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Effect of *in-ovo* injection of critical nutrients and early post hatch feeding of early chick nutritional supplement on the chick quality characteristics of commercial broiler chicken

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Abstract
An experiment was carried out to investigate the effect of *in-ovo* injection of critical nutrients and early post hatch feeding of early chick nutritional supplement (ECNS) on the chick quality characteristics of commercial broiler chicken. *In-ovo* feeding trial was carried out with five hundred and forty hatching eggs were randomly assigned to six treatments each with three replicates of 30 eggs. On the 18th day of incubation, each group of 90 eggs were injected with 0.5 ml of 10% glucose solution (T3), 0.5 ml of 0.5% lysine solution (T4), 0.5 ml of 0.5% threonine (T5), 0.5 ml of 0.5% β-hydroxy-β-methylbutarate (HMB) (T6) along with 90 eggs served as non-injected control (T1) and 90 eggs as kepts as injected control. On the day of hatching, the hatched-out broiler chicks (288 numbers) were individually weighed, wing banded and randomly allotted into six treatment groups each with three replicates of 16 chicks each. The feeding of ECNS was started immediately after hatch in the hatcher tray of the incubator and continued till the chicks were placed in the chick transport box except control (T1) which was subjected to fasting for 24 hours to simulate field conditions. The data on hatching egg weight on the day of setting and on the day of injection, hatchability, hatch weight, egg/chick weight ratio, chick transit weight loss and early chick mortality were recorded and statistically analysed by SPSS software. The results revealed that the combined *in-ovo* feeding and post hatch early feeding of ECNS feeding had significantly ($P< 0.01$) Increased the hatch weight from 1.04 to 1.28 g than the control chicks. The transit weight loss of chicks was significantly ($P< 0.05$) affected between treatments with least weight loss of 4.66 per cent was observed in HMB *in-ovo* fed chicks followed by other treatments (5.75 to 6.46 per cent) when compared with control (6.60 per cent). Significant ($P< 0.05$) difference was recorded in egg/chick weight ratio of broilers in this study (1.40 to 1.43) than 1.44 of control. The Early chick mortality was 'nil' in normal saline, glucose, lysine, threonine fed *in-ovo* and ECNS post hatch when compared to control. The hatchability was not affected by *in-ovo* injection of different critical nutrients suggested that this technology can be adopted under field conditions to improve chick quality and subsequent performance.

Keywords: *in-ovo* injection**,** early post hatch feeding, transit weight loss, hatch weight, early chick mortality

Introduction
Under field conditions, most chicks receive feed and water 24-36 hours after hatching, which results in mobilization of body reserves to support metabolism, thermo regulation; decreases body weight and impairs overall performance. Body weight is increased up to threefold during the first week (Jin *et al*., 1998) [1] and delayed feeding in the first few days of life reduces final body weight. Early access to feed and water (early chick nutrition) as quickly as possible post-hatch is one of the most effective tool in stimulating early digestive function and sustaining performance throughout the growing period of the chick. The first week represents an ever-increasing proportion of the broiler production cycle. A good start leads to a uniform flock of chicks with a good 7th day weight which is positively correlated to the slaughter weight of the birds

Early chick feeding has an overall, long-term beneficial effects in broilers as it reduces mortality and transit weight loss; minimizes slow starters; eliminate ketosis, improves appetite, increases average daily gain, growth performance, enhances immunity, improves liveability thereby reduces mortality, decreases carcass condemnations, improves dressed weight and breast meat yield in broilers (Panda, 2006). Rapid growth of broiler chicken within a shorter period has provoked the interest of poultry nutritionists in early chick nutrition during the perinatal period. Keeping the above factors in mind a biological study was carried out to investigate the effect of *in-ovo* feeding and early chick nutritional supplement on growth performance of hybrid broiler chicken.

Materials and Methods

In-ovo injection of nutrient solutions was done as per modified Noor *et al*. (1995) [3] method. The *in-ovo* injection was carried out in empty incubation cabinet where the temperature and humidity was maintained at 37.5 °C and 60 per cent respectively.

On 18th day of incubation, the eggs were illuminated and infertile eggs were discarded. All fertile eggs were removed from the incubation tray and placed in plastic egg flats. Five hundred and forty hatching eggs were randomly assigned to six treatments each with three replicates of 30 eggs each and marked with permanent marker for identification. Then, each egg was candled and earmarked to identify the site of the injection. After disinfection of egg shell surface with 99.90% ethyl alcohol-laden swab, a pin head size (0.30 mm diameter) hole was made just below the air cell opposite to head spot using a sharp modified egg shell driller dipped in 99.90 per cent ethanol to sterilize the tip. Through this hole 0.5 ml of (*in-ovo* injection) one of the following nutritive solutions was injected into the amnion using a insulin syringe with 31 gauge needle (0.25 mm x 8 mm) to a depth of about 8 mm without disturbing the air cell.

1. Non injected control
2. Injected control (0.5 ml of normal saline solution)
3. 0.5 ml of 10% glucose solution
4. 0.5 ml of 0.5% lysine solution
5. 0.5 ml of 0.5% threonine
6. 0.5 ml of 0.5% β-hydroxy-β-methylbutarate (HMB)

Control eggs were removed from the incubator together with the treated groups, and kept in the same environment for 15 minutes (Time utilized to complete the *in-ovo* injection procedure for each group) to equalize the conditions for all treatment groups. The group of eggs designated as injected controls were injected with 0.5 ml of 0.9% Normal Saline to rule out a possible negative response caused by the stress of injection and handling. Prior to each injection (between eggs) the needle was immersed in 99.90% ethanol and replaced between treatments. The injection area was disinfected with 99.90% ethyl alcohol and the hole was sealed with melted paraffin wax and transferred to hatching trays. A validation test using a water-soluble dye was carried out to confirm the site (Amnion) of deposition of nutrient solution.

After completion of *in-ovo* injection, all eggs were transferred and incubated in hatching trays at the dry bulb temperature of 36.3 °C and the wet bulb temperature of 30.2°C without turning from 19- 21 days. The hatch was taken on day 21. The hatch weight of the chick was individually recorded, treatment wise.

The hatched-out broiler chicks (288 numbers) were randomly allotted into six treatment groups each with three replicates of 16 chicks each. The feeding of Steamed corn-soyabean and fish meal based early chick nutritional supplement (CSF WS) was done immediately after hatch in the hatcher tray of the incubator and continued till the chicks were placed in the chick transport box.

Table 1: Design of biological experiment

Treatments	Number of 18th day incubated fertile eggs utilized for *in-ovo* injection	Post hatch ECNS feeding (No. of birds utilized)
Non injected control	90	48
0.5 ml of normal saline + ECNS	90	48
0.5 ml of 10% glucose + ECNS	90	48
0.5 ml of 0.5% lysine + ECNS	90	48
0.5 ml of 0.5% threonine + ECNS	90	48
0.5 ml of 0.5% β-hydroxy-β-methylbutarate (HMB) + ECNS	90	48

*ECNS- Early Chick Nutritional Supplement

The broiler chicks were reared up to two weeks in cages and from third week to sixth week of age in deep litter house. The feed and water were provided *ad libitum*. commercial broiler birds were fed ad libitum with early chick nutritional supplement immediately after hatch in the hatcher tray and continued till the chicks were placed in chick transport box. After reaching the farm the birds were fed ad libitum with broiler pre starter, starter and finisher mash as per BIS (2007) specifications from 1-7, 8-21 and 22-42 days of age respectively.

The growth parameters on hatch weight, transit weight loss, body weight, weight gain, feed consumption, feed conversion ratio (FCR), and liveability were recorded. The body weight of the broiler chickens was recorded at hatch, at farm after transportation, at biweekly interval by using an electronic balance with 0.2 g accuracy. Feed consumption up to the six weeks of age was recorded. Feed conversion ratio was calculated by dividing average feed consumption by average body weight gain. Data recorded in this biological experiment was subjected to one way analysis of variance (ANOVA) with the Statistical Package for Social Science (SPSS, 1999) [5].

Results and Discussion

The hatch weight, egg/chick weight ratio, hatchability and transit weight loss of broiler chicks received both *in-ovo* feeding and early post hatch feeding of ECNS is presented in table. 2.

The hatching egg weight on setting was ranged from 68.77 to 68.89 g revealed the uniformity of egg size utilized in this experiment to avoid the experimental errors. In most poultry species egg weight and hatch weight are positively correlated. Thus, increased egg weight usually results in both a heavier chick and increased yolk sac size. Hatch weight of broiler chicks in our study did not show any significant difference due to selection of fertile eggs with uniform weight (68±1 g) from a single day collection at the time of setting. On 18th day of incubation, the egg weight was ranged from 60.47 to 60.59 g with 1.45 to 1.65 per cent physiological weight loss due to incubation process.

In-ovo feeding had resulted in highly significant ($P< 0.01$) improvement in hatchability than control (99.67 vs 92.22 per cent). Significant improvement in hatchability 7.45 per cent) due to *in-ovo* feeding was observed compared to control

(92.22 per cent) in this experiment, and was supported by Kulandaivel (2007) [6] who fed his experimented broilers with both *in-ovo* and post hatch ECNS and found that the per cent hatchability was improved by 83 per cent than 76 per cent in control.

In-ovo feeding with glucose, lysine, HMB produced significantly ($P< 0.05$) heavier chicks with hatch weight of 48.69 to 49.01 g than normal saline and threonine and control (47.73 to 48.39 g), same trend was reflected in egg/chick weight ratio (1.40 to 1.42 and 1.44). Hatch weight of broiler chicks recorded in this study was significantly higher (48.69 to 49.01g) to control (47.73 g), the hatch weight obtained by Kulandaivel (2007) [6] in a similar experiment was (37.66 g) in treatment groups to (37.76 to 37.85 g) than in control.

Though there was significant ($P< 0.05$) difference in transit weight loss of chicks, least weight loss of 4.66 per cent was observed in HMB *in-ovo* fed treatment than other treatments (5.75 to 6.46 per cent) and control (6.60 per cent). Significant difference was recorded in egg/chick weight ratio of broilers in this study (1.40 to 1.43) than 1.44 of control. Kulandaivel (2007) [6] recorded the same trend in his study by recording 1.32 to 1.39 in treatment chicks than 1.39 in control. Significant reduction in chick transit weight loss was recorded in this study in both *in-ovo* and ECNS fed groups (6.46 to 4.66 per cent) than control (6.60 per cent).

Early chick mortality was 'nil' in normal saline, glucose, lysine, threonine fed *in-ovo* and ECNS post hatch. In control group it was 1.54 per cent and in HMB *in-ovo* and ECNS it was 1.19 per cent. In control group it was 1.54 per cent and in HMB *in-ovo* and ECNS it was 1.19 per cent. Day-old chick weight showed significant ($P< 0.05$) difference between treatments. The combination feeding programme of *in-ovo* and ECNS feeding produced significantly heavier chicks (45.48 to 46.72 g) than control (44.58 g) at day old in this study. The literature on both *in-ovo* and ECNS feeding in broiler chicken is scanty possibly due to the fact that this is one of the emerging / newer areas of research.

Table 2: Mean (±SE) hatching egg weight, hatch weight and egg/chick weight ratio and transit chick weight loss as influenced by *in-ovo* feeding on 18th day of incubation and early post hatch ECNS feeding in broiler chicken

Treatments	Hatching egg weight (g) on setting	Hatching egg weight (g) On 18th d of incubation	Hatchability (%)	Hatch weight (g)	Egg / Chick weight ratio	Transit weight loss (%)	Early chick mortality (%)
	(n=90)	(n=90)	(n=3)	(n=48)	(n=48)	(n=48)	
Control	68.81 ± 0.05	60.51 ± 0.05	92.22^{b} ± 0.02	47.73^{b} ± 0.29	1.44^{a} ± 0.01	6.60^{a} ± 0.25	1.54 ± 0.90
0.5 ml of 0.5% Normal saline + ECNS	68.77 ± 0.05	60.47 ± 0.05	96.67^{a} ± 0.00	48.39^{ab} ± 0.30	1.42^{ab} ± 0.01	6.02^{a} ± 0.24	0.00 ± 0.00
0.5 ml of 10% Glucose + ECNS	68.86 ± 0.05	60.56 ± 0.05	96.67^{a} ± 0.00	48.69^{a} ± 0.17	1.42^{bc} ± 0.00	6.46^{a} ± 0.25	0.00 ± 0.00
0.5 ml of 0.5% Lysine + ECNS	68.85 ± 0.05	60.55 ± 0.05	96.67^{a} ± 0.00	48.93^{a} ± 0.32	1.41^{bc} ± 0.01	6.05^{a} ± 0.22	0.00 ± 0.00
0.5 ml of 0.5% Threonine + ECNS	68.89 ± 0.05	60.59 ± 0.05	96.67^{a} ± 0.00	48.34^{ab} ± 0.31	1.43^{ab} ± 0.01	5.75^{a} ± 0.30	0.00 ± 0.00
0.5 ml of 0.5% HMB + ECNS	68.78 ± 0.07	60.48 ± 0.07	96.67^{a} ± 0.00	49.01^{a} ± 0.22	1.40^{c} ± 0.01	4.66^{c} ± 0.23	1.19 ± 0.19
Significant	NS	NS	**	*	*	*	NS

n - Number of observations
NS - Not Significant * Significant ($P< 0.05$) ** Highly significant ($P< 0.01$)
Mean values sharing any one common superscript in a column do not differ significantly

Conclusion

Based on the results obtained from this biological experiment involving *in-ovo* feeding combined with early post hatch ECSN feeding in broilers, it was concluded that the egg/chick weight ratio, hatch weight and day-old weight of chicks were significantly increased. However, the hatchability was not affected by this *in-ovo* feeding technology. The early chick mortality was significantly reduced by the combined *in-ovo* and early post hatch ECNS feeding in broiler chicks. This indicates that this technology can be adopted under field conditions to improve the chick quality and other associated production performances in commercial broilers.

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