Original Research

Effect of *In-ovo injection* of Glucose, Lysine, Threonine and β-hydroxy-β-methylbutarate (HMB) on the Morphometry of Digestive Organs in Commercial Broilers

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Abstract

Under field conditions, most of the broiler chicks receive feed and water 24-36 hours after hatching results in decreases body weight and impairs overall performance. Hence, a biological trial was carried out in broilers to investigate the effect of In-ovo injection of glucose, lysine, threonine and β-hydroxy-β-methylbutarate (HMB) on the morphology of digestive organs in commercial broilers. On the 18th day of incubation, of total 540 embryonated, each 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 as non-injected control (T_1) and 90 eggs as injected control. A pin head size hole was made just below the air cell and 0.5 ml of (In-ovo injection) nutrient solution was injected into the amnion using an insulin syringe. The hole was sealed and the period of incubation was completed. Biological feeding trial was continued up to 42 days with 288 broiler chicks from different In-ovo treated groups each with three replicates of sixteen chicks each. The chicks were fed ad libitum with broiler pre-starter, starter and finisher mash as per BIS (2007) specifications from 1-10, 11-21 and 22-42 days of age respectively. Data on absolute weight of digestive organs at sixth week of age was recorded by using an electronic balance with 0.2 g accuracy and subjected to statistical analysis. Results revealed that In-ovo injection of glucose, lysine, threonine and HMB on 18th of incubation produced significantly heavier liver and pancreas on absolute weight basis. However, other digestive organ absolute and relative weights were not significantly influenced in this study. In-ovo injection of vital nutrients increased only accessory digestive organs like liver and pancreas of broilers whereas other digestive organs weight was not affected.

Key words: Broiler Chicken, Digestive Organs, Glucose, HMB, *In-ovo*Injection, Lysine, Morphometry, Threonine

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Introduction

Under field conditions, most of the broiler 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. During immediate post-hatch period, glycogen reserves decline rapidly and adversely affect the growth and livability. Chick's first meal occurs when they consume the amnion fluid before internal pipping (about 18th day of incubation in broilers). Injecting appropriate nutrients in to amnion is a novel way to feed critical dietary components to embryos. *In-ovo* technology may also provide a precision nutrition at the specific time for peak absorption of specific nutrients, cofactors or metabolic modulators by the embryo. Glucose (GLUC) provides immediate source of energy for piping. Bhanja and Mandal (2005) reported that *In-ovo injection* of limiting amino acids in different combinations at 14 d of incubation did not have any influence on the length and weight of the digestive and immune organs. Whereas Santos *et al.* (2010) demonstrated *In-ovo* feeding of different nutrient solutions and sodium chloride (control) into broiler breeder eggs on 18th d of incubation resulted in more absolute and relative weight of digestive organs and intestine at day old and up to 3 weeks of age. The development and health of digestive system is of prime importance in broilers to express its full genetic potential. Hence, the present study was planned to investigate the effects of *In-ovo* injection of glucose, lysine, threonine and β-hydroxy-β-methylbutarate (HMB) on morphology of digestive organs of hybrid broiler chicken.

Materials and Methods

In-ovo injection of nutrient solutions was done as per the modified Noor *et al.* (1995) 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 candled and infertile eggs were discarded. 540 fertile eggs were removed from the incubation tray and placed in plastic egg flats. 90 eggs were randomly assigned to each treatment 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. Though this hole 0.5 ml of (*In-ovo* injection) one of the following nutritive solution was injected into the amnion using an insulin syringe with 31 gauge needle (0.25 mm x 8 mm) to a depth of about 8 mm without disturbing the air cell.

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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. A validation test was also carried out to confirm the site (Amnion) of deposition of nutrient solution. 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 hatchability and hatch weight of the chick was recorded treatment wise and statistically analysed.

The hatched out 288 broiler chicks from were randomly allotted into 6 treatment groups each with three replicates of sixteen chicks for each replicate. The chicks 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. Data on sixth week body weight, FCR, absolute weight of digestive organs at sixth week of age was recorded by using an electronic balance with 0.2 g accuracy. The relative weight was calculated based on live body weight and expressed as per cent of live weight. Six birds from each treatment were subjected to slaughter as per the method of Arumugam and Panda (1970) following overnight feed withdrawal. The weight of the digestive organs viz. proventriculus, gizzard, liver, pancreas, small intestine and large intestine all without contents were recorded and expressed as g/100 g live weight. The broiler chickens were fed *ad libitum* feed during the experimental period. Feed consumption up to 6th week was recorded. Feed conversion ratio was calculated by dividing average feed consumption by average body weight gain.

Data recorded in the biological experiments were subjected to one way analysis of variance (ANOVA). The statistical analyses were carried out with the Statistical Package for Social Science (SPSS, 1999 for windows version 17; SPSS GmbH, Munich, Germany) to determine analysis of variance between groups.

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Means were compared by Duncan multiple range comparison test (Steel and Torrie, 1981) with level of significance (P<0.05).

All the experimental procedures were assessed and approved by the Institutional Animal Ethics Committee from the Tamil Nadu Veterinary and Animal Sciences University, Chennai -600 051 and all the institutional guidelines were followed.

Result and Discussion

Data on hatchability, hatchling weight, marketing body weight and feed conversion ratio and relative weigh of digestive organs of sixth week age broiler chicken as influenced by *In-ovo* feeding on 18th d incubation is presented in Table 1 and 2.

Table 1: Mean (±SE) hatchability, hatchling weight, marketing body weight and feed conversion ratio of broiler chicken as influenced by *In-ovo* feeding of various vital nutrients on 18th d of incubation.

Treatments		Hatchability (%) (n=6)	Hatchling Weight (g) (n=96)	6th week Body weight (g)	Cumulative FCR (n=6)
Control		92.22^{c}	47.25^{b}	2080.6 (33)	2.21^{a}
In-ovo feeding of **0.5 ml**	0.5% Normal Saline	94.44^{b}	47.85^{ab}	2135.1 (36)	2.12^{a}
	10% Glucose	96.67^{a}	48.79^{a}	2159.9 (35)	2.04^{ab}
	0.5% Lysine	96.67^{a}	48.53^{a}	2116.6 (34)	2.03^{ab}
	0.5% Threonine	96.67^{a}	48.14^{ab}	2241.9 (34)	1.91^{b}
	0.5% HMB	96.67^{a}	48.14^{ab}	2212.4 (33)	1.93^{b}
Pooled SEM		0.83	0.32	34.35	0.08
Significant		*	*	NS	*

*Value in each cell is mean of six observations; NS – Non significant, * Significant (P<0.05); Mean values sharing any one common superscript in a column do not differ significantly*

In-ovo feeding significantly (P<0.05) improved hatchability and ranged from 94.44 to 96.67 per cent than control (92.22 per cent). *In-ovo* feeding with lysine or threonine produced significantly (P<0.05) heavier day old chicks (45.79 and 46.04 g) and was comparable with *In-ovo* glucose and normal saline fed groups. Control (44.96 g) was significantly (P<0.05) lowest body weight and this was comparable with glucose and normal saline groups. Hatchability significantly improved due to *In-ovo feeding* in this study was in agreement with Santos *et al.* (2010) who recorded improved hatchability of 89.04 to 91.25 % in maltose and vitamins injected groups and in control 90.49 %. Salmanzadeh (2012) confirmed the present findings of increased hatch weight by In-ovo injection of glucose. However, Bhanja and Mandal (2005) and Uni *et al.* (2005) reported no significant difference between *In-ovo* feeding and control. The sixth week body weight was a numerically increased in threonine fed group (2195.86 g), while the control birds had the lowest body weight of 2035.64 g. Kadam *et al*. (2013) and Lotfi *et al.* (2013) recorded increased body weight and weight gain in broilers injected with In-ovonutrients of betaine and gherline while Bello *et al.*

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(2014) observed no significant difference. Sixth week cumulative FCR in broiler chicken fed with *In-ovo*nutrients was significantly (P<0.05) improved than control. While the threonine (1.91) and HMB (1.93) injected chicks registered significantly better FCR compared to other treatments and control. The improved FCR obtained in the present study was conferred by Salmanzadeh *et al.* (2012) who found that the 6th week mean cumulative feed conversion ratio of broilers was better (1.88, 1.87 and 1.88) in the glucose *In-ovo* fed groups (15, 20 and 25 per cent) compared to control (1.91) and sham group (1.93).

Table 2: Mean (±SE) relative weight of digestive organs of broiler chicken at 6th week of age as influenced by *In-ovo feeding* of various vital nutrients on 18th d of incubation

Treatments		Relative Weight of the Digestive Organs (Percentage of Live Weight)							
		Liver	**Pancreas**	**Proventriculus + Gizzard**	**Total Intestine**	**Duodenum**	**Jejunum**	**Ileum**	**Caecum**
Control		2.02	0.27	2.33	5.07	0.86	1.64	1.34	0.82
***In-ovo feeding* of 0.5 ml**	0.5% Normal Saline	1.77	0.22	2.4	5.02	0.96	1.52	1.48	0.83
	10% Glucose	1.78	0.28	2.27	5.18	1.07	1.61	1.3	1.04
	0.5% Lysine	2.27	0.2	2.3	5.75	0.94	2.15	1.67	0.79
	0.5% Threonine	1.88	0.29	2.42	5.06	1.1	1.75	1.35	0.71
	0.5% HMB	1.79	0.28	2.26	4.85	0.96	1.66	1.37	0.89
Pooled SEM		0.17	0.02	0.15	0.19	0.07	0.16	0.11	0.09
Significant		**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

*Value in each cell is mean of six observations; NS – Non significant, * Significant (P<0.05); Mean values sharing any one common superscript in a column do not differ significantly*

In-ovo feeding produced significantly (P<0.05) heavier liver and pancreas on absolute weight basis. However, other digestive organ showed no significant difference both in absolute and relative weight basis in this study.

Control chicks had small liver (36.07 g) than *In-ovo* fed groups (38.27 to 50.47 g) while lysine produced heaviest liver, normal saline yielded the lightest liver. Whereas, the relative weight of liver was not affected by *In-ovo* treatments and ranged from 1.77 to 2.27 per cent of live weight. Increased liver weight obtained in this study was also reported by Maiorka *et al.* (2003) and Faye *et al.* (2006). Whereas, Pedroso *et a.,* (2006) reported that liver weight was not affected by *In-ovo* feeding. *In-ovo* injection of lysine, threonine, HMB produced significantly (P< 0.05) heavier pancreas (5.75 to 6.42 g) than glucose (5.58 g), normal saline (4.77g) and control (4.28 g) but did not affect the relative weight of pancreas (0.20 to 0.028 per cent). The present finding was contrary to Pedroso *et al.* (2006) who found that *In-ovo* feeding of glucose, linoleic acid and glutamine in different experiments did not affect the mean weight of the pancreas.

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The absolute and relative weight of the proventriculus and gizzard weight was not affected by different *In-ovo* treatments and the absolute weighted ranged between 45.89 to 52.30 g and the relative weight ranged from 2.26 to 2.42 per cent. Similarly, the absolute weight (106.42 to 130.52 g) and relative weight (4.85 to 5.75 per cent) of intestine, the absolute weight (18.95 to 23.00 g) and relative weight (0.86 to 1.10 per cent) of duodenum, the absolute weight (32.95 to 48.25 g) and relative weight (1.52 to 2.15 per cent) of jejunum, the absolute (26.53 to 32.68 g) and relative weight (1.30 to 1.67 per cent) ileum and the absolute (15.40 to 21.03 g) and relative weight (0.71 to 1.04 per cent) of ceaca was also not affected by *In-ovo* injection of glucose, lysine, threonine and HMB in this experiment. In all these, the *In-ovo* fed chicks registered numerically higher weight than control. Similar findings were also reported by Pedroso *et al* (2006) concluded that *In-ovo f*eeding produced no difference in digestive organs (proventriculus and gizzard and, duodenum, jejunum and ileum) weight. Similar findings were also reported by Chen *et al*. (2009) studied *In-ovo* feeding of ducks and reported no change in liver, proventriculus and gizzard weight and also intestinal weight when compared in control.

Contrary findings of increased intestinal weight by earlier workers Uni and Ferket, (2003), Salahi *et al.* (2011) and Rocha *et al.* (2013). Heavier digestive organs were obtained in *In-ovo feeding* by Santos *et al.* (2010) and Lotfi *et al.* (2013). Increased proventriculus, gizzard weight was also recorded by Rocha *et al.* (2013). *In-ovo* feeding of chicken had resulted in heavier duodenum, jejunum, ileum as reported by Maiorka *et al.* (2003) and Salahi *et al.* (2011).

Conclusion

In-ovo injection of glucose, lysine, threonine and HMB on 18th of incubation produced significantly heavier liver and pancreas on absolute weight basis. However, other digestive organ absolute and relative weights were not significantly influenced in this study.

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