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## Delayed post-hatch feeding affects the performance and immunocompetence differently in male and female broiler chickens

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ICAR – Central Avian Research Institute, Bareilly, India

### ABSTRACT

The effect of post-hatch (PH) feed deprivation for 6, 12, 24 and 36 h was studied in male and female broiler chickens. At 21 d, lower body weight (BW) was recorded in 36 h feed-deprived (FD) birds; however, at 42 d PH, only 36 h FD female birds had lower BW compared to control and other FD birds. Feed intake during 0–21 d PH was lower in 36 h FD birds, but feed conversion ratio did not differ between control and FD birds. The H:L ratio significantly increased in 12–36 h FD male birds and 24–36 h FD females. The humoral immune response was similar in FD and control birds, but the cellular immune response was higher in 12 and 24 h FD female birds. At 36 h the expression of interleukin 6 (IL-6), toll-like receptor-2 (TLR-2) and tumour necrosis factor- $\alpha$  gene was down-regulated in male birds only. However, the expression of IL-6 and TLR-2 was up-regulated in 12–36 h FD female birds at 7 and 14 d PH. It may be concluded that PH feed deprivation for the first 24 h did not affect growth performance but improved immune response in slow-growing broiler females.

### ARTICLE HISTORY

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### KEYWORDS

Broiler chickens; cytokines; feed deprivation; growth; immunocompetence

### 1. Introduction

It has been a common practice in every hatchery where all the chicks are generally pulled out when majority of them hatched. This causes the early hatched chicks to be deprived of feed for a longer duration. Besides this, other hatchery and management operations such as sex determination, vaccination, packaging and transportation to farm are also responsible for the delay in feeding (Batal and Parsons 2002). An increased post-hatch (PH) holding period has detrimental effects on chicks due to potential dehydration and energy depletion. Delayed placement along with delayed feeding of chicks was related to lower weight gain and higher mortality, poor response to vaccination, slow gastrointestinal tract and immune system development, poor resistance to diseases and pathogens, and adverse effects on long-term performance in newly hatched chicks (Panda et al. 2015). Early feed intake (FI) is one of the factors responsible for early development of immune system in newly hatched chicks. Feed provides nutrients which are essential for the growth and development of both primary and secondary lymphoid organs. The immune system of hatchlings, particularly the mucosal immune system, also requires oral FI for rapid development. PH feed restriction is harmful to the immune system (Juul-Madsen et al. 2004) as well as to the growth of the birds (Noy and Sklan 1999).

During the first week PH, intestines increase their weight more quickly than the body mass, which is accompanied by elevation in numbers of enterocytes due to the dramatic increase in villus length. Feed deprivation for more than 24 h decreases the villi height significantly, thus affecting the enter-

ocyte population (Geyra et al. 2001). A rapid development of the gut-associated lymphoid tissue (GALT) occurs concomitantly with the development of digestive structures and functions. The intestinal epithelial lining (IEL) has a diverse population of lymphocytes including natural killer (Gobel et al. 2001), T cell receptor  $\alpha\beta$  and T cell receptor  $\gamma\delta$  (Lillehoj and Chung 1992) bearing cells. The major T cell populations in the IEL are further divided according to the T cell co-receptors CD4 and CD8. These innate populations affect immunity by the immediate release of cytokines following activation (Lillehoj et al. 2004).

We hypothesized that delayed feed placement will affect the maturation of the general or GALT-associated immune system in the birds. In the present study, we have evaluated the effects of PH feed deprivation on PH performance, the development of immune organs such as bursa of Fabricius and spleen, *in vivo* immune response and expression profiles of cytokines/chemokines associated with the immunocompetence of broiler chickens.

### 2. Materials and methods

#### 2.1. Experimental design, birds and treatments

For this study, fertile eggs were collected from coloured synthetic broiler parent line females (35 weeks old) over a period of 7 days and set in a forced draft incubator. The coloured broilers have been developed by selective breeding and crossing of Cornish, Plymouth Rock and New Hampshire birds based on their economic traits. The base populations were stabilized after two generation of cross-breeding and two generations of

random mating. The experimental design was a completely randomized design with five treatments, eight replications per treatment and eight birds (equal sex) per replication with a similar group mean weight. A total of 320 sexed broiler chicks from a single hatch were randomly assigned to 40 cages (45 × 60 × 37.5 cm each) in a four-tier battery brooder housed in a naturally ventilated open-sided room. The average initial body weight (BW) of the chicks was 43.09 ± 0.22 g. Each pen was equipped with a feeder and waterer placed outside the cage. The initial brooding temperature was 32.0°C, but this was gradually reduced to 24 ± 1°C at 21 d PH. A light period of 24 h was maintained throughout the trial period. Access to feed and water was *ad libitum* throughout the trial period. All management and procedures in this study were carried out as per the code of practice approved by the Institute of Animal Ethics Committee. The experimental treatments included immediate access to feed (control) and delayed feed placement at 6, 12, 24 and 36 h PH. Broiler starter mash [22.0% crude protein (CP); 2950 kcal/kg of metabolizable energy (ME)] and finisher mash (20.0% CP; 3050 ME/kg) as per the standard of ICAR (2013) were offered to the birds for a period of 42 days.

## 2.2. Response criteria

The BW of individual chicks and the FI of all the birds in a pen (replicate wise) were recorded at 7 d intervals throughout the experimental period. The feed conversion ratio (FCR) was calculated for 0–21 d, 22–42 d and the overall period (0–42 d) by dividing the feed consumption by the corresponding weight gain. Mortality of the chicks (if any) during the experimental period was noted and expressed as percentage of total chicks reared in the particular treatment group.

## 2.3. Haematological parameters

Blood samples from the jugular vein of six birds (both the sex) were collected at 36 h PH in plastic vials containing Ethylenediaminetetraacetic acid (15 µl/ml blood) kept in ice. The haematological parameters such as per cent haemoglobin (Hb) and packed cell volume (PCV) were obtained manually by the Sahli haemoglobinometer and micro-haematocrit method. To obtain the heterophil to lymphocyte ratio, one drop of blood was smeared on a glass slide. The smear was stained using Giemsa stains (Lucas and Jamroz 1961) and fixed approximately 1–2 h later by the application of methyl alcohol. One hundred leukocytes, including granular (heterophils, eosinophils and basophils) and non-granular (lymphocytes and monocytes), were counted on every slide (duplicate each for six broiler chicks) under the microscope at 100×, and then heterophil to lymphocyte ratio was calculated following the method described by Gonzales et al. (2003).

## 2.4. Immune responses

### 2.4.1. Humoral response

Sheep red blood cells (SRBCs) suspended in Alsever's solution were washed three times in an isotonic phosphate-buffered saline (PBS: pH 7.2) using centrifugation (700 g) and adjusted to

provide a 1% suspension, (v/v) which was stored at 4°C prior to use. The 28-d-old chicks (12 birds/treatments and 6 from each sex) were injected intravenously with 1 ml of the SRBC suspension. Blood sample (2 ml) was obtained from the jugular vein of each chick five days later. Each blood sample was allowed to clot for serum collection and sera were stored at –20°C until analysis. The antibody response to SRBC was determined using a standard haemagglutination assay (Siegel and Gross 1980). The reciprocal of highest dilution showing a clear agglutination was the end point of titre and the values were expressed as log 2.

### 2.4.2. Cell-mediated immune response

The cellular immune response was assessed in 21-d-old chicks (12 birds/treatments and 6 from each sex) using the *in vivo* cutaneous basophilic hypersensitivity response to the mitogen phytohaemagglutinin from *Phaseolus vulgaris* (PHAP). The toe web thickness, between the third and fourth digits, of both the left and right feet was measured using a micrometer. Immediately afterwards 100 µg PHAP, dissolved in 0.1 ml PBS, was injected into the same inter-digital space of the right foot. The toe web of the left foot was used as the control and injected only with PBS. The inflammatory response was determined 24 h later by measuring the thickness of the respective toe webs and subtracting the earlier measurements: values for the right toe web were compared with those for the left (control) to determine the response (Corrier and DeLoach 1990).

## 2.5. Immune organ development, tissue collection and immune gene expression

At 36 h, 7 and 14 d PH, six birds (both the sex) from each treatment group were killed by cervical dislocation. The weight of bursa, spleen and thymus were recorded and expressed as per cent of live weight. Approximately 150 mg of spleen tissue was collected from each bird and total RNA isolation and cDNA synthesis were carried out as per the procedure of Bhanja et al. (2014). The oligonucleotide sequences of the primers used for the gene expression study are presented in Table 1. The expression of immune-related gene encoding *IL-6* (interleukin 6), *TLR-2* (toll-like receptor-2) and *TNF-α* (tumour necrosis factor) was analysed by real-time PCR, using an iQ5 cyclor following a previously described procedure (Bhanja et al. 2014).

The relative expression ratio (ER) of each target gene was computed, based on its real-time PCR efficiencies (E) or a static efficiency of 2, and the cycle threshold (Ct) difference (Δ) of mean control versus each unknown sample (ΔCt control – treatment) as described below (Pfaffli 2001) using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as the reference housekeeping gene:

$$ER = \frac{(E \text{ target})^{-\Delta C_t \text{ target (control-treatment)}}}{(E \text{ ref})^{-\Delta C_t \text{ ref (control-treatment)}}}$$

## 2.6. Statistical analysis

The statistical analysis and interpretation of data were done using the SPSS software package Version 16.0 (2007). The response criteria, haematological parameters and immune response were

**Table 1.** Oligonucleotide sequence of immunity-related gene primers.

S no.	Gene	Primer (5'-3')	Annealing temp. (°C)	Size of amplicon (bp)	Putative biological role	Accession number
1	<i>IL-6</i>	F-gaaatccctctcgcaatctga R-tgaaacggaacaactgcatct	57	281	Humoral immunity	NM001007079
2	<i>TLR-2</i>	F-gtggccatgtcgatcagagaac R-tcagcggagagtcacagatgtagc	56	202	Innate immunity	NM_204278.1
3	<i>TNF-α</i>	F-agaccagatgggaagggaatgaa R-gaagagggccaccacagacag	55	219	Humoral immunity	JN942589
4	<i>GAPDH</i>	F-ccgtctctctggcaaatgcc R-agccccagccttctccatg	57.5	266	Housekeeping	NM_204305

*IL-6*: interleukin-6; *TLR-2*: toll-like receptor-2; *TNF-α*: tumour necrosis factor-α and *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase.

analysed by one-way ANOVA. The mRNA expression levels (ER) of immunity-related genes were analysed by REST 2009 software. The difference in mean values was considered as significant at the level of 95% ( $P < .05$ ) and 99% ( $P < .01$ ).

### 3. Results

#### 3.1. PH growth response

There was no difference in male and female BW on the day of hatch (D0), but at 21 d PH both the male and female birds feed deprived (FD) for 36 h had significantly lower ( $P < .05$ ) BW than the control and those FD for 6 or 24 h. At 42 d, PH FD male birds had similar BW as that of control birds. The effect of feed deprivation was more prominent in 36 h FD female birds as they had about 76 g lower market weight ( $P = .005$ ) than the control birds. The FI of 36 h FD birds during 0–21 d PH was lower ( $P = .004$ ) compared to that of the control and other FD birds. However, FI in the FD birds was similar to that of the control during 22–42 and 0–42 d PH. The FCR did not differ between the control and FD birds during 0–21, 22–42 and 0–42 d PH (Table 2). No mortality was recorded during the experimental period of 42 days.

#### 3.2. Haematological parameters

The effect of feed deprivation on the haematological parameters of 36-h-old male and female broiler birds is presented in Table 3. The male birds showed similar, but the female birds FD for 24 and 36 h had higher content of PCV ( $P = .002$ ) and Hb ( $P = .001$ ). Heterophil count and H/L ratio were higher ( $P < .05$ ) in 12–36 h FD male birds and in 24–36 h FD female birds.

#### 3.3. Immune organ weight and in vivo immune response

The immune organ weight (g/100 g of live weight) of the FD and control male and female birds at 36 h, 7d and 14 d PH is presented in Table 4. At 36 h PH male birds FD for 24 and 36 h had lower ( $P = .039$ ) spleen weight than the control birds. However, at 7 and 14 d PH, no difference was observed in the bursa, spleen and thymus weight of the FD and control birds. The antibody titre to SRBCs in both male and female birds was apparently higher, but not significant in 12 and 24 h FD birds compared to the control or 36 h FD birds. Foot web index in response to PHAP injection was significantly higher ( $P = .006$ ) in 12 and 24 h FD female birds, but similar in male birds when compared with that of the control birds (Table 5).

#### 3.4. Expression of immune-related genes

At 36 h PH, the relative expressions of *IL-6* was down-regulated (DR) in 12 and 24 h FD male and 12 h FD female birds; however, at 7 d PH the expression was up-regulated (UR) in 6 and 24 h FD female birds compared to the control birds. At 14 d PH, the expression was UR in 36 h FD male and 12 and 36 h FD female birds (Figure 1(a,b)).

At 36 h and 7 d PH, *TLR-2* expression was DR in most of the FD male birds, but reached a significant level in 6, 12 and 36 h FD birds. At 14 d PH, 36 h FD males had UR expression as compared to the control birds. The *TLR-2* expression was DR ( $P < .05$ ) in 6, 12 and 36 h FD female birds at 36 h PH; however, the expression was UR at 7 and 14 d PH (Figure 2(a,b)).

*TNF-α* expression was DR in FD male birds except in 6 and 12 h FD birds at 7 d PH, where it was UR ( $P < .05$ ). In female birds also *TNF-α* expression was DR at 36 and 7 d PH in FD birds, but at 14 d PH it was non-significantly UR in 24 and 36 h FD birds (Figure 3(a,b)).

### 4. Discussion

#### 4.1. PH growth response

Feed deprivation for the first 24 h did not affect the performance of male and female birds; however, 36 h FD females showed 6.36% lower market weight (at 42 d PH). An earlier study indicated that chicks fed immediately after hatch gained 10.5% more weight than those FD after 48 h (Bhanja et al. 2009). Previously in egg-type chickens, we had also found about 12.45 BW reductions in 36 h FD birds at 42 d PH (Shinde et al. 2015). Fasting reduces the intestinal villi height and surface area for the absorption of nutrients (Shinde et al. 2015) and this might be the reason for the reduction in the overall growth performance of the FD birds (Panda et al. 2010). The coloured broilers normally attain a BW in the range of 1400–1500 g at 42 days of age. Because of their low BW and plumage colour, there is a high demand for coloured broilers in the Indian subcontinent (Bhanja and Mandal 2007). In the present study, the BW of coloured broilers was lower than that reported by Bhanja and Mandal (2007). This may be due to the handling stress experienced during recording of immunological and haematological parameters or the season of study.

This also correlates with the feed consumption pattern as observed in 36 h FD birds. Similar findings were also reported by Tabeidian et al. (2011) and Pourreza et al. (2012), wherein it was found that a short period of FD (12 h) could impact the growth performance, FI and FCR in broilers. Research in broiler chickens and turkey poults reported a decrease in the

**Table 2.** Mean BW, FI and FCR of broiler birds subjected to 0, 6, 12, 24 and 36 h delay in PH feed placement.

Attributes	Delay in PH feed placement					SEM	P-value	
	Control	6 h	12 h	24 h	36 h			
<i>Body weight (g)</i>								
Day 0	Male	43.9	43.5	42.7	44.3	43.9	0.3	.494
	Female	42.3	43.8	43.7	42.8	44.1	0.32	.334
21 d	Male	503.1 <sup>c</sup>	504.7 <sup>c</sup>	472.9 <sup>ab</sup>	496.3 <sup>bc</sup>	465.1 <sup>a</sup>	4.67	.013
	Female	467.0 <sup>b</sup>	455.7 <sup>b</sup>	451.2 <sup>ab</sup>	463.5 <sup>b</sup>	427.9 <sup>a</sup>	3.95	.019
42 d	Male	1346.5	1373.4	1306.4	1333.3	1303.5	9.44	.127
	Female	1200.7 <sup>c</sup>	1182.3 <sup>bc</sup>	1151.9 <sup>ab</sup>	1195.7 <sup>bc</sup>	1124.3 <sup>a</sup>	7.46	.005
<i>Feed intake (g)</i>								
0–21 d		788.5 <sup>bc</sup>	801.2 <sup>c</sup>	754.7 <sup>ab</sup>	780.9 <sup>bc</sup>	723.0 <sup>a</sup>	7.69	.004
22–42 d		1917.4	1915.4	1904.4	1937.1	1898.3	13.65	.93
0–42 d		2705.9	2716.6	2659.1	2718.1	2621.3	19.1	.432
<i>Feed conversion ratio</i>								
0–21 d		1.78	1.84	1.79	1.8	1.77	0.01	.148
22–42 d		2.42	2.41	2.45	2.46	2.41	0.01	.449
0–42 d		2.19	2.21	2.21	2.23	2.19	0.01	.552

Note: Values have been analysed by one-way ANOVA.

<sup>a,b,c</sup>Means bearing different superscripts in a row differ significantly ( $P < .05$ ).

**Table 3.** Effect of feed deprivation on haematological parameters of 36-h-old birds subjected to 0, 6, 12, 24 and 36 h delay in feed placement.

Attributes	PCV	Haemoglobin	Heterophil	Lymphocytes	H/L ratio
<i>Male birds</i>					
Control	29.00	6.00	19.50 <sup>a</sup>	59.50	0.34 <sup>a</sup>
6 h	32.00	6.50	22.00 <sup>a</sup>	58.00	0.40 <sup>ab</sup>
12 h	31.00	6.25	27.50 <sup>b</sup>	48.00	0.57 <sup>bc</sup>
24 h	32.00	7.25	27.00 <sup>b</sup>	46.00	0.59 <sup>c</sup>
36 h	32.00	7.75	29.50 <sup>b</sup>	51.50	0.58 <sup>bc</sup>
SEM	0.61	0.30	1.31	2.37	0.04
P-value	0.550	0.362	0.010	0.303	0.042
<i>Female birds</i>					
Control	30.00 <sup>a</sup>	6.25 <sup>a</sup>	22.50 <sup>a</sup>	55.50	0.40 <sup>a</sup>
6 h	29.00 <sup>a</sup>	6.00 <sup>a</sup>	25.50 <sup>ab</sup>	56.50	0.45 <sup>ab</sup>
12 h	30.00 <sup>a</sup>	6.50 <sup>a</sup>	23.50 <sup>a</sup>	53.50	0.44 <sup>ab</sup>
24 h	35.00 <sup>b</sup>	7.25 <sup>b</sup>	29.00 <sup>b</sup>	52.50	0.56 <sup>c</sup>
36 h	37.00 <sup>b</sup>	9.00 <sup>b</sup>	28.50 <sup>b</sup>	53.50	0.54 <sup>bc</sup>
SEM	1.09	0.37	0.96	1.01	0.02
P-value	0.002	0.001	0.048	0.813	0.045

Values have been analysed by one-way ANOVA.

<sup>a,b,c</sup>Means bearing different superscripts in a column differ significantly ( $P < .05$ ).

BW of birds which had no access to feed compared to those fed the starter diet immediately after hatching (Noy and Sklan 1999; Saki 2005). The present result also supports the finding (Juul-Madsen et al. 2004) that broilers can be subjected to first 24 h feed deprivation without affecting the normal performance.

Standard size fertile eggs were collected from the breeder hen of 35 weeks old. The chicks that hatched from those eggs were randomly distributed so that there was no significant difference in the day-old chick weight. However, there was significantly higher residual yolk sac weight at 36 h PH (data not presented) in 24–36 h FD chicks (4.3–4.53% of day-old chick weight) as compared to that of control chicks (2.24% of day-old chick weight). It is reported that the yolk sac content is used up by the chicks that have access to feed immediately after hatching (Noy et al. 1996). Following hatching, the yolk is transported both to the circulation through the vascular system and to the intestine through the yolk stalk (Noy et al.

**Table 4.** Immune organ weight (expressed as g/100 g) of birds subjected to 0, 6, 12, 24 and 36 h delay in PH feed placement.

Attributes	Delay in PH feed placement					SEM	P-value	
	Control	6 h	12 h	24 h	36 h			
<i>At 36 h</i>								
Spleen	Male	0.06 <sup>b</sup>	0.06 <sup>b</sup>	0.05 <sup>ab</sup>	0.04 <sup>a</sup>	0.04 <sup>a</sup>	0.00	.039
	Female	0.06	0.05	0.04	0.05	0.04	0.00	.133
Bursa	Male	0.12	0.12	0.10	0.10	0.10	0.01	.854
	Female	0.12	0.15	0.12	0.09	0.15	0.01	.374
Thymus	Male	0.16	0.12	0.15	0.15	0.11	0.01	.216
	Female	0.16	0.16	0.10	0.13	0.11	0.01	.324
<i>7 d PH</i>								
Spleen	Male	0.10	0.09	0.12	0.12	0.08	0.01	.328
	Female	0.10	0.08	0.10	0.11	0.09	0.00	.614
Bursa	Male	0.26	0.21	0.21	0.30	0.22	0.02	.520
	Female	0.21	0.23	0.17	0.15	0.23	0.01	.279
Thymus	Male	0.08	0.08	0.09	0.10	0.07	0.01	.702
	Female	0.10	0.09	0.11	0.07	0.07	0.01	.410
<i>14 d PH</i>								
Spleen	Male	0.11	0.12	0.09	0.10	0.09	0.01	.799
	Female	0.09	0.09	0.12	0.08	0.09	0.01	.126
Bursa	Male	0.32	0.28	0.23	0.25	0.32	0.01	.160
	Female	0.33	0.23	0.28	0.23	0.28	0.02	.736
Thymus	Male	0.07	0.09	0.07	0.07	0.09	0.00	.459
	Female	0.10	0.07	0.10	0.10	0.12	0.01	.240

Values are the mean of three observations and have been analysed by one-way ANOVA.

<sup>a,b,c</sup>Means bearing different superscripts in a row differ significantly ( $P < .05$ ).

**Table 5.** Immune response of birds subjected to 0, 6, 12, 24 and 36 h delay in PH feed placement.

Attributes	Delay in PH feed placement					SEM	P-value
	Control	6 h	12 h	24 h	36 h		
<i>SRBC titre (log 2)</i>							
Male	11.00	10.00	11.67	12.50	10.80	0.29	.131
Female	10.33	11.50	12.67	11.75	11.00	0.27	.141
<i>Foot web index (mm)</i>							
Male	0.66	0.59	0.57	0.74	0.52	0.05	.695
Female	0.39 <sup>a</sup>	0.64 <sup>ab</sup>	0.76 <sup>b</sup>	0.80 <sup>b</sup>	0.42 <sup>a</sup>	0.05	.006

Values are the mean of six observations and have been analysed by one-way ANOVA.

<sup>a,b,c,d</sup>Means bearing different superscripts in a row differ significantly ( $P < .05$ ).

1996). Therefore, it is expected that yolk nutrients would be utilized more quickly by the fasted than the fed chicks, allowing young chicks to maintain their physiological functions. On the contrary, yolk utilization was more rapid in the fed chicks than the fasted chicks, suggesting that yolk transfer is facilitated by the intestinal motility of fed chicks (Bhanja et al. 2009).

#### 4.2. Haematological parameters

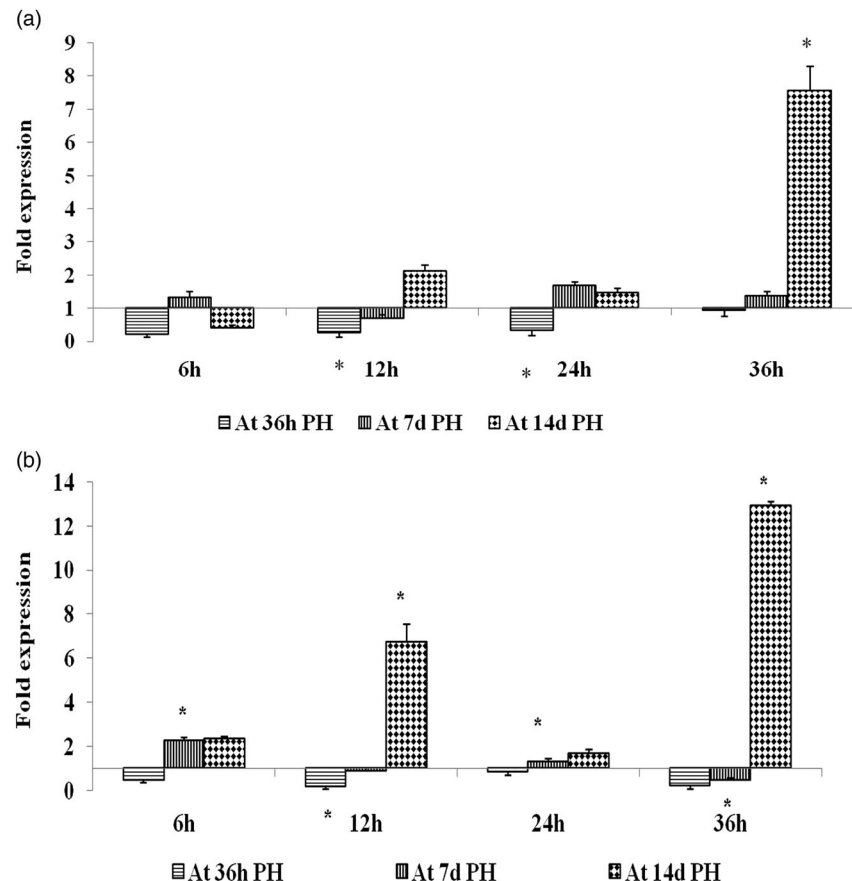
The present study revealed a linear increase in the PCV and Hb as the feed deprivation period increased from 12 to 36 h PH. Earlier studies in chickens (Nakage et al. 2006) and in ducks (Herbert et al. 1989) have also reported that birds submitted to feed and water fasting presented higher PCV and Hb

values than the fed birds, suggesting that these lower volume may be due to lower plasmatic volume caused by water fasting.

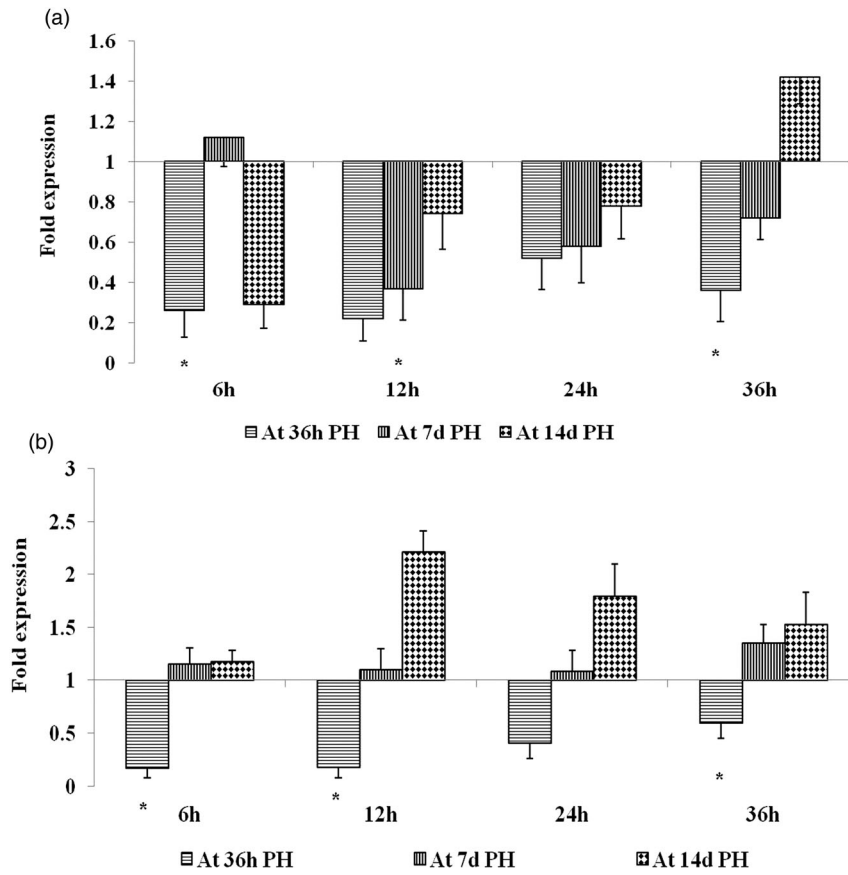
In the present study, birds FD for 24 and 36 h showed significantly higher heterophil count and lower lymphocytes count compared to the control birds and an increasing trend in H/L ratio along with FD period than the control, which is concomitant with Maxwell et al. (1992) and Gross and Siegel (1986). H/L ratio is used as an indication of stress. PH feed deprivation causes blood changes (Shinde et al. 2015), indicating stress and thus depressed immune function (Gross and Siegel 1986). However, De Jong et al. (2002) did not observe such changes in adult broilers submitted to food restriction, suggesting that the type and the intensity of immune reaction depend on stress type, fasting degree, stress duration and age at which the birds are submitted to stress.

#### 4.3. Immune organ development and in vivo immune response

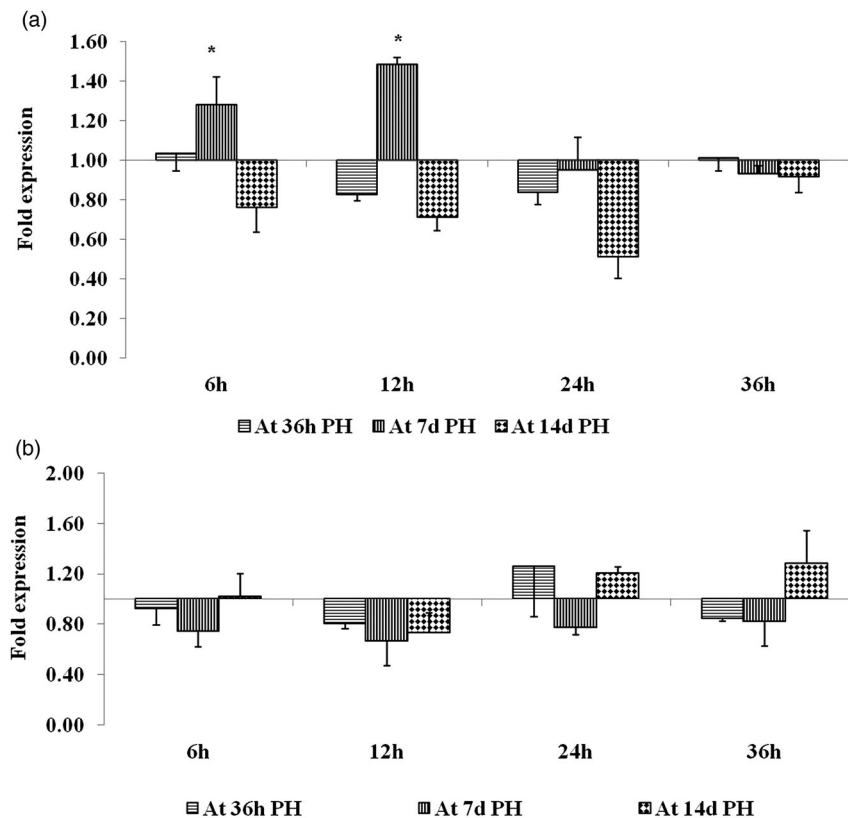
No significant difference was observed in the bursa, spleen and thymus weight of FD and control birds at 36 h, 7 and 14 d PH, except at 36 h, when spleen weight was lower in 36 h FD male birds. In post-hatching bursa development begins after 72 h of hatching, which explain the absence of the effects of fasting on this organ during the experimental



**Figure 1.** Relative fold expression of *IL-6* gene at 36 h, 7d and 14 d PH in the spleen tissues of control and FD broiler (a) male chicks and (b) female chicks. Expression of the control group is taken as 1.0. \*Significant expression at the level of 95% ( $P < .05$ ).



**Figure 2.** Relative fold expression of *TLR-2* gene at 36 h, 7d and 14 d PH in the spleen tissues of control and FD broiler (a) male chicks and (b) female chicks. Expression of the control group is taken as 1.0. \*Significant expression at the level of 95% ( $P < .05$ ).



**Figure 3.** (a) Relative fold expression of *TNF-α* gene at 36 h, 7d and 14 d PH in the spleen tissues of control and FD broiler male chicks. (b) Relative fold expression of *IL-6*, *TLR-2* and *TNF-α* gene in the spleen tissues of control and FD broiler female chicks. Expression of the control group is taken as 1.0. \*Significant expression at the level of 95% ( $P < .05$ ).

period of 0–42 d PH (Pires et al. 2007). Tabeidian et al. (2011) had also reported no significant differences in bursa, spleen and heart weights due to PH FD between experimental treatments at 21 and 42 d PH. However, they found heavier spleen in the fed than in fasted chicks (48–72 h), which is concomitant with the present finding. Data on the chemical composition of residual yolk sac (data not presented) revealed a higher lipid and ash, but lower protein content in the residual yolk sac of 24 and 36 h FD chicks as compared to the control chicks. This suggested that protein (not fat) could be a limiting nutrient for the newly hatched chicks (Noy and Sklan 2001; Bhanja et al. 2009). The residual yolk contributes to the maintenance of young birds during the first few days after hatching (Anthony et al. 1989). Proteins are needed for the development and sustainability of life as they exert most biological functions and confer structure and function to cells and tissue architecture. In the present study, lower utilization of yolk sac contents in 36 h FD chicks might have contributed to lower immune response, especially of cell-mediated immunity.

Humoral immune response was significantly higher in 12 and 24 h FD birds compared to the control or 36 h FD birds. In line with the present findings, Panda et al. (2010) reported that the humoral immune response was significantly higher in the chicks that had access to feed immediately or 12 h after placement compared to those FD for either 24 or 48 h. Cellular immune response was significantly better in chicks FD for 24 h than in the control or other FD chicks. In our earlier study with egg-type chicken, it was found that feed deprivation for 6–24 h enhanced *in vivo* response to SRBC and PHAP (Shinde et al. 2015). In the present study also, 12–24 h FD female birds showed higher cell-mediated immune response, but the response reduced in 36 h FD birds. Panda et al. (2010) reported no difference in the cell-mediated immune response to PHAP inoculation due to PH feed deprivations up to 48 h, but the lymphocyte proliferation ratio decreased significantly in birds FD for 24 and 48 h compared to those that had access to feed immediately or after 12 h of hatch. Non-availability of nutrients during early life hampers the immune system which is incomplete at the time of hatching (Dibner et al. 1998). Further, in fasting chicks there is secretion of corticosteroids, which inhibits immune cell proliferation, resulting in low immune response (Dibner et al. 1998). Feeding on the day of hatch may provide an early antigen stimulus and thus facilitate rapid differentiation of the humoral response (Lupelti et al. 1984). Juul-Madsen et al. (2004) have also reported that feed withdrawal for more than 24 h affects immunological response.

#### 4.4. Expression of immune-related genes

The B and T lymphocyte cells play an important role in immunity as native T cells differentiate into Th1 cells (cellular immunity) and Th2 cells (humoral immunity). The development of these cells is initiated during embryogenesis and continues till PH. Glick et al. (1983) reported that the thymus is very sensitive to periods of food deprivation, which causes a rapid decline in CD4<sup>+</sup> T cells, resulting in lower IgG production. The CD4<sup>+</sup> cells act as the source for IL-6, a pro-inflammatory cytokine, which induces the final maturation of B cells into antibody-secreting

plasma cells (Jones 2005) causing the proliferation and differentiation of immunoglobins. In the present study also we have seen apparently reduced thymus weight in FD birds. There was DR of IL-6 gene expression in 12–24 FD birds at 36 h PH, but UR in 6–24 h FD females. Greiner et al. (1994) reported that glucose is an essential fuel for proliferating Th2 cells and was found to be reduced in chicks subjected to 24 h or more fasting (Shinde et al. 2015). Bakyaraj et al. (2012), Bhanja, Goel et al. (2015) and Bhanja, Hotowy et al. (2015) revealed that *in ovo* injection of amino acid, glucose and trace elements not only leads to the enhancement of cellular as well as humoral immunity, but also increases the IL-6 and TNF- $\alpha$  gene expression in PH chicks or embryos, suggesting their role in immunity development in broiler chickens. IL-6 is the pro-inflammatory cytokine which induces the final maturation of B cells, thereby increasing the secretion of immunoglobins (Mosmann and Coffman 1989). In the present study, *in vivo* immune response to SRBC was apparently higher when the birds were subjected to lesser feed deprivation (12–24 h).

Toll-like receptors (TLRs) play an important role in innate immunity. In the present study, TLR-2 expression was DR in FD chicks at early ages. Upon pathogen infection, pathogen-associated molecular patterns are signalled by TLRs present on antigen-presenting cells and initiate a signalling cascade for the production of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, chemokines and IL-8) and up-regulating co-stimulatory molecules (He et al. 2010; Mackinnon et al. 2010). In the present study also, TLR-2 expression was associated with increased expression of IL-6, especially in FD female birds.

## 5. Conclusion

The present study reveals that PH feed deprival for the first 24 h did not affect the growth performance, but improved *in vivo* cellular immune response in slow-growing coloured broiler females. However, feed withdrawal for 36 h or beyond may affect the market weight of slow-growing broilers. Moreover, the results of this study are also applicable to fast-growing broilers.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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