

Impacts of in Ovo-Juvenile Nutrition on Optimal Growth and Feed Efficiency in Poultry

P.K. Shukla¹, Raju Kushwaha²

Author Affiliation

¹Professor and Head, Department of Poultry Science

²Assistant Professor, Department of Animal Nutrition, College of Veterinary Science, DUVASU, Mathura-281001, Uttar Pradesh, India.

Reprint Request

Raju Kushwaha,
Assistant Professor,
Department of Animal Nutrition,
College of Veterinary Science,
DUVASU, Mathura-281001,
Uttar Pradesh, India.
E-mail: pkmathura@yahoo.co.in

Received on 16.11.2017
Accepted on 24.11.2017

Abstract

The perinatal chick makes the metabolic and physiological transition from nutrition supplied in the egg to feed, and it is functionally 'programmed' to adapt to its environment. Early feed access to chicks after hatching had significant impact on the growth and development, increases feed utilization efficiency. Alternative to early feed access *in ovo* feeding is developed, where essential nutrients are directly injected in to developing embryo. Some critical nutrients in the amnion may be limited because of parental nutrition constraints related to weight control and egg production rate. As the productivity of poultry increased, the demand of embryos for nutrients changed. However, the chemical composition of an egg has remained practically unchanged, and therefore, it began providing the embryos with suitable substances with the help of *in ovo* technology. The primary focus of the current review is to address effect of different nutrients over the hatchability, growth and development of chicks after *in ovo* feeding.

Keywords: *In Ovo*-Nutrition; Growth; Feed Efficiency; Hatchability.

Introduction

Commercial poultry production is among the most efficient and progressively successful of all food production sectors. What factors does it take for continued success in efficient poultry production? It takes the right genetics, combined with optimum health and management practices, and an optimized nutrition and feeding program. Efficiency and sustainability depends on the ability of a poultry production company to achieve competitive production indicators, including average daily gain, days to market weight, feed (caloric) conversion, livability, flock uniformity, and processing yields. After proper management of commercial genetic stock, nutrition and feed is the most variable

component of economic efficiency and profitability, as it represents 70 to 80% of live production costs. The period of embryonic and neonatal development is approaching 50% of the productive life of modern broilers and turkeys.

The perinatal period - the last four days before hatch through the first four days after hatch is most critical for development and survival of commercial broilers; and it is the period during which nutritional conditioning and perinatal programming can occur. The perinatal chick makes the metabolic and physiological transition from nutrition supplied in the egg to feed, and it is functionally 'programmed' to adapt to its environment. A chick's first meal occurs when it consumes the amnion fluid before internal pipping at about 18 days and 25 days of incubation

in broilers and turkeys, respectively. Some critical nutrients in the amnion may be limited because of parental nutrition constraints related to weight control and egg production rate. Incubation distress can also adversely affect perinatal development by altering nutrient partitioning during the plateau stage of oxygen consumption. When oxygen availability to the late term embryo is limited by low egg conductance or poor incubator ventilation, embryos and hatchlings may suffer low glycogen status and impaired enteric development.

Advantages

Supplementing the amnion with appropriate nutrients is a novel way to feed critical dietary components to embryos. Indeed this in-ovo feeding may 'jumpstart' development, improving the nutritional status of the perinatal chick or poul by in ovo feeding yields several advantages. These advantages include: improved digestive capacity; increased growth rate and feed efficiency; reduced post-hatch mortality and morbidity; improved immune response to enteric antigens; reduced incidence of developmental skeletal disorders; and increased muscle development and breast meat yield. The benefits of in-ovo feeding on early growth and development of poultry have been demonstrated by several experiments in the authors' laboratories. In-ovo feeding broilers and turkeys has increased hatching weights by one to seven per cent over controls, and this advantage has been observed to sustain until at least 35 days. The degree of response to in-ovo feeding may depend upon genetics, breeder hen age, egg size and incubation conditions. In-ovo feeding of chicken, turkeys and duck embryos consistently accelerated the digestive and nutrient uptake capacity of the digestive tract during the perinatal period.

Positive Effects

Positive effects have been observed with solutions containing salt, sucrose, maltose, and dextrin β -hydroxy- β -methyl butyrate, arginine, egg white protein, and zinc methionine. In ovo feeding has been observed to:

1. Improve glycogen and metabolic energy status
2. Increase hatchability
3. Advance morphometric development of the intestinal tract and mucin barrier enhance expression of genes for brush border enzymes (sucrase-isomaltase, leucine aminopeptidase) and their biological activity

4. Enhance expression of nutrient transporters, SGLT-1, PEPT-1 and NaK ATPase
5. Improve bone development and reduce leg asymmetry of hatchlings, and
6. Increase breast muscle at hatch.

Nutrients for in Ovo Administration

Carbohydrates: The natural resource of carbohydrates is not able to meet the metabolic needs of an embryo, especially during late embryogenesis. In the homeostatic regulation of the glucose level in the blood, an embryo is forced to generate energy using a variety of metabolic processes, such as gluconeogenesis, wherein the substrate is the glycerol or amino acid available from lipolysis and proteolysis (Klasing, 1998). However, at the time of hatching, embryos prefer glucose instead of fatty acids for energy production, since during internal hatching, the availability of oxygen is limited, and with the same amount of consumed oxygen, glucose oxidation provides more energy than the catabolism of lipids. The limited amount of glucose forces an embryo to start the lipolytic and proteolytic processes. Proteolysis is an unfavourable process, as it involves the degradation of proteins, which adversely affect embryo development (Pearce and Brown, 1971). The positive effects of carbohydrates administered *in ovo* on the development of enteritis of turkeys was also found by Bohórquez et al. (2007). Providing lactose hydrate (1.5 ml, 9% solution) to the amnion on the 22nd day of incubation resulted in the elongation of intestinal villi and an expansion of the surface of the intestine.

In studies by Bhanja et al. (2008), no effect of carbohydrates on body weight gain in one-day-old chicks and during breeding was found. However, *in ovo* injection of glucose (50 mg) on the 18th day of incubation affected the level of development of the digestive organs and the biochemical blood profile in day-old chicks, as well as in birds during breeding. On the first day of a chick's life, it was found that the experimental group had a higher level of glucose and protein in the plasma and a higher weight of the liver, glandular stomach (proventriculus) and gizzard (ventriculus), as well as small intestine. On the 10th day of life, the chicks treated with glucose had significantly lowered glucose and uric acid in the plasma and a higher weight of the spleen and small intestine.

Amino Acids: Supplementation of amino acids, such as arginine and glutamine, may be used for the synthesis of other amino acids. Experiments have shown that embryos given arginine and lysine on

the 18th day hatched better, and on the 42nd day, gained greater body weight compared with the control group; the administration of only arginine reduced the chicks' hatching time (Shafey et al., 2014). In embryonic development, all amino acids are essential; the absence of any of them causes protein synthesis impairment and a disturbance of the homeostasis of the embryo, which in turn results in impaired growth and development of the young growing organism. Kadam et al. (2009) demonstrated the positive impact of injecting threonine (Thr) into the yolk sac. This resulted in a weight gain for the chicks and a higher weight gain of 2.4% compared with the group, where the amino acids were injected into the egg's albumen. Coskun et al. (2014), who conducted experiments on broiler embryos, demonstrated the positive impact of DL-methionine supplementation, administered to the amniotic fluid. A higher chick weight to total egg weight was shown (72.7%) in comparison to the control group (70.0%). The move to feeding from the external environment and, therefore, faster adaptation of the gastrointestinal tract to an exogenous diet seems necessary for increased survival abilities and growth.

Vitamins: Vitamins counteract the negative effects of free radicals and thereby protect the embryo against damage (Surai, 2000). The positive effect of vitamins on the final body weight was found in the studies by Selim et al. (2012), which were conducted on duck eggs. Both the females and males were characterised by higher body weight at the end of breeding after the administration of vitamin E (10 mg) and vitamin C (3 mg) on the 12th day of hatching. The birds from the experimental group, compared to the control group, gained on average 335 g more body weight. However, these groups were characterised by a higher feed conversion ratio of 0.4 (FCR) on average. Nowaczewski et al. (2012), examining the effects of vitamin C on the hatching of chicken and duck eggs, found that a positive effect of the compound was observed only in the latter birds' eggs. In the case of chicken eggs, the injection of vitamin C did not significantly impact the improvement of hatching. Bhanja et al. (2007) administered vitamins A (100UI), E (0.5 UI) C (50 mg), B1 (100 ug) and B6 (100 g), an impact on hatchability rates was shown. From this group of vitamins, B6 contributed to improving the percentage of hatched eggs. In the group that received vitamin B6, hatchability was 81.5% compared to the control group, where it was only 72%.

Other Nutrients: Many other substances that have been used with the *in ovo* technique can be found in the available literature. Most studies have focused

on the insulin like growth factor (IGF-I). This interest in IGF-1 is associated with its broad spectrum of activity. The Liu (2011) studies, which were performed on duck eggs, confirmed the effect of IGF-1 on the proliferation of muscle fibres. In studies conducted by Keralapurath et al. (2010), experimental group, the use of L-carnitine prolonged the process of hatching, and the treatment decreased the hatching rate.

In Ovo Feeding Jump-Starts Perinatal Development

Poultry can be programmed to succeed with the desired phenotypic traits by modifying nutritional modification during the perinatal period: the first 3 days before and after hatch. The chick's first meal occurs when it imbibes the amnion prior to hatch, and so this is the first opportunity for nutritional programming. By *in ovo* feeding (Uni and Ferket, 2003), nutrient balance and key metabolic co-factors of the amnion meal can be modified and influence subsequent phenotypic traits of economic importance for the poultry industry.

In ovo feeding has increased hatchling weights by 3% to 7% ($P < .05$) over controls, and this advantage is often sustained at least until 14 days post-hatch. The degree of response to *in ovo* feeding may vary. Above all, IOF solution formulation has the most profound effect on the neonate. Positive effects have been observed with IOF solutions containing NaCl, sucrose, maltose, and dextrin (Uni et al., 2005), β -hydroxy- β -methyl butyrate, egg white protein, and carbohydrate (Foye et al., 2006b), Arginine (Foye et al., 2007), and zinc methionine (Tako et al., 2005). In addition to the increased body weights typically observed at hatch, the positive effects of *in ovo* feeding may include increased hatchability (Uni and Ferket, 2004); advanced morphometric development of the intestinal tract (Tako et al., 2004) and mucin barrier (Smirnov et al., 2006); enhanced expression of genes for brush boarder enzymes (sucrase-isomaltase, Leucine aminopeptidase) and their biological activities, along with enhanced expression of nutrient transporters, SGLT-1, PEPT-1, and NaK ATPase (Foye et al., 2007); increased liver glycogen status (Foye et al., 2006a); enhanced feed intake initiation behavior (de Oliveira, 2007); and increased breast muscle size at hatch (Foye et al., 2006a).

In ovo feeding clearly advances the digestive capacity, energy status, and development of critical tissues of the neonate by about 2 days at the time of hatch. Using scanning electron microscopy, Bohórquez et al. (2008) observed that *in ovo* feeding significantly increased functional maturity and

mucus secretion of goblet cells of villi of ileum and ceca of turkey poults. Associated with these goblet cells was the colonization of lactobacilli. Therefore, in ovo feeding may help improve the colonization resistance of enteric pathogens of neonatal chicks and poults.

In ovo feeding offers promise of sustaining the progress in production efficiency and welfare of commercial poultry. Although selection for fast growth rate and meat yield may favor the modern broiler to become a more altricial, proper early nutrition and in ovo feeding may help these birds adapt to a carbohydrate-based diet and metabolism typical of a precocial bird at hatch.

In Ovo Nutrition and Hatchability

Many studies have been conducted concerning the effect of in ovo feeding and its effect on hatchability and early growth performance. In an earlier study, when amino acids injected into the yolk sac at 7 days of incubation, hatchability was not affected, and body weight of chicks increased relative to egg weight prior to incubation (Ohta et al. 1999). Embryos which received the same treatment at 14 days of incubation through the yolk sac route had higher hatchability and improved post-hatch growth (29 to 79 g) at 28 day of age. Pre-hatch birds naturally consume the amniotic fluids towards hatch (Kadam et al., 2008). Therefore, addition of a nutrient solution to the embryonic amniotic fluid would deliver essential nutrients into the embryo's intestine (Romanoff, 1960). The best in ovo injection time may be 453 h of incubation (Salahi et al., 2011). It should be noted that embryonated eggs are transferred from setter to hatcher at 18 days of incubation. Practically, this would be an ideal time to administer vaccines and nutrients, in an in ovo feeding programme. In several experiments and recorded increased hatchling weights by 3 to 7 % over controls, and this advantage has been observed to be sustained at least until 35 days of age (Uni and Ferket, 2004). In addition to the increased body weights at hatch, better hatchability (Uni et al., 2005); advanced development of the intestinal mucosa (Uni and Ferket, 2004), and increased breast muscle size at hatch (Foye et al., 2005) have all been associated with in ovo feeding of different nutrients.

In ovo Nutrition and Post-Hatch Growth

In ovo feeding clearly enhances the digestive capacity and development of critical tissues of the neonate by about 2 days at the time of hatch. In ovo feeding of a dextrin solution containing 18%

maltodextrin and 10% potato starch dextrin with 75 µg/mL of iodinated casein and reported improvement in hatchability, hatch weight, early poult growth under commercial turkey production practices (Bottje et al., 2010). In ovo feeding of carbohydrates and HMB at 17.5d of incubation can improve the energy status of late-term broiler embryos and improve early growth, to enhance the genetic potential for late embryonic and early post-hatch growth (Uni et al., 2005). In ovo feeding of carbohydrates and β-Hydroxy-β-methylbutyrate led to higher body weight of all in ovo-fed hatchlings was greater than controls, and these differences were sustained until the end of the experiment (Tako et al., 2004). When L-carnitine administered into fertile chicken eggs at 17 or 18 d of incubation but this did not affect hatchability, yolk sac weight, or body weight (Zhai et al., 2008).

In Ovo Nutrition and Gut Development

The digestive tract of the hatchlings has limited ability to digest and utilize diets rich in proteins and carbohydrates (Uni and Ferket, 2004). The yolk and albumen provide insufficient carbohydrates to meet the metabolic demand of the embryo or chick. It was also reported that *in ovo* injection of 1 mL of saline containing carbohydrate at 18 days of incubation resulted in significant increase in jejunal villus height by over 45 % only after 48 hours of injection (Uni and Ferket, 2004). The relative weight of the liver, proventriculus, gizzard and small intestine was higher in glucose-injected embryos than un-injected control on the day of hatch. Tako et al., 2004, observed that the administration of nutrients into the amnion stimulated the intestinal development of hatching chicks, as evident from an increase in villus length and improvement in disaccharide digestion. These studies suggest that the presence of carbohydrate in the intestinal lumen of the chick embryo might be a trigger for the proliferation of goblet cells and their production of acidic mucin. *In ovo* injection of 0.5% conc. of all common 20 amino acids on the 14th day had comparatively larger digestive organs than un-injected control at 3 weeks of age (Bhanja et al., 2004).

In Ovo Nutrition and Embryo Metabolism

During the course of embryonic development, the embryo increases in size exponentially, but the rate of growth decreases as it approaches hatching; which may be due to the reduced rate of nutrient and energy assimilation from the yolk, changes in the gas exchange relative to the oxygen requirements of

the embryo and/or changes in the growth potential of the embryonic tissues. The last phase of embryo development is marked by dramatic physiological and metabolic changes. It is characterized by oral consumption of the amnion by the embryo, accumulation of glycogen reserves in muscle and liver tissues and glycogenolysis, initiation of pulmonary respiration, abdominal internalisation of remaining yolk, shell pipping and emergence. Anything that hinders or promotes growth and development during this period will have a marked effect on overall performance and health of poultry. The few days pre- and post-hatch are critical for the development of the broiler hatchlings. Yolk is the primary source of energy and phospholipids for cell membrane growth in the embryo and hatchling, whereas the albumen remaining in the amnion together with body protein serves as a source of energy for hatching and post hatch thermal regulation. Glycolysis rather than fatty acid oxidation is needed by hatchlings to provide energy because oxygen is limited during the transition from chorioallantois to pulmonary respiration (Hoiby et al., 1987). Extensive lipid metabolism also occurs in the liver, like exporting stored and *de novo* synthesized cholesterol, triacylglycerol and phospholipids packaged or repackaged as low-density lipoproteins (LDL), very low

In Ovo Nutrition and Immunity

Current research is being focused to link early growth with immunocompetence through *in ovo* nutrition. The first such report was by Gore and Qureshi, 1997, who observed that *in ovo* injection of vitamin E into the amnion of turkey embryos three days prior to hatch enhanced antibody and macrophage response to sheep red blood cells and improved post-hatch poult and broiler quality. Kidd, 2004, suggested the use of nutrients (amino acids, fat or water-soluble vitamins, and trace metals) at amounts in excess of requirements to confer immune benefits to poultry. Supplying natural nutrients by *in ovo* feeding sustains and accelerates enteric development, providing the bird with an ideal starting point towards improved immune response to enteric antigens. Recently, Bhanja *et al.* 2010, reported higher expression of genes associated with humoral immunity, IL-6 and TNF- α in chicks injected with lysine, threonine or methionine and cystine. Bhattacharyya *et al.* 2007, found that *in ovo* injection of 10% glucose on the 21st day of incubation into the developing embryo may enhance the post-hatch poult growth and also elicit better humoral immune response. better understanding of key

immunomodulatory nutrient needs will enable nutritionists to formulate *in ovo* diets to improve immunity and overall health in birds.

Conclusion

Nutritional science is no longer a matter of supplying minimally required nutrients in the ideal balance to achieve desired production and welfare goals. We now know that nutrition is a process that can be programmed to succeed by strategic perinatal diet manipulation by *in ovo* and post-hatch feeding. Early Feeding' is a potent managerial intervention that can improve growth, performance as well as immune competence of the birds. *In ovo* approaches need to be standardized for ensuring earliest of the earliest feeding to harness production potential of the poultry to its utmost. Rapid growth rate, feed conversion efficiency, reaching marketable bodyweight can be enhanced through the *in ovo* feeding. *In-ovo* feeding clearly advances the digestive capacity, energy status, and development of critical tissues of the perinatal chick or poultry about two to three days. Thus, *in ovo* feeding technology has established a new science of perinatal nutrition that will open opportunities for greater production efficiency and animal welfare; it may be a necessary means to optimize poultry production.

References

1. Bhanja S.K., Mandal A.B., Agarwal S.K., Majumdar S., Bhattacharyya A. Effect of *in ovo* injection of vitamins on the chick weight and post-hatch growth performance in broiler chickens. Proc. XVI European Symposium on Poultry Nutrition, 26-30.08.2007, Strasbourg, France. 2007.
2. Bhanja S. K., Sudhagar M, Pandey N, Goel A, Mehra M, Majumdar S and Agarwal SK, Modulation of immunity genes through *in ovo* supplemented amino acids in broiler chickens. In *Proceedings XIII th European Poultry Conference* 2010;23-27,161-123.
3. Bhanja S.K., Mandal A.B., Agarwal S.K., Majumdar S. Effect of *in ovo* glucose injection on the post hatch-growth, digestive organ development and blood biochemical profiles in broiler chickens. *Indian J. Anim. Sci.*, 2008;78:869-872.
4. Bhanja SK, Mandal AB and Johri TS, Standardization of injection site, needle length, embryonic age and concentration of amino acids for *in ovo* injection in broiler breeder eggs. *Indian Journal of Poultry Science* 2004;39:105-111.

5. Bhattacharya D, Boppana V, Roy R and Roy J, Method for automated design of integrated circuits with targeted quality objectives using dynamically generated building blocks, Ed. Google Patents (2007).
6. Bohorquez D., Santos A. Jr, Ferket P. *In ovo*-fed lactose augments small intestinal surface and body weight of 3 day-old turkey poults. *Poultry Sci.*, 2007;86(S1):214-215.
7. Bohorquez, D. V., Santos, Jr., A. A., and Ferket, P. R. In ovo feeding and dietary β -hydroxy- β -methylbutyrate effects on poultry quality, growth performance and ileum microanatomy of turkey poults from 1 to 11 days of age. *Poultry Sci.* 2008;87(Supplement 1):139.
8. Bottje W, Wolfenden A, Ding L, Wolfenden R, Morgan M, Pumford N, Lassiter K, Duncan G, Smith T, Slagle T and Hargis B, Improved hatchability and posthatch performance in turkey poults receiving a dextrin-iodinated casein solution in ovo. *Poult Sci* 2010;89:2646-2650.
9. Copkun Y., Erener G., Pahin A., Karadavut U., Altop A., Okur A.A. Impacts of *in ovo* feeding of DL-methionine on hatchability and chick weight. *Turkish JAF Sci. Tech.*, 2014;2:47-50.
10. De Oliveira, J.E., P.R. Ferket, C.M. Ashwell, Z. Uni, and C. Heggen-Peay. Changes in the late term turkey embryo metabolism due to *in ovo* feeding. *Poultry Sci.* 2007;86(Supplement 1):214.
11. Foye O, Ferket P and Uni Z, The effects of *in ovo* feeding of arginine and/or beta-hydroxy-beta-methylbutyrate (HMB) on glycogen metabolism and growth in turkey poults. *Poult Sci* 84:9 (2005).
12. Foye, O.T., Uni, Z., and Ferket, P.R. Effect of *in ovo* feeding egg white protein, beta-hydroxy beta-methylbutyrate, and carbohydrates on glycogen status and neonatal growth of turkeys. *Poult. Sci.* 2006a;85:1185-1192.
13. Foye, O.T., Uni, Z., and Ferket, P.R. The effects of *in ovo* feeding arginine, β -hydroxy- β -methyl butyrate, and protein on jejunal digestive and absorptive activity in embryonic and neonatal turkey poults. *Poult. Sci.* 2007;86:2343-2349.
14. Foye, O.T., Uni, Z., McMurtry, J.P., and Ferket, P.R. The effects of amniotic nutrient administration, "in ovo feeding" of arginine and/or beta-hydroxy-beta-methyl butyrate (HMB) on insulin-like growth factors, energy metabolism and growth in turkey poults. *Int. J. Poult. Sci.* 2006b;5(4): 309-317.
15. Gore A and Qureshi M, Enhancement of humoral and cellular immunity by vitamin E after embryonic exposure. *Poult Sci* 1997;76:984-991.
16. Høiby M, Aulie A and Bjønnes PO, Anaerobic metabolism in fowl embryos during normal incubation. *Comparative Biochemistry and Physiology Part A: Physiology* 1987;86:91-94.
17. Kadam M.M., Bhanja S.K., Mandal A.B., Tyagi P.K., Patil A.R. Influence of *in ovo* threonine injection site on early post-hatch growth and digestive organ development of broiler chicken. *J. Anim. Physiol. Anim. Nutr. (Berl.)*, 2009;44:203-205.
18. Keralapurath M., Keirs R.W., Corzo A., Bennett L.W., Pulikanti R., Peebles E.D. Effects of *in ovo* injection of L-carnitine on subsequent broiler chick tissue nutrient profiles. *Poultry Sci.*, 2010;89:335-341.
19. Kadam DMM, Bhanja S, Mandal A, Thakur R, Vasan P, Bhattacharyya A and Tyagi J, Effect of *in ovo* threonine supplementation on early growth, immunological responses and digestive enzyme activities in broiler chickens. *Br Poult Sci* 2008;49:736-741.
20. Kidd MT, Nutritional modulation of immune function in broilers. *Poult Sci* 2004;83:650-657..
21. Klasing K.C. Carbohydrates in comparative avian nutrition. New York, USA, CAB Int., 1998.p.201-209.
22. Liu H.H., Wang J.W., Chen X., Zhang R.P., Yu H.Y., Jin H.B., Li L., Han C.C. *In ovo* administration of rhIGF-1 to duck eggs affects the expression of myogenic transcription factors and muscle mass during late embryo development. *J. Appl. Physiol.*, 2011;111:1789-1797.
23. Nowaczewski S., Kontecka H., Krystianiak S. Effect of *in ovo* injection of vitamin C during incubation on hatchability of chickens and ducks. *Folia Biol. (Krakow)*, 2012;60:93-97.
24. Ohta Y, Tsushima N, Koide K, Kidd M and Ishibashi T, Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. *Poult Sci* 1999;78:1493-1498.
25. Pearce J., Brown W.O. Carbohydrate metabolism in physiology and biochemistry of the domestic fowl, D.J. Bell and B.M. Freeman (eds.). Academic Press, London, UK, 1971;1:295-319.
26. Romanoff AL, The avian embryo. Structural and functional development. *The avian embryo Structural and functional development* (1960).
27. Selim S.A., Gaafar K.M., El-Ballal S.S. Influence of *in ovo* administration with vitamin E and ascorbic acid on the performance of Muscovy ducks. *Emir. J. Food Agric.*, 2012;24:264-271.
28. Shafey T.M., Mahmoud A.H., Alsobayel A.A., Abouheif M.A. Effects of *in ovo* administration of amino acids on hatchability and performance of meat chickens. *S. Afr. J. Anim. Sci.*, 2014;44:123-130.
29. Surai P.F. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. *Brit. Poultry Sci.*, 2000;41:235-243.
30. Salahi A, Mozhdeh MK and Seyed NM, Optimum time of *in ovo* injection in eggs of young broiler breeder flock, in *18th European Symposium on Poultry Nutrition*, Ed, Cesme, Izmir, Turkey (2011).
31. Smirnov, A., Tako, E., Ferket, P. R., and Uni, Z. Mucin gene expression and mucin content in the chicken

- intestinal goblet cells are affected by in ovo feeding of carbohydrates. *Poult. Sci.* 2006;85:669-673.
32. Tako, E., Ferket, P. R., and Uni, Z. Effects of in ovo feeding of carbohydrates and beta-hydroxy beta-methylbutyrate on the development of chicken intestine. *Poult. Sci.* 2004;83:2023-2028.
 33. Tako, E., Ferket, P. R., and Uni, Z. Changes in chicken intestinal zinc exporter mRNA expression and small intestine functionality following intraamniotic zinc-methionine administration, *J. Nutr. Biochem.* 2005;15:339-346.
 34. Uni, Z., and Ferket, P.R. Enhancement of development of oviparous species by in ovo feeding. United States Patent No. 6, 2003.p.592,878.
 35. Uni, Z., and Ferket, P.R. Methods for early nutrition and their potential. *World's Poultry Science Journal* 2004;60:101-111.
 36. Uni, Z., Ferket, P. R., Tako, E., and Kedar, O. (In ovo feeding improves energy status of late-term chicken embryos. *Poult. Sci.* 2005;84(5):764-770.
 37. Zhai W, Neuman S, Latour MA and Hester PY, The effect of in ovo injection of L-carnitine on hatchability of white leghorns. *Poult Sci* 2008;87:569-572.
-