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Research Article

Impact of *in ovo* Injection of Certain Vitamins to Improve the Physiological Conditions of Hatching Chicks

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Abstract

Background and Objective: Exposure of breeder hens to hyperthermia causes disturbances in the breeder's eggs due to insufficient nutrient deposition from heat-stressed hen into the egg. Therefore, this experiment was conducted to test the hypothesis that the adverse effects of heat stress on hatchability, embryonic growth and hatchling health status could be ameliorated by *in ovo* injection of certain vitamins. **Materials and Methods:** A total of 270 fertile eggs from Fayoumi breeders (45 weeks) were randomly assigned to 6 groups (45 eggs/group). During the egg collection period in the summer season, birds were maintained at 9-13°C above the standard thermo-neutral temperature. The experimental groups were: negative control (eggs not injected either with vitamins or Sterile Deionized Water (SDW)), positive control (eggs injected with 0.1 mL of SDW/egg) and four other treatments in which vitamins A, E, D_3 and folic acid were injected (1 mg of each vitamin dissolved in 0.1 mL of SDW/egg). The eggs were incubated at 37.7°C and 65% relative humidity in an automatic incubator. **Results:** The residual yolk, embryo length and weight and hatchability% were improved ($p<0.01$) in all vitamins *in ovo* injected groups compared with both controls. Hatchling weight and the assessed health status indices were increased ($p<0.01$) in all vitamins *in ovo* injected groups compared with both controls. **Conclusion:** It is concluded that direct injection of vitamins into eggs laid by heat-stressed breeders is an effective way of reducing the disturbance in eggs resulting from inadequate nutrient deposition from hen to egg.

Key words: Breeders, hatchability, heat stress, *in ovo*, vitamins, flock stock, embryonic development

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Recently, poultry production has grown dramatically with most of this growth as a result of demand intensification[1]. Normally developed and grown avian embryos are considered as a worthy possession for the poultry industry as they are the future flock stock. For normal embryonic development and growth, all needed nutrients should be available in the egg. Nutrient supply begins with the utilization of maternal diet, further, it is entirely depending on the efficiency of these nutrients being deposited into the egg[2]. Indeed, extreme challenges threaten the poultry production in terms of adverse effects on growth performance and general health which in turn affects the eggs' fertility and hatchability[3]. One challenge, particularly, Heat Stress (HS), is one of the most significant environmental stressors that stresses breeder hens, reflecting low hatchability and aberrant embryonic development[4]. Moreover, under normal environmental conditions, earlier literature reported that the newly hatched chicks are often over-stressed because of the rapid growth of the immune system at the critically early stage of their lives[5]. Hence, from a nutritional point of view, the nutritional status of embryos is very crucial to maintain their healthy development, growth and immunocompetence. Thus, "*in ovo* feeding" as one of the egg manipulation technologies is entirely a valuable approach used to assure an effectively available supply of nutrients to sustain and enhance enteric embryonic growth and general health[6]. Earlier research on *in ovo* injection with different nutrients such as minerals, vitamins, amino acids and fatty acids was conducted with findings that suggested an improvement in embryonic development, post-hatch growth and immune response[7,8]. Nevertheless, in the case of hens exposed to HS due to the metabolic peroxidation to yolk lipids, the accumulated fat-soluble vitamins in the egg yolk were found to be of lower concentrations[9]. likewise, high levels of malondialdehyde as a lipid-peroxidation indicator, were detected in eggs from heat-stressed hens, which in turn results in eggs of low nutritional value[10]. So far, the supply of embryos from heat stressed breeders for the nutrients has to be precisely controlled[11]. Chicks from Egypt's local strains are weak relative to other strains, besides, Egypt is known for its almost hot-arid climate throughout the year, which causes breeders to suffer from maternal hyperthermia, which in effect increases nutritional insufficiency from the eggs into embryos[12]. This experiment was therefore performed to test the hypothesis that vitamin A, E, D_3 and folic acid *in ovo* administration could improve the hatchability and health status of newly hatched embryos from eggs laid by local Fayoumi stain chickens raised at high ambient temperatures.

MATERIALS AND METHODS

Study area: The current experiment was carried out at the Research Poultry Farm, National Research Center, Egypt from July-August, 2019. The animal protocol was approved by the Institutional Animal Care and Use Committee of the National Research Center, Egypt.

Experimental design and treatments: A total of 270 freshly laid eggs by a flock of 45-weeks-old Fayoumi breeders were used in this experiment at the summer season. The breeders were reared at an average minimum and maximum temperature ranged from 86.62-94.80°F (in comparison with the standard 70°F at the age of 45-weeks-old).

The eggs were individually weighed and divided into 6 groups (45 eggs/group) in a completely randomized design. The experimental treatments were: negative control (eggs not injected, either with the vitamins or sterile deionized water), positive control (eggs were injected with 0.1 mL sterile deionized water/egg) and four other treatments in which vitamin A, vitamin E, vitamin D_3 and folic acid were injected (1 mg of each vitamin dissolved in 0.1 mL sterile deionized water/egg). Retinol $\geq$95%, α-Tocopherol $\geq$95.5%, Cholecalciferol $\geq$98% and Folic acid $\geq$97% were obtained from Sigma (Sigma-Aldrich, Inc., St. Louis, MO, USA). The eggs were set for incubation in an automatic incubator with a temperature 37.7°C and 65% Relative Humidity (RH). The *in ovo* injection was done by using 18-G sterile needle in a laminar flow system before the incubation of the eggs[13].

Embryo and hatchling measurements: On the 12th day of incubation, fertility was verified through candling the eggs with a hand ultraviolet lamp. On the 17th day of incubation, a total of 10 eggs per treatment were selected randomly for embryo weight, embryo length[14] and residual yolk determination[15].

For chick's identification at the time of hatch, on the morning of the 19th day of incubation, eggs were transferred into hatching sections in the incubator which were adjusted at 37.2°C and 70% RH till the end of the incubation period (embryonic day 21.5). Chicks that were still quite wet down, revealing they had just hatched, were selected randomly for the measurements. This was obtained at 3 points during the hatching period: early, (465 hrs), mid-term, (480 hrs) and late, (493 hrs) period of incubation. The hatched chicks/hatching time/treatment were collected and named a hatch group. A total of 10 chicks/hatch group were selected randomly for hatchling body weight determination just after the down

dried, then, the hatchability was determined as "the number of healthy chicks hatched as a percentage of injected eggs with living embryos"[16].

Blood collection and hematological analysis: Total 10 chicks per hatch group were decapitated and blood was collected into 3 mL vacutainer tubes (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ) for hematological and biochemical analysis. About 10 µL of heparin was added to the tubes before the blood collection to prevent blood from coagulation. For hematological analysis, few drops of blood were taken for hemoglobin (Hb) and Packed Cell Volume (PCV) determination according to the methods of Tietz[17]. The remaining of the blood was then centrifuged for 13 min at 3000 xg. The plasma samples were collected into Eppendorf tubes and stored at -20°C until further biochemical analysis.

Plasma Health Indicators: Total Protein (TP) and albumin were determined in plasma according to Weichselbaum[18] and Bartholomew and Delaney[19], respectively. Globulin was calculated by the difference between TP and albumin. Plasma triiodothyronine (T_3) and thyroxin (T_4) concentrations were determined using the available commercial kits (Byk-Sangtec Diagnostica, Dietzenbach-Germany, Immulite 2000, DPC, LA)[20]. The immune-reactive insulin-like growth factor1 (IGF1) was determined using the commercially available kits (Elabscience, Wuhan, Hubei, China)[21].

Statistical analysis: The analysis of variance on all the data was conducted using the SAS General Linear Model (GLM) procedure (SAS Institute)[22]. Individual embryo or hatchling was considered the experimental unit. Tukey's honestly significant difference test was used to test the significant differences among the mean values. For all analysis, the pooled Standard Error of the Mean (SEM) was listed. The significant level for differences was set as $p<0.05$.

RESULTS

Hatchability, embryo and hatchling characteristics: As shown in Table 1, all vitamins *in ovo* injected groups improved the embryo and hatchling characteristics. Compared with both control groups, all *in ovo* injected treatments with vitamin A, E, D_3 and folic acid at a level of 1 mg/egg led to an increase ($p<0.01$) in the embryo length, residual yolk, embryo weight, percent of hatchability and hatchling weight. The highest ($p<0.01$) significant increase in hatchability percentage was observed in folic acid *in ovo*-injected treatment (84.4 compared with 65.4 and 66.6 in negative and positive controls, respectively).

Hematological and plasma health indices: The effects of different vitamins *in ovo* injection on the assessed hematological and plasma health indicators are presented in Table 2 and 3. An increase ($p<0.01$) in blood Hb and PCV was observed in all *in ovo* injected treatments with vitamin A, E, D_3 and folic acid, compared with both control groups (Table 2). The highest ($p<0.01$) significant increase in Hb was observed in folic acid *in ovo*-injected treatment (9.9 compared with 8.6 and 8.7 in negative and positive controls, respectively).

On the other hand, plasma TP, albumin, globulin, thyroid hormones and IGF1 were assessed as indicators of the hatchling health status. The results showed that all *in ovo* injected treatments with vitamin A, E, D_3 and folic acid increased ($p<0.01$) the plasma TP, globulin, T_3, T_4 and IGF1, in comparison with both controls (Table 3). T_4 level showed

Table 1: Effects of *in ovo* injection of vitamin A, E, D_3 and folic acid on embryo measurements

	Treatments							
Items	Control (-)	Control (+)	Vitamin A	Vitamin E	Vitamin D_3	Folic acid	SEM	p-value
Embryo length (cm)	8.9^{c}	9.0^{c}	12.4^{b}	12.7^{b}	13.1^{ab}	13.5^{a}	0.25	<0.0001
Residual yolk (% egg weight)	25.2^{b}	26.4^{b}	32.4^{a}	33.2^{a}	34.2^{a}	34.9^{a}	0.84	<0.0001
Embryo weight (% egg weight)	26.0^{c}	27.0^{c}	38.2^{b}	39.8^{ab}	40.7^{a}	41.1^{a}	0.72	<0.0001
Hatchability (%)	65.4^{d}	66.6^{d}	75.6^{c}	80.1^{b}	82.2^{b}	84.4^{a}	0.76	<0.0001
Hatchling weight (g)	22.3^{d}	22.7^{d}	25.5^{c}	27.0^{b}	28.8^{a}	29.1^{a}	0.34	<0.0001

$^{a-c}$Means with different superscripts within the same row differ significantly ($p<0.05$), [1]SEM: Standard error of the means

Table 2: Effects of *in ovo* injection of vitamin A, E, D_3 and folic acid on hematological measurements

	Treatments							
Items	Control (-)	Control (+)	Vitamin A	Vitamin E	Vitamin D_3	Folic acid	SEM	p-value
Hb (g dL^{-1})	8.6^{d}	8.7^{d}	9.2^{c}	9.4^{bc}	9.7^{ab}	9.9^{a}	0.16	<0.0001
PCV (%)	0.330^{c}	0.331^{b}	0.340^{ab}	0.341^{ab}	0.342^{a}	0.345^{a}	0.003	0.0052

$^{a-d}$Means within a row with different superscripts are significantly different ($p<0.05$). SEM: Standard error of the means, Hb: Hemoglobin, PCV: Packed cell volume

Table 3: Effects of *in ovo* injection of vitamin A, E, D_3 and folic acid on plasma measurements

	Treatments							
Items	Control (-)	Control (+)	Vitamin A	Vitamin E	Vitamin D_3	Folic Acid	SEM	p-value
Total protein (g dL^{-1})	4.20^{c}	4.34^{c}	5.22^{b}	5.46^{ab}	5.78^{ab}	5.97^{a}	0.23	<0.0001
Albumin (g dL^{-1})	1.79^{c}	1.87^{b}	2.09^{bc}	2.31^{ab}	2.41^{ab}	2.49^{a}	0.13	0.0005
Globulin (g dL^{-1})	2.41^{b}	2.48^{b}	3.13^{a}	3.15^{a}	3.37^{a}	3.49^{a}	0.18	<0.0001
T_3 (ng dL^{-1})	2.38^{c}	2.44^{c}	2.96^{b}	3.01^{b}	3.30^{a}	3.36^{a}	0.05	<0.0001
T_4 (ng dL^{-1})	12.16^{d}	12.26^{d}	13.69^{c}	13.76^{c}	14.53^{b}	15.03^{a}	0.16	<0.0001
IGF-I (ng mL^{-1})	11.48^{c}	11.67^{c}	15.42^{b}	15.90^{b}	17.61^{a}	17.74^{a}	0.34	<0.0001

$^{a-c}$Means with different superscripts within the same row differ significantly ($p<0.05$). SEM: Standard error of the means, T_3: Triiodothyronine, T_4: Thyroxin, IGF1: Insulin-like growth factor1

highest ($p<0.01$) significant increase in folic acid *in ovo*-injected treatment compared with all other experimental groups.

DISCUSSION

In the current study, our investigations demonstrated that *in ovo* injections of vitamins A, E, D_3 and folic acid significantly improved hatchability percentage, embryo and hatchling characteristics and health status indices of hatchlings that originated from eggs laid by heat-stressed hens. It is generally agreed that eggs from heat-stressed breeders are of low nutritional value, in particular, they are found to have a lower concentration of fat-soluble vitamins due to the metabolic peroxidation of yolk lipids[10]. Such deleterious changes will adversely affect the embryonic development and hatchability even if the following egg incubation period is normal. Previous research reported beneficial effects on hatchability traits, production performance and immune status as a result of *in ovo* injections of various nutrients such as vitamins, fatty acids, amino acids, trace minerals[23,24], however, eggs used in earlier research were laid under normal environmental conditions. To our best knowledge, our study was the first attempt to test the hypothesis that embryonic growth and hatchling health status from eggs laid by heat-stressed hens could be enhanced by *in ovo* injection with specific vitamins. The first step towards explained our positive findings in vitamins injected treatments over the control ones may be the failure of stressed breeders to deposit all the components essential for normal embryonic development and growth in their eggs due to the disturbance in body metabolism and temperature regulation under heat stress conditions[11], thus, our injected vitamins aid in correcting the disturbance in the laid eggs, which is reflected in the normal development and hatching of viable chicks. Vitamin A, E, D_3 and folic acid are well-known as essential nutrients for normal hatchability and hatchling quality[2], even in certain limits, hatchability improves in proportion to the amount of these vitamins' supplementation to maternal diet[25]. Along with the established notion of the disturbed body metabolism of the stressed dam that could adversely affect their nutrients utilization and the deposition of these nutrients into eggs, it would be better to *in ovo* supplement the embryos by these essential vitamins to ensure normal embryonic development and growth, which was truly attained in our study.

In addition, our findings showed an improvement in the measured biochemical and hematological parameters of the vitamins-*in ovo* injected treatments. The previous findings[26] about response to folic acid *in ovo* injection agree with ours regarding the plasma T_3, T_4 and IGF1 levels. As far as we know, no previous data were recorded regarding impacts of *in ovo* injections of vitamin A, E and D_3 on T_3, T_4, IGF1, TP, albumin and globulin blood levels. IGF1 is well-known for its ability to boost growth through its crucial role in regulating cell differentiation and proliferation[27]. Besides, T_3 and T_4 are recognized for their role in controlling metabolic processes that are critical for normal development and growth, also, the thyroid hormone profile found to correlate with body weight and general health status[28]. Accordingly, our enhanced embryo and hatchling characteristics may be owed to the improvement in these assayed hormones as a health status indicator in response to vitamins *in ovo* injection. Likewise, our results regarding the increase in TP, albumin and globulin levels in the blood of hatchling from vitamins-*in ovo* injected treatments agree with El-Azeem *et al.*[26], who reported improvement in these parameters in response to folic acid *in ovo* injection. As mentioned earlier, there is a lack of previous data on hematological and biochemical blood parameters for *in ovo* injected vitamins A, E and D_3. However, these fat-soluble vitamins are well-known by wide variety of physiological functions in the animal body, ranging from the critical vitamin A to embryonic stem cell development and differentiation[29], the regulation of the metabolism of macronutrients[30], into the essential vitamin E for normal erythrocytes morphology[31]. So far, increasing of such measured parameters in the current study may provide

indications of the role of the used fat-soluble vitamins in regulating different physiological functions in the body, besides, the specific function of folic acid as a methyl donor in one-carbon metabolism that plays a vital role in regulating protein metabolism[32].

CONCLUSION

Based on the obtained findings, supplementing the embryo by different vitamins via *in ovo* injection improved the hatchability percentage, embryo and hatchling characteristics and hatchling blood parameters. Thus, direct injection of vitamin A, E, D_3 and folic acid into eggs laid by heat-stressed Fayoumi breeders at a level of 1 mg/egg is an effective method to mitigate the disturbance in the eggs caused by inadequate nutrient deposition from the dam to the egg. These findings could have implications for *in ovo* injection to boost poultry production. Further still, different doses of the injected vitamins need to be tested

SIGNIFICANCE STATEMENT

This study revealed that *in ovo* injection of vitamins A, E, D_3 and folic acid into eggs laid by heat-stressed breeders may be considered as an effective method for early nutritional manipulation which provides the potential for increased poultry production efficiency. These findings will aid researchers to uncover the crucial research area for improving hatchability percentage, embryo and hatchling characteristics and health status and suggest a possible notion of the ameliorative effect of vitamins *in ovo* injection for egg disturbance resulting from inadequate deposition of nutrients from the heat stressed-hen into the egg.

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REFERENCES

1. Adesogan, A.T., A.H. Havelaar, S.L. McKune, M. Eilittä and G.E. Dahl, 2020. Animal source foods: Sustainability problem or malnutrition and sustainability solution? Perspective matters. Glob. Food Secur., Vol. 5. 10.1016/j.gfs.2019.100325.
2. Vieira, S.L., 2007. Chicken embryo utilization of egg micronutrients. Revista Brasileira Ciencia Avicola, 9: 1-8.
3. Lara, L.J. and M.H. Rostagno, 2013. Impact of heat stress on poultry production. Animal, 3: 356-369.
4. Zhu, Y., L. Lu, X. Liao, W. Li and L. Zhang, 2017. Maternal dietary manganese protects chick embryos against maternal heat stress via epigenetic-activated antioxidant and anti-apoptotic abilities. Oncotarget, 8: 89665-89680.
5. Bakyaraj, S., S.K. Bhanja, S. Majumdar and B. Dash, 2012. Modulation of post-hatch growth and immunity through *in ovo* supplemented nutrients in broiler chickens. J. Sci. Food Agric., 92: 313-320.
6. Siwek, M., A. Slawinska, K. Stadnicka, J. Bogucka, A. Dunislawska and M. Bednarczyk, 2018. Prebiotics and synbiotics–*in ovo* delivery for improved lifespan condition in chicken. BMC Vet. Res., Vol. 14. 10.1186/s12917-018-1738-z.
7. Bakyaraj, S., S.K. Bhanja, S. Majumdar and B. Dash, 2012. Modulation of post-hatch growth and immunity through *in ovo* supplemented nutrients in broiler chickens. J. Sci. Food Agric., 92: 313-320.
8. Shafey, T.M., H.A. Al-Batshan, A.N. Al-Owaimer and K.A. Al-Samawei, 2010. Effects of *In ovo* administration of L-carnitine on hatchability performance, glycogen status and insulin-like growth factor-1 of broiler chickens. Br. Poult. Sci., 51: 122-131.
9. Lin, Y.F., H.L. Tsai, Y.C. Lee and S.J. Chang, 2005. Maternal vitamin E supplementation affects the antioxidant capability and oxidative status of hatching chicks. J. Nutr., 135: 2457-2461.
10. Fouad, A.M., W. Chen, D. Ruan, S. Wang, W.G. Xia and C.T. Zheng, 2016. Impact of heat stress on meat, egg quality, immunity and fertility in poultry and nutritional factors that overcome these effects: A review. Int. J. Poult. Sci., 15: 81-95.
11. Kirk, S., G.C. Emmans, R. McDonald and D. Arnot, 1980. Factors affecting the hatchabili of eggs from broiler breeders. Br. Poult. Sci., 21: 37-53.
12. Kottek, M., J. Grieser, C. Beck, B. Rudolf and F. Rubel, 2006. World map of the Koppen-geiger climate classification updated. Meteorol. Z., 15: 259-263.
13. Ohta, Y. and M.T. Kidd, 2001. Optimum site for *in ovo* amino acid injection in broiler breeder eggs. Poult. Sci., 80: 1425-1429.
14. Willemsen, H., N. Everaert, A. Witters, L. De Smit and M. Debonne *et al*., 2008. Critical assessment of chick quality measurements as an indicator of posthatch performance. Poult. Sci., 87: 2358-2366.
15. Tucker, J.K., N.I. Filoramo, G.L. Paukstis and F.J. Janzen, 1998. Residual yolk in captive and wild-caught hatchlings of the red-eared slider turtle (Trachemys scripta elegans). Copeia, 1998: 488-492.

16. Sgavioli, S., C. Domingues, E. Santos, T. de Quadros and L. Borges, 2016. Effect of *in-ovo* ascorbic acid injection on the bone development of broiler chickens submitted to heat stress during incubation and rearing. Braz. J. Poult. Sci., 18: 153-162.
17. Turkson, P.K. and E.Y. Ganyo, 2015. Relationship between haemoglobin concentration and packed cell volume in cattle blood samples. Onderstepoort J. Vet. Res., Vol. 82. 10.4102/ojvr.v82i1.863.
18. Weichselbaum, T.E. and C.A.U.S. Sn, 1946. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am. J. Clin. Pathol., 16: 40-49.
19. Suzuki, Y., 2007. Protein error of pH indicators in the presence of detergents. Anal. Sci., 23: 733-738.
20. Sánchez-Carbayo, M., M. Mauri, R. Alfayate, C. Miralles and F. Soria, 1999. Analytical and clinical evaluation of TSH and thyroid hormones by electro chemiluminescent immunoassays. Clin. Biochem., 32: 395-403.
21. Houston, B. and I.E. O'Neill, 1991. Insulin and growth hormone act synergistically to stimulate insulin-like growth factor-I production by cultured chicken hepatocytes. J. Endocrinol., 128: 389-393.
22. Bosiers, M., G. Torsello, H.M. Gißler, J. Ruef and S. Müller-Hülsbeck *et al*., 2009. Nitinol stent implantation in long superficial femoral artery lesions: 12-month results of the Durability I study. J. Endovasc. Ther., 16: 261-269.
23. Bhattacharyya, A., S. Majumdar, S.K. Bhanja, A.B. Mandal and M.M. Kadam, 2017. Effect of maternal dietary manipulation and *in ovo* injection of nutrients on the hatchability indices, post-hatch growth, feed consumption, feed conversion ratio and immunocompetence traits of turkey poults. Indian J. Anim. Sci., 46: 287-294.
24. Gonzales, E., C.P.d. Cruz, N.S.M. Leandro, J.H. Stringhini and A.B.d. Brito, 2013. In ovo supplementation of 25 (OH) D3 to broiler embryos. Braz. J. Poult. Sci., 15: 199-202.
25. Subramaniyan, S.A., D. Kang, S.H. Siddiqui, J. Park, W. Tian, B. Park and K. Shim, 2020. Effects of *In Ovo* supplementation with nanonutrition (L-Arginine conjugated with Ag NPs) on muscle growth, immune response and heat shock proteins at different chicken embryonic development stages. Animals, Vol. 10. 10.3390/ani10040564.
26. Abd El-Azeem, N.A., M.S. Abdo, M. Madkour and I. El-Wardany, 2014. Physiological and histological responses of broiler chicks to *in ovo* injection with folic acid or L-carnitine during embryogenesis. Global Veterinaria, 13: 544-551.
27. Yu, M., H. Wang, Y. Xu, D. Yu and D. Li, 2015. Insulin like growth factor 1 (IGF 1) promotes myoblast proliferation and skeletal muscle growth of embryonic chickens via the PI3K/Akt signalling pathway. Cell Biol. Int., 39: 910-922.
28. Gregory A. Brent., 2012. Mechanisms of thyroid hormone action. J. Clin. Invest., 122: 3035-3043.
29. Herrmann, W. and R. Obeid, 2011. Vitamins in the prevention of human diseases. 1st Ed., Vol. 5, Walter de Gruyter, Germany, ISBN: 9783110214482, Pages: 726.
30. Chen, W. and G.Chen, 2014. The roles of vitamin A in the regulation of carbohydrate, lipid and protein metabolism. J. Clin. Med., 3: 453-479.
31. Garg, D.P., A.K. Bansal, A. Malhotra, R. Kiran and D. Dhawan, 2009. Methomyl induced hematological and biochemical alterations–protection by vitamin E. Pestic. Biochem. Physiol., 93: 127-132.
32. Liu, J.J. and R.L. Ward, 2010. Folate and one-carbon metabolism and its impact on aberrant DNA methylation in cancer. Adv. Genet., 71: 79-121.