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Effect of Peri-natal Supplementation of Amino acids on Post-hatch Performance of Broiler Chicken

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ABSTRACT

An experiment was conducted to assess the effect of peri-natal supplementation of amino acids in broiler chicken. Three hundred and eighty Cobb broiler eggs set for incubation, were divided into un-supplemented and *in ovo* supplemented (lysine, methionine, arginine, threonine, and glutamine at 2.2, 1, 2.5, 1.6 and 2.5 mg/egg) groups on 18 days of incubation. Following hatching, un-supplemented group was again sub-divided into un-supplemented (control) and post-hatch supplemented group. The post hatch supplemented diet consisted of 25 % extra amino acids (lysine, methionine, threonine, arginine, glutamine) than that in starter diet for first 3 days. Results indicated poor hatchability of fertile eggs on *in ovo* supplementation. The body weight gain, feed intake and feed conversion ratio did not differ significantly (P>0.05) among treatments. At first week of age, longer (P<0.008) length of jejunal villus was observed in post-hatch amino acid supplemented group (161 µm) as compared to *in ovo* supplemented (93.5 µm) and control (117 µm) groups. Humoral immune response was better (P<0.013) in broiler chicks on *in ovo* and post-hatch supplementation. The ileal digestibility coefficient of crude protein, calcium and phosphorus were not influenced (P>0.05) by *in ovo* and post-hatch supplementation of amino acids. It could be concluded that *in ovo* supplementation of (lysine, methionine, arginine, threonine, and glutamine at 2.2, 1, 2.5, 1.6 and 2.5 mg/egg) or post-hatch supplementation of 25 % extra amino acids (lysine, methionine, threonine, arginine, glutamine) did not influence the growth performances of broiler chicken. However, humoral immune response was better on *in ovo* and post-hatch supplementation.

Key words: Amino acids, Broiler, Growth performance, Hatchability, *in ovo*, Immunity

INTRODUCTION

The gut development is of great importance during the last period of broiler embryonic development and the early post-hatch period. *In ovo* administration of nutrients in amnion prepares the opportunity for chicks to orally consume supplemented nutrients and develop their digestive and absorptive ability prior to hatch. It has been reported that approximately 2 to 5 % of the hatchlings do not survive the critical post-hatch period and many survivors exhibit stunted growth, poor feed utilization, reduced immunity and lower meat yield (Uni and Ferket, 2004). There may be a great demand of amino acids such as glycine, proline, lysine and arginine during early period of embryonic growth (Kadam *et al.,* 2008). Threonine (Thr) being the only precursor of glycine, plays an important role for pre-hatch embryonic growth. Foye *et al.*(2006) observed that *in ovo* injection of arginine enhanced hepatic reserves providing the fuel needed for rapid subsequent growth during the critical post-hatch period. Samli *et al.* (2007) reported that glutamine stimulates intestinal cell proliferation, leading to increase in the absorption through gastrointestinal mucosa and consequently the accretion of nutrients. *In ovo* feeding and post-hatch supplementation help the bird during the transition from embryo to chick and help the broiler to achieve its full genetic potential. Supplying the embryo with amino acids through *in ovo* feeding would allow the gastro-intestinal tract to develop the structures and functionality to properly digest and absorb nutrients immediately when exogenous nutritional supplementation is provided after hatch (Uni and Ferket, 2003). These nutrients, along with the yolk sac reserves, can contribute not only to maintaining the systems and

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metabolism already established, but also to continuing growth, development, and proper nutritional status (Noy and Sklan, 1998). Post- hatch supplementation of amino acids has an overall long-term beneficial effect in broilers as it reduces mortality and transitional weight loss, improves growth performance, enhances immunity, and improves breast meat yield in broilers (Panda, 2008). The current focus of broiler management needs to be shifted to the fortification during the embryonic to first few days of post- hatch phase so that the early growth impetus results in achieving the targeted growth in less time. Keeping the above information in view, the present study was designed to check the effect of peri- natal supplementation of amino acids on post hatch performance of broiler chicken.

MATERIALS AND METHODS

The experimental procedure was approved by Ethical committee of ICAR-National Institute of Animal Nutrition and Physiology, Bangalore, India.

Three hundred and eighty uniform sized eggs (Cobb broiler) were procured from commercial hatchery and incubated with the dry bulb temperature

Table 1. Ingredient and nutrient composition of experimental diet

	Post- hatch	Starter	Finisher
Duration and target groups	0-3 d: Post-hatch	0-21 d: Control & *in ovo* 4-21d: post-hatch	22-35 d: All groups
Ingredients (%)			
Maize	57.19	58.06	61.81
Soybean meal	36	36	32
Sunflower oil	2	2	2.25
Lime stone	1	1	1
Dicalcium phosphate	1.75	1.75	1.5
Salt	0.35	0.35	0.35
Celite	0	0	0.5
L-Lysine	0.59	0.37	0.2
DL-Methionine	0.33	0.22	0.14
L-Threonine	0.2	0	0
L-Arginine	0.34	0	0
L-Glutamine	0.70	0	0
Vitamin mineral premix *	0.25	0.25	0.25
Nutrient composition (%)			
ME (kcal /kg)**	2990	2975	3047
Crude protein	22.7	22.1	20.5
Lysine	1.68	1.34	1.11
Methionine	0.63	0.5	0.41
Threonine	0.97	0.77	0.73
Arginine	1.74	1.4	1.28
Glutamine	3.5	2.8	
Calcium	1.04	1.04	0.98
Available P**	0.45	0.45	0.4

*Trace mineral premix 0.1%, Vitamin Premix 0.1%, choline 0.05 %. Trace mineral premix supplied mg/ kg diet: Mn, 75; Se, 0.2; Fe, 40; Zn, 70; Cu, 10. The vitamin premix supplied per kg diet: vitamin A, 8250 IU; vitamin. D_3, 1200 ICU; vitamin. K, 1 mg; vitamin. E, 40 IU; vitamin B_1, 2 mg; vitamin B_2, 4 mg; vitamin B_{12}, 10 mcg; niacin, 60 mg; pantothenic acid, 10 mg; ** Calculated values

ranging from 99-100°F and wet bulb temperature of 85-87°F from day 1 to 18. On 18th day, 340 fertile eggs were assigned to two groups: control group and *in ovo* group. *In ovo* group was supplemented with amino acid combination (lysine, methionine, arginine, threonine, and glutamine at 2.2, 1, 2.5, 1.6 and 2.5 mg/egg). Following hatching, chicks from without *in ovo* supplemented group were further divided into control (Group T_1) and post-hatch supplemented group (Group T_3). Chicks from *in ovo* supplementation were continued as *in ovo* supplemented group (Group T_2). Group T_1 and Group T_2 were fed normal starter diet without any supplementation for 0-21 days. The post hatch-supplemented diet (with 25 % extra amino acids of lysine, methionine, threonine, arginine, glutamine than the starter diet) was fed to the birds of Group T_3 for first 3 days and subsequently from 4-21 days they were fed normal starter diet. Finisher diet was same for all the groups. Ingredient and nutrient composition of experimental diets are presented in Table 1.

After hatching, chicks were randomly distributed, into battery cages (6 replicates with 9 chicks in each replicate). Battery cages were arranged with feeders, waterer, dropping trays, and necessary heating arrangements. Twenty-four hours light and proper air ventilation was provided. The temperature inside the cage was maintained at 33°C on day 1 and gradually reduced to 24-25°C by the end of the third week and maintained. The feed and fresh drinking water were provided *ad lib.*

Body weight was recorded every week to ascertain the weekly and overall body weight gain. The experimental diets were given *ad lib.* and the residue was weighed at weekly interval in order to arrive at feed intake. Based on the data pertaining to the feed intake and body weight gain, the feed conversion ratio (FCR) was calculated.

On 7, 21 and 35 days of age, one chick from each replicate was sacrificed by cervical dislocation for gut development studies. Functional development of gut was measured by histological examination of jejunal villi. About 2-3 cm long jejunal samples were collected in 10% formal saline after washing the contents with normal saline. The paraffin embedded sections were stained with Haematoxylin and Eosin (H&E). The weight of the immune organs was recorded at 21 and 35 days of age.

The humoral immune response was measured on day 28 post-hatch (12 chicks per treatment) using 1 ml of 1% (V/V) suspension of sheep red blood cells (SRBC) as per Siegal and Gross (1980) and Vander Zijpp (1983). The cell mediated immune response on day 21 was assessed by cutaneous basophilic hypersensitivity test *in vivo* by using phytohaemagglutinin lectin from *Phaseolus vulgaris* (PHA-P) as described by Corrier and Deloach (1990).

After 35 days of the experiment, the ileal contents from Meckel's diverticulum to ileo-caecal junction (2 chicks / replicate) were collected replicate wise and dried in an oven at 60°C. The digesta samples were ground and stored in air-tight containers for the estimation of crude protein (CP), acid insoluble ash (AIA) (AOAC, 1990). After ashing, the ash samples were digested with dilute hydrochloric acid (1:2) and the mineral extract was used for estimation of minerals by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Perkin Elmer instrument. The apparent digestibility coefficient of nutrients was calculated from the equation:

1 – [(ileal nutrient/ ileal AIA) / (diet nutrient/ diet AIA)]

The data pertaining to the various parameters were subjected to one way analysis of variance for completely randomized design and tested for significance among the dietary treatment means (SPSS Version 16.0, SPSS Inc, Chicago, USA).

RESULTS AND DISCUSSION

Hatchability of fertile eggs was poor in *in ovo* supplemented group (37 %) as compared to control (88 %) (Table 2). Egg and chick weight did not differ significantly (P>0.05) between control and *in ovo* group. In the present study, *in ovo* supplementation of amino acid (lysine, methionine, arginine, threonine and glutamine) in combinations lowered the hatchability. As such, a possible explanation for poor results might be

Table 2. Hatchability of eggs on *in ovo* supplementation

Group	Treatment	Egg weight (g)	Chick weight (g)	Hatchability (%)
I	Control	69.08	50.85	88 (151/ 170)
II	*In ovo* supplementation	68.92	52.65	37 (63/170)
SEM		0.246	0.542	
P-value		0.33	0.10	

probably due to the type, number and amino acid used in combinations for *in ovo* supplementation. Toghyani *et al.* (2012) supplemented 35 mg Arg, 25 mg Thr and 35 mg Arg+25 mg Thr per egg individually and in combination, and reported decreased hatchability in Arg (76.3%) and Arg+Thr (73.8%) supplemented groups in comparison to control (88.2%) and Thr injected group (88.8%). Bhanja *et al.* (2012) on supplementation of 25 mg each of limiting amino acids *viz.* Lys, Met, Thr, Arg, Gly, Ile individually on 14th d of incubation observed that hatchability was not affected. Chick weight at hatch was similar in comparison to un-injected control group (Lys, 48.21; Thr 48.92; Met, 50.11; Arg, 48.09 vs non-injected control 48.58 g/b). Shafey *et al.* (2013) on *in ovo* injection of graded dose of glutamine at 5, 7.5, and 10 mg/egg at 17 day of incubation did not observe any significant difference on hatchability between treatment groups (Gln 5 mg, 89.3; 7.5 mg, 88.5; 10 mg, 88.8%; Control: 93.6%). Chick weight at hatch did not differ significantly among treatment groups (Gln 5 mg, 40.90; 7.5 mg, 40.80; 10 mg, 41.03 vs 40.98 g/b control group). Al-Asadi *et al.* (2013) reported that *in ovo* injection of 2 % lysine or arginine in broiler chicken had significantly higher hatchability in compared to non-injected group (Lys, 87.52; Arg, 91.75 vs control grp, 79.59 g/b), with higher chick weight in *in ovo* injected amino acid group in comparison to un-injected group (Lys, 50.20; Arg, 51.03 vs control 47.70g/b). Coskun *et al.* (2014) observed that *in ovo* injection of 1 ml of DL-methionine reduced the hatchability (84.7%) compared to the un-injected control group (90.2%). Awachat *et al.* (2018) injected combination of Lys 22, Met 10 and Thr 16 mg and reported reduced hatchability on *in ovo* injected group (67.8%) as compared to (93.3%) control. Kadam *et al.* (2009) recorded poor hatchability when Thr (20 mg/egg) was injected into the albumen either through broad or narrow end, however better hatchability was recorded when Thr was injected into the yolk sac of the egg. Many of the earlier studies (Kadam *et al.,* 2008; Bhanja *et al.,* 2012; Shafey *et al.,* 2014; Nayak *et al.,* 2016; Awachat *et al.,* 2017; Coskun *et al.,* 2018; Sogunle *et al.,* 2018) have reported better or similar hatchability on *in ovo* amino acid supplementation. In all these studies, they have supplemented mostly individual amino acids or combination of not more than 3 amino acids with lower doses.

There was no significant difference (P>0.05) in body weight gain, feed intake or feed conversion ratio due to either *in ovo* or post-hatch supplementation (Table

Table 3. Growth performance of broiler chicken

	Body weight gain (g/bird)			Feed intake (g/bird)			FCR		
	0-3	3-5	0-5	0-3	4-5	0-5	0-3	4-5	0-5
T_1	788	1065	1854	977	1771	2748	1.24	1.66	1.48
T_2	825	1036	1862	1066	1653	2719	1.30	1.60	1.46
T_3	804	1069	1863	1041	1714	2756	1.30	1.60	1.47
SEM	11.4	18.9	14.8	23.1	44.2	44.42	1.277	1.619	1.470
P-value	0.45	0.76	0.87	0.28	0.58	0.32	0.61	0.52	0.92

[a,b]Means with different superscripts in a column differed significantly; T_1, Control; T_2, *in ovo* injection; T_3, post-hatch supplementation

Table 4. Digestive organ weight (% of live weight) and length (cm / 100 g live weight) at 1st and 5th week of age

Group	Duodenum		Jejunum		Ileum		Caecum		Liver	Proventri	Gizzard
	Length	Weight	Length	Weight	Length	Weight	Length	Weight	Weight	Weight	Weight
1st week											
T_1	11.7	2.69	29.4	4.73	26.4	3.57	5.34	1.21	4.92	1.16	8.96
T_2	12.3	2.79	28.7	4.51	5.1	3.18	5.00	1.02	4.66	1.09	6.83
T_3	11.0	2.60	27.6	5.11	24.4	3.60	4.71	1.07	4.36	1.11	7.63
SEM	0.49	0.09	0.98	0.17	1.09	0.16	0.18	0.08	0.24	0.04	0.51
P-value	0.58	0.75	0.77	0.36	0.65	0.52	0.38	0.63	0.65	0.79	0.23
5th week											
T_1	1.13	1.36	2.99	3.01	1.63	2.95	0.83	0.80	2.30	0.384	2.14
T_2	0.96	1.35	1.87	3.3	1.69	3.28	0.87	0.88	2.09	0.368	2.07
T_3	0.98	1.33	2.09	3.27	1.74	3.37	0.9	0.93	2.07	0.376	2.16
SEM	3.34	0.041	0.29	0.087	0.05	0.076	0.03	0.025	0.05	0.008	0.04
P-value	0.36	0.96	0.25	0.327	0.7	0.051	0.67	0.11	0.13	0.72	0.67

T_1, Control; T_2, *in ovo* injection; T_3, post-hatch supplementation

3). There are earlier reports of *in ovo* amino acids supplementation especially on single or fewer (two or three) amino acid combinations at lower doses in broiler chicken. Bhanja *et al.* (2012) reported that supplementation of 25 mg of each limiting amino acids *viz.* lysine, methionine, threonine, arginine, glycine and isoleucine, did not affect feed conversion ratio but there was an improvement in body weight gain of broiler chicken. Shafey *et al.* (2014) reported that amino acid mixtures injected group had higher body weight gain than un-injected control group. Toghyani *et al.* (2012) reported that *in ovo* injection of Arg, 35; Thr, 25 mg and Arg, 35 + Thr 25 mg/ egg individually and in combination increased body weight in comparison to un-injected control group (2155.6 g). Awachat *et al.* (2017) supplemented combination of 3 amino acids, Arg 22 mg+ Glu 25 mg + Thr 30 mg/egg on 18 days of incubation and did not observe any effect on growth performance in broiler chicken. Coskun *et al.* (2018) supplemented Lys and Met and reported non-significant difference between live weight gain, feed intake, and feed conversion ratio in broiler chicken. The positive response in earlier work may be due to the stock used, which might have been more responsive to *in ovo*

Table 5. Development of jejunal villus at 1, 3 and 5 week of age

Group	1 week of age			3 week of age			5 week of age		
	0-3	3-5	0-5	0-3	4-5	0-5	0-3	4-5	0-5
	Length (µm)	Breadth (µm)	Crypt Depth (µm)	Length (µm)	Breadth (µm)	Crypt Depth (µm)	Length (µm)	Breadth (µm)	Crypt Depth (µm)
T_1	117[b]	13.5	22.1	143	23.9	21.8	205	31.0	31.2
T_2	93.5[b]	28.1	16.2	120	25.0	10.9	152	30.7	25.9
T_3	161[a]	26.3	20.9	118	21.6	17.4	187	39.1	26.3
SEM	10.4	3.02	1.45	8.92	2.98	2.66	15.0	1.83	2.88
P-value	0.008	0.08	0.22	0.49	0.92	0.27	0.37	0.09	0.75

Means with different superscripts are differed significantly; T_1, Control; T_2, *in ovo* injection; T_3, post-hatchsupplementation

Table 6. Immune response and weight of immune organs (% of live weight)

Group	FPI (mm)	HA titre (log 2)	3 wk of age		5 wk of age	
			Bursa	Spleen	Bursa	Spleen
T_1	0.52	7.00[b]	0.247	0.100	0.248	0.115
T_2	0.57	7.57[a]	0.208	0.092	0.202	0.127
T_3	0.65	7.60[a]	0.234	0.109	0.254	0.116
SEM	0.043	0.100	0.016	0.006	0.014	0.006
P-value	0.45	0.01	0.63	0.53	0.25	0.65

[a,b]Means with different superscripts in a column differed significantly; T_1, Control; T_2, *in ovo* injection; T_3, post-hatch supplementation

amino acid supplementation as compared to the current commercial stocks.

A significant (P<0.008) increase in the length of jejunal villus on post-hatch amino acid supplemented group (161 µm) was observed as compared to *in ovo* supplemented (93.5 µm) and control (117 µm) group at first week of age. The rest of the gut parameters were similar in all treatments (Table 5). Bhanja and Mandal (2005) reported that supplementation of different combination of essential and non-essential amino acids had no influence in the digestive organs weight. Bhanja *et al.* (2012) reported that supplementation of 25 mg each of Lys, Met, Thr, Arg and Gly did not cause any significant difference in weight of digestive organs of day-old chick. Bartell and Batal (2007) reported that addition of 1 or 4% glutamine to the feed or water, or both for 4 days post-hatch had heavier relative intestinal weights and longer intestinal villi as compared to the chicks fed the corn-SBM control diet (1% Gln, 838.6 um vs 778.3 um duodenal villi height). Salmanzadeh *et al.* (2016) recorded increased villus height, villus width and crypt depth in the jejunum as a result of in *ovo* Glu supplementation as compared to the non-injected and sham controls in newly hatched and 10-day old chick. Awachat *et al.* (2017) observed that *in ovo* supplementation of Arg 22 mg+ Glu 25 mg + Thr 30 mg/egg significantly (P<0.05) increased the weights of duodenum (1.61 vs 1.30), jejunum (2.29 vs 1.68), proventriculus (1.13 vs 0.84) gizzard (9.81 vs 8.21) and the length of jejunum (44.75 vs 38.34) on the day of hatch.

Humoral immune response was significantly (P<0.013) better on *in ovo* and post-hatch supplementation (Table 6). Cell-mediated immune response and weight of immune organs did not differ significantly among the groups. Few of the earlier studies reported a beneficial effect of *in ovo* amino acid supplementation on immune response in broiler chicken. Bhanja and Mandal (2005) reported higher cell-mediated immunity in amino acid injected (14 d injection) group in comparison to (0.22 mm) non-injected group. Humoral immune response was also improved on *in ovo* injection. Bhanja *et al.* (2014) reported that arginine and threonine on 14-day of injection enhanced the expression of growth-related genes, while threonine and Met+Cys modulated the expression of immune genes

Table 7. Ileal digestibility coefficient of nutrients

Group	Crude protein	Ca	P
T_1	0.812	0.535	0.439
T_2	0.827	0.517	0.423
T_3	0.823	0.548	0.444
SEM	0.004	0.008	0.009
P-value	0.31	0.31	0.66

T_1, Control; T_2, *in ovo* injection; T_3, post-hatch supplementation

in broiler chickens. Bakayraj *et al.* (2012) found that cell-mediated immunity (0.38) was higher in chicks injected *in ovo* amino acid as compared to control (0.20 mm). Awachat *et al.* (2018) reported that *in ovo* supplementation of amino acid combination (lysine, methionine and threonine) did not have any influence in cell mediated and humoral immunity in broiler chicken. There was no significant difference (P>0.05) in ileal digestibility of CP, Ca and P among different treatments (Table 7). Jose *et al.* (2016) did not observe any significant difference between treatment groups in apparent digestibility of CP and on *in ovo* supplementation of zinc.

CONCLUSIONS

In ovo supplementation of amino acids (lysine, methionine, arginine, threonine, and glutamine at 2.2, 1, 2.5, 1.6 and 2.5 mg/egg) or post-hatch supplementation of 25 % extra amino acids (lysine, methionine, threonine, arginine, glutamine) did not influence the growth performances of broiler chicken. However, humoral immune response was better on *in ovo* and post-hatch supplementation.

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