Indian J Anim Health (2024), 63(2): 326- 333
DOI: https://doi.org/10.36062/ijah.2024.13323

Research Article

Effect of L-ascorbic acid and α-tocopherol on biochemical changes and mRNA expression of IGF-I, caspase-2 and survivin genes in Japanese quail during induced stress

N. Shit[1*], K. V. H. Sastry[1], G. Singh[2] and J. Mohan[1]

[1]Division of Physiology and Reproduction, Central Avian Research Institute, Izatnagar- 243 122, Uttar Pradesh, India; [2]Division of Veterinary Physiology and Climatology, Indian Veterinary Research Institute, Izatnagar- 243 122, Uttar Pradesh, India

Abstract

Anti-oxidant vitamins evidently alleviate stress-regulated changes in avian biology, but the underlying molecular mechanism is still yet to be explored. Aiming to this, the study assessed the effect of anti-oxidant vitamins on expression patterns of stress-regulated genes in Japanese quail during stress. One hundred and forty-four Japanese quails (10 weeks) were equally divided into four groups, *i.e.* Gr I (control), Gr II (positive control), Gr III and IV, subjected to induced stress for 10 days. Anti-oxidant vitamins, *i.e. L*-ascorbic Acid (L-AA) and α-tocopherol (α-TP) @ 250ppm, were supplied through drinking water to Gr-II and Gr-IV for a period of 10 days. Six birds were sacrificed each on 1, 2, 4, 6, 8 and 10 days. Samples were screened for biochemical changes and gene expression patterns using Quantitative RT-PCR. The mean glucose concentration differed significantly ($p<0.05$) among the treatment groups. The corticosterone was significantly ($p<0.05$) higher in Gr-III and Gr-IV, while the mean T_3 concentration declined significantly in stressed birds with evidently low magnitude in Gr-IV. Though the fold expression of the IGF-1 gene was significantly ($P<0.05$) downregulated in hierarchical (F_1, F_2 and F_3) follicles, it was obviously less severe in Gr-IV. The expression pattern of caspase-2 was equally sensitive and showed highest magnitude in largest follicle from Gr-IV. The expression pattern of the surviving gene was significantly ($P<0.05$) upregulated since the intensity of expression was noticeably less in Gr-IV. This result concludes that anti-oxidant vitamins may impact the biochemical pathways and alleviate the stress-regulated molecular changes in Japanese quail.

Keywords: Anti-oxidant vitamins, Gene-expression, Japanese quail, Stress

Highlights

- The mean serum glucose level was initially increased, but it declined thereafter in stressed birds.
- The corticosterone concentration was higher in Gr-III and IV, but the change was less severe in later groups.
- Since, T_3 concentration was reduced significantly but T_4 level remains at par among the treatments.
- The fold expression of IGF-1 gene was down-regulated in hierarchial follicle while reverse pattern was noticed in caspase-2 expression.
- The smallest follicle revealed the highest magnitude of expression in survivin gene.

INTRODUCTION

Japanese quail (*Coturnix coturnix japonica*) has added diversification in the chicken dominated poultry industry and shares 0.13% (0.29×10^6) of the total poultry population of the country (GoI, 2012). This small creature has also been considered a model animal for bio-medical research for its early genetic makeup, resistance to emerging and re-emerging chicken diseases, etc. Under standard husbandry practices, they attain highest body weight at 64 days of age with highest amplitude of mRNA expression of IGF-1 and survivin gene (Shit *et al*., 2014). Interestingly, like other domesticated avian species, quails are equally sensitive, susceptible to stress, and adversely affected for reproductive functions. Stress-induced corticosteroids and catecholamines decrease glucose utilization and alter the hormonal profile, which subsequently reduces the rate of fertility and hatchability (Ahmed *et al*., 2008). The induced stress evidently reveals a significant reduction of both tri-iodothyronine (T_3) and sex steroids concentration with an increasing trend of corticosterone in White Leghorn hens (Agarwal *et al*., 2013) and Japanese quail (Shit *et al*., 2017). Sahin and Kucuk (2001) reported that dietary supplementation of natural antioxidants has been found to be effective in increasing feed efficiency

*Corresponding Author, E-mail: drnonigopal@gmail.com

and ameliorating detrimental effects of stress on reproduction in Japanese quail. Literally, vitamin-C (L-ascorbic acid) is synthesised in the kidneys of birds and reptiles and plays a critical role in stress alleviation because of its reducing properties and functions as an electron donor and participation in redox and hydroxylation pathways acting as a cofactor for cytochrome p450 dependent hydroxylase. L-ascorbic acid is essential to avian species during stress, assuming as a co-factor for the bio-conversion of vitamin D to 1,25 dihydroxy cholecalciferol to compensate for the homeostasis mechanism during stress. Stress damages cells by ROS induced lipid peroxidation of PUFA and accelerates cellular atresia. It suppresses the induction of atresia induced by adrenalin mediating alpha-receptors through *in-vitro* controlled experimentation in chicken (Moudgal *et al*., 1985). Co-incidence to this phenomenon, vitamin E (α-tocopherol) also inhibits the lipid peroxidation of PUFA by its intra-membrane antioxidant properties and alleviates the stress effect. They improve general performance, innate immunity, and reproductive efficiency in various poultry species, such as ducks, turkeys, chickens, and Japanese quail, upon dietary supplementation. Shit *et al*. (2017) has concluded a significant upregulation in relative expression of caspase-2 and Bcl-XL in Japanese quail under stress. Research revealed a synergistic effect of L-ascorbic acid and α-tocopherol in alleviating the negative impact of heat stress on growth performance and immunity in chickens. The L-ascorbic acid and α-tocopherol have synergistically counteracted the negative impact of stress on reproductive function (Shit *et al*., 2020). In-Surk *et al*. (2014) reported that dietary supplementation with vitamin C (L-AA) resulted in a significant decrease in the mRNA expression of pro-inflammatory cytokines and HSP70, and higher antioxidant parameters over the control birds under summer conditions. Studies indicated that HSPs undergo downregulation in response to dietary antioxidant vitamins, which could be associated with the modulation of pro-inflammatory cytokine expression. Shit *et al.* (2012) reported a noticeable change in the egg quality parameters and fertility percentage in Japanese quail subjected to dietary supplementation of L-ascorbic acid at low ambient temperature. However, the underlying endocrine and molecular mechanism associated with antioxidant vitamins for stress alleviation is yet to be investigated in Japanese quail. So, the present study was designed to explore the impact of antioxidant vitamins on physio-biochemical changes and expression profile of stress allied genes in Japanese quail.

MATERIALS AND METHODS

Experimental birds and management: A total of one hundred forty-four Japanese quail hens (10 weeks) from the same hatch were used for this study. They were split equally into four groups, i.e. Group I, II, III and IV, with an account of 36 hens/group. They were maintained in individual cages with 14:10 h photo-schedule. The experiment was conducted in accordance with the guidelines of "The Animal Ethics Monitoring Committee" of the Institute ICAR-Central Avian Research Institute.

Induction of stress: Birds from Group-I received *ad-libitum* quail layer ration (ME-2716 Kcal/kg, CP-20.03%, Ca-3.06%, P-0.33%, lysin-1.0% and methionine-0.45%) and served as untreated control. Positive control was maintained (Group-II) by fortified layer ration @ 250 ppm each of L-ascorbic acid and α-tocopherol. Stress was induced by feed withdrawal (Bell, 2003) for the period of 10 days assuming complete cessation of production and regression of reproductive tissue with *ad-libitum* drinking water in birds for both Group-III and IV while L-AA and α-TP were available @ 250 ppm each to the birds of Group-IV only.

Sampling for biochemical and expression studies: Following cervical dislocation, samples *i.e*. serum for biochemical assay and stress regulated estrogen dependent follicular tissues (F_1, F_2 and F_3) for expression study were collected from six birds per group on 1, 2, 4, 6, 8 and 10 days of the experiment. Immediately after the draining of the yolk material, the follicular membrane was rinsed with ice-cold saline to spill out the remnants of yolk material and used for expression study. Without unravelling granulosa and theca layers, tissue samples were incubated separately in RNA stabilization solution (RNAlater, Ambion Inc., USA) at 4°C overnight and processed for RNA isolation following the manufacturer's guidelines.

Gene-expression by RT-qPCR: Total RNA extraction from each follicle was performed in follicular tissues using Trizol reagent according to the manufacturer's protocol (Invitrogen, USA). The RNA integrity was checked by 1% denaturing agarose gel electrophoresis. The concentration and purity of RNA preparations were measured through NanoDrop (Thermo2000), and the ratio of 260/280 was more than 2.0 for all the samples. Each RNA sample (5 µg) was treated with 5U of RNase-free DNase (Biogene, USA) at 37°C for 1 h to make it free from traces of genomic DNA contamination and

Table 1. Primer pair sequences with the amplicon size (bp) and annealing temperature (°C) used for expression study by real-time PCR

Gene	Primer sequences	Annealing temp. (°C)	Amplicon size (bp)	Reference/ Accession no
IGF-1	F-5´TGTATTGTGCTCCAATAAAGC R-5´CTGTTTCCTGTGTTCCCTCTACTTG	58	127	Guernec *et al*., 2003
Caspase 2	F-5´TTGATTGTGGAACATTCTTTGG R-5´CACTGCTGAAATGGATATTGC	56	163	HM587999
Survivin	F-5´TCGAAGATGTAGCCAAGG R-5´CAGCGTGGCAGTGTC	56	98	HM588003
Beta-actin	F-5´GGAAGTTACTCGCCTCTG R-5´AAAGACACTTGTTGGGTTAC	58	114	GU230784

subsequently inactivated by incubation at 65°C for 10 min. Each DNase-treated total RNA sample (1 µg) was reverse transcribed using the "Revert Aid First strand cDNA synthesis kit" (MBI Fermentas, USA) according to the manufacturer's instructions. The resultant cDNA was stored frozen at -20°C till used. Negative controls were performed using all components except reverse transcriptase.

After reverse transcription into cDNA, it was quantified using real-time quantitative polymerase chain reaction (RT-qPCR) using Syber Green master mix in IQ5 cycler (Bio-Rad). The amplification of mRNAs was carried out by gene-specific primers designed from coding region of chicken mRNA sequences available in Gene Bank considering the close relationship between chicken and quail in phylogeny and sequences. The sequence of forward and reverse primers for IGF-1, caspase-2, survivin and β-actin are shown in Table 1.

For RT-qPCR assay, the reaction was set in triplicate with each cDNA sample. The amplification was carried out in 25 µL volume containing 1x QuantiTect SYBR Green PCR master mix (Syber Green1dye, Hot Start Taq DNA polymerase and dNTPs in optimized buffer components; QIAGEN GmBH), a 0.2-mM concentration of each gene-specific primer and 1 µL of cDNA template. The cycling conditions of RT-qPCR include initial denaturation of 95°C for 10 minutes followed by 40 cycles of denaturation of 95°C for 30 seconds, annealing for 30 seconds and extension of 72°C for 45 seconds. For each gene of interest, negative and positive controls were included. A melting curve was performed for each sample after completion of amplification and analyzed in comparison with negative and positive controls to determine the specificity of polymerase chain reaction. Each RT-qPCR experiment contained triplicates of test samples, one no-template control and a $\log_{10}$ dilution series. Regression analysis of the standard curve was used to calculate the slopes of the gene-specific $\log_{10}$ dilution series. The relative expression was determined following the delta C_t method (Pfaffl, 2002).

Biochemical assay: Serum was extracted following standard procedure and used for biochemical assay. The biochemical value of serum glucose was determined by GOD-POD method. The serum concentration of thyroid hormones (T_3 and T_4) and corticosterone were assessed by enzyme immunoassay kits (United Biotech Inc.) following the manufacturer's instructions.

Statistical analysis: The data generated during the course of the study were analysed using one-way ANOVA (v. 20) and means compared using Duncan's multiple range tests (Duncan, 1955). The relative mRNA expression was normalized against ß-actin, the reference gene and the fold expression of the gene of interest was calculated according to Pfaffl (2002).

RESULTS

Effect of glucose: The mean serum glucose concentration ranges between 213.85 and 277.71 mg/dL (Table 2). An increasing trend of the concentration was noticed up to day 4 in Gr-III and Gr-IV which was decreased gradually towards the end of the study. A significant difference ($P<0.05$) was evident among the treatment groups.

Effect of corticosterone: The assessment of serum corticosterone showed a change in concentration from 1.10 – 2.88 ng/mL among the studied groups (Table 3). The mean concentration was significantly

Table 2. The serum concentration of glucose (mean±SE) in control and vitamins supplemented groups of Japanese quail during induced stress (n=6)

Glucose concentration (mg/dL)				
Days	**Group I**	**Group II**	**Group III**	**Group IV**
Day 1	228.86±14.11	226.93±4.62	235.3±9.4	224.29±13.31
Day 2	226.66^{p}±13.09	217.86^{p}±5.04	270.85^{r}±12.91	239.79^{q}±8.01
Day 4	213.85^{p}±13.73	221.96^{p}±4.91	277.71^{r}±10.42	256.2^{q}±18.17
Day 6	222.27^{p}±11.71	218.51^{p}±12.71	260.9^{q}±16.21	252.4^{q}±8.02
Day 8	226.79^{p}±6.99	214.08^{p}±12.04	254.49^{q}±7.35	243.59^{pq}±11.05
Day 10	225.99^{p}±13.12	215.44^{p}±5.15	251.97^{q}±7.04	237.16^{pq}±9.63

$^{p\text{-}r}$Means bearing different superscripts (group-wise) differ significantly (P<0.05)

Table 3. The serum concentration of corticosterone (mean±SE) in control and vitamins supplemented groups of Japanese quail during induced stress (n=6)

Corticosterone concentration (ng/mL)				
Days	**Group I**	**Group II**	**Group III**	**Group IV**
Day 1	1.33±0.29	1.31±0.22	1.48±0.24	1.53±0.29
Day 2	1.37^{p}±0.05	1.21^{p}±0.06	2.71^{r}±0.34	2.13^{q}±0.23
Day 4	1.25^{p}±0.12	1.17^{p}±0.14	2.88^{r}±0.26	1.93^{q}±0.25
Day 6	1.27^{p}±0.19	1.33^{p}±0.27	2.79^{r}±1.04	1.93^{q}±0.24
Day 8	1.23^{p}±0.09	1.13^{p}±0.11	1.85^{r}±0.11	1.67^{q}±0.18
Day 10	1.36^{p}±0.07	1.10^{p}±0.13	1.65^{q}±0.27	1.59^{pq}±0.12

$^{p\text{-}r}$Means bearing different superscripts (group-wise) differ significantly (P<0.05)

Table 4. The serum concentrations of tri-iodothyronine (mean±SE) in control and vitamins supplemented groups of Japanese quail during induced stress (n=6)

Tri-iodothyronine concentration (ng/dL)				
Days	**Group I**	**Group II**	**Group III**	**Group IV**
Day 1	123.29±3.41	129.49±3.33	119.48±5.31	121.00±7.51
Day 2	108.13±1.11	120.12±1.72	118.82±4.13	109.04±7.04
Day 4	126.01^{qr}±1.41	139.24^{r}±3.01	101.9^{p}±1.21	112.75^{q}±6.02
Day 6	129.22^{r}±2.4	123.16^{r}±2.14	93.34^{p}±1.87	106.17^{q}±8.73
Day 8	125.11^{qr}±3.2	135.65^{r}±3.01	74.43^{p}±2.71	109.47^{q}±3.42
Day 10	113.07^{qr}±4.7	126.45^{r}±2.63	73.69^{p}±2.47	102.88^{q}±1.24

$^{p\text{-}r}$Means bearing different superscripts (group-wise) differ significantly (P<0.05)

Table 5. The serum concentrations of thyroxine (mean±SE) in control and vitamins supplemented groups of Japanese quail during induced stress (n=6)

Thyroxine concentration (ng/mL)				
Days	**Group I**	**Group II**	**Group III**	**Group IV**
Day 1	13.65±0.34	14.93±1.55	11.54±0.68	12.8±0.38
Day 2	11.38±0.66	16.39±1.01	13.12±0.16	14.15±0.91
Day 4	12.98±0.28	16.5±1.07	11.59±0.20	14.55±0.33
Day 6	14.26±0.26	12.44±0.52	12.8±0.22	12.57±0.99
Day 8	12.39±0.37	15.03±0.73	15.11±0.38	13.95±2.26
Day 10	13.27±0.74	11.62±0.41	13.96±0.42	13.1±1.21

(P<0.05) increased in both the stressed groups, i.e. Gr-III and IV, till the 4th day of the experiment, and was found to be comparatively higher than the untreated and positive control groups throughout the study.

Effect of thyroid hormones: A significant (P<0.05) reduction in mean T_3 concentration was recorded among the birds in Gr-III and IV from the 4th day of the experiment, and the change was drastic in the earlier group at any point in time (Table 4). The mean concentration between untreated and positive control ranged from 108.13 – 139.24 ng/dL and changed invariably throughout the study. The mean serum thyroxine (T_4) concentration estimated among the groups ranged between 11.38 – 16.39 ng/mL (Table 5). However, no statistical changes were observed in any of the groups throughout the study period.

Expression of IGI-1: The fold expression of the IGF-1 gene was significantly (P<0.05) downregulated in all the hierarchical (F_1, F_2 and F_3) follicles from Gr-III and Gr-IV on the 2nd and 4th day of the experiment, respectively (Fig. 1). However, due to follicular atresia, the expression study in Gr-III could not be performed on the 6th day onwards since a similar trend of changes was noticed in Gr-IV till the 8th day of the experiment. No statistical changes in mRNA expression of IGF-1 gene were observed among untreated and positive control groups.

Expression of caspase-2: The caspase-2 was significantly (P<0.05) upregulated in F_1 and F_2 follicle of group III from day 2

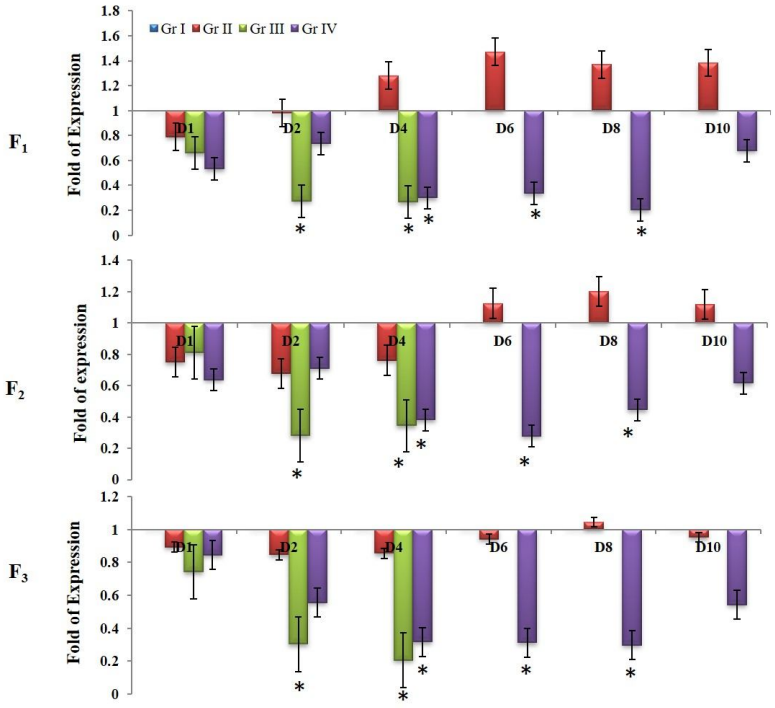

Fig. 1. The mRNA expression of IGF-1 gene in hierarchial follicles (F_1, F_2 and F_3) of Japanese quail under different treatments during induced stress (mean±SE, N=6). *Significant at p<0.05

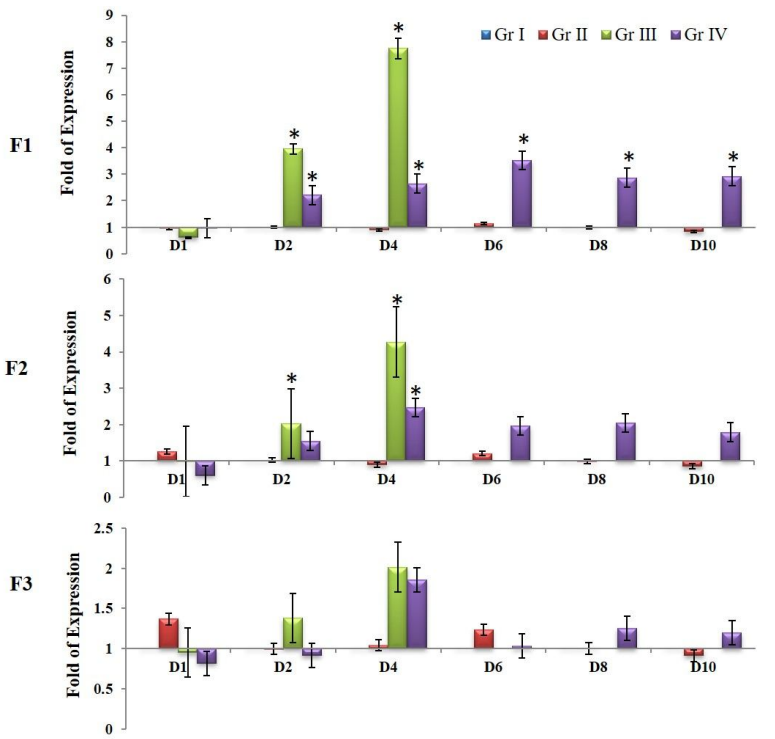

Fig. 2. The mRNA expression of caspase-2 gene in hierarchial follicles (F_1, F_2 and F_3) of Japanese quail under different treatments during induced stress (mean±SE, N=6). *Significant at p<0.05

(Fig. 2). The magnitude of expression was highest in F_1 follicle and reached to 7.75-fold. The F_1 follicle received from group IV revealed an equal sensitivity and showed a significant up-regulation in the mRNA expression of caspase-2 gene. However, no statistical difference was seen in the F_3 follicle in both groups III & IV. The expression patterns in all hierachial follicles under control and positive control did not show any noticeable change throughout the study.

Expression of survivin: The survivin was significantly (P<0.05) upregulated in hierarchical follicles of group IV from day 1 onwards, and evidently, the smaller follicles showed higher sensitivity (Fig. 3). The result showed the maximum upregulation on day 4 with the highest magnitude of expression (14-fold) in the F_3 follicle. However, noticeable changes were absent in all other studied groups.

DISCUSSION

The unpredictable component of life, so-called stress, causes an activation of HPA axis that adversely affects avian reproduction by altering neuro-endocrine balance (McEwen and Wingfield, 2003). Stress response causes follicular atresia in birds by eliciting the downstream pathways of caspase cascade (Anish *et al.*, 2008). The effect of anti-oxidant vitamins, i.e. vitamins C & E, have been commonly used as ameliorating agents to counter the adverse effects of stress on production and reproductive performance (Gous and Morris, 2005).

The mean circulating glucose level was recorded higher up to day 4 in both the vitamin-supplemented groups, *i.e.* Gr-III and Gr-IV, which decreased thereafter towards the end of this study (Table 2). According to Dupont *et al*. (2004), avian species have fewer pancreatic ß-cells and low levels of plasma insulin that may elicit increased levels of serum glucose. It is established that the rates of glucose utilization per unit body mass in fasted chickens have been found to be lower than in the fed state, which bears evidence

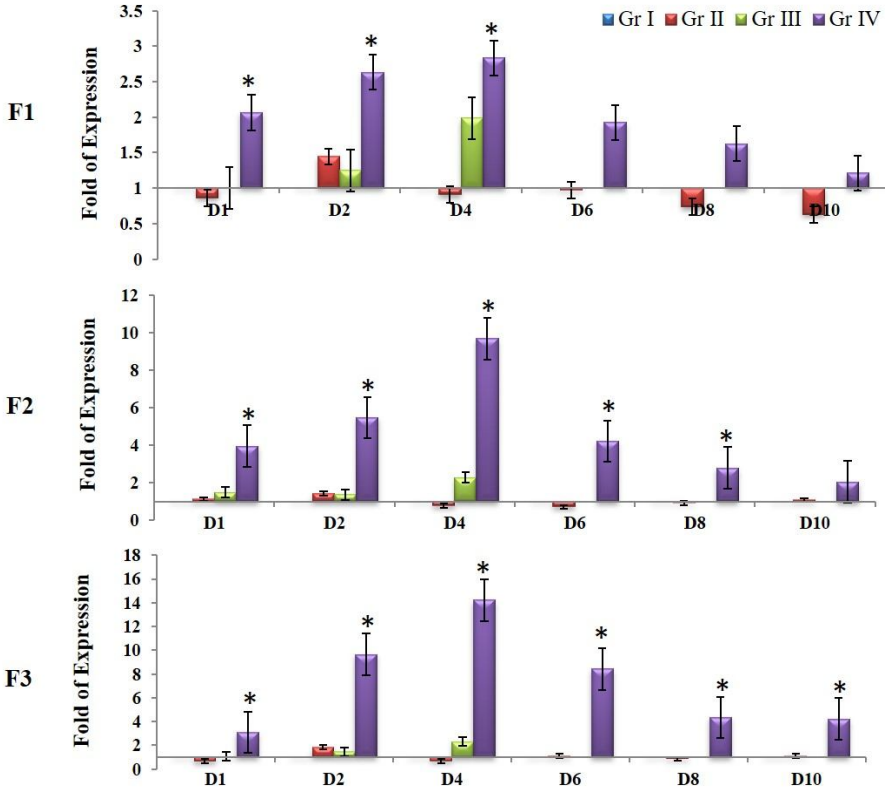

Fig. 3. The mRNA expression of survivin gene in hierarchial follicles (F_1, F_2 and F_3) of Japanese quail under different treatments during induced stress (mean±SE, N=6). *Significant at p<0.05

of higher glucose levels during stress (Brady *et al*., 1978). The stress-mediating events accelerates metabolic changes such as hepatic glycogenolysis, protein catabolism and gluconeogenesis to mobilize energy reserves to assist an individual in evading the stressors (Razdan, 2003) that could be correlated with the present findings. Seyrek *et al*. (2004) showed a marked decrease in blood glucose levels by vitamin C and E supplementation in heat-stressed Japanese quail.

Corticosterone is the principal steroid hormone of the avian adrenal cortex which increases during stress and extends adjustment to stressors by affecting locomotion, feed intake, fluid balance and energy mobilization (Cockrem *et al*., 2004). In the present study, a higher level of serum corticosterone was noticed in the birds from Gr-III and Gr-IV supplemented with antioxidant vitamins throughout the experimental period (Table 3). However, the mean corticosterone levels are relatively lower in the un-supplemented groups. Ascorbic acid inhibits glucocorticoid synthesis by inhibiting mitochondrial steroid 11b-hydroxylation and microsomal 21-hydroxylation in beef adrenals. Therefore, the vitamin supplementation in the present investigation might have helped partially with the ameoleration of stress effects in the Japanese quail.

Thyroid hormones (T_3 and T_4) are the prime metabolic hormones in vertebrates and are involved in a wide range of fundamental physiological activities including thermoregulation. It is well established that stress-regulated metabolic alteration suppresses the feed intake, which could be co-related to the report that starvation downregulates the content of TSH- mRNA in the pituitary gland and causes a rapid decrease of T_3 but not T_4 in male Japanese quail (Kobayashi and Ishii, 2002). The mean serum T_3 concentration in both vitamin-supplemented treatment groups (III and IV) reduced significantly ($P<0.05$) on the 2nd, 8th and 10th day of the experiment (Table 4) while the T_4 levels varied inconsistently throughout the experimental period (Table 5). It is established that stress decreases T_3 and increases T_4 levels, respectively (Kobayashi and Ishii, 2002). However, in comparison to un-supplemented birds (Table 4), the reduction in T_3 levels was less in both the supplemented treatment groups. Such improvement in hormone levels could be due to the positive effect of vitamins in restoring type 2 deiodinase (Dio2) enzyme activity which helps in spontaneous conversion of thyroid hormones (Yoshimura *et al*., 2003).

IGF-1 is synthesized by various tissues via autocrine and paracrine mechanisms to affect an array of anabolic pathways (McMurtry *et al*., 1997). In the present study, the expression of IGF-1 was significantly downregulated in group III on the 2nd and 4th days of the experiment, while group IV followed a similar trend in the expression on days 6 and 8 (Fig. 1). No conclusive effect of vitamins was noticed in the expression of IGF-1 gene. This observation could be due to the short duration of vitamin supplementation, which is unable to counteract the negative effect of

stress on IGF-1 expression in hierarchical follicles. However, exact underlying mechanism of such findings needs further study.

Caspase-2 is a nuclear resident protein which triggers apoptosis by release of mitochondrial cytochrome c and other apoptogenic factors into the cell cytoplasm (Guo *et al*., 2002). Evidently, stress-induced intra-cellular nitric oxide (NO) accumulation proportionately accelerates the activation of the caspases cascade, which directly leads to reproductive tissue regression via apoptosis in mammals (Skarzynski *et al*., 2005). The caspase-2 was significantly ($P<0.05$) upregulated in the hierarchical follicles of both vitamin-supplemented treatment groups. However, the maximum expression level was noticed in group III on day 4 when follicles were atretic than in group IV (Fig. 2). This result suggests an insufficient response of vitamins to counteract the adverse effect of stress in group III, resulting in activation of downstream caspase cascade pathways and follicular atresia (Anish *et al*., 2008; Sundaresan *et al*., 2008). Supplementation of vitamin C and E (group IV) might have reduced the follicular atresia by reducing caspase-mediated apoptosis during immobilization stress.

Survivin is a bifunctional inhibitor of apoptosis protein with single baculovirus inhibitor of apoptosis protein (IAP) repeats. Evidently, this protein seemed to be involved in replacing smac/DIABLO death domain from the binding site of initiator caspases. Further, because of its BIR domain, these IAPs bind to the initiator caspases and inhibit receptor dependent extrinsic pathways of apoptosis (Wheatley and McNeish, 2005). Evidently, survivin has been found to inhibit apoptosis either by inhibiting the cytochrome-c induced proteolytic processing events in the cytoplasm or by directly interfering with the activities of terminal effector cell death protease such as caspases-3, -7 and -9 (Shin *et al*., 2001). The over expression of survivin is only reported during embryonic and foetal tissues, whereas it remains to be downregulated or un-detectable in many normal adult tissues (Ambrosini *et al*., 1997). In this study, the mRNA of survivin was significantly upregulated in hierarchical follicles of group IV from day 1 till 4th day of the experiment. It is noteworthy to mention that a similar pattern of expression was noticed in the F_2 and F_3 follicles of group III on the 4th day of the experiment (Fig. 3). However, no conclusive effect of anti-oxidant vitamins was perceived in the mRNA expression of survivin in hierarchical follicles from both the supplemental treatment groups.

The findings of the present study suggested that the mean serum glucose level was increased initially, which declined thereafter in stressed birds. The corticosterone concentration was higher in Gr-III and IV, but the change was less severe in later groups because of the antioxidant properties of the vitamins. Since, T_3 concentration was reduced significantly but T_4 level remains at par among the treatments. The fold expression of IGF-1 gene was downregulated in hierarchial follicle while reverse pattern was noticed in caspase-2 expression. The smallest follicle revealed the highest magnitude of expression in survivin gene. This result depicts that anti-oxidant vitamins may impact the biochemical pathways and alleviate the stress-regulated biochemical and molecular changes in Japanese quail.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: NS: Investigation, data generation, preparing original draft; KVHS: Conceptualization, data curation, supervision and final editing; GS, JM: Statistical analyses, methodology and editing.

Data availability statement: Data sets generated during this research are available with the corresponding author on reasonable request.

Ethical statement: The study was approved by the Animal Ethics Monitoring Committee of Central Avian Research Institute, Izatnagar (U.P.).

ACKNOWLEDGEMENT

The authors like to thank the Director, ICAR-CARI, Izatnagar for patronizing the research work. Dr. (Mrs) Mohini Saini is thankfully acknowledged for extending logistic support to carry out the experiment.

REFERENCES

Agarwal R, Sastry KVH, Tripathi V, Singh R, Saxena R *et al*., 2013. Expression profile of luteinizing hormone receptor gene in hierarchal follicles and regressing oviduct tissues of White Leghorn hens during moulting. Reprod Domest Anim, 48: 278-283, doi: 10.1111/j.1439-0531.2012. 02145.x

Ahmed W, Ahmed S, Ul-Hau A and Kamran Z, 2008. Response of laying hens to vitamin C supplementation through drinking water under sub-tropical conditions. Avian Biol Res, 1(2): 59-63, doi: 10.3184/175815508X360461

Ambrosini G, Adida C and Altieri DC, 1997. A novel anti-apoptosis gene, Survivin, expressed in cancer and lymphoma. Nat Med, 3: 917-921, doi: 10.1038/nm0897-917

Anish D, Sastry KVH, Sundaresan NR, Saxena VK, Singh R *et al*., 2008. Reproductive tissue regression: involvement of caspases, inducible nitric oxide synthase and nitric oxide during moulting in White Leghorn hens. Anim Reprod Sci, 104: 329-343, doi: 10.1016/j.anireprosci.2007.02.020

Bell DD, 2003. Historical and current molting practices in the U.S. table egg industry. Poult Sci, 82: 965-970, doi: 10.1093/ps/82.6.965

Brady LJ, Romsos DR, Brady PS, Bergen WG and Leveille GA, 1978. The effects of fasting on body composition, glucose turnover, enzymes and metabolites in the chicken. J Nutr, 106: 648-657, doi: 10.1093/jn/108.4.648

Cockrem JF, Adams DC, Bennett EJ, Candy EJ, Henare SJ *et al*., 2004. Endocrinology and the conservation of New Zealand birds. In: Experimental Approaches to Conservation Biology (Gordon MS, Bartol SM, eds). University of California Press, Los Angeles, pp 101-121

Duncan, BD, 1955. Multiple range and multiple F tests. Biometrics, 11: 1-12

Dupont J, Dagou C, Derouet M, Simon J and Taouis M, 2004. Early steps of insulin receptor signaling in chicken and rat: apparent refractoriness in chicken muscle. Dom Anim Endocrinol, 26: 127-142, doi: 10.1016/j.domaniend.2003.09.004

GoI, 2012. 19th Livestock Census – 2012 All India Report. Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries, Krishi Bhawan, New Delhi

Gous RM and Morris TR, 2005. Nutritional interventions in alleviating the effects of high temperatures in broiler production. World's Poult Sci J, 61(3): 463-466, doi: 10.1079/WPS200568

Guernec A, Berri C, Chevalier B, Wacrenier-Cere N, Le-Bihan-Duval E *et al*., 2003. Muscle development, insulin-like growth factor-I and myostatin mRNA levels in chickens selected for increased breast muscle yield. Growth Horm IGF Res, 13(1): 8-18, doi: 10.1016/s1096-6374(02)00136-3

Guo Y, Srinivasula SM, Druilhe A, Fernandes-Alnemri T and Alnemri ES, 2002. Caspase-2 induces apoptosis by releasing proapoptotic proteins from mitochondria. J Biol Chem, 227(16): 13430-13437, doi: 10.1074/jbc.m108029200

In-Surk J, Young-Hyun K, Yang-Soo M and Sea-Hwan S, 2014. Effects of vitamin C or E on the pro-inflammatory cytokines, heat shock protein 70 and antioxidant status in broiler chicks under summer conditions. Asian-Australas J Anim Sci, 27(5): 749-756, doi: 10.5713/ajas.2013.13852

Kobayashi M and Ishii S, 2002. Effect of starvation on gonadotrophin and thyrotropin subunit mRNA levels and plasma hormone levels in the male Japanese quail (*Coturnix coturnix japonica*). Zoolog Sci, 19(3): 331-342, doi: 10.2108/zsj.19.331

McEwen BS and Wingfield JC, 2003. The concept of allostasis in biology and biomedicine. Horm Behav, 43(1): 2-15, doi: 10.1016/s0018-506x(02)00024-7

McMurtry JP, Francis GL and Upton Z, 1997. Insulin-like growth factors in poultry. Domest Anim Endocrinol, 14(4): 199-229, doi: 10.1016/s0739-7240(97)00019-2

Moudgal RP, Razdan MN, Kajal S and Singal SP, 1985. Effect of ascorbic acid and adrenergic receptor blockers on adrenaline induced *In vitro* follicular atresia in White Leghorn hens. Indian J Exp Biol, 23(6): 343-344

Pfaffl MW, Graham W, Horgan L and Dempfle L, 2002. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in realtime PCR. Nucleic Acids Res 30: 62-66, doi: 10.1093/nar/30.9.e36

Razdan P, 2003. Stress and early pregnancy in sows. Effects on endocrinology, ova transport and embryo development. In Doctoral thesis, Veterinaria 153, Acta Universitatis Agriculturae Sueciae, Swedish University of Agricultural Sciences, Uppsala

Sahin K and Kucuk O, 2001. Effects of vitamin C and vitamin E on performance, digestion, of nutrients and carcass characteristics of Japanese quails reared under chronic heat stress (34°C). J Anim Physiol Anim Nutr, 85: 335-341, doi: 10.1046/j.1439-0396.2001.00339.x

Seyrek K, Yenisey C, Serter M, Kargin-Kiral F, Ulutas PA *et al*., 2004. Effect of vitamin C supplementation on some serum biochemical parameters of laying Japanese quails exposed to heat stress (34.8°C). Revue Med Vet, 155(6): 339342

Shin S, Sung BJ, Cho YS, Kim HJ, Ha NC *et al*., 2001. An anti-apoptotic protein human survivin is a direct inhibitor of caspase-3 and -7. Biochemistry, 40(4): 1117-1123, doi: 10.1021/bi001603q

Shit N, Sastry KVH, Singh G and Mohan J, 2020. Effect of L-ascorbic acid and alpha-tocopherol on ovarian regression, hormonal changes and gene expression in Japanese quail during stress. Explor Anim Med Res, 10(1): 32-41

Shit N, Sastry KVH, Singh G, Mandal R and Mohan J, 2017. Studies on metabolic hormones, sex steroids and mRNA expression of caspase 2, Bcl-XL gene in ovarian follicles of Japanese quail hens during stress. Indian J Poult Sci, 52(1): 70-75, doi: 10.5958/0974-8180.2017.00017.4

Shit N, Sastry KVH, Singh RP, Pandey NK and Mohan J, 2014. Sexual maturation, serum steroid concentrations, and mRNA expression of IGF-1, luteinizing and progesterone hormone receptors and survivin gene in Japanese quail hens. Theriogenology, 81: 662-668, doi: 10.1016/j.theriogenology.2013.12.011

Shit N, Singh RP, Sastry KVH, Agarwal R, Singh R *et al*., 2012. Effect of dietary L-ascorbic acid (L-AA) on production performance, egg quality traits and fertility in Japanese quail (*Coturnix japonica*) at low ambient temperature. Asian Australas J Anim Sci, 25(7): 1009-1014, doi: 10.5713/ajas.2011.11254

Skarzynski DJ, Jaroszewski JJ and Okuda K, 2005. Role of tumor necrosis factor-alpha and nitric oxide in luteolysis in cattle. Domest Anim Endocrinol, 29: 340-346, doi: 10.1016/j.domaniend.2005.02.005

Sundaresan NR, Saxena VK, Sastry KVH, Nagarajan K, Jain P *et al*., 2008. Cytokines and chemokines in postovulatory follicle regression of domestic chicken (*Gallus gallus domesticus*). Dev Comp Immunol, 32: 253-264, doi: 10.1016/j.dci.2007.05.011

Wheatley SP and McNeish IA, 2005. Survivin: A protein with dual roles in mitosis and apoptosis. Int Rev Cytol, 247: 35-88, doi: 10.1016/S0074-7696(05)47002-3

Yoshimura T, Yasuo S, Watanabe M, Ligo M, Yamamura T *et al*., 2003. Light-induced hormone conversion of T_4 to T_3 regulates photoperiodic response of gonads in birds. Nature, 426: 178-181, doi: 10.1038/nature02117

Received- 07.12.2023, Accepted- 26.10.2024, Published- 13.11.2024 (Online), 01.12.2024 (Print)
Section Editor: Dr. A. K. Patra, Associate Editor