Full Length Article

EFFECT OF NANO VITAMIN C SUPPLEMENTATION ON SERUM BIOCHEMICAL PROFILE OF JAPANESE QUAILS

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ABSTRACT

The present study was conducted to study the effect of dietary supplementation of vitamin C and nano vitamin C on the serum biochemical profile of Japanese quail during the summer season. The experiment was carried out on 96 day-old Japanese quail chicks which were divided randomly into three treatment groups. Each treatment contained two replicates with sixteen birds per replicate. The experiment was carried out for a period of six weeks during the summer season in May and June. Feed and water were provided ad libitum to study the effect of the inclusion of vitamin C (200 mg/kg) and nano vitamin C (20 mg/kg) on serum biochemical profile of Japanese quail during the summer season. The study revealed that the mean serum levels of total protein, albumin, globulin, HDL cholesterol and SGPT/ALT increased significantly (P<0.01); whereas, serum levels of total cholesterol, LDL cholesterol and triglycerides decreased significantly (P<0.01) in treatment groups supplemented with nano vitamin C. No significant differences (P>0.05) were observed in serum SGOT/AST among treatment groups.

Keywords: Cholesterol, Japanese quails, Nano vitamin C, Serum protein, SGOT and SGPT

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INTRODUCTION

Quails are popular because their flesh is high in protein (26%) and low in fat (3%) (Shinde *et al.,* 2014). Quails compete with broilers as a source of meat as the demand for animal protein has increased

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(Jaap, 1964). Quails experience heat stress when exposed to high outdoor temperatures, particularly during the summer months in India, which exhibit a higher frequency of heat waves with temperatures exceeding 35 °C. Elevated environmental temperatures have adverse effects on the physiological responses of poultry, leading to changes in blood chemistry, reduced productivity, and an increased mortality rate (Attia *et al.,* 2016 and Barrett *et al*., 2019). Vitamin C (ascorbic acid) is required for numerous biosynthetic reactions as well as for the regulation of body temperature and immune system activation. Under normal conditions, adult poultry can synthesize enough vitamin C to meet their needs. However, it has been noted that vitamin C requirements are increased during stress and various studies have shown that increasing ascorbic acid in poultry feeds has a favourable effect. Nanotechnology has received a great deal of attention in the last decade in the fields of diagnosis, medicine, and nutrition (Nabi *et al.,* 2020). Nano vitamin C, or nanoscale vitamin C, refers to a form of vitamin C that has been reduced to very small particle sizes at the nanometre scale. This technology involves breaking down vitamin C into nano particles, which can have unique properties and potential benefits. In the context of poultry farming, nano vitamin C is sometimes used as a dietary supplement for chicken and quails.

Therefore, a study was undertaken to observe the effect of vitamin C and nano vitamin C supplementation on serum biochemical profile in Japanese quails.

MATERIALS AND METHODS

The research work was conducted during the summer season in May and June to study the effect of dietary supplementation of nano vitamin C on the serum biochemical profile of Japanese quails. The biological experiment was carried out at the Department of Poultry Science, College of Veterinary Science, Tirupati. Lab analysis was carried out in the Departments of Veterinary Physiology, Department of Veterinary Biochemistry and Veterinary Anatomy of College of Veterinary Science, Tirupati and Frontier Institute of Technologies, Regional Agricultural Research Station (R.A.R.S), Tirupati. Commercial quail feed was procured from M/S. Suvera Hatcheries Private Limited. The nano form of vitamin C was prepared in Nanotechnology laboratories, R.A.R.S, Tirupati. The experiment was carried out on 96 day-old Japanese quail chicks obtained from Manvi Quails Breeding Farm and Hatchery. The quail chicks on arrival were weighed individually and distributed randomly into three treatment groups as given in Table 1. Each treatment contained two replicates with sixteen birds per replicate. The experiment was carried out for six weeks. Feed and water were provided *ad libitum*. The birds were housed in battery cages during the experiment period.

Ionic gelation was used for the preparation of chitosan-encapsulated vitamin C nanoparticles with minor modification in the method described by Duse *et al.* (2018). In a nutshell, 1 $mg \cdot mL^{-1}$ of chitosan was dissolved in 0.5 per cent acetic acid solution and stirred

Table 1. Experimental diets

S. No	Group	Quails/group	Treatment	Dose rate and schedule
1	C1	16x2	Basal diet (as per BIS, 2007)	No supplementation
2	T1	16x2	Basal diet + vitamin C	200 mg/kg
3	T2	16x2	Basal diet + nano vitamin C	20 mg/kg

continuously at 120 rpm throughout the night at room temperature. An aqueous solution of sodium tripolyphosphate (NaTPP) at a concentration of 1 mg·mL^{-1} was prepared. Vitamin C was dissolved in methanol at a concentration of 2 mg·mL^{-1} solution. The vitamin C encapsulated solution was prepared by stirring the chitosan-NaTPP solution at 600 rpm using a magnetic stirrer, and dropwise vitamin C solution was added, and the obtained nanoparticle suspension was agitated for 30 min and then was used in the further studies. Serum biochemical parameters estimated with MISPAVIVA chemistry analyzer by using the kits supplied by ERBA diagnostics (Mannheim, Germany) and Agappe Diagnostics Ltd., (Kerala, India) as per the manufacturer instructions. Two millilitre of whole blood was collected from jugular vein into a clot accelerator vacutainer. The samples were brought to laboratory and centrifuged at 2000 rpm for 5 minutes and the serum was separated in to a sterile eppendorf tube and stored.

Statistical analysis of the data was carried out according to the procedures suggested by Snedecor and Cochran (1989). The data obtained were subjected to one way ANOVA. Differences between means were tested at the 5% probability level using Duncan's LSD test. (Duncan 1955).

RESULTS AND DISCUSSION

Serum biochemical profile (g/dl)

The mean serum biochemical profile of Japanese quails reared from day old to 6 weeks of age by supplementation of vitamin C and nano vitamin C are presented in the Table 2.

The serum total protein levels in C1, T1 and T2 groups were 4.73, 5.21 and 5.31 (g/dl) respectively. Significantly (P<0.01) higher serum total protein was observed in T1 and T2 group compared to C1 group. There was no significant difference between the T1 and T2 groups.

The serum albumin levels in C1, T1 and T2 groups were 1.66, 1.73 and 1.92 g/dl, respectively. Significantly (P<0.01) higher serum albumin was observed in T2 group compared to C1 and T1 groups.

The serum globulin levels in C1, T1 and T2 groups were 3.07, 3.39 and 3.48 g/dl, respectively. Significantly (P<0.01) higher

serum globulin was observed in T2 and T1 groups compared to C1 group. There was no significant difference between T1 and T2 groups.

Serum lipid profile

The mean serum total cholesterol of Japanese quails reared from day-old to 6 weeks of age by supplementation of vitamin C and nano vitamin C are presented in the Table. 3.

The mean serum total cholesterol levels of Japanese quails in C1, T1 and T2 groups were 198.42, 190.51 and 180.40 mg/dl respectively. Significantly (P<0.01) lower serum total cholesterol was observed in T2 group compared to T1 group; while, highest (P<0.01) serum total cholesterol was observed in C1 group.

The mean serum HDL cholesterol levels of Japanese quails in C1, T1 and T2 groups were 99.45, 104.09 and 108.78 mg/dl, respectively. Significantly (P<0.01) higher serum HDL cholesterol was observed in T2 compared to T1 groups and significantly (P<0.01) lowest value of serum HDL cholesterol among all treatments was observed in C1 group.

The mean serum LDL cholesterol levels of Japanese quail in C1, T1 and T2 groups were 71.10, 59.40 and 46.47 mg/dl, respectively. Significant (P<0.01) differences existed between the mean values with C1 recording the highest value followed by T1 and T2.

The mean serum triglycerides of Japanese quail in C1, T1 and T2 groups were 140.13, 131.86 and 128.13 mg/dl, respectively. Significantly (P<0.01) lowest serum triglycerides levels was observed in T2 group with increasing (P<0.01) values in T1 and C1 groups.

Serum enzymes

The mean serum SGOT and SGPT levels of Japanese quails reared from day

Table 2. Mean (± S.E) serum biochemical profile of Japanese quails supplemented with vitamin C and nano vitamin C on 6th week

Treatment	Serum total protein(g/dl)	Serum albumin(g/dl)	Serum globulin(g/dl)
C1	4.73±0.06^{b}	1.66±0.04^{b}	3.07±0.09^{b}
T1	5.21±0.07^{a}	1.73±0.05^{b}	3.39±0.09^{a}
T2	5.31±0.04^{a}	1.92±0.06^{a}	3.48±0.05^{a}
SEM	0.05	0.03	0.05
n	10	10	10
SS	**	**	**

Values in column bearing different superscripts differ significantly; ** (P<0.01)

Table 3. Mean (±S.E) serum lipid profile of Japanese quail supplemented with vitamin C and nano vitamin C on 6th week.

Treatment	SERUM LIPID PROFILE			
	Serum total Cholesterol (mg/dl)	Serum HDL Cholesterol (mg/dl)	Serum LDL Cholesterol (mg/dl)	Serum Triglycerides (mg/dl)
C_1	198.42±1.2^{5}a	99.45±0.5^{0}c	71.10±1.3^{4}a	140.13±1.2^{2}a
T_1	190.51±1.3^{3}b	104.09±1.0^{3}b	59.40±2.0^{5}b	131.86±1.5^{2}b
T_2	180.40±1.1^{6}c	108.78±0.^{9}4a	46.47±1.^{6}4c	128.13±0.^{9}6c
SEM	1.53	0.85	2.09	1.16
n	10	10	10	10
SS	**	**	**	**

Values in columns bearing different superscripts differ significantly; ** (P<0.01)

Table 4. Mean (±S.E) SGOT and SGPT of Japanese quail supplemented with vitamin C and nano vitamin C from day old to 6 weeks.

Treatment	SGOT (AST) (IU/L)	SGPT (ALT) (IU/L)
C1	249.87±0.71	17.70±0.5^{1}a
T1	249.90±0.80	16.50±0.3^{4a}b
T2	248.64±0.88	15.20±0.6^{1}b
SEM	0.46	0.33
n	10	10
SS	NS	**

Values in column bearing different super scripts differ significantly ** (P<0.01), NS (P>0.05).

old to 6 weeks of age by supplementation of vitamin C and nano vitamin C are presented in the Table. 4.

The SGOT levels in C1, T1 and T2 groups were 249.87, 249.90 and 248.64 IU/L respectively. There was no significant difference among C1, T1 and T2 groups.

The SGPT levels in C1, T1 and T2 groups were 17.70, 16.50 and 15.20 IU/L respectively. Low level of serum SGPT was observed in T2 group when compared with C1 group. But T1 group had statistically similar serum SGPT compared to other two groups.

The serum biochemical profiles of total protein, albumin and globulin in serum of the quails supplemented with vitamin C (200 mg/kg) and nano vitamin C (20 mg/kg) increased significantly (P<0.01) compared to that of control group (Table 2). In agreement with the present findings, Khazaei *et al.* (2021) and Morsy (2018) reported significant (P<0.05) increase in the serum total protein with supplementation of vitamin E @ 250 mg/kg and vitamin C @ 500 mg/kg in the feed of Japanese quails. Singh *et al.* (2023) reported no significant difference in the serum albumin and globulin levels (g/dL) in birds supplemented with 200 mg/ltr vitamin E + 200 mg/ltr vitamin C, which may be due to variation in vitamins used and period of supplementation. Whereas, Seyrek *et al.* (2004) reported that, the albumin concentration was increased significantly (P<0.01), while globulin serum concentrations did not differ significantly in Japanese quails due to vitamin C supplementation. In contrary, Sigolo *et al.* (2019) reported a significant (P<0.05) decrease in serum total protein with supplementation of vitamin E and vitamin C @ 600 mg/kg feed in Japanese quails. The increase in serum total protein in the study may be attributed to the role of vitamin C in synthesis of collagen, a structural protein in connective tissues. An improvement in collagen synthesis might contribute to changes in total protein levels. Further, vitamin C enhances the absorption of non-heme iron from the gastrointestinal tract. This could potentially affect hemoglobin levels and, consequently, total protein levels.

In present study, the serum lipid profile such as serum total cholesterol, LDL cholesterol and serum triglycerides level of the birds supplemented with vitamin C (200 mg/kg) and nano vitamin C (20 mg/kg) decreased significantly (P<0.01) compared to control group whereas serum HDL cholesterol increased significantly (P<0.01) (Table 3). In agreement with the present findings, Sigolo *et al.* (2019) reported that supplementation of vitamin C at 800 or 1000 mg/kg level decreased serum total cholesterol and serum triglycerides (P< 0.01); whereas, serum HDL and LDL cholesterol are increased significantly (0.05) in Japanese quails. Chougule *et al.* (2020) reported that supplementation of vitamin C @ 250 mg/kg significantly (P<0.05) reduced the serum cholesterol levels when compared to the control group of broilers. In agreement with the present findings, Sheikh-Samani *et al*. (2016) reported that nano-multivitamin supplementation on broiler chickens' basal diet without vitamins premix supplementation significantly (P<0.05) reduced the triglyceride

levels of serum. It may be due to the antioxidant properties of vitamin C. vitamin C also influences cholesterol metabolism by inhibiting the synthesis of cholesterol in the liver.

The serum SGOT (AST) levels of the birds supplemented with vitamin C (200 mg/kg) and nano vitamin C (20 mg/kg) were not altered in the present study; whereas, SGPT (ALT) decreased significantly (P<0.01) due to vitamin C supplementation. Serum concentrations of transaminase enzymes (*i.e.*, AST and ALT), are commonly used as an index of liver disease so that increased AST and ALT levels are associated to hepatic damage (Scholtz *et al.,* 2009). In contrary to our results, Singh *et al.* (2023) reported that vitamin C supplemented group had significantly (P<0.05) higher ALT (U/L) and AST (U/L) values than the control broilers. Sigolo *et al.* (2019) also reported a significant (P<0.05) decrease in AST and ALT with supplementation of vitamin E and vitamin C @ 600 mg/kg feed in Japanese quails.

CONCLUSION

The present study revealed that the mean serum total protein, serum albumin, serum globulin, serum HDL cholesterol, serum SGPT/ALT increased significantly (P<0.01), whereas serum total cholesterol, serum LDL cholesterol and serum triglycerides decreased significantly (P<0.01) in treatment groups supplemented with nano vitamin C. No significant differences (P>0.05) were observed in serum SGOT/AST among treatment groups.

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