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Response of *In ovo* Supplementation of Amino Acid and Minerals on Egg Hatchability of Broiler Chicken

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ABSTRACT

Background: *In ovo* supplementation of nutrients either amino acids or minerals in broiler chicken has been common more often individually or few nutrients with positive results. In view of the above, *in ovo* supplementation with combination of amino acids and minerals as a complete nutrient capsule for the gut and immune system development was tested for hatchability since the modern commercial broilers are very fragile to any changes in the internal environment.

Methods: Four trials were conducted to study the response of *in ovo* supplementation of combinations of Lys, Met, Arg, Thr, Glu, Zn, Se and Cu on hatchability of broiler eggs. In trial I, the fertile eggs on day 18 were divided into five groups of 32 eggs in each group. Group I served as control without any amino acids and mineral supplementation. Group II, III, IV and V supplemented with Lys, Met, Arg, Thr, Glu, Zn, Se and Cu @ 2.2, 1, 2.5, 1.6, 2.5 mg, 80, 0.3, 16 µg; 4.4, 2, 5, 3.2, 5 mg, 80, 0.3, 16 µg; 11, 5, 12.5, 8, 12.5 mg, 80, 0.3, 16 µg and 22, 10, 25, 16, 25 mg, 80, 0.3, 18 µg respectively. In trial II, seventy fertile eggs were divided into seven groups, Group I served as control. Group II, III, IV, V, VI and VII were supplemented with graded levels (1x to 6x) of amino acids. The 1x consisted of Lys, Met, Arg, Thr and Glu @ 2.2, 1, 2.5, 1.6 and 2.5 mg, respectively. On day 18, 10 eggs per group were supplemented with graded levels of AA combination into the amniotic fluid. In trail III and IV it was similar to trial II but was without Lys and Met, respectively.

Result: Results indicated that hatchability (Trial 1) in Group V, IV, III, II and I were 0, 18, 21.8, 71 and 90.6%, respectively. The Combination of five amino acids (Trial II) (Lys, Met, Thr, Arg, Glu) for *in ovo* supplementation reduced the hatchability, whereas, combination of four amino acids (Trial III) (Met, Thr, Arg, Glu) without Lys at lower doses resulted in good hatchability (86.6%). *In ovo* supplementation of amino acid or mineral-amino acid combinations in commercial broiler eggs should be restricted to few amino acids.

Key words: Amino acids, Broiler, Hatchability, *In ovo*, Mineral.

INTRODUCTION

A fertile egg is a complete nutrient capsule for the development of the embryo until hatch; however, it has sufficient fat in form of yolk whereas available amino acids level may be lower at the later phase of incubation for embryonic growth and the embryo utilizes amino acids for tissue growth at a much higher rate during incubation (Ohta *et al.* 1999, 2001).

The increase in the mineral concentration in the breeder's diet has no influence on mineral concentrations in the egg (Naber, 1979; Angel, 2007). Most of the minerals in the egg were consumed during day 11 to day 17 of incubation which leads to lower levels of P, Fe, Zn, Cu, and Mn in yolk (Yair and Uni, 2011). Residual yolk is the main source of these minerals during early growth hence deficiency of this micro minerals in yolk will adversely affect the embryonic growth.

The *in ovo* supplementation employing nutrients either individually or in combination reported variable results on hatchability, either positively (Bhanja and Mandal, 2005; Shafey *et al.* 2014; Kanagaraju and Rathnapraba, 2019), negatively (Jose *et al.* 2018; Haonan *et al.* 2019) or without any effect (Bhanja *et al.* 2012; Coskun *et al.* 2017; Sogunle *et al.* 2018) depending on the dose and combination of nutrients. In view of the above, *in ovo* supplementation with combination of amino acids and minerals as a complete nutrient capsule for the gut and immune system development was tested for hatchability since the modern commercial broilers are very fragile to any changes in the internal environment.

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MATERIALS AND METHODS

The experiment was carried out during 2018-19 at ICAR-National Institute of Animal Nutrition and Physiology and the animal experimental procedure was approved by Ethical committee of ICAR-National Institute of Animal Nutrition and Physiology, Bangalore, India.

Trial I

One hundred and eighty uniform sized eggs (Cobb broiler) were incubated with the dry bulb temperature ranging from 99 to 100°F and wet bulb temperature of 85-87°F from day 1 to 18. From day 18 onwards, the relative humidity was increased by setting the wet bulb thermometer reading of more than 88°F till hatch. The fertile eggs on day 18 were divided into five groups of 32 eggs in each group. Group I served as control without any amino acids and mineral supplementation. The required amount of crystalline amino acids and trace elements were weighed and dissolved in the sterile water in such a concentration that 0.5 ml contained the required amount of selected nutrient to be supplemented in one egg. Group II, III, IV and V supplemented with Lys, Met, Arg, Thr, Glu, Zn, Se and Cu @ 2.2, 1, 2.5, 1.6, 2.5 mg, 80, 0.3, 16 µg; 4.4, 2, 5, 3.2, 5 mg, 80, 0.3, 16 µg; 11, 5, 12.5, 8, 12.5 mg, 80, 0.3, 16 µg and 22, 10, 25, 16, 25 mg, 80, 0.3, 18 µg, respectively. Several pre standardization of the *in ovo* techniques with sterile water was done and thus sham control was avoided. *In ovo* supplementation was performed under sterile condition of laminar air flow at a temperature of around 35°C. The prior standardization with ink was carried out in each trial to confirm injection is into the amniotic fluid. The supplementation solution was warmed to 30°C and administered through the broad end into the amniotic fluid using a 24 gauge needle. After injections, the hole was sealed using paraffin and the eggs were transferred to hatching boxes. In ovo injection procedure was completed within 20 minutes starting from taking out the egg from the incubator and keeping it back into the hatcher. The hatchability was recorded. The day old body weight of chick and gut development parameters was also recorded only in control and group II (better hatch).

Trial II

Cobb broiler eggs was procured from commercial hatchery and incubated. On day 18, seventy fertile eggs were divided into seven groups. Group I served as control without any amino acid supplementation. Group II, III, IV, V, VI and VII supplemented with graded levels (1x to 6x) of amino acids. The 1x consisted of Lys, Met, Arg, Thr and Glu @ 2.2, 1, 2.5, 1.6 and 2.5 mg, respectively. After 2 h of injection, the eggs were opened to check the livability (either alive or dead) of the embryos.

Trial III

Trial III was conducted by supplementing graded level of amino acids similar to trail II. In this trial, Lys was excluded from five amino acid combination. Hatchability was recorded.

Trial IV

Trial IV was conducted by supplementing graded level (1x, 5x, 10x) of amino acids as in trail II. In this trial, Met was excluded from five amino acid combination. Hatchability was recorded.

RESULTS AND DISCUSSION

Trial I

The results indicated *in ovo* supplementation of different doses of amino acid and mineral combination influenced the hatchability drastically (Table 1). There was a gradual decreasing trend in the hatchability as the dosage of supplementation increased. The hatchability decreased with the increase in the osmolarity. There was no difference ($P>0.05$) in the chick weight and gut development parameters between treatment groups (Table 2).

Trial II

Livability percentages of the embryo's were lower in all the treatment groups (Fig 1). As the dose of amino acids increased the livability decreased drastically and gradually

Table 1: Hatchability of eggs on *in ovo* supplementation of amino acids and minerals.

Group	Osmolarity (mosmol/kg)	Hatchability %
I Control	-	90.6
II	208.5	71
III	420.5	21.8
IV	1030.4	18
V	2083	0

Table 2: Chick weight (g/b) and gut development (cm/100 g or % of live weight) parameters.

		Group I	Group II	SEM	Significance
Chick weight		44.5	43.3	0.57	0.15
Duodenum	Length	1.56	1.35	0.06	0.07
	Weight	20.5	20.9	0.90	0.82
Jejunum	Length	1.19	0.96	0.07	0.10
	Weight	36.8	32.1	1.74	0.19
Ileum	Length	0.93	1.01	0.04	0.27
	Weight	31.8	36.1	1.31	0.10
Caecum	Length	0.90	0.84	0.05	0.52
	Weight	8.80	8.86	0.41	0.95
Liver	Weight	2.66	2.48	0.12	0.46
Proventriculus	Weight	0.94	0.93	0.03	0.93
Gizzard	Weight	5.50	5.27	0.18	0.54
Yolk	Weight	7.79	9.24	0.56	0.21

from 50% in 1x group to 20% in 5x group. The livability percentage of 6x group was zero.

Trial III

Hatchability of eggs was 93.3% in control group and was 86.6% in 1x concentration group (Fig 2). From 2x to 6x concentration, the hatchability percentage was zero with none of the chicks hatching out.

Trial IV

Hatchability of eggs was 30.76% in 1x concentration group, whereas, it was 0% in 5x and 10x concentration (Fig 3). Hatchability of eggs was 100% in control group.

Earlier study from our laboratory conducted by Awachat *et al.* (2018) observed that supplementation of combination

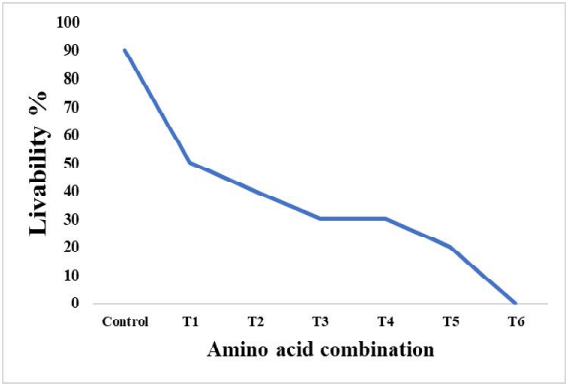

Fig 1: Livability (%) of embryo's on *in ovo* supplementation (n=10).
Control (without amino acid); T1 (Lys 2.2, Met 1, Arg 2.5, Thr 1.6, Glu 2.5 mg); T2 (Lys 4.4, Met 2, Arg 5, Thr 3.2, Glu 5 mg); T3 (Lys 6.6, Met 3, Arg 7.5, Thr 4.8, Glu 7.5 mg); T4 (Lys 8.8, Met 4, Arg 10, Thr 6.4, Glu 10 mg); T5 (Lys 11, Met 5, Arg 12.5, Thr 8, Glu 12.5 mg); T6 (Lys 13.2, Met 6, Arg 12.5, Thr 9.6, Glu 15 mg).

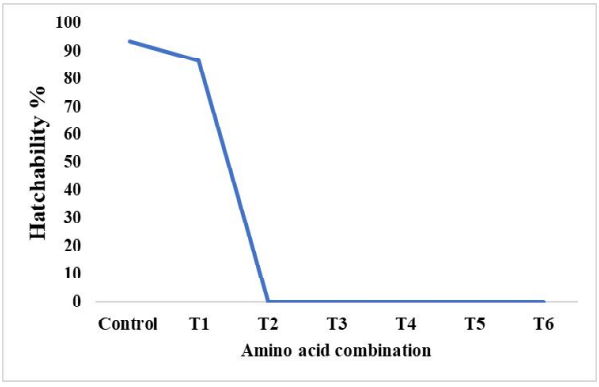

Fig 2: Hatchability of trial III (n=15) eggs on *in ovo* supplementation of four amino acids (without Lys).
Control (without amino acids); T1(Met 1, Arg 2.5, Thr 1.6, Glu 2.5 mg); T2 (Met 2, Arg 5, Thr 3.2, Glu 5 mg); T3 (Met 3, Arg 7.5, Thr 4.8, Glu 7.5 mg); T4 (Met 4, Arg 10, Thr 6.4, Glu 10 mg); T5 (Met 5, Arg 12.5, Thr 8, Glu 12.5 mg); T6 (Met 6, Arg 15, Thr 9.6, Glu 15 mg).

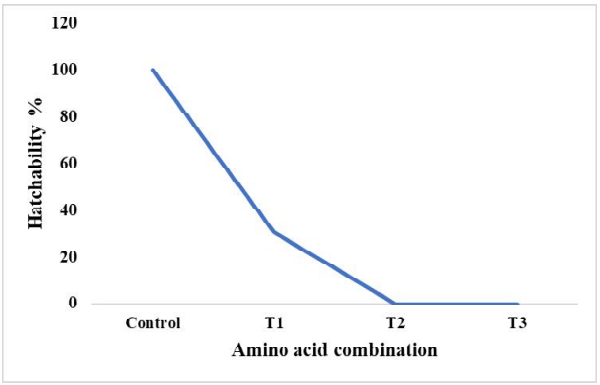

Fig 3: Hatchability of trial IV (n=10) eggs on *in ovo* supplementation four amino acids (without Met).
Control (without amino acid supplementation); T1 (Lys 2.2, Arg 2.5, Thr 1.6, Glu 2.5 mg); T2 (Lys 11, Arg 12.5, Thr 8, Glu 12.5 mg); T3 (Lys 22, Arg 25, Thr 16, Glu 25 mg).

of 3 amino acids (Arg, 22; Glu, 25 and Thr 30 mg/egg) resulted in good hatchability (94%). However, in the present study, *in ovo* supplementation of amino acids in combination with minerals (Lys, Met, Arg, Thr, Glu, Zn, Se and Cu) or only with amino acids (Lys, Met, Arg, Thr and Glu) involving more than three amino acids led to lower hatchabilities. As such, a possible explanation for the lowered hatchability might be due to the type, number, amino acid concentrations and interactions between amino acid and minerals. The results of the Trial I showed that the hatchability decreased as the dosage amino acids and minerals (Lys, Met, Arg, Thr, Glu, Zn, Se, Cu) and osmolarity of the solutions increased. To rule out any possible amino acid and mineral interaction causing the reduction in hatchability, more trials were conducted without minerals and with only amino acid combinations (Trial II to IV). *In ovo* supplementation of five amino acids (Lys, Met, Thr, Arg, Glu) and four amino acids (Lys, Thr, Arg, Glu) combinations even at lower doses (1x) resulted in poor hatchability (30%). The hatchability was so low in treatment-2 of Trial-IV while in treatment-2 of Trial-I (almost similar but had more minerals) had higher hatchability. The hatchability did not follow a particular pattern, with mineral combination, hatchability was little better. The cause needs to be further probed. However, more nutrient injection is definitely a concern for poor hatchability. *In ovo* supplementation of combination of four amino acids (Met, Thr, Arg and Glu) without lysine at lower doses resulted in good hatchability (86.6%) suggesting it is better to restrict up to this dosage. These results also indicated there was no influence of osmolarity since If dose is lower osmomolarity (around 200 mosmol/kg) will also be lower. Thus, the study indicated that higher concentrations of amino acids should be avoided in combinations with more than three amino acids. It also indicated Lys in combinations affected more than Met for *in ovo* supplementation.

Varied hatchabilities have been reported due to the combination of different aspects of *in ovo* procedure, day of injection, site and the concentration of amino acids used

for *in ovo* injection. Ohta *et al.* (1999) reported hatchability was lower (13.3%) when injected on 0th day, 18 different AA (Asp, 5.31; Thr, 2.53; Ser, 3.86; Glu, 6.99; Gly, 1.77; Ala, 3.01; Val, 3.34; Cys,1.10; Met,1.91; Ile, 2.71; Leu, 4.53; Tyr, 1.84; Phe, 2.81; Lys,3.78; His, 1.35; Arg, 3.24; Pro,1.96; Trp, 0.95 mg/0.5 ml) into the yolk sac or air cell, whereas, hatchability was zero and similar to control when the AA solution was supplemented on day 7 into the air cell and yolk sac, respectively. Chick weight did not differ significantly among all treatments. Ohta *et al.* (2001) also found that injection of 18 different amino acid solutions to fertile broilers eggs on day 5 of incubation increased hatchability from 84.5 to 90.9%.

Bhanja *et al.* (2012) reported hatchability was not affected on supplementation of 25 mg each of limiting amino acids *viz.* Lys, Met, Thr, Arg, Gly and Ile individually on 14th day of incubation. 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.* (2014) reported *in ovo* supplementation of amino acid mixtures in groups, AA1 group 23.7 mg of Lys, 5.16 + Glu,12.10 + Gly, 3.22 + Pro, 3.24; AA2 group 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 did not affect the hatchability (AA1 - 97%; AA2 - 94.4% and AA3- 94.7% vs. control group 93.3%). Kadam *et al.* (2008) reported *in ovo* injection of graded level of Thr (10, 20, 30 or 40 mg per egg) had similar hatchability between different treatment groups (10 mg, 94.54%; 20 mg, 98.04%; 30 mg, 91.84%; 40 mg, 96.08%). Coskun *et al.* (2014) reported better hatchability in Lys and Met supplemented group. Nayak *et al.* (2016) observed similar hatchability (96.42%) as compared to the control (94.28%) on supplementation of Arg 2.5 mg/egg.

There are only very few reports available on the reduced hatchability on *in ovo* supplementation of amino acids. Coskun *et al.* (2014) observed that *in ovo* injection of 1 ml of Met reduced the hatchability (84.7%) compared to un-injected control group (90.2%). 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 supplemented group (88.8%). Awachat *et al.* (2018) supplemented Lys 22 mg, Met 10 mg and Thr 16 mg and reported reduced hatchability on *in ovo* supplemented group (67.8%) compared to (93.3%) control. Kadam *et al.* (2009) recorded poor hatchability when Thr (20 mg/egg) was supplemented into the albumen either through broad or narrow end, however better hatchability was recorded when Thr was supplemented into the yolk sac of 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.* 2014) have reported good hatchability 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. Except in the study of Bakayraj *et al.* (2012) wherein good hatchability was observed in amino acid supplemented group [AA for CMI (Lys, 22 + Met, 10 + Arg, 25 + leu, 24 + Ile, 16 mg); AA for HI] (Met,10 + Thr,16 + Arg, 25 + Gly, 12.5 + Ser, 12.5 + Val, 18 mg) even with higher doses. This probably may be due the broiler stock used in the above study was a slow growing and probably more resistant in comparison to the present commercial broiler which are highly sensitive. However, in the present study, amino acids doses similar to the above study resulted in poor hatchability.

Modern broilers have tremendous genetic potential for rapid growth and feed conversion efficiency, which would have made the present commercial stocks to be more susceptible for any modification or changes in terms of dosage or combination of amino acids for *in ovo* supplementation or any changes in the internal environment.

CONCLUSION

The *in ovo* supplementation of five amino acids (Lys, Met, Thr, Arg, Glu) with or without minerals or four amino acids combination (Lys,Thr, Arg, Glu) with Lys reduced the hatchability. The combination of four amino acids (Met, Thr, Arg, Glu) without Lys at lower doses (1x) resulted in good hatchability (86.6%). Our results indicated *in ovo* supplementation of amino acid or mineral-amino acids combinations in commercial broiler eggs should be carried out with caution and restricted to only few amino acids.

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