Tropical Animal & Poultry Science

Publisher: Tropican Animal Research Services, Armidale NSW, Australia. www.tropican.com.au. Email: trop.sci@gmail.com
ISSN: 2208-8431

Influence of perinatal amino acid supplementation on hatchability, gastro-intestinal tract development and growth performance of broiler chicks.

V. B. Awachat[1], A. V. Elangovan[1], N. Jose[1], C. G. David[1], J. Ghosh[1], S. K. Bhanja[2] and S. Majumdar[2]

[1]ICAR - National Institute of Animal Nutrition and Physiology, Bangalore, India

[2]ICAR - Central Avian Research Institute, Izatnagar, Bareilly, India.

Corresponding author: avelango@gmail.com or avelango@yahoo.co.in

Abstract

An experiment was designed to determine the effect of perinatal administration of amino acids on hatchability, growth performance and gastrointestinal tract development of broiler chicks. Two hundred and ninety eight uniform sized Cobb broiler eggs were set for incubation. The fertile eggs were divided into two groups, one group without any injection and the other group was administered *in ovo* with amino acid solution containing arginine (22 mg), glutamine (25 mg) and threonine (30 mg) per egg on day 18 of incubation into the amniotic cavity. After hatching, the chicks (n=240) were further divided into four groups (6 replicates with 10 chicks in each replicate), namely, Group I: without *in ovo* and without post hatch supplemented diet (Wo*INOVO*-WoPHS); Group II: without *in ovo* and with post hatch supplemented diet (25% higher level of lysine, 1.68 mg, methionine,0.63 mg andthreonine,0.99 mg) (Wo*INOVO*-WPHS); Group III: with*in ovo* and without post hatch supplemented diet (W*INOVO*-WoPHS); Group IV: with*in ovo* and with post hatch supplemented diet (W*INOVO*-WPHS). The results indicated that *in ovo* administration of amino acids did not show any significant difference in both hatchability and chick weight. Live weight gain, feed intake and feed conversion ratio during 0-3 wk, 3-5 wk and overall phase were not affected (P>005) by either *in ovo* supplementation and post hatch supplemented diet or their interaction. *In ovo* supplementation significantly (P<005) increased the weight (% of live weight) of duodenum, proventriculus and gizzard at day of hatch. It is concluded that *in ovo* supplementation of arginine, glutamine and threonine was beneficial in the early gut development at hatch, but such improvements were not significantly reflected in the growth performance of broiler chicks following post hatch amino acids dietary supplementation.

Adoptable findings

While there has been much progress in the development of procedure for *in ovo* feeding of poultry, the identification of suitable products is still an on-going process. Amino acids may be the most suitable supplements for *in ovo* feeding but it is not certain which are the most important ones and how they should be combined. This study assesses a supplement containing concentrations of key amino acids.

Keywords: Broiler, growth, *in ovo*, post hatch, amino acid, perinatal

Introduction

The peri-natal period is the most crucial time in the life of a young chick as it undergoes metabolic and physiological shifts from the utilization of egg nutrients to exogenous feed (Ferket, 2012). The development of the gut occurs throughout incubation, but the functional abilities only begin to develop from 18th day of embryo formation. Therefore, gut development is of great importance during the last period on poultry embryonic development and the early post hatch period. *In ovo* administration of nutrients into the amnion gives an opportunity for chicks to orally consume supplemented nutrients and develop their digestive and absorptive abilities prior to hatch. There is a great need of amino acids such as glycine, proline, lysine and arginine during early period of embryonic growth (Kadam *et al*., 2008). Threonine being the only precursor of glycine, plays an important role for pre-hatch embryonic growth. Foye *et al*. (2006) demonstrated that *in ovo* administration of arginine enhances hepatic liver reserves providing the nutrients needed for subsequent rapid 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 access to nutrients. The current focus of broiler management deals with nutrient fortification during peri-natal (last few days of pre hatch to first few days post hatch) phase, with the aim of achieving the targeted growth in less time. Thus, the present study was designed to formulate a pre starter diet for broiler chicken for the first three days of post hatch and to evaluate whether such pre-conditioning areas effective as *in ovo* administration of nutrients in relation to gut development and growth performance.

Materials and methods

Experimental design

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

Incubation

Two hundred and ninety eight uniform sized cobb broiler eggs were procured from commercial hatchery and incubated. On day 18, all the unfertile eggs were removed after candling, the fertile eggs were shifted to hatching trays. 282 fertile eggs were divided into two groups, one group (n=140 eggs) without any injection and another (n=142 eggs) was administered *in ovo* with amino acid solution containing arginine (22 mg), glutamine (25 mg) and threonine (30 mg) per egg into the amniotic cavity. The required amount of crystalline amino acids (Sigma Research Laboratories Pvt Ltd, India) were weighed and dissolved in sterile water in such a concentration that 0.5 ml contained the required amount of amino acids to be injected in one egg. On day 18 of incubation, 0.5 ml of the amino acid solution was administered under laminar flow system. The broad end of the egg was suitably sterilized with 70% ethanol and amino acid solution administered using 25 mm needle, the pinhole site was sealed with sterile paraffin wax immediately and eggs were transferred to the incubator. The entire *in ovo* procedure was completed within 20 minutes after the eggs were removed from the incubator.

Birds housing

The chicks hatched from the different treatment groups were randomly distributed into battery cages (6 replicates with 10 chicks in each replicate) fitted with heating arrangements: feeders, waterer and dropping trays with 24 hours light and proper air ventilation, were reared under standard management conditions. 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. The chicks were further divided into four groups, group I: without *in ovo* and without post hatch supplemented diet (Wo*INOVO*-WoPHS); Group II: without *in ovo* and with post hatch supplemented diet (25% higher level of lysine, 1.68 mg, methionine, 0.63 mg and threonine, 0.99 mg) (Wo*INOVO*-WPHS) for first three days; Group III: with *in ovo* and without post hatch supplemented diet (W*INOVO*-WoPHS); Group IV: with *in ovo* and with post hatch supplemented diet (W*INOVO*-WPHS) for first three days. Feed and water were provided *ad lib* during the entire experimental period.

Experimental diets

Experimental diets were prepared with maize and soybean meal as major ingredients (Table 1 and 2). The dietary treatments consisted of one normal pre-starter diet for Group I (Wo*INOVO*-WoPHS) and Group III (W*INOVO*-WoPHS) and another with post hatch supplemented diet for Group II (Wo*INOVO*–WPHS) and Group IV (W*INOVO*-WPHS).

Table 1: Ingredient and nutrient composition of experimental diets

	Post hatch	**Starter**	**Finisher**
	(0-3 days)	**(0 or 4-21 days)**	**(22- 35 days)**
Ingredient (%)			
Maize	57.53	58.06	62.31
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
L Lysine HCl	0.37	0.37	0.2
DL Methionine	0.22	0.22	0.14
L Threonine	0.20	0	0
L Arginine	0.34	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
L-Lysine	1.34	1.34	1.11
DL-Methionine	0.5	0.5	0.41
L-Threonine	0.97	0.77	0.73
L-Arginine	1.74	1.4	1.28
Calcium	1.04	1.04	0.98
Available phosphorus*	0.45	0.45	0.4

*Trace mineral premix 0.1%, Vit. 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: Vit. A, 8250 IU; Vit. D_3,1200 ICU; Vit. K, 1mg; Vit. E, 40 IU; Vit. B_1, 2mg; Vit. B_2 4mg; Vit. B_{12}, 10 mcg; niacin, 60 mg; pantothenic acid, 10 mg.

Measurements

Body weight changes were recorded every week to ascertain weekly and overall body weight gain. The experimental diets were given *ad lib* and the residue was weighed at weekly interval to determine the

feed intake. , The weekly and period-wise cumulative feed conversion ratio (FCR) were calculated using feed intake and body weight gain records.

Table 2: Amino acid analysis (g/kg) of maize and soybean meal

	Maize	**Soybean meal**
Methionine	1.51	5.98
Cysteine	1.68	6.87
Lysine	2.28	28.2
Threonine	2.54	17.43
Tryptophan	0.57	6.08
Arginine	3.6	33.79
Isoleucine	2.37	20.38
Leucine	8.15	34.44
Valine	3.38	21.37
Histidine	2.12	12.07
Phenylalanine	3.4	23.15

AA: Amino acid, CP: Crude protein. * Figures standardized to a dry matter content of 88 %.

Analysed at Amino Lab, Evonik Industries.

Digestive organs

Six birds from each treatment were sacrificed by cervical dislocation at weekly interval (0-4 wk of age) and twelve birds from each treatment at 5 wk of age. Gut development was measured by recording the weights of gizzard, proventriculus and liver, as well as, weight and length of duodenum, jejunum, ileum and caecum.

Histology

Functional development of gut was measured by histological examination of duodenal villi at 35 days of post hatch: 2-3 cm long duodenal 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).

Statistical analysis

The data were subjected to two way analysis of variance (ANOVA) for completely randomized design (SPSS, 2010 Version 18.0), except for egg weight and chick weight which were analysed by Independent T test.

Growth performance

Live weight gain, feed intake and feed conversion ratio during 0-3 wk, 3-5 wk and overall phase werenot affected (P>0.05) due to either main effect of *in ovo* and post hatch supplementation or their interaction (Table 4).

Results

Hatchability

Hatchability of fertile eggs, egg weight and chick weight did not differ significantly (P>0.05) due to *in ovo* supplementation (Table 3).

Table 3: Hatchability

Groups	Treatments	Egg wt (g)	Chick wt (%)	Hatchability (%)
I	Wo*INOVO*	70.6	49.06	90.84 (129/142)
II	*WINOVO*	70.5	48.49	94.24 (132/140)
	Pooled SEM	0.264	0.235	
	Significance	0.91	0.23	

SEM: Standard error of means. *Wo*INOVO:* Without *INOVO;*WINOVO :* With *INOVO*

Gastrointestinal tract development

Gastrointestinal tract development at day of hatch, digestive organ weight (% of live weight) and length (cm/100g live weight) differed significantly (P<0.05) due to *in ovo* supplementation of amino acid. *In ovo* supplementation 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) (Table 5). Most of the weight and length of digestiveorgans did not differ significantly (P>0.05) due to *in ovo* administration of amino acid, post hatch supplementation or their interaction during 1 to 5 wk of age (Table5, 6 &7).

Table 4: Growth performance of broiler chicken

		Live weight gain (g/bird)			Feed intake (g/bird)			Feed conversion ratio		
		0 - 3 wk	3 - 5 wk	0 - 5 wk	0-3 wk	3 - 5 wk	0-5 wk	0-3 wk	3 - 5 wk	0-5 wk
Effect of *in ovo* supplementation (*IN OVO*)										
1	Wo*INOVO*	735	1048	1795	958	1797	2754	1.3	1.72	1.54
2	*WINOVO*	744	1059	1792	991	1793	2785	1.33	1.71	1.56
	Significance	0.9	0.81	0.8	0.88	0.94	0.91	0.79	0.82	0.76
Effect of post hatch supplemented diet (PHS)										
1	WoPHS	739	1059	1787	977	1796	2773	1.32	1.7	1.55
2	WPHS	740	1048	1799	972	1794	2765	1.31	1.72	1.54
	Significance	0.53	0.79	0.95	0.29	0.98	0.65	0.42	0.72	0.56
Interaction effect (*IN OVO* × PHS)										
1	Wo*INOVO* – WoPHS	725	1056	1781	944	1790	2734	1.3	1.71	1.54
2	Wo*INOVO*-WPHS	753	1040	1793	1010	1803	2813	1.34	1.73	1.57
3	W*INOVO*-WoPHS	745	1062	1808	973	1801	2774	1.31	1.7	1.54
4	W*INOVO*-WPHS	736	1055	1791	971	1785	2757	1.32	1.71	1.55
	SEM	6.88	20.73	21.99	14.70	22.52	31.95	0.02	0.03	0.02
	Significance	0.2	0.92	0.77	0.27	0.77	0.48	0.68	0.9	0.76

Table 5: Digestive organ weight (% of live weight) and length (cm / 100g live weight) at day of hatch

		Duodenum		**Jejunum**		**Ileum**		**Caecum**		**Liver**	**Proventriculus**	**Gizard**
	Treatment	Length	Weight	Length	Weight	Length	Weight	Length	Weight	Weight	Weight	Weight
1	**Wo*INOVO***	17.96	1.30[b]	38.34[b]	1.68[b]	34.18	1.39	7.99	0.83	3.01	0.84[b]	8.28[b]
2	***WINOVO***	19.09	1.61[a]	44.75[a]	2.29[a]	36.98	1.68	8.00	0.94	3.00	1.13[a]	9.81[a]
	SEM	0.547	0.066	1.224	0.147	1.841	0.106	0.438	0.068	0.095	0.054	0.287
	Significance	0.31	0.01	0.01	0.03	0.46	0.17	0.99	0.40	0.97	0.001	0.001

* Wo*INOVO* -WoPHS : Without *in ovo* and without post hatch supplemented diet.

* Wo*INOVO*-WPHS : Without *in ovo* and without post hatch supplemented diet.

* W*INOVO*-WoPHS : With *in ovo* and without post hatch supplemented diet.

* W*INOVO*-WPHS : With *in ovo* and with post hatch supplemented diet.

Table 6. Digestive organ weight (% of live weight) and length (cm / 100g live weight) at 3rd wk

	Duodenum		Jejunum		Ileum		Caecum		Liver	Proventriculus	Gizard
	Length	Weight	Length	Weight	Length	Weight	Length	Weight	Weight	Weight	Weight
Effect of *in ovo* supplementation (*IN OVO*)											
1 Wo*INOVO*	3.43	1.25	7.68[b]	3.02	7.42[b]	2.44	1.56	0.84	2.83	0.63	4.52
2 *WINOVO*	3.42	1.23	8.37[a]	2.96	8.08[a]	2.40	1.63	0.84	2.74	0.62	4.80
Significance	0.92	0.79	0.03	0.65	0.04	0.80	0.31	0.95	0.49	0.89	0.36
Effect of post hatch supplemented diet (PHS)											
1 WoPHS	3.42	1.21	8.02	3.03	7.56	2.55	1.51[b]	0.78	2.75	0.63	4.79
2 WPHS	3.43	1.26	0.24	0.06	0.26	0.11	1.68[a]	0.90	0.09	0.03	0.26
Significance	0.95	0.41	0.95	0.49	0.22	0.08	0.02	0.18	0.61	0.92	0.42
Interaction effect (*IN OVO* × *PHS)*											
1 Wo*INOVO* – WoPHS	3.40	1.20	7.80	3.05	7.43	2.47	1.50	0.74	2.76	0.61	4.64
2 Wo*INOVO*-WPHS	3.47	1.29	7.57	2.99	7.41	2.40	1.61	0.93	2.91	0.65	4.39
3 W*INOVO*-WoPHS	3.44	1.22	8.24	3.02	7.70	2.62	1.51	0.81	2.74	0.64	4.93
4 W*INOVO*-WPHS	3.39	1.24	8.51	2.89	8.46	2.18	1.75	0.87	2.74	0.61	4.68
SEM	0.07	0.03	0.16	0.06	0.17	0.07	0.04	0.04	0.06	0.02	0.15
Significance	0.71	0.64	0.43	0.77	0.21	0.20	0.36	0.47	0.59	0.35	0.99

* Wo*INOVO* -WoPHS : Without *in ovo* and without post hatch supplemented diet.

* Wo*INOVO*-WPHS : Without *in ovo* and without post hatch supplemented diet.

* W*INOVO*-WoPHS : With *in ovo* and without post hatch supplemented diet.

* W*INOVO*-WPHS : With *in ovo* and with post hatch supplemented diet.

Table 7. Digestive organ weight (% of live weight) and length (cm / 100g live weight) at 5th wk

	Duodenum		Jejunum		Ileum		Caecum		Liver	Proventriculus	Gizard
	Length	Weight	Length	Weight	Length	Weight	Length	Weight	Weight	Weight	Weight
Effect of *in ovo* supplementation (*IN OVO*)											
1 Wo*INOVO*	1.71	0.91	3.58	2.94	3.59	1.74	0.81	0.83	2.06	0.39	2.79
2 *WINOVO*	1.61	0.77	3.69	1.66	3.49	1.59	0.80	0.73	1.81	0.63	3.29
Significance	0.26	0.06	0.63	0.23	0.78	0.55	0.49	0.91	0.05	0.36	0.27
Effect of post hatch supplemented diet (PHS)											
1 WoPHS	1.66	0.81	3.66	1.70	3.46	1.74	0.81	0.73	1.82	0.63	3.15
2 WPHS	1.66	0.87	3.62	2.90	3.63	1.59	0.80	0.82	2.05	0.40	2.93
Significance	0.92	0.40	0.82	0.26	0.58	0.19	0.77	0.94	0.15	0.39	0.99
Interaction effect (*IN OVO* × *PHS)*											
1 Wo*INOVO* – WoPHS	1.73	0.90	3.62	1.83	3.42	1.86	0.83	0.71	1.89	0.39	2.95
2 Wo*INOVO*-WPHS	1.69	0.92	3.55	4.05	3.76	1.63	0.79	0.95	2.24	0.40	2.62
3 W*INOVO*-WoPHS	1.58	0.73	3.70	1.58	3.50	1.63	0.79	0.76	1.76	0.86	3.34
4 W*INOVO*-WPHS	1.64	0.81	3.68	1.74	3.49	1.56	0.81	0.69	1.86	0.41	3.24
SEM	8.984	5.484	16.135	6.941	13.812	7.122	3.562	2.337	7.618	1.880	14.384
Significance	0.59	0.71	0.87	0.32	0.54	0.39	0.99	0.44	0.92	0.70	0.37

* Wo*INOVO* -WoPHS : Without *in ovo* and without post hatch supplemented diet.

*Wo*INOVO*-WPHS : Without *in ovo* and without post hatch supplemented diet.

* W*INOVO*-WoPHS : With *in ovo* and without post hatch supplemented diet.

* W*INOVO*-WPHS : With *in ovo* and with post hatch supplemented diet.

Table 8. Development of duodenal villus at 5th wk of age

		Length (um)	Breadth (um)	Crypt Depth (um)
Effect of *in ovo* supplementation (*IN OVO*)				
1	Wo*INOVO*	1571.4	141.7	43.8
2	*WINOVO*	1435.5	148.2	42.7
	Significance	0.52	0.60	0.80
Effect of post hatch supplemented diet (PHS)				
1	WoPHS	1614.0	146.7	45.0
2	WPHS	1398.2	143.9	41.7
	Significance	0.30	0.79	0.55
Interaction effect (*IN OVO* × PHS*)*				
1	Wo*INOVO*-WoPHS	1451.3^{ab}	147.3	45.9
2	Wo*INOVO*-WPHS	1661.5^{ab}	137.6	42.3
3	W*INOVO*-WoPHS	1736^{a}	146.2	44.3
4	W*INOVO*-WPHS	1135^{b}	150.1	41.0
	SEM	102.46	4.87	2.50
	Significance	0.05	0.54	0.98

Histology

The villus length, breadth and crypt depth did not vary significantly (P>0.05) due to either main effect of *in ovo* and post hatch supplementation (Table 8). Due to interaction of *in ovo* and post hatch supplementation, length of dudeonal villi was significantly higher in W*INOVO*-WoPHS (1736.0µm) in comparison to WoINOVO-WoPHS group (1451.3 µm).

Discussion

Hatchability and chick weight

The percent hatchability on fertile egg set basis and chick weight did not differ with*in ovo* administration of amino acid enriched solution group and control group, respectively. Earlier studies have shown that *in ovo* administration of amino acids either individually or in combination affects hatchability and chick weight positively (Ohta and Kidd, 2001; Bhanja and Mandal, 2005; Bakyaraj *et al.*,2012; Al- Asadi *et al.*,2013; Shafey *et al.*, 2014); negatively (Ohta *et al.*,1999; Toghyani *et al.*, 2012) or without any effect (Bhanja *et al.*,2012; Kadam *et al.*,2008; Shafey *et al.*,2013).A high variability in hatchability has been observed in earlier studies and may not be only related to amino acid, but also due to other external factors, such as time and site of administration and needle length (Ohta *et al.*,1999; Ohta and Kidd, 2001; Bhanja and Mandal, 2005).

Growth performance

There was no influence of peri-natal amino acid supplementation on growth performances of broiler chicks in the present study. Positive effect of *in ovo* amino acid supplementation on performance of

broiler chicken has been previously documented by some researchers (Bhanja and Mandal,2005; Bhanja *et al.*,2012;Toghyani *et al.*,2012;Shafey *et al.*, 2014). On the other hand, Bakyaraj *et al.* (2012) reported no significant improvement in body weights of broiler chicks hatched out of *in ovo* amino acid supplemented eggs, except for better feed conversion ratio (FCR). Shafey et al. (2014) reported significantly higher feed intake but not FCR in *in ovo* amino acid supplemented broiler chicks. Interestingly, Kadam *et al*. (2008) reported a significantly higher feed intake and FCR in broilers following *in ovo* administration of graded doses of threonine. Similarly, *in ovo* administration of 2% Arginine significantly increased body weight gain and feed intake of broiler chicks (Al- Asadi *et al.*, 2013). Bhanja and Mandal (2005) (in their study of *in ovo* injection with a different combination of essential and non-essential amino acid (Lys,5.84+Arg,5.01; Lys.5.84+ Met,2.95+Cys,1.70; Thr,3.91+ Gly, 2.74+Ser,5.97; Ile,4.19+ Leu,7.00+ Val,5.16; Gly,2.74 + Pro,3.03)) reported that body weight gain was significantly higher in the amino acid injected group, only in case of Ile+Leu+Val (87.8 g/b) and Gly+Pro (78.8 g/b) in comparison to the control group (72.63g/b) at 1st wk of age. Bhanja *et al.* (2012) reported that the amino acid injected groups had higher body weight gain (Met, 539.9; Arg, 534; Gly, 515.9 g/b) than un-injected control (485.2 g/b) chicks at four week of age and that there was no significant difference in the FCR of birds injected with amino acids. Bakayraj *et al.* (2012) observed in a separate study that *in ovo* supplementation combination of nutrients i.e. amino acid [AA for cell mediated immunity (Lys,22 + Met,10+ Arg,25+ leu,24+ Ile,16 mg); AA for humoral immunity (Met,10+ Thr,16+ Arg,25+ Gly,12.5+ Ser,12.5+ Val,18mg)]in broiler chicken did not affect their body weight gain, but an improved FCR was observed in the group injected AA for humoral immunity. Shafey *et al.* (2014)conducted a study on *in ovo* administration of amino acid mixtures in groupsAA1 23.7 mg of Lys, 5.16+ Glu,12.10+ Gly,3.22+ Pro,3.24; AA2 23.6 mg of Arg,5.04+ Glu,12.10+ Gly,3.22; and AA3 group 28.76 mg of Arg,5.04+ Lys,5.16+ Glu,12.10+ Gly,3.22+ Pro,3.24. The reports of Shafey *et al*. (2014) revealed that amino acid injected group (AA1,1900;AA2,1927;AA3,1879 g/b) had higher body weight gain than un-injected control group (1815g/b). In our study, feed intake was significantly higher in amino acid injected group (AA1, 3246; AA2, 3291; AA3, 3195 g/b) in comparison to un-injected control (3056 g/b) during the entire experimental (1 - 35 day of age) period. FCR was non-significant between amino acid injected groups in comparison to un-injected control group. Kadam *et al.* (2008) reported in their study of *in ovo* injection graded level of threonine (10, 20, 30 or 40 mg per egg), that between 0-7 and 7-14 day of age, there was no difference in body weight gain between the different treatments. *In ovo* Threonine (Thr) injected group had greater body weight gain than untreated chicks during the periods 14 to 21 (10 mg Thr, 211.79; 20 mg, 216.71; 30mg, 215; 40mg, 210.03 g/b vs un-injected control group 201.62 g/b) and during 21 to 28 d (10 mg Thr, 340.95; 20 mg, 364.64; 30mg, 344.44; 40mg, 340.53 g/b vs un-injected control group 304.82 g/b). *In ovo* injected 20 mg of threonine (343.57) had higher feed intake in comparison to un-injected control groups (301.08 g/b). Compared with *in ovo* injected group (10, 20 and 30 mg Thr) and un-injected group, 40 mg treated group had better FCR. Toghyani *et al.* (2012) reported in (their study of *in ovo* injection of Arg, 35; Thr, 25 mg and Arg, 35 + Thr 25 mg/ egg in individually and in combination) that body weight was significantly higher in the amino acid injected groups (Arg, 2322.1; Thr, 2569.4 and Arg+ Thr, 2279.4 g/b) in comparison to un-injected control group (2155.6). In our study, *in ovo* amino acid injected group (Arg, 94.7; Thr, 103.2 and Arg+ Thr, 97.1 g/b) had higher feed intake in comparison to non-injected group (86.3 g/b/d). Feed conversion ratio was not significantly affected by *in ovo* injection of amino acid. However, Al- Asadi *et al.* (2013) reported in their study of effect of *in ovo* injection of 2 % lysine and 2% arginine in broiler chicken, that *in ovo* injection with lysine and arginine significantly increased body weight as compared to non-injected control group (Lys,2738.43; Arg,2748.20 g/b vs control 2636.63 g/b) at 6th wk of age and weekly feed intake was significantly higher in amino acid injected group in comparison to un-injected control group.

Gastrointestinal tract development

In ovo supplementation of amino acid (Thr, Arg, Glu) had positive influence on the development ofthe digestive organ at hatch, but not during post hatch growing periods, probably because the post hatch diet did not have any influence on post hatch growth rate of chicks. Bhanja *et al.* (2012) reported that on injection of 25 mg each of Lys, Met, Thr, Arg and Gly, that there was no significant difference in the weight of digestive organs and intestine in day-old chicks. Similarly, Bhanja and Mandal (2005) reported that *in ovo* injection of essential and non-essential amino acids did not significantly alter the weights of digestive organs compared to the control birds. Toghyani *et al.* (2012) reported that liver, gizzard and ceaca weight and length of intestine were not affected by treatment of *in ovo* injection of Arg, Thr, and Arg, + Thr per egg either individually or in combination, but that ileum weight increased in Thr and pancreas weight decreased in treatment with Arg+Thr injection. Overall results from this study indicate that certain amino acids have fundamental roles in gut development, however, there are pertinent needs for further investigation on their dietary effects on post hatch growth performances in chicks.

Histology

There was no influence on villus length, breadth and crypt depth either due to *in ovo* and post hatch supplementation of threonine, arginine and glutamine. Villus height in duodenum and ileum were increased in Arg and Arg+Thr injection group. Bartell and Batal (2007) reported (in their study of the effect of 1 or 4% glutamine addition to the feed, water or both for 4 days of post hatch in comparison with a corn-soybean meal control group) that chicks fed diets with 1% glutamine had heavier intestinal relative weights and longer intestinal villi as compared to the chicks fed the corn-SBM diet (1% Gln, 838.6 um vs 778.3 um duodenal villi height).

In the present study, hatching eggs were obtained from commercial hatchery with genetic potential for higher growth performance and reared on quality breeder ration. The non-significant beneficial effect of *in ovo* supplemented amino acids on growth performance of chicks in our study may be due to optimal level of nutrient present in egg obtained from the commercial hatchery. For instance, Bozbay and Ocak (2015) observed in experiment of *in ovo* injection of branched chain amino acids on eggs having optimal levels of nutrient and found that *in ovo* injection had no effect on the hatchability, chick quality and the rateof growth performance. It therefore, stands to reason that healthy chicks may not respond to *in ovo* supplements (Schulte-Drüggelte, 2015) and the degree of limiting protein synthesis of these amino acids may depend on the ratios and antagonistic relationship between each of these amino acids in poultry diet (Burnham, 1992), coupled with the protein content and quality of poultry diets (Corzo,2010). Based on these observations, it is concluded that *in ovo* supplementation of arginine, glutamine and threonine was beneficial in the gut development at hatch. However, such improvements were not significantly reflected in the growth performance of broiler chicks following post hatch amino acids dietary supplementation.

Conclusion

In ovo supplementation of amino acids (threonine, arginine and glutamine) increased the gut development at the time of hatch. However, *in ovo* supplementation and post hatch supplementation of amino acids (threonine, arginine and glutamine) did not influence the growth performances of broiler chicken.

References

Al-Asadi, A. N. O.; 2013: Effect of early feeding (*in ovo* injection) of amino acids on hatchability, some productive and physiology traits of broiler. International Journal of Science and Technology 8, 6-13.

Bakyaraj, S.; Bhanja, S. K.; Majumdar, S.; Dash, B., 2011: Modulation of post-hatch growth and immunity through *in ovo* supplemented nutrients in broiler chickens. Journal of Science and Food Agriculture 92, 313–320.

Bartell, S. M.; Batal, A. B.; 2007: The effect of supplemental glutamine on growth performance, development of the gastrointestinal tract and humoral immune response of broilers. Poultry Science 86, 1940-1947.
Bertolo, F. P.; Chen, C. I.; Law, G.; Pencharz, P. B.; Ball, R. O., 1998: Threonine requirement of neonatal piglets receiving total parental nutrition is considerably lower than that of piglets receiving an ideal diet intragastrically. Journal of Nutrition 128, 1752-1758.
Bhanja, S. K.; 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 Australian* Journal of Animal Science 18, 524-531.
Bhanja, S. K.; Mandal, A. B.; Agarwal, S. K.; Majumdar, S., 2012: Modulation of post hatch-growth and immunocompetence through *in ovo* injection of limiting amino acids in broiler chickens. Indian Journal of Animal Sciences 82, 993-998.
Bozbay, C K.; Ocak, N., 2015: Growth, digestive tract and muscle weights in slow-growing broiler is not affected by a blend of branched-chain amino acids injected into different sites of egg. Journal of Agriculture and Environmental Sciences 4, 261-269.
Burnham, D.; Emmans, G. C.; Gous, R.M., 1992: Isoleucine requirements of the chicken: The effect of excess leucine and valine on the response to isoleucine. British Poultry Science 33, 71–87.
Corrier, D. E.; Deloavh, J. R., 1990: Evaluation of cell mediated, cutaneous basophil hypersensitivity in young chickens by an interdigital skin test. Poultry Science 69, 403-408.
Corzo, A.; Dozier III, W. A.; Loar, R. E.; Kidd, M. T.;Tillman, P. B., 2010: Dietary limitation of isoleucine and valine in diets based on maize, soybean meal and meat and bone meal for broiler chickens. British Poultry Science 51, 558-563.
Dozier III, W. A.; Corzo, A.; Kidd, M. T.; Tillman, P. B.; Branton, S. L., 2011: Determination of the 4th and 5th limiting amino acids of broilers fed diets containing maize, soybean meal and poultry by-product meal from 28 to 42 days of age. British Poultry Science 52, 238-244.
Ferket, P. R.; Uni, Z., 2006: Early Feeding - *in ovo* feeding enhances of early gut development and digestive capacity of poultry. *In*: 12th European Poultry Conference, Verona, Italy. pp. 10-14.
Foye, O.T.; Uni, Z.; Ferket, P.R., 2006: Effect of *in ovo* feeding egg white protein, P-hydroxyl-β-methylbutyrate and carbohydrates on glycogen status and neonatal growth of turkeys. Poultry Science 85, 1185-1192.
Geyra, A.; Uni, Z.; Sklan, D., 2001a: The effect of fasting at different ages on growth and tissue dynamics in the small intestine of the young chick. British Poultry Science 86, 776-782.
Geyra, A.; Uni, Z.; Sklan, D., 2001b: Enterocyte dynamics and mucosal development in the post hatch chick. Poultry Science 80, 776-782.
Kadam, M. M.; Bhanja, S. K.; Mandal, A. B.; Thakur, R.; Vasan, P.; Bhattacharyya, A., 2008. Effect of *in ovo* threonine supplementation on early growth, immunological responses and digestive enzyme activities in broiler chickens. British Poultry Science 49, 736-741.
Noy, Y.; Sklan, D., 1999: Energy utilization in newly hatched chicks. *Poultry Science* 78, 1750-1756.
Noy, Y.; Sklan, D., 1998: Metabolic response to early nutrition. Journal of Applied Poultry Research 7, 437- 451.
Ohta, Y.; Kidd, M. T., 2001. Optimum site for *in ovo* amino acid injection in broiler breeder eggs. Poultry Science 80, 1425-1429.
Ohta, Y.; Kidd, M.; Ishibashi, T., 2001: Embryo growth and amino acid concentration profiles of broiler breeder eggs, embryos, and chicks after *in ovo* administration of amino acids. Poultry Science 80, 1430 -1436.
Ohta, Y.; Tsushima, N.; Koide, K.; Kidd, M. T.; Ishibashi, T., 1999: Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks.Poultry Science 78, 1493-1498.
Ospina-Rojas, I. C.; Murakami, A. E.; Do Amaral Duarte, C. R.; Eyng, C.; Lopes De Oliveira, C. A.; Janeiro, V., 2014: Valine, isoleucine, arginine and glycine supplementation of low-protein diets for broiler chickens during the starter and grower phases. British Poultry Science 55, 766–773.

Pophal, S.; Mozdziak, P. E.; Vieira, S. L., 2004: Satellite cell mitotic activity of broilers fed differing levels of lysine. International Journal of Poultry Science 3, 758–763.
Saki, A.; Haghighat, M.; Khajali, F., 2013: Supplemental arginine administered *in ovo* or in the feed reduces the susceptibility of broilers to pulmonary hypertension syndrome. British Poultry Science 54, 575-580.
Samli, H. E.; Senkoylu, N.; Koc, F.; Kanter, M.; Agma, A., 2007: Effects of Enterococcus faecium dried whey on broiler performance gut histomorphology microbiota Archive of Animal Nutrition 61,42-49.
Schulte-Druggelte, R., 2015: The importance of quality nutrition and management on the breeder farm. International Hatchery Practice 29, 625-626.
Schutte, J. B.; Jong, J. D.; Swink, W.; Pack, M., 1997: Replacement value of betaine for DL methionine in male broiler chicks. Poultry Science 76, 321-325.
Siegel, P.B.; Gross, W.B., 1980: Production and persistency of antibodies in chickens to sheep erythrocytes.1. Directional selection. Poultry Science 59, 1-5.
SPSS Version 18.0., 2010: *SPSS Software products, Marketing Department*, SPSS Inc. Chicago, IL, USA.
Tako, E.; Ferket, P.R.; Uni, Z., 2004: Effects of *in ovo* feeding of carbohydrates and beta-hydroxy-beta-methyl butyrate on the development of chicken intestine. Poultry Science 83, 2023-2028.
Toghyani, M.; Tohidi, M.; Gheisari, A.; Tabeidian, A.; Toghyani, M., 2012: Evaluation of oyster mushroom (Pleurotus ostreatus) as a biological growth promoter on performance, humoral immunity and blood characteristics of broiler chicks. Poultry Science 49, 183-190.
Tong, B.C.; Barbul, A., 2004: Cellular and physiological effects of arginine. Mini Review Medical Chemistry 4, 823-832.
Uni, Z.; Ferket, P. R.; Tako, E.; Kedar, O., 2005: *In ovo* feeding improves energy status of late-term chicken embryos. Poultry Science 84, 764-770.
Uni, Z.; Platin, R; Sklan, D., 1998: Cell proliferation in chicken intestinal epithelium occurs both in the crypt and along the vill