



## Comparative effect of arginine and/or tryptophan *in ovo* feeding on hatchability percentage, growth performance and economic importance of commercial broiler

NIBEDITA NAYAK<sup>1</sup>, R ASHA RAJINI<sup>2</sup>, S EZHILVALAVAN<sup>3</sup>, AMIYA RANJAN SAHU<sup>4</sup> and J JOHN KIRUBAHARAN<sup>5</sup>

Madras Veterinary College, Chennai, Tamil Nadu 600 007 India

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### ABSTRACT

*In ovo* feeding is the administration of exogenous nutrients into the amnion of the late-term avian embryo so that the avian embryo orally consumes the amniotic fluid (primarily water and albumen protein) prior to pipping the air cell. So the study was carried out to find out the effect of *in ovo* nutrition on post-hatch performances in broilers. Total 700 fertile eggs of Cobb 400 with uniform egg weight were divided into 5 groups: non-injected control, injected control (0.5 ml of 0.9% normal saline), arginine (0.5 ml of 0.5% arginine), tryptophan (0.5 ml of 0.5% tryptophan) and combination (0.25 ml of 0.5% each of arginine and tryptophan); and injected on 18 d of incubation into amnion. On the 21d, 108 chicks were randomly selected from each treatment in total accounting 540 chicks. The hatchability and hatch weight were studied where arginine had shown a highly significant improved hatch weight and placement weight. There was no significant difference in feed efficiency, but cumulative feed intake was higher in tryptophan and combination groups. Body weight and body weight gain had also resulted significantly in arginine fed *in ovo* groups up to 21d of age. The broiler farm economy index (BFEI) and broiler feed price ratio (BFPR) were best in tryptophan and arginine *in ovo* fed groups, respectively.

**Key words:** Arginine, Broiler production, Hatchability, *In ovo* nutrition, Tryptophan

India is the fourth largest producer of poultry meat in the world, but the per capita availability of poultry meat (2.15 kg) is much behind as per the recommendation by Indian Council of Medical Research (11 kg) which necessitates need of huge growth of poultry industry in the near future. Unlike the mammalian embryo, the avian embryo develops in a carbohydrate free environment with finite amount of energy i.e. closed nutrition (Foye *et al.* 2006). The embryonic developmental period as well as the first week after hatching represents a larger proportion (45%) of the whole life span. Thus early nutrition through *in ovo* feeding not only affects embryo survival and disease resistance, but also the ultimate attainment of genetic potential (Bhanja *et al.* 2012). *In ovo* feeding improves the energy status and gut development of perinatal chick as avian embryo orally consumes the amniotic fluid prior to pipping the air cell (about 18 d of incubation in broilers) supplementing the amnion with nutrients. Arginine is a

candidate nutrient for *in ovo* feeding as it influences hatchability, growth and immunity (Al-Daraji *et al.* 2012, Al-Asadi 2013). The *in ovo* supplementation of L-arginine stimulates myoblast differentiation in broiler chicks, but decreases post-hatch muscle fibre diameter (Li *et al.* 2016). Moreover, due to lack of a full complement of enzymes of the urea cycle, chickens are unable to synthesize arginine *de novo* and have an absolute dietary requirement for arginine (Wu and Morris 1998, Ball *et al.* 2007). In addition to arginine, tryptophan was chosen because it was reported by Corzo *et al.* (2005) that it influences feed intake, immunity, meat yield and overall health status.

However, there have been no studies regarding the regulatory effects of tryptophan and the combination of arginine and tryptophan through *in ovo* feeding method. Hence, the present study was undertaken to evaluate the potential effects of *in ovo* arginine and tryptophan on hatchability, production and economic parameters.

### MATERIALS AND METHODS

*In ovo injection:* Total 700 fertile broiler eggs were obtained from a commercial flock of Cobb 400 breeders at 34 wk of age. Fertile eggs were randomly selected and disinfected with 99.90% ethyl alcohol. 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. The

Present address: <sup>1</sup>Ph.D Scholar (drnibeditavet@gmail.com), Division of Poultry Science, CARI, Bareilly. <sup>2</sup>Retd. Professor (professorasha@gmail.com). <sup>3</sup>Associate Professor (ezhilvalavan@tanuvas.org.in), Poultry Research Station, TANUVAS, Chennai. <sup>4</sup>Ph.D Scholar (dramiyavet@gmail.com), Animal Genetics and Breeding. <sup>5</sup>Professor and Head (john@tanuvas.org.in), Department of Veterinary Microbiology.

treatment solutions were injected into the amnion of 18 d old embryo through the pinhole made at broad end of egg using 25 mm needle as per Uni and Ferket (2003). Prior to *in ovo* injection, the injectants were warmed up to room temperature of 30°C. The pinhole site was immediately sealed with sterile paraffin wax and eggs were returned to the incubator. On 19 d of incubation, the eggs were transferred to a hatcher and placed in marked pedigree hatching boxes so as to identify chicks from a particular treatment.

**Birds and housing:** Total 540 chicks were randomly selected from the hatching box, with 108 numbers in each treatment. Chicks were weighed individually in a 0.1g accuracy balance and hatch weight was recorded. Chicks hatched from various treatment groups were distributed randomly and placed in experimental brooding cages in a well-lit and ventilated open-sided house. Each treatment group had six replicates having 18 birds each and chicks were transferred to growing cages on the 15 d and reared up to 35 d. Birds were provided with *ad lib.* water and feed based on guide of Vencobb standard in Table 1 (Teeter and Wiernusz 2003). Throughout the experiment, standard management practices were followed in test and control groups. All experimental protocols were approved by the Institutional Animal Ethical Committee at Madras Veterinary College, Chennai (TANUVAS), India.

**Hatchability and production performances:** The number

of chicks hatched in each treatment was recorded and the hatchability percentage calculated based on fertile egg set basis. Each chick was individually weighed at farm after transporting from hatchery and placement weight was recorded. The transit loss was calculated based on the hatch weight and placement weight. Birds were individually weighed every week up to 35 d for calculating body weight gain. Mortality among birds was recorded during the experimental period and the per cent livability was worked out. The broiler farm economy index (BFEI) and broiler feed price ratios (BFPR) were calculated.

**Statistical analysis:** Results were analyzed based on ANOVA using V.20 SPSS software. Differences between treatments were detected by the Duncan's multiple range tests following ANOVA, and values were considered statistically different at  $P < 0.05$ .

## RESULTS AND DISCUSSION

**Hatchability, hatch weight and chick to egg weight ratio:** Effect of *in ovo* feeding of arginine and tryptophan and their combination on the 18 d of incubation through amnion in broiler breeder chicken egg on hatchability, hatch weight and chick to egg weight ratio is presented in Table 2. *In ovo* feeding with arginine, tryptophan and their combination had no significant effect on hatchability. However, a numerically higher percentage of hatchability was observed in the *in ovo* fed arginine and tryptophan groups as compared to positive and negative control groups, respectively. This agreed with findings of Ohta *et al.* (2004), Bhanja and Mandal (2005), Bhanja *et al.* (2012), Chamani *et al.* (2012) and Shafey *et al.* (2014). However, Foye *et al.* (2006) observed better hatchability with 0.7% arginine in comparison to 0.2% arginine *in ovo* fed group of poults. *In ovo* feeding with arginine significantly ( $P < 0.01$ ) improved hatch weight and was comparable with injected control while the effect of tryptophan was comparable with non-injected control. Arginine has been understood to be conditionally essential amino acid during embryonic stress. It has been known to improve growth and performance in young ones, as it is the most potential nitrogen carrier and a powerful secretagogue, increasing the release of insulin, growth hormone and IGF-A in blood stream. This could have resulted in an increase in weight of embryo; mostly

Table 1. Ingredient and nutrient composition of the experimental broiler diet

Name of the ingredient (per cent)	Per cent inclusion level		
	Broiler pre-starter (0-7 days)	Broiler starter (8-21 days)	Broiler finisher (22-35 days)
Maize (Corn)	47.09	49.99	57.00
Cumbu (Bajra)	11.00	9.00	5.00
Soyabean meal	30.00	29.71	25.50
Dry fish	7.70	6.50	7.00
Oil (Fat)	1.80	2.60	3.40
Mineral mixture	2.00	1.80	1.80
Vitamin mix (AB <sub>2</sub> D <sub>3</sub> K)	0.01	0.01	0.01
Feed additives**	0.40	0.39	0.29
Total	100	100	100
<i>Nutrient composition of the experimental ration</i>			
Crude protein*	22.19	21.5	20.09
Metabolisable energy (Kcal/Kg)+	2976	3055	3158
Lysine+	1.29	1.23	1.13
Methionine+	0.39	0.55	0.45
Calcium*	1.33	1.17	1.20
Av. Phosphorus*	0.47	0.43	0.43
Arginine+	1.54	1.53	1.42
Tryptophan+	0.25	0.24	0.22

\*\* Feed additive includes - Vetroliv, Ultracil, Ultra B<sub>12</sub>, Perivac plus, Lysine, Methionine, Enzyme, Choline chloride and Unicox. +, Calculated value; \*, Analysed value (AOAC 1997).

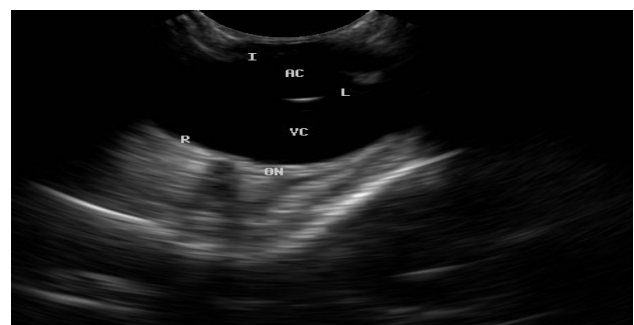


Fig. 1. Per cent variation in hatch weight relative to arginine treatment.

Table 2. Effect of *in ovo* feeding of arginine, tryptophan and their combination on hatchability, hatch weight, chick/egg weight ratio, placement weight and transit weight loss (Mean±SE)

Variable	Treatment				
	Control	Injected control	Arginine (ARG)	Tryptophan (TRP)	Combination (ARG+TRP)
Hatchability percentage <sup>NS</sup> (n=140)	95.71	94.28	96.42	95.71	93.57
Hatch egg weight (g) <sup>NS</sup> (n=140)	60.54±0.004	60.53±0.011	60.55±0.004	60.54±0.008	60.53±0.004
Hatch weight (g)** (n=108)	43.96 <sup>bc</sup> ±0.29	44.50 <sup>ab</sup> ±0.26	45.18 <sup>a</sup> ±0.24	44.35 <sup>bc</sup> ±0.22	43.59 <sup>c</sup> ±0.30
Chick/Egg weight ratio** (n=108)	72.6 <sup>bc</sup> ±0.48	73.5 <sup>ab</sup> ±0.43	74.62 <sup>a</sup> ±0.39	73.25 <sup>bc</sup> ±0.37	72.0 <sup>c</sup> ±0.49
Placement weight (g)** (n=108)	40.26 <sup>c</sup> ±0.28	40.9 <sup>bc</sup> ±0.25	42.24 <sup>a</sup> ±0.23	41.10 <sup>b</sup> ±0.21	40.26 <sup>c</sup> ±0.28
Transit weight loss (g) <sup>NS</sup> (n=108)	3.69±0.39	3.58±0.12	2.94±0.10	3.25±0.15	3.32±0.11

<sup>NS</sup>Not significant, \*\*Highly significant ( $P<0.01$ ), Mean values within each row bearing common superscripts do not differ significantly. n, Number of observations.

towards later part of its growth. The per cent difference in hatch weight between treatments relative to arginine is furnished in graph (Fig.1) which had the best hatchability and was taken as 100%. The hatch weight of chicks showed a highly significant ( $P\leq 0.01$ ) difference between treatments. Arginine fed groups had highest hatch weight as compared to injected control groups. This agreed with findings of Al-Murrani (1982), Ohta *et al.* (2004), Bhanja and Mandal (2005), Foye *et al.* (2006), Kulandaivel (2007) and Shafey *et al.* (2014); but Ohta and Kid (2001) found no significant difference in hatch weight due to *in ovo* amino acids feeding. *In ovo* feeding with arginine significantly improved egg to chick weight ratio than other groups. Chick to egg weight ratio also followed a similar trend as hatch weight. This effect was also noticed by Ohta *et al.* (2004), Bhanja and Mandal (2005) and Shafey *et al.* (2014), whereas Ohta *et al.* (2004) did not find significant difference in hatching body weight relative to egg weight. These findings suggest that the amino acid content of egg is sufficient to support hatching process but not to maximize embryonic growth (John *et al.* 1988).

**Placement weight and transit weight loss:** Effect of *in ovo* feeding of arginine, tryptophan and their combination on placement weight and transit weight loss is presented in Table 2. Body weight of chicks (first day at farm) specified as placement weight showed a highly significant difference between treatments, with arginine *in ovo* fed chicks having significant ( $P<0.01$ ) higher body weight. No significant difference was observed between treatments in transit weight loss, however, numerically arginine fed groups had lower transit weight loss compared to all other treatment groups. Placement weight was significantly higher in arginine *in ovo* fed group and no relevant literature was available for comparison except Coles *et al.* (2001) who observed no significant difference in treatment with respect to placement weight. It has been noticed that amino acid

fed groups had numerically lower transit loss compared to others. When compared with negative control the difference with respect to transit weight loss between arginine and negative control was 20.33% and this in turn had influenced placement weight of chicks. This could be attributed to climate and period of our study.

#### Effect of *in ovo* feeding on production performance

**Effect of *in ovo* feeding on weekly body weight (g) and body weight gain (g):** Mean weekly body weight and body weight gain of broilers as influenced by *in ovo* feeding at day old and at weekly intervals up to five weeks of age is presented in Tables 3, 4. The body weight gain from 0–3, 3–5 and 0–5 weeks is shown in graphical presentation (Fig. 2). The results indicated a highly significant difference ( $P<0.01$ ) between treatments at first week and significant difference ( $P<0.05$ ) in second and third week. However, the weight advantage was sustained up to 21 d only between treatments due to *in ovo* feeding. *In ovo* feeding with arginine produced significantly ( $P<0.01$ ) heavier birds in first, second and third week continuously as compared to

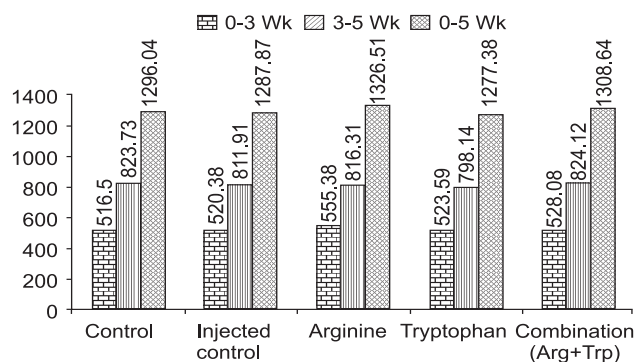


Fig. 2. Effect of *in ovo* feeding of arginine, tryptophan and their combination on body weight gain in three periods (0–3, 3–5 and 0–5 weeks).

injected control. Birds fed with *in ovo* arginine had significantly ( $P<0.05$ ) higher body weight gain in second week and highly significant ( $P<0.01$ ) higher gain in third week. All other treatments had significantly lowered body weight gain up to three weeks of age. From 0–3 week of age, arginine fed groups showed the best weight gain; nevertheless in weight gain of 3–5 weeks the combination group gave better weight gain, but the overall weight gain from 0–5 wks, arginine groups rallied ahead of all other groups. Among the literature reviewed, two types of results were noticed. Coles *et al.* (2001), Bhanja and Mandal (2005), Foye *et al.* (2006), Abdukalykova and Ruiz-Feria (2006), Chamani *et al.* (2012) and Kanagaraju (2014) had observed that the amino acids had improved body weight only up to first and second week; while Al-Murrani (1982) and Bhanja *et al.* (2012) working on broilers and Al-Daraji *et al.* (2012), who studied quails, observed increased body weight in amino acid fed birds right up to the end of the study mainly 56 and 42 d of age, respectively. Our result seems to fall between these two categories, where body weight increased up to 21 d and later the effect of *in ovo* feeding with arginine, waned into insignificance. It could be assumed that *in ovo* treatment of arginine had probably supported chick weight up to 21<sup>st</sup> d of age as a result of increasing amino acids which could have enhanced protein synthesis after hatching. Post hatch, glucose is synthesized from fat and protein (Elwyn and Bursztein 1993) and gluconeogenesis from protein metabolism is very likely to be the only source of glucose. De oliveira *et al.* (2007) summarized that the effect of different *in ovo* feed solution

ingredients do not always include increased body weights or better growth performance.

**Feed consumption (g) and feed efficiency:** The influence of *in ovo* feeding on cumulative feed consumption of birds and feed efficiency were analysed and presented in Tables 5,6. The results revealed that feed consumption in broilers was not influenced by *in ovo* feeding and had no significant difference between treatments; however, a numerically better feed consumption was observed in the *in ovo* amino acid groups. There was no significant difference in feed efficiency, but it has been noticed that cumulative feed intake was numerically higher in tryptophan and in the combination group. Bhanja *et al.* (2012), Saki *et al.* (2013) and Al-Daraji *et al.* (2012) found no significant treatment effect on feed intake but they observed difference with respect to feed conversion ratio, while Bhanja and Mandal (2005), Gaffer *et al.* (2013), Shafey *et al.* (2014), Eslami *et al.* (2014) and Kanagaraju (2014) observed similar results with no significant difference for feed efficiency. However, it has been observed that birds fed with tryptophan had higher feed intake. Emadi *et al.* (2011) had found a complementary effect with increased feed intake due to the precursor function of tryptophan for neurotransmitter serotonin (found in brain) which increases feed intake.

**Livability:** No significant difference was observed in livability percentage between all the treatment groups. However, it may be noted that arginine and tryptophan *in ovo* fed groups had no mortality. Uni and Ferket (2004) have stated *in ovo* feeding of nutrients improved feed utilization at an early age, immune response to enteric

Table 3. Effect of *in ovo* feeding of arginine, tryptophan and their combination on weekly body weight (g) (Mean±SE)

Week	Treatment				
	Control	Injected control	Arginine	Tryptophan	Combination (ARG+TRP)
First (n=96)**	86.88 <sup>b</sup> ±1.07	89.65 <sup>a</sup> ±0.95	90.06 <sup>a</sup> ±0.99	86.7 <sup>b</sup> ±0.88	86.56 <sup>b</sup> ±0.73
Second (n=96)*	251.97 <sup>b</sup> ±4.52	259.95 <sup>ab</sup> ±3.99	271.43 <sup>a</sup> ±3.59	256.56 <sup>b</sup> ±3.97	259.82 <sup>ab</sup> ±4.06
Third (n=94)*	560.46 <sup>b</sup> ±10.94	564.88 <sup>b</sup> ±7.83	600.56 <sup>a</sup> ±7.89	567.94 <sup>b</sup> ±8.56	571.65 <sup>b</sup> ±9.59
Fourth <sup>NS</sup> (n=84)	1019.9±13.58	1029.13±12.60	1062.26±11.73	1014.80±13.67	1038.29±14.11
Fifth (n=82) <sup>NS</sup>	1340.23±18.23	1332.37±18.02	1371.69±16.63	1321.73±19.95	1352.20±20.14

<sup>NS</sup>Not significant, \*Significant ( $P<0.05$ ), \*\*Highly significant ( $P<0.01$ ). Mean values within each row bearing common superscripts do not differ significantly. n, number of observations.

Table 4. Effect of *in ovo* feeding of arginine, tryptophan and their combination on weekly body weight gain (g) (Mean±SE)

Week	Treatment				
	Control	Injected control	Arginine	Tryptophan	Combination (ARG+TRP)
First (n=96) <sup>NS</sup>	43.33±0.94	45.04±0.88	44.68±0.90	42.40±0.81	43.08±0.64
Second (n=96)*	164.35 <sup>b</sup> ±4.04	170.30 <sup>b</sup> ±3.49	181.37 <sup>a</sup> ±2.95	169.84 <sup>b</sup> ±3.43	173.26 <sup>ab</sup> ±3.82
Third (n=94)**	308.36 <sup>b</sup> ±7.89	305.28 <sup>b</sup> ±5.36	334.15 <sup>a</sup> ±5.03	310.94 <sup>b</sup> ±6.07	312.90 <sup>b</sup> ±6.15
Fourth (n=84) <sup>NS</sup>	457.69±6.72	462.76±7.59	468.13±5.94	448.53±10.31	468.92±6.88
Fifth (n=82) <sup>NS</sup>	317.24±10.30	304.68±9.43	309.84±9.93	307.90±10.59	313.62±9.26

<sup>NS</sup>Not significant, \*Significant ( $P<0.05$ ), \*\*Highly significant ( $P<0.01$ ). Mean values within each row bearing common superscripts do not differ significantly. n, number of observations.

Table 5. Effect of *in ovo* feeding of arginine, tryptophan and their combination on cumulative feed consumption (g) (Mean±SE) (n=6)

Weekly feed consumption	Treatment				
	Control	Injected control	Arginine	Tryptophan	Combination (ARG+TRP)
First <sup>NS</sup>	98.85±0.96	105.23±3.34	101.73±2.24	98.84±1.86	101.72±1.21
Second <sup>NS</sup>	344.47±3.71	335.59±5.39	345.48±2.94	342.90±1.86	342.97±2.09
Third <sup>NS</sup>	792.95±5.59	783.00±9.40	784.47±4.84	787.00±1.51	791.51±3.25
Fourth <sup>NS</sup>	1517.82±11.89	1520.56±12.58	1512.50±4.12	1533.06±3.19	1535.55±1.83
Fifth <sup>NS</sup>	2427.72±11.58	2432.07±12.63	2418.75±5.89	2440.80±6.90	2451.02±2.53

<sup>NS</sup>Not significant. n, number of observations.

Table 6. Effect of *in ovo* feeding of arginine, tryptophan and their combination on feed efficiency (Mean±SE) (n=6)

Week	Treatment				
	Control	Injected control	Arginine	Tryptophan	Combination (ARG+TRP)
First <sup>NS</sup>	1.14±0.02	1.17±0.03	1.12±0.01	1.14±0.01	1.17±0.02
Second <sup>NS</sup>	1.38±0.07	1.29±0.03	1.27±0.01	1.33±0.02	1.32±0.03
Third <sup>NS</sup>	1.42±0.06	1.38±0.03	1.30±0.02	1.38±0.02	1.39±0.03
Fourth <sup>NS</sup>	1.49±0.04	1.47±0.02	1.42±0.02	1.51±0.02	1.48±0.03
Fifth <sup>NS</sup>	1.81±0.02	1.82±0.02	1.76±0.03	1.74±0.10	1.77±0.02

<sup>NS</sup>Not significant. n, number of observations.

antigens, intestinal development and digestive capacity, thereby reduced mortality. The same was observed by Saki *et al.* (2013) and Kanagaraju (2014).

**Broiler economy:** The broiler farm efficiency index (BFEI) and broiler feed price ratio (BFPR) are presented in Table 7, where BFEI reveals the overall efficiency of a broiler farm expected to be 2.0 (Narahari and Rajini 2005). BFEI is based on average live weight, livability per cent, feed efficiency and growing period in days. All the treatments in this experiment showed a value of above 2.0 as body weight and livability was higher in the *in ovo* fed groups. This proves that the birds have been managed well and performed to the optimum. While checking on the broiler feed price ratio (BFPR), a clear difference was observed with arginine fed group giving a better BFPR of 1.52. As per the statement of Narahari and Rajini (2005), a BFPR of 2.0 and above would be ideal, however, this was calculated during the period when the broiler feed cost ranged between ₹ 5 to 18, but now as the feed cost has sky rocketed and the BFPR ratio of 2.0 or more is unimaginable. It could be stated that assuming even if we could get broiler feed at a lowest cost of ₹ 26/kg the best BFPR for the year 2015 would be above 1.45. Based on this value, we could state that the BFEI value obtained in all the *in ovo* treatments were good.

Table 7. Effect of *in ovo* feeding of arginine, tryptophan and their combination on broiler economics (Mean±SE) (n=108)

Treatment	Feed cost/kg (₹)	Sale price/kg live weight (₹)	Feed consumption/bird (kg)	Body weight of bird at 5 week (kg)	BFEI <sup>NS</sup>	BFPR
Control	28.00	75.00	2.42	1.34	2.07±0.08	1.48
Injected control	28.02	75.00	2.43	1.33	2.06±0.05	1.46
Arginine	28.04	75.00	2.41	1.37	2.22±0.09	1.52
Tryptophan	28.04	75.00	2.44	1.32	2.48±0.43	1.45
Combination (ARG+TRP)	28.04	75.00	2.45	1.35	2.21±0.05	1.47

<sup>NS</sup>Not significant. n, number of observations. Mean values within each column bearing common superscripts do not differ significantly.

In the present study, *in ovo* feeding provides enough nutrition to improve hatchability, hatch weight, body weight establishing a new science of perinatal nutrition for greater production efficiency. Since the avian embryo develops in carbohydrate free environment, the injected amino acids act as source of glucose for synthesis of glycogen that aids in hatching activity. *In ovo* feeding stimulates intestinal digestive and absorptive capacity than the control chicks, which may correlate to improve post-hatch growth performance. It may be concluded that arginine and tryptophan are critical amino acids for the growth of avian embryo as well as during post-hatch period. The BFEI and BFPR indicate the economic viability of the *in ovo* feeding strategy.

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