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Influence of *In-ovo* Arginine Feeding on Post-hatch Growth Performance and Economics of Broilers

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ABSTRACT

A total of 420 fertile broiler eggs (Cobb) with uniform egg weight were collected and divided into three groups: non-injected control, injected control (0.5 ml of 0.9% normal saline) and arginine (0.5 ml of 0.5% arginine); and injected on 18 day (d) of incubation into amnion. On the 21 d, 108 chicks were randomly selected from each treatment totaling to 324 chicks. The hatchability, hatch weight and body weight, body weight gain were studied. Arginine had shown a highly significant (P<0.01) effect on hatch weight (45.18±0.24 g) and placement weight (42.24±0.23 g). Body weight (600.56±7.89 g) and body weight gain (334.15±5.03 g) were significantly (P<0.05) improved in arginine fed *in ovo* groups up to 21 d of age. Broiler farm economy index (BFEI) and broiler feed price ratio (BFPR) were best in arginine *in ovo* fed groups. *In ovo* administration of limiting amino acid (arginine) influences the growth of embryo and ultimately improves the post-hatch production performance in broilers. It can also be concluded that the reduction in weight loss during transportation is due to the action of conditionally essential amino acid arginine against stress.

Keywords: Arginine, broiler production, hatchability, *in ovo* nutrition

The major achievement of poultry industry is improved feed efficiency from 2.5 to 1.6 and the same could be stated for body weight which is realized in 35 instead of 56 days. Today India is the fourth largest producer of poultry meat in the world, but the per capita availability of poultry meat is only 2.15 kg as against 11 kg recommended by ICMR which necessitates need of huge growth of poultry industry in the near future. Poultry nutrition plays a critical role in improving hatchability, body weight and feed conversion ratio (FCR). *In ovo* feeding is the administration of exogenous nutrients into the amnion of the late-term avian embryo (Uni and Ferket, 2003). As the avian embryo orally consumes the amniotic fluid (primarily water and albumen protein) prior to pipping the air cell (about 18 d of incubation in broilers), *in ovo* feeding will improve the energy status and gut development of the perinatal chick by supplementing the amnion with nutrients.

The avian embryo grows in a carbohydrate free environment with finite amount of energy i.e. closed nutrition and nutrients for growth and development invested by the broiler breeder hen to support embryonic growth and hatching. The aim of *in ovo* feeding is to increase hatchability and hatch weight and ultimately body weight by supporting the embryos with different nutrients at incubation. Among amino acids arginine (Arg) has been proven to positively influence the avian systemic immune response which ultimately influences body growth. Wu and Morris (1998) described Arg as one of the most versatile amino acids in animal cells. Birds have the highest requirement of Arg (Ball *et al.*, 2007) which is due to lack of endogenous Arg synthesis, high protein deposition rate due to the fast growth of current broiler lineages and the antagonistic metabolic interaction between lysine (Lys) and Arg (Edmonds and Baker, 1987). However, there have been limited studies regarding the regulatory effects of arginine through *in ovo* feeding method. Hence, the study was undertaken to evaluate the potential effects of *in ovo* Arg on hatchability, hatch weight, production parameters and to analyse the economics of *in ovo* feeding strategies in broilers.

MATERIALS AND METHODS

In ovo injection

Total 420 numbers of fertile broiler eggs with average weight (68±0.1 g) were obtained from a commercial flock of Cobb 400 breeders (34 wk of age). The eggs were cleaned with egg shell sanitizer and incubated with broad end up in forced draft automatic chicken incubator. The dry bulb (99.5±0.50°F) and wet bulb temperature (82.94±0.40°F) were maintained from day one to 18 d of incubation. Hatching eggs in the incubator were turned by 45^0 angles on either side at hourly interval until they were transferred to the hatcher. On 18 d of incubation, eggs were candled and infertile eggs were discarded. Fertile eggs were randomly assigned to each treatment and marked for identification, later disinfection of egg shell surface with 99.90% ethyl alcohol was carried out, 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% ethanol to sterilize the tip. Non-injected control (negative control) directly kept for incubation whereas 0.5 ml of *in ovo* injection was injected into the amnion of the 18 d old embryo, through a pinhole made at the broad end of the egg, using 25 mm needle as per Uni and Ferket (2003) in other two treatment groups which consists control (0.5ml of normal saline solution as positive control) and 0.5ml of 0.5% arginine solution (5 mg/ml). Prior to *in ovo* injection the injectants were warmed up to 30°C. The pinhole site was sealed with sterile paraffin wax immediately 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

A total of 324 nos. of chicks were randomly selected from the hatched chicks with 108 numbers in each treatment and 18 in each replicate. Per cent hatchability was calculated for each treatment. Chicks were weighed individually to 0.1 g accuracy and hatch weight was recorded. Chicks hatched from various treatment groups were distributed randomly and placed in experimental brooding cages and kept in a well-lit and ventilated open sided house. Each treatment group had six replicates of 18 birds each, chicks were later transferred to growing cages on the 15 d and reared up to 35 d.

Experimental birds were provided with nutritional guide of Vencobb standard with similar nutrient composition and birds were provided with *ad lib* feed and water. Standard management practices were followed in the test and control groups throughout the experiment. All experimental protocols were approved by the Institutional Animal Ethical Committee in Madras Veterinary College, Chennai (TANUVAS).

Hatchability and production performances

Number of chicks hatched in each treatment was recorded and the hatchability per cent was calculated. As all the eggs incubated were fertile eggs, the calculated hatchability was "fertile hatchability".

After transportation from hatchery to farm each chick was individually weighed at and placement weight was recorded. Based on the hatch weight and placement weight the transit loss was calculated. Birds were individually weighed every week up to 35 d and the body weight gain was calculated. The broiler performance indices such as broiler farm economy index (BFEI) and broiler feed price ratio (BFPR) was calculated using the following formula:

$$\text{BFEI} = \frac{\text{Average live weight} \times \text{per cent Livability}}{\text{Feed efficiency} \times \text{growing period in days}}$$

$$\text{BFPR} = \frac{\text{Total value of meat/live broilers produced}}{\text{Total value of feed consumed}}$$

Statistical Analysis

Results were analyzed by 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. Hatchability was analysed by chi-square test.

Table 1: Effect of *in ovo* arginine feeding on hatchability, hatch weight, chick/egg weight ratio, placement weight and transit weight loss (Mean±S.E)

Variable	Treatments			F value
	Control	Injected control	Arginine (ARG)	
Hatchability percentage [NS] (n = 140)	95.71	94.28	96.42	0.826 (χ^2 value)
Hatch egg weight (g) [NS] (n = 140)	60.54±0.004	60.53±0.011	60.55±0.004	1.358
Hatch weight (g) ** (n =108)	43.96[bc]±0.29	44.50[ab]±0.26	45.18[a]±0.24	5.065
Chick/Egg weight ratio** (n= 108)	72.6[bc]±0.48	73.5[ab]±0.43	74.62[a]±0.39	5.048
Placement weight (g) ** (n = 108)	40.26[c]±0.28	40.9[bc]±0.25	42.24[a]±0.23	10.216
Transit weight loss (g)[NS] (n =108)	3.69±0.39	3.58±0.12	2.94±0.10	2.029

[NS]- 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 2: Effect of *in ovo* arginine feeding on weekly body weight (g) (Mean±S.E)

Week	Treatments			F value
	Control	Injected control	Arginine	
First (n=96)**	86.88[b]±1.07	89.65[a]±0.95	90.06[a]±0.99	3.416
Second (n=96)*	251.97[b]±4.52	259.95[ab]±3.99	271.43[a]±3.59	3.170
Third (n=94)*	560.46[b] ±10.94	564.88[b]±7.83	600.56[a]±7.89	3.087
Fourth (n=84)[NS]	1019.95±13.58	1029.13±12.60	1062.26±11.73	2.018
Fifth (n=82)[NS]	1340.23±18.23	1332.37±18.02	1371.69±16.63	1.063

[NS]- 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 3: Effect of *in ovo* arginine feeding on weekly body weight gain (g) (Mean±S.E)

Week	Treatments			F value
	Control	Injected control	Arginine	
First (n=96)[NS]	43.33±0.94	45.04±0.88	44.68±0.90	1.700
Second (n=96)*	164.35[b]±4.04	170.30[b]±3.49	181.37[a]±2.95	3.047
Third (n=94) **	308.36[b]±7.89	305.28[b]±5.36	334.15[a] ±5.03	3.425
Fourth (n=84)[NS]	457.69±6.72	462.76±7.59	468.13±5.94	1.211
Fifth (n=82)[NS]	317.24±10.30	304.68±9.43	309.84±9.93	0.244

[NS]- 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

RESULTS AND DISCUSSION

Hatchability, hatch weight and chick to egg weight ratio

The effect of *in ovo* feeding of arginine on 18 d of incubation through amnion in broiler breeder chicken egg on hatchability, hatch weight and chick to egg weight ratio is presented (Table 1). *In ovo* feeding with arginine had not significantly influenced hatchability. However, a numerically higher percentage of hatchability was observed in the *in ovo* fed arginine (96.42%) group as compared to negative and positive control groups (94.28 and 95.71%) 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 poults. *In ovo* feeding with arginine significantly ($P<0.01$) improved hatch weight (45.18±0.24) and was comparable with injected control (44.50±0.26). The hatch weight of chicks showed a highly significant ($P\leq0.01$) difference between treatments and arginine fed group had highest hatch weight. 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). Chick to egg weight ratio also showed significant ($P<0.05$) difference indicating *in ovo* arginine fed group significantly improved egg to chick weight ratio than un-injected and injected control. The effect of chick to egg weight ratio was also noticed by Ohta *et al*. (1999, 2001 and 2004), Bhanja and Mandal (2005) and Shafey *et al*. (2014). However Ohta *et al.* (2001 and 2004) found no significant difference in hatched 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

The effect of *in ovo* feeding of arginine on placement weight and transit weight loss is presented (Table 1). Body weight of chicks (first day at farm) specified as placement weight showed a highly significant difference ($P<0.01$) between treatments with arginine *in ovo* fed chicks having highest 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 other 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. 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. Kanagaraju (2014) observed significantly lower transit weight loss in lysine and threonine fed groups which averaged to 4.99% as compared to control. The higher per cent weight loss could be attributed to the climate and period of study.

Effect of *in ovo* feeding on weekly body weight (g) and body weight gain (g)

The mean weekly body weight and body weight gain of broilers (g) as influenced by *in ovo* feeding of arginine at day old and at weekly intervals up to five weeks of age is presented (Table 2 and 3). The results indicated a highly significant difference ($P<0.01$) between treatments at 1st week and significant difference ($P<0.05$) in 2nd and 3rd week between treatments; however, the weight advantage was sustained up to 21 d only. *In ovo* feeding with arginine produced significantly ($P<0.01$) heavier birds in 1st, 2nd and 3rd week continuously (90.0±0.99, 271.43±3.59 and 600.56±7.89) and was comparable with injected control (89.65±0.95, 259.95±3.99 and 564.88±7.89), but no effect was observed during 4th and 5th week of age. *In ovo* arginine fed birds had significantly ($P<0.05$) higher body weight gain in 2nd week and highly significant ($P<0.01$) gain in 3rd week and did not show any significant changes in fourth and fifth week in all the groups studied.

Among the literature reviewed two types of results were noticed of which work done by 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 1st and 2nd week, while Al-Murrani (1982) and Bhanja *et al*. (2012) working on broilers and Al-Daraji *et al.* (2012), who studied on quails, observed increased body weight in amino acid fed birds right up to the end of the study mainly 56 and 42 day of age respectively. Our result seems to fall between these two categories,

Table 4: Effect of *in ovo* arginine feeding on cumulative feed consumption (g) (Mean±S.E) (n=6)

Weekly feed consumption	Treatments			F value
	Control	Injected control	Arginine	
First[NS]	98.85±0.96	105.23±3.34	101.73±2.24	1.572
Second[NS]	344.47±3.71	335.59±5.39	345.48±2.94	1.276
Third[NS]	792.95±5.59	783.00±9.40	784.47±4.84	0.600
Fourth[NS]	1517.82±11.89	1520.56±12.58	1512.50±4.12	1.504
Fifth[NS]	2427.72±11.58	2432.07±12.63	2418.75±5.89	1.997

[NS]- Not significant, n = Number of observations

Table 5: Effect of *in ovo* arginine feeding on feed efficiency (Mean ± S.E) (n=6)

Week	Treatments			F value
	Control	Injected control	Arginine	
First[NS]	1.14±0.02	1.17±0.03	1.12±0.01	1.004
Second[NS]	1.38±0.07	1.29±0.03	1.27±0.01	1.098
Third[NS]	1.42±0.06	1.38±0.03	1.30±0.02	1.202
Fourth[NS]	1.49±0.04	1.47±0.02	1.42±0.02	1.194
Fifth[NS]	1.81±0.02	1.82±0.02	1.76±0.03	0.358

[NS]- Not significant, n = Number of observations

Table 6: Effect of *in ovo* arginine feeding on broiler economics (Mean±S.E) (n=108)

Treatments	Feed cost/ kg (₹)	Sale price/kg live weight (₹)	Feed consumption/ bird (kg)	Body weight of bird at 5 week (kg)	BFEI[NS]	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

[NS]- Not significant, n = Number of observations
Mean values within each column bearing common superscripts do not differ significantly

where body weight increased up to 21 d and later the effect of *in ovo* feeding with arginine became insignificant. With regards to body weight gain a significant (P<0.05) result during 2nd week and a highly significant (P<0.01) during 3rd week was obtained; with arginine *in ovo* fed groups obtaining the best body weight gain and this agreed with the findings of Bhanja *et al.* (2012) and Shafey *et al.* (2014). Shafey *et al*. (2014) had provided three groups of amino acid combination and they observed that the combination with arginine, glutamine, glycine and proline gave the best result and they attributed this increased body weight gain to arginine. It could be assumed that *in ovo* treatment of arginine had probably supported chick weight up to 21 d of age as a result of increasing amino acids which could have enhanced protein synthesis after hatching.

Effect of *in ovo* feeding on feed consumption (g) and feed efficiency

Cumulative feed consumption of birds (g) and feed efficiency as influenced by *in ovo* feeding of different treatments were analysed and presented (Table 4 and 5). 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* arginine group. Also there was no significant difference among treatment groups with regards to feed efficiency. Among the literature studied Bhanja *et al*. (2004 and 2012), Saki *et al*. (2013) and Al-Daraji *et al*. (2012) found no significant effect on feed intake with regards to amino acid *in ovo* feeding but observed difference with respect to feed conversion ratio, while Bhanja and Mandal (2005), Gaafar *et al*. (2013), Shafey *et al*. (2014), Eslami *et al*. (2014) and Kanagaraju (2014) observed similar results with no significant difference in treatments for feed efficiency.

Effect of *in ovo* feeding on broiler economy

The economic parameters like broiler farm efficiency index (BFEI) and broiler feed price ratio (BFPR) are presented (Table 6). Broiler farm economy index reveals the overall efficiency of a broiler farm (Narahari and Rajini, 2005) is expected to be 2.0, and in our study arginine treatment also showed this value of above 2.0, which 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) the BFPR of 2.0 and above would be ideal, however this was calculated during the period when the broiler feed cost ranged between Rs.15 to 18, but since the feed cost has sky rocketed and a 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 Rs. 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 the *in ovo* arginine treatment was good.

CONCLUSION

Arginine is the most potential nitrogen carrier which improves growth and performance of young ones. It has increased in weight of embryo mostly towards later part of its growth. *In ovo* feeding of arginine had improved body weight and weight gain up to 21 d of age. *In ovo* feeding of arginine had significantly (P<0.01) improved hatch weight, chick/egg weight ratio and placement weight and provides enough nutrition for the weight to be sustained up to at least 35 d establishing a new science of perinatal nutrition for greater production efficiency. The higher percentage of weight loss may be due to climate and period of study. The broiler farm efficiency index (BFEI) and broiler feed price ratio (BFPR) was much better in arginine fed group which indicates the economic viability of the *in ovo* feeding strategy.

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