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Research Article
Immuno-pathological Study of a Deprivation Effect on the Immune Response of Post-hatched Chicks

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Abstract

Objective: This study was conducted to investigate the effect of deprivation during the first few days of the post-hatch period on the immune response of chicks. **Materials and Methods:** One hundred and twenty one days old Ross 308 birds were used and they were divided into four groups, beginning with group 1 (G1): Direct feed supply, which was divided into 2 subgroups at 11 days of age with G1.a as a positive control and G1.b as a negative control. Groups 2, 3 and 4 experienced deprivation for 24, 48 and 72 h, respectively. **Results:** The results showed a significant ($p<0.05$) increase in antibody titre in G1 compared to that in G2 and G3 at 11 days of age, whereas at 21 days of age, G2 and G4 recorded a significant increase ($p<0.05$) in antibody titre compared to that in G1 and G3. The mortality rate significantly ($p<0.05$) increased in G1b compared to that in other groups. Histopathological study of the duodenum at 14 days of age showed that there were no developmental and functional differences between G1 and G2. In contrast, deprivation for 48 h (G3) showed a regenerative process, which was indicated by newly formed crypt cells in addition to the presence of congested mucosa. Deprivation for 72 h (G4) showed some compensatory growth in the form of hyperplasia of crypt cells and a congested muscular layer with inflammatory cells, but the cells did not attain their full size during G1 and G2. **Conclusion:** The study showed that an early feed supply had a direct effect on the immune status of the birds within 1-3 weeks. In addition, the study showed that an early supply creates a concrete communication with the digestive function of the gut but not at an older age.

Key words: Chicks, feed, deprivation, yolk sac, immunity, digestive system

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A new strategy of the poultry industry is determining how to keep birds free from diseases and how to reduce time to market with highly productive traits. To accomplish the first objective, special attention has been dedicated to study the effect of deprivation within the first few days of a post-hatched period on innate immunity and at the end of the experimental period (32 days). It is well known that the early days of newly hatched chicks are critically important and producers may feel that feeding is not essential during this period because conventional wisdom says that a bird can survive on its residual yolk[1]. The content of the yolk sac (YS) in most cases is entirely absorbed over the first 7 days after hatching and the YS itself is transformed into scar tissue[2,3]. During the initial 48 h, which is the post-hatch period, the yolk contributes to maintenance and small intestinal development[4]. The anti-peristaltic movement that transfers the yolk from the yolk stalk to the duodenum appears to be stimulated by the presence of feed in the gut[5]. The YS represents the maternal antibodies that have to be transformed from the mother to the newly hatched chicks. Certain exogenous factors are needed to initiate this transformation and this factor is represented by a direct feed supply that has been proven to evoke intestinal movement and cell proliferation in intestinal mucosa, as well as the function of the immune system in broiler hatching[1]. All these points should be considered for enhancing or interfering with the MI, since the latter process plays an important role in the failure of vaccination[6]. This study aimed to investigate the effect of deprivation during the first few days of the post-hatch period on the immune response of chicks.

MATERIALS AND METHODS

Newly hatched Ross 308 (n = 120) chicks were placed in a convenient place. They were divided into 4 groups and placed in separate boxes to be treated, as shown in Table 1.

Immunological assay:

Haemagglutination inhibition test: A haemagglutination inhibition (H.I) test was performed in the following periods:

- At 1 day old to detect maternal immunity (MI)
- At 11 days old to detect the decline in MI
- At 21 days old to detect the response to the first vaccination
- At 31 days old to detect the effect of the second vaccination

The test was conducted according to the methods of Marthedal *et al.*[7].

All birds received the same basic scientific diet that is specific for broilers.

Histopathological study: Birds from different groups were sacrificed at 72 h post-housing and at 14 days of age. Samples were taken from the duodenum and jejunum and stored in 10% formalin to be stained according to the methods of Luna[8]. The sectioning of slices were 5 μm and a haematoxylin and eosin stain was used.

Challenge test: All of the groups were exposed to a challenge infection with a local virulent strain of the Newcastle disease virus. The lesion was taken from affected birds with a local virulent strain. The sample was ground and then, the tissue was suspended and centrifuged. The supernatant fluid was inoculated in egg chick embryos (ECE) at an age of 9 days via the allantoic sac. The eggs were incubated and daily candling was performed. The incubated eggs showed dead embryos in less than 50 h. Allantoic fluid was harvested to be tested for an agglutination test[9,10]. The isolated virus was evaluated for virulence through a preliminary study by estimating the intracerebral pathogenicity index (ICPI) and intravenous pathogenicity index (IVPI)[11,12]. The ICPI was 1.91, while the IVPI was 2.56. The isolated virus was used as a challenge virus, which was introduced through an intranasal route at an age of 32 days.

Table 1: Study design

Groups	No. of bird	Treatment	Sub-group	Notes
G1	38	Direct feed supply	A = (20 bird) +ve control	Vaccinated at 12 days and re-vaccinated at 21 days with NDV*
		At 11 days old were sub-divided	B = (18 bird) -ve control	Treated at 12 and 22 days with distilled water
G2	27	Deprivation time 24 h		Vaccinated at 12 days and re-vaccinated at 21 days with NDV
G3	28	Deprivation time 48 h		Vaccinated at 12 days and re-vaccinated at 21 days with NDV
G4	27	Deprivation time 72 h		Vaccinated at 12 days and re-vaccinated at 21 days with NDV

One hundred and twenty one days old Ross 308 birds/average hatched weight: 40±3 g. *NDV: Newcastle disease vaccine, vaccination was done via drinking water (D.W.)

Statistical analysis: The data were expressed as the means and standard error and then, they were subjected to statistical analysis using one-way analysis of variance (ANOVA). The least significant differences (LSD) between groups were found using SPSS (SPSS, Inc., IBM, Chicago, Illinois, USA) program[13]. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Absorption of yolk sac and production traits: It has been shown in our previous study that the absorption index and feed conversion ratio were correlated with early access to feed, while fasting of newly hatched chicks for more than 48 h had a negative effect on growth rate, body weight gain, feed consumption and feed conversion ratio[14]. These factors play an important role in the initiation of development of the digestive tract[15]. As a result of these different treatments, the digestive tract was highly developed in groups that received early access to feeding, which means there was active peristaltic movement of the intestine. This trend plays an important role in the transport of yolk to blood circulation because of highly functioning absorptive villi, which showed a linear correspondence with feeding time. This result provides an explanation for the high level of immunoglobulin in chicks that received early access to feed, since the yolk contains maternally derived Abs (IgY)[16,17].

Immuno-pathological study: The maternally derived antibodies (MDA_s) were 292±81 at 1 day old. These antibodies play an important role in the immune response to the vaccination process because the immunoglobulin will act as a neutralizing agent for the vaccine A_g[18]. Table 2 shows that G_1 had the highest Ab level at an age of 11 days (64±120), whereas at an age of 21 days, it was the lowest among the other groups (22.0±4.0). This is because, a highly functional digestive tract facilitates the passage of yolk to the intestine, where it travels to the blood stream. Figure 1 shows hyperplasia of crypt cells as well as an increase in villus length. The main constituent of the yolk is immunoglobulin, which will neutralize the primary dose of the administered vaccine due to functional absorptive villi. In addition, we had to wait for a long time to give the primary dose of the vaccine since high maternal immunity interferes with the vaccine. A similar approach was used by Faulkner *et al.*[19].

The G_2 showed an Ab titre of 30.0±60 at the age of 11 days, which was significantly increased ($p<0.05$) to 78.0±18.0 at an age of 21 days (Table 2). This result occurred because the Ab level at an age of 11 days does not interfere with the primary dose of the vaccine[20]. In addition, the functional status of the digestive tract is not as active as it is observed in G_1 and the villus length is shorter with no proliferative activity (Fig. 2). The G3 showed no significant difference from G2 and shared some of the characteristics of

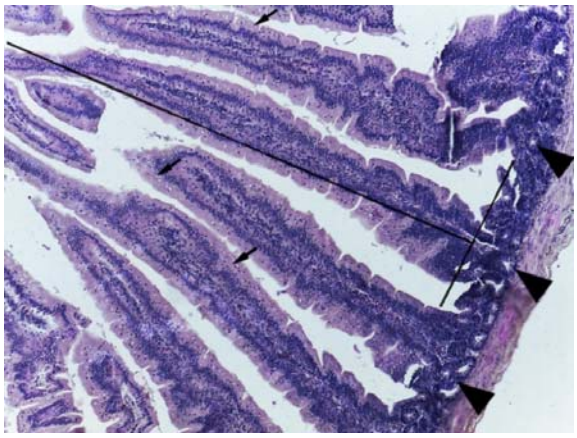

Fig. 1: Group 1: Highly functional digestive tract indicated by the length of absorptive villi and hyperplastic crypt cells (arrowhead) (14 days of age), H and E, X10

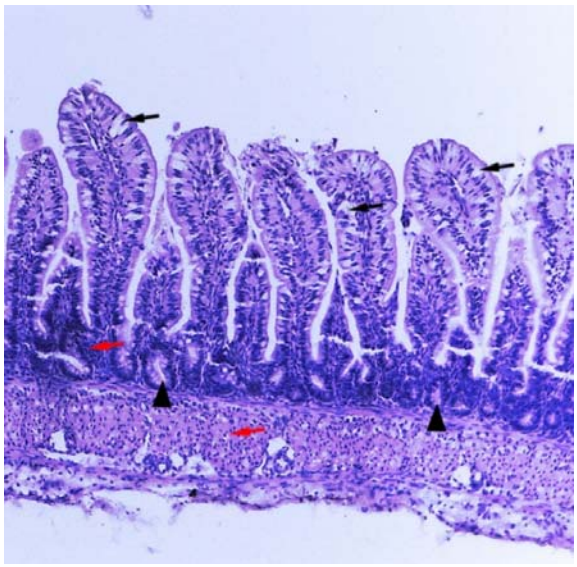

Fig. 2: Group 2: The villi are still functioning but to a lesser extent compared to G1. Their length is shorter and there is a considerable number of goblet cells (small arrow) (14 days of age), H and E, X10

Table 2: Different Ab levels in different groups, as estimated by an H.I. test at ages 11 and 21 days

Groups	11 days	21 days
1	$64.0±12.0^{A}$	$22.0±4.0^{B}$
2	$30.0±6.0^{B}$	$78.0±18.0^{A}$
3	$26.0±8.0^{B}$	$36.0±5.0^{B}$
4	$50.0±6.0^{AB}$	$71.0±16^{A}$

The different capital letters refer to significant differences between different groups at ($p<0.05$)

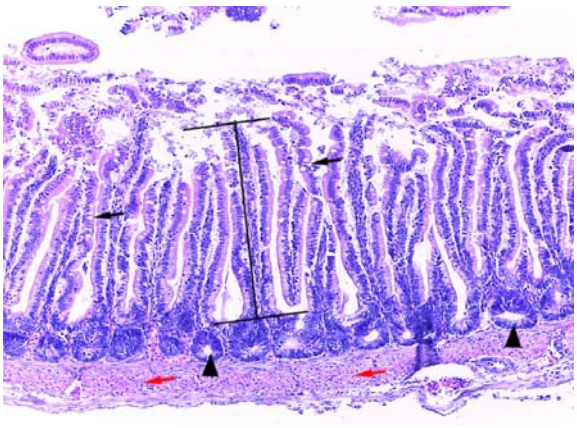

Fig. 3: Group 3: Newly formed crypt cells (arrowhead) indicate a regenerative process and an area of congestion in the mucosa. Villi are truncated at their tips. The muscular layer showed area of congestion (red arrow) (14 days of age), H and E, X10

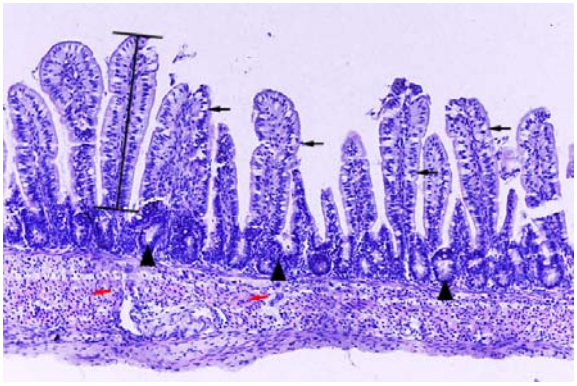

Fig. 4: Group 4: Regenerative process in absorptive villi (small arrow), including hyperplasia of crypt cells (arrowhead), increase in the number of goblet cells (small arrow), a congested muscular layer (red arrow) and inflammatory cells infiltration (14 days of age), H and E, X10

G4, especially for the level of the Ab titre (50.0±8.0). In addition to the proliferative capacity and regenerative process in the epithelial mucosa, some villi are truncated and atrophied (Fig. 3). The muscular layer showed an area of congestion. This outcome shows that there was low absorptive capacity due to a low value for the Ab titre, since maturation of the immune system would be impeded if the gut did not develop[21,22]. The G4 showed a significant increase ($p<0.05$) in Ab titre as compared to G3 and G1 and this outcome could be attributed to the regenerative process in absorptive villi due to crypt hyperplasia to compensate the loss in body weight due to early deprivation (Fig. 4). This result agrees with the findings of Gonzales *et al.*[23]. There were inflammatory cells in the muscular layer and an increase in the number of goblet cells.

Table 3: Different Ab levels in different groups, as estimated by an H.I. test at an age of 31 days

Groups	31 days
1a	512.0 ± 256^{A}
1b	2.0 ± 2^{C}
2	128.0 ± 24^{BC}
3	280.0 ± 83.0^{B}
4	310.0 ± 56^{AB}

The different capital letters refer to significant differences between different groups at ($p<0.05$)

Table 4: Mortality rate after a challenge with a local virulent Newcastle disease virus at 32 days old

Groups	Percentage
1a	5.5 ± 0.09^{B}
1b	88.0 ± 1.90^{A}
2	0.0 ± 0.00^{D}
3	3.7 ± 0.15^{C}
4	0.0 ± 0.00^{D}

The different capital letters refer to significant differences between different groups at ($p<0.05$)

Table 3 shows that all groups possess a protective level of Ab against a Newcastle disease virus at an age of 31 days with the exception of G1b (negative control), which was 2±2 and that result may be due to the contagious characteristics of the LaSota strain[24]. Birds in the G1b that resist challenge virus, despite the low Ab titre (2±2) might be due to the presence of a nonspecific inhibitor in their serum and this result agreed with a previous study[25], who suggested that Ab level might not represent the immune status of the bird.

Challenge test: Table 4 shows that all groups possess high protective level with a challenge virus. The mortality rates were 5.5±0.09, 0.0, 3.7±15 and 0.0±0.0% in G1a, G2 and G3 and G4, respectively. The G1b differs significantly ($p<0.05$) from other groups with a mortality rate of 88±1.9%. This discrepancy can be explained by the low Ab level titre that represents maternal immunity.

Post-mortem examination of the dead birds revealed ulcerative lesions in a different part of the digestive tract, especially in the lower third of the ileum, as well as in different parts of the payer's patches and caecal tonsils. This outcome provides an indication that the current strain in our country is a highly velogenic viscerotropic virus (Fig. 5, 6), which has been explained by other researchers[25,26].

Fig. 5: Hemorrhagic ulcer in the lower third of the ileum

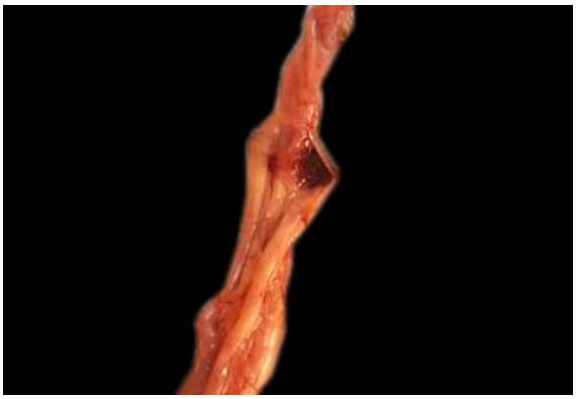

Fig. 6: Necrotic ulcer in the caecal tonsils

CONCLUSION

The study concluded that a post-hatched deprivation period is critical and had an important role in the development of the digestive and immune system for a period of 2 weeks. Re-feeding likely initiates palatability, which causes compensatory growth but does not attain the same production traits as animals that received direct access to feed. The immune response showed a protective level against a challenge with a field strain in all groups in the experiment. From the results, we recommend that feeding and its effect on growth performance should not be used at the expense of the immune status of the bird and the ideal deprivation period in endemic places should not exceed more than 24 h.

SIGNIFICANCE STATEMENT

Most poultry producers believe that it is necessary to deprive chicks for the first 48 h to prevent adverse effects of the retained yolk sac. This study shows that deprivation has a negative impact on production traits, including decreased body weight and in addition, most researchers confirmed that there is a reciprocal relationship between body weight and the immune response. This study indicated the adverse effect of deprivation is critical during the first 3 weeks and because of the regenerative process, all treated and non-treated groups had the same immune levels at the end of experimental period. Thus, the theory could help poultry producers keep chicks free from diseases while enhancing their immune response.

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