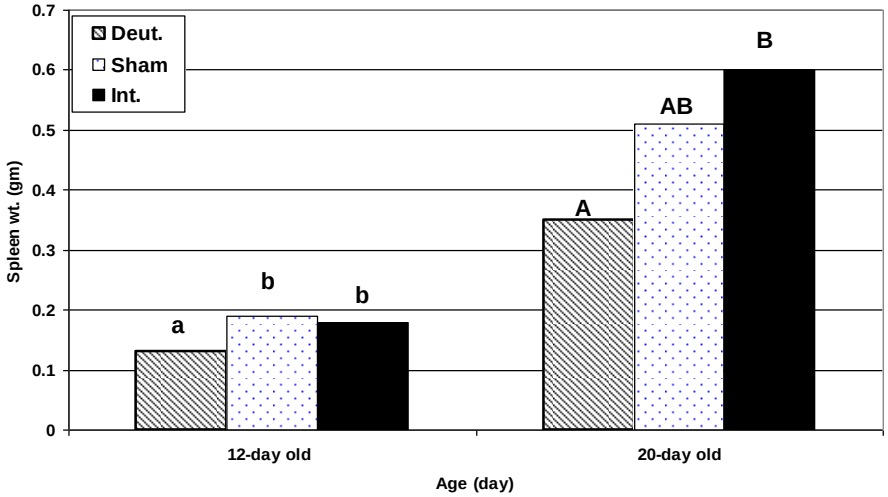

Figure 1 - Effect of deutectomy on Spleen weight (g) of 12- and 20-day old broiler chicks

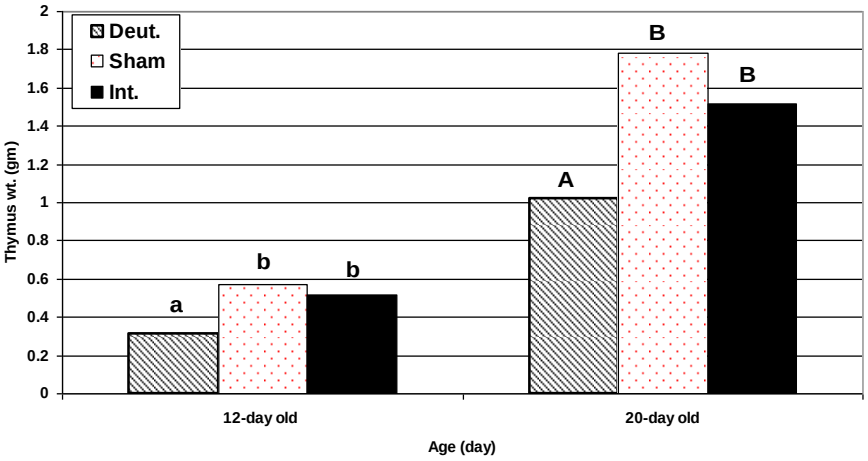

Figure 2 - Effect of deutectomy on thymus weight (g) of 12- and 20-day old broiler chicks

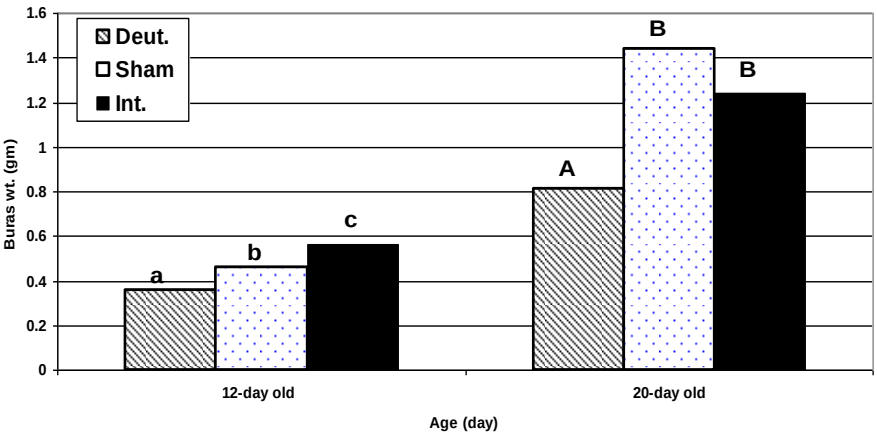

Figure 3 - Effect of deutectomy on Bursa of Fabricius weight (g) of 12- and 20-day old broiler chicks

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Table 7 - Geometric mean titers of antibodies against 10% sheep RBCs suspension injected I/V in 2-day and 12-day old deutectomized, sham operated or intact chicks

Days post-hatch / Status of residual yolk sac	Day 12 (primary immune response)	Day 20 (secondary immune response)
Deutectmized	2.5	5.3
Sham operated	4.3	8.0
Intact	4.6	8.6

CONCLUSION

This study showed that the residual yolk sac is very critical for the development of the digestive system and the immune response in broiler chicks during the early days pos-hatch.

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