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Research Article

Stress Levels, Mortality, Intestinal Morphometry and Histomorphology of Chabro Broiler Birds Subjected to Varying Degrees of Post Hatch Delay in Feeding

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Abstract

Background and objective: Kashmir is a part of Jammu and Kashmir State of India where a large chunk of day old chicks are procured from outside the state and these chicks are transported across a distance of hundreds of kilometers over a period of several days. The long distance transport without any access to feed not only subjects the birds to early life stress but also affects their gut morphological development. Therefore, a study was conducted to evaluate the effect of delayed feeding on stress levels, mortality, intestinal morphometry and histomorphology of chabro broiler chicken. **Materials and Methods:** A total of 400 day old chabro chicks were randomly divided into 5 groups, each group comprising of four replicates of 20 birds. Chicks allotted to group-1 (G_1) were offered feed at hatchery itself whereas feeding in groups G_2, G_3, G_4 and G_5 were initiated at the farm after the delay of 12, 24, 48 and 72 h, respectively. **Results:** The results revealed that the heterophil count showed a steady increase from G_1-G_5 and significantly ($p<0.05$) higher count was found in G_3, G_4 and G_5 compared to G_1. However, the lymphocyte count showed a steady decrease as delay in feeding increased. H:L ratio followed a regular increase from G_1-G_5 and was significantly ($p<0.05$) higher in G_3, G_4 and G_5 compared to G_1. An overall mortality of 13% was recorded during the trial and all of it occurred during first 2 weeks. The highest overall mortality of 22.50±3.23% was recorded in group G_5 followed by 17.50±6.61% in G_4. The villus height of duodenum and jejunum was significantly ($p<0.05$) higher in G_2, G_3, G_4 and G_5 as compared to G_1. Crypt depth and muscular is thickness of duodenum was significantly ($p>0.05$) lower in G_4 and G_5 compared to G_1. **Conclusion:** The duration of post hatch feeding delay gradually increased the stress level and mortality of birds and also decreased the length of different segments of small intestine in birds. No adverse effect on histomorphology was observed at the end of trial. Feeding at hatchery itself or feeding during transportation of birds would be a viable strategy to overcome the negative effects of delayed feeding in chicken.

Key words: Chabro chicken, delayed feeding, histomorphology, intestinal morphometry, stress

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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

In commercial operations, chicks hatch over a period of time and are only taken out of the incubator once the maximum number of the birds have hatched out[1]. Most of the birds may have to even wait up to 2 days in the hatchery and also in the transit to the farms before they are given access to food and water[2]. Sexing, vaccinations and transportation of chicks further increases their delay in access to feed[3]. Moreover, there is an inevitable spread of 30-48 h in a hatch for late versus early hatchers[4]. This delay in initial access to feed and water can have lasting negative effects on birds[5] as the magnitude of growth a broiler chicken attains indicates that every day in the life of a broiler chicken is very important[6]. In order to maximize the genetic potential and profitability of fast growing broiler chicken, post hatch feeding delay access must be minimized as possible[7]. The detrimental effects of delayed feeding in neonatal chicks could be overcome by reducing the transport time to the poultry farm, so as to provide them early access to feed[8]. The negative effects include reduction in growth and increase in early mortality, primarily due to dehydration and shortage of available energy[9]. Further, it causes immunosuppression and retarded gastrointestinal development in birds[10].

The time period from the hatch of first chick to the hatch of the last chick (known as hatch window) may range from 24-48 h or more[11]. Several factors have been reported to influence hatch window viz., age of breeder flock, egg characteristics, sex of the embryo and length and temperature of egg storage before incubation. It has been reported that chicks from old aged breeder hens hatch earlier than those from young breeder hens[8]. Likewise, Ulmer-Franco *et al.*[12] suggested that chicks from smaller or lighter eggs may hatch earlier than chicks from larger or heavier eggs. Moreover, female chicks have been reported to hatch earlier than male chicks[13]. Early hatching of chicks from eggs stored for short periods of time compared to eggs stored for a week or more has been documented[14]. Similarly, storage temperature affect hatch window for eggs stored for longer than 3 days[15].

Kashmir is a part of Jammu and Kashmir State of India. Out of 140 lac broiler chicken raised in Kashmir valley annually[16], a large chunk of day old chicks are procured from outside the state and these chicks are transported across a distance of hundreds of kilometers over a period of several days. The long distance transport without any access to feed not only subjects the birds to early life stress but also affects their gut morphological development. In view of the negative impacts of post-hatch delay in feeding, a study was conducted to evaluate its effect on stress levels, mortality, intestinal morphometry and histomorphology of chabro broiler chicken.

MATERIALS AND METHODS

Experimental site: The study was conducted in the Teaching and Research Farm of Center for Research on Poultry, Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama.

Birds and experimental design: The experiment was carried out on relatively slow growing meat type chabro chicks procured from a local hatchery. Chabro is a broiler bird with colored plumage developed by Central Poultry Development Organisation, Northern Region. It attains a body weight of 1400-1500 g at 7 weeks of age with FCR[17] of 2.48. A total of 400 day old chabro chicks were procured. The chicks were randomly divided into 5 groups, each group comprising of four replicates of 20 birds. Chicks allotted to group-1 (G_1) were offered feed at hatchery itself whereas feeding in groups G_2, G_3, G_4 and G_5 were initiated at the farm after the delay of 12, 24, 48 and 72 h, respectively. The chick boxes were transported from the hatchery to the poultry farm where the chicks were brooded and reared up to 6 weeks of age in deep litter system using saw dust as litter material. The chicks were housed replicate wise in separate pens providing a floor space of one square feet per chick. Feed (commercially available pre-starter and starter mash) and water was offered *ad libitum*. Standard management and healthcare (vaccination) protocol was followed.

Parameters recorded

Stress levels and mortality: Stress levels was estimated on the basis of heterophil:lymphocyte (H:L) ratio. A drop of blood was collected randomly from 10 birds from each treatment group at the time of initiation of feeding and a smear was formed on glass slide. The smear was air dried and fixed with methanol. Later it was stained using Wright Giemsa stain and subjected to Differential Leukocyte Count (DLC) from which H:L ratio was worked out. Individual pens were inspected daily and mortality if any was recorded. Moreover, individual pens were inspected daily and mortality if any was recorded.

Intestinal morphometry and histomorphology: The intestines of the slaughtered birds were collected and

duodenum, jejunum and ileum lengths were measured (cm) using measuring scale. Tissue samples from these parts were preserved and subjected to histomorphology. The intestinal samples were processed by paraffin embedding technique for histo-morphology. The formalin fixed intestinal samples were handled for processing in capsules labelled with identification number and processed by routine technique. Briefly, the samples were washed under running tap water for 6-8 h, dehydrated in acetone, three changes of 20 min each, followed by clearing in benzene (100%), two changes of 20 min each. Paraffin impregnation was achieved by giving one change in benzene-paraffin (1:1) [melting point of paraffin 54°C] for 20 min followed by three changes of 1 h each in paraffin wax maintained at 56°C in oven. Casting of blocks was carried out using 2 L shaped moulds which facilitates manipulation of size as per the requirement. The tissue was placed in the blocks so as to facilitate sectioning across all layers. The blocks were labeled alongside for identification. The paraffin blocks were cut using rotary microtome. Approximately 60 sections of 5-6 µm thickness were cut per sample and every fifth section was retained for staining. Twelve sections per samples were stained with Harris' haematoxylin and eosin method[18]. The slides were observed under the microscope equipped with the camera for photomicrography. The microscopic images recorded were analysed using video test-5 image analyzing software calibrated for the purpose.

Ethics statement: All procedures performed in this study involving birds were approved by Institutional Animal Ethics Committee.

Statistical analysis: The data obtained was statistically assessed by one-way ANOVA as per the standard methods of Snedecor and Cochran[19] using the general linear model procedure of Statistical Package for the Social Sciences, Base 10.0, 1999 (SPSS Software products, Marketing Department, SPSS Inc. Chicago, USA). To test the significance of difference between means Duncan's multiple range test[20] was used and differences were considered significant at 5% level.

RESULTS

Stress level of chabro birds: Stress levels in terms of heterophil:lymphocyte (H:L) ratio is given in Table 1 and Fig. 1. The heterophil count showed a steady increase from G_1-G_5 and significantly ($p<0.05$) higher heterophils were found in G_3, G_4 and G_5 compared to G_1. However, on the other hand the

Table 1: Effect of delayed feeding on heterophil, lymhocyte count and ratio in chabro chicken

Parameters	G_1 feeding soon after hatch	G_2 12 h delay in feeding	G_3 24 h delay in feeding	G_4 48 h delay in feeding	G_5 72 h delay in feeding
Heterophil count	26.90±0.82^a	27.56±1.48^a	38.44±1.69^b	43.40±1.37^c	50.20±1.67^d
Lymphocyte count	69.40±1.40^c	68.67±1.53^c	55.33±2.00^b	52.90±1.02^b	46.00±1.52^a
H:L ratio	0.39±0.02^a	0.41±0.03^a	0.71±0.04^b	0.83±0.04^b	1.11±0.06^c

Means across the columns in a particular row bearing similar superscript did not differ significantly ($p<0.05$)

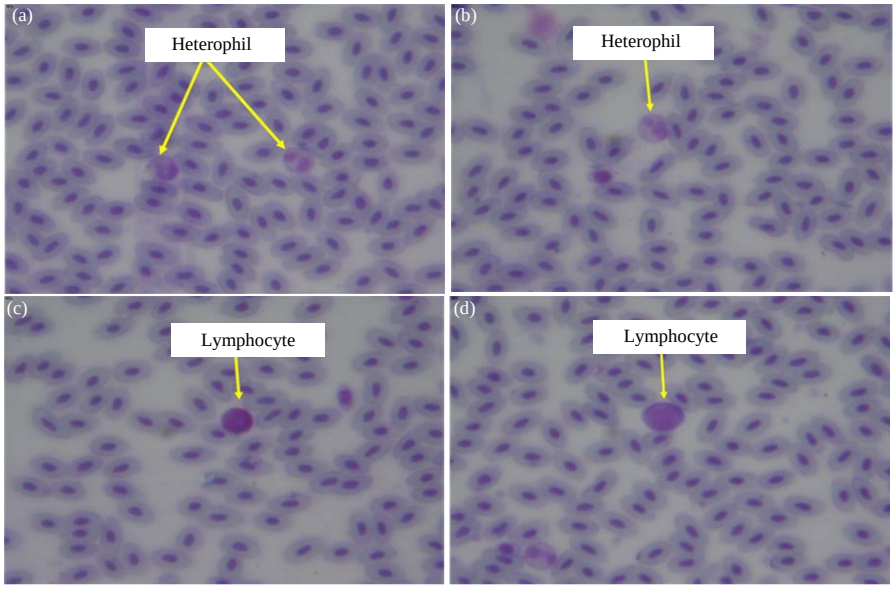

Fig. 1(a-d): Heterophils and lymphocytes in blood smears of chabro chicken [Wright Giemsa X1000 (OZx4)]

Table 2: Effect of delayed feeding on mortality pattern of chabro chicken

Weeks	G_1 feeding soon after hatch	G_2 12 h delay in feeding	G_3 24 h delay in feeding	G_4 48 h delay in feeding	G_5 72 h delay in feeding
0-1	2.50±1.44^{a}	3.75±1.25^{a}	5.00±0.00^{a}	11.25±6.25^{a}	16.25±3.15^{b}
1-2	6.25±4.73	8.75±3.15	0	6.25±3.15	6.25±3.75
2-3	0	0	0	0	0
3-4	0	0	0	0	0
4-5	0	0	0	0	0
5-6	0	0	0	0	0
0-6	8.75±5.54^{ab}	12.50±3.23^{ab}	5.00±0.00^{a}	17.50±6.61^{ab}	22.50±3.23^{b}

Means across the columns in a particular row bearing similar superscript did not differ significantly (p<0.05)

Table 3: Effect of delayed feeding on intestinal morphometry (cm) of chabro chicken

Parameters	G_1 feeding soon after hatch	G_2 12 h delay in feeding	G_3 24 h delay in feeding	G_4 48 h delay in feeding	G_5 72 h delay in feeding
Duodenum	24.63±0.42^{b}	24.63±0.42^{b}	22.63±0.68^{ab}	21.00±0.38^{a}	21.00±0.58^{a}
Jejunum	67.38±1.27^{c}	64.38±2.74b^{c}	61.50±1.88^{abc}	60.63±1.45^{ab}	57.00±2.35^{a}
Ileum	64.88±1.36^{c}	60.25±1.74b^{c}	57.38±1.93^{b}	56.63±1.18^{ab}	52.00±0.41^{a}

Means across the columns in a particular row bearing similar superscript did not differ significantly (p<0.05)

Table 4: Effect of delayed feeding on villus height (μm) of chabro chicken

Parameters	G_1 feeding soon after hatch	G_2 12 h delay in feeding	G_3 24 h delay in feeding	G_4 48 h delay in feeding	G_5 72 h delay in feeding
Duodenum	1114.82±17.18^{a}	1245.36±9.60^{b}	1336.71±16.61^{c}	1404.59±19.95^{d}	1267.54±57.75^{b}
Jejunum	1024.73±11.63^{a}	1122.12±16.19^{b}	1193.52±15.13^{c}	1272.82±8.02^{d}	1140.82±13.39^{b}
Ileum	830.96±18.02	850.76±5.81	837.71±8.12	849.56±8.24	827.42±9.31

Means across the columns in a particular row bearing similar superscript did not differ significantly (p<0.05)

Table 5: Effect of delayed feeding on crypt depth (μm) of chabro chicken

Parameters	G_1 feeding soon after hatch	G_2 12 h delay in feeding	G_3 24 h delay in feeding	G_4 48 h delay in feeding	G_5 72 h delay in feeding
Duodenum	172.18±1.95^{b}	171.83±1.74^{b}	174.12±6.81^{b}	159.57±9.64^{a}	158.83±9.56^{a}
Jejunum	150.86±1.45	152.08±1.46	151.68±1.63	155.75±1.67	152.93±2.26
Ileum	154.83±1.37	154.60±1.27	155.23±1.14	155.32±2.48	157.34±2.43

Means across the columns in a particular row bearing similar superscript did not differ significantly (p<0.05)

Table 6: Effect of delayed feeding on muscularis thickness (μm) of chabro chicken

Parameters	G_1 feeding soon after hatch	G_2 12 h delay in feeding	G_3 24 h delay in feeding	G_4 48 h delay in feeding	G_5 72 h delay in feeding
Duodenum	169.40±2.28^{b}	153.34±7.47^{a}	168.22±2.60^{b}	159.87±2.54^{ab}	163.97±2.53^{ab}
Jejunum	155.75±2.24	156.58±2.66	152.72±2.67	152.91±2.26	159.62±2.67
Ileum	144.14±2.02	143.06±2.43	142.86±2.27	144.04±2.49	143.86±1.96

Means across the columns in a particular row bearing similar superscript did not differ significantly (p<0.05)

lymphocyte count showed a steady decrease as delay in feeding increased. The H:L ratio was found to be 0.39±0.02, 0.41±0.03, 0.71±0.04, 0.83±0.04 and 1.11±0.06 in groups G_1, G_2, G_3, G_4 and G_5, respectively. It followed a regular increase from G_1-G_5 and was significantly (p<0.05) higher in G_3, G_4 and G_5 compared to G_1.

Effect on mortality: The results of mortality in various chabro birds subjected to different durations of post-delay hatching are shown in Table 2. An overall mortality of 13% was recorded during the trial and all of it occurred during first 2 weeks. A mortality of 7.75% in 1st week and 5.5% in 2nd week was recorded. The highest overall mortality of 22.50±3.23% was recorded in group G_5 followed by 17.50±6.61% in G_4. In all the groups mortality whatsoever occurred in 1st 2 weeks only and no mortality was recorded in any of the groups from 3 weeks onwards. Between the groups significantly higher mortality was recorded during the 1st week in group G_5 (16.25±3.15%) followed by G_4 (11.25±6.25%) and least in G_1 (2.50±1.44%).

Intestinal morphometry and histomorphology of birds: Table 3 depicts the morphometric results of different parts of small intestine. Duodenum and jejunum length showed a steady decrease from G_1-G_5. Although G_2 and G_3 showed no significant difference, the groups G_4 and G_5 were having significantly lower duodenal and jejunal length respectively as compared to G_1. Similarly, length of ileum also followed a steady decreasing trend from G_1-G_5. Though G_2 did not show any significant difference from G_1, however, G_3, G_4 and G_5 had significantly (p<0.05) lower length of ileum as compared to G_1.

The effect of delayed feeding on histomorphological changes in different intestinal segments of chabro birds is presented in Table 4-6 and Fig. 2. The villus height of

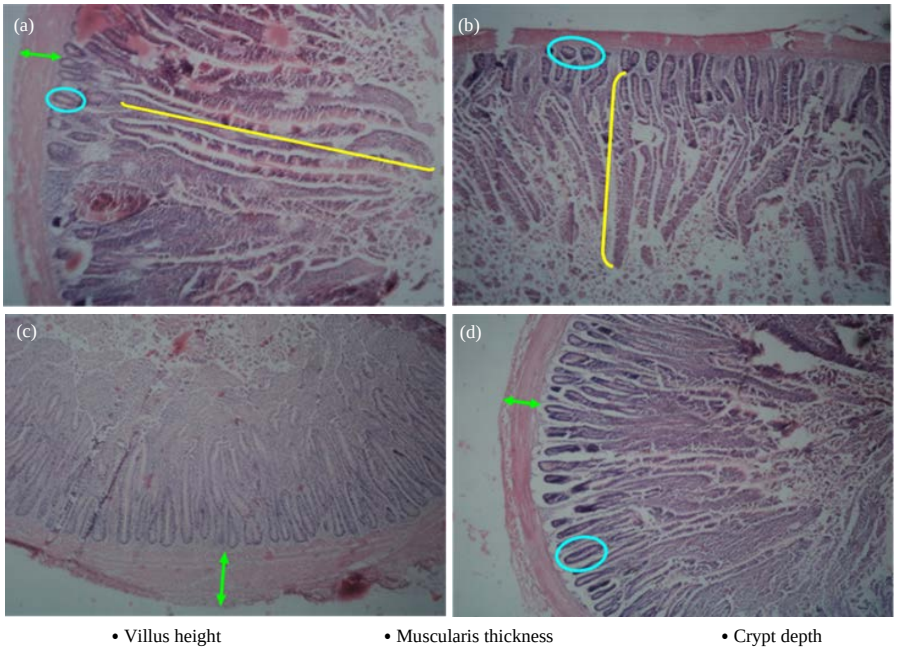

Fig. 2(a-d): Small intestinal histomorphology of chabro chicken, (a) Treatment fed soon after hatch was pulled out, (b) 24 h delay in feeding, (c) 48 h delay in feeding and (d) 72 h delay in feeding {H.EX40 (OZx3)}

duodenum and jejunum was significantly ($p<0.05$) higher in G_2, G_3, G_4 and G_5 as compared to G_1. However, the villus height of ileum did not show any significant ($p>0.05$) difference among various groups. Crypt depth and muscularis thickness of duodenum was significantly ($p>0.05$) lower in G_4 and G_5 compared to G_1, however, the jujenal and ileal crypt depths and muscularis thickness showed no significant ($p>0.05$) difference among various treatment groups.

DISCUSSION

The heterophil lymphocyte ratio has been reported to be good indicator of different types of stress including the feed deprivation[21]. Increase in the H:L ratio in the present study as a result of progressive increase in the delay of feeding is suggestive of increased stress levels in the birds. Cengiz *et al.*[22] also found that delayed feed access significantly increased the H:L ratio at day 6. Chicks that had been deprived of feed for 48 h post-hatch had less lymphocyte synthesis after 72 h, lower bursa weight at 21 days of age, retarded lymphoid development at 21 days of age and lowered disease resistance[10]. Feed deprivation during first 12 h reportedly has no effect on immune response of layer chicken[23]. Bursa of Fabricius (Bursa) is an immune organ unique to avian species that produces antibodies in response to pathogen invasion[24]. Furthermore, 48 h of post-hatch delayed feeding lowers the immune capacity of broilers up to 42 days of age by way of reduced humoral and cellular immune capacity[5].

The present study revealed that delayed feeding resulted in higher mortality and it was significantly higher in groups with increased extent of delay in feeding. Delayed access to water and feed post-hatch dehydrates the chicks[25] resulting in depressed immune response[26], increased early mortality[27]. Very high mortality to the extent of 60% was also reported by Mbajiorgu *et al.*[28] in the broiler chicken subjected to feeding delay of 36 h between 1-3 days of age. The severe shortage of energy in the birds subjected to feed and water deprivation for 48 h causing alterations in body composition has been suggested as a reason for high mortality in turkey poults[29].

All the segments appeared to be shorter in the groups subjected to delayed feeding as compared to control and more the duration of delay shorter was the intestinal length. It has been demonstrated that the intake of exogenous feed can stimulate the growth and development of gastrointestinal tract in newly hatched chicks. It has been reported that when chicks were subjected to delayed feeding of 24-72 h, GIT growth was stunted[30]. Gonzales *et al.*[31] also reported that the delayed feeding delays GIT development. Maiorka *et al.*[32] also suggested the need to feed chicks immediately after hatch to ensure proper development of gastrointestinal tract[33]. Similarly, birds that had access to feed immediately after hatch have been reported to exhibit more rapid development of the

intestine during immediate post-hatch period[34]. Other studies in turkey poults have also indicated that early access to feed is critical for the development of intestinal tract[35].

In the present study, the histomorphology of different segments of small intestine did not reveal any harmful effect of delayed feeding on villus height, crypt depth or muscularis thickness except for some changes in the duodenum but those too did not follow any definitive trend. This is in contrast to the results of Maiorka *et al.*[32] who reported that feed deprivation of chicks depressed the intestinal villi, enterocyte proliferation and development of mucosal layer. Ganjali *et al.*[8] reported that villus height of duodenum and jejunum at 0, 2, 4, 8 and 12 days of age in broiler chicken decreased significantly. Similarly, Shinde *et al.*[23] reported that delay in feeding resulted in reduced duodenal, jejunal and ileal villus height in layer chicken at 36 h and 7 days post hatch. However, no such results were found in the present study which might be due to the fact that the histomorphological study was performed on 6 week old chicks and it appears that the difference if any at histomorphological level might have got masked with age. To our surprise, the villus height of duodenum and jejunum increased in 6 week old birds in all the groups subjected to delayed feeding, thus further studies in this regard are warranted.

CONCLUSION

It could therefore be concluded that with increase in the duration of post hatch feeding delay, the stress levels increased gradually and so did the mortality. A progressive decrease in the length of different segments of small intestine was noticed with increase in the duration of post hatch feeding delay. However, no adverse effect on histomorphology of small intestine was observed at the end of 6 week trial. In view of negative effects of delayed feeding on broiler chicken, early feeding strategies which may include hatchery feeding or feeding during transportation of birds need to be developed.

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