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Shwetha HS
Veterinary College, Bengaluru, Karnataka, India

Narayana Swamy M
Veterinary College, Bengaluru, Karnataka, India

Srinivas RB
Veterinary College, Bengaluru, Karnataka, India

Kalmath GP
Veterinary College, Bengaluru, Karnataka, India

Byregowda TR
Veterinary College, Bengaluru, Karnataka, India

Veena MP
Veterinary College, Bengaluru, Karnataka, India

Anitha MM
Veterinary College, Bengaluru, Karnataka, India

Rajendran D
National Institute of Animal Nutrition and Physiology, Adugodi, Karnataka, India

Corresponding Author:
Shwetha HS
Veterinary College, Bengaluru, Karnataka, India

Effect of dietary supplementation of nano-selenium and nano-curcumin on liver and kidney histology in broiler chickens

Shwetha HS, Narayana Swamy M, Srinivas RB, Kalmath GP, Byregowda TR, Veena MP, Anitha MM and Rajendran D

Abstract
The present study was designed to evaluate the dietary influence of nano-selenium (Nano-Se) and nano-curcumin on histology of liver and kidney in Ven-Cobb broiler chickens. A total of 360 day-old broiler chicks were randomly divided into four groups with each group comprising 90 chicks. Group I chicks stood as control with standard broiler diet. While chickens kept in groups II, III and IV were supplemented with nano-Se (0.3 mg / kg diet), nano-curcumin (200 mg / kg diet) and combination of both nano-Se (0.15 mg / kg diet) and nano-curcumin (100 mg / kg diet) along with basal diet, respectively. At day, 42 the experimental birds were scarified to collect liver and kidney for histological examination. The his to-pathological photomicrographs of the liver and kidney sections in Group II and IV revealed very well-maintained cellular architecture, whereas, degenerative changes in the hepatocytes and kidney tubular epithelial cells were noted in samples from Group I. Thus, Nano-Se and nano-curcumin were found to be more effective in ameliorating oxidative stress with maintenance of cellular architecture that imparts physiological well-being of broiler chickens.

Keywords: Chickens, liver, kidney, histology, nano-curcumin, nano-selenium

Introduction
Nanotechnology, an emerging technology, has been implicated to revolutionize the poultry industry. Thus, the feed particle size acts as a tool to optimize feed utilization and improve production efficiency in broilers has attracted more attention in poultry industry (Zaefarian *et al*., 2016) [17]. Broiler chickens are the fastest-growing and the most efficient species among meat-producing animals and very susceptible to oxidative stress (Zhang *et al*., 2015) [18]. Phytogenic mixtures are given individually or in groups as dietary supplements in poultry rations is a new strategy to ameliorate the adverse effects (Ahmadifar *et al.*, 2021) [2].
The Selenium (Se) is an important microelement with numerous positive effects on human health and body cells require appropriate concentration of Se for the maintenance of homeostasis (Kieliszek *et al.*, 2022) [12]. Selenium plays an important role in protein folding and maintenance of liver physiology of experimental chickens (Abbas *et al.*, 2017) [1]. Selenium nano-particles (Se-NPs) can antagonize the oxidative stress and acts as potential hepatocyte protective therapeutic agent (Hao *et al.*, 2021; Jun *et al.*, 2022) [9, 11]. The bioavailability of dietary Se can be improved by converting it to nanoparticles and by providing it a carrier (Gangadoo *et al.*, 2018) [19].
Curcumin is considered as "wonder drug of life" obtained from the rhizome of the medicinal plant *Curcuma longa* (L) and its structure contains carbonyl, methoxy and hydroxyl groups and which are involved in antioxidant activities to scavenge free radicals especially proxy radicals and may improve broiler growth (Gera *et al*., 2017) [8]. Chickens fed with a diet containing curcuma powder (5 g / kg) have a positive impact on liver enzymes (Emadi and Kermanshahi, 2007) [6]. Histopathology of liver tissues showed normal portal triads and hepatic veins with normal liver morphology upon supplementation with turmeric extract at 0.5% and 1.0% (Johannah *et al.*, 2018) [10]. Curcumin remarkably took part in the reduction of damage in renal tissue to improve the function of kidney and it also exhibited reduction of oxidative stress (Wu *et al.*, 2017) [16].
However, scientific information available on the effect of supplementation of nano-selenium and nano-curcumin on histopathology in broiler chickens is scarce. Therefore, the present work was executed to clarify the impact of nanoselenium and nanocurcumin supplementation

On histological changes of liver and kidney in broiler chickens.

Materials and Methods

All experimental procedures of the study were performed according to the guidelines set by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Registration number 493 / GO / ReBiBt-S / Re-L / 01) and the research was approved by the Institutional Animal Ethical Committee (Number: VCH/IAEC/2019/83) of Veterinary College, Bengaluru, Karnataka, India.

Experimental design

A total of three hundred sixty, commercial broiler (Ven Cobb strain) chicks, aged 1-day-old, were procured from Venkateshwara Hatcheries Pvt Ltd, Bengaluru, India. The experimental birds were allotted to four dietary supplementation groups. Each group was having 90 birds with 30 chicks in one replicate. Group I birds were fed on basal broiler diet as control group. The supplemented groups such as Group II, III and IV birds were fed with nano-Se at 0.3 mg/kg, nano-curcumin at 200 mg / kg and combination of both nano-Se and nano-curcumin at 0.15 mg / kg + 100 mg/kg along with basal diet, respectively. Feeding of test diets commenced at day one and continued till the six weeks of age. From day 1 to 42, the chicks were offered a corn soya-based diet formulated as per the recommendation of National Research Council (NRC 1994) guidelines to fulfil the nutrient requirements of poultry (Table 1). In the 42 days of the experiment, 6 chickens were randomly selected from each group and sacrificed to collect the liver and kidney and were fixed in 10 per cent neutral buffered formalin (NBF) solution for histological examination.

Table 1: Ingredient composition (kg / 100 kg of feed) of the experimental diets

Ingredients	Pre-starter diet (0 -14 days)	Starter diet (15-28 days)	Finisher diet (29-42 days)
Yellow maize	53.47	57.00	60.47
Soybean meal	41.0	35.77	31.00
Vegetable oil	2.2	4.0	5.5
*Mineral mixture	1.5	1.5	1.5
Di calcium phosphate	1.0	0.9	0.8
Common Salt	0.3	0.3	0.3
**Vitamin Premix	0.2	0.2	0.15
DL-Methionine	0.2	0.2	0.18
B complex	0.1	0.1	0.1
***Antibiotic	0.03	-	-
Total	100	100	100
Nutrient Composition			
ME (kcal/kg)a	2948.5	3076.65	3129.61
Crude Protein (%)	22.89	20.02	17.99
Calcium (%)a	1.01	0.91	0.855
Phosphorus (%)a	0.46	0.37	1.0.355
Lysine (%)a	1.4	1.18	1.03
Methionine (%)	0.49	0.39	0.342
Selenium (ppm)	0.08	0.10	0.08

*Mineral Mixture: Each 100 g contains: Magnesium Oxide: 1.48 g, Ferrous Sulphate: 6.0 g, Copper Sulphate: 0.05 g, Manganese Sulphate: 0.04 g, Potassium Iodide: 0.001 g, Zinc Sulphate: 1.0 g, Potassium Chloride:17.09 g and Sodium Selenite: 0.001 g **Vitamin Premix: Each 100 gm contains Vitamin AD_3 (Vitamin A: 10,00,000 IU / g, Vitamin D: 200000 IU / g) - 0.165 g, Vitamin K_3: 0.103 g, Vitamin E: 2.4 g, Thiamine mononitrate: 0.206 g, Riboflavin: 0.513 g, Pyridoxine hydrochloride: 0.309 g, Cyanocobalamin: 0.00031 g, Folic Acid: 0.103 g, Niacin: 4.124 g, Ca-D-pantothenate: 1.031 g, Biotin: 1.5 g, Malta dextrin: 89.545 g. ******* Antibiotic: Ox tetracycline

Haematoxylin and eosin staining of liver and kidney

At day 42, experimental birds were scarified and representative tissue samples of 3-5 mm thickness were collected from liver and kidney in 10 per cent neutral buffered formalin (NBF) solution for histological examination. The sections were washed and dehydrated with graded ethanol series in the processor. After processing, tissue sections were embedded in paraffin blocks. The tissues were processed by routine paraffin embedding technique and 4 microns size sections were cut and subjected for routine Haematoxylin and Eosin (H and E) staining method (Luna, 1968) [13].

Results

Microscopically liver and kidney from Group I and Group III birds revealed degenerative changes in the hepatocytes and kidney tubular epithelial cells, respectively. Birds fed with nano-selenium in Group II revealed normal appearance of liver and kidney architecture. Birds fed with combination of both nano-selenium and nano-curcumin (Group IV) revealed very well-maintained normal architecture of both liver and kidney, (Plate 1 & 2).

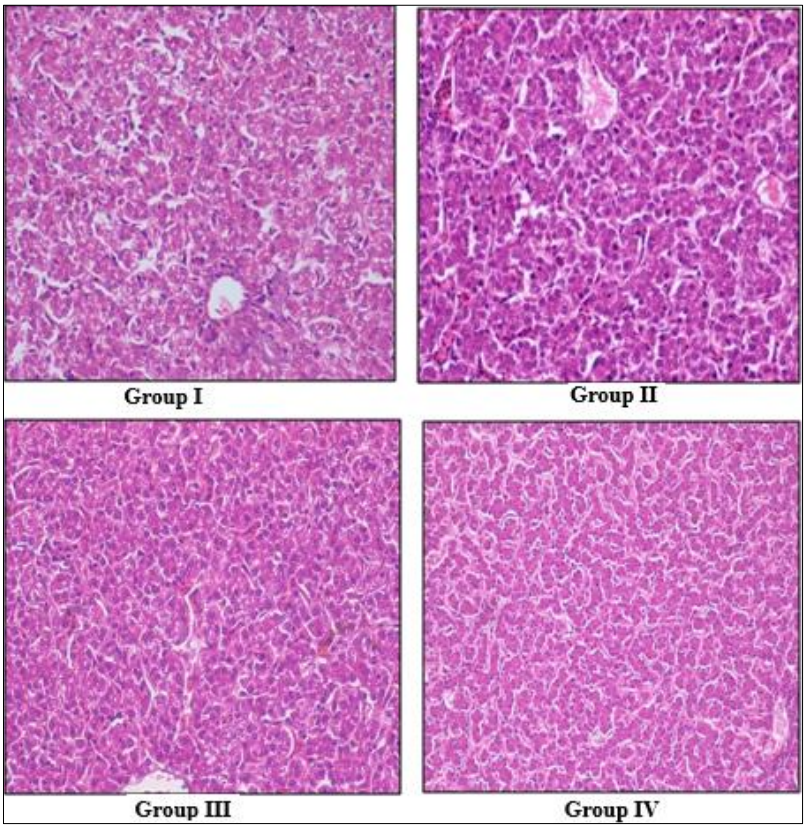

Plate 1: Histological structure of liver in various experimental groups of broiler chickens

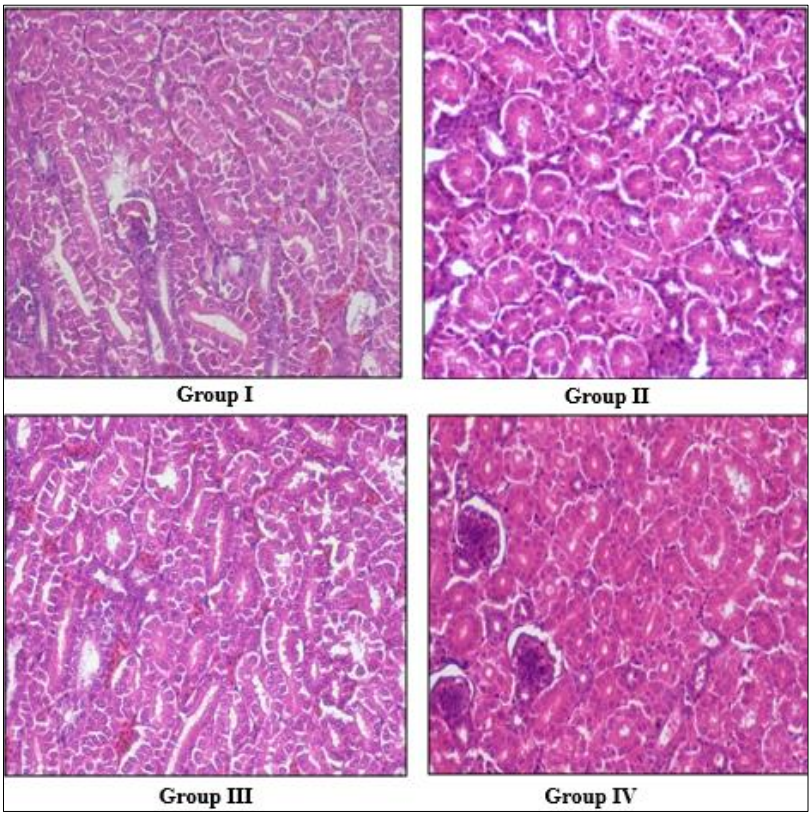

Plate 2: Histological structure of kidney in various experimental groups of broiler chickens

Discussion

Multiple studies have reported the positive impact of selenium nanoparticles on the histology of liver and kidney in different species (Gangadoo *et al*., 2020; Abbas *et al*., 2017) [7, 1]. Selenium helped to change the peroxide into non-hazardous substances in the liver to maintain the normal cellular architecture in Rhode Island Red chickens (Abbas *et al*., 2017) [1]. Histo-pathological analysis showed that nano-Se did not cause any damaging effects to the tissues (Gangadoo *et al*., 2020) [7]. The extracellular and intracellular structure of the liver was preserved and degeneration of fat and cell apoptosis was significantly decreased in rats fed with nano-

selenium (Sohrabi *et al*., 2020) [14].
The histological sections of liver and kidney in chicks receiving curcumin exhibit cellular texture and appearance based on the level of curcumin in the diet (Al-Aameli *et al.*, 2020) [3]. Moreover, Emadi *et al*. (2015) [5] reported the hepato protective properties of curcumin in Japanese quail. The addition of nano-curcumin alone to the Aflatoxin-contaminated diet ameliorated the toxic effects of aflatoxin B1 on the liver tissue histopathology (Ashry *et al*., 2022) [4]. Previous studies have shown that curcumin detoxification mechanisms, phase II enzyme activity up-regulation and positive regulation of cells in liver and kidney (Wang *et al*., 2018) [15]. Therefore, the present study found that the photomicrograph of the liver and kidney with selenium and curcumin nanoparticles fed group (Group IV) showed normal histology. However, limited information is available in the literature regarding the effect of Se and curcumin NP supplementation in combination to chickens.

Conclusion
The study revealed that dietary nano-Se and nano-curcumin maintain the cellular architecture of liver and kidney to maintain the physiological wellbeing of broiler chickens.

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Conflicts of interest
The authors declare no conflict of interest.

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