Vol. 19, No. 3, Autumn, 2023, 81-90
DOI: 10.22055/IVJ.2023.377882.2534
Journal Homepage: http://www.ivj.ir/ ***Original Article***

Effects of *in ovo* injection of nanocurcumin on intestinal development and serum parameters in chicken embryo

Reza Sayrafi[1*] and Saeed Seifi[2]

[1]Assistant Professor, Department of Basic Sciences, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran
[2]Associate Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

Received: 24.12.2022 Accepted: 01.10.2023

Abstract

The objective of the present study was to investigate the effects of in ovo injection of nanocurcumin on intestinal histomorphology and some serum components of the hatched chicks. The chicken eggs were injected with 1, 10, 100, 1000, and 10000 ppm of nanocurcumin, and the other two groups were used as control and injected control (saline). At the end of the experiment, blood and tissue samples were collected for biochemical and histological examination. For histological examination, small intestine was sampled and routine histological methods containing fixation, dehydration, clearing and paraffin embedding were used. Sections were stained with hematoxylin & eosin for light microscopy evaluation. Blood biochemical parameters were analyzed by an automatic analyzer. Results indicated that in ovo injection of saline significantly reduced albumin, globulin, total protein and AST (aspartat aminotransferase) compared to the control group, so that injection of nanocurcumin corrected the reduction of these factors. Uric acid was increased by injection of 1, 10 and 100 ppm nanocurcumin compared to the other groups. Blood glucose levels decreased slightly with increasing the dose of nanocurcumin, and in the 1000 ppm nanocurcumin group, the levels were significantly lower than those of the control. *In ovo* injection of 1000 ppm nanocurcumin significantly increased the villi height and villus height/crypt depth ratio compared to the saline and 1 ppm nanocurcumin groups. Although the primary purpose of this study was to evaluate the effects of *in ovo* administration of nanocurcumin, it was demonstrated that the use of saline in the *in ovo* injection was inappropriate. In conclusion, *in ovo* injection of nanocurcumin corrected saline-altered serum factors, lowered blood sugar, and increased the intestinal absorption surface.

Key words: Nanocurcumin, *In ovo* injection, Intestinal histomorphometry, Blood biochemical parameters

Introduction

The use of turmeric in food and as a traditional medicine derived from the root of *Curcuma longa* has been used in Asia for thousands of years (Aggarwal et al, 2007). The powdered extracts of dried roots may contain volatile and nonvolatile oils, proteins, fat, minerals, carbohydrates, moisture, and curcuminoids. The curcuminoids of turmeric are a mixture of three principal compounds: curcumin, demethoxycurcumin, and bisdemethoxycurcumin. In addition, the major component is curcumin (77%), which is a polyphenolic compound (Goel et al, 2008). Polyphenols have recently received considerable attention and are studied in disease prevention and treatment due to their biological capabilities (Yang et al,

* **Corresponding Author**: Reza Sayrafi, Assistant Professor, Department of Veterinary Basic Sciences, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran
E-mail: r.sayrafi@ausmt.ac.ir

2020). Curcumin has been reported to have a wide spectrum of biological actions, such as anti-inflammatory, antioxidant, antiangiogenic, anticarcinogenic, antidiabetic, antiallergic, and antimicrobial activities (Aggarwal and Harikumar, 2009; Mendes et al, 2020).

Although curcumin has beneficial biological effects, it appears that its poor systemic bioavailability compromises the pharmacological efficacy. The main reasons contributing to the low bioavailability of curcumin include rapid systemic elimination and poor absorption (Moniruzzaman and Min, 2020). Only less than one percent of curcumin is transferred to the blood circulation, and the rest is metabolized by the liver (Shehzad et al, 2010). Several studies also have reported that curcumin is conjugated in the liver, rapidly metabolized, and excreted in the feces; hence, its systemic bioavailability is limited. Several studies have investigated the improvement of the bioavailability of curcumin to make better use of its potential. Today, new methods to enhance the bioavailability of curcumin are ongoing. Nanoparticles encapsulating curcumin are one of these approaches. Utilization of nanoparticles of curcumin enhances its bioavailability, increasing solubility in the water while improving the utilization of curcumin properties (Moniruzzaman and Min, 2020).

The Chick embryo is a valuable model independent from external influences, easy to maintain, and fast growing. The egg model is used in nutritional, toxicological, pharmaceutical, and medical experiments as a primary study, often carried out before experiments with animals or humans (Yoshiyama et al, 2005; Nassar, 2018). The chicken embryo is also a popular and sensitive model used to assess the developmental toxicity of various nanoparticles (Patel et al, 2018). Furthermore, birds have a limited source of nutrients for embryonic development (Uni et al, 2012). Therefore, the nutrient package and antioxidant capacity may not be commensurate with the needs of the embryo, leading to poor embryo growth, reduced hatchability, and chick quality. This deficiency may be remedied by providing extra sources of nutrients and antioxidants through *in ovo* administration (Urso et al, 2015). Antioxidants are components of natural foods protecting cells against free radicals and oxidative damage. The presence of antioxidants is essential for embryo health, especially in the last 3 days of the embryonic period (Malheiros et al, 2012). Thus, the chick embryo model was chosen in our study to assess the impact of nanocurcumin on intestinal development and serum parameters.

Materials and Methods

Experimental design

A total of 70 fertilized Ross 308 eggs (62±1.1 g) were purchased from a commercial hatchery. The experimental design adhered to the guidelines of the Animal Ethics Committee of Amol University of Special Modern Technologies (Ethical code: ir.ausmt.rec.1399.11.21). The eggs were randomly divided into seven groups according to the following protocols:

1. Control (uninjected), 2. Injected control (injected with normal saline), 3. Injected with 1 ppm nanocurcumin, 4. Injected with 10 ppm nanocurcumin, 5. Injected with 100 ppm nanocurcumin, 6. Injected with 1000 ppm nanocurcumin, 7. Injected with 10000 ppm nanocurcumin. Nanocurcumin used in this experiment was obtained from Exir Nano Sina Company (SinaCurcumin®). The eggs were incubated for 21 days under standard conditions. During the first 18 days of incubation period, relative humidity and temperature were maintained at 65% and 37.5 °C, respectively. From day 19 onwards, the relative humidity and temperature were 75% and 37 °C, respectively. On day 3 of incubation, all eggs were light-candled and

the injection site of each egg was cleaned. The calculated doses of nanocurcumin dissolved in normal saline. Then, eggs were injected as *in ovo* via egg yolk route with 0.1 ml of mentioned dosages. The injection sites were covered by paraffin and the eggs were taken back to the incubator. The eggs were light-candled two days after injection for checking dead embryos.

Histologic Examination

For histomorphometric analysis, after hatching, 1-cm tissue samples of the duodenum (n=8 cases in each group) were transected and fixed in 10% buffered formalin. Tissues were dehydrated by transferring through a series of alcohols with increasing concentrations, placed into xylol, and embedded in paraffin. Six to seven micrometer thick sections were cut by microtome and were stained with hematoxylin & eosin. The morphometrical parameters of villous height, crypt depth, villus height to crypt depth ratio, and muscle thickness were measured. Later, the average of 20 values obtained for each chick was taken.

Determination of Serum Components

After hatching, all chicks were bled. Serum was obtained by centrifugation of the coagulated blood (3000 rpm for 10 min) and stored at -20 °C. Glucose, Uric acid, Creatinine, Globulin, Albumin, Aspartate Amino Transferase (AST), and total protein was analyzed by an automatic analyzer (Clima, Ral. Co, Spain).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) and for posthoc comparisons, Duncan's multiple range test was applied. All statements of significance were based on a probability of $P<0.05$.

Results

Blood serum indices

Table 1 shows the effects of nanocurcumin *in ovo* injection on some serum components of newly hatched chicks. During this experiment, in the last group (Injected with 10000 ppm nanocurcumin), only one chick was hatched. Therefore, no sample was taken from this group. Different levels of nanocurcumin did not have a significant impact on some serum biochemical parameters as compared to the uninjected control. We did not find any significant effect of nanocurcumin on total protein, creatinine, globulin, and AST in hatched chicks as compared to the uninjected control. In the injected control (Injected with normal saline) group, the levels of albumin, total protein, globulin, and AST exhibited a decrease compared to the control group ($P>0.05$). The use of different levels of nanocurcumin increased these parameters compared to the injected control group ($P<0.05$). Nanocurcumin at doses of 10, 100, and 1000 ppm increased uric acid levels compared to the control and injected control groups ($P<0.001$). Blood glucose levels at doses of 1, 10, and 100 ppm nanocurcumin did not change significantly compared to the control and injected control groups ($P>0.05$), while in the 1000 ppm nanocurcumin group, a significant decrease was observed compared to the control group and other levels of nanocurcumin ($P<0.05$).

Table 1. The effect of in ovo injection of nanocurcumin on serum components

Groups	Albumin (g/dL)	Uric acid (mg/dL)	Total protein (g/dL)	Creatinine (mg/dL)	Globulin (g/dL)	Glucose (mg/dL)	AST (IU/L)
Control	0.94[ab]	6.5[c]	2.4[a]	0.20	1.45[a]	252[a]	243[a]
Injected control	0.54[d]	6.0[c]	1.6[b]	0.20	1.05[b]	215[ab]	186[b]
1 ppm NC	0.75[c]	5.4[c]	2.2[a]	0.27	1.49[a]	247[a]	221[a]
10 ppm NC	0.83[bc]	7.2[b]	2.3[a]	0.21	1.53[a]	232[a]	231[a]
100 ppm NC	1.00[a]	12.9[a]	2.8[a]	0.26	1.80[a]	223[a]	250[a]
1000 ppm NC	0.82[bc]	8.0[b]	2.3[a]	0.20	1.58[a]	199[b]	260[a]
SEM	0.45	0.75	0.10	0.019	0.094	6.5	16
P-Value	<0.001	<0.001	<0.001	NS	0.03	0.03	0.01

[a, b, c, d] Means within each column with different letters are significantly (P < 0.05). NS: Not significant (*P* > 0.05). NC: Nanocurcumin.

Intestinal histomorphology

Table 2 shows the effects of the *in ovo* injection of nanocurcumin on intestinal histomorphometry. Nanocurcumin at a dose of 1000 ppm significantly increased the villi height and villus height/crypt depth ratio compared to the injected control and 1 ppm nanocurcumin groups (P<0.05). There was no significant difference among the other groups. In addition, the crypts depth and the Muscle thickness were not affected by the treatments (P>0.05) (Table 2; Figure 1).

Table 2. The effect of in ovo injection of nanocurcumin on Histomorphometrical parameters of duodenum (μm)

Groups	Villus height	Crypt depth	Villus height/crypt depth ratio	Muscle thickness
Control	388[ab]	54	7.1[ab]	123
Injected control	363[b]	53	6.6[b]	133
1 ppm NC	369[b]	58	6.3[b]	132
10 ppm NC	410[ab]	57	7.1[ab]	150
100 ppm NC	406[ab]	55	7.0[ab]	122
1000 ppm NC	490[a]	59	8.2[a]	156
SEM	35	2.0	0.40	12
P-Value	0.048	NS	0.047	NS

[a, b] Means within each column with different letters are significantly (P < 0.05). NS: Not significant (*P* > 0.05). NC: Nanocurcumin.

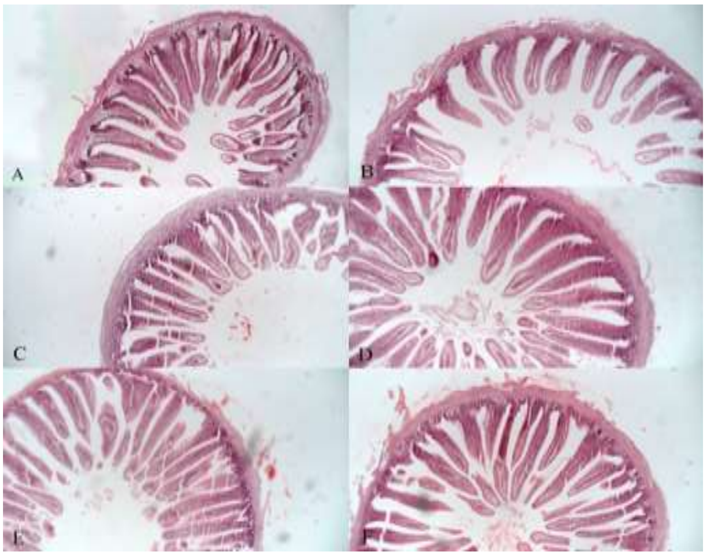

Figure 1: Photomicrographs of small intestine sections from the A: control group, B: injected control group, C: 1 ppm NC, D: 10 ppm NC, E: 100 ppm NC, F: 1000 ppm NC. H&E (10X).

Discussion

Other studies have reported changes in some of the serum parameters due to saline injection into eggs. The results of the study conducted by El-Kholy et al. (2019) indicated that by injecting saline into quail eggs, the levels of total protein and serum globulin were decreased significantly compared to the uninjected group, but the level of albumin was increased significantly. In agreement with the present results, Elwan et al. (2019) demonstrated that *in ovo* injection of the saline solution had no significant effect on the serum uric acid level in newly hatched broiler chicks exposed to heat stress during incubation. Furthermore, *in ovo* concurrent injection of methionine and cysteine increased the serum uric acid concentration compared to the control and saline injection groups, but there were no significant differences among all groups in serum protein profiles (total protein, globulin, and albumin).

El-Kholy et al. (2019) showed a decrease in hatchability after *in ovo* injection of saline in Japanese quails. With the injection of vitamins C, B6, and B12, the hatching rate was also improved, and it was the same as the rate in the uninjected group. Other studies have reported the reduction of hatchability owing to egg injection of saline (Selim et al, 2012; El-Kholy, 2013). The decrease in the levels of some serum biochemical parameters after *in ovo* injection of saline may be owing to the different osmolality of the injection solution. In another study, the osmolality of the injected solution was an important factor affecting turkey hatching (Ferket et al, 2005).

Sodium and chloride are the major ions of the extracellular fluid, functioning primarily in the control of fluid balance, water distribution, and osmotic pressure of body fluids. However, the entry of foreign electrolytes may disrupt the natural concentration gradients necessary for the influx of water to fetal tissues (McGruder et al, 2011). There is a possible relationship between the saline inoculated and the gastrointestinal function. Sodium and chloride may play a role in the organogenesis of the gastrointestinal system and then in the absorption of amino acids and glucose (Foye et al, 2007; Chen et al, 2009; Rahardja et al, 2019). Physiological saline is used for intravenous injection, since its osmolarity is close to that of sodium chloride in blood and does not cause blood cells to lysis (McGruder et al, 2011). Although in previous *in ovo* injection studies, saline has been the usual carrier for injected agents, according to these results, it does not appear that saline is the best carrier for the *in ovo* inoculation of agents into the embryo.

Some studies have investigated the effect of curcumin or nano curcumin on serum parameters in birds. Arshami et al. (2013) reported that the levels of cholesterol, triglycerides, and LDL were decreased by increasing curcumin doses in *Hy-line* hens. However, the levels of albumin, total protein, and glucose decreased slightly and curcumin did not affect creatine kinase. Reda et al. (2020) showed that nanocurcumin supplementation in Japanese quails significantly increased serum total protein, albumin, and globulin.

In the present study, blood glucose was slightly reduced by injection of 1, 10 and 100 ppm nanocurcumin, respectively, and in the 1000 ppm nanocurcumin group, it was significantly lower than the control. The effect of curcumin on lowering serum glucose levels has been proven. Curcumin can reduce blood glucose levels in several ways, such as by suppressing the hyperglycemia-induced inflammatory state, reducing the hepatic glucose production, stimulating the pancreatic cells to secrete more insulin, and reducing the insulin resistance (Ghorbani et al, 2014). Moreover, various studies have been conducted to evaluate the effects of curcumin or nanocurcumin on blood sugar and insulin resistance in related disorders like diabetes. It has been reported that turmeric, curcumin, or nanocurcumin exhibits hypoglycemic effects in type 2 diabetic mellitus (Na et al, 2013; Selvi et al, 2015; Rahimi et al, 2016). It is well known that type 2 diabetic mellitus is highly related to oxidative stress and inflammatory cytokines; therefore, due to the antioxidative and anti-inflammatory action of curcumin or nanocurcumin, it might be an effective therapeutic agent. The ability of curcumin to lower blood sugar levels, along with its other therapeutic effects, is limited due to its low bioavailability in body tissues. Nanocurcumin is further dissolved in water, which increases its absorption in the small intestine and can easily penetrate the cell membrane barrier; hence, it is more effective than curcumin. Thus, according to the present study and other previous studies, nanocurcumin can reduce and regulate blood glucose levels in healthy subjects or diabetic patients.

In the present study, *in ovo* injection of saline reduced AST levels, and no difference was observed in the nanocurcumin groups compared to the control group. Akbarian et al. (2012) reported that AST was not affected in broiler chickens by dietary turmeric rhizome powder. Gholami-Ahangaran et al. (2016) expressed similar results. Heidary et al. (2020) reported that *in ovo* injection of nanocurcumin had no significant effects on the blood serum lipids, MDA (malondialdehyde) concentration, liver GPX (glutathione peroxidase) and liver total antioxidant activities of broiler chickens exposed to heat stress on 10 days of age. Moreover, *in ovo* injection of 0.05 mL/egg nanocurcumin increased the liver SOD (superoxide dismutase) activity compared to both control and sham groups. However, Suvanated et al. (2003) showed that diets containing turmeric powder reduced the rate of MDA production and the oxidative reactions in chickens. Sayrafi et al. (2017) revealed that the decrease in liver enzyme activity in broiler chickens following nanocurcumin supplementation might be due to its antioxidant potential. In another study, dietary supplementation with turmeric rhizome powder increased AST activities in chickens (Emadi and Kermanshahi, 2007). Moreover, Rahmani et al. (2018) reported that AST activities were significantly higher in broiler chickens fed on a nanocurcumin supplementation diet than in broiler chickens fed on curcumin diets. In addition, the AST plasma level in chickens was increased when nanocurcumin was increased from 200 to 400 mg/kg diet.

It has been shown that nanoparticles of curcumin have higher tissue distribution and bioavailability than those of curcumin (Ma et al, 2007). Under certain conditions, an antioxidant may even act as a pro-oxidant producing various types of toxic oxygen (Haider Iqbal and Singh, 2019). High levels of nanocurcumin and its role as a pro-oxidant, cause liver cell damage and increase the liver enzyme activity (Halliwell, 2000). Inconsistency in data from different studies may be due to the concentration of curcumin or in the method of administration (Heidary et al, 2020). The effectiveness of antioxidants in normal conditions and diseases can also be different. Moreover, Steiner stated that dietary phytogenics in poultry might not always show the expected response (Steiner, 2009). Since this study was conducted under normal conditions, the lack of change in serum AST levels compared to the control group can be justified.

The effects of curcumin or nanocurcumin on the morphology of birds' small intestine have been investigated in a number of studies. Our results were consistent with those obtained by Elwan et al. (2019) who found that *in ovo* injection of saline did not exhibit a significant difference in the intestinal histomorphometry of chickens compared to the control group.

Rajput et al. (2012) demonstrated the positive effect of curcumin supplementation on intestinal histomorphology and showed that villus height and villus height/crypt depth were improved in broiler chicks fed on a diet supplemented with curcumin compared to the control group. In their study, significant effects of curcumin on villus height and villus height/crypt depth are in agreement with our results, but the crypt depth was decreased with the use of curcumin. Similarly, Rahmani et al. (2018) showed that supplementation of curcumin or nanocurcumin in diets improved the villus height and villus surface area of broiler chickens compared to those fed on a control diet and reared under normal and cold stress conditions. In accordance with our results, the study conducted by Heidary et al. (2020) revealed that *in ovo* injection of nanocurcumin had no significant effects on the intestinal crypt depth of chickens exposed to heat stress on day 10. The villous height in the chicks hatched from the sham group (pierced without injection) and 0.01 and 0.03 mL/egg nanocurcumin treatments were lower than those in the control group, and *in ovo* injection of 0.05 mL/egg nanocurcumin increased the villous height similar to the control group. Contrary to our results, the villus height/crypt depth in nanocurcumin treatments was lower than controls.

Regarding small intestine histomorphometric changes, the *in ovo* injection of nanocurcumin improved small intestine histomorphometric indexes like the villi height and increased the villus height/crypt depth ratio. These results indicate that *in ovo* inoculation of nanocurcumin improves the surface area of villi as an indicator of intestinal developmental and functional alterations.

The architecture of the intestinal mucosa can provide useful information about intestinal function. The structure of villi in the gastrointestinal tract can describe and provide information regarding nutrient absorption in animals. An increase in the villi height is an indication of an increase in the level of nutrient absorption in the intestine (Sieo et al, 2005; Sayrafi et al, 2011).

In addition, the ratio villus height/crypt depth is an indicator to estimate the likely digestive capacity of the small intestine. An increase in this ratio is considered useful for digestion or absorption, and vice-versa (Montagne et al, 2003).

The epithelial cells of the intestine are changed constantly, compensate for the villi cell loss through proliferation and maturation inside intestinal crypts, and migrate to the villus tip (Montagne et al, 2003). The effect of nanocurcumin on increased villus height may be due to increased intestinal epithelial cells turnover and then longer villus, which can provide a larger villus surface area, may improve birds' performance owing to improved absorption of nutrients in the intestine (Awad et al, 2008).

Increased villi height has been reported due to the use of various growth promoters, including prebiotic and antibiotic growth promoters (Sayrafi et al, 2011). Curcumin is also considered a growth promoter (Liu et al, 2020). According to the results of the present study, nanocurcumin can also be used as a growth promoter, and after hatching and starting oral feeding, it can be useful for birds' performance.

This study, for the first time, revealed the effect of *in ovo* administration of nanocurcumin against the harmful effects of *in ovo* injection of saline. The results showed that *in ovo* injection of saline significantly reduced some serum factors compared to the control group, so that injection of nanocurcumin corrected the reduction of these factors. Blood glucose levels were decreased with increasing the dose of nanocurcumin, which in the 1000 ppm nanocurcumin group, they were decreased significantly compared to the control group. *In ovo* injection of 1000 ppm nanocurcumin significantly increased the villi height and villus height/crypt depth ratio compared to the saline group.

Although the primary purpose of this study was to evaluate the effects of *in ovo* administration of nanocurcumin, it was shown that the *in ovo* injection of saline was inappropriate. It is concluded that *in ovo* injection of nanocurcumin corrected saline-altered serum factors, lowered blood sugar, and increased the intestinal absorption surface.

Conflict of interest

There was not any conflict of interest in this paper.

Funding

This study was funded by Research Council of Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran (IR.AUSMT.REC.1402.16).

References

Aggarwal, B.B. & Harikumar, K.B. (2009). Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *The international journal of biochemistry & cell biology*, 41(1), 40-59.

Aggarwal, B.B., Sundaram, C., Malani, N. & Ichikawa, H. (2007). Curcumin: the Indian solid gold. *Advances in Experimental Medicine and Biology*, 595, 1-75.

Akbarian, A., Golian, A., Kermanshahi, H., Gilani, A. & Moradi, S. (2012). Influence of turmeric rhizome and black pepper on blood constituents and performance of broiler chickens. *African Journal of Biotechnology*, 11(34), 8606-8611.

Arshami, J., Pilevar, M., Azghadi, M.A., Raji, A.R. (2013). Hypolipidemic and antioxidative effects of curcumin on blood parameters, humoral immunity, and jejunum histology in Hy-line hens. *Avicenna journal of phytomedicine*, 3(2), 178.

Awad, W., Ghareeb, K. & Böhm, J. (2008). Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing *Enterococcus faecium* and oligosaccharides. *International Journal of Molecular Sciences*, 9(11), 2205-2216.

Chen, W., Wang, R., Wan, H.F., Xiong, X.L., Peng, P. & Peng, J. (2009). Influence of *in ovo* injection of glutamine and carbohydrates on digestive organs and pectoralis muscle mass in the duck. *British Poultry Science*, 50(4), 436-442.

El-Kholy, M.S. (2013). Physiological studies on Japanese quail as affected by some nutritional treatments (PhD Thesis). Zagazig University, Egypt.

El-Kholy, M.S., Ibrahim, Z.A.E.G., El-Mekkawy. M.M. & Alagawany, M. (2019). Influence of *in ovo* administration of some water-soluble vitamins on hatchability traits, growth, carcass traits and blood chemistry of Japanese quails. *Annals of Animal Science*, 19(1), 97-111.

Elwan, H.A., Elnesr, S.S., Xu, Q., Xie, C., Dong, X. & Zou, X. (2019). Effects of *in ovo* methionine-cysteine injection on embryonic development, antioxidant status, IGF-I and TLR4 gene expression, and jejunum histomorphometry in newly hatched broiler chicks exposed to heat stress during incubation. *Animals*, 9(1), 25.

Emadi, M. & Kermanshahi, H. (2007). Effect of turmeric rhizome powder on the activity of some blood enzymes in broiler chickens. *International Journal of Poultry Science*, 6(1), 48-51.

Ferket, P., De Oliveira, J., Ghane, A. & Uni, Z. (2005). Effect of *in ovo* feeding solution osmolality on hatching turkeys. *Poultry Science*, 84, 118-119.

Foye, O.T., Ferket, P.R.& Uni, Z. (2007). 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. *Poultry Science*, 86(11), 2343-2349.

Gholami-Ahangaran, M., Rangsaz, N. & Azizi, S. (2016). Evaluation of turmeric (Curcuma longa) effect on biochemical and pathological parameters of liver and kidney in chicken aflatoxicosis. *Pharmaceutical biology*, 54(5), 780-787.

Ghorbani, Z., Hekmatdoost, A. & Mirmiran, P. (2014). Anti-hyperglycemic and insulin sensitizer effects of turmeric and its principle constituent curcumin. *International journal of endocrinology and metabolism*, 12(4), e18081.

Goel, A., Kunnumakkara, A.B. & Aggarwal, B.B. (2008). Curcumin as "Curecumin": from kitchen to clinic. *Biochemical pharmacology*, 75(4), 787-809.

Halliwell, B. (2000). The antioxidant paradox. *The Lancet*, 355(9210), 1179-1180.

Haider Iqbal, D. & Singh, D. (2019). Antioxidants: A Brief Review. *South Asian Research Journal of Medical Sciences*, 1(2), 36-39.

Heidary, M., Hassanabadi, A. & Mohebalian, H. (2020). Effects of *in ovo* injection of nanocurcumin and vitamin E on antioxidant status, immune responses, intestinal morphology and growth performance of broiler chickens exposed to heat stress. *Journal of Livestock Science and Technologies*, 8(1), 17-27.

Liu, M., Lu, Y., Gao, P., Xie, X., Li, D., Yu, D. & Yu, M. (2020). Effect of curcumin on laying performance, egg quality, endocrine hormones, and immune activity in heat-stressed hens. *Poultry Science*, 99(4), 2196-2202.

Ma, Z., Shayeganpour, A., Brocks, D.R., Lavasanifar, A. & Samuel, J. (2007). High-performance liquid chromatography analysis of curcumin in rat plasma: application to pharmacokinetics of polymeric micellar formulation of curcumin. *Biomedical Chromatography*, 21(5), 546-552.

Malheiros, R.D., Ferket, P.P. & Goncalves, F.M. (2012). Oxidative stress protection of embryos by "*In ovo*" supplementation. In XXIV World's Poultry Congress Salvador, Bahia, Brazil.

Mendes, T.S., Novais, E.A., Badaró, E., de Oliveira Dias, J.R., Kniggendorf, V., Lima-Filho, A.A.S. & Rodrigues, E.B. (2020). Antiangiogenic effect of intravitreal curcumin in experimental model of proliferative retinopathy. *Acta Ophthalmologica*, 98(1), e132-e133.

McGruder, B.M., Zhai, W., Keralapurath, M.M., Bennett, L.W., Gerard, P.D. & Peebles, E.D. (2011). Effects of *in ovo* injection of electrolyte solutions on the pre-and post-hatch physiological characteristics of broilers. *Poultry Science*, 90(5), 1058-1066.

Moniruzzaman, M. & Min, T. (2020). Curcumin, curcumin nanoparticles and curcumin nanospheres: A review on their pharmacodynamics based on monogastric farm animal, poultry and fish nutrition. *Pharmaceutics, 12*(5), 447.

Montagne, L., Pluske, J.R. & Hampson, D.J. (2003). A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Animal feed science and technology*, 108(1-4), 95-117.

Na, L.X., Li, Y., Pan, H.Z., Zhou, X.L., Sun, D.J., Meng, M. & Sun, C.H. (2013). Curcuminoids exert glucose-lowering effect in type 2 diabetes by decreasing serum free fatty acids: A double-blind, placebo-controlled trial. *Molecular nutrition and food research*, 57(9), 1569-1577.

Nassar, F.S. (2018). Poultry as an experimental animal model in medical research and pharmaceutical industry. *Biomed Journal*, 2(4), 1-4.

Patel, S., Jana, S., Chetty, R., Thakore, S., Singh, M. & Devkar, R. (2018). TiO 2 nanoparticles induce omphalocele in chicken embryo by disrupting Wnt signaling pathway. *Scientific Reports*, 8(1), 1-11.

Rahardja, D.P., Hakim, M.R., Yusuf, M. & Lestari, V.S. (2019). Hatching and post-hatching performances of Indonesian native chicken eggs infused saline solution. In IOP Conference Series: *Earth and Environmental Science*, 247(1), 012014.

Rahimi, H.R., Mohammadpour, A.H., Dastani, M., Jaafari, M.R., Abnous, K., Mobarhan, M.G. & Oskuee, R.K. (2016). The effect of nano-curcumin on HbA1c, fasting blood glucose, and lipid profile in diabetic subjects: a randomized clinical trial. *Avicenna journal of phytomedicine*, 6(5), 567.

Rahmani, M., Golian, A., Kermanshahi, H. & Bassami M.R. (2018). Effects of curcumin or nanocurcumin on blood biochemical parameters, intestinal morphology and microbial population of broiler chickens reared under normal and cold stress conditions. *Journal of applied animal research*, 46(1), 200-209.

Rajput, N., Muhammah, N., Yan, R., Zhong, X. & Wang, T. (2012). Effect of dietary supplementation of curcumin on growth performance, intestinal morphology and nutrients utilization of broiler chicks. *The Journal of Poultry Science*, 50, 44–52.

Reda, F.M., El-Saadony, M.T., Elnesr, S.S., Alagawany, M. & Tufarelli, V. (2020). Effect of dietary supplementation of biological curcumin nanoparticles on growth and carcass traits, antioxidant status, immunity and caecal microbiota of Japanese quails. *Animals*, 10(5), 754.

Sayrafi, R., Shahrooz, R., Soltanalinejad, F. & Rahimi, S. (2011). Histomorphometrical study of the prebiotic effects on intestine morphology and growth performance of broiler chickens. *Veterinary Research Forum*, 2(1), 45-51.

Sayrafi, R., Hosseini, S.M. & Ahmadi, M.A. (2017). The protective effects of nanocurcumin on liver toxicity induced by salinomycin in broiler chickens. *Revue de Médecine Vétérinaire*, 168(7/9), 136-142.

Selim, S.A., Gaafar, K.M. & El-ballal, S.S. (2012). Influence of in-ovo administration with vitamin E and ascorbic acid on the performance of Muscovy ducks. *Emirates Journal of Food and Agriculture*, 24(3), 264-271.

Selvi, N.M.K., Sridhar, M.G., Swaminathan, R.P. & Sripradha, R. (2015). Efficacy of turmeric as adjuvant therapy in type 2 diabetic patients. *Indian Journal of Clinical Biochemistry,* 30(2): 180-186.

Shehzad, A., Wahid, F. & Lee, Y.S. (2010). Curcumin in cancer chemoprevention: molecular targets, pharmacokinetics, bioavailability, and clinical trials. *Archiv der Pharmazie*, 343(9), 489-499.

Steiner, T. (2009). Phytogenics in animal nutrition. Nottingham University Press. Nattingham. UK.

Suvanated, C., Kijparkorn, S. & Angkanaporn, K. (2003) Effect of turmeric (Curcuma longa linn.) as an antioxidant on immune status and growth performances of stressed broilers (PhD Thesis). Chulalongkorn University, Bangkok, Thailand.

Sieo, C.C., Abdullah, N., Tan, W.S. & Ho, Y.W. (2005). Influence of β-glucanase-producing Lactobacillus strains on intestinal characteristics and feed passage rate of broiler chickens. *Poultry Science*, 84(5): 734-741.

Uni, Z., Yadgary, L.& Yair, R. (2012). Nutritional limitations during poultry embryonic development. *Journal of Applied Poultry Research*, 21(1), 175-184.

Urso, U.R.A., Dahlke, F., Maiorka, A., Bueno, I.J.M., Schneider, A.F., Surek, D. & Rocha, C. (2015). Vitamin E and selenium in broiler breeder diets: Effect on live performance, hatching process, and chick quality. *Poultry Science*, 94(5), 976-983.

Yang, Q.Q., Cheng, L.Z., Zhang, T., Yaron, S., Jiang, H.X., Sui, Z.Q. & Corke, H. (2020). Phenolic profiles, antioxidant, and ant proliferative activities of turmeric (*Curcuma longa*). *Industrial Crops and Products*, 152, 112561.

Yoshiyama, Y., Sugiyama, T. & Kanke, M. (2005). Experimental diabetes model in chick embryos treated with streptozotocin. *Biological and Pharmaceutical Bulletin*, 28(10), 1986-1988.

Received: 24.12.2022
Accepted: 01.10.2023

مقاله کامل

دوره نوزدهم ـ شماره ۳ ـ پاییز ۱۴۰۲

DOI: 10.22055/IVJ.2023.377882.2534

اثرات تزریق داخل تخم مرغی نانوکورکومین بر توسعه روده و شاخص‌های سرمی جنین جوجه

رضا صیرفی[1]* و سعید سیفی[2]

[1] استادیار گروه علوم پایه، دانشکده دامپزشکی، دانشگاه تخصصی فناوری‌های نوین آمل، آمل، ایران

[2] دانشیار گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه تخصصی فناوری‌های نوین آمل، آمل، ایران

تاریخ دریافت: ۱۴۰۱/۱۰/۳ تاریخ پذیرش: ۱۴۰۲/۷/۹

چکیده

هدف مطالعه حاضر بررسی اثرات تزریق داخل تخم مرغی نانوکورکومین بر هیستومورفولوژی روده و برخی از اجزای سرمی در جوجه‌های تفریخ شده بود. به تخم مرغ‌ها ۱، ۱۰، ۱۰۰، ۱۰۰۰ و ۱۰۰۰۰ ppm نانوکورکومین تزریق شد و دو گروه دیگر به عنوان شاهد و شاهد تزریقی (سالین) استفاده شدند. در پایان آزمایش، نمونه‌های خون و بافت برای بررسی بیوشیمیایی و بافت‌شناسی جمع‌آوری شد. برای آزمون بافت‌شناسی، از روده کوچک نمونه‌برداری شد و از روش‌های بافت‌شناسی معمول شامل تثبیت، آب‌گیری، شفاف‌سازی و قالبگیری با پارافین استفاده شد. مقاطع با هماتوکسیلین-ائوزین برای ارزیابی میکروسکوپ نوری رنگ‌آمیزی شدند. پارامترهای بیوشیمیایی خون توسط دستگاه آنالایزر اتوماتیک مورد بررسی قرار گرفت. نتایج نشان داد که تزریق داخل تخم مرغی سالین باعث کاهش معنی‌دار آلبومین، گلوبولین، پروتئین تام و AST (آسپارتات آمینوترانسفراز) نسبت به گروه کنترل شد، به طوری که تزریق نانوکورکومین کاهش این موارد را اصلاح کرد. اسید اوریک با تزریق ۱، ۱۰ و ۱۰۰ ppm نانوکورکومین، نسبت به سایر گروه‌ها افزایش یافت. با افزایش دوز نانوکورکومین سطح گلوکز خون اندکی کاهش یافت و در گروه ۱۰۰۰ ppm نانوکورکومین، سطوح به طور معنی‌داری کمتر از گروه شاهد بود. تزریق داخل تخم مرغی ۱۰۰۰ ppm نانوکورکومین به طور قابل توجهی ارتفاع پرز و نسبت ارتفاع پرز به عمق کریپت را در مقایسه با گروه‌های سالین و ۱ ppm نانوکورکومین افزایش داد. اگر چه هدف اولیه این مطالعه بررسی اثرات تجویز داخل تخم مرغی نانوکورکومین بود، اما نشان داده شد که استفاده از سالین در تزریق داخل تخم مرغی نامناسب است. در نتیجه، تزریق داخل تخم مرغی نانوکورکومین، فاکتورهای سرمی تغییر یافته با سالین را اصلاح کرد، قند خون را کاهش و سطح جذب روده را افزایش داد.

کلمات کلیدی: نانوکورکومین، تزریق داخل تخم مرغی، هیستومورفومتری روده، پارامترهای بیوشیمیایی خون

* نویسنده مسئول: رضا صیرفی، استادیار گروه علوم پایه، دانشکده دامپزشکی، دانشگاه تخصصی فناوری‌های نوین آمل، آمل، ایران

E-mail: r.sayrafi@ausmt.ac.ir