Indian Journal of Animal Sciences **87** (4): 473–479, April 2017/Article

In ovo nutrient supplementation improves the performance and intestinal morphology in early feed deprived egg type chickens

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Received: 30 July 2016; Accepted: 26 September 2016

ABSTRACT

The effect of *in ovo* feeding (IOF) in ameliorating the adverse effects of feed deprivation (FD) was studied in egg type chickens (White leghorn). The experimental design was a 3 × 3 factorial design with three treatments (*in ovo*, sham and un-injected control) and three types of feeding (immediate fed or fasted for 24 h and 36 h) as factors. Response criteria were embryonic weight, digestive organ development, growth performance, intestinal and villi morphology. Embryonic weight at 20 d (% of pre incubated egg) in IOF group was significantly higher compared to un-injected control group. IOF chicks had higher body weight (BW), proventriculus weight, duodenal villi length (VL) and villi width (VW) at 24 and 36 h post hatch (PH) than the sham control and un-injected control chicks. Immediately fed and 24 h FD chicks had significantly higher BW than 36 h FD chicks, while organ weight was higher in immediately fed chicks compared to FD chicks. The IOF and 24 h FD chicks had higher digestive organ weight, BW and VL than the un-injected and immediately fed chicks. IOF and 36 h FD chicks had comparable performances with un-injected control and immediately fed chicks. It is concluded that *in ovo* supplemented chicks can withstand early post-hatch feed deprivation and had improved PH performance.

Key words: Egg type chickens, Feed deprivation, *In ovo* feeding, Intestinal morphology, Post-hatch performance

Poultry production in India has gained significant momentum and contributes nearly 0.7% to the national GDP. Despite such phenomenal growth, there are many challenges which need to be addressed for filling up the existing wide gap between the demand and supply of eggs and meat for our nutritionally starved masses (Rokade *et al*. 2016). One such challenge is juvenile nutrition and care; the incubation period of 21 days and early 7 days post-hatch period in chick comprises about 50% of a 2 kg broiler's lifespan (Uni and Ferket 2010). This period have a marked effect on the overall performance and health of the poultry. During this period, there is higher caloric demand for the hatching process and maintenance of basal metabolism (Ferket and Uni 2002). Further, delay in early feeding due to common hatchery operations like sex determination, vaccination, packaging and transportation to production facilities is also responsible either directly or indirectly for decrease in the performance of the birds. Delayed access to feed and water also makes the hatchlings more susceptible to pathogens, reduces their response to vaccination and slows down the development of the gastrointestinal tract and immune system (Noy and Sklan 1999). Now, many of the poultry researchers realize that future gains in genetic and production potential of poultry will be possible through the advancements made during incubation period and embryogenesis.

These adverse effects can be minimized by providing immediate feed to hatched chicks. Early access to feed stimulates growth and development of the small intestine, while feed deprivation results in a reduction of villus size and gut development (Shinde *et al*. 2015). Though there are few early feeding system like pre-starter feeding, hatchery feeding and supplementation with specialist feedstuffs, but *in ovo* feeding (IOF), i.e. injection of nutrients in to the egg during incubation, finds a promising alternative. Nutrients like carbohydrates, amino acids, vitamins and trace minerals were found to be most effective for the purpose of IOF (Bhanja *et al.* 2008, Bhanja *et al.* 2014, 2015; Goel *et al*. 2013, 2016).

This study was undertaken to examine whether *in ovo* feeding can be applied to alleviate the negative effect of delayed feed placement mostly in the restoration of intestinal morphology in egg type chickens.

MATERIALS AND METHODS

In ovo treatments: Fertile eggs (540), from the layer breeders (White leghorn) maintained on the adequate nutritional plane were collected, weighed and distributed into 9 groups of 60 eggs each in a 3 × 3 factorial design. There were three treatments (*in ovo*, sham and un-injected control) and three types of feeding (immediate fed, 24 h

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FD and 36 h FD) as factors. The eggs were set in a forced draft incubator at 37.5°C and 60% relative humidity. The first 3 groups were injected with critical nutrient solution. The dose of IOF used for such injection per egg was based on the requirement of critical nutrients like carbohydrates, amino acids, vitamins and trace elements required during first three days in PH chicks (NRC 1994, recommendations for 30g feed) and is presented in Table 1. The required amount of critical nutrients were weighed and dissolved in 5% ethanol solution in such a concentration that 0.5 ml contained the required amount of critical nutrients to be injected in one egg on 18 d of incubation using a 24 gauge 25 mm needle at the broad end of egg following the method standardized by Bhanja *et al*. (2004). Rest six groups were used as sham control (0.5 ml of 5% ethanol/egg) or un-injected control. Immediately after injection, the eggs were shifted to a hatcher and kept in pedigree hatching boxes.

Housing and management: The chicks hatched from the respective treatment groups were distributed in to three groups (immediate fed, 24 h FD and 36 h FD) which were then weighed, wing banded and transferred to 4-tier battery brooder cages. Each treatment group had 4 replicates of 12 birds each and the chicks were reared up to 42 d of age. Layer starter mash (20% CP, 2800 Kcal/kg ME as per the standard of Indian Council of Agricultural Research 2013) were offered to the egg type chickens for a period of 42 day PH.

Digestive organ development and yolk-sac utilization: At 24 and 36 h PH, four birds (of both sexes) were selected randomly from each treatment (one from each replicate), weighed and killed by cervical dislocation for measurement of digestive organ weights. The residual yolk sac, proventriculus plus gizzard without contents, small intestine without contents and liver were weighed and expressed as g/100g of body weight (BW).

Table 1. Dose of selected critical nutrients for *in ovo* supplementation per egg on 18th ED in egg type chickens

Critical nutrient	Percentage/ 100g of feed	Consumption during first-3 days*	*In ovo* dose per egg (mg) @ 10%
Glucose			25.00
Arginine	1.00	0.30 (g)	30.00
Lysine	0.85	0.255 (g)	25.50
Threonine	0.68	0.204 (g)	20.40
Methionine	0.30	0.09 (g)	9.00
Iodine	0.35	0.105 (mg)	10.50
Iron	80 (mg)	24 (mg)	2.40
Copper	8.00	2.40 (mg)	0.24
Zinc	40 (mg)	12 (mg)	1.20
Selenium	0.15 (mg)	0.045 (mg)	4.5 mcg
Vit A	1500 IU	450 IU	45 IU
Vit E	10 IU	3 IU	0.3 IU
Vit B_{12}	0.009 (mg)	0.0027 (mg)	0. 27 mcg
Biotin	0.15	0.045 (mg)	4.5 mcg
Choline	1300	390 (mg)	39.00
Folacin	0.55	0.165 (mg)	16.5 mcg
Niacin	27	8.1 (mg)	0.81
Panto. acid	10	3.00 (mg)	0.30
Pyridoxine	3.00	0.9 (mg)	90 mcg
Riboflavine	3.60	1.08 (mg)	108 mcg
Thiamin	1.00	0.3 (mg)	30 mcg

*As per NRC, 1994 requirement and calculated based on consumption of 30 g mash feed.

Response criteria: The body weight of individual chicks and the feed intake (FI) of all the birds in a pen (replicate wise) were recorded at 7 d intervals throughout the experimental period. The feed conversion ratio was calculated for the period of 0–14, 0–28 and 28–42 d by dividing the feed consumption to the corresponding weight gain.

Intestinal morphology: For histological analysis of the intestine; the duodenum, jejunum and ileum were collected aseptically after sacrificing four experimental birds (of both sexes) from each treatment group at 24 h and 36 h PH. All the procedure of tissue processing and slide staining was carried out as described by Franco *et al.* (2006). After staining, each stained cross section sample was observed at two to three villi areas for villus height (VH) and villus width (VW). VH and VW were measured using the Image-Pro Plus microscope equipped with a video camera (Motic BA210, Xiamen, China).

Statistical analysis: The statistical analysis and interpretation of data was done using the SPSS software package version 16.0 (2007). The body weight, organ weight and intestinal morphology were analyzed by one-way ANOVA using standard procedure as described by Snedecor and Cochran (1980). Duncan Multiple Range Test (Duncan 1955) was used for verifying significant difference among treatment means.

RESULTS AND DISCUSSION

Embryo weight (20th day) in egg type chicken: The 20 d embryo weight (% of pre incubated egg) of *in ovo* fed group was higher (P=0.019) compared to un-injected control group in egg type chickens (Table 2). Present result is in concomitant with Uni *et al*. (2006), who demonstrated that

Table 2. Effect of *in ovo* feeding on 20th day embryo weight and yolk weight in egg type chickens

Attribute	18th day egg weight (g)	20th day embryo weight (% of egg weight)	Yolk weight (% of embryo weight)
In ovo supplemented (IOF)	49.72	60.42^{b}	11.77
Sham control	50.41	58.73^{ab}	12.39
Un-injected control	49.76	56.71^{a}	12.04
SEM	0.48	0.56	0.36
P-value	0.814	0.019	0.784

*Values are the mean of four observations and have been analyzed by one-way ANOVA. ^{a,b}Means bearing different superscripts in a column differ significantly (P<0.05).

in ovo feeding of carbohydrates and β-hydroxy-β-methyl butyrate (HMB) at 17.5 days of incubation improved the energy status of late term broiler embryos and early growth, to enhance the genetic potential for late embryonic and early post-hatch growth. Ohta and Kidd (2001) and Bhanja *et al.* (2004a) reported that *in ovo* administration of all twenty amino acids increased the chick weight by 3.6% and 2.1%, respectively.

Body weight changes, yolk utilization and digestive organ weight during the first 24 and 36 h PH: At 24 and 36 h PH; irrespective of period of fasting, weight gain was higher (P=0.001) in un-injected control chicks compared to the IOF chicks. Irrespective of treatments at 24 and 36 h PH; the weight gain in immediate fed group chicks was 3.30 and 5.68% while the FD chicks had 1.66 and 2.93% weight loss, respectively. Interaction effect was found to be significant (P=0.001) for weight gain or loss between treatments and periods in 24 h FD chicks, while sham fed and control fed chicks had higher weight gain than the IOF fed chicks. Higher weight gain in sham and un-injected control group might be due to increased FI immediately after feed deprival period to satisfy energy needs. FI was normal during early post-hatch period in IOF supplemented group as the energy needs were satisfied from *in ovo* supplemented nutrients. Not much literature is available on the effect of IOF on weight gain of chicks from hatch to 24 or 36 h PH. Weight loss in 24 h FD chicks did not differ among treatment groups. No interaction effect was reported among treatment and periods in weight gain and loss in 36 h PH feed deprived chicks (Table 3).

Irrespective of treatments, yolk sac weight was lower (P<0.05) in immediate fed group compared to FD chicks at 24 and 36 h PH. Irrespective of period of fasting, yolk sac weight was apparently lower in IOF and sham control than the un-injected control chicks. There was no interaction effect between treatments and periods, though it was lower in IOF and sham control fed group (Table 3). Dong *et al*. (2013) also reported that *in ovo* injection of carbohydrates increased the yolk sac nutrient utilization compared to the control group suggesting that *in ovo* feeding may enhance the capacity for intestinal absorption. In the present study, irrespective of treatments, yolk sac utilization was significantly increased in immediate fed group in comparison to 24 and 36 h FD chicks. This corroborates the earlier studies of Noy *et al*. (1996) where they reported more rapid utilization of yolk in the fed chicks than the

Table 3. Effect of *in ovo* feeding on digestive organ development and intestine morphology of 24 and 36 h feed deprived layer chicks

Attribute	24 h post-hatch feed deprivation						36 h post-hatch feed deprivation					
	Wt gain or loss	Yolk	Liver	Proventriculus (%)	Gizzard (%)	Intestine (%)	Wt gain or loss	Yolk	Liver	Proventriculus (%)	Gizzard (%)	Intestine (%)
Group												
In ovo suppl. + Imd. fed	2.07^{b}	2.39	3.79	1.15	7.59	5.26	4.47	1.81	3.84	1.17	7.29	4.75
Sham control + Imd. fed	3.79^{c}	2.23	3.51	1.00	7.14	4.92	5.75	1.76	3.27	0.92	6.43	4.65
Un-injected control + Imd. fed	4.20^{c}	3.34	3.51	1.12	6.82	4.85	6.90	1.91	3.30	1.05	6.90	4.68
In ovo suppl. + FD	-1.77^{a}	3.10	3.31	1.24	7.19	4.84	-3.24	2.16	2.83	0.90	6.89	4.90
Sham control + FD	-1.46^{a}	3.75	2.69	0.98	6.40	4.72	-2.52	1.99	2.41	0.72	6.45	4.22
Un-injected control + FD	-1.75^{a}	3.67	2.98	1.02	6.94	4.08	-3.00	2.87	2.59	0.86	6.72	4.32
SEm	0.18	0.19	0.10	0.03	0.13	0.12	0.33	0.12	0.14	0.03	0.14	0.10
Treatment												
In ovo supplemented	0.41^{a}	2.74	3.55	1.20^{b}	7.39	5.05	1.24^{a}	1.98	3.34	1.03^{b}	7.09	4.82
Sham control	1.13^{b}	2.99	3.10	0.99^{a}	6.77	4.82	1.61^{a}	1.88	2.84	0.82^{a}	6.44	4.44
Un-injected control	1.71^{c}	3.50	3.24	1.07^{a}	6.88	4.46	2.40^{b}	2.39	2.95	0.95^{b}	6.81	4.50
Periods of fasting												
Immediate fed	3.30^{b}	2.65^{a}	3.60^{b}	1.09	7.18	5.01^{b}	5.68^{b}	1.83^{a}	3.47^{b}	1.04^{b}	6.87	4.69
FD	-1.66^{a}	3.51^{b}	2.99^{a}	1.08	6.84	4.55^{a}	-2.93^{a}	2.34^{b}	2.61^{a}	0.83^{a}	6.68	4.48
Significance												
Treatments	0.001	0.197	0.056	0.001	0.102	0.113	0.001	0.127	0.165	0.001	0.198	0.227
Periods of fasting	0.001	0.020	0.001	0.805	0.168	0.047	0.001	0.023	0.001	0.001	0.51	0.278
Treatments × Periods	0.001	0.366	0.583	0.182	0.348	0.561	0.063	0.327	0.851	0.673	0.834	0.397

*Values are the mean of four observations and have been analyzed by two-way ANOVA. ^{a,b,c} Means bearing different superscripts in a column differ significantly (P<0.05).

fasted chicks. The yolk, which contributes to nourish the young bird during first few days after hatching transports the nutrients to the circulation through vascular system and to the intestine through the yolk stalk (Noy *et al*. 1996). Yolk being the largest source of nutrients for chick during their early days of life outside the egg, therefore, it would be expected that yolk nutrients were utilized more quickly by fasted chicks in comparison to fed chicks, allowing young chicks to maintain their physiological functions. But yolk utilization was more rapid in the fed chicks than the fasted chicks, suggesting that transfer of yolk was facilitated by intestinal motility of fed chicks (Bhanja *et al.* 2009).

Irrespective of period of fasting; at 24 and 36 h PH, higher (P=0.001) proventriculus weight was found in IOF chicks compared to sham control chicks. No difference was observed in liver, gizzard and intestine weight. Irrespective of treatments; at 24 h PH, liver and intestine weight was higher (P<0.05) in immediate fed chicks than the 24 h FD chicks, while at 36 h PH, liver and proventriculus weight was higher in immediate fed chicks than 36 h FD chicks. No interaction effect was observed between treatments and periods for digestive organs at both the periods (Table 3). This corroborates the earlier studies of Bhanja *et al*. (2004a) where they reported IOF of 0.5% concentration of all common twenty amino acids on 14th day of incubation led to higher digestive organs at 21 day PH suggesting that *in ovo* nutrients utilized by the developing embryo accelerates enteric development for greater digestive and nutrient absorptive capacity. It was also observed that, irrespective of treatments; liver, spleen and intestinal weight was higher while gall bladder weight was lower (P=0.001) in immediate fed chicks than the FD chicks in egg type chicks. The present finding is in accordance with Bhanja *et al*. (2009) who reported that the development of gastrointestinal tract and organs was directly related to feed intake. Moreover, Maiorka *et al*. (2003) also reported the negative effects of FD for 24, 48 and 72 h PH and recommended the need to feed chicks immediately after hatch to ensure proper Development of gastrointestinal tract, liver and pancreas (Noy and Sklan 2001, El-Husseiny *et al*. 2008).

Post hatch performance in egg type chicken: Irrespective of period of fasting; at 14, 28 and 42 d PH, chicks receiving IOF had higher (P=0.001) BW than the sham control and un-injected control (Table 4). Irrespective of treatments (*in ovo*, sham or un-injected control) chicks fed immediately or FD for 24 h had higher (P=0.001) BW at 14 and 28 d PH

Table 4. Effect of *in ovo* feeding on mean body weight (BW), feed intake (FI) and feed conversion ratio (FCR) of egg type chickens having immediate fed, 24 and 36 h delay in PH feed placement

Attribute	Body weight				Feed intake			FCR		
	0d	14d	28d	42d	0-14d	15-28d	29-42d	0-14d	0-28d	29-42d
Group										
In ovo suppl. + Immediate fed	30.46	116.5^{e}	258.7	454.9	155.1	378.6	602.9	1.93^{ab}	2.45	3.18
In ovo suppl. + 24 h FD	30.24	110.8^{d}	257.0	449.8	146.3	399.9	614.1	1.84^{ab}	2.41	3.28
In ovo suppl. + 36 h FD	30.14	98.2^{ab}	233.2	433.6	141.6	369.8	615.6	2.15^{c}	2.55	3.13
Sham control + Immediate fed	31.34	102.3^{bc}	240.3	426.6	138.8	364.9	577.7	1.99^{b}	2.47	3.32
Sham control + 24 h FD	30.17	105.2^{c}	238.3	439.2	141.1	369.8	602.1	1.85^{ab}	2.41	2.93
Sham control + 36 h FD	29.35	93.8^{a}	219.6	409.7	110.8	351.4	551.1	1.79^{a}	2.52	2.88
Un-injected control + Imd. fed	30.70	103.6^{bc}	238.4	430.3	149.2	374.8	609.3	1.89^{ab}	2.45	3.49
Un-injected control + 24 h FD	30.50	101.2^{bc}	238.8	410.5	137.5	368.7	602.6	1.90^{ab}	2.40	3.64
Un-injected control + 36 h FD	30.97	102.6^{bc}	215.6	422.5	136.1	358.1	608.5	1.90^{ab}	2.68	2.82
SEM	0.14	0.74	1.74	2.89	2.92	3.64	5.95	0.03	0.02	0.06
Treatment										
In ovo supplemented (IOF)	30.31	109.7^{b}	251.0^{b}	447.4^{b}	148.7^{b}	382.2^{b}	609.7^{b}	1.97	2.46	3.19
Sham control	30.28	100.4^{a}	232.6^{a}	424.5^{a}	130.3^{a}	362.0^{a}	576.9^{a}	1.88	2.47	3.04
Un-injected control	30.73	102.7^{a}	231.7^{a}	422.1^{a}	142.1^{b}	368.3^{a}	607.2^{b}	1.9	2.50	3.34
Period of fasting										
Immediate fed	30.77	108.0^{b}	246.4^{b}	439.3	148.8	373.7	599.0	1.93	2.46^{a}	3.33^{b}
24 h FD	30.30	105.9^{b}	244.9^{b}	433.3	141.6	379.5	606.3	1.87	2.41^{a}	3.28^{b}
36 h FD	30.18	98.4^{a}	222.5^{a}	421.7	129.5	359.8	591.7	1.95	2.58^{b}	2.94^{a}
Significance										
Treatments	0.329	0.001	0.001	0.001	0.002	0.042	0.050	0.082	0.656	0.126
Periods of fasting	0.103	0.001	0.001	0.074	0.001	0.070	0.613	0.144	0.025	0.011
Treatments × Periods	0.089	0.001	0.982	0.164	0.070	0.635	0.507	0.009	0.657	0.082

*Values are the mean of four observations and have been analyzed by two-way ANOVA. ^{a,b,c}Means bearing different superscripts in a Column differ significantly (P<0.05).

in comparison to those FD for 36 h. There was interaction effect between treatments and fasting period at 14 d, where IOF fed and 24 h FD chicks had higher (P=0.001) BW than sham control or un-injected control fed immediately. Similar trend was also observed at 28 and 42 d PH but found to be non-significant. In concomitant with present results, Bhanja *et al*. (2004b) and Uni *et al*. (2005) also reported 33 to 60g higher BW in *in ovo* fed chicks at 21 d and 25 d PH, respectively. Moreover, previous studies conducted on IOF of either vitamin E (Bhanja *et al*. 2006) or combination of vitamin B1, B2 and E (Bakyaraj *et al*. 2012) also resulted in 12 to 23g (5.3 to 13.3%) higher PH BW in chickens.

It was observed that IOF fed and 24 h FD chicks had significantly higher BW than sham control or un-injected control and immediate fed chicks, which show that *in ovo* fed nutrients are sufficient to ameliorate feed deprival effect of first 24 h. In accordance to present results, Uni and Ferket (2004) concluded that if early feeding is beneficial for early development of PH poultry, then feeding the embryo during incubation by *in ovo* administration would be expected to facilitate the development of better digestive tract, increased BW and thus improved nutritional status of *in ovo* fed hatchling. The potential benefits of IOF would be profound if both practices were combined, and would minimize the adverse effects of post-hatch holding of chicks. In the present study, chicks fed immediately or FD for 24 h had significantly higher BW in comparison to those FD for 36 h. This corroborates the earlier studies of Saki (2005) who reported decreased BW by chickens which were not allowed to access feed compared with chickens fed by starter diet immediately after hatching.

Irrespective of period of fasting, feed intake (FI) at 0–14 d and 29–42 d was found to be similar in IOF and un-injected control but were significantly higher (P<0.05) than the sham control. While at 15–28 d, FI was significantly higher (P=0.042) in IOF chicks compared to the sham and un-injected control (Table 4). The feed conversion ratio (FCR) derived for 0–14 d, 0–28 d and 29–42 d, was similar in all the treatments (IOF, sham control and un-injected control). Irrespective of treatments, FCR at 0–28 d was significantly better (P=0.025) in immediate fed and 24 h FD chicks compared to 36 h FD chicks; but at 29–42 d, it was better (P=0.011) in 36 h FD chicks (Table 4). Similar to this, Bhanja and Mandal (2005) also reported increased feed intake in the AA injected groups compared to control, though the FCR was numerically better in the chicks injected with Gly + Pro and Lys +Met + Cys. However, Bhanja *et al*. (2004b) reported that there was no difference in feed consumption and feed conversion ratio (FCR), but apparently better FCR was observed in amino acid injected chicks.

Intestinal villi morphology in egg type chicken: Irrespective of periods of fasting, in duodenum (P=0.001), jejunum (P=0.001) and ileum; villi height (VH) was found to be higher in IOF chicks than sham and un-injected control chicks. The *in ovo* supplemented and 24 and 36 h FD chicks had higher villi height and width compared to un-injected and immediately fed chicks. Intestinal morphology reflects

Table 5. Effect of *in ovo* feeding on intestinal villi morphology (in micro meter) of 24 h old egg type chickens subjected to immediate feed and 24 h delay in PH feed placement

Attribute	Duodenum		Jejunum		Ileum	
	Height (VH)	Width (VW)	Height (VH)	Width (VW)	Height (VH)	Width (VW)
Group						
In ovo suppl. + Immediate (Imd) fed	419.7	69.3^{c}	391.5^{c}	56.1	263.6	52.1
Sham control + Immediate fed	305.3	63.2^{bc}	262.2^{b}	58.2	246.3	56.0
Un-injected control + Imd. fed	265.7	62.7^{bc}	225.4^{b}	50.9	226.2	52.8
In ovo suppl. + 24 h FD	326.1	53.2^{ab}	178.3^{a}	41.7	192.6	43.8
Sham control + 24 h FD	259.5	50.5^{a}	149.3^{a}	44.0	160.3	38.1
Un-injected control + 24 h FD	232.0	47.8^{a}	146.2^{a}	42.5	163.8	38.7
SEM	13.5	1.76	17.15	1.46	8.49	1.38
Treatments						
In ovo supplemented (IOF)	372.9^{b}	60.7	293.1^{b}	49.3	231.3	48.3
Sham control	279.9^{a}	56.8	229.9^{a}	53.5	200.0	47.1
Un-injected control	250.4^{a}	56.6	189.4^{a}	46.7	194.9	45.4
Periods of fasting						
Immediate fed	327.6^{b}	64.7^{b}	300.2^{b}	55.6^{b}	245.3^{b}	53.6^{b}
24 h FD	272.5^{a}	50.6^{a}	161.5^{a}	42.4^{a}	170.4^{a}	40.1^{a}
Significance						
Treatments	0.001	0.570	0.001	0.815	0.086	0.748
Periods of fasting	0.009	0.001	0.001	0.016	0.001	0.009
Treatments × Periods	0.621	0.013	0.002	0.895	0.609	0.784

*Values are the mean of four observations and have been analyzed by two-way ANOVA. ^{a,b,c}Means bearing different superscripts in a column differ significantly (P<0.05).

Table 6. Effect of *in ovo* feeding on intestinal villi morphology (in micro meter) of 36 h old egg type chickens subjected to immediate feed and 36 h delay in PH feed placement

Attribute	Duodenum		Jejunum		Ileum	
	Height (VH)	Width (VW)	Height (VH)	Width (VW)	Height (VH)	Width (VW)
Group						
In ovo suppl. + Immediate fed	434.1	91.2	490.4	86.8	258.7	65.2
Sham control + Immediate fed	434.6	92.5	368.8	73.6	249.9	61.1
Un-injected control + Immediate fed	396.5	81.1	390.6	81.8	329.6	55.4
In ovo suppl. + 36 h FD	324.9	62.3	436.9	70.6	348.7	62.5
Sham control + 36 h FD	300.3	66.2	299.5	53.9	245.8	47.9
Un-injected control + 36 h FD	284.1	62.5	243.0	47.3	241.0	40.8
SEM	16.30	2.99	16.28	2.45	10.9	1.81
Treatment						
In ovo supplemented (IOF)	406.8	84.0	449.3[b]	74.3	285.7	64.4
Sham control	359.9	78.3	334.2[a]	63.1	247.7	54.5
Un-injected control	340.3	73.1	316.8[a]	59.7	296.4	49.9
Periods of fasting						
Immediate fed	421.7[b]	88.0[b]	402.7[b]	79.9[b]	276.9	61.0
36 h FD	297.6[a]	64.1[a]	351.5[a]	60.5[a]	270.4	49.5
Significance						
Treatments	0.172	0.572	0.048	0.702	0.229	0.637
Periods of fasting	0.008	0.015	0.008	0.002	0.234	0.089
Treatments × Periods	0.844	0.984	0.223	0.143	0.359	0.683

*Values are the mean of four observations and have been analyzed by two-way ANOVA. [a,b,c]Means bearing different superscripts in a column differ significantly (P<0.05).

the health status of the intestine of chickens and villus height, crypt depth and villus surface area indicate the functional condition of the small intestine (Laudadio *et al*. 2012). The *in ovo* feed nutrients are supposed to be exposed to tissues of the GIT when amniotic fluid is consumed by the embryo prior to pipping, which improves the nutritional status of the chicks by accelerating enteric development for greater digestive and nutrient absorptive capacity (Kadam *et al*. 2013). Uni and Ferket (2004) reported that *in ovo* injection of 1 ml of saline containing carbohydrate at 18 d of incubation significantly increased jejunum villus height by over 45% within 48 h after injection. Jia *et al*. (2011) also reported that *in ovo* feeding of 200 g/l maltose solutions to the chicken embryo enhances the absorption of nutrients and thus the development of the jejunum villus and finally the weight of hatchlings. After *in ovo* feeding, the gastrointestinal tract (GIT) of hatchlings becomes functionally similar to that of conventional 2 day old chicks offered feed immediately after hatch and helps in accelerating the enteric development for greater digestive and nutrient absorptive capacity (Uni *et al*. 2003, Uni and Ferket 2004).

In conclusion, irrespective of period of fasting, *in ovo* supplemented chicks had better growth performance, digestive organ development and intestinal morphology than the sham control and un-injected control chicks. *In ovo* supplemented and 24 h FD chicks had significantly better performance, while *in ovo* supplemented and 36 h FD chicks had comparable results with that of un-injected control and immediately fed chicks.

ACKNOWLEDGEMENT

The first author is grateful to Indian Veterinary Research Institute and Central Avian Research institute, Izatnagar for providing necessary facilities to carry out the research work in accordance with the regulations and recommendations of ICAR.

REFERENCES

Bakyaraj S, Bhanja S K, Majumdar S and Dash B. 2012. Modulation of post-hatch growth and immunity through *in ovo* supplemented nutrients in broiler chickens. *Journal of Science Food and Agriculture* **92**: 313–20.

Bhanja S K and Mandal A B. 2005. Effect of *in ovo* injection of critical amino acids on pre and post hatch growth, immunocompetence and development of digestive organs in broiler chickens. *Asian Australasian Journal of Animal Science* **18**: 524–31.

Bhanja S K, Devi C A, Panda A K and Sunder G S. 2009. Effect of post hatch feed deprivation on yolk-sac utilization and performance of young broiler chickens. *Asian Australasian Journal of Animal Science* **22**: 1174–79.

Bhanja S K, Mandal A B and Goswami T K. 2004b. Effect of *in ovo* injection of amino acids on growth, immune response, development of digestive organs and carcass yields of broiler. *Indian Journal of Poultry Science* **39**(3): 212–18.

Bhanja S K, Mandal A B and Johri T S. 2004a. Standardization of injection site, needle length, embryonic age and concentration of amino acids for *in ovo* injection in broiler breeder eggs. *Indian Journal of Poultry Science* **39**(2): 105–11.

Bhanja S K, Mandal A B, Agarwal S K and Majumdar S. 2006. Modulation of post-hatch growth and immunocompetence

through *in ovo* injection of vitamin E and linoleic acid. *Worlds Poultry Science Journal* **62**: 325.

Bhanja S K, Mandal A B, Agarwal S K, Majumdar S, Bhattacharyya A and Kadam M M. 2008. *In ovo* glucose injection for higher chick weight and gastrointestinal tract development. *Indian Veterinary Journal* **85**: 289–92.

Bhanja S K, Sudhagar M, Goel A, Pandey N, Mehra M, Agarwal S K and Mandal A B. 2014. Differential expression of growth and immunity related genes influenced by in ovo supplementation of amino acids in broiler chickens. *Czech Journal Animal Science* **59**(9): 399–408.

Bhanja S K, Goel A, Pandey N, Mehra M, Majumdar S and Mandal A B. 2015. *In ovo* carbohydrate supplementation modulates growth and immunity related genes in broiler chickens. *Journal of Animal Physiology and Animal Nutrition* **99**(1): 163–73.

Dong X Y, Jiang Y J, Wang M Q, Wang Y M and Zou X T. 2013. Effects of *in ovo* feeding of carbohydrates on hatchability, body weight and energy status in domestic pigeons (*Columba livia*). *Poultry Science* **92**(8): 2118–23.

Duncan D B. 1955. Multiple ranges and multiple F test. *Biometrics* **11**: 1–42.

El-Husseiny O M, Abou El-Wafa S and El -Komy H M A. 2008. Influence of fasting or early feeding on broiler performance. *International Journal of Poultry Science* **7**: 263–71.

Ferket P R and Uni Z. 2002. Early enteric development of turkeys. *Proceedings of the 25th Technical Turkey Conference*, pp. 59–64.

Franco J R G, Murakami A E, Natali M R M, Garcia E R M and Furlan A C. 2006. Influence of delayed placement and dietary lysine levels on small intestine morphometrics and performance of broilers. *Brazilian Journal of Poultry Science* **8**: 233–41.

Goel A, Bhanja S K, Mehra M, Mandal A B and Pande V. 2016. *In ovo* trace element supplementation enhances expression of growth genes in embryo and immune genes in post-hatch broiler chickens. *Journal of the Science of Food and Agriculture* **96**(8): 2737–45.

Goel A, Bhanja S K, Pandem V, Mehra M and Mandal A B. 2013. Effects of *in ovo* administration of vitamins on post hatch-performance, immunocompetence and blood biochemical profiles of broiler chickens. *Indian Journal of Animal Sciences* **83**(9): 916–21.

Jia C L, Wei Z H, Yu M, Wang X Q and Yu F. 2011. Effect of *in ovo* feeding maltose on the embryo growth and intestine development of broiler chicken. *Indian Journal Animal Sciences* **81**(5): 503–06.

Kadam M M, Barekatain M R, Bhanja S K and Iji P A. 2013. Prospects of *in ovo* feeding and nutrient supplementation for poultry: the science and commercial applications-a review. *Journal of Science Food Agricultural* **93**: 3654–61.

Laudadio V, Passantino L, Perillo A, Lopresti G, Passantino A, Khan R U and Ufarelli V. 2012. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. *Poultry Science* **91**: 265–70.

Maiorka A, Santin E, Dahlke F, Boleli I C, Furlan R L and Macari M. 2003. Post hatching water and feed deprivation affect the gastrointestinal tract and intestinal mucosa development of broiler chicks. *Journal of Applied Poultry Research* **12**: 483–92.

Noy Y and Sklan D. 1999. Energy utilization in newly hatched chicks. *Poultry Science* **78**: 1750–56.

Noy Y and Sklan D. 2001. Exogenous feed utilization in the post hatch chick. *Poultry Science* **80**: 1490–95.

Noy Y, Uni Z and Sklan D. 1996. Routes of yolk utilization in the newly-hatched chick. *British Poultry Science* **37**: 987–95.

NRC. 1994. Nutrient requirements of poultry. Ninth Revised Edition, 1994, National Academy Press, Washington DC.

Ohta Y and Kidd M T. 2001. Optimum site for *in ovo* amino acid injection in broiler breeder eggs. *Poultry Science* **80**: 1425–29.

Rokade J J, Bhanja S K, Shinde A S, Sajad, Mukesh Kapgate and Mandal A B. 2016. Evaluation of mannan-oligosaccharides (MOS) in broiler chicken during hot humid summer using zoo technical, molecular and physio-biochemical tools. *Indian Journal of Animal Sciences* **86**(4): 460–67.

Saki A A. 2005. Effect of post- hatch feeding on broiler performance. *International Journal of Poultry Science* **4**: 4–6.

Shinde A S, Goel A, Mehra M, Rokade J J, Bhadauria P**,** Mandal A B and Bhanja S K. 2015. Delayed post hatch feeding affects performance, intestinal morphology and expression pattern of nutrient transporter genes in egg type chickens. *Journal Nutrition and Food Science* **5**: 372.

Snedecor G W and Cochran W G. 1989. Statistical Methods. 8th edition, Iowa State University Press, Ames, Iowa. Reprinted in 1994. East-West Press Pvt. Ltd., New Delhi.

Uni Z, Ferket R P, Tako E and Kedar O. 2005. *In ovo* feeding improves energy status of late-term chicken embryos. *Poultry Science* **84**: 764–70.

Uni Z and Ferket R P. 2004. Methods for early nutrition and their potential. *Worlds Poultry Science Journal* **60**: 101–11.

Uni Z and Ferket R P. 2010. *In ovo* feeding - Impact on intestinal development, energetic status and growth. 47th National Conference of Worlds Poultry Science Association.

Uni Z, Ferket P R and Tako E. 2006. *In ovo* feeding: impact on gut development, energetic status, gene expression and growth performance. The 29th Technical Turkey Conference, Turkey. pp 47–48.

Uni Z, Tako E, Gal-Garber O and Sklan D. 2003. Morphological, molecular and functional changes in the chicken small intestine of the late-term embryo. *Poultry Science* **82**: 1747–54.