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Effects of *in ovo* Feeding of L-Glutamine on Hatchability Performance and Hatching Time of Meat-Type Breeder Eggs

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Abstract: The effects of *in ovo* feeding of L-Glutamine (GLN) at 17th day of incubation on hatchability traits, hatching time and chick weight at hatch (CWT and CWT%, expressed as an absolute value and as a percentage of egg weight, respectively) of Ross meat-type breeder eggs were studied at 26 and 32 weeks of age in trials 1 and 2, respectively. The treatments were a non-injected control, a positive control where saline was injected or saline with GLN at 5, 7.5 and 10 mg/egg. *In ovo* feeding of GLN did not influence hatchability traits, CWT, CWT% and hatching time. *In ovo* feeding of 10 mg GLN/egg increased percentage of hatched chicks at 468 h of incubation and reduced percentage of hatched chicks at 492 h of incubation when compared with those of the control treatments. Age of birds did not influence hatchability traits and percentages of hatched chicks at 468 and 492 h of incubation and CWT%. Eggs produced at 26 weeks of age had lower weight, percentage of hatched chicks at 504 h of incubation, hatching time and CWT when compared with those produced at older age. It is concluded that *in ovo* of up to 10 mg GLN/egg at 17th day of incubation of layer-type breeder eggs did not influence hatchability traits, chick weight at hatch and hatching time and eggs produced at older age had higher egg weight, chick weight at hatch and hatched later than those produced at younger age.

Key words: *In ovo* feeding, L-glutamine, broiler breeders, hatchability performance, eggs

INTRODUCTION

The growth of chicken embryo is completely reliant on the nutrient content of the egg for energy production and tissue synthesis. Providing sufficient available nutrients in the egg will enable a good starting point for the hatching chicks. *In ovo* administration of liquid nutrients of carbohydrates, amino acids, proteins and L-carnitine few days before hatching improved chick hatching weight and reduced time required to reach target market weight (Uni *et al*., 2005; Shafey *et al*., 2009, 2010, 2012). The embryo utilizes amino acids for tissue growth at a much higher rate during the latter half of incubation. However at this stage of incubation, some amino acids may be deficient to meet embryonic requirements for development especially in small size eggs (Ohta *et al*., 1999; Sunny and Bequette, 2010). Small size eggs produce embryos with higher rates of gluconeogenesis earlier in the development stage resulting from diverting greater supplies of amino acids and consequently produce smaller size embryos when compared with those produced from large size eggs (Sunny and Bequette, 2010). Deficient supply of amino acids could reduce embryonic growth and development. The synthesis of such amino acids may result from other amino acids such as Glutamine (GLN), a non-essential amino acid that is found in higher levels in muscles and plasma. It is an important energy substrate for rapidly dividing cells (Smith, 1990). It plays an essential role in several important metabolic pathways (Marliss *et al*., 1971; Smith, 1990), regulates nutrient metabolism, gene expression and protein synthesis (Wu, 2009) and improves the humoral immune response (Newsholme, 2001). Therefore, it is important to ensure adequate amount available of this amino acid to meet the increased physiological demands of the embryo, especially in the last stage of incubation.

In ovo feeding of GLN to chicken embryos has been investigated by PedrosoI *et al*. (2006) who found that *in ovo* feeding of GLN did not influence chick weight at hatch but reduced hatching time. However, Tavassoli *et al*. (2011) reported that *in ovo* administration of GLN increased chick weight at hatch. Also, *in ovo* feeding of GLN and carbohydrates to duck embryos improves small intestine development and pectoralis mass and weight gain in the early days of post-hatch (Chen *et al*., 2009). Several factors may influence the amount of available nutrients in the egg and consequently the outcome of *in ovo* administration of

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nutrients. These factors include egg size. This study was designed to examine the effects of *in ovo* feeding of GLN on hatchability performance, hatching time and chick weight at hatch from meat-type breeder eggs at 26 and 32 weeks of age.

MATERIALS AND METHODS

A total of 550 fresh laid eggs produced by a layer-type breeder, Ross flock at 26 weeks of age (Al-Wady Company, Al-Riyadh, Saudi Arabia) were used in the first trial. Eggs were numbered, weighed individually and distributed into weight classes. Eggs were set in a Maino, force-draft incubator (Model II, Maino Enrico, Co., Rome, Italy) and incubated at 37.5°C (99.5°F) and 55% relative humidity. Eggs were turned every 2 h. Eggs were examined by candling at 17th day of incubation and infertile eggs and eggs containing dead embryos were removed. After examination, 375 eggs were evenly assigned into 25 replicates of 15 eggs of equal weight per replicate. Five replicates were randomly assigned to each of the 5 experimental treatments. There were two control treatments: non-injected (negative control) and injected with 0.9% sterilised saline (positive control) and 3 experimental treatments in which sterilised saline containing GLN at 50, 100 or 150 mg/egg. The wide end of the eggshell was disinfected with 70% isopropyl alcohol and pierced for an injection hole. The 0.5 mL of solution was injected into the amniotic fluid using a 25 mm 21-G needle. After injection, the hole was sealed with melted paraffin wax and the eggs were returned to the incubator. Eggs were transferred to separate compartments in the hatching tray on the morning of day 19 of incubation, for chick identification at hatch. The hatching tray was divided into hatching compartments using thin sheets of mesh wires. The hatching compartment was set at 37°C (98.6°F) and 65% relative humidity until the end of day 21 of incubation, at which time; chicks, pips (unhatched eggs with live or dead chicks) and dead embryos (unhatched eggs with unbroken shell) were counted. Percent hatchability was calculated on the basis of the number of hatched chicks as a percentage of the *in ovo* treated eggs per replicate. Hatching time was recorded every 12 h. Chicks were observed at 12 h intervals from 444-504 h of incubation and hatching weight was recorded to the nearest 0.1 g.

The incubation trial was repeated with 400 fresh laid eggs produced at 32 weeks of age from the same flock. After candle examination of eggs at 17th day of incubation, 300 eggs were evenly assigned into 20 replicates of 15 eggs of equal weight per replicate. Four replicates were randomly assigned to each of the 5 experimental treatments. Measurements of percent hatchability and hatchability failures (pips with live embryos, pips with dead embryos, dead embryos), chick weight at hatch expressed as an absolute value or as a percentage of egg weight, hatching period were determined in trials 1 and 2.

Data analysis: There were no significant differences among the *in ovo* treatments in the hatchability traits, hatching time of eggs and chick weight at hatch so data from trials 1 and 2 were combined together for final analysis. Data were arranged in a 5×2 factorial arrangement for *in ovo* treatment and trial as main effects and their interaction fitted into the model. All percent data were transformed using arc sine square root percentage transformation before analysis. When significant variance ratios were detected, differences between treatment means were tested using the Least Significant Difference (LSD) procedure. All statistical analysis was performed using the Statistical Analysis System (SAS, 2006).

RESULTS AND DISCUSSION

The effects of *in ovo* feeding of GLN and trial on hatchability and hatchability failures, hatching time and chick weight at hatch are shown in Table 1-3, respectively. *In ovo* feeding of GLN did not significantly influence hatchability, hatchability failures, chick weight at hatch, percentage of hatched chicks at 504 h of incubation and hatching time. *In ovo* feeding of 10 mg GLN/egg significantly ($p<0.05$) increased percentage of hatched chicks at 468 h of incubation and reduced percentage of chicks hatched chicks at 492 h of incubation when compared with those of the control (negative (non-injected) and positive (saline injected), respectively) or *in ovo* feeding of 5 mg GLN/egg treatments.

There was no significant difference between the two trials (26 and 32 weeks of age, tria1 1 and 2, respectively) in hatchability, hatchability failures and chick weight at hatch when expressed as a percentage of egg weight and percentages of hatched chicks at 468 and 492 h of incubation. Eggs produced at 26 weeks of age (trial 1) had significantly ($p<0.01$) lower weight, percentage of hatched chicks at 504 h of incubation, hatching time and chick weight at hatch as an absolute value when compared with those produced at 32 weeks of age (Trial 2).

The potential use of *in ovo* administration of GLN in chicken eggs has been investigated by Pedrosol *et al.* (2006) who reported that *in ovo* feeding of up to 30 mg GLN/egg at 16th day of incubation did not influence

Table 1: Mean percent of hatchability and hatchability failures of meat-type breeder eggs subjected to *in ovo* administration of L-Glutamine (GLN) during the late stage of incubation[1]

Main effects	Hatch of fertile eggs (%)[2]	Early embryo deaths (%)	Late embryo deaths (%)	Pipped with live embryos (%)	Pipped with dead embryos (%)
***In ovo* administration of GLN (T, mg/egg)[2]**					
Control (-)[3]	93.6±1.59	4.7±1.14	1.6±1.61	0.0±0.00	0.0±0.00
Control (+)[4]	92.2±1.61	6.2±0.83	0.0±0.00	0.0±0.00	1.5±1.00
5	89.3±3.28	5.9±1.13	3.6±1.88	1.2±1.00	0.0±0.00
7.5	88.5±1.29	7.3±0.93	2.2±0.93	0.0±0.00	1.9±0.87
10	88.8±2.36	7.7±1.34	2.7±1.35	0.0±0.00	0.8±0.80
Trial (R)[5]					
1 (26 weeks of age)	89.1±1.82	6.5±0.87	3.3±1.12	0.4±0.41	0.6±0.44
2 (32 weeks of age)	91.4±0.96	6.3±0.56	1.1±0.71	0.1±0.16	1.1±0.44
Source of variation					
T	NS	NS	NS	NS	NS
R	NS	NS	NS	NS	NS
T×R	NS	NS	NS	NS	NS

[1]Values are Mean±SE; [2]GLN was dissolved in distilled sterilized saline water (0.9% NaCl); [3]Non-injected control eggs; [4]Eggs were subjected to *in ovo* injection of sterilized distilled saline water (0.9% NaCl); [5]Eggs obtained from a Ross flock at the age of 26 and 32 weeks for trial 1 and 2, respectively; NS = Non Significantly different ($p>0.05$)

Table 2: Mean percent of hatched chicks during after 468, 492 and 504 h of incubation and hatching time of meat-type breeder eggs subjected to *in ovo* administration of L-Glutamine (GLN) during the late stage of incubation[1]

	Percentage of hatching chicks after hours of incubation			
Main effects	468	492	504	Hatching time (h)
***In ovo* administration of GLN (T, mg/egg)[2]**				
Control (-)[3]	41.5±7.06^{b}	55.7±5.69^{ab}	2.8±1.87	489.8±0.62
Control (+)[4]	38.9±3.97^{b}	58.7±4.39^{a}	1.8±0.80	487.7±2.24
5	42.3±4.04^{b}	56.7±4.13^{ab}	1.0±0.70	498.6±0.25
7.5	50.7±3.31^{ab}	46.9±3.72^{bc}	1.7±0.74	485.7±3.53
10	57.7^{a}±2.98^{ab}	41.4±2.72^{c}	1.0±0.66	488.7±0.23
Trial (R)[5]				
1 (26 weeks of age)	48.0±2.82	51.6±2.86	0.0±0.00	486.2±1.90
2 (32 weeks of age)	45.0±3.00	52.0±2.80	2.8**±0.72	489.9**±0.81
Source of variation				
T	*	*	NS	NS
R	NS	NS	**	NS
T×R	NS	NS	NS	NS

[1]Values are Mean±SE; [2]GLN was dissolved in distilled sterilized saline water (0.9% NaCl); [3]Non-injected control eggs; [4]Eggs were subjected to *in ovo* injection of sterilized distilled saline water (0.9% NaCl); [5]Eggs obtained from a Ross flock at the age of 26 and 32 weeks for trial 1 and 2, respectively; NS = Non Significantly different ($p>0.05$); *Significantly different ($p<0.05$); **Significantly different ($p<0.01$)

Table 3: Chick hatching weight express as an absolute and percentage values (chick hatching weight*100/egg weight) of meat-type breeder eggs subjected to *in ovo* administration of L-Glutamine (GLN) during the late stage of incubation[1]

Main effects	Egg weight (g)	Chick weight (g)	Chick weight (%)
***In ovo* administration of GLN (T, mg/egg)[2]**			
Control (-)[3]	60.42±0.37	40.98±0.32	67.78±0.30
Control (+)[4]	60.31±0.34	40.84±0.25	67.74±0.28
5	60.07±0.39	40.90±0.34	68.05±0.34
7.5	60.06±0.37	40.80±0.31	67.95±0.32
10	60.56±0.36	41.03±0.35	67.73±0.34
Trial (R)			
1 (26 weeks of age)	58.52±0.12	39.74±0.13	67.91±0.16
2 (32 weeks of age)	63.90±0.19**	43.30**±0.23	67.73±0.27
Source of variation			
T	NS	NS	NS
R	**	**	NS
T×R	NS	NS	NS

[1]Values are Mean±SE; [2]GLN was dissolved in distilled sterilized saline water (0.9% NaCl); [3]Non-injected control eggs; [4]Eggs were subjected to *in ovo* injection of sterilized distilled saline water (0.9% NaCl); [5]Eggs obtained from a Ross flock at the age of 26 and 32 weeks for trial 1 and 2, respectively; NS = Non Significantly different ($p>0.05$); *Significantly different ($p<0.05$); **Significantly different ($p<0.01$)

embryonic mortality, hatchability and chick weight at hatch but reduced hatching time. In the present study, *in ovo* feeding of up to 10 mg GLN/egg at 17th day of incubation did not influence hatchability, hatchability failures, hatching time of chicks and chick weight at hatch. However, Tavassoli *et al.* (2011) reported that *in ovo* administration of up to 10 mg GLN/egg at 16th day of incubation had no effect on hatchability but 5 mg GLN/egg increased chick weight at hatch. However, the researchers did not report the weight of eggs used or the relative weight of the hatching chick to egg weight in their study. Differences in results of chick weight at hatch and hatching time among these studies are more likely related to differences in the experimental procedures used in these studies. These results may suggest that these traits may be influenced by a number of factors including *in ovo* feeding of GLN. Generally, the idea of *in ovo* feeding of amino acids to embryos is to reduce the catabolism of albumin derived amino acids for gluconeogenesis, thus sparing them for muscle and tissue protein synthesis and consequently improving body weight at hatch (Uni *et al.*, 2005). *In ovo* administration of an amino acid mixture increased amino acids content of the embryo (Ohta *et al.*,

1999) and yolk and weight of the hatched chicks (Ohta *et al*., 2001) and consequently improved the utilization of amino acids by the embryos (Al-Murrani, 1982). Nevertheless, age, strain, egg size or flock broiler breeder conditions may influence the performance of *in ovo* administration of nutrients. Therefore, it can be suggested that the effect of *in ovo* GLN should be examined considering those factors.

The difference in egg weight between the two age groups (26 and 32 weeks of age, trial 1 and 2, respectively) was reflected on the absolute value of chick weight at hatch and hatching time. Egg size has been shown to correlate with chick weight at hatch and growth rate in poults and chicks (Skoglund *et al*., 1952; Wilson, 1991; Abiola, 1999; Applegate *et al*., 1999; Applegate and Lilburn, 1999; Lourens *et al*., 2006; Traldi *et al*., 2011). Lourens *et al*. (2006) concluded that the differences in body weight at hatch of chicks from small vs. large eggs is due to the surplus supply of nutrients in the large eggs and the size of the residual yolk sac at hatch. Consistent with this relationship, Speake *et al*. (1998) observed an increase in subcutaneous fat in large egg embryos compared to their smaller counterparts and suggested that these larger fat stores could provide an easily available energy source during the first 2 days of post hatch when feed intake is limited. The chick weight at hatch depends on the egg weight which is determined mainly by strain and age of laying hens. However, relative chick hatching weight to egg weight was not significantly different between the 2 trials. The positive correlation between egg weight and hatching time was reported by Smith and Bohren (1975) and Bohren (1978) who reported shorter incubation period associated with small eggs.

CONCLUSION

It is founded that *in ovo* of up to 10 mg GLN/egg at 17th day of incubation of layer-type breeder eggs did not influence hatchability and hatchability failures, chick weight at hatch, percentage of hatched chicks at 504 h of incubation and hatching time. Eggs produced at 32 weeks of age had a higher egg weight, chick weight at hatch and hatched later than those eggs produced at 26 weeks of age.

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