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Nano-Selenium and Nano-Curcumin Supplementation Effect on Liver Function in Broiler Chickens

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ABSTRACT

The present study was designed to compare the impact of dietary supplementation of nano-selenium and nano-curcumin on biochemical parameters response of 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 served as control with standard broiler diet. 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. Blood samples were collected on 21st, 28th, 35th and 42nd day and processed for serum samples. Liver enzymes, glucose and total protein profile was estimated by using spectrophotometric method. Serum alanine transaminase (ALT) activities were significantly lower in supplemented groups compared to control groups. Significantly lower serum aspartate amino transferase (AST) activities were observed in Group III and Group IV when compared to Group I. Non-significant ($P<0.05$) differences were observed in activity of serum gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), glucose, total protein and albumin between the nanoparticles supplemented groups and control group at different days of observation. Thus, the inclusion of Nano-Se and nano-curcumin has a hepatoprotective effect in broiler chickens. The evidence in this study can help to design nutritional research trials in poultry industry.

KEYWORDS: Antioxidants, Chickens, Liver Enzymes, Nano-Curcumin, Nano-Selenium

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INTRODUCTION

Supplementation of selenium (Se) preserves beta cells activity in pancreas thus maintaining blood glucose levels during cellular oxidative stress (Habibian et al., 2013). Selenium plays an important role in protein folding and maintenance of liver physiology of experimental chickens (Abbas et al., 2017). Curcumin is considered as "wonder drug of life" obtained from the rhizome of the medicinal plant Curcuma longa (L) and involved in antioxidant activities to scavenge free radicals especially peroxyl radicals and may improve broiler growth (Gera et al., 2017). Turmeric supplementation improved serum metabolites such as total protein, albumin, globulin and assist in better utilization of glucose in broiler chickens (Ahmadi, 2010). Chickens fed with a diet containing curcuma powder (5 g/kg) have a positive impact on liver enzymes (Emadi and Kermanshahi, 2007). However, scientific information available on the effect of supplementation of selenium and curcumin in nano-form on biochemical parameters in broiler chickens is scarce.

MATERIALS AND METHODS

The broiler chickens were reared in the Department of Poultry Science, Veterinary College, Bengaluru, India. The laboratory examinations were conducted at the Department of Veterinary Physiology, Veterinary College, Bengaluru, and National Institute of Animal Nutrition and Physiology, Bengaluru, India.

Animal ethics and welfare

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.

Preparation of nano-selenium

Selenium nanoparticles (SeNPs) were prepared based on the reduction of selenious acid (H_2SeO_3) by employing sodium alginate ($C_6H_9NaO_7$) as a template (Tan et al., 2009). The sodium alginate and ascorbic acid ($C_6H_8O_6$) were purchased from Himedia, India. Selenious acid was purchased from Merk Milli Pore, USA. For the synthesis of SeNPs, 2ml of (0.2 %) Sodium alginate was mixed with 8 ml of 30 mM selenious acid (Kumar et al., 2014), to which 200 µl of 40 mM ascorbic acid was added to initiate the reaction (Preedia et al., 2017). The mixtures were allowed to react with each other in the concentrated form and kept for incubation for 48 hours at room temperature. After 48 hours of incubation, the precipitate solution was centrifuged at 10,000 g for 30 minutes to get Se pellets at ambient temperature (25 °C). The resulting pellet was washed with double distilled water followed by absolute ethanol three times (Preedia et al., 2017). The obtained pellet was dried at room temperature and the powder form of SeNPs was used for DLS analysis revealed that the average particle size of nano-Se is ranges from 37 to 60 nm.

Preparation of nano-curcumin

The curcumin nano-suspension was prepared by solvent-antisolvent precipitation method. Curcumin was dissolved in ethanol (100 mg/mL), which acted as a solvent phase. This solvent phase was added to the water (1:10) which acted as anti-solvent phase (Aditya et al., 2019). The mixture of curcumin, ethanol and water was subjected to homogenization for 20 minutes using IKA's T25 digital *Ultra-Turrax* homogenizer. The solvent-antisolvent precipitation was kept overnight in a hot air oven at 80°C to reduce the supernatant. Thereafter, it was transferred to a freezing chamber and freeze drier at -110 °C for 24 hours (Yadav and Neeraj, 2014). Then it was transferred to a vacuum drying chamber till it dried into fine nano-curcumin powder. The synthesized triplicate sample of curcumin nanoparticles was transferred to airtight container. The DLS analysis data revealed that the nano-curcumin had an asymmetric shape and average hydrodynamic size in the range of 9-98 nm.

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).

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
Dicalcium 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)	2948.5	3076.65	3129.61
Crude Protein (%)	22.8	20.0	17.9
Calcium (%)a	1.01	0.91	0.855
Phosphorus (%)	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 Selenate: 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, Cyanocobalamine : 0.00031 g, Folic Acid : 0.103 g, Niacin: 4.124 g, Ca-D-pantothenate: 1.031 g, Biotin : 1.5 g, Maltodextrin : 89.545 g.

***Antibiotic :Oxytetracycline

The nutrient requirement figures published in Nutrient Requirements of Poultry (National Research Council, 1994) are the most recent available and viewed as minimal nutrient needs for poultry. Blood samples were collected from six randomly selected birds from each group, on days 21, 28, 35 and 42. Separated serum samples were stored at -80 °C for analysis.

Biochemical analysis

Glucose, total protein, albumin, AST, ALT, LDH and GGT level in serum was estimated using commercially available reagent kits manufactured by Erba Lachema marketed by Transasia Biomedicals, Ltd., Mumbai, India. All measurements were performed spectrophotometrically using a Konelab T20xt biochemical analyser (Thermo Fisher Scientific, Waltham, MA, USA). The measurement conditions were set up according to the manufacturer's assay protocol.

Statistical analysis

All data obtained were analysed statistically by Two-way ANOVA with the application of Bonferroni post-test using 'GraphPad Prism' version 5.01 (2007) computerized software. The values are presented as Mean ± Standard Error and the level of significance ($P < 0.05$) or non-significance ($P > 0.05$) was determined at P value of 0.05.

RESULTS AND DISCUSSION

Effects of dietary supplementation of nano-selenium and nano-curcumin on biochemical profile of liver are shown in Table 2.

Table 2.Effect of dietary supplementation of nano-selenium and nano-curcumin on biochemical parameters in broiler chickens.

Parameter	Groups	21 Days	28 Days	35 Days	42 Days
Glucose (mg/dL)	Group I	201.1 ± 9.89	209.7 ± 12.11	200.0 ± 10.72	194.2 ± 3.41
	Group II	171.2 ± 7.71	195.7 ± 18.94	186.8 ± 18.03	175.0 ± 16.13
	Group III	179.6 ± 8.03	200.5 ± 14.25	183.5 ± 15.19	185.7 ± 14.18
	Group IV	174.3 ± 11.62	179.6 ± 15.39	171.8 ± 12.32	174.4 ± 11.35
Total protein (g/dL)	Group I	2.78 ± 0.19	2.89 ± 0.09	3.03 ± 0.06	3.06 ± 0.07
	Group II	2.83 ± 0.11	3.03 ± 0.06	3.11 ± 0.08	3.06 ± 0.10
	Group III	2.79 ± 0.15	2.90 ± 0.06	3.03 ± 0.06	3.05 ± 0.09
	Group IV	2.92 ± 0.08	3.09 ± 0.03	3.04 ± 0.11	3.08 ± 0.11
Albumin (g/dL)	Group I	1.16 ± 0.04	1.22 ± 0.04	1.16 ± 0.05	1.11 ± 0.03
	Group II	1.24 ± 0.04	1.23 ± 0.05	1.10 ± 0.09	1.17 ± 0.05
	Group III	1.33 ± 0.06	1.28± 0.07	1.27 ± 0.06	1.23 ± 0.06
	Group IV	1.25 ± 0.07	1.20 ± 0.05	1.09 ± 0.09	1.11 ± 0.11
AST (IU/L)	Group I	223.6 ±12.84^{y}	224.3 ±20.94^{y}	237.1 ± 19.71^{y}	227.0 ±26.22^{y}
	Group II	180.5 ±12.10^{xy}	190.4 ±15.41^{xy}	181.8 ± 14.18^{xy}	183.6 ±15.59^{xy}
	Group III	160.9 ±15.04^{x}	153.7 ±14.31^{x}	177.1 ±15.78^{x}	151.9 ±12.35^{x}
	Group IV	157.1 ±13.45^{x}	162.4 ±16.59^{x}	174.4 ±15.00^{x}	162.0 ±15.17^{x}
ALT (IU/L)	Group I	18.8 ± 3.50^{y}	20.9 ± 3.41^{y}	21.0 ± 4.02^{y}	23.6 ± 2.76^{y}
	Group II	9.88 ± 1.79^{x}	10.9 ± 1.42^{x}	11.6 ± 1.22^{x}	13.6 ± 2.27^{x}
	Group III	9.86 ± 1.20^{x}	10.5 ± 2.14^{x}	10.2 ± 1.97^{x}	14.1 ± 3.39^{x}
	Group IV	9.19 ± 1.21^{x}	11.8 ± 2.06^{x}	11.6 ±1 .77^{x}	13.7 ± 1.95^{x}
LDH (IU/L)	Group I	3106.1 ± 319.75	3423.1 ± 178.45	3197.1±154.7	3783.6 ± 165.8
	Group II	2978.5 ± 195.43	2948.5 ± 179.05	2914.0±356.3	3097.6 ± 255.9
	Group III	2820.8 ± 261.41	2848.5 ± 207.37	2681.5±309.5	3462.8 ± 222.4
	Group IV	2859.1 ± 191.07	3075.6 ± 152.29	2855.1±135.9	3311.6 ± 320.2
GGT (IU/L)	Group I	10.8 ± 1.16	11.7 ± 1.05	11.5 ± 0.75	14.0 ± 1.55
	Group II	9.31 ± 1.65	10.6 ± 1.38	10.2± 0.66	12.4 ± 1.62
	Group III	8.94 ± 1.69	10.2 ± 0.75	10.24 ± 0.63	12.2 ± 1.25
	Group IV	9.49 ± 0.82	9.80 ± 0.87	9.86 ± 0.51	12.6 ± 1.50

The values with different superscripts within a column (x, y) for a parameter differ significantly ($P<0.05$). None of the values in a row differ significantly.

Serum ALT activities were significantly low in treatment groups compared to control group. Significantly lower serum aspartate aminotransferase (AST) activities were observed in Group III and Group IV when compared to Group I. No significant ($p < 0.05$) differences were observed in activity of serum GGT and LDH in between the

nanoparticles treated groups and control group at different days of observation. Glucose, total protein and albumin concentrations in serum were not significantly different among the experimental groups (Table 2). The reduced serum AST and ALT levels in nano-selenium supplemented group resultscorroborate the findings of Dalia et al. (2017), who reported basal diets supplemented with 0.3 mg/kg of Se up-regulated selenoproteins mRNA levels and reduces AST and ALT activities. Biogenic Se-NPs showed a protective effect on liver parenchyma and inhibition of elevated level of liver enzymes (Li et al., 2017). Similarly, Placha et al. (2009) reported that the excessive selenium supplementation through the diet do not have any role on GGT status in broilers. Nano-selenium supplementation reduced the activities of GGT, LDH and it plays a protective role by preventing the oxidative stress in broiler chickens (Xueting et al., 2018). Thus, in the present trial, there was a significant decrease in the ALT and AST activities in the nano-curcumin supplemented group, as reported by previous studies (Dalia et al., 2017; Al-Aameli et al., 2020; Reda et al., 2020). Reda et al. (2020) reported that the supplementation of curcumin could decrease the AST activity in Japanese quails. *Curcuma longa* antioxidant properties can scavenge oxygen free radicals and alleviate the lipid peroxidation by minimizing the levels of liver enzymes in chickens (Al-Aameli et al., 2020; Dalia et al., 2017). Sayrafi et al. (2017) demonstrated that the supplementation of nano-curcumin @ 200 mg/kg of diet declined the serum AST enzyme level in broiler chickens due to its antioxidant properties. Antioxidant property of curcumin against aflatoxin has been previously reported by Gowda et al., 2008 & 2009). Akbarian et al. (2012) and Qasem et al. (2016) who reported non-significant variation in LDH and the gamma-glutamyl transferase activities by supplementing curcumin to broiler chickens which is similar to Group III results. Rahman et al. (2020) observed significant decrease of AST and ALP in the broiler chickens due to the free radical scavenging activity of nano-curcumin. As there is a significant reduction in the serum liver enzyme activity in chickens fed with dietary additives of nano-curcumin and nano-selenium might be attributed to antioxidant role of these nano-nutraceuticals which may have positive role on liver enzymes.

Our results also agree with the findings of other researchers who observed no difference on total protein and albumin by supplementing chemical nano-selenium (Che-Se-NPs) up to 0.4 g/kg in quails (Alagawany et al., 2021). Circulatory concentration of glucose was not influenced by supplementation of different sources of selenium, i.e., organic, inorganic, and nano-selenium in broiler chickens (Boostani et al., 2015). Selenium supplementation in the form of organic selenium and sodium selenite at 0.3 ppm in feed did not affect serum total protein, albumin, globulin and albumin/globulin ratio in broiler chicken (Dalia et al., 2017). Supplementation of nano-curcumin in Group III did not alter significantly on the glucose, total protein and albumin concentration is in concurrence with the studies of Arshami et al. (2013) in chickens.

CONCLUSION

The study revealed that combined effects of nano-selenium and nano-curcumin could exert hepato-protective effect in broiler chickens.

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